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Full Length Article

Algae Removal of Nitrogen and Phosphorus from Biogas Slurry Coupled with the Nutritional Value of Algae

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Abstract

The purpose of this study is to study the effect of algae removal of nitrogen and phosphorus from the liquid of chicken manure to improve nutritional value of algae. In this experiment, the biogas slurry was diluted into 15%, 30%, 45% and 60% concentration gradients. We studied the growth of algae in different concentrations of methane and the effect of nitrogen and phosphorus removal from them, analyzed the nutrients of algae from different concentrations of biogas slurry. The results showed that: (1) Algae grow best in 15% biogas slurry, and the removal rate of nitrogen and phosphorus was the highest, with the increase of biogas slurry concentration, the growth of algae was inhibited, and the removal rate of nitrogen and phosphorus was also reduced. (2) The nutrient content of microalgae in 4 concentrations of biogas slurry was basically the same, there was no significant difference between them, and the content of harmful heavy metals was lower than that of Chinese feed standard, so it is a safe feed resource. Thus microalgae can be used to remove the nitrogen and phosphorus from the low concentration biogas liquid, so it can be used to treat the cultured sewage and produce high quality algae feed.

Keywords: Algae; Biogas slurry; Nitrogen and phosphorus; Algal feed

Introduction

With the rapid development of large-scale aquaculture, a large number of livestock manure has been generated, as an effective way to treat livestock manure and reuse it, biogas engineering is widely used in large and medium-sized farms. However, a large number of biogas fluid is also produced, because of the rich N, P and other nutrients in the biogas slurry, it does not meet the national emission standards, so it needs to be dealt with further, so more and more attention has been paid to the further treatment of biogas slurry. At present, the main treatment methods of biogas slurry are: natural treatment and industrial treatment. In the natural treatment method, the most widely used method is the artificial wetland treatment system. In constructed wetlands, plants, protozoans and microorganisms are used to deal with too many nutrients in the wastewater, its advantages are low running cost, easy operation, good quality of effluent, and beautifying the environment, but there are many disadvantages, but there are many disadvantages, for example, low efficiency, much time and large area of land. It is difficult for practical application of scarce land. The main methods of industrial treatment include physical and chemical treatment and biological treatment, Physicochemical treatment is to separate pollutants from wastewater by physical and chemical processes, or to transform them into harmless substances, so that water quality can be purified, its main features are fast processing and good treatment, but it will cause secondary pollution due to the use of a large number of chemical reagents. Biological treatment is to treat wastewater with microorganism and combine with engineering technology, so as to fully degrade the contaminants in anaerobic fermentation of biogas slurry, its characteristic is that the treated sewage reaches the national discharge standard, but a large amount of sludge will be produced during the treatment, which is difficult to deal with and will become another source of pollution.

We found that in the sewage pond, there is always a layer of algae in the warm season, it can not only eliminate the odor of ponds around but also can purify sewage, inspired by this, we assume that we can grow algae in the slurry in order to purify the biogas slurry. By consulting the relevant information, we know that: microalgae have the advantages of large production potential, fast propagation speed, easy cultivation and strong adaptability, and the ability to adapt to bad environment is widely used, its strong ability to adapt to the harsh environment is particularly concerned. Using kitchen waste water to grow algae in shake flask, it was found that algal growth rate, biomass and yield of algae can reach more than 80% in Zarrouk culture medium (Huang *et al.*, 2009). Navicula and Cyclotella can remove 87.09% and 88.18% COD in Papermaking Wastewater (Dong *et al.*, 2010). The results have shown that

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microalgae can grow in domestic sewage or organic waste water. According to the characteristics of algae can grow in wastewater, we combine microalgal culture with biogas slurry purification ,we hope that the biogas slurry can be processed to meet the national emission standards and obtain high quality algae feed ,and achieve double effects of biotransformation of nutrients and biogas slurry treatment, this method can provide a feasible way for advanced treatment and resource utilization of biogas slurry, and also provide new ideas for the development of feed resources.

