Cost effective and economic method for cultivation of *Chlorella pyrenoidosa* for the simultaneous treatment of biogas digester wastewater and biogas production

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ABSTRACT:

Microalgae have recently received a lot of attention as a new biomass source for the production of bio fuels and for the treatment of waste water. In this work, Chlorella pyrenoidosa was cultivated in biogas digester wastewater. The growth kinetics of the algae as well as the bio-remediation effect on the waste water was studied. The Chlorella pyrenoidosa can utilize the nitrogen content present in biogas digester wastewater as a substrate for its growth. The growth of microalgae was found to follow the Monod growth model satisfactorily.

Under the optimal condition in biogas waste water medium of microalgae, a maximum biomass of 1.5 gm/l was obtained in fifteen days. The net specific growth rate of microalgae Chlorella pyrenoidosa was found to be 0.1 D^{-1}. The growing algae also removed 76 % of nitrate nitrogen (NO_3-N) from the biogas wastewater. Treated Biogas waste water can be further used for the anaerobic digestion of algal biomass for the production of biogas. This suggests that the cultivation of Chlorella pyrenoidosa in biogas wastewater would be efficient, saving water as well as producing digestible biomass. Thus, on one hand the biogas waste water is being treated and on the other, the alga is showing substantial growth. The same algal species can then be used to produce biogas or other corresponding bio fuel.

Keywords -Chlorella pyrenoidosa, anaerobic digester outlet slurry, cultivation, nitrogen nitrate, Lipids,

I. INTRODUCTION

Microalgae are a diverse group of photosynthetic microorganisms that have a potential to grow fast in nutrient medium and yields more biomass in a small surface. They have a ability to produce biofuels in an economically effective and environmentally sustainable manner. The production of these biofuels can be engaged with flue gas CO_2 mitigation, wastewater treatment, and the production of high-value chemicals.

Chlorella vulgaris was cultured in wastewater discharged from a steelmaking plant with the plan of developing ancost-effectivelysufficient system to eliminate ammonia from wastewater and CO_2 from Flue gas at the same time [1].

The growth of green algae Chlorella sp. on wastewaters sampled from four different points of the treatment process flow of a local municipal wastewater treatment plant (MWTP) was done. They investigate how well the algal growth removed nitrogen, phosphorus, chemical oxygen demand (COD), and metal ions from the wastewaters [2].

Numerical model for glycerine concentration-dependent *Chlorella minutissima* UTEX2341 biomass and lipids production was developed [3].They found that the initial concentration of glycerine was the most significant factor as a carbohydrate source for algae growth and lipid production. The effects of temperature and light on the growth of *Ankistrodesmusfalcatus, Phormidiumbohneri*and *Oscillatoriaagardhii*have been compared and mathematically formulated in the waste water [4].

The mathematical modeling and effect of nitrogen and phosphorus on the growth of blue-green algae population was done. This modeling investigates how to use nitrogen and phosphorus more effectively in the control and elimination of blue-green algae blooms [5].

Bioremediation of wastewater by microalgae can provide the microalgae feedstock for their biomass energy, as well as reduce the material cost of the biofuel [6] [9] [10]. Microalgae are autotrophic organisms in water that play a vital role in the elimination of pollutants in water. They have strong survival abilities and can utilize nitrogen and phosphorus in wastewater for their growth. Chlorella can remove nitrogen and phosphorus in wastewater [7] [11].

The work greatly diminishes the direct pollution caused by the waste. However, large amount of biogas and industrial wastewater produced in the process is always a problem consequently owing to its high concentrations of nitrogen, which would results in the eutrophication of the environmental water if the wastewater is discharged directly to the environment [8]. Therefore, cultivation of the microalgae *Chlorella pyrenoidosa*in the biogas wastewater can not only eliminate the excess nitrogen and phosphorus, but also obtain large amount of the valuable microalgae, which can be used for the exploitation of fertilizers and feed additives.

