



Impact of Microalgae in Domestic Wastewater Treatment: A Lab-Scale Experimental Study

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Article Info	ABSTRACT
Article type: Research Article	In most developing nations, municipal wastewater treatment is limited to aerobic secondary treatments, expensive and ineffective in removing nutrients from treated effluents before discharge, resulting in eutrophication and imbalance in receiving bodies. As a result, the effectiveness of <i>Chlorella vulgaris</i> for primarily treated wastewater collected from a sewage treatment plant during an 8-hour detention time was investigated in this study. Microalgae have been found to efficiently remove organics and nutrients to levels far below the desired limit in the present research. After algal treatment concentration of COD, phosphate and ammonia reduced to 12.43 mg/L (93.75%), 0.04 mg/L (98.40%) and below detectable limit (100%) respectively. In addition, remarkable reduction was found in solids (TSS, TS and TDS) and EC concentration. The use of microalgae resulted in an increase in DO concentration. As a result, introducing <i>Chlorella vulgaris</i> into a wastewater treatment system can lower nutrient and organics contents without any additional treatment.
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INTRODUCTION

Wastewater production on a wide scale is unavoidable in all modern societies. The composition of sewage reflects the way people live and the technologies they use (Gonçalves et al., 2017). Wastewater treatment is critical because it removes contaminants from wastewater and produces effluent that can be discharged or reused. Contamination issues can arise from the continuous transfer of wastewaters without adequate treatment (Moondra et al., 2021a). However, in the current situation, the essential requirement is to remove high levels of N and P, which can cause eutrophication. Apart from polluting freshwater, the spread of these blooms can be viewed as a threat to the overall health of the population. This demonstrates the need for effective treatment strategies to reduce nitrogen and phosphorus concentrations in wastewaters before discharge into receiving bodies. Biological treatments, on the other hand, can do this because chemical treatments increase difficulty, process costs and energy input (Whitton et al., 2016). The most environmentally friendly and least expensive type of wastewater treatment is a biological treatment, which uses microorganisms to break down contaminants in wastewater and valorize the remains through the synthesis of value-added compounds (Chaudhry et al., 2005; Rawat et al., 2011; Bhattacharjee and Siemann, 2015; Saxena et al., 2016).

Sewage treatment Plant (STP) secondary treatment units are ineffective at treating nutrients

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(Kaya et al., 1995), and their release alters pH and lowers DO, affecting fish populations (Abdel-Raouf et al., 2012) and degrading freshwater habitats (Renuka et al., 2013). Secondary treatments are expensive in capital and operations since they generate a lot of sludge (Arbib et al., 2014). In developing nations, these constraints are a hurdle for secondary treatment (Cabanelas et al., 2013). The blowers/aerators that meet aerobic bacteria's oxygen need to break down organic waste consume nearly half of the electricity used in the activated sludge process (Samori and Samori, 2012). With a greater understanding of the impact of wastewater on the environment, more advanced and eco-friendly technology is needed to improve water quality and reduce untreated wastewater. Developing clean, sustainable and renewable technologies is the need of the hour to reduce the pollution load in the receiving bodies.

Algal wastewater treatment can consume nutrients from wastewater, increase dissolved oxygen, and reduce pathogens in the wastewater. The mutual relationship between microorganisms and algae in the effluent could meet CO₂ and O₂ requirement for the treatment (Quijano *et al.*, 2017). The ultimate goal of wastewater management is to protect the ecology while addressing general well-being and financial concerns. Wastewater treatment is a critical initiative that needs to be prioritized for the betterment of society and our future (Boretti and Rosa, 2019). Wastewater is an excellent place for microalgae to thrive because it is a low-cost medium (Delrue *et al.*, 2016).

Photo bioreactors (PBRs) are adaptable systems that can be streamlined by the biological and physiological qualities of the algal species being developed. In PBR, direct transfer of gases and contaminants between the mature cells and climate is not permitted by the reactor wall. In regards to their shape or structure, PBRs are considered to have a few merits over open ponds as they offer better control over culture conditions and development parameters (pH, temperature, blending, CO₂ and O₂), leading to lesser evaporation, diminished CO₂ losses, permit to achieve higher microalgae densities, higher volumetric productivities, offer a progressively sheltered and ensured condition, preventing pollution or limiting attack by microorganisms (Hoh *et al.*, 2016). PBRs also experience ill-effects such as overheating, bio-fouling, oxygen accumulation, trouble in scaling up, the significant expense of building, working and developing algal biomass, cell harm by shear pressure and decay of material utilized for the photo-stage (Mata *et al.*, 2010; Hwang *et al.*, 2016; Goncalves *et al.*, 2017).

