Comparative study for the treatment of domestic wastewater using *Chlorella Vulgaris*

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Abstract

Tertiary treatment using chemicals frequently prompts secondary contamination of sludge, making additional issues of safe disposal. Thus, vitality and cost required for tertiary treatment of wastewater stay an issue for industries and municipalities. In this study, different microalgal concentrations 360 mL (20%), 450 mL (25%), 540 mL (30%), 630 mL (35%), 720 mL (40%) and 810 mL (45%) were studied to treat domestic wastewater for 11 hours of detention time for both filtered and non-filtered effluent. During the study, removal was observed in Total Solids (TS), Total Dissolved Solids (TDS), Total Suspended Solids (TSS), Electrical Conductivity (EC), phosphate, ammonia and Chemical Oxygen Demand (COD) for all the microalgal concentrations mentioned. 30% microalgal concentration showed maximum removal efficiency among different microalgal concentrations studied. Maximum removal efficiency found in non-filtered effluents when treated with 30% microalgal concentration was 96.60, 91.73 and 84.71% respectively for ammonia, phosphate and COD concentration. However, the removal efficiency increased to 97.62, 92.47 and 88.75% respectively for ammonia, phosphate and COD in the case of filtered effluents. In the case of TSS and EC, removal efficiency reached up to 30.87 and 24.31% respectively for non-filtered effluents while it was 48.00 and 25.88% respectively, for the filtered effluents. One-way ANOVA was also conducted to determine the statistical significance of the study. The study showed that an algae-based system could accomplish a more affordable and environment-friendly way to treat domestic wastewater without tertiary treatment to a desirable limit.

1. Introduction

The world will confront 40% water deficiency by 2030, which is a challenge for sustainable development [1,2]. This shortfall may emerge from the expanding interest for water, degradation of water assets and the absence of innovations to recover the used water [3]. The untreated wastewater contains fluctuating degrees of organic and inorganic mixes.

In India, 61,754 Million Liters per Day (MLD) sewage is generated against the sewage treatment capacity of 22,963 MLD. Owing to the distinct gap between sewage generation and treatment capacity, about 38,791 MLD of untreated sewage (62% of total sewage) is directly discharged into nearby water bodies [4]. The wastewaters originating from these point or nonpoint sources, if disposed of in a nearby water body without any proper treatment, adversely affect the water quality and aquatic ecosystem [5]. Thus, the current need is to minimize the consumption of water as well as to return it to the earth with minimum possible pollution because of the limited potential of self-purification in water bodies [6].

However, most existing sewage treatment processes in developing countries are confronting difficulties with nitrogen and phosphorus removal to meet the necessities for disposal and water reuse, at low expenses [4,7]. Besides, the downsides of conventional treatments are high vitality utilization, flimsiness treatment impact, lengthy procedure, carbon emanation and excess sludge discharge [8,9].

The most eco-friendly and economical technique for wastewater treatment is with biological methods, where chemical break down and treatment of residues is done by microorganisms also with the production of value-added compounds [10,11]. Many studies have proposed microalgae as an alternative biological

treatment that efficiently removes nutrients from wastewater even when nutrient concentrations are high [12,13,14,15,16] as municipal wastewater is enriched with essential nutrients which support algal biomass production [17,18].

Literature has been reported with nitrogen (>90%) and phosphorus (>50%) removal using microalgae [11]. *Chlorella Vulgaris* can effectively remove nitrogen (up to 81–85%) and phosphorus (32–36%), respectively [19], *Galdieria sulphuraria* when used removed ammoniacal nitrogen (63–89%) and phosphorus (71–95%), respectively [20]. The green algae *Micractinium sp.*, when used for nutrient removal, gave efficiency to the system about >90% in the case of both phosphorus and nitrogen, respectively [15]. *Chlorella Vulgaris* was used in the present study because of its characteristics features like (1) high growth rate, (2) fast nutrient removal rate; (3) strong adaptability to different types of wastewater and local climate; and (4) high biomass productivity [21]. Microalgae treatment acts as a disinfectant by the release of antibacterial substances with an increase in pH [22], causes no secondary pollution and is an effective solution to global warming [23] with low capital and operational expenses [13] these advantages make it more prominently acceptable globally [24]. However, the treatment is also associated with some challenges as studies are mostly confined to controlled conditions with artificial illumination [22], high detention time is required for treatment [25], and the major cost is associated with the harvesting of algal biomass for value-added products [26].

