Wastewater Substrate Disinfection for Application of *Synechococcus Elongatus* PCC 7942 Cultivation as Tertiary Treatment

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Abstract

The cultivation of microalgae or/and cyanobacteria in nutrient-rich wastewaters presents a significant opportunity for enhancing sustainability of tertiary wastewater treatment processes via resources/energy recovery/production. However, maintaining a monoculture in wastewater-media constitutes a significant challenge to be addressed, as a plethora of antagonistic and predating microorganisms exist is such media. In this regard, the present work assesses the efficiency of the low-cost wastewater substrate disinfection techniques of filtration, use of NaClO, H₂O₂ or Fe(VI), in terms of antagonistic or/and predating microbial species growth inhibition in *Synechococcus elongatus* PCC 7942 cultivations. Nitrates and phosphates removal rates were also experimentally assessed. The results showed that filter thickness has a greater effect on disinfection efficiency than that of filter's pore size. Furthermore, the disinfection efficiency of Fe(VI), which was produced on-site by electrosynthesis via a Fe⁰/Fe⁰ cell, was greater than that of NaClO and H₂O₂. Filtration at ≤ 1.2 µm pore size coupled with chemical disinfection leads to unhindered S7942 growth and efficient nitrates and phosphates removal rates, at dosages of CT ≥ 270 mg min L⁻¹ for NaClO and CT ≥ 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 S7942 harvesting and may enhance photosynthesis rate. Thus, the utilization of wastewaters for S7942 cultivation as tertiary treatment seems a promising and novel alternative to common nutrient removal processes that can reduce environmental footprint and operational costs of wastewater treatment plants.

Introduction

Preservation of freshwater quality and inversion 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) and respond to the challenge of a carbon neutral future, as set in Paris Agreement (2016). Thus, the implementation of the best available technologies, practices and techniques (BAT) for the protection of the environment, the recovery of resources and the mitigation of greenhouse gases emissions must be the response to the challenge of sustainability.

Due to the high nutrient and energy content of wastewaters, the wastewater treatment sector offers significant opportunities for the application of BAT that align with the concept of circular economy (Hoek et al., 2016). Wastewater treatment processes that are based on phycée or/and cyanobacteria for the removal/recovery of nutrients suggest the evolution of activated sludge (AS) systems. In particular, the utilization of cyanobacteria for wastewater treatment coupled with resources recovery have received much attention as a novel and alternative resource for carbon emission mitigation and the production of third generation biofuels and/or biochemicals (Vassilev and Vassileva 2016; Maurya et al. 2021). According to this, many studies have been carried out where cyanobacteria are used as a bioremediation tool in order to treat wastewater effluents of various origins including pig slurries, domestic activities,
aqua culture systems e.t.c (Ansari et al. 2017; Morillas-Esparza et al. 2021; Winayu et al. 2021; Samiotis et al. 2021). Worth mentioning that some cyanobacteria strains may produce cyanotoxins, while other species such as *Synechococcus elongatus* PCC 7942 (hereafter *S*7942) do not produce cyanotoxins and under saline stress can increase its intracellular sucrose accumulation leading to increased yields of biofuels (biodiesel, bioethanol, biogas, bio-hydrogen) or/and relevant industrial products from *S*7942 (Stamatakis et al. 1999; Vayenos et al. 2000; Nowruzi et al. 2021). The cultivation of cyanobacteria in wastewater streams is considered a sustainable alternative to common nutrient removal processes and most promising for reducing the additional costs associated with cyanobacteria cultivation in full-scale (Arias et al. 2017; Samiotis et al. 2021). Cyanobacteria fixate significant quantities of CO$_2$ and produce O$_2$ and high-value biomass that can be used for the production biofuels, hydrocarbons, proteins, pigments and biopolymers for pharmaceutical, chemical and food industry (Trivedi et al. 2015; Pathak et al., 2018). The cultivation of cyanobacteria for the production of biofuels outperforms, in terms of sustainability, the conventional technologies that utilize sugar, starch, vegetable or animal fats due to the competitive consumption of food resources and the associated commitment of arable land, especially if it is combined with wastewater and/or flue gases treatment (Rosegrant et al. 2006; Gonsalves et al. 2016; Almomani et al., 2019). However, there is a big gap regarding the design, implementation and operation of cyanobacteria-based wastewater treatment processes. Their implementation in full-scale applications constitutes a challenge, because it depends on 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).

