Treatment of Sugar Process Waste Water and Biogas Production using Algal Biomass

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***Abstract* -Present study was to evaluate the growth of green algae *Chlorella* sp in sugar process waste waters samples. Algae growth pattern was studied using three diff technique viz… Growth determination by optical density, centrifugation and Cell count by Hemocytometer and compared with BBM media. During this nutrient removal, COD and BOD reduction was also recorded. Many researchers working in the field of wastewater treatment, in this a newly developed wastewater treatment by algae is gaining much importance. Microalgae are also used for phytoremediation to reduce the nutrient content in the wastewater due to their (algae's) ability to assimilate nutrients into the cells. In this present study, when *Chlorella* sp was grown in sugar process waste water (open system) it reduced Total kjeldhal nitrogen by 64.28%, Phosphorus 61%, COD 85% and BOD 88%. Based on the laboratory scale study it can be concluded that *Chlorella* sp has the potential to utilize nutrient content of wastewater for its mass growth. Thus obtained algal biomass was co digested with undigested sludge from sugar industry. The results showed that the biogas production and methane content was improved after addition of microalgae. The demand for biogas is continuously growing and the biogas substrate, such as food waste, may soon become limited and it is therefore important for biogas producers to expand the range of substrates. One way to enhance the biogas may be co-digestion of algae with any substrate.**

Keywords— Anaerobic digestion, Chlorella, Co-digestion, Sludge, Waste water

I. INTRODUCTION

Wastewater is a general term used to represent the water with poor quality that contains more amounts of pollutants and microbes. The wastewater discharged into the water bodies are hazardous to environment and cause eutrophication. To remove these nutrients, several processes are used, but the disadvantages of this type of treatment are high cost and increased sludge production. As an alternative to the conventional treatment methods, microalgae are suggested to remove the nutrients from wastewater. Microalgae are eukaryotic microscopic aquatic plants that carry out the same process and mechanism of photosynthesis as higher plants in converting sunlight, H_2O + CO_2 into biomass + O_2. Algae provide an efficient way to consume nutrients and provide oxygen needed for the growth of aerobic bacteria. Microalgae biomass has several advantages over conventional energy crops. They are able to double their biomass within 24 h and the land area needed to cultivate microalgae can be non-fertile which decreases the competition of land for human food crops. Also through the process of algae wastewater treatment very large amounts of algal biomass can be grown Furthermore, algae do not necessarily need fresh water but can also be cultivated in salt water or wastewater. In addition, microalgae are rich in lipids, starch and protein, which can be utilized as a non-food-based feedstock for biofuels(mainly in the form of biodiesel, bio ethanol and biogas).Biogas is an environmentally friendly fuel and the expansion of biogas production systems will be an important contribution to the global conversion from fossil to renewable energy systems[1].Since the demand for biogas is continuously growing in the society and the biogas substrate, for example food waste, may soon become limited .One way to do this is to use microalgae as a substrate in the digestion process and co-digest it with the sludge Algae consist of polysaccharides (alginate, laminaran and mannitol), with zero lignin and low cellulose content [2] which make them an easy material to convert to methane by anaerobic digestion processes. In general, the biogas composed of methane (CH_4) 55-75%, carbon dioxide (CO_2) 25-45% and the rest are hydrogen sulfide (H_2S), nitrogen (N_2) and oxygen (O_2).

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II. MATERIALS AND METHODS

A. *Inoculam development*

Based on the available literature *Chlorella* sp is widely used for waste water treatment hence in the present study pure culture of *Chlorella* sp was collected from Department of Biotechnology, BEC Bagalkot. Mass culturing of algae is carried out using Bolds Basal Media (BBM) [14]. The pH of the media was adjusted to 6.8 and sterilized at 15 lb pressure for 15min in an autoclave. The fresh cultures from the BBM were further used in the study.

B. *Collection of waste water samples*

Wastewater (sugar process waste) samples were collected from Godavari biorefinaries ltd, Sameerwadi. Total

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3 different samples were used sample1 was from laggon1, Sample2 was from lagoon2, Sample3 from Digestor outlet. The undigested sludge used for anaerobic digestion was from the same industry.

C. Preliminary screening of algal growth

The waste water samples(1,2,3) diluted to different concentrations using tap water to observe the algal growth in 250ml glass bottles.

