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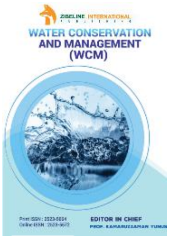

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RESEARCH ARTICLE

MICROALGAL-BACTERIAL CONSORTIA: AN ALLURING AND NOVEL APPROACH FOR DOMESTIC WASTEWATER TREATMENT

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ABSTRACT

The algal-bacterial process has received more attention in the present scenario as a new low-cost method towards pollutant removal from wastewater because symbiotic relationship between algae and bacteria is efficient when compared to traditional secondary treatments. The present work evaluated the optimum concentration of microalgal-bacterial consortia for significant reductions in physicochemical parameters of raw domestic wastewater. In the study, three different concentrations (20%, 30% and 40%) of microalgal-bacterial consortia were studied at 8 hours and 16 hours HRT. Among the different concentrations of consortia studied, 30% consortia gave maximum removal efficiency at both the HRTs. The maximum removal efficiency of phosphate, ammonia, BOD and COD was about 99.79%, 94.85%, 89.02% and 88.96%, respectively, at 8 hours HRT. However, at 16 hours, HRT maximum removal efficiency observed was 97.40%, 94.05%, 83.52% and 88.40% for phosphate, ammonia, BOD and COD respectively. The study depicts that microalgal-bacterial consortia can efficiently remediate nutrients and organic matter from domestic wastewater in both cases when sunlight was ample and even with minimal / no sunlight; hence this system can work effectively throughout the day with much lesser HRT and higher removal efficiency.

KEYWORDS

Biological treatment, Phycoremediation, *C. Vulgaris,* Organic matter, Nutrient Removal, Eco-friendly.

1. INTRODUCTION

Urbanization and industrialization in developing countries like India, with a minimum focus on the environment, has resulted in rapid degradation of the environment. Pollution is not just a challenge but synergistically a public health issue as well. Though pollution is controlled to some extent, the environment gets more and more polluted because of an increase in the number of industries leading to the generation of domestic and industrial wastewater. The reduction in water quality would also lead to water scarcity, which is a major issue around the globe. With time the ever-degrading environmental quality has become an issue of concern, making it necessary to identify the polluting agents and develop the techniques to reduce them to such an extent that it does not harm the environment.

A major requirement of wastewater treatment nowadays is to remove high concentrations of nutrients, which otherwise can lead to risks of eutrophication if these nutrients accumulate in rivers and lakes (Pittman et al., 2011). However, finding remedial outcomes for the treatment and safe disposal of the wastewater is a troublesome task as it incorporates procedures that are specialized and costly. In this study, an attempt was made to determine the effectiveness of microalgal-bacterial consortia as a treatment system for domestic wastewater. Thus removal efficiency of nitrogen and phosphorus in the treatment process could be improved by taking advantage of the synergy between multiple species of algae and microbes when compared to traditional single and multi-step treatments (Brenner et al., 2008).

Wastewater constitutes an excellent opportunity for microalgal–bacterial consortia as it can be considered as a medium for growing them at a low-cost and as a new potential market (Gou et al., 2020). In algae-assistant systems, microalgae mainly supply dissolved oxygen for bacteria to remove organic matter and algae themselves actively uptake nutrients for self-growth (Karya et al., 2013; Jia and Yuan, 2016). Benefits like low operating costs, ability to reduce atmospheric CO_2 level and/or capture of CO_2 and production of valuable end bio-products has supported the potential of microalgal-bacterial based treatments (Subashchandrabose et al., 2011; Karya et al., 2013). In microalgae–bacteria consortia systems, microalgae can produce various organic substances that bacteria can assimilate (Munoz and Guieysse, 2006).

