# Wastewater treatment and valorization coupled with cyanobacterium *Synechococcus elongatus* PCC 7942 cultivation

Χρήση του κυανοβακτηρίου *Synechococcus elongatus* PCC 7942 στην επεξεργασία και αξιοποίηση υγρών αποβλήτων

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*This dissertation is submitted for the degree of*

*Doctor of Philosophy*

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We must trust to nothing but facts; these are presented to us by Nature and cannot deceive. We ought, in every instance, to submit our reasoning to the test of experiment, and never to search for truth but by the natural road of experiment and observation.

***Antoine Lavoisier***

**Acknowledgements**

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I dedicate this work to my father who I hope sees me from the heavens and be proud of his son.

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## List of Abbreviations and Acronyms

| Symbol | Description |
|---|---|
| UN | United Nations |
| EU | European Union |
| SDG | Sustainable Development Goals |
| IPCC | Intergovernmental Panel on Climate Change |
| GHG | Greenhouse gases |
| MDBs | Multilateral Development Banks |
| PBR | Photobioreactor |
| WWTP | Wastewater treatment plant |
| ATP | Adenosine triphosphate |
| ADP | Adenosine diphosphate |
| PAR | Photosynthetically active radiation |
| BG-11 | Blue-green cultivation medium |
| Chl *a* | Chlorophyll *a* |
| APC | Allophycocyanin |
| PC | Phycocyanin |
| *S*7942 | *Synechococcus elongatus* PCC 7942 |
| GAE | Galic acid equivalent |
| DW | Dry weight |
| AC | Activated carbon |
| CB | Carbon Black |
| CL | Cloisite 30B |
| BAT | Best available technologies, practices and techniques |
| AS | Activated sludge |
| BTWW | Biologically treated wastewater |
| DMF | N, N-dimethylformamide |
| UV | Ultraviolet |
| MCRT | Mean cell residence time |
| SRT | Solids retention time |
| HWW | Hydroponic wastewater |
| OHS | Open hydroponic system |
| CHS | Closed hydroponic system |

List of Symbols

| Symbol | Description | Units |
|---|---|---|
| TS | Total solids | mg $L^{-1}$ |
| TVS | Total volatile solids | mg $L^{-1}$ |
| TSS | Total suspended solids | mg $L^{-1}$ |
| VSS | Volatile suspended solids | mg $L^{-1}$ |
| $O.D._{750nm}$ | Optical density at 750 nm | AU |
| TKN | Total Kjeldhal Nitrogen | mg $L^{-1}$ |
| COD | Chemical oxygen demand | $mgO_2$ $L^{-1}$ |
| Cp | Cell productivity | mg VSS $L^{-1}$ $d^{-1}$ |
| $L_i$ | Light intensity | µmol-photons $m^{-2}$ $s^{-1}$ |
| NUR | Nitrates utilization rate | $mgNO_3$_N $L^{-1}$ $d^{-1}$ |
| PUR | Phosphates utilization rate | $mgPO_4^{3-}$ $L^{-1}$ $d^{-1}$ |
| SNUR | Specific nitrates utilization rate | $mgNO_3$_N $mgVSS^{-1}$ |
| SPUR | Specific phosphates utilization rate | $mgPO_4$ $mgVSS^{-1}$ |
| TVC | Total viable count | CFU $mL^{-1}$ |
| CT | Dosage in terms of concentration-time | mg min $L^{-1}$ |
| $RGR_{chl\ a}$ | Relative chlorophyll a growth rate | % |
| $RRR_{NO3\_N}$ | Relative nitrates removal rate | % |
| $RRR_{PO4\_P}$ | Relative phosphates removal rate | % |
| HRT | Hydraulic retention time | days |
| EC | Electric conductivity | mS $cm^{-1}$ |
| $BOD_5$ | 5-day biochemical oxygen demand | $mgO_2$ $L^{-1}$ |
| TP | Total phosphorous | mg $L^{-1}$ |
| $U_{DN}$ | Specific nitrate denitrification rate | $kgNO_3$_N $KgVSS^{-1}$ $d^{-1}$ |
| $Y_{max}$ | Maximum biomass yield coefficient | kgVSS $kgBOD_{removed}^{-1}$ |
| $K_d$ | Cell decay coefficient | $d^{-1}$ |
| MLVSS | Mixed liquor volatile suspended solids | mg $L^{-1}$ |
| $V_{DN}$ | Denitrification tank volume | $m^3$ |
| D.O. | Dissolved oxygen | mg $L^{-1}$ |
| $\lambda^o_+$ | Cation equivalent conductance | mho $cm^2$ $equivalent^{-1}$ |
| $\lambda^o_-$ | Anion equivalent conductance | mho $cm^2$ $equivalent^{-1}$ |

## Summary

Environmental conservation and climate change mitigation impose the need to investigate new routes for the treatment of wastewaters towards their valorization and mitigation of $CO_2$ emissions. Wastewater streams contain valuable resources, such as water and nutrients, which are expected to become increasingly scarce for the foreseeable future. Due to their composition, wastewaters can be used as substrate for the cultivation of cyanobacteria, a sustainable feedstock for the production of biofuels and added value products.

Cyanobacteria present higher photosynthetic activity than terrestrial plants and can remove/recover nitrogen and phosphorous from wastewater via assimilation into biomass, while fixating significant quantities of $CO_2$ from atmospheric air or flue gases. This creates the opportunity for the development of processes that can offer tertiary wastewater treatment (nutrients removal) and $CO_2$ emissions mitigation, while the generated biomass can be utilized in downstream valorization processes (Figure 1).

From the plethora of cyanobacteria species that can be potentially used for wastewater treatment and valorization, this work focuses on the cultivation of freshwater cyanobacterium *Synechococcus elongatus* PCC 7942 monocultures in various nutrient-rich wastewater media. *Synechococcus elongatus* PCC 7942 is a cyanobacterium that does not produce cyanotoxins and can be used as a more sustainable feedstock alternative for the production of fuels, food and chemicals. Furthermore, *Synechococcus elongatus* PCC 7942 can increase its intracellular sucrose synthesis when exposed to saline conditions present in some wastewater streams, hence it can be manipulated to produce added value biomass. The synthesized sucrose is a considered a valuable resource, as it can be utilized in various industrial applications, including bioethanol production. Moreover, sucrose can also be metabolically transformed by *Synechococcus elongatus* PCC 7942 to biohydrogen via anaerobic dark fermentation.

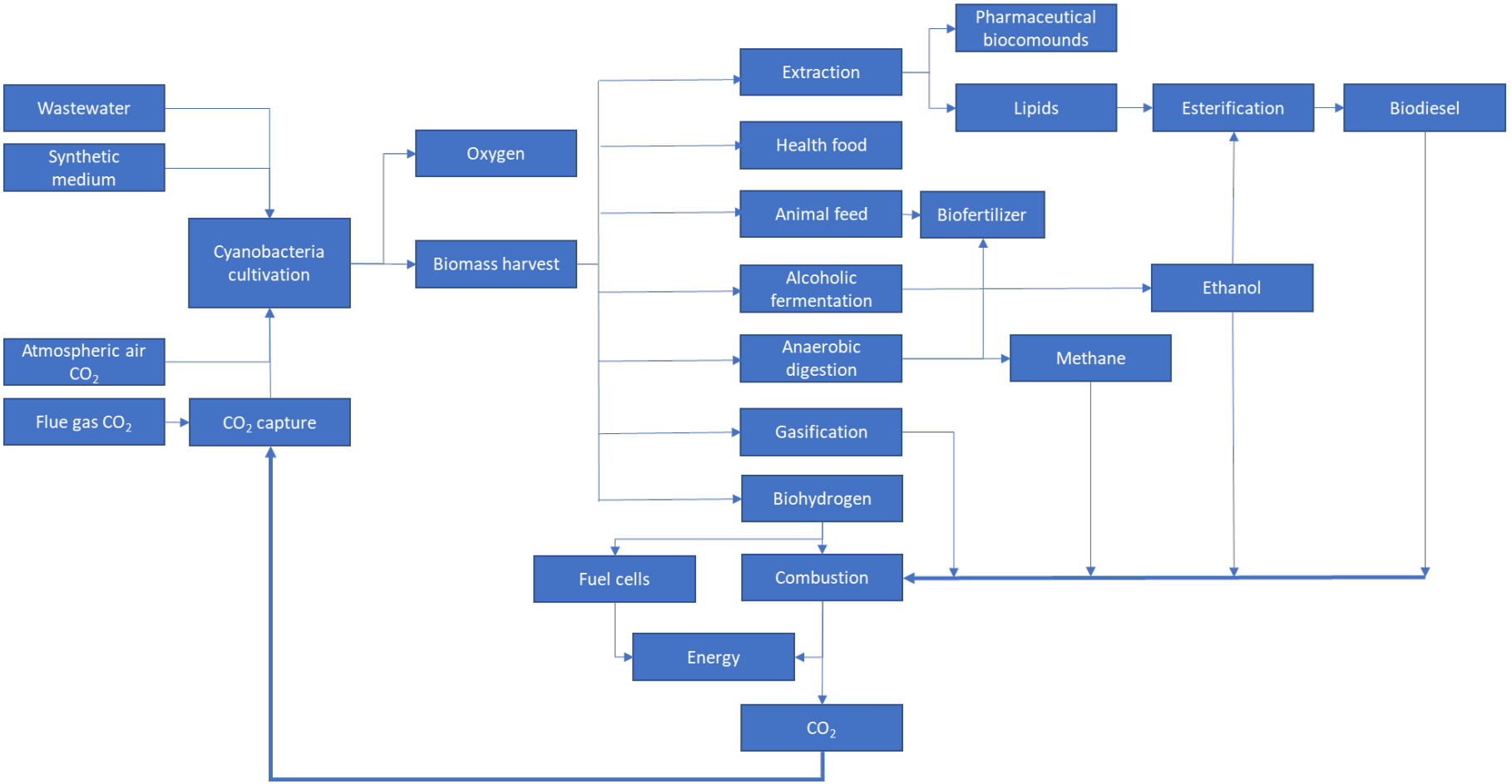


**Figure 1. S**chematic overview of wastewater treatment/valorization and $CO_2$ mitigation via cyanobacteria cultivation.

However, cultivation of cyanobacteria in wastewater substrate and maintenance of monoculture constitutes a challenge for full scale application since both physicochemical parameters (temperature, nutrients depletion, etc.) and biological parameters (antagonistic species, predation etc.) may hinder culture growth. Moreover, the lack of extensive literature regarding the use of cyanobacteria for wastewater treatment/valorization and especially the lack of fundamental design and operational parameters for cyanobacteria-based processes renders their scaling up and implementation a difficult task.

In this regard, an effort was made in this work (a) to propose proper low-cost disinfection techniques for the utilization of wastewater in the production of valuable cyanobacterial biomass, (b) to assess *Synechococcus elongatus* PCC 7942 adequacy for the treatment of secondary (biologically) treated industrial wastewater substrates (c) to provide critical design and operational data for the implementation of *Synechococcus elongatus* PCC 7942-based wastewater treatment process, as a supplementary or alternative tertiary wastewater treatment stage (d) to demonstrate the applicability of a *Synechococcus elongatus* PCC 7942-based wastewater treatment stage as a component for sustainable treatment and reuse of wastewaters that cannot be secondary treated due to the lack of organic constituents.

In the first series of experimental setups, an attempt was made to propose efficient and low-cost wastewater media disinfection techniques for cyanobacteria cultivation. The disinfection techniques of filtration and chemical disinfection using NaClO or $H_2O_2$, as well as the novel and considered environmentally friendly disinfection method that is based on in-situ electrosynthesis of hexavalent iron species (ferrates), were evaluated individually or as a synergetic couple by measuring the removal of total viable count at 22ºC (TVC). TVC is an indicative parameter for the presence of biological contaminants in an aqueous medium. The effect of filtration medium characteristics, of chemicals dosage and contact time were assessed as a guideline for the selection of filtration medium or/and chemicals' dosage in full scale cyanobacteria cultivation applications, with special emphasis on the multilevel action of ferrates as disinfectant, as oxidant of organic compounds and as coagulant for the removal of turbidity and harvesting of cultivated biomass.

The results showed that efficient disinfection of wastewater media for unhindered *Synechococcus elongatus* PCC 7942 proliferation can be achieved by coupling filtration at cutoff size ≤ 1.2 μm with chemical disinfection, preferably using ferrates produced via a $Fe^0$/ $Fe^0$ electrochemical cell in highly basic solution and by applying a dosage in terms of concentration-time (*CT*) ≥ 157 mg min $L^{-1}$. Ferrates presented higher disinfection efficiency compared to the commonly used chemical disinfectants NaClO and $H_2O_2$ (Figure 2), while assisting in the removal of organic compounds (71-84% COD removal) and turbidity (89-97% removal) from wastewater media. Lowering turbidity increases light transfer efficiency in a photobioreactor (PBR), thus photosynthetic activity and biomass production. The respective NaClO and $H_2O_2$ dosage in the filtrated wastewater media for complete disinfection are *CT* ≥ 270 mg min $L^{-1}$, significantly higher than that of ferrates.

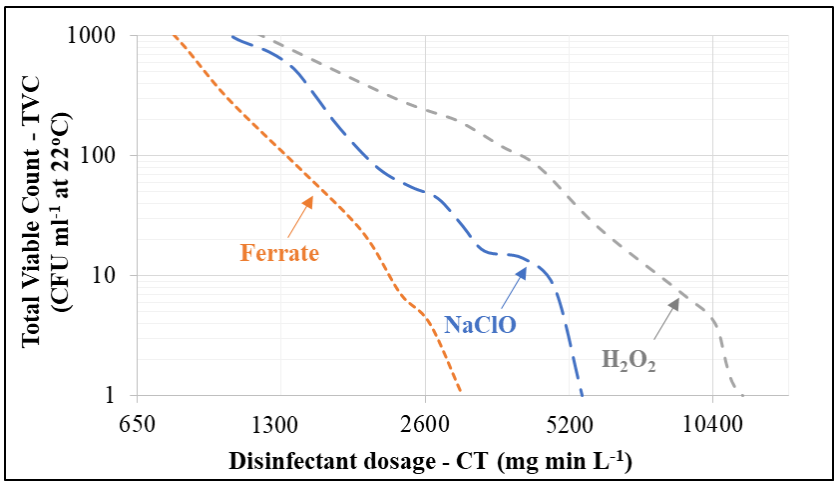


**Figure 2.** Disinfection efficiency of sodium hypochlorite, hydrogen peroxide and ferrates in terms of TVC removal at different disinfectant dosages (*CT*).

Neither the technique of filtration nor that of chemical disinfection alone could address the challenge of maintaining a *Synechococcus elongatus* PCC 7942 monoculture in the PBR. A combined application of filtration followed by chemical disinfection proved efficient for maintaining a *Synechococcus elongatus* PCC 7942 monoculture. Disinfection efficiency presented moderate positive correlation (Corr. Coeff. = 0.611) with pore-size, having high statistical significance ($p = 0.020$), as well as moderate to very strong negative correlation (Corr. Coeff. = -0.700) with filter thickness, having very high statistical significance ($p = 0.005$). It is thus concluded that filter thickness, which affects filtration duration, has a greater effect on disinfection efficiency than that of filtering media's pore size. Thus, in full scale applications, an ultrafiltration

configuration or a slow sand filtration technique could be used as a low-cost preliminary disinfection process for the significant minimization of biological contaminants in wastewater-media. The followed chemical disinfection stage requires the lowest dosages of used chemicals. An illustration of the proposed downstream process for secondary wastewater treatment and valorization is presented in Figure 3.

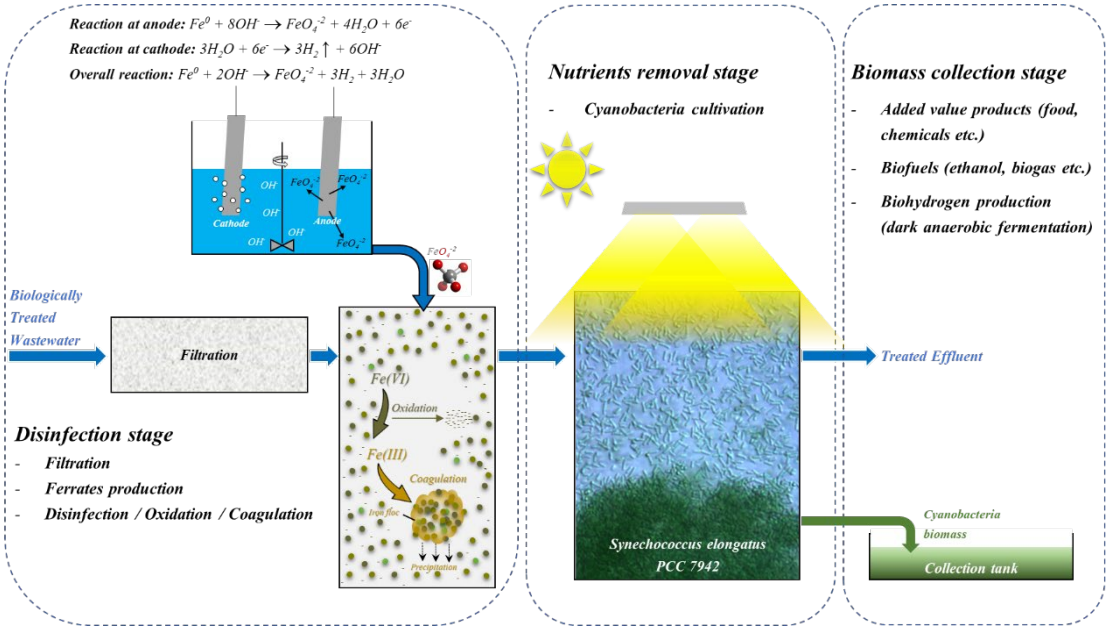


**Figure 3.** Proposed wastewater substrate disinfection process for cyanobacteria cultivation and tertiary treatment applications.

In the second series of experimental setups, properly disinfected high strength wastewater of different composition that had been subjected to aerobic biological treatment were used for the assessment of *Synechococcus elongatus* PCC 7942's adequacy as a biological component for sustainable wastewater treatment applications. The growth rate of *Synechococcus elongatus* PCC 7942 was evaluated in relation to cultivation temperature, in order to identify the temperature thresholds for its efficient cultivation.

Moreover, the impact of cultivation duration i.e., of hydraulic residence time (HRT), on growth and nutrients assimilation rates was evaluated. Valuable correlation coefficients (conversion factors) between the parameters that can express biomass concentration (chlorophyll *a*, total suspended solids, volatile suspended solids and optical density at 750 nm) were experimentally obtained. They are of great importance in the assessment, interpretation and implementation of research results by scholars and engineers, as well as for the dimensioning and adoption of the proposed technology.

Figure 4 ilustrates the linear correlations between chlorophyll *a* (Chl *a*), total suspended solids (TSS), volatile suspended solids (VSS) and optical density at 750 nm of *Synechococcus elongatus* PCC 7942 cultures in BG-11 media.

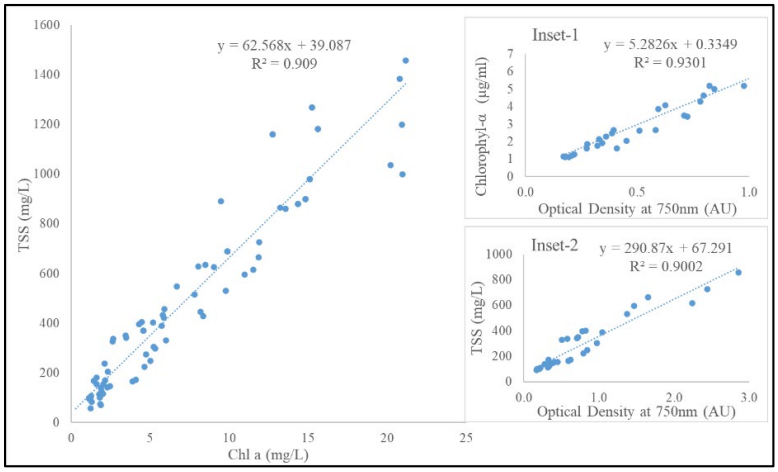


**Figure 4.** Correlation between parameters expressing biomass concentration of *Synechococcus elongatus* PCC 7942 cultures in BG-11 media

The obtained conversion factors and correlation coefficients from the study of *Synechococcus elongatus* PCC 7942 cultivation setups are presented in Table 1.

**Table 1.** Experimentally obtained conversion factors and correlation coefficients.

| **Pair** | **Value** | **Unit** |
|---|---|---|
| Chl *a* to TSS | 62.57 | mgTSS mgChl $a^{-1}$ |
| Chl *a* to VSS | 56.31 | mgVSS mgChl $a^{-1}$ |
| TSS to VSS | 0.90 | mgVSS $mgTSS^{-1}$ |
| $O.D._{750nm}$ to Chl *a* | 5.28 | mgChl *a* $AU^{-1}$ |
| $O.D._{750nm}$ to TSS | 290.87 | mgTSS $AU^{-1}$ |

*AU = absorbance unit*

*Synechococcus elongatus* PCC 7942 cultures in BG-11 media was assessed as a potent biological component for novel and sustainable biological nutrient removal processes for industrial wastewater treatment and valorization, as it can efficiently proliferate:

- in properly disinfected industrial wastewaters that have been subjected to biological treatment
- at a wide temperature range (16-32°C)
- at relatively high salinities of approximately 0.2 M NaCl

This is evident in Figure 5, where the average relative growth rate ($RGR_{Chl\ a}$) evolution in *Synechococcus elongatus* PCC 7942 cultures with BG-11 media, with treated, relatively saline (0.2 M NaCl) dairy wastewater and treated snack wastewater at 20°C

to 27ºC are presented. The impact of temperatures from 16ºC to 37ºC on $RGR_{Chl\ a}$ is illustrated in the inset of Figure 5.

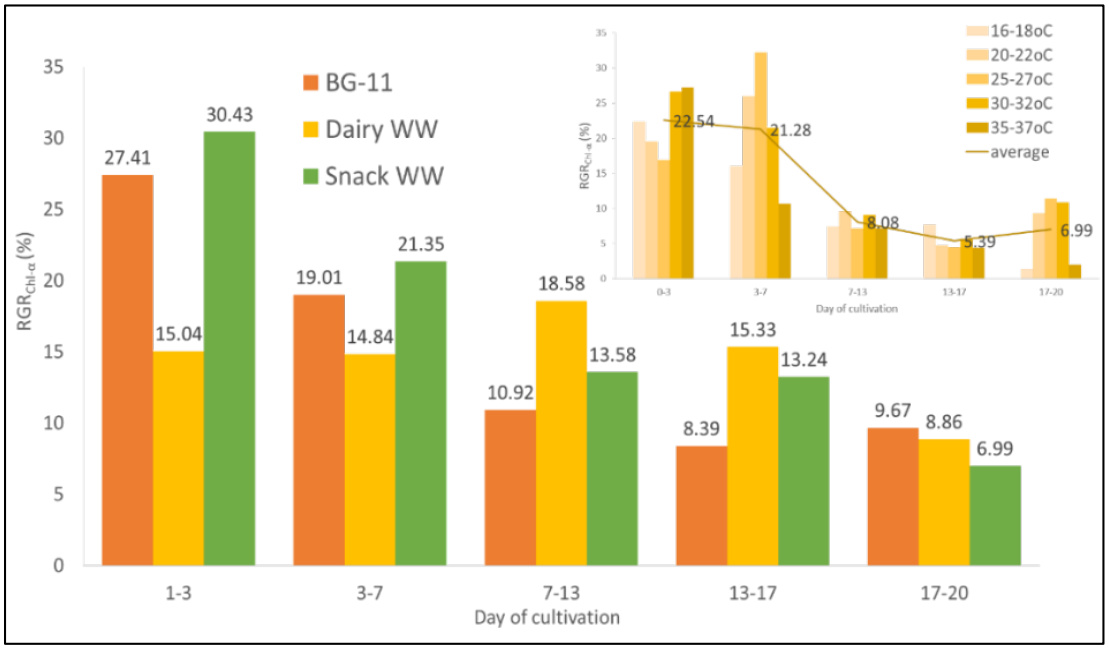


**Figure 5.** *Synechococcus elongatus* PCC 7942's $RGR_{Chl\ a}$ in relation to wastewater composition, temperature and cultivation duration.

The observed in Figure 5 lag in the growth rate maxima of *Synechococcus elongatus* PCC 7942 cultivation setups with dairy wastewater (Dairy WW) is attributed to species' acclimatization to saline conditions. Those initialy low $RGR_{Chl\ a}$ values are compensated by the reatarded growth maxima that is observed during the second week of cultivation.

The relative growth rate of all *Synechococcus elongatus* PCC 7942 cultures decreased over time regardless the growth media and the culture temperature, which is attributed to the obstruction of photosynthesis due to increased optical density in the PBRs and the subsequent intermittent flux of light as a result of mutual shading. This means that while increasing hydraulic residence time (HRT) i.e., PBR volume, leads to higher biomass yields, the efficiency of cultivation drops as an effect of increased culture density. Thus, the cultivation duration is managed by wastewater characteristics, PBR design and lighting configuration and must be carefully selected at case.

The obtained results regarding the optimal operating temperature and salinity tolerance for *Synechococcus elongatus* PCC 7942 cultivation are considered suitable towards advantageous full-scale implementation of *Synechococcus elongatus* PCC 7942-based

wastewater treatment technologies, since a plethora of wastewater streams have temperatures and salinities at these ranges. However, a balance between efficient nutrient removal/recovery, biomass production and PBR volume has to be established, which is highly affected by the effect of cultivation duration on *Synechococcus elongatus* PCC 7942's growth rate and its nutrients assimilation rate. In this regard, not only the effect of cultivation period on biomass yields was evaluated, but its effect on nitrates removal by assimilation was additionally studied. Figure 6 illustrates the average (a) relative nitrates removal rate ($RRR_{NO3\_N}$) and (b) $RGR_{Chl\,a}$ of *S*7942 cultures, for the first week of cultivation and the overall duration of cultivation (20 days). It is evident that $RRR_{NO3\_N}$ and $RGR_{Chl\,a}$ are similar or even greater in experimental setups with wastewater as growth media indicating that the properly disinfected, biologicaly treated wastewaters from activated sludge processes provide all the necessary nutrients for *Synechococcus elongatus* PCC 7942's cultivation.

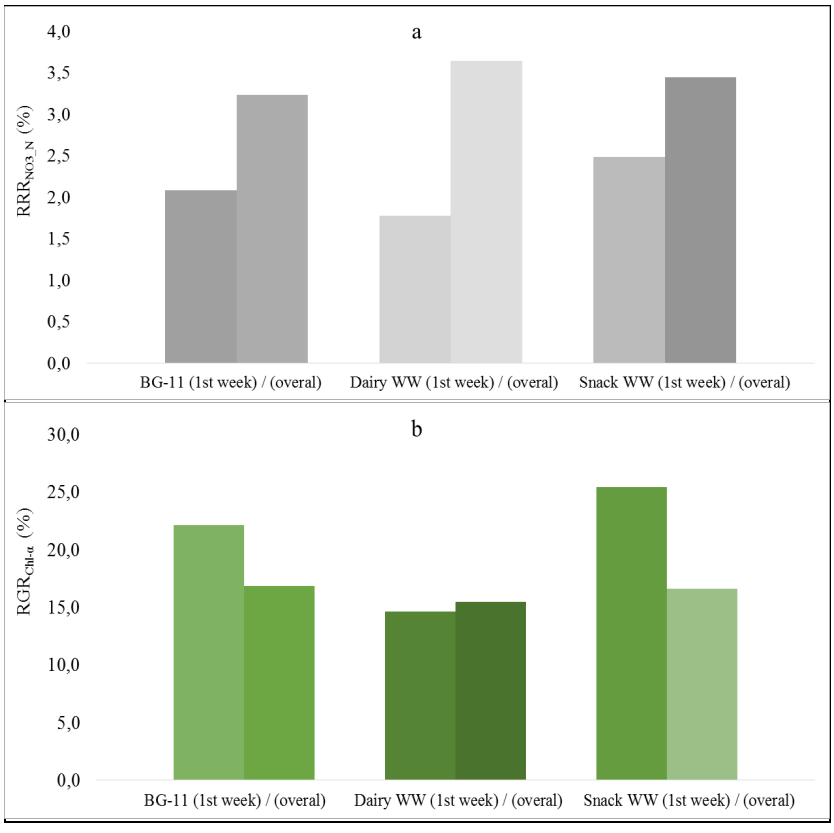


**Figure 6.** $RRR_{NO3\_N}$ (a) and $RGR_{Chl\,a}$ (b) of *Synechococcus elongatus* PCC 7942 test setups and control setups for a week and 20 days of cultivation.

An opportunity to elevate sustainability of WWTPs via implementation of a *Synechococcus elongatus* PCC 7942-based nutrient removal stage, individually or as a synergetic couple with denitrification process, is at site, since according to the

comparative results between PBR and denitrification tank volume requirements, both volumes could be in the same order of magnitude.

However, the lack of fundamental design and operational parameters hinders development, upscaling and implementation of cyanobacteria processes that are in compliance with circular economy and sustainability principles. In this regard, further study was conducted for dimensioning of a *Synechococcus elongatus* PCC 7942 cultivation PBR for different salinity wastewaters. The effect of salinity was examined in terms of its impact on cell productivity and nutrients assimilation rate, since both parameters affect PBR volume. Moreover, the salinity threshold of cultivation media may have significant implications regarding process' applicability region, as well as its viability in terms of increased value of obtained phototrophic biomass. The response of cyanobacteria into stressors, such as salinity and nutrients availability can trigger metabolic responses that increase the intracellular assimilation of valuable products (metabolites) that can be used in green energy production, such as direct biohydrogen production, as well as in other economic sectors.

The study revealed that cultivation of cyanobacterium *Synechococcus elongatus* PCC 7942 can serve as tertiary treatment for nitrogen and phosphorous removal/recovery from wastewaters with salinities as high as 0.45 M NaCl offering the possibility of enhanced valorization of wastewater and of the produced biomass. At increased wastewaters salinities up to the experimentally obtained salinity threshold value of 0.45 M NaCl, despite the observed decline of cell productivity, nitrates removal rate remains constant, which is attributed to the metabolic changes of *Synechococcus elongatus* PCC 7942 leading to higher nitrogen assimilation in biomass. Figure 7 ilustrates *Synechococcus elongatus* PCC 7942's nitrates removal rate, specific nitrates utilization rate (*SNUR*), doubling time and biomass nitrogen content in relation to salinity levels of growth medium, which were set up to 600 mmol $L^{-1}$ (sea water salinity levels).

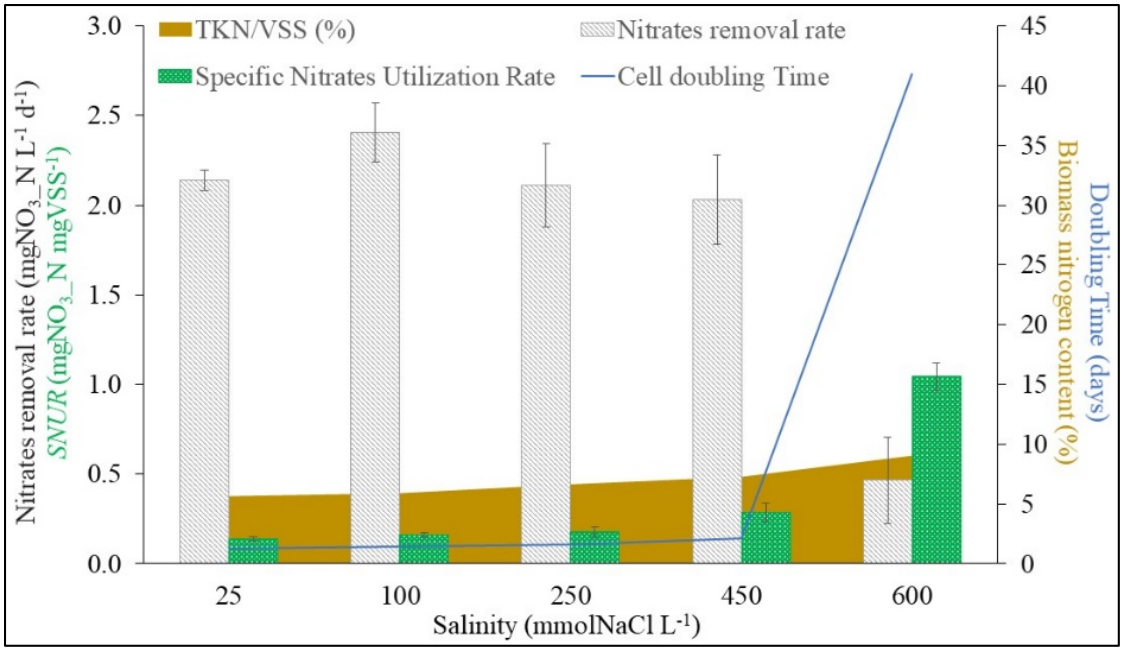


**Figure 7.** Effect of salinity on *Synechococcus elongatus* PCC 7942 proliferation, nitrates removal and assimilation.

As evident in Figure 7, salinity negatively affects cell productivity up to the point of completely inhibiting *Synechococcus elongatus* PCC 7942's proliferation at salinities over 450 mmolNaCl $L^{-1}$. Nonetheless, up until the salinity threshold of 450 mmolNaCl $L^{-1}$, nitrates removal is relatively steady as a result of increased nitrogen assimilation in biomass, which is expressed as % TKN $VSS^{-1}$ or *SNUR*.

The higher *SNUR* values that are observed with increasing salinity, which along with cell productivity is one of the two experimentally obtained parameters for PBR dimensioning according to the proposed formula (Equation (1)), compensates the lower cell productivity observed at saline conditions (Figure 8) in a way that the PBR volume remains relatively constant regardless of salinity (volume change < 12 %). On the other hand, in the widely applied activated sludge (AS) denitrification processes the respective volume increases up to 260% analogously to salinity.

$$V_{PBR} = \frac{N\_Denitr}{SNUR * cell\ productivity} \quad (1)$$

Where: $V_{PBR}$ is the photobioreactor volume ($m^3$); *N_Denitr* is the daily nitrate-nitrogen load ($kgNO_3\_N\ d^{-1}$); *SNUR* is the specific (nitrate) nitrogen utilization rate ($kgNO_3\_N\ kgVSS^{-1}$) and *cell productivity* ($kgVSS\ m^{-3}\ d^{-1}$).

Figure 8 illustrates the effect of salinity on *cell productivity* of *Synechococcus elongatus* PCC 7942 in BG-11 growth medium (control setups) or in properly disinfected wastewaters from industrial wastewater treatment plant (test setups).

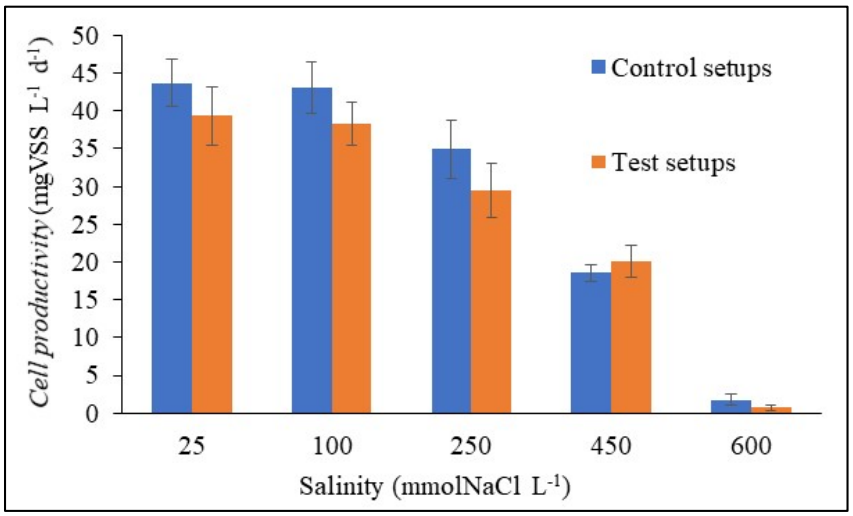


**Figure 8.** Impact of salinity on *Synechococcus elongatus* PCC 7942 cell productivity.

It is concluded that PBR volume can be at the same order of magnitude to an activated sludge denitrification reactor given an optimal *cell productivity* and favorable light intensity. Even at non-favorable growth conditions of limited lighting (5-30 μmol $m^{-2}$ $s^{-1}$) the resulting PBR volume in comparable to activated sludge denitrification reactors.

The salinity induced metabolic changes of *Synechococcus elongatus* PCC 7942 that lead to increase of *SNUR* are attributed to higher protein synthesis for the production of intracellular osmolyte (sucrose). The enrichment in sucrose content enhances biomass use as a raw material both for the industrial sector and the renewable energy sector. Therefore, a *Synechococcus elongatus* PCC 7942-based process can be adapted as a supplementary or an alternative treatment stage to the widely applied activated sludge denitrification processes.

Moreover, a *Synechococcus elongatus* PCC 7942-based treatment stage could be used for the treatment of wastewaters that do not contain the necessary quantities of organic carbon for biological treatment in activated sludge treatment plants (BOD:N:P ratio of 100:5:1), such as those from hydroponic farms (HWW). A *Synechococcus elongatus* PCC 7942-based treatment stage can be a component of integrated management systems that are based on the principles of circular economy. Such a system was conceptualized and studied regarding the treatment of wastewater from hydroponic

farms. These wastewater streams may constitute another substrate for cyanobacteria cultivation and the production of added value products or/and bioenergy, whereas if not properly managed they can become a serious threat for the environment.

The proposed solution for the integrated management of HWW is based on (i) the reuse of HWW for the preparation of new feeding solution via an Electric Conductivity (EC)-control tool that rapidly quantifies chemicals dosage for enrichment, (ii) the treatment/utilization of HWW for the cultivation of cyanobacteria in a PBR and (iii) the advanced treatment of HWW or PBR effluents for reuse or disposal in the environment (Figure 9).

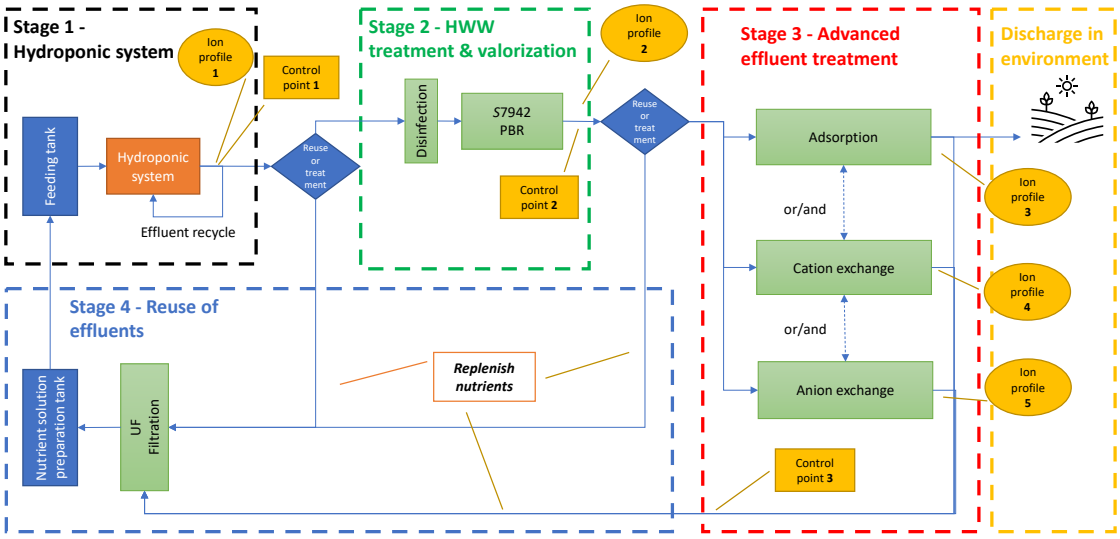


**Figure 9.** Flowchart of proposed EC-controlled process for integrated management of hydroponic wastewater.

In more detail, the hydroponic drainage is recycled to the feeding tank up until the EC-control tool indicates increased presence of plant growth inhibiting ions. Then, recycle ceases, ions concentration is estimated via EC and effluent is either used in the preparation of new nutrient solution or is treated/utilized in *Synechococcus elongatus* PCC 7942 cultivation PBRs. When the recycled and utilized effluents can no longer used in the preparation of new nutrient solution i.e., when the concentration of hindering ions in the nutrient solution preparation tank is estimated to exceed the set threshold values, then advanced treatment is applied for complete water recycle.

The results showed that properly disinfected via filtration/chemical disinfection HWW from open or closed hydroponic system (OHS or CHS) constitute a potent cultivation substrate for unhindered and efficient *Synechococcus elongatus* PCC 7942 proliferation

(Figure 10). Figure 10 illustrates the similar average cell productivity (*Cp*) values and the corresponding standard deviations of the studied control setups (BG-11 and diluted 1:2 BG-11 media) and test setups with properly disinfected OHS and CHS HWW.

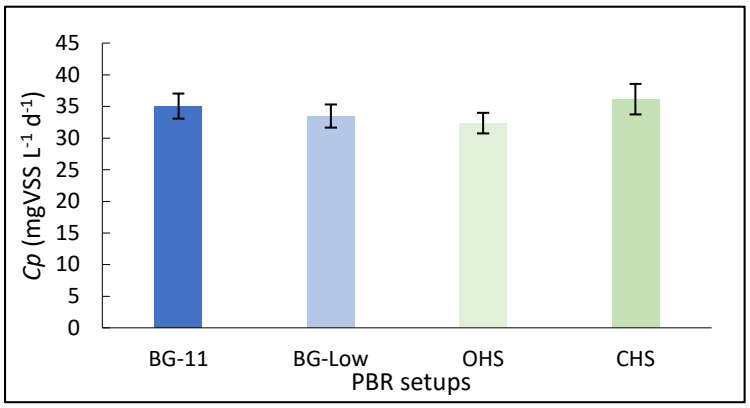


**Figure 10.** Average cell productivity (Cp) values and corresponding standard deviations of control setups and test setups with properly disinfected HWW.

Hydroponic wastewater substrates proved to be an efficient medium for *Synechococcus elongatus* PCC 7942 cultivation. Additionally, in the case of CHS's effluent its higher nutrient content triggered higher nitrogen assimilation rate and higher phosphorous assimilation rate (Figure 11), expressed as specific nitrate or phosphate utilization rates (*SNUR*, *SPUR*).

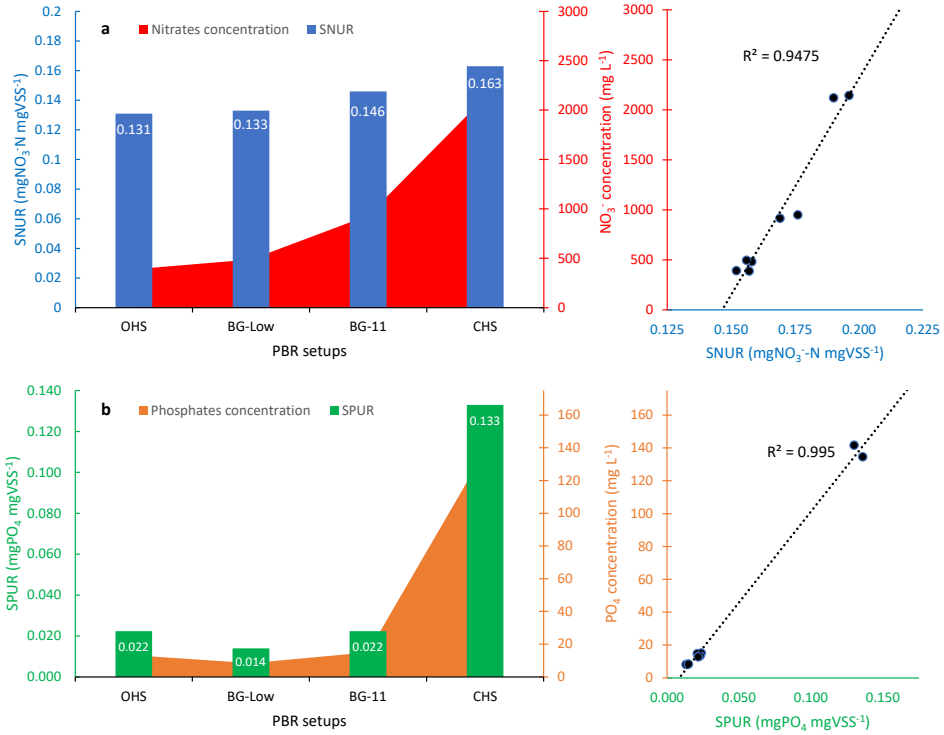


**Figure 11.** Specific nitrates utilization rate (SNUR) and specific phosphates utilization rate (SPUR) in relation to nitrates and phosphates concentration in experimental setups.

The higher the initial nitrates and phosphates concentrations the higher the assimilation of nutrients in the *Synechococcus elongatus* PCC 7942 biomass. *SNUR* has significant impact on PBR volume, since nitrogen is the predominant nutrient in wastewaters. The use of the proposed EC-control tool coupled with cyanobacteria or/and microalgae cultivation as a biological component for tertiary wastewater treatment applications, constitutes the basic stages for hydroponic drainage wastewaters' integrated management.

*Synechococcus elongatus* PCC 7942 cultivation can be incorporated in most high strength and high salinity wastewater treatment plants. PBR dimensioning must take into account nutrients assimilation rate. PBR volume calculation is a necessary step for the design and implementation of *Synechococcus elongatus* PCC 7942 cultivation. The increase of cell productivity in relation to PBR design characteristics and operating conditions must be further studied.

It is concluded that *S*7942 cultivation coupled with wastewater treatment can present an innovative nutrients removal stage with high potential for commercial and energy valorization of high salinity and/or high nutrient load wastewater. The proposed photobioreactor sizing methodology, which follows the design philosophy of activated sludge systems, can assist in the development, design and full-scale implementation of such innovative wastewater treatment and utilization stages following the principles of circular economy and sustainable development. Improving cell productivity by controlling culture conditions and exploiting intracellular sucrose of *S*7942 for the production of bio-hydrogen and/or other biofuels and/or chemicals, as well as co-processing of flue gases for $CO_2$ capture/utilization are the next challenges that should be addressed.

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# 1. Chapter 1. Introduction

## 1.1. Environmental conservation

Environmental conservation constitutes one of the greatest challenges of humankind in the 21st century, towards a sustainable future. More than half of the Sustainable Development Goals (SDG) of the 2030 Agenda for Sustainable Development (UN General Assembly, 2015), which has been adopted by all United Nations Member States, have an environmental focus or address the sustainability of natural resources (UN General Assembly, 2018). By taking into consideration the fact that the progress of one of the SDG (Figure 1.1) is inexplicably linked to the progress of another, it can be concluded that environmental conservation is a cornerstone of sustainable development.


**Figure 1.1.** Sustainable Development Goals of the 2030 Agenda for Sustainable Development. Source: UN Department of Global Communications (2020)

Therefore, all disciplines and sciences and by extension universities have the responsibility of producing knowledge that allows humans to lessen the impact of their lifestyle and even to modify the civilising logic that has caused environmental problems (Estermann, 2012), which are also social problems (Nunez and Moreno, 2016). The two major aspects of environmental conservation that must be thoroughly studied are (i) the control of pollution and mitigation of anthropogenic impacts on air, soil and water quality characteristics, as well as (ii) the sustainable consumption of natural resources, which both affect biodiversity and human health.

The increasing needs for food, energy and natural resources on a global scale increase the pressure on the environment by means of air, soil and water quality deterioration or/and depletion of resources. The adverse effect of environmental degradation on climate, resources availability and biodiversity has forced governments to take actions towards environmental conservation and sustainable development. That been said, international treaties and resolutions (Paris agreements, 2015; UN General Assembly, 2018) have been adopted by most countries to address the challenge of environmental conservation and sustainable development, with emphasis on (i) climate change mitigation, (ii) conservation, recovery, reuse of natural resources and (iii) green energy and sustainable food production.

## 1.2. Climate change and GHG emissions mitigation

According to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC, 2022), there are ever closer linkages between climate change mitigation, development pathways and the pursuit of sustainable development goals (Figure 1.1, since development pathways largely drive GHG emissions and hence shape the mitigation challenge and the portfolio of available responses.

In order to assess the impact of anthropogenic activities on GHG emissions and evaluate the response to the mitigation challenge, global GHG emissions sources are usually categorized to five broad sectors: (i) energy systems for electricity and heat production, (ii) industry, (iii) buildings, (iv) transportation and (v) agriculture, forestry and other land uses (AFOLU). The contribution of each sector on global and EU GHG emissions is illustrated in Figures 1.2 and 1.3 respectively.

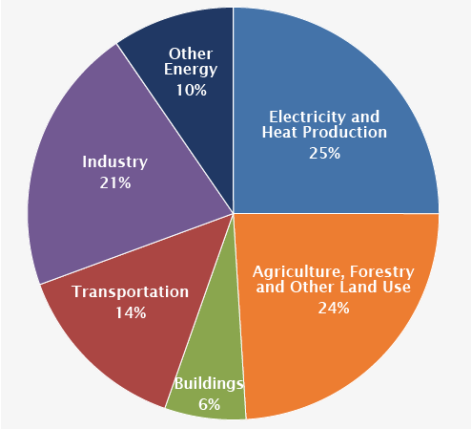


**Figure 1.2.** Global GHG emissions by economic sector based on global emissions from 2010. Source: IPCC (2014)

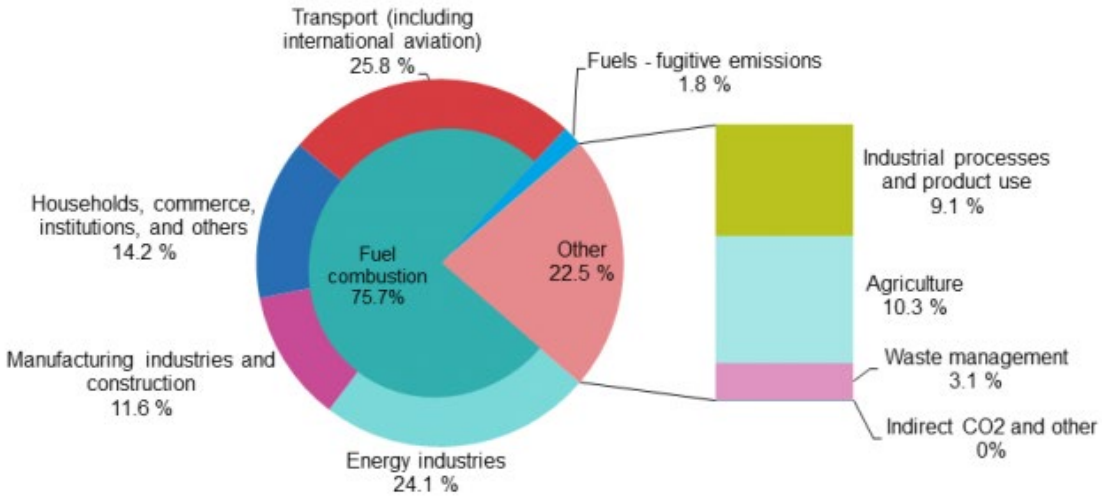


**Figure 1.3.** GHG emissions by economic sector in EU based on emissions from 2019. Source: Eurostat (2021)

All these sectors are responsible for the large increase in anthropogenic GHG emissions during the past century and each sector encounters its own challenges in terms of climate change mitigation. To elucidate, GHS emissions in energy systems sector are emitted in highly polluting units and the emissions are dominated by fossil fuels combustion; in transport and building sector are more diffuse and spread across many actors; in industry sector are linked to the production of metals, chemicals, cement and other basic materials demanded by economies (Lamb et al., 2021). Worth mentioning that the IPCC has defined Waste and Wastewater as a separate sector, while in the USEPA's Sources of Greenhouse Gas Emissions page (2021), waste and wastewater emissions are attributed to the Commercial and Residential sector. According to IPCC (2022) report, the management of waste generated in the food system (including food waste, wastewater, packaging waste etc.) leads to biogenic GHG emissions and contributed $GtCO_2$-eq $yr^{-1}$ to food systems' GHG emissions in 2018. Of these emissions, 55% were from domestic and commercial wastewater (30 $MtCH_4$ $yr^{-1}$ and 310 $ktN_2O$ $yr^{-1}$), 36% from solid waste management (20 $MtCH_4$ $yr^{-1}$ 17 and 310 $ktN_2O$ $yr^{-1}$), and 8% from industrial wastewater (4 $MtCH_4$ $yr^{-1}$ and 80 $ktN_2O$ $yr^{-1}$). Emissions from waste incineration and other waste management systems contributed 1%.

The IPPC (2022) report presented evidence which show that countries can grow their economies while reducing GHS emissions. That been said, the main GHG mitigation options for achieving the goal of limiting the global temperature increase to 1.5 ˚C and

2 ˚C above pre-industrial levels (Paris agreements, 2015) are reduction/prevention, sequestration and substitution (Table 1.1.).

**Table 1.1.** GHG mitigation options (IPCC, 2014).

| **GHG mitigation option** | **Description** |
|---|---|
| reduction/prevention | Reduction/Prevention of emissions to the atmosphere by conserving existing carbon pools in soils or vegetation that would otherwise be lost or by reducing emissions of $CO_2$, $CH_4$ and $N_2O$ |
| sequestration | Enhancing the uptake of carbon in terrestrial reservoirs and thereby removing $CO_2$ from the atmosphere |
| substitution | Reducing $CO_2$ emissions by substitution of biological products for fossil fuels or energy-intensive products. Demand-side options (e.g., by lifestyle changes, reducing losses and wastes of food, changes in human diet, changes in wood consumption) |

In general, there are three conceptually different modes of atmospheric $CO_2$ reduction, which include the reduction of fossil fuel consumption, the removal of $CO_2$ from the atmosphere and $CO_2$ capturing (Benemann, 1997). Well established climate change mitigation approaches that are employed and carry an acceptable level of managed risk include decarbonization technologies and techniques that reduce $CO_2$ emissions (Figure 1.4), such as renewable energy, fuel switching, efficiency gains, nuclear power, and carbon capture storage and utilization (Shinnar and Citro 2008; Ricke et al., 2017; Victor et al., 2018; Bataille et al., 2018; Mathy et al., 2018; Bustreo et al., 2019; Fawzy et al., 2020). Moreover, novel technologies and techniques that capture and sequester $CO_2$ from the atmosphere, referred as negative emission or as $CO_2$ removal technologies, have been development during the past decades, such as bioenergy carbon capture and storage, biochar, enhanced weathering, direct air carbon capture and storage, ocean fertilization, ocean alkalinity enhancement, soil carbon sequestration, afforestation and reforestation, wetland construction and restoration, as well as mineral carbonation and the use of biomass in construction (McLaren, 2012; McGlashan et al., 2012; Ricke et al., 2017; Lawrence et al., 2018; Lenzi, 2018; Palmer, 2019; Yan et al., 2019; Lin, 2019; Pires, 2019; Goglio et al., 2020).

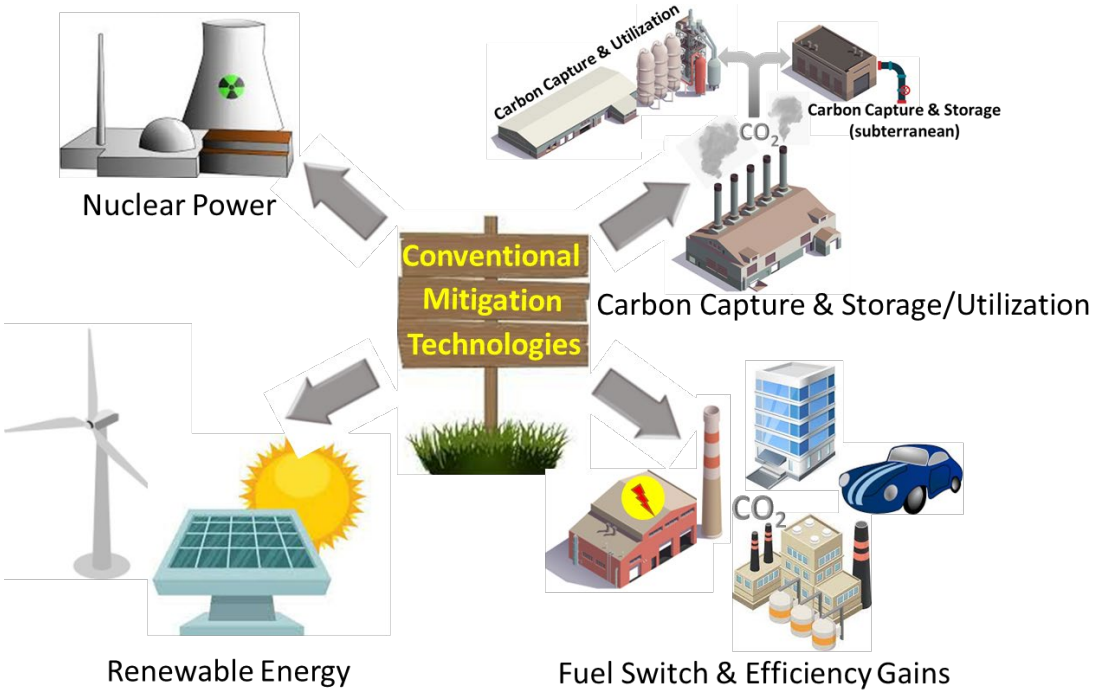


**Figure 1.4.** Major decarbonization technologies that focus on the reduction of $CO_2$ emissions related to the supply and demand sides of energy. (Modified from Fawzy et al., 2020)

At the international level, Finance from Multilateral Development Banks (MDBs) is a major source of GHG mitigation finance in developing countries (MDB, 2015; Ha et al., 2016; Bhattacharya et al., 2018). Sectorwise, the MDBs mitigation finance for 2018 is allocated to renewable energy (29%), transport (18%), energy efficiency (18%), lower-carbon and efficient energy generation (7%), agriculture, forestry and land use (8%), waste and wastewater (8%), and other sectors (12%) (MDB, 2019). According to IPCC (2022), at the national level, applied research has shown that integrated modelling of land, energy and water resources not only has the potential to identify superior solutions, but also reveals important differences in terms of investment requirements and required financing arrangements compared to the traditional sectorial financing toolkits (Welsch et al., 2014).

Of the existing technologies, processes, techniques for removing and capturing $CO_2$ emissions, particular interest has been given to those based on the cultivation of cyanobacteria or/and microalgae. Bio-mitigation of $CO_2$ emissions with microalgae and especially with cyanobacteria provides a complementary function that may moderate the cost of biofuels production and of industrial relevant products (Bothe, 1982; Mata

et al., 2010; Singh et al., 2014; Lau et al., 2015, Chittora et al., 2020), while the use of waste $CO_2$ from power plants and other industries to enhance production of phototrophic biomass has been shown to be technically feasible, and hence, may be deployed to reduce production costs and for GHG emission control (Razzak et al., 2013; Fawzy et al., 2020). The cultivation of cyanobacteria is preferable to other phototrophic species due to their increased tolerance to high $CO_2$ concentrations and increased carbon fixation rates (Viswanaathan et al., 2022), their enhanced light energy utilization efficiency (Khan et al., 2018), as well as due to their content in valuable metabolites, such as fatty acids, carbohydrates, phicobiliproteins and polyhydroxybutyrates (Robles et al., 2020), which are easier to obtained due to the softer cellular wall of cyanobacteria for their utilization in industrial and energy sector (Arias et al., 2017).

It is worth mentioning at this point that according to life cycle analysis, biofuel from cyanobacteria or/and microalgae biomass is identified as one of the major renewable energy sources for sustainable development, with potential to replace the fossil-based fuels (Medipally et al., 2015) via production of biofuels, such as biohydrogen, biodiesel, bioethanol, biomethanol (Robles et al., 2020). In this regard, the production of biohydrogen from cyanobacteria biomass via intracellular fermentative conversion has gained great interest during the last decade, since it can be used as a low carbon source for heat and electricity production, as well as an efficient transportation fuel (Mishra, et al., 2019). Moreover, phototrophic biomass and cyanobacteria biomass in particular, is considered a renewable feedstock for obtaining a plethora of biopharmaceutical and nutraceutical products, such as pigments, fatty acids, proteins, vitamins (Trivedi et al., 2015; Carrido-Cardenas et al., 2018; Khan et al., 2018).

### 1.3. Resources conservation, reuse, recovery

Responding to climate change is one series of measures for achieving environmental conservation and sustainable development. Additionaly, the conservation of natural resources constitutes another critical factor towards sustainability, which during the past decade has been approached following the practitioner-led principles of circular economy (Figure 1.5).

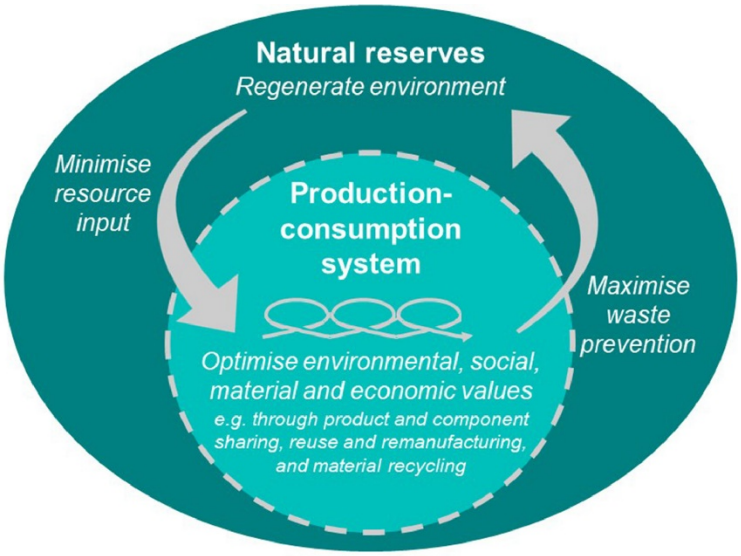


**Figure 1.5.** Circular economy principles. Source: Velenturf and Purnell (2021)

It is widely established that the depletion of natural resources on a global scale and the associated carbon emissions continues to accelerate while paradoxically mountains of waste (and associated pollution) are still piling up (Velenturf and Purnell, 2017), and it is hence logical that circular economy strives to minimize resource exploitation and maximize waste prevention (Figure 1.5).

Of the IPCC economic sectors, the wastewater treatment subsector presents significant opportunities for the application of technologies that are based on circular economy principles. Wastewaters contain water, valuable nutrients (nitrogen and phosphorous), micronutrients (metallic ions) and chemical energy (Hoek et al., 2016). Their recovery, recycle and utilization via nutrient removal/recovery technologies is a promising strategy for reducing the depletion of non-renewable resources and the environmental impact linked to their extraction and manufacture, but such technologies are not yet fully mature (Robles et al., 2020).

Similarly to $CO_2$ removal and capture processes, the phototrophic processes are those that are considered as a more sustainable and cost-effective alternative for nutrients recovery in waste streams in the context of circular economy (García et al., 2019). In this content, the cultivation of photosynthetic species in wastewater can result to an up to 90% recovery of nutrients (Romero-Villegas et al., 2018). Different types of wastewater and various photosynthetic species have been studied, at a 20-fold increasing rate during the past decade (Garrido-Cardenas et al., 2018), which all confirm nitrogen and phosphorus uptake and recovery by phototrophic biomass

(Guldhe et al., 2017). As aforementioned, phototrophic processes can utilize $CO_2$ from flue gases, which further enhances viability of such remediation/recovery processes in terms of cost and environmental impact minimization (Guldhe et al., 2017). Thus, the adaptation of cyanobacteria-based processes in wastewater treatment sector, as well as in other economic sectors, can enhance sustainability of applied technologies and techniques (Singh and Ahluwalia, 2013) in terms of GHG emissions mitigation, production of green energy and recovery of natural resources.

## 1.4. Application of cyanobacteria for sustainable processes

Cyanobacteria in particular are rich in organic compounds that can be utilized in the production of biofuels, health and food supplements, pharmaceuticals and cosmetics (Figure 1.6) by providing a wide range of bioproducts, including polysaccharides, lipids, pigments, proteins, vitamins, bioactive compounds, and antioxidants (Pulz and Gross, 2004; Das et al., 2011; Borowitzka, 2013; Lau et al., 2015; Chittora et al., 2020).

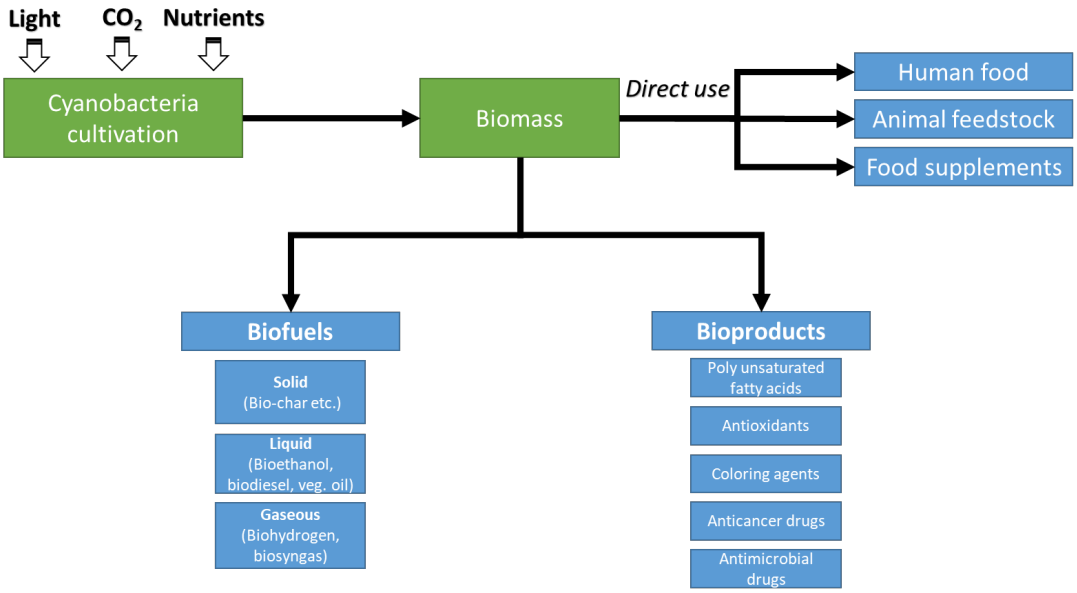


**Figure 1.6.** Illustration of potential uses of biomass from cyanobacteria or/and microalgae. (Modified from Khan et al., 2018)

According to Wang and Yin (2018), in terms of chemical composition, cyanobacterial biomass is mainly composed of proteins, carbohydrates and lipids, with proteins account for 40–60% of dry biomass, followed by carbohydrate (20–30%) and lipids (10–20%). In Table 1.2 presents the general compositions of different cyanobacteria and microalgae species are presented.

**Table 1.2.** Protein, carbohydrate and lipid content, in percentage of dry weight basis, of different cyanobacteria and microalgae species (Becker, 2007).

| **Cyanobacteria and/or microalgae species** | **Proteins** (%w/w) | **Carbohydrates** (%w/w) | **Lipids** (%w/w) |
|---|---|---|---|
| *Anabaena cylindrica* | 43-56 | 25-30 | 4-7 |
| *Aphanizomenon flos-aquae* | 62 | 23 | 3 |
| *Chlamydomonas rheinhardii* | 48 | 17 | 21 |
| *Chlorella pyrenoidosa* | 57 | 26 | 2 |
| *Chlorella vulgaris* | 51-58 | 12-17 | 14-22 |
| *Dunaliella salina* | 57 | 32 | 6 |
| *Euglena gracilis* | 39-61 | 14-18 | 14-20 |
| *Porphyridium cruentum* | 28-39 | 40-57 | 9-14 |
| *Scedesmus obliquus* | 50-56 | 10-17 | 12-14 |
| *Spirogyra sp.* | 63 | 15 | 11 |
| *Anthospira maxima* | 60-71 | 13-16 | 6-7 |
| *Spirulina platensis* | 46-63 | 8-14 | 4-9 |
| *Synechococcus sp.* | 63 | 15 | 11 |

One can easily notice from Table 1.2 that most of the cyanobacteria species contain large amounts of proteins, dominantly enzymatic proteins or crude proteins (mainly amino acids), thus can provide high quality nutrients commonly found in foods and feeds for animals (Razzak et al., 2013). It is worth mentioning at this point that the lipid content of cyanobacteria and microalgae species is one to two orders of magnitude grater that of typical macroalgae species that can be cultivated and/or harvested for valorization (Cai et al., 2013). To elucidate, the lipid content of Laminaria sp. (brown seaweed) and Ulva sp. (green seaweed), two representative macroalgae species, is only 2 %w/w and 0.6 %w/w respectively, significantly lower than the 11 %w/w and 14-22 %w/w lipid content of the cyanobacterium Synechoccocus sp. and the microalgae Chlorella vulgaris respectively. This renders cyanobacterial biomass a great feeding stock for the production of biofuels via the potential paths presented in Figure 1.7, or/and for the provision of added value products, giving the additional merit of $CO_2$ capture.

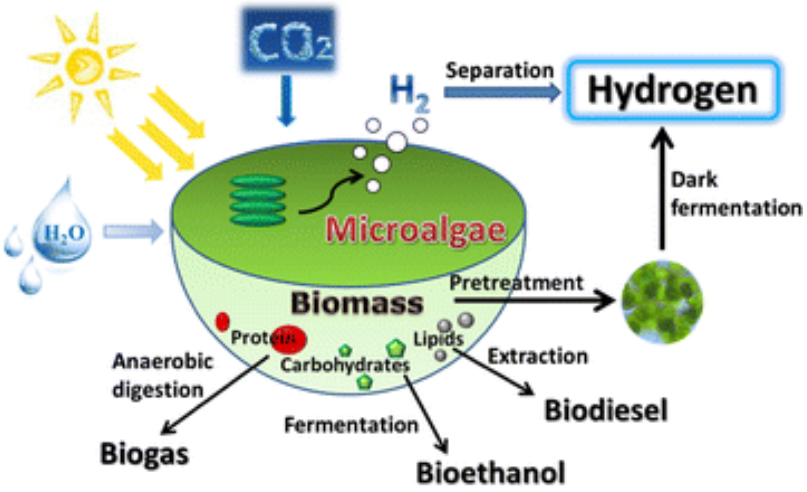


**Figure 1.7.** Potential pathways from cyanobacteria to biofuels (Wang and Yin, 2018).

Cyanobacterial and microalgae cultures are of increasing value given that: (i) they can be cultivated on non-agricultural land using wastewater, (ii) they can provide a high yield on a per unit of light irradiated area, (iii) their growth requires $CO_2$ and nutrients that can be obtained from fossil fuel combustion and wastewater respectively and (iv) they contain high oil and starch making possible the production of high-quality biodiesel. It is worth mentioning at this point that salt tolerant cyanobacteria species can be used to produce biomass for biodiesel production by utilizing saline wastewater, addressing both challenges of saline wastewater treatment and provision of cultivation nutrients. A comparison of the sources (feedstock) used for biodiesel production, in terms of oil yield and land area requirements is presented in Table 1.3.

