Assessment of *Synechococcus elongatus* PCC 7942 as an option for sustainable wastewater treatment

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ABSTRACT

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 (S7942) 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 S7942 biomass concentration are presented. The results show that at typical industrial wastewater temperatures, S7942 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 S7942-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.

Key words: cyanobacteria, disinfection, growth rate, industrial wastewater, nitrates removal, saline wastewater

HIGHLIGHTS

- *Synechococcus elongatus* PCC 7942 efficiently grows in treated industrial wastewater.
- High rates of nitrates removal and growth at typical wastewater temperatures.
- Efficient growth and high nitrates removal in saline industrial wastewater.
- Filtration coupled with chlorination/dechlorination is effective for pre-treatment.

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 greenhouse gases (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 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 km³ 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 (Zhan & 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 CO₂ equivalent emissions and up to 5.2% of the global total non-CO₂ GHG emissions (mainly CH₄ and N₂O) (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 CO₂ 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 and/or 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 CO₂ 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. 2010). It has been suggested that process engineering may enable the complete use of nutrients present in wastewaters for carbon recovery and capture of exogenous CO₂ (Valverde-Pérez et al. 2015; Gardner-Dale et al. 2017; Lu et al. 2018). The resulting biomass from CO₂ 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 S7942), can follow the fermentative hydrogen production pathway, i.e. the metabolic production of biohydrogen from their sucrose. Worth mentioning is that the S7942 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, S7942 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, S7942 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 S7942 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 S7942 growth and nitrates removal rate was examined. Emphasis has been given on the identification of the appropriate parameters expressing S7942 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 S7942-based nutrient removal process is conducted in terms of reactor volume requirements.

2. MATERIALS AND METHODS

2.1. Microbial strain, growth media and culture conditions

The autotroph, phototrophic microbial strain used in this study was the single-celled, freshwater cyanobacterium S7942, 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 5,000 rpm and single rinsing with deionized water. In order to evaluate the adequacy of S7942 for industrial wastewater treatment, cultures of S7942 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 to 1.73 mg/L. The duration of S7942 cultivation was up to 20 days and 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/m².s. Cultivation temperature was controlled and set to values between 16 and 37 °C.

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 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.

A wastewater media may inhibit or reduce S7942 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 S7942 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.

| Table 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 | 3,540 | 109 | 4,295 | 46 |
| 5-day biochemical oxygen demand (BOD₅), mg/L | 1,718 | 22 | 2,275 | 9 |
| Total Kjeldahl nitrogen (TKN), mg/L | 77.3 | 3.5 | 107 | 2.0 |
| Nitrate nitrogen, mg/L | 0.371 | 57.3 | 7.3 | 84.11 |
| Ammonia nitrogen, mg/L | 7.1 | 3.4 | 12.7 | 1.1 |
| Total (dissolved) phosphorous, mg/L | 40.91 | 8.3 | 17.2 | 1.3 |
| Electric conductivity, μS/cm at 25 °C | 13,740 | 12,580 | 1,814 | 1,717 |
In this study, the two types of wastewater that were used as the media for \textit{S7942} growth (snack and dairy industry BTWW) were assessed regarding potential inhibition of PAR transfer via photometric analysis (scanning) at wavelengths between 400 to 700 nm (photosynthetically active spectral range). The light absorption spectrums of the BTWW were measured and compared to that of \textit{S7942} cultures in various Chl \textit{a} concentration (cultivation duration up to 20 days). It was evident that the light absorption spectrum of \textit{S7942} has absorption peaks consistent to the absorption maxima of \textit{S7942}'s photosynthetic pigments, i.e. chlorophyll \textit{a} at around 440 and 680 nm (Cinque \textit{et al.} 2000), allophycocyanin at around 650 and 620 nm (Murakami \textit{et al.} 1981) and phycocyanin at around 631 nm (Espinosa \textit{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 \textit{S7942} cultivation in wastewater was conducted using (i) unfiltered BTWW, (ii) filtered BTWW, (iii) chlorinated and dechlorinated BTWW and (iv) filtered and chlorinated/dechlorinated BTWW. Fiberglass filters of 1.2 pore-size and cellulose filters of 0.45 \textmu m pore-size were used for filtration. Chlorination was performed with sodium hypochlorite until free chlorine concentration reached values of approximately 4 mg/L, maintained for 60 min, and dechlorination obtained with sodium thiosulphate to concentrations below the detection limit of (0.01 mg/L) of the applied analytical method (APHA 4500-Cl-G).

