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DOMESTIC WASTEWATER TREATMENT USING CHLORELLA VULGARIS AND SCENEDESMUS QUADRICAUDA (ALGAL TREATMENT)

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ABSTRACT

The Microalgae has the ability of treating the wastewater with high nutrient content and yields good quantity of biomass. The biofuel extracted from algae is considered to be the third generation biofuel. This study involves Phycoremediation of domestic wastewater using Chlorella Vulgaris and ScenedesmusQuadricauda.the COD removal efficiency of ScenedesmusQuadricauda is 57.8 %, 73.0 % and 86.5 % during 2nd, 8th and 13th day respectively and the COD removal efficiency of Chlorella vulgaris is 55.6 %, 72.2 % and 83.3% during 2nd, 8th and 13th day respectively. The growth rate of Chlorella Vulgaris is higher than ScenedusmusQuadricauda. The Phosphate removal efficiency of ScenedesmusQuadricauda is 27.04 % and 65.39 % during 2nd and 8th day respectively and the Phosphate removal efficiency of Chlorella vulgaris is 59.65 %, 72.27 % and 86.36 % during 2nd, 8th and 13th day respectively. The algal biomass is observed to have large quantity of Formic acid and N Heptonic Acid among Volatile fatty acid.

Keywords— Microalgae, Phycoremediation, Chlorella Vulgaris, Scenedesmus Quadricauda and Biofuel.

I. INTRODUCTION

Microalgae are simple photosynthetic microorganisms that utilises CO_2, nutrients in water and sunlight for the growth and produces biomass rich in lipids, carbohydrates and protein over short period of time. The natural oil content in some species of microalgae is as high as 80%[1]. Microalgae may be eukaryotic or prokaryotic. These photosynthetic microorganisms can survive and even multiply quickly even under difficult and harsh condition. The growth rate of algae is much higher than that of oil crops[2]. The algae can be also grown in barren land or even dessert using raceway ponds or photobioreactors. The production and harvesting techniques of algae has to be improved and made economical for commercialization[3]. The third generation biofuels extracted from algal biomass is potent and viable replacement for the first generation biofuel extracted from crop. The usage of wastewater for cultivating algae and using flue gas to supply CO_2 reduces the Greenhouse gas emission to environment[4].

Microalgae can be cultivated in open ponds, high rate algal ponds, photobioreactors etc. Photobioreactors are supreme significant devices for the large production algal biomass. Most of the Photo-bioreactors are still designed semi-empirically[5]. The real-world options that improves the algal biomass production in HRAP while treating the wastewater are found to be addition of CO_2, controlling the parasite species like grazers and bio-flocculation[6]. The combination of anaerobic digestion and algae cultivation provides many environmental benefits along production of large amount of biomass that can be used to extract biofuel[7]. Dual purpose microalgae cultivation for wastewater treatment coupled with biofuel generation is an attractive option in terms of reducing the energy cost [8]. The treatment of wastewater using macro algae or micro algae and removing organic or inorganic pollutants is termed as Phycoremediation[9]. The growth of photosynthetic microorganisms is affected by many environmental factors such as light intensity, mixing, mass transfer, temperature and pH[5].

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II. MATERIALS AND METHODS

A. Domestic Wastewater

The domestic wastewater generated at National Institute of Technology Karnataka is collected from the wastewater treatment plant before treatment during different periods.The physiochemical characteristics of the wastewater is studied in laboratory and shown in Table 1.

Table 1: Analysis of NITK Domestic waste water

Sl.No	Parameter	Value
1	pH	5.3 to 6.9
2	Conductivity	270 to 430 mS/cm^2
3	Temperature	27 to 30 ^oC
4	COD	420 to 500 mg/l
6	DO	1.2 to 2 mg/l
7	Nitrate	38 to 52 mg/l
8	Ammonia Nitrogen	7 to 12 mg/l
9	Phosphate	6 to 10 mg/l

The wastewater is diluted in 1:3 ratio with distilled water and added with the algae in certain proportion and batch study is done.