It is important to highlight that many studies on cultivation of microalgae have been adapted to laboratory settings and thereby somewhat have been detached from real world conditions. This included nutrient addition (Ansari et al. 2017), use of synthetic wastewater (Barnharst et al. 2018), or very short cultivation periods (Halfhide et al. 2014). Wastewater quality may directly affect the growth rate and nutrient removal efficiency of microalgae. This has been confirmed by the study of Tejido-Nuñez et al. (2019) that used *Chlorella vulgaris* and *Tetraselmis obsilus* to treat effluents from fish aquaculture systems. Their results showed that the efficiency of nitrate and phosphate removal was highly depended on the choice of the species involved and the pretreatment of wastewater using sterile filtration prior to cultivation, which enhanced the growth of the aforementioned species.

In addition, there are many limitations resulting from microalgae implementation including the use of wastewater as a culture medium and the generation of biomass in the presence of other microorganisms that compete for nutrients. The co-presence of other microorganisms needs to be limited by the application of several common disinfection methods including filtration or the addition of chemical disinfectants comparing to simple lab techniques such as autoclave that cannot be applied due to high cost and energy demands. Sodium hypochlorite (NaClO) in concentrations of 0.2 mg L$^{-1}$ to 2 mg L$^{-1}$ has shown good disinfection, with 1.9 mg L$^{-1}$ being recommended. This dose allows disinfection and at the same time chlorine residues of less than 0.2 mg L$^{-1}$, after 24 hours still remain in the wastewater media (Medrano-Barboza et al. 2021).
Besides disinfection with NaClO, novel and considered environmentally friendly disinfection techniques have been developed, with those based on hexavalent iron (ferrates) production showing a particular interest. The merits of using hexavalent iron for disinfection is that it presents high oxidative ability and it is transformed completely to the non-toxic trivalent form, simultaneously acting as a coagulant (Al Umairi et al. 2021). Due to their high oxidative and aggregation ability, ferrates can oxidize organic compounds from wastewaters, as well as microorganisms and their spores leading to clearer growth media, where photosynthetically active radiation (PAR) can travel unhindered by turbidity (Škulcová et al. 2021). Furthermore, the aggregative action of ferrates can assist on the collection of microalgae or/and cyanobacteria from a photobioreactor, 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). On the other hand, ferrates stability in environmental conditions is minimal rendering it non proper for commercial use neither in terms of provision (scarce and highly-priced) nor of storage (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).

It is well known that ultrafiltration and sand filtration are simple techniques that have been used in water and wastewater treatment as low-cost particle and pathogen removal filters (Kyzas and Mitropoulos 2019; Freitas et al. 2021). Although their main use is particulates removal, ultrafiltration and sand filtration have been also used as a post treatment exhibiting >90% removal of bacteria (Farooq and Al-Yousef 1993; Bray et al. 2021). In the study of Wood et al. (2019) is well documented the application of sand filters for the removal of Cryptosporidium in commercial swimming pools. Their review clearly explains that sand filters efficiency is not only depended on pore size but also on filter depth. Particles which are too small to be screened could be retained by the filter media as a result of weak intermolecular binding forces that come into play if the particles can get very close to the surface of the sand grains. Young-Rojanschi and Madramootoo (2014) also observed that increased filter media depths resulted in improvements of performance for E. coli reduction.

Aim of this study is to assess the possibility of utilizing biologically treated wastewaters as substrate for cultivation of S7942 monoculture in a photobioreactor, which can be used for tertiary treatment applications. More specifically, the efficiency of low-cost wastewater substrate disinfection techniques for antagonistic or/and predating microbial species growth inhibition are evaluated. For this aim, S7942 growth rate and nutrients removal in properly disinfected wastewater-media were studied. This work is considered of great importance for the implementation of cyanobacteria-based wastewater treatment processes, as sustainable alternative or supplementary treatment stage to conventional biological nutrient removal processes.

**Materials And Methods**

For the assessment of biologically treated wastewaters adequacy as S7942 growth-media in terms of growth inhibition by the presence of antagonistic or/and predating microbial species, a series of cultivation photobioreactors were set up using sterilized 500 ml Erlenmeyer flasks. Sterile cotton caps
were used for the uninhibited air transfer inside the photobioreactors avoiding the risk of air borne contamination. Moreover, nutrients removal rate was evaluated. A culture of \textit{S7942} in BG-11 growth-medium (Rippka et al. 1979) was used as inoculum for control and test setups. The inoculums were separated from media via centrifugation at 5000 rpm for 10 minutes. The photobioreactors were kept under continuous agitation and artificial lighting (fluorescent lamps at light intensities of 5 – 30 \( \mu \)mol-photons m\(^{-2}\) s\(^{-1}\)), at controlled temperatures of 20\(^\circ\)C to 26\(^\circ\)C.

