African Journal of Biotechnology Vol. 10(61), pp. 13115-13120, 10 October, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.358

Full Length Research Paper

Cultivation of the microalga, *Chlorella pyrenoidosa*, in biogas wastewater

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Accepted 1 June, 2011

Biogas wastewater is always a problem as a result of its extremely high concentrations of nitrogen and phosphorus, which is the main reason for the eutrophication of the surrounding water. The microalga, *Chlorella pyrenoidosa*, can utilize the nitrogen and phosphorus in wastewater for its growth. Therefore, the microalga was introduced to be cultivated in the biogas wastewater, which could not only bioremediate the wastewater, but also produce plenty of the microalga biomass that could be used for the exploitation of fertilizers, feed additives and biofuels. This study showed that the microalga, *C. pyrenoidosa* could grow well in the biogas wastewater under optimal condition: initial cell density of 0.15 (OD_{680}), pH 8 and illumination intensity of 10000 LX. Under the optimal condition, the dry cell weight of the microalgae reached 0.1 g/L after cultivation in the wastewater for fourteen (14) days; in the meantime, the microalga also removed 71.8% of phosphorus, 100% of ammoniacal nitrogen (NH_4^+-N), 52.8% of nitrate nitrogen (NO_3-N) and 23.0% of nitrite nitrogen (NO_2-N) from the biogas wastewater, suggesting that the cultivation of *C. pyrenoidosa* in biogas wastewater would be efficient for the treatment of wastewater. This study also provided a low-cost way to produce the microalga and its relevant products.

Key words: *Chlorella pyrenoidosa*, biogas wastewater, cultivation, phosphorus, nitrogen.

INTRODUCTION

Biogas projects are widely established to deal with the waste produced in the feeding of poultry and animal. The projects greatly reduce the direct pollution caused by the waste, especially in countryside. However, large amount of biogas wastewater produced in the process is always a problem as a result of its high concentrations of nitrogen and phosphorus, which would cause the eutrophication of the environmental water if the wastewater is discharged directly to the environment. Although, many microorganic and chemical technologies have been used to treat the biogas wastewater, the expensive operation cost and large early investment limit their applications.

Microalgae are autotrophic organisms in water that play an important role in the elimination of pollutants in water environment. They have strong survival abilities and can absorb nitrogen and phosphorus in wastewater for their growth. Nowadays, many kinds of microalgae have been widely used in the bioremediation of water environment, especially the treatment of wastewater (Carlos et al., 1997; Luz et al., 2002; Zheng et al., 2011). *Chlorella, Cyanobacteria* and some diatom species were the microaglae usually used in the treatment of wastewater and *Chlorella* has relatively higher removal of nitrogen and phosphorus in wastewater (Wang et al., 2010). Therefore, cultivation of the microalga *Chlorella* in the biogas wastewater cannot only eliminate the excess nitrogen and phosphorus in the wastewater to avoid the eutrophication of the environmental water, but also obtain large amount of the valuable microalga, which can be

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used for the exploitation of fertilizers and feed additives.

In addition, biomass energy of microalga is one of the most potential renewable energy with the advantages of rapid reproduction, simple pyrolysis process and no environmental pollution (Hu et al., 1999). However, the over expensive material limits the industrial application of the biomass energy (Hu et al., 1999; Ashish et al., 2009). Bioremediation of wastewater by microalgae cannot only provide the microalgae feedstock for their biomass energy, but also greatly reduce the material cost of the biofuel (Zheng et al., 2011). Therefore, cultivation of *Chlorella* in the biogas wastewater also provides a low-cost way for the exploitation of the microalgae biomass energy.

This study examined the growth of *Chlorella pyrenoidosa* in the biogas wastewater and optimized the cultivating condition of the microalga in the wastewater. The bioremediation of the wastewater by the microalga was also discussed, which would be useful for the treatment of the biogas wastewater and the production of the microalga biomass.