In the present study, an attempt was made to analyze the effectiveness of the microalgal system if incorporated in wastewater treatment regarding the removal of the different physico-chemical parameters along with nutrients at much lower detention time and in natural conditions to address few of the challenges of microalgal treatment in the Indian context.

2. Materials And Methods

Nutrient and organic removal batch study were performed using *Chlorella Vulgaris* in 2L glass beakers with a working volume of 1,800 mL for 11 hours of detention time with different microalgal concentrations as shown in Fig. 1. The algae strain used in the study is a native strain that has been isolated by Phycolinc Technologies Pvt. Ltd from an effluent treatment site of an electroplating industry in Vadodara, Gujarat and the stock algal solution was provided for the present study. *Chlorella Vulgaris* provided was spherical eukaryotic green algae with cell size and thickness ranging between 4 to 10 µm in diameter and 120 to 200 µm respectively.

The study was conducted in an open atmosphere under natural light conditions. External aeration was provided for 10 hours to keep microalgae in suspension, followed by an hour of settling. After the settling period, the supernatant was drawn for physico-chemical analysis. The time slot for an 11-hour study was 10:00 a.m.– 21.00 p.m., which has both the effect of light and dark phase. The sunlight available during this phase was for about 6 hours.

The raw domestic wastewater used for the study was collected from the nearby sewage pumping station at Surat. Combination of different microalgal concentrations and raw domestic wastewater for the study is shown in Table 1. Various parameters analyzed during the study (for both the non-filtered and filtered

effluents) were pH, Electrical Conductivity (EC), Total Dissolved Solids (TDS), Total Solids (TS), Total Suspended Solids (TSS), Chemical Oxygen Demand (COD), phosphate, ammonia, nitrate and Dissolved Oxygen (DO) as prescribed in APHA, 2012 manual [27]. Duplicate samples were analysed for each parameter, and the average was considered for analysis. The study was conducted from September to November in the year 2019, and 24 samples were analyzed for each microalgal concentration. During the initial phase of the study, a faint green tint was observed in the effluent due to the presence of filamentous algae. But with the time, the microalgal settleability improved, and the intensity of color decreased to nil. Hence the study was also conducted with the effluent filtered with a coarse filter (4-5.5 μm) to get an idea about the color impartment (true or apparent). The study was conducted to analyze the sustainability of the microalgae system if used in Sewage Treatment Plant (STP) for an all-day round with maximum and nil light availability during the day and night hours, respectively. One-way ANOVA analysis ($p=0.05$) was also conducted using IBM-SPSS-25 software to determine the significance of the treatment on various parameters statistically.

3. Results And Discussion

During the experimental process, various physico chemical parameters were measured for 11 hours of detention time for all the concentrations of *C. Vulgaris*. The average concentration of raw domestic wastewater collected from a nearby sewage pumping station for analysis of various parameters at different microalgal concentration is tabulated in Table 2.

After the treatment of the raw domestic wastewater with microalgae, it was found that even though with low detention time of 11 hours in the present study, efficient removal was observed in all the parameters analyzed at different microalgal concentration when compared with various literature where the HRTs varied in days to weeks [28,29,30]. Removal in different parameters was observed in each concentration studied. However, maximum removal was found in the effluent of 30% microalgae.