**Table 1.3.** Comparison of feedstock for biodiesel production (Chisti, 2007).

| Crop | Oil yield (L/ha) | Land area requirements (Mha)[a] | Percent of existing US cropping area[a] |
|---|---|---|---|
| Corn | 172 | 1540 | 846 |
| Soybean | 446 | 594 | 326 |
| Canola | 1190 | 223 | 122 |
| Jatropha | 1892 | 140 | 77 |
| Coconut | 2689 | 99 | 54 |
| Oil palm | 5950 | 45 | 24 |
| Microalgae[b] | 136900 | 2 | 1.1 |
| Microalgae[c] | 58700 | 4.5 | 2.5 |

*[a] For meeting 50% of all transport fuel needs of the United States; [b] 70% oil (by wt) in biomass; [c] 30% oil (by wt) in biomass.*

By taking into consideration that the environmental and economic sustainability of phototrophic processes is directly linked to the selection of biomass feedstock and its potential as raw material for the production biofuels, food, feed and valuable chemicals production in industrial scale (Ho et al., 2010; Abishek et al., 2014; Cerri et al., 2017; Khan et al., 2018), the merits of cyanobacterial or/and microalgae biomass cultivation are evident and undisputable.

The selection of cyanobacteria for example, is preferable to conventional energy plants, since they can grow at a faster rate, they do not compete for arable land, they do not require fresh water for their growth and can assist in $CO_2$ mitigation, as well as in the treatment of wastewater with positive energy balance (Park and Lee, 2001; Zamora et al., 2008; Singh and Ohlsen, 2011; Paniagua-Michel et al., 2011; Pandey, 2017). Moreover, the absence of lignocellulosic cellular membrane in cyanobacteria cell wall facilitates the pretreatment process and reduces the overall cost of extraction processes for the acquirement of valuable bio-compounds (Arias et al., 2017; Khan et al., 2018). A summary of various cyanobacteria, as well as microalgae production, harvesting and processing alternatives are presented in Figure 1.8.

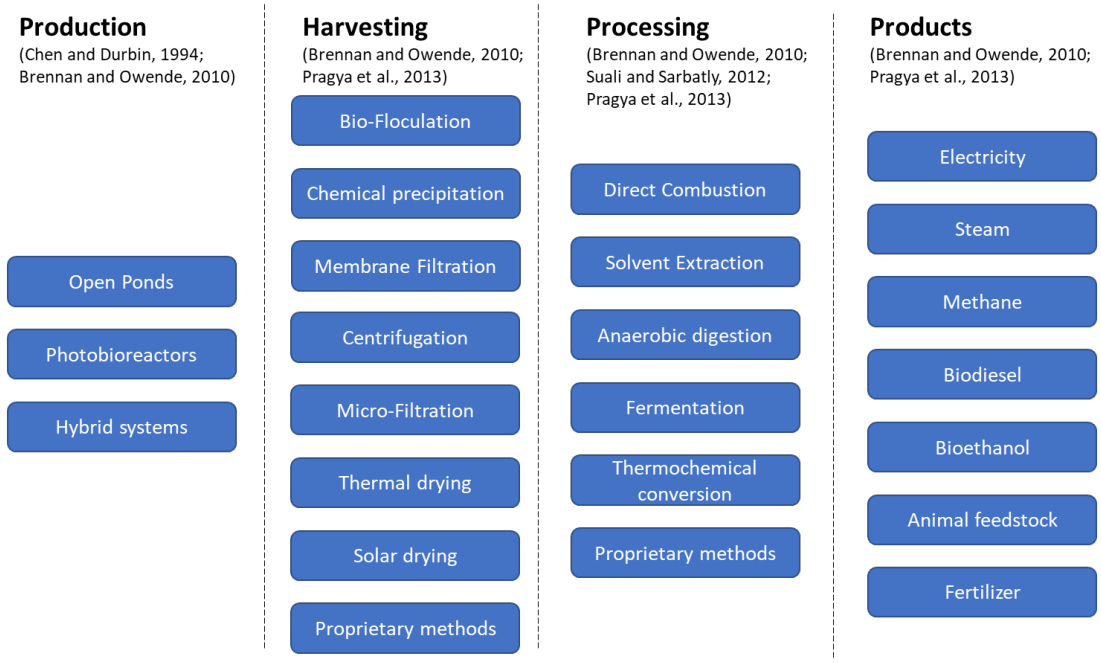


**Figure 1.8.** Cyanobacteria and microalgae production, harvesting and processing alternatives. (modified from Juneja et al., 2013)

It should be emphasized that the cultivation of phototrophic biomass must neither compromise food output nor the use of natural resources, such as water, nutrients and

minerals used in synthetic nutrient medium. In this accord, one of the biggest practical challenges in mass cultivation of phototrophic species has to do with low land requirements of cultivation installations, the availability of water and the consumption of nutrients. In order to cultivate phototrophic species in open ponds for example, large land area requirements and enormous amounts of water (~11–13 million liters per hectare) and macronutrients (N and P) are required contributing to 10–20% of the total biomass production cost that is estimated at EUR 10 $kg^{-1}$, of which a considerable part accounts to nutrient inputs (Viswanaathan et al., 2022). Moreover, the enhancement of cellular growth in cultivation reactors is another significant challenge that needs to be addressed by means of photobioreactor design and optimization of operation conditions, such as culture mixing, fluid dynamics and hydrodynamic stress, culture depth, gas bubble size and distribution, gas exchange, mass transfer, dilution rate, toxic chemicals and pathogens, competition by other algae, harvest frequency (Razzak et al., 2013). Thus, for large scale cultivation, phototrophic species should have desirable features. These are summarized in Table 1.4, which was obtained from Borowitzka (1992).

**Table 1.4.** Desired characteristics of phototrophic species for mass culturing (Borowitzka, 1992).

| Species characteristic | Advantages | Disadvantages |
|---|---|---|
| (1) Growth in extreme environments | Reduced problems with competing species and predators | Only limited numbers of species available. Culture difficult to maintain on a large scale under extreme environments (i.e. cold weather) |
| (2) Rapid growth rate | Provides competitive advantage over competing species and predators. It has a reduced pond area required | Growth rate is usually inversely related to cell size; i.e. fast growing cells are usually very small in size |
| (3) Large cell size, colonial or filamentous morphology | Reduces harvesting costs | Large cells usually grow slower than smaller size cells |
| (4) Wide tolerance of environmental conditions | Less control of culture conditions required for reliable culture | - |
| (5) Tolerance of shear force | Allows cheaper pumping and mixing methods to be used | - |
| (6) High cell product content | Higher value of biomass | Products are usually secondary metabolites (not directly involved in normal growth). High concentrations of secondary metabolites normally mean slower growth |

Although many phototrophic species have been evaluated and utilized for the production of valuable metabolites, the productive potential of cyanobacterial species has remained largely unexplored despite the advantages they offer in bioprocess engineering, such as (i) simple input requirements, (ii) tolerance of marginal agricultural environments, (iii) rapid genetics, and (iii) carbon-neutral applications that could be leveraged to address global climate change concerns (Ducat et al., 2011). In the context of global resource shortages and increasing environmental pollution, microalgal photosynthetic biomanufacturing provides a highly promising route for achieving sustainable development (Luan et al., 2020).

Cyanobacteria are the only known photoautotrophic prokaryotes i.e., single-celled organisms lacking of a nucleus and other organelles (vacuole, chloroplasts etc.) that are capable of utilizing light to convert inorganic carbon ($CO_2$, $HCO_3^-$) and water into organic compounds via oxygenic photosynthesis (Hamilton et al., 2016). Cyanobacteria presumably provided the first large-scale biotic source of oxygen on early Earth's history (estimated from 3.7 to 2.3 Gyr) (Ettwing et al., 2010) and are considered primary producers of organic carbon on Earth, estimated that approximately 20-30% of organic carbon is derived from photosynthetic $CO_2$ fixation by cyanobacteria (Waterbury et al., 1979).

Cyanobacteria are prime movers of global nitrogen and carbon cycling, responsible for an astonishing 50% of the planet's biological nitrogen fixation (Welsh et al., 2008) and present higher growth rates and photosynthetic efficiencies than terrestrial plants due to their simple structures (Li et al., 2008). The photosynthesis efficiency of cyanobacteria is several times higher than that of terrestrial plants, with reported efficiency values of 3-9% compared to ≤0.25-3% of terrestrial plants (Ducat et al., 2011). Moreover, cyanobacteria are considered a more sustainable alternative feedstock for the production of biofuels and chemical compounds than plant-based feedstocks, since they do not require arable land for their cultivation and are high-yield sources for lipids (20-50% DW), starch and glycogens (20-50% DW) (Sheehan et al., 1998; Dismukes et al., 2008; Luan et al., 2020)

### 1.4.1. Bioenergy

Nowadays, 35% of the world's energy consumption is met by oil, 25% by natural gas, 28% by coal, 7% by hydroelectric power and the remaining 5% by nuclear energy and

alternative energy sources (Kamshybayeva et al., 2022). Biofuels are considered an excellent alternative to fossil fuels and can be produced from several biomass sources, such as food crops, crop wastes or disposed fruits, woody parts of plants, garbage and phototrophic species including cyanobacteria and microalgae (Harun et al., 2010; Ho et al., 2010; Juneja et al., 2013; Elkatory et al., 2022). The current trend in the production of bioethanol, which is the most common biofuel that is now mainly produced from sugars of corn and sugarcane, is its production using renewable and sustainable feedstock, such as carbohydrates from cyanobacteria or/and microalgae biomass (Bothast and Schlicher, 2005; Goldemberg, 2009; Elkatory et al., 2022). This trend is attributed to the vigorous increase of bioethanol demand during the past decade (Licht, 2006) and the limited arable land availability, as well as on the fact that cyanobacteria or/and microalgae-based fuels are considered ecofriendly, nontoxic, with a strong potential of fixing $CO_2$, since 1 kg of algal biomass is estimated that can fix approximately 1.83 kg of $CO_2$ (Gendy and El-Temtanmy, 2013). Most of the cyanobacteria and microalgae species are favorable for biodiesel production due to high lipids contents 50–70% and may reach to 80% (Chisti, 2007; Powell and Hill, 2009; Mata et al., 2010). They also contain a significant amount of carbohydrates, such as glycogen, starch, agar and cellulose, compounds, which according to the review work Khan et al. (2018) can reach up to 60 % of the total dry biomass weight and can be easily converted to fermentable sugars for bioethanol production (Ueda et al., 1996).

Nevertheless, there are significant drawbacks in the use of cyanobacteria and microalgae biomass as a competitive feedstock for biofuels production, which are associated with operational and maintenance cost for their cultivation (nutrient feeding solution, energy consumption for lighting, agitation etc.), cost of biomass dewatering and harvesting (cost of coagulants, energy consumption for filters, filter replacements etc.), pretreatment of biomass and ensuring maximum biomass growth and fermentation yields (Singh and Gu, 2010; Khan et al., 2018).

Using new and innovative techniques for cultivation, cyanobacteria or/and microalgae biomass may allow the biodiesel production to achieve the price and scale of fabrication required to compete with, or even replace, petroleum (Razzak et al., 2013). According to Gendy and El-temtamy (2013), at least a decade of research and development is necessary for achieving economic viability of such biofuels. However, Yusuf and Yan (2011) estimated that the production of biofuels from cyanobacterial or/and microalgal

biomass can be economically viable if crude oil sell higher than 100 $ per barrel, a price that has been recently (March 2022) reached because of COVID-19 inflation-related economic crisis, as well because of the Russian-Ukrainian war that triggered a global energy crisis.

Hence, an opportunity is presented for economically viable implementation of cyanobacteria or/and microalgae cultivation processes for sustainable biofuels production, as long as the utilized biomass is obtained in a sustainable manner and it is technically feasible (Juneja et al., 2013; Elkatory et al., 2022).

#### 1.4.1.1. Cyanobacteria biohydrogen production

The cultivation of cyanobacteria is of increasing interest during the past decades due to their ability of metabolically producing hydrogen gas ($H_2$) by diverging the electrons emerging from the two primary reactions of oxygenic photosynthesis directly into the production of $H_2$ (Ghirardi et al., 2009; Kamshybayeva et al., 2022). Cyanobacteria can be cultivated in inexpensive media with high energy conversion efficiency (Angermayr et al., 2009) and are able to produce $H_2$ via three main routes: (i) directly from the native bidirectional hydrogenase; (ii) from a native nitrogenase; (iii) from an introduced hydrogenase (Tamagnini et al., 2007). Nonetheless, all cyanobacteria species absorb energy from light (photons) by utilizing their antenna pigments (chlorophyll *a,* carotenoids and phycobiliproteins present in thylakoid membrane) in the photosystem 2 (PSII) complex, which is transferred to pfotosystem 1 (PSI) via linear electron transport proteins, leading to an increase in the number of electrons and the accumulation of cellular energy in the form of ATP molecules by the protein ferredoxin (Fd). If required, these electrons are used to activate $H_2$ enzymes (hydrogenase or nitrogenase) (Kamshybayeva et al., 2022).

Due to fact that $O_2$ is a bioproduct of oxygenic photosynthesis and that the presence of $O_2$ at saturations over 0.1% reduces/stops the activity of the hydrogenase (Zhang et al., 2015), two processes for biohydrogen production have been developed, the direct and the indirect biophotolysis (Figure 1.9).

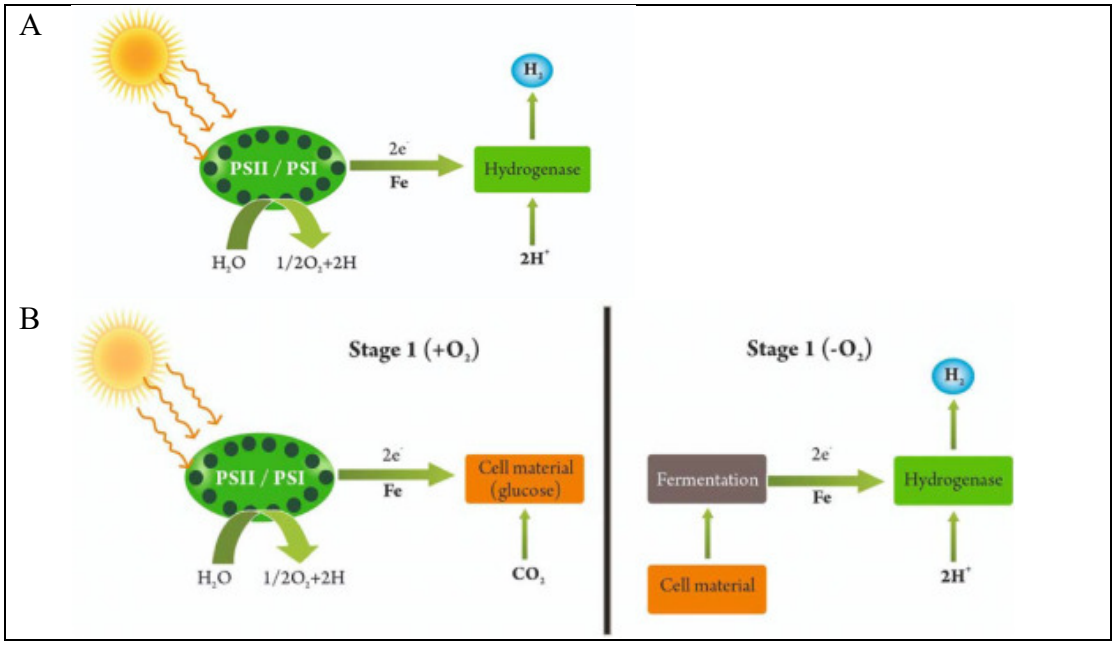


**Figure 1.9.** Scheme of direct (A) and indirect (B) photolysis by cyanobacteria cells (Elsevier License Number 5384190635646).

As shown in Figure 1.9, the steps of photosynthesis and of $H_2$ production via hydrogenase (Equations 1 and 2 respectively) are the same both in direct and indirect process (Acar and Dincer, 2018).

Photosynthesis:

$$2H_2O \rightarrow 4H^+ + 4e^- + O_2 \qquad \text{Equation 1}$$

Hydrogen production:

$$4H^+ + 4e^- \rightarrow 2H_2 \qquad \text{Equation 2}$$

In direct biophotolysis, under anaerobic conditions or upon excessive absorption of solar energy, some microorganisms release excess electrons with the help of hydrogenases, resulting in the conversion of extracted from water hydrogen ions into $H_2$ gas (Kamshybayeva et al., 2022). On the other hand, indirect biophotolysis produces $H_2$ from electrons derived from the breakdown of biosynthesised carbohydrates, as described in Equations 3 and 4 (Harwood, 2020).

Biosynthesis of carbohydrates (sugars) during cyanobacteria oxygenic photosynthesis:

$$12H_2O + 6CO_2 \rightarrow C_6H_{12}O_6 + 6O_2 \qquad \text{Equation 3}$$

Fermentative cyanobacterial $H_2$ production:

$$C_6H_{12}O_6 + 6H_2O \rightarrow 12H_2 + 6O_2 \qquad \text{Equation 4}$$

What is different on a technical level is that in indirect biophotolysis the levels of $O_2$ can be better controlled because it occurs in another process stage, thus the activity of hydrogenase is not reduced. This two-stage process but it comes with a cost regarding the efficiency of converting solar energy into $H_2$ (Bolatkhan et al., 2019), which is already considered low, arguably at 0.05% efficiency (Brenner et al., 2006). Some cyanobacterial strains that have been used in biohydrogen production are presented in Table 1.5.

**Table 1.5.** Active cyanobacterial strains used in $H_2$ production studies (modified from Kamshybayeva et al., 2022).

| Organism name | Organism description | Maximum $H_2$ production | Growth condition |
|---|---|---|---|
| *Anabaena siamensis* TISTR 8012 | Filamentous, hupS mutant | 3 μmol $mg^{-1}$ chl *a* $h^{-1}$ | Air, under light, BG-11 medium |
| *Anabaena siamensis* TISTR 8012 | Filamentous, hupS-deficient mutant | 30 μmol $mg^{-1}$ chl *a* $h^{-1}$ | Air, under light, BG-11 medium |
| *Anabaena sp.* PCC 7120 | Filamentous, hupL mutant | 60 μmol $mg^{-1}$ chl *a* $h^{-1}$ | Air, under light |
| *Anabaena sp.* PCC 7120 | Filamentous, hupL mutant | 35 μmol $mg^{-1}$ chl *a* $h^{-1}$ | Air, under light, BG-11 medium |
| *Anabaena sp.* PCC 7120 | Filamentous, Hup mutant | 29 μmol $mg^{-1}$ chl *a* $h^{-1}$ | Air, under light, BG-11 medium |
| *Anabaena sp.* PCC 7120 | Filamentous, hupW mutant | 3.3 μmol $mg^{-1}$ chl *a* $h^{-1}$ | Air, under light, BG-11 medium |
| *Anabaena sp.* PCC 7120 | Filamentous, Hup mutant | 63 μmol $mg^{-1}$ chl *a* $h^{-1}$ | Air, under light, BG-11 medium |
| *Anabaena sp.* PCC 7120 | Filamentous, hupW mutant | 6.2 mL $L^{-1}$ $h^{-1}$ | Air, under light |
| *Anabaena sp.* UTEX 1448 | Filamentous, wild-type strain | 67 μmol $mg^{-1}$ chl *a* $h^{-1}$ | Air, under light, BG-11 medium |
| *Cyanothece sp.* ATCC 51142 | $N_2$-fixing, unicellular, wild-type strain | 465 μmol $mg^{-1}$ chl *a* $h^{-1}$ | Air, ASP2 medium, 25 μE (photons) $m^{-2}$ $s^{-1}$ |
| *Cyanothece sp.* Miami BG 043511 | $N_2$-fixing, unicellular, wild-type strain | 15.8 mL $L^{-1}$ $h^{-1}$ | Air, ASP2 medium, 30ºC, 30 μE (photons) $m^{-2}$ $s^{-1}$ |
| *Cyanothece sp.* ATCC 51142 | $N_2$-fixing, unicellular, wild-type strain | 300 μmol $mg^{-1}$ chl *a* $h^{-1}$ | ASP2 medium, 30ºC, 30 μE (photons) $m^{-2}$ $s^{-1}$, 50 mmol glycerol |
| *Synechocystis* M55 | Unicellular, ndhB mutant | 200 nmol $mg^{-1}$ chl *a* $min^{-1}$ | Air, 30ºC, 50 μE (photons) $m^{-2}$ $s^{-1}$ |
| *Synechocystis* sp. PCC 6803 | Unicellular, ctal/cyd mutant | 190 nmol $mg^{-1}$ chl *a* $min^{-1}$ | Air, modified Allen's medium, 34ºC, 70 μE (photons) $m^{-2}$ $s^{-1}$ |
| *Synechocystis* sp. PCC 6803 | Unicellular, ctal/cyd mutant | 115 nmol $mg^{-1}$ chl *a* $min^{-1}$ | Air, 28ºC, 50 μE (photons) $m^{-2}$ $s^{-1}$ |
| *Synechocystis* sp. PCC 6803 | Unicellular, narB/nirA mutant | 300 nmol $mg^{-1}$ chl *a* $min^{-1}$ | Air, 25-40 μE (photons) $m^{-2}$ $s^{-1}$ |
| *Synechocystis* sp. PCC 6803 | Unicellular, wild-type strain | 3.1 μl $mg^{-1}$ chl *a* $h^{-1}$ | Air, 20 μE (photons) $m^{-2}$ $s^{-1}$ |
| *Synechocystis* sp. PCC 6803 | Unicellular, wild-type strain | 37 μmol $mg^{-1}$ chl *a* $h^{-1}$ | Air, 20 μE (photons) $m^{-2}$ $s^{-1}$ |
| *Oscillatoria* sp. Miami BG7 | Filamentous, wild-type strain | 5.9 μl $mg^{-1}$ DW $h^{-1}$ | Air, $NH_4Cl$ as combuined $N_2$ source, 100 μE (photons) $m^{-2}$ $s^{-1}$ |
| *Oscillatoria limosa* 23 | Filamentous, wild-type strain | 19.83 μl $mg^{-1}$ chl *a* $h^{-1}$ | Air, nitrate-free medium, 1.2 lux photons |
| *Oscillatoria* sp. Miami BG7 | Filamentous, wild-type strain | 260 μmol $mg^{-1}$ chl *a* $h^{-1}$ | Air, 30-50 μE (photons) $m^{-2}$ $s^{-1}$ |
| *Sybechococcus* sp. Miami BG 043511 | Unicellular, wild-type strain | 220 μmol $mg^{-1}$ chl *a* $h^{-1}$ | Air, 28ºC, 150 μE (photons) $m^{-2}$ $s^{-1}$ |

| | | | |
|---|---|---|---|
| *Synechococcus* sp. Miami BG 043511 | Unicellular, wild-type strain | 140 μmol $mg^{-1}$ chl *a* $h^{-1}$ | Air, 200 μE (photons) $m^{-2}$ $s^{-1}$ |
| *Synechococcus* PCC 602 | Unicellular, wild-type strain | 15.77 μl $mg^{-1}$ chl *a* $h^{-1}$ | Air, 20 μE (photons) $m^{-2}$ $s^{-1}$ |
| *Synechococcus* PCC 7942 | Unicellular, hydA mutant | 162.52 μmol $mg^{-1}$ chl *a* $h^{-1}$ | Air, 20-50 μE (photons) $m^{-2}$ $s^{-1}$ |
| *Synechococcus* PCC 112 | Unicellular, wild-type strain | 0.019 μmol $mg^{-1}$ chl *a* $h^{-1}$ | Air, 27°C, 30 μE (photons) $m^{-2}$ $s^{-1}$ |
| *Phormidium valderianum* BDU 20041 | Filamentous, wild-type strain | 0.22 μl $mg^{-1}$ DW $h^{-1}$ | Air, 30 W(photons) $m^{-2}$ fluorescent light |
| *Phormidium corium* B-26 | Filamentous, wild-type strain | 0.003 μmol $mg^{-1}$ chl *a* $h^{-1}$ | Air, 35 μE (photons) $m^{-2}$ $s^{-1}$ |
| *Desertifilum* sp. IPPAS B-1220 | $N_2$-fixing, filamentous, wild-type strain | 348 μmol $mg^{-1}$ chl *a* $h^{-1}$ | Air, 30 μE (photons) $m^{-2}$ $s^{-1}$ |

Freshwater cyanobacteria strains, such as Anabaena sp. PCC 7120 and *Synechococcus elongatus* PCC 7942, with tolerance to salt stress up to 0.6 M NaCl, accumulate sucrose and/or trehalose as major compatible solutes to compensate osmotic pressure at increasing salinities (Zhang et al., 2020). Worth mentioning that sucrose is also synthesized and accumulated as a complementary carbon pool in cyanobacteria (carbon sink) and used to adapt to the circadian rhythm and environmental changes during day–night cycles (Damrow et al., 2016).

Recently, many approaches have been investigated towards the improvement of cyanobacteria biohydrogen production efficiency, mainly by metabolic, genetic and technical means (Figure 1.10).

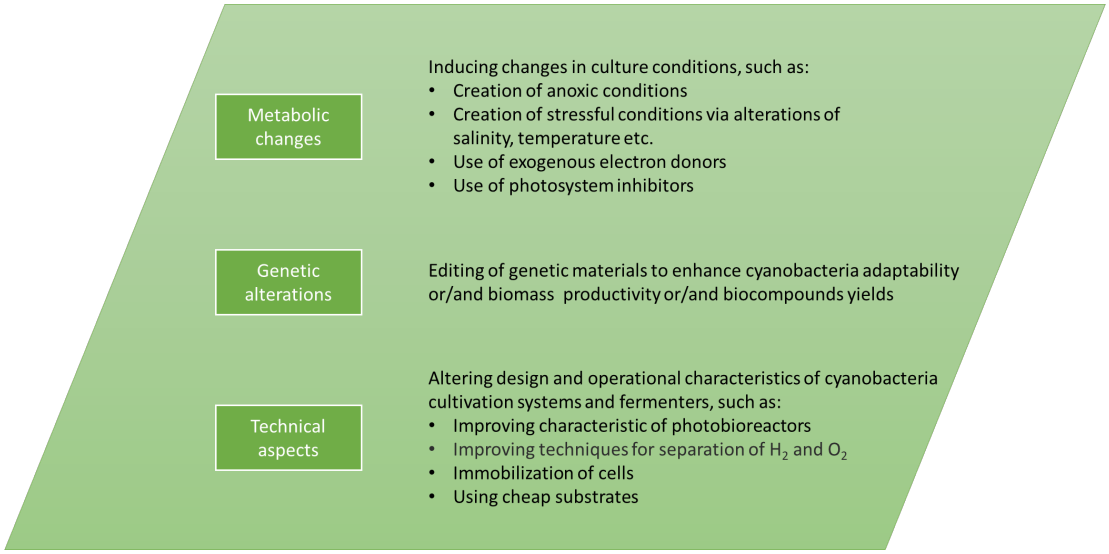


**Figure 1.10.** Approaches for increasing cyanobacteria biohydrogen production (Dutta et al., 2005; Singh et al., 2016).

Nonetheless, what remains an issue toward sustainable cultivation of cyanobacteria and other phototrophic species is the selection of cultivation medium, which should be of

low environmental and economic cost. Thus, the selection of a cultivation medium that aligns with the principles of circular economy (reduce-reuse-recycle) is of major importance towards the development and adaptation of novel cyanobacteria-based processes. In this accord, waste streams, such as wastewater from agroindustrial sector that contain significant amounts of nutrients, could be utilized as a sustainable cultivation medium.

### 1.4.2. Waste streams treatment and valorization

In accordance with the prevailing linear economic model, current production and consumption practices only consider treating waste streams when they involve a potential risk to the environment, whilst recovering valuable resources is only considered when it entails an economic benefit to the production process (Robles et al., 2020). Moreover, the expense of nutrient feedstocks has motivated researchers to explore the integration of cheaper nutrient streams such as those from wastewater (Abdel-Raouf et al., 2012).

Recycle and valorization of wastewater can provide additional sources of income for citizens and local authorities, which can sell energy generated from wastewater recycling to compensate for the wastewater management costs (Colenbrander et al., 2017; Gondhalekar and Ramsauer, 2017; IPCC, 2022). Furthermore, recycling and valorization of wastewater can minimize the costs associated with the renewal of centralized wastewater treatment plants (Bernstad Saraiva Schott and Cánovas, 2015; Gharfalkar et al., 2015; Gonzalez-Valencia et al., 2016; Herrero and Vilella, 2018; Matsuda et al., 2018; Nisbet et al., 2019).

Regarding resources sustainability for cyanobacteria or/and microalgae species cultivation, the use of wastewater, brackish water or sea water in open ponds or in closed PBRs, in which the evaporative water losses are negligible, are both potential solutions to the challenge of sustainable biomass production (Juneja et al., 2013). These substrates and especially wastewater could substitute the agricultural fertilizers that are commonly used to maintain steady growth in phototrophic cultures (Weissman and Tillett, 1992; Becker, 1994; Dalrymple et al., 2013), as well as the freshwater amended with chemical components (Bhatnagar et al., 2011). Water and nutrients contained in wastewater are expected to become increasingly scarce for the foreseeable future, while environmental conservation and climate change mitigation impose the need to

investigate new routes for the utilization of wastewater and $CO_2$ to produce fuels, food and chemicals (Bich et al., 1999; Martinez et al., 2000; Wilkie and Mulbry, 2002; Aslan and Kapdan, 2006; Juneja et al., 2013; Ding et al., 2015; El-Sheekh et al., 2022). Using wastewater as a nutrient source for cyanobacteria or/and microalgae cultivation can decrease the overall biomass production cost by 30% and by 44% when they are cultivated in raceway ponds and thin layer cascade reactors respectively (Acien Fernandez et al., 2019).

Moreover, the use of cyanobacteria or/and microalgae for removal/recycle of nutrients from wastewater, especially those from agroindustry (Figure 1.11), provides a sustainable approach for wastewater treatment, bioenergy production, as well as the production of food and added value products (Andrade et al., 2020; de Carvalho et al., 2020, Melo et al., 2022). There is a vast potential for utilization of wastewater as substrate for their cultivation, since about 359 billion cubic meters of wastewater are produced globally due to household activities, industrial processes, farming and agriculture (Jones et al., 2021). In this context and according to Pahunang et al. (2021) algal photobioreactors can be used as a carbon sink in WWTPs by capturing the $CO_2$ generated during wastewater treatment, the $CO_2$ generated from biogas combustion, as well as the $CO_2$ contained in atmospheric air or in biogas streams (Shoener et al., 2014; Nayak et al., 2016; Vo Hoang Nhat et al., 2018; Corpuz Vermi et al., 2021).

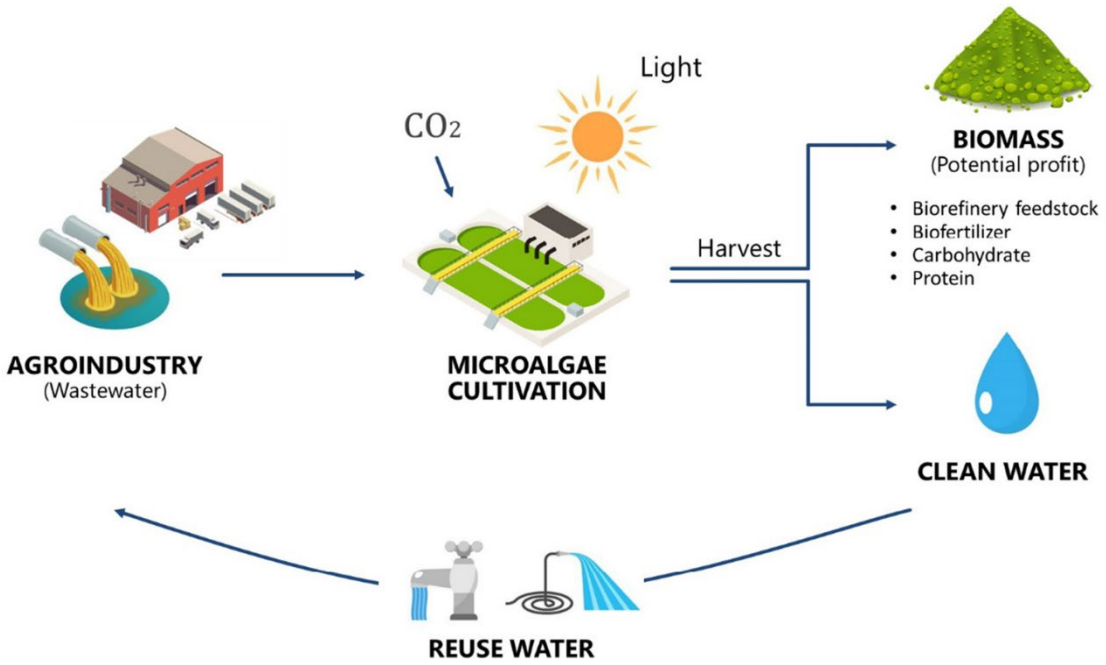


**Figure 1.11.** Scheme highlighting a sustainable approach system for the integration

between microalgae cultivation and agroindustry wastewater treatment (Source: Melo et al., 2022; Springer Nature License Number 5360131217586)

Both raw and secondary-treated municipal and industrial wastewater can be utilized as substrate for cyanobacteria or/and microalgae cultivation, since they usually contain in excess all the required nutrients for their growth (Razzak et al., 2013). In this regard, wastewater streams from agro food industries present a high potential for cyanobacteria cultivation, since they are characterized by increased concentrations of readily utilizable by cyanobacteria nutrients, such as nitrates and phosphates.

The removal or remediation of nutrients by cyanobacteria takes place through the metabolic pathways for nutrient uptake and assimilation during photosynthesis for biomass growth (Whitton et al., 2015). Nitrogen is assimilated into amino acids for the formation of proteins (Apandi et al., 2018), while phosphorous is removed via assimilation for the formation ATP (adenosine triphosphate) and ADP (adenosine diphosphate) (Gani et al., 2016) or/and via precipitation in cases of elevated pH levels (Powell and Hill, 2009). Cyanobacteria contain several times greater nitrogen and phosphorus contents than that of plants, approximately 10% and 11% respectively on a dry weight basis (Razzak et al., 2013). Due to the high phosphates assimilation rates, cyanobacteria can address the common challenge of biological treatment process that is the removal of phosphorous from wastewater streams (Pittman et al., 2011; Whitton et al., 2015), offering a simple and cost-effective solution to tertiary treatment processes (Tam and Wong, 1995).

Worth mentioning at this point that nitrates and phosphates are the predominant form of nutrients after secondary aerobic biological treatment i.e., biological oxidation usually in widely applied activated sludge (AS) wastewater treatment plants (WWTPs). Thus, cyanobacteria-based systems can be employed as a tertiary wastewater treatment stage for the removal of nutrients from livestock wastes, agro-industrial wastes, industrial wastes, municipal wastewater and domestic wastewater (Abdel-Raouf et al., 2012; Maizatul et al., 2017). According to Pahunang et al. (2021), the implementation of such systems can mitigate GHG emissions from WWTPs, which are estimated to produce 0.77 Gt of $CO_2$ for the year 2010, equivalent to 1.57% of the global annual emissions of GHGs (IPCC, 2015). That been said, a WWTP coupled with cyanobacteria or/and microalgae tertiary treatment stage can lead to efficient wastewater treatment

and minimization of biomass production cost (Rosso and Stenstrom, 2008; Tseng et al., 2016). Worth mentioning at this point that the use of wastewater as feedstock (substrate) for cyanobacteria or/and microalgae biomass production is expected to half the cultivation cost from EUR 10 $kg^{-1}$ to EUR 5 $kg^{-1}$ (Ranglova et al., 2020; Navarro-Lopez et al., 2020; Bauer et al., 2021).

Regarding the sustainable use of resources, wastewater, as well as flue gases can be used as feedstock for the production of cyanobacteria biomass through utilization of nutrients and $CO_2$ via photosynthetic assimilation and carbon fixation respectively. International Energy Agency (2010) declared that the energy from wastes and combustible sources has higher potential as alternative fuel compared to other renewable sources. Due to their inherent ability to tolerate high concentrations of $CO_2$, such as those of flue gases from power plants, cyanobacteria are considered important in $CO_2$ sequestration programs and the development of $CO_2$ capturing technologies (Viswanaathan et al., 2022).

As illustrated in Figure 1.12, cyanobacteria and microalgae can remove/recover via assimilation into biomass nitrogen and phosphorous from wastewater and can fixate significant quantities of $CO_2$, while producing biomass that can be further applied in downstream processes for the production of, fuels, food and chemicals (Whang and Yin, 2018). Worth mentioning at this point that the removal/recovery of nutrients from waste streams via assimilation into cyanobacterial or/and microalgal biomass, especially of phosphorous that is considered a non-renewable resource, is a strategy to address the serious challenges of sustainability, while addressing other ecological issues such as eutrophication (Juneja et al., 2013). However, maximizing biomass production and minimizing costs associated with cultivation medium and harvesting are critical to cost-effective nutrient removal system development (Hoffmann, 1998).

Wastewater treatment processes that are based on cyanobacteria or/and microalgae cultivation are considered advantageous compared to the common treatment processes due to their minimal $CO_2$ footprint, the significant cost saving and energy production potential, the effective nutrients and pathogen removal (Posadas et al., 2017), the minimization of oxygen requirements for aeration, which constitutes the major operational cost of a wastewater treatment plant (Mallick, 2002; Munoz and Guieysse, 2006), and economic value addition to the algal by-products (Shashirekha et al., 2019).

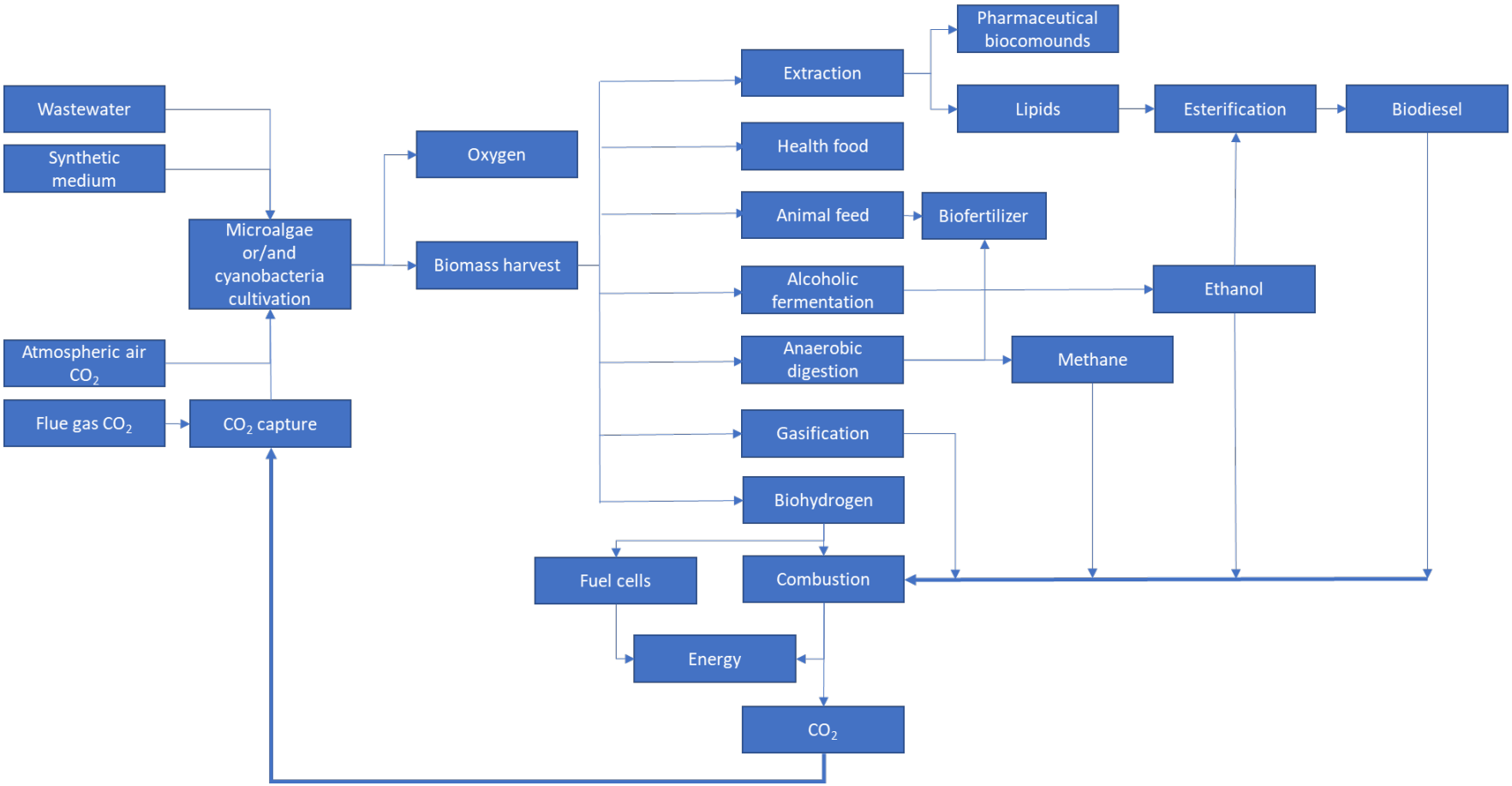


**Figure 1.12.** Schematic overview of energy, food and chemicals production from cyanobacteria or/and microalgae cultivation in wastewater or synthetic media and with flue gases or atmospheric air $CO_2$ utilization in downstream processes. (modified from Costa and Morais, 2011).

The potential use of sun's photosynthetically active radiation (PAR) and wastewaters, as well as the lower area requirements for cyanobacteria or/and microalgae cultivation than those of traditional plant crops, gives a competitive advantage compared to conventional energy, food and chemical production processes (Markou and Georgakakis 2011; Khan et al., 2018; Gonzalez-Moralez et al., 2020; Elkatory et al., 2022). Nevertheless, studies regarding cyanobacteria or/and microalgae cultivation in wastewater coupled with flue gases treatment are limited and there is a lack of fundamental design and operational parameters for their full-scale implementation.

Various types of phototrophic biomass have been used and evaluated in terms of their molecular components and their potential for bioenergy or/and industrial applications, with increased interest on wastewater treatment and valorization using cyanobacteria or/and microalgae species (Table 1.6). Cyanobacteria and microalgae are able to effectively grow in wastewater conditions due to the abundance of nutrient inorganic species, as well as to efficiently remove nitrogen, phosphorus and other toxic materials from wastewater (Razzak et al., 2013). Worth mentioning at this point that cyanobacteria present the comparative advantages of being able to produce valuable compounds, such as phicobiliproteins and polyhydroxybutyrate (Robles et al., 2020), as well as being easier to digest due to their soft cell wall (Arias et al., 2017).

**Table 1.6**. Summary of indicative published studies using wastewater for cyanobacteria or microalgae cultivation and of the main objective or outcomes.

| Wastewater type | Cyanobacteria or microalgae species | Objective/outcome | Reference |
|---|---|---|---|
| Agricultural run-off | *Chlorella vulgaris* | Nutrients removal / Carbon sequestration | Anand, 2010 |
| Agro-industrial wastewater | *Chlorella vulgaris* | Biofuels / Bioproducts | Posadas et al., 2017 |
| Aquaculture wastewater | *Chlorella vulgaris, Scenedesmus obliquus* | Nutrients removal / Biomass | Gao et al., 2016 |
| Brewery effluent | *Chlorella vulgaris; Scenedesmus obliquus* | Nutrients removal / Biomass | Raposo et al., 2010; Mata et al., 2010 |
| Cafeteria effluent | *Scenedesmus sp.* | Nutrients removal / Elements removal | Mohamed et al., 2015 |
| Dairy | *Chlorella saccharophila UTEX 2911, Chlamydomonas pseudococcum UTEX 214, Scenedesmus sp. UTEX 1185, Chlorella protothecoides, Tetradesmus obliquus; Rhizoclonium sp., Stigeoclonium sp., Cladophora sp. Gomphonema sp. Oscillatoria sp.; C. vulgaris. Synechococcus elongatus, Spirulina (Arthrospira) platensis; Euglena gracilis WZSL mutant; C. sorokiniana (2); C. pyrenoidosa; Scenedesmus, Pseudanabaena galeata, Scenedesmus dimorphus, C. polypyrenoideum; C. sorokiniana. C. protothecoides; S. obliquus* | Biofuels / Biomass / Bioproducts / Nutrients removal | Viegas et al. (2021); Audu et al. (2021); Khalaji et al. (2021); Pishbin et al. (2021); Zapata et al. (2021); Zkeri et al. (2021); Barsanti et al. (2021); Santos et al. (2021); Ahmad et al. (2020); Asadi et al. (2020); Feng et al. (2020); Handayani et al. (2020); Lorentz et al. (2020); Ouhsassi et al. (2020); Espinosa-Gonzalez et al. (2014); Girard et al. (2014); Kothari et al. (2013); González et al. (1997); Lincoln et al. (1996) |
| Dairy and fish | *Chroococcus sp., Haematococcus pluvialis, Dunaliella sp., Coelastrella saipanensis, Chlorella sp.* | Bioproducts / Lipid production | Vidya et al. (2021) |
| Dairy and poultry | *Chlorella sp.* | Bioproducts / Lipid production | Gumbi et al. (2021) |
| Dairy and winery | *Leptolyngbya sp.* | Biomass / Biofuels | Tsolcha et al. (2021) |
| Digested distillery effluent | *Spirulina platensis* | Biomass | Kaushik et al., 2006 |
| Food waste and municipal | *Chlorella sorokiniana* | Nutrients removal / Lipid production | Chi et al., 2011 |
| Industrial (brewing, soy sauce pulp and paper, dairy and poultry) | *Dunaliella* | Nutrients removal / Biomass | AL-Rajhia et al., 2012 |

| | | | |
|---|---|---|---|
| Municipal | *Chlorella minutissima, Auxenochlorella protothecoides UMN280, Scenedesmus sp. AMDD* | Nutrients removal / Biofuels | Bhatnagar and Chinnasamy, 2011; McGinn et al., 2011; Zhou et al., 2012 |
| Municipal and dairy | *Arthrospira jenneri, Coccomonas sp., Polytomella sp.; Polytoma tetraolare, Chlamydomonas caeca, Geitlerinema, Synechocystis, Cyanobium, Glaucospira, Carteria sp., Lepocynclis ovum, Euglena clavate, Actinastrum, Scenedesmus, Chlorella, Spirogyra, Nitzschia, Micractinium, Golenkinia, Chlorococcum, Closterium* | Biomass / Nutrients removal / Lipid production | Woertz et al. (2009); Bernal et al. (2008) |
| Palm oil mill effluent | *Chlorella sorokiniana, Chlorella sp.* | Lipid production / Biofuels | Putri et al., 2011; Hadiyanto and Nur, 2012 |
| Paper and pulp mill effluent | *Oscillatoria chlorina, Scenedesmus quadricauda, Scenedesmus sp.* | Biomass / Nutrients removal | Saikia et al., 2011; Usha et al., 2016 |
| Potato, fish animal feed, yeast and coffee | *Phormidium, Oocystis, Microspora* | Nutrients removal | Posadas et al. (2014) |
| Poultry, swine, cattle, brewery, urban and dairy | *Scedesmus obliquus* | Biomass / Biofuels / Nutrients removal | Ferreira et al. (2018) |
| Poultry litter anaerobic digestion effluent | *Chlorella minutissima, Chlorella sorokiniana, Scenedesmus bijuga* | Nutrients removal | Singh et al., 2011 |
| Slaughterhouse, municipal and dairy | *C. vulgaris, C. minutissima, C. pyrenoidosa, Chroococcus sp., Spirulina sp.* | Biomass / Nutrient removal | Chawla et al. (2020) |
| Sugar mill effluent | *Scenedesmus obliquus* | Nutrients removal | Shashirekha et al., 2016 |
| Sweetmeat factory waste media | *Scenedesmus obliquus* | Live feedstock | Toyub et al., 2008 |
| Swine wastewater | *Chlorella sp., Chlorella vulgaris, Chlamydomonas reinhardtii, Chlamydomonas debaryana, Scenedesmus sp.* | Biomass / Biofuels / Nutrients removal | Hu et al., 2012; Hasan et al., 2014; Kim et al., 2014; Michelon et al., 2016 |
| Tannery—soak liquor | *Spirulina sp., Nannochloropsis sp.* | Biomass | Abinandan et al., 2014 |
| Tapioca and cassava ethanol | *S. platensis; C. pyrenoidosa* | Bioelectricity / Biomass | Hadiyanto et al., 2019; Yang et al., 2008 |
| Wet market effluent | *Scenedesmus sp.* | Nutrients removal | Apandi et al., 2018 |

### 1.4.3. $CO_2$ Capture and Fixation

Similarly to terrestrial plants, cyanobacteria and microalgae present a net positive $CO_2$ uptake, fixating $CO_2$ only during daytime, while overnight some $CO_2$ is emitted back to the environment (Razzak et al., 2013). Cyanobacteria in particular have an enormous biotechnological potential and play a key role in carbon fixation process on a global scale, since they present at least 10-fold higher $CO_2$ fixation ability compared to terrestrial plants, rapid growth rates and tolerance to extreme environments (Bhola et al., 2014; Singh et al., 2014; Sanchez-Baracaldo and Tanai, 2020). Due to these attributes, cyanobacteria, along with microalgae are responsible for approximately 50% of the total global carbon fixation (Field et al., 1998; Swarnalatha et al., 2019), capable of sequestering approximately 513 tons of $CO_2$ and converting it into approximately 280 tons of dry biomass $ha^{-1}$ $year^{-1}$ by utilizing about 10% of solar energy (Sydney et al., 2010). More than 65 GT of carbon per year is been estimated to be fixed globally, corresponding to the carbon output of 65000 power plants of 500MW each (Weerahandi et al., 2012).

It has been shown in a plethora of studies that several cyanobacteria and microalgal strains are capable of efficiently growing in the presence of flue gases that also contain other contaminants (SOx and NOx), while fixating 50% of the $CO_2$ (Wang et al., 2008). *Botryococcus braunii, Chlorella sorokiniana, Chlorella vulgaris, Chlorococcum littorale, Dunaliella tertiolecta, Nannochloropsis oculata, Scenedesmus dimorphus, Scenedesmus obliquus,* and *Spirulina platensis* are some species that present extraordinary capability to fix $CO_2$ (Cheah et al., 2015). Table 1.7 lists some of the studied species in relation to their $CO_2$ fixation potential (rate).

**Table 1.7.** $CO_2$ tolerance and uptake by various cyanobacteria and microalgae (Viswanaathan et al., 2022).

| **Cyanobacteria and microalgae species** | **$CO_2$ tollerance (% concentration)** | **$CO_2$ fixation rate (% concentration)** |
|---|---|---|
| *Chlamydomonas sp.* | 15 | - |
| *Chlorella sp.* | 0.03 - 15 | 0.46 - 1.62 |
| *Chlorella kessleri* | 18 | 0.16 |
| *Chlorella (marine)* | 2 - 15 | 2.14 - 4.69 |
| *Chlorella ryrenoidosa SjTU-2* | 5 - 50 | 0.029 - 0.071 |
| *Chlorella vulgaris* | 2 - 18 | 0.25 - 0.43 |
| *Chlorococcum littorale* | 20 - 60 | 0.246 |
| *Chlorococcum cohaerens* | 0.03 | 0.78 |
| *Cyanidium caldarium* | 100 | - |

| | | |
|---|---|---|
| *Dunaliella sp.* | 3 | 0.31 |
| *Dunaliella tertiolecta* | 10-15 | 0.27-5.82 |
| *Eudorina sp.* | 20 | - |
| *Euglena gracilis* | 45 | - |
| *Haematococcus pluvialis* | 34 | 0.14 |
| *Microcystis aeruginosa* | 15 | 0.134 |
| *Microcystis ichthyoblabe* | 15 | 0.142 |
| *Nannochloris sp.* | 15 | - |
| *Phaeodactylum tricornitum* | 15 | 0.59 |
| *Phormidium sp.* | 15 | 7.39 |
| *Scenedesmus sp.* | 15 - 80 | 0.61 |
| *Scenedesmus dimorphus* | 0.03 | 1.27 |
| *Scenedesmus incrassatulus* | 0.03 | 1.50 |
| *Scenedesmus obliquus* | 2.5 - 18 | 0.55-4.6 |
| *Synechococcus elongatus* | 60 | - |
| *Spirulina sp.* | 20 | 0.14 |
| *Spirulina platensis* | 15 | 0.92 |
| *Tetraselmis sp.* | 14 | - |

Of the species presented in Table 1.7, it is evident that cyanobacteria show increased tolerance to $CO_2$ concentrations, which can have significant implications in the development of carbon sequestration technologies for flue gases remediation. Indicative cyanobacteria $CO_2$ sequestration applications are presented in Table 1.8.

**Table 1.8.** $CO_2$ sequestration and applications of cyanobacteria (Dalvi et al., 2021).

| **Cyanobacteria** | **Biotechnological application** |
|---|---|
| *Thermosynechococcus* CL–1 (TCL–1) | $CO_2$ biofixation and bioethanol production |
| *Phormidium valderianum* BDU 2004 | $CO_2$ sequestration |
| *Synechocystis salina* and *Microcystis aeruginosa* | $CO_2$ biofixation |
| *Phormidium* sp. | Bioethanol production |
| *Synechococcus aquatilis* | $CO_2$ sequestration |
| *Synechococcus lividus* and *Mastigocladus laminosus* | $CO_2$ sequestration |

Although able to withstand high concentrations of $CO_2$, cyanobacteria and microalgae exhibit enhanced cell productivity only at $CO_2$ concentrations of 10–40%, correlated to factors such as their tolerance limit to $CO_2$ and $CO_2$ utilization rate (Nagase et al., 1998; de Morais and Costa, 2007; Ramirez-Perez and Janes, 2009; Ramanan et al., 2010; Viswanaathan et al., 2022). There are different tolerance limits and fixation rates of $CO_2$ present in flue gas, since among different species cultivated species in similar conditions, considerable variations in $CO_2$ fixation rates are observed ranging from

200-1000 mg L−1 day−1 (Sydney et al., 2010; Larsson and Lindblom, 2011; Lizul et al., 2014; Cheah et al., 2015).

The efficiency of $CO_2$ removal or fixation in a closed culture system depends on algal species, $CO_2$ concentration, photobioreactor design and operating conditions (Cheng et al., 2006; de Morais and Costa, 2007). The exact relation between $CO_2$ concentration and fixation efficiency has not yet been established, since there is an apparent species dependence on the $CO_2$ fixation efficiency, probably attributed to physiological conditions of algae, such as potential for cell growth and $CO_2$ metabolism (Razzak et al., 2013).

## 1.5. Parameters affecting biomass growth and composition

Cyanobacteria and microalgae can rapidly grow with a theoretical yield of about 77 g $m^{-2}$ $day^{-1}$ which is about 280 ton $ha^{-1}$ $year^{-1}$ (Melis, 2009; Formighieri et al., 2012), being significantly lower in large scale culture systems and PBRs due to lower PAR transfer phenomena (Rodolfi et al., 2009; Bchet et al., 2013; Medipally et al., 2015). Their size usually ranges between 0.2 μm to 20 μm and their growth rate is determined by external factors such as light intensity and spectrum, alkalinity, salinity, nutrients availability and temperature (Mata et al., 2010). In general, cyanobacteria and microalgae thrive in neutral to alkaline conditions and at temperatures above 20 ºC and below 40 ºC, while light intensity, $CO_2$ and nutrients availability play major role on their growth rate. Cyanobacteria can generally tolerate lower temperatures, higher $CO_2$ concentrations and can thrive in relatively alkaline environments and in conditions of limited lighting (Singh et al., 2015; Viswanaathan et al., 2022). Other important factors that determine the success of cultivation include stirring and mixing, width and depth of the bioreactor, harvest frequency and dilution rate (Khan et al., 2018).

The interest in cyanobacteria and microalgae as a renewable and sustainable feedstock for biofuels production has inspired a new focus in biorefinery pushing research towards the study of potential applications and of growth enhancement techniques (Khan et al., 2018). Extensive study is necessary to increase biomass productivity by means of optimizing cultivation conditions, as well as to obtain the required design and operational characteristics required for upscaling and implementing cyanobacteria cultivation processes on industrial scale. The critical factors that affect cyanobacteria and microalgae growth rate and composistion, such as culture lighting, temperature,

nutrients availability, pH and salinity, must be extensively studied in order to extract fundamental design and operational parameters for upscaling such processes.

When cyanobacteria and microalgae are imposed to nutrients or salinity changes, cellular mechanisms are triggered as a response, which in most cases increase intracellular accumulation of target compounds, thus biomass value, but at a cost on cell productivity. There are stressors, such as changes of lighting conditions, pH, temperature or the presence of antagonistic or growth hindering microbial species, which alter synthesis and accumulation rate of target carbonaceous and nitrogenous compounds, such as carbohydrates, lipids, proteins, protein-based compounds and pigments (Markou et al., 2019). In Table 1.9, indicative cases of high-value compounds production using cyanobacteria and microalgae species under stress conditions are presented.

**Table 1.9.** Indicative cases of high-value compounds production under different stress conditions (modified from Markou et al., 2012).

| Compound | Strain | Stressor | Content (%w/w) |
|---|---|---|---|
| Astaxanthin | *Haematococcus pluvialis* | Nitrogen limitation, high light | 21% |
| | *Haematococcus pluvialis* | High light, mixotrophy | 10% |
| | *Haematococcus pluvialis* | High temperature | 5.5% |
| | *Chlorella sp.* | 0.2 mM NaCl | 4% |
| | *Chlorella sp.* | High light | 1.5 |
| β-carotene | *Dunaliella salina* | Hight light | 3.1% |
| | *Dunaliella salina* | Nitrogen limitation | 2.7% |
| Phycocyanin | *Arthrospira* sp. | 0.6 M NaCl | 17.5% |
| | *Arthrospira* sp. | Two-stage culture process, 10 μmol E $m^{-2}$ $s^{-1}$ + 0.5 g $L^{-1}$ NaNO3 + 11.7 g $L^{-1}$ NaCl + 2.68 g $L^{-1}$ $K_2HPO_4$ | 16.09% |
| Lipids | *Chlorella* sp. | Nitrogen limitation | 65.1% |
| | *Chlorella* sp. | Phosphorus limitation | 47.7% |
| EPA (eicosapentaenoic acid) | *Chaetoceros brevis* | Temperature and light (30°C, 75 μmol photons $m^{-2}$ $s^{-1}$) | 31.5% of total fatty acids |
| EPS (exopolysaccharides) | *Arthrospira* sp. | Two-stage culture process, 10 μmol E $m^{-2}$ $s^{-1}$ + 40 g $L^{-1}$ NaCl | 1.02 g $g^{-1}$ DW |
| | *Chlorella* sp. | 50 μmol E $m^{-2}$ $s^{-1}$, 50°C | 1.8 g $L^{-1}$ |
| | *Nostoc* sp. | 160 μmol E $m^{-2}$ $s^{-1}$, 30°C, 1% Glucose (w/v) | 1.8 g $L^{-1}$ |

| | | | |
|---|---|---|---|
| Phenolics | *Spirulina platensis* | Light intensity (120 µmol photons $m^{-2}$ $s^{-1}$) | 49.83 mg $g^{-1}$ DW |
| | *Selenastrum capricornutum* | Temperature (12°C) | 42.8 µg $g^{-1}$ DW |
| | *Arthrospira plantensis* | 35°C, 2.5 g $L^{-1}$ Nitrogen | 5 mg GAE $g^{-1}$ |
| Sucrose/biohydrogen | *Synechococcus elongatus* PCC 7942* | Salinity up to 0.4 M NaCl, 0.04% to | Sucrose: molar ratio 6.5 sucrose Chl $a^{-1}$ Biohydrogen: 0.76 nM $H_2$ mgChl $a^{-1}$ $h^{-1}$ |
| Sucrose/biohydrogen | *Synechococcus elongatus* PCC 7942 (PAMCOD)* | Salinity up to 0.4 M | Sucrose: molar ratio 13.5 sucrose Chl $a^{-1}$ Biohydrogen: 2.0 nM $H_2$ mgChl $a^{-1}$ $h^{-1}$ |

*Vayenos et al., (2020)

Cyanobacteria in particular have evolved a plethora of cellular mechanisms to address changes in their environment (Table 1.10) and cope with the stressful conditions leading to physiological and biochemical alterations (Table 1.11). These mechanisms affect physiological processes of cyanobacteria, such as growth rate, pigments content, lipids and carbohydrates content, net photosynthetic rate and nitrogen metabolism. Thus, there are opportunities for the manipulation of cyanobacteria biosysthesis to an extent that will increase the value of cultivated biomass and render cyanobacteria-based technologies economically viable.

**Table 1.10.** Molecular mechanisms employed by cyanobacteria for adaptation to major abiotic stress factors including high and low temperatures, UV radiation and salinity stress (modified from Rosic, 2021).

| **Abiotic stressor** | **Mechanisms** | **Molecule** | **Features** |
|---|---|---|---|
| Temperature | Osmotic balance; Membrane fluidity | Shorter chain polyunsaturated fatty acids | Accumulation of shorter chain polyunsaturated fatty acids within the membrane |
| | Osmotic balance | Trehalose | Accumulations of compatible solutes allow tolerance to cold by decreasing the intracellular fluid freezing temperature and cell desiccation |
| | Adhesion, cellular protection | Extracellular polysaccharides (EPS) | Nostoc commune macroscopic colonies have cells embedded within the EPS improving cold and desiccation tolerance |
| | Differential gene expression/protein synthesis | Heat shock proteins (HSPs) | HSPs affect protein folding, unfolding, aggregation, degradation and transport |
| UV radiation | Photoprotection; Scavenging ROS | Scytonemin | Absorbing radiation in the UV-A range (315–400 nm) with max at 370 nm; antiproliferative, anti-inflammatory and antioxidant activities |
| | | Mycosporine-like amino acids (MAAs) | Absorbing radiation in the UVA and UVB (280–315 nm) ranges with max from 310 to 362 nm; antiproliferative, anti- |

| | | | |
|---|---|---|---|
| | | | inflammatory; antiageing features; antioxidant activities |
| | | Carotenoid pigments | Absorbing radiation in the UVR and visible range 300–600 nm; light-harvesting molecules; antioxidant, anti-inflammatory and antiproliferative activities |
| | Scavenging ROS | Polyphenols | Antioxidant properties |
| Hypersalinity | Osmotic balance (via accumulation of compatible solutes) | Disaccharides (sucrose and trehalose) | In freshwater cyanobacteria (low-salt tolerance strains), protection against desiccation |
| | | Glucosylglycerol (GG) | In cyanobacteria from marine environments (moderate-salt tolerance); filamentous (e.g. Coleofasciculus) and unicellular (Synechocystis) halophilic cyanobacteria |
| | | Glycine betaine (GB) | In hypersaline environments, GB has accumulated plus GG, disaccharides at lower concentrations |
| | | MAAs | MAA secondary metabolites in the cytoplasm play a role in osmotic regulation and drought protection |

**Table 1.11.** Effects of various stress conditions on specific and common physiological and biochemical parameters in cyanobacteria (modified from Pandey et al., 2021).

| Stressor | Substance | Specific alterations | Common alterations | Cyanobacteria species |
|---|---|---|---|---|
| Metal toxicity | $Cd^{2+}$ | Increase in the thickness of the sheath layer, increase in number and size of polyphosphate granules to incorporate heavy metals into them | Deterioration of thylakoid membranes | *Anabaena flos-aquae* |
| | $Pb^{2+}$ | | Decrease in heterocyst frequency | *Anabaena* sp. |
| | $Cr^{2+}$ | Decline in EPS, exo-polysaccharide, the polymer of carbohydrates, and protein contents | | *Nostoc muscorum* ATCC 27893 and *Anabaena* sp. PCC 7120 |
| | $As^{5+}$ | EPS content increased at lower dose, depressions, and grooves on the surface for binding of the metal ions. White crusts over the apertures present more in *Anabaena* sp. than *Nostoc muscorum* | Decline in phycobiliproteins (PBPs) | *Nostoc muscorum*, *Anabaena* sp. |
| | $Al^{3+}$ | | Decrease in EPS secretion, decline in photosynthesis rate | *Anabaena* PCC 7120 |
| | $Cu^{2+}$ | Excretion of siderophores in huge quantities to alter chemical speciation of surface waters for either sequestration or decrease in copper toxicity source | Nitrogen fixation affected | Cyanobacteria |
| Insecticide | Deltamethrin (2.8%) | | Induced carbohydrate accumulation, decline in nitrogen fixation | *Calothrix sp.* (GUEco1002) |
| | Cypermethrin | Decline in PS II photochemistry | Enzymatic antioxidant activity increased as defense mechanism | *Nostoc muscorum* |
| Salinity | NaCl | Osmotically derived hydrostatic pressure creates tension; thus, cell wall is expanded and stretched at junction of the septum and nascent | | *A. cylindrica* |

| | | | | |
|---|---|---|---|---|
| | | pole, increased plastoquinone, and a subsequent decrease in chl *a* | | |
| | NaCl | | Decrease in heterocyst frequency, increase in carbohydrate contents under low stress as adaptive measure | *A. cylindrica* |
| Drought | | Accumulation of considerable amount of proline that may act as osmoregulant | Secretion of EPS to protect cell walls from damage during swelling and shrinkage associated with drought stress | L. boryana |
| Radiation | UV-B | | Decrease in heterocyst frequency | *Anabaena* sp., *Nostoc* sp., *Nostoc carneum*, *Scytonema* sp. |
| | UV-A + UV-B | Phycobilisomes are disarranged and form amorphous aggregates dispersed in cytoplasm. Polyphosphate granules get converted into amorphous structure from round structures | Drastically damaged thylakoids | *Cylindrospermopsis raciborskii* CYRF-01 |
| Microcystin toxicity | | PSII is direct target site of microcystin and inhibits it | | *Synechococcus elongatus* |

### 1.5.1. Light

By definition, photosynthesis (Figure 1.13) is the process of complex organic compounds synthesis by utilizing the energy of light. It is the biological pathway that all plants, algae and cyanobacteria follow for their growth by converting light, inorganic or/and organic carbon, nutrients and micronutrients to organic molecules used in cellular synthesis. Consequently, light availability, in fact light intensity, light frequency and photoperiods, is one of the major factors that affect the growth rate of phototrophic species (Carvalho et al., 2011; Stockenreiter et al., 2013; Gris et al., 2014; Liao et al., 2017; Abu et al., 2016; Binnal and Babu, 2017; Ferro et al., 2018; Iasimone et al., 2018; Lehmuskero et al., 2018; Mehan et al., 2018).

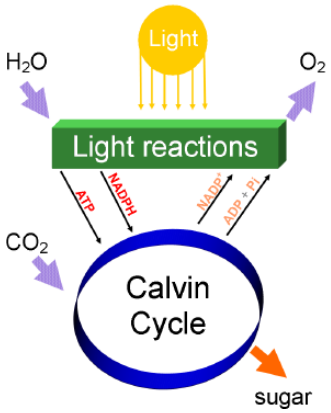


**Figure 1.13.** Simplified illustration of photosynthesis process (Mayer, 2008).

Light intensity, usually expressed in μmol of photons that impact on a square meter per second (μmol $m^{-2}$ $s^{-1}$), proportionally affects the growth rate of phototrophic species until reaching a saturation point at which the photosynthetic activity of microalgae

achieves its maximum value (Raeisossadati et al., 2019). According to Schuurmans et al. (2015), the optimum level of light intensities for most cyanobacteria and microalgae species is 200 to 400 µmol $m^{-2}$ $s^{-1}$, whereas the optimal light intensity for cyanobacterial biomass production was computationally determined to be 261 µmol $m^{-2}$ $s^{-1}$. When light intensity exceeds the saturation point, the phenomenon of photoinhibition appears, which limits cellular growth due to light induced damages in photosystems I (PSI) and II (PSII) (Binnal and Babu, 2017; Ramanna et al., 2017). The three distinguished phases of the photosynthetic light-response curve are depicted in Figure 1.14.

Moreover, the excess of absorbed photons is emitted as heat or fluorescence, which results in reduced photosynthetic efficiency (Baker, 2008; Behera et al., 2018; Lehmuskero et al., 2018). Cyanobacteria and microalgae may respond to the light induced reduction of photosynthetic efficiency via photoadaptation/photoacclimation process and the plethora of mechanisms involved, such as the changing the types and quantities of pigments, cellular growth rate, dark respiration rate or the availability of essential fatty acids (Fabregas et al., 2004).

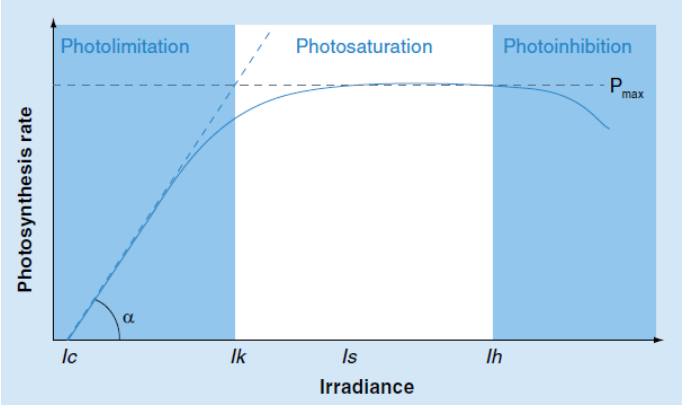


**Figure 1.14**. PI-curve that relates photosynthesis rate to irradiance. Ic: Compensation irradiance, the point at which photosynthetic oxygen production balances respiratory oxygen consumption; Ih: Irradiance value at which photoinhibition sets in and light become injurious; Ik: The irradiance at which control passes from light absorption and photochemical energy conversion to reductant utilization; Is: Saturation value, the point at which P does not increase despite the increased irradiance. (Source: Tredici, 2010)

Photoinhibition can occur both in cases of natural or artificial lighting and is highly associated with cultivation bioreactor (photobioreactor, PBR) design and light transfer efficiency. Light distribution significantly differs depending on light source intensity,

optical light path and the physicochemical characteristics of culture solution. Light intensity is higher near the light source, usually in the surface of tank bioreactors or at the walls of a PBR and is reduced towards their bottom or their center respectively. The rate at which light intensity reduces follows the inverse square law, while is highly affected by light scatter and light adsorption phenomena (Ogendal, 2019). Thus, photoinhibition may occur locally, whereas in deeper areas within the PBR dark zones may appear, where light intensity is significantly lower limiting photosynthesis (Gris et al., 2014; Barcelo-Villalobos et al., 2019). Moreover, the increase of biomass content in a PBR induces a phenomenon called shelf-shading which is caused by scattering of light upon impact on particulate matter, such as cyanobacteria and microalgae biomass (Park and Lee, 2001; Martinez et al., 2018). The volume of the dark zone depends on light intensity, light path, particulate matter concentration, type of photosynthetic pigments in cells, culture turbidity and color, as well as PBR opacity (Lehmuskero et al., 2018; Abu-Ghosh et al., 2016; Wagner et al., 2018; Martinez et al., 2018; Bauer et al., 2021).

