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Coupling of the hydrogen and polyhydroxyalkanoates (PHA) production through anaerobic digestion from Taihu blue algae

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ABSTRACT

Coupling bio-production of hydrogen and polyhydroxyalkanoates (PHA) from Taihu blue algae through metabolites circulation was investigated. It was found that the pH adjustment, especially basification was more practical and efficient than other methods for the pretreatment of blue algae before anaerobic digestion. On this occasion, SCOD, biogas accumulation and hydrogen content reached 26 mg/gTS, 500 mL and 37.2%, and which were 4.3, 1.3 and 14.4 times of those of the control group, respectively. Secondly, amounts of both butyric acid and hydrogen could be further increased when blue algae was alkali pretreated at pH 13, as the accumulation of butyric acid, acetic acid and hydrogen reached 1.7, 1.4 and 3.8 times compared to those of the control, respectively. Finally, the coupling bio-production of hydrogen and PHA was conducted through pumping organic residues into PHA fermenter from anaerobic digester. Remarkably, it was found that the larger the pumping rate of carbon and nitrogen sources supply, the higher the yield of DCW and PHA could be expected by *Bacillus cereus*.

1. Introduction

Cyanobacteria blooms are mainly associated with eutrophicating and poorly flushing of water bodies. As a typical large, shallow inland lake located in Yangtze River Delta, China, Taihu Lake also suffered from the frequent outbreak of the Blue Green Algae, a kind of cyanobacteria due to the conflict between the fast economic development and regional environmental pollution (Qin et al., 2007). Additionally, algal blooms in Taihu Lake led to the water supply crisis of the lakeside Wuxi city during the summer of 2007 (Xinhua, 2007).

In order to reduce the eutrophication of Taihu Lake, refloatation of blue algae after blooming was considered as the most efficient approach to retrieve nitrogen and phosphorus from the lake. However, without further management, large amounts of refloated blue algae would result in serious secondary environmental pollution. Thus, many resource recovery methods, such as incineration, composting, making bio-energy (hydrogen/methane) and even high-valued products via anaerobic digestion were all put forward to reclaim the Taihu blue algae. However, the bio-production of hydrogen and methane seems to be more practical, as there might be several hundred thousand tons of blue algae refloated annually from Taihu Lake (CCTV, 2008).

Generally, it is believed that the methane production would be enhanced if the hydrogen accumulation was conducted first, as which was the main gas byproducts during the acidification stage of the anaerobic digestion process. In addition, hydrogen accumulation could be intensified with butyric and acetic acid being produced as the end-products during this stage (Hawkes et al., 2007). At the same time, if not being disposed of timely, organic acids and ammonium nitrogen accumulated during the biohydrogen process would in turn inhibit the hydrogen production (Hallenbeck, 2009). Therefore, it might be instructive to produce methane and other value added products such as polyhydroxyoalkanoates (PHA), etc. with these organic residues accumulated from biohydrogen process (Valdez-Vazquez and Poggi-Varaldo, 2009). Meanwhile, as a family of thermo polyesters produced by many microorganisms grown on renewable resources such as sugars or fatty acids, PHA is biodegradable and biocompatible, and now is widely applied in polymers, biofuels, materials, pharmaceuticals and fermentation industries (Chen, 2008). Moreover, butyric acid was found to be a more favorable acid substrate for PHA formation with comparison to any other organic acid, such as acetate, propionate, lactate (Yan et al., 2003). Thus, it may be more attractive to conduct the coupling bio-production of hydrogen and PHA through the metabolites circulation. Namely, hydrogen and butyric acid were generated

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from Taihu blue algae via anaerobic digestion. Afterwards, butyric acid, ammonia nitrogen and other organic residues were pumped into another fermenter as carbon and nitrogen sources to produce PHA by *Balillus cereus* (Yan et al., 2007). So far, no publications could be tracked about this field.

To demonstrate the potential of the coupling production of both bio-energy and bio-materials from refloated Taihu blue algae, the effectiveness of pretreatment on blue algae to obtain as more organic contents, the further enhanced biohydrogen production from blue algae with basification pretreatment, and the coupling bioproduction of PHA with metabolites circulation from biohydrogen process were investigated in this study.

2. Methods

2.1. Materials, microorganism and fermentation cultures

2.1.1. Taihu blue algae

Blue algae used in this study were freshly collected from the blue algae refloating sites alongside Taihu Lake and cryopreserved for future use, with the moisture content as 95–98%, carbon, nitrogen, phosphate and ash content at about 440, 68, 6.7 and 82 mg/g, respectively.

Anaerobic granular sludge used in this study was from DSM Citric Acid (Wuxi) Ltd., China. Prior to inoculating for biohydrogen production, the anaerobic granular sludge was autoclaved at 121 °C for 15 min to eliminate non-spore-harboring methanogens (Kapdan and Kargi, 2006).

2.1.2. Microorganism

Bacillus cereus used in this study was isolated from activated sludge of Taipo Wastewater Sewage, Hong Kong, and identified as GC subgroup B by Chinese University of Hong Kong (Yan et al., 2007).