Previously, most of the researchers worked on the treatment of municipal and industrial waste water but this work examined the growth of *Chlorella pyrenoidosa*in the biogas wastewater and optimized its cultivating condition in the wastewater. The bioremediation of the wastewater by the microalgae was also discussed, which would be vital for the treatment of the biogas wastewater and the production of the microalgae biomass. This biomass and treated water is used for the production of biogas. Thus, cultivation of Chlorella in the biogas or industrial wastewater also provides a low-cost way for the exploitation of the microalgae biomass energy.

II. MATERIAL & METHODS

2.1 Microalgae, culture medium & condition

The *Chlorella pyrenoidosa* was purchased from *National Collection of Industrial Microorganisms* (*NCIM*). The culture medium was fog medium which consist of $MgSO_4.7H_2O$ 0.1 gm, K_2HPO_4 0.1gm, $Cacl_2.H_2O$ 0.500 gm, Fe-EDTA (In hot water dissolve 745.0 mg of Na_2EDTA and then add 557.0 mg of $FeSO_4.7H_2O$ Boil the solution for few minutes and make the volume to 100.0 ml.) 2.5 ml, Agar 6 gm, Micronutrient solution (H_3BO_3 286.0 mg, $MnCl_2.4H_2O$ 181.0 mg, $ZnSO_4.7H_2O$ 22.0 mg, $Na_2MoO_4.2H_2O$ 39.0 mg $CuSO_4.5H_2O$ 8.0 mg, Distilled water 100.0 ml) 0.5 ml. C. pyrenoidosa was cultivated in the fog medium at the temperature of 21±2°C each day before the microalgae was inoculated to the biogas wastewater.

2.2 Digestion Process

300 ml of biogas wastewater was inoculated with *Chlorella pyrenoidosa*in a 500 ml flask. Then, they were cultivated at 21°C with pH 5. Their growth was determined daily by testing the absorbance at 680 nm in order to study the growth of *Chlorella pyrenoidosa* in the biogas wastewater.

Microalgae growth process can be explained Monod equation. This is based on mass balance. There are various terms associated with Monod kinetics with the contribution of biomass and substrate to the environment. S represents the substrate concentration and X represents the biomass concentration. The rate of substrate and biomass growth is ds/dt anddx/dt. Rate equation for algal growth is

$$\frac{dX}{dt} = \frac{\mu_{max} S}{K_s + S} X$$

μ_{max} is the maximum specific growth rate of the microalgae; X is the conc. of microalgae in the medium; S is the substrate concentration. To learn the bioremediation of the biogas wastewater by *Chlorella pyrenoidosa*the microalga was cultivated in 300 ml wastewater for two weeks under the optimal condition. The growth of the microalga was detected every 3^{rd} day and the concentrations of nitrate nitrogen (NO_3-N) content in the wastewater was analyzed at the beginning & the end of the experiment using the standard methods.

2.3 Nitrate consumption

Bioremediation of biogas waste water was analyzed by calculating the concentration of nitrates in the initial and the final day of inoculation. Nitrate nitrogen is used as a feedstock for the cultivation of algal biomass. The nitrate nitrogen (NO_3-N) content consumption kinetics may be expressed as substrate conversion to product

$$-\frac{dS}{dt} = \frac{1}{Y_{X/S}} \frac{dx}{dt}$$

Where – dS/dt is the total consumption rate of nitrate nitrogen; t is total time for microalgae growth and substrate consumption; $Y_{x/s}$ is the maximum microalgae Yield coefficient; S is nitrate nitrogen concentration.

III. RESULTS & DISCUSSION

3.1 Growth of *Chlorella pyrenoidosa*in Biogas waste water

Algal growths in terms of optical density OD_{680} in the Biogas wastewaters under axenic condition were plotted in Fig. 1. C. pyrenoidosa could grow well in the biogas wastewater. Their growth increased rapidly in the first fifth days, then slowed down in the next ten days, and almost stopped in the last five days. This could be due to the gradual consumption of certain nutrient elements like nitrogen in the biogas wastewater. 0.45 gm of microalga *Chlorella pyrenoidosa*was cultivated in 300 ml of medium in fifteen days of cultivation period in biogas waste water. This also proved that the *Chlorella pyrenoidosa*could grow well in biogas wastewater and might be used for the treatment of the wastewater.