Major studies so far done in the field of phycoremediation are under artificial lighting and on synthetic wastewater for long HRTs (Caporgno et al., 2015; Quijano et al., 2017; Li et al., 2019). In the present study, microalgal species *C. Vulgaris* was employed to treat the effluent collected from the Primary Sedimentation Tank (PST) of 135 MLD sewage treatment plant. The study was carried out at the same detention period as the secondary treatment, i.e., 8 hours, to study the impact of microalgal treatment in reducing the physicochemical parameters assessed to the acceptable disposal limit without any additional treatment.

MATERIAL AND METHODS

Experiments were conducted to determine the efficiency of the system to study its effect when incorporated into a sewage treatment plant. Domestic wastewater treatment was carried out in this study utilizing *C. Vulgaris*, which was provided by Phycolinc Technologies Pvt. Ltd., an Ahmedabad-based consultancy. Every day 30 Litres of primary treated wastewater was collected from the PST outlet of the STP. During the previous analysis, the best removal of physico-chemical parameters was observed at 30% microalgal concentrations compared to other concentrations studied (Moondra et al., 2020). Hence 30 % microalgal concentration was used in the present study.

The batch study was conducted in two 15 L containers; one was filled with 30% (4.5 L) of microalgal solution (added only at the start of the experiment) and the rest with the primary

treated sewage. The other container was filled with 15 L of primary treated sewage (a control system), as shown in Figure 1. External aeration was provided in both the reactors. The study was conducted for 8 hours in which the aeration was provided in both the reactors for the first 3 hours. Then the mix was allowed to settle for an hour, i.e., intermittent settling, after which the mix was aerated for 2 hours, followed by final settling of 2 hours. After the final settling, the supernatant was taken for physicochemical analysis. The intermittent mixing during the study was done to maintain the DO concentration of 3 mg/L in the control system.

The mixture (microalgae and domestic wastewater) in both beakers was aerated for the first 24 hours before starting the experiment to acclimate the microalgae to the new environment. pH, ammonia, phosphate, chemical oxygen demand (COD), electrical conductivity (EC), total solids (TS), total dissolved solids (TDS), total suspended solids (TSS), nitrate, and dissolved oxygen (DO) were measured using standard testing protocols as outlined in APHA 2012. The effluent collected was filtered using a coarse filter with a pore size of 4.0 - 5.5 μm since filtration is the cost-efficient microalgae harvesting technique (Hwang et al., 2016). The change in effluent parameters was investigated for both pre and post-filtration. The analysis during the study was continued for 35 days. Each parameter for a sample was analyzed twice, and the average was considered for analysis.

RESULTS AND DISCUSSION

Large variation was observed in the influent wastewater characteristics during the study. The removal efficiency of the different parameters analyzed during the study was the key factor in deciding the impact of microalgae in wastewater treatment. The mean and standard deviation of influent and effluent wastewater for all parameters (except pH) in both situations, i.e., microalgal treatment and control (just aeration) for both non-filtered and filtered effluent, is shown in Table 1.

The study observed that microalgae effectively removed organics and nutrients from the influent. Microalgal treatment increased pH and DO concentration in the effluent compared to the control system. During the study, pH of the primary treated wastewater varied from 6.87 to 7.87. After treatment with *C. Vulgaris*, an increment in pH was found to be majorly due to photosynthetic CO_2 assimilation. (Schumacher and Sekoulov, 2003; Goncalves *et al.*, 2017). After microalgal treatment pH in the non-filtered effluent was ranging between 7.85 to 8.72, whereas in case of filtered effluent pH varied between 7.75 to 8.65. However, in the control

