Highly effective removal of presence of toxic metal concentrations in the wastewater using microalgae and pre-treatment processing

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ORIGINAL ARTICLE

Highly effective removal of presence of toxic metal concentrations in the wastewater using microalgae and pre-treatment processing

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Abstract
Cultivation of the microalgae in the wastewater has gained profound interest in recent years owing to the significant advantages like removal of nutrients from the wastewater. In general, the growth of the microalgae *Coelastrella* sp. on the swine wastewater is effective despite the presence of pollutants. The current study discusses the removal of concentration of nitrate, ammonia and phosphorous in the wastewater. Further, the biomass productivity and lipid content of *Coelastrella* sp. were also determined. Based on the procured results, the concentrations of the total phosphorus and ammonia nitrogen were decreased with the culturing duration. On the other hand, the copper oxide recorded the spike pattern along the cultured time. In contrast with the content of the ammonia and copper inhibiting the growth of the *Coelastrella* sp., with regard to the biomass and lipid productivity rate, the pre-treated wastewater reported superior growth rate of microalgae then untreated water. Aeration pre-treatment reduced the ammonia and copper contents in the wastewater which enable the impressive growth rate. The obtained average biomass and lipid productivity were 81.5 mg/ld and 19.5 mg/ld, respectively.

Keywords Microalgae · Heavy metal · Wastewater · Lipid content · Nitrate

Introduction

Handling of the swine wastewater remains challenging during the purification process owing to the chemical oxygen demand (COD) and biological oxygen demand (BOD). Due to the presence of highly suspended solids and sludge, the pre-treatment procedure is environmentally hazardous (Anushree and Sharma 2017; Brindhadevi et al. 2020). Further, the use of the swine wastewater to the value-added purpose, such as agriculture, is not viable due to the presence of heavy metal. Thus, removal of the toxic heavy metals is seemingly important to re-use the wastewater. One of the best and inexpensive techniques to absorb the metals in the wastewater is the cultivation of microalgae. Microalga is an important source for the production of the biomass to satisfy the energy demand via bio-fuel production (Sekar et al. 2021; Ganesan et al. 2020). As the fossil fuels are depleting, many scholars believe that the utilization of renewable resources found to be crucial source for environmental protection and promote sustainability, microalga is considered to be better solution for its impressive productivity and its environmentally friendly nature (Praveenkumar et al. 2019; Meddah et al. 2020; Anderson et al. 2020). Besides, the rate of the photosynthesis is nearly 50 times higher than the terrestrial plants. Another key thing to remember is that the algae can be used as the major food source when they are commercialized sufficiently but the microalga has not been treated as the primary source of energy due to the high requirements of nutrients and water requirements (Kim et al. 2021; Brindhadevi et al. 2021). Hence, cultivating them in the wastewater in an optimized way expected to be phenomenal. Typically, microalgae grow in all sorts of the wastewaters without any difficulty. From the municipal wastewater to industrial wastewater, the algae have the capacity to grow without any difficultly. However, the growth is affected due to the presence of high toxic metal content, such as ammonia, nitrogen and phosphorous (Rashid et al. 2019; Sliesarenko et al. 2020; Zhu et al. 2018). The growth rate of the microalgae is hindered due to the large consumption of the harmful pollutants. Many investigations proved the production of bio-fuel from the biomass and use of them as

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the energy source to power the automotive engines (Gaikwad et al. 2021).