Materials and Methods

The biogas solution used in the experiment is derived from the anaerobic fermentation pool of Shandong Hao Tai experimental animal breeding Co., Ltd., and the recovered biogas liquid is stored in the 4e 4dongee refrigerator. Microalgae used in the experiment were selected and domesticated by the research team, for the genus alga. The instrument comprises a shaker (HQL300, Wuhan KYKY technology development limited liability company), autoclave (LDZX-75KB, Shanghai Shenan medical equipment factory), light incubator (BSG-300, Shanghai Boxun (H-2050R), refrigerated centrifuge instrument Co. Ltd., Xiangyi Centrifuge, freeze dryer (FD5) the United States, SIM), light reactor (KLF2000, Switzerland Bioengineering), gas chromatography-mass spectrometry (Agilent 7890A/5975C).

Pretreatment of biogas slurry: Take 12 L biogas slurry, remove large particle impurities in biogas slurry by yarn filtration, and divide them into 4 parts. They are divided into 4 parts, diluted with distilled water to different concentrations, respectively. After diluted, the biogas slurry concentration is 15%, 30%, 45% and 60%, respectively, and it is sterilized 15 min at 121 degrees Celsius as a culture medium.

Microalgae Culture Method

The initial OD680 was 0.1 by inoculating Chlorella in 5000 m L conical bottle with 3000 mL of various concentrations. The conical bottle was placed in the incubator of constant temperature, and the value of P^H was 7, and it was incubated in the incubator for 14 days. The conditions were as follows: the temperature was 28 conical bottles with 3000 mL of various concentrations. The conical bottle was placed in the incubator of constant temperature$_{680}$ of the culture medium were measured every 24 h, and 4800r/min centrifugation 20 min were taken per 24 h, and the water quality parameters were measured by the proper multiple of supernatant dilution.

The determination of P^H value uses Mettler Toledo 320-S P^H meter, Ultraviolet visible spectrophotometer for the determination of optical density, The method for the determination of total phosphorus using the national standard GB11893-89, determination of total nitrogen using the national standard HJ 636-2012, determination of ammonia nitrogen using the national standard HJ535-2009, the determination of COD using the national standard GB11914-89, determining the solid suspension by the national standard GB11901-89. The algal composition is entrusted to the Chinese Ministry of agriculture for the determination of the quality of the feed quality supervision and test center.

Results

Analysis of Composition in Biogas Slurry

The results showed that the total nitrogen, ammonia nitrogen, total phosphorus and chemical oxygen demand in biogas slurry did not meet the national emission standards, which were 4.4, 6.2, 6.4 and 5.9 times of the standard value respectively (Table 1).

The Effect of Different Concentrations of Biogas Slurry on the Growth of Chlorella

From Fig. 1 and 2 it can be seen that the growth of *Chlorella* is not the same in the biogas liquid with different dilution concentration, it has the best growth trend in 15% biogas slurry, and it grows slower in other biogas slurry because of its higher concentration of ammonia nitrogen has inhibitory effect on *Chlorella vulgaris*. The results show that the higher the concentration of biogas slurry, the slower the growth rate of Chlorella.

The Removal Rate of Ammonia Nitrogen from Different Concentrations of Methane

As can be seen from Fig. 3, the removal rate of ammonia nitrogen in the 15% biogas slurry is the highest and the removal rate is up to 96.2%, it because the concentration of the slurry is low, and its ammonia concentration does not inhibit the growth of the *Oedogonium*, they can use ammonia nitrogen as a nitrogen source to synthesize its own protein and chlorophyll and other nutrients, so the removal rate of ammonia nitrogen is very high. In 30% of the biogas liquid, the ammonia nitrogen also decreased quite a lot, and the removal rate was close to 90%. In the biogas slurry of 45% and 60%, the concentration of ammonia nitrogen is still high, which will still inhibit the growth of *Oedogonium.* Therefore, the absorption and utilization rate of ammonia nitrogen is also low, but the removal rate of ammonia nitrogen is still high due to aeration.

The Removal Rate of Total Nitrogen in Different Concentrations of Algae by Alga

The total nitrogen in the biogas liquid includes ammonia nitrogen, nitrate nitrogen, nitrite nitrogen and a part of nitrogen containing organic matter, and the main component is ammonia nitrogen (Fig. 4), so the total nitrogen removal rate is similar to that of ammonia nitrogen.