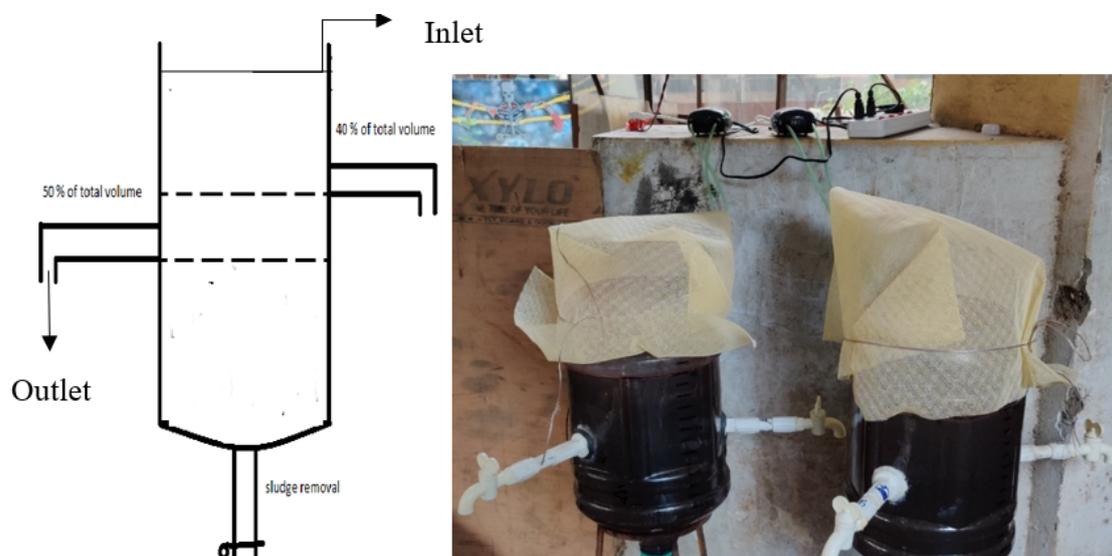


Fig. 1. Experimental setup of microalgal treatment for primary treated STP wastewater

system where only aeration was provided, pH varied from 6.60 to 8.56 in non-filtered effluent and was in the range of 6.65 to 8.48 for filtered effluent.

High pH also helps optimize cyanobacteria and pathogen disinfection (Goncalves *et al.*, 2017). Auto-flocculation is observed at high pH, contributing to removing suspended algae from the effluent and lessening the phosphorus concentration via interaction between cations and PO_4^{3-} -P to precipitate as an algal–mineral complex (Hoffmann, 1998). pH also has a significant role in various cellular processes such as energy metabolism, the functioning of enzymes, proteins uptake and nutrient uptake.

An increase in DO concentration was observed in the reactors i.e., the microalgal system and the control system. DO concentration was higher in the microalgal system because of photosynthesis (Park *et al.*, 2011) and external aeration. DO concentration in the influent was below the detectable limit. However, the DO concentration reached to 7.30 mg/L in the algal system and up to 3.80 mg/L in the control system. Variation in pH and DO concentration in influent and effluent is presented in Figure 2.

An adequate amount of DO leads to an increase in organics and nutrient removal. High pH, contributes to lessening the phosphorus concentration via interaction between cations and PO_4^{3-} -P to precipitate as an algal–mineral complex (Hoffmann, 1998). pH also has a significant role

Table 1. Variations in influent and effluent wastewater characteristics

Parameter	Raw W/W	30% M (NF)	30%M (F)	Aeration (NF)	Aeration (F)
pH	7.29	8.29	8.19	7.95	7.86
EC (mS/cm)	2.21±0.20	1.81±0.16	1.74±0.16	1.86±0.21	1.80±0.19
TS (mg/L)	3226.17±551.63	2270.37±413.00	2005.96±392.72	2404.24±455.69	2080.13±416.34
TDS (mg/L)	1095.20±99.73	898.29±78.98	864.25±79.95	927.04±103.13	890.67±92.12
TSS (mg/L)	2130.97±558.81	1372.09±406.18	1109.43±377.04	1477.20±434.02	1189.46±395.63
DO (mg/L)	0.00±0.00	6.30±0.79	6.20±0.78	3.40±0.23	3.40±0.21
COD (mg/L)	233.41±33.80	37.87±24.71	28.21±19.82	116.55±21.27	93.44±21.27
PO_4^{3-} -P (mg/L)	2.75±0.42	0.26±0.24	0.20±0.21	0.80±0.34	0.70±0.32
NO_3^- -N (mg/L)	0.73±0.48	5.19±2.54	5.09±2.56	3.37±2.23	3.27±2.18
NH_4^+ -N (mg/L)	15.63±1.54	1.68±2.33	1.21±1.94	6.00±3.43	5.22±2.95