The ammonia percentage removal efficiency at different concentration considered in the study was 59.9 ± 24.3 for 20% concentration, 67.4 ± 22.4 for 25% concentration, 73.7 ± 24.8 for 30% concentration, 69.6 ± 27.9 for 35% concentration, 49.8 ± 19.5 for 40% concentration and 54.4 ± 20.1 for 45% concentration in case of non-filtered effluent respectively. However, when effluent was filtered with coarse filter to remove filamentous microalgae if any, percentage removal efficiency further increased to 65.6 ± 23.5 for 20% concentration, 71.1 ± 21.9 for 25% concentration, 77.7 ± 22.6 for 30% concentration, 75.3 ± 24.6 for 35% concentration, 55.9 ± 18.4 for 40% concentration and 58.5 ± 19.1 for 45% concentration respectively shown in Fig.2(a). An increase in nitrate concentration was observed during the study as ammonia was converted to nitrate in aerobic conditions via nitrification [31]. Nitrate concentration raised to 3.5, 3.8, 5.8, 5.8, 3.8 and 3.8 mg L^{-1} , when wastewater treated with 20, 25, 30, 35, 40 and 45% microalgae, respectively. Nitrogen is transformed into N_2 gas in conventional nitrogen removal methods, whereas in the algal treatment system, nitrogen compounds are taken up for their growth [32]. The uptake of nitrate is light energy-dependent, and also microalgae prefer to utilize already reduced nitrogen, such as ammonia, in comparison to nitrate, which

is less energy-intensive conversion [33]. Ammonia was efficiently removed in the system as it is incorporated into protein via protein anabolism for microalgal growth [34,35].

The phosphate percentage removal efficiency at different concentration considered during the study was 60.5 ± 18.6 for 20% concentration, 64.0 ± 16.2 for 25% concentration, 71.5 ± 18.0 for 30% concentration, 69.3 ± 18.2 for 35% concentration, 54.6 ± 13.2 for 40% concentration and 56.1 ± 13.2 for 45% concentration in case of non-filtered effluent. However, for filtered effluents percentage removal efficiency further increased to 66.3 ± 16.0 for 20% concentration, 70.0 ± 13.5 for 25% concentration, 74.6 ± 17.0 for 30% concentration, 71.3 ± 18.1 for 35% concentration, 57.8 ± 12.7 for 40% concentration and 60.1 ± 12.9 for 45% concentration. Removal efficiency for phosphate at different microalgal concentrations is shown in Fig.2(b). Microalgae have shown the great potential to utilize phosphorus from wastewater, but mainly in the form of orthophosphate [36], and it is incorporated into organic compounds such as nucleic acids, phospholipids and proteins [21] which resulted in phosphate removal in the system.

The COD percentage removal efficiency for different concentration considered in the study was 52.4 ± 17.3 for 20% concentration, 55.1 ± 18.9 for 25% concentration, 65.1 ± 20.2 for 30% concentration, 62.5 ± 22.1 for 35% concentration, 57.5 ± 15.0 for 40% concentration and 59.4 ± 13.8 for 45% concentration in case of non-filtered effluent. However, when effluent was filtered with a coarse filter the percentage removal efficiency further increased to 59.9 ± 16.3 for 20% concentration, 63.6 ± 15.1 for 25% concentration, 69.9 ± 20.5 for 30% concentration, 66.0 ± 21.6 for 35% concentration, 62.0 ± 12.9 for 40% concentration and 64.5 ± 12.9 for 45% concentration as shown in Fig.3. COD removal is performed in the microalgae system in symbiotic relation with the heterotrophic bacteria [37], as bacteria utilize the oxygen produced by algae as an end product for their growth and survival. Low F/M ratio of the system also contributes to COD removal [38].