Both photoinhibition and shading phenomena can be resolved on the basis of PBR design and operation i.e., by means of reactor geometry and lighting orientation, lighting cycle alteration, culture agitation and regulation of optical density through biomass harvesting (Wang et al., 2012; Iluz and Abu-Gosh, 2016; Barcello-Villalobos et al., 2019; Gonzalez-Camejo et al., 2019).

It is worth mentioning at this point that among the phototrophic species, cyanobacteria and red algae present a comparative advantage that allows their growth even at very low light conditions, as they possess antennae structures arranged as complexes, termed phycobilisomes, on top of the membrane near the reaction centers of photosystem II (Figure 1.15) that can collect light of very low intensity (Heldt and Piechulla, 2011; Viswanaathan et al., 2022).

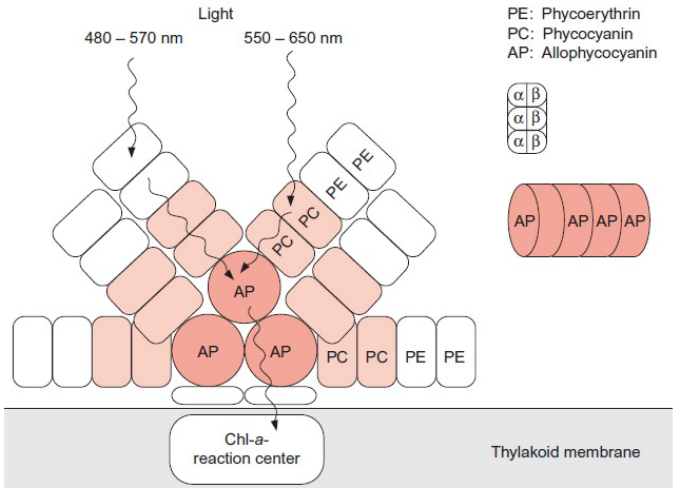


**Figure 1.15.** Schematic presentation of phycobilisome structure as proposed by Bryant et al. (1979) and illustrated by Heldt and Piechulla (2011). (Elsevier license number: 5357621118400)

The phycobilisomes or phycobiliproteins (PBPs) play prominent role in absorption of the light energy and transfer it to photosystems for initiating the photochemistry and they are responsible for the bluish-green color of cyanobacteria when cultivated under low-light conditions, since PBPs can constitute up to 40–50% of the total proteins in the cell (Book 3.2 Tiwari et al.,). PBPs are classified into three major categories: allophycocyanin (APC), phycocyanin (PC), and phycoerythrin (PE) with characteristics spectrum referred as blue-green (650–655 nm), blue (610–620 nm), and pink (540–570 nm), respectively, in both cyanobacteria and red algae (Moraes and Kalil 2009; Sonani et al., 2014a, b). It is estimated that around 50% of absorbed light by PBPs is transferred to photosystems that involve in $CO_2$ fixation during the process of photosynthesis (Tavanandi et al., 2018). In order to protect their photosynthetic apparatus, cyanobacteria have photoprotective pigments named carotenoids, which minimize oxidative stress by quenching singlet oxygen, while performing a role as accessory light-harvesting pigments that are also involved in photochemical quenching (Melnicki et al., 2016). Moreover, To overcome oxidative stress, cyanobacteria endowed antioxidant defense system involving enzymatic antioxidants, viz. superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POD), ascorbate peroxidase (APX), glutathione reductase (GR), dehydroascorbate reductase (DHAR), glutathione-S-transferase (GST), and nonenzymatic antioxidants; and cysteine (Cys), proline (Pro), and NP-SH (Kumar et al., 2018). These substances are of high-value and can have various applications in pharmaceutical, cosmetics and food industry.

### 1.5.1.1. Light frequency and photosynthetic pigments

The duration and intensity of light are not the only factors affecting photosynthetic activity of cyanobacteria and microalgae, as the frequency of the emitted light significantly affects light energy attenuation from photosynthetic pigments. Although rate of growth under increasing light intensity is a function of strain and culture temperature, the growth rate of algae is maximal at saturation intensity and decreases with both increase or decrease in light intensity (Sorokin and Krauss, 1958). Different phototrophic species have different pigments or have different pigments ratio for capturing photons, thus utilize different light wavelengths from the PAR spectrum. As evident in Figure 1.16, the absorption spectrum among photosynthetic pigments significantly differs. Chlorophylls present an affinity for the lower wavelengths of PAR spectrum (400 nm to 500 nm), while phycobilines (phycierythrin, phycocyanin, allophycocyanin) for higher wavelengths and up to 680 nm. It is worth mention at this point that the energy content of blue light (400–480 nm) is greater than that of red light (620–750 nm), fewer photons of blue light are required to achieve an equivalent magnitude of energy intensity using red light. Selecting light sources that emit at specific desirable wavelengths for achieving efficient photosynthesis (Emerson and Lewis, 1943) and enhances specific cellular processes, such as cellular division (Voigt and Munzner, 1994), polysaccharides synthesis (You et al., 2004), starch formation (Miyachi and Kamiya, 1978).

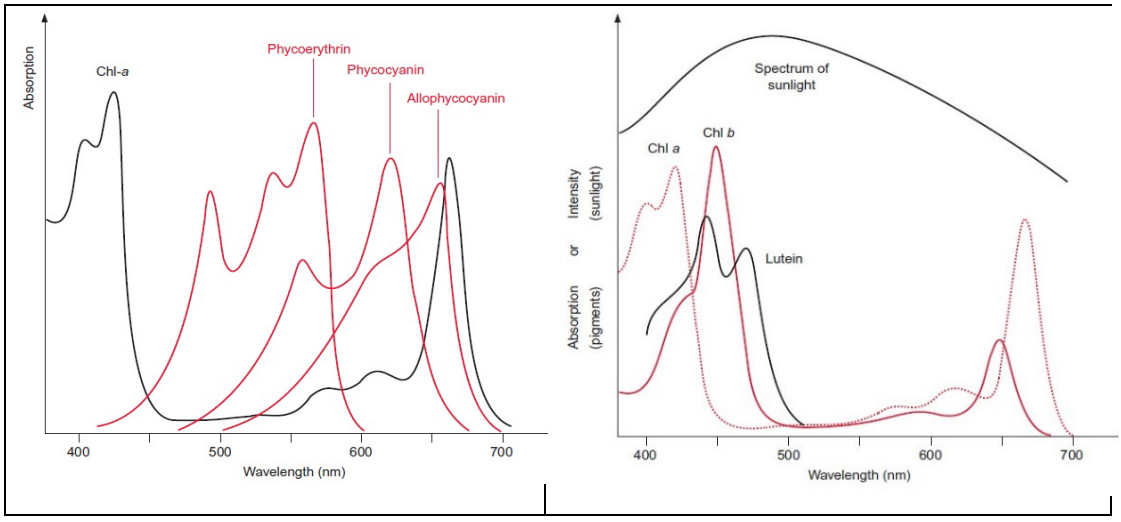


**Figure 1.16.** Absorption spectrum of cyanobacteria photosynthetic pigments (left) and of microalgae (right) (Heldt and Piechulla, 2011). (Elsevier license number: 5357621118400)

Thus, when artificial lighting is implemented as the sole or supplementary lighting source of a PBR, the light source spectrum in relation to the cultivated phototrophic species' photosynthetic pigments must be taken into serious consideration for the selection of the most efficient solution for cyanobacteria and microalgae cultivation. The selection of lighting source is one of the most critical components for cyanobacteria and microalgae cultivation systems, as it must ensure increased cell productivity via emission of specific light intensity and wavelengths, with relative low energy consumption, low heat dissipation, high reliability and low installation/operational cost (Carvalho et al., 2011). Table 1.12, obtained from Gonzalez-Camejo et al. (2019) briefly summarizes some advantages and disadvantages of different artificial light sources applied in cyanobacteria and microalgae cultivation.

**Table 1.12.** Advantages and disadvantages of different artificial light sources (Gonzalez-Camejo et al., 2019).

| Light source | Advantages | Disadvantages | Reference |
|---|---|---|---|
| Incandescent light bulbs | Low cost | - Light emitted in infrared region<br>- Light radiated in all directions | (Matthijs et al., 1996; Carvalho et al., 2011) |
| Halogen lamps | Better energetic efficiency than light bulbs | - Light emitted in infrared region<br>- Light radiated in all directions | (Matthijs et al., 1996; Carvalho et al., 2011) |
| Fluorescent lamps | Similar spectrum to daily light | More expensive than light bulbs and halogen lamps | (Matthijs et al., 1996) |
| LED lamps | Narrow wavelength<br>High efficiency<br>Long lifespan<br>Reduce light stress<br>Dissipate less energy | High cost | (Matthijs et al., 1996; Carvalho et al., 2011; Yan et al., 2013; Singh et al., 2015; Nwoba et al., 2019) |

The phicobiliproteins of cyanobacteria and red algae give them an evolutionary advantage over other algae and higher plants, as they allow phycobilisomes to absorb green light very efficiently (Figure 1.16), while phycocyanine and phycoerythrin enhance the absorbance of shorter and longer wavelengths respectively (Heldt and Piechulla, 2011). The optimum light absorbance spectrum for cyanobacteria and microalgal growth differs from strain to strain, however, blue (420–470 nm) and red (660 nm) wavelengths seem to be more suitable for green algae growth and lipid accumulation, whereas cyanobacteria tend to prefer red, yellow, or green light (Markou et al., 2019). It is worth mentioning at this point that the cultivation medium should

allow the efficient light penetration of specific wavelengths into culture in order to increase cell densities (Teo et al., 2014; Schulze et al., 2014).

The photosynthetic pigments of cyanobacteria and microalgae, especially phycobiliproteins and carotenoids, are the most successful commercial products derived from such biomass (Markou et al., 2019), having many applications in bifunctional food production, cosmetics industry and other economic sectors (Leu and Boussiba, 2014; Sonani et al., 2017). The global market value of phycocyanin and carotenoids is at an increasing trend in view of the environmental and health consumers advantages (Ranga Rao et al., 2018a,b), estimated at $138.6 million for phycocyanin and $1.5 billion for carotenoids regarding the years 2020 (AMR, 2020) and 2017 (BCC, 2018) respectively.

Production of cyanobacterial pigments has already a firm position in the market and gains increased interest especially in view of environmental and health consumers advantages (Ranga Rao et al., 2018a,b). -carotene, astaxanthin, and phycocyanin available in markets are commercialized under classical forms such as powders, tablets, or liquids. The price of phycobiliproteins varies from US$3/mg to US$25/mg (Pandey et al., 2019). The global market was estimated to reach approximately US$60 million in 2019. Recently, advanced forms are emerging, especially for phycocyanin, and include microcapsules, nanoparticles, and nanofibers (de Morais et al., 2018). The global market value of carotenoids reached $1.5 billion in 2017 and should reach $2.0 billion by 2022 (BCC, 2018), while the phycocyanin market value in 2018 was estimated at $122.3 million and estimated to touch a value more than $233 million by 2028 (BCC, 2018).

In order to enhance the prospects of economic feasibility of cyanobacteria or/and microalgae production, nutrient starvation has been one step applied to the process. For example, astaxanthin content gets enhanced in Haematococcus when exposed to nutrient starvation, such as nitrogen, phosphorus, or sulfur, or under salt stress, high temperature, and high light intensity (Dominguez-Bocanegra et al., 2004; He et al., 2007). Another example is the production of β-carotene from Dunaliella under nutrient starvation with probable combination with salinity stress (Ben-Amotz, 1999; Phadwal and Singh, 2003).

### 1.5.2. Temperature

Temperature is one of the most influential environmental factors that can affect algal growth rate, cell size, biochemical composition, as well as nutrient solubility, requirements and uptake (Juneja et al., 2013; Viswanaathan et al., 2022). In general, cyanobacteria and microalgae (i) can grow in a wide temperature range from 15 to 40 $^{o}$C, with optimal temperature usually between 20 to 35 $^{o}$C depending on species and other environmental conditions (Viswanaathan et al., 2022), (ii) their growth rate increases with increasing temperature but declines markedly above the species- or strain- specific optimum as the increased photorespiration impacts cell productivity (Konopka and Brock, 1978; Renaud et al., 2002; Singh and Singh, 2015), while (iii) at optimal growth temperature their cell size is minimal (Rhee, 1982; Harris, 1986), whereas (iv) at non-optimal temperatures their efficiency for carbon and nitrogen utilization decreases (Darley, 1982), which is probably attributed to cytoplasmic viscosity changes (Hope and Walker, 1975; Raven and Geider, 1988). Moreover, it has been suggested that temperature has a significant effect (v) on photoinhibition threshold light intensity value (Vonshak and Torzillo, 2004), as well as (vi) on the level of unsaturation of fatty acids in the lipid membrane, the accumulation of polysaccharides (Kalacheva et al., 2002; Thompson, 1996; Harwood, 2004; Guschina and Harwood, 2009) and starch content (Emerson et al., 1944). Increasing temperature beyond the optimum reduces protein synthesis and leads to decreased growth rates, cellular death and lysis (Konopka and Brock, 1978). Although the algal productivity increases with increasing temperature, beyond the optimum range, photorespiration negatively impacts its productivity (Harker et al., 1995). It is worth mentioning at this point that exceeding the optimum temperatures by only 2–4 °C may result in the total culture loss (Richmond, 1999).

As previously mentioned, (§1.5), temperature can impose stressful conditions in cyanobacteria and/or microalgae proliferation. In this regard, cyanobacteria species have evolved mechanisms to cope with temperature changes, some of them been able to withstand even extreme temperatures. During temperature adaptation, the metabolic changes in cyanobacteria may result in the synthesis of valuable compounds. For example, during temperature stress of *Synechococcus elongatus* PCC 7942, the production and accumulation of carotenoids increases, since the specific pigment performs the protection mechanism against peroxidation process (Latifi et al., 2009).

Thus, temperature not only affects cyanobacteria growth rate, but may also alter the biochemical composition of biomass and subsequently its uses and value.

### 1.5.3. pH

In most cases, fresh water eukaryotic algae can grow well in slightly acidic environments (with pH around 6.5–6.8); however, alkaline conditions (pH 7–9) are mostly desired by cyanobacteria (Viswanaathan et al., 2022), such as the alkaliphilic cyanobacterium *Synechococcus elongatus* PCC 7942 (Billini et al., 2008). The impact of pH on algae growth differs among species, while it is been established that pH determines the solubility and availability of $CO_2$ and nutrients in the cultivation medium and can have a significant impact on cyanobacteria and microalgae metabolism (Goldman, 1973; Chen and Durbin, 1994). In cyanobacteria and microalgae cultures, pH determines the speciation of carbon in the solution, thus the availability of carbon that is usually bioaccumulated in the form of $CO_2$ via passive flux or $HCO_3^-$ via active transport (Nielsen, 1975; Azov, 1982; Chen and Durbin, 1994; Moazami-Goudarzi and Colman, 2012). Figure 1.16 illustrates the speciation of inorganic carbon in water in relation to pH. Thus, the control of pH in cyanobacteria and microalgae cultures can assist in $CO_2$ feeding and the minimization of $CO_2$ losses (Razzak et al., 2013).

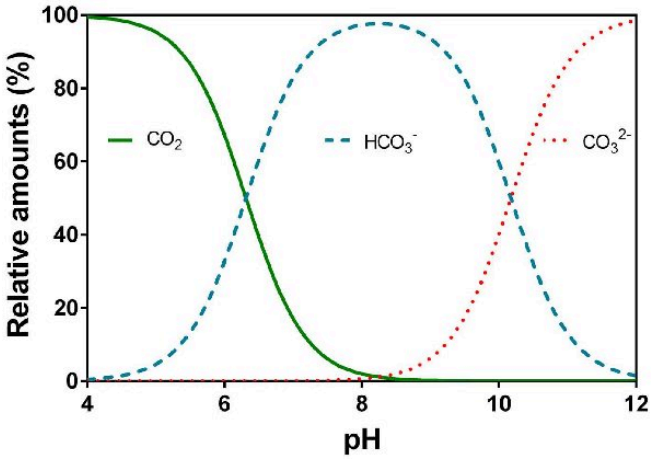


**Figure 1.16.** Relative speciation (%) of carbon dioxide ($CO_2$), bicarbonate ($HCO_3^-$), and carbonate ($CO_3$) in water as a function of pH (Pedersen et al., 2013). (Elsevier license number: 5357640490547)

Regarding the effect of pH on nutrients availability, it is widely established that at alkaline conditions and high dissolved oxygen concentrations, the availability of phosphates and of metallic ions may be compromised due to their potential transformation to precipitating salts (Cai et al., 2013). On the other hand, at acidic pH

the uptake of nutrients can be disturbed or metal toxicity may be induced (Sunda, 1975; Anderson and Morel, 1978). Cyanobacteria species in particular present significant decrease in photosynthetic activity when exposed to pH levels below 6.0 (Coleman and Coleman, 1981), but they may adapt in such conditions (Goldman and Azov, 1982), while pH values continuously rising during the active growth phase due to the release of $OH^-$ ions (Kaplan et al., 1980; Hansen, 2002).

### 1.5.4. Microbial contaminants

The presence of microbial contaminants in cyanobacteria or/and microalgae cultures has a significant impact on biomass yields, since microbial stains may hinder the growth of the cultivated phototrophic specie(s) due to cell shading phenomena, the antagonistic consumption of nutrients or/and light, as well as due to the predating behavior of various protozoan and metazoan species. There are two ways that microbial contaminants can infestate a culture, either by being a component of a non-properly disinfected cultivation medium or by external contamination, such as airborne microbial contaminants.

Microbial contamination poses a serious threat to cultivation systems, especially the open pond systems that are susceptive to external contamination, thus must be quickly and effectively controlled, as these organisms can rapidly cause complete loss of production batches (Yu et al., 2015; Jaiswal et al., 2018; Gonzalez-Morales et al., 2020). In this regard, closed photobioreactors (PBR), which allow for better control of cultivation conditions, can provide a more sterile environment that enables the optimized growth of a specie(s) for producing a desired product (Kuan, 2015).

In order to control biological contaminants, various disinfection treatment techniques are often used, such chemical treatment (use of herbicides, antibiotics, detergents, hypochlorite, phenol etc.) and filtration/separation techniques (Da Silva and Reis, 2015; Gupta et al., 2019). Another strategy to deal with contamination is the use of selective culture environments, such as pH control and the regulation of salt concentrations for halotolerant strains (Bacellar et al., 2013).

The practice has shown that the disinfection efficiency, the installation and operation cost, as well as the complexity of the applied process or technique are the parameters that dictate the applied disinfection protocol in full-scale installations, such as in water refineries and wastewater treatment plants. That been said, on full-scale imthe

commonly applied techniques of filtration or chemical disinfection will always be preferred to those based on UV radiation, ozonation or the strict control of culture environment.

### 1.5.5. Salinity and Nutrients

Salinity, which is usually expressed as sodium chloride concentration unless otherwise specified, is a physichochemical parameter that can affect the growth rate of cyanobacteria and microalgae, as well as nutrients assimilation rate and biomass composition (Juneja et al., 2013; Ravishankar and Ranga Rao, 2020). Each species has a specific range of salinities where it can proliferate. Moreover, cyanobacteria and microalgae may respond to changes of salinity by altering their biochemical composition, such as changing their content in lipids, proteins, carbohydrates and pigments (Ben-Amotz et al., 1985; Renaud and Parry, 1994; Takagi and Yoshida, 2006; Zhila et al., 2011; Vayenos et al., 2020).

In the case of freshwater cyanobacteria, which are less tolerant of high salinity conditions, under salt stress they accumulate additional quantities of disaccharides, such as sucrose and trehalose, as compatible solutes (organic osmotic solutes) to counter high salinity conditions by maintain osmotic balance (Hagemann, 2011; Oren, 2015). In more detail, in response to salt stress, which is caused by the increased ionic strength leading to influx of potentially harmful ions intracellularly (Allakhverdiev et al., 2000), as well as by the high external osmotic pressure leading to loss of water and reduced cell turgor that inhibits cell growth, cyanobacteria have adopted the salt-out strategy (Liang et al., 2020) i.e., the maintenance of relatively low intracellular ion concentration by actively pumping out inorganic ions and accumulate high levels of compatible solutes, which are small water-soluble organic compounds such as sucrose, glycerol, glycine betaine, ectoine, etc., by either de novo biosynthesis or uptake from the environment to ensure osmotic balance (Galinski and Truper, 1982; Hagemann, 2011).

Similarly, the bioavailability of essential nutrients for cyanobactria and microalgae growth, mainly of carbon, nitrogen and phosphorous, can control growth rate and biomass composition by altering metabolic processes (Markou et al., 2017). Typically, under nutrient limitation/starvation carbon fixation pathways are altered and cells synthesize and accumulate carbon-rich macromolecules such as carbohydrates or lipids

(Ravishankar and Ranga Rao, 2020). Additionally, nutrient limitations may affect the synthesis rate of photosynthetic pigments of cyanobacteria and microalgae, such as accumulating or downregulating their content in β-carotene, astaxanthin, chloropyll *a* and phycobilines (Yeh and Chang, 2011; Praveenkumar et al., 2012; Chen et al., 2015; Panis and Carreon, 2016; Vayenos et al., 2020). Worth mentioning at this point that any limitations of bioavailable micronutrients, such as iron and magnesium, induce the phenomenon of chlorosis i.e., the loss of photosynthetic pigments, leads to inhibition of cyanobacteria and microalgae growth.

Thus, cyanobacteria and microalgae cultures can be manipulated towards the synthesis of desired bioproduct for specific downstream processes by selecting appropriate cultivation medium that will trigger specific metabolic responses. For example, cultivating the freshwater cyanobacterium *Synechococcus elongatus* PCC 7942 in saline conditions up to 0.4 M NaCl results in increased sucrose synthesis (Vayenos et al., 2020). Nonetheless, the golden section between trigerring metabolic changes that enhance the synthesis of specific target compounds and maintaining an efficient culture growth rate must be approached (Robles et al., 2020). That been said, the threshold values for each stressor have to be evaluated at case i.e., for each cultivated microbial strain and for specific operational conditions and photobioreactor design.

## 1.6. Cyanobacterium *Synechococcus elongatus* PCC 7942

Cyanobacteria are prokaryotes responsible for 20 to 30 % of organic carbon on the earth by photosynthetic $CO_2$ fixation (Waterbury et al., 1979) having a photosynthetic efficiency tenfold higher than that of plants and algae (Ducat, 2011). They evolved after the Great Oxidation Event (2.32–2.4 billion years ago) and since then have played a significant role on earth's oxygen levels (Sanchez-Baracaldo and Cardona, 2020). Cyanobacteria are photosynthetic producers of organic compounds (including hydrocarbons, alcohols, and fatty acids) of possible commercial interest (Vayenos et al., 2020). Moreover, they present special interest as they can photosyntheticaly produce $H_2$ by reducing $H^+$ by hydrogenases, an enzyme present in green algae and cyanobacteria (Bothe 1982) and by nitrogenases that are present only in cyanobacteria (Saper et al., 2018).

Of the presented phototrophic species, the cyanobacterium *Synechococcus elongatus* PCC 7942 presents significant interest, since it is a widely studied cyanobacterium

regarding its biology that can be utilized for the production of sucrose and biohydrogen. *Synechococcus elongatus* PCC 7942 is a unicellular, obligate photoautotroph cyanobacterium that requires only inorganic nutrients and light for its growth (Collier and Grossman, 1992). Under salt stress, the fresh water unicellular cyanobacterium *Synechococcus elongatus* PCC 7942 responses by producing and accumulating sucrose as the only compatible solute and in order to balance out the external hyperosmolarity (Blumwald et al., 1983; Reed et al., 1986; Qiao et al., 2018).

This organic carbon source may constitute a novel source of sugar feedstock in microbial fermentation processes for green energy production (Ducat et al., 2011; Duan et al., 2016) or/and may be directly used intracellularly by cyanobacteria for $H_2$ gas production by means of microbial dark fermentation, which may offer an economically promising approach (Vayenos et al., 2020). According to Mishra et al. (2019), the stoichiometrically feasibility of dark fermentation yields close to 12 mol of hydrogen as stored in a glucose molecule and the thermodynamic prospective of glucose metabolism produces 2 mol of acetate including 4 mol of hydrogen molecule during acetate-type of dark fermentative (Equation 5). Furthermore, based on Gibbs free energy change, butyrate-type fermentation is more dominant than acetate reaction, producing only 3.3 ATP molecules and having a maximum $H_2$ production of 2.5 mol of $H_2$ per mole of glucose stoichiometrically (Equation 6). The reactions describing the production of $H_2$ from propionate and lactate are presented in Equations 7 and 8 respectively (Baeyens et al., 2020).

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3\,COOH + 4H_2 + 2CO_2 \quad \text{(Acetate Fermentation)} \qquad (5)$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow CH_3\,CH_2\,CH_2COOH + 2H_2 + 2CO_2 \quad \text{(Butyrate Fermentation)} \qquad (6)$$

$$\text{Propionate: } C_3H_6O_2 + 4H_2O + light\ energy \rightarrow 7H_2 + 3CO_2 \qquad (7)$$

$$\text{Lactate: } C_3H_6O_2 + 3H_2O + light\ energy \rightarrow 6H_2 + 3CO_2 \qquad (8)$$

The freshwater cyanobacterium *Synechococcus elongatus* PCC 7942 presents a notable acclimation to nutrient stresses and adaptation to variations in temperature, light intensity and salinity (Kuan et al., 2015). It grows best in BG-11 (Rippka et al., 1979) nutrient composition (Suh et al., 1998), as well as at temperatures and light intensities of approximately 33°C (Kuan, 2015) and 194 μmol $m^{-2}$ $s^{-1}$ respectively (Silva et al., 2014). Its upper salinity tolerance limit (threshold value) is 0.4 M NaCl (Nomura et al.,

1995; Deshnium et al., 1995; Vayenos et al., 2020) and the optimal pH values for its proliferation is around 8.5-9.0 (Farias-Silva et al., 2017; Vayenos et al., 2020).

*Synechococcus elongatus* PCC 7942 utilizes light using the photosynthetic pigments chlorophyll *a* (Chl *a*) and the phycobiliproteins allophycocyanin (APC) and phycocyanin (PC), which according to Collier and Grossman (1992) are associated with the photosynthetic apparatus in the thylakoid membranes (Figure 1.17).

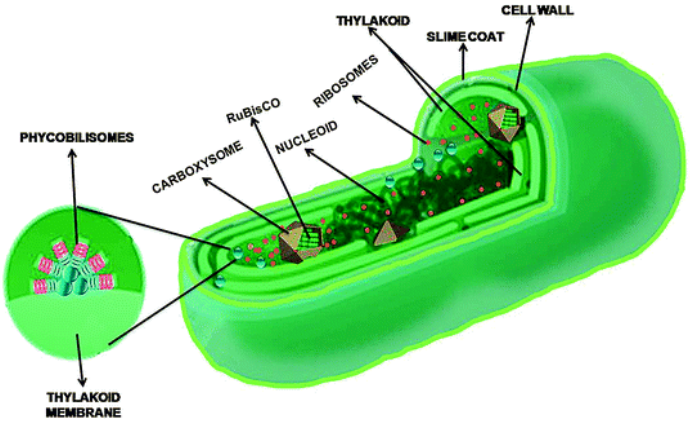


**Figure 1.17.** Cyanobacterial structure (Source: Singh et al., 2014; Springer Nature License Number 5360150458208).

Like other cyanobacteria, *Synechococcus elongatus* PCC 7942 exhibits a complex suite of morphological and physiological changes when deprived of an essential nutrient, a general aspect of which appears to be the phenomenon of chlorosis, or bleaching, i.e. the change in cell appearance from normal blue-green to chlorotic yellow or yellow-green due to changes in photosynthetic pigments (Collier and Grossman, 1992). Bleaching of Synechococcus spp. in response to nutrient deprivation was first documented in detail by Allen and Smith in 1969. According to Collier and Grossman (1992), many investigators have quantified changes in various cell components during bleaching relative to culture volume, protein, Chl, cell volume, and optical density at 750 nm, where it was evident that bleaching was less severe in P-deprived cells than in N- or S-deprived cells, attributed to the fact that P-deprived cells show little evidence of net phycobilisomes degradation and maintain a higher phycocyanin level, and therefore a higher phycocyanin to chlorophyll ratio, than N- or S- deprived cells (Ihlenfeld and Gibson, 1975). The rate of phycobilisomes degradation and synthesis is different under P deprivation than under N or S deprivation because *Synechococcus elongatus* PCC 7942 and his ancestors evolved strategies to survive under P-limiting conditions (most likely to occur in freshwater ecosystems) by storing polyphosphate

reserves intracellularly (Wetzel, 1983; Collier and Grossman, 1992). Worth mentioning that a small percentage of nutrient-deprived Synechococcus cells can survive in the chlorotic state for weeks by entering a dormant state until nutrients are once again available (Collier and Grossman, 1992; Forchhammer and Schwarz, 2018).

In *Synechococcus elongatus* PCC 7942 cultures, degradation of phycocyanin is observed with increasing salinity. Moreover, under salt stress, increases its cytoplasmic sucrose concentration at up to approximately 5 mol $mol^{-1}$ Chl *a*, or 300 mM $cell^{-1}$ in order to balance out the external osmotic pressure (Ladas and Papageorgiou 2000a, b; Suzuki et al., 2010; Klähn and Hagemann, 2011). *Synechococcus elongatus* PCC 7942 intracellular sucrose accumulation, thus the potential for biohydrogen production via dark fermentation of sucrose to $H_2$, can be remarkably improved by means of genetic engineering, such as its genetic transformation with the *cod*A gene of *Arthrobacter globiformis*, a gene that enables the engineered strain (strain designated as PAMCOD) to convert choline to glycine betaine enhancing this way its photosynthetic functions and stabilizing its photosynthetic structures (Deshnium et al., 1995; Ladas and Papageorgiou, 2000a,b; Vayenos et al., 2020). At the near optimal pH in the cell suspension and in the presence of 0.4 M NaCl, Syn7942 cells yielded by means of dark fermentation 0.76 nmol $H_2$ mg Chl $a^{-1}$ $h^{-1}$, while PAMCOD cells yielded 2 $H_2$ mg Chl $a^{-1}$ $h^{-1}$ (Vayenos et al., 2020).

While the biology of *Synechococcus elongatus* PCC 7942 is widely studied and even though its biomass can be utilized for the production of added-value products and/or bioenergy, it has not been studied with similar vigor regarding its potential biotechnological applications, especially those that can utilize/treat waste streams for its cultivation.

## 1.7. Challenges in application of cyanobacteria processes in wastewater treatment

Despite that decades of research regarding wastewater treatment via cultivation of phototrophic species, scaling-up and industrial applications of cyanobacteria and/or microalgae processes requires key breakthroughs to achieve economical application (McGaughy et al., 2019). Their full-scale implementation is challenging, since it depends on the successful combination of key principles of engineering, biology and biochemistry (Scott et al., 2010), as well as the ability to reliably and accurately

simulate full-scale performance in response to reactor and process design, influent composition, environmental conditions and operating parameters (Shoener et al., 2019).

That is to say, there is a big gap regarding the full-scale implementation of algae-based wastewater treatment systems, since there is a lack of fundamental design and operational parameters that could address all considerations related to process efficiency. In more detail, there are several issues that have to be addressed prior full-scale implementation, namely the applicability boundaries of the process that are associated with wastewater characteristics (temperature, salinity etc.); maintaining monoculture growth in the PBR; assessing cell productivity and nutrients assimilation rate in relation to the physicochemical profile of cultivation medium and interpreting design and operational data of other relevant studies; calculating the PBR volume requirements based on a standard methodology (Abinandan and Shanthakumar, 2015; Wang and Curtis, 2016). Moreover, according to Vayenos et al. (2020) the efficient means to export a targeted metabolite, i.e. biomass harvesting and the recovery of cytosolic products, represents a significant technical barrier to the economic feasibility of microbially synthesized commodities, since cell harvesting and lipid extraction may represent up to 30% of the total production costs (Kovacevic and Wesseler, 2010).

Collection and concentration of biomass from cultivation systems contribute heavily to the operation cost of the overall process and therefore, more efficient and economical harvesting technology should be developed to enhance the commercial viability of the microalgae biofuels industry (Razzak et al., 2013). The challenge of biomass harvesting/recovery must be addressed towards the viable full-scale application of cyanobacteria and microalgae cultivation, as it contributes up to 30% of the overall operational cost (Grima et al., 2003). According to Viswanaathan et al. (2022), sedimentation, flocculation, centrifugation filtration, sonication, precipitation and flotation are some of the techniques usually applied for biomass harvesting/recovery, with every technique exhibiting its own advantages and disadvantages, such as time, economy, damage to the biomass, ease of operation, continuous operations, occupation of space, energy, chemicals, fouling, clogging of machines, etc. (Grima et al., 2003; Bosma et al., 2003; Munoz and Guieysse, 2006; Brennan and Owende, 2010). Further study is necessary to achieve high biomass recovery efficiency of species that may have size in the order of micrometers and to minimize harvesting processes' cost and environmental footprint, a harvesting system is the major limiting factor for adoption

of cyanobacteria or/and microalgae-based wastewater treatment by industries (de-Bashan and Bashan, 2010).

Another challenge that has to be addressed to achieve success on a commercial scale is the fermentative hydrogen production from cyanobacteria, which despite being a process that shows great potential in sustainable energy generation, its efficiency has to be improved by means of operational design control and a proper combination of different treatment methods (Wang and Yin, 2018). The major challenges in current biological hydrogen production are summarized in the work of Gupta et al. (2013) and is presented in graphical format in Figure 1.18.

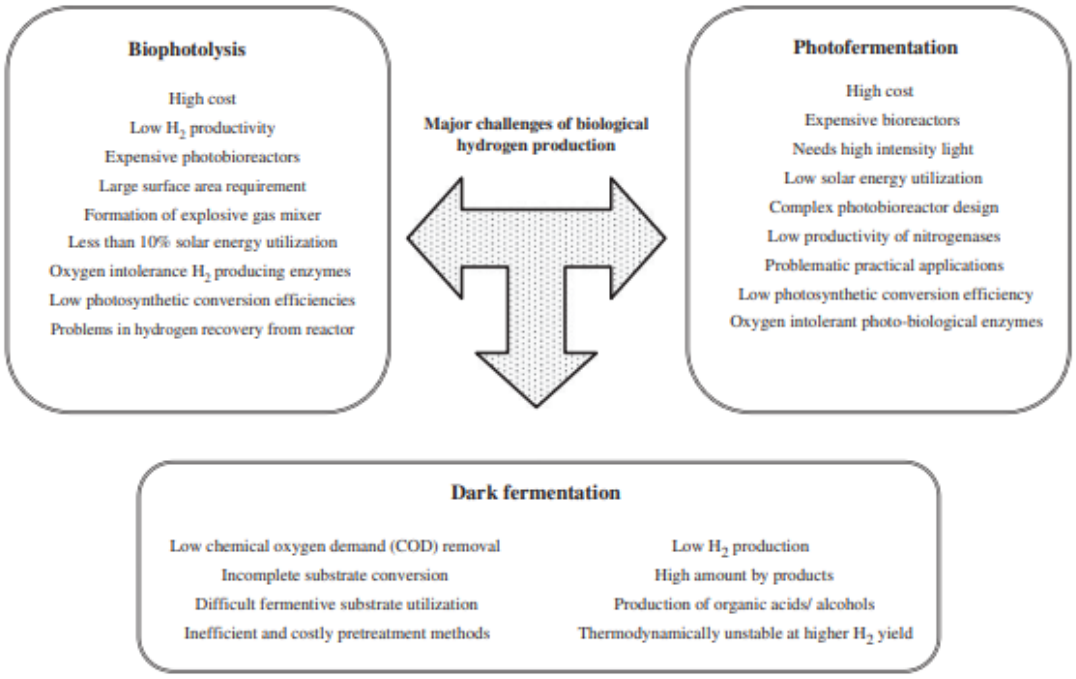


**Figure 1.18.** Major challenges in current biological hydrogen production technologies.

According to Nagarajan et al. (2021), to scale up biohydrogen production to a commercial level, the light conversion efficiency to hydrogen must be in the range of 5–10%, while in reality, the highest light conversion efficiency attained is around 3% even though the theoretical maximum is indicated at approximately 12–14%.

Finally, the invasion of contaminant organisms has long being recognized as a major constraint for large-scale algae cultivation (Gonzalez et al., 2013), which occurs not only in open cultivation systems, but also on closed and hybrid systems that have been specially designed to decrease contamination risks (Wang et al., 2013; Mcbride et al., 2014). The need for disinfection of the growth media so as to maintain the desired

monoculture increases operating costs, thus the study for efficient low-cost disinfection techniques is essential for process viability.

## 1.8. Scope of the thesis

Now more than ever, there is an imperative need to reduce carbon emissions, to recover resources and to sustainably produce energy and goods by following the principles of circular economy. Hence, the cultivation of cyanobacteria for the production of added value products or/and bioenergy has attracted a lot of attention during the last decade due to the higher biomass yields compared to that of plant crops, their tolerance and adaptability, as well as their increased $CO_2$ fixation ability. Moreover, increased interest is been observed regarding the application of cyanobacteria or/and microalgae for the treatment and utilization of wastewater streams or/and flue gases. It has been suggested by various studies that the implementation of microalgae and especially of cyanobacteria-based processes in various industrial sectors can enhance their sustainability. Cyanobacteria species, such as *Synechococcus elongatus* PCC 7942, have shown increased potential for sustainable production of resources and bioenergy. Their cultivation, especially if it is combined with resources recovery, could constitute a sustainable alternative process not only in agro-food and chemicals production sectors, but also in the wastewater treatment sector. The nutrient content of a plethora of wastewater could be potentially utilized for the cultivation of cyanobacteria and the removal/recovery of nitrogen and phosphorous. However, there are limited data regarding the technical aspects of applying cyanobacteria-based processes in wastewater treatment, especially regarding the application of cyanobacteria monocultures. Such applications could constitute a sustainable alternative to commonly applied nutrients removal processes that have been criticized regarding their inability to recover resources and energy.

That been said, the objective of the present work is to evaluate the applicability of a *Synechococcus elongatus* PCC 7942-based nutrients removal/recovey process from biologically treated industrial wastewaters and hydroponic wastewaters for the production of biomass and the acquisition of added value products or/and green energy. The proposed process will be evaluated in terms nitrates and phosphates removal rates by assimilation, as well as regarding biomass productivity. Fundamental design and operational parameters will be obtained from the evaluation of growth and nutrients removal/recovery rates on various wastewater substrates, which can be applied for

adopting and upscaling the proposed process. An effort to present the necessary steps for the utilization of industrial wastewater or wastewater from hydroponic crops for the cultivation of *Synechococcus elongatus PCC* 7942 will be made, as the later cannot be treated with common biological processes and contain significant quantities of nutrients that may result to environmental degradation if not properly managed.

## 1.9. References


1. Abinandan S, Shanthakumar S (2015). Challenges and opportunities in application of microalgae (Chlorophyta) for wastewater treatment: A review Renew Sustain Energy Rev, 52, 123–132. DOI: 10.1016/j.rser.2015.07.086
2. Abinandan S, Subashchandrabose SR, Venkateswarlu K, Megharaj M (2018). Nutrient removal and biomass production: Advances in microalgal biotechnology for wastewater treatment. Critic. Rev. Biotechnol, 38, 1244–1260. https://doi.org/10.1080/07388551.2018.1472066
3. Abishek PM, Patel J, Rajan PA (2014). Algae oil: a sustainable renewable fuel of future. Biotechnol Res Int, 272814. https://doi.org/10.1155/2014/272814.
4. Abu-Ghosh S, Fixler D, Dubinsky Z, Iluz D (2016). Flashing light in microalgae biotechnology Bioresour Technol, 203, 357-363. https://doi.org/10.1016/j.biortech.2015.12.057
5. Acién Fernández FG, Fernández Sevilla JM, Molina Grima E (2019). Costs Analysis of Microalgae Production. In Biofuels from Algae, 2nd (ed), Pandey A, Chang JS, Soccol CR, Lee DJ, Chisti Y, (Ed.), Elsevier: Amsterdam, The Netherlands, 551–566.
6. Ahmad S, Pathak VV, Kothari R, Kumar A, Naidu Krishna SB (2018). Optimization of nutrient stress using C. pyrenoidosa for lipid and biodiesel production in integration with remediation in dairy industry wastewater using response surface methodology. 3 Biotech, 8, 326–326. https://doi.org/10.1007/s13205-018-1342-8
7. Allakhverdiev SI, Sakamoto A, Nishiyama Y, Murata N (2000a). Inactivation of photosystems I and II in response to osmotic stress in Synechococcus: Contribution of water channels. Plant Physiol 122, 1201–1208. https://doi.org/10.1104/pp.122.4.1201
8. Allen MM, Smith AJ (1969). Nitrogen chlorosis in blue-green algae. Arch Mikrobiol, 69, 114-120. https://doi.org/10.1007/BF00409755
9. AMR (2020). Carotenoids Maket. https://www.alliedmarketresearch.com/carotenoids-market
10. An JY, Sim SJ, Lee JS, Kim BW (2003). Hydrocarbon production from secondarily treated piggery wastewater by the green alga Botryococcus braunii. J Appl Phycol, 15, 185–191. https://doi.org/10.1007/s10811-010-9577-8
11. Anand MN (2010). Microalgal bioremediation of nutrients in wastewater and carbon-dioxide in flue gas. Master's Thesis, Environmental Engineering, Missouri University of Science and Technology, Rolla, MO, USA.
12. Anderson DM, Morel FMM (1978). Copper sensitivity of Gonyaulax tamarensis. Limnol Oceanogr, 23, 283–295.
13. Andrade DS, SantosTelles T, Castro GHL (2020). The Brazilian microalgae production chain and alternatives for its consolidation. J Clean Prod, 250, 119526. https://doi.org/10.1016/j.jclepro.2019.119526

14. Anne PM, Velenturf, Purnell P (2021). Principles for a sustainable circular economy. Sustainable Production and Consumption, 27, 1437-1457. https://doi.org/10.1016/j.spc.2021.02.018.
15. Apandi N, Mohamed RMSR, Al-Gheethi, A, Gan P, Ibrahim A, Kassim, AHM (2018). Scenedesmus biomass productivity and nutrient removal from wet market wastewater, A bio-kinetic study. Waste and Biomass Valorization, 1–18. https://doi.org/10.1007/s12649-018-0313-y
16. Arias DM, Uggetti E, García-Galán MJ, García J (2017). Cultivation and selection of cyanobacteria in a closed photobioreactor used for secondary effluent and digestate treatment. Sci Total Environ, 587–588, 157-167. https://doi.org/10.1016/j.scitotenv.2017.02.097
17. Asadi P, Rad HA, Qaderi F (2020). Lipid and biodiesel production by cultivation isolated strain Chlorella sorokiniana pa.91 and Chlorella vulgaris in dairy wastewater treatment plant effluents. J Environ Health Sci Eng, 18, 573–585. https://doi.org/10.1007/s40201-020-00483-y
18. Aslan S, Kapdan IK (2006). Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. Ecol Eng, 28, 64–70. https://doi.org/10.1016/j.ecoleng.2006.04.003
19. Audu M et al (2021). Ash-Pretreatment and Hydrothermal Liquefaction of Filamentous Algae Grown on Dairy Wastewater. Algal Res, 57, 102282. https://doi.org/10.1016/j.algal.2021.102282
20. Azov Y (1982). Effect of pH on inorganic carbon uptake in algal cultures. Appl. Environ. Microbiol, 43, 1300–1306. https://doi.org/10.1128/aem.43.6.1300-1306.1982
21. Bacellar Mendes LB, Vermelho AB (2013). Allelopathy as a potential strategy to improve microalgae cultivation. Biotechnol Biofuels, 6(1), 152. https://doi.org/10.1186/1754-6834-6-152
22. Baeyens J, Zhang H, Nie J, Appels L, Dewil R et al (2020). Reviewing the potential of biohydrogen production by fermentation. Renewable and Sustainable Energy Reviews, 131. DOI: 10.1016/j.rser.2020.110023
23. Baker NR (2008). Chlorophyll fluorescence: a probe of photosynthesis in vivo. Annu Rev Plant Biol, 59, 89-113. https://doi.org/10.1146/annurev.arplant.59.032607.092759
24. Barceló-Villalobos M, Fernández-del Olmo P, Guzmán JL, Fernández-Sevilla JM, Acién Fernández FG (2019). Evaluation of photosynthetic light integration by microalgae in a pilot-scale raceway reactor. Bioresour Technol, 280, 404-411. https://doi.org/10.1016/j.biortech.2019.02.032
25. Barsanti L, Ciurli A, Birindelli L, Gualtieri P (2021). Remediation of Dairy Wastewater by Euglena Gracilis WZSL Mutant and β-glucan production. J Appl Phycol, 33, 431–441. https://doi.org/10.1007/s10811-020-02314-x
26. Bashan LE, Bashan Y (2010). Immobilized microalgae for removing pollutants: Review of practical aspects. Bioresour Technol, 101, 1611-1627. https://doi.org/10.1016/j.biortech.2009.09.043
27. Bataille C et al (2018). A review of technology and policy deep decarbonization pathway options for making energy-intensive industry production consistent with the Paris Agreement. J Clean Prod. 187, 960–973. https://doi.org/10.1016/j.jclepro.2018.03.107
28. Bauer L, Ranglová K, Masojídek J, Drosg B, Meixner K (2021). Digestate as sustainable nutrient source for microalgae—Challenges and Prospects. Appl. Sci., 11, 1056. https://doi.org/10.3390/app11031056
29. BCC (2018). Business Communication Company Phycocyanin Market: Food & Beverage Application to Hold Close to 85% Value Share Throughout the Forecast Period:

Global Industry Analysis (2013–2017) & Opportunity Assessment (2018–2028). www.futuremarketinsights.com/reports/phycocyanin-market-. Accessed 16 February 2020.
30. Bchet Q, Munoz R, Shilton A, Guieysse B (2013). Outdoor cultivation of temperature-tolerant Chlorella sorokiniana in a column photobioreactor under low power-input. Biotechnol Bioeng., 110, 118–26. https://doi.org/10.1002/bit.24603
31. Becker EW (1994). Microalgae: Biotechnology & Microbiology; Cambridge University Press: Cambridge, UK.
32. Behera B, Acharya A, Gargey IA, Aly N, Balasubramanian P (2018). Bioprocess engineering principles of microalgal cultivation for sustainable biofuel production. Bioresour Technol Reports, 5, 297-316. https://doi.org/10.1016/j.biteb.2018.08.001
33. Ben-Amotz A (1999). Dunaliella β-carotene. From science to commerce. In: Seckbach J (ed). Enigmatic Microorganisms and Life in Extreme Environments, 1, 401.
34. Ben-Amotz, A, Tornabene TG, Thomas WH (1985). Chemical profile of selected species of microalgae with emphasis on lipids. J. Phycol., 21, 72–81.
35. Bernal CB, Vázquez G, Quintal IB, Bussy AL (2008). Microalgal dynamics in batch reactors for municipal wastewater treatment containing dairy sewage water. Water Air Soil Pollut., 190, 259–270. https://doi.org/10.1007/s11270-007-9598-3
36. Bernstad Saraiva Schott A, Cánovas A (2015). Current practice, challenges and potential methodological improvements in environmental evaluations of food waste prevention – A discussion paper. Resour. Conserv. Recycl., 101, 132–142. https://doi.org/10.1016/j.resconrec.2015.05.004
37. Bhatnagar M, Chinnasamy S (2010). Chlorella minutissima—A promising fuel alga for cultivation in municipal wastewaters. Appl Biochem Biotechnol, 161, 523–536. https://doi.org/10.1007/s12010-009-8771-0
38. Bhatnagar, A, Chinnasamy S, Singh M, Das KC (2011). Renewable biomass production by mixotrophic algae in the presence of various carbon sources and wastewaters. Appl Energy, 88, 3425–3431. https://doi.org/10.1016/j.apenergy.2010.12.064
39. Bhattacharya AK, Mark HP, Annalisa P(2018). The new global agenda and the future of the multilateral development bank system. Economics Discussion Papers, No 2018-26, Kiel Institute for the World Economy. http://www.economics-ejournal.org/economics/discussionpapers/2018-26
40. Bhola V, Swalah, F, Ranjith Kumar R, Singh, Bux F (2014). Overview of the potential of microalgae for CO2 sequestration. Intl. J. Environ. Sci. Technol., 11, 2103-2118. DOI 10.1007/s13762-013-0487-6
41. Bich NN, Yaziz MI, Bakti NAK (1999). Combination of Chlorella vulgaris and Eichhornia crassipes for wastewater nitrogen removal. Water Res., 33, 2357–2362.
42. Billini M, Stamatakis K, Sophianopoulou V (2008). Two members of a network of putative Na+/H+ antiporters are involved in salt and pH tolerance of the freshwater cyanobacterium Synechococcus elongatus. Journal of Bacteriology, 190(19), 6318-6329. https://doi.org/10.1128/JB.00696-08
43. Binnal P., Babu PN (2017). Optimization of environmental factors affecting tertiary treatment of municipal wastewater by Chlorella protothecoides in a lab scale photobioreactor. Journal of Water Process Engineering, 17, 290–298. https://doi.org/10.1016/j.jwpe.2017.05.003
44. Blumwald E, Mehlhorn RJ, Packer L (1983). Ionic osmoregulation during salt adaptation of the cyanobacterium Synechococcus 6311. Plant Physiol, 73, 377–380
45. Bolatkhan K, Kossalbayev BD, Zayadan BK, Tomo T, Veziroglu TN, Allakhverdiev SI (2019). Hydrogen production from phototrophic microorganisms: Reality and perspectives,

International Journal of Hydrogen Energy, 44(12), 5799-5811. https://doi.org/10.1016/j.ijhydene.2019.01.092
46. Borowitzka MA (1992). Algal biotechnology products and processes: matching science and economics. Journal of Applied Phycology, 4, 267-279. https://doi.org/10.1007/BF02161212
47. Bosma R, van Spronsen WA, Tramper J, Wijffels RH (2013). Ultrasound, a new separation technique to harvest microalgae. J Appl Phycol, 15, 143–153.
48. Bothast RJ, Schlicher MA (2005). Biotechnological processes for conversion of corn into ethanol. Appl Microbiol Biotechnol., 67, 19–25. https://doi.org/10.1007/s00253-004-1819-8
49. Bothe H (1982). Nitrogen fixation. In: Carr NG, Whitton BA (eds) Botanical monographs, the biology of cyanobacteria. Blackwell Scientific Oxford Publications, Oxford, 87–104.
50. Brennan L, Owende P, (2010). Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sustain Energy Rev, 14, 557–577. https://doi.org/10.1016/j.rser.2009.10.009
51. Brenner N, Farkash K, Braun E (2006). Dynamics of protein distributions in cell populations. Physical Biology, 3, 172. https://doi.org/10.1088/1478-3975/3/3/002
52. Bryant D, Guglielmi G, de Marsac NT (1979).The structure of cyanobacterial phycobilisomes: a model. Arch. Microbiol, 123, 113–127. https://doi.org/10.1007/BF00446810
53. Bustreo C et al (2019). How fusion power can contribute to a fully decarbonized European power mix after 2050. Fusion Eng Des, 146, 2189–2193. https://doi.org/10.1016/j.fusengdes.2019.03.150
54. Canan A, Dincer I (2018). Hydrogen Energy. 1.13.7. Vols. 1 - Part A. Energy Fundamentals, in Comprehensive Energy Systems, by Ibrahim Dincer, 568-604. Oshawa, Ontario: Elsevier, 2018.
55. Carvalho AP, Silva SO, Baptista JM, Zalcata FX (2011). Light requirements in microalgal photobioreactors: an overview of biophotonic aspects. Appl Microbiol Biotechnol, 89, 1275-1288. https://doi.org/10.1007/s00253-010-3047-8
56. Cerri CEP, You X, Cherubin MR, et al (2017). Assessing the greenhouse gas emissions of Brazilian soybean biodiesel production. PLoS ONE, 12(5), e0176948. https://doi.org/10.1371/journal.pone.0176948.
57. Chawla P, Malik A, Sreekrishnan TR, Dalvi V, Gola D (2020). Selection of optimum combination via comprehensive comparison of multiple algal cultures for treatment of diverse wastewaters. Environ Technol Innov, 18, 100758. https://doi.org/10.1016/j.eti.2020.100758
58. Cheah, WY, Show PL, Chang JS, Ling TC, Juan JC (2015). Biosequestration of atmospheric CO2 and flue gas containing CO2 by microalgae. Bioresour Technol, 184, 190–201. https://doi.org/10.1016/j.biortech.2014.11.026
59. Chen CY, Durbin EG (1994). Effects of pH on the growth and carbon uptake of marine phytoplankton. Mar Ecol-Prog Ser, 109, 83–94.
60. Chen Y, Tang X, Kapoore RV, Xu C, Vaidyanathan S (2015). Influence of nutrient status on the accumulation of biomass and lipid in Nannochloropsis salina and Dunaliella salina. Energy Convers Manag, 106, 61–72. https://doi.org/10.1016/j.enconman.2015.09.025
61. Cheng L, Zhang L, Chen H, Gao C (2006). Carbon dioxide removal from air by microalgae cultured in a membrane-photobioreactor. Separation and Purification Technology, 50, 324-329. https://doi.org/10.1016/j.seppur.2005.12.006

62. Chi Z, Zheng Y, Jiang A, et al (2011). Lipid Production by Culturing Oleaginous Yeast and Algae with Food Waste and Municipal Wastewater in an Integrated Process. Appl Biochem Biotechnol, 165, 442–453. https://doi.org/10.1007/s12010-011-9263-6
63. Chisti Y (2007). Biodiesel from microalgae. Biotechnol Adv, 25(3), 294–306. https://doi.org/10.1016/j.biotechadv.2007.02.001
64. Chisti Y (2013). Constraints to commercialization of algal fuels. J Biotechnol, 167, 201–214. https://doi.org/10.1016/j.jbiotec.2013.07.020
65. Chittora D, Meena M, Barupal T, Swapnil P (2020). Cyanobacteria as a source of biofertilizers for sustainable agriculture. Biochem Biophys Rep, 22, 100737. https://doi.org/10.1016/j.bbrep.2020.100737
66. Coleman JR, Colman B (1981). Inorganic carbon accumulation and photosynthesis in a blue-green alga as a function of external pH. Plant Physiol, 67, 917–921.
67. Colenbrander S, Dodman D, Mitlin D (2017). Using climate finance to advance climate justice: the politics and practice of channelling resources to the local level. Climate Policy, 1-14. https://doi.org/10.1080/14693062.2017.1388212
68. Collier JL, Grossman A (1992). Chlorosis induced by nutrient deprivation in Synechococcus sp. Strain PCC 7942: Not all bleaching is the same. J Bacteriol, 174, 4718–4726. https://doi.org/10.1128/jb.174.14.4718-4726.1992
69. Corpuz Vermi AM, Borea L, Senatore V, Castrogiovanni F, Buonerba A, Oliva G, Ballesteros F, Zarra T, Belgiorno V, Choo K, Hasan SW, Naddeo V (2021). Wastewater treatment and fouling control in an electro algae-activated sludge membrane bioreactor. Science of the Total Environment, 786. https://doi.org/10.1016/j.scitotenv.2021.147475
70. Costa JAV, deMorais MG (2011). The role of biochemical engineering in the productions of biofuels from microalgae. Bioresource Technology, 102, 2–9. https://doi.org/10.1016/j.biortech.2010.06.014
71. Da Silva TL, Reis A (2015). Scale-up problems for the large scale production of algae algal biorefinery: an integrated approach. Berlin: Springer, 125–49. DOI: https://doi.org/10.1007/978-3-319-22813-6_6
72. Dalrymple OK, Halfhide T, Udom I, Gilles B, Wolan J, Zhang Q, Ergas S (2013). Wastewater use in algae production for generation of renewable resources: A review and preliminary results. Aquat Biosyst, 9, 2. https://doi.org/10.1186/2046-9063-9-2
73. Damrow R, Maldener I, Zilliges Y (2016). The multiple functions of common microbial carbon polymers, glycogen and PHB, during stress responses in the non-diazotrophic cyanobacterium Synechocystis sp. PCC 6803. Front Microbiol, 7, 966. https://doi.org/10.3389/fmicb.2016.00966
74. Darley WM (1982). Algal Biology: A Physiological Approach; Blackwell: Oxford, UK, 9, 1–168.
75. Das P, Aziz SS, Obbard JP (2011). Two phase microalgae growth in the open system for enhanced lipid productivity. Renew Energy, 36(9), 2524–8. https://scholarbank.nus.edu.sg/handle/10635/59243
76. de Carvalho JC et al (2020). Microalgal biomass pretreatment for integrated processing into biofuels, food, and feed. Bioresour Technol, 300, 122719. https://doi.org/10.1016/j.biortech.2019.122719
77. De Farias Silva, C.E., Sforza E, Bertucco A (2017). Effects of pH and Carbon Source on Synechococcus PCC 7002 Cultivation: Biomass and Carbohydrate Production with Different Strategies for pH Control. Appl Biochem Biotechnol, 181, 682–698. https://doi.org/10.1007/s12010-016-2241-2

78. de Morais MG, Costa JAV (2007). Carbon dioxide fixation by Chlorella kessleri, C. vulgaris, Scenedesmus obliquus and Spirulina sp. cultivated in flasks and vertical tubular photobioreactors. Biotechnology Letters, 29(9), 1349-1352. https://doi.org/10.1007/s10529-007-9394-6
79. de Morais MG, Costa JAV (2007). Carbon-dioxide fixation by Chlorella kessleri, C. vulgaris, Scenedesmus obliquus and Spirulina sp. cultivated in flasks and vertical tubular photobioreactors. Biotechnol Lett, 29, 1349–1352. https://doi.org/10.1007/s10529-007-9394-6
80. de Morais MG, da Fontoura Prates D, Moreira JB, Duarte JH, Costa JAV (2018). Phycocyanin from microalgae: Properties, extraction and purification, with some recent applications. Ind Biotechnol, 14 (1), 30–37. https://doi.org/10.1089/ind.2017.0009
81. Deshnium P, Los DA, Hayashi H, Mustardy L, Murata N (1995). Transformation of Synechococcus with a gene for choline oxidase enhances tolerance to salt stress. Plant Mol Biol, 29, 897–902. https://doi.org/10.1007/BF00014964
82. Ding J, Zhao F, Cao Y, Xing L, Liu W, Mei S, Li S (2015). Cultivation of microalgae in dairy farm wastewater without sterilization. Int J Phytoremediation, 17, 222–227. https://doi.org/10.1080/15226514.2013.876970
83. Dismukes GC, Carrieri D, Bennette N, Ananyev GM, Posewitz MC (2008). Aquatic phototrophs: efficient alternatives to land-based crops for biofuels Curr. Opin Biotechnol, 19, 235-240. https://doi.org/10.1016/j.copbio.2008.05.007
84. Dodd JC (1979). Algae production and harvesting from animal wastewaters. Agric Wastes, 1, 23–37.
85. Domínguez-Bocanegra AR, Guerrero Legarreta I, Martinez Jeronimo F, Tomasini Campocosio A (2004). Influence of environmental and nutritional factors in the production of astaxanthin from Haematococcus pluvialis. Bioresour Technol, 92 (2), 209–214. https://doi.org/10.1016/j.biortech.2003.04.001
86. Duan Y, Luo Q, Liang F, Lu X (2016). Sucrose secreted by the engineered cyanobacterium and its fermentability. J Ocean Univ China, 15, 890–896. https://doi.org/10.1007/s11802-016-3007-8
87. Ducat DC, Avelar-Rivas JA, Way JC, Silvera PA (2012). Rerouting carbon flux to enhance photosynthetic productivity. Appl Env Microbiol, 78, 2660–2668. https://doi.org/10.1128/AEM.07901-11
88. Ducat DC, Way JC, Silver PA (2011). Engineering cyanobacteria to generate high-value products. Trends Biotechnol, 29(2), 95-103. https://doi.org/10.1016/j.tibtech.2010.12.003
89. Dutta D, De D, Chaudhuri S, Bhattacharya SK (2005). Hydrogen production by cyanobacteria. Microbial Cell Factories, 4, 36-47. https://doi.org/10.1186/1475-2859-4-36
90. Elkatory MR, Hassaan MA, El Nemr A (2022). Chapter 12 - Algal biomass for bioethanol and biobutanol production, Editor(s): Mostafa El-Sheekh, Abd El-Fatah Abomohra, Handbook of Algal Biofuels, Elsevier, Pages 251-279.
91. El-Sheekh M, El-Dalatony M.M, Thakur N et al (2022). Role of microalgae and cyanobacteria in wastewater treatment: genetic engineering and omics approaches. Int J Environ Sci Technol, 19, 2173–2194. https://doi.org/10.1007/s13762-021-03270-w
92. Emerson R, Lewis CM (1943). The dependence of the quantum yield of Chlorella photosynthesis on wave lenghth of light. Am J Bot, 30, 165–178.
93. Emerson R, Stauffer J, Umbreit W (1944). Relationships between phosphorylation and photosynthesis in Chlorella. Am J Bot, 31, 107–120.
94. Espinosa-Gonzalez I, Parashar A, Bressler DC (2014). Heterotrophic growth and lipid accumulation of Chlorella protothecoides in whey permeate, a dairy by-product stream, for

biofuel production. Bioresour Technol, 155, 170–176. https://doi.org/10.1016/j.biortech.2013.12.028
95. Estermann J (2012). "Crisis civilizatoria y Vivir Bien". Polis, 11 (33), 149-174. http://www.scielo.cl/scielo.php?pid=S0718-65682012000300007&script=sci_arttext
96. Fábregas J, Maseda A, Domínguez A (2004). Otero, A. The cell composition of Nannochloropsis sp. changes under different irradiances in semicontinuous culture. World J Microbiol Biotechnol, 20, 31–35.
97. Fawzy S, Osman AI, Doran J et al (2020). Strategies for mitigation of climate change: a review. Environ Chem Lett, 18, 2069–2094. https://doi.org/10.1007/s10311-020-01059-w
98. Feng S et al (2020). Performance of a microalgal-bacterial consortium system for the treatment of dairy-derived liquid digestate and biomass production. Bioresour Technol, 306, 123101. https://doi.org/10.1016/j.biortech.2020.123101
99. Ferreira A et al (2018). Combining biotechnology with circular bioeconomy: from poultry, swine, cattle, brewery, dairy and urban wastewaters to biohydrogen. Environ Res, 164, 32–38. https://doi.org/10.1016/j.envres.2018.02.007
100. Ferro L, Gorzsás A, Gentili FG, Funk C (2018). Subarctic microalgal strains treat wastewater and produce biomass at low temperature and short photoperiod. Algal Res, 35, 160-167. https://doi.org/10.1016/j.algal.2018.08.031
101. Field CB, Behrenfeld MJ, Randerson JT, Falkowski P (1998). Primary Production of the Biosphere: Integrating Terrestrial and Oceanic Components. Science, 281 (5374), 237–240. https://doi.org/10.1126/science.281.5374.237
102. Forchhammer K, Schwarz R (2018). Nitrogen chlorosis in unicellular cyanobacteria – a developmental program for surviving nitrogen deprivation. Environ Microb, 21(4), 1173-1184. https://doi.org/10.1111/1462-2920.14447
103. Formighieri C, Franck F, Bassi R (2012). Regulation of the pigment optical density of an algal cell: filling the gap between photosynthetic productivity in the laboratory and in mass culture. J Biotechno,162, 115–23. https://doi.org/10.1016/j.jbiotec.2012.02.021
104. Galinski EA, Trüper Betaine HG (1982). A compatible solute in the extremely halophilic phototrophic bacterium Ectothiorhodospira halochloris FEMS. Microbiol Lett, 13, 357-360.
105. Gani, P, Sunar NM, Matias-Peralta H, Jamaian SS, Latiff, AA (2016). A. Effects of different culture conditions on the phycoremediation efficiency of domestic wastewater. Journal of Environmental Chemical Engineering, 4(4), 4744–4753. https://doi.org/10.1016/j.jece.2016.11.008
106. Gao F, Li C, Yang ZH, Zeng GM, Feng LJ, Liu JZ, Liu M, Cai HW (2016). Continuous microalgae cultivation in aquaculture wastewater by a membrane photobioreactor for biomass production and nutrients removal. Ecol Eng, 92, 55–61. http://ee.hnu.edu.cn/__local/E/3D/E2/dx.doi.org/10.1016/j.ecoleng.2016.03.046
107. García D, de Godos I, Domínguez C, Turiel S, Bolado S, Muñoz R (2019). A systematic comparison of the potential of microalgae-bacteria and purple phototrophic bacteria consortia for the treatment of piggery wastewater Bioresour. Technol, https://doi.org/10.1016/j.biortech.2018.12.095
108. Gharfalkar M, Court R, Campbell C, Ali Z, Hillier G (2015). Analysis of waste hierarchy in the European waste directive 2008/98/EC. Waste Manag, 39, 305–313. https://doi.org/10.1016/j.envc.2022.100469
109. Ghirardi M, Dubini A, Yu J, Maness P (2009). Photobiological hydrogen-producing systems. Chem Soc Rev, 38(1), 52–61. doi:10.1039/b718939g

110. Girard J-M et al (2014). Mixotrophic cultivation of green microalgae scenedesmus obliquus on cheese whey permeate for biodiesel production. Algal Res, 5, 241–248. https://doi.org/10.1016/j.algal.2014.03.002
111. Goglio P et al (2020). Advances and challenges of life cycle assessment (LCA) of greenhouse gas removal technologies to fight climate changes. J Clean Prod, 244, 118896. https://doi.org/10.1016/j.jclepro.2019.118896
112. Goldemberg J (2009). Ethanol for a sustainable energy future. Science, 315, 808–10. https://doi.org/10.1126/science.1137013
113. Goldman JC (1973) Letter: Carbon dioxide and pH: Effect on species succession of algae. Science, 182, 306–307.
114. Goldman JC, Azov Y, Riley CB, Dennett MR (1982). The effect of pH in intensive microalgal cultures. I. biomass regulation. J Exp Mar Biol Ecol, 57, 1–13.
115. Gondhalekar D, Ramsauer T (2017). Nexus city: operationalizing the urban water-energy-food nexus for climate change adaptation in Munich, Germany. Urban Clim, 19, 28-40, https://doi.org/10.1016/j.uclim.2016.11.004
116. González LE, Cañizares RO, Baena S (1997). Efficiency of ammonia and phosphorus removal from a colombian agroindustrial wastewater by the microalgae Chlorella vulgaris and Scenedesmus dimorphus. Bioresour Technol, 60, 259–262. https://doi.org/10.1016/S0960-8524(97)00029-1
117. González-Camejo J, Viruela A, Ruano MV, Barat R, Seco A, Ferrer J (2019). Effect of light intensity, light duration and photoperiods in the performance of an outdoor photobioreactor for urban wastewater treatment. Algal Research, 40, 101511, https://doi.org/10.1016/j.algal.2019.101511.
118. González-Camejo J, Viruela A, Ruano MV, Barat R, Seco A, Ferrer J (2019). Dataset to assess the shadow effect of an outdoor microalgae culture. Data in Brief, 25, 104143. https://doi.org/https://doi.org/10.1016/j.dib.2019.104143
119. González-Morales SI, Pacheco-Gutiérrez N, Ramírez-Rodríguez CA et al (2020). Metabolic engineering of phosphite metabolism in Synechococcus elongatus PCC 7942 as an effective measure to control biological contaminants in outdoor raceway ponds. Biotechnol Biofuels, 13, 119. https://doi.org/10.1186/s13068-020-01759-z
120. Gonzalez-Valencia R, Magana-Rodriguez F, Cristóbal J, Thalasso F (2016). Hotspot detection and spatial distribution of methane emissions from landfills by a surface probe method. Waste Manag, 55, 299–305. https://doi.org/10.1016/j.wasman.2016.03.004
121. Grima EM, Belarbi EH, Fernández FA, Medina AR, Chisti Y (2003). Recovery of microalgal biomass and metabolites: Process options and economics. Biotechnol Adv, 20, 491–515. https://doi.org/10.1016/S0734-9750(02)00050-2
122. Gris B, Morosinotto T, Giacometti GM, Bertucco A, Sforza E (2014). Cultivation of Scenedesmus obliquus in photobioreactors: effects of light intensities and light–dark cycles on growth, productivity, and biochemical composition. Appl Biochem Biotechnol, 172, 2377-2389. https://doi.org/10.1007/s12010-013-0679-z
123. Guldhe S, Kumari L, Ramanna P, Ramsundar P, Singh I, Rawat F (2017). Prospects, recent advancements and challenges of different wastewater streams for microalgal cultivation. J Environ Manage, 203, 299-315. https://doi.org/10.1016/j.jenvman.2017.08.012.
124. Gumbi SfT, Mutanda T, Olaniran AO (2021). Nutrient removal from dairy and poultry wastewater with simultaneous biomass and biodiesel production by Chlorella sp. T4 isolated from a freshwater stream in South Africa. Waste Biomass Valorization. https://doi.org/10.1007/s12649-021-01492-0