\section*{2.2. Monitoring parameters and analytical methods}

\subsection*{2.2.1. Culture growth}

The growth rate of \textit{S7942} 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 \textit{S7942} biomass. Therefore, evaluating the impact of operating parameters on growth rate is the first goal towards utilization of \textit{S7942} for wastewater treatment applications.

In this study, the growth rate of \textit{S7942} was evaluated in terms of (i) chlorophyll \textit{a} (Chl \textit{a}) concentration (duplicate samples), (ii) optical density at 750 nm (O.D.-750 nm, single determination) and (iii) total and volatile suspended solids concentration (TSS, VSS, duplicate samples). Chlorophyll \textit{a}, which is the major photosynthetic pigment of \textit{S7942} strain along with the phycobiliproteins allophycocyanin and phycocyanin (Collier & 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 \textit{S7942} cultures, in terms of Chl \textit{a} concentration ($RGR_{\text{Chl a}}$), was calculated by Equation (1) (Vayenos \textit{et al.} 2020).

$$RGR_{\text{Chl a}} = \left[ \frac{[\text{Chl a}(n \ d)]}{[\text{Chl a}(0 \ d)]} \right]^{1/3} - 1 \times 100$$

(1)

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

Equation (1) is considered a universal formula for calculating relative (%) evolution of a parameter, whether it expresses biomass (TSS, VSS, Chl \textit{a}, O.D.750 nm, 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 \textit{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 \textit{a} concentration, since Chl \textit{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. 750 nm were also measured in order to obtain correlation coefficients (conversion factors) between all these parameters that can express biomass concentration (Figure 1). This is considered of great importance in the assessment, interpretation and implementation of research results by scholars and engineers, which may express growth rates or biomass concentration in different format.

The relationship between Chl \textit{a} and TSS concentration (Figure 1) is evident through the linear correlation between these two parameters ($R^2 = 0.91$), with slope of 62.57 mgTSS/mgChl \textit{a}. Similar slope value was reported from Dechatiwongse \textit{et al.}
(2014) regarding the relationship between dry cell concentration of cyanobacterium *Cyanothece* ATCC 51,142 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.750 nm, 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 1: inset 1 and inset 2, respectively). There is an evident linear correlation between Chl $a$ concentration and O.D.750 nm ($R^2 = 0.93$), as well as between TSS concentration and O.D.750 nm ($R^2 = 0.90$), with resulting slopes of 5.28 mgChl $a$/AU and 290.87 mgTSS/AU, respectively. Similar slope value was obtained in Kuan et al.’s (2015) study, regarding the relationship between $S7942$ dry cell concentration (total solids at 90 °C) and optical density at 600 nm.

The above obtained conversion factors of 62.57 mgTSS/mgChl $a$, 5.28 mgTSS/mgChl $a$ and 290.87 mgTSS/AU are considered indicative for uncontaminated $S7942$ cultures. Furthermore, regarding the relationship between VSS concentration and TSS concentration, the obtained VSS/TSS ratios in uncontaminated $S7942$ 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.

### 2.2.2. 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.

$$RRR_{NO3-N} = - \left[ \left( \frac{[NO_3N](t_d)}{[NO_3N](0_d)} \right) - 1 \right] \times 100$$

(2)

Replicate samples were used in order to accurately evaluate nitrates removal rate by $S7942$, 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 S7942 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 the separation column, thus not evaluated.

The evaluation of the potentiometric nitrates' determination method revealed its inadequacy for monitoring nitrates concentration in algae cultures due to various interferences. Specifically, the ion selective electrode gave false and non-replicable readings, attributed to the presence of various interfering ions in the S7942 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) and/or in cases of persistent color in the filtrated sample. Consequently, 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 color 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).

2.2.3. 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 S7942 microbial species was made based on their morphological characteristics and on relevant databases (Oyadomari 2001; APHA 2017).

2.2.4. 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 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).