The control setups contained BG-11 media, while the test setups contained biologically treated industrial wastewater (wastewater-media) that had been subjected or not to a single or a combination of low-cost disinfection techniques. The wastewater-media was obtained from the nitrification tank of a dairy industry's activated sludge (AS) wastewater treatment plant (WWTP), after suspended solids removal via sedimentation. Both control and test setups had an initial \textit{S7942} biomass content, in terms of chlorophyll \( a \) concentration, of approximately 1 mg L\(^{-1}\).

The disinfection performance of filtration (at pore size of 0.45 \( \mu \)m up to 5-13 \( \mu \)m), the use of NaClO (chlorination), hydrogen peroxide (\( \text{H}_2\text{O}_2 \)) and ferrates (Fe(VI)) in wastewater-media were evaluated prior the \textit{S7942} growth assessment. The disinfection efficiency of each technique was determined by microbiological examination (total viable count - TVC at 22\(^\circ\)C). 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 inhibitory action of different concentration disinfectants on \textit{S7942} growth rate was also studied. The growth of antagonistic or/and predating microbial species in the test setups was checked via microscopic examination using a Leica phase contrast microscope (x40 up to x1000 magnification).

The disinfection with ferrate, which is considered a novel and environmentally friendly disinfection technique (Al Umairi et al. 2021) was evaluated additionally in terms of its coagulation ability by assessing organic compounds (chemical oxygen demand, COD), phosphates and turbidity removal. For the production of ferrate solution, an electrochemical \( \text{Fe}^0/\text{Fe}^0 \) cell in 25 M NaOH was set up. 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). In this method, a volume of Fe(VI) solution is transferred to a concentrated Cr(III) solution, where Fe(VI) oxidises Cr(III) to Cr(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.

\textit{S7942} biomass growth rates, expressed as relative (\%) growth rate (RGR\(_{\text{Chl} \ a}\)), were calculated on the basis of chlorophyll-\( a \) concentration (Moran 1982) and according to equation (1) (Vayenos et al. 2020).

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

(1)
Where, \( n \) is the days of cultivation; \( [\text{Chl } a](n\ d) \) is the chlorophyll \( a \) concentration after the \( n^{th} \) day; \( [\text{Chl } a](0\ d) \) is the initial chlorophyll \( a \) concentration.

Equation (1) was also used for the calculation of relative (%) nitrates removal rate \( (RRR_{\text{NO}_3,N}) \) and relative (%) phosphates removal rate \( (RRR_{\text{PO}_4,P}) \), 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. 2012; Trikilidou et al. 2020).

Results And Discussion

Assessment of filtration

The porosity and the thickness of a filter-media 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 regarding their ability to remove the existing microbial load of the wastewater-media for \textit{S7942} cultivation. More specifically, filters with pore size of 0.45 \( \mu \)m, 0.7 \( \mu \)m, 1 \( \mu \)m, 1.2 \( \mu \)m 2-4 \( \mu \)m, 3-5 \( \mu \)m, 5-7 \( \mu \)m and 5-13 \( \mu \)m were tested for TVC reduction in the filtrates. Their thickness and respective material are presented in Table 1, along with the results from TVC measurements.
Table 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°C) |
|-----------------------|----------------|----------------------|-------------------------------------|
| unfiltered            | -              | -                    | >5000                               |
| 0.45                  | cellulose      | 0.135                | 280                                 |
| 0.7                   | fiber glass    | 0.45                 | 4                                   |
| 1                     | fiber glass    | 0.70                 | 2                                   |
| 1.2                   | fiber glass    | 0.26                 | 65                                  |
| 2-4                   | paper          | 0.15                 | 1256                                |
| 3-5                   | paper          | 0.17                 | 814                                 |
| 5-7                   | paper          | 0.32                 | 540                                 |
| 5-13                  | paper          | 0.15                 | >2000                               |

As shown in Table 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 of the fiber-glass, presented lower disinfection efficiency, despite having the smallest filter pore size. The paper filters with 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).