Numerous studies have stated the benefits of consortia, either bacterial-microalgal consortia or consortia between multiple microalgae strains, along with its economic viability (Subashchandrabose et al., 2011; Pires et al., 2013; Renuka et al., 2013; Gonclaves et al., 2017). The release of carbon dioxide through bacterial heterotrophy and oxygen through algal photosynthesis ensures a gaseous equilibrium in the water, which benefits both the algal and bacterial flora. Several studies stated the advantage of consortia over microalgae alone, such as consortia are less subjected to fluctuations in the environmental conditions and more resistant to contaminations. Moreover, microalgal consortia flocs settled more quickly when compared with microalgal flocs, thereby creating a natural bio flocculation phenomenon, which is very important for efficient harvesting of the biomass (Wang et al., 2015; Delrue et al., 2016).

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Furthermore, microalgae growth can be promoted by bacteria (Delrue et al., 2016; Wang et al., 2016). Additionally, the best removal rates for NH_4^+ and TP were obtained for the co-cultures in comparison to single cultures. When treating an urban polluted river with *Neochlorisoleoabundans*, a natural bacterial consortium would develop itself with other native green microalgae and diatoms (Olguin et al., 2013). The microalgal consortium of various strains showed removal in TOC (86%), TN (90%), Ammonia (89%), TP (70%) and Orthophosphates (76%) from sewage wastewater (Mahapatra et al., 2014; Delrue et al., 2016). The present study focuses on the performance of various microalgal bacterial consortia concentrations in an open atmosphere for the removal of nutrients, organic matter and solids from the urban raw wastewater at lower HRT in the presence and absence of sunlight to determine the proficiency of the method, if incorporated in the sewage treatment plants of developing countries.

2. Materials and Methods

In this study, the consortia used were a blend of freshwater species *C. Vulgaris* and bacteria profoundly found in the activated sludge process. The consortia culture was kept in a natural environment and was provided with nutrients to keep them in active mode. A batch lab-scale study was conducted in 2000 mL glass beaker with a working volume of 1800 mL. Different glass beaker with varying consortia concentration 360 mL (20%), 540 mL (30%) and 720 mL (40%) while the remaining volume was made up with the raw domestic wastewater respectively collected from a sewage pumping station of an urban city of Gujarat, India as shown in Figure 1. The study was performed in two phases, under natural light conditions in an open atmosphere. In the first phase, the study was conducted with different consortia concentrations at 8 hours HRT. Artificial aeration was provided for 7 hours to keep consortia in suspension, followed by an hour of settling during which artificial aeration was stopped. The supernatant was drawn for physicochemical analysis after the settling period. The time slot for 8 hours study was 10:00 hours – 18.00 hours. The light availability during this phase was about 6 hours.

In the second phase, the study was conducted with different consortia concentration at 16 hours HRT. The time slot for a 16-hour study was 17.00 hour – 9.00 hour with light availability of 3 hours in this phase. Artificial aeration was provided for 15 hours to keep consortia in suspension. The supernatant was drawn for physicochemical analysis after the settling period of an hour. Various parameters analyzed during the study (for both the phases) were ammonia, phosphate, nitrate, Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), pH, Total Dissolved Solids (TDS), Total Solids (TS), Total Suspended Solids (TSS), Electrical Conductivity (EC) and Dissolved Oxygen as prescribed in APHA, 2012 manual.

Duplicates were performed for each parameter studied for a sample, and the average was considered for analysis. The parameters were analyzed for effluent in two ways, i.e., one is non-filtered, and the other is filtered (through a coarse filter of pore size 4.0 - 5.5μm) to get an idea about the filamentous algae in the effluent. However, after the initial days of experiments, a good settablity of the consortia was observed during the study giving a clear supernatant. The study was conducted in two phases (different HRTs) for three months, i.e., March-May 2019 (45 samples for each microalgal bacterial concentration was examined at both the HRTs), to analyze the sustainability of the consortia system to have the effect of both light and dark phases when used in day to day practices.