3. RESULTS AND DISCUSSION

3.1. Growth of S7942

3.1.1. Effect of temperature on S7942 growth

In order to evaluate the temperature boundaries for S7942 growth, thus determine if temperature adjustment would be needed at full-scale applications, duplicate culture setups with BG-11 growth media and at different controlled temperatures of approximately 16–18 °C, 20–22 °C, 25–27 °C, 30–32 °C and 35–37 °C were deployed. S7942 doubling time, in terms of Chl a concentration, was calculated for a cultivation period of 20 days (n = 20 days) and presented in Figure 2. Worth mentioning is that a relatively constant growth rate was observed in all S7942 cultures that were used for obtaining the doubling times versus temperature chart (Figure 1) regardless the operational temperature, with strong linear correlations (0.90 < R2 < 0.98) between Chl a concentration and time.

Doubling time was relatively similar at temperatures from approximately 20 °C to 32 °C, ranging from 1.86 to 1.76 days. At the lowest cultivation temperatures of approximately 16 °C to 18 °C, a relatively small increase of 35% to 41% in Chl a doubling time was observed. A suppression of such magnitude on S7942 growth at temperatures lower than 20 °C was expected and being in accordance with the results of Deshnium et al. (1997). At operating temperatures of 35 °C to 37 °C a significant increase in Chl a doubling time of 223% to 235% was observed, suggesting inhibition of S7942 growth due to temperature-stress and/or increased decay rate (Han et al. 2016; Aguilera & Giannuzzi 2018). Consequently, temperatures of 30 °C to 32 °C
are considered optimal for S7942 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 20 °C to 27 °C.

Worth mentioning 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 S7942 cell, resulting in 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 or optical density are proposed for accurately expressing S7942 growth, as otherwise an up to three times greater doubling time may be falsely estimated.

The obtained results regarding the optimal operating temperature for S7942 cultivation are considered suitable towards advantageous full-scale implementation of S7942-based wastewater treatment technologies, since a plethora of industrial wastewater streams have temperatures at this range (Alekseiko et al. 2014).

### 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 25 and 27 °C cell productivity, in terms of TSS, was approximately 57 mg/L/d. This value is significantly lower than the optimal S7942 cell productivity in BG-11 growth-media (194 mg/L/d, at 25 ± 1 °C, 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/m²/s, which is significantly lower than of Silva’s et al. (2014) study that ranged from 50 to 150 μmol/m²/s.

### 3.1.3. Antagonistic and/or predating microorganisms’ growth in S7942 cultures

#### 3.1.3.1. Cultures in BG-11 media

All S7942 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.

#### 3.1.3.2. Cultures in BTWW

On the contrary, S7942 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 S7942 as feed substrate.

#### 3.1.3.3. Cultures in filtered BTWW

S7942 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 h, resulting to a decrease of S7942 population. Similar growth of protozoan and metazoan species, but at a lower count, was observed in S7942 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.
3.1.3.4. Cultures in chlorinated/dechlorinated BTWW. Similarly, the S7942 cultures with chlorinated/dechlorinated BTWW presented growth of antagonistic and/or predating microbial species. The cultures with dairy wastewater exhibited this growth after the first 24 h, while the setups with salty snack industry’s wastewater after the 48 h. 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 1) and the subsequent consumption of residual free chlorine for oxidation of soluble organics and not for complete disinfection of the wastewater.

3.1.3.5. Cultures in filtered and chlorinated/dechlorinated BTWW. The cultures in filtered with 0.45 $\mu$m pore-size cellulose filter and chlorinated/dechlorinated BTWW maintained homogenous population of S7942 throughout approximately a week, followed by limited growth of yeast and small-size ciliates (smaller or even-sized to S7942). Worth noting is that the growth of these microbial species did not seem to hinder S7942 growth rate, since S7942 cultures in chlorinated/dechlorinated BTWW exhibited similar and even greater overall $RGR_{Chl\ a}$ to that of S7942 cultures in BG-11 media (Figure 3, Table 2). Furthermore, a considered novel and environmentally friendly (Ghernaout & 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 & Eddy 2003).

3.1.3.6. Assessment of S7942 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 the seventh day. These $RGR_{Chl\ a}$ values, as well as the greater than 40% drop during the second week of S7942 cultivation is consistent with the results regarding S7942 culture in BG-11 media and at temperatures from 16 to 37 °C (Figure 3).