Li et al. (2018) studied the *Coelastrella* sp. growth in the swine wastewater. It is revealed that the concertation of Zn (II) reduced the growth of the microalgae and nutrient removal. The presence of the Zn (II) in the wastewater reduces the value of pH and volatilization. On the other hand, the *Coelastrella* sp. reported the 62.3% removal efficiency of NH_3–N for the lower concentration of Zn (II) (Li et al. 2018). Besides that, the growth of the algae also dropped the level of total phosphorous to 77% for 8 mg/l. Cheng et al. reported the toxic contents in the wastewater that impact the microalgae growth and cultivation. Alga in the swine wastewater depends on the nutrients, toxic level and cultivation. The above review presented the various types of the microalgae species and its treatment for higher productivity rate (Cheng et al. 2019). Pengfeicheng et al. cultivated the microalgae *Tribonema* sp. and *Synechocystis* which spin the wastewater with rich lipid and protein contents. Cultivation of the microalgae typically reduces the requirement of the nutrient intake and make the microalgae economical and inexpensive. Besides, the growth of microalgae removes the toxic metal presence, such as ammonia, phosphorus, copper and nitrogen. To enhance the process of the treatment, the TiO_2 nanoparticles were employed. On the other hand, the growth of microalgae with the pre-treated wastewater generates higher biomass productivity compared to non-treated wastewater (Salama et al. 2017). Meanwhile, the pre-treated wastewater reduces the pollutant NH_3–N, TP, and COD concentrations at higher rates than the non-intense pulsed light wastewater (Cheng et al. 2020a). From the above, it is clear that growing the microalgae serves two purposes with the removal of toxic metallic content in wastewater by the absorption of nutrients abilities and production of the biomass. In this study, microalgae strains were cultivated in the swine wastewater to capture the heavy metals' presence in the wastewater.

Materials and methods

Microalgae cultivation and wastewater collection

Initially, the microalgae were isolated from the local pond near the piggery farm located in Tamil Nadu, India. After procuring the strain, they were identified based on the morphology and rDNA sequencing. The obtained microalgae were cultivated in incubator at 26 ± 1 °C using the blue–green BG11 medium in a 500 ml flask (Luo et al. 2016; Wan et al. 2019). Twice in a day, they were shaken to radiate light evenly. The collected swine wastewater was pre-treated before the cultivation of the microalgae. Three types of the wastewater are considered, such as ordinary, aerated wastewater and diluted wastewater. The swine wastewater was diluted with distilled water and forms DSW. On the other hand, the collected water was aerated in an open atmosphere for one week to form ASW. All three collected samples underwent sedimentation and filtration. After the filtration (using the 100 µm sieve mesh), they were centrifuged at 8000 rpm for 8–10 min and autoclaved at 120 °C for at least 40 min. The concentration of the pollutants was measured and the treated water was maintained for future process at 3 °C.

Analytical methods

The concentrations of the NH_3–N and TP were measured using standard wastewater monitoring and analysis procedure was done using the atomic absorption spectroscopy (Franchino et al. 2016). The mass of the algal biomass yield and productivity in mg/l day were measured using the below Eqs. (1) and (2).

$$\text{Algal biomass yield(mg/l)} = X_{mc} - X_{ic} \tag{1}$$

$$\text{Algal biomass productivity(mg/ld)} = \frac{X_{mc} - X_{ic}}{dt} \tag{2}$$

$$\mu\left(\frac{1}{d}\right) = \frac{\ln W_2 - \ln W_1}{\text{Final time} - \text{beginning time}}$$

where X_{mc} maximum concentration, X_{ic} initial concentration, dt duration of days, *W* dry weight of the microalgae.

The ammonia nitrogen and total phosphorus were obtained from the first-order kinetic model based on the earlier studies (Li et al. 2020). Further, the fatty acid methyl ester (FAME) and lipid were also determined. The FAME content of the dried algae samples was found using transesterification technique (Gao et al. 2018).

Results and discussion

Effect of cultivation time on removal of total nitrogen (TN)

Figure 1 represents the *Coelastrella* sp. strain on the removal of the toxic pollutant NH_3–N. From the obtained results, it is evident that the NH_3–N content has been decreased significantly as the number of cultivation days increased. Until 8 days, the content of the NH_3–N was dropped massively. Once the cultivation day goes beyond the day 10, the removal efficiency is dropped for all test samples (Luo et al. 2016; Li et al. 2020). In specific, the ASW sample reported higher removal of the pollutant compared to DSW and SW. Besides, the DSW and SW are not creating high