Table 1: Initial values of biogas slurry indexes

Parameter	Numerical Value
pH	8.55
Total solid suspended matte (mg/L)	44
Total nitrogen (mg/L)	309
Ammonia nitrogen (mg/L)	249
Total phosphorus (mg/L)	32
Chemical oxygen demand (mg/L)	890

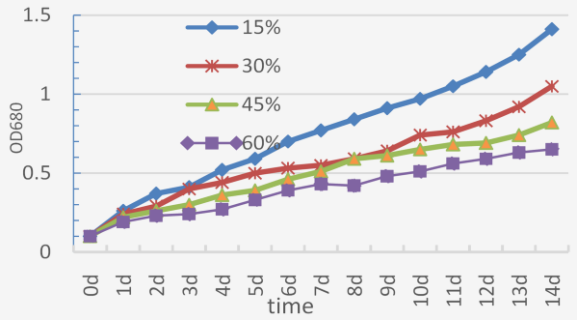

Fig. 1: The growth curves of Chlorella in different dilutions of biogas slurry

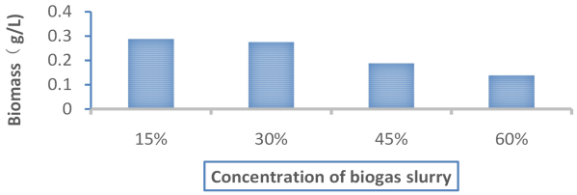

Fig. 2: The biomass of oedogonium in different dilutions of biogas slurry

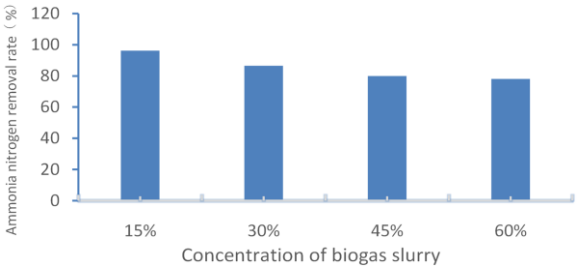

Fig. 3: The removal rate of ammonia nitrogen from different concentrations of algae by alga

However, the removal rate of total nitrogen is slightly lower than that of ammonia nitrogen because some organic compounds such as nitrogen containing organic matter cannot be used by the alga.

The Removal Rate of Total Phosphorus in Different Concentrations of Algae by Alga

As can be seen from Fig. 5, when the concentration of biogas slurry is low, the content of total phosphorus is also low. When the concentration of biogas slurry is 15%, the total phosphorus content is about 4.8 mg/L, and the removal rate is as high as 72.1%. That is, the utilization rate of phosphorus is the highest. The removal rate of total phosphorus is also relatively high in 30% of the biogas solution, which is 60.05%.When the concentration gradually increased, the removal rate of total phosphorus in biogas slurry decreased gradually, This is because the phosphorus needed to maintain the growth of algae is not too much, appropriate amount of phosphorus can promote the growth of scabbard, and excessive phosphorus can inhibit the growth of *Oedogonium.*

Composition of Alga from Different Concentrations of Biogas Slurry

The results are shown in Table 2 and 3, we found that the nutrient content of the alga from different concentrations of biogas slurry was not significant, but with the increase of the concentration of biogas slurry, the content of all components increased. It can also be seen that the content of protein and fat is abundant in the alga, the contents of Cd, Cr, Hg and Pb are in accordance with the Chinese feed hygiene standards (GB13078-2001) (Cd = 0.5, Cr = 10, Hg = 0.5, Pb = 5), according to this, we can conclude that the alga is a high protein energy feed resource.

Discussion

Microalgae are important producers in the ecosystem, with the maturation of microalgae culture technology, scale culture microalgae can be carried out in sewage, it can not only reduce the cost of microalgae, realize the recycling of waste, but also can purify the sewage and protect the water source. According to Yang (2015) reports, under laboratory conditions, the removal of total nitrogen and total phosphorus in the 50, 75 and 100% biogas liquid by *Chlorella vulgaris* is very good, after 8 days, the removal rates of total nitrogen in the three concentrations were 84.84, 90.11 and 91.50%, respectively, and the total phosphorus removal rates were 88.55, 91.56 and 93.27%, respectively. In a pipeline photo bioreactor, a purification experiment for 50 and 75% biogas slurry was carried out, it was found that the removal rates of nitrogen and phosphorus in 50% biogas slurry were 36.64 and 33.42%, in 75% of the biogas slurry, it was 46.88 and 57.81%, respectively. Experiments on the treatment of 50 and 75% biogas slurry in a runway aquaculture pool show that, the removal rate of total nitrogen from 50 and 75% biogas slurry was 69.74 and 95.51%, the total phosphorus removal rate was 66.38 and 85.32%, which reached the two grade emission standard of aquaculture pollutants. The pollution resistance of Spirulina was measured (Chen *et al.*, 2013), it was proved that Spirulina could not grow in high concentration of biogas liquid (more than 20% of the concentration of biogas slurry) studies.