![Figure 3](https://example.com/figure3.png) Figure 3 | Average $RGR_{Chl\ a}$ evolution in S7942 cultures with BG-11 media (eight cultures), with treated dairy wastewater (three cultures) and treated snack wastewater (three cultures) at 20 °C to 27 °C (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 RGR\textsubscript{Chl a} values during the first week of cultivation. The relatively low initial RGR\textsubscript{Chl a} value was due to an approximately two-day lag in S7942 growth observed in two out of three cultures. This lag is attributed to the acclimatization of S7942 to the ‘new’ relatively saline environment. After the seventh day of cultivation and up to the 17th day, RGR\textsubscript{Chl a} values were relatively high indicating that acclimatization leads to a late-start and growth maxima retardation (Figure 3). Nevertheless, the survival and growth of S7942 in such, relative saline, wastewater-media indicates that S7942 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 \textit{et al.} 2020). Worth mention is that S7942 is able to tolerate maximally salinity of approximately 0.5 M NaCl (Ladas & Papageorgiou 2000; Du \textit{et al.} 2013), a salinity (2.9%) lower than that of seawater (3.5%) (Liang \textit{et al.} 2020). The high tolerance of S7942 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 \textit{et al.} 2020). This adds a technical and economical bottleneck for the broad and sustained adoption of phototrophic treatment processes. As Shoener \textit{et al.} (2019) highlighted, low-cost filtration systems, such as sand-filters, alone cannot solve the problem of photobioreactor contamination. Filtration at 0.45 \textmu m 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 S7942-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, an S7942-based treatment stage is proposed as an alternative nutrient removal technology.

### 3.1.3.7. Effect of cultivation period

The effect of cultivation period on biomass yields can be evaluated based on the obtained results regarding RGR\textsubscript{Chl a} evolution in S7942 cultures (Figure 3). These data are extremely important for

| Culture media                  | Statistic | Relative (%) NO\textsubscript{3} \_N removal rate (\textit{RRRNO3_N}) | Relative (%) S7942 growth rate (\textit{RGRChl a}) |
|-------------------------------|-----------|-------------------------------------------------|-----------------------------------------------|
|                               |           | Overall (20 days)                             | 1\textsuperscript{st} week                        |
| BG-11                         | Average   | 3.23                                           | 2.08                                           |
|                               | Min       | 1.16                                           | 1.37                                           |
|                               | Max       | 6.70                                           | 3.83                                           |
|                               | Std. deviation | 1.68                                           | 0.74                                           |
| Treated snack wastewater      | Average   | 3.45                                           | 2.48                                           |
|                               | Min       | 2.56                                           | 1.27                                           |
|                               | Max       | 4.41                                           | 3.27                                           |
|                               | Std. deviation | 0.93                                           | 1.07                                           |
| Treated dairy wastewater      | Average   | 3.64                                           | 2.36                                           |
|                               | Min       | 2.26                                           | 1.55                                           |
|                               | Max       | 4.69                                           | 3.07                                           |
|                               | Std. deviation | 1.25                                           | 0.77                                           |

Table 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
upsampling and implementing S7942-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 S7942 growth rates in relatively small photobioreactors.

As evident in Figure 3, the relative growth rate of all S7942 cultures decreased over time regardless the growth media and the culture temperature. The drop in RGRChl a that is observed at the 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 & Richmond 1996). A shift of maximum growth rate at longer cell retention time could be obtained via increase of mixing rate (agitation) and/or 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 seven days.

3.2. S7942 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 S7942, thus the volume of a photobioreactor and the investment/operating cost.

The calculated relative (%) nitrates removal rate (RRRNO3_N) in the S7942 cultures and their respective RGRChl a are presented in Table 2, categorized in terms of growth-media, i.e. cultivation in BG-11 or in BTWW wastewater. The S7942 cultures with BG-11 at 16 °C to 18 °C, 30 °C to 32 °C and 35 °C to 37 °C were excluded from the calculations, since they are incomparable to the cultures with wastewater-media that operated at 20 °C to 27 °C.

The average RRRNO3_N and RGRChl 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.

As evident in Figure 4, RRRNO3_N and RGRChl a are similar or even greater in experimental setups with wastewater as growth media. This indicates that the BTWW do not inhibit S7942 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 S7942 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 3.