125. Gupta S, Pawar SB, Pandey RA (2019). Current practices and challenges in using microalgae for treatment of nutrient rich wastewater from agro-based industries. Sci Total Environ, 687, 1107–26. https://doi.org/10.1016/j.scitotenv.2019.06.115
126. Gupta SK, Kumari S, Reddy K, Bux F (2013). Trends in biohydrogen production: major challenges and state-of-the-art developments, Environmental Technology, 34, 1653-1670. https://doi.org/10.1080/09593330.2013.822022
127. Guschina IA, Harwood JL (2009). Algal Lipids and Effect of the Environment on Their Biochemistry. In Lipids in Aquatic Ecosystems, Springer: Berlin, Germany, 1–24. https://doi.org/10.1007/978-0-387-89366-2_1
128. Hadiyanto H, Christwardana M, da Costa C (2019). Electrogenic and biomass production capabilities of a Microalgae–Microbial fuel cell (MMFC) system using tapioca wastewater and Spirulina platensis for COD reduction. In: Energy Sources, Part A: Recovery, Utilization, and Environmental Effects, 1–12. https://doi.org/10.1080/15567036.2019.1668085
129. Hadiyanto H, Nur MMA (2012). Potential of palm oil mill effluent (POME) as medium growth of Chlorella sp. for bioenergy production. Intl J Environ Bioenergy 3, 67–74.
130. Handayani T, Mulyanto A, Priyanto FE, Nugroho R (2020). Utilization of dairy industry wastewater for nutrition of microalgae Chlorella vulgaris. J Phys Conf Ser, 1655, 1–7. https://doi.org/10.1088/1742-6596/1655/1/012123
131. Hansen PJ (2002). Effect of high pH on the growth and survival of marine phytoplankton: Implications for species succession. Aquat Microb Ecol, 28, 279–288. doi:10.3354/ame028279
132. Harker M, Tsavalos AJ, Young AJ (1996). Factors responsible for astaxanthin formation in the chlorophyte Haematococcus pluvialis. Bioresour Technol, 55, 207–214. https://doi.org/10.1016/0960-8524(95)00002-X
133. Harris GP (1986). Phytoplankton Ecology: Structure, Function and Fluctuation; Chapman and Hall: New York, NY, USA.
134. Harun R, Danquah MK, Forde GM (2010). Microalgal biomass as a fermentation feedstock for bioethanol production. J Chem Technol Biotechnol, 85, 199–203. https://doi.org/10.1002/jctb.2287
135. Harwood CS (2020). Iron-only and vanadium nitrogenases: fail-safe enzymes or something more?. Annu Rev Microbiol, 74, 247-266. https://doi.org/10.1146/annurev-micro-022620-014338
136. Harwood JL (2004). Involvement of Chloroplast Lipids in the Reaction of Plants Submitted to Stress. In Lipids in Photosynthesis: Structure, Function and Genetics; Siegenthaler PA, Murata N, (Eds). Springer Berlin, Germany, 6, 287–302.
137. Hasan R, Zhang B, Wang LJ, Shahbazi A (2014). Bioremediation of swine wastewater and biofuel potential by using Chlorella vulgaris, Chlamydomonas reinhardtii and Chlamydomonas debaryana. J Pet Environ Biotechnol, 5, 175. DOI:10.4172/2157-7463.1000175
138. He P, Duncan J, Barber J (2007). Astaxanthin accumulation in the green alga Haematococcus pluvialis: Effects of cultivation parameters. J Integr Plant Biol, 49 (4), 447–451. https://doi.org/10.1111/j.1744-7909.2007.00468.x
139. Heldt HW, Piechulla B (2011). The use of energy from sunlight by photosynthesis is the basis of life on earth. Editor(s): Hans-Walter Heldt, Birgit Piechulla, Plant Biochemistry (Fourth Edition), Academic Press, 43-64. https://doi.org/10.1016/B978-0-12-384986-1.00002-8

140. Herrero A, Vilella M (2018). 'We have a right to breathe clean air': the emerging environmental justice movement against waste incineration in cement kilns in Spain. Sustain Sci, 13(3), 721–731. https://doi.org/10.1007/s11625-017-0473-x
141. Ho SH, Chen WM, Chang G (2010). Scenedesmus obliquus CNW-N as a potential candidate for CO2 mitigation and biodiesel production. Bioresour Technol, 101(22), 8725–30. https://doi.org/10.1016/j.biortech.2010.06.112
142. Hoffmann J.P (1998). Wastewater treatment with suspended and nonsuspended algae. Journal of Phycology, 34, 757-763. https://doi.org/10.1046/j.1529-8817.1998.340757.x
143. Hope AB, Walker NA (1975). The Physiology of Giant Algal Cells; Cambridge University Press: London, UK.
144. Hu B, Min M, Zhou W, Du Z, Mohr M, Chen P, Zhu J, Cheng Y, Liu Y, Ruan R (2012). Enhanced mixotrophic growth of microalga Chlorella sp. on pretreated swine manure for simultaneous biofuel feedstock production and nutrient removal. Bioresour Technol, 126, 71–79. https://doi.org/10.1016/j.biortech.2012.09.031
145. Iasimone F, Panico A, De Felice V, Fantasma F, Iorizzi M, Pirozzi F (2018). Effect of light intensity and nutrients supply on microalgae cultivated in urban wastewater: biomass production, lipids accumulation and settleability characteristics. J Environ Manag, 223, 1078-1085. https://doi.org/10.1016/j.jenvman.2018.07.024
146. Ihlenfeldt MJA, Gibson J (1975). Phosphate utilization and alkaline phosphatase activity in Anacystis nidulans (Synechococcus). Arch Microbiol, 102, 23-28. https://doi.org/10.1007/BF00428340
147. Iluz D, Abu-Ghosh S (2016). A novel photobioreactor creating fluctuating light from solar energy for a higher light-to-biomass conversion efficiency. Energy Convers Manag, 126, 767-773. https://doi.org/10.1016/j.enconman.2016.08.045
148. IPCC (2014). Climate Change 2014: Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
149. IPCC (2015). Intergovernmental Panel on Climate Change Climate Change 2014: Mitigation of Climate Change: Working Group III Contribution to the IPCC Fifth Assessment Report Cambridge University Press, Cambridge. https://doi.org/10.1017/CBO9781107415416
150. Jaiswal D, Sengupta A, Sohoni S (2018). Genome Features and Biochemical Characteristics of a Robust, Fast Growing and Naturally Transformable Cyanobacterium Synechococcus elongatus PCC 11801 Isolated from India. Sci Rep, 8, 16632. https://doi.org/10.1038/s41598-018-34872-z

151. Jinyue Yan, Michael Obersteiner, Kenneth Möllersten, Jose Roberto Moreira, Negative Emission Technologies – NETs, Applied Energy, Volume 255, 2019, 113749, ISSN 0306-2619, https://doi.org/10.1016/j.apenergy.2019.113749
152. Jones ER, Van Vliet MTH, Qadir M, Bierkens MFP (2021). Country-level and gridded estimates of wastewater production, collection, treatment and reuse. Earth Syst Sci. Data, 13, 237–254. https://doi.org/10.5194/essd-13-237-2021
153. Juneja A, Ceballos RM, Murthy GS (2013). Effects of Environmental Factors and Nutrient Availability on the Biochemical Composition of Algae for Biofuels Production, A Review Energies, 6, 4607-4638. https://doi.org/10.3390/en6094607
154. Kalacheva G, Zhila N, Volova T, Gladyshev M (2002). The effect of temperature on the lipid composition of the green alga Botryococcus. Microbiology, 71, 286–293. https://doi.org/10.1023/A:1015898426573

155. Kamshybayeva GK, Kossalbayev BD, Sadvakasova AK, Zayadan BK, Bozieva AM, Dunikov D, Alwasel S, Allakhverdiev SI (2022). Strategies and economic feasibilities in cyanobacterial hydrogen production. International Journal of Hydrogen Energy, 47(69), 29661-29684. https://doi.org/10.1016/j.ijhydene.2022.06.277
156. Kaplan A, Badger MR, Berry JA (1980). Photosynthesis and the intracellular inorganic carbon-pool in the blue green algae Anabaena variabilis. Response to external CO2 concentration. Planta, 149, 219 – 226. https://doi.org/10.1007/BF00384557
157. Kausik R, Prasanna R, Joshi HC (2006). Utilization of anaerobically digested distillery effluent for the production of Spirulina platensis (ARM730). Journal of Scientific and Industrial Research, 53, 521-525.
158. Khalaji M, Hosseini SA, Ghorbani R, Agh N, Rezaei H, Kornaros M, Koutra E (2021). Treatment of dairy wastewater by microalgae Chlorella vulgaris for biofuels production. Biomass Convers Biorefin. https://doi.org/10.1007/s13399-021-01287-2
159. Khan MI, Shin JH, Kim JD (2018). The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. Microb Cell Fact, 17, 36. https://doi.org/10.1186/s12934-018-0879-x
160. Klähn S, Hagemann M (2011). Compatible solute biosynthesis in cyanobacteria. Environ Microbiol, 13, 551–562. https://doi.org/10.1111/j.1462-2920.2010.02366.x
161. Konopka A, Brock TD (1978). Effect of temperature on blue-green algae (cyanobacteria) in Lake Mendota. Appl Environ Microbiol, 36, 572–576. https://doi.org/10.1128/aem.36.4.572-576.1978
162. Kothari R, Prasad R, Kumar V, Singh DP (2013). Production of biodiesel from microalgae Chlamydomonas polypyrenoideum grown on dairy industry wastewater. Bioresour Technol, 144, 499–503. https://doi.org/10.1016/j.biortech.2013.06.116
163. Kovacevic V, Wesseler J (2010). Cost-effectiveness analysis of algae energy production in the EU. Energy Policy, 38(10), 5749-5757. https://doi.org/10.1016/j.enpol.2010.05.025
164. Kuan D, Duff S, Posarac D, Bi X (2015). Growth optimization of Synechococcus elongatus PCC7942 in lab flasks and a 2-D photobioreactor. The Can J Chem Eng, 93, 640-647. https://doi.org/10.1002/cjce.22154
165. Ladas N, Papageorgiou CG (2000a). The salinity tolerance of freshwater cyanobacterium Synechococcus sp. PCC 7942 is determined by its ability for osmotic adjustment and presence of osmolyte sucrose. Photosynthetica, 38(3), 343–348. DOI: 10.1023/A:1010957117237
166. Ladas NP, Papageorgiou GC (2000b). Cell turgor: a critical factor for the proliferation of cyanobacteria at unfavorable salinity. Photosyn Res, 65, 155–164. https://doi.org/10.1023/A:1006423221150
167. Larsson M, Lindblom J (2011). Algal flue gas sequestration and wastewater treatment: An industrial experiment. Master's Thesis, KTH Industrial Engineering and Management Machine Design, Industrial Ecology, Royal Institute of Technology, Stockholm, Sweden.
168. Latifi A, Ruiz M, Zhang CC (2009). Oxidative stress in cyanobacteria. FEMS Microbiology Reviews, 33, Issue 2, 258–278. https://doi.org/10.1111/j.1574-6976.2008.00134.x
169. Lau NS, Matsui M, Abdullah AA (2015). Cyanobacteria: photoautotrophic microbial factories for the sustainable synthesis of industrial products. Biomed Res Int, 2015, 754934. https://doi.org/10.1155/2015/754934

170. Lawrence MG, Schäfer S, Muri H (2018). Evaluating climate geoengineering proposals in the context of the Paris Agreement temperature goals. Nat Commun, **9**, 3734. https://doi.org/10.1038/s41467-018-05938-3
171. Lehmuskero MS, Chauton T (2018). Boström Light and photosynthetic microalgae: a review of cellular- and molecular-scale optical processes. Prog Oceanogr, 168, 43-56. https://doi.org/10.1016/j.pocean.2018.09.002
172. Lenzi D (2018) The ethics of negative emissions. Glob Sustain 1:e7. https://doi.org/10.1017/sus.2018.5
173. Leu S, Boussiba S (2014). Advances in the production of high-value products by microalgae. Ind Biotechnol, 10(3), 169–183. https://doi.org/10.1089/ind.2013.0039
174. Li Y, Horsman M, Wu N, Lan CQ, Dubois-Calero N (2008). Biofuels from microalgae. Biotechnol Prog, 24, 815–882. https://doi.org/10.1021/bp070371k
175. Liang Y, Zhang M, Wang M, Zhang W, Qiao C, Luo Q, Lu X (2020). Freshwater Cyanobacterium Synechococcus elongatus PCC 7942 adapts to an environment with salt stress via ion-induced enzymatic balance of compatible solutes. Appl and Envir Microbiol, 86, 2904-2919. https://doi.org/10.1128/AEM.02904-19
176. Liao Q., Sun Y., Huang Y., Xia A., Fu Q., Zhu X. (2017). Simultaneous enhancement of Chlorella vulgaris growth and lipid accumulation through the synergy effect between light and nitrate in a planar waveguide flat-plate photobioreactor Bioresour Technol, 243, 528-538. https://doi.org/10.1016/j.biortech.2017.06.091
177. Licht FO (2006). World ethanol markets: the outlook to 2015. Agra Europe Special Report: Tunbridge Wells, UK.
178. Lin A (2019). Carbon dioxide removal after Paris. Ecol Law Q, 45, 533. https://doi.org/10.15779/Z386M3340F
179. Lincoln EP, Wilkie AC, French BT (1996). Cyanobacterial process for renovating dairy wastewater. Biomass Bioenerg, 10, 63–68. https://doi.org/10.1016/0961-9534(95)00055-0
180. Lizzul AM, Hellier P, Purton S, Baganz F, Ladommatos N, Campos L (2014). Combined remediation and lipid production using Chlorella sorokiniana grown on wastewater and exhaust gases. Bioresour Technol, 151, 12–18. https://doi.org/10.1016/j.biortech.2013.10.040
181. Lorentz JF, Calijuri ML, Assemany PP, Alves WS, Pereira OG (2020). Microalgal biomass as a biofertilizer for pasture cultivation: plant productivity and chemical composition. J Clean Prod, 276, 124130. https://doi.org/10.1016/j.jclepro.2020.124130
182. Luan G, Zhang S, Lu X (2020). Engineering cyanobacteria chassis cells toward more efficient photosynthesis. Curr Opin Biotechnol, 62, 1–6. https://doi.org/10.1016/j.copbio.2019.07.004
183. Maizatul AY, Radin Mohamed R, Al-Gheethi AA (2017). An overview of the utilisation of microalgae biomass derived from nutrient recycling of wet market wastewater and slaughterhouse wastewater. Int Aquat Res, 9, 177–193. https://doi.org/10.1007/s40071-017-0168-z
184. Mallick N (2002). Biotechnological potential of immobilized algae for wastewater N, P and metal removal: A review. BioMetals, 15, 377–390. https://doi.org/10.1023/A:1020238520948
185. Markou G (2012). Alteration of the biomass composition of Arthrospira (Spirulina) platensis under various amounts of limited phosphorus. Bioresour Technol, 116, 533–535. https://doi.org/10.1016/j.biortech.2012.04.022

186. Markou G (2014). Effect of various colors of light-emitting diodes (LEDs) on the biomass composition of Arthrospira platensis cultivated in semi-continuous mode. Appl Biochem Biotechnol, 172(5), 2758–2768. https://doi.org/10.1007/s12010-014-0727-3
187. Markou G, Dao LHT, Muylaert K, Beardall J (2017). Influence of different degrees of N limitation on photosystem II performance and heterogeneity of Chlorella vulgaris. Algal Res, 26, 84–92. https://doi.org/10.1016/j.algal.2017.07.005
188. Markou G, Georgakakis D (2011). Cultivation of filamentous cyanobacteria (blue-green algae) in agro-industrial wastes and wastewaters: a review. Appl Energy, 88, 3389–3401. https://doi.org/10.1016/j.apenergy.2010.12.042
189. Markou G, Kougia E, Kefalogianni I, Tsagou V, Arapoglou D, Chatzipavlidis I (2019). Effect of glycerol concentration and light intensity on growth and biochemical composition of Arthrospira (Spirulina) Platensis: a study in semi-continuous mode with non-aseptic conditions. Appl Sci, 9(21), 4703. https://doi.org/10.3390/app9214703
190. Markou G, Nerantzis E (2013). Microalgae for high-value compounds and biofuels production: A review with focus on cultivation under stress conditions. Biotechnol Adv, 31(8), 1532–1542. https://doi.org/10.1016/j.biotechadv.2013.07.011
191. Martínez C, Mairet F, Bernard O (2018). Theory of turbid microalgae cultures. J Theor Biol, 456, 190-200. https://doi.org/10.1016/j.jtbi.2018.07.016
192. Martınez, M.E.; Sánchez, S.; Jiménez, J.M.; El Yousfi, F.; Muñoz, L. Nitrogen and phosphorus removal from urban wastewater by the microalga Scenedesmus obliquus. Bioresource Technology Volume 73, Issue 3, 2000, 263-272 https://doi.org/10.1016/S0960-8524(99)00121-2
193. Masojídek J, Torzillo G, Koblížek M (2013). Photosynthesis in Microalgae, in: Handb. Microalgal Cult., JohnWiley & Sons, Ltd, Oxford, UK, 21–36. https://doi.org/10.1002/9781118567166.ch2
194. Mata TM, Martins AA, Caetano NS (2010). Microalgae for biodiesel production and other applications: a review. Renew Sust Energy Rev, 14, 217–32. https://doi.org/10.1016/j.rser.2009.07.020
195. Mathy S, Menanteau P, Criqui P (2018). After the Paris Agreement: measuring the global decarbonization wedges from national energy scenarios. Ecol Econ, 150, 273–289. https://doi.org/10.1016/j.ecolecon.2018.04.012
196. Matsuda T, Hirai Y, Asari M, Yano J, Miura T, Ii R, Sakai S (2018). Monitoring environmental burden reduction from household waste prevention. Waste Manag, 71, 2–9. https://doi.org/10.1016/j.wasman.2017.10.014
197. Matthijs HCP, Balke H, van Hes UM, Kroon BMA, Mur LR, Binot RA (1996). Application of light-emitting diodes in bioreactors: flashing light effects and energy economy in algal culture (Chlorella pyrenoidosa). Biotechnol Bioeng, 50, 98-107. DOI: 10.1002/(SICI)1097-0290(19960405)50:1<98::AID-BIT11>3.0.CO;2-3
198. Mcbride RC, Lopez S, Meenach C, Burnett M, Behnke C (2014). Contamination management in low cost open algae ponds for biofuels production. Ind Biotechnol, 10(3), 221–7. https://doi.org/10.1089/ind.2013.0036
199. McGaughy K, Abu Hajer A, Drabold E, Bayless D, Reza MT (2019). Algal Remediation of Wastewater Produced from Hydrothermally Treated Septage. Sustainability, 11, 3454. https://doi.org/10.3390/su11123454
200. McGinn PJ, Dickinson KE, Bhatti S,; Frigon JC, Guiot SR, O'Leary SJB (2011). Integration of microalgae cultivation with industrial waste remediation for biofuel and bioenergy production: Opportunities and limitations. Photosynth Res, 109, 231–247. https://doi.org/10.1007/s11120-011-9638-0

201. McLaren D (2012). A comparative global assessment of potential negative emissions technologies. Process Saf Environ Prot, 90, 489–500. https://doi.org/10.1016/j.psep.2012.10.005
202. MDB, 2015, Progress Report (2014–2015) of the MDB Working Group on Sustainable Transport Institutional Document | November 2015
203. Medipally SR, Yusoff FMd, Banerjee S, Shariff M (2015). Microalgae as Sustainable Renewable Energy Feedstock for Biofuel Production. BioMed Research International, Article ID 519513. https://doi.org/10.1155/2015/519513
204. Mehan L, Verma R, Kumar R, Srivastava A (2018). Illumination wavelengths effect on Arthrospira platensis production and its process applications in River Yamuna water treatment. Journal of Water Process Engineering, 23, 91-96. https://doi.org/10.1016/j.jwpe.2018.03.010
205. Melis A. (2009). Solar energy conversion efficiencies in photosynthesis: minimizing the chlorophyll antennae to maximize efficiency. Plant Sci, 177, 272–80. https://doi.org/10.1016/j.plantsci.2009.06.005
206. Melo JM, Ribeiro MR, Telles TS (2022). Microalgae cultivation in wastewater from agricultural industries to benefit next generation of bioremediation: a bibliometric analysis. Environ Sci Pollut Res, 29, 22708–22720. https://doi.org/10.1007/s11356-021-17427-0
207. Michelon W, Da Silva MLB, Mezzari MP, Pirolli M, Prandini JM, Soares HM (2016). Effects of nitrogen and phosphorus on biochemical composition of microalgae polyculture harvested from phycoremediation of piggery wastewater digestate. Appl. Biochem. Biotechnol, 178, 1407–1419. https://doi.org/10.1007/s12010-015-1955-x
208. Mishra AN, Tiwari DN, Rai AN (2019). Cyanobacteria: from basic science to applications. Chapter 5. Elsevier Publishers Academic, Dordrecht, pp 79–130
209. Mishra P, Krishnan S, Rana S, Singh L, Sakinah M, Ab Wahid Z (2019). Outlook of fermentative hydrogen production techniques: An overview of dark, photo and integrated dark-photo fermentative approach to biomass. Energy Strategy Reviews, 24, 27-37. https://doi.org/10.1016/j.esr.2019.01.001
210. Miyachi S, Kamiya A (1978). Wavelength effects on photosynthetic carbon metabolism in Chlorella. Plant Cell Physiol, 19, 277–288. https://doi.org/10.1093/oxfordjournals.pcp.a075594
211. Moazami-Goudarzi M, Colman B (2012). Changes in carbon uptake mechanisms in two green marine algae by reduced seawater pH. J Exp Mar Biol Ecol, 413, 94–99. https://doi.org/10.1016/j.jembe.2011.11.017
212. Muñoz R, Guieysse B (2006). Algal–bacterial processes for the treatment of hazardous contaminants: A review. Water Res, 40, 2799–2815. https://doi.org/10.1016/j.watres.2006.06.011
213. Nagarajan D, Dong CD, Chen CY, Lee DJ, Chang JS (2021).. Biohydrogen production from microalgae – major bottlenecks and future research perspectives. Biotechnol J, 16:e2000124. https://doi.org/10.1002/biot.202000124
214. Nagase H, Eguchi K, Yoshihara K, Hirata K, Miyamoto K (1998). Improvement of microalgal NOx removal in bubble column and airlift reactors. J Ferment Bioeng, 86, 421–423. https://doi.org/10.1016/S0922-338X(99)89018-7
215. Navarro-López E, Ruíz-Nieto A, Ferreira A, Acién FG, Gouveia L (2020). Biostimulant potential of Scenedesmus obliquus grown in brewery wastewater. Molecules, 25, 664. https://doi.org/10.3390/molecules25030664

216. Nayak M, Karemore A, Sen R (2016). Sustainable valorization of flue gas $CO_2$ and wastewater for the production of microalgal biomass as a biofuel feedstock in closed and open reactor systems RSC Adv, 6, 91111-91120. https://doi.org/10.1039/C6RA17899E
217. Nielsen ES (1975). Marine Photosynthesis: With Special Emphasis on the Ecological Aspects; Elsevier: Amsterdam, The Netherlands, 1975; Volume 13.
218. Nisbet EG et al. (2019). Very Strong Atmospheric Methane Growth in the 4 Years 2014–2017: Implications for the Paris Agreement. Global Biogeochem. Cycles, 33(3), 318–342. https://doi.org/10.1029/2018GB006009
219. Nomura M, Ishitani M, Takabe T, Rai AK, Takabe T (1995). Synechococcus sp. PCC7942 transformed with Escherichia coli bet genes produces glycine betaine choline and acquires resistance to salt stress. Plant Physiol, 107, 703–708. https://doi.org/10.1104/pp.107.3.703
220. Nwoba G, Parlevliet DS, Laird DW, Alameh K, Moheimani NR (2019). Light management technologies for increasing algal photobioreactor efficiency. Algal Res, 39, 101433. https://doi.org/10.1016/j.algal.2019.101433
221. Ogendal L (2019). Light Scattering a brief introduction. University of Copenhagen. https://www.nbi.dk/~ogendal/personal/lho/LS_brief_intro.pdf
222. Oren, A. (2015). Pyruvate: A key nutrient in hypersaline environments? Microorganisms, 3, 407–416. https://doi.org/10.3390/microorganisms3030407
223. Ouhsassi M, Khay EO, Bouyahya A, El Ouahrani A, Harsal AE, Abrini J (2020). Evaluation of self-purifying power of cyanobacteria *Pseudanabaenagaleata*: case of dairy factory effluents. Appl Water Sci, 10, 181. https://doi.org/10.1007/s13201-020-01270-8
224. Pahunang RR, Buonerba A, Senatore V, Oliva G, Ouda M, Zarra T, Muñoz R, Puig S, Ballesteros FC, Li CW, Hasan SW, Belgiorno V, Naddeo V (2021). Advances in technological control of greenhouse gas emissions from wastewater in the context of circular economy. Science of The Total Environment, 792, 148479. https://doi.org/10.1016/j.scitotenv.2021.148479
225. Palmer C (2019). Mitigating climate change will depend on negative emissions technologies. Engineering, 5, 982–984. https://doi.org/10.1016/j.eng.2019.10.006
226. Paniagua-Michel J, Farfan BC, Buckle Ramirez LF (2011). Culture of marine microalgae with natural biodigested resources. Aquaculture, 64, 249–56. https://doi.org/10.1016/0044-8486(87)90330-9
227. Panis G, Carreon JR (2016). Commercial astaxanthin production derived by green alga Haematococcus pluvialis: A microalgae process model and a techno-economic assessment all through production line. Algal Res, 18, 175–190. https://doi.org/10.1016/j.algal.2016.06.007
228. Park KH, Lee CG (2001). Effectiveness of flashing light for increasing photosynthetic efficiency of microalgal cultures over a critical cell density Biotechnol. Bioprocess Eng, 6, 189-193. https://doi.org/10.1007/BF02932549
229. Pedersen O, Colmer T, Sand-Jensen K (2013). Underwater Photosynthesis of Submerged Plants – Recent Advances and Methods. Frontiers in Plant Science, 4. https://doi.org/10.3389/fpls.2013.00140
230. Phadwal K, Singh PK (2003). Effect of nutrient depletion on β-carotene and glycerol accumulation in two strains of Dunaliella sp. Bioresour Technol, 90(1), 55–58. https://doi.org/10.1016/S0960-8524(03)00090-7
231. Pires JCM (2019). Negative emissions technologies: a complementary solution for climate change mitigation. Sci Total Environ, 672, 502–514. https://doi.org/10.1016/j.scitotenv.2019.04.004

232. Pishbin M, Sarrafzadeh M-H, Faramarzi MA (2021). Nitrate and phosphate removal efficiency of Synechococcuselongatus under mixotrophic and heterotrophic conditions for wastewater treatment. IJST-T CIV ENG 45:1831–1843. https://doi.org/10.1007/s40996-020-00514-6
233. Pittman JK, Dean AP, Osundeko O (2011). The potential of sustainable algal biofuel production using wastewater resources. Bioresour Technol, 102, 17–25. https://doi.org/10.1016/j.biortech.2010.06.035
234. Posadas E, Alcántara C, García-Encina PA, Gouveia L, Guieysse B, Norvill Z, Acién FG, Markou G, Congestri R, Koreiviene J (2017). Microalgae cultivation in wastewater. Chapter 3. In Microalgae-Based Biofuels and Bioproducts, Gonzalez-Fernandez C. Muñoz R. (Ed.).; Woodhead Publishing Series in Energy, Woodhead Publishing: Chicago, IL, USA.
235. Posadas E, Bochon S, Coca M, García-González MC, García-Encina PA, Muñoz R (2014). Microalgae-based agro-industrial wastewater treatment: a preliminary screening of biodegradability. J Appl Phycol, 26, 2335–2345. https://doi.org/10.1007/s10811-014-0263-0
236. Powell EE, Hill GA (2009). Economic assessment of an integrated bioethanol-biodiesel-microbial fuel cell facility utilizing yeast and photosynthetic algae. Chem Eng Res Des, 87(9), 1340–8. https://doi.org/10.1016/j.cherd.2009.06.018
237. Pragya N, Pandey KK, Sahoo P (2013). A review on harvesting, oil extraction and biofuels production technologies from microalgae. Renew Sustain Energy Rev, 24, 159–171. https://doi.org/10.1016/j.rser.2013.03.034
238. Praveenkumar R, Shameera K, Mahalakshmi G, Akbarsha MA, Thajuddin N (2012). Influence of nutrient deprivations on lipid accumulation in a dominant indigenous microalga Chlorella sp., BUM11008: Evaluation for biodiesel production. Biomass Bioenerg, 37, 60–66. https://doi.org/10.1016/j.biombioe.2011.12.035
239. Pulz O, Gross G (2004). Valuable products from biotechnology of microalgae. Appl Microbiol Biotechnol, ;65, 635–48. https://doi.org/10.1007/s00253-004-1647-x
240. Putri EV, Din MFM, Ahmed Z, Jamaluddin H, Chelliapan S (2011). Investigation of Microalgae for High Lipid Content Using Palm Oil Mill Effluent (POME) as Carbon Source; IACSIT Press: Singapore, 12, 85–89. [Google Scholar]
241. Qiao C, Duan Y, Zhang M, Hagemann M, Luo Q, Lu X (2018). Effects of reduced and enhanced glycogen pools of salt-induced sucrose production in a sucrose-secreting strain of Synechococcus elongatus PCC 7942. Appl Environ Microbiol, 84, 1–11. https://doi.org/10.1128/AEM.02023-17
242. Ramanan R, Kannan K, Deshkar A, Yadav R, Chakrabarti T (2010). Enhanced algal $CO_2$ sequestration through calcite deposition by Chlorella sp. and Spirulina platensis in a mini-raceway pond. Bioresour Technol, 101, 2616–2622. https://doi.org/10.1016/j.biortech.2009.10.061
243. Ramanna L, Rawat I, Bux F (2017). Light enhancement strategies improve microalgal biomass productivity. Renew Sust Energ Rev, 80, 765–773. DOI: 10.1016/j.rser.2017.05.202
244. Ramirez-Perez JC, Janes HW (2009). Carbon-dioxide sequestration by Spirulina platensis in photo-bioreactors. Habitation, 12, 65–77. https://doi.org/10.3727/154296610X12686999887328
245. Ranga Rao A, Deepika G, Ravishankar GA, Sarada R, Narasimharao BP, Su Y, Lei B (2018a). Botryococcus as an alternative source of carotenoids and its possible applications- an overview. Crit Rev Biotechnol 38, 541–558. https://doi.org/10.1080/07388551.2017.1378997
246. Ranga Rao A, Deepika G, Ravishankar GA, Sarada R, Narasimharao BP, Lei B, Su Y (2018b). Industrial potential of carotenoid pigments from microalgae: Current trends and future prospects. Crit Rev Food Sci Nutr, 25, 1–22. https://doi.org/10.1080/10408398.2018.1432561

247. Ranga Rao A, Ravishankar GA (2020). Global Microalgal-Based Products for Industrial Applications. Chapter 22 in Hanbook of Algal Technologies and Phytochemicals. CRC Press, Ravishankar G.A. and Ranga Rao A. (ed.)
248. Ranglová K, Lakatos GE, Câmara Manoel JA, Grivalský T, Suárez Estrella F, Acién Fernández FG, Molnár Z, Ördög V, Masojídek J (2020). Growth, biostimulant and biopesticide activity of the MACC-1 Chlorella strain cultivated outdoors in inorganic medium and wastewater. Algal Res, 102136. https://doi.org/10.1016/j.algal.2020.102136
249. Raposo MFdJ, Oliveira SE, Castro PM, Bandarra NM, Morais RM (2010). On the Utilization of Microalgae for Brewery Effluent Treatment and Possible Applications of the Produced Biomass. Journal of the Institute of Brewing, 116, 285-292. https://doi.org/10.1002/j.2050-0416.2010.tb00433.x
250. Raven JA, Geider RJ (1988). Temperature and algal growth. New Phytol, 1988, 110, 441–461. https://doi.org/10.1111/j.1469-8137.1988.tb00282.x
251. Razzak SA, Hossain MM, Lucky RA, Amarjeet S, Hugo de Lasa (2013). Integrated CO2 capture, wastewater treatment and biofuel production by microalgae culturing—A review, Renewable and Sustainable Energy Reviews, 27, 622-653, https://doi.org/10.1016/j.rser.2013.05.063
252. Reed RH, Borowitzka LJ, Mackay MA, Chudek JA, Foster R, Warr SRC, Moore DJ, Stewart WDP (1986). Organic solute accumulation in osmotically stressed cyanobacteria. FEMS Microbiol Rev, 39, 51–56. https://doi.org/10.1016/0378-1097(86)90060-1
253. Renaud SM, Parry DL (1994). Microalgae for use in tropical aquaculture II: Effect of salinity on growth, gross chemical composition and fatty acid composition of three species of marine microalgae. J Appl Phycol, 6, 347–356. https://doi.org/10.1007/BF02181949
254. Renaud SM, Thinh LV, Lambrinidis G, Parry DL (2002). Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. Aquaculture, 211, 195–214. https://doi.org/10.1016/S0044-8486(01)00875-4
255. Rhee GY (1982). Effects of environmental factors and their interactions on phytoplankton growth. Adv Microb Ecol, 6, 33–74.
256. Richmond A (1999). Physiological principles and modes of cultivation in mass production of photoautotrophic microalgae Z. Cohen (Ed.), Chemicals from microalgae, Taylor & Francis, London.
257. Ricke KL, Millar RJ, MacMartin DG (2017). Constraints on global temperature target overshoot. Sci Rep, 7, 14743. https://doi.org/10.1038/s41598-017-14503-9
258. Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RT (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J Gen Microbiol, 111, 1–61. DOI: 10.1099/00221287-111-1-1
259. Robles A, Aguado D, Barat R, Borrás L, Bouzas A, Giménez JB, Martí N, Ribes J, Ruano MV, Serralta J, Ferrer J, Seco A (2020). New frontiers from removal to recycling of nitrogen and phosphorus from wastewater in the Circular Economy, Bioresource Technology, 300, 122673. https://doi.org/10.1016/j.biortech.2019.122673.
260. Rodolfi L, Chini Zittelli G, Bassi N, Padovani G, Biondi N, Bonini G, Tredici MR (2009). Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnol Bioeng, 102, 100–12. https://doi.org/10.1002/bit.22033
261. Román Núñez YC, Cuesta Moreno OJ (2016). Communication and environmental conservation: advances and challenges in Latin America. Revista Latina de Comunicación Social, 71, 015-039. DOI: 10.4185/RLCS-2016-1082en

262. Romero-Villegas GI, Fiamengo M, Acién-Fernández FG, Molina-Grima E (2018). Utilization of centrate for the outdoor production of marine microalgae at the pilot-scale in raceway photobioreactors. J Environ Manage, 228, 506-516. https://doi.org/10.1016/j.jenvman.2018.08.020
263. Rosso D, Stenstrom MK (2008).The carbon-sequestration potential of municipal wastewater treatment. Chemosphere, 70, 1468-1475.https://doi.org/10.1016/j.chemosphere.2007.08.057
264. Saikia MK, Kalita S, Sarma GC (2011). An experimental investigation on growth stimulation (+) and inhibition (−) of algae (Oscillatoria chlorina and Scenedesmus quadricauda) treated with pulp and paper mill effluents. Int J Appl Biol Pharm Technol, 2, 87–94. [Google Scholar]
265. Sánchez-Baracaldo P, Cardona T (2020). On the origin of oxygenic photosynthesis and Cyanobacteria. New Phytologist, 225, 1440-1446. https://doi.org/10.1111/nph.16249
266. Santos MJBdA, Andrade DS, Bosso A, Murata MM, Morioka LRI, Silva JBd, Suguimoto HH (2021). Chlorella sorokiniana cultivation in cheese whey for β-galactosidase production Res, Soc Dev, 12. https://doi.org/10.33448/rsd-v10i12.20727
267. Saper G, Kallmann D, Conzuelo F, Zhao F, Tóth NT, Liveanu V, Meir S, Szymanski S, Aharoni A, Schuhmann W, Rothschild A, Schuster G, Adir N (2018). Live cyanobacteria produce photocurrent and hydrogen using both the respiratory and photosynthetic systems. Nat Commun, 9, 2168. https://doi.org/10.1038/s41467-018-04613-x
268. Sara AR, Raut N, Fatma AQ, Qasmi M, Al Saadi A (2012). Treatments of industrials wastewater by using microalgae. In: International Conference on Environmental, Biomedical and Biotechnology IPCBEE, 41, IACSIT Press, Singapore.
269. Schulze PSC, Barreira LA, Pereira HGC, Perales JA, Varela JCS (2014). Light emitting diodes (LEDs) applied to microalgal production Trends Biotechnol, 32, 422-430. DOI: 10.1016/j.tibtech.2014.06.001
270. Scott SA, Davey MP, Dennis JS, Horst I, Howe CJ, Lea-Smith DJ, Smith AG (2010). Biodiesel from algae: challenges and prospects. Current Opinion in Biotechnology, 21, 277–286. https://doi.org/10.1016/j.copbio.2010.03.005
271. Shashirekha V, Sudhakar MP (2019). Microalgae: Potential agents for $CO_2$ mitigation and bioremediation of wastewaters. Chapter 8. In New and Future Developments in Microbial Biotechnology and Bioengineering: Microbes in Soil, Crop and Environmental Sustainability; Singh, J.S., Singh, D.P., Eds.; Elsevier: Amsterdam, The Netherlands, pp. 129–148. https://doi.org/10.1016/b978-0-12-818258-1.00008-x
272. Shashirekha, V.; Sivakumar, M.; Seshadri, S. Effective C-N-P ratio for growth and nutrient removal efficiency of Scenedesmus obliquus in sugar mill effluent. Energy Ecol. Environ. **2016**, 1, 283–295. [Google Scholar] [CrossRef]
273. Sheehan J, Dunahay T, Benemann J, Reossler P (1998). A Look Back at the U.S. Department of Energy's Aquatic Species Program – Biodiesel from Algae. Golden, CO: U.S. Department of Energy, National Renewable Energy Laboratory.
274. Shinnar R, Citro F (2008). Decarbonization: achieving near-total energy independence and near-total elimination of greenhouse emissions with available technologies. Technol Soc, 30, 1–16. https://doi.org/10.1016/j.techsoc.2007.10.006
275. Shoener B, Bradley I, Cusick R, Guest J (2014). Energy positive domestic wastewater treatment: the roles of anaerobic and phototrophic technologies. Environ. Sci Proc Imp, 16, 1204–1222. https://doi.org/10.1039/C3EM00711A
276. Shoener B, Schramm SM, Beline F, Bernard O, Martínez C, Plosz BG, Snowling S, Steyer JP, Perez BV, Wagner D, Guest JS (2019). Microalgae and cyanobacteria modelling in

water resource recovery facilities: A critical review. Water Res X, 2, 100024. https://doi.org/10.1016/j.wroa.2018.100024
277. Shoener BD, Bradley IM, Cusick RD, Guest JS (2014). Energy positive domestic wastewater treatment: the roles of anaerobic and phototrophic technologies. Environ Sci Process Impacts, 16, 1204-1222. https://doi.org/10.1039/C3EM00711A
278. Silva CSP, Silva-Stenico ME, Fiore MF, De Castro HF, Da Rós PCM (2014). Optimization of the cultivation conditions for Synechococcus sp. PCC7942 (cyanobacterium) to be used as feedstock for biodiesel production. Algal Res, 3, 1-7. https://doi.org/10.1016/j.algal.2013.11.012
279. Sing SF, Isdepsky A, Borowitzka MA, Lewis DM (2014). Pilot-scale continuous recycling of growth medium for the mass culture of a halotolerant Tetraselmis sp. in raceway ponds under increasing salinity: a novel protocol for commercial microalgal biomass production. Bioresour Technol, 161, 47–54. https://doi.org/10.1016/j.biortech.2014.03.010
280. Singh A, Ohlsen SI (2011). A critical review of biochemical conversion, sustainability and life cycle assessment of algal biofuels. Appl Energy, 88, 3548–55. DOI: 10.1016/j.apenergy.2010.12.012
281. Singh D, Basu C, Meinhardt-Wollweber M, Roth B (2015). LEDs for energy efficient greenhouse lighting Renew. Sustain. Energy Rev, 49, 139-147. https://doi.org/10.1016/j.rser.2015.04.117
282. Singh JS, Kumar A, Rai AN, Singh DP (2016). Cyanobacteria: a precious bio-resource in agriculture, ecosystem, and environmental sustainability. Front Microbiol, 7, 529. https://doi.org/10.3389/fmicb.2016.00529
283. Singh SK, Sundaram S, Kishor K (2014). Carbon-Concentrating Mechanism of Cyanobacteria. In: Photosynthetic Microorganisms. Springer Briefs in Materials. Springer, Cham. https://doi.org/10.1007/978-3-319-09123-5_3
284. Singh UB, Ahluwalia AS (2013). Microalgae: a promising tool for carbon sequestration. Mitig Adapt Strateg Glob Change, 18, 73–95. https://doi.org/10.1007/s11027-012-9393-3
285. Sonani RR, Patel S, Bhastana B, Jakharia K, Chaubey MG, Singh NK, Madamwar D (2017). Purification and antioxidant activity of phycocyanin from Synechococcus sp. R42DM isolated from industrially polluted site. Bioresour Technol, 245, 325–331. https://doi.org/10.1016/j.biortech.2017.08.129
286. Sorokin C, Krauss RW (1958). The Effects of light intensity on the growth rates of green algae. Plant Physiol, 33, 109–113. https://doi.org/10.1104/pp.33.2.109
287. Stockenreiter M, Haupt F, Graber AK, Seppälä J, Spilling K, Tamminen T, Stibor H (2013). Functional group richness: implications of biodiversity for light use and lipid yield in microalgae. J Phycol, 49, 838-847. https://doi.org/10.1111/jpy.12092
288. Suali E, Sarbatly R (2012). Conversion of microalgae to biofuel. Renew. Sustain Energy Rev, 16, 4316–4342. https://doi.org/10.1016/j.rser.2012.03.047
289. Suh IS, Park C.B, Han J, Lee SB (1998). Cultivation of cyanobacterium in various types of photobioreactors for biological CO2 fixation. Studies in surface science and catalysis, 114, 471-474. https://doi.org/10.1016/S0167-2991(98)80798-2
290. Sunda W (1975). The Relationship between Cupric Ion Activity and the Toxicity of Copper to Phytoplankton. Ph.D. Thesis, Massachusetts Institute of Technology, Cambridge, MA, USA.
291. Suzuki E, Ohkawa H, Moriya K, Matsubara T, Nagaike Y, Iwasaki I, Fujiwara S, Tsuzuki M, Nakamura Y (2010). Carbohydrate metabolism in mutants of the cyanobacterium

Synechococcus elongatus PCC 7942 defective in glycogen synthesis. Appl Environ Microbiol, 76, 3153–3159. https://doi.org/10.1128/AEM.00397-08
292. Swarnalatha GV, Shekh A, Sijil PV, Madhubalaji CK, Chauhan VS, Sarada R (2019). Carbon Dioxide Sequestration by Microalgae. https://el.gr1lib.org/book/6026713/e93720
293. Sydney EB, Sturm WS, de Carvalho JC, Thomaz-Soccol V, Larroche C, Pandey A, Soccol CR (2010). Potential carbon dioxide fixation by industrially important microalgae. Bioresour Technol, 101, 5892–5896. https://doi.org/10.1016/j.biortech.2010.02.088
294. Tahani SG, El-Temtamy SA (2013). Commercialization potential aspects of microalgae for biofuel production: An overview. Egyptian Journal of Petroleum, 22, 43-51, https://doi.org/10.1016/j.ejpe.2012.07.001
295. Takagi M., Karseno, and Yoshida T (2006). Effect of salt concentration on intracellular accumulation of lipids and triacylglyceride in marine microalgae Dunaliella cells. J Biosci Bioeng, 101, 223–226. https://doi.org/10.1263/jbb.101.223
296. Tam NFY, Wong YS (1995). Wastewater treatment with microorganisms. The commercial Press (H.K.) Ltd. 2D Finnie St. Quarry Bay, Hong Kong.
297. Tamagnini P, Leitão E, Oliveira P, Ferreira D, Pinto F, Harris DJ, Heidorn T, Lindblad P (2007). Cyanobacterial hydrogenases: diversity, regulation and applications. *FEMS Microbiology Reviews*, 31(6), 692–720. https://doi.org/10.1111/j.1574-6976.2007.00085.x
298. Teo CL, Atta M, Bukhari A, Taisir M, Yusuf AM, Idris A (2014). Enhancing growth and lipid production of marine microalgae for biodiesel production via the use of different LED wavelengths**.** Bioresour Technol, 162, 38-44. https://doi.org/10.1016/j.biortech.2014.03.113
299. Thompson GA (1996). Lipids and membrane function in green algae. Biochim Biophys Acta, 1302, 17–45. https://doi.org/10.1016/0005-2760(96)00045-8
300. Ting Cai, Stephen Y. Park, Yebo Li (2013). Nutrient recovery from wastewater streams by microalgae: Status and prospects, Renewable and Sustainable Energy Rev, 19, 360-369. https://doi.org/10.1016/j.rser.2012.11.030
301. Toyub MA, Miah MI, Habib MAB, Rahman MM (2008). Growth performance and nutritional value of Scenedesmus obliquus cultured in different concentrations of sweetmeat factory waste media. Bangladesh J Anim Sci, 37, 86–93. https://doi.org/10.3329/bjas.v37i1.9874
302. Tredici MR (2010). Photobiology of microalgae mass cultures: understanding the tools for the next green revolution. Biofuels, 1, 143–162. https://doi.org/10.4155/bfs.09.10
303. Tseng LY, Robinson AK, Zhang X, Xu X, Southon J, Hamilton AJ, Sobhani R, Stenstrom MK, Rosso D (2016). Identification of preferential paths of fossil carbon within water resource recovery facilities via radiocarbon analysis. Environ Sci Technol, 50, 12166-12178. https://pubs.acs.org/doi/abs/10.1021/acs.est.6b02731
304. Tsolcha ON, Patrinou V, Economou CN, Dourou M, Aggelis G, Tekerlekopoulou AG (2021). Utilization of biomass derived from cyanobacteria-based agro-industrial wastewater treatment and raisin residue extract for bioethanol production. Water, 13, 486. https://doi.org/10.3390/w13040486
305. Ueda R, Hirayama S, Sugata K, Nakayama H (1996). Process for the production of ethanol from microalgae. US Patent 5,578,472
306. UN (2015). General Assembly: Transforming our world: the 2030 Agenda for Sustainable Development, 21 October 2015, A/RES/70/1, available at: https://www.refworld.org/docid/57b6e3e44.html [accessed 18 March 2022]
307. UN (2020). United Nations Department of Global Communications: Sustainable Development Goals (SDG), available

at:https://www.un.org/sustainabledevelopment/news/communications-material [accessed 18 March 2022]
308. UN Environment Assembly: A contribution to the global follow-up and review in the 2016 high level political forum (hlpf) on the work of the united nations environment programme, available at: https://wedocs.unep.org/20.500.11822/26458 [accessed 18 March 2022]
309. Usha MT, Chandra TS, Sarada R, Chauhan VS (2016). Removal of nutrients and organic pollution load from pulp and paper mill effluent by microalgae in outdoor open pond. Bioresour Technol, 214, 856–860. https://doi.org/10.1016/j.biortech.2016.04.060
310. Van der Hoek JP, De Fooij H, Struker A (2016) Wastewater as a resource: strategies to recover resources from Amsterdam's wastewater. Resour. Conserv. Recyl. 113:53–64. https://doi.org/10.1016/j.resconrec.2016.05.012
311. Vayenos D, Romanos EmG, Papageorgiou GC, Stamatakis K (2020). The freshwater cyanobacterium Synechococcus sp. PCC7942 under salt stress: A cell factory for sucrose and hydrogen production. Photosynth Res, 146, 235-245. https://doi.org/10.1007/s11120-020-00747-6
312. Victor N, Nichols C, Zelek C (2018) The U.S. power sector decarbonization: investigating technology options with MARKAL nine-region model. Energy Econ, 73,410-425. https://doi.org/10.1016/j.eneco.2018.03.021
313. Vidya D, Nayana K, Sreelakshmi M, Keerthi KV, Mohan KS, Sudhakar MP, Arunkumar K (2021). A Sustainable cultivation of microalgae using dairy and fish wastes for enhanced biomass and bio-product production. Biomass Convers Biorefin. https://doi.org/10.1007/s13399-021-01817-y
314. Viegas C, Gouveia L, Gonçalves M (2021). Bioremediation of cattle manure using microalgae after pre-treatment with biomass ash. Bioresour Technol Rep, 14, 100681. https://doi.org/10.1016/j.biteb.2021.100681
315. Viswanaathan S, Perumal PK, Sundaram S (2022). Integrated Approach for Carbon Sequestration and Wastewater Treatment Using Algal–Bacterial Consortia: Opportunities and Challenges. Sustainability, *14*, 1075. https://doi.org/10.3390/su14031075
316. Vivek Dalvi, Krutika Patil, Harshita Nigam, Rahul Jain, Sunil Pabbi, Anushree Malik (2021). Chapter 14 of Ecophysiology and Biochemistry of Cyanobacteria, R.P. Rastogi (ed.), Springer. https://doi.org/10.1007/978-981-16-4873-1_14
317. Vo Hoang Nhat P., Ngo HH, Guo WS, Chang SW, Nguyen DD, Nguyen PD (2018). Can algae-based technologies be an affordable green process for biofuel production and wastewater remediation? Bioresour Technol, 256, 491-501. https://doi.org/10.1016/j.biortech.2018.02.031
318. Voigt J, Münzner P (2004). Blue light-induced lethality of a cell wall-deficient mutant of the unicellular green alga Chlamydomonas reinhardtii. Plant Cell Physiol, *35*, 99–106.
319. Vonshak A, Torzillo G (2004). Environmental stress physiology. In: Richmond, A. (ed.): Handbook of Microbial Culture, Biotechnology and Applied Phycology. Blackwell Science, Oxford, 57-82.
320. Wang B, Lan CQ, Horsman M (2012). Closed photobioreactors for production of microalgal biomasses. Biotechnol Adv, 30 (4), 904-912. https://doi.org/10.1016/j.biotechadv.2012.01.019
321. Wang B, Li Y, Wu N, Lan CQ (2008). $CO_2$ bio-mitigation using microalgae. Appl Microbiol Biotechnol, 79, 707–718. https://doi.org/10.1007/s00253-008-1518-y

322. Wang H, Zhang W, Chen L, Wang J, Liu T (2013). The contamination and control of biological pollutants in mass cultivation of microalgae. Bioresour Technol, 128, 745–50. https://doi.org/10.1016/j.biortech.2012.10.158
323. Wang J, Curtis WR (2016). Proton stoichiometric imbalance during algae photosynthetic growth on various nitrogen sources: toward metabolic pH control. J Appl Phycol, 28, 43–52. https://doi.org/10.1007/s10811-015-0551-3
324. Wang J, Yin Y (2018). Fermentative hydrogen production using pretreated microalgal biomass as feedstock. Microb Cell Fact, 17, 22. https://doi.org/10.1186/s12934-018-0871-5
325. Waterbury JB, Watson SW, Guillard RRL, Brand LE (1979). Widespread occurrence of a unicellular, marine, planktonic, cyanobacterium. Nature, 277, 293–294. https://doi.org/10.1038/277293a0
326. Weerahandi N, Ngo V, Fagan JM (2012). Alternative Fuels for Vehicle. An overview of various approaches for reducing greenhouse gas emissions and ending oil dependence. https://rucore.libraries.rutgers.edu/rutgers-lib/42878/PDF/1/
327. Weissman JC, Tillett DM, (1992). Design and Operation of an Outdoor Microalgae Test Facility: Large-Scale System Results. In Aquatic Species Project Report (FY 1989–90); NREL/TP-232-4174; Brown, L.M., Sprague, S., Eds.; National Renewable Energy Laboratory: Golden, CO, USA, 32–56.
328. Wetzel R.G (1983). Limnology, 2nd ed., p. 255-297. Saunders College Publishing, Philadelphia. https://www.scirp.org/(S(351jmbntvnsjt1aadkposzje)
329. Whitton R, Ometto F, Pidou M, Jarvis P, Villa R, Jefferson B (2015). Microalgae for municipal wastewater nutrient remediation: mechanisms, reactors and outlook for tertiary treatment, Environ Technol Rev, 4, 133-148. https://doi.org/10.1080/21622515.2015.1105308
330. Wilkie AC, Mulbry WW (2002). Recovery of dairy manure nutrients by benthic freshwater algae. Bioresour Technol, 84, 81–91. https://doi.org/10.1016/S0960-8524(02)00003-2
331. Woertz I, Feffer A, Lundquist T, Nelson Y (2009). Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock. J Environ Eng, 135, 1115–1122. https://doi.org/10.1061/(ASCE)EE.1943-7870.0000129
332. Wu X, Merchuk JC (2001). A model integrating fluid dynamics in photosynthesis and photoinhibition processes. Chem Eng Sci, 56, 3527–3538. https://www.infona.pl/resource/bwmeta1.element.elsevier-e95d5cc7
333. Yan C, Zhang L, Luo X, Zheng Z (2013). Effects of various LED light wavelengths and intensities on the performance of purifying synthetic domestic sewage by microalgae at different influent C/N ratios. Ecol Eng, 51, 24-32. https://doi.org/10.1016/j.ecoleng.2012.12.051
334. Yan J, Obersteiner M, Möllersten K, Moreira JR (2019). Negative emission technologies NETs. Appl Energy, 255, 113749. https://doi.org/10.1016/j.apenergy.2019.113749
335. Yang C, Ding Z, Zhang K (2008). Growth of Chlorella pyrenoidosa in wastewater from cassava ethanol fermentation. J Microbiol Biotechnol, 24, 2919–2925. https://doi.org/10.1007/s11274-008-9833-0
336. Yeh KL, Chang JS (2011). Nitrogen starvation strategies and photobioreactor design for enhancing lipid content and lipid production of a newly isolated microalga Chlorella vulgaris ESP-31: Implications for biofuels. Biotechnol J, 6 (11), 1358–1366. https://doi.org/10.1002/biot.201000433

337. You T, Barnett SM (2004). Effect of light quality on production of extracellular polysaccharides and growth rate of Porphyridium cruentum. Biochem Eng J, *19*, 251–258. https://doi.org/10.1016/j.bej.2004.02.004
338. Yu J, Liberton M, Cliften P et al. (2015). Synechococcus elongatus UTEX 2973, a fast growing cyanobacterial chassis for biosynthesis using light and $CO_2$. Sci Rep, 5, 8132 (2015). https://doi.org/10.1038/srep08132
339. Zamora Castro JE, Paniagua-Michel J, Lezama-Cervantes CA (2008). Novel approach for bioremediation of a coastal marine wastewater effluent based on artificial microbial mats. Mar Biotechnol, 10, 181–9. https://doi.org/10.1007/s10126-007-9050-0
340. Zapata D, Arroyave C, Cardona L, Aristizábal A, Poschenrieder C, Llugany M (2021). Phytohormone production and morphology of *Spirulina platensis* grown in dairy wastewaters. Algal Res, 59, 102469. https://doi.org/10.1016/j.algal.2021.102469
341. Zhang D, Dechatiwongse P, del Rio-Chanona EA, Maitland GC, Hellgardt K, Vassiliadis VS (2015). Modelling of light and temperature influences on cyanobacterial growth and biohydrogen production. Algal Research, 9, 263-274. https://doi.org/10.1016/j.algal.2015.03.015.
342. Zhila NO, Kalacheva GS, Volova TG (2011). Effect of salinity on the biochemical composition of the alga Botryococcus braunii Kütz IPPAS H-252. J Appl Phycol, 23, 47–52. https://doi.org/10.1007/s10811-010-9532-8
343. Zhou W, Li Y, Min M, Hu B, Zhang H, Ma X, Li L, Cheng Y, Chen P, Ruan R (2012). Growing wastewater-borne microalga Auxenochlorella protothecoides UMN280 on concentrated municipal wastewater for simultaneous nutrient removal and energy feedstock production. Appl Energy, 98, 433–440. [https://doi.org/10.1016/j.apenergy.2012.04.005
344. Zkeri E, Iliopoulou A, Katsara A, Korda A, Aloupi M, Gatidou G, Fountoulakis MS, Stasinakis AS (2021). Comparing the use of a two-stage MBBR system with a methanogenic MBBR coupled with a microalgae reactor for medium-strength dairy wastewater treatment. Bioresour Technol, 323, 124629. https://doi.org/10.1016/j.biortech.2020.124629

# 2. Chapter 2. Experimental procedures

This chapter introduces in brief the main experimental strategy followed in this Thesis. In more detail, (a) the procedures for cyanobacteria inoculum preparation and inoculation are described, (b) the analytical methods and the used apparatus are presented in brief and (c) the methodology for assessing disinfection efficiency, as well as the methodology for obtaining the measured parameters that describe biomass growth and nutrients removal and assimilation rates are presented. Moreover, (d) the procedure for the in-situ electroproduction of ferrates is presented, as well as the procedures followed for the advanced treatment of effluents.

## 2.1. *Synechococcus elongatus* PCC 7942 cultivation setups

In this work, control and test *Synechococcus elongatus* PCC 7942 cultivation photobioreactors were setup using sterilized glass flasks with sterile cotton caps for uninhibited air transfer inside the PBR and protection from airborne biological contamination (Figure 2.1). The sterilization of glassware, of prepared solutions and of dilution water was performed using an ASTEL autoclave at 120ºC.

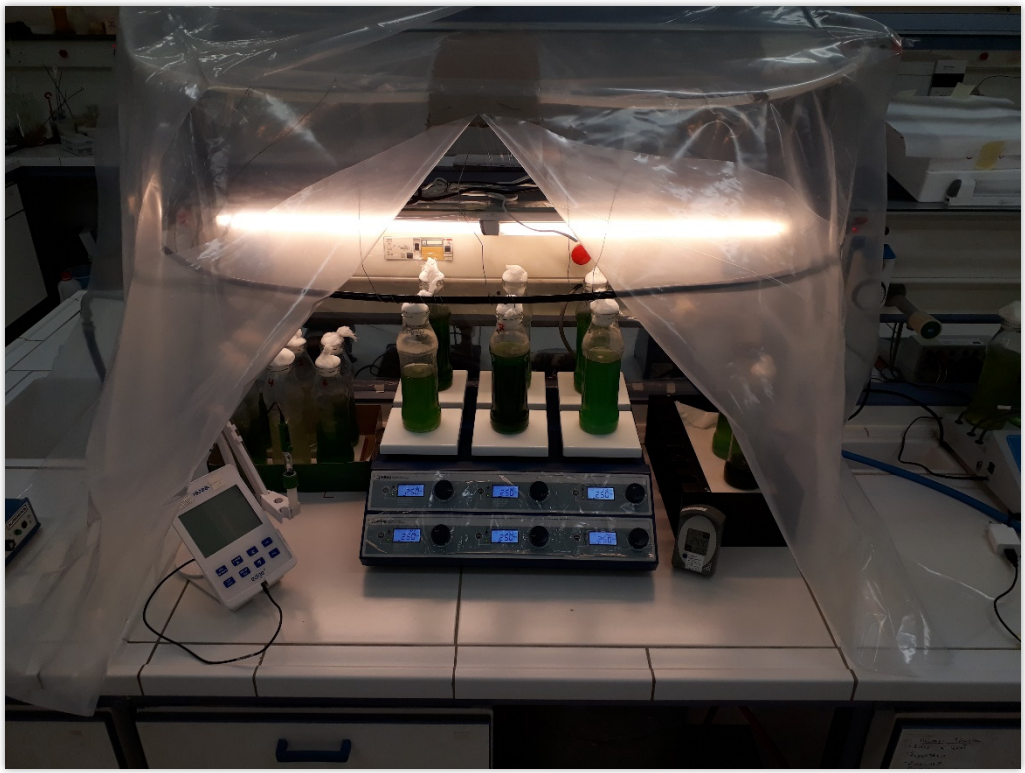

**Figure 2.1.** *Synechococcus elongatus* PCC 7942 cultivation setups.

For control setups, BG11 cultivation media (Rippka et al., 1979) was used as substrate, whereas for test setups four types of wastewaters were used, two from the secondary biological treatment stage of a dairy industry wastewater treatment plant (WWTP) and of a snack industry WWTP after sedimentation, and two from the drainage of an open and a closed hydroponic system (OHS and CHS).

Each individual component (solution) used in the preparation of BG11 growth medium was prepared separately, at sterile conditions and then sterilized again in autoclave. Wastewater substrates were used in test setups both raw i.e., as obtained from the WWTP, and disinfected via the techniques described in later section.

The nutrient content of BG11 growth medium and of the four wastewater substrates that were used in this study are presented in Table 2.1, along with their pH and electric conductivity (EC). The presented values are obtained from 8 batches of BG11 growth medium, three batches of dairy and snack industry's wastewater and one batch of open and closed hydroponic systems drainage that were used in this study.

**Table 2.1.** BG11 growth medium and wastewater substrates composition.

| Parameter | BG11 growth medium | Dairy wastewater substrate | Snack wastewater substrate | OHS wastewater substrate | CHS wastewater substrate |
|---|---|---|---|---|---|
| pH | 7.0 - 7.3 | 7.1 – 7.5 | 7.2 – 7.4 | 7.5 | 4.4 |
| Electric Conductivity (EC) (mS $cm^{-1}$ at 25°C) | 1.78 – 2.06 | 8.71 – 12.22 | 1.67 – 1.93 | 1.32 | 5.11 |
| Nitrate nitrogen (mg $L^{-1}$) | 208.4 – 230.9 | 34.3 – 69.8 | 54.1 – 91.3 | 87.8 | 482.6 |
| Ammonia nitrogen (mg $L^{-1}$) | 0.01 – 0.08 | 0.9 – 3.8 | 0.5 – 2.3 | 0.1 | 9.8 |
| Total (dissolved) phosphorous, mg $L^{-1}$ | 8.7 – 13.6 | 8.3 | 1.3 | 4.6 | 45.0 |

Both control setups and control setups were inoculated with the cyanobacterium *Synechococcus elongatus* PCC 7942 from previously isolated cultures in BG11 growth medium. After approximately four days of cultivation in thermostatic conditions at 30°C, the inoculum cultures were centrifuged at 5000 rpm for 10 to 15 minutes, the supernatant was discarded and the condenced biomass was resuspended using deionized water. Thereafter, from these suspended *Synechococcus elongatus* PCC 7942 cultures, specific volume was used as inoculum in the new control or test setups in order to obtain a specific initial biomass concentration.

The initial cell concentration in the setups, expressed in terms of chlorophyll *a* (Chl *a*) concentration, ranged from 0.81 mg $L^{-1}$ to 1.73 mg $L^{-1}$. The duration of *Synechococcus elongatus* PCC 7942 cultures systematic monitoring was up to 21 days, but cultures with cultivation duration over two months were kept and periodically assessed. All cultures were kept under continuous (24 h/d) agitation/artificial lighting and exposed to ambient room light. Light intensities in the cultures ranged from 5 to 30 μmol $m^{-2}$ $s^{-1}$. Cultivation temperature was controlled and set to values between 16 °C and 37 °C. In order to minimize power requirements for lighting, all culture setups were exposed to ambient light and to constant low-light intensity (5 - 10 μmol-photons $m^{-2}$ $s^{-1}$) artificial lighting using fluorescent lamps. Thus, light intensity during night hours was minimal, while during daytime and depending on weather conditions, the ambient light increased light intensities up to 30.2 μmol-photons $m^{-2}$ $s^{-1}$. All setups and analyses run in duplicates, i.e. two identical PBR configurations for each substrate used for *Synechococcus elongatus* PCC 7942 cultivation and duplicate analysis of the collected samples.

## 2.2. Monitoring parameters and calculations

The fact that phototrophic biomass concentration is expressed in different formats by researchers and engineers, such as in terms of chlorophyll a, optical density (usually at 730 nm or 750 nm), total solids (TS) or total volatile solids (TVS), total suspended solids (TSS) or total volatile suspended solids (VSS), renders it difficult for engineers involved in the design of WWTPs to adopt and implement phototrophic-based wastewater treatment processes. Thus, in this study, in order to assist the interpretation of the results and the adaptation/scaling-up of the proposed S7942-based treatment process, the parameters of (i) chlorophyll *a* (Chl *a*), (ii) optical density at 750 nm (O.D.750 nm) and (iii) total and volatile suspended solids (TSS, VSS) were measured, pair plotted and presented in later chapter.

All *Synechococcus elongatus* PCC 7942 cultivation media that were used in this study contained nitrogen in the form of nitrates with insignificant quantities of nitrite and ammonium ions. Thus, nitrogen assimilation was assessed by monitoring nitrate nitrogen removal from the solution, along with biomass concentration and its organic nitrogen content in terms of Total Kjeldhal Nitrogen (TKN), i.e. the sum of ammonium and organic (cell-bound) nitrogen. The effect of wastewater salinity on *Synechococcus elongatus* PCC 7942 growth was examined via comparison of control setups in BG-11

growth media versus the test setups with NaCl addition. The soluble organic content of the photobioreactors, in terms of chemical oxygen demand (COD), was also monitored.

The evaluation of *S*7942 growth rate was made in terms of chlorophyll *a* (Chl *a*) concentration and the cell productivity rate was calculated using Equation (5).

$$Cp = \frac{VSS_1 - VSS_0}{t} \quad (5)$$

Where, *Cp* (mg VSS $L^{-1}$ $d^{-1}$) = the cell productivity
$VSS_0$ (mg $L^{-1}$) = the initial VSS concentration
$VSS_1$ (mg $L^{-1}$) = the final VSS concentration in (mg $L^{-1}$)
*t* (days) = the duration between initial and final VSS concentration.

The calculation of *S*7942's growth rate, *nitrates utilization rate* (mg$NO_3$_N $L^{-1}$ $d^{-1}$) and phosphates utilization rate (mg$PO_4^{3-}$ $L^{-1}$ $d^{-1}$), resulted from slope of the linear regression of Chl *a* concentration, nitrate-nitrogen and phosphates concentration over time respectively.

Specific nitrates utilization rate (*SNUR*), expressed as mg$NO_3$_N $mgVSS^{-1}$, was calculated according to Equation (6) by dividing *nitrates utilization rate* with cell productivity (*Cp*).

$$SNUR = \frac{Nitrates\ utilization\ rate}{Cp} \quad (6)$$

Similarly, specific phosphates utilization rate (*SPUR*), expressed as mg$PO_4$ $mgVSS^{-1}$, was calculated according to Equation (7) by dividing *nitrates utilization rate* with cell productivity (*Cp*).

$$SPUR = \frac{Phosphates\ utilization\ rate}{Cp} \quad (7)$$

In this study, a Metrohm (Switzerland) ion chromatographer (model 881 compact IC pro equipped with Metrosep A Supp 5 - 250/4.0 and Metrosep C 4 - 250/4.0 columns) was used for the determination of nitrates, ammonium, phosphates, potassium, calcium, magnesium, sulphates, chloride and sodium, according to standard methods (ISO 14911:1998; ISO 10304-1:2007). The measurement of EC and pH was conducted following standard methods for the examination of water and wastewater (APHA, 2017 2510 B & 4500-H+ B), using benchtop meters of Thermos Scientific (USA) (pH-meter:

model ORIONSTAR A111 equipped with a UZ1-11066 electrode; EC-meter: model ORION 3 STAR equipped with an Orion013005MD electrode).

### 2.3. Culture contamination and disinfection

The evaluation of *Synechococcus elongatus* PCC 7942 culture contamination and the identification of growth hindering microbial species in the PBRs was conducted via microscopical examination of cultures using a Leica DM100 phase contrast microscope and based on relevant databases (Oyadomari 2001; APHA 2017).

The disinfection performance of filtration (at pore size of 0.45 μm up to 5–13 μm), the use of NaClO (chlorination), hydrogen peroxide ($H_2O_2$) and ferrates (Fe(VI)) in wastewater-media were evaluated prior the *Synechococcus elongatus* PCC 7942 growth assessment. The disinfection efficiency of each technique was determined by microbiological examination (total viable count—TVC at 22 °C) and microscopic examination of *Synechococcus elongatus* PCC 7942 cultures. Moreover, the effectiveness of the disinfectants was evaluated in terms of Concentration-Time (CT) calculation and TVC measurement (ISO 6222/99). CT is defined as the disinfectant residual concentration (C) multiplied by the effective contact time (T), expressed as mg min L−1. The growth of biological contaminants in the test setups was checked via microscopic examination using a Leica phase contrast microscope (× 40 up to × 1000 magnification).

For the production of ferrate solution, an electrochemical $Fe^0/Fe^0$ cell in 25 M NaOH solution was set up. In more detail, a DC power generator, set at 15 V and 2A, was connected to two iron plate electrodes (16 cm x 9 cm x 0.1 cm) having approximately 2 cm distance between them. The two plate electrodes were submerged 4 cm deep into a 500 mL vessel containing 250 mL 25 M NaOH solution and agitated at 50 rpm with magnetic stirrer. The concentration of hexavalent iron (Fe(VI)) in the prepared solution was determined based on a modified indirect volumetric analytical method (Schreyer et al., 1950). This method is based on Cr(III) to Cr(VI) oxidation by Fe(VI). The resulting chromates are titrated against a known concentration of divalent iron solution (0.025 N Ferrous ammonium sulphate solution) in order to determine Fe(VI) concentration stoichiometrically.

*Synechococcus elongatus* PCC 7942 biomass growth rates, expressed as relative (%) growth rate ($RGR_{Chl\ a}$), were calculated on the basis of chlorophyll *a* concentration (Moran 1982) and according to equation (1) (Vayenos et al., 2020).

$$RGR_{chl\ a} = [(\frac{[Chl\ a](n\ d)}{[Chl\ a](0\ d)})^{(1/n)} - 1] \times 100 \quad (10)$$

Where, *n* is the days of cultivation; *[Chl a](n d)* is the chlorophyll *a* concentration after the $n^{th}$ day; *[Chl a](0 d)* is the initial chlorophyll *a* concentration.

Equation (1) was also used for the calculation of relative (%) nitrates removal rate ($RRR_{NO3\_N}$) and relative (%) phosphates removal rate ($RRR_{PO4\_P}$), by replacement of Chl *a* concentration values with the corresponding measured values of nitrate-nitrogen (APHA 4500-NO3-b) or phosphate-phosphorous concentrations (APHA 4500-P-c) respectively.

All physicochemical and microbiological analyses were performed at the accredited according to ISO 17025 Environmental Chemistry & Water and Wastewater Treatment Laboratory of University of Western Macedonia, Greece, by following standard methods and having calculated measurement uncertainties (Amanatidou et al., 2011; Amanatidou et al., 2012; Trikilidou et al., 2020).

## 2.4. Advanced effluent treatment

For the advanced treatment of effluents, the widely applied techniques of ion-exchange and adsorption were evaluated based on experimentally obtained data. Different types of commercially available ion-exchange resins and adsorption materials and nanomaterials were evaluated in terms of ion removal efficiency in relation to the imposed operational conditions. In more detail, a regerenable anion exchange resin in OH- form, a regerenable cation exchange resin in H+ form and a non-regerenable mixed bed resin were evaluated regarding their selectivity and efficiency for anions or cations removal.

Moreover, the adsorption efficiency of Activated Carbon (AC) particles of different diameter (granular 3 mm, powder 0.6 mm, fine powder <0.075 mm) were evaluated, as well as of two commercially available nanomaterials (Carbon Black and Cloisite 30B) that had been previously studied for the fabrication of nanocomposites (Stimoniaris et al., 2012; Stergiou et al., 2015). Furthermore, the effect of sonication in the adsorption

efficiency of AC and of nanomaterials was evaluated by using either pre-sonicated adsorption nanomaterials or by applying ultrasounds during the treatment process in order to suppress the possible formation of agglomerates via a 200W Hielscher (Germany) ultrasonic processor, model UP200S. The applied contact time, as well as sonication time ranged from 1 min up to 60 min.