This shows the growth of microalgae as per Monod growth kinetics model for microalgae. As in substrate limited Monod growth kinetics

Table1. Model state variables and initial and final concentration of nitrogen source

Parameter	*Values*
μ_g (d^{-1})	0.1002
X_0 (mg/l)	0.35
X_f (mg/l)	1.40
Initial nitrogen nitrate S_o (mg/l)	84
Final nitrogen nitrate S_f (mg/l)	20.16

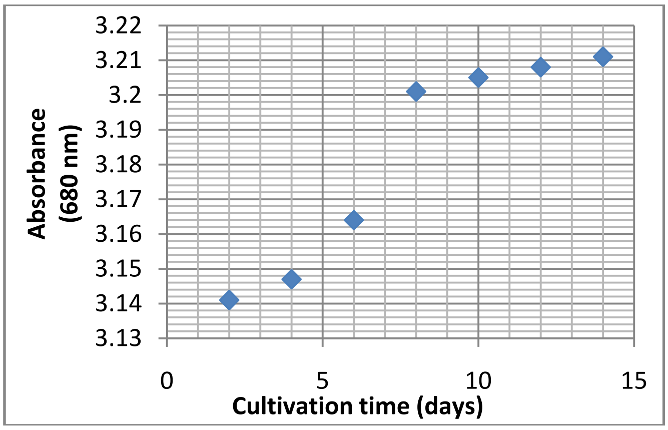

Fig. 1 Growth of *Chlorella pyrenoidosa*in the biogas wastewater under the optimal condition

3.2 Bioremediation of the biogas wastewater by C. pyrenoidosa

With the growth of *Chlorella pyrenoidosa,* the concentrations of nitrate nitrogen decreases. The NO_3-N in the biogas wastewater was eliminated by 76%, respectively, within fifteen (15) days. The nitrite concentration in the biogas wastewater was tested initially, during the introduction of the inoculum, and subsequently after fifteen days. This clearly indicated a 76percent reduction in the nitrate concentration, proving its consumption by the microalgae. Table 2 shows the utilization of nitrate by microalgae in the biogas waste water slurry.

Table2. Elimination of nitrate nitrogen in the biogas wastewater by *Chlorella pyrenoidosa*

Initial nitrogen nitrate S_o (mg/l)	*Final nitrogen nitrate S_f (mg/l)*	*% Removal*
84	20.16	76 %

IV. CONCLUSION

he cultivation of the microalga, *Chlorella pyrenoidosa*in the biogas wastewater was studied and analyzed.The results from this study demonstrated the feasibility of cultivating *Chlorella pyrenoidosa* in biogas wastewaters outlet slurry. *Chlorella pyrenoidosa* could adapt well in biogas wastewaters outlet slurry with no lag phases observed. Algal growth was significantly enhanced in the centrate because of its much higher levels of nitrogen content in biogas wastewaters outlet slurry. The microalgae *Chlorella pyrenoidosa*had good growth in the biogas wastewater and its dry weight reached 0.45 gm in 300 ml after cultivation for fifteen (15) days, which was more than three times of the initial dry weight. Although, the concentrations of nitrate nitrogen in the wastewater were extremely high, the *Chlorella pyrenoidosa* could still grow well in the wastewater. Biogas waste water is the suitable method for cultivation of microalgae.

Microalgae C. pyrenoidosa removes around 76 percent of the concentration of nitrate in the biogas waste water in the 15 days of inoculation. This work treat the biogas waste water for further use and produced microalgae may be further undergo an-aerobic digestion in a lab scale continuous stirrer batch process anaerobic digester for the production of bio gas as a renewable source of energy. Treated waste water was used as slurry for the anaerobic digestion and hydrolysis process of the microalgae biomass. This concludes that the cultivation of C. pyrenoidosa in biogas wastewater would be efficient, economic and saving water as well as producing digestible biomass.

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