Fuel 117 (2014) 1-4

Contents lists available at ScienceDirect

Fuel

journal homepage: www.elsevier.com/locate/fuel

Short communication

Iron-enhanced anaerobic digestion of cyanobacterial biomass from Lake Chao



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ARTICLE INFO

Article history: Received 24 June 2013 Received in revised form 30 August 2013 Accepted 5 September 2013 Available online 17 September 2013

Keywords: Anaerobic digestion Cyanobacterial biomass Eutrophication Iron

1. Introduction

In China cyanobacterial blooms in the lakes of Chao, Tai, and Dianchi, are becoming more and more serious and regular over the last few decades [1]. Currently the refloatation and collection of the cyanobacterial biomass was considered to be the most efficient approach. However, decomposition of harvested cyanobacterial biomass would result in serious secondary environmental pollution [1]. Various physical, chemical and biological methods have been developed to make valuable products such as the fertilizer, animal feed, methane, ethanol and bio-oil [2–6]. Compared to the other processes, anaerobic digestion not only can convert the cyanobacterial biomass into methane, but also does not require advanced dewatering or further chemical extraction processes [5–9]. However, the reported methane yield was much lower than the theoretical value [9].

External addition of enzyme, surfactant, micro and macro elements, inorganic minerals, etc., was normally effective to enhance the methane yield [10–16]. As one of the macro elements for microbial growth, a suitable dosage of iron would balance substrate-specific deficiencies and improve the anaerobic digestion efficiency of fruit waste, sewage sludge, volatile fatty acids [14– 17]. Thus, in the current study the feasibility of FeCl₃ as the additive to improve the methane yield of cyanobacterial biomass was tested.

ABSTRACT

Effects of FeCl₃ on anaerobic digestion of cyanobacterial biomass from Lake Chao, one of the most serious eutrophic freshwater lakes in China, were investigated. Results showed that FeCl₃ was one feasible additive for the anaerobic process and methane generation rate and yield were both enhanced. The maximum methane yield reached 234 mL g⁻¹ based on the degraded volatile solid when the dosed FeCl₃ was 0.25 g L⁻¹, while the methane yield was only 131 mL g⁻¹ in the control reactor. Decomposition efficiency of cyanobacterial biomass and release of element *P* were promoted significantly (*P* < 0.05).

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2. Materials and methods

2.1. Substrate and Inoculums

Cyanobacterial biomass from Lake Chao was air-dried and deposited at -20 °C. Carbohydrate and protein contents were $32.7 \pm 0.5\%$ and $34.7 \pm 2.1\%$, while a relatively small amount of lipid $(3.4 \pm 0.3\%)$ based on the total solid (TS) was detected. Elemental analysis of the cyanobacteial biomass was performed and shown in Table 1. Digester sludge was collected from the anaerobic reactors at China Resources Snow Breweries and was passed through a sieve of 40 mm before inoculation.

2.2. Batch tests

Anaerobic digestion tests were performed in 250 mL serum bottles. The initial biomass concentration was fixed at 20 g volatile solid (VS) L^{-1} and the inoculums sludge was 2.5 g VS L^{-1} . After all nutrients were added, FeCl₃ was added to make a series of dosages of 0.05, 0.10 and 0.25 g L^{-1} , respectively, based on a working volume of 150 mL. The reactors were named as Fe(0.05), Fe(0.10) and Fe(0.25) in the following text. The initial pH was adjusted to 7.0 ± 0.1 and distilled water was added up to the working volume. The reactors were purged with N₂–CO₂ gas mixture with 40 volume% N₂ for 30 s and then sealed with aluminum/rubber plugs. The reactors were placed in an air bath shaker at 120 rpm and temperature was maintained at 35 ± 1 °C. Blank tests with digester sludge only were also performed in order to determine endogenous methane production. All batches were carried out in duplicate.



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 Table 1

 Element components of cyanobacterial biomass from Lake Chao.

Terms	Content (%TS)
С	27.5
Н	8.6
Ν	5.5
0	48.7
S	0.47

2.3. Analytical methods

Ammonia, Fe²⁺, Fe³⁺, phosphorus were measured according to the standard method [18]. The maximum methane production and generation rate were calculated according to the modified Gompertz equation [13]. Determination of CH₄, acetate, propionate, TS, VS and cyanobacterial compositions of carbohydrate, protein and lipid were measured according to the process previously reported [13,19].