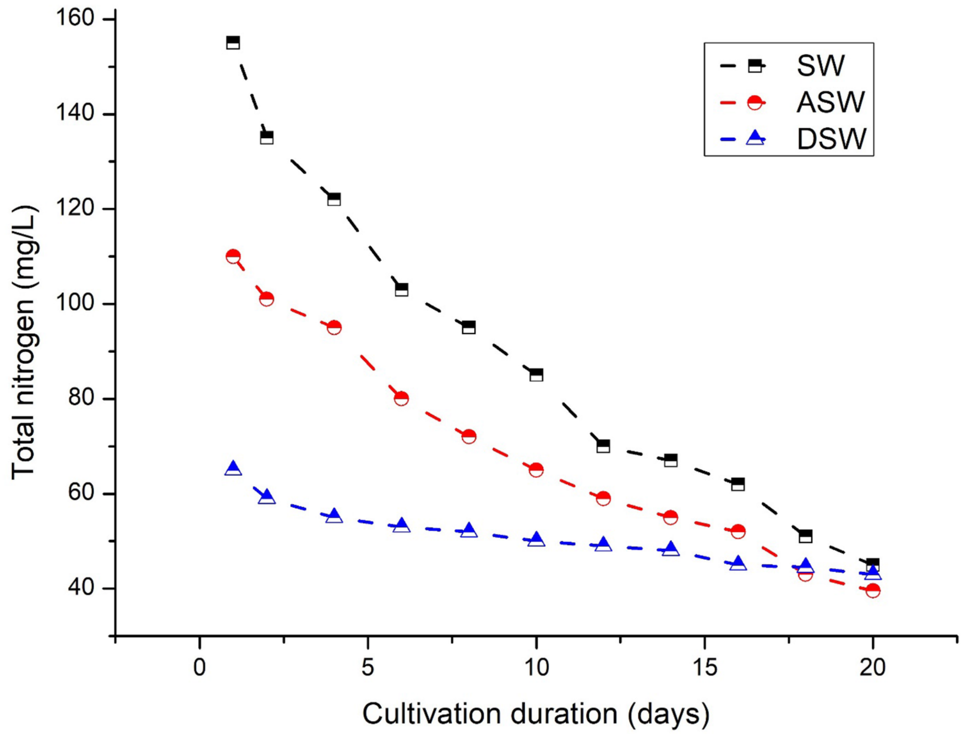


Fig. 1 Changes in total nitrogen concentration at various wastewater pretreatment

impact on the removal of the toxic pollutants like DSW due to the volatilization and dominant removal mechanism. For instance, at day 4, the contents of the NH_3–N for SW, DSW and ASW were 100 mg/l, 60 mg/l and 120 mg/l, respectively. On comparing with the DSW, the ASW reported 50% higher removal efficiency. The obtained results revealed that the pre-treated wastewater is essential for the removal of toxic metallic contents in the swine wastewater (Cheng et al. 2020b; Bhatia et al. 2021). The ASW increased the removal of NH_3–N content due to the better removal ability of the *Coelastrella* sp. cells (Tong et al. 2020). Meanwhile, the distilled water reduces the volatilization of the pollutants (Muñoz and Guieysse 2006; Gonçalves et al. 2017). On the other hand, as the cultivation time increased *Coelastrella* sp., performance was lowered as represented in Fig. 2. For example, the removal of TN for the day 4 of SW, DSW and ASW was 122 mg/l, 55 mg/l and 95 mg/l which is 23%, 20%, and 14% of the removal efficiency compared to day 1. However, from the day 4 to day 10, values were not significant. The ASW reported superior removal efficiency.

Effect of cultivation time on the total phosphorus (TP) removal

Figure 2 represents the effect of *coelastrella* sp. strain on phosphorus removal. The content of the phosphorous in the wastewater restricts the growth of microalgae. Removal of the P-loads from the wastewater increases the growth and biomass productivity of the microalgae. Initially, the concentrations of the total phosphorous were impressively dropped until day 8. Once the cultivation time reached beyond the day 8, the removal of TP decreased. Furthermore, the role of pre-treatment was crucial. At day 4, the TP contents for the SW, ASW and DSW were 35 mg/l, 19 mg/l and 11 mg/l which were further reduced to 26.55 mg/l, 10.55 mg/l and 9 mg/l on day 10. The TP content was decreased as the cultivation time was increased. After the keen observation, it is noted that the TP content was not dropped below 3.2 mg/l despite the treated test wastewater samples.

The removal efficiency of the phosphorous is low due to the significance of the presence of zinc in the wastewater. In general, zinc concentration on the wastewater decreases the growth rate of the microalgae and also decreases the P recovery from the wastewater. According to the previous report, the TP can be removed through sedimentation process. Thus, the aeration treatment considered to be the effective method in the removal of the pollutants. However, with regard to the P recovery, the ASW method is considered to be more effective since the recovery of the TP, which is twofold times higher than DSW. However, compared to ASW and DSW, the neat SW reported the profound response on removal of the TP pollutants in the swine wastewater.