## 2.5. References


1. Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RT (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J Gen Microbiol 111:1–61

2. APHA (American Public Health Association), 2017. Standards Methods for the Examination of Water and Wastewater (23rd ed.), American Water Works Association and Water Environmental Federation, Washington, DC.

3. Oyadomari, J.K., 2001. Keweenaw Algae: A Collection of Freshwater Algae (and Protozoans) from the Keweenaw Peninsula, MI. educational website, www.keweenawalgae.mtu.edu, hosted by Michigan Technological University, Houghton, MI.

4. ISO 6222/99 (1999). EN ISO 6222:1999 Water Quality - Enumeration of European Standard. National Standards Authority of Ireland., 6.

5. Schreyer JM, Thompson GW, Ockerman LT (1950) Oxidation of Chromium(lll) with Potassium Ferrate(VI). Anal Chem 22:1426-1427

6. Vayenos D, Romanos EmG, Papageorgiou GC, Stamatakis K (2020) The freshwater cyanobacterium Synechococcus sp. PCC7942 under salt stress: A cell factory for sucrose and hydrogen production. Photosynth Res 146:235-245. https://doi.org/10.1007/s11120-020-00747-6

7. Moran P (1982) Formulae for determination of chlorophyllous pigments extracted with N,N-dimethyl-formamide. Plant Physiol 69:1376-1381. https://doi.org/10.1104/pp.69.6.1376

8. Amanatidou E, Trikoilidou E, Samiotis G, Benetis NP, Taousanidis N (2012) An easy uncertainty evaluation of the COD titrimetric analysis in correlation with quality control and validation data. Method applicability region Anal Methods 4:4204–4212. https://doi.org/ 10.1039/c2ay25687h

9. Amanatidou E, Trikoilidou E, Tsikritzis L, Katsiouli F (2011). Uncertainty in spectrophotometric analysis - “Error propagation break up”, a novel statistical method for uncertainty management. Talanta 85:2385-2390. https://doi.org/10.1016/j.talanta.2011.07.084

10. Trikilidou E, Samiotis G, Tsikritzis L, Amanatidou E (2020) Performance of Semi-Micro-Kjeldahl Nitrogen Method – uncertainty and nitrate interference. Int J Environ Anal Chem 1–11. https://doi.org/10.1080/03067319.2020.1807967

11. Stergiou, C. A., Stimoniaris, A. Z., & Delides, C. G. (2015). Hybrid Nanocomposites With Organoclay and Carbon-Based Fillers for EMI Suppression.

IEEE Transactions on Electromagnetic Compatibility, 57(3), 470–476. https://doi.org/10.1109/TEMC.2014.2384014

12. Stimoniaris, A. Z., Stergiou, C. A., & Delides, C. G. (2012). A detailed study of α-relaxation in epoxy/carbon nanoparticles composites using computational analysis. Express Polymer Letters, 6(2), 120–128.

13. ISO - ISO 10304-1:2007 - Water quality — Determination of dissolved anions by liquid chromatography of ions — Part 1: Determination of bromide, chloride, fluoride, nitrate, nitrite, phosphate and sulfate. (n.d.). Retrieved March 28, 2022, from https://www.iso.org/standard/46004.html

14. ISO - ISO 14911:1998 - Water quality — Determination of dissolved Li+, Na+, NH4+, K+, Mn2+, Ca2+, Mg2+, Sr2+ and Ba2+ using ion chromatography — Method for water and wastewater. (n.d.). Retrieved March 28, 2022, from https://www.iso.org/standard/25591.html

## 3. Chapter 3. Wastewater substrate disinfection for cyanobacteria cultivation as tertiary treatment

Cultivation of cyanobacteria or/and microalgae in nutrient-rich wastewaters offers an opportunity for enhancing sustainability of tertiary wastewater treatment processes via resources/energy recovery/production, mitigation of emitted GHGs and provision of added value products. However, maintaining a monoculture in wastewater-media constitutes a significant challenge to be addressed. In this regard, the present work assesses the efficiency of the low-cost wastewater substrate disinfection techniques of filtration, use of NaClO, $H_2O_2$ or Fe(VI), as a preliminary treatment stage upstream a cyanobacteria cultivation photobioreactor. The growth rate of cyanobacterium *Synechococcus elongatus* PCC 7942, as well as nitrates and phosphates removal rates were experimentally assessed in cultivation setups with biologically treated dairy wastewater that had been subjected to a single or a synergetic couple of disinfection techniques. The results showed that filter thickness has a greater effect on disinfection efficiency than filter pore size. Furthermore, the disinfection efficiency of Fe(VI), which was produced on-site by electrosynthesis via a $Fe^0/Fe^0$ cell, was greater than that of NaClO and $H_2O_2$. Filtration at ≤ 1.2 μm pore size coupled with chemical disinfection led to unhindered *Synechococcus elongatus* PCC 7942 growth and efficient nitrates and phosphates removal rates, at dosages of $CT \geq 270$ mg min $L^{-1}$ for NaClO and $CT \geq 157$ mg min $L^{-1}$ for Fe(VI). The coagulation action of Fe(III) species that result from Fe(VI) reduction and the oxidation action of Fe(VI) can assist in turbidity, organic compounds and phosphorous removal from wastewater-media. Moreover, the residual iron species can assist in *Synechococcus elongatus* PCC 7942 harvesting and may enhance photosynthesis rate by increasing light transfer efficiency. Thus, a filtration configuration coupled with chemical disinfection, preferably using ferrates, downstream of sedimentation tank of a secondary biological wastewater treatment stage is proposed as a necessary, efficient and low-cost disinfection technique for full-scale scale implementation of cyanobacteria cultivation as tertiary wastewater processes.

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## *3.1. Introduction*

The preservation of freshwater quality and mitigation of climate change are inextricably linked to the development and full-scale application of sustainable technologies that align with the concept of circular economy i.e., reduce-reuse-recycle (Sakai et al., 2011). Thus, the implementation of the best available technologies, practices and techniques (BAT) for the protection of the environment, the recovery of resources and the control of greenhouse gases emissions must be the response to the challenge of sustainability. In this regard, the wastewater treatment sector offers significant opportunities for application of BAT that align with the concept of circular economy, due to the high nutrient and energy content of wastewaters (Hoek et al., 2016).

Wastewater treatment processes that are based on algae or/and cyanobacteria for the removal/recovery of nutrients suggest the evolution of activated sludge (AS) systems. In particular, cyanobacteria have received much attention as a novel and alternative approach for carbon footprint mitigation in wastewater treatment and for the production of third generation biofuels, hydrocarbons, proteins, pigments and biopolymers for pharmaceutical, chemical and food industries (Trivedi et al., 2015; Vassilev and Vassileva 2016; Pathak et al., 2018; Maurya et al., 2021; Goswami et al., 2022; Melo et al., 2022). Moreover, the utilization of wastewater streams is considered promising for reducing the cost of full-scale cyanobacteria cultivation (Arias et al., 2017; Samiotis et al., 2021). Cyanobacteria cultivation for the production of biofuels, especially if it is combined with wastewater and/or flue gases treatment, outperforms in terms of sustainability the conventional technologies that utilize sugar, starch, vegetable or animal fats, because of the competitive consumption of food resources and the associated commitment of arable land (Rosegrant et al., 2006; Gonaalves et al., 2016; Almomani et al., 2019). However, full-scale application of cyanobacteria in wastewater treatment constitutes a challenge due to the plethora of parameters that may influence their performance, such as influent composition, environmental conditions and operating parameters (Shoener et al., 2019).

Many studies on cultivation of phototrophic species in wastewaters have been adapted to laboratory settings and thereby somewhat have been detached from real world conditions, as in many cases (i) external source of nutrients is provided, (ii) synthetic wastewater as cultivation medium is used, (iii) very short cultivation duration is applied, and (iv) costly wastewater sterilization techniques are applied, ignoring this

way the possible challenges such as unbalanced nutrients or chemical and biological contaminants (Tejido-Nuñez et al., 2019). Biological contaminants of wastewaters mainly consist of procaryotic bacteria (eubacteria and archaebacteria) and eucariotes (fungi, protozoa, rotifers and nematodes) (Gerardi 2006). These biological contaminants constitute a serious threat to phototrophic species cultivation, as they may compete for nutrients (Sorensen et al., 2021), they can hinder photosynthesis due to cell-shading phenomena (Flynn 2021) or may result to population demise of cultivated species due to predating phenomena (Day et al., 2017). Thus, co-presence of other microorganisms needs to be limited by the application of low-cost disinfection techniques, which usually include filtration or the addition of chemical disinfectants, such as sodium hypochlorite (NaClO) and hydrogen peroxide ($H_2O_2$) (Collivignarelli et al., 2018).

Besides disinfection with NaClO or $H_2O_2$, novel and considered environmentally friendly disinfection techniques have been developed, with those based on hexavalent iron (ferrates) production showing a particular interest due to their benign nature (Sharma et al., 2022). The merits of using ferrates ($FeVIO_4^{2-}$, Fe(VI); $FeVO_4^{3-}$, Fe(V); and $FeIVO_4^{4-}$) for disinfection are that they present high oxidation ability and that they are completely transformed to non-toxic trivalent iron species, simultaneously acting as a coagulant (Al Umairi et al., 2021). Ferrates can oxidize organic compounds, microorganisms and spores that are present in wastewaters. Furthermore, the aggregation action of resulting trivalent iron species can assist in organic compounds and turbidity removal from wastewater cultivation media, as well in the collection (harvesting) of cyanobacteria or/and microalgae from a photobioreactor (PBR), a process that has been criticized due to its low efficiency and/or the high associated cost (Addison et al., 2021; He et al., 2021). Thus, the application of ferrates in wastewater-media can remove biological contaminants that may hinder monoculture cultivations and lead to clearer growth media, where photosynthetically active radiation (PAR) can travel unhindered by turbidity (Škulcová et al., 2021). On the other hand, the minimal stability of ferrate compounds in environmental conditions renders them non proper for commercial use both in terms of storage (poor shelf life) and provision (scarce and highly priced substance) (Wang et al., 2021). Nevertheless, on-site ferrates production via a low-cost Fe0/Fe0 electrochemical cell can alleviate both drawbacks (Mácová et al., 2009).

The aim of this work is to assess the possibility of utilizing biologically treated wastewaters as substrate for cultivation of *Synechococcus elongatus* PCC 7942 monocultures in a PBR, which can be used for tertiary treatment applications. For this aim, the efficiency of low-cost wastewater substrate disinfection techniques for the removal of biological contaminants is being evaluated and the growth rate of *Synechococcus elongatus* PCC 7942, as well as the nutrients removal rate in properly disinfected wastewater-media are being studied. This work is of great importance for the implementation of cyanobacteria-based or/and microalgae-based wastewater treatment processes, as sustainable alternative or supplementary treatment stage to conventional biological nutrient removal processes.

## 3.2. Materials and Methods

Sterilized 500 mL Erlenmeyer flasks were used as *Synechococcus elongatus* PCC 7942 cultivation PBRs and the assessment of biologically treated wastewaters' adequacy as *Synechococcus elongatus* PCC 7942 growth medium. Sterile cotton caps were applied for uninhibited air transfer inside the PBRs and protection from airborne biological contamination. A culture of *Synechococcus elongatus* PCC 7942 in BG-11 growth-medium (Rippka et al., 1979) was used as inoculum for control and test setups. The inoculums were separated from BG-11 growth-medium via centrifugation at 5000 rpm for 10 minutes. The PBRs were kept under continuous agitation and lighting, at controlled room temperatures ranging from 20.4 ºC to 26.2 ºC. The average wastewater substrate temperature was 22.9 ºC, having a standard deviation of 1.3 ºC. Agitation at approximately 200 rpm was provided using magnetic stirrers and 5 cm magnetic rods. In order to minimize power requirements for lighting, PBRs were exposed to ambient light and to constant low-light intensity (5 μmol-photons $m^{-2}$ $s^{-1}$) artificial lighting using fluorescent lamps (Phillips model XX). Thus, light intensity during night hours was minimal, while during daytime and depending on weather conditions, the ambient light increased light intensities up to 30.2 μmol-photons $m^{-2}$ $s^{-1}$.

The control and the test setups had an initial *Synechococcus elongatus* PCC 7942 biomass content, in terms of chlorophyll *a* concentration, of approximately 1 mg $L^{-1}$ and were monitored for 20 days regarding the growth rate of *Synechococcus elongatus* PCC 7942 and the achieved nutrients (nitrogen and phosphorous) removal rate. All setups and analyses run in duplicates i.e., two identical PBR configurations for each substrate used for *Synechococcus elongatus* PCC 7942 cultivation and duplicate

analysis of the collected samples. The control setups contained BG-11 medium, while the test setups contained biologically treated industrial wastewater (wastewater-medium) that had been subjected or not to a single or a combination of low-cost disinfection techniques. The wastewater-medium was obtained from the nitrification tank of a dairy industry's activated sludge (AS) wastewater-treatment plant (WWTP), after suspended solids removal via sedimentation. Biologically treated dairy wastewaters are considered suitable microalgae growth medium (Asadi et al., 2019) and high strength wastewaters. Their high nitrogen load and increased salinity can result in significant yields of phototrophic biomass and added value products (Samiotis et al., 2022; Melo et al., 2022). Moreover, if phototrophic processes are combined with novel and more sustainable biological treatment processes, such as the complete solids retention process (Amanatidou et al., 2016 a, b), minimization of WWTPs' ecological footprint can be achieved via excess sludge minimization, production of added value products from phototrophic biomass and carbon emission mitigation. Furthermore, dairy wastewaters are characterized by increased salinity, which may have significant implications regarding the utilization of *Synechococcus elongatus* PCC 7942 for the treatment of wastewater and the production of added value products, since increased salinities may lead to increased nitrogen removal via assimilation (Samiotis et al., 2022) and increased intracellular sucrose production for direct biohydrogen production via the anaerobic dark fermentation metabolic path (Vayenos et al., 2020).

The disinfection performance of filtration (at pore size of 0.45 μm up to 5-13 μm), the use of NaClO (chlorination), hydrogen peroxide ($H_2O_2$) and ferrates (Fe(VI)) in wastewater-media were evaluated prior the *Synechococcus elongatus* PCC 7942 growth assessment. The disinfection efficiency of each technique was determined by microbiological examination (total viable count - TVC at 22ºC) and microscopic examination of *Synechococcus elongatus* PCC 7942 cultures. Moreover, the effectiveness of the disinfectants was evaluated in terms of *CT* calculation and TVC measurement (ISO 6222/99). *CT* is defined as the disinfectant residual concentration (*C*) multiplied by the effective contact time (*T*), expressed as mg min $L^{-1}$. The growth of biological contaminants in the test setups was checked via microscopic examination using a Leica phase contrast microscope (x40 up to x1000 magnification).

The disinfection technique with ferrate is considered environmentally friendly (Al Umairi et al., 2021) and presents coagulation ability, which was assessed in this study

in terms of organic compounds (chemical oxygen demand, COD), phosphates and turbidity removal from the wastewater-media. For the production of ferrate solution, an electrochemical $Fe^0/Fe^0$ cell in 25 M NaOH solution was set up. In more detail, a DC power generator, set at 15 V and 2A, was connected to two iron plate electrodes (16 cm x 9 cm x 0.1 cm) having approximately 2 cm distance between them. The two plate electrodes were submerged 4 cm deep into a 500 mL vessel containing 250 mL 25 M NaOH solution and agitated at 50 rpm with magnetic stirrer. The concentration of hexavalent iron (Fe(VI)) in the prepared solution was determined based on a modified indirect volumetric analytical method (Schreyer et al., 1950). This method is based on Cr(III) to Cr(VI) oxidation by Fe(VI). The resulting chromates are titrated against a known concentration of divalent iron solution (0.025 N Ferrous ammonium sulphate solution) in order to determine Fe(VI) concentration stoichiometrically.

*Synechococcus elongatus* PCC 7942 biomass growth rates, expressed as relative (%) growth rate ($RGR_{Chl\ a}$), were calculated on the basis of chlorophyll *a* concentration (Moran 1982) and according to equation (1) (Vayenos et al., 2020).

$$RGR_{chl\ a} = [(\frac{[Chl\ a](n\ d)}{[Chl\ a](0\ d)})^{(1/n)} - 1] \times 100 \quad (1)$$

Where, *n* is the days of cultivation; *[Chl a](n d)* is the chlorophyll *a* concentration after the $n^{th}$ day; *[Chl a](0 d)* is the initial chlorophyll *a* concentration.

Equation (1) was also used for the calculation of relative (%) nitrates removal rate ($RRR_{NO3\_N}$) and relative (%) phosphates removal rate ($RRR_{PO4\_P}$), by replacement of Chl *a* concentration values with the corresponding measured values of nitrate-nitrogen (APHA 4500-NO3-b) or phosphate-phosphorous concentrations (APHA 4500-P-c) respectively.

All physicochemical and microbiological analyses were performed at the accredited according to ISO 7025 Environmental Chemistry & Water and Wastewater Treatment Laboratory of University of Western Macedonia, Greece, by following standard methods and having calculated measurement uncertainties (Amanatidou et al., 2011; Amanatidou et al., 2012; Trikilidou et al., 2020).

## 3.3. Results and discussion

### 3.3.1. Wastewater biological contaminants in wastewater media

Microscopic examination of *Synechococcus elongatus* PCC 7942 cultures with inefficiently disinfected wastewater media from a dairy industry and from a salty-snack industry revealed the presence and growth of biological contaminants. As shown in Figure 3.1 – inset 2, fungi were the dominant biological contaminant species to *Synechococcus elongatus* PCC 7942 in the dairy wastewater test setups, while protozoan (mainly ciliates) and metazoan (mostly rotifers) dominated in the salty-snack wastewater test setups (Figure 3.1 – inset 3). Beside the growth of non-phototrophic microorganisms, antagonistic to *Synechococcus elongatus* PCC 7942 phototrophic species were also developed, especially in experimental setups with non-filtrated wastewater-medium (Figure 3.1 – inset 1). In Figure 3.1 – inset 1, an uninfected *Synechococcus elongatus* PCC 7942 culture is depicted.

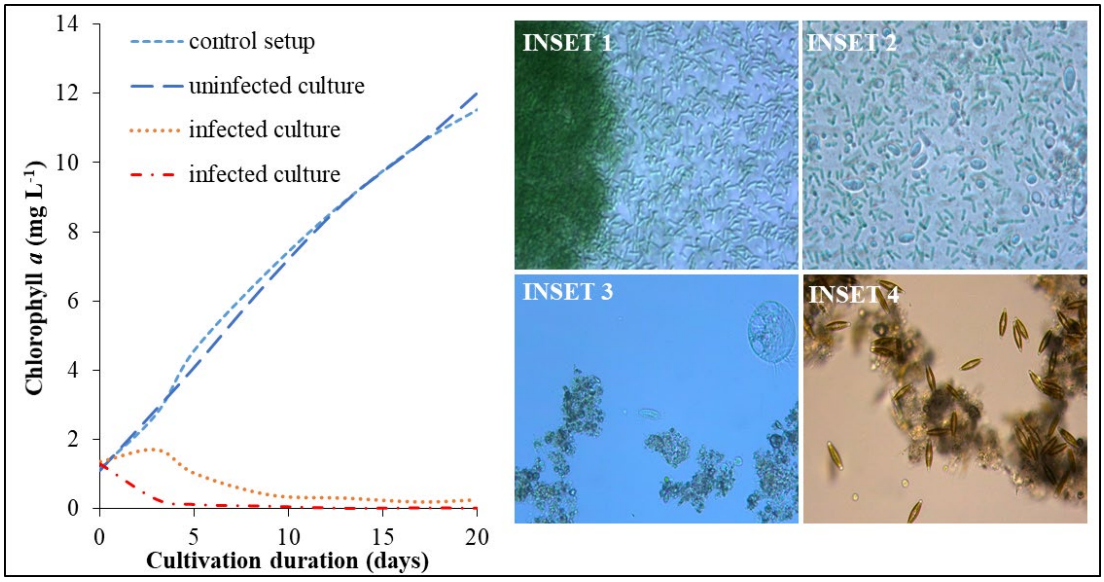


**Figure 3.1.** Indicative *Synechococcus elongatus* PCC 7942 growth curves in uninfected and infected experimental culture setups (Microscopic depiction of *Synechococcus elongatus* PCC 7942 cultures at x1000: Inset 1 = uninfected culture; Inset 2-4 = infected culture).

These preliminary experimental results indicate the necessity of biological contaminants control, since they pose a serious threat to cyanobacteria or/and microalgae cultivation. According to Day et al. (2017) even small numbers of herbivorous protozoa can rapidly proliferate and thus destroy a culture of microalgae.

Tejido-Nuñez et al. (2019) who studied the effect of sterile filtration as a pretreatment on *Chlorella vulgaris* and *Tetradesmus obliquus* growth, found that protozoa were observed in all non-sterile water samples resulting in the decline of *C. vulgaris* biomass and therefore reducing the nutrients removal efficiency of the process.

### 3.3.2. Assessment of filtration

The porosity and the thickness of a filter-medium are two key parameters that dictate filtration performance. The small pore size of a filter introduces higher retention rate, whereas the high porosity is defined by a higher permeability capability. Furthermore, filter thickness affects the duration of filtration and subsequently filtration efficiency (Fahimirad et al., 2021). In our study, filters of different pore size and thickness were evaluated in duplicate experiments regarding their ability to remove the existing microbial load of the wastewater-medium for *Synechococcus elongatus* PCC 7942 cultivation. More specifically, filters with pore size of 0.45 μm, 0.7 μm, 1 μm, 1.2 μm, 2-4 μm, 3-5 μm, 5-7 μm and 5-13 μm were tested in terms of TVC reduction in the wastewater-media filtrates. Their thickness and respective material are presented in Table 3.1, along with the results from TVC measurements.

**Table 3.1.** Disinfection efficiency of filtration at different pore size.

| **Filter pore size (μm)** | **Filter material** | **Filter thickness (mm)** | **Total Viable Count (CFU mL$^{-1}$ at 22$^{o}$C)** |
|---|---|---|---|
| unfiltered | - | - | >5000 |
| 0.45 | cellulose | 0.135 | 280 ± 28.3 |
| 0.7 | fiber glass | 0.45 | 4 ± 2.8 |
| 1 | fiber glass | 0.70 | 2 ± 1.4 |
| 1.2 | fiber glass | 0.26 | 65 ± 15.6 |
| 2-4 | paper | 0.15 | 1256 ± 152.7 |
| 3-5 | paper | 0.17 | 814 ± 65.1 |
| 5-7 | paper | 0.32 | 540 ± 56.6 |
| 5-13 | paper | 0.15 | >2000 |

As shown in Table 3.1, the highest disinfection efficiency was achieved with the fiber glass filters, which are characterized by increased thickness (0.26 mm to 0.70 mm). The

0.45 μm pore size cellulose filters, which had significantly smaller thickness than that of the fiber-glass, presented lower disinfection efficiency, despite having the smallest filter pore size. The paper filters, which all had pore sizes greater than 2 μm presented worst disinfection efficiency than the fiber-glass and cellulose filters. Among these paper filters, the thickest one (0.32 mm) presented the highest disinfection efficiency, in contrast to the two smaller pore sizes paper filters (2-4 μm and 3-5 μm).

Bivariate analysis (Spearman's rank correlation) between pore-size, thickness and TVC revealed a moderate positive correlation (Corr. Coeff. = 0.611) between pore-size and TVC, with high statistical significance ($p = 0.020$). Furthermore, filter thickness and TVC exhibited a moderate to very strong negative correlation (Corr. Coeff. = -0.700) with higher statistical significance ($p = 0.005$). It is worth mentioning that the resulting ratios between each filter's pore-size and thickness presented very strong to perfect correlation (Corr. Coeff. = |0.913|) with TVC, having very high statistical significance ($p < 0.001$). This almost perfect correlation is indicative of the cumulative effect of filter's pore size and thickness on disinfection efficiency. The interpretation of the results from the aforementioned statistical analysis was conducted according to the Akoglu's (2018) guide.

Besides the assessment of filters' disinfection efficiency in terms of TVC reduction in the wastewater-medium filtrates, the presence of biological contaminants in *Synechococcus elongatus* PCC 7942 cultivations PBRs was microscopically examined and its impact on culture growth was evaluated in duplicate experimental setups (two control and fourteen test setups). According to the microscopic examination of cultures, biological contaminants were present in all test setups with filtrated wastewater medium. As evident in Figure 3.2, their presence suppressed to the decline *Synechococcus elongatus* PCC 7942 growth leading to almost complete extinction of its population in terms of chlorophyll *a*. Each test setup's chlorophyll *a* concentration dropped from the initial value of approximately 1 mg $L^{-1}$ to 0.03 - 0.19 mg $L^{-1}$ after 20 days, while the latter did not increase even after approximately 40 days of cultivation (chlorophyll $a < 0.1$ mg $L^{-1}$ in all test setups, not shown in Figure 3.2). The filters that presented the highest disinfection efficiency (0.7 μm and 1 μm pore size fiber-glass filters) presented a 5-days delay in the decline of chlorophyll *a* concentration, attributed to the low initial population of biological contaminants in the filtrates.

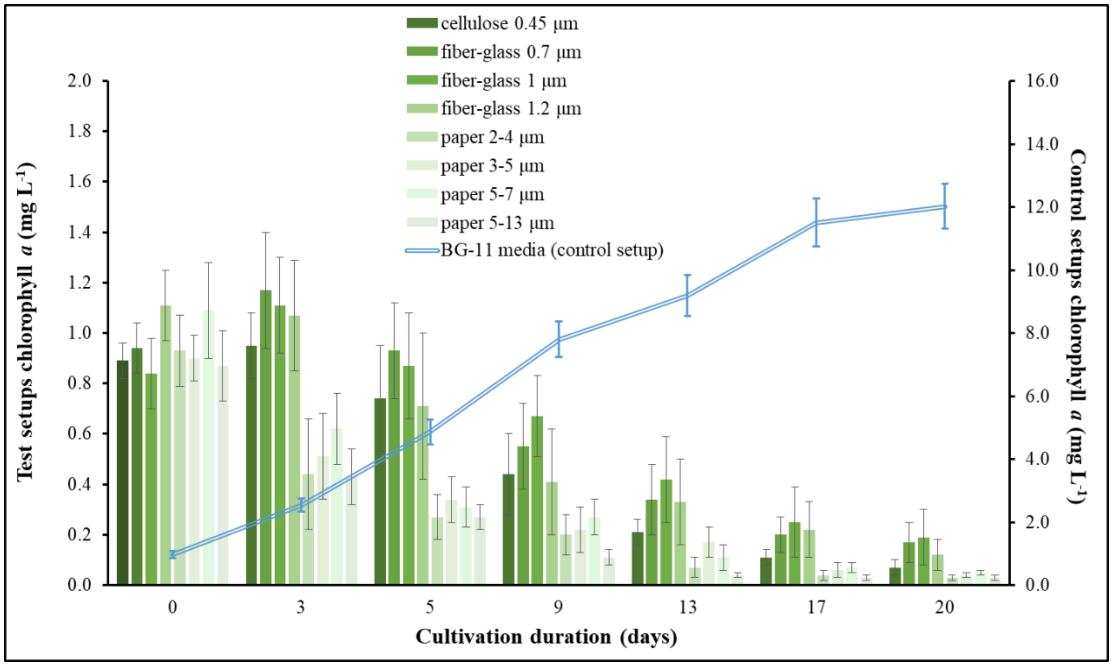


**Figure 3.2.** Cholorophyll *a* concentration evolution in *Synechococcus elongatus* PCC 7942 control setups and in test setups with filtrated wastewater-media.

It is concluded that none of the applied filtration techniques is efficient to sustain a monoculture of *Synechococcus elongatus* PCC 7942 in the PBRs. It is evident from the results presented in Table 3.1 and Figure 3.1, as well as from the performed statistical analysis (Spearman's rank correlation tests), that both pore size and filter thickness have a significant effect on disinfection efficiency. However, the thickness of the filter, i.e. the duration of filtration, seems to have a greater effect on disinfection efficiency than that of filter's pore size. This is based both on the results of the statistical analysis and on the observations showing that (a) the smaller TVC count was obtained using the thickest filter (0.70 mm thickness, 1 μm pore size) and not when using the smallest pore size filters of 0.70 μm or 0.45 μm and (b) the thickest paper filter (0.32 mm, 5-7 μm pore size) presented the highest disinfection efficiency when compared to filters with >2 μm pore size. Thus, in real scale applications, an ultrafiltration configuration or a slow sand filtration technique could be used as a low-cost preliminary disinfection process for the significant minimization of viable microorganisms in a wastewater-media, but further polishing would be required for complete disinfection.

### 3.3.3. Assessment of chemical disinfection by Sodium hypochlorite, Hydrogen peroxide, Hexavalent iron

Similarly to filtration, chemical disinfection alone, using NaClO or $H_2O_2$ or Fe(VI), is proven to not be able to alleviate the problem of *Synechococcus elongatus* PCC 7942 culture contamination. While higher dosages of these disinfectants, in terms of *CT,* led to lower TVC at 22ºC, complete disinfection could not be achieved. Even at considerably high NaClO or $H_2O_2$ or Fe(VI) dosages of 5530 mg min $L^{-1}$, 12000 mg min $L^{-1}$, 3105 mg min $L^{-1}$ respectively, viable microorganisms are still accounted in the wastewater-medium (Figure 3.3). The effect of NaClO on wastewater disinfection has been also studied by Medrano-Barboza et al., 2021. According to their study and similarly to our results, total inactivation of bacterial load could not be achieved at NaClO concentrations up to 60 mg $L^{-1}$, despite the observed significant decrease in TVC. In the present study, high efficiency disinfection was achieved at NaClO concentrations of 92 mg $L^{-1}$ and 184 mg $L^{-1}$ for 30 min and 60 min application respectively. Sodium hypochlorite solution presented approximately 2.2 times higher disinfection efficiency compared to $H_2O_2$, thus the use of NaClO is suggested. The highest disinfection efficiency was observed using the freshly prepared via electrosynthesis Fe(VI) solution (Figure 3.3).

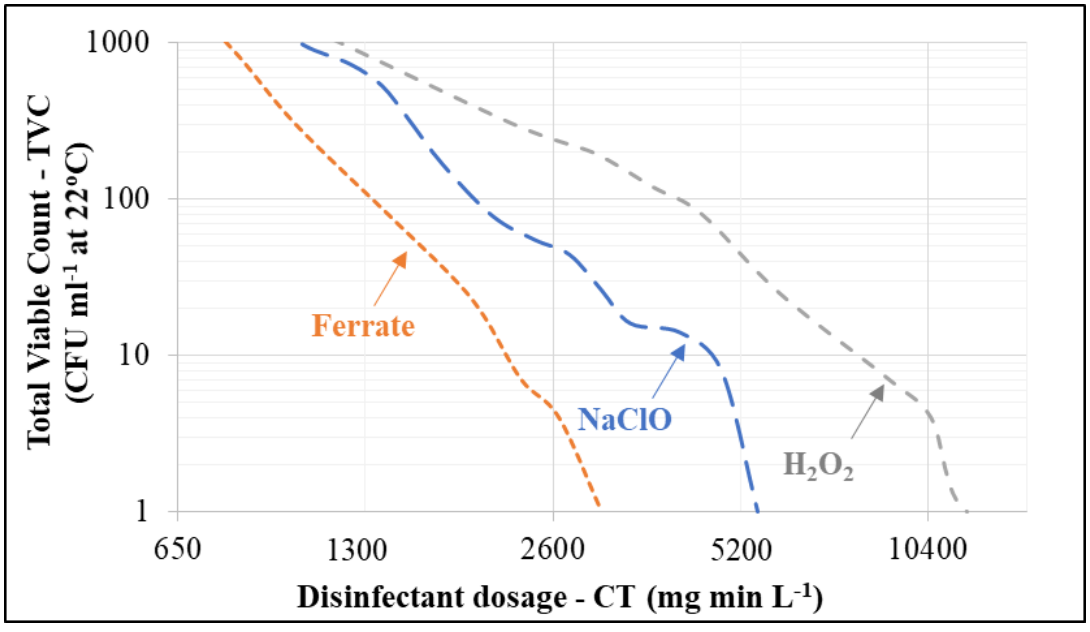


**Figure 3.3.** Disinfection efficiency of sodium hypochlorite (pH from 7.2 to 8.1), hydrogen peroxide (pH from 7.2 to 6.5) and ferrates (pH from 7.2 to 8.3) in terms of TVC removal at different disinfectant dosages (*CT*).

It is worth mentioning at this point that there was no pH adjustment during the application of any of the three disinfectants, thus they were evaluated at the efficiency imposed by the wastewater characteristics and the impact of disinfectant solution on critical parameters, such as pH and redox potential. Hydrogen potential in particular can significantly affect oxidising power of disinfectants, as well as the speciation of chloride and ferrates. Oxidizing power of NaClO increases with increasing pH (Fukuzaki et al., 2007), while the opposite behavior is observed with $H_2O_2$ (Torres et al., 2014). This could explain the significant difference in disinfection efficiency between NaClO and $H_2O_2$ observed at the conditions of the experiments i.e. at an initial pH of 7.2 (Figure 3.3). Ferrates on the other hand, which presented the highest disinfection efficiency, change their structure in relation to pH (Figure 3.4) and exhibit an interesting behavior. Their oxidizing power increases with a decrease in pH and is related to protonation of $Fe^{VI}O_4^{2-}$ and $Fe^{V}O_4^{3-}$ (Henry-Chase and Tewari 2013), but the optimal performance of ferrate as an oxidizing chemical may correspond to pH conditions of between 9 and 10, representing the overall combined effect of its lower oxidation potential but greater stability (C. Li et al., 2005).

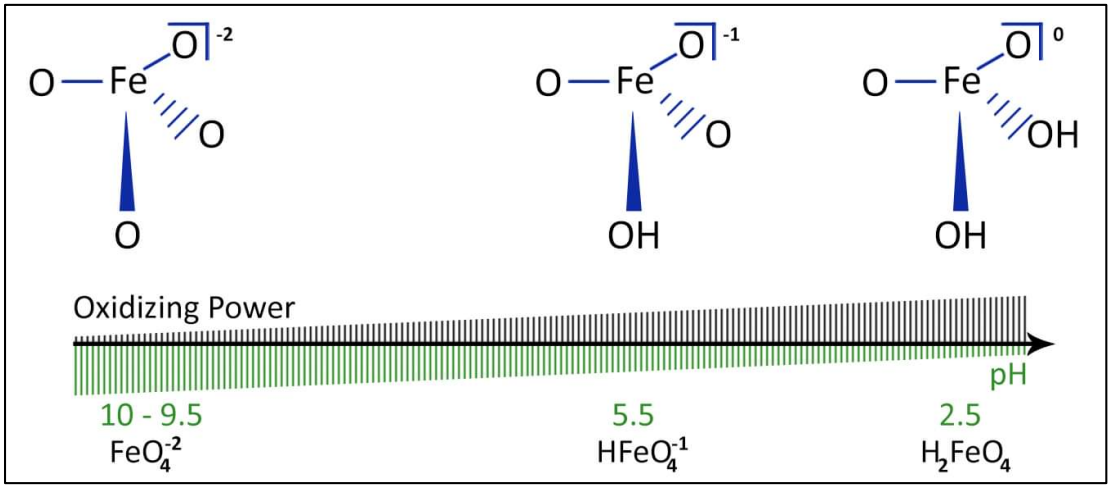


**Figure 3.4.** Changes in ferrate structure and oxidizing power in relation to pH (modified from Henry-Chase and Tewari 2013 based on Delaude and Laszlo 1996).

The oxidation state of $FeO_4^{-2}$ can be changed from +6 to +3 in acidic and alkaline conditions (Wood 1958), as shown in equations (2) and (3), having a standard half-cell reduction potential from +2.20 V to +0.72 V in acidic and basic solution respectively. It is worth noting that the oxidation potential of ferrates in acidic solution is the strongest of all the oxidants/disinfectants used in water and wastewater treatment,

including chlorine, hypochlorite, chlorine dioxide, ozone, hydrogen peroxide, dissolved oxygen, and permanganate (Jiang and Lloyd, 2002).

$$FeO_4^{2-} + 8H^+ + 3e^- \leftrightarrows Fe_3^+ + 4H_2O \quad (2)$$

$$FeO_4^{2-} + 8H_2O + 3e^- \leftrightarrows Fe(OH)_3\downarrow + 8H_2O \quad (3)$$

Moreover, during ferrates reduction to Fe(III) (Eq. 2) particulate matter and colloidal organic compounds that may be present in wastewater-media are removed by coagulation and adsorption on flocs of iron (III) hydroxide that are formed at neutral and alkaline pH (Eq. 3) (Thomas et al., 2022). The removal of suspended solids and organic compounds has significant impact on light transfer efficacy in a PBR, as PAR transfers in the substrate uninhibited (Škulcová et al., 2021).

According to Tschobanoglous, et al. (2003) and Sulistyo (2012), the in situ generated ferric (hydr)oxides by the Fe(VI) reduction, possesses excellent coagulation and adsorption ability and thus has immense potential in eliminating contaminants and reaction byproducts by adsorbing particles and building up bridges to combine colloids and suspended particles into bigger ones. Meanwhile, a ferric ions coagulant, when added in water will undergo hydrolysis as described below:

$$Fe^{+3} + 12\ H_2O \rightarrow 2\ [Fe(H_2O)_6]^{3+} \quad (4)$$

Mononuclear species

$$8\ [Fe(H_2O)_6]^{3+} + 20\ OH^- \rightarrow [Fe_8(OH)_{20}]^{4+}(aq) + 6\ H_2O \quad (5)$$

Polynuclear species

$$[Fe_8(OH)_{20}]^{4+}(aq) + 4\ OH^- + 24\ H_2O \rightarrow 8\ [Fe(OH)_3(H_2O)_3]\ (s) \quad (6)$$

The coagulation steps upon reduction of ferrates to ferric species include (i) their hydrolysis and subsequent polymerization, (ii) adsorption of hydrolysis species at the solid-solution interface to accomplish destabilization of the colloid, (iii) aggregation of destabilized particles and (iv) precipitation or/and floatation of aggregates.

For Fe(VI) concentrations of 4.2 mg $L^{-1}$ to 51.7 mg $L^{-1}$, the process efficiency, in terms of turbidity and COD removal, ranged from 89% to 97% and 71% to 84% respectively. COD removal can be also attributed to organic compounds oxidation by radicals formed during Fe(VI) reduction, which can also enhance recalcitrant organic compounds

removal from wastewaters (Kokarovtseva et al., 1972; Sharma et al., 2015; Drzewicz et al., 2019). Our results are in accordance with the findings of other researchers who presented the dual-function of ferrates as a coagulant and as a potent oxidant that is able to perform efficient disinfection and oxidize a large number of organic compounds measured in COD determination, thus can be applied for chemically-enhanced primary treatment (De Luca et al., 1992; Delaude and Laszlo 1996; Sharma 2002; Al Umairi et al., 2021). Al Umairi et al. (2021) found that a ferrate dose of 0.5 mg $L^{-1}$ Fe removed 80% of total suspended solids (TSS), 57% of chemical oxygen demand (COD), whereas higher concentrations of ferrate at 15 mg $L^{-1}$ as a disinfectant were necessary to achieve a five orders of magnitude higher removal of *E. coli.* Zhang et al. (2020) also demonstrated that ferrate (VI) addition at 6 mg $L^{-1}$ can effectively serve as a core treatment process removing simultaneously turbidity (98%) and inactivating 100% total coliforms in one single dose.

The results of our study suggest the use of ferrate solution as a disinfectant/coagulant is a novel and effective treatment approach for disinfection of wastewaters meant to be used as substrate for cyanobacteria monoculture, offering the additional benefit of collectively removing TSS, turbidity and microbial load. These results add up to the potential applications of ferrates in aqueous solutions, such their use for in energy materials and green organic synthesis, as they can be used for disinfection, oxidation and coagulation making a meaningful contribution to addressing the challenging demands of sustaining the water supply in the 21$^{st}$ century (Sharma et al., 2015).

It is worth mentioning that the evident coagulation action of ferrates can assist with the efficient harvest of cyanobacteria or/and microalgae from PBRs (Addison et al., 2021), which constitutes a challenge for engineers that is usually addressed by coagulation-flocculation techniques (Choy et al., 2018). An additional merit of a ferrate-based disinfection technique is that the resulting Fe(III) from ferrates reduction could enhance photosynthesis in the PBR, as Fe(III) species have been shown to enhance phototrophic biomass production (Rana and Prajapati, 2021). Thus, a ferrate solution could replace NaClO or $H_2O_2$ as disinfectant, offering a more sustainable solution to wastewater-media disinfection. The in situ electrochemical preparation of ferrate solution in a strongly basic environment using a simple $Fe^0/Fe^0$ cell is evaluated as an easy to apply method that produces a relatively stable product, since ferrates are relatively stable in basic solutions and very unstable in neutral and acidic solutions (Henry-Chase and

Tewari, 2013). Nevertheless, further study regarding optimal $Fe^0/Fe^0$ cell configuration and current density is considered essential towards energy consumption minimization for Fe(VI) production.

### 3.3.4. Synergy of studied disinfection techniques

As previously presented, each of the studied disinfection technique alone could not address the challenge of maintaining a growing *Synechococcus elongatus* PCC 7942 monoculture in the PBR. Hence, the suggested low-cost disinfection technique of filtration at pore size ≤1.2 μm was evaluated as synergetic couple with the use of NaClO or Fe(VI), in terms of disinfection efficiency and *Synechococcus elongatus* PCC 7942 growth in duplicate experimental setups (two control and eighteen test setups) (Figure 3.3).

The assessment of disinfection efficiency showed that complete wastewater-medium disinfection, in terms of TVC, can be achieved with minimized NaClO or Fe(VI) dosages, if a preliminary filtration stage is applied. No viable microorganisms were accounted at wastewater-media filtrated with the ≤1.2 μm pore size filters (Table 3.1) and at disinfectant dosages of $CT \geq 270$ mg min $L^{-1}$ or $CT \geq 157$ mg min $L^{-1}$ for NaClO or Fe(VI) respectively. The obtained wastewater-medium with this procedure is considered properly disinfected, since neither colony forming units were detected in cultivation plates nor fungi and since the microscopic examination of the resulting cultures revealed no presence of protozoa or rotifers and nematodes. It is noteworthy that in properly disinfected wastewater-medium with filtration using cellulose filters with pore size 0.45 μm and 0.135 mm thickness, as well as using fiber-glass filters with pore size 1.2 μm and 0.26 mm thickness, limited growth of yeast and small-size ciliates (smaller or even-sized to *Synechococcus elongatus* PCC 7942) was observed after the first week of cultivation. However, as illustrated in Figure 3.3 their growth did not hinder *Synechococcus elongatus* PCC 7942 growth.

In the case of NaClO disinfection, dechlorination of the wastewater-medium with sodium thiosulphate is necessary prior its use to prevent the inhibition of *Synechococcus elongatus* PCC 7942 growth by residual chlorine. Hence, in this study, dechlorination was performed and the absence of residual chlorine in the wastewater-media was confirmed by measuring free chlorine concentration (DPD colorimetric method) after its dechlonination with sodium thiosulphate.

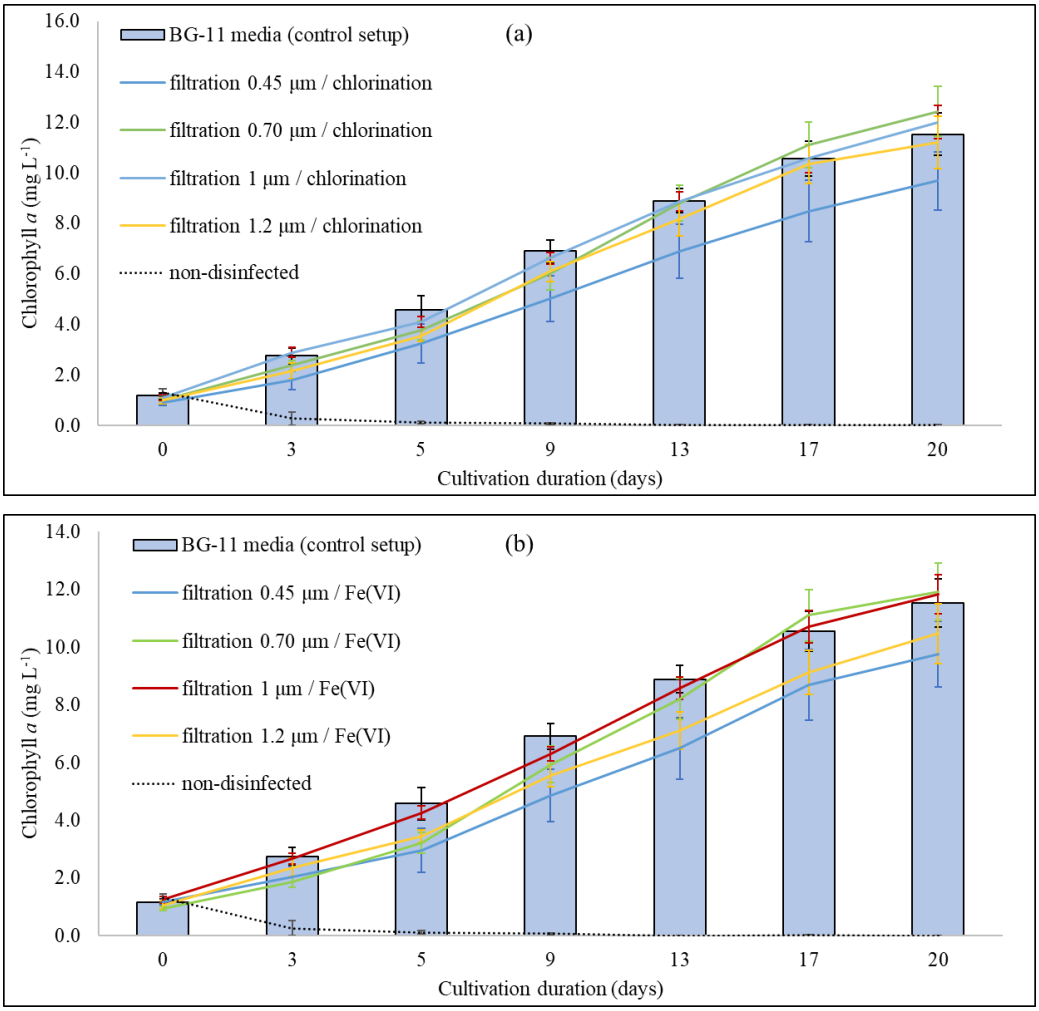


**Figure 3.5.** Growth curves of control setups and test setups in wastewater-media after filtration followed by chlorination (a) or ferrate-based disinfection (b).

The properly disinfected wastewater-media were used for the study of *Synechococcus elongatus* PCC 7942 growth in 8 duplicate test setups. Their growth rate in comparison with a duplicate of control setups and a duplicate setup with non-disinfected wastewater-medium for a 20-days cultivation period is presented in Figure 3.5. After the 20 days of cultivation, the population of *Synechococcus elongatus* PCC 7942 continued increasing in both control and test setups but with a decreasing growth rate. According to Samiotis et al. (2021), in similar cultivation conditions, the drop of growth rate after 20 days can reach up to 75 %, which is attributed to the obstruction of photosynthesis due to increased optical density in the PBR and the subsequent intermittent flux of light as a result of mutual shading. Moreover, the hydraulic residence time (HRT) dictates the volume of the PBR (Samiotis et al., 2022), thus a cultivation period past 20-days would result in large PBR volumes, rendering the full-scale application of the proposed process challenging. As shown in Figure 3.5,

*Synechococcus elongatus* PCC 7942 cultures in test setups present comparable to the control cultures' growth rates. This implies that filtration coupled with disinfection using NaClO or Fe(VI) is effective. The fiber-glass filters with pore size 0.7 μm and 0.45 mm thickness, as well as those with pore size 1 μm and 0.70 mm thickness were proved more effective. It should be noted that with the applied dosages of Fe(VI) solution, neither the quantities of residual Fe(VI) nor of total iron had an impact on *Synechococcus elongatus* PCC 7942 growth rate. There was no residual action of NaClO, due to the applied dechlorination of wastewater-media. Thus, low-cost filtration coupled with NaClO or preferable with Fe(VI) disinfection is an efficient procedure prior to the use of wastewaters as *Synechococcus elongatus* PCC 7942 cultivation substrate and tertiary treatment (Figure 3.6).

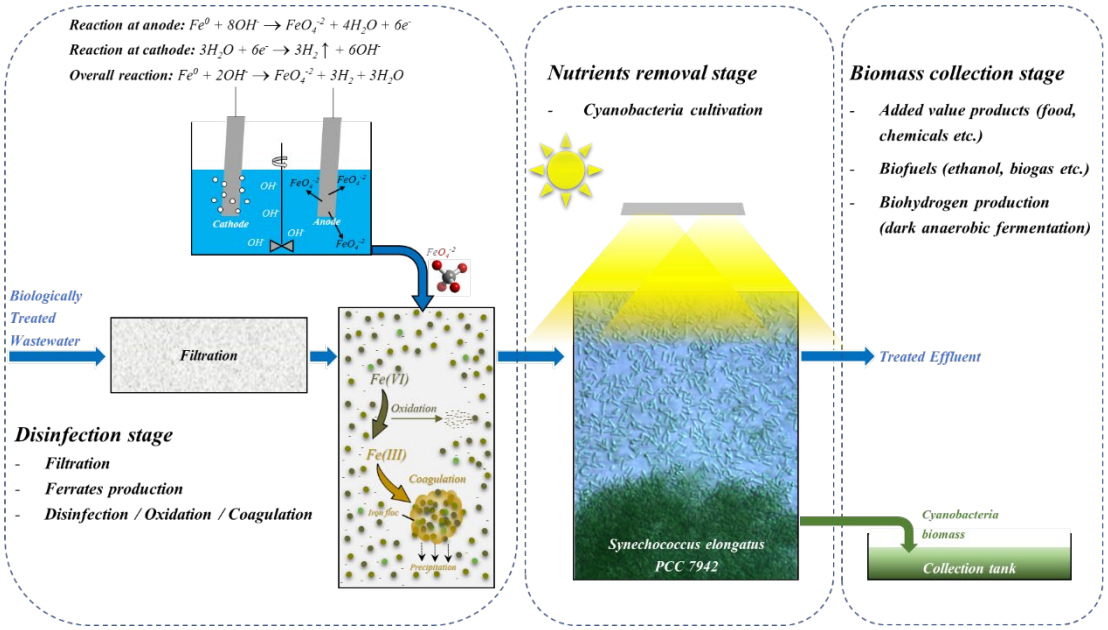


**Figure 3.6.** Proposed wastewater substrate disinfection process for cyanobacteria cultivation and tertiary treatment applications.

### 3.3.5. *Synechococcus elongatus* PCC 7942's growth rate and nutrients removal rate in disinfected wastewater-media

The properly disinfected wastewater-media, presented in Figure 3.3, were used for the study of *Synechococcus elongatus* PCC 7942 growth. Based on equation (1), the % specific growth rate of *Synechococcus elongatus* PCC 7942, as well as the % specific nitrates and phosphates removal rates were evaluated in control and test setups (Figure 3.7). For this purpose, 2 control setups, 4 duplicate test setups with wastewater-media filtrated at ≤1.2 μm pore size and chlorinated/dechlorinated and 4 duplicate test setups

with wastewater-media filtrated at ≤1.2 μm pore size and disinfected with Fe(VI) were monitored for a 20 days period. The average values of % relative *Synechococcus elongatus* PCC 7942 growth rate, as well as the average values of nitrates and phosphates removal rates of each setup group are presented in Figure 3.7. Nutrients removal rates in the test setups were similar or even greater to those obtained from control setups, with significantly higher phosphates removal rate observed when Fe(VI) is used, attributed to the coagulation action of the resulting Fe(III). This is confirmed by the statistical analysis (independent t-test) between relative growth rates and relative nutrients removal rates of control and test setups, which revealed that the mean score is not significantly different between the control and test setups (Sig.>0.05), except for the parameter of $RRR_{PO4\_P}$ between control setups and test setups with ferrate as disinfectant (Sig. = 0.041).

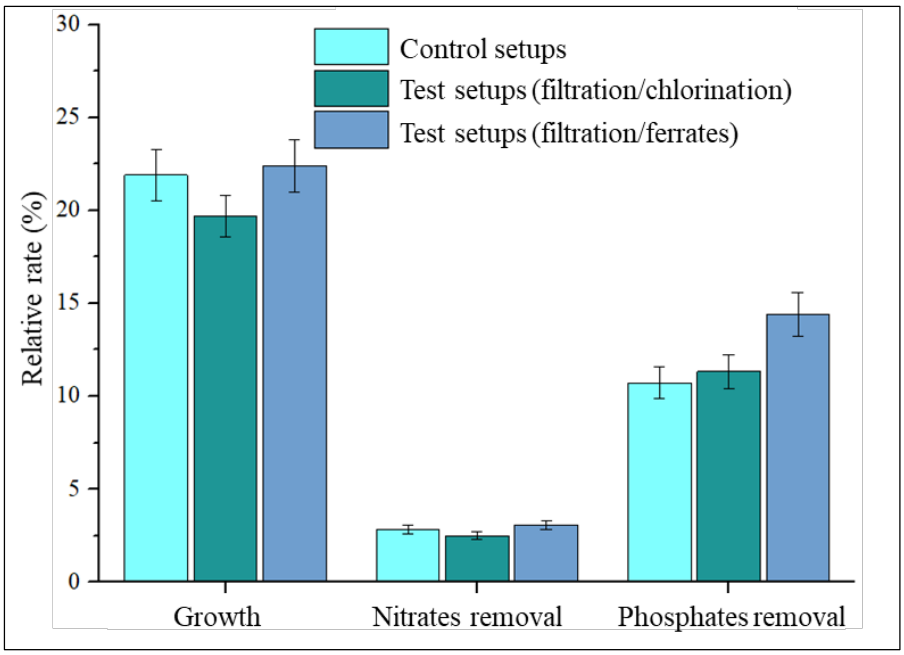


**Figure 3.7.** Average values and standard deviation of % relative growth, nitrates and phosphates removal rates in control and test setups.

According to Figure 3.7, the average relative growth rates ($RGR_{(chl\ a)}$) in the test setups with properly disinfected wastewater-media was 19.7% when filtration/chlorination was applied and 22.4% when filtration/Fe(VI) chemical disinfection was applied. These values indicate unhindered growth of *Synechococcus elongatus* PCC 7942 in wastewater media, since they are similar to the $RGR_{(chl\ a)}$ average value of 21.9% obtained in the control setups with BG-11 medium. The sufficient growth of

*Synechococcus elongatus* PCC 7942 is also evident from the average values of nitrates relative reduction rate ($RRR_{NO3\_N}$) and phosphates relative reduction rate ($RRR_{PO4\_P}$), which ranged from 2.51% to 3.07% and 11.33% to 14.40% respectively, compared to those obtained in the control setups ($RRR_{NO3\_N}$ = 2.84% and $RRR_{PO4\_P}$ = 10.72% respectively). It is evident that $RRR_{NO3\_N}$ of control and test setups is analogous to their respective growth rate, having a relatively constant $RRR_{NO3\_N}$ /$RGR_{(chl\ a)}$ ratio of approximately 0.13. Thus, it can be concluded that nitrates removal is attributed solely to assimilation of nitrate-nitrogen into the *Synechococcus elongatus* PCC 7942 biomass and not on additional physicochemical processes. On the other hand, the $RRR_{PO4\_P}$ /$RGR_{(chl\ a)}$ ratio is significantly different in the control setups and the test setups, which indicate that apart from phosphates assimilation, parallel physicochemical processes occur in the PBR. The $RRR_{PO4\_P}$ /$RGR_{(chl\ a)}$ ratio of the control setups was 0.49, while of the test setups with filtrated/chlorinated wastewater-media or filtrated/chemically disinfected with Fe(VI) wastewater-media, were 0.58 and 0.64 respectively. The higher phosphates removal rates of test setups is attributed to increased coagulation induced by elevated pH levels (da Silva Cerozi and Fitzsimmor 2016), since both NaClO solution and Fe(VI) solution were highly alkaline, as well as to the coagulation action of Fe(III) species resulting from Fe(VI) reduction (Zhang et al., 2020). The initial pH values of control setups was approximately 7.1, while the test setups with filtrated/chlorinated or filtrated/chemically disinfected with Fe(VI) wastewater-media had initial pH values of approximately 7.7 and 8.1 respectively.

The results regarding $RRR_{NO3\_N}$ led to the conclusion that at the imposed cultivation conditions, which are considered non-favorable due to the relatively low applied light intensities (5 – 30 μmol-photons $m^{-2}$ $s^{-1}$), nitrates removal up to 42% can be achieved for a cultivation period of 20 days. At favourable conditions of 150 μmol photons $m^{-2}$ $s^{-1}$, 3.4 times higher cell productivities are obtained (Silva et al., 2014; Samiotis et al., 2021), thus nitrates removal up to 85% can be achieved. The respective phosphates removal is approximately 90% at non-favourable conditions, reaching to complete phosphates removal at favourable conditions. Thus, at typical wastewater nutrient ratios (N:P = 5:1, Curtin et al., 2011), phosphates might become the limiting factor for *Synechococcus elongatus* PCC 7942 growth, a phenomenon that has to be addressed via nutrients addition or/and pH control (Di Termini et al., 2011; Mennaa et al., 2019).

## 3.4. Conclusions

*Synechococcus elongatus* PCC 7942 (*Synechococcus elongatus* PCC 7942) can be cultivated in properly disinfected wastewaters, thus used in tertiary treatment applications. Efficient disinfection of wastewater-media can be achieved by coupling filtration with chemical disinfection using NaClO or the more environmentally friendly Fe(VI). Filtration at ≤1.2 μm pore size and chemical disinfection at $CT \geq 270$ mg min $L^{-1}$ and $CT \geq 157$ mg min $L^{-1}$ for NaClO and Fe(VI) respectively lead to unhindered *Synechococcus elongatus* PCC 7942 growth. Fe(VI) dosages up to $CT \geq 157$ mg min $L^{-1}$ do not hinder *Synechococcus elongatus* PCC 7942 growth and can assist with the removal of turbidity (improve light transfer efficacy), of organic compounds and of phosphorous from wastewater-media due to the coagulation and oxidation action. On-site electrosynthesis of Fe(VI) via a $Fe^0/Fe^0$ cell addresses the problem of provision and storage of the chemically unstable ferrate compounds. For a cultivation period of 20 days and at non-favorable lighting conditions (5-30 μmol-photons $m^{-2}$ $s^{-1}$), nitrates removal is 42%, which could be increased up to 85% at favourable lighting conditions (150 μmol-photons $m^{-2}$ $s^{-1}$). The respective phosphates removal is significantly higher (>92%), attributed to coagulation phenomena induced by elevated pH levels and the coagulation action of Fe(III) species from Fe(VI) reduction. The results of this study indicate that the use of *Synechococcus elongatus* PCC 7942 can play a significant role towards the design of sustainable and carbon-negative tertiary wastewater treatment technologies. An ultrafiltration configuration coupled with chemical disinfection downstream the sedimentation tank of a secondary biological wastewater treatment stage is proposed as a necessary, efficient and low-cost disinfection technique for full-scale scale implementation of *Synechococcus elongatus* PCC 7942-based wastewater treatment processes.

## 3.5. References


1. Addison EL, Gerlach KT, Spellman CD, Santilli G, Fairbrother AR, Shepard Z, Dudle JD, Goodwill JE (2021) Physicochemical implications of cyanobacteria oxidation with Fe(VI). Chemosphere 266:128956. https://doi.org/10.1016/j.chemosphere.2020.128956.
2. Akoglu H (2018) User's guide to correlation coefficients. Turk. J Emerg Med 18:91-93. https://doi.org/10.1016/j.tjem.2018.08.001
3. Al Umairi, AR, How ZT, El-Din MG (2021) Enhanced primary treatment during wet weather flow using ferrate as a coagulant, coagulant aid and disinfectant. J Environ Manage 290:112603. https://doi.org/10.1016/j.jenvman.2021.112603

4. Almomani F, Judd S, Bhosale RR, Shurair M, Aljaml K, Khraisheh M (2019) Intergraded wastewater treatment and carbon bio-fixation from flue gases using Spirulina platensis and mixed algal culture. Process Saf Environ Prot 124:240-250. https://doi.org/10.1016/j.psep.2019.02.009
5. Amanatidou E, Samiotis G, Trikoilidou E, Pekridis G, Tsikritzis L (2016a) Complete Solids Retention Activated Sludge Process. Water Sci Technol 73:1364–1369. https://doi.org/10.2166/wst.2015.614
6. Amanatidou E, Samiotis G, Trikoilidou E, Tsikritzis L (2016b) Particulate organics degradation and sludge minimization in aerobic, complete SRT bioreactors. Water Res 94:288–295. https://doi.org/10.1016/j.watres.2016.02.008
7. Amanatidou E, Trikoilidou E, Samiotis G, Benetis NP, Taousanidis N (2012) An easy uncertainty evaluation of the COD titrimetric analysis in correlation with quality control and validation data. Method applicability region Anal Methods 4:4204–4212. https://doi.org/ 10.1039/c2ay25687h
8. Amanatidou E, Trikoilidou E, Tsikritzis L, Katsiouli F (2011). Uncertainty in spectrophotometric analysis - "Error propagation break up", a novel statistical method for uncertainty management. Talanta 85:2385-2390. https://doi.org/10.1016/j.talanta.2011.07.084
9. APHA (American Public Health Association) 2017 Standards Methods for the Examination of Water and Wastewater, 23rd edn. American Water Works Association and Water Environmental Federation, Washington, DC, USA
10. Arias DM, Uggetti E, García-Galán MJ, García J (2017) Cultivation and selection of cyanobacteria in a closed photobioreactor used for secondary effluent and digestate treatment, Sci Total Environ 587–588:157-167. https://doi.org/10.1016/j.scitotenv.2017.02.097
11. Asadi P, Amini Rad H, Qaderi F (2019) Comparison of Chlorella vulgaris and Chlorella sorokiniana pa.91 in post treatment of dairy wastewater treatment plant effluents. Environ Sci Pollut Res 26:29473-29489. https://doi.org/10.1007/s11356-019-06051-8
12. Choy SY, Prasad KMN, Wu TY, Raghunandan ME, Phang SM, Juan JC, Ramanan RN (2018) Separation of Chlorella biomass from culture medium by flocculation with rice starch. Algal Res 30:162-172. https://doi.org/10.1016/j.algal.2017.11.012
13. Collivignarelli MC, Abbà A, Benigna I, Sorlini S, Torretta V (2018) Overview of the Main Disinfection Processes for Wastewater and Drinking Water Treatment Plants. Sustainability 10:86. https://doi.org/10.3390/su10010086
14. Curtin K, Duerre S, Fitzpatrick B, Meyer P (2011) Biological nutrient removal, Minnesota Pollution Control Agency
15. Da Silva Cerozi B, Fitzsimmons K (2016) The effect of pH on phosphorus availability and speciation in an aquaponics nutrient solution. Bioresour Technol 219:778-781. https://doi.org/10.1016/j.biortech.2016.08.079
16. Day JG, Gong Y, Hu Q (2017) Microzooplanktonic grazers – a potentially devastating threat to the commercial success of microalgal mass culture. Algal Res 27:356–365. https://doi.org/10.1016/j.algal.2017.08.024
17. De Luca SJ, Cantelli M, De Luca MA (1992) Ferrate vs. traditional coagulants in the treatment of combined industrial wastes. Water Sci Technol 26:2077-2080. https://doi.org/10.2166/wst.1992.0665
18. Delaude, L., Laszlo, P., 1996. A novel oxidizing reagent based on potassium ferrate(VI). J. Org. Chem. 61, 6360–6370. https://doi.org/10.1016/j.chemosphere.2005.02.027

19. Di Termini I, Prassone A, Cattaneo C, Rovatti M (2011) On the nitrogen and phosphorus removal in algal photobioreactors. Ecol Eng 37:976-980. https://doi.org/10.1016/j.ecoleng.2011.01.006
20. Drzewicz P, Drobniewska A, Sikorska K, Nałęcz-Jawecki G (2019) Analytical and ecotoxicological studies on degradation of fluoxetine and fluvoxamine by potassium ferrate. Environ Technol 40:3265–3275. https://doi.org/10.1080/09593330.2018.1468488
21. Fahimirad S, Fahimirad Z, Sillanpää M (2021) Efficient removal of water bacteria and viruses using electrospun nanofibers. Sci Total Environ 751:141673. https://doi.org/10.1016/j.scitotenv.2020.141673
22. Flynn KJ (2021) Enhancing Microalgal Production - constructing decision support tools using system dynamics modelling. Zenodo https://doi.org/10.5281/zenodo.5036605
23. Fukuzaki S, Urano H, Yamada S (2007) Effect of pH on the Efficacy of Sodium Hypochlorite Solution as Cleaning and Bactericidal Agents. Journal of The Surface Finishing Society of Japan 58:465. https://doi.org/10.4139/sfj.58.465
24. Gerardi MH (2006) Wastewater Bacteria. John Wiley & Sons, Inc DOI:10.1002/0471979910.
25. Gonaalves AL, Rodrigues CM, Pires JCM, Simaes M (2016) The effect of increasing CO2 concentrations on its capture, biomass production and wastewater bioremediation by microalgae and cyanobacteria. Algal Res 14:127-136. https://doi.org/10.1016/j.algal.2016.01.008
26. Goswami RK, Agrawal K, Mehariya S, Verma P (2021) Current perspective on wastewater treatment using photobioreactor for Tetraselmis sp.: an emerging and foreseeable sustainable approach. Environ Sci Pollut Res https://doi.org/10.1007/s11356-021-16860-5
27. He H-Y, Qiu W, Liu Y-L, Yu H-R, Wang L, Ma J (2021) Effect of ferrate pre-oxidation on algae-laden water ultrafiltration: Attenuating membrane fouling and decreasing formation potential of disinfection byproducts. Water Res 190:116690. https://doi.org/10.1016/j.watres.2020.116690
28. Henry-Chase A, TewariBB (2013) Use to Ferrate (VI) a green chemical for the environmental remediation. Revista Boliviana de Quimica 30:13-23.
29. Jiang JQ, Lloyd B (2002) Progress in the development and use of ferrate(VI) salt as an oxidant and coagulant for water and wastewater treatment. Water Res 36:1397–1408. https://doi.org/10.1016/S0043-1354(01)00358-X
30. Kokarovtseva IG, Belyaev IN, Semenyakova LV (1972) Oxygen compounds of iron (VI), (V) and (IV). Russ Chem Rev 41:929–937. http://dx.doi.org/10.1070/RC1972v041n11ABEH002104
31. Li C, Li XZ, Graham N (2005) A study of the preparation and reactivity of potassium ferrate. Chemosphere 61:537–543.
32. Mácová Z, Bouzek K, Hives J, Sharma V K, Terryn R J, Baum J C (2009). Research progress in the electrochemical synthesis of ferrate (VI). Electrochim Acta 54:2673–2683. https://doi.org/10.1016/j.electacta.2008.11.034
33. Maurya PK, Mondal S, Kumar V, Singh SP (2021) Roadmap to sustainable carbon-neutral energy and environment: can we cross the barrier of biomass productivity? Environ Sci Pollut Res 28:49327–49342. https://doi.org/10.1007/s11356-021-15540-8
34. Medrano-Barboza J, Aguirre-Bravo AA, Encalada-Rosales P, Yerovi R, Ramírez-Iglesias JR (2021) Slaughtering and piggery wastewater for cultivation and biomass generation of Chlorella vulgaris. Revista Bionatura 6:1824-1831
35. Melo JM, Ribeiro MR, Telles TS, Amaral HF, Andrande DS (2022) Microalgae cultivation in wastewater from agricultural industries to benefit next generation of

bioremediation: a bibliometric analysis. Environ Sci Pollut Res https://doi.org/10.1007/s11356-021-17427-0
36. Mennaa FZ, Arbib Z, Perales JA (2019) Urban wastewater photobiotreatment with microalgae in a continuously operated photobioreactor: growth, nutrient removal kinetics and biomass coagulation–flocculation. Environ Technol 40:342-355. https://doi.org/10.1080/09593330.2017.1393011
37. Moran P (1982) Formulae for determination of chlorophyllous pigments extracted with N,N-dimethyl-formamide. Plant Physiol 69:1376-1381. https://doi.org/10.1104/pp.69.6.1376
38. Pathak J, Rajneesh, Maurya PK, Singh SP, Häder DP, Sinha1 RP (2018) Cyanobacterial Farming for Environment Friendly Sustainable Agriculture Practices: Innovations and Perspectives. Front Environ Sci. 6:7. https://doi.org/10.3389/fenvs.2018.00007
39. Rana MS, Prajapati SK (2021) Resolving the dilemma of iron bioavailability to microalgae for commercial sustenance. Algal Res 59:102458. https://doi.org/10.1016/j.algal.2021.102458
40. Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RT (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J Gen Microbiol 111:1–61
41. Rosegrant MW, Msangi S, Sulser T, Santos RV (2006) Biofuels and the global food balance. Washington DC:International Food Policy Research Institute
42. Sakai S, Yoshida H, Hirai Y, Asari M, Takigami H, Takahashi S, Tomoda K, Peeler MP, Wejchert J, Unterseh TS, Douvan AR, Hathaway R, Hylander LD, Fischer C, Oh GJ, Jinhui L, Chi NK (2011) International comparative study of 3R and waste management policy developments. J Mater Cycles Waste Manag 13:86-102
43. Samiotis G, Stamatakis K, Amanatidou E (2021) Assessment of Synechococcus elongatus PCC 7942 as an option for sustainable wastewater treatment. Water Sci Technol 84:1438. https://doi.org/ 10.2166/wst.2021.319
44. Samiotis G, Stamatakis K, Amanatidou E (2022) Dimensioning of Synechococcus elongatus PCC 7942 cultivation photobioreactor for valorization of wastewater resources. Chem Eng J 435:134895. https://doi.org/10.1016/j.cej.2022.134895
45. Schreyer JM, Thompson GW, Ockerman LT (1950) Oxidation of Chromium(lll) with Potassium Ferrate(VI). Anal Chem 22:1426-1427
46. Sharma VK, Feng M, Dionysiou DD, Zhou H-C, Jinadatha C, Manoli K, Smith MF, Luque R, Ma X, Huang C-H (2022) Reactive High-Valent Iron Intermediates in Enhancing Treatment of Water by Ferrate. Environ Sci Technol 56:30-47. https://doi.org/10.1021/acs.est.1c0461
47. Sharma VK, Zboril R, Varma RS (2015) Ferrates: Greener Oxidants with Multimodal Action in Water Treatment Technologies. Acc Chem Res 48:182−191. https://doi.org/ 10.1021/ar5004219
48. Shoener B, Schramm SM, Beline F, Bernard O, Martínez C, Plosz BG, Snowling S, Steyer JP, Perez BV, Wagner D, Guest JS (2019) Microalgae and cyanobacteria modelling in water resource recovery facilities: A critical review. Water Res X 2:100024. https://doi.org/10.1016/j.wroa.2018.100024
49. Silva CSP, Silva-Stenico ME, Fiore MF, De Castro HF, Da Rós PCM (2014) Optimization of the cultivation conditions for Synechococcus sp. PCC7942 (cyanobacterium) to be used as feedstock for biodiesel production. Algal Res 3:1-7. https://doi.org/10.1016/j.algal.2013.11.012
50. Škulcová AB, Tamášová K, Staňová AV, Bírošová L, Krahulcová M, Gál M, Konečná B, Janíková M, Celec P, Grabicová K, Grabic R, Filip J, Belišová N, Ryba J, Kerekeš K, Špalková V, Híveš J, Mackuľak T (2021) Effervescent ferrate(VI)-based tablets as

an effective means for removal SARS-CoV-2 RNA, pharmaceuticals and resistant bacteria from wastewater. J Water Process Eng 43:102223. https://doi.org/10.1016/j.jwpe.2021.102223
51. Sorensen E, Capo E, Farnelid H, Lindehoff E, Legrand C (2021) Temperature Stress Induces Shift From Co-Existence to Competition for Organic Carbon in Microalgae-Bacterial Photobioreactor Community – Enabling Continuous Production of Microalgal Biomass. Front Microbiol 12. https://doi.org/10.3389/fmicb.2021.607601
52. Sulistyo H, Sediawan WB, Sarto S, Yusuf Y, Nainggolan R (2012) Water Treatment by Coagulation-Flocculation Using Ferric Sulphate as Coagulant. Asean journal of chemical engineering 12:42–50. https://doi.org/10.22146/ajche.49754
53. Tchobanoglous G, Burton FL, Stensel HD (2003) Wastewater Engineering: Treatment and reuse, Metcalf & Eddy Inc. McGraw Hill Book Co, New York
54. Tejido-Nuñez Y, Aymericha E, Sancho L, Refardt D (2019) Treatment of aquaculture effluent with Chlorella vulgaris and Tetradesmus obliquus: The effect of pretreatment on microalgae growth and nutrient removal efficiency. Ecol Eng 136:1–9. https://doi.org/10.1016/j.ecoleng.2019.05.021
55. Thomas M, Drzewicz P, Więckol-Ryk A, Panneerselvam B (2022) Effectiveness of potassium ferrate (VI) as a green agent in the treatment and disinfection of carwash wastewater. Environ Sci Pollut Res 29:8514–8524. https://doi.org/10.1007/s11356-021-16278-z
56. Torres CRG, Crastechini E, Feitosa FA, Pucci CR, Borges AB (2014) Influence of pH on the effectiveness of hydrogen peroxide whitening. Oper Dent 39:261-268. doi: 10.2341/13-214-L
57. Trikilidou E, Samiotis G, Tsikritzis L, Amanatidou E (2020) Performance of Semi-Micro-Kjeldahl Nitrogen Method – uncertainty and nitrate interference. Int J Environ Anal Chem 1–11. https://doi.org/10.1080/03067319.2020.1807967
58. Trivedi J, Aila M, Bangwal DP, Kaul S, Garg MO (2015) Algae based biorefinery-How to make sense? Renew Sust Energ Rev 47:295–307. https://doi.org/10.1016/j.rser.2015.03.052
59. Van der Hoek JP, De Fooij H, Struker A (2016) Wastewater as a resource: strategies to recover resources from Amsterdam's wastewater. Resour Conserv Recyl 113:53–64. https://doi.org/10.1016/j.resconrec.2016.05.012
60. Vassilev SV, Vassileva CG (2016) Composition, properties and challenges of algae biomass for biofuel application: An overview Fuel 181:1-33. https://doi.org/10.1016/j.fuel.2016.04.106
61. Vayenos D, Romanos EmG, Papageorgiou GC, Stamatakis K (2020) The freshwater cyanobacterium Synechococcus sp. PCC7942 under salt stress: A cell factory for sucrose and hydrogen production. Photosynth Res 146:235-245. https://doi.org/10.1007/s11120-020-00747-6
62. Virender K, Sharma (2002) Potassium ferrate(VI): an environmentally friendly oxidant. Advances in Environmental Research 6:143-156. https://doi.org/10.1016/S1093-0191(01)00119-8
63. Wang S, Shao B, Qiao J, Guan X (2020) Application of Fe(VI) in abating contaminants in water: State of art and knowledge gaps. Front Env Sci Eng 15:80. https://doi.org/10.1007/s11783-020-1373-3
64. Wood RH (1958) The heat, free energy and entropy of the ferrate(VI) ion. J Am Chem Soc 80:2038–2040. https://doi.org/10.1021/ja01542a002
65. Zhang H, Zheng L, Li Z, Pi K, Deng Y (2020) One-step Ferrate(VI) treatment as a core process for alternative drinking water treatment. Chemosphere 242:125134. https://doi.org/10.1016/j.chemosphere.2019.125134

# 4. Chapter 4. Assessment of *Synechococcus elongatus* PCC 7942 as an option for sustainable wastewater treatment

Industrial wastewaters are recognized as a valuable resource, however their disposal without proper treatment can result in environmental deterioration. The associated environmental/operational cost of wastewater treatment necessitates upgrade of applied processes towards the goals of sustainability and mitigation of climate change. The implementation of cyanobacteria-based processes can contribute to these goals via resources recovery, production of high-value products, carbon fixation and green-energy production. The present study evaluates the cyanobacterium *Synechococcus elongatus* PCC 7942 (*S*7942) as a biological component for novel and sustainable alternatives to typical biological nutrient removal processes. Valuable results regarding cultivation temperature boundaries, applied disinfection techniques and analytical methods, as well as regarding relations between parameters expressing *S*7942 biomass concentration are presented. The results show that at typical industrial wastewater temperatures, *S*7942 efficiently grew and removed nitrates from treated snack-industry's wastewater. Moreover, in cultures with treated and relatively saline dairy wastewater, its growth rate slightly decreased, but nevertheless nitrates removal rate remained efficiently high. A comparison between typical denitrification processes and the proposed nutrient removal process indicated that a *S*7942-based system may constitute an alternative or a supplementary to denitrification process. Thus, *Synechococcus elongatus* PCC 7942 proved to be a potent candidate towards sustainable industrial wastewater treatment applications.