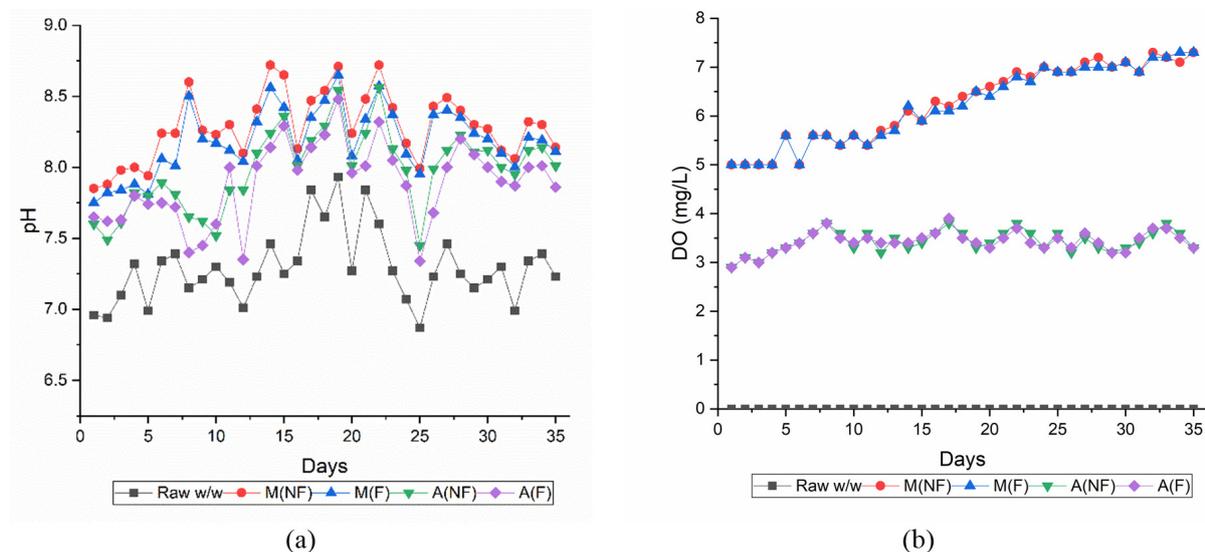


Fig. 2. Variation in (a) pH concentration and (b) DO concentration in influent and effluent

in various cellular processes such as energy metabolism, the functioning of enzymes, protein uptake and nutrient uptake (Moondra et al., 2021b).

COD concentration in the primary treated STP wastewater ranged between 196.50 mg/L to 297.60 mg/L. After algal treatment, COD concentration in the non-filtered effluent reduced and was within the permissible range and varied between 12.43 mg/L to 128.00 mg/L, with the maximum removal efficiency of 93.75%. Reduction observed in filtered effluent was slightly higher than non-filtered, with removal efficiency reaching 95.32%. However, in the control system, COD removal for non-filtered and filtered effluent reached 63.84% and 72.84%, respectively, with the lowest COD concentration in non-filtered and filtered effluent as 83.20 mg/L and 67.20 mg/L. In the mixotrophic mode i.e., CO₂ and other organic matter like glucose or acetate acted as carbon sources for *Chlorella vulgaris* (Gao et al., 2016), contributing to COD removal. The low food to microorganism (F/M) ratio is also the reason for the high removal efficiency of organic matter by algae (Moondra et al., 2020); photosynthetic capability also contributes to the removal of organic matter (Wang et al., 2010). Variation in COD concentration in influent and effluent and their respective removal efficiency is shown in Figure 3.

Microalgal treatment is also efficient in removing nutrients. Phosphate concentration in the influent wastewater varied from 1.87 mg/L to 3.32 mg/L. After phycoremediation, the phosphate concentration in the non-filtered effluent reduced to 98.40%, with phosphate concentration varying from 0.04 mg/L to 1.09 mg/L. Whereas for the filtered effluent the phosphate concentration was reduced to below the detectable limit (BDL) with 100% removal efficiency. Algae have excellent sorption capacity and precipitate due to high pH and DO concentrations (Hoffmann, 1998; Schumacher and Sekoulov, 2003). Bio-assimilation (Wagner and Loy, 2002), adsorption, chemical precipitation above pH 8.50 (De-Bashan and Bashan, 2010; Ding et al., 2012; Wang et al., 2014) are the metabolisms that help in the removal of phosphorus.

In the control system, phosphate was not removed effectively. Phosphate concentration in the non-filtered and filtered effluent of the control system ranged between 0.40 mg/L and 1.45 mg/L and 0.30 mg/L to 1.26 mg/L respectively. Variation in phosphate concentration in influent and effluent and their respective removal efficiency is illustrated in Figure 4.

Ammonia concentration in the influent wastewater varied from 12.60 mg/L to 17.58 mg/L. After phycoremediation, the ammonia concentration in the non-filtered effluent reduced to 100%, with ammonia concentration varying from BDL to 8.96 mg/L. The ammonia concentration