Table 1: Dilution of the samples

Sample1			Sample2			Sample3		
No of Dilutions	*Effluent (%)*	*Tap Water (%)*	*No of dilutions*	*Effluent (%)*	*Tap Water (%)*	*No of Dilutions*	*Effluent (%)*	*Tap Water (%)*
1	*100*	*00*	*1*	*100*	*00*	*1*	*100*	*00*
2	*75*	*25*	*2*	*75*	*25*	*2*	*75*	*25*
3	*50*	*50*	*3*	*50*	*50*	*3*	*50*	*50*
4	*25*	*75*	*4*	*25*	*75*	*4*	*25*	*75*

D. Treatement of waste water samples

Depending on the results of preliminary screening, 25% of sample1, 25% of sample2 and 100% of sample3 were selected to inoculate the algae for the treatment. 40ml of algal suspension (9mg/ml by wet basis) from fresh cultures of *Chlorella* grown in BBM media (7 days old) was inoculated in each 5L samples. pH of all the samples were adjusted to 6.8 before inoculation .The treatment was of an open system.

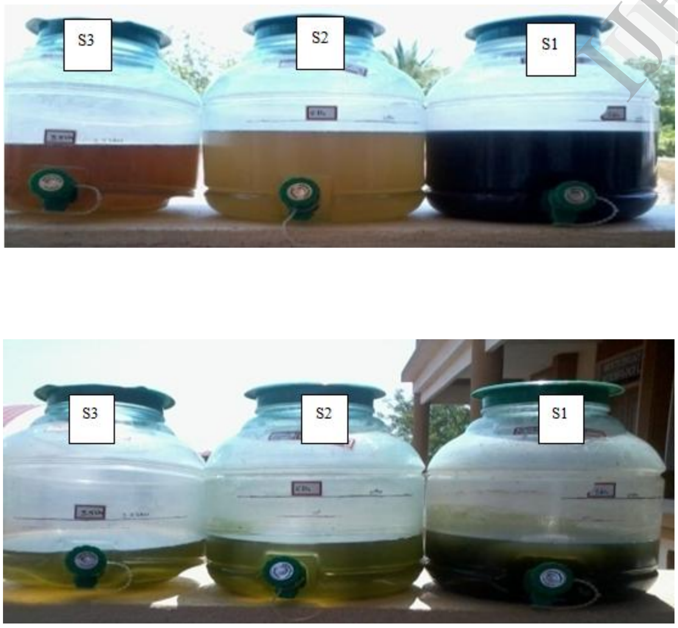


Fig1: Initial and final samples after treatment

a) Algal growth determination

Algal growth in the waste water was determined using the 3 different methods

- ***Cell count using Hemocytometer***

The center large square of the Hemocytometer was focused under the microscope before filling the sample in the field .The cell suspension is added into the counting chamber, numbers of cells are counted. Average number of cells in one large square is multiplied by 10^{4}.

No of cells/ml= Average no of cellsx10^4

- ***Cell growth by Optical density***

Algal cell multiplication is measured using spectrophotometer. Absorbance of the samples (1, 2, and 3 was read at 680nm at regular interval, Absorbance was plotted against time (day).

- ***Cell growth by Centrifugation method***

At regular intervals to check the growth of algal cells 1ml of sample was centrifuged at 10000 rpm for 10min. The supernatant was discarded and the pellet (algal biomass) was quantified by measuring the weight.

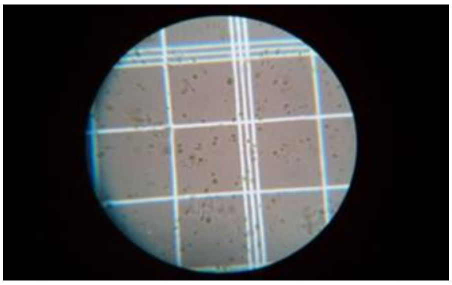


Fig 2: Cell count by Hemocytometer

b) Reduction of nutrients COD and BOD.

Samples for nutrient reduction were collected at regular intervals during the treatment. The collected sample was centrifuged at 10,000 rpm for 10 min and the supernatant is collected for analyses of COD, BOD, Total kjeldhal nitrogen and phosphorus. The measurements of BOD, COD and phosphorus were performed using standard methods (APHA 1998). Measurement of Total kjeldhal nitrogen was performed using Kjeldahl Distillation Method (UHS, Bagalkot).

E. Co digestion of algae for biogas production

The algae biomass could be processed in anaerobic digesters. Algae typically yield less methane than wastewater sludge (~0.3 vs. 0.40 L CH_4/g volatile solids introduced). Ammonia toxicity and recalcitrant cell walls are commonly cited causes of the lower yields. Ammonia toxicity might be counteracted by co-digesting algae with high-carbon organic wastes. Carbon-rich feed stocks that are available near major wastewater pond systems include primary and secondary municipal sludge, sorted municipal organic solid waste, waste fats-oils greases (FOGs), food industry waste, waste paper, and various agricultural residues. Acclimation of the digester microbial community to algae digestion may also improve the yield.

a) Harvesting of algae biomass

Harvesting of algal biomass was carried out using cross flow filtration and followed by centrifugation.