2.1.3. Cultures for PHA fermentation

The seed culture medium for *B. cereus* contained (g/L): glucose 20, $(NH_4)_2SO_4$ 3, $MgSO_4$ 1.2, citric acid 1.7, KH_2PO_4 13.3, 10 ml mineral solution (per L HCl (1 mol/L): $FeSO_4{\cdot}7H_2O$ 10, $ZnSO_4{\cdot}7H_2O$ 2.25, $CuSO_4{\cdot}5H_2O$ 2.25, $MnSO_4{\cdot}5H_2O$ 0.5, $CaCl_2{\cdot}2H_2O$ 2, H_3BO_4 0.3, $(NH_4)_6Mo_7O_{24}$ 0.1) and the pH was adjusted to 7.0. Fermentation culture was nearly the same as seed culture except that the ammonia sulfate was 1.5 g/L, and no glucose was added.

2.2. Experimental apparatus and operating procedures

2.2.1. Experimental apparatus

A 5 L anaerobic digester equipped with pH detector, nitrogen cylinder and hydrogen collector was connected to a 3 L fermenter (KBT, South Korea) via a peristaltic pump (BT100-2J, Longer, China) and silicone tube. Within the PHA fermenter, the tube was replaced by dialysis membrane (MD 25-7, MEMBRA-CEL™, USA) to transfer carbon and nitrogen metabolites (MWCO $\geqslant$ 7000).

2.2.2. Biohydrogen biosynthesis from Taihu blue Algae through anaerobic digestion

Biohydrogen production was carried out with an initial volume of 3 L within the anaerobic digester. The mass ratio of Taihu blue algae and anaerobic granular sludge was 6:1, pH was adjusted to about 7.5, and the reaction temperature was maintained at 35 °C with a water bath. During the anaerobic digestion process, the reactor was first flushed with nitrogen to maintain the anaerobic environment, and the hydrogen collecting bottle was filled with 2 mol/L of NaOH to absorb CO_2 and measure biogas.

2.2.3. Simultaneous production of PHA with metabolites circulation

Seed was cultivated in the 250 mL flask containing 75 mL of seed culture at 30 °C and 200 rpm for 24 h. Fermentation was conducted for about 48–60 h with an initial volume of 2 L and the inoculation volume of 10%. The fermentation temperature was maintained at 30 °C. pH was controlled at 7.0 ± 0.1 using 3 mol/L of NaOH and HCl, respectively. The aeration rate was 1.5 L/min and agitation speed was 400 rpm (Yan et al., 2005).

2.3. Pretreatment methods

Control: Blue algae used for anaerobic digestion were not pretreated, with the original pH value as about 7.5.

Acidification: Blue algae were diluted to about 1% of TS (total solids), then adjusted to pH 2, 3, 4, 5 and 6 with 6 mol/L of HCl, respectively. Afterwards, the well blended blue algae were kept for 12 h at room temperature to maintain the pretreatment effect.

Basification: Blue algae were diluted to about 1% of TS (total solids), then adjusted to pH 9, 10, 11, 12 and 13 with 6 mol/L of NaOH, respectively. Afterwards, the well blended blue algae were kept for 12 h at room temperature to maintain the pretreatment effect.

Thermal pretreatment: Blue algae were autoclaved at 121 °C for 30 min.

Microwave radiation: Blue algae were microwaved at 70 W for 5 min.

2.4. Analytical procedures

2.4.1. COD, SCOD, VSS and ammonium nitrogen

According to the State Environmental Protection Administration of China (SEPA) standard, COD and soluble COD (SCOD) were determined with the method of potassium dichromate oxidation; TS and VSS was determined with the gravimetric method; and $(NH_4)_2SO_4$ was detected using the Nessler's reagent colorimetric method (SEPA, 2002).

2.4.2. Hydrogen

The hydrogen of biogas composition was detected using gas chromatograph (GC910, Kechuang, China) equipped with a thermal conductivity detector and a stainless packed column (with Porapak N 60-80 as carrier). The operating temperatures of column and detector were kept at 90 and 100 °C, respectively. Argon was used as the carrier gas at a flow rate of 15 mL/min, and the injection volume of sample was 100 μL.

2.4.3. Organic acids

Organic acids were detected using HPLC (Agilent 1100, USA) equipped with a UV detector at the wavelength of 210 nm, and with an ZORBAX SB-A column (300 × 7.8 mm, Biorad, USA) at the column temperature of 60 °C acetonitrile (0.5%) and 0.02 mol/L of KH_2SO_4 (99.5%) were used as the mobile phase with a flow rate of 0.5 mL/min. pH of the samples was adjusted to 2–3 with H_3PO_4 and the injection volume was 10 μL. The eluted time for lactate, acetate, propionate and butyrate was at about 10.7, 12.7, 14.9 and 18.1 min, respectively.

2.4.4. DCW and PHA content

DCW (dry cell weight) of *B. cereus* was determined after lyophilization, and the PHA content was determined after chloroform extraction. After centrifugation of at 6500 rpm for 10 min, precipitates were washed twice with tap water, and then centrifuged again at 6500 rpm for 10 min, and then the cell pellets were lyophilized (FTS systems, USA) for 24 h. The dry cell weight (g/L) was expressed as the biomass weight divided by the volume of sampling volumes within the centrifugal tube. As for PHA estimation, about 0.2 g DCW was extracted in 10 mL chloroform in a tightly sealed