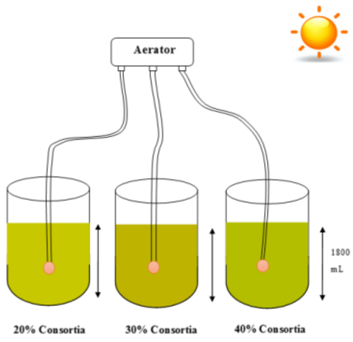

Figure 1: Pictorial view of the experimental setup

3. Results and Discussion

3.1 Treatment with 20%, 30% and 40% consortia at 8 hours HRT

During this phase of the study, nutrient removal, along with various physicochemical parameters, were measured at 8 hours HRT for all three concentrations of consortia (20%, 30% and 40%). The variation in raw domestic wastewater collected from nearby sewage pumping station for concentrations of phosphate, ammonia, nitrate, BOD and COD were 2.08 ± 0.35 mg/L, 11.84 ± 1.46 mg/L, 0.51 ± 0.16 mg/L, 157.06 ± 19.88 mg/L and 288.2 ± 20.65 mg/L respectively. Similarly, average concentration of pH, EC, TDS, TSS, TS and DO were found to be 7.42 ± 0.25, 2.35 ± 0.24 mS/cm, 1162.8 ± 118.35 mg/L, 1302.91 ± 173.55 mg/L, 2465.71 ± 192.29 mg/L and 0.08 ± 0.11 mg/L. After the treatment of raw wastewater with different consortia concentrations, maximum removal was found in the effluent (both non-filtered and filtered) of 30% consortia. Variation in the removal efficiency for different parameters after treatment with 20%, 30% and 40% consortia concentration at 8 hours HRT is shown in Table 1.

Table 1: Variation in percentage reduction in different parameters after treatment with 20%, 30% and 40% microalgal- bacterial consortia at 8 hours HRT for non-filtered and filtered effluent

Parameter	Non-filtered Effluents			Filtered Effluents		
	20%C	30%C	40%C	20% C	30%C	40%C
EC	6.04±2.56	7.87±3.25	8.23±3.55	7.46±3.09	9.76±4.16	9.43±3.98
TS	18.46±6.47	33.07±11.38	33.17±11.18	22.87±8.09	35.69±11.30	35.30±11.03
TDS	5.60±2.36	7.45±3.05	7.80±3.36	7.03±2.88	9.34±3.98	9.01±3.79
TSS	29.86±9.54	55.56±17.36	55.51±16.93	36.84±13.49	58.86±16.29	58.46±16.10
BOD	64.45±12.62	70.42±12.83	66.02±12.91	68.21±11.69	74.73±12.44	70.80±12.42
COD	65.02±13.27	70.86±13.44	66.54±13.55	68.68±12.34	75.00±12.97	71.25±12.86
Ammonia	68.74±11.69	81.01±10.73	74.81±8.92	73.61±12.03	85.29±10.45	79.41±9.99
Phosphate	83.12±7.74	89.38±6.91	85.59±6.89	86.57±7.01	93.88±6.41	88.95±6.71

After the treatment, it was found that even though with such a low HRT, removal was observed in all the parameters, analysed at different consortia concentrations, which was higher than found in the literature where the HRTs varied from 4 days to 9 days (De-Bashan et al., 2004; Perez-Garcia et al., 2010; Silva-benavides and Torzillo, 2012). After treatment, the removal efficiency for phosphate and ammonia reached to 89.71% and 78.36% for non–filtered effluent, and 92.10% and 84.28% for filtered effluent when treated with 20% consortia. However, the maximum removal efficiency for phosphate and ammonia when treated with 30% consortia for the non-filtered samples was 95.69% and 91.28%, respectively, while for filtered samples, it was about 99.79% and 94.85%, respectively. When treatment was done with 40% consortia maximum reduction found in phosphate and ammonia concentration was 90.59% and 83.21% for non–filtered samples, while reductions were 94.71% and 88.63%, respectively, for the filtered samples.