During the study, removal in TS and TSS was also observed to a certain extent due to the formation of algal bacterial biomass that settles down and leaves a clear supernatant. The TS percentage removal efficiency at different microalgal concentration considered in the study was 11.7 ± 7.2 for 20% concentration, 12.4 ± 6.6 for 25% concentration, 13.7 ± 4.1 for 30% concentration, 11.1 ± 4.9 for 35% concentration, 11.2 ± 4.8 for 40% concentration and 11.5 ± 5.0 for 45% concentration in case of non-filtered effluent. The TS removal efficiency further increased to 18.2 ± 6.7 for 20% concentration, 17.6 ± 6.7 for 25% concentration, 22.3 ± 5.7 for 30% concentration, 20.0 ± 6.6 for 35% concentration, 18.4 ± 5.5 for 40% concentration and 18.7 ± 5.5 for 45% concentration for filtered effluents as shown in Fig.4(a). The TSS removal efficiency at different concentration considered in the study was 15.3 ± 11.7 for 20% concentration, 16.6 ± 10.2 for 25% concentration, 19.5 ± 6.9 for 30% concentration, 16.0 ± 7.8 for 35% concentration, 16.1 ± 7.7 for 40% concentration and 15.5 ± 8.3 for 45% concentration for non-filtered effluent. However, when effluent was filtered with a coarse filter, removal efficiency further increased to 25.7 ± 11.0 for 20% concentration, 24.6 ± 11.2 for 25% concentration, 32.7 ± 10.2 for 30% concentration, 29.8 ± 11.0 for 35% concentration, 26.5 ± 8.0 for 40% concentration and 25.5 ± 7.9 for 45% concentration as shown in Fig.4(b). The problem of separating algae from water may be solved by using attached algae because these attached algae are often observed in the form of algal biofilm in the presence of sufficient sunlight [39].

When compared to TS and TSS removal in TDS was low. The TDS percentage removal efficiency at different concentration during the study was 7.3 ± 4.0 for 20% concentration, 7.1 ± 4.5 for 25% concentration, 5.8 ± 2.6 for 30% concentration, 4.5 ± 2.1 for 35% concentration, 3.6 ± 1.6 for 40% concentration and 5.5 ± 2.4 for 45% concentration in case of non-filtered effluent. However, when effluent was filtered with a coarse filter, the percentage removal efficiency was observed up to 8.7 ± 4.1 for 20% concentration, 9.0 ± 4.2 for 25% concentration, 8.1 ± 2.6 for 30% concentration, 6.6 ± 3.0 for 35% concentration, 6.1 ± 2.1 for 40% concentration and 8.7 ± 2.6 for 45% concentration as shown in Fig.5(a). The EC percentage removal efficiency at different microalgal concentration was 7.0 ± 4.3 for 20% concentration, 6.4 ± 3.7 for 25% concentration, 7.3 ± 7.0 for 30% concentration, 5.7 ± 6.3 for 35% concentration, 3.9 ± 2.5 for 40% concentration and 5.5 ± 3.4 for 45% concentration in case of non-filtered effluent. However, for filtered effluent, removal efficiency further increased 8.8 ± 4.1 for 20% concentration, 8.8 ± 3.5 for 25% concentration, 9.3 ± 7.0 for 30% concentration, 7.0 ± 6.4 for 35% concentration, 6.1 ± 2.5 for 40% concentration and 7.1 ± 3.1 for 45% concentration as shown in Fig.5(b). Maximum removal efficiency observed in different parameters at different microalgal concentrations is tabulated in Table 3.

From the study, it was found that microalgal treatment was efficient in removing nutrients and organic matter to much lesser concentrations than the desirable limits. The study also gave the understanding that the removal efficiency in the effluent increases with the increase in the microalgal concentration up to the optimum concentration. The TDS removal was least when compared to other parameters but was higher in lesser microalgal concentrations, i.e., 20% in comparison to higher microalgal concentrations. In the case of TS, lesser microalgal concentration had higher removal efficiency. Ammonia removal was maximum among all the parameters as nitrogen contributes to 10%, followed by phosphorus (1%) of algal biomass [40]. Higher removal observed in filtered effluents for different parameters was the result of the removal of organic content and also microalgal species present in suspension to a certain extent when passed through a coarse filter.