Fig 3: Cross flow filtration

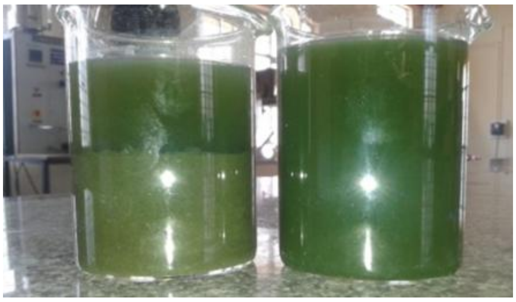


Fig 4: Harvested algal biomass

b) Co digestion of algae

The harvested algal biomass along with sludge was co-digested for bio gas production and analyzed quantitatively by water displacement method and qualitatively using portable methane detector.Set up of small reactor with working volume of 100ml was designed for biogas studies. Three sets of experiments were conducted, first set was control with sludge alone the next two sets were varied with algal concentration 20% algae+80% sludge and 40% algae+60% sludge (1g and 2g by dry weight). The pH for all the digestion samples was maintained at 6.8-6.9.Total solids (TS), volatile solids VS, carbohydrate, protein content were estimated using standard methods.

Fig 5: Biogas production setup

III. RESULTS AND DISCUSSION

A) Preleminary survey for algal growth

Sugar process waste water was having high COD and BOD where algae could not growth. Hence samples are diluted to check the growth of algae. In sample1 and 2 growths was not obtained. But in sample 3 algae was grown without dilution .Where as in sample1 and 2 algae growth was observed with dilution .Based on this results 25% dilution of sample1 and 2, undiluted sample 3 were selected for further study.

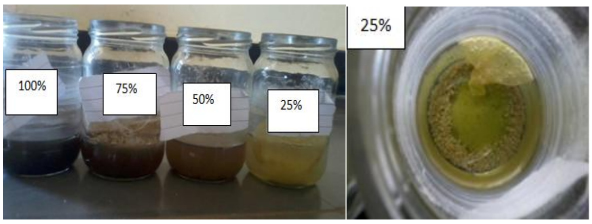
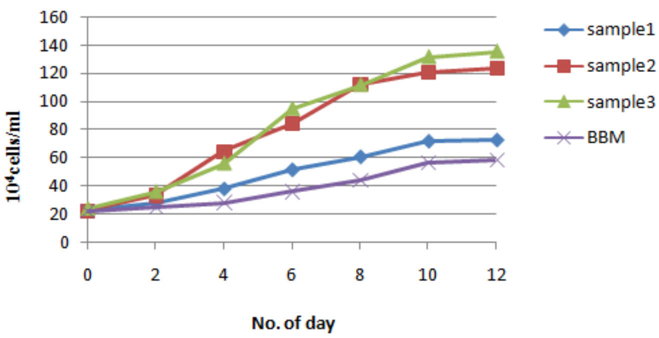


Fig 6: Algae growth in 25% dilution

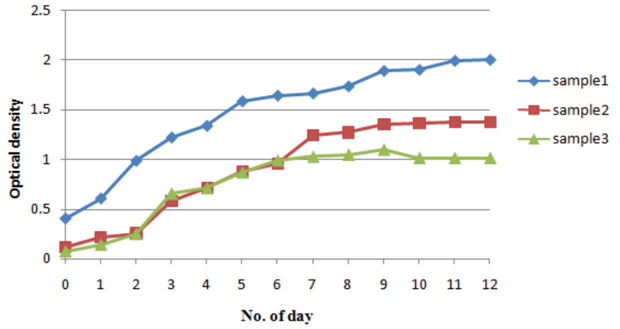
In undiluted dairy waste water algal growth was very poor, then it was diluted to 10% and 25% to grow the algae, where as in municipal waste water algae could grow without dilution[12].*Chlorella* grown very fast in 50% municipal waste water [24]. In sewage treatment plant at IIUM campus *chlorella* was grown without dilution [14].Recalcitrant waste water was diluted to 10% and then treated using *Chlorella vulgaris* followed by macrophyte [23].