Effect of the microalgae biomass on pretreatment

Figure 3 represented the dynamic changes in the cultivation of the biomass based on the pre-treatment process. The

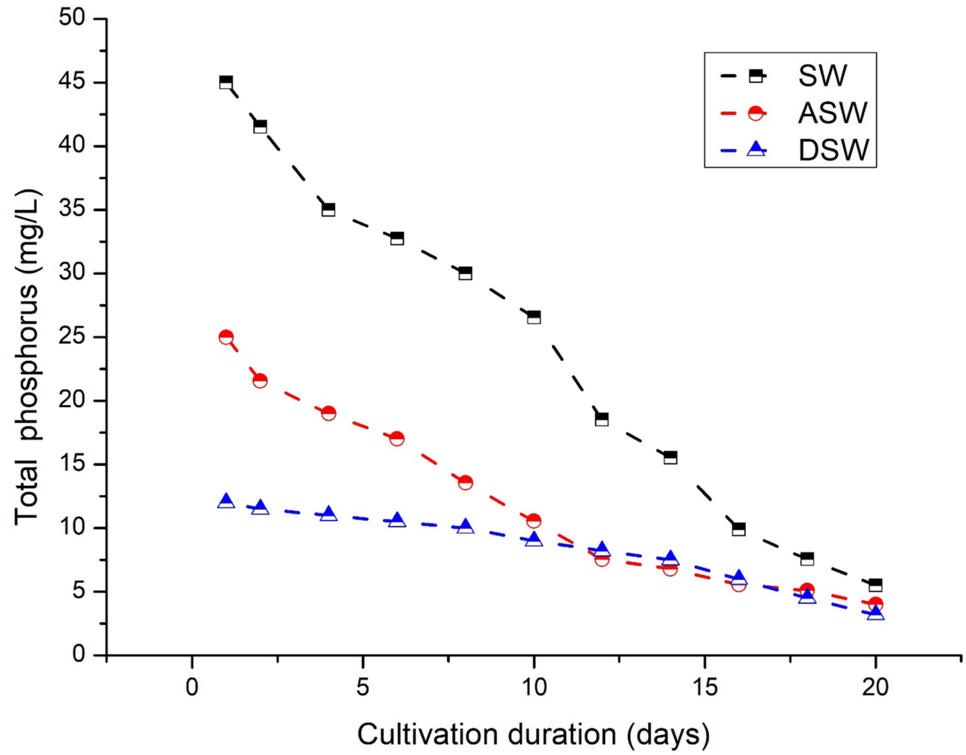


Fig. 2 Changes in total phosphorous concentration at various wastewater pretreatment