In the test setups, the growth of antagonistic and predating microbial species suppressed to the decline *S7942* growth leading to almost complete extinction of *S7942* population in terms of chlorophyll *a* (Figure 1). Chlorophyll *a* concentrations dropped from the initial concentration of 1 mg L⁻¹ to 0.03 mg L⁻¹ up to 0.19 mg L⁻¹ after 20 days. 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 antagonistic or and predating microbial species in the filtrates.

It is concluded that none of the applied filtration techniques is efficient to sustain a monoculture of *S7942* in the photobioreactors. It is evident from Table 1 and Figure 1 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 on the observations 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.

**Assessment of chemical disinfection by Sodium hypochlorite, Hydrogen peroxide, Hexavalent iron**

Similarly to filtration, chemical disinfection alone, using NaClO or H₂O₂ or Fe(VI), is proven to not be able to alleviate the problem of S7942 culture contamination. While higher dosages of these disinfectants, in terms of $CT$, led to lower TVC at 22°C, complete disinfection could not be achieved. Even at considerably high NaClO or H₂O₂ 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-medias (Figure 2). The effect of the suggested NaClO solution for 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 NaOCl concentrations up to 60 mg L⁻¹, despite the observed significant decrease in TVC.

In our study high efficiency disinfection was achieved at NaOCl 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 H₂O₂, thus the use of NaClO is suggested.

The highest disinfection efficiency was observed using the freshly prepared via electrosynthesis Fe(VI) solution (Figure 2). Moreover, the resulting Fe(III) species from ferrates reduction proved to be a potent coagulant for the removal of particular matter and colloidal organic compounds that may be present in wastewater-media, which has a significant impact on light transfer efficacy in a photobioreactor (Škulcová et al. 2021). 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 89–97% and 71–84% respectively. Worth mentioning that COD removal can be also attributed to organic compounds oxidation by Fe(VI) (Sharma et al. 2015).

Our results are in accordance with the findings of other researchers. More specifically, the dual-function of ferrate as a coagulant and disinfectant for chemically-enhanced primary treatment has been also reported by Al Umairi et al. (2021). They 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 in order to achieve a 5-log 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. Worth mentioning that the evident coagulative action of ferrates can assist on the
emerging problem of microalgae or/and cyanobacteria harvesting from the photobioreactors (Addison et al. 2021). An additional merit of a ferrate-based disinfection technique is that the resulting Fe(III) from ferrates reduction could enhance photosynthesis in the photobioreactor. Fe(III) species have been shown to enhance biomass production (Rana and Prajapati 2021). Thus, a ferrate solution could replace NaClO or H₂O₂ as disinfectant, offering a more sustainable solution to wastewater-media disinfection. Nevertheless, further study regarding optimal Fe⁰/Fe⁰ cell configuration and current density is considered essential towards energy consumption minimization for Fe(VI) production.

**Synergy of studied disinfection techniques**

As previously presented, each of the studied disinfection technique alone could not address the challenge of maintaining a growing S7942 monoculture in the photobioreactor. Hence, the suggested low-cost disinfection technique of filtration was evaluated as synergetic couple with the use of NaClO or Fe(VI), in terms of disinfection efficiency and S7942 growth in the experimental setups (Figure 3).

The assessment of disinfection efficiency showed that complete wastewater-media 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 all wastewaters filtrated with ≤1.2 µm pore size filters (Table 1) and at disinfectant dosages of $CT \geq 270 \text{ mg min L}^{-1}$ and $CT \geq 157 \text{ mg min L}^{-1}$ for NaClO and Fe(VI) respectively. The obtained wastewater-media with this procedure are considered properly disinfected. In the case of NaClO disinfection, dechlorination of the wastewater-media with sodium thiosulphate is necessary prior its use, in order to prevent the inhibition of S7942 growth by residual chlorine.

The properly disinfected wastewater-media were used for the study of S7942 growth in 8 test setups (Figure 3). The results showed that S7942 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 with pore size 1 µm and 0.70 mm thickness were proved more effective. It should be noted that at the applied dosages of Fe(VI) solution, neither the quantities of residual Fe(VI) nor of total iron had an impact on S7942 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 Fe(VI) disinfection is an efficient procedure prior the use of wastewaters as S7942 cultivation substrate and tertiary treatment.