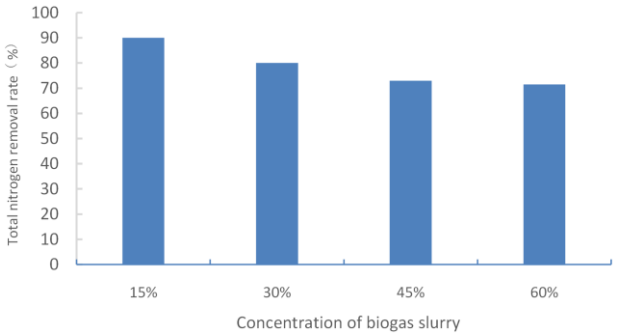

Fig. 4: The removal efficiency of total nitrogen in different dilutions of biogas slurry

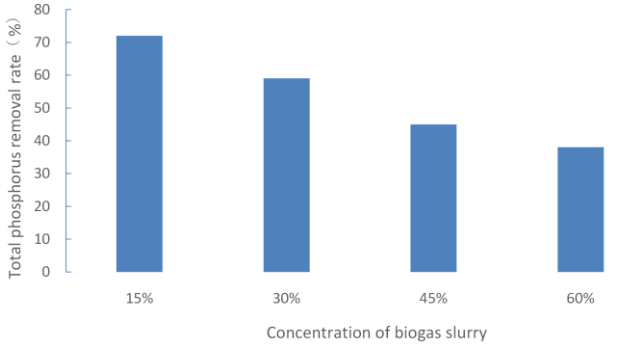

Fig. 5: Concentration variety of total phosphorus

It was found that the 10% concentration of biogas solution is the most suitable for the growth of Spirulina (Cheunbarn and Peerapornpisal, 2010). Used tubular light bioreactor was used to treat municipal wastewater (the total nitrogen content in the sewage is about 25–26 mg/L, and the total phosphorus content is about 2 mg/L), the removal rate of nitrogen and phosphorus is 89.68 and 86.71%, respectively (Arbib *et al.*, 2013). The municipal wastewater were treated (the total nitrogen and total phosphorus content in the wastewater were 55 mg/L and 3.0 mg/L), and the removal rates were 90.9–93.6% and 89.9–91.8%, respectively (Li *et al.*, 2013). The high nutrient content mixed sewage (total nitrogen and total content of 290 mg/L and 530 mg/L respectively) was to used culture sheath algae, the results showed that removal rate of total nitrogen and total phosphorus was 61% (Min *et al.*, 2011). We found that the removal rate of total nitrogen, ammonia nitrogen and total phosphorus in the 15% biogas slurry of alga was 90, 72.1 and 96.2%, respectively. Moreover, the removal rate of total nitrogen, ammonia nitrogen and total phosphorus gradually decreased with the increase of the concentration of biogas slurry. What is common in the results of this and other studies is that microalgae can effectively remove nitrogen and phosphorus in wastewater and other wastewater, the difference is that the removal rate is high or low, the difference is that the removal rate of nitrogen and phosphorus is different, some are high and some are low. The main reason is that microalgae are different, the concentration of nitrogen and phosphorus in the sewage is different and the culture conditions of microalgae are different.

Table 2: Nutrient content of oedogonium

Component	Sample 1	Sample 2	Sample 3	Sample 4	P value
HHV (MJ/kg)	15.5	15.6	15.9	15.9	<0.05
Ash (%)	19.0	19.5	19.3	19.6	
Protein (%)	16.1	16.5	16.8	16.9	
Total lipid (%)	25.3	26.3	26.7	26.9	

Note: sample 1, sample 2, sample 3, and sample 4 are derived from 15%, 30%, 45%, 60%, respectively