MATERIALS AND METHODS

Microalga, culture medium and culture condition

The microalga, *C. pyrenoidosa* was provided by Professor Zhou Lihong in Fisheries College of Jimei University. The culture medium was the f/2 medium which consist of $NaNO_3$ 74.8 mg/L, KH_2PO_4 4.4 mg/L, Na_2SiO_3 10 mg/L, ferric citrate 3.9 mg/L, $ZnSO_4$ 23 μg/L, $MnCl_2 \cdot 4H_2O$ 178 μg/L, $CaCl_2 \cdot 6H_2O$ 12 μg/L, $Na_2MoO_4 \cdot 2H_2O$ 7.3 μg/L, $CuSO_4 \cdot 5H_2O$ 10 μg/L and $Na_2EDTA \cdot 2H_2O$ 4.35 μg/L. The f/2 medium was sterilized before use. *C. pyrenoidosa* was cultivated in the f/2 medium at the temperature of 21±1°C and under the illumination intensity of 5000 LX for 12 h each day before the microalga was inoculated to the biogas wastewater.

Experimental methods

C. pyrenoidosa of different volumes were centrifugated respectively under 4000 rpm for 10 min. After the microalga sedimentations were washed by distilled water and centrifugated twice, they were inoculated to 50 ml biogas wastewater in a 150 ml flask, respectively, to form different initial densities. Then, they were cultivated at 21°C under the illumination intensity of 5000 LX for 12 h each day, and their growth were determined daily by testing the optical densities at 680 nm as previously described (Yang et al., 2008) in order to study the growth of *C. pyrenoidosa* with different initial densities in the biogas wastewater. The dry cell weight of the microalgae was calculated according to the linear equation between OD_{680} and the dry cell weight of the microalga, which was: dry weight (mg/mL) = $0.207OD_{680} + 0.0022$ (the linear-fitting coefficient $R^2 = 0.9984$), which was figured out according to the previous method (Yang et al., 2008).

The cultivation condition of *C. pyrenoidosa* in the biogas wastewater was optimized according to the orthogonal experiment designed in Table 1. The inoculation method and the determination of the growth were the same as earlier mentioned, and the microalga was cultivated in the wastewater for 7 days.

To learn the bioremediation of the biogas wastewater by *C. pyrenoidosa*, the microalga was cultivated in 600 ml wastewater for two weeks under the optimal condition in the previous orthogonal experiment. The growth of the microalga was detected every day as previously described (Yang et al., 2008) and the concentrations of nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N), ammoniacal nitrogen (NH_4^+-N) and phosphorus (P) in the wastewater were analyzed every two days using the standard methods (Lei, 2006).

RESULTS AND DISCUSSION

Growth of *C. pyrenoidosa* with different initial densities

As shown in Figure 1, *C. pyrenoidosa* of different initial densities could grow in the biogas wastewater and their growth increased rapidly in the first three days, then slowed down in the next ten days, and almost stopped in the last five days, which could be due to the gradual consumption of certain nutrient elements like nitrogen and phosphorus in the wastewater. Microalgae have been widely used in the treatment of wastewater for their strong survival abilities and efficient utilization of nitrogen and phosphorus in wastewater (Carlos et al., 1997; Luz et al., 2002; Zheng et al., 2011). This study also proved that the microalga *C. pyrenoidosa* could grow well in biogas wastewater and might be a good candidate microalga for the treatment of the wastewater. Although, *C. pyrenoidosa* with all different initial densities could grow well in the wastewater, lower initial density would be preferred for lower cost. Therefore, the initial densities of OD_{680} 0.05, 0.1 and 0.15 were considered in the subsequent orthogonal experiments.

Optimization of cultivation condition for *C. pyrenoidosa* in the biogas wastewater

According to the analysis of orthogonal experiments (Table 2), the optimal condition for the growth of *C. pyrenoidosa* in the biogas wastewater were obtained: initial density (OD_{680}) of 0.15, pH 8 and illumination intensity of 10000 LX. Among the three factors, pH had the largest influence on the growth of the microalga in the biogas wastewater. Under the optimal condition, the microalga, *C. pyrenoidosa* could grow very well in the biogas wastewater (Figure 2) and the production of the microalga was about 0.1 g/L (dry weight) after cultivation in the wastewater for fourteen (14) days, which was more than three times of the initial dry weight.

Chlorella is one of the most important microalgae usually used in the treatment of wastewater. Luz et al. (2002) reported the removal of ammonium and phosphorus ions from synthetic wastewater by the microalgae *Chlorella vulgaris*, Yang et al. (2008) studied the growth of *C. pyrenoidosa* in wastewater from cassava ethanol fermentation and Ashish et al. (2010) studied the cultivation of *Chlorella minutissima* in the municipal wastewater for the biofuel. These researches showed that the microalgae had good growth in the wastewaters, but their growth highly depended on the cultivation modes, conditions and so on. Therefore, it is necessary to

Table 1. Design of the orthogonal experiment.