B. Culturing of Algae

The algal cultures of Chlorella Vulgaris and Scenedusmus were provided by Dr. V Sivasubramanian, Director of Phycospectrum Environmental Research Centre (PERC), Chennai. The algae were cultured in Bold Basalt Media (BBM). The composition for 1 litre of BBM is 0.075 g of $MgSO_4.7H_2O$, 0.075 g of K_2HPO_4, 0.09 g of $NaNO_3$,0.014 g of KH_2PO_4, 0.025 g of NaCl, 0.025 g of $CaCl_2.2H_2O$, 0.05 g of EDTA-Na_4, 0.00498 g of $FeSO_4.7H_2O$, 0.232 mg of $MnCl_2.4H_2O$, 0.01142 g of H_3BO_3, 1.41 mg of $ZnSO_4.7H_2O$, 0.252 mg of $CuSO_4.5H_2O$, 0.192 mg of $NaMoO_4.5H_2O$ and 0.080 mg of $CoCl_2.6H_2O$ dissolved in 1 litre distilled water[10].

C. Batch study

The 300 ml Borosilicate glass is taken for the batch study. In Reactor A 30 ml of ScenedesmusQuadricauda and 120 ml of diluted (1:3) wastewater is studied and in Reactor B 30 ml of Chlorella Vulgaris and 120 ml of diluted (1:3) wastewater is studied. The Light/Dark ratio is maintained as 10/14. The tube light is used for providing lighting with 2800 lumen.

Cell count of the micro algal culture is found using Haemocytometer line method[11]. The no. of cells present in the matrix on the haemocytometer was observed under microscope with 40X resolution.The Spectrophotometer is used to determine the Phosphate concentration. The Gas Chromatography (GC- FID BP 21) is used to determine the Volatile Fatty acid content.The settled biomass is collected after 3 days and the setup is aerated using 3 lit/min capacity aquatic air pump for 10 hours per day from 9th day to 12th day.The algae are allowed to settle and the supernatant liquid is collected for performing the analysis and test. For the cell count the wastewater is well mixed and counting is done using Haemocytometer.

Figure 1: Reactor A Before & After

Figure 2: Reactor B Before and After

Figure 1 and Figure 2 clearly shows the development of algae in wastewater during the starting of batch study and after completion of batch study. The increase in cell count of algae makes the water turn

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greener. The biomass settled at the bottom is collected and stored in separate container with water as shown in Figure 3 and Figure 4.

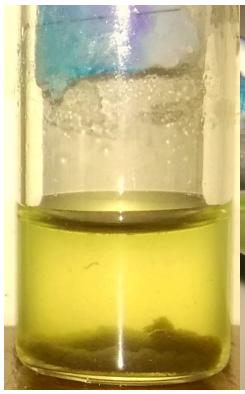

Figure 3: Biomass Collected from Reactor A

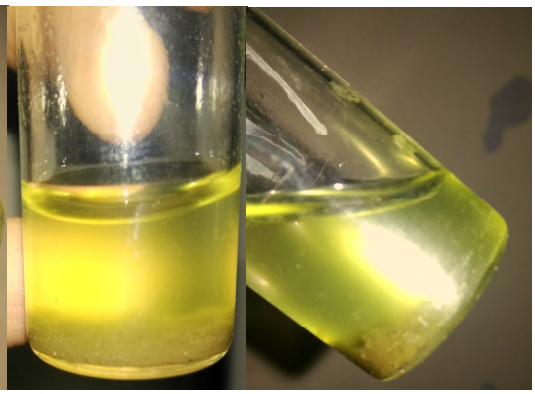

Figure 4: Biomass Collected from Reactor B

III. RESULTS AND DISCUSSION

A. pH

From the Figure 5 and Figure 6 it is clear that initially there is an increase in the pH because of the consumption of bicarbonates from the wastewater as it acts as Carbon source for the for the photosynthesis and growth of algae[11]. After the aeration from 9th day the algae uses Carbon dioxide from the air pumped into the reactor and thus pH reduces.