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## 4.1. Introduction

Protection of the environment and climate change mitigation is a significant challenge humanity faces through the 21st century. Responding to this challenge by implementing sustainable technologies, practices and techniques for environmental protection, resources recovery and GHGs emission mitigation, should be the "Ithaca" of engineers and researchers.

Although many infrastructure sectors, such as energy, industry, transportation and building systems, have been extensively studied with regards to GHGs emission reduction and sustainability, studies regarding the sector of wastewater treatment have been sparse (Lu et al., 2018), despite the fact that wastewaters are recognized as a valuable resource and their treatment is associated with significant environmental and operational/energy cost. Wastewaters are considered a valuable resource as they contain water, organic matter, nutrients (nitrogen and phosphorous), mineral nutrients and chemical energy (Hoek et al., 2016), but if not properly treated they can cause environmental deterioration and produce potent greenhouse gases (GHGs).

The sector of wastewater treatment offers significant opportunities for resources and energy recovery/production, as well as for GHGs emissions mitigation, since annually more than 360 $km^3$ of wastewater is generated on a global scale (Sato et al., 2013). The estimated potential energy gain from wastewater utilization is over 805 billion kWh as equivalent of electricity per year, i.e. more than 8.53% of global renewable energy (Zhang and Ma, 2020). However, substantial improvements in wastewater treatment plants (WWTPs) will be necessary, as it is expected that more advanced and energy intensive treatment will be required to adhere to future demands and quality standards and to adapt to climate change (Frijns et al., 2013). WWTPs have been recognized as one of the largest emitters of GHGs, since it is estimated that only the degradation of organics during wastewater treatment contributes to approximately 1.57% of global $CO_2$ equivalent emissions and up to 5.2% of the global total non-$CO_2$ GHG emissions (mainly $CH_4$ and $N_2O$) (USEPA, 2012; IPCC, 2014). Furthermore, wastewater treatment accounts for 3% of global electricity consumption, a proportion that is expected to rise in the next decades due to the increasing number of WWTPs constructed worldwide (Li et al., 2015).

Great progress has been made to increase energy efficiency of WWTPs and recover renewable energy from wastewater using technologies such as anaerobic digestion, anaerobic membrane bioreactors and bio-electrochemical systems (Heidrich et al., 2010). In spite of it, these approaches reduce only fossil fuel consumption and its associated carbon emissions, whereas few of them have been investigated for the additional possibility of active and direct $CO_2$ capture and utilization (Lu et al., 2018). Furthermore, these technologies present limitations regarding nutrients removal, thus failing to achieve environmental standards regarding effluent quality if not combined with further tertiary treatment.

On the other hand, phototrophic wastewater treatment technologies, which are based on green-algae or/and blue-green algae (cyanobacteria), could address the limitations of anaerobic treatment, while increasing the energetic potential of wastewater resources by up to three times through leveraging nutrients for biomass growth and organic carbon storage (Shoener et al., 2014). Nevertheless, it should be noted that artificial lighting of phototrophic reactors constitutes a significant challenge, in terms of technical application and operational cost, which needs to be addressed. Life cycle analysis suggests that algae cultivation would be highly economically viable when linked to wastewater treatment (Yang, et al., 2011). Therefore, the application of phototrophic processes can elevate sustainability of WWTPs through resources and energy recovery/production, as well as $CO_2$ fixation.

One of the most promising organisms for such applications is cyanobacteria, as they can efficiently utilize nutrients from wastewater and fixate carbon converting them to industrially relevant compounds and fuels (Zhang et al., 2017). Nutrient-rich environment of wastewaters, especially of high-strength industrial wastewaters, can provide the media for large-scale cultivation of cyanobacteria in WWTPs. The treatment tanks of WWTPs may be an ideal place for cultivation of cyanobacteria, because they can provide sufficient nutrients, good operating temperatures and significant light exposure (Martins et al., 2011). It has been suggested that process engineering may enable the complete use of nutrients present in wastewaters for carbon recovery and capture of exogenous $CO_2$ (Valverde-Perez et al., 2015; Gardner-Dale et al., 2017; Lu et al., 2018). The resulting biomass from $CO_2$ mitigation and nutrient utilization is considered of high-value due to the fact that it can be used for the production of biofuels (biodiesel, bioethanol, biogas, biohydrogen), as well as

hydrocarbons, proteins, pigments and biopolymers for pharmaceutical, chemical and food industry (Trivedi et al., 2015). Direct photosynthetic production of sucrose by cyanobacteria is also considered a potential strategy to provide abundant sugar feedstock for biorefineries (Song et al., 2016). Thus, cyanobacteria-based wastewater treatment may not only offer a response to the challenge of nutrients removal and resources recovery from WWTPs, but can also assist reaching the target of climate change inversion via carbon neutral or negative carbon technologies.

Recent research results (Vayenos et al., 2020) have shown that some monocyte cyanobacteria, such as the freshwater cyanobacterium *Synechococcus elongatus* PCC 7942 (hereafter *S*7942), can follow the fermentative hydrogen production pathway, i.e. the metabolic production of biohydrogen from their sucrose. Worth mentioning is that the *S*7942 strain does not produce cyanotoxins and can be manipulated to increase biohydrogen production, since under certain stressful conditions, such as of those imposed by salinities up to 0.4 M NaCl, *S*7942 increases its sucrose levels (Stamatakis et al., 1999; Vayenos et al., 2020). Of the numerous stressors that can trigger biopolymer (lipid and carbohydrate) storage in cyanobacteria cells, such as intense light and salinity stress (Barry et al., 2016), saline conditions are often encountered in industrial wastewater streams. Thus, *S*7942 may prove to be a great candidate towards more sustainable industrial wastewater treatment applications, as its cultivation in relatively saline conditions offers the opportunity of yielding cyanobacterial biomass of increased value.

However, full-scale implementation of algae-based wastewater treatment technologies is challenging, since it depends on the ability to reliably and accurately simulate full-scale performance in response to reactor and process design, influent composition, and environmental conditions and operating parameters (Shoener et al., 2019). That is to say, there is a big gap regarding the design and operation of algae-based wastewater treatment systems. Such is the lack of fundamental design data and operational parameters that are necessary towards their full-scale implementation.

The present study evaluates *S*7942 regarding its potential use for industrial wastewater treatment as the biological component for novel and sustainable alternatives to typical biological nutrient removal processes (nitrification/denitrification, anaerobic phosphorous removal) or for novel supplementary treatment stages that can elevate the

sustainability of WWTPs. The effect of wastewater, e.g. the impact of salinity or cultivation temperature, on *S*7942 growth and nitrates removal rate was examined. Emphasis has been given on the identification of the appropriate parameters expressing *S*7942 biomass concentration and on the extraction of relevant conversion factors and specific rates, as important for the process-design and the assessment of results between different studies. Furthermore, the drawbacks of the applied analytical and disinfection methods are presented and discussed in order to avoid the misinterpretation of results regarding growth and nitrates removal rates, as well as to prevent the growth of antagonistic or/and predating species in a photobioreactor. Finally, a comparison between the typical nitrates removal processes (post and pre denitrification AS processes) and the proposed *S*7942-based nutrient removal process is conducted in terms of reactor volume requirements.

## 4.2. Materials and Methods

### 4.2.1. Microbial strain, growth media and culture conditions

The autotroph, phototrophic microbial strain used in this study was the single-celled, freshwater cyanobacterium *S*7942, which was obtained from the pure cultures of the Institute of Biosciences and Applications, NCSR "Demokritos". The obtained cultures were transferred in sterilized borosilicate glass vessels and thereafter used in pure cultures (backup and control cultures in standard growth media), as well as in experimental culture setups (treated wastewater media) after centrifugation at 5000 rpm and single rinsing with deionized water. In order to evaluate the adequacy of *S*7942 for industrial wastewater treatment, cultures of *S*7942 were setup in Erlenmeyer flasks containing (a) standard blue-green eleven growth media (BG-11) and (b) treated industrial wastewaters. The initial cell concentration in the setups, expressed in terms of chlorophyll a (Chl *a*) concentration, ranged from 0.81 mg $L^{-1}$ to 1.73 mg $L^{-1}$. The duration of *S*7942 cultivation was up to 20 days and all cultures were kept under continuous (24h/d) agitation/artificial lighting and exposed to ambient room light. Light intensities in the cultures ranged from 5 to 30 μmol $m^{-2}$ $s^{-1}$. Cultivation temperature was controlled and set to values between 16ºC and 37ºC.

Regarding the cultures cultivated in wastewater, two types of industrial wastewater that had been subjected to nitrification in an aerated nitrification bioreactor (thereafter biologically treated wastewaters, BTWW) were used. A BTWW from an AS WWTP

of a snack industry and a relatively saline (approximately 0.4 M NaCl) BTWW from an AS WWTP of a dairy industry. The physicochemical characteristics of both BTWW, as well as of the raw influent of each AS WWTP are presented in Table 4.1. It is evident that organic compounds and ammonia are efficiently oxidised in the aeration tanks resulting to BTWW with high nitrate and phosphate content, a form of nutrients that can be readily utilized by cyanobacteria.

**Table 4.1.** Physicochemical characteristics of wastewater growth media.

| Parameter | Wastewater from dairy industry | | Wastewater from snack industry | |
|---|---|---|---|---|
| | Raw | Biologically treated | Raw | Biologically treated |
| pH | 6.8 | 7.2 | 7.3 | 7.4 |
| Chemical Oxygen Demand (COD), mg $L^{-1}$ | 3540 | 109 | 4295 | 46 |
| 5-day Biochemical oxygen demand ($BOD_5$), mg $L^{-1}$ | 1718 | 22 | 2275 | 9 |
| Total Kjeldahl Nitrogen (TKN), mg $L^{-1}$ | 77.3 | 3.5 | 107 | 2.0 |
| Nitrate Nitrogen, mg $L^{-1}$ | 0.371 | 57.3 | 7.3 | 84.11 |
| Ammonia Nitrogen, mg $L^{-1}$ | 7.1 | 3.4 | 12.7 | 1.1 |
| Total (dissolved) Phosphorous, mg $L^{-1}$ | 40.91 | 8.3 | 17.2 | 1.3 |
| Electric Conductivity, μS/cm at 25ºC | 13740 | 12580 | 1814 | 1717 |

A wastewater media may inhibit or reduce *S*7942 growth rate due to various reasons, such as the inhibition of photosynthesis due to absorption of photosynthetically active radiation (PAR) by wastewater, growth of antagonistic or predating microbial species and stressful environmental conditions (pH, salinity, toxic substances etc.). The *S*7942 growth rate response to saline wastewater is of particular interest, since it can result in higher sucrose production, thus higher potential for production of bioenergy and bioproducts, with simultaneous nutrients removal/recovery and carbon fixation.

In this study, the two types of wastewaters that were used as the media for *S*7942 growth (snack and dairy industry BTWW) were assessed regarding potential inhibition of PAR transfer via photometric analysis (scanning) at wavelengths between 400nm to 700nm (photosynthetically active spectral range). The light absorption spectrums of the BTWW were measured and compared to that of *S*7942 cultures in various Chl *a* concentration (cultivation duration up to 20 days). It was evident that the light absorption spectrum of *S*7942 has absorption peaks consistent to the absorption maxima of *S*7942's photosynthetic pigments, i.e. chlorophyll *a* at around 440nm and 680nm (Cinque et al., 2000), allophycocyanin at around 650nm and 620nm (Murakami et al., 1981) and phycocyanin at around 631nm (Espinosa et al., 2007). On the other hand, the light absorption spectrums of the BTWW presented insignificant absorbance in corresponding spectrum regions. Consequently, there is negligible obstruction in the passage of photosynthetically active light wavelengths inside the wastewater photobioreactors. This is attributed to the nature of the BTWW, which were almost transparent and colorless due to the fact that (a) most of their organic compounds that may absorb light in visible spectrum have been oxidized by AS bacteria and (b) photosynthetic microorganisms are negligible in typical AS wastewater treatment processes.

Thereafter, a series of experimental setups for *S*7942 cultivation in wastewater was conducted using (i) unfiltered BTWW, (ii) filtered BTWW, (iii) chlorinated and dechlorinated BTWW and (iv) filtered & chlorinated/dechlorinated BTWW. Fibber-glass filters of 1.2 pore-size and cellulose filters of 0.45 μm pore-size were used for filtration. Chlorination was performed with sodium hypochlorite until free chlorine concentration reached values of approximately 4 mg $L^{-1}$, maintained for 60 minutes, and dechlorination obtained with sodium thiosulphate to concentrations below the detection limit of (0.01 mg $L^{-1}$) of the applied analytical method (APHA 4500-Cl- G).

### 4.2.2. Monitoring parameters and analytical methods

*Culture growth:* The growth rate of *S*7942 is of great importance as it determines nitrates removal rate, thus the volume of a photobioreactor for nutrients removal/recovery. It also sets the level of economic and ecological benefit via exploitation of *S*7942 biomass. Therefore, evaluating the impact of operating parameters on growth rate is the first goal towards utilization of *S*7942 for wastewater treatment applications.

In this study, the growth rate of *S*7942 was evaluated in terms of (i) chlorophyll *a* (Chl *a*) concentration (duplicate samples), (ii) optical density at 750 nm ($O.D._{750nm}$, single determination) and (iii) total and volatile suspended solids concentration (TSS, VSS, duplicate samples). Chlorophyll *a*, which is the major photosynthetic pigment of *S*7942 strain along with the phycobiliproteins allophycocyanin and phycocyanin (Collier and Grossman, 1992), was determined in N,N-dimethylformamide (DMF) extracts of cell pellets according to Moran (1982), while TSS and VSS were obtained using the standard gravimetric method (APHA 2540-Solids B, E). The relative growth rate of *S*7942 cultures, in terms of Chl *a* concentration ($RGR_{Chl\ a}$), was calculated by Equation (1) (Vayenos et al., 2020).

$$RGR_{chl\ a} = [(\frac{[Chl\ a](n\ d)}{[Chl\ a](0\ d)})^{(1/n)} - 1] \times 100$$

Equation (1)

Where, *n* stands for the days of culture growth; *[*Chl *a](n d)* is the Chl *a* concentration after $n^{th}$ day; *[Chl a](0 d)* is the initial (day 0) Chl *a* concentration.

Equation (1) is considered a universal formula for calculating relative (%) evolution of a parameter, whether it expresses biomass (TSS, VSS, Chl a, O.D.750nm, etc.) or nutrients (nitrogen, phosphorous, carbonates etc.) concentration. Thus, equation (1) can be also used for expressing relative growth rate in terms of biomass concentration or/ optical density, by replacement of Chl *a* concentration values with the corresponding values of TSS or VSS concentrations or optical densities.

In this study, growth rate, cell doubling time and cell (biomass) productivity was accounted on the basis of Chl *a* concentration, since Chl *a* concentration is considered as one of the most reliable parameters for expressing photosynthetic species' growth. Nevertheless, the parameters of TSS, VSS and O.D.750nm were also measured in order to obtain correlation coefficients (conversion factors) between all these parameters that can express biomass concentration (Figure 4.1). This is considered of great importance in the assessment, interpretation and implementation of research results by scholars and engineers, whichmay express growth rates or biomass concentration in different format.

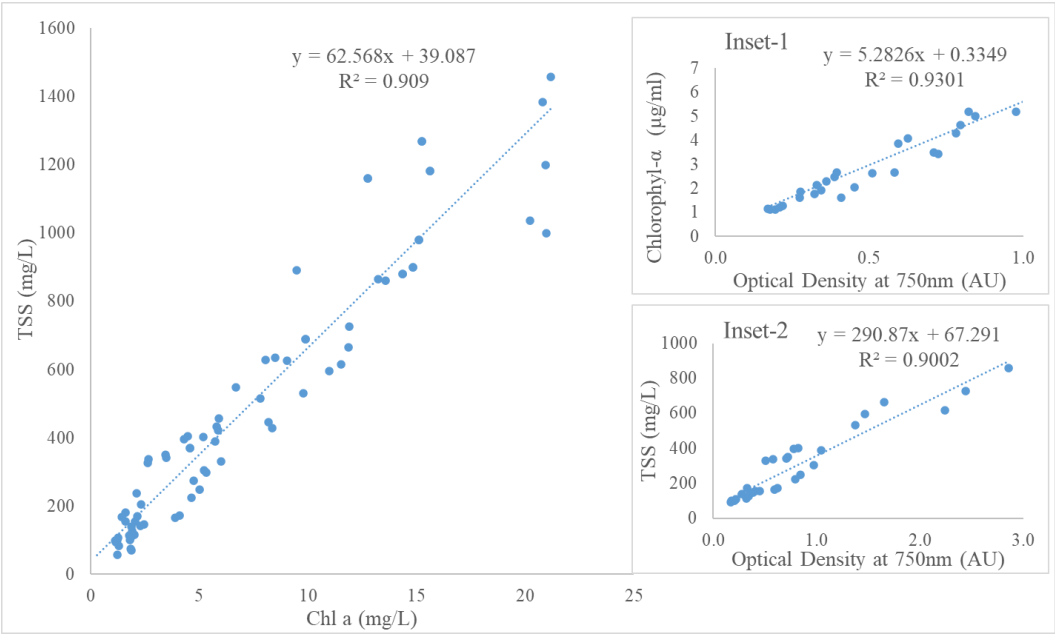


**Figure 4.1.** Chl *a* concentration (mg $L^{-1}$) versus TSS concentration (mg $L^{-1}$) in twelve (12) uncontaminated *S*7942 cultures in BG-11 media (79 data points; up to 20 days cultivation duration); Inset-1: Chl *a* concentration (mg/L) versus $O.D._{750nm}$ (AU) (33 data points); Inset-2: TSS concentration (mg/L) versus $O.D._{750nm}$ (AU) (33 data points).

The relationship between Chl *a* and TSS concentration (Figure 4.1) is evident through the linear correlation between these two parameters (R2 = 0.91), with slope of 62.57 mgTSS mgChl $a^{-1}$. Similar slope value was reported from Dechatiwongse et al. (2014) regarding the relationship between dry cell concentration of cyanobacterium *Cyanothece* ATCC 51142 and optical density at 750 nm. Worth mentioning is that a linear correlation >0.9 was observed during the overall 20 days monitoring period in all uncontaminated culture setups.

The correlations of Chl *a* or TSS concentration with $O.D._{750nm}$, which is another expression of biomass concentration, was considered important to be additionally established. Thus, a series of measurements was also conducted for obtaining the relevant conversion factors regarding optical density at 750 nm (Figure 4.1: inset 1 and inset 2 respectively). There is an evident linear correlation between Chl *a* concentration and $O.D._{750nm}$ (R2 = 0.93), as well as between TSS concentration and $O.D._{750nm}$ (R2 = 0.90), with resulting slopes of 5.28 mgChl *a* $AU^{-1}$ and 290.87 mgTSS $AU^{-1}$ respectively. Similar slope value was obtained in Kuan's (2015) study, regarding the relationship between *S*7942 dry cell concentration (total solids at 90ºC) and optical density at 600nm.

The above obtained conversion factors of 62.57 mgTSS mgChl $a^{-1}$, 5.28 mgTSS mgChl $a^{-1}$ and 290.87 mgTSS $AU^{-1}$ are considered indicative for uncontaminated *S*7942 cultures. Furthermore, regarding the relationship between VSS concentration and TSS concentration, the obtained VSS/TSS ratios in uncontaminated *S*7942 cultures ranged from 0.75 to 0.98 with mean value of 0.89, median value of 0.90 and standard deviation of 0.06. Therefore, a VSS/TSS ratio of 0.90 is considered representative for converting VSS measurements to TSS and vice versa.

***Nitrates removal:*** In this study, nitrates removal is expressed in terms of relative (%) nitrates removal rate ($RRR_{NO3\_N}$) and was calculated according to Equation (2), which is obtained by exchanging Chl *a* concentration by nitrate-nitrogen concentration in Equation (1) and converting the results to positive values.

$$RRR_{NO3\_N} = -[\left(\frac{[NO_{3_N}](n\ d)}{[NO_{3_N}](0\ d)}\right)^{(1/n)} - 1] \times 100 \qquad \text{Equation (2)}$$

Replicate samples were used in order to accurately evaluate nitrates removal rate by *S*7942, while two photometric and one potentiometric standard analytical methods (APHA, 2017) for nitrates determination were deployed and their performance was evaluated. The three analytical methods that were deployed for nitrates determination in *S*7942 cultures were (i) potentiometric determination of nitrates with ion selective electrode (APHA 4500-NO3- D), (ii) photometric determination of nitrates at ultraviolet (UV) spectrum (APHA 4500-NO3- B) and (iii) photometric determination of nitrates after their reduction to nitrites with cadmium. High performance liquid chromatography was not considered suitable for samples that may contain organic residues that could damage separation column, thus not evaluated.

The evaluation of the potentiometric nitrates' determination method revealed its inadequacy for monitoring nitrates concentration in algae culturesdue to various interferences. Specifically, the ion selective electrode gave false and non-replicable readings, attributed to the presence of various interfering ions in the *S*7942 culture samples, such as bicarbonates, chloride, chlorate, perchlorate and nitrite. The UV photometric analytical method exhibited interferences only in cases of relatively high organics content in the sample (more than 10% absorption at 275 nm compared to that at 220 nm) or/and in cases of persistent colour in the filtrated sample. Concequently, in

cases of low organic content in the culture, the relatively fast and easy to apply APHA 4500-NO3-B method is proposed for regular monitoring.

In cases of relatively high organic content or/and persistent colour in the culture filtrates, the cadmium reduction method (APHA 4500-NO3-E) is proposed. This method exhibited acceptable results and the only interfering agent is the presence of suspended solids (removed after filtration) and the high concentrations (above several milligrams per liter) of metallic ions (APHA, 2017).

*Microscopic observation of cultures:* In order to evaluate culture contamination levels by antagonistic to cyanobacteria microbial species and/or by predating microorganisms, the microscopic characteristics of cultures were regularly monitored with a digital phase contrast microscope (Leica Model DM1000). The identification of competitive to *S*7942 microbial species was made based on their morphological characteristics and on relevant databases (Oyadomari, 2001; APHA, 2017).

*Other analyses:* All culture setups were monitored regarding their pH, electric conductivity and temperature, as well as the dissolved total organic and total inorganic carbon (DOC and DIC) concentration according to standard methods (APHA 4500-H+ B; APHA 2510-Conductivity B; APHA 2550-Temperature B; APHA 5310-TOC B). Wastewater composition (Table 4.1) was also determined by standard methods (APHA 5220-COD C; APHA 5210-BOD D; APHA-Norg C; APHA-NH3 C; APHA 4500-P E).

All analyses were conducted at the accredited according to ISO 17025 "Environmental Chemistry & Water and Wastewater Treatment Laboratory", Department of Chemical Engineering, University of Western Macedonia, Greece. The laboratory calculates measurement uncertainties in all the applied methods (Amanatidou et al., 2012, Trikilidou et al., 2020).

## 4.3. Results and Discussion

### 4.3.1. Growth of S7942

#### 4.3.1.1. Effect of temperature on S7942 growth

In order to evaluate the temperature boundaries for *S*7942 growth, thus determine if temperature adjustment would be required at full-scale applications, duplicate culture setups with BG-11 growth media and at different controlled temperatures of

approximately 16-18ºC, 20-22ºC, 25-27ºC, 30-32ºC and 35-37ºC were deployed. *S*7942 doubling time, in terms of Chl *a* concentration, was calculated for a cultivation period of twenty days ($n$ = 20 days) and presented in Figure 4.2. Worth mentioning is that a relatively constant growth rate was observed in all *S*7942 cultures that were used for obtaining the doubling times versus temperature chart (Figure 4.1) regardless the operational temperature, with strong linear correlations (0.90 < R2 <0.98) between Chl *a* concentration and time.

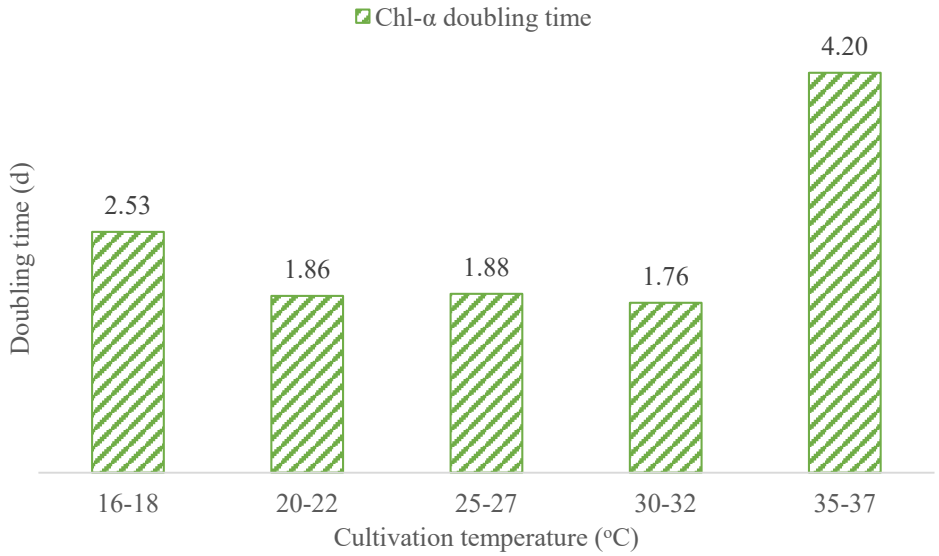


**Figure 4.2.** Average doubling-time in terms of Chl *a* in relation to temperature (*S*7942 in BG-11).

Doubling-time was relatively similar at temperatures from approximately 20ºC to 32ºC, ranging from 1.86 to 1.76 days. At the lowest cultivation temperatures of approximately 16ºC to 18ºC, a relatively small increase of 35% to 41% in Chl *a* doubling time was observed. A suppression of such magnitude on *S*7942 growth at temperatures lower than 20ºC was expected and being in accordance with the results of Deshnium et al., 1997. At operating temperatures of 35ºC to 37ºC a significant increase in Chl *a* doubling time of 223% to 235% was observed, suggesting inhibition of *S*7942 growth due to temperature-stress and/or increased decay rate (Han et al., 2016; Giannuzzi, 2018). Consequently, temperatures of 30ºC to 32ºC are considered optimal for *S*7942 cultivation, which is in accordance to other studies (Kuan et al., 2015; Rillema et al., 2020), but having insignificant differences (approximately 6%) with the growth rates obtained at 20ºC to 27ºC.

Worth mentioning is at this point is that while biomass concentration in terms of TSS and VSS exhibited similar or even greater linear correlation with time (0.96 < R2 <0.99), the corresponding doubling times were significantly higher (approximately 2-3 times greater) than the respective ones calculated in terms of Chl *a*. This suggests a systematic underestimation of biomass based on TSS or VSS measurements, probably due to the relatively small size of *S*7942 cell, resulting loss of biomass retention on the 1.2 μm pore-size, fiber-glass filter proposed by the applied standard method (APHA, 2540-D, G). Therefore, the parameters of Chl aor optical density are proposed for accurately expressing *S*7942 growth, as otherwise an up to three times greater doubling time may be falsely estimated.

The obtained results regarding the optimal operating temperature for *S*7942 cultivation are considered suitable towards advantageous full-scale implementation of *S*7942-based wastewater treatment technologies, since a plethora of industrial wastewater streams have temperatures at this range (Alekseiko et al.2014).

#### 4.3.1.2. Assessment of S7942 cell productivity

Cell productivity was calculated based on the Chl *a* measurements, after conversion from Chl *a* concentration to TSS using the established in study conversion factor (62.57 mgTSS/mgChl *a*). At cultivation temperatures between 25ºC to 27ºC cell productivity, in terms of TSS, was approximately 57 mg $L^{-1}d^{-1}$. This value is significantly lower than the optimal *S*7942 cell productivity in BG-11 growth-media (194 mg $L^{-1}d^{-1}$, at 25 ± 1ºC, for 10 days cultivation period) reported in Silva's et al. (2014) study. This difference is attributed to the lower light intensity applied in the present study, which is the variable that has the most significant effect on cell productivity in non-limited nutrient conditions (Silva et al., 2014). The applied light intensity, calculated from measurements of illuminance, ranged from approximately 5 to 30 μmol $m^{-2}s^{-1}$, which is significantly lower than of Silva's et al. (2014) study that ranged from 50 to 150 μmol $m^{-2}s^{-1}$.

#### 4.3.1.3. Antagonistic and/or predating microorganisms' growth in S7942 cultures

*Cultures in BG-11 media:* All *S*7942 cultures with BG-11 growth media maintained a homogenous population throughout the monitoring periods (up to 20 days monitoring), with limited presence of yeast contamination in some photobioreactors.

*Cultures in BTWW:* On the contrary, *S*7942 cultures with non-further processes BTWW as growth media could not survive for more than two days, due to the antagonistic action of bacteria and algae present in such substrate, as well as due to the growth of protozoan and metazoan populations in the aerobic conditions of the photobioreactor that use *S*7942 as feed substrate.

*Cultures in filtered BTWW:* *S*7942 cultures in filtered with 1.2 μm pore-size fiber-glass filter, BTWW exhibited significant growth of antagonistic and predating microbial species after 24 to 48 hours, resulting to a decrease of *S*7942 population. Similar growth of protozoan and metazoan species, but at a lower count, was observed in *S*7942 cultures in filtered with 0.45 μm pore-size cellulose filter BTWW. Nevertheless, by the eighth day of cultivation there was an over 44% and up to 86% drop of Chl *a* concentration in all photobioreactors with filtered wastewater media. Consequently, filtration alone cannot ensure disinfection of the BTWW prior its inflow in the photobioreactor.

*Cultures in chlorinated/dechlorionated BTWW:* Similarly, the *S*7942 cultures with chlorionated/dechlorionated BTWW presented growth of antagonistic and/or predating microbial species. The cultures with dairy wastewater exhibited this growth after the first 24 hours, while the setups with salty snack industry's wastewater after the 48 hours. Nevertheless, this growth resulted again to a significant drop of Chl *a* concentration by day eight (over 55% and up to 73%). The earlier growth of antagonistic and/or predating microbial species in the cultures with dairy wastewater is attributed to its higher organic content in terms of COD (Table 4.1) and the subsequent consumption of residual free chlorine for oxidation of soluble organics and not for complete disinfection of the wastewater.

*Cultures in filtered and chlorinated/dechlorinated BTWW:* The cultures in filtered with 0.45 μm pore-size cellulose filter and chlorinated/dechlorinated BTWW maintained homogenous population of *S*7942 throughout approximately a week, followed by limited growth of yeast and small-size ciliates (smaller or even-sized to *S*7942). Worth noting is that the growth of these microbial species did not seem to hinder *S*7942 growth rate, since *S*7942 cultures in chlorinated/dechlorinated BTWW exhibited similar and even greater overall $RGR_{Chl\ a}$ to that of *S*7942 cultures in BG-11 media (Figure 4.3, Table 4.2). Furthermore, a considered novel and environmentally friendly (Ghernaout

and Naceur, 2011) disinfection technique that is based on hexavalent iron (ferrate) production via low-cost electrochemical cell was evaluated. This technique provided similar disinfection efficiency to chlorination, meaning that it could replace the proposed chlorination technique as a more environmentally friendly disinfection process. Nevertheless, further study must be performed regarding optimization of in-situ ferrate production process in terms of energy efficiency. UV radiation was not selected as an alternative disinfection technique, since the possible presence of particulate matter in BTWW may reduce its disinfection efficiency (Metcalf and Eddy, 2003).

Assessment of S*7942* growth rate in properly disinfected BTWW*:* The three cultures with filtered and chlorinated/dechlorinated BTWW from the snack industry exhibited relatively high $RGR_{Chl\ a}$ of approximately 21.4% to 30.4% during the first week of cultivation, dropping to less than 13.6% after seventh day. These $RGR_{Chl\ a}$ values, as well as the greater than 40% drop during the second week of *S*7942 cultivation is consistent with the results regarding *S*7942 culture in BG-11 media and at temperatures from 16ºC to 37ºC (Figure 4.3).

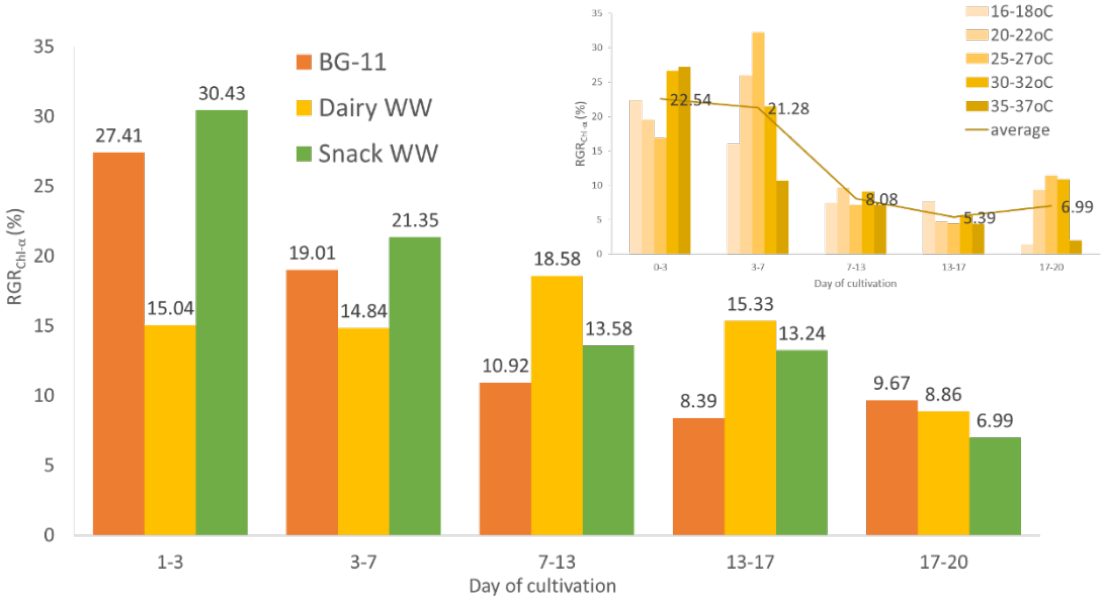


**Figure 4.3.** Average $RGR_{Chl\ a}$ evolution in *S*7942 cultures with BG-11 media (8 cultures), with treated dairy wastewater (3 cultures) and treated snack wastewater (3 cultures) at 20ºC to 27ºC (insert: BG-11 media at controlled temperatures).

The cultures with filtered and chlorinated/dechlorinated, relatively saline (approximately 0.4 M NaCl) BTWW from a dairy industry exhibited approximately

15% lower *RGRChl a* values during the first week of cultivation. The relatively low initial $RGR_{Chl\ a}$ value was due to an approximately two-day lag in *S*7942 growth observed in two out of three cultures. This lag is attributed to the acclimatization of *S*7942 to the "new" relatively saline environment. After the seventh day of cultivation and up to the seventeenth day, $RGR_{Chl\ a}$ values were relatively high indicating that acclimatization leads to a late-start and growth maxima retardation (Figure 4.3).

Nevertheless, the survival and growth of *S*7942 in such, relative saline, wastewater-media indicates that *S*7942 could constitute an alternative to AS biological component for the treatment of industrial wastewater treatment with increased salinity. It is shown further that this treatment is in addition coupled with yielding added value from cyanobacteria biomass production with increased sucrose levels. It is triggered by the natural reaction of S7942 to osmotic changes due to salinization of the environment via increased sucrose levels in the cytoplasm, regulating the inner-cell turgor pressure (Vayenos et al., 2020). Worth mention is that *S*7942 is able to tolerate maximally salinity of approximately 0.5 M NaCl (Ladas and Papageorgiou, 2000; Du et al., 2013), a salinity (2.9%) lower than that of seawater (3.5%) (Liang et al., 2020). The high tolerance of *S*7942 in relative saline conditions and the increase of its sucrose levels renders this specific strain an excellent candidate towards eco-friendly and resource/energy recovering treatment of industrial wastewaters. In particular, because among many applications the augmented sucrose production can be used for fermentative hydrogen production.

The hitherto studies however indicate the need for disinfection of wastewater prior the photobioreactor for nutrient removal (Arias et al., 2020). This adds a technical and economical bottleneck for the broad and sustained adoption of phototrophic treatment processes. As Shoener et al. (2019) highlighted, low-cost filtration systems, such as sand-filters, alone cannot solve the problem of photobioreactor contamination. Filtration at 0.45 μm cut-off size, chlorination and dechlorination seems promising, but nevertheless, further investigation regarding low-cost disinfection techniques and technologies is essential towards implementation of algal-based wastewater treatment systems.

Moreover, the loss of alkalinity during biological oxidation, which is compensated to a degree by denitrification process, constitutes and additional challenge in implementing

a *S*7942-based treatment stage for nutrients recovery, as it may result in low pH and inhibition of phototrophic processes. Thus, in cases of BTWWs with relatively low alkalinity, a supplementary to denitrification treatment stage is proposed for elevating WWTPs sustainability, while in cases where the pH of the BTWW remains in a relatively neutral to alkaline region, a *S*7942-based treatment stage is proposed as an alternative nutrient removal technology.

*Effect of cultivation period:* The effect of cultivation period on biomass yields can be evaluated based on the obtained results regarding $RGR_{Chl\,a}$ evolution in *S*7942 cultures (Figure 4.3). These data are extremely important for upscaling and implementing *S*7942-based wastewater treatment stages. They are considered necessary in order to achieve a balance between efficient nutrient removal/recovery, biomass production and photobioreactor volume. The goal is to achieve efficient nutrients removal/recovery in relatively short time, i.e. obtain high *S*7942 growth rates in relatively small photobioreactors.

As evident in Figure 4.3, the relative growth rate of all *S*7942 cultures decreased over time regardless the growth media and the culture temperature. The drop in $RGR_{Chl\,a}$ that is observed at second and third week of culture is attributed to the obstruction of photosynthesis due to increased optical density in the photobioreactors and the subsequent intermittent flux of light as a result of mutual shading (Qiang and Richmond, 1996). A shift of maximum growth rate at longer cell retention time could be obtained via increase of mixing rate (agitation) or/and photobioreactor light transfer configuration. Nevertheless, the results indicate that under the specific operational conditions, maximum growth rate could be maintained in the photobioreactor, given a mean cell residence time (MCRT) or solids retention time (SRT) of approximately 7 days.

### 4.3.2. *S*7942 nitrates removal rate

Nitrates removal rate is the fundamental parameter for assessing the applicability of the proposed technology for nutrients removal/recovery, due to the fact that it defines the required MCRT of *S*7942, thus the volume of a photobioreactor and the investment/operating cost.

The calculated relative (%) nitrates removal rate ($RRR_{NO3\_N}$) in the *S*7942 cultures andtheir respective $RGR_{Chl\,a}$ are presented in Table 4.2, categorized in terms of growth-

media i.e., cultivation in BG-11 or in BTWW wastewater. The *S*7942 cultures with BG-11 at 16ºC to 18ºC, 30ºC to 32ºC and 35ºC to 37ºC were excluded from the calculations, since they are incomparable to the cultures with wastewater-media that operated at 20ºC to 27ºC.

**Table 4.2.** Statistics of relative nitrates removal rate and relative growth rate at cultures with BG-11 growth-media, treated snack wastewater and treated dairy wastewater.

| Culture media | Statistic | Relative (%) $NO_3$_N removal rate ($RRR_{NO3\_N}$) | | Relative (%) *S*7942 growth rate ($RGR_{Chl\ a}$) | |
|---|---|---|---|---|---|
| | | Overall (20 days) | 1st week | Overall (20 days) | 1st week |
| BG-11 | Average | 3.23 | 2.08 | 16.8 | 22.1 |
| | Min | 1.16 | 1.37 | 12.0 | 12.6 |
| | Max | 6.70 | 3.83 | 22.4 | 31.8 |
| | Std.Deviation | 1.68 | 0.74 | 3.8 | 5.5 |
| Treated Snack wastewater | Average | 3.45 | 2.48 | 16.6 | 25.5 |
| | Min | 2.56 | 1.27 | 14.5 | 18.4 |
| | Max | 4.41 | 3.27 | 18.8 | 30.1 |
| | Std.Deviation | 0.93 | 1.07 | 2.1 | 6.2 |
| Treated Dairy wastewater | Average | 3.64 | 2.36 | 15.5 | 14.6 |
| | Min | 2.26 | 1.55 | 10.2 | 11.2 |
| | Max | 4.69 | 3.07 | 18.3 | 19.7 |
| | Std.Deviation | 1.25 | 0.77 | 4.6 | 4.5 |

The average $RRR_{NO3\_N}$ and $RGR_{Chl\ a}$ values were calculated for the first cultivation week, as well as for the overall duration of cultivation (20 days) and are graphically presented in Figure 4.4.

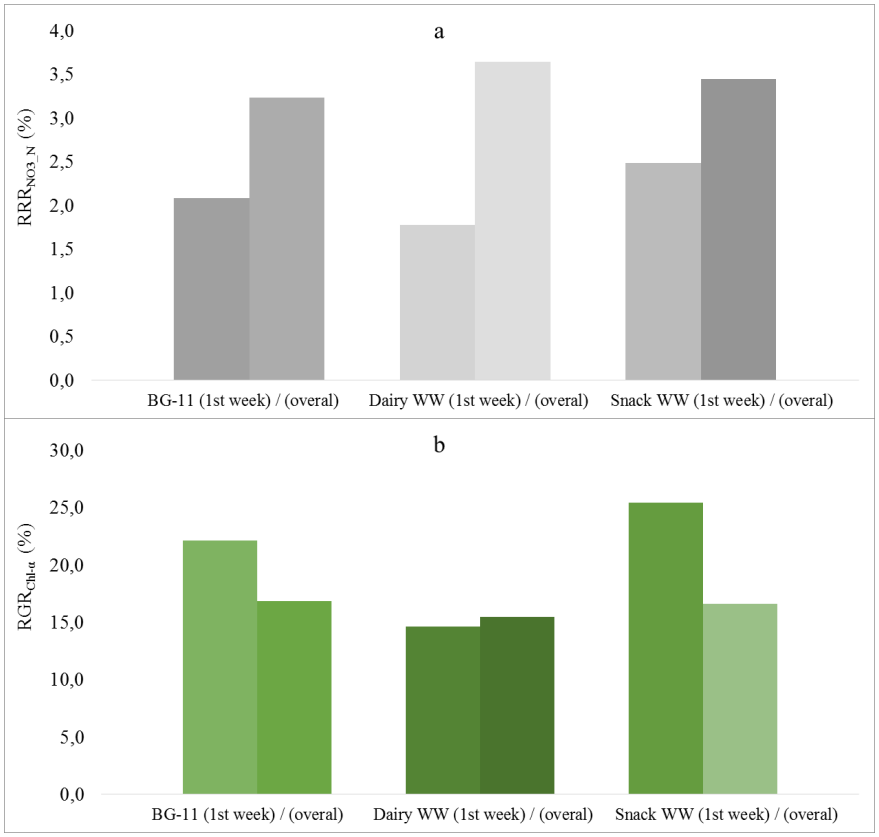


**Figure 4.4.** Average $RRR_{NO3\_N}$ (a) and $RGR_{Chl\ a}$ (b) of *S*7942 cultures, for the first week of cultivation and the overall duration of cultivation (20 days).

As evident in Figure 4.4, $RRR_{NO3\_N}$ and $RGR_{Chl\ a}$ are similar or even greater in experimental setups with wastewater as growth media. This indicates that the BTWW do not inhibit *S*7942 growth and provide all the necessary nutrients.

As previously reported, limited growth of yeast and small-size ciliates was observed in filtrated/chlorinated BTWW growth media. In order to assess their impact on the observed nutrient consumption, an additional series of experiments was conducted using duplicate setups with BG11 media or BTWW, both previously subjected to sterilization by autoclave. The results showed that growth rates and nutrient removal rates in terms of nitrates and phosphates removal, were similar to those obtained from filtrated/chlorinated BTWW. This indicates that nutrient consumption in cultures with filtrated/chlorinated BTWW is attributed to the growth of *S*7942 and the subsequent utilization of nutrients and not to consumption by antagonistic microbial species. The duration of this experimental series was 14 days and the average obtained rates are presented in Table 4.3.

**Table 4.3.** Average growth and nutrient removal rates in autoclaved media.

| Parameter / Growth Media | Autoclaved BG-11 | Autoclaved dairy BTWW | Autoclaved snack BTWW |
|---|---|---|---|
| Average S7942 growth rate (mg $L^{-1}$ $d^{-1}$) | 0.299 | 0.218 | 0.283 |
| Average nitrates removal rate (mg $L^{-1}$ $d^{-1}$) | 3.73 | 3.44 | 3.26 |
| Average phosphates removal rate (mg $L^{-1}$ $d^{-1}$) | 1.78 | 2.32 | 1.97 |

These results indicate that if coupled with aerobic AS process, *S*7942 may constitute a promising biological component for novel nutrient removal processes and the treatment of similar industrial wastewaters.

An opportunity to elevate sustainability of WWTPs is at site via implementation of a *S*7942-based nutrient removal stage individually or as a synergetic couple with denitrification process. Key elements for successful implementation of a *S*7942-based nutrient removal stage in AS treatment is the investment and operational cost, which is usually associated with land usage, energy consumption for lighting and the low-tech or high-tech disinfection technologies (Arias et al., 2020). While there is an undisputable benefit of cultivating prototrophic biomass in wastewaters, there is a significant challenge towards full-scale implementation of phototrophic-based wastewater treatment regarding the optimal size of a photobioreactor for nutrients removal/recovery.

Due to the nature of biological nitrogen removal in AS process (nitrification/denitrification), the resulting volume of a nitrogen removal bioreactor (denitrification tank) has to be considerably smaller than that of a respective photobioreactor. This is attributed to the fact that nitrogen removal in a phototrophic process is based only in synthesis of biomass, while in AS process is based additionally on aerobic respiration of biomass. (Denitrification process: utilization of released $O_2$ from reduction of nitrates/nitrites to $N_2O$, NO and $N_2$ gases).

Denitrification occurs mainly as a response to changes in the $O_2$ concentration of AS bacteria's immediate environment, as well as a result of direct nitrates uptake by activated sludge bacteria. Despite being beneficial process for efficiently removing nitrates from wastewater, it has a negative effect in removing valuable nitrogen fertilizer from the soil to form $N_2$ gas, while releasing significant quantities of the potent

greenhouse gas $N_2O$ and the tropospheric pollutant NO (Skiba, 2008). On the contrary, a phototrophic process recovers nitrogen from wastewaters, transforms it to valuable biomass, fixates $CO_2$ and does not directly release GHGs. Nevertheless, the volume requirements of a photobioreactor for nutrients removal should be at a magnitude that does not prohibits its construction in terms of land usage and investment cost. Worth mentioning is that minimization of photobioreactor's volume could assist in addressing the challenge that is maintaining monoculture in open air configurations. It is because a photobioreactor of similar volume to that of typical denitrification tanks, could be designed and constructed as a closed system unaffected by airborne contamination.

The volume of a photobioreactor is inextricably linked to the growth rate of the cultivated phototrophic organism. Thus, minimization of investment cost can be achieved by increasing the growth rate in the photobioreactor. The means for enhancing growth rate at longer *S*7942 cultivation times are the maintenance of optimal temperature and the unintermittent flux of light in the culture.

### 4.3.3. Evaluation of *S*7942 as alternative to AS de-nitrification process

There is an undisputable benefit of cultivating phototrophic biomass for nutrients recovery from wastewaters, as it is considered a valuable raw material for many applications. Though, there is a significant drawback towards full-scale implementation of algal-based wastewater treatment regarding the optimal size of a photobioreactor for nutrients removal/recovery.

In order to quantify this problem and evaluate *S*7942 adequacy as alternative to AS de-nitrification process, which constitutes the typically applied nitrogen removal process in AS WWTPs, a comparative scenario between AS de-nitrification process and *S*7942-based nitrogen removal process was employed. This scenario accounts the treatment of a BTWW with specific characteristics. More specifically, a BTWW with volumetric load of 120 $m^3\ d^{-1}$, biodegradable organic substrate concentration ($BOD_5$) of 2000 mg $L^{-1}$ and available nitrate-nitrogen for denitrification at a concentration of 100 mg $L^{-1}$, which is been subjected to AS de-nitrification process or to *S*7942-based nitrogen removal process.

The necessary denitrification bioreactor volume is calculated for the two typical variations of the process, (i) the post-denitrification process and (ii) the pre-denitrification process. The calculation of denitrification reactors based on literature

data (Crites and Tchobanoglous, 1998; Metcalf and Eddy, 2003) regarding specific nitrate denitrification rates ($U_{DN}$), maximum biomass yield coefficient ($Y_{max}$) and decay coefficient ($k_d$), as well as on the presumption that biomass concentration in terms of mixed liquor volatile suspended solids (MLVSS) is 3000 mg $L^{-1}$ and that operating conditions in denitrification bioreactor are 0.2 mg $L^{-1}$ of dissolved oxygen and 20ºC water temperature. The $U_{DN}$ values of 0.03 $kgNO_3$_N $KgVSS^{-1}$ $d^{-1}$ and 0.06 $kgNO_3$_N $KgVSS^{-1}$ $d^{-1}$ were selected for calculating the volume of the denitrification bioreactor in post-denitrification and pre-denitrification process respectively, while the $Y_{max}$ value of 0.65 kgVSS $KgBOD_{removed}^{-1}$, $k_d$ value of (0.05 $d^{-1}$) and a dissolved oxygen concentration of 0.2 mg $L^{-1}$ were selected.

The resulting bioreactor volume in the post-denitrification configuration is 166.7 $m^3$, corresponding to a hydraulic retention time (HRT) of approximately 1.4 days. In the pre-denitrification configuration, the resulting volume and HRT are significantly lower having values of 83.3 $m^3$ and 0.7 days respectively.

The volume of the respective photobioreactor is calculated based on the experimental estimation of specific nitrates utilization rate (SNUR), which is the mass of nitrates removed per mass of cyanobacteria growth for a specific time period. In the employed scenario, SNUR was estimated based on the average *S*7942 growth rate of all properly disinfected cultures in BTWW and for a 20 days period, as well as on the respective average nitrate removal rate. The growth rate of *S*7942 is expressed in terms of VSS concentration, obtained from Chl *a* measurements and the established in this study Chl *a* to VSS transformation coefficient.

According to the resulting average SNUR of 0.196 $mgNO_3$_N $mgVSS^{-1}$ and the data from the employed scenario, a daily *S*7942 biomass production of approximately 61.2 kg $d^{-1}$ should be cultivated in order to achieve complete nitrate nitrogen removal. By taking into account an average calculated cell productivity of approximately 40 mgVSS $L^{-1}d^{-1}$ the necessary volume of the photobioreactor is estimated at approximately 1530 $m^3$, corresponding to a hydraulic retention time (HRT) of 12.75 days. The estimated photobioreactor volume is approximately 9 times and 18 times larger than the denitrification bioreactor of post-denitrification and pre-dentitrification processes respectively. The volume of the photobioreactor could be significantly decreased via optimization of cultivation conditions, mainly in terms of optimal lighting conditions. An increase of cell productivity up to the reported optimal value of 194 mg

$L^{-1}d^{-1}$ (Silva et al., 2014) would correspond to a minimization of photobioreactor's volume down to 315 $m^3$, i.e. approximately 80% volume reduction (1.9 to 3.8 times larger than typical denitrification reactors). These results are considered promising, since the necessary photobioreactor volume for efficient nitrogen removal can be in the same order of magnitude to the typical denitrification tank volumes. Nevertheless, focus must be given towards optimization of *S*7942 cell productivity in wastewater media in order to minimize photobioreactor's investment cost, with special emphasis on increased photosynthetic activity.

## 4.4. Conclusions

*S*7942 is assessed as a potent biological component for novel and sustainable biological nutrient removal processes for industrial wastewater treatment. A S7942-based process could be implemented as an alternative or supplementary treatment stage in typical AS WWTPs offering the possibility of obtaining valuable products and renewable energy. *S*7942 can efficiently grow in properly disinfected, via low cost and/or environmentally friendly techniques, industrial wastewaters that have been subjected to biological oxidation. Furthermore, *S*7942 showed the ability to adapt and grow in treated, relatively saline industrial wastewater and being promising in terms of increasing nutrients removal efficiency in WWTPs. The unhindered growth of *S*7942 in treated industrial wastewaters, at typical wastewater temperatures, renders this specific cyanobacterium a promising candidate for sustainable industrial wastewater treatment applications. The implementation of a *S*7942-based wastewater treatment stage could elevate sustainability of WWTPs and assist in climate change mitigation via (a) nutrients recovery/reuse of high-value products, (b) renewable energy production and (c) reduction of carbon emissions of activated sludge process through utilization of external carbon sources.

## 4.5. References


1. Aguilera, A., Giannuzzi, L., 2018. Book Review: Cyanobacteria Growth Kinetics. Front. Mar. Sci. 5, 242.
2. Amanatidou, E., Trikoilidou, E., Samiotis, G., Benetis, N.P., Taousanidis, N., 2012. An easy uncertainty evaluation of the COD titrimetric analysis in correlation with quality control and validation data. Method applicability region. Anal. Methods 4, 4204-4212.
3. APHA (American Public Health Association), 2017. Standards Methods for the Examination of Water and Wastewater (23rd ed.), American Water Works Association and Water Environmental Federation, Washington, DC.
4. Arias, D.M., Garcia, J., Uggeti, E., 2020. Production of polymers by cyanobacteria grown in wastewater: Current status, challenges and future perspectives. New Biotechnol. 55, 46-57.
5. Barry, C.D., Renou-Wilson, F., Wilson, D., Muller, C., Foy, R.H., 2016. Magnitude, form and bioavailability of fluvial carbon exports from Irish organic soils under pasture. Aquat. Sci. 78, 541–560.
6. Cinque, G., Croce, R., Bassi, R., 2000. Absorption spectra of chlorophyll a and b in Lhcb protein environment. Photosynth. Res. 64, 233–242.
7. Collier, J.L., Grossman, A.R., 1992. Chlorosis induced by nutrient deprivation in Synechococcus sp. Strain PCC 7942: Not all bleaching is the same. J. Bacteriol. 174, 4718–4726.
8. Crites, R., Tchobanoglous, G., 1998. Small and Decentralized Wastewater Management Systems. McGraw –Hill, Inc, NY.
9. Dechatiwongse, P., Srisamai, S., Maitland, G., Hellgardt, K., 2014. Effects of light and temperature on the photoautotrophic growth and photoinhibition of nitrogen-fixing cyanobacterium Cyanothece sp. ATCC 51142. Algal Res. 5, 103-111.
10. Deshnium, P., Gombos, Z., Nishiyama, Y., Murata, N., 1997. The action in vivo of glycine betaine in enhancement of tolerance of Synechococcus sp. strain PCC 7942 to low temperature. J Bacteriol., 179, 339-44.
11. Du, W., Liang F., Duan Y., Tan X., Lu X., 2013. Exploring the photosynthetic production capacity of sucrose by cyanobacteria. Metab. Eng., 19, 17–25.
12. Espinosa, J., Forchhammer, K., Contreras, A. 2007. Role of the synechococcus PCC 7942 nitrogen regulator protein PipX in NtcA-controlled processes. Microbiology 153, 711- 718.
13. Frijns, J., Hofman, J., Nederlof, M., 2013. The potential of (waste)water as energy carrier. Energ. Convers. Manage. 65, 357-363.
14. Gardner-Dale, D., Bradley, I., Guest, J., 2017. Influence of solids residence time and carbon storage on nitrogen and phosphorus recovery by microalgae across diel cycles. Water Res. 121, 231–239.
15. Ghernaout, D., Naceur, M.W., 2011. Ferrate(VI): In situ generation and water treatment – A review. Desal. Water Treatment, 30, 319-332.
16. Han, F., Pei, H., Hu, W., Han, L., Zhang, S., Ma, G., 2016. Effect of high-temperature stress on microalgae at the end of the logarithmic phase for the efficient production of lipid. Environ. Technol. 37, 2649-2657.
17. Heidrich, E., Curtis, T., Dolfing, J., 2010. Determination of the internal chemical energy of wastewater. Environ. Sci. Technol. 45, pp.827–832.
18. IPCC, 2014. Climate Change 2014: Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

19. Kuan, D., Duff, S. Posarac, D., Bi, X., 2015. Growth optimization of Synechococcus elongatus PCC7942 in lab flasks and a 2-D photobioreactor. The Can. J. Chem. Eng. 93, 640-647.
20. Ladas, N.P., Papageorgiou, G.C., 2000. Cell turgor: a critical factor for the proliferation of cyanobacteria at unfavorable salinity. Photosynth Res., 65, 155–164.
21. Leow, S., Witter, J.R., Vardon, D.R., Sharma, B.K., Guesta, J.S., Strathmann, T. J., 2015. Prediction of microalgae hydrothermal liquefaction products from feedstock biochemical composition. Green Chem. 17, 3584–3599.
22. Li, W.W., Yu, H.Q., Rittmann, B.E., 2015. Chemistry: reuse water pollutants. Nature 528, 29–31.
23. Lu, L., Guest, J.S., Peters, Zhu, X., Rau, G.H., Ren, J.R., 2018. Wastewater treatment for carbon capture and utilization. Nat. Sustain. 1, 750–758.
24. Martins, J., Peixe, L., Vasconcelos, V., 2010. Cyanobacteria and bacteria co-occurrence in a wastewater treatment plant: absence of allelopathic effects. Water Sci. Technol. 62, 1954-1962.
25. Metcalf and Eddy (revised by: Tchobanoglous G., Burton L.F., Stensel H.D.), (2003). Wastewater Engineering: Treatment and Reuse. Metcalf and Eddy Inc., McGraw Hill, 473 Boston, USA.
26. Moran, R., 1982. Formulae for Determination of Chlorophyllous Pigments Extracted with N,N-Dimethylformamide. Plant Physiol. 69, 1376-1381.
27. Murakami, A., Mimuro, M., Ohki, K., Fujitta, Y., 1981. Absorption Spectrum of Allophycocyanin Isolated from Anabaena cylindrical Variation of the Absorption Spectrum Induced by Changes of the Physico-Chemical Environment. J. Blochem. 89, 79-86.
28. Oyadomari, J.K., 2001. Keweenaw Algae: A Collection of Freshwater Algae (and Protozoans) from the Keweenaw Peninsula, MI. educational website, www.keweenawalgae.mtu.edu, hosted by Michigan Technological University, Houghton, MI. Accessed online at June 2020.
29. Qiang, H., Richmond, A., 1996. Productivity and photosynthetic efficiency of Spirulina platensis as affected by light intensity, algal density and rate of mixing in a flat plate photobioreactor. J. Appl. Physiol. 8, 139–145.
30. Rillema, R., MacCready, J.S., Vecchiarelli, A.G., 2020. Cyanobacterial growth and morphology are influenced by carboxysome positioning and temperature. bioRxiv, 2020.06.01.127845; doi.org/10.1101/2020.06.01.127845.
31. Rosegrant, M.W., Msangi, S., Sulser, T.B., Valmonte-Santos, R., 2006. Biofuels and the global food balance: bioenergy and agriculture promises and challenges. 2020 vision briefs 14 (3), International Food Policy Research Institute (IFPRI).
32. Sato, T., Qadir, M., Yamamoto, S., Endo, T., Ahmad, Z., 2013. Global, regional, and country level need for data on wastewater generation, treatment, and use. Agr. Water Manage. 130, 1–13.
33. Shoener, B., Bradley, I., Cusick, R., Guest, J., 2014. Energy positive domestic wastewater treatment: the roles of anaerobic and phototrophic technologies. Environ. Sci. Proc. Imp. 16, 1204–1222.
34. Shoener, B., Schramm, S.M., Beline, F., Bernard, O., Martínez, C., Plosz, B.G., Snowling, S., Steyer, J.P., Perez, B.V., Wagner, D., Guest, J.S., 2019. Microalgae and cyanobacteria modeling in water resource recovery facilities: A critical review. Water Res. X. 2, 100024.
35. Silva, C.S.P., Silva-Stenico, M.E., Fiore, M.F., de Castro, H.F., Da Rós, P.C.M., 2014. Optimization of the cultivation conditions for Synechococcus sp. PCC7942 (cyanobacterium) to be used as feedstock for biodiesel production. Algal Res. 3, 1-7.

36. Singh, S.P., Singh, D., 2010. Biodiesel production through the use of different sources and characterization of oils and their esters as the substitute of diesel: a review. Renew. Sust. Energ. Rev. 14, 200–216.
37. Sivonen, K., Jones, G., 1999. Cyanobacterial Toxins, in Chorus, I., Bartram, J. (Eds.), Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring, and Management. E & FN Spon, London, pp. 41-111.
38. Skiba, U., 2008. Denitrification, in Editor(s): Jørgensen, S.E., Fath, B.D. (Eds.), Encyclopedia of Ecology, Academic Press, 866-871.
39. Song, K., Tan, X., Liang, Y., Lu, X., 2016. The potential of Synechococcus elongatus UTEX 2973 for sugar feedstock production. Appl. Microbiol. Biotechnol. 100, 7865–7875.
40. Stamatakis, K., Ladas, N.P., Alygizaki-Zorba, A., Papageorgiou, G.C., 1999. Sodium chloride-induced volume changes of freshwater cyanobacterium Synechococcus sp PCC 7942 cells can be probed by chlorophyll a fluorescence. Arch. Biochem. Biophys. 370, 240-249.
41. Trikilidou, E., Samiotis, G., Tsikritzis, L., Amanatidou, E., 2020. Performance of Semi-Micro-Kjeldahl Nitrogen Method – uncertainty and nitrate interference. Int. J. Environ. Anal. Chem. 1–11.
42. Trivedi, J., Aila, M., Bangwal, D.P., Kaul, S., Garg, M.O., 2015. Algae based biorefinery-How to make sense?. Renew. Sust. Energ. Rev. 47, 295–307.
43. USEPA, (United States Environmental Protection Agency), 2012. Global anthropogenic non-CO2 greenhouse gas emissions: 1990–2030.
44. Valverde-Pérez, B., Ramin, E., Smets, B.F., Plósz, B.G., 2015. EBP2R–an innovative enhanced biological nutrient recovery activated sludge system to produce growth medium for green microalgae cultivation. Water Res. 68, 821–830.
45. Van der Hoek, J.P., De Fooij, H., Struker, A., 2016. Wastewater as a resource: Strategies to recover resources from Amsterdam's wastewater. Resour. Conserv. Recy. 113, 53-64.
46. Vayenos, D., Romanos, G.E., Papageorgiou, G.C., Stamatakis, K., 2020. Synechococcus elongatus PCC7942: a cyanobacterium cell factory for producing useful chemicals and fuels under abiotic stress conditions. Photosynth Res. 146, 235–245.
47. Yajing Liang, Mingyi Zhang, Min Wang, Wei Zhang, Cuncun Qiao, Quan Luo, Xuefeng Lu, 2020. Freshwater Cyanobacterium Synechococcus elongatus PCC 7942 Adapts to an Environment with Salt Stress via Ion-Induced Enzymatic Balance of Compatible Solutes. Appl. and Envir. Microbiol., 86, 2904-2919.
48. Yang, J., Xu M., Zhang, X., Hu, Q., Sommerfeld, M., Chen, Y., 2011. Life-cycle analysis on biodiesel production from microalgae: water footprint and nutrients balance. Bioresource Technol. 102, 159–165.
49. Zhan, M., Ma, Y., 2020. Energy use and challenges in current wastewater treatment plants, in Liu, Y., Gu, J., Zhang, M. (Eds.), A-B Processes: Towards Energy Self-sufficient Municipal Wastewater Treatment. IWA Publishing, pp. 1–28.
50. Zhang, A., Carroll, A.L., Atsumi, S., 2017. Carbon recycling by cyanobacteria: improving CO2 fixation through chemical production. FEMS Microbiol. Lett. 364, fnx165.