Removal efficiency for COD and BOD reached up to 75.20% and 76.30% in non–filtered effluent, 78.63% and 78.62% for filtered effluent when treated with 20% consortia. However, maximum removal efficiency for COD and BOD when treated with 30% consortia for the non-filtered samples was 85.36% and 85.96%, respectively, for filtered samples, it was about 88.96% and 89.02%, respectively. When treated with 40% consortia, maximum reduction obtained in COD and BOD concentration was 78.96% and 79.64% for non–filtered samples, and for filtered samples, the maximum reduction was 83.61% and 84.34%, respectively. However, nitrate concentration increased after treatment with 20%, 30% and 40% consortia, which might be because denitrification was not taking place in the system as the system was having high DO concentration. Variation in different parameters before and after treatment during the study is shown in Figure 2.

An increase in DO and pH were also found during the study. pH reached 8.69 and 8.52 in non-filtered and filtered effluent when treated with 20% consortia, up to 8.78 and 8.73 when treated with 30% consortia and was up to 8.72 and 8.69 when treated with 40% consortia. The concentration of DO was a maximum of 6.1 mg/L when 20% consortia were used for treatment and 6.6 mg/L in the case of 30% and 40% consortia. During the initial stage of the experimental study, reduction in solids was significantly less, but with time the system acclimatized, and higher reductions in solids were observed, which can be due to improved settling. The maximum reduction observed in TDS, TSS and TS concentration when treated with 20% consortia was 9.36%, 46.58% and 29.41%, respectively, for non-filtered effluents. Similarly, the maximum reduction in effluent when treated with 30% consortia for TDS, TSS and TS concentrations was 11.76%, 74.58% and 45.23%, respectively. The maximum reduction observed in TDS, TSS and TS when treated with 40% consortia was 12.80%, 75.12% and 47.56%, respectively, for non-filtered effluent.

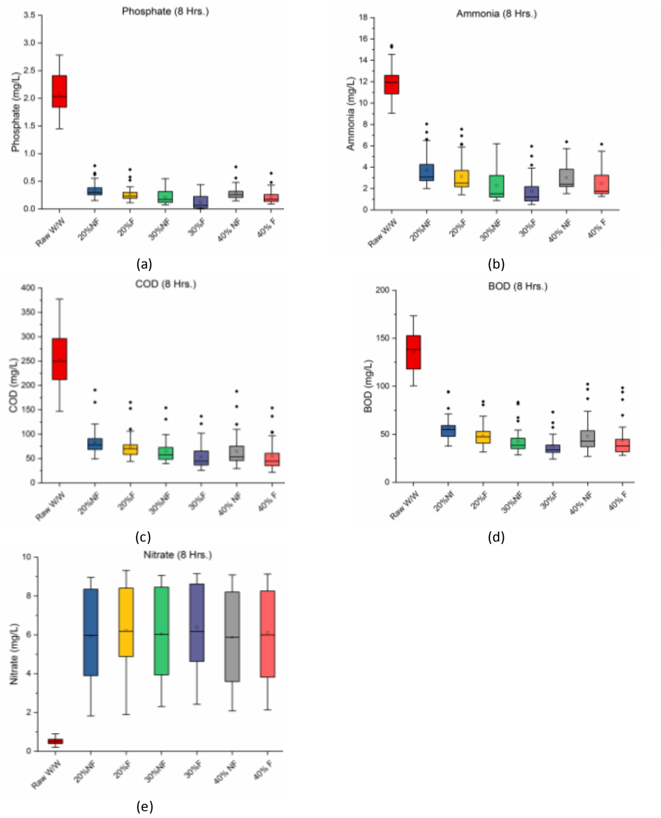

Figure 2: Variation in (a) Phosphate (b) Ammonia (c) COD (d) BOD (e) Nitrate concentration before and after the treatment with microalgal-bacterial consortia at 8 hours HRT

3.2 Study with various consortia concentration at 16 hours HRT

During this phase of the experimental study, removal in various physicochemical parameters was determined at 16 hours HRT for all three concentrations of microalgal-bacterial consortia (20%, 30% and 40%). Variation in the concentration of influent and effluents (after treatment with consortia) at 16-hour HRT of various parameters is tabulated in Table 2 for both on-filtered (NF) and filtered (F) (through a coarse filter of pore size 4.0 - 5.5 µm) effluent.