Level	Factor		
	Initial density (OD_{680})	pH	Illumination intensity (LX)
1	0.05	6	2500
2	0.10	7	5000
3	0.15	8	10000

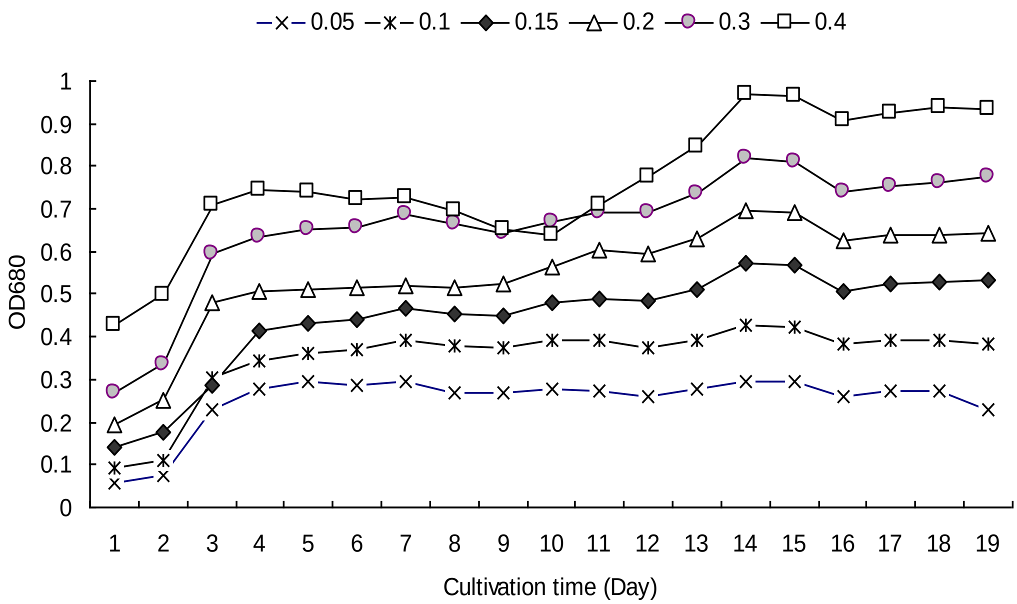

Figure 1. Growth of *C. pyrenoidosa* with different initial densities in the biogas wastewater.

Table 2. Analysis of orthogonal experiment.

Level		Factor			
		Initial density (OD_{680})	pH	Illumination intensity (LX)	Final density (OD_{680})
1		0.05	6	2500	
2		0.10	7	5000	
3		0.15	8	10000	
Experiment numbers	1	1	1	1	0.052
	2	1	2	2	0.322
	3	1	3	3	0.486
	4	2	1	2	0.124
	5	2	2	3	0.423
	6	2	3	1	0.437
	7	3	1	3	0.162
	8	3	2	1	0.375
	9	3	3	2	0.543
X1		0.29	0.11	0.29	
X2		0.33	0.38	0.33	
X3		0.36	0.49	0.36	
Range		0.07	0.37	0.07	

X1, X2 and X3: the average values of final density at levels 1, 2 and 3, respectively, and the range is equal to the maximum difference among the three levels.