Table 3: The content of metal elements in the alga Unit: mg/kg

Element	Sample 1	Sample 2	Sample 3	Sample 4	P value
Al	1566	1714	1791	1909	<0.05
Ba	128	130	138	136	
Ca	3364	3396	3717	3863	
Cd	0.04276	0.04632	0.051	0.056	
Co	0.46	0.494	0.474	0.506	
Cr	1.52	1.58	1.29	1.33	
Cu	3.22	3.38	3.56	3.84	
Fe	374	380	405	417	
Hg	≦0.5	≦0.5	≦0.5	≦0.5	
K	23813	24187	24525	25875	
Mg	3699	3721	3952	3988	
Mn	144	118	128	136	
Mo	0.1588	0.16	0.174	0.188	
Na	1917	1943	2286	2314	
Ni	0.338	0.356	0.451	0.475	
P	4984	5036	5193	5287	
Pb	0.829	0.881	0.834	0.888	
S	919	1081	1324	1456	
Se	≦1	≦1	≦1	≦1	
Sr	93.2	99	99.6	107	
V	1.16	1.2	1.16	1.2	
Zn	18.7	18.6	19.6	20.1	

This study shows that the growth of alga in different dilution is different, and the best growth is in 15% dilution. In other concentrations of biogas slurry, due to the inhibitory effect of high ammonia nitrogen concentration, results in slower growth. The higher concentration of biogas slurry, the slower the growth rate of *Oedogonium* will be. Yang Yi studies show that cultivate nuclear protein in 50, 75 and 100% biogas slurry for 8 days, OD value were 2.252, 2.736 and 2.046, in the 100% concentration of biogas liquid, the period of stagnation was longer than that in the concentration of 50 and 75%, and the number of days to reach the same concentration was more longer (Yang, 2015). Chen *et al.* (2013) showed that Spirulina could not grow in high concentration of biogas slurry (more than 20%). Cheunbarn and Peerapornpisal (2010) studies showed that 10% of the biogas liquid was the most suitable for the growth of Spirulina.

Yuan *et al*. (2011) studies show that high ammonia nitrogen has a strong toxic effect on the growth of algae in the process of algal culture. Li Bo. studies have

shown that high concentrations of organic compounds can also inhibit the growth of Spirulina (Li, 2005). For microalgae growth; the nutrients in biogas slurry are generally not balanced, such as the lower C/N ratio and the imbalance of N/P ratio. The imbalance of nutrient elements will limit the growth of microalgae (Aslan and Kapdan, 2006; Xin *et al*., 2010). In conclusion, biogas slurry concentration, nutrient balance, pH and culture conditions have great influence on the growth of similar microalgae, which leads to different conclusions. For the same biogas slurry or sewage, different microalgae species have different growth effects.

Xu (2014) reported in the nuclear Chlorella powder, crude protein, fat, ash, chlorophyll a and lutein were 62.56, 5.2, 5.76, 0.05 and 0.03%, respectively but also contains a variety of minerals, such as Zn, Mg, Ca etc. It is worth mentioning that Pb, Cd, As, Hg content not exceeds the standard; scenedesmus alga has a cumulative effect on oil, and the content of oil is up to 22%. Chen (2014) found that the composition of the alga is different from the different medium, the total lipid content and essential amino acid index of Chlorella cultivation of 20% biogas slurry is 52.38 and 14.3% higher than that of BG11 medium. Wang *et al.* (2009) reports, per kilogram of Spirulina contains beta carotene 1700 mg, vitamin B1.6 mg, calcium pantothenate 11 mg, 0.5 mg of folic acid, inositol 350 mg, niacin 118 mg, vitamin B 55 mg, 190 mg of vitamin E, calcium containing 1050~4000 mm, phosphorus 7617~8 940 mg, potassium 400 13305~15 mg, and it also contains a variety of other bioactive substances; Chlorophyll content can be as high as 4%~6% in Alga. Our results showed that the crude protein content of scabbard cultured in different concentrations of biogas slurry was greater than 16%, the total fat was around 26%, and the harmful heavy metals was lower than the national standard. Zhang (2014) studied the effects of common organic carbon sources, inorganic nitrogen sources, organic nitrogen sources and environmental pollutants paraquat on the growth and biochemical characteristics of the *Oedogonium*, the results shows that two monosaccharides, sugar and starch will affect the fat and protein content of Oedogoniales, inorganic nitrogen can significantly reduce the content of protein and increase carbohydrate, paraquat reduced the protein content and increased the content of carbohydrates.

Conclusion

To sum up, the composition of various microalgae was different, which was closely related to the species of microalgae and the composition of sewage.

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