3. Results and discussion

3.1. Gas production

When the dosage of FeCl₃ was increased from 0.05 to 0.25 g L⁻¹, the biogas production increased from 872 ± 115 to 1053 ± 63 mL while the control was only 696 ± 95 mL (Fig. 1a). The accumulated methane production of reactor Fe(0.05) was about 252 ± 11 mL and had no significant difference from that (230 ± 65 mL) of control (p > 0.05). When 0.1 and 0.25 g L⁻¹ FeCl₃ was added, the methane production was significantly increased to 310 ± 9 and 417 ± 4 mL, respectively (Fig. 1b). Despite a considerable increase in methane production, no significant change was observed in the total gas



Fig. 1. Gas production during the batch process.

production in the reactors Fe(0.05) and Fe(0.1). This could be attributed to reduced CO_2 release due to the formation of $FeCO_3$. Both the methane production and formation rate in the Fe-doseded reactors were enhanced significantly and the lag time were shortened compared to the control (Table 2). Iron is an essential trace element for anaerobic microorganisms in regard to its function as an energy carrier for methanogenic microorganism, and as the constituent of many enzymes [20–22].

3.2. Hydrolysis product

In the control reactor, soluble carbohydrate and protein, two main hydrolysis products of cyanobacterial biomass, were 0.34 ± 0.03 and 6.80 ± 0.53 g L⁻¹, respectively (Table 2). Carbohydrate and protein in the iron-dosed reactors varied in the range of 0.22–0.25 g L⁻¹, and 4.42–5.12 g L⁻¹, respectively. This meant that the anaerobic fermentation and acidification of carbohydrate and protein, especially protein was enhanced significantly (p < 0.05). Activity analysis of hydrolytic enzyme including cellulase and proteinase showed that the microbe metabolizing carbohydrate and protein was not enhanced (data was not shown). Long-chain fatty acids (LCFA), hydrolysis product of lipid, had been reported to inhibit the growth and metabolism of a wide variety of microorganisms, including fermentative acidogens, hydrogen producing acetogens and the acetate consuming methanogens [23-25]. Fe²⁺ could react with LCFA and generate insoluble product [26]. This would decrease the negative effect of LCFA on the fermentative acidogens and enhance the transformation of carbohydrate and protein in the iron-dosed reactors.

3.3. Release of ammonia and phosphate

In the anaerobic digestion process of cyanobacterial biomass, nutrients of ammonia and phosphate were released into the solution (Fig. 2). High ammonia would influence the activity of acetate utilizing methanogenic archaea, hydrogen-utilizing methanogens and syntrophic bacteria [5,9]. In the current research a relative lower ammonia concentration was detected in the digesters and slightly fluctuated around 52.0 mg L⁻¹ (Fig. 2). This suggested that the addition of Fe had no significant influence on the nitrogen release (p > 0.05). Ammonia concentration in the anaerobic digester was not high as expected, but was in accordance with other reports [5–6].

Phosphate concentrations in the reactors of control, Fe(0.05), Fe(0.1) and Fe(0.25) were 5.9 ± 0.1 , 8.3 ± 0.1 , 10.1 ± 0.9 and 6.5 ± 0.1 mg L⁻¹, respectively. Compared with the control, addition of iron had significantly influenced the *P* release (p < 0.05) when the initial Fe was below 0.1 g/L. A higher Fe dosage might react with the PO₄³⁻ and reduce its concentration which has been applied extensively for the *P* removal in the biological wastewater treatment process [27].

3.4. Availability of Fe

As shown in Fig. 3, the soluble Fe was only about 10% of the initial dosages and was mainly available as the Fe²⁺. This indicated that most of iron existed in the solid phases. In the iron dosed reactors, biological iron reduction process would generate Fe²⁺ which was metabolized by the microbes and reacted with released inorganic salts from cyanobacterial biomass, e.g. S²⁻, CO₃²⁻, PO₄³⁻, generating the product of FeCO₃ and Fe₃(PO₄)₂ which would reduce the soluble Fe²⁺ concentration (Fig. 3). However, these potential products couldnot be proved and identified using the traditional X-ray diffraction and scanning electron microscopy/energy dispersive spectroscopy (date was not shown).

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