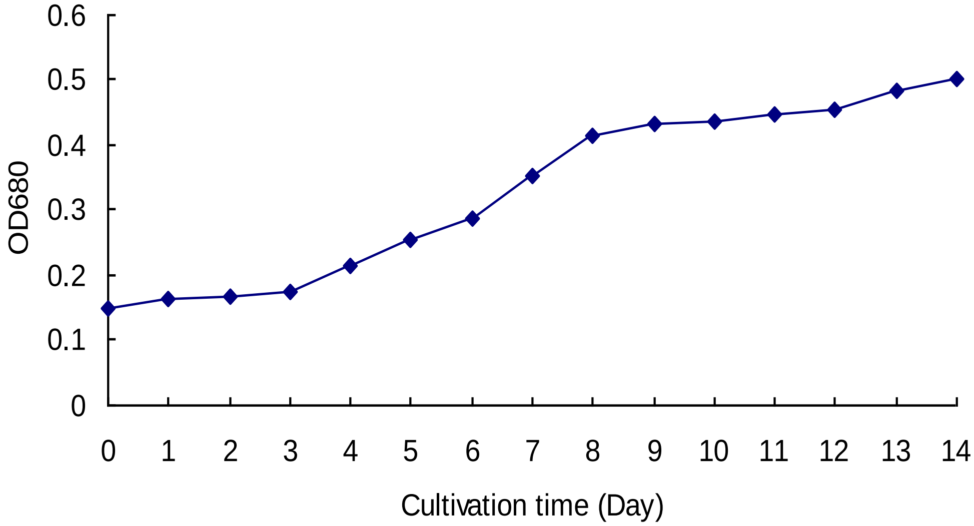

Figure 2. Growth of *C. pyrenoidosa* in the biogas wastewater under the optimal condition.

optimize the cultivation condition of the microalga in the biogas wastewater, and the results showed that the microalga had much better growth under the optimal condition (Figure 2).

Bioremediation of the biogas wastewater by *C. pyrenoidosa*

With the growth of *C. pyrenoidosa,* the concentrations of phosphorus (P), nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N) and ammoniacal nitrogen (NH_4^+-N) in the biogas wastewater decreased gradually (Figures 2 and 3). The P, NO_3-N and NO_2-N in the biogas wastewater were eliminated by 71.8, 52.8 and 23.0%, respectively, within fourteen (14) days, while the NH_4^+-N in the wastewater was completely eliminated in the first four days after the microalga was inoculated to the wastewater. In the first eight days, with the rapid growth of the microalga, the concentrations of P, NO_2-N, NO_3-N and NH_4^+-N in the wastewater decreased very quickly. In the next days, both the growth rate of the microalga and the decreasing rate of the concentration of P in the wastewater slowed down, but the consumption rate of the NO_3-N in the wastewater accelerated remarkably. The rapid growth of *C. pyrenoidosa* in the first eight days could be due to the rich N and P in the wastewater. The slow growth in the next days could be due to the low concentration of P in the wastewater, so that P became the limiting factor to the growth of the microalga. Therefore, the growth of the microalga in the following days mainly depended on the NO_3-N in the wastewater and the concentration of the NO_3-N decreased rapidly in the following days.

Quite a few researches have been done on the bioremediation of wastewaters by the microalga, *Chlorella* and the results showed that the microalga had good eliminations of the elements N and P in wastewaters (Luz et al., 2002; Nirupama, 2002; Zheng and Wang, 2010; Ashish et al., 2010). Luz et al. (2002) also found that the microalga *C. vulgaris* could remove 93% of ammonium from the synthetic wastewater within 2 days and 99% of ammonium within 6 days, which were similar to our results. In addition, the diluted wastewater was often used for the cultivation of microalga in many experiments (Nguyen et al., 1999; Luz et al., 2002) because most mciroaglae could not grow directly in the wastewater with high concentrations of nitrogen and phosphorus. However, our study showed that *C. pyrenoidosa* could grow directly in the biogas wastewater that had higher concentration of N or P than that of other wastewaters (Nguyen et al., 1999; Luz et al., 2002; Zheng et al., 2011), which suggested that the microalga *C. pyrenoidosa* had much higher survival in the biogas wastewater and could make better use of nitrogen and phosphorus in the wastewater.

Conclusion

The cultivation of the microalga, *C. pyrenoidosa* in the biogas wastewater was studied. Although, the concentrations of nitrogen and phosphorus in the wastewater were extremely high, the microalga could still grow well in the wastewater. With the orthogonal experiment, the optimal condition for the growth of *C. pyrenoidosa* in the biogas wastewater was obtained: initial density (OD_{680}) of 0.15, pH 8 and illumination intensity of 10000 LX. Under the optimal condition, the microalga had good growth in the biogas wastewater and its dry weight reached 0.1 g/L after cultivation for fourteen (14) days, which was more than three times of the initial dry weight. With the rapid growth of *C. pyrenoidosa* in the wastewater, the microalga also removed 71.8% of phosphorus, 100% of ammoniacal