These results indicate that if coupled with aerobic AS process, S7942 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 S7942-based nutrient removal stage individually or as a synergetic couple with denitrification process. Key elements for successful implementation of a S7942-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 low-tech or high-tech disinfection technologies (Arias et al. 2020). While there is an indisputable benefit of cultivating phototrophic 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 O2 from reduction of nitrates/nitrites to N2O, NO and N2 gases).

Denitrification occurs mainly as a response to changes in the O2 concentration of AS bacteria’s immediate environment, as well as a result of direct nitrates uptake by activated sludge bacteria. Despite being a beneficial process for efficiently removing nitrates from wastewater, it has a negative effect in removing valuable nitrogen fertilizer from the soil to form N2 gas, while releasing significant quantities of the potent greenhouse gas N2O and the tropospheric pollutant NO (Skiba 2008).
On the contrary, a phototrophic process recovers nitrogen from wastewaters, transforms it to valuable biomass, fixes CO₂ 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 S7942 cultivation times are the maintenance of optimal temperature and the unintermittent flux of light in the culture.

![Figure 4](image_url) Average $R_{RR_{NO2-N}}$ (a) and $R_{GR_{CHL-a}}$ (b) of S7942 cultures, for the first week of cultivation and the overall duration of cultivation (20 days).

| Parameter/growth media | Autoclaved BG-11 | Autoclaved dairy BTWW | Autoclaved snack BTWW |
|------------------------|------------------|------------------------|-----------------------|
| Average S7942 growth rate (mg/L°d) | 0.299 | 0.218 | 0.283 |
| Average nitrates removal rate (mg/L°d) | 3.75 | 3.44 | 3.26 |
| Average phosphates removal rate (mg/L°d) | 1.78 | 2.32 | 1.97 |
3.2.1. Evaluation of S7942 as alternative to AS de-nitrification process

There is an indisputable 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 S7942 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 S7942-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 m³/d, biodegradable organic substrate concentration (BOD₅) of 2,000 mg/L and available nitrate-nitrogen for denitrification at a concentration of 100 mg/L, which has been subjected to AS de-nitrification process or to S7942-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 & Tchobanoglous 1998; Metcalf & Eddy 2003) regarding specific nitrate denitrification rates (UDN), maximum biomass yield coefficient (Ymax) and decay coefficient (kd), as well as on the presumption that biomass concentration in terms of mixed liquor volatile suspended solids (MLVSS) is 3,000 mg/L and that operating conditions in denitrification bioreactor are 0.2 mg/L of dissolved oxygen and 20 °C water temperature. The UDN values of 0.05 kgNO₃-N/KgVSS/d and 0.06 kgNO₃-N/KgVSS/d were selected for calculating the volume of the denitrification bioreactor in post-denitrification and pre-denitrification process, respectively, while the Ymax value of 0.65 kgVSS/KgBODremoved, kd value of (0.05/d) and a dissolved oxygen concentration of 0.2 mg/L were selected.

The resulting bioreactor volume in the post-denitrification configuration is 166.7 m³, 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 m³ 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 S7942 growth rate of all properly disinfected cultures in BTWW and for a 20-day period, as well as on the respective average nitrate removal rate. The growth rate of S7942 is assessed as a potent biological component for novel and sustainable biological nutrient removal processes for industrial wastewaters that have been subjected to AS de-nitrification process or to S7942-based nitrogen removal process.

According to the resulting average SNUR of 0.196 mgNO₃-N/mgVSS and the data from the employed scenario, a daily S7942 biomass production of approximately 61.2 kg/d 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/d, the necessary volume of the photobioreactor is estimated at approximately 1,530 m³, corresponding to a hydraulic retention time (HRT) of approximately 12.75 days. The estimated photobioreactor volume is approximately 9 times and 18 times larger than the denitrification bioreactor of post-denitrification and pre-denitrification 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/d (Silva et al. 2014) would correspond to a minimization of photobioreactor's volume down to 315 m³, 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 S7942 cell productivity in wastewater media in order to minimize photobioreactor’s investment cost, with special emphasis on increased photosynthetic activity.

4. CONCLUSIONS

S7942 is assessed as a potent biological component for novel and sustainable biological nutrient removal processes for industrial wastewater treatment. An 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. S7942 can efficiently grow in properly disinfected, via low cost and/or environmentally friendly techniques, industrial wastewaters that have been subjected to biological oxidation. Furthermore, S7942 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 S7942 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 S7942-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.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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