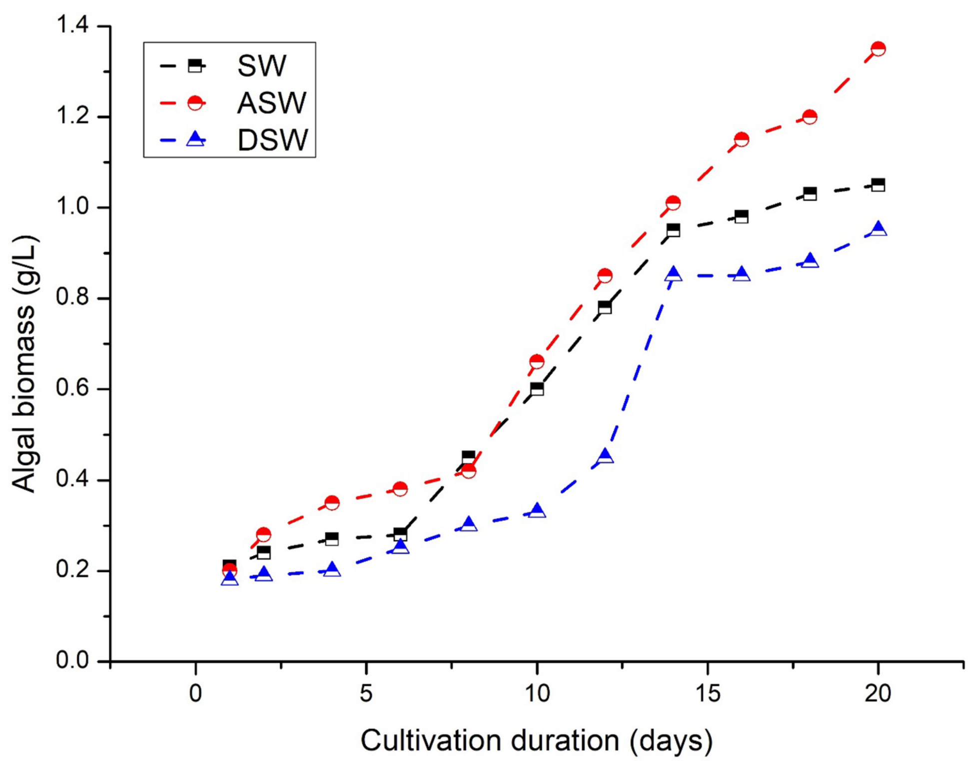


Fig. 3 Algal biomass productivity for various wastewater pretreatment

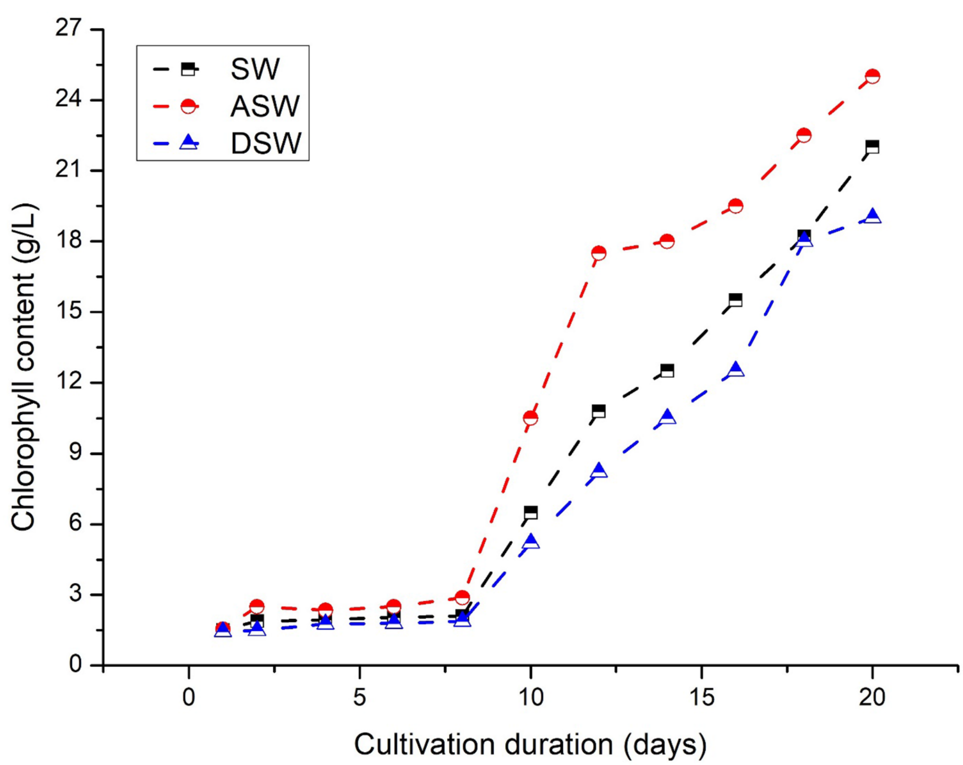
biomass production rate was calculated for different pretreatment procedures and compared to each other to analyze the effects of the metallic content affecting the growth of the microalgae (Tong et al. 2020). From the representation, it is crystal clear that the algal biomass was tremendously increased at the initial stage of the cultivation time, i.e., 0–4 days. As the cultivation days exceeded beyond 10 days, the accumulation of the biomass was dropped massively. This effect was observed for all types of the treated and non-treated samples. The DSW sample reported less biomass production compared to ASW and SW. On the other hand, the ASW reported the higher biomass yield and biomass productivity which was more than 0.5-fold times large. Another key thing to remember is the concentration of the TP and TN resisting the growth of the *Coelastrella* sp. (Luo et al. 2016). Compared to the other treatment procedures, the ASW reported high biomass productivity than SW and DSW. For instance, at day 2, the ASW, SW and DSW biomass productivities were 0.28 g/l, 0.24 g/l and 0.19 g/l which were not increased until the day 6. After day 6, the productivity of the biomass took a huge spike in the algal biomass rate. For better clarity, compared to the day 2, the day 8 reported 72.7%, 70.9% and 50% increase in the algal biomass. Compared to the SW and DSW, the ASW reported 25% and 34% increase in the productivity.