The microalgal system led to an increase in the DO concentration, which raised to 6.6, 6.8, 7.2, 7.6, 7.6 and 7.8 mg L⁻¹, respectively, when influent was treated with a microalgal concentration of 20, 25, 30, 35, 40 and 45% respectively. The increase in DO was mainly because of the photosynthetic effect of algae in addition to external aeration provided for the mixing of algae in the influent. Algal growth increases alkalinity to a remarkable level regardless of the initial pH [41]. The rise of pH by photosynthesis was impeded due to the production of H⁺ ions by nitrification and by the use of ammonium as a nitrogen source for the photosynthesis process itself [42]. The elevated pH also enhances Ammonical-N removal. pH increased to 8.40 when treated with 20% microalgal concentration and 8.56 when treated with both 25 and 30% microalgae, whereas pH reached 8.81, 8.53 and 8.57 when treated with 35, 40 and 45% microalgal concentration respectively. One-way ANOVA conducted for different parameters analyzed revealed that microalgal concentrations had a significant impact on various parameters studied, as shown in Table 4.

After the treatment of the raw domestic wastewater with microalgae, it was found that coupling domestic wastewater treatment with algae can be a reasonable, cost-effective and viable opportunity for wastewater

treatment, with an opportunity for clean water production for different purposes without the need for tertiary treatment.

4. Conclusions

Algal treatment of domestic wastewater, mediated through a combination of nutrient uptake, raised pH and high dissolved oxygen concentration, can offer an ecologically safe and efficient means to expel nutrients in comparison to conventional wastewater treatment plants. The study found that 30% concentration of *Chlorella Vulgaris* is the optimum concentration among all the concentration studied to remove the nutrients and organic content effectively. Maximum removal efficiency found in non-filtered effluents when treated with 30% microalgal concentration was 96.60, 91.73 and 84.71% respectively for ammonia, phosphate and COD concentration. However, the removal efficiency increased to 97.62, 92.47 and 88.75% respectively for ammonia, phosphate and COD in the case of filtered effluents without causing secondary sludge unlike other conventional and chemical treatments used for domestic wastewater treatment. Algae in wastewater can significantly contribute to the management of freshwater ecosystems by providing a more environmentally sound approach to deal with lessen eutrophication capacity of the point source and can be the solution to the problems associated with secondary treatments with a practical advancement in wastewater treatment with low-carbon, low vitality utilization and asset reuse.

Declarations

Availability of data and materials

All data generated or analyzed during this study are incorporated into the manuscript itself.

Competing interests

The authors declare they have no competing interests.

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Authors' contributions

NM has done in detailed literature survey to find research gaps that lead to the way for this study. She also collected samples, conducted all the experiments, along with data interpretation and statistical analysis single-handedly during the study.

NDJ provided administrative and technical support in addition to critical comments and revisions in the manuscript as being the co-supervisor for the study.

RAC gave administrative and technical support along with the critical comments for the experimental setup initially as well as during the study. The study was conducted under his supervision. All authors read and approved the final manuscript.

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Tables

Table 1 Different microalgal concentration and corresponding raw wastewater volume used for the study.

Microalgal Concentration			Volume of Raw Wastewater
Percentage	Volume	Dry Weight	
20%	360 ml	1.80 mg L ⁻¹	1440 ml
25%	450 ml	2.25 mg L ⁻¹	1350 ml
30%	540 ml	2.75 mg L ⁻¹	1260 ml
35%	630 ml	3.15 mg L ⁻¹	1170 ml
40%	720 ml	3.60 mg L ⁻¹	1080 ml
45%	810 ml	4.05 mg L ⁻¹	990 ml

Table 2 Raw wastewater characteristics for the study with different microalgal concentration

Parameter	Set 1 (20% and 25%)	Set 2 (30% and 35%)	Set 3 (40% and 45%)
pH	7.2 ± 0.2	7.2 ± 0.2	7.3 ± 0.3
EC (mS cm ⁻¹)	2.2 ± 0.1	2.2 ± 0.2	2.2 ± 0.2
TDS (mg L ⁻¹)	1133.3 ± 127.5	1120.8 ± 97.37	1090.8 ± 118.4
TSS (mg L ⁻¹)	1429.5 ± 265.4	1587.5 ± 254.5	1682.4 ± 243.0
TS (mg L ⁻¹)	2587.8 ± 306.1	2699.3 ± 295.4	2719.2 ± 282.7
COD (mg L ⁻¹)	251.8 ± 46.8	281.6 ± 26.0	285.6 ± 22.9
Phosphate (mg L ⁻¹)	2.4 ± 0.7	2.8 ± 0.4	2.4 ± 0.5
Ammonia (mg L ⁻¹)	11.2 ± 1.1	12.5 ± 1.7	13.7 ± 2.4
Nitrate (mg L ⁻¹)	1.0 ± 0.4	1.1 ± 0.3	1.0 ± 0.3
DO (mg L ⁻¹)	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.1

