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A novel closed-circuit circulation system about integrated ethanol-methane fermentation process based on the subcritical water pretreatment of corn stover

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ABSTRACT

For the purpose to fully reutilize wastewater originated in second-generation bioethanol production from corn stover, a novel closed-circuit circulation system of integrated ethanol-methane fermentation process was successfully established based on the subcritical water pretreatment, in which the wastewater was fully recycled for 20 batches. It was indicated that the pH of recycling liquid decreased with the increasing of cycle numbers, which led to the constant enhancement of the pretreatment efficiency as approximately 60% hemicellulose and 50% lignin degradation as well as more than 80% cellulose reserved. Meanwhile, the performance of both ethanol (2.76–6.94 g/L) and methane (382.56 −2631.24 mL/L) fermentation reached stability and trended to be self-improved during the 20 batches, which indicated that the designed integrated process was stable and applicable. Furthermore, the variety of microbial community and other processes showed intimate correlation with the physiochemical properties of the recycling liquid. It was promising to apply the system in bioenergy industries to fully reutilize of the wastewater in the future.

1. Introduction

Corn stover is one of the most abundant agriculture residues as well as the important feedstock for the production of second-generation bioethanol derived from lignocellulosic wastes, which has gained increasing interesting in recent years (Chen and Fu, 2016; Gladis et al., 2015). It is reported that corn stover contained more than 30% cellulose and 20% hemicellulose coupled with approximately 15% lignin (all in dry matter) (Chen et al., 2011). Although the hemicellulose and the amorphous cellulose are easily hydrolyzed to fermentable sugars, the lignin and crystalline cellulose are still significantly resistant to the hydrolyzation, which need to be eliminated or structurally modified by pretreatment (Cesaro and Belgiorno, 2015). The pretreatment methods fall into three main categories: a. Chemical methods: the raw materials were pretreated with different chemical reagents, such as dilute acids, organic solvents, metal chlorides, plasma and alkaline solutions (Divya et al., 2015); b. Physical methods: the steam explosion, wet oxidation, microwave, thermal explosion and hot water (Jiang et al., 2015; Srinivasan and Ju, 2012; Yu et al., 2015) were also applied to corn stover for pretreatment; c. Biological methods: fungi, bacteria, microbial consortium and enzyme had been studied likewise for the delignification of corn stover (Oliva-Taravilla et al., 2015; Schilling et al., 2009; Zhang et al., 2011). Although some of the conventional chemical or physio-chemical methods were effective for the pretreatment of corn stover, some problems were emerged at the same time, such as high process cost, complicated technical requirements for the downstream process (e.g. enzymolysis and fermentation) and environment problems, especially the wastewater disposal. Therefore, environment-friendly pretreatment methods and reutilization of wastewater are urgently required (Capolupo and Faraco, 2016; Xu and Huang, 2014).

Subcritical water, also called hot-compressed water or superheated water, is water heated under pressure from its atmospheric boiling temperature (100 °C) to its supercritical point (374 °C) (Ravber et al., 2015). Under these conditions, the physiochemical properties of water were greatly changed, for instance, the increase of thermal motion and diffusion rate as well as the decrease of permittivity (polarity) and self-ionization (Smith, 2002), which is

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suitable for the application to extraction or pretreatment (Di Girolamo et al., 2013). Moreover, without any chemical reagents used in the whole process, subcritical water pretreatment method makes it possible for the reutilization of wastewater generated in the conversion of lignocellulose materials (such as corn stover and wheat straw) to bioethanol.

The second-generation bioethanol production is a water-intensive and energy consuming process, a large amount of wastewater from pretreatment and distillation needed to be disposed of (Cheng et al., 2010). The wastewater has high chemical oxygen demand (COD) and biological oxygen demand (BOD) (Cheng et al., 2010), thus anaerobic digestion is generally used for the wastewater treatment to meet the environment criteria (Divya et al., 2015). However, the wastewater is a valuable resource which was laid in a wrong position in our opinion. Although researches have been done to recycle stillage in both ethanol and methane fermentation (Bahmani et al., 2016; Wang et al., 2014; Yang et al., 2016), the recycling of wastewater in the whole bioethanol process based on the lignocellulose (corn stover) has not been found. To establish zero wastewater bioethanol conversion process, a closed-circuit circulation system for fully reutilizing of wastewater was designed and carried out.

In this study, a novel integrated ethanol-methane fermentation system was established based on the subcritical water pretreatment of corn stover. We conducted 20 batches recycling process with zero waste discharge to investigate the stability and availability of the process on a laboratory scale. During this process, pretreatment, ethanol and methane fermentation was systematically monitored and analyzed. In addition, high-throughput sequencing was conducted to study the microbial community variation of methane sludge.