**Antagonistic and predating species growth in S7942 culture setups**

Microscopic examination of S7942 cultures setups with different non-properly disinfected wastewater media, one from a dairy industry and another from a salty-snack industry, were conducted. As shown in Figure 4 – inset 2, yeasts were the dominant antagonistic species to S7942 in the dairy wastewater test setups. On the other hand, protozoan (mainly ciliates) and metazoan (mostly rotifers) dominated in in the salty-snack wastewater test setups (Figure 4 – inset 3). Beside the growth of non-phototrophic
microorganisms, antagonistic to S7942 phototrophic species were also developed, especially in experimental setups with non-filtrated wastewater-media (Figure 4 – inset 4). In Figure 4 – inset 1, an uninfected S7942 culture is depicted.

The results indicate the necessity of biological contaminants control, since they pose a serious threat to microalgae or cyanobacteria cultivation. According to Day et al. (2017) even small numbers of herbivorous protozoa can rapidly multiply in 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 Tetraselmis 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.

It is noteworthy that in properly (coupled) disinfected wastewater-media 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 S7942) was observed after the first week of cultivation. However, the growth of these microbial species did not hinder S7942 growth.

**S 7942's growth rate and nutrients removal rate in disinfected wastewater-media**

The properly disinfected wastewater-media, presented in Figure 3, were used for the study of S7942 growth. Based on equation (1), the % specific growth rate of S7942, as well as the % specific nitrates and phosphates removal rates were evaluated in control and test setups (Figure 5). For this purpose, 2 control setups, 4 test setups with wastewater-media filtrated at ≤1.2 µm pore size and chlorinated/dechlorinated and 4 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 S7942 growth rate, as well as the average values of nitrates and phosphates removal rates of each setup group are presented in Figure 5. 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 coagulative action of the resulting Fe(III).

According to Figure 5, 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 S7942 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 S7942 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 2.51–3.07% and 11.33–14.40% respectively, compared to those obtained in the control setups ($RRR_{NO3_N} = 2.84\%$ and $RRR_{PO4_P} 0.72\%$ 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 S7942 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 photobioreactors. 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 ($5 - 30 \mu\text{mol-photons m}^{-2}\text{s}^{-1}$), nitrates removal up to 42% can be achieved for a cultivation period of 20 days. At favourable conditions of 150 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$, 3.4 times higher cell productivities are obtained (Silva's 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 $S7942$ growth, a phenomenon that has to be addressed via nutrients addition or/and pH control (Di Termini et al. 2011; Mennaa et al. 2019).

Conclusions

*Synechococcus elongatus* PCC 7942 ($S7942$) can be cultivated in properly disinfected wastewaters, thus used for 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 $\leq 1.2 \mu\text{m}$ pore size and chemical disinfection at $CT \geq 270 \text{ mg min L}^{-1}$ and $CT \geq 157 \text{ mg min L}^{-1}$ for NaClO and Fe(VI) respectively leads to unhindered $S7942$ growth. Fe(VI) dosages up to $CT \geq 157 \text{ mg min L}^{-1}$ do not hinder $S7942$ growth and can assist in 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 ($5-30 \mu\text{mol-photons m}^{-2}\text{s}^{-1}$), nitrates removal is 42%, which could be increased up to 85% at favourable lighting conditions ($150 \mu\text{mol-photons m}^{-2}\text{s}^{-1}$). 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 $S7942$ can play a significant role towards the design of sustainable and carbon-negative tertiary wastewater treatment technologies. An ultrafiltration configuration coupled with chemical disinfection after the sedimentation tank of secondary biological treatment stage is proposed.
as a necessary, efficient and low-cost disinfection technique for full-scale scale implementation of S7942-based wastewater treatment processes.

Declarations

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**Figures**

![Chlorophyll a concentration evolution in S7942 control setup and in test setups with filtrated wastewater substrate.](image-url)

**Figure 1**

Chlorophyll a concentration evolution in S7942 control setup and in test setups with filtrated wastewater substrate.
**Figure 2**

Disinfection efficiency of chlorination and Fe(VI)-based disinfection techniques at different disinfectant dosages ($CT$).

**Figure 3**

Growth curves of control setup in BG-11 medium and of test setups in wastewater substrate after filtration followed by chlorination or ferrate-based disinfection.
Figure 4

Indicative S7942 growth curves in uninfected and infected experimental culture setups (Microscopic depiction of S7942 cultures at x1000: Inset1 = uninfected; Inset 2-4 = infected).

Figure 5
Average values of % relative growth, nitrates and phosphates removal rates in control and test setups.