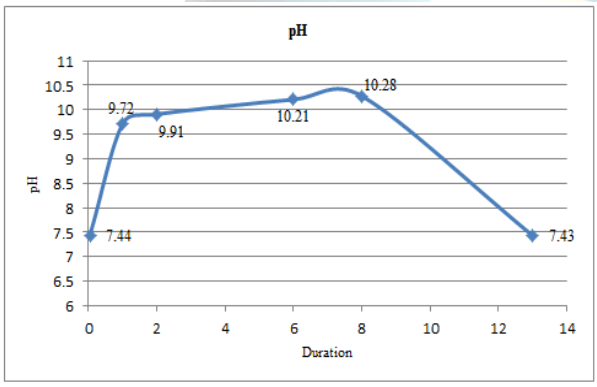

Figure 5: pH of Reactor A

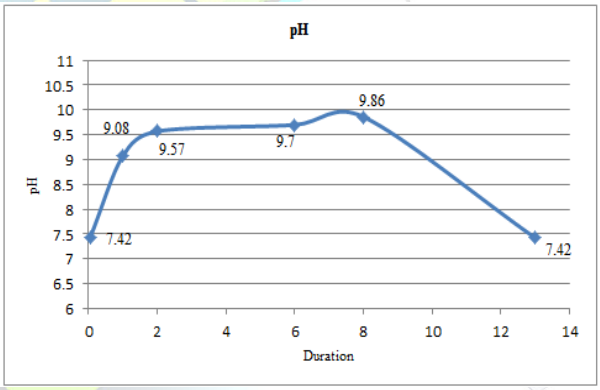

Figure 6: pH of Reactor B

B. Cell Count

Biological organism follow logarithmic trend during the growth period after lag phase. From the Figure 9 and Figure 10, the cell count shows that the growth of the algae is logarithmic in case of no aeration (R^2 value in Figure 9 and Figure 10 assuming logarithmic growth). The aeration from 9th day to 12th day has resulted in sudden growth of algae on 12th day. The 13th day cell count of Reactor A and Reactor B is 56 x 10^6 cells/ml and 125.6 x 10^6 cells/ml respectively. The growth of Chlorella Vulgaris is very high during aeration when compared to ScenedesmusQuadricauda, thus reactor B has high growth rate during the period of aeration.

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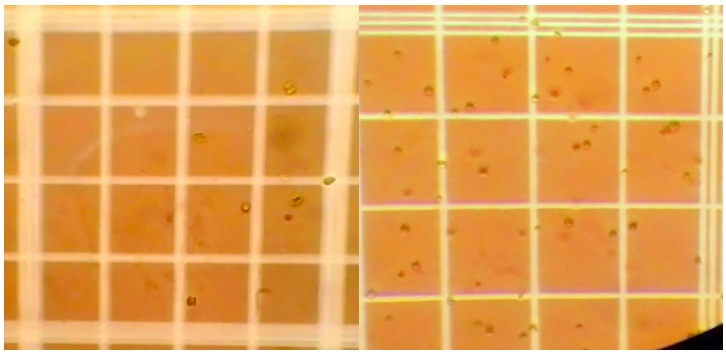

Figure 7: Initial observation of Chlorella Vulgaris **Figure 8: Final observation of Chlorella Vulgaris**

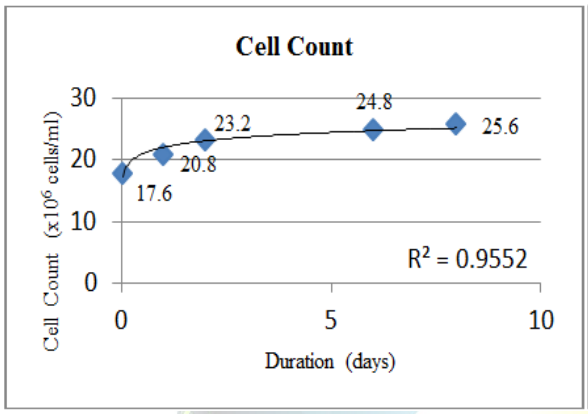

Figure 9: Cell Count of Reactor A

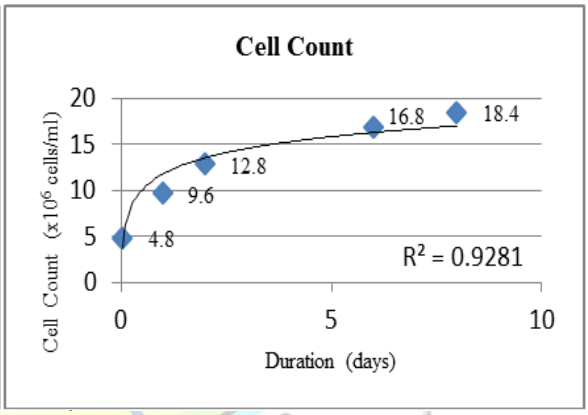

Figure 10: Cell Count of Reactor B

C. **COD**

The COD removal efficiency for Reactor A and Reactor B is 57.8 % and 55.6 % respectively on 2nd day. The COD removal efficiency for Reactor A and Reactor B is 73.0 % and 72.2 % on 8th day respectively. ScenedesmusQuadricauda and Chlorella Vulgaris were observed to have nearly same removal efficiency while Scenedesmus is slightly having greater removal efficiency. After the aeration of reactors the 13th day removal efficiency of Reactor A and Reactor B is 86.5 % and 83.3 % respectively. The removal of COD follows logarithmic trend as shown in Figure 11 and Figure 12 with R^2 value greater than 0.95 (assuming logarithmic trend) .