B) Algal growth determination

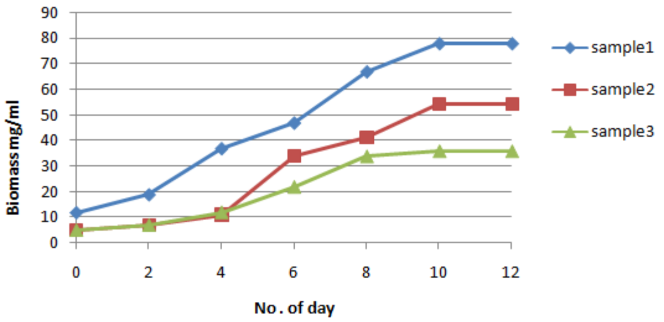
Growth of algae was determined by the following three methods. Whereas cell count using Hemocytometer found to be suitable method in the present study (*Chlorella).* Whereas the other 2 methods were not accurate due to the presence of suspended solids in the samples. Growth pattern of *Chlorella* studied in these 3 samples and compared it with BBM media. Growth was started on 2nd day in BBM media and continued upto 18 days. Whereas *Chlorella* inoculated in waste water samples reached the stationary phase .



Graph 1: Cell count by Hemocytometer



Graph 2: Growth determination by optical density



Graph 3: Growth determination by Centrifugation

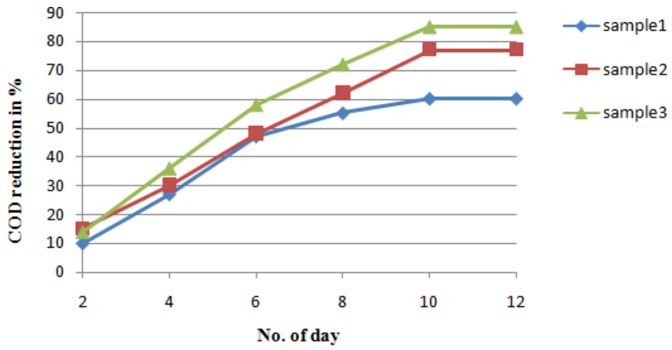
Algal growth is measured using spectrophotometer [24] [22] [32]. And also using Hemocytometer [23] [24].

C) Reduction Of Nutrients, COD And BOD After Treatment

BOD,COD,Total kjeldhal nitrogen and phosphorus reduction were studied in all 3 different waste water samples after 12 days of inoculatio.Sample1 showed the maximum TN and phosphorus reduction followed by sample2, sample 3 showed the maximum reduction of BOD and COD, followed by sample2.

Table2: Reduction in COD, BOD and other nutrients

Samples	**% of Reduction (After 12 days)**			
	BOD (mg/L)	***COD (mg/L)***	***TN (mg/L)***	***Phosphorus (mg/L)***
Sample 1	78	60	64.28	61
Sample2	82	77	62.9	50
Sample3	88	85	56.52	47



Graph 4: Reduction of COD in 3 samples

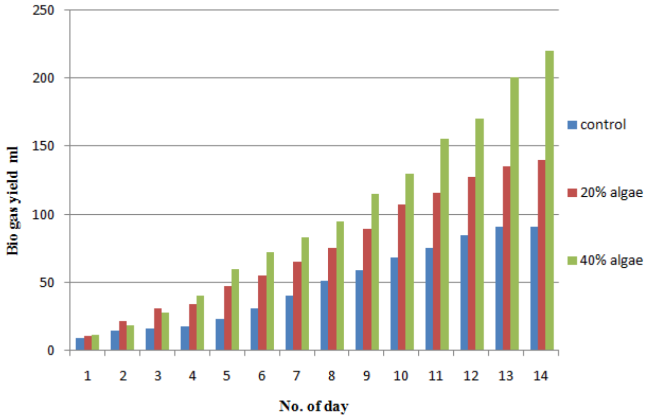
In brewery waste water BOD reduced by 27% and COD 15% using *Chlorella*. BOD, COD was able to reduce by 68.49 and 58.06% respectively in rice mill waste water using oedogonium and chara [18], where as *scenedesmus* in brewery effluent reduced COD by 57.5% [11]. Significant reduction of BOD and COD was recorded in domestic waste water using *chlorella* by 70.91 and 80.64% and using *scenedesmus* COD and BOD was reduced by 89.21% 70.97% [13].Phosphorus and nitrogen reduction in rice mill waste water was 71.59% and 56.42 respectively [18].

Nitrogen reduction by 20.8% obtained in brewery waste using *scenedesmus* [11]. Recalcitrant waste water was treated using *Chlorella vulgaris* followed by macrophyte, this culture reduced ammonium ions 71.6% phosphorus 28%[23]. Municipal waste water was treated with a combination of microalgae and a micro algae growth promoting bacteria reaching removal of up to100% ammonia, 15% nitrate, 36% phosphorus[47].