2. Methods and materials

2.1. Materials and reagents

The corn stover used in this study was collected from local farms (Yangling, Shannxi, China) and chopped to 4–5 cm size, and then washed and dried at room temperature. Where after the raw materials were ground to pass through a 40-mesh sieve and dried in an oven at 105 °C, and then which was stored at desiccator for further study.

Kluyveromyces marxianus (*K. marxianus*) was obtained from Northwest A&F University (Yangling, China) and cellulases (complex cellulase-NS50013 and cellobiase-NS-50010) were kindly provided by Novozymes Biotechnology Co., Ltd, Denmark, which were used for ethanol fermentation. The previous investigation showed that the enzyme activity of NS50013 and NS50010 were 205 FPU/mL and 307 CBU/mL, respectively. Anaerobic granular sludge was provided by Henan Zhongzheng Environmental Engineering Co., LTD, China. All the chemicals and solvents were analytical grade unless otherwise specified.

2.2. Pretreatment process

A high temperature-high pressure reactor with 500 mL working volume was used for pretreatment of corn stover by subcritical water, in which a pressure gage and a thermocouple were used to assay the pressure and temperature inside the reactor during the pretreatment process. Corn stover powder (10 g) and distilled water (400 mL) were added into the reactor, and then pretreated at 190 °C for 0 min (starting the cooling system as soon the temperature reached to 190 °C) which was optimized in our previous study (Yuze and Xin, 2013). In addition, the pressure was self-generated during heating process to keep the water in liquid state. After subcritical water pretreatment, the water-soluble portion was collected by filtration with a qualitative filter paper (30–50 μm pore size) for methane fermentation and the obtained residue was dried at 105 °C for 24 h. The yield of residue was calculated according to the following equation:

$$Yield\ of\ Residue\,(\%) = Residue\,(g) / Raw\ materials\,(g) \times 100\,\%$$

where “Raw material” represented the corn stover used in the pretreatment and “Residue” represented the water-insoluble portion obtained after pretreatment.

2.3. Ethanol fermentation

2.3.1. Seed medium

K. marxianus was inoculated into a 250 mL Erlenmeyer flask containing 100 mL medium consisted of glucose 2 g, peptone 2 g and yeast extract 1 g, which was incubated in a shaker (200 rpm) at 30 °C for 20 h as the seed broth.

2.3.2. Simultaneous saccharification and fermentation (SSF) process

Fermentation medium was composed of (g/L): $(NH4)_2HPO_4$ 0.5, $MgSO_4$ 0.025, $CaCO_3$ 0.1 and yeast extract 1, dissolved in 0.05 mol/L citrate buffer (pH 5.0). The pH was adjusted to 5.0 using 0.1 mol/L citric acid.

SSF process: Firstly, 1.5 g pretreated substrate mixed intensively with 0.6 mL NS50013 (15 FPU/g) and 0.4 mL NS50010 (15 CBU/g) in a 50 mL Erlenmeyer flask which was kept in a shaker (50 rpm) at 50 °C for 24 h for the initial enzymatic hydrolysis; Secondly, the temperature was cooled down to 35 °C and 1.2 mL seed broth was added into the mixture to start the semi-anaerobic fermentation without shaking for 72 h. The total volume of SSF was 16.2 mL and the concentration of substrate was 9.26% (w/v). Every 24 h, 0.1 mL solution was sampled and diluted to 1 mL for the assay of glucose, ethanol and yeast growth.

2.4. Methane fermentation

The water-soluble portion (400 mL) obtained from subcritical water pretreatment was mixed with seed sludge (150 g) and sealed in an anaerobic sludge reactor (as shown in Supplementary Fig. 1) which was then kept in a thermostat at 35 °C for the methane fermentation. Thereafter, drainage method was applied to detect the production and productive rate of biogas until the fermentation was finished (no more water drained out). And the total reducing sugar and pH of the fermentation liquor were determined before and after every fermentation batch.

2.5. Analysis of microbial communities of sludge during the recycling process

The sludges of the 1st, 14th, 17th, 19th and 20th batch were sampled for the analysis of microbial communities. All the investigation and data process were carried out at the Novogene Bioinformatics Institute according to the protocol established by the manufacturer. Briefly, total genome DNA from samples were extracted using CTAB/SDS (Hexadecyl Trimethyl Ammonium Bromide/Sodium Dodecyl Sulfate) method, and then the V4 region of the 16S rRNA gene was amplified with the primer set 515F (5′-GTGCCAGCMGCCGCGGTAA-3′)/806R (5′-GGACTACHVGGGTWTCTAAT-3′) and all PCR reactions were carried out with Phusion® High-Fidelity PCR Master Mix (New England Biolabs). After purification with Qiagen Gel Extraction Kit (Qiagen, Germany), sequencing libraries were generated using TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) following the