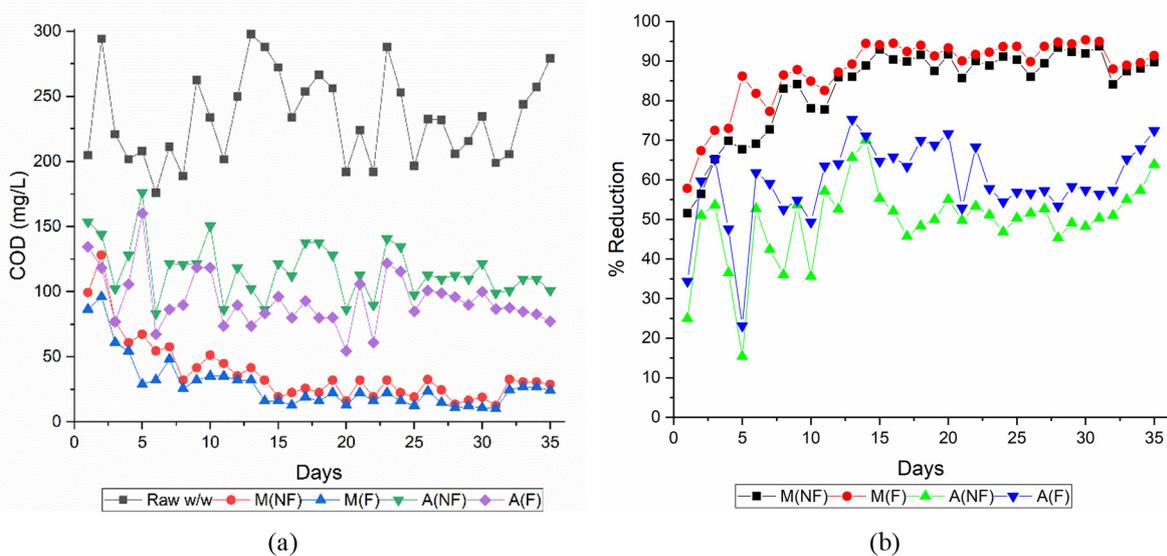


Fig. 3. Variation in (a) COD concentration and (b) Removal efficiency in microalgal and control system

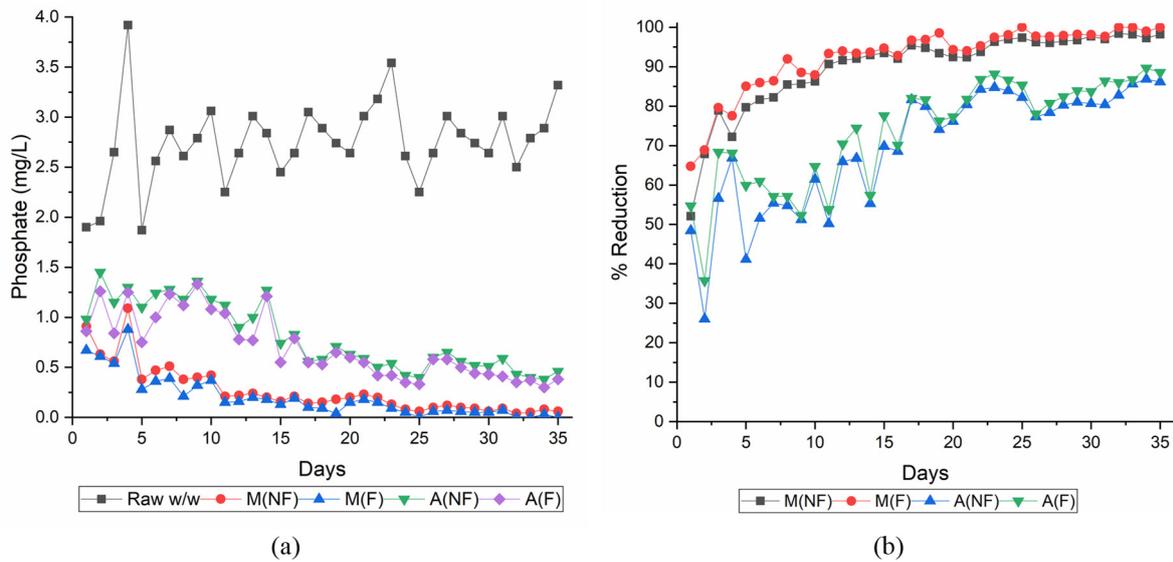


Fig. 4. Variation in (a) Phosphate concentration and (b) Removal efficiency in microalgal and control system

