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Enhancing anaerobic digestion of lignocellulosic materials in excess sludge by bioaugmentation and pre-treatment

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ABSTRACT

This study attempted to enhance anaerobic conversion of lignocellulosic materials in excess sludge by bioaugmentation and pretreatment. The results reveal that highly active lignocellulolytic microorganisms (*Clostridium stercorarium* and *Bacteroides cellulosolvens*) could be enriched from anaerobic sludge in ordinarily operated anaerobic digester (AD). Inoculating these microorganisms into AD could substantially enhance the degradation of cellulose and hemicellulose. However, this effect of bioaugmentation was shielded for raw excess sludge due to lignin incrustation in native biosolids. For this problem, pretreatments including acid, alkali, thermal and ultrasonic methods were effectively used to deconstruct the lignin incrustation, in which thermal pretreatment was demonstrated to be the most effective one. Then, pretreatment associated with bioaugmentation was successfully used to enhance the energy conversion of lignocellulosic materials, which resulted in the degradation of cellulose, hemicellulose and lignin to 68.8–78.2%, 77.4–89% and 15.4–33.7% respectively and thus increased the CH_4 production by 210–246%, compared with ordinary AD.

1. Introduction

Originating from residues of exogenous materials (toilet tissue, vegetable remnants, leaves, etc.) in wastewater streams (Parnaudeau and Dignac, 2007), lignocellulosic materials comprise a large fraction (14–44%) of raw excess sludge (Hao et al., 2013b). Lignocellulosic materials consist of mainly three different types of polymers, namely cellulose, hemicellulose and lignin (Fengel and Wegener, 1984). Cellulose exists of D-glucose subunits, linked by b-1,4 glycosidic bonds. Hemicellulose consists mainly of xylans (in hardwood) or glucomannans (in softwood), with side chains of acetyl, gluconuryl, or arabinofuranosyl units. Lignin has a complex amorphous and recalcitrant structure consisting of three different phenylpropane units (p-coumaryl, coniferyl and sinapyl alcohol) that are held together by different kind of linkages (Gallert and Winter, 1999; Hendriks and Zeeman, 2009). Both cellulose and hemicellulose are anaerobically biodegradable when they exist independently, with hemicellulose more easily hydrolysable. However, they very often are lignin-encrusted and form a kind of quite stable polymers, which are quite resistant to anaerobic degradation (Adney et al., 1991; Gallert and Winter, 1999; Hendriks and Zeeman, 2009; Taherzadeh and Karimi, 2008) and likely left over in digested sludge. Literatures have reported that lignocellulose could account for 8–24% of dry mass in digested sludge (Hao et al., 2013b). Clearly, the poor digestibility of the lignocellulosic materials hinders not only the reduction of excess sludge, but also the energy conversion of biosolids.

Like all other biopolymers, anaerobic digestion (AD) of lignocellulose takes place through four subsequent and synergistic actions: hydrolytic, fermentative, acidogenic, and methanogenic processes. Among them, hydrolysis has been well identified as a rate-limiting step (Adney et al., 1991). Hydrolysis is performed by extracellular hydrolytic enzymes excreted by lignocellulolytic microorganisms. Therefore, the hydrolysis rate depends on the enzyme loading (Hui et al., 2013). The generally slow hydrolysis rate indicates that the enzyme level is low in AD under normal operational conditions. For this reason, it is a prerequisite to increase the enzyme level for improving the hydrolysis. Several studies have demonstrated already that adding hydrolytic enzymes to anaerobic digester could indeed increase the hydrolysis rate (Adney et al., 1991; Romano et al., 2009; Yang et al., 2010; Ziemiński et al., 2012). Romano et al. (2009) investigated the effects dosing enzyme products containing cellulase, hemicellulase, and β-glucosidase to anaerobic digestion of wheat grass, and they observed that all the enzyme products showed positive effects on the solubilization of the wheat grass. Yang et al. (2010) achieved a 40–50% increase in

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VSS reduction by applying commercial enzyme preparation containing alpha amylase and neutral protease to enhance the hydrolysis of excess sludge. Similarly, Ziemiński et al. (2012) demonstrated enhanced biogas production (by 13–19%) in anaerobic digestion of lignocellulosic wastes after enzymatic pretreatment. However, enzymatic hydrolysis by purified enzyme products could contribute to a substantial part to the cost, making lignocellulosic biomass utilization not a commercially viable process (Hui et al., 2013). Alternatively, bioaugmentation of lignocellulolytic microorganisms in AD could provide a cost-effective method to substantially increase the enzyme loading and eventually promote the degradation of lignocellulose.

As mentioned above, although cellulose and hemicellulose are hydrolysable, they are often covered by lignin in native biosolids and thus prevented from enzymatic attack. This means hydrolysis is likely still ineffective even if the enzyme loading is augmented. Therefore, pretreatment for deconstructing lignin incrustation prior to enzymatic hydrolysis becomes essential. Various pretreatment methods have been introduced for this purpose, which can be classified into physical pretreatment (milling, irradiation, ultrasonic, etc.), physico-chemical pretreatment (thermal, explosion, etc.), chemical pretreatment (acid, alkali, oxidizing agents, etc.) and biological pretreatment (fungi and actinomycetes) (Taherzadeh and Karimi, 2008). These methods could improve the accessibility of lignocellulosic materials for hydrolytic enzymes through various mechanisms, such as breaking the covalent associations between the lignocellulosic components, de-polymerizing lignin, decreasing cellulose crystallinity and dissolving hemicellulose (Hendriks and Zeeman, 2009; Jiao et al., 2012; Taherzadeh and Karimi, 2008).

