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Enhancement of Taihu blue algae anaerobic digestion efficiency by natural storage

Hengfeng Miao, Minfeng Lu, Mingxing Zhao, Zhenxing Huang, Hongyan Ren, Qun Yan, Wenquan Ruan *

School of Environmental and Civil Engineering, Jiangnan University, Wuxi 214122, China

HIGHLIGHTS

- Blue algae storage led to cell death, microcystins release and VS reduction.
- Short-time storage as pretreatment was beneficial to blue algae methanation for AD.
- AD of blue algae stored for 15 d led to the highest CH_4 yield of 287.6 mL g^{-1} VS.
- VS removal, VFA and enzymes variation proved higher efficiencies of stored algae AD.
- AD presented significant biodegradation potential for microcystins from blue algae.

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ABSTRACT

Taihu blue algae after different storage time from 0 to 60 d were anaerobic fermented to evaluate their digestibility and process stability. Results showed that anaerobic digestion (AD) of blue algae under 15 d natural storage led to the highest CH_4 production of 287.6 mL g^{-1} VS at inoculum substrate ratio 2.0, demonstrating 36.69% improvement comparing with that from fresh algae. Storage of blue algae led to cell death, microcystins (MCs) release and VS reduction by spontaneous fermentation. However, it also played an important role in removing algal cell wall barrier, pre-hydrolysis and pre-acidification, leading to the improvement in CH_4 yield. Closer examination of volatile fatty acids (VFA) variation, VS removal rates and key enzymes change during AD proved short storage time ($\leqslant$15 d) of blue algae had higher efficiencies in biodegradation and methanation. Furthermore, AD presented significant biodegradation potential for MCs released from Taihu blue algae.

1.Introduction

Depleting supplies of fossil fuel as well as growing environmental concern of the public towards global warming has driven increasing focus on bioenergy, such as methane (CH_4), hydrogen (H_2) and biodiesel production from biomass (Khoo et al., 2013). Thanks to their high-potential growth rates and high content of carbohydrates, microalgae have recently received considerable attention as an alternative feedstock for bioenergy generation, especially for biomethanation (Alzate et al., 2012). Anaerobic digestion (AD), coupled with renewable-energy production in the form of biogas and waste treatment, has been responsible for degrading most of the carbonaceous material and regarded as a significant technology for the future (Hnain et al., 2011). Using microalgae such as *Microcystis* (Wei et al., 2013; Zhong et al., 2013), *Scenedesmus* (González-Fernández et al., 2013), *Phaeodactylum*, *Spirulina* (Lakaniemi et al., 2013), *Melosira* (Frigon et al., 2013) and *Oscillatoria* (Alzate et al., 2012) as substrates for AD envisioned a more energy productive, economical and energetical approach. Especially in Wuxi (southeast of China), Taihu blue algae (mainly consist of cyanobacteria) was salvaged from Taihu Lake every summer, as one of the most efficient measures to reduce Taihu Lake's eutrophication. Meanwhile, because of the lower operating cost than other methods such as incineration, composting and protein extraction, AD of Taihu blue algae investigations were carried out for biogas (Zeng et al., 2010; Yuan et al., 2011; Zhong et al., 2012), biohydrogen and polyhydroxyalkanoate (PHA) production (Yan et al., 2010).

However, bloom of blue algae in Taihu Lake is seasonal and occurs mainly from May to October each year. Biomass salvaged from the water body was depending upon the extent of algae

Abbreviations: AD, anaerobic digestion; AK, acetate kinase; AMPTS, Automatic Methane Potential Test System; Chl-a, chlorophyll a; ISR, inoculum to substrate ratio; MCs, microcystins; P, cumulative specific methane yield; PHA, polyhydroxyalkanoate; P_{max}, maximum CH_4 potential; R_{max}, CH_4 production rate; SEM, scanning electron microscopy; TAN, total ammonia nitrogen; TC, total carbon; TCD, thermal conductivity detector; TFA, trifluoroacetic acid; TKN, total Kjeldahl nitrogen; TN, total nitrogen; TP, total phosphate; TS, total solids; VFA, volatile fatty acids; VS, volatile solids; λ, lag phase.

* Corresponding author. Tel.: +86 510 85918265; fax: +86 510 85197091.
E-mail address: wqruanjn@gmail.com (W. Ruan).

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blooming in Taihu Lake. During the time when the climate was in favor of cyanobacteria growth, especially in the summer, an enormous quantity of algal biomass was salvaged from Taihu Lake (Yan et al., 2012). The final disposal of the biomass by its anaerobic co-digestion with pig manure for biogas production could not completely utilize all the salvaged biomass, and the rest of blue algae was collected and naturally stored for up to several months before AD. On the other hand, preliminary studies on AD of microalgae have shown low CH_4 productivities compared with municipal solid waste or fruit and vegetable wastes (Liu et al., 2012). The main reason has been attributed to the tough and protective cell walls of microalgae, which make them highly resistant to bacterial attack (Passos et al., 2013). Besides, recalcitrant compounds like polyaromatics, heteropolysaccharides, algaenan, sporopollenin, silica, uronic acid, lignin as well as toxins (microcystins, MCs) were also threatening to deterioration of AD performance (Sialve et al., 2009). Pretreatments such as enzyme hydrolysis, hydrothermal (Grala et al., 2012) and ultrasound (Alzate et al., 2012) treatment has been applied to disrupt the microalgae cells and remove inhibitions for improving CH_4 yield. So far, very little work has been done on the effect of natural storage as a means of pretreatment on living microalgae AD, especially for Taihu blue algae.