Effects of pre-treatment on the chlorophyll contents, lipid content and productivity

Figure 4 shows the variation of the chlorophyll according to the cultural time. Initially the chlorophyll contents for the collected samples were 1.51 mg/l, 1.55 mg/l and 1.45 mg/l. The requirement of the chlorophyll content was close until day 6. After day 6, there was a drastic change in the chlorophyll content on day 10. For instance, on day 10, the chlorophyll was threefold, fivefold, and 2.5-fold higher compared to the day 2. This is due to the cultural growth of the microalgae. However, the chlorophyll content was close to each other at the end of the cultivation time of 22 g/l, 25 g/l and 19 g/l, respectively, for SW, ASW and DSW (Narayanan et al. 2018).

The lipid content for the algal biomass was determined using the sulpho-phospho-vanillin (SPV) colorimetric method. Figure 5 shows the lipid content and productivity of *Coelastrella* sp. cultured in the SW, DSW and ASW. The samples SW, DSW and ASW reported 6.55 mg/ld, 12.55 mg/ld and 18.5 mg/ld, respectively. The productivity of the lipids for the ASW-treated swine wastewater was threefold times higher than the SW and twofold times higher than DSW (Luo et al. 2016). Despite the huge difference in the productivity, the lipid content of the SW, DSW and ASW was close to one another. For instance, the SW, ASW and DSW lipid content was 17.2%, 16.5% and 15.5%. Due to the higher growth of algal in ASW, the lipid productivity was impressively higher than other samples reaching 18.5 mg/ld. Compelling all the above, the aeration pre-treatment procedure is one of the best practices compared to dilution process.