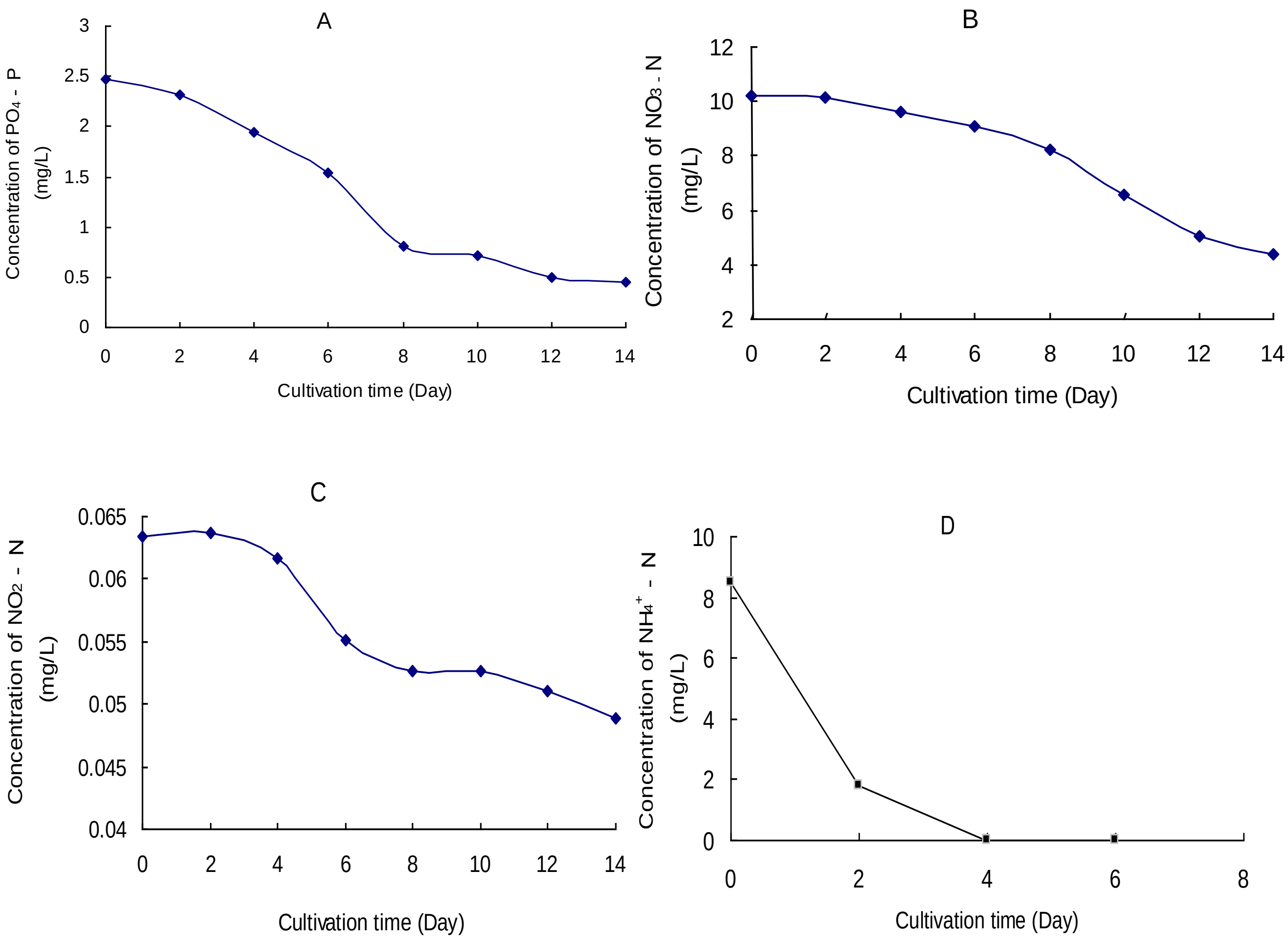

Figure 3. Eliminations of phosphorus (A), nitrate nitrogen (B), nitrite nitrogen (C) and ammoniacal nitrogen (D) in the biogas wastewater by *C. pyrenoidosa.*

nitrogen (NH_4^+-N), 52.8% of nitrate nitrogen (NO_3-N) and 23.0% of nitrite nitrogen (NO_2-N) from the biogas wastewater within fourteen days, indicating that *C. pyrenoidosa* might be a good candidate microalga for the treatment of biogas wastewater. This study also provides a low-cost way for the exploitation of microalgae biomass for biofuel.

ACKNOWLEDGEMENTS

This study was supported by the Key Scientific and Technological Project of Fujian Province (2010Y0039), the Spark Project of Fujian Province (2010S0068) and the Foundation for Innovative Research Team of Jimei University, China (2010A004).

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