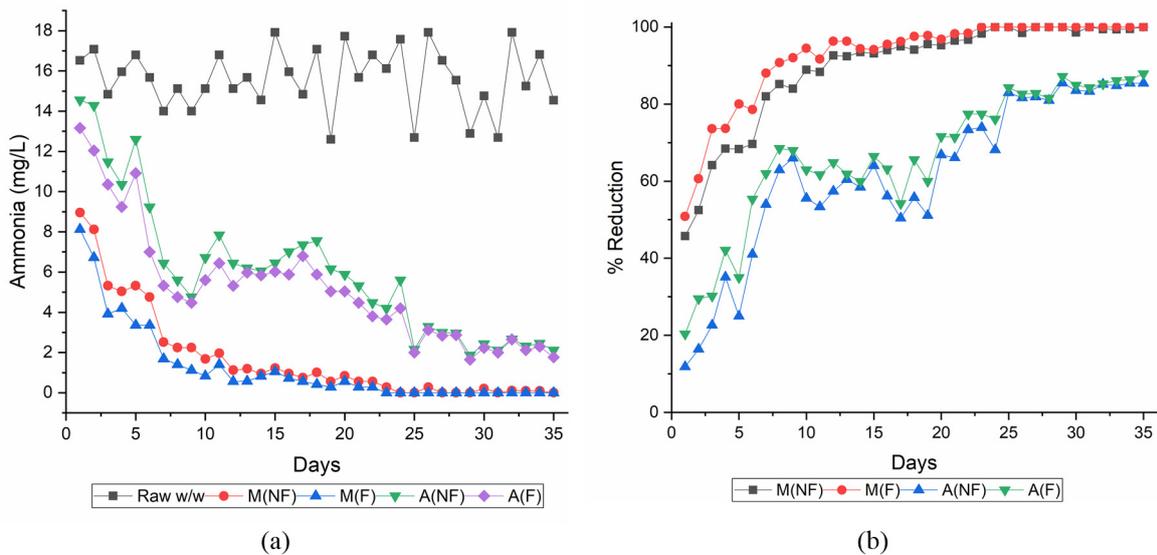


Fig. 5. Variation in (a) Ammonia concentration and (b) Removal efficiency in microalgal and control system

varied from BDL to 8.12 mg/L for the filtered effluent. In the control system, ammonia was not removed effectively. Ammonia concentration in the non-filtered and filtered effluent of the control system ranged between 1.87 mg/L and 14.56 mg/L and 1.65 mg/L to 13.16 mg/L respectively. Variation in ammonia concentration in influent and effluent and their respective removal efficiency is depicted in Figure 5. Nitrogen is required to form genetic material, enzymes, proteins, hormones, vitamins, alkaloids, amides, and energy transfer molecules in algal cells. Cell uptake, followed by algal biomass wasting, is the way that helps in the elimination of nitrogen. Depending on the treatment system and conditions, direct assimilation and volatilization help NH_4^+ removal. It is reported that microalgae uptake nitrogen as $\text{NH}_4^+\text{-N}$ first than $\text{NO}_3^-\text{-N}$ (Markou and Georgakakis, 2011).

During the study, it was observed that ammonia reduction led to an increase in nitrate concentration. Nitrate concentration in the influent varied from 0.11 mg/L to 1.62 mg/L. Due

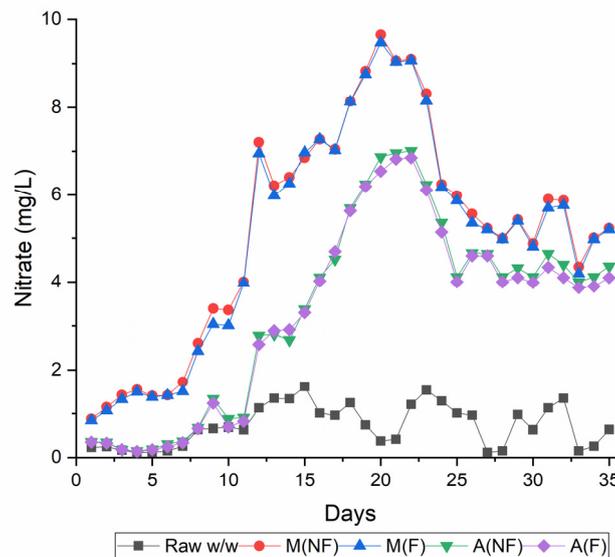


Fig. 6. Variation in nitrate concentration in microalgal and control system

to nitrification after treatment, nitrate concentration increased to 9.10 mg/L and 9.07 mg/L for non-filtered and filtered effluent, respectively, after algal treatment. However, in the control system, nitrate concentration increased to 7.01 mg/L and 6.85 mg/L for non-filtered and filtered effluent, respectively, as illustrated in Figure 6.

During the study, nitrate concentration increased from the start of the experiment and by the end of the study, nitrate concentration reduced from its highest concentration. This change in the nitrate concentration in the effluent because the nitrate act as a source of nitrogen in the absence of ammonia concentration. Microalgae use nitrogen sources in the following order: $\text{NH}_4^+ > \text{NO}_3^- > \text{N}_2$, and when NH_4^+-N is abundant, algae do not use other nitrogen sources until the NH_4^+-N is exhausted (Renuka *et al.*, 2013). Reduction in $\text{NO}_3^- -\text{N}$ concentration was observed when the NH_4^+-N concentration was negligible. Nearly all nitrogen is incorporated into the algal biomass for protein synthesis. In addition, the sludge production during the study was negligible as the microalgal system was working at a low F/M ratio.