# 5. Chapter 5. Dimensioning of Synechococcus elongatus PCC 7942 cultivation photobioreactor for valorization of wastewater resources

In compliance with circular economy and sustainability principles, novel wastewater treatment processes based on cyanobacteria and/or microalgae cultivation should be developed and applied. The lack of fundamental design and operational parameters hinders upscaling and implementation of such phototrophic wastewater treatment processes. In this regard, the present study sheds light on the dimensioning of a *Synechococcus elongatus* PCC 7942 (*S*7942) cultivation photobioreactor for nitrogen removal from different salinity wastewaters and for biomass-derived added value products. To attain photobioreactor dimensioning, *S*7942's *cell productivity*, nitrates removal rate and *specific nitrates utilization* rates were calculated based on experimental data under non-favorable growth conditions of limited lighting (5 to 30 μmol $m^{-2}$ $s^{-1}$). Chlorophyll *a* and phycocyanin concentrations, as well as biomass nitrogen content were measured to assess nitrogen assimilation in relation to salinity. Under conditions of limited lighting, *S*7942's *cell productivity* ranged from 43.7 to 20.0 mgVSS $L^{-1}$ $d^{-1}$. Increasing salinity up to the obtained threshold value of 450 mmolNaCl $L^{-1}$, *specific nitrogen utilization* rate ranged from 0.140 to 0.286 $mgNO_3$_N $mgVSS^{-1}$. The resulting photobioreactor volume is 10 to 20 times larger compared to an activated sludge denitrification reactor, thus it does not hinder the application of the proposed process. Given an optimal *cell productivity* obtained by applying light intensity of 150 μmol $m^{-2}$ $s^{-1}$, photobioreactor volume can be at the same order of magnitude (1.5 to 4 times larger). In contrast to activated sludge denitrification reactors, the volume of *S*7942 photobioreactor remains relatively constant regardless of the wastewater salinity. The study promotes the implementation of phototrophic wastewater treatment processes contributing to GHGs emission mitigation, resources recovery, as well as added value products and green energy obtainment.

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## 5.1. Introduction

Effluents from secondary biological wastewater treatment, especially from the treatment of agro-food industrial wastewater, contain high concentrations of nitrogen, which may lead to eutrophication of water bodies (Abdel-Raouf et al., 2012). Most of the applied wastewater treatment technologies, such as the widely applied activated sludge (AS) process, are not economical for the efficient treatment of such wastewaters and fail to recover valuable resources (Cai et al., 2013; Wicker and Bhatnagar, 2020). Furthermore, AS process has been criticized for the emission of greenhouse gases (GHGs), such as the potent in terms of global warming potential $N_2O$ gas produced during biological nitrification and denitrification processes. There is an increasing interest regarding the mitigation of $N_2O$ emissions in AS processes, as $N_2O$ accounts for the majority (up to 83 %) of the entire carbon footprint of a wastewater treatment plant (WWTP) (Duan et al., 2021) corresponding globally to about 94 % of the waste sector (Adouani et al., 2015). $N_2O$ emissions account for 7.9 % of the total anthropogenic GHG emissions, increased by 44 % from 1990 to 2014 (USEPA, 2021) and wastewater treatment sector is responsible for 3.2 % to 10 % of the total $N_2O$ emissions (Law et al., 2012), ranked as the sixth larger contributor by USEPA (2021).

On the other hand, cyanobacteria and microalgae offer a low cost and effective approach to remove/recover the excess nutrients from wastewaters and tertiary wastewater treatment, because of their high capacity for inorganic nutrient uptake (Priyadharshini et al., 2021), while producing potentially valuable biomass (De la Noue et al., 1992).

Many researches have indicated that phototrophic-based wastewater treatment processes are promising for tertiary treatment and resources recovery via biomass valorization (Zhang et a., 2008; markou and Georgakakis, 2011; Srora et al., 2021). The derived from wastewater treatment cyanobacteria or/and microalgae biomass has the potential to be an environmentally friendly biofuel feedstock (Abdel-Raouf et al., 2012) and can be used for the production of industrial relevant products (Khan et al., 2018), while during its synthesis fixates significant quantities of carbon dioxide (chia et al., 2021).

Cyanobacteria specifically, which is a group of organisms that includes a large number of species, exhibit high nutrient removal efficiency (Leow et al., 2015) and allow

recovery and reuse of resources (Khan et al., 2018), while they can fixate significant quantities of $CO_2$ and produce $O_2$ and biomass (Pathak et al., 2018). The resulting biomass from $CO_2$ mitigation and nutrients utilization is considered of high-value, since it can be used for the production of biofuels (biodiesel, bioethanol, biogas, biohydrogen), as well as hydrocarbons, proteins, pigments and biopolymers for pharmaceutical, chemical and food industry (Jayati et al., 2015).

Recent research results (Vayenos et al., 2020) have shown that some monocyte cyanobacteria, such as the freshwater cyanobacterium *Synechococcus elongatus* PCC 7942 (hereafter *S*7942), can follow the fermentative hydrogen production pathway, i.e. the metabolic production of biohydrogen from their sucrose. It is worth mentioning that the *S*7942 strain does not produce cyanotoxins and that it can be manipulated to increase biohydrogen production, since under certain stressful conditions *S*7942 increases its sucrose levels (Stamatakis et al., 1999; Vayenos et al., 2020). There are numerous stressors that can trigger biopolymer (lipid and carbohydrate) storage, such as intense light and salinity stress (Barry et al., 2016), resulting to cyanobacterial biomass of higher value. Increased salinity is a common characteristic of a plethora of industrial wastewater streams, thus *S*7942 may be proven a great candidate for wastewater treatment applications towards sustainable WWTPs, as pointed out in recent study (Samiotis et al., 2021).

Similarly to a various autotrophic cyanobacteria, *S*7942 can assimilate nitrogen directly in the form of ammonium via ammonium transporters or indirectly via cellular reducing mechanisms that reduce nitrate and nitrite ions to ammonium ions (Cai et al., 2013). Thereafter, *S*7942 incorporates the directly or indirectly uptaken nitrogen into amino acids mainly through the glutamine synthetase-glutamate synthase cycle (Herrero et al., 2001). When ammonium ion concentration exceeds a threshold value, the cellular nitrate and nitrite reduction mechanisms may be inhibited and subsequently only assimilation of ammonium nitrogen occurs (Aichi et al., 2006). Phosphorous on the other hand is assimilated by *S*7942 only in the form of phosphates (Ritchie et al., 1997; González-Morales et al., 2020), which are the dominant soluble phosphorous species in aerobically, biologically treated wastewaters. Of the three nitrogen forms that are usually encountered in such wastewaters (nitrate ions, ammonium ions and nitrite ions) only nitrate ions are present at high concentrations. Both ammonium and nitrite ions are relatively absent due to their transformation to nitrate ions via biological oxidation

(nitrification) by nitrifying bacteria. Thus, in sufficiently aerated, biologically treated wastewaters, the phenomenon of ammonium-promoted inhibition of nitrate assimilation (Aichi et al., 2006) or/and ammonium induced growth inhibition of *S*7942 (Sakamoto et al., 2021) is not to be expected. Given the unhindered by antagonistic or predating microbial species growth of *S*7942, such as activated sludge bacteria, protozoan and metazoan, a *S*7942-based wastewater treatment process presents an opportunity for the development of alternative and more sustainable wastewater treatment technologies (Samiotis et al., 2021). Nowadays, phototrophic processes are used mainly for obtaining added value products or/and green energy, but have limited applications in wastewater treatment and are associated with increased land requirements and operational cost. These challenges can be addressed via photobioreactor dimensioning and design optimization (Leow et al., 2015).

Aim of this work is to promote the implementation of a novel cyanobacterium-based wastewater treatment stage by providing a straightforward approach for photobioreactor volume calculation, targeting to nitrogen removal associated with resources recovery, obtaining of added value products or/and green energy from industrial wastewater of different salinity, as well as GHGs emission mitigation in WWTPs. For this aim, valuable results are experimentally obtained and presented regarding the removal of nitrogen by assimilation in *S*7942 biomass cultivated in biologically treated industrial wastewater. The impact of salinity on design and operational parameters is assessed and the feasibility of implementing the proposed process is discussed based on a comparative assessment with widely applied AS denitrification processes.

## 5.2. Materials and Methods

A series of *S*7942 cultures, using as growth-media biologically treated and properly disinfected by filtration and chlorination (Samiotis et al., 2021) industrial wastewater from a dairy industry, were setup. The biologically treated wastewater media (substrate) that were used in this study contained nitrogen in the form of nitrates with insignificant quantities of nitrite and ammonium ions (<0.5 mg $L^{-1}$). Thus, nitrogen assimilation was assessed by monitoring nitrate nitrogen removal from the solution, along with biomass concentration and its organic nitrogen content in terms of Total Kjeldhal Nitrogen (TKN), i.e. the sum of ammonium and organic (cell-bound) nitrogen. The effect of

wastewater salinity on *S*7942 growth was examined via comparison with cultures in standard BG-11 growth media. The soluble organic content of the photobioreactors, in terms of chemical oxygen demand (COD), was also monitored.

For the implementation of a *S*7942-based process and for photobioreactor dimensioning, the fundamental design and operational parameters, i.e. biomass growth rate and specific nitrogen utilization rate (*SNUR*) were estimated based on respective physicochemical measurements. The threshold of wastewater salinity was determined based on its effect on *S*7942 growth.

The fact that phototrophic biomass concentration is expressed in different formats by researchers and engineers, such as in terms of chlorophyll *a*, optical density (usually at 730 nm or 750 nm), total solids (TS) or total volatile solids (TVS), total suspended solids (TSS) or total volatile suspended solids (VSS), renders it difficult for engineers involved in the design of WWTPs to adopt and implement phototrophic-based wastewater treatment processes. Thus, in this study, in order to assist the interpretation of the results and the adaptation/scaling-up of the proposed *S*7942-based treatment process, the parameters of (i) chlorophyll *a* (Chl *a*), (ii) optical density at 750 nm ($O.D._{750\ nm}$) and (iii) total and volatile suspended solids (TSS, VSS) were measured, pair plotted and presented.

### 5.2.1. *S*7942 culture setups

Towards the assessment of nutrients recovery/removal rate by *S*7942 cyanobacterium, a series of laboratory scale, batch work experiments were conducted. Ten cylindrical glass vessels of 750 mL volume (diameter of 12 cm; height of 21 cm) were used as photobioreactors, five containing BG-11 medium (control setups) and five containing biologically treated dairy wastewater (test setups). Cultivation lasted 20 days, at average temperature of 23.55 ºC, with continuous agitation at 200 rpm and light intensities of 5 μmol $m^{-2}$ $s^{-1}$ to 30 μmol $m^{-2}$ $s^{-1}$. It is worth mentioning that the applied light intensity is relatively low compared to an optimal value of 150 μmol $m^{-2}$ $s^{-1}$ (Silva et al., 2014) in order to assess *S*7942's growth and photobioreactor dimensioning under non-favorable conditions. Sterilized cotton cover-caps were placed in every photobioreactor in order to avoid airborne contamination and not hinder gas exchange and $CO_2$ transfer from atmospheric air to the reactors.

A pure *S*7942 culture (initial culture) was used for the preparation of the inoculation cultures in BG-11 medium. After approximately 4 days of cultivation, these cultures were used as inoculum in the control setups and in the test setups to obtain a Chl *a* concentration of approximately 1 mg $L^{-1}$. The *S*7942 inoculation cultures were separated via centrifugation at 5000 rpm for 10 min.

Test setups contained biologically treated dairy wastewater as growth media (substrate) subjected to subsequent disinfection after sedimentation (Samiotis et al., 2021). More specifically, wastewater was obtained from the aerobic compartment of a dairy industry's AS WWTP, where approximately 99 % of nitrogen is in its oxidized form (nitrates). Substrate disinfection was conducted by filtration with 1.2 nm pore-size fiberglass filters followed by chlorination and dechlorination with sodium hypochlorite and sodium thiosulphate respectively.

Addition of NaCl in BG-11 and in wastewater growth media was made in order to assess the salinity threshold for *S*7942 growth. To obtain different salinity levels in the photobioreactors, ranging from approximately 25 mmolNaCl $L^{-1}$ up to 600 mmolNaCl $L^{-1}$, saturated NaCl solution was added in four out of five control setups and four out of five test setups.

### 5.2.2. Analytical methods

The physicochemical parameters of pH, nitrates, phosphates, TSS and VSS were determined by applying standard methods for the examination of water and wastewater (Rice et al., 2017). Chl *a* concentration was determined in N,N-dimethylformamide (DMF) extracts of cell pellets according to Moran et al. (1982). Optical density at 750 nm ($O.D._{750nm}$) and light absorbance (Abs) at specific wavelengths, indicative of *S*7942's main photosynthetic pigments (Chl *a* at 680 nm and phycocyanin at 625 nm) (Ruffing and Jones, 2009), were measured using a properly calibrated Shimadzu UV1900 spectrophotometer. Phycocyanin concentration was determined from Lambert-Beer law, using the molar absorption coefficient of 1.54 x $10^5$ $m^2$ $mol^{-1}$ (Boussida and Richmond, 1980) and the molecular weight of 36,700 Da (Glazer and Fang, 1972).

*S*7942 growth rates were determined by measuring chlorophyll *a* concentration and its viability was assessed by additional microscopic examination of biomass using a Leica

DM1000 phase contrast microscope. The identification of antagonistic to *S*7942 or/and predating microbial species was conducted based on their morphological characteristics and relevant databases (Oyadomari, 2001; Amanatidou et al., 2015; Rice et al., 2017).

All analyses were conducted at the accredited according to ISO 17025 "Environmental Chemistry & Water and Wastewater Treatment Laboratory", Department of Chemical Engineering, University of Western Macedonia, Greece. The laboratory calculates measurement uncertainties in all the applied methods (Amanatidou et al., 2012; Trikilidou et al., 2020).

## 5.3. Results and Discussion

### 5.3.1. Nitrogen removal and assimilation

Nitrogen removal in the culture setups is attributed to nitrates uptake by *S*7942 and their subsequent utilization mostly for the synthesis of amino acids and proteins (Koksharova et al., 2005). Among the synthesized proteins there are the photosynthetic pigments of *S*7942, which are phycocyanin, chlorophyll *a* and allophycocyanin (Ungerer et al., 2018).

The calculation of *S*7942's growth rate in terms of doubling time (days) and the calculation of nitrates removal rate ($mgNO_3\_N\ L^{-1}\ d^{-1}$) resulted from slope of the linear regression of chlorophyll *a* concentration and nitrate-nitrogen concentration over time respectively (Clark et al., 2018; Princiotta et al., 2019). *SNUR* ($mgNO_3\_N\ mgVSS^{-1}$) was calculated according to equation (1) by dividing nitrates removal rate with biomass growth (*cell productivity*). *Cell productivity* can be experimentally determined at case, as it depends on operating conditions and photobioreactor design, by dividing the difference of VSS concentration for a specific monitoring period with the duration of monitoring. *Cell productivity* can be measured or estimated using robust prediction models that minimize uncertainty induced by the complexity of biochemical processes, as presented in the work of Coutinho et al. (2019). Nevertheless, the uncertainty of model predictions may increase due to the assumptions that are made in order to simplify the expressions for the process rates and to reduce the number of the model components and parameters (Zambrano et al., 2016; Coutinho et al., 2019).

$$SNUR = \frac{Nitrates\ utilization\ rate}{Cell\ productivity} \quad (1)$$

Biomass nitrogen content was calculated by dividing TKN of biomass with VSS mass and is expressed as % nitrogen content. The results of the aforementioned calculations are presented in relation to salinity levels in Figure 5.1.

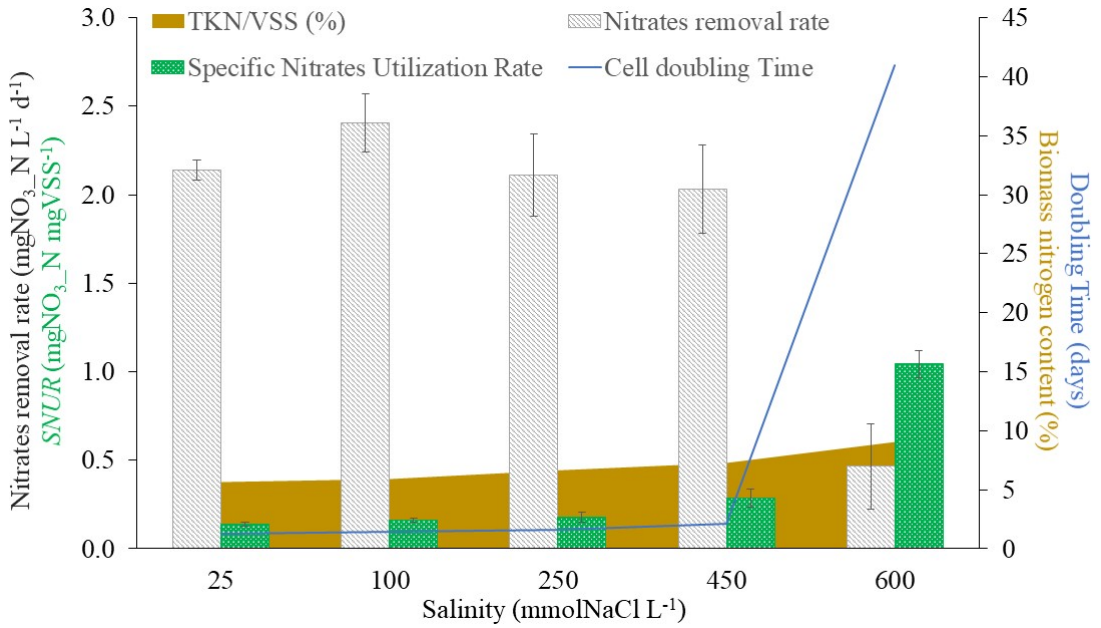


**Figure 5.1.** *S*7942's nitrates removal rate, specific nitrogen utilization rate (*SNUR*), doubling time and biomass nitrogen content (%) in relation to salinity levels of growth medium.

Figure 5.1 shows that biomass growth rate in terms of cell doubling time remained relatively constant for salinities up to 250 mmolNaCl $L^{-1}$, with a low increasing trend from 1.24 days to 1.62 days. Thereafter, an increase of cell doubling time to 2.09 days was observed at approximately 450 mmolNaCl $L^{-1}$, followed by a dramatic increase to 41 days at approximately 600 mmolNaCl $L^{-1}$. On the other hand, *SNUR* remained relatively constant (0.140 to 0.179 $mgNO_3$_N $mgVSS^{-1}$) for salinities up to 250 mmolNaCl $L^{-1}$, increased to 0.286 $mgNO_3$_N $mgVSS^{-1}$ at 450 mmolNaCl $L^{-1}$ and had a highest value of 1.045 $mgNO_3$_N $mgVSS^{-1}$ at approximately 600 mmolNaCl $L^{-1}$ (Figure 5.1), when *cell productivity* is minimum. It should be noted that at negligible *cell productivity, SNUR* has no practical significance. Thus, at salinity levels of 600 mmolNaCl $L^{-1}$, *SNUR* values bear no practical meaning on nitrogen assimilation and subsequently on the dimensioning of the photobioreactor.

Furthermore, nitrates removal rate, calculated by the slope of the linear regression of nitrates concentration over time, was relatively high (2.139 to 2.031 $mgNO_3$_N $L^{-1}$ $d^{-1}$) and remained relatively constant for salinities up to 450 mmolNaCl $L^{-1}$, despite the

increasing *S*7942 doubling time (Figure 5.1). From equation (1) is evident that when *cell productivity* declines in relation to salinity, *SNUR* increases, especially at salinities over 250 mmolNaCl $L^{-1}$. It would be expected that the rate of biomass synthesis would dictate nitrates removal rate, but nitrates removal rate remains relatively constant up to 450 mmolNaCl $L^{-1}$ despite the increasing *S*7942 doubling time (decreasing *cell productivity*). From all the above it is concluded that the salinity threshold of a *S*7942-based wastewater treatment process for efficient biomass growth and nitrogen assimilation is up to 450 mmolNaCl $L^{-1}$, corresponding to an electric conductivity of 38 mS $cm^{-1}$ (correlation coefficient, Table 5.2). Most saline industrial wastewater present salinities below this threshold. No addition of NaCl is provided in the S7942-based treatment of saline industrial wastewater thus, no secondary pollution is caused. Furthermore, no secondary pollution from organic compounds, expressed as soluble COD, is generated by the described autotrophic process. The slight increase (<5%) of soluble COD observed after 20 days of cultivation is attributed to the decay and lysis of *S*7942 and the dissolution of cellular matter in the growth media. Nevertheless, following harvesting of biomass the separated liquid could be recirculated in the aeration tank of the treatment plant if COD concentration exceeds acceptable values.

*Cell productivity* rate along with *SNUR* are the fundamental design parameter used for the dimensioning of a photobioreactor. As shown in Table 5.1 and Figure 5.2, *cell productivity* rates are similar in both control and test setups ranging from 43.7 mgVSS $L^{-1}$ $d^{-1}$ to 20.0 mgVSS $L^{-1}$ $d^{-1}$ for salinities up to 450 mmolNaCl $L^{-1}$, declining significantly to values of approximately 1 mgVSS $L^{-1}$ $d^{-1}$ at approximately 600 mmolNaCl $L^{-1}$, indicative of *S*7942's cell death. The (%) decline rate of *cell productivity* in control and test setups is presented in Table 5.1.

**Table 5.1.** *Cell productivity and* (%) decline rate in relation to salinity, at control and test setups.

| Salinity (mmolNaCl $L^{-1}$) | *Cell productivity* in control setups (mg $L^{-1}$ $d^{-1}$) | *Cell productivity* decline rate at control setups (%) | *Cell productivity* in test setups (mg $L^{-1}$ $d^{-1}$) | *Cell productivity* decline rate at test setups (%) |
|---|---|---|---|---|
| 25 | 43.7 | - | 39.3 | - |
| 100 | 43.1 | 1.4 | 38.3 | 2.5 |
| 250 | 35.0 | 19.9 | 29.5 | 24.9 |
| 450 | 18.6 | 57.4 | 20.0 | 49.1 |
| 600 | 1.7 | 96.1 | 0.7 | 98.2 |

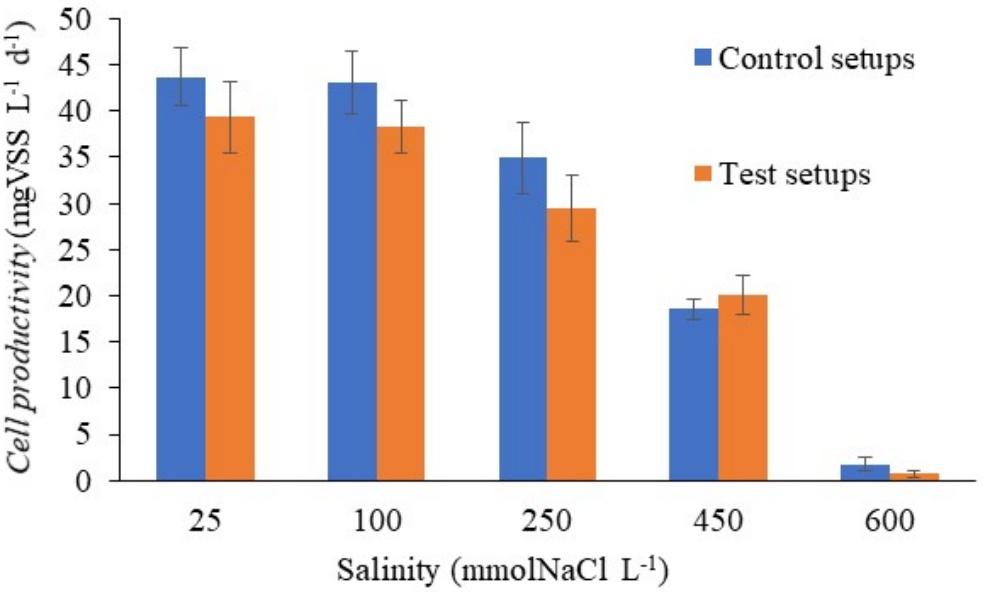


**Figure 5.2**. *S*7942 *cell productivity* in relation to salinity in control and test setups.

Since, up to the salinity threshold, nitrates removal rate remains constant and *cell productivity* decreases, it is concluded that there is an increase of nitrogen assimilation into biomass attributed to *S*7942 metabolic response to saline conditions (Stamatakis et al., 1999; Vayenos et al., 2020). Saline conditions result to higher intracellular sucrose content that is triggered by the natural response of *S*7942 to salinity driven osmotic changes for inner-cell turgor pressure regulation (Vayenos et al., 2020). In the intracellular sucrose synthesis, sucrose-phosphate synthase coupled to sucrose-phosphate phosphatase are the mainly involved enzymes (Ehira et al., 2014). Both enzymes consist of homotetrameric proteins with an average monomeric molecular weight of about 90 kD (about 800 amino acids long) (Stein and Granot, 2019). High salinity levels lead to lower cellular growth rate (Stamatakis et al., 1999), but higher amino acids production rate (Herrero et al., 2001), which are the structural components of the aforementioned enzymes involved in sucrose synthesis (Nunes-Nesi et al., 2010; Sakamoto et al., 2021). The enrichment in sucrose content enhances biomass use as a raw material both for the industrial sector and the renewable energy sector. The biomass can be utilized for the extraction of biopolymers (proteins) and for obtaining sucrose that can be used for industrial purposes or for the production of biofuels (Jayati et al., 2015). Furthermore, the high sucrose content in *S*7942 cell can lead to high yields of direct fermentative bio-hydrogen production (Vayenos et al., 2020), avoiding the costly cellulosic biomass pretreatment requirements for the production of renewable fuels and chemicals (Ehira et al., 2014).

With increasing salinity, degradation of phycocyanin is observed (Ladas and Papageorgiou, 2001). This phenomenon is of significant interest for the assessment of nitrogen assimilation in *S*7942 cell. For this reason, phycocyanin degradation in relation to salinity was studied to assess its impact on nitrates removal rate in *S*7942 photobioreactor by measuring phycocyanin to chlorophyll *a* absorption ratio ($Abs_{625nm}/Abs_{680nm}$) as indicator of phycocyanin degradation (Figure 5.3). It is evident that higher salinities lead to lower phycocyanin/chlorophyll *a* ratio, thus to phycocyanin degradation.

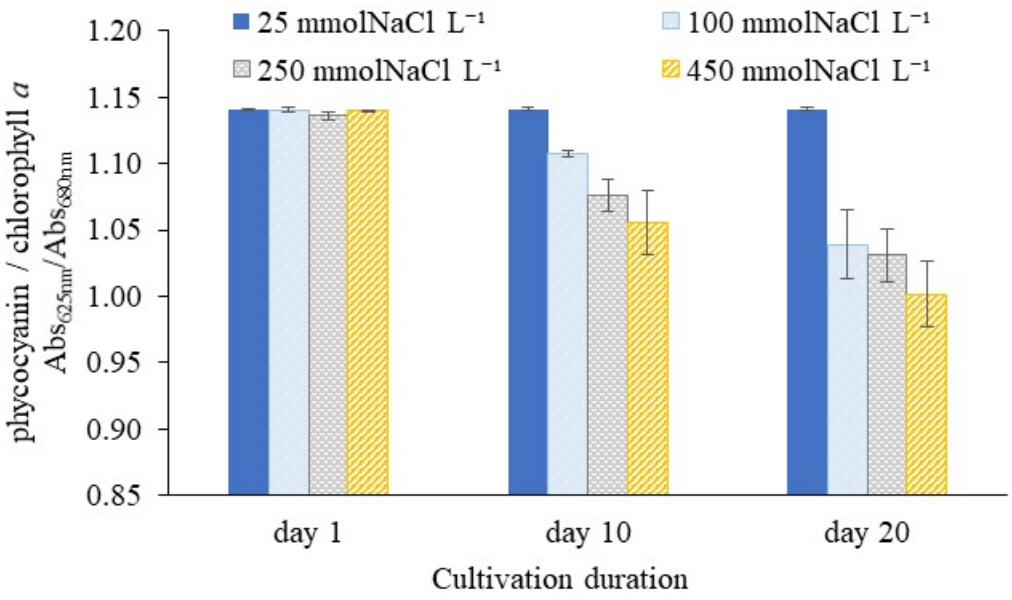


**Figure 5.3.** Phycocyanin to chlorophyll *a* absorption ratio variation in relation to cultivation duration and salinity levels up to threshold value.

Compared to chlorophyll *a*, phycocyanin presents significant differences regarding its nitrogen content per molecule. Chlorophyl *a* nitrogen content, calculated from its molecular formula (Sonani et al., 2019), is 6.3 %, whereas phycocyanin exhibits a significantly higher average nitrogen content of 10.1 %, calculated from three molecular formulas obtained from literature (Kim et al., 2018; Sonani et al., 2019; Alam et al., 2020). Thus, phycocyanin assimilates 38.2 % more nitrogen per pigment molecule synthesized resulting in 3.7 times higher nitrogen assimilated into phycocyanin per unit of *S*7942 mass than in chlorophyll *a*. This value slightly drops to a minimum of 3.2 after 20 days of cultivation to salinities up to approximately 450 mmolNaCl $L^{-1}$. The proportions were calculated based on chlorophyll *a* and phycocyanin molecule nitrogen content and their concentrations in the culture setups.

It is therefore concluded that at increasing salinities up to 450 mmolNaCl $L^{-1}$, where relatively constant nitrogen removal rate is observed (average value 2.139 ± 0.162 $mgNO_3$ $L^{-1}$ $d^{-1}$), the increase of *SNUR* is attributed to the low phycocyanin degradation and the activation of sucrose synthesis mechanism.

### 5.3.2. Dimensioning of *S*7942 photobioreactor

The volume of a reactor is the most critical parameter in the design of a WWTP, since it defines process efficiency, implementation cost and land requirements. Thus, the volume of a *S*7942 photobioreactor for nitrates removal/recovery from wastewaters and for *S*7942 cultivation determines the applicability of the proposed technology. Since the proposed technology removes nitrogen solely by assimilation and does convert it to non-recoverable $N_2$ or $N_2O$ gases, a photobioreactor volume of comparable size to AS denitrification reactors would constitute the *S*7942-based wastewater treatment a feasible supplementary or alternative solution for WWTPs' environmental footprint minimization and resources recovery (added-value products and green energy).

In general, the volume of a AS denitrification reactor is considerably smaller than that of a respective photobioreactor, which is attributed to the fact that nitrogen removal in phototrophic processes is based only on biomass synthesis, while in AS denitrification process is based additionally on anaerobic respiration of biomass. Quantification of these volume differences has not been achieved until today. In this study, following the calculation of photobioreactor volume, a comparative assessment between the volume of a *S*7942 photobioreactor and the volume of widely applied AS denitrification processes has been conducted.

In Table 5.2, experimentally obtained conversion factors between the parameters Chl *a*, $O.D._{750nm}$, TSS and VSS, as well as a correlation coefficient between salinity levels and conductivity of the *S*7942 growth medium are presented. Monitoring of these parameters is a direct way of assessing phototrophic biomass growth, while biomass concentration can be directly estimated by TSS and VSS measurement. By correlating Chl *a*, $O.D._{750nm}$ with TSS and VSS, *cell productivity* ($kgVSS\ m^{-3}\ d^{-1}$) can be calculated using Chl *a*, $O.D._{750nm}$ measurements and the respective conversion factors (Table 5.2). These conversion factors are considered of significant importance for the dimensioning, assessment and adoption of the proposed technology.

**Table 5.2.** Experimentally obtained conversion factors and correlation coefficients (Jayati et al., 2015).

| Pair | Value | Unit |
|---|---|---|
| Chl *a* to TSS | 62.57 | mgTSS mgChl $a^{-1}$ |
| Chl *a* to VSS | 56.31 | mgVSS mgChl $a^{-1}$ |
| TSS to VSS | 0.90 | mgVSS $mgTSS^{-1}$ |
| $O.D._{750nm}$ to Chl *a* | 5.28 | mgChl *a* $AU^{-1}$ |
| $O.D._{750nm}$ to TSS | 290.87 | mgTSS $AU^{-1}$ |
| Conductivity to Salinity* | 0.647 | mgNaCl cm $\mu S^{-1}$ $L^{-1}$ |

*AU = absorbance unit; *from experimental data of present study*

As shown in Table 5.2, for every unit increase of Chl *a* concentration an increase of *S*7942 biomass in terms of TSS or VSS is observed, equal to 62.57 mg $L^{-1}$ and 56.31 mg $L^{-1}$ respectively. Furthermore, for every unit (AU) increase of $O.D._{750nm}$ an increase of Chl *a* concentration equal to 5.28 mgChl $L^{-1}$ and an increase of TSS concentration equal to 290.87 mgTSS $L^{-1}$ is observed.

#### 5.3.2.1. Denitrification reactor volume calculation in activated sludge process

According to literature (Tchobanoglous et al., 2003; EPA, 2010), the volume of a denitrification reactor - $V_{DN}$ ($m^3$) is calculated on the basis of nitrate-nitrogen load - *N_Denitr* ($kgNO_3\_N$ $d^{-1}$), activated sludge (biomass) concentration – *MLVSS* ($kgVSSm^{-3}$) and the specific denitrification rate – $U'_{DN}$ ($kgNO_3\_N$ $kgVSS^{-1}$ $d^{-1}$), as described in equation (2).

$$V_{DN} = \frac{N\_Denitr}{U'_{DN} * MLVSS} \quad (2)$$

It is evident from equation (2) that for a given nitrate-nitrogen load, the parameters of $U'_{DN}$ and *MLVSS* concentration define $V_{DN}$. The value of the specific denitrification rate ($U'_{DN}$) results from equation (3) taking into account the effect of temperature ($T$, ºC), the impact of dissolved oxygen (*D.O.,* mg $L^{-1}$) concentration and an experimental specific denitrification rate ($U_{DN}$) obtained from literature and presented in Table 5.3.

Equation (3) shows that at *D.O.* concentrations of 1 mg $L^{-1}$ denitrification process is practically inhibited. A typical *D.O.* concentration value in denitrification reactor is 0.2 mg $L^{-1}$ (EPA, 2010), since absence of D.O. cannot occur due to the release of $O_2$ during nitrates reduction in biological denitrification process. The value of 1.09 in equation (3) is a considered a realistic estimate of the Arrhenius "θ" factor according to the widely cited work of Dold et al. (2008).

$$U'_{DN} = U_{DN} * 1.09^{(T-20)} * (1 - D.O.) \quad (3)$$

$U'_{DN}$ is the measure of nitrates removal rate in relation to biomass concentration in a bioreactor in biological denitrification processes, where nitrates are mainly reduced to nitrogen gas ($N_2$) via metabolic paths of activated sludge denitrifying bacteria. On the other hand, *SNUR* is the measure of nitrates removal per unit of produced biomass weight. In assimilative nitrate removal processes, such as the proposed *S*7942-based process, *SNUR* expresses the rate at which nitrate-nitrogen assimilates into the cultivated biomass.

The presence of high salt content may also inhibit denitrification. Significant drop of denitrification rate occurs with increasing salinity, especially at salt concentrations higher than 180 mmolNaCl $L^{-1}$ approximately (1 %), as described by equation (4) (Dincer and Kargi, 1999):

$$R_{DN} = 29 * \frac{15200}{15200 + C_{salt}} \quad (4)$$

Where $R_{DN}$ is the denitrification rate in mg $L^{-1}$ $h^{-1}$ and $C_{salt}$ is the salt concentration in mgNaCl $L^{-1}$.

$R_{DN}$ expresses the denitrification rate per volume unit of a denitrification bioreactor, while $U'_{DN}$ is the denitrification rate per mass unit of biomass in a denitrification bioreactor. At steady state operation of a denitrification bioreactor, where relatively constant biomass concentration occurs, both $U'_{DN}$ and $R_{DN}$ are influenced by the same parameters, such as D.O. concentration, pH and salinity.

Equation (4) can assist in the estimation of salinity induced inhibition of denitrification in AS process. The verification of equation (4), using experimental data of other studies regarding the impact of salinity in denitrification rate (Glass and Silverstein, 1999;

Garcia-Ruiz et al., 2018), showed a relative error up to 12 % compared to the results of the reviewed studies.

The parameters of $U_{DN}$ and *MLVSS* for AS "post" and "pre" denitrification processes, which are necessary for calculating $V_{DN}$, are presented in Table 5.3.

**Table 5.3.** $U_{DN}$ and *MLVSS* values of AS denitrification processes (Tchobanoglous et al., 2003; EPA, 2010).

| **Denitrification process** | **Design parameter** | |
|---|---|---|
| | $U_{DN}$ (kg $NO_3$_N kgVSS$^{-1}$ d$^{-1}$) | *MLVSS* (kgVSS m$^{-3}$) |
| Post-denitrification | Range: 0.017 – 0.048<br>Typical value: 0.03 | Range: 1.6 – 4.8<br>Typical value: 3.0 |
| Pre-denitrification | Range: 0.03 – 0.11<br>Typical value: 0.06 | |

### 5.3.2.2. Photobioreactor volume calculation in autotrophic processes

As aforementioned, in AS denitrification processes, biomass content plays a significant role on the dimensioning of a reactor. On the other hand, because in phototrophic processes nitrates removal is associated with biomass growth, *cell productivity* (kgVSS m$^{-3}$ d$^{-1}$) and *SNUR* were taken into account for the photobioreactor volume calculation (Equation 5).

The calculation of photobioreactor volume with the provided dimensioning equation takes into account nitrogen load, *SNUR* and *cell productivity*, which can be either experimentally or theoretically obtained. Consequently, if *SNUR* and *cell productivity* are evaluated at case, the provided dimensioning formula can be used for straightforward photobioreactor volume calculation and scaling up the proposed process.

$$V_{PBR} = \frac{N\_Denitr}{SNUR * cell\ productivity} \quad (5)$$

Where: $V_{PBR}$ is the photobioreactor volume (m$^3$); *N_Denitr* is the daily nitrate-nitrogen load (kg$NO_3$_N d$^{-1}$); *SNUR* is the specific (nitrate) nitrogen utilization rate (kg$NO_3$_N kgVSS$^{-1}$) and *cell productivity* (kgVSS m$^{-3}$ d$^{-1}$).

According to the conducted experiments, *SNUR* and *cell productivity* rates are affected by the salinity of wastewater media (Figure 5.1). Up to the obtained salinity threshold

of approximately 450 mmolNaCl $L^{-1}$, *SNUR* rises with increasing salinity at a rate of approximately 0.0006 $mgNO_3$_N $mgVSS^{-1}$ $mmolNaCl^{-1}$ ($R^2$=0.901), attributed to increased protein synthesis for the production of intracellular osmolyte (sucrose). On the other hand, *cell productivity* declines at a rate of 0.0553 mgVSS $L^{-1}$ $d^{-1}$ $mmolNaCl^{-1}$ ($R^2$=0.981) as salinity increases beyond threshold value. Consequently, the denominator of equation (4), which includes both rates that are essential for the dimensioning of a *S*7942 photobioreactor, presents relatively constant values, ranging from 6.53 $mgNO_3$_N $L^{-1}$ $d^{-1}$ to 6.64 $mgNO_3$_N $L^{-1}$ $d^{-1}$ for salinities up to approximately 250 mmolNaCl $L^{-1}$ and having its highest value of 7.75 $mgNO_3$_N $L^{-1}$ $d^{-1}$ at the salinity threshold of approximately 450 mmol $L^{-1}$. This is considered of significant importance because, for the obtained salinity threshold, the resulting $V_{PBR}$ remains relatively constant (volume change < 12 %) regardless of the salinity of the treated wastewater media (Table 5.4). On the contrary, the resulting $V_{DN}$ increases up to 260 % as salinity increases from approximately 25 mmol $L^{-1}$ to the salinity threshold of 450 mmol $L^{-1}$.

In Table 5.4, $V_{DN}$ per kg of nitrate-nitrogen removed for "pre" and "post" AS denitrification are presented and compared to $V_{PBR}$. $V_{PBR}$ was calculated using the experimentally obtained *SNUR* and *cell productivity* rates, under non-favorable conditions, as well as using an optimal *S*7942 *cell productivity* rate of 0.198 mg $L^{-1}$ $d^{-1}$ obtained under favorable experimental conditions (Silva et al., 2014). This optimal *S*7942 *cell productivity* rate was adjusted in relation to salinity levels according to the obtained decline rate of *S*7942 test setups (Table 5.4).

Under non-favorable conditions, $V_{PBR}$ in relation to wastewater salinity ranges between 176.2 $m^3$ to 199.4 $m^3$ per kg of nitrate-nitrogen load (*N_Denitr*). In favorable conditions $V_{PBR}$ ranges between 35.7 $m^3$ to 40.4 $m^3$ per kg of *N_Denitr*. $V_{DN}$ was calculated at average cultures temperature, at *D.O.* of 0.2 mg $L^{-1}$ (EPA, 2010) and by taking into account the effect of salinity levels (equation 4) (Garcia-Ruiz et al., 2018). $V_{DN}$ for "post" and "pre" denitrification configurations and for salinities up to the threshold value of 450 mmolNaCl $L^{-1}$, ranged from 10.2 $m^3$ to 26.6 $m^3$ and 5.1 $m^3$ to 13.3 $m^3$ respectively.

**Table 5.4.** $V_{DN}$ and $V_{PBR}$ per kg of nitrate-nitrogen removed or/and recovered, in relation to salinity.

| Salinity (mmolNaCl $L^{-1}$) | $V_{DN}$ Post-denitrification ($m^3$) | $V_{DN}$ Pre-denitrification ($m^3$) | $V_{PBR}$ at non-favorable conditions ($m^3$) | $V_{PBR}$ at favorable conditions ($m^3$) |
|---|---|---|---|---|
| 25 | 10.2 | 5.1 | 176.2 | 35.7 |
| 100 | 13.8 | 6.9 | 154.4 | 31.3 |
| 250 | 18.9 | 9.5 | 171.5 | 34.8 |
| 450 | 26.6 | 13.3 | 199.4 | 40.4 |
| 600 | 33.0 | 16.5 | - | - |

These results show that with an optimal *cell productivity* rate, achievable at higher light intensities (150 μmol $m^{-2}$ $s^{-1}$), $V_{PBR}$ is at the same order of magnitude to $V_{DN}$ having 2 to 4 times larger volume (1.5 to 3.0 at threshold salinity value). Moreover, *cell productivity* rate can be increased by means of controlling operational parameters linked to the design of photobioreactors, such as is the installation of baffle for better atmospheric air $CO_2$ transfer in the photobioreactor, which according to Rahman et al. (2021), can lead up to 2.17 times higher growth rate of *Synechococcus* sp. HS-9.

In addition to photobioreactor volume, which remained constant in relation to salinity, operational cost in terms of energy consumption is important for assessing the applicability of the *S*7942-based treatment process. A preliminary comparison of energy consumption between the proposed process and the widely applied AS pre-denitrification process was conducted. This comparison is based on the experimental data of this study and on literature data from two full-scale WWTPs (ENERWATER, 2020) and takes into account the energy consumption for agitation, lighting and nitrates recirculation.

The results showed that energy consumption per mass of nitrates removed/recovered of the *S*7942-based process is approximately 10.1 kWh $kgNO_3\text{-}N^{-1}$ and expected to be lower if energy consumption is compensated by biomass valorization. On the other hand, for the biological pre-denitrification process, energy consumption per mass of nitrates removed ranged from 5.8 kWh $kgNO_3\text{-}N^{-1}$ to 6.7 kWh $kgNO_3\text{-}N^{-1}$ and expected to be higher at saline wastewater.

It is thus concluded that implementation of a *S*7942-based phototrophic-based treatment stage in WWTPs is a tangible solution for more sustainable wastewater treatment.

Ongoing studies are conducted regarding (i) the optimization of *S*7942 process in low-cost tank photobioreactors, (ii) the performance of biohydrogen production, (iii) the life cycle assessment of AS wastewater treatment processes incorporating the *S*7942-based denitrification stage and (iv) the application of an *S*7942-based nutrient removal process from hydroponic effluents.

## 5.4. Conclusions

Cultivation of cyanobacteria *Synechococcus elongatus* PCC 7942 can serve as tertiary treatment for nitrogen removal/recovery from wastewaters offering the possibility of valorizing the produced biomass. Fundamental design parameters for the dimensioning of a photobioreactor are specific nitrogen utilization rate (*SNUR*), *cell productivity* rate along with available nitrogen. At increased wastewaters salinities up to a threshold value of 450 mmolNaCl $L^{-1}$, despite the observed decline of *cell productivity,* nitrates removal rate remains constant, which is attributed to the metabolic changes of *S*7942 leading to higher nitrogen assimilation in biomass. This results to constant photobioreactor volume (volume change < 12 %), while in widely applied AS denitrification processes the respective volume increases up to 260 % analogously to salinity. Thus, the photobioreactor volume can be at the same order of magnitude to an AS denitrification reactor given an optimal *cell productivity* and favorable light intensity. Even under non-favorable growth conditions of limited lighting (5-30 μmol $m^{-2}$ $s^{-1}$) the resulting photobioreactor volume is comparable to AS denitrification reactors. Moreover, metabolic changes of *S*7942, caused by salinity, lead to increasing specific nitrogen utilization rate, attributed to higher protein synthesis for the production of intracellular osmolyte (sucrose). The enrichment in sucrose content enhances biomass use as a raw material, both for the industrial sector and the renewable energy sector. Therefore, a *S*7942-based process can be adapted as a supplementary or an alternative treatment stage to the widely applied AS denitrification processes towards the minimization of wastewater treatment plant's ecological footprint and obtaining of added value products.

## 5.5. References


1. N. Abdel-Raouf, A.A. Al-Homaidan, I.B. Ibraheem, Microalgae and wastewater treatment, Saudi J. Biol Sci. 19 (2012) 257-275. https://doi.org/10.1016/j.sjbs.2012.04.005.
2. R. Wicker, A. Bhatnagar, Application of Nordic microalgal-bacterial consortia for nutrient removal from wastewater, Chem. Eng. J. 398 (2020) 1385-8947. https://doi.org/10.1016/j.cej.2020.125567.
3. T. Cai, Y.S. Park, Y. Li, Nutrient recovery from wastewater streams by microalgae: Status and prospects, Renew. Sust. Energ. Rev. 19 (2013) 360–369. https://doi.org/10.1016/j.rser.2012.11.030.
4. H. Duan, Y. Zhao, K. Koch, G.F. Wells, M. Zheng, Z. Yuan, L. Ye, Insights into Nitrous Oxide Mitigation Strategies in Wastewater Treatment and Challenges for Wider Implementation, Environ. Sci. Technol. 55 (2021) 7208-7224. https://pubs.acs.org/doi/10.1021/acs.est.1c00840.
5. N. Adouani, L. Limousy, T. Lendormi, O. Sire, N2O and NO emissions during wastewater denitrification step: Influence of temperature on the biological process, C. R. Chim. 18 (2015) 1631-0748. https://doi.org/10.1016/j.crci.2014.11.005.
6. USEPA (United States Environmental Protection Agency), Inventory of U.S. Greenhouse Gas Emissions and Sinks, accessed online December 2021. https://www.epa.gov/ghgemissions/inventory-us-greenhouse-gas-emissions-and-sinks.
7. Y. Law, L. Ye, Y. Pan, Z. Yuan, Nitrous oxide emissions from wastewater treatment processes. Philos. Trans. R. Soc. 367 (2012) 1265–1277. https://doi.org/10.1098/rstb.2011.0317.
8. S.D. Priyadharshini, P.S. Babu, S. Manikandan, R. Subbaiya, M. Govarthanan, N. Karmegam, Phycoremediation of wastewater for pollutant removal: A green approach to environmental protection and long-term remediation, Environ. Pollut. 290 (2021) 117989. https://doi.org/10.1016/j.envpol.2021.117989.
9. J. De la Noue, G. Laliberte, D. Proulx, Algae and waste water, J. Appl. Phycol. 4 (1992) 247–254. https://doi.org/10.1007/BF02161210.
10. E. Zhang, B. Wang, Q. Wang, S. Zhang, B. Zhao, Ammonia–nitrogen and orthophosphate removal by immobilized Scenedesmus sp. isolated from municipal wastewater for potential use in tertiary treatment, Bioresour. Technol. 99 (2008) 3787–3793. https://doi.org/10.1016/j.biortech.2007.07.011.
11. G. Markou, D. Georgakakis, Cultivation of filamentous cyanobacteria (blue-green algae) in agro-industrial wastes and wastewaters: a review, Appl. Energy. 88 (2011) 3389–3401. https://doi.org/10.1016/j.apenergy.2010.12.042.
12. K. Arora, P. Kaur, P. Kumar, A. Singh, S.K.S. Patel, X. Li, Y. Yang, S.K. Bhatia, S. Kulshrestha, Valorization of wastewater resources into biofuel and value-added products using microalgal system, Front. Energy Res. 9 (2021) https://doi.org/10.3389/fenrg.2021.646571.
13. M.I. Khan, J.H. Shin, J.D. Kim, The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products, Microb. Cell Fact. 17 (2018) 36. https://doi.org/10.1186/s12934-018-0879-x.
14. S.R. Chia, K.W. Chew, H.Y. Leong, S.H. Ho, H.S.H. Munawaroh, P.L. Show, CO2 mitigation and phycoremediation of industrial flue gas and wastewater via microalgae-bacteria consortium: Possibilities and challenges, Chem. Eng. J. 425 (2021) 131436. https://doi.org/10.1016/j.cej.2021.131436.

15. S. Leow, J.R. Witter, D.R. Vardon, B.K. Sharma, J.S. Guest, T.J. Strathmann, Prediction of microalgae hydrothermal liquefaction products from feedstock biochemical composition, Green Chem. 17 (2015) 3584-3599. https://doi.org/10.1039/C5GC00574D.
16. J. Pathak, Rajneesh, P.K. Maurya, S.P. Singh, D.P. Häder, R.P. Sinha, Cyanobacterial farming for environment friendly sustainable agriculture practices: innovations and perspectives. Front. Environ. Sci. 6 (2018) 7. https://doi.org/10.3389/fenvs.2018.00007.
17. T. Jayati, A. Mounika, D.P. Bangwal, K. Savita, M.O. Garg, Algae based biorefinery-How to make sense?, Renew. Sust. Energ. Rev. 47 (2015) 295–307. https://doi.org/10.1016/j.rser.2015.03.052.
18. D. Vayenos, G.E. Romanos, G.C. Papageorgiou, K. Stamatakis, Synechococcus elongatus PCC7942: a cyanobacterium cell factory for producing useful chemicals and fuels under abiotic stress conditions, Photosynth. Res.146 (2020) 235–245. https://doi.org/10.1007/s11120-020-00747-6.
19. K. Stamatakis, N.P. Ladas, A. Alygizaki-Zorba, G.C. Papageorgiou, Sodium chloride-induced volume changes of freshwater cyanobacterium Synechococcus sp PCC 7942 cells can be probed by chlorophyll a fluorescence, Arch. Biochem. Biophys. 370 (1999) 240-249. https://doi.org/10.1006/abbi.1999.1366.
20. C.D. Barry, F. Renou-Wilson, D. Wilson, C. Muller, R.H. Foy, Magnitude, form and bioavailability of fluvial carbon exports from Irish organic soils under pasture, Aquat. Sci. 78 (2016) 541–560. https://doi.org/10.1007/s00027-015-0464-x.
21. G. Samiotis, K. Stamatakis, E. Amanatidou, Assessment of Synechococcus elongatus PCC 7942 as an option for sustainable wastewater treatment, Water Sci. Technol. 84 (2021) 1438-1451. https://doi.org/10.2166/wst.2021.319.
22. A. Herrero, A.M. Muro-Pastor, E. Flores, Nitrogen control in cyanobacteria, J. Bacteriol.183 (2001) 411-425. https://doi.org/10.1128/JB.183.2.411-425.2001.
23. M. Aichi, S. Yoshihara, M. Yamashita, S. Maeda, K. Nagai, T. Omata, Characterization of the nitrate-nitrite transporter of the major facilitator superfamily (the nrtP gene product) from the Cyanobacterium Nostoc punctiforme strain ATCC 29133, Biosci. Biotechnol. Biochem. 70 (2006) 2682–2689. https://doi.org/10.1271/bbb.60286.
24. R.J. Ritchie, D.A. Trautman, A.W.D. Larkum, Phosphate Uptake in the Cyanobacterium Synechococcus R-2 PCC 7942, Plant Cell Physiol. 38 (1997) 1232-1241. https://doi.org/10.1093/oxfordjournals.pcp.a029110.
25. S.I. González-Morales, N.B. Pacheco-Gutiérrez, C.A. Ramírez-Rodríguez, A.A Brito-Bello, P. Estrella-Hernández, L. Herrera-Estrella, D.L López-Arredondo, Metabolic engineering of phosphite metabolism in Synechococcus elongatus PCC 7942 as an effective measure to control biological contaminants in outdoor raceway ponds, Biotechnol. Biofuels 13 (2020) 119. https://doi.org/10.1186/s13068-020-01759-z.
26. T. Sakamoto, N. Takatani, K. Sonoike, H. Jimbo, Y. Nishiyama, T. Omata, Dissection of the mechanisms of growth inhibition resulting from loss of the PII protein in the Cyanobacterium Synechococcus elongatus PCC 7942, Plant Cell Physiol. 62 (2021) 721–731. https://doi.org/10.1093/pcp/pcab030.
27. C.S.P. Silva, M.E. Silva-Stenico, M.F. Fiore, H.F. de Castro, P.C.M. Da Rós, Optimization of the cultivation conditions for Synechococcus sp. PCC7942 (cyanobacterium) to be used as feedstock for biodiesel production, Algal Res. 3 (2014) 1–7. https://doi.org/10.1016/j.algal.2013.11.012.
28. E.W. Rice, R.B. Baird, A.D. Eaton, APHA Standard Methods for the Examination of Water and Wastewater, 23nd Ed., Washington, DC: American Public Health

Association/American Water Works Association/Water Environment Federation, 2017.
29. R. Moran, Formulae for determination of chlorophyllous pigments extracted with N,N-Dimethylformamide, Plant Physiol. 69 (1982) 1376–1381. https://doi.org/10.1104/pp.69.6.1376.
30. A.M. Ruffing, H.D.T. Jones, Physiological effects of free fatty acid production in genetically engineered Synechococcus elongatus PCC 7942, Biotechnol. Bioeng. 109 (2009) 2190-2199. https://doi.org/10.1002/bit.24509.
31. S. Boussiba, A.E. Richmond, C-phycocyanin as a storage protein in the blue-green alga Spirulina platensis, Arch. Microbiol. 125 (1980) 143–147. https://doi.org/10.1007/BF00403211.
32. A.N. Glazer, S. Fang, Chromophore Content of Blue-Green Algal Phycobiliproteins, J. Biol.Chem. 248 (1972) 659-662.
33. J.K. Oyadomari, Keweenaw Algae: A Collection of Freshwater Algae (and Protozoans) from the Keweenaw Peninsula. http://www.keweenawalgae.mtu.edu, 2001 (accessed 20 June 2020).
34. E. Amanatidou, G. Samiotis, E. Trikoilidou, D. Tzelios, A. Michailidis, Influence of wastewater treatment plants operational conditions on activated sludge microbiological and morphological characteristics, Environ. Technol. 37 (2015) 265-278. https://doi.org/10.1080/09593330.2015.1068379.
35. E. Amanatidou, E. Trikoilidou G. Samiotis, N.P. Benetis, N. Taousanidis, An easy uncertainty evaluation of the COD titrimetric analysis in correlation with quality control and validation data. Method applicability region. Anal. Methods 4 (2012) 4204–4212. https://doi.org/10.1039/C2AY25687H.
36. E. Trikilidou, G. Samiotis, L. Tsikritzis, E. Amanatidou, Performance of Semi-Micro-Kjeldahl Nitrogen Method – uncertainty and nitrate interference. Int. J. Environ. Anal. Chem. (2020) 1–11. https://doi.org/10.1080/03067319.2020.1807967.
37. O.A. Koksharova, J. Klint, U. Rasmussen, The first protein map of Synechococcus sp. PCC 7942, Microbiology 75 (2005) 765-774. https://doi.org/10.1134/S0026261707010195.
38. J. Ungerer, P. Lin, H. Chen, H.B. Pakrasi, Adjustments to photosystem stoichiometry and electron transfer proteins are key to the remarkably fast growth of the Cyanobacterium Synechococcus elongatus UTEX 2973, mBio. 9 (2018) https://doi.org/10.1128/mBio.02327-17.
39. R.L. Clark, L.L. McGinley, H.M. Purdy, T.C. Korosh, J.L. Reed, T.W. Root, B.F. Pfleger, Light-Optimized Growth of Cyanobacterial Cultures: Growth Phases and Productivity of Biomass and Secreted Molecules in Light-Limited Batch Growth, Metab. Eng. 47 (2018) 230–242. https://doi.org/10.1016/j.ymben.2018.03.017.
40. S.D. Princiotta, S.P. Hendricks, D.S. White, Production of Cyanotoxins by Microcystis aeruginosa Mediates Interactions with the Mixotrophic Flagellate Cryptomonas, Toxins 11(2019) 223. https://doi.org/10.3390/toxins11040223.
41. D. Coutinho, A. Vargas, C. Feudjio, M. Benavides, A. Vande Wouwer, A robust approach to the design of super-twisting observers – application to monitoring microalgae cultures in photo-bioreactors, Comput Chem Eng 121 (2019) 46-56. https://doi.org/10.1016/j.compchemeng.2018.07.006.
42. J. Zambrano, I. Krustok, E. Nehrenheim, B. Carlsson, A simple model for algae-bacteria interaction in photo-bioreactors, Algal Res. 19 (2016) 155-161. https://doi.org/10.1016/j.algal.2016.07.022.
43. Ehira S, Kimura S, Miyazaki S, Ohmori M. Sucrose synthesis in the nitrogen-fixing Cyanobacterium Anabaena sp. strain PCC 7120 is controlled by the two-component

response regulator OrrA, Appl Environ Microbiol. 80 (2014) 5672-5679. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4178590/.
44. O. Stein, D. Granot, An Overview of Sucrose Synthases in Plants, Front. Plant Sci. 10 (2019) 95. https://www.frontiersin.org/articles/10.3389/fpls.2019.00095/full.
45. A. Nunes-Nesi, F. Alisdair R., S. Mark, Metabolic and Signaling Aspects Underpinning the Regulation of Plant Carbon Nitrogen Interactions, Mol. Plant 3 (2010) 973-996. https://doi.org/10.1093/mp/ssq049.
46. N.P. Ladas, G.C. Papageorgiou, The salinity tolerance of freshwater cyanobacterium Synechococcus sp. PCC 7942 is determined by its ability for osmotic adjustment and presense of psmolyte sucrose, Photosynthetica 38 (2001) 343-348.
47. R.R. Sonani, R.P. Rastogi, S.N. Patel, M.G. Chaubey, N.K. Singh, G.D. Gupta, V. Kumar, D. Madamwar, Phylogenetic and crystallographic analysis of Nostoc phycocyanin having blue-shifted spectral properties, Sci. Rep. 9 (2019) 9863. https://doi.org/10.1038/s41598-019-46288-4.
48. K.M. Kim, J.Y. Lee, A.-R. Im, S. Chae, Phycocyanin Protects Against UVB-induced Apoptosis Through the PKC α/βII-Nrf-2/HO-1 Dependent Pathway in Human Primary Skin Cells, Molecules 23 (2018) 478-490. https://doi.org/10.3390/molecules23020478.
49. Md.A. Alam, J.-L. Xu, Z. Wang, Microalgae Biotechnology for Food, Health and High Value Products, 1st ed., Springer, Biomedical Sciences, 2020.
50. G. Tchobanoglous, L.F. Burton, H.D. Stensel, Wastewater Engineering: Treatment and Reuse. Metcalf and Eddy Inc., 4th ed., McGraw Hill, Boston, MA, USA. 2003.
51. EPA -Environmental Protection Agency, Nutrient Control Design Manual. State of Technology, Review Report, Washington, http://www.epa.gov, 2010 (accessed October 2021).
52. P. Dold, I. Takács, Y. Mokhayeri, A. Nichols, J. Hinojosa, R. Riffat, C. Bott, W. Bailey, S. Murthy, Denitrification with Carbon Addition—Kinetic Considerations, Water Environ. Res. 80 (2008) 417-427. https://doi.org/10.2175/106143007X221085.
53. A.R. Dincer, F. Kargi, Salt inhibition of nitrification and denitrification in saline wastewater, Environ. Technol. 20 (1999) 1147-1153. https://doi.org/10.1080/09593332008616912.
54. C. Glass, J. Silverstein, Denitrification of high-nitrate, high salinity wastewater, Water Res. 33 (1999) 223-229. https://www.sciencedirect.com/science/article/pii/S0043135498001778.
55. M.J. Garcia-Ruiz, A. Castellano-Hinojosa, J. Gonzalez-Lopez, F. Osorio, Effects of salinity on the nitrogen removal efficiency and bacterial community structure in fixed-bed biofilm CANON bioreactors, Chem. Eng. J. 347 (2018) 156-164. https://www.sciencedirect.com/science/article/pii/S138589471830634X.
56. A. Rahman, J. Dwi Putra, N. Betawati Prihantini, T.M.I. Mahlia, M. Aziz, Deendarlianto, N. Nasruddin, C ultivation of Synechococcus HS-9 in a novel rectangular bubble column photobioreactor with horizontal baffle, Case Stud. Eng. 27 (2021) 101264. https://doi.org/10.1016/j.csite.2021.101264.
57. ENERWATER, Methodology for Rapid Audit (RA) - D3.4, Horizon 2020 ENERWATER Project; European Commission: Brussels, Belgium (2015). https://ec.europa.eu/research/participants/documents/downloadPublic?documentIds=080166e5bef0e5c6&appId=PPGMS.

# 6. Chapter 6. Integrated management of hydroponic wastewater for complete water recycle and cyanobacteria cultivation using an electric conductivity-based tool

This work presents an integrated management process for total hydroponic wastewater (HWW) reuse, by developing an electric conductivity-based tool that calculates enrichment requirements of recycled streams and indicates their proper treatment method. Treatment incudes advanced methods and *Synechococcus elongatus* PCC 7942 (*S*7942) cyanobacteria cultivation for HWW nutrients removal and valorization. The results showed that HWW can be utilized as substrate for *S*7942 cultivation. Increasing initial substrate nitrate-nitrogen concentration from 389 ±3 mg $L^{-1}$ to 2132 ±17 mg $L^{-1}$ increases nitrogen assimilation from 0.131 mg$NO_3$-N mg$VSS^{-1}$ to 0.163 mg$NO_3$-N mg$VSS^{-1}$. Similarly, increasing initial substrate phosphate-phosphorous concentration from 8.4 ±0.2 mg $L^{-1}$ to 138.2 ±5.0 mg $L^{-1}$ increases phosphorous assimilation from 0.022 mg$PO_4$ mg$VSS^{-1}$ to 0.133 mg$PO_4$ mg$VSS^{-1}$. Ion exchange advanced treatment exhibited efficiency greater than 98%. Adsorption with activated carbon and nanomaterials showed selective removal of nitrates, phosphates, calcium and magnesium. Upon application of sonication, the ion selectivity of nanomaterials increased.

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## 6.1. Introduction

The conservation of water resources both for human use and for the production of agricultural products, to meet the needs of a constantly increasing global population, constitutes a challenge for the traditional agriculture i.e. the cultivation in soil, due to the climate change, urban expansion and soil-water contamination (Surendran et al., 2017; Souza et al., 2019; Gentry 2019; Richa et al., 2020). During the past decades, soilless agricultural techniques have been applied to increase crop productivity (Mahjoor et al., 2016). Due to the worldwide increased of hydroponic farming, which is almost exclusively applied in greenhouse crops (Lopez-Galvez et al., 2014), the challenge regarding the management of generated wastewater emerged (Richa et al., 2020).

Nutrients concentration in the initial nutrient solution, which is used for plants fertigation, depends on many parameters such as plants species, plants growth stage, greenhouse climate, type of substrate as well as the applied hydroponic technique i.e., open hydroponic system" (OHS) where the nutrient solution run off to the environment after each irrigation event or "closed hydroponic system" (CHS) where usually a portion of the nutrient solution is reused for crop irrigation (Mohammed 2018). CHSs present the advantage of reducing water and fertilizer consumption, as well as the production of lower volume of wastewater that may pollute water resources (Rufí-Salís et al., 2020). On the other hand, the constant recirculation of effluent leads to the gradually increase of salts concentration, especially that of NaCl, in the nutrient solution (Savvas et al., 2007; Mielcarek et al., 2019), which eventually becomes unsuitable for use and must be replaced. Nevertheless, CHSs is barely implemented in the Mediterranean region countries, where the lack of water and the deterioration of its quality is already a major problem, mainly due to the high investment costs, the high salinity of the groundwater used for the nutrient solution preparation (Magan et al., 2008) and the fear for plants root system infections.

Effluents of both OHS and CHS contain excessive amounts of organic and inorganic compounds that may lead to degradation of the environment, if not properly treated prior their disposal (Isozaki et al., 2004; Richa et al., 2020). The main problem in the management and reuse of the nutrient solution resulting from hydroponic systems, is

its unstable composition. In general, several nutrient elements have significant higher concentration in hydroponic wastewater than in the initial nutrient solution used for plants fertigation.

Hence, due to their composition and in compliance with the principals of circular economy (reduce-reuse-recycle), secondary cultivation systems (cascade crop) of salt-tolerant species have been proposed as the most promising alternative solution for the treatment and utilization of hydroponic wastewaters (HWW) to remove/recover their nitrogen and phosphorous content before discharge the used nutrient solution to the environment (Dorais and Dube, 2011; Santos and Pires, 2018; Li et al., 2018; Richa et al., 2020). It is worth mentioning that reuse of HWW is a complicated procedure when it is based on physicochemical measurements and the quantification of specific ions present in the solution. Hence, OHS are usually applied by producers despite the significant quantities of generated HWW. However, as cascade crops are usually cultivated plant species of low commercial value (García-Caparrós et al., 2018; Avdouli et al., 2021), which occupy valuable space in the greenhouse, they require different cultivation treatment and grower specialization, while their products are addressed to a different market than the main crop.

Another perspective could be the management of the HWW using cyanobacteria and/or microalgae. Since Oswald and Golueke (1960) first pointed out the possibility of using microalgae for bioremediation of wastewater and with increasing interest during the past decade, several reports have been published on the subject of cyanobacteria or/and microalgae cultivation in wastewater streams, highlighting the potential merits (Cuellar-Bermudez et al., 2017; Melo et al., 2021). Due to the composition of various wastewater streams, such as HWW that contain significant quantities of nutrients, they can be an excellent substrate for cyanobacteria or/and microalgae cultivation. Their cultivation can result in the acquisition of added value products and/or biofuels, offering both environmental and economic benefits (Kumar and Cho, 2014; Richa et al., 2020).

The use of cyanobacteria or/and microalgae in order to recover nutrients and water for HWW (Hultberg et al., 2013; Huo et al., 2020; Salazar et al., 2021) can be combined with biodiesel, food supplements and cosmetics production (Chisti, 2007; Bertoldi et al., 2009; Huang et al., 2010; Mata et al., 2010; Lee et al., 2018). Furthermore, some cyanobacteria species, such as *Synechococcus elongatus* PCC 7942 (hereafter *S*7942),

can be manipulated for direct biohydrogen production via the metabolic path of anaerobic dark fermentation of its intracellular osmolyte (sucrose). In specific, when *S*7942 is exposed to increased salinity levels (up to 450 mmolNaCl $L^{-1}$), its growth is practically inhibited (Samiotis et al., 2022a). Under these conditions *S*7942 increases its sucrose levels to compensate osmotic changes by regulating its inner turgor pressure (Vayenos et al., 2020). This phenomenon increases the value of the cultivated biomass and may enhance the viability of a *S*7942-based HWW treatment system that utilizes the relatively saline HWW of CHSs. In addition, microalgae seem to buffer several parameters of the nutrient solution such as pH, dissolved oxygen, concentration of phytohormons and nutrients consumption. For these reasons co-cultivation of cyanobacteria and/or microalgae may be beneficial for hydroponically grown crops since they can act as biofertilizers, promoting plants growth (especially during the early growth stages), germination, and root development (de Camargo et al., 2015; La Bella et al., 2022).

A HWW management concept that can be easily controlled by means of Electric Conductivity (EC) and that can incorporate a cyanobacteria cultivation HWW treatment stage and advanced treatment methods seems a tangible goal towards sustainable HWW treatment and complete water recovery. For a given HWW composition (ion profile) EC can indicate ions concentration and assist in the calculation of chemicals dosage and enrichment requirements for HWW reuse in the preparation of new nutrient solution. Moreover, EC can indicate when HWW cannot be further recycled and must be transferred for advanced treatment and reuse. Cyanobacteria cultivation is one desirable treatment stage that can result in minimization of HWW treatment cost due to the potential acquisition of added value product and/or green energy.

The aim of this work is to propose an EC-controlled integrated management process for HWW reuse, treatment and valorization. An EC-based tool that indicates if HWW will be reused, treated, or disposed in the environment is developed and presented. For the treatment and reuse of HWW, the application of ion-exchange and/or adsorption advanced methods was evaluated. For HWW valorization i.e., the acquisition of added value products or/and green energy, the cultivation of *Synechoccoccus elongatus* PCC 7942 (*S*7942) cyanobacteria was studied in terms of cell productivity and nutrients removal by assimilation.

## 6.2. Materials and Methods

The proposed solution for the integrated management of HWW is based on (i) the reuse of HWW for the preparation of new nutrient solution via an EC-control tool that rapidly quantifies chemicals dosage for enrichment, (ii) the treatment/valorization of HWW for the cultivation of cyanobacteria in a photobioreactor (PBR) and (iii) the advanced treatment of HWW or PBR effluents for reuse or disposal in the environment. The downstream process for the reuse, treatment and valorization of HWW is illustrated in Figure 6.1.

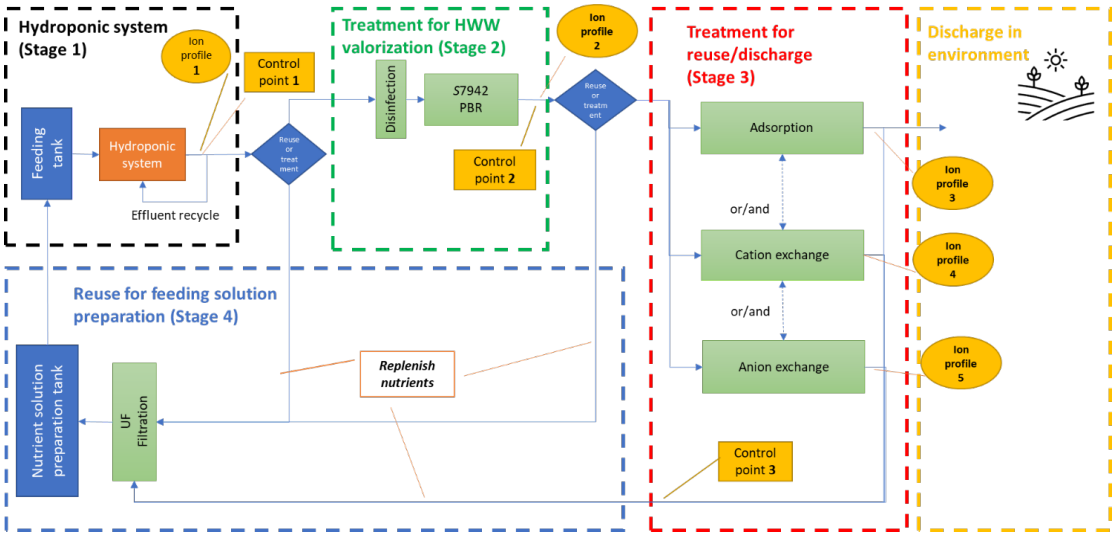


**Figure 6.1.** Flowchart of proposed process for integrated management of hydroponic wastewater.

As illustrated in Figure 6.1, the process consists of four distinct stages: Stage 1 – Hydroponic system; Stage 2 – HWW treatment/valorization via cyanobacteria cultivation; Stage 3 – Advanced effluent treatment; Stage 4 – Reuse of effluents.