Table 2: Variation in concentration of different parameters before and after treatment with 20%, 30% and 40% microalgal bacterial consortia at 16 hours HRT

Parameter	pH	EC (mS/cm)	TS (mg/L)	TDS (mg/L)	TSS (mg/L)	Phosphate (mg/L)	Ammonia (mg/L)	Nitrate (mg/L)	BOD (mg/L)	COD (mg/L)	DO (mg/L)
Raw W/W	7.26± 0.35	2.15± 0.30	2294.93± 195.80	1073.56± 144.22	1221.38± 194.31	2.01± 0.43	11.37± 1.46	0.52± 0.25	135.17± 21.32	253.43± 54.28	0.04± 0.08
20% C (NF)	8.35± 0.27	2.06± 0.31	1898.05± 248.96	1023.52± 153.18	874.53± 156.25	0.48± 0.24	3.65± 1.65	5.67± 2.50	57.46± 15.61	85.79± 33.83	4.77± 0.74
20% C (F)	8.31± 0.27	2.04± 0.30	1817.01± 268.17	1013.22± 150.62	803.79± 164.61	0.4± 0.25	3.18± 1.65	5.76± 2.50	50.30± 13.77	75.88± 31.09	4.77± 0.75
30% C (NF)	8.34± 0.28	2.02± 0.31	1712.90± 276.73	1000.81± 152.87	712.09± 155.52	0.35± 0.26	2.62± 1.98	5.79± 2.46	44.23± 14.89	67.09± 30.36	5.57± 1.03
30% C (F)	8.35± 0.25	2.00± 0.31	1552.47± 295.89	994.22± 154.65	558.25± 165.53	0.31± 0.26	2.09± 1.89	5.88± 2.44	38.72± 12.93	55.70± 31.13	5.53± 1.03
40% C (NF)	8.41± 0.21	2.03± 0.31	1760.06± 282.69	1008.79± 153.91	751.27± 168.43	0.40± 0.24	3.12± 1.54	5.90± 2.42	50.53± 20.37	67.53± 39.17	5.42± 0.96
40% C (F)	8.35± 0.26	2.02± 0.31	1669.65± 270.84	1005.22± 152.93	664.43± 143.36	0.36± 0.23	2.64± 1.56	5.97± 2.40	45.61± 18.89	57.43± 35.06	5.39± 0.94

After the study, it was found that after increasing the HRT to 16 hours and at low light intensity and duration, maximum removal was found in 30% consortia effluent. After treatment, removal efficiency for phosphate and ammonia reached up to 89.52% and 78.36 % in non–filtered effluents, 92.08% and 83.96% in filtered effluent when treated with 20% consortia. However, maximum removal efficiency for phosphate and ammonia when treated with 30% consortia in the non-filtered sample was 95.50% and 92.52%, respectively, for the filtered sample, it was about 97.40% and 94.85%. When treated with 40% consortia maximum reduction obtained in phosphate and ammonia concentration was 94.10% and 83.25% for non–filtered sample, for filtered sample reductions was 96.40% and 88.05%, respectively, as shown in Figure 3.

Removal efficiency for COD and BOD after treatment reached 74.86% and 65.20% for non–filtered effluent, 78.10% and 70.12% for filtered effluent when treated with 20% consortia. However, maximum removal efficiency for COD and BOD when treated with 30% consortia for the non-filtered sample was 83.70% and 78.41%, respectively, for the filtered sample, it was about 88.40% and 83.52%. When treated with 40% consortia, maximum reduction obtained in COD and BOD concentration was 83.60 % and 76.50% for non–filtered sample for filtered sample reductions were 86.70% and 78.52%, respectively, as shown in Figure 3. However, increase in the concentration of nitrate was observed to 8.72 mg/L, 8.99 mg/L and 8.99 mg/L after treatment with 20%, 30% and 40% consortia; this may be due because denitrification is not taking place and was a rate-limiting step in the system as the DO was higher in all the systems and anoxic condition was deficient (Li et al., 2008).