Fig. 4 Chlorophyll content for various wastewater pre-treatment



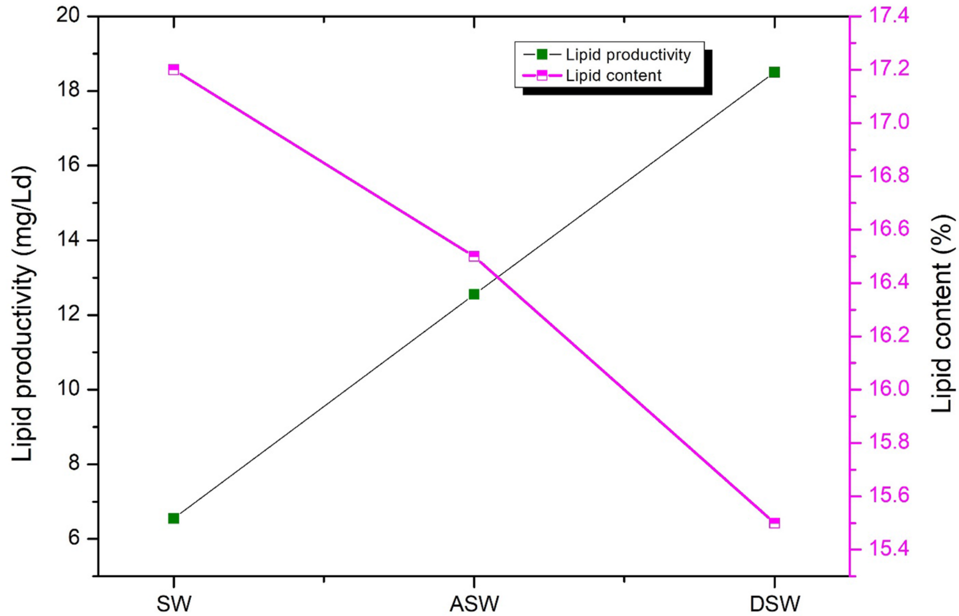


Fig. 5 Lipid productivity and lipid content for various wastewater pretreatment

Conclusion

This study demonstrated the removal of the toxic pollutants from the swine wastewater using the *Coelastrella* sp. On the other hand, the effects of the pretreatment procedures on the total nitrogen, total phosphorous, biomass yield, chlorophyll and lipid content were analyzed. From the findings, it is clear that the SW reported higher total nitrogen and total phosphorus removal compared to the ASW and DSW. Pre-treatment procedures reduced the volatilization and assimilation of the total nitrogen. On the other hand, the growth of the *Coelastrella* sp. was limited due to the presence of the TN and TP and the long cultivation time. Besides, *Coelastrella* sp. growing on the ASW reported the higher biomass yield than DSW and SW. The maximum biomass production was 1.35 mg/l for ASW. Furthermore, the lipid productivity was 40% and 89% higher than the DSW and SW. With regard to the chlorophyll content, the ASW recorded 27% and 12% higher values than DSW and SW.

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Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

Anderson A, Karthikeyan A, Kumar CR, Ramachandran S, Praveenkumar TR (2020) Lowest emission sustainable aviation biofuels as the potential replacement for the Jet-a fuels. Aircr Eng Aerosp Technol. https://doi.org/10.1108/AEAT-07-2020-0135

Anushree A, Kumar S, Sharma C (2017) Synthesis, characterization and catalytic performance of $ZnO–CeO_2$ nanoparticles in wet oxidation of wastewater containing chlorinated compounds. Appl Nanosci. https://doi.org/10.1007/s13204-017-0596-5

Bhatia SK, Mehariya S, Bhatia RK, Kumar M, Pugazhendhi A, Awasthi MK et al (2021) Wastewater based microalgal biorefinery for bioenergy production: progress and challenges. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2020.141599

Brindhadevi K, Shanmuganathan R, Pugazhendhi A, Gunasekar P, Manigandan S (2020) Biohydrogen production using horizontal and vertical continuous stirred tank reactor—a numerical optimization. Int J Hydrogen Energy. https://doi.org/10.1016/j.ijhydene.2020.06.155

Brindhadevi K, Anto S, Rene ER, Sekar M, Mathimani T, Chi NTL et al (2021) Effect of reaction temperature on the conversion of algal biomass to bio-oil and biochar through pyrolysis and hydrothermal liquefaction. Fuel. https://doi.org/10.1016/j.fuel.2020.119106

Cheng DL, Ngo HH, Guo WS, Chang SW, Nguyen DD, Kumar SM (2019) Microalgae biomass from swine wastewater and its conversion to bioenergy. Bioresour Technol. https://doi.org/10.1016/j.biortech.2018.12.019

Cheng P, Chen D, Liu W, Cobb K, Zhou N, Liu Y et al (2020a) Autoflocculation microalgae species *Tribonema* sp. and *Synechocystis* sp. with T-IPL pretreatment to improve swine wastewater nutrient removal. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2020.138263

Cheng HH, Narindri B, Chu H, Whang LM (2020b) Recent advancement on biological technologies and strategies for resource recovery from swine wastewater. Bioresour Technol. https://doi.org/10.1016/j.biortech.2020.122861

Franchino M, Tigini V, Varese GC, Mussat Sartor R, Bona F (2016) Microalgae treatment removes nutrients and reduces ecotoxicity of diluted piggery digestate. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2016.06.100

Gaikwad PU, Gnanamani S, Subramani N (2021) Experimental and numerical simulation of the piston engine fueled with alternative fuel blends: CFD approach. Aircr Eng Aerosp Technol. https://doi.org/10.1108/AEAT-12-2020-0291

Ganesan R, Manigandan S, Samuel MS, Shanmuganathan R, Brindhadevi K, Lan Chi NT et al (2020) A review on prospective production of biofuel from microalgae. Biotechnol Rep. https://doi.org/10.1016/j.btre.2020.e00509

Gao F, Peng YY, Li C, Yang GJ, Deng YB, Xue B et al (2018) Simultaneous nutrient removal and biomass/lipid production by *Chlorella* sp. in seafood processing wastewater. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2018.05.380

Gonçalves AL, Pires JCM, Simões M (2017) A review on the use of microalgal consortia for wastewater treatment. Algal Res. https://doi.org/10.1016/j.algal.2016.11.008