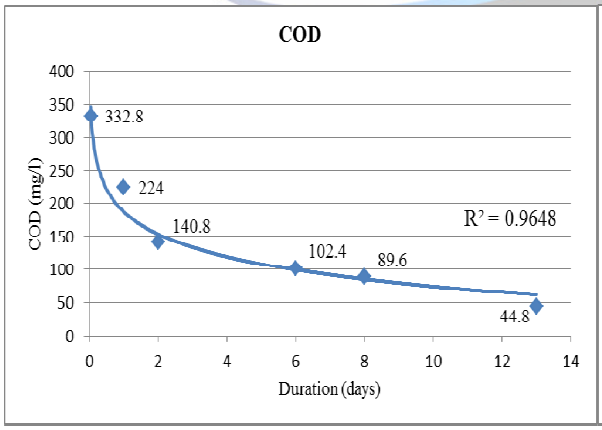

Figure 11: COD of Reactor A

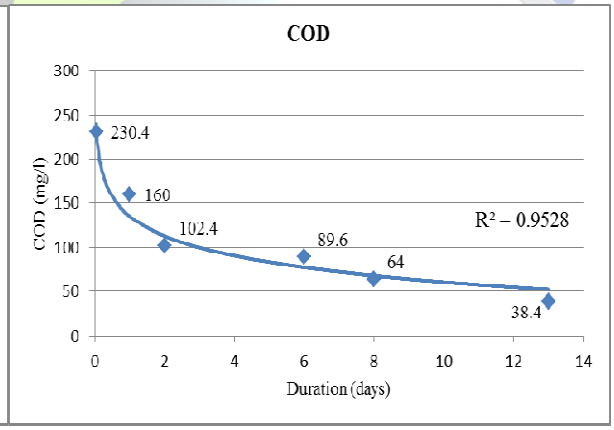

Figure 12: COD of Reactor B

D. **Phosphate**

The Phosphate is a vital nutrient required for the growth of algae and hence it is removed very efficiently. The Phosphate removal efficiency for Reactor A and Reactor B is 27.04 % and 59.65 % respectively

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on 2nd day. The Phosphate removal efficiency for Reactor A and Reactor B is 65.39 % and 72.27 % on 8th day respectively. Chlorella Vulgaris was observed to be superior to ScenedesmusQuadricauda in removing phosphate. After the aeration of reactors the 13th day removal efficiency of Reactor B is 86.36 %. In the reactor A, during the period of aeration there was no much reduction in phosphate. This may be due to the suspension of algae in the water without settling and increasedgrowth of microalgae. Centrifuging the sample would have given lower phosphate concentration in reactor.

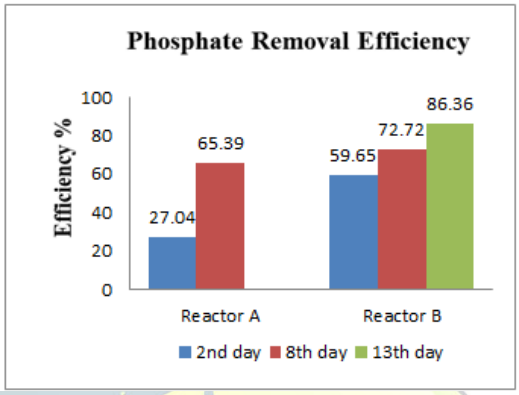

Figure 13: Removal Efficiency of Phosphate

E. **Volatile Fatty Acid**

The Algal biomass is analysed in Gas chromatography (GC-FIS BP 21) to estimate the VFA content by Manni and Caron procedure[12]. 3ml of aqueous biomass (biomass with water) is taken and mixed with few drops of Nitric acid to bring the pH 2. Then 5ml of diethyl ether is added and the sample is mixed for 10 minute. The supernatant is collected and small amount of anhydrous sodium sulphate is added. The sample is then kept in refrigerator in air tight tube after 10 minutes injected into GC and VFA is found. The composition of various volatile fatty acids and their proportions in algal biomass are shown in Figure 14. The Formic Acid and Heptanoic Acid forms major area of around38.326 % and 35.447% respectively as shown in Table 2. Formic acid can be used in tanning industries and dye industries. Heptanoic acid can be used to produce ester and fragrance.