HWW originated from nutrient solution which was used for the irrigation of greenhouse rose crop, grown in open and closed hydroponic system. The plants were irrigated for three minutes every hour, from 8:00 to 17:00 daily. The initial nutrient solution composition used for plants irrigation is presented in Table 6.2.

### 6.2.1. Determination of ions content via EC measurement

The developed EC-based tool for the determination of ions content in effluent streams from each stage takes into consideration EC measurements, once measured ions concentration and the calculated contribution of each ion on EC. By using ion

contribution, EC can be used as effluent composition indicator. The use of EC for approximate quantification of ions concentration can assist in the preparation of new nutrient solution and the selection of treatment stage for reuse or discharge in the environment. EC is measured at defined control points. When EC reaches a critical value at a control point (Figure 1), the hydroponic nutrient solution must be discharged for further treatment. EC critical value is the point where salts accumulation is estimated to exceed threshold values for plants growth and cannot be reused.

EC measurements was used for the approximation of cations or anions concentration (Equation 1) by taking into account equivalent conductances ($\lambda^{o}_{+}$ and $\lambda^{o}_{-}$, mho cm$^2$ equivalent$^{-1}$) of ions (Table 6.1), the monovalent ion conductivity coefficient ($y$) (Equation 2) and the contribution of $i$ ion on total measured $EC$ ($a_i$). To obtain the proposed Equation (1), the equations presented in 2510 B standard method for the examination of water and wastewater (APHA, 2017) were used.

**Table 6.1.** Equivalent conductances for specific cations and anions at 25ºC.

| Cation | $\lambda^{\circ}_{+}$ | Anion | $\lambda^{\circ}_{-}$ |
|---|---|---|---|
| $H^+$ | 350 | $OH^-$ | 198.6 |
| $1/2Ca^{2+}$ | 59.5 | $HCO_3^-$ | 44.5 |
| $1/2Mg^{2+}$ | 53.1 | $1/2CO_3^{2-}$ | 72 |
| $Na^+$ | 50.1 | $1/2SO_4^{2-}$ | 80.0 |
| $K^+$ | 73.5 | $Cl^-$ | 76.4 |
| $NH_4^+$ | 73.5 | $F^-$ | 54.4 |
| $1/2Fe^{2+}$ | 54 | $NO_3^-$ | 71.4 |
| $1/2Fe^{3+}$ | 68 | $H_2PO_4^-$ | 33 |
| | | $1/2HPO_4^{2-}$ | 57 |

Hence, in this study, the total EC and ion content of each stage effluent was measured and the partial EC ($k_{calc}$) of $i$ ion was calculated (Equation 3). The % contribution ($a_i$) of $i$ ion on total measured EC was calculated by dividing $k_{calc}$ with measured total EC. Thereafter, the $i$ ion concentration of each stage effluent was calculated according to Equation (1).

$$C_i = \frac{EC * a_i^{-2} * MW_i}{y^2 * \lambda_i * z_i} \quad (1)$$

Where, $EC$ is the measured total electric conductivity of effluent (µS cm$^{-1}$ at 25ºC); $a_i$ is the experimentally obtained % contribution of $i$ ion on $EC$; $MW_i$ is the molecular weight of $i$ ion (g mol$^{-1}$); $y$ is the monovalent ion conductivity coefficient; $\lambda_i$ is the equivalent conductance of $i$ ion (mho cm$^2$ equivalent$^{-1}$); $z_i$ is the absolute value of the charge of $i$ ion.

The monovalent ion activity coefficient ($y$) for $k_{calc}$ and $C_i$ determination was calculated using Equation (3) for $IS < 0.5$ M and for temperatures from 20 ºC to 30 ºC (Robinson and Stokes, 1959; Davies, 1962).

$$y = 10^{-0.5[IS^{1/2}/(1+IS^{1/2})-0.3IS]} \quad (2)$$

Where, $IS$ is the ion strength of the solution in molar units calculated according to the Equation 2.1.

$$IS = \Sigma z_i^2 (mM_i)/2000. \quad (2.1)$$

The $k_{calc}$ mathematical formula is presented as follow:

$$k_{calc} = k^{\circ} y^2 \quad (3)$$

Where, $y$ is the monovalent ion activity coefficient; $k^o$ is the infinite dilution conductivity calculated according to Equation 3.1.

$$k^{\circ} = \Sigma |z_i| (\lambda_{+i}^{\circ})(mM_i) + \Sigma |z_i| (\lambda_{-i}^{\circ})(mM_i) \quad (3.1)$$

*Where,* $|z_i|$ is the absolute value of the charge of the $i$ ion; $mM_i$ is the millimolar concertation of the $i$ ion; $\lambda^{\circ}_{+i}$, $\lambda^{\circ}_{-i}$ is equivalent conductance of the $i$ ion.

In this study, a Metrohm (Switzerland) ion chromatographer (model 881 compact IC pro equipped with Metrosep A Supp 5 - 250/4.0 and Metrosep C 4 - 250/4.0 columns) was used for the determination of nitrates, ammonium, phosphates, potassium, calcium, magnesium, sulphates, chloride and sodium, according to standard methods (ISO 14911:1998; ISO 10304-1:2007). The measurement of EC and pH was conducted following standard methods for the examination of water and wastewater (APHA, 2017 2510 B & 4500-H+ B), using benchtop meters of Thermos Scientific (USA) (pH-meter: model ORIONSTAR A111 equipped with a UZ1-11066 electrode; EC-meter: model ORION 3 STAR equipped with an Orion013005MD electrode). All analyses were conducted at the accredited according to ISO 17025 "Environmental Chemistry & Water and Wastewater Treatment Laboratory", Department of Chemical Engineering, University of Western Macedonia, Greece. The laboratory calculates measurement

uncertainties in all the applied analytical methods (Amanatidou et al., 2011; Amanatidou et al., 2012; Trikilidou et al., 2020).

### 6.2.2. Stage 1 – Hydroponic system

During the first stage, the ion content of the nutrient solution drained from the OHS or the CHS was evaluated by EC measurement using the once experimentally established ion profile of HWW stream i.e., the measurement of ions concentration and the calculation of their contribution on EC. In the case of CHS, the effluent nutrient solution was continuously recycled, without addition of water and nutrients and pH correction, until their EC exceeded 3 mS $cm^{-1}$. This EC threshold was selected by operator empirically in order to assure that no plant growth hindering ion is accumulated into the nutrient solution. Samples from the OHS effluent and the nutrient solution remaining in the recycling tank of the CHS (when its conductivity exceeded 3 mS $cm^{-1}$) were collected and used in this study. Thereafter, depending on the samples' ion content and the threshold concentration values for unhindered plant growth, HWW were either recycled or used in the preparation of new nutrient solution to minimize chemicals and water consumption or subjected to further treatment. The HWW specific ions concentration threshold values (thus EC critical values) depend on many factors, such as the cultivated plant species, plants growth stage, climate conditions etc. and set at case by the hydroponic system operator.

### 6.2.3. Stage 2 – HWW treatment/valorization via S7942 cultivation

Once reaching the critical EC value, the HWW is discarded into a cyanobacteria cultivation PBR for the removal of nitrogen and phosphorous and the potential production of added value products and/or the of green energy. In this study, the selected cyanobacteria strain was *S*7942, which was cultivated in a series of duplicate control setups with BG11 growth media or duplicate test setups with properly disinfected HWW from OHS and from CHS. Culture setups with diluted 1:2 in water BG11 medium (BG-low) were also used in order to evaluate the impact of lower nutrients concentration on *S*7942 growth and on nutrients assimilation rates. Disinfection of HWW via filtration coupled with chemical disinfection was applied prior the feeding of *S*7942 PBR, as described in Samiotis et al. (2021), since it was considered essential for preventing the growth of microbial species that hinder cyanobacteria growth. The necessity of HWW disinfection was demonstrated by

additional experiments using duplicate control setups and duplicate test setups with non-disinfected OHS or CHS wastewater.

The PBRs were made of a 750 mL cylindrical glass vessel (hight = 27 cm; diameter = 6 cm) tapped with sterilized cotton caps for unhindered air transfer and avoidance of airborne contamination. Active volume of PBR was 500 mL, which gradually reduced to approximately 350 mL after 20 days of monitoring due to samplings. Continuous agitation (magnetic stirring at 200 rpm) and lighting (11 Watt fluorescent light and ambient light) was applied at light intensities of 5 to 35 μmol $m^{-2}$ $s^{-1}$ for a duration period of 20 days, at controlled temperatures of 21.3 to 23.7 ºC. Nitrates and phosphates reduction rates along with cellular growth rates were experimentally determined in all setups.

The evaluation of *S*7942 growth rate was made in terms of chlorophyll *a* (Chl *a*) concentration and the cell productivity rate was calculated using Equation (6). The concentration of volatile suspended solids (VSS) was calculated based on Chl *a* concentration and the conversion factor of 56.31 mgVSS mgChl $a^{-1}$ (Samiotis et al., 2021).

$$Cp = \frac{VSS_1 - VSS_0}{t} \quad (6)$$

Where, *Cp* (mg VSS $L^{-1}$ $d^{-1}$) is the cell productivity; $VSS_0$ (mg $L^{-1}$) is the initial VSS concentration; $VSS_1$ (mg $L^{-1}$) is the final VSS concentration in (mg $L^{-1}$); *t* (days) = the duration between initial and final VSS concentration.

The calculation of *S*7942's growth rate, nitrates utilization rate (mg$NO_3$_N $L^{-1}$ $d^{-1}$) and phosphates utilization rate (mg$PO_4^{3-}$ $L^{-1}$ $d^{-1}$), resulted from slope of the linear regression of Chl *a* concentration, nitrate-nitrogen and phosphates concentration over time respectively (Samiotis et al., 2021; Samiotis et al., 2022a).

Specific nitrates utilization rate (*SNUR*), expressed as mg$NO_3$_N $mgVSS^{-1}$, was calculated according to Equation (7) by dividing nitrates utilization rate with cell productivity (*Cp*).

$$SNUR = \frac{Nitrates\ utilization\ rate}{Cp} \quad (7)$$

Similarly, specific phosphates utilization rate (*SPUR*), expressed as mg$PO_4$ mg$VSS^{-1}$, was calculated according to Equation (8) by dividing phosphates utilization rate with cell productivity (*Cp*).

$$SPUR = \frac{Phosphates\ utilization\ rate}{Cp} \quad (8)$$

Statistical analysis between control and test setups was performed using IBM's (USA) software "SPSS Statistics 20" and by conducting (a) independent t-test for evaluating statistical differences among data sets and (b) bivariate analysis (Spearman's rank correlation) for identifying potential statistical correlations among data sets.

Finally, the evaluation of *S*7942 culture contamination and the identification of growth hindering microbial species in the PBRs was conducted via microscopical examination of cultures using a Leica DM100 phase contrast microscope and based on relevant databases (Oyadomari 2001; APHA 2017).

At the end of Stage – 2, the ion content of PBR effluent was evaluated in control point 2 (Figure 6.1), according to the methodology described in section 2.1., in order to decide whether it will be reused or be further treated, which was set by operator at 3 mS $cm^{-1}$.

### 6.2.4. Stage 3 – Advanced effluent treatment

In this stage, when EC of HWW from control points 1 or 2 indicates high concentration of specific ions, to a level that may hinder plant growth or exceed legislation limits in cases of reuse or discharge into the environment, they are subjected to advanced treatment techniques for the selective removal of specific ions.

For the advanced treatment of effluents, the widely applied techniques of ion-exchange and adsorption were evaluated based on experimentally obtained data. Different types of commercially available ion-exchange resins and adsorption materials and nanomaterials were evaluated in terms of ion removal efficiency in relation to the imposed operational conditions. In more detail, a regenerable anion exchange resin in $OH^-$ form, a regenerable cation exchange resin in $H^+$ form and a non-regenerable mixed bed resin were evaluated regarding their selectivity and efficiency for anions or cations removal. Moreover, the adsorption efficiency of Activated Carbon (AC) particles of different diameter (granular 3 mm, powder 0.6 mm, fine powder <0.075 mm) were evaluated, as well as of two commercially available nanomaterials (Carbon Black and

Cloisite 30B) that had been previously studied for the fabrication of nanocomposites (Stimoniaris et al., 2012; Stergiou et al., 2015). Furthermore, the effect of sonication in the adsorption efficiency of AC and of nanomaterials was evaluated by using either pre-sonicated adsorption nanomaterials or by applying ultrasounds during the treatment process in order to suppress the possible formation of agglomerates via a 200W Hielscher (Germany) ultrasonic processor, model UP200S. The applied contact time, as well as sonication time ranged from 1 min up to 60 min.

### 6.2.5. Stage 4 – Reuse of effluents

This step can be applied after control point 1, 2 and 3 (Figure 6.1) for effluent reuse in the preparation of new nutrient solution when EC exceeds set point value. Enrichment requirements were evaluated based on effluent composition in the respective control point, determined via EC measurement (section 2.1.). It should be noted that all recycle streams used for the preparation of fresh nutrient solution are usually subjected to ultrafiltration or similar techniques prior their enrichment with nutrients to avoid possible contamination spread in the hydroponic system. In this study, ultrafiltration was avoided because disinfected effluents where used.

Same steps for evaluating the composition of effluents by EC measurements were followed in all three control points regardless the composition of the solution.

The first step for the preparation of nutrient solution using HWW or treated HWW was to evaluate its composition via EC measurement and by using Equation (1) for each ion of interest. Thereafter, the final concentration of ions in the nutrient solution preparation tank was calculated based on tank volume and on the physicochemical characteristics of the dilution water.

## 6.3. Results and discussion

### 6.3.1. Stage 1 – Hydroponic system

According to the conducted measurements for the establishment of ion profile in control point 1 and as evident in Table 6.2, the OHS effluent presented lower EC to that of nutrient solution due to the significantly lower concentration of nitrates, phosphates potassium and calcium (plant uptake) and despite the increased concentration of other ions. On the other hand, EC in CHS effluent was approximately 3 and 4 times greater than that of nutrient solution and OHS effluent respectively. The scale of difference in

EC is indicative of ions accumulation in the solutions. The increase of specific ions concentration in OHS and CHS effluents is attributed to plant water transpiration and water evaporation. In CHS, both plant water transpiration and water evaporation increase analogously to the times of recirculation. Hence, different type of crop or/and different number of effluent recirculations generate HWW of different composition, in which the contribution of each ion on EC significantly differs. This is evident in Table 6.2, where the measured ion content and the calculated according to methodology described in section 2.1. contribution of each ion on EC of nutrient solution, OHS HWW and CHS HWW significantly differs (independent t test, Sig.<0.05). Nonetheless, once the concentration of specific ions and their contribution on EC of the HWW has been initially established (ion profile) for a given crop and at specific operating conditions, the expected composition can be evaluated based only on EC measurement.

**Table 6.2.** Nutrient solution characteristics - OHS and CHS effluent composition and ions contribution on EC.

| Physicochemical parameter | Nutrient Solution | % contribution on EC | OHS HWW | % contribution on EC | CHS HWW | % contribution on EC |
|---|---|---|---|---|---|---|
| EC (mS/cm at 25°C) | 1.7 | - | 1.3 | - | 5.1 | - |
| pH | 5.5 | < 1 | 7.5 | < 1 | 4.4 | < 1 |
| Nitrates (mg $L^{-1}$) | 762 | 38.8 | 389 | 27.0 | 2132 | 32.0 |
| Ammonium (mg $L^{-1}$) | 18.0 | 3.2 | 0.1 | < 1 | 9.8 | < 1 |
| Phosphates (mg $L^{-1}$) | 94 | 1.4 | 14 | < 1 | 138 | < 1 |
| Potassium (mg $L^{-1}$) | 250 | 20.8 | 57 | 6.5 | 552 | 13.5 |
| Calcium (mg $L^{-1}$) | 160 | 21.0 | 48 | 8.6 | 99 | 3.8 |
| Magnesium (mg $L^{-1}$) | 24 | 4.6 | 64 | 16.9 | 260 | 14.8 |
| Sulphates (mg $L^{-1}$) | 72 | 5.3 | 56 | 5.6 | 188 | 4.1 |
| Chloride (mg $L^{-1}$) | 23 | 2.2 | 80 | 3.0 | 126 | 3.5 |
| Sodium (mg $L^{-1}$) | 15 | 1.4 | 44 | 5.8 | 85 | 2.41 |

Worth mentioning at this point that the determination of the HWW ion profile is a procedure that should be performed only once for every type of crop and only repeated when significant differences on ion profile are expected i.e., during distinguished phases of plant growth or when environmental conditions dramatically change.

### 6.3.2. Stage 2 – HWW treatment/valorization via *S*7942 cultivation

#### 6.3.2.1. HWW adequacy as cyanobacteria cultivation medium

According to the results of physicochemical analyses of OHS and CHS HWW, both effluents were considered adequate for cyanobacteria cultivation in terms of nitrogen

and phosphorous availability. The resulting nitrogen to phosphorous (N:P) ratio in the OHS and CHS were 16:1.3 and 16:5.2 respectively. Both ratios are lower than Redfield's average ratio of 16:1, considered sufficient for cyanobacteria growth (Richmond and Hu, 2013), indicating high phosphorous content. N:P ratios lower than 16:1 can enhance assimilation of phosphorous into cyanobacteria biomass by triggering luxury P uptake (Solovchenko et al., 2020). Moreover, the concentration of essential elements in both effluents, such as metallic ions are sufficient for plant growth. Finally, it must be mentioned that CHS effluent presents negligible alkalinity (4 $mgCaCO_3$ $L^{-1}$), as indicated by its acidic pH of 4.4. Absence of alkalinity implies absence of bicarbonates and carbonates that are inorganic carbon sources for the growth of photoautotrophic species. On the other hand, OHS effluent had a relatively high alkalinity of 214 $mgCaCO_3$ $L^{-1}$, indicative of the increased presence of bicarbonates in the solution. The absence of alkalinity in CHS HWW did not had implications on *S*7942 growth, due to solubilization of atmospheric $CO_2$ into the nutrient solution. Furthermore, the production of $OH^-$ during photosynthesis increased pH in PBR. After 20 days of cultivation, an increase of alkalinity and pH was observed from 4 $mgCaCO_3$ $L^{-1}$ to 173 $mgCaCO_3$ $L^{-1}$ and from 4.4 to 7.9 respectively.

In this study, as well as in previous studies (Samiotis et al., 2021), the presence of biological contaminants in HWW presents a significant challenge for maintaining a monoculture in the *S*7942 PBRs. Hence, in this work the necessity of HWW disinfection was demonstrated by the test setups with non-disinfected HWW. As evident from the evolution of Chl *a* concentration in Figure 6.2, the proliferation of competitive to *S*7942 and of predating microbial species hindered its growth and resulted to its demise. More specifically, the microscopic examination revealed that fungi where the dominant species that suppressed the growth of *S*7942, with significant presence of protozoan (mainly free-swimming ciliates) in non-disinfected HWW. There are two major implications of hindered *S*7942 growth by biological contaminants. The first is the low nutrients assimilation due to the lower cell productivities (Figure 6.2), thus low HWW treatment efficiency and larger PBR volume requirements. The second is the possibility of cyanotoxins producing bacteria proliferation in the *S*7942 cultures. *S*7942 monoculture via proper disinfection (Samiotis et al., 2022b) ensures that cyanotoxins are not produced.

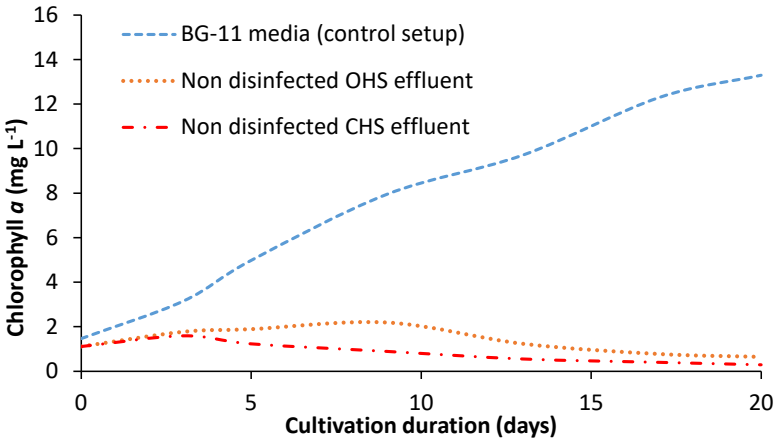


**Figure 6.2.** Evolution of Chl *a* concentration (average values) in control setups and in test setups with non-disinfected HWW.

The test setups with properly disinfected via filtration/chlorination (Samiotis et al., 2022b) wastewater substrate presented unhindered *S*7942 growth, despite the limited presence of fungi in the *S*7942 PBRs. The unhindered growth of *S*7942 in test setups with properly disinfected HWW is evident in Figure 6.3, where the growth rates of test setups, in terms of cell productivity (*Cp*), are similar to those obtained in control setups with BG-11 media or to the BG-Low media presenting difference of -3.05 % to 7.73 %. This similarity of *Cp* values is confirmed by the statistical analysis (independent t-test) between relative growth rates of control setups and properly disinfected test setups, which revealed that the mean score is not significantly different (Sig.>0.05).

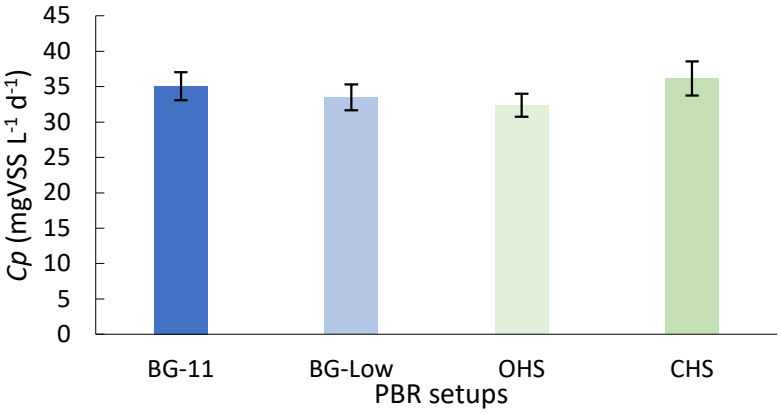


**Figure 6.3.** Average cell productivity (*Cp*) values and corresponding standard deviations of control setups and test setups with properly disinfected HWW.

Worth mentioning at this point that due to the possible formation of secondary toxic compounds during chlorination (chloramines), the disinfection process could be

unsuitable for cultivating cyanobacteria in food industry, but suitable for other application, such as green energy production. That been said, an alternative to chlorination, environmentally friendly disinfection technique that is based on in-situ electroproduction of ferrates via a $Fe^0//Fe^0$ cell, which has been evaluated in previous study with excellent results (Samiotis et al., 2022b), can be applied.

The obtained *Cp* values of the test setups with disinfected OHS or CHS wastewater media were 32.37 ± 1.63 mgVSS $L^{-1}$ $d^{-1}$ and 36.15 ± 2.43 mgVSS $L^{-1}$ $d^{-1}$ respectively, while of those with BG-11 or BG-Low media were 35.08 ± 1.97 mgVSS $L^{-1}$ $d^{-1}$ and 33.49 ± 1.84 mgVSS $L^{-1}$ $d^{-1}$ respectively. These results show that properly disinfected HWW can be utilized for the cultivation of cyanobacteria or/and microalgae and the acquisition of added value products or/and green energy.

#### 6.3.2.2. Assessment of nitrates and phosphates removal in *S*7942 cultivation PBRs

The effect of nutrients concentration on *SNUR* and *SPUR* was examined in this study based on the comparison between the obtained values in control and test setups. In the proposed *S*7942-based photoautotrophic treatment process, nitrogen and phosphates are removed from HWW by assimilation into biomass. At the imposed operational conditions of limiting lighting (5 to 35 μmol $m^{-2}$ $s^{-1}$) and no external addition of $CO_2$ in the *S*7942 setups, nitrates removal for a 20-day cultivation period was 59.90 ± 4.67 % and 23.11 ± 1.07 % in the OHS and CHS setups, corresponding to an average nitrates removal of 233 mg $L^{-1}$ and 490 mg $L^{-1}$ respectively. Phosphates removal for a 20-day cultivation period was 67.71 ± 9.80 % and 47.86 ± 3.51 % in the OHS and CHS setups, corresponding to an average phosphates removal of 9.5 mg $L^{-1}$ and 66.1 mg $L^{-1}$ respectively. The lower nitrates and phosphates % removal efficiency of CHS setups is attributed to the higher nitrates and phosphates concentration. The concentration of nitrates and phosphates in CHS setups was approximately 5.5 times and 10 times higher respectively compared to OHS setups.

Increased assimilation rate of nitrogen and phosphorous in the biomass was observed with increased nutrients concentration as it is evident in Figure 6.4. Figure 6.4a illustrates a linear correlation ($R^2$ = 0.9475) between nitrates concentration in the experimental setups and *SNUR* values. The higher the nitrates concentration, the higher the *SNUR*. Increase of nitrates concentration from 389 ± 3 mg $L^{-1}$ to 2132 ± 17 mg $L^{-1}$ resulted in increase of *SNUR* values from 0.155 mg$NO_3$-N mgVSS$^{-1}$ to 0.193 mg$NO_3$-N mgVSS$^{-1}$. This may have significant implications for a *S*7942-based nitrogen-rich

wastewater treatment stage implementation, since it leads to proportionally lower PBR volume requirements (Samiotis et al., 2022a).

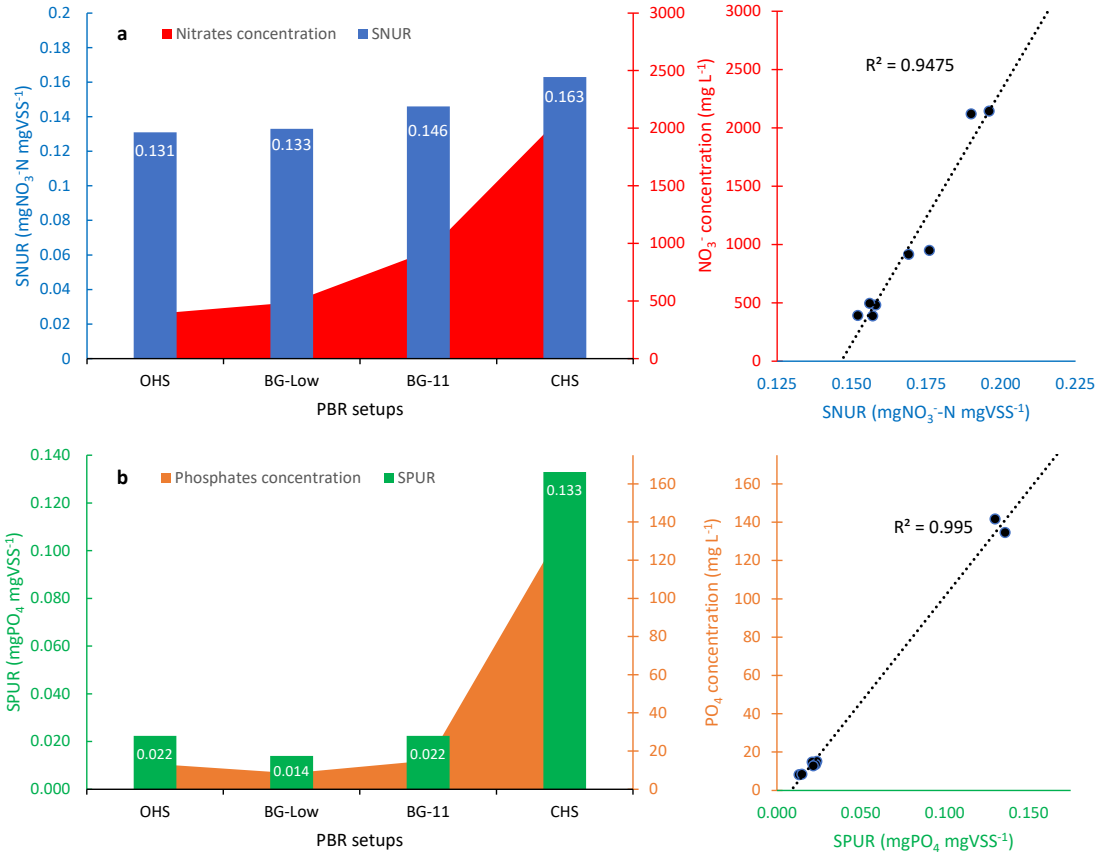


**Figure 6.4.** Specific nitrates utilization rate (SNUR) and specific phosphates utilization rate (SPUR) in relation to nitrates and phosphates concentration in experimental setups.

Regarding phosphorous assimilation into *S*7942 biomass, increasing phosphates concentration from 8.4 ± 0.2 mg $L^{-1}$ to 138 ± 5.0 mg $L^{-1}$, *SPUR* values increase up to an order of magnitude (from 0.014 $mgPO_4$ $mgVSS^{-1}$ to 0.133 $mgPO_4$ $mgVSS^{-1}$). A linear correlation $R^2$ = 0.995 (Figure 6.4b) between phosphates concentration and *SPUR* values was observed, which has been also reported in literature, described as luxury phosphorous uptake phenomenon. This phenomenon is observed in most cyanobacteria species when exposed in phosphate-rich environments (Solovchenko et al., 2020), such as those of CHS effluents. The biochemical process of luxury phosphorous uptake involves polyphosphate synthesis under phosphorus supply in excess, which does not affect cell productivity but the intracellular storage/accumulation of phosphorous that can be utilized during nutrient stresses (Crocetti et al., 2000; Li and Dittrich, 2018).

This study showed that the cultivation of *S*7942 in CHS effluents enhances both nitrogen and phosphate assimilation into the biomass, resulting to higher value biomass as a raw material for industrial relevant products, as well as for the recovery of nitrogen and the considered non-renewable phosphorous.

As demonstrated in this work, *SNUR* increases in faction to nitrates concentration. *SNUR* can also increase in faction to salinity. According to Samiotis et al. (2022a), at saline conditions over 250 mmolNaCl $L^{-1}$, *SNUR* is significantly increased, attributed to the activation of sucrose synthesis mechanism. In the present work, given that the salinity levels of OHS and CHS HWW were lower than 100 mmolNaCl $L^{-1}$ and 30 mmolNaCl $L^{-1}$ respectively, the increase of SNUR is solely attributed to increased nitrates concentration. It was evident that the concentration of nitrates is another factor, along with salinity, which enhances nitrogen assimilation in cells.

### 6.3.3. Stage 3 – Advanced effluent treatment

PBR effluent should be discharged for advanced treatment when it cannot be further recycled/reused. The average concentration values of major anions and cations present in the PBRs' effluent from OHS and CHS HWW, as well as the average pH and EC values are presented in Table 6.3. Furthermore, Table 6.3 presents the contribution of each ion species on EC, calculated according to the previously presented methodology (section 2.1).

**Table 6.3.** Physicochemical characteristics of *S*7942 PBR effluents using OHS or CHS HWW at HRT of 20 days - Contribution of ion species on the resulting effluent EC.

| Physicochemical parameter | OHS PBR effluent | Std. Dev. | % contribution on EC | CHS PBR effluent | Std. Dev. | % contribution on EC |
|---|---|---|---|---|---|---|
| EC (mS/cm at 25°C) | 1.44 | 0.08 | - | 5.25 | 0.17 | |
| pH | 9.8 | 0.39 | < 0.1 | 7.9 | 0.24 | < 0.1 |
| pOH | 4.2 | 0.39 | < 0.1 | 6.1 | 0.24 | < 0.1 |
| Calcium (mg $L^{-1}$) | 43.5 | 3.18 | 6.32 | 93.2 | 7.64 | 3.15 |
| Magnesium (mg $L^{-1}$) | 49.6 | 2.24 | 10.61 | 237.3 | 8.68 | 11.80 |
| Sodium (mg $L^{-1}$) | 153 | 7.8 | 16.32 | 347 | 12.9 | 8.61 |
| Potassium (mg $L^{-1}$) | 58.9 | 3.54 | 5.42 | 566 | 24.04 | 12.11 |
| Ammonium (mg $L^{-1}$) | 0.05 | 0.02 | <0.1 | 0.07 | 0.03 | < 0.1 |
| Lithium (mg $L^{-1}$) | 0.01 | 0.00 | < 0.1 | 0.02 | 0.01 | < 0.1 |
| Iron (mg $L^{-1}$) | 0.03 | 0.01 | < 0.1 | 0.11 | 0.03 | < 0.1 |
| Nitrates (mg $L^{-1}$) | 182 | 17.0 | 10.26 | 1730 | 36.77 | 22.68 |
| Phosphates (mg $L^{-1}$) | 4.52 | 1.46 | <0.1 | 72.1 | 5.78 | 0.28 |
| Chloride (mg $L^{-1}$) | 83.1 | 5.42 | 8.76 | 118.4 | 16.23 | 2.90 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Sulphates (mg $L^{-1}$) | 43.5 | 6.36 | 3.55 | 232.4 | 12.73 | 4.41 |
| Bicarbonates (mg$CaCO_3$ $L^{-1}$) | 213.5 | 8.31 | 7.62 | 173.0 | 6.14 | 1.44 |
| Carbonates (mg$CaCO_3$ $L^{-1}$) | 17.4 | 2.04 | 2.04 | < 0.1 | 0.00 | < 0.1 |

The total contribution of measured ions on the EC of the PBR effluent is 74.0 % and 65.7 % for OHS and CHS respectively. Alkalinity contributes on the EC of OHS and CHS PBR effluents by approximately 9.7 % and 1.5 % respectively. The other ion species, which are not quantified in this study, contribute on the EC of the PBR effluents by 16.3 % and 32.9 % respectively. The ions that have the major contribution on EC are nitrates, sodium, potassium, chloride and magnesium.

Application of adsorption or/and ion exchange is proposed for the advanced treatment of PBR effluent, as well as of the other stages effluents.

#### 6.3.3.1. Treatment via adsorption techniques

In this study, all the adsorption materials that were evaluated for the treatment of HWW or PBR effluents showed similar (independent t test, Sig. > 0.05) and selective removal efficiency for nitrate, phosphate, calcium and magnesium ions, having limited efficiency regarding the removal of other major ions present in the CHS PBR effluent (sodium, chloride, potassium, sulphates). Nonetheless, as illustrated in Table 6.4, after the application of sonication both carbon black (CB) and cloisite 30b (CL) were able to additionally remove sodium, chloride, potassium, sulphates, with similar (independent t test, Sig. > 0.05) removal efficiencies to that of nitrate, phosphate, calcium and magnesium ions removal. This behavior was not observed with any of the three evaluated activated carbon (AC) materials.

**Table 6.4.** Ion selectivity of adsorption materials

| | Nitrates | Phosphates | Sodium | Chloride | Potassium | Calcium | Magnesium | Sulphates |
|---|---|---|---|---|---|---|---|---|
| AC | ✓ | ✓ | | | | ✓ | ✓ | |
| AC & Sonication | ✓ | ✓ | | | | ✓ | ✓ | |
| CB | ✓ | ✓ | | | | ✓ | ✓ | |
| CB & Sonication | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| CL | ✓ | ✓ | | | | ✓ | ✓ | |
| CL & Sonication | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |

AC = activated carbon; CB = carbon black; CL = cloisite 30b

Hence, the application of sonication and of different contact time, resulted to significant differences in specific ion removal rate of experimental setups, which in this study is

expressed in terms of a specific ion's concentration decrease per mg of adsorption material (mg $L^{-1}$ $mg^{-1}$).

In more detail, AC and non-sonicated AC, CB and CL could efficiently remove nitrates, phosphates, calcium and magnesium from PBR effluent having specific removal rate ranging from 63.8 to 246.8 mg $L^{-1}$ $mg^{-1}$, 16.3 to 46.1 mg $L^{-1}$ $mg^{-1}$, 2.5 to 18.4 mg $L^{-1}$ $mg^{-1}$, 8.0 to 28.1 mg $L^{-1}$ $mg^{-1}$ respectively. It should be mentioned that higher specific ions removal rates can be achieved by means of adsorption column design and operating conditions.

Bivariate analysis (Spearman's rank correlation) revealed that the observed differences in specific removal rates are attributed to the effect of contact time and in the case of AC additionally on mesh size. Moderate to strong positive correlation (Corr. Coeff. = 0.627 to 0.755) between contact time and nitrates, phosphates, calcium and magnesium specific removal rates, with high statistical significance (p= 0.001 to 0.05), was observed. Furthermore, strong to very strong negative correlation (Corr. Coeff. = -0.765 to -0.822) between AC mesh size and nitrates, phosphates, calcium and magnesium specific removal rates with very high statistical significance ($p < 0.001$) was observed.

On the other hand, sonicated CB and sonicated CL showed significantly different (independent t test, Sig. = 0.021) and higher nitrates, phosphates, calcium and magnesium ion removal efficiencies than that of AC and non-sonicated CB and CL. Sonicated CB and CL presented specific removal efficiencies ranging from 458.4 to 1774.6 mg $L^{-1}$ $mg^{-1}$, 65.0 to 253.2 mg $L^{-1}$ $mg^{-1}$, 40.2 to 157.6 mg $L^{-1}$ $mg^{-1}$, 19.8 to 73.8 mg $L^{-1}$ $mg^{-1}$ respectively. Both sonicated and non-sonicated nanomaterials presented significant differences in specific ion removal rates, which in their case is attributed to the effect of contact time, as well as to the effect of sonication time. Moderate to strong positive correlation (Corr. Coeff. = 0.668 to 0.741) between contact time and ion removal efficiencies with high statistical significance (p= 0.001 to 0.05) was observed. Furthermore, strong to very strong positive correlation (Corr. Coeff. = 0.795 to 0.880) between sonication time and ion removal efficiencies with very high statistical significance ($p < 0.001$) was observed.

The results showed that sonication of CB and CL increased ion selectivity and removal efficiency. The positive effect on ion selectivity and removal efficiency shown upon application of ultrasounds may be attributed to the increase of the active surface due to

the disaggregation of the nanoparticles. The breakdown of the aggregates into smaller particles potentially alters or changes the electrostatic field around the new smaller sized particles, resulting in enhanced ion approach and increasing absorbency. In addition, the ultrasound process may contribute to a better distribution of ions on the surface of the nanoparticles, thus increasing their selectivity and adsorption capacity, an effect that is similarly observed for other ions (Baltazar, 2022; Negris et al., 2022). The increased nanomaterials' efficiency upon application of ultrasounds could compensate the cost difference between AC and adsorption nanomaterials. Nonetheless, further research regarding the enhancement of nanomaterial's adsorption efficiency and the minimization of installation and operational cost of such treatment processes must be performed to render them economically viable.

The positive effect of sonication on CB or CL ion removal efficiency and on their selectivity indicated that an nanomaterials adsorption configuration coupled with sonication could be used for the treatment of HWW or PBR effluents. Moreover, it is concluded that AC is not a suitable adsorption material for ions removal from HWW.

#### 6.3.3.2. Treatment via ion exchange techniques

The alternative to adsorption process that was evaluated in this work regarding ions removal and recycle/reuse of effluents was based on the selective removal of anions or/and cations via ion-exchange resins. Gnomon for the selection of cation or/and anion exchange treatment stage is the concentration of plant growth hindering ions, which may vary from crop to crop.

Both evaluated commercially available regenerable cation and anion exchange resins exhibited almost complete removal of cations and anions respectively. Their efficiency was higher than 98 % and reached up to 99.9 %, which according to bivariate analysis (Spearman's rank correlation) is attributed to the effect of flow rate in ion exchange column. A strong to very strong negative correlation (Corr. Coeff. = -0.681 to -0.805) between ions removal efficiency and flow rate i.e., contact time, was observed with very high statistical significance ($p < 0.001$).

While the examined mixed bed resin presented similar ion removal efficiencies to that of cation and anion exchange resins (independent t test, Sig. = 0.033), its use as the sole advanced treatment step is not suggested. It is considered incompatible with the

principles of circular economy to indistinctly remove every ion present in the effluents and since they are necessary components of nutrient solution. Hence, minimization of chemicals usage at that stage can only be achieved if selectively applying a cation or an anion removal stage, which depends on HWW or PBR effluent composition evaluated by EC measurements.

After their advanced treatment, the treated effluents can be recycled and enriched with ions according to the methodology described in section 2.1.

### 6.3.4. Stage 4 – Reuse of effluents

The methodology applied for reuse of effluents is the same for all three control points of the studied process and is based on the effluent ion profile and on EC measurement at specific control points. The application of this methodology is presented for control point 1 of CHS effluent, by taking into consideration the threshold concentration values of 150 mg $L^{-1}$ and 100 mg $L^{-1}$ for chloride and sodium (plant growth hindering ions) respectively. The chloride and sodium threshold concentration values correspond to EC of 6.1 mS $cm^{-1}$ and 6.0 mS $cm^{-1}$ respectively. Thus, these threshold values can define the critical EC value and the number of recirculations in CHS without nutrients addition or dilution. Consequently, the EC value of 3 mS $cm^{-1}$ that was empirically set by the operator, can now be adjusted to a higher value. When EC exceeds 6.0 mS $cm^{-1}$, effluent can be either diluted for the preparation of new nutrient solution or sent for treatment/valorization in *S*7942 PBR or/and sent for advanced treatment. The calculated ions concentration after dilution in the nutrient solution preparation tank are presented in Table 6.5. The necessary ions addition for the preparation of new nutrient solution is then easy to be estimated following the dilution law and based on the quantities of drainage and feeding solution preparation tank volume. In the case of CHS, the drainage is 0.05 $m^3$ and the tank volume is 1 $m^3$. The measured physicochemical characteristics of the dilution water used for the preparation of new nutrient solution, the desired characteristics of the nutrient solution and the savings in chemicals usage are presented in Table 6.5. The ions concentration that corresponds to the critical EC of 6.0 mS $cm^{-1}$ were calculates and presented in Table 6.5. As evident, the reuse of nutrients results in significant reduction of chemicals usage for the preparation of new nutrient solution. Hence, the reuse of HWW may reduce water consumption, reduce operational cost of hydroponic farming and compensate a fraction of costs associated

with advanced treatment. Hydroponic farming viability can be further enhanced, as cyanobacteria biomass can be utilized for the production of green energy, fertilizers, feedstock, industrial relevant products (biopolymers, pharmaceuticals etc.).

**Table 6.5.** Measured composition of dilution water; defined nutrient solution characteristics; calculated CHS HWW characteristics at the critical EC of 6 mS $cm^{-1}$; calculated composition of CHS HWW after dilution in nutrient solution preparation tank; expected savings in chemicals usage.

| | Measured / Defined ion concentrations | | Calculated ion concentrations | | |
|---|---|---|---|---|---|
| Parameter | Dilution water (groundwater) | Nutrient solution | CHS HWW at threshold EC | CHS HWW after dilution | Chemicals savings |
| EC (mS/cm at 25ºC) | 0.65 | 1.7 | 6 | 1.19 | - |
| Nitrates (mg $L^{-1}$) | 25 | 763 | 2508 | 273 | 35.8 % |
| Sodium (mg $L^{-1}$) | 4 | 15 | 100 | 14 | * |
| Phosphates (mg $L^{-1}$) | 5 | 97 | 162 | 21 | 21.3 % |
| Chloride (mg $L^{-1}$) | 9 | 29 | 148 | 20 | * |
| Potassium (mg $L^{-1}$) | 1 | 253 | 649 | 66 | 26.2 % |
| Calcium (mg $L^{-1}$) | 68 | 160 | 116 | 73 | 45.5 % |
| Magnesium (mg $L^{-1}$) | 18 | 36 | 306 | 47 | 100 % |
| Sulphates (mg $L^{-1}$) | 11 | 72 | 221 | 32 | 44.4 % |

**chemicals (salts) components not necessary in nutrient solution*

Future goal should be the automation of the HWW management process via online EC monitoring and the creation of a process control software. The adaptation by hydroponic farmers of such a low-cost tool is an important step towards the application of novel and sustainable HWW management.

## 6.4. Conclusions

EC-controlled HWW management elevates hydroponic farming sustainability via total water reuse, saving chemicals and/or HWW effluents valorization by cyanobacteria cultivation. EC can be used in ions concentration and enrichment requirements calculation for HWW reuse, as well as for the selection of proper treatment. A critical EC can be calculated indicating the number of effluent recirculations taking into account the plant growth hindering concentrations of specific ions. Higher nitrates and phosphates concentrations increase assimilation of nitrate-nitrogen and phosphate-phosphorous in the *S*7942 biomass. Ion exchange can efficiently (>98%) remove growth hindering ions. Adsorption nanomaterials coupled with ultrasounds seems a promising alternative treatment.

## 6.5. References


1. Amanatidou, E., Trikoilidou, E., Samiotis, G., Benetis, N. P., & Taousanidis, N. (2012). An easy uncertainty evaluation of the COD titrimetric analysis in correlation with quality control and validation data. Method applicability region. Analytical Methods, 4(12), 4204–4212. https://doi.org/10.1039/C2AY25687H
2. Amanatidou, E., Trikoilidou, E., Tsikritzis, L., & Katsiouli, F. (2011). Uncertainty in spectrophotometric analysis—"Error propagation break up", a novel statistical method for uncertainty management. Talanta, 85(5), 2385–2390. https://doi.org/10.1016/J.TALANTA.2011.07.084
3. APHA, American public health association, American Water Works Association, W. E. F. (2017). Standard Methods for the Examination of Water and Wastewater, 23rd Edition.
4. Avdouli, D., Max, J. F. J., Katsoulas, N., & Levizou, E. (2021). Basil as Secondary Crop in Cascade Hydroponics: Exploring Salinity Tolerance Limits in Terms of Growth, Amino Acid Profile, and Nutrient Composition. Horticulturae 2021, Vol. 7, Page 203, 7(8), 203. https://doi.org/10.3390/HORTICULTURAE7080203
5. Bertoldi, F. C., Sant'anna, E., & Barcelos-Oliveira, J. L. (2009). Chlorella vulgaris cultivated in hydroponic wastewater. Acta Horticulturae, 843, 203–210. https://doi.org/10.17660/ACTAHORTIC.2009.843.26
6. Chisti, Y. (2007). Biodiesel from microalgae. Biotechnology Advances, 25(3), 294–306. https://doi.org/10.1016/J.BIOTECHADV.2007.02.001
7. Crocetti, G. R., Hugenholtz, P., Bond, P. L., Schuler, A., Keller, J., Jenkins, D., & Blackall, L. L. (2000). Identification of polyphosphate-accumulating organisms and design of 16S rRNA-directed probes for their detection and quantitation. Applied and Environmental Microbiology, 66(3), 1175–1182. https://doi.org/10.1128/AEM.66.3.1175-1182.2000
8. Cuellar-Bermudez, S. P., Aleman-Nava, G. S., Chandra, R., Garcia-Perez, J. S., Contreras-Angulo, J. R., Markou, G., Muylaert, K., Rittmann, B. E., & Parra-Saldivar, R. (2017). Nutrients utilization and contaminants removal. A review of two approaches of algae and cyanobacteria in wastewater. Algal Research, 24, 438–449. https://doi.org/10.1016/J.ALGAL.2016.08.018
9. De Camargo, E.C., Casali, C. A., Lombardi, A. T., Inês, M., & Lima, S. (2015). Coupling microalgal cultures with hydroponics: prospection for clean biotechnology processes. J. Algal Biomass Utln, 6(1), 88–94. ISSN: 2229 – 6905
10. Dorais, M., & Dubé, Y. (2011). Managing greenhouse organic wastes: A holistic approach. Acta Horticulturae, 893, 183–198. https://doi.org/10.17660/ACTAHORTIC.2011.893.12
11. García-Caparrós, P., Llanderal, A., Maksimovic, I., & Lao, M. T. (2018). Cascade Cropping System with Horticultural and Ornamental Plants under Greenhouse Conditions. Water 2018, Vol. 10, Page 125, 10(2), 125. https://doi.org/10.3390/W10020125
12. Gentry, M. (2019). Local heat, local food: Integrating vertical hydroponic farming with district heating in Sweden. Energy, 174, 191–197. https://doi.org/10.1016/J.ENERGY.2019.02.119
13. Huang, G. H., Chen, F., Wei, D., Zhang, X. W., & Chen, G. (2010). Biodiesel production by microalgal biotechnology. Applied Energy, 87(1), 38–46. https://doi.org/10.1016/J.APENERGY.2009.06.016
14. Hultberg, M., Carlsson, A. S., & Gustafsson, S. (2013). Treatment of drainage solution from hydroponic greenhouse production with microalgae. Bioresource Technology, 136, 401–406. https://doi.org/10.1016/J.BIORTECH.2013.03.019
15. Huo, S., Liu, J., Addy, M., Chen, P., Necas, D., Cheng, P., Li, K., Chai, H., Liu, Y., & Ruan, R. (2020). The influence of microalgae on vegetable production and nutrient removal in greenhouse hydroponics. Journal of Cleaner Production, 243, 118563. https://doi.org/10.1016/J.JCLEPRO.2019.118563

16. ISO - ISO 10304-1:2007 - Water quality — Determination of dissolved anions by liquid chromatography of ions — Part 1: Determination of bromide, chloride, fluoride, nitrate, nitrite, phosphate and sulfate. (n.d.). Retrieved March 28, 2022, from https://www.iso.org/standard/46004.html
17. ISO - ISO 14911:1998 - Water quality — Determination of dissolved Li+, Na+, NH4+, K+, Mn2+, Ca2+, Mg2+, Sr2+ and Ba2+ using ion chromatography — Method for water and waste water. (n.d.). Retrieved March 28, 2022, from https://www.iso.org/standard/25591.html
18. Isozaki, M., Konishi, N., Kuroki, M., Nomura, Y., & Tanaka, K. (2004). Growth of Tomato Plants and Changes in Nutrient Element Concentrations in the Rockwool System with a Device to Recycle Used Nutrient Solutions. Journal of the Japanese Society for Horticultural Science, 73(4), 354–363. https://doi.org/10.2503/JJSHS.73.354
19. Kumar, R. R., & Cho, J. Y. (2014). Reuse of hydroponic waste solution. Environmental Science and Pollution Research, 21(16), 9569–9577. https://doi.org/10.1007/S11356-014-3024-3/TABLES/2
20. La Bella, E., Baglieri, A., Fragalà, F., & Puglisi, I. (2022). Multipurpose Agricultural Reuse of Microalgae Biomasses Employed for the Treatment of Urban Wastewater. Agronomy 2022, Vol. 12, Page 234, 12(2), 234. https://doi.org/10.3390/AGRONOMY12020234
21. Lee, J. Y., Rahman, A., Behrens, J., Brennan, C., Ham, B., Kim, H. S., Nho, C. W., Yun, S. T., Azam, H., & Kwon, M. J. (2018). Nutrient removal from hydroponic wastewater by a microbial consortium and a culture of Paracercomonas saepenatans. New Biotechnology, 41, 15–24. https://doi.org/10.1016/J.NBT.2017.11.003
22. Li, J., & Dittrich, M. (2018). Dynamic polyphosphate metabolism in cyanobacteria responding to phosphorus availability. https://doi.org/10.1111/1462-2920.14488
23. Lopez-Galvez, F., Allende, A., Pedrero-Salcedo, F., Alarcon, J. J., & Gil, M. I. (2014). Safety assessment of greenhouse hydroponic tomatoes irrigated with reclaimed and surface water. International Journal of Food Microbiology, 191, 97–102. https://doi.org/10.1016/J.IJFOODMICRO.2014.09.004
24. Magán, J. J., Gallardo, M., Thompson, R. B., & Lorenzo, P. (2008). Effects of salinity on fruit yield and quality of tomato grown in soil-less culture in greenhouses in Mediterranean climatic conditions. Agricultural Water Management, 95(9), 1041–1055. https://doi.org/10.1016/J.AGWAT.2008.03.011
25. Mahjoor, F., Ghaemi, A. A., & Golabi, M. H. (2016). Interaction effects of water salinity and hydroponic growth medium on eggplant yield, water-use efficiency, and evapotranspiration. International Soil and Water Conservation Research, 4(2), 99–107. https://doi.org/10.1016/J.ISWCR.2016.04.001
26. Mata, T. M., Martins, A. A., & Caetano, N. S. (2010). Microalgae for biodiesel production and other applications: A review. Renewable and Sustainable Energy Reviews, 14(1), 217–232. https://doi.org/10.1016/J.RSER.2009.07.020
27. Melo, J. M., Ribeiro, M. R., Telles, T. S., Amaral, H. F., & Andrade, D. S. (2021). Microalgae cultivation in wastewater from agricultural industries to benefit next generation of bioremediation: a bibliometric analysis. Environmental Science and Pollution Research 2021 29:15, 29(15), 22708–22720. https://doi.org/10.1007/S11356-021-17427-0
28. Mielcarek, A., Rodziewicz, J., Janczukowicz, W., & Dobrowolski, A. (2019). Analysis of Wastewater Generated in Greenhouse Soilless Tomato Cultivation in Central Europe. Water 2019, Vol. 11, Page 2538, 11(12), 2538. https://doi.org/10.3390/W11122538
29. Mohammed, S. (2018). Tomorrow's agriculture : "NFT Hydroponics"-- grow within your budget. https://doi.org/10.1007/978-3-319-99202-0
30. Negris, L., Santos, H. N., Picoloto, R. S., Alves, F. E. A., Flores, E. M. M., Santos, M. F. P., & Vicente, M. A. (2022). Ultrasound-assisted adsorption on porous ceramic for removal of iron in water. Environmental Technology (United Kingdom), 43(8), 1211–1224.

https://doi.org/10.1080/09593330.2020.1822923/SUPPL_FILE/TENT_A_1822923_SM4638.DOCX
31. Oswald, W. J., & Golueke, C. G. (1960). Biological Transformation of Solar Energy. Advances in Applied Microbiology, 2(C), 223–262. https://doi.org/10.1016/S0065-2164(08)70127-8
32. Oyadomari, J.K., Keweenaw Freshwater Algae. (n.d.). Retrieved July 15, 2022, from http://www.keweenawalgae.mtu.edu/
33. Richa, A., Touil, S., Fizir, M., & Martinez, V. (2020). Recent advances and perspectives in the treatment of hydroponic wastewater: a review. Reviews in Environmental Science and Biotechnology, 19(4), 945–966. https://doi.org/10.1007/s11157-020-09555-9
34. Rufí-Salís, M., Petit-Boix, A., Villalba, G., Sanjuan-Delmás, D., Parada, F., Ercilla-Montserrat, M., Arcas-Pilz, V., Muñoz-Liesa, J., Rieradevall, J., & Gabarrell, X. (2020). Recirculating water and nutrients in urban agriculture: An opportunity towards environmental sustainability and water use efficiency? Journal of Cleaner Production, 261, 121213. https://doi.org/10.1016/J.JCLEPRO.2020.121213
35. Salazar, J., Valev, D., Näkkilä, J., Tyystjärvi, E., Sirin, S., & Allahverdiyeva, Y. (2021). Nutrient removal from hydroponic effluent by Nordic microalgae: From screening to a greenhouse photobioreactor operation. Algal Research, 55, 102247. https://doi.org/10.1016/J.ALGAL.2021.102247
36. Samiotis, G., Stamatakis, K., & Amanatidou, E. (2021). Assessment of Synechococcus elongatus PCC 7942 as an option for sustainable wastewater treatment. Water Science and Technology, 84(6), 1438–1451. https://doi.org/10.2166/WST.2021.319
37. Samiotis, G., Stamatakis, K., & Amanatidou, E. (2022a). Dimensioning of Synechococcus elongatus PCC 7942 cultivation photobioreactor for valorization of wastewater resources. Chemical Engineering Journ
38. Samiotis, G., Ziagova, M. G., & Amanatidou, E. (2022b). Wastewater substrate disinfection for cyanobacteria cultivation as tertiary treatment. Environmental Science and Pollution Research, 1, 1–13. https://doi.org/10.1007/S11356-022-20369-W/FIGURES/7
39. Santos, F. M., & Pires, J. C. M. (2018). Nutrient recovery from wastewaters by microalgae and its potential application as bio-char. Bioresource Technology, 267, 725–731. https://doi.org/10.1016/J.BIORTECH.2018.07.119
40. Savvas, D., Mantzos, N., Barouchas, P. E., Tsirogiannis, I. L., Olympios, C., & Passam, H. C. (2007). Modelling salt accumulation by a bean crop grown in a closed hydroponic system in relation to water uptake. Scientia Horticulturae, 111(4), 311–318. https://doi.org/10.1016/J.SCIENTA.2006.10.033
41. Solovchenko, A., Gorelova, O., Karpova, O., Selyakh, I., Semenova, L., Chivkunova, O., Baulina, O., Vinogradova, E., Pugacheva, T., Scherbakov, P., Vasilieva, S., Lukyanov, A., & Lobakova, E. (2020). Phosphorus Feast and Famine in Cyanobacteria: Is Luxury Uptake of the Nutrient Just a Consequence of Acclimation to Its Shortage? Cells 2020, Vol. 9, Page 1933, 9(9), 1933. https://doi.org/10.3390/CELLS9091933
42. Souza, S. V., Gimenes, R. M. T., & Binotto, E. (2019). Economic viability for deploying hydroponic system in emerging countries: A differentiated risk adjustment proposal. Land Use Policy, 83, 357–369. https://doi.org/10.1016/J.LANDUSEPOL.2019.02.020
43. Stergiou, C. A., Stimoniaris, A. Z., & Delides, C. G. (2015). Hybrid Nanocomposites With Organoclay and Carbon-Based Fillers for EMI Suppression. IEEE Transactions on Electromagnetic Compatibility, 57(3), 470–476. https://doi.org/10.1109/TEMC.2014.2384014
44. Stimoniaris, A. Z., Stergiou, C. A., & Delides, C. G. (2012). A detailed study of α-relaxation in epoxy/carbon nanoparticles composites using computational analysis. Express Polymer Letters, 6(2), 120–128. https://doi.org/10.3144/EXPRESSPOLYMLETT.2012.13
45. Surendran, U., Chandran, C., & Joseph, E. J. (2017). Hydroponic cultivation of Mentha spicata and comparison of biochemical and antioxidant activities with soil-grown plants.

Acta Physiologiae Plantarum, 39(1), 1–14. https://doi.org/10.1007/S11738-016-2320-6/FIGURES/8
46. Trikilidou, E., Samiotis, G., Tsikritzis, L., & Amanatidou, E. (2020). Performance of Semi-Micro-Kjeldahl Nitrogen Method – uncertainty and nitrate interference. Https://Doi.Org/10.1080/03067319.2020.1807967. https://doi.org/10.1080/03067319.2020.1807967
47. Vayenos, D., Romanos, G. E., Papageorgiou, G. C., & Stamatakis, K. (2020). Synechococcus elongatus PCC7942: a cyanobacterium cell factory for producing useful chemicals and fuels under abiotic stress conditions. Photosynthesis Research, 146(1–3), 235–245. https://doi.org/10.1007/S11120-020-00747-6/TABLES/5

# 7. Chapter 7. Conclusions and perspectives

Cyanobacteria and microalgae assimilate into their biomass significant quantities of nutrients, while fixating $CO_2$ in its gaseous form, as well as in its dissolved $HCO_3^-$ form. They synthesize a variety of organic compounds, such as hydrocarbons, proteins, lipids, polymers and pigments, which constitute their biomass a valuable product with various industrial applications (biofuels, medicines, cosmetics, food etc.). This has led on the increasing cultivation of cyanobacteria and microalgae, which is mostly done in open pond configurations that have significant land requirements and/or by feeding cultures with synthetic nutrient medium in open pond or PBR configurations.

When is not competing for arable land and the use of resources, the cultivation of cyanobacterial or/and microalgal biomass as feedstock for the aquisition of industrial relevant products or/and bioenergy offers a sustainable alternative to traditional feedstocks (plants, animals, fossils and minerals etc.). In this regard, the cultivation of cyanobacteria and/or microalgae in wastewater treatment plants (WWTPs) using nutrient-rich effluents offers the opportunity of elevating sustainability of both phototrophic biomass cultivation processes and wastewater treatment procecess. The incorporation of tank photobioreactor (PBR) in a WWTP may alleviate the increased land requirements associated with cyanobacteria and/or microalgae cultivation, whereas the nutrient content of wastewaters can minimize the necesities for nutrients addition in cultivation medium.

In this work, the cyanobacterium *Synechococcus elongatus* PCC 7942 was assesed as a potent biological component for sustainable wastewater treatment/valorization biotechnological applications that align with the principles of circular economy. The

study of a Synechococcus elongatus PCC 7942-based wastewater treatment/valorization process, led on (a) the identification of process' applicability boundaries in terms of cultivation media characteristics, pre-treatment requirements and cultivation conditions, (b) the extraction of design and operational parameters for the adoption and upscale of the process, (c) the evaluation of process' efficiency and of the potential advantages compared to commonly applied processes in terms of reactor volume requirements and biomass valorization.

The study showed that wastewater must be properly disinfected prior its use as a cultivation media, since the contained biological contaminants hinder the proliferation of *Synechococcus elongatus* PCC 7942, thus nitrogen and phosphorous removal via assimilation in the PBR. Filtration coupled with chemical disinfection can provide a low-cost approach for efficient disinfection and unhindered growth of *Synechococcus elongatus* PCC 7942 cultures. The disinfection efficiency of filtration is linked both on filter medium pore size and on filter thickness i.e., the duration of filtration. Similarly, the disinfection efficiency of chemical disinfection is linked both on disinfectant dosage (C) and contact time (T), with efficient disinfection of wastewater media achieved after filtration and chlorination at dosages in terms of Concentreation-Time (CT) product, of $CT \geq 270$ mg min $L^{-1}$ for NaClO and $CT \geq 157$ mg min $L^{-1}$ for ferrates (FeVI).

While the commonly applied disinfectants sodium hypochlorite and hydrogen peroxide can efficiently remove biological contaminants of filtrated wastewaters, the use of environmentally friendly ferrates can offer a better alternative. The application of ferrates, produced in-situ via a low-cost $Fe^0/Fe^0$ electrochemical cell, can additionally offer significant removal of organic compounds and turbidity from the wastewaters due to its oxidation action, as well as the coagulation action of the resulting FeIII. Coagulation can also assist in biomass harvesting from the PBR, a challenge that has not been sufficiently addressed by engineers in terms of low-cost and efficient biomass collection.

Upon proper disinfection of the evaluated wastewater media, which were obtained from the secondary biological treatment stage of a dairy industry and a snack industry, as well as from the drainages of an open and a closed hydroponic system, *Synechococcus elongatus* PCC 7942 can efficiently proliferate and remove nitrates and phosphates via assimilation (Figure 7.1).

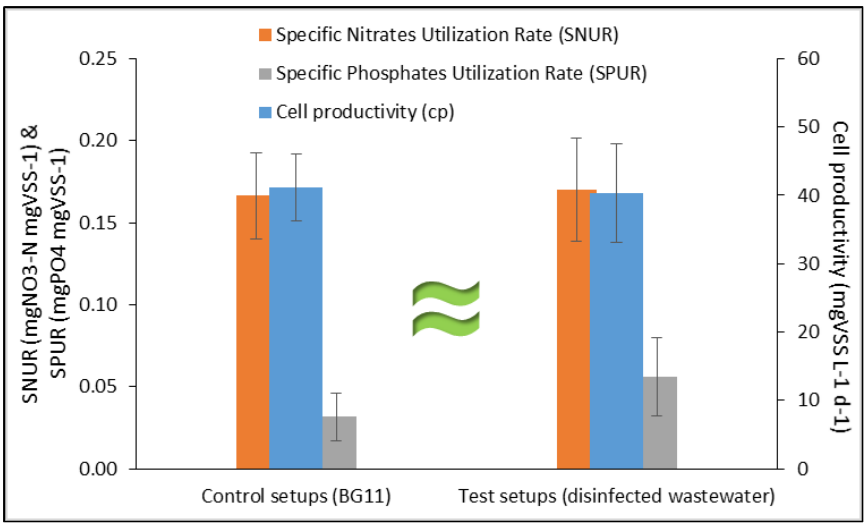


**Figure 7.1** Assessment of *Synechococcus elongatus* PCC 7942 cultivation in wastewater media.

Temperature and salinity oppose thresholds for *Synechococcus elongatus* PCC 7942 cultivation. Nevertheless, it is able to proliferate at a temperature range of 16ºC to 32ºC and at salinities up to 450 mmolNaCl $L^{-1}$.

The study revealed that salinity in particular, which in many wastewater streams may be as high as the threshold value, positively correlates with nitrogen assimilation into Synechococcus elongatus PCC 7942 biomass. Moreover, higher nutrient concentrations in cultivation media not only trigger luxury phosphorus uptake, but also increase nitrogen assimilation into the cyanobacteria (Figure 7.2).

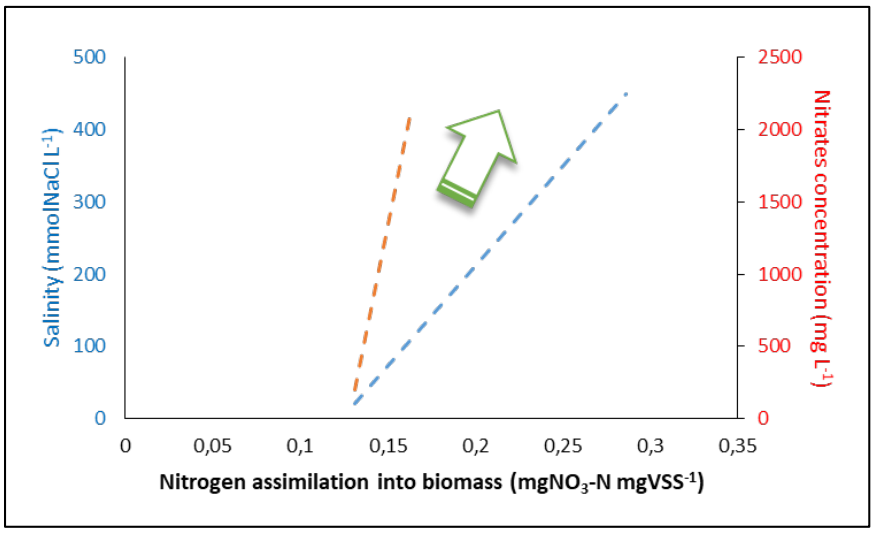


**Figure 7.2** Effect of salinity and nutrient content on nitrogen assimilation.

These findings have significant implications on dimensioning of a photobioreactor for the treatment of high strength and relatively saline wastewater, whose treatment constitutes a challenge in common biological wastewater treatment processes.

Based on the proposed by this study photobioreactor dimensioning equation (Eq. 7.1), which takes into account nitrate-nitrogen load (*N_Denitr*, $kgNO_3\_N\ d^{-1}$), *SNUR* (and *cell productivity*, a photobioreactor ($V_{PBR}$) volume can be of comparable volume to a common biological denitrification bioreactor. The key element for reactor volume minimization is cell productivity enhancement via bioreactor design and control of operational conditions.

$$V_{PBR} = \frac{N\_Denitr}{SNUR * cell\ productivity} \qquad \text{Eq. 7.1}$$

Therefore, a *Synechococcus elongatus* PCC 7942-based wastewater treatment process can be adapted as a supplementary or an alternative treatment stage to the widely applied biological denitrification processes towards the minimization of wastewater treatment plant's ecological footprint and obtaining of added value products or/and energy.

Moreover, the study revealed that a photoautotrophic-based process can offer the only biotechnological answer to the challenge of treating nutrient-rich wastewater with low organic compounds content, in which activated sludge process cannot be applied. In this accord, a wastewater treatment stage based on *Synechococcus elongatus* PCC 7942 cultivation was evaluated as potent biological process for the integrated management of hydroponic wastewater. An integraded management that is based on (i) a developed electric conductivity-based tool for straightforward characterization of hydroponic wastewater (HWW) and the selection of nutrient requirements or of the nessessary treatment stage for their reuse, (ii) a Synechococcus elongatus PCC 7942 cultivation treatment stage for the removal of nutrients via assimilation into biomass that can be used for HWW valorization, and (iii) an advanced treatment stage for selective removal of plant growth hindering ions.

In conclusion, *Synechococcus elongatus* PCC 7942 presents significant characteristics for biotechnological applications, as it is able to proliferate in various wastewater media. Moreover, it can be used for the removal of $CO_2$ from flue gases.

In this work, the methodology for photobioreactors' volume calculation provides a solution for scaling up of phototrophic species cultivation for wastewater treatment and biomass valorization.

As a follow up of this work, attention should be focused on the manipulation of *Synechococcus elongatus* PCC 7942 to increase its sucrose yields for bioethanol or direct biohydrogen production, or/and to enhance other synthesized compounds such as proteins, lipids and pigments and bioactive compounds.

## Appendix A

In this Appendix, an overview of the publications originated from this Thesis, is discussed.

| **Thesis publications** | |
|---|---|
| Chapter 3 | Environmental Science and Pollution Research, (2022) 1, 1–13 |
| Chapter 4 | Water Science and Technology, (2021) 84(6), 1438–1451 |
| Chapter 5 | Chemical Engineering Journal, (2022) 435(2), 134895 |
| Chapter 6 | Bioresource Technology Reports, (2022) XX, XX-XX |
| **Oral presentations** | |
| *Synechococcus elongatus* PCC 7942 - Ένα κυανοβακτήριο για πράσινη επεξεργασία βιομηχανικών υγρών αποβλήτων | 1ο Διαδικτυακό Συνέδριο Νέων Επιστημόνων «Ορυκτοί Πόροι-Περιβάλλον-Χημική Μηχανική» |
| Cultivation of cyanobacterium *Synechococcus elongatus* PCC 7942 in wastewater substrate – A challenge to be addressed | 8$^{th}$ International Conference on Sustainable Solid Waste Management, 23-26 JUNE 2021, Thessaloniki, Greece |
| Wastewater treatment coupled with *Synechococcus elongatus* PCC 7942 cultivation | 1$^{st}$ International Conference on Sustainable Chemical and Environmental Engineering 31$^{st}$ August to 4$^{th}$ September 2022, Rethymno, Crete, Greece |