In addition to organics and nutrients, the study also observed solids reduction. Among all the solids (TS, TSS and TDS) studied, the maximum reduction was observed in TSS. A huge variation of TSS was found in the influent during the study. TSS concentration varied from 960.00 mg/L to 2055.00 mg/L in the primary treated STP wastewater. After the microalgal treatment, TSS concentration reduced to 55.34% (715.00 mg/L) and 63.06% (510.00 mg/L) for non-filtered and filtered effluent, respectively. Whereas in the absence of microalgae (control system), TSS concentration was reduced to 54.89% (809.00 mg/L) and 60.06% (623.00 mg/L) for non-filtered and filtered effluent, respectively.

Similar to TSS, the removal of TS also showed the same trend. TS concentration varied from 2220.00 mg/L to 4162.00 mg/L in the influent. After the microalgal treatment, TS concentration was reduced to 48.84% (1655.00 mg/L) and 55.57% (1408.00 mg/L) for non-filtered and filtered effluent, respectively. Whereas in the absence of microalgae (control system), TS concentration was reduced to 47.12% (1791.00 mg/L) and 51.23% (1575.00 mg/L) for non-filtered and filtered effluent, respectively. Microalgae, when mixed with bacterial or other microorganisms in the wastewater, leads to flocs formation, forming a bio-flocculation phenomenon that helps in biomass formation and solid removal (Delrue *et al.*, 2016). Variation in TSS and TS concentration in influent and effluent is shown in Figure 7.