Table 2: Result of Gas Chromatography

Component Name	Chemical Formula	Area %	Retention Time (min)
Formic Acid	CH_2O_2	38.326	5.677
Acetic Acid	$C_2H_4O_2$	1.130	6.663
Propionic Acid	$C_3H_6O_2$	1.239	6.983
Isobutyric Acid	$C_4H_8O_2$	2.178	7.682
Butyric Acid	$C_4H_8O_2$	3.038	8.143
Isovaleric Acid	$C_5H_{10}O_2$	3.435	8.923
Valeric Acid	$C_5H_{10}O_2$	4.348	9.638
Isocaproic Acid	$C_6H_{12}O_2$	4.557	10.098
Hexanoic Acid	$C_6H_{12}O_2$	6.301	11.215
N Heptonic Acid	$C_7H_{14}O_2$	35.447	18.280

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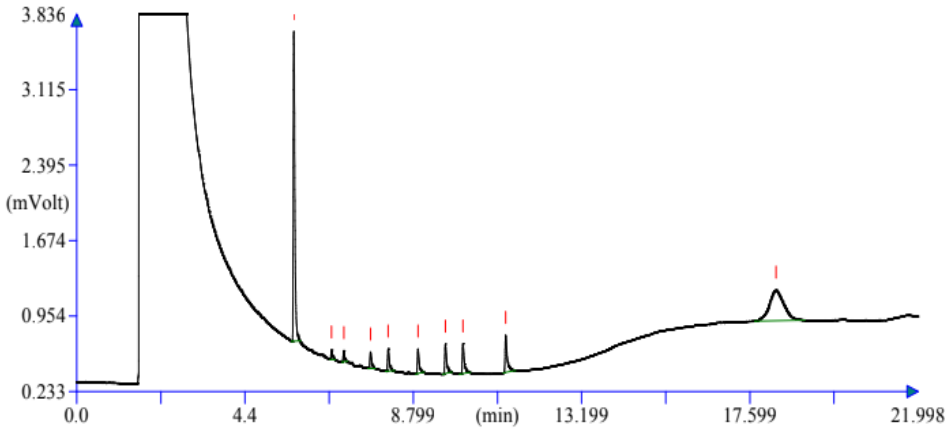

Figure 14: VFA of Algal biomass

F. **SEM Analysis**

The wastewater is filtered using 40 micron filter paper and the filter paper is dried in open air as shown in the Figure 15. The filter paper is cut into small piece and scanned under Scanning Electron Microscope for details as shown in the Figure 16.

Figure 15: Filter Paper

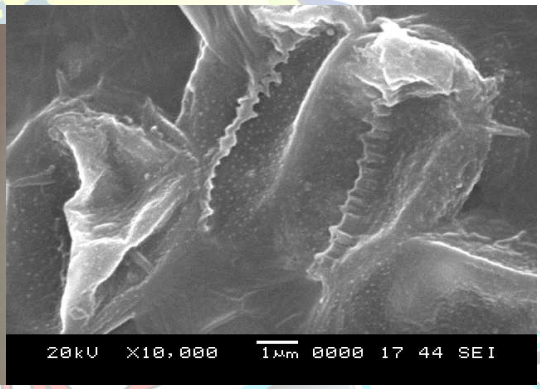

Figure 16: SEM image of ScenedesmusQuadricauda

CONCLUSION

This study demonstrates that Chlorella Vulgaris and ScenedesmusQuadricauda can be used for the treatment of domestic wastewater. During the batch study, the COD removal efficiency of ScenedesmusQuadricauda is 57.8 %, 73.0 % and 86.5 % during 2nd, 8th and 13th day respectively. While the COD removal efficiency of Chlorella vulgaris is 55.6 %, 72.2 % and 83.3% during 2nd, 8th and 13th day respectively. The Phosphate removal efficiency of ScenedesmusQuadricauda is 27.04 % and 65.39 % during 2nd and 8th day respectively. While the Phosphate removal efficiency of Chlorella vulgaris is 59.65 %, 72.27 % and 86.36 % during 2nd, 8th and 13th day respectively. The algal biomass is observed to have large quantity of Formic acid (38.326 %) and N Heptonic Acid (35.447 %) among Volatile fatty acid. The algal biomass can be used for production of biofuel or other useful products after processing.

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