In this study, the effect of natural storage on Taihu blue algae with its physicochemical, morphological and components variation, further on CH_4 yield efficiency and process stability was investigated. The objectives of this study were to (1) evaluate the algal biomass change by natural storage; (2) analyze the AD efficiency and CH_4 yield of blue algae after natural storage; and (3) discuss the feasibility of natural storage as Taihu blue algae pretreatment before AD.

2. Methods

2.1. Substrate and inoculum

The substrate of algal biomass was freshly collected from Bogong Island, Taihu Lake (120° 23′ N, 31° 54′ E) as a mixture of algae bloom and lake water. The genera *Microcystis*, *Cyclotella*, *Cryptomonas* and *Scenedesmus* were dominant species in the mixture, contributing to 42.6%, 21.0%, 12.7% and 8.3% of the total biomass, respectively. Microalgae identification was carried out by microscopical examination (Olympus IX 70, Japan) according to the Phytoplankton Manual (Sournia, 1978). Before AD experiments, the collected Taihu blue algae biomass was mixed homogenously and then equally transferred into separate 1000 mL beakers with volume of 500 mL each. The samples were stored for 0, 7, 15, 30 and 60 d, respectively, in the glasshouse at room temperature. The water loss during the process was adjusted with distilled water to keep net weight. After storage of different time, the biomass in each beaker was mixed homogenously in shaking incubator and sampled for physicochemical parameters determination. Specifically, Table 1 shows the chemical parameters of each substrate under different storage time.

The inoculum of anaerobically digested swine manure was from Wuxi Nanyang Workstock Industry Co., Ltd. (Wuxi, China) operating at 35 °C, with a 30 d retention time. Before use, the inoculum was sieved with a 1 mm mesh to remove large suspended particles and grit.

2.2. Batch laboratory AD tests

The bench-scale AD tests for determining the anaerobic biodegradability and ultimate CH_4 yield of blue algae were carried out by using Automatic Methane Potential Test System (AMPTS) II (Bioprocess, Sweden) with software of AMPTS v5.0 (Badshah et al., 2012). The AMPTS II has three main units. A water-bath incubation unit which allows up to 15 glass bottles of 500 mL containing the substrate and/or inoculum which is incubated at 35 °C. The media in each bottle is mixed with a mechanical agitator setting to be 30 s on and 120 s off at 46 rpm during the entire experiment. The 80 mL vials containing a 3 M solution of sodium hydroxide (NaOH) absorbs non-methane gases such as carbon dioxide (CO_2) and hydrogen sulphide (H_2S). Besides, the biogas produced and CH_4 content was measured online periodically using the automated data-acquisition system.

Batch experiments were conducted in triplicate to determine the biogas production rates of algae for 22 d, while another reactor was carried out under the same condition to study the process stability and parameters variation. Five substrates of blue algae after different storage times were added to each bottle at concentration of 2 g VS L^{-1}, and the inoculum to substrate ratio (ISR) of 2.0 was used based on VS ratio. By contrast, biogas production rate of anaerobically digested swine manure was tested to decide the background CH_4 production by inoculum as control. The working volumes in the reactors were adjusted to 400 mL with distilled water and flushed with nitrogen gas to ensure anaerobic conditions. During AD, the biogas samples were daily collected while the liquid samples were measured at 2-day intervals from the control digester for process stability investigation. Scheme S1 shows the schematic diagram of the experimental procedure. CH_4 production potential (P_{max}), CH_4 production rate (R_{max}) and lag phase (λ) were modeled using the modified Gompertz equation (Kafle and Kim, 2013),

$$P = P_{\max} \times \exp\left\{-\exp\left[\frac{R_{max} \times e}{P_{\max}}(\lambda - t) + 1\right]\right\} \quad (1)$$

where, P is the cumulative specific methane yield (mL CH_4 g^{-1} VS) for a given time t; P_{max} is the maximum CH_4 potential (mL CH_4 g^{-1} VS) at the end of digestion time; R_{max} is the CH_4 production rate (mL CH_4 g^{-1} VS d^{-1}); λ is the lag phase (d); t is time (d) and e is exp (1), i.e. 2.71828.

2.3. Analytical methods

2.3.1. CH_4

CH_4 concentration in the biogas was analyzed using a gas chromatograph (GC 910, Kechuang, China) equipped with a thermal conductivity detector (TCD) and argon as the carrier gas. The injector, oven and detector temperatures were 100, 90 and 100 °C, respectively. Flow rate of carrier gas was 15 mL min^{-1}, and the injection volume of sample was 100 μL (Yan et al., 2008).

2.3.2. Physicochemical parameters

The total solids (TS), volatile solids (VS), total carbon (TC), total nitrogen (TN), total phosphate (TP) and total ammonia nitrogen (TAN) were measured by using the APHA standard methods (Eaton et al., 2005). The chlorophyll a (Chl-a) concentration of blue algae was assayed according to the Chinese national standard methods (GB 17378.7/8.1-1998) by ultraviolet–visible spectrophotometer (Model U-3000, Hitachi, Japan). The protein content was determined based on the total Kjeldahl nitrogen (TKN) measurement using the correction factor 6.25 (Msuya and Neori, 2008). The total lipid content was analyzed gravimetrically from the extract obtained with diethyl ether in a Soxtec System HT (HT2 1045, Tecator, Sweden) (Barje et al., 2008). Carbohydrate was estimated as the remaining fraction of VS after the determination of protein and lipid.

2.3.3. MCs

The extracellular toxins (MC-LR and MC-RR) in the substrates (Taihu blue algae) were extracted with 50% methanol three times,