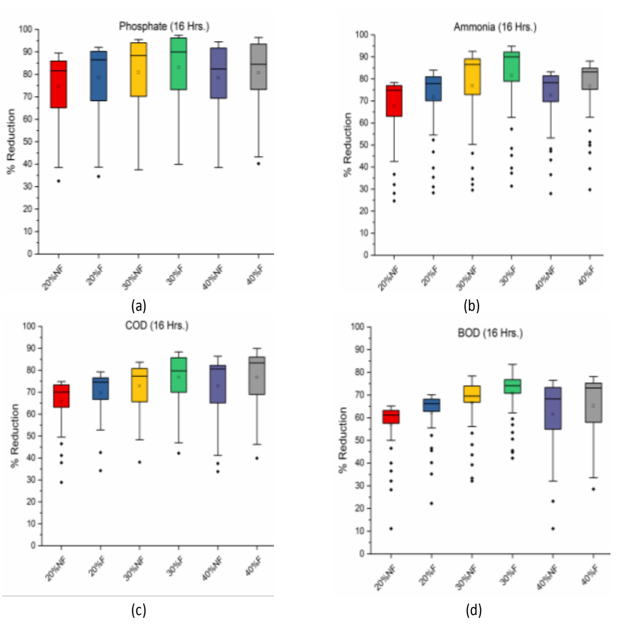

Figure 3: Variation in removal efficiency of (a) Phosphate (b) Ammonia (c) COD (d) BOD after the treatment with microalgal-bacterial consortia at 16 hours HRT

An increase in DO and pH were also found during this phase. pH reached up to 8.74 and 8.65 for non-filtered and filtered effluent when treated with 20% consortia, up to 8.71 and 8.65 when treated with 30% consortia and was up to 8.75 and 8.69 when treated with 40% consortia. The concentration of DO reached up to 5.7 mg/L when 20% consortia were used for treatment and 6.9 mg/L in the case of 30% and 40% consortia. During the initial stage of the experimental study at 16 hours, HRT removal efficiency in solids was relatively less, but with time as the system acclimatized and reductions in solids were observed to improve because of the improved settling. The maximum reduction observed in TDS, TSS and TS when treated with 20% consortia was 7.51%, 45.58% and 28.23%, respectively, for non-filtered effluent. Similarly, the maximum reduction in effluent when treated with 30% consortia for TDS, TSS, and TS was 10.11%, 61.20% and 41.00%, respectively. The maximum reduction observed in TDS, TSS and TS when treated with 40% consortia was 9.85%, 56.99% and 43.06%, respectively, for non-filtered effluent.

The study gave the understanding that microalgal-bacterial consortia treatment can be quite useful in removing nutrients along with the organic load and solids. Algal growth and nutrient uptake are affected by the availability of nutrients, along with physical factors such as pH, light intensity, temperature, and biotic factors. Mixing is another essential growth parameter since it homogenizes the cell distribution by preventing microalgal settling, heat, metabolites and facilitates the transfer of gases (Goncalves et al., 2017). When all the three different concentration of microalgal-bacterial consortia i.e. 20%, 30% and 40% were compared in context to the removal efficiency of the different parameters taken into consideration during the study, it was found that 30% microalgal-bacterial consortia concentration is most efficient during the study at both the HRTs.

However, it was found that removal efficiency was slightly higher at 8-hour HRT, might be because, during that phase, sunlight intensity and availability were more leading to higher photosynthetic activities, thus having a combining effect of both algal-bacterial consortia in the system along with the external aeration provided to keep the consortia in suspension and for homogenous mixing. It was also found that organic matter and nutrient removal efficiency did not reduce to a greater extent at 16-hour HRT where there was lesser photosynthetic effect due to lesser/no sunlight available, as bacteria in coordination with microalgae compensated the effect of light to much extent. Thus, the system can work well throughout the day with higher pollutant removal and effluent with negligible pollutant concentration and hence making it safe for disposal.