Kim DH, Yoon JJ, Kim SH, Park JH (2021) Effect of conductive material for overcoming inhibitory conditions derived from red algae-based substrate on biohydrogen production. Fuel. https://doi.org/10.1016/j.fuel.2020.119059

Li X, Yang WL, He H, Wu S, Zhou Q, Yang C et al (2018) Responses of microalgae *Coelastrella* sp. to stress of cupric ions in treatment of anaerobically digested swine wastewater. Bioresour Technol. https://doi.org/10.1016/j.biortech.2017.12.058

Li X, Yang C, Zeng G, Wu S, Lin Y, Zhou Q et al (2020) Nutrient removal from swine wastewater with growing microalgae at various zinc concentrations. Algal Res. https://doi.org/10.1016/j.algal.2020.101804

Luo L, He H, Yang C, Wen S, Zeng G, Wu M et al (2016) Nutrient removal and lipid production by *Coelastrella* sp. in anaerobically and aerobically treated swine wastewater. Bioresour Technol. https://doi.org/10.1016/j.biortech.2016.05.059

Meddah MS, Praveenkumar TR, Vijayalakshmi MM, Manigandan S, Arunachalam R (2020) Mechanical and microstructural characterization of rice husk ash and Al_2O_3 nanoparticles modified cement concrete. Constr Build Mater. https://doi.org/10.1016/j.conbuildmat.2020.119358

Muñoz R, Guieysse B (2006) Algal-bacterial processes for the treatment of hazardous contaminants: a review. Water Res. https://doi.org/10.1016/j.watres.2006.06.011

Narayanan GS, Kumar G, Seepana S, Elankovan R, Arumugan S, Premalatha M (2018) Isolation, identification and outdoor cultivation of thermophilic freshwater microalgae *Coelastrella* sp. FI69 in bubble column reactor for the application of biofuel production. Biocatal Agric Biotechnol. https://doi.org/10.1016/j.bcab.2018.03.022

Praveenkumar TR, Vijayalakshmi MM, Meddah MS (2019) Strengths and durability performances of blended cement concrete with TiO_2 nanoparticles and rice husk ash. Constr Build Mater. https://doi.org/10.1016/j.conbuildmat.2019.05.045

Rashid J, Azam R, Kumar R, Ahmad M, Rehman A, Barakat MA (2019) Sulfonated polyether sulfone reinforced multiwall carbon nanotubes composite for the removal of lead in wastewater. Appl Nanosci. https://doi.org/10.1007/s13204-019-00953-2

Salama ES, Kurade MB, Abou-Shanab RAI, El-Dalatony MM, Yang IS, Min B et al (2017) Recent progress in microalgal biomass production coupled with wastewater treatment for biofuel generation. Renew Sustain Energy Rev. https://doi.org/10.1016/j.rser.2017.05.091

Sekar M, Mathimani T, Alagumalai A, Chi NTL, Duc PA, Bhatia SK et al (2021) A review on the pyrolysis of algal biomass for biochar and bio-oil—bottlenecks and scope. Fuel. https://doi.org/10.1016/j.fuel.2020.119190

Sliesarenko V, Tomina V, Dudarko O, Bauman M, Lobnik A, Melnyk I (2020) Functionalization of polymeric membranes with phosphonic and thiol groups for water purification from heavy metal ions. Appl Nanosci. https://doi.org/10.1007/s13204-019-01170-7

Tong M, Li X, Luo Q, Yang C, Lou W, Liu H et al (2020) Effects of humic acids on biotoxicity of tetracycline to microalgae *Coelastrella* sp. Algal Res. https://doi.org/10.1016/j.algal.2020.101962

Wan L, Wu Y, Zhang X, Zhang W, Zhang W (2019) Nutrient removal from pickle industry wastewater by cultivation of *Chlorella pyrenoidosa* for lipid production. Water Sci Technol. https://doi.org/10.2166/wst.2019.217

Zhu Y, Rong J, Zhang T, Xu J, Dai Y, Qiu F (2018) Controlled and facile synthesis of a self-assembled enzyme–inorganic catalyst based on flexible metal-coated fiber for an excellent removal of synthetic pollutants from aqueous environment. Appl Nanosci. https://doi.org/10.1007/s13204-018-0791-z