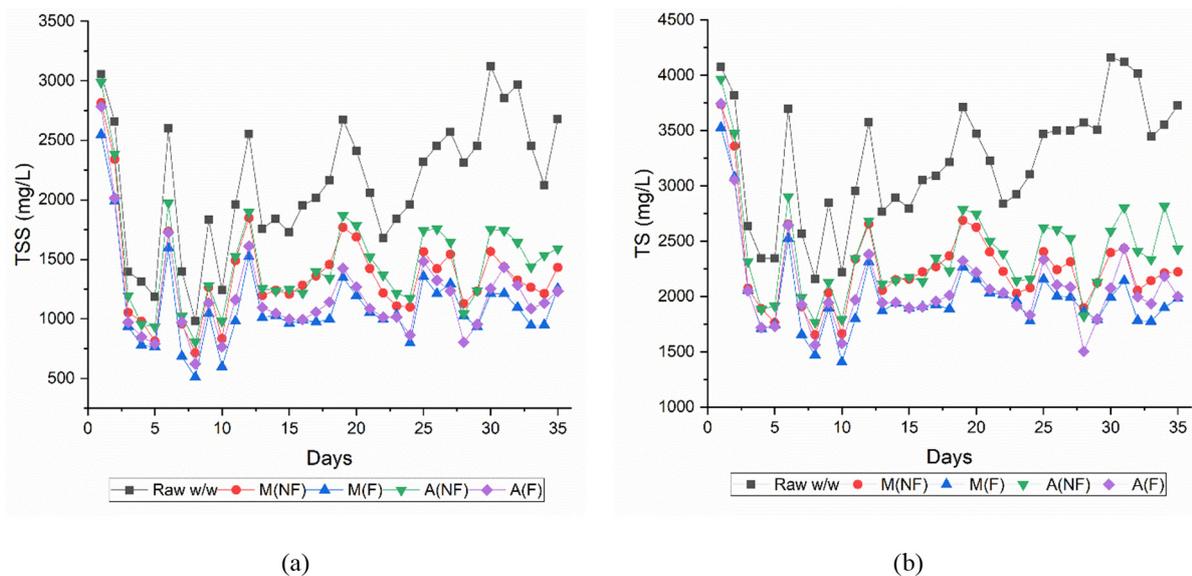


Fig. 7. Variation in (a) TSS and (b) TS concentration in influent and effluent

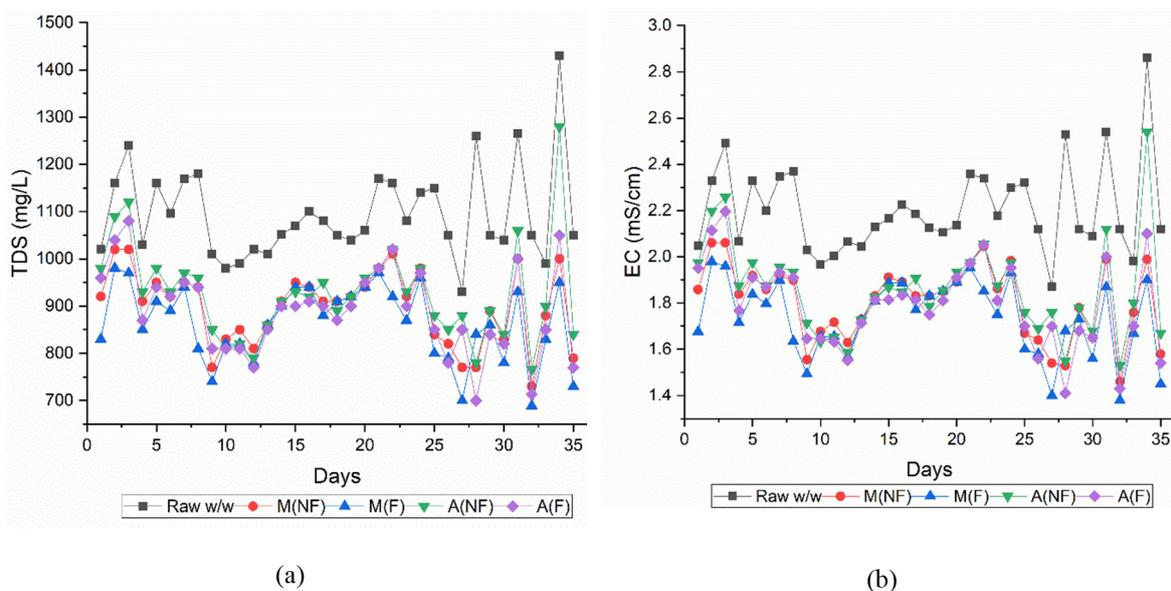


Fig. 8. Variation in (a) TDS and (b) EC concentration in influent and effluent

EC and TDS followed a similar trend during the study. TDS concentration varied from 930.00 mg/L to 1430.00 mg/L in the STP wastewater. After the microalgal treatment, TDS concentration reduced to 38.89% (730.00 mg/L) and 44.44% (689.00 mg/L) for non-filtered and filtered effluent respectively. Whereas in the absence of microalgae (control system), TDS concentration was reduced to 33.33% (767.00 mg/L) and 38.09% (714.00 mg/L) for non-filtered and filtered effluent, respectively.

EC concentration varied from 1.87 mS/cm to 2.49 mS/cm in the STP wastewater. After the microalgal treatment, EC concentration reduced to 39.53% (1.46 mS/cm) and 44.27% (1.38 mS/cm) for non-filtered and filtered effluent respectively. Whereas in the absence of microalgae (control system), EC concentration was reduced to 33.60% (1.76 mS/cm) and 38.74% (1.70 mS/cm) for non-filtered and filtered effluent, respectively. Figure 8 depicts the variation in TDS and EC concentrations in influent and effluent. Biological nutrient processes are the key processes

that lower conductivity in wastewater treatment. During the aerobic phase, the sludge absorbs phosphate, lowering the concentration of dissolved phosphorus to a lower level than at the start of the cycle. Sludge releases and absorbs ions such as K and Mg together with phosphate. In aerobic conditions, nutrient uptake causes a drop in conductivity. Conductivity changes during biological nitrogen removal via nitrification and denitrification, as hydroxide consumption during transformation causes a drop in conductivity (Levlin, 2010).

External aeration during the study was provided to keep the algae in suspension. Mixing homogenizes the cells' distribution, and warmth encourages the transfer of gases and prevents settling (Goncalves et al., 2017), thus empowering the disintegration of nutrients (Khan *et al.*, 2018). Likewise, a specific disturbance level is necessary for the quick circulation of microalgae cells from the dark to the light zone, leading to the uniform dispersion of light (Mata *et al.*, 2010).

CONCLUSION

C. Vulgaris has demonstrated tremendous ability to remove organic matter and nutrients from primary treated wastewater at such a low detention time. The microalgal system has effectively eliminated organics and nutrients to much lesser than the desirable limit. After algal treatment concentration of COD, phosphate and ammonia reduced to 12.43 mg/L (93.75%), 0.04 mg/L (98.40%) and below detectable limit (100%) respectively. Sludge generation during the study was negligible; hence no additional sludge handling issues. A high concentration of DO in the microalgal-treated effluent, when disposed to the nearby receiving body, will not harm the aquatic ecosystem leading to eutrophication. Thus if *C. Vulgaris* is incorporated in the sewage treatment plant, it can enhance its efficiency by minimizing the operating cost.

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The present research did not receive any financial support.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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