In the light of these considerations, this study firstly attempted to enhance anaerobic digestion of cellulose and/or hemicellulose under bioaugmentation of lignocellulolytic microorganisms, which were enriched from anaerobic sludge in ordinarily operated anaerobic digester. Then, pretreatments including acid, alkali, thermal and ultrasonic methods were tested to deconstruct lignocellulosic materials for facilitating the bioaugmentation. Finally, bioaugmentation associated with pretreatment was applied to achieve a substantial improvement in anaerobic degradation of lignocellulose and the subsequent energy conversion.

2. Material and methods

2.1. Experiments

2.1.1. Enriching lignocellulolytic microorganisms

Enriching lignocellulolytic microorganisms was conducted in three parallel anaerobic reactors (250-mL serum bottles), using xylan as the sole substrate and anaerobic sludge from a laboratory-scale anaerobic digester as the bacterial source. Xylan was selected as the substrate because it is simpler in structure than cellulose, so that the enrichment of the lignocellulolytic microorganisms could be achieved easily. The 5-L digester was operated at $T = 35$ °C, SRT = 20 d and pH = 7.0, receiving lignocellulose-free excess sludge from a lab-scale SBR system fed with synthetic wastewater that contained no lignocellulosic compositions (Hao et al., 2013a).

The reactors were operated in a batch mode with a cycle time of 6 days and 10 continuous cycles for culturing. Initially, 100 mL of the cultured anaerobic sludge (MLSS = 22.38 g/L and MLVSS = 17.69 g/L) was inoculated into the testing bottles. At the beginning of each cycle, 0.25 g of xylan was added into each bottle together with the culture mediums (DIN EN ISO 11734 L47 and DIN EN ISO 11734) for supplementing nutrient and minerals (Weiß et al., 2010). An additional serum bottle with the same inoculum and culture mediums but without xylan was set as a control. Complete anaerobic condition was achieved by dosing $Na_2S{\cdot}9H_2O$ (500 mg/L) and monitored with Resazurin (1 mg/L). The pH level was identically regulated at 6.8 with a buffer solution (Hepes: 50 mmol/L; (Hao et al., 2012)). The bottles were then flushed with N_2 gas for 5 min to expel any residual O_2 and sealed with rubber stoppers. Finally, the bottles were put into an air thermostatic shaker ($T = 35$ °C, 100 rpm) for cultivating. At the end of each cycle, biogas volume was measured before replacing 200 mL of supernate with a new batch of substrate and culture mediums. The enrichment function of the lignocellulolytic microorganisms was evaluated by the biogas production and the xylanase activity in the supernate. The enriched bacteria were further identified by microscopic observation and molecular techniques.

2.1.2. Pretreatment

Pretreatment with acid, alkali, thermal and ultrasonic methods was applied respectively when raw excess sludge was used as the substrate. The pretreatment of acid and alkali was performed in 1-L beakers. Raw excess sludge was suspended and exposed respectively to the acidic (pH = 2, 3, 4) and alkaline (pH = 11, 12, 13) conditions for 2 h by respectively dripping HCl and NaOH solutions (4 mol/L). After the pretreatment, the pH value was adjusted back to the neutral (7.0). Thermal pretreatment was conducted in a pressurized sterilization pot. Raw excess sludge was respectively steamed at 100, 120 and 150 °C for 2 h. An ultrasonic cleaner was used for the ultrasonic pretreatment. Raw excess sludge was put into 50-mL centrifugal tubes, and then vibrated in the ultrasonic cleaner at $P = 500$ W for 0.5 and 2 h respectively.

2.1.3. Anaerobic digestion (AD) of lignocellulosic materials

The effectiveness of bioaugmentation and pretreatment in enhancing lignocellulose degradation was investigated in a series of anaerobic batch tests (Table 1). Firstly, synthetic excess sludge was used as the substrate to investigate the effectiveness of bioaugmentation in enhancing degradation of cellulose and hemicellulose when they existed separately (without lignin incrustation, Test 1 in Table 1). The synthetic excess sludge was prepared by blending lignocellulose-free excess sludge (as described in Section 2.1.1) with xylan (representing hemicellulose) and carboxymethylcellulose sodium (CMC, representing hemicellulose).

Following that, raw excess sludge from a wastewater treatment plant in Beijing was used as the substrate in Test 2 (Table 1) to investigate the effectiveness of bioaugmentation in enhancing degradation of lignocellulosic materials with lignin incrustation. In the above two tests, the enriched lignocellulolytic microorganisms were dosed into the anaerobic reactors (bottles) in different existing forms (suspended and immobilized) and at different dosages (Table 1) to determine the optimal operational conditions. The degradation of lignocellulose was evaluated by both biogas production and xylanase activity.

In the tests with the raw excess sludge after different pretreatments (Tests Nos. 3–6, Table 1), the enriched lignocellulolytic microorganisms were dosed in the immobilized form at the dosage of 60% (predetermined in Test 1 and Test 2). Besides the biogas production and xylanase activity, content changes of the lignocellulosic materials were also measured to assess the synergic effects of pretreatment and bioaugmentation on the degradation of lignocellulose.

2.2. Apparatus and materials

All the AD tests were conducted in 500-mL serum bottles with the same working volume of 250 mL. Initially, anaerobic sludge and the enriched lignocellulolytic microorganisms were inoculated into each bottle according to Table 1. A control test without adding