Nutrient removal was observed to a greater extent as microalgae are capable of acclimatizing in nitrogen-rich wastewaters and adsorbing them into biomass as it contributes to the production of protein, chlorophyll, and amino acids (Foladori et al., 2018). In the literatures, it was found that microalgae/consortia take up nutrients in the form of $NH_4>NO_3>N_2$, and when NH_4^+ is accessible, microalgae don't use other nitrogen sources until ammonia is exhausted (Renuka et al., 2013). The increase in pH accelerated nitrogen removal. The removal of nutrients was enhanced by the presence of nitrifying bacteria and phosphate-accumulating organisms in consortia. In the present study, phosphorus removal was due to the assimilation of phosphorus for algal growth and P uptake and stored within the algal biomass as polyphosphates (Whitton et al., 2015; Hwang et al., 2016). The removal for COD and BOD was attributed by the attached growth of microorganism and biomass, external aeration and also consortia was disintegrating the complex form of impurities into simpler ones. BOD removal was marginally higher when compared to COD.

An increase in pH concentration was observed during the study at both the HRTs; this change in the pH increased the capability of algae to assimilate nutrients by changing the form of nutrients available and also algal-bacterial cell physiology (Kube et al., 2018; Delgadillo-mirquez et al., 2016). The elevated pH also contributed to significant removal of Ammoniacal-N and phosphorus via ammonia volatilization and phosphate precipitation (Park et al., 2011) as auto flocculation respectively, which also aids in the removal of the suspended algae from the effluent leads to optimal productivity of the cyano-bacterium thus increasing the overall efficiency of the treatment system (Satpal and Khambete, 2016; Goncalves et al., 2017). It was also found that light is an essential operating factor in algal culture in terms of light intensity and cycle (Hoh et al., 2016). Light and temperature fluctuations affect the algal-bacterial wastewater treatment system by affecting the proportions of specific algal groups/species in biofilm (Schnurr and Allen, 2015).

Within sight of NH_4, the oxygen required for nitrification outperformed the amount produced by photosynthesis, DO concentration increased gradually if no external aeration was provided (Foladori et al., 2018). The dissolved oxygen concentration increment during the examination under the various conditions as a result of algal development, showing a prevalence of photosynthetic action over heterotrophic carbon-oxidation, nitrification and external aeration (Delgadillo-mirquez et al., 2016). Dissolved oxygen was adequate in the system beyond saturation, thus contributing to the removal of organic contents. Reduction in solids was observed with time in microalgal-bacterial consortia as microalgae and bacteria in combination have better settleability leading to auto-flocculation in the system. However, the reduction in TDS was least when compared with TS and TSS. Variation in EC was similar to TDS.

4. Conclusions

Microalgal-bacterial consortia can effectively treat wastewater by taking up nutrients for their growth and reducing various organic and physicochemical compounds. Algae, a water-purifying agent acting as a pollution indicator, can be a better alternative toward bioremediation. It was found that microalgae, in combination with bacteria forming consortia, can treat domestic wastewater efficiently in lesser time and is a promising alternative for contaminants removal and could represent an alluring expansion to existing biological treatments used to treat wastewaters. Among all the concentration of consortia studied (20%, 30% and 40%), 30% gave the maximum reduction for all the physicochemical parameters studied at both HRTs. Efficient reduction in organic content (> 80%) and nutrient (>90%) was observed in both the case when sunlight was ample and secondly with minimal/no sunlight; hence this system can work effectively throughout the day with much lesser HRT and higher removal efficiency can lead to renewed interest in the development of alternative wastewater treatment methods, i.e., using microalgal consortia for domestic wastewater. However, the present study was confined to the physico-chemical parameters only. In contrast, biological parameters such as biomass generation, change in Chlorophyll content etc. can also be determined for various cases as the future work. In addition to this, determining the optimum HRT for the study with different combinations of algae and bacteria can be helpful for researchers in the future. Feasibility of the treatment can be carried for various other wastewater other than sewage such as industrial wastewater, greywater (light and dark) etc. to explore the advantages and limitations of the phycoremediation technique when applied in the real world.

Conflict of Interest

There is no conflict of interest in this manuscript.

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