Table 3 Maximum percentage removal observed in different parameters at various microalgal concentration for both non-filtered (NF) and filtered (F) sample

Parameter	20%	20%	25%	25%	30%	30%	35%	35%	40%	40%	45%	45%
	(NF)	(F)	(NF)	(F)	(NF)	(F)	(NF)	(F)	(NF)	(F)	(NF)	(F)
EC	13.1	15.6	9.9	12.2	24.3	25.9	23.5	24.7	9.0	10.2	10.2	11.4
TDS	13.3	14.2	12.4	13.3	11.2	12.4	8.0	12.5	6.1	11.2	7.1	13.3
TSS	39.3	43.7	34.7	41.6	30.9	48.0	33.7	50.7	27.2	36.3	28.2	35.8
TS	28.5	31.6	25.4	30.3	21.1	30.3	21.8	32.6	18.5	24.7	19.7	25.7
COD	79.2	81.9	84.7	86.1	84.7	86.3	84.3	86.4	73.9	76.1	72.9	77.7
Phosphate	80.0	82.8	82.8	85.5	91.3	93.0	88.4	90.4	73.6	76.1	75.4	77.5
Ammonia	84.6	88.9	91.0	93.7	96.6	97.6	96.3	96.9	77.1	78.4	78.0	80.2

Table 4 One-way ANOVA for influent and effluent of various parameters ($p = 0.05$)

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
COD	Between Groups	1642920.911	12	136910.076	58.480	.000
	Within Groups	812372.690	347	2341.132		
	Total	2455293.601	359			
Phosphate	Between Groups	184.929	12	15.411	63.141	.000
	Within Groups	84.691	347	0.244		
	Total	269.620	359			
Nitrate	Between Groups	293.005	12	24.417	24.797	.000
	Within Groups	341.679	347	0.985		
	Total	634.684	359			
TS	Between Groups	13305605.361	12	1108800.447	12.811	.000
	Within Groups	30033821.028	347	86552.798		
	Total	43339426.389	359			
Ammonia	Between Groups	4804.201	12	400.350	79.171	.000
	Within Groups	1754.704	347	5.057		
	Total	6558.905	359			
DO	Between Groups	2232.563	12	186.047	547.729	.000
	Within Groups	117.865	347	0.340		
	Total	2350.428	359			
pH	Between Groups	56.144	12	4.679	105.934	.000
	Within Groups	15.326	347	0.044		
	Total	71.470	359			
EC	Between Groups	1.676	12	0.140	2.199	.011
	Within Groups	22.041	347	0.064		
	Total	23.718	359			
TSS	Between Groups	9507222.778	12	792268.565	12.578	.000
	Within Groups	21857134.944	347	62988.862		
	Total	31364357.722	359			
TDS	Between Groups	402673.333	12	33556.111	2.343	.007

Within Groups	4970366.667	347	14323.823
Total	5373040.000	359	

Figures

Figure 1

Pictorial view of experimental setup.

Figure 2

Variation in removal efficiency in (a) Ammonia and (b) Phosphate at different microalgal concentration for non-filtered (NF) and filtered effluents (F).

Figure 3

Variation in removal efficiency in COD at different microalgal concentration for non-filtered (NF) and filtered effluents (F).

(a)

(b)

Figure 4

Variation in removal efficiency in (a) TS and (b) TSS at different microalgal concentration for non-filtered (NF) and filtered effluents (F).

(a)

(b)

Figure 5

Variation in removal efficiency in (a) TDS and (b) EC at different microalgal concentration for non-filtered (NF) and filtered effluents (F).