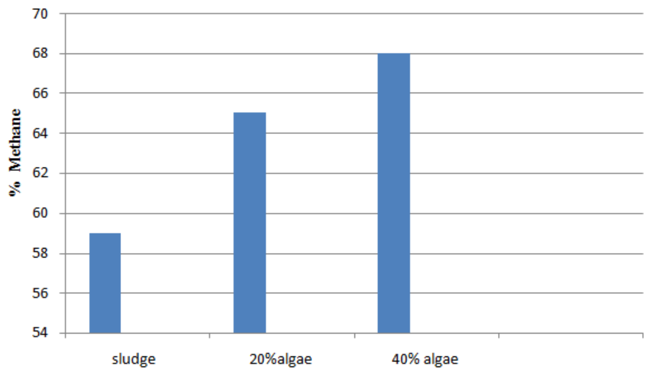
Removal of ammonium and orthophosphate from the batch dairy wastewater was 96% and 99%, respectively by Day 15 .For the 25% dilution experiment, initial concentrations of total ammonia nitrogen were 30 mg/L and were reduced to 5 mg/L in 6 days .The initial orthophosphate phosphorus concentration of 2.6 mg/L was reduced to 0.6 mg/L in 9 days, and it was completely removed by Day 12. Nitrate concentrations were consistently below 0.3 mg/L [12].

D) Co digestion of harvested of harvested algal biomass

The harvested algal bio mass with different concentration was co digested with sludge for biogas production.



Graph 5: Biogas yield



Graph 6: Methane content

Graph 5 represents the bio gas yield. There was an increase in bio gas with increase in algal concentration when compared to digestion of sludge alone as control. Graph 6 represent the methane content which was increased with increasing concentration of algae. Methane content was more for algae40%+ sludge 60% when compared with algae 20%+ sludge 80% and control (sludge alone).

Cyanobacterial biomass and seaweed biomass when utilized as slurry along with cow dung and slaughter house waste has yield up to 842ml on 10^{th} day when compared with the yield of in control (186ml) where cow dung used as sole slurry source [44].

The green alga, *Chaetomorpha litorea* collected from the backwaters of Muttukadu near Chennai for its potential in production of biogas. The biogas obtained through anaerobic digestion was 80.5 L /kg seaweed under the pressure of 21 kg, which had 65% of methane and it burnt for nearly 30 minutes in the biogas stove reaching the high boiling temperature in the short duration of time [34].

Cyanobacteria and *Chlorella* were aerobically digested with different organic loading rate, the highest methane production rates for Cyanobacteria and *Chlorella* were 78±25 mL (L.d) and 100±mL (L.d) [43].

The addition of 50% of waste paper in algal feed stock increased the methane production rate to 1170±75ml/L day as compared to 573±28ml/L day of algal sludge digestion alone[45]. Similarly 50% of waste paper was added to aquaculture microalgae sludge to adjust C:N which in turn doubled the methane production rate from 0.6L/L to 1.2L/L[38].

Methane production (309±46) rate was increased when micro algae and sewage sludge was co digested at 12% algae [46]. Sewage sludge was digested with 50% of algae, 40% algae and 18.9%algae respectively and bio gas yield was 290.3, 312.1 and 240.3 ml/g VS [40][41].

E) Proximate and ultimate analysis

Algae and sludge are characterized separately using standard methods. Total solids was more in algae compared to sludge were as Volatile solids was found more in sludge compared to algae. Protein, carbohydrate and lipid content were also estimated, carbohydrate and protein content is found more in algae when compared sludge.

Table 3: Characteristics of algae and sludge

Samples	**Total solids mg/L**	**Volatile solids mg/L**	**Protein mg/ml**	**Carbohydrate mg/ml**
Algae	13.04	227.4	0.76	11
Sludge	8.5	482.02	0.51	3

IV. CONCLUSION

Algae can be grown in sugar process effluent where COD, BOD, TN and phosphorus can be reduced. The effluent from lagoon1,lagoon 2 (without dilution) could not support algal growth may be due to high level of COD, at dilution with 25% effluent(75% water) algal growth may be obtained , where as in sample3 (without dilution) supported the growth of algae may be due to lower level of COD. The growth rate of algae increased with decreasing concentration of COD level. Algal based waste water treatment reduced COD upto 85%, BOD up to 88%, Total kjeldahl Nitrigen upto 64.28%, phosphorus upto 61% within 12 days . In the present study co-digestion of microalgae and sludge increased 58.63% of bio gas and 8% methane content when compared to sludge alone. Hence microalgae like *Chlorella* sp may be inoculated in sample3 directly and with dilution in sample 1 and 2 to reduce the load simultaneously this biomass obtained may be used to the bio gas unit to enhance the methane.

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