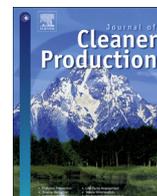




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Establishment and assessment of a novel bioethanol and efficient biogas coupling fermentation system integrated with the pretreatment of a cellulolytic microbial consortium

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ABSTRACT

A novel bioethanol and biogas coupling fermentation system integrated with the pretreatment of a cellulolytic microbial consortium was proposed to treat cassava distillage. In the present study, 8 batches of ethanol fermentation integrated with 7 batches of biogas fermentation were successively proceeded. The recycled water after two-stage of anaerobic digestion was reused for raw material mixing in the later 7 batches of ethanol fermentation, whereas only tap water was used in the first batch. It was found the ethanol yield, starch utilization ratio, fermentation time can be kept steady at about 12.6%(v/v), 90% and 48 h respectively during the recycling process, which were very close to those of the conventional process using tap water. The organic substrates, volatile fatty acids, total and ammonia nitrogen reached steady states after 2–5 batches of recycling, while the total ion and alkalinity revealed a little decrease tendency with the increment of the recycling batches. Further, the total methane yield and methane producing velocity of each recycling batch in the proposed coupling system could reach 180–206 L and 40–45 L CH₄ L⁻¹ d⁻¹ (litres of methane generate per litre of reactor volume per day) respectively, which was 16.1–32.9% and 25–40.6% higher than those of the original one. In addition, the total mass yield obtained by the combination in the proposed coupling system was 317 g ethanol plus 68.7 g methane/kg cassava, and 8.3% higher energy was generated compared with the original system. In conclusion, the insertion of a biological pretreatment step into the proposed coupling system revealed obvious superiorities and applicable potential.

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1. Introduction

Bioethanol production has the potential for reducing the negative environmental impacts generated by fossil fuels, attracts worldwide attention (Rosillo-Calle and Walter, 2006; Nguyen and Gheewala, 2008). The production of bioethanol using cheaper substrates, such as starchy or cellulosic materials, can obviously reduce the production cost. However, to date, many technical problems have still hindered the commercial application of cellulosic materials (Szymanowska-Powalowska et al., 2014). Therefore, utilization of cheap starchy materials could be another effective approach for reducing the cost of bioethanol production. Among

these materials, cassava (*Manihot esculenta* Crantz) is believed as one of the most appropriate substrates for bioethanol production because it owns the nature of abundant starchy content, relative cheap and high yield (Papong and Malakul, 2010; Leng et al., 2008), and can grow in dry and poor soils, avoiding land competition with other major food crops (Jansson et al., 2009).

However, a large number of distillage (containing high concentration of organic materials and low pH) was generated during the pilot scale production of cassava-bioethanol. The traditional treatment strategy was sequentially combined anaerobic and aerobic digestion to treat these distillage, and the effluent was then further treated by physical or chemical methods to reach the national disposal criterion (Kim et al., 1997). However, such kinds of treatment have the disadvantages of complicated operation and energy consumption, and face the problem of secondary pollution (Sun et al., 2010).

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A full recycling process of bioethanol production through two-stage anaerobic treatment was proposed to treat cassava distillate in our previous study (Zhang et al., 2010). In this process, bioethanol was produced by the fermentation of cassava starch and the distillate generated was treated through two-stage anaerobic digestion which could produce a certain amount of biogas, and the resultant anaerobic effluent was reused for bioethanol production. Such kind of operation can result in zero wastewater discharge and little energy consumption (Mao and Zhang, 2008). However, cassava fibres contained in the distillate can not be effectively digested in this process due to their complex lignocellulosic structures, and the discharge of these substrates in solid–liquid separation step will result in secondary environmental pollution and wasting of the lignocellulosic resources (Zhang et al., 2011b). Therefore, how to effectively utilize these resources and enhance the final methane yield of the recycling process is the key to realize the ideal model of bioethanol production with zero wastewater discharge and energy consumption (Mao and Zhang, 2008).

Pretreatment of lignocellulosic biomass can accelerate anaerobic process and promote methane formation (Zieminski et al., 2012). Some pretreatment strategies including chemical (Zhang et al., 2011b), mechanical (Pommier et al., 2010), biological (Mshandete et al., 2005) or combinations thereof (Bruni et al., 2010) have been utilized to treat lignocellulosic materials for efficient methane fermentation. Among these strategies, biological pretreatment, with advantages of simplicity and low capital investment attracts more attention (Mshandete et al., 2008). In a previous study, a cellulolytic microbial consortium with high cellulose degradation ability that can effectively degrade cassava fibres was successfully constructed (Zhang et al., 2011a). Therefore, in this study, the anaerobic digestion of cassava distillate with a high solid content (i.e., cassava residues) was designed to be pre-hydrolyzed by this cellulolytic microbial consortium, and then be digested in two-stage anaerobic digesters to strengthen the anaerobic efficiency and eventually increase the methane yield. Such a systemic integration can result in the enhancement of stability and efficiency of the anaerobic process (Zhang et al., 2013).

In the present study, cassava based bioethanol production was performed with the integration of a two-stage anaerobic digestion process combined with a previous hydrolysis step of a constructed cellulolytic microbial consortium. The main goals of this study are to verify the excellences of inserting a hydrolysis step with a cellulolytic microbial consortium into an ethanol-biogas coupling fermentation system and to discuss the applicability of such a systemic integration for simultaneously efficient bioethanol and methane production with zero wastewater discharge and little energy consumption.

2. Materials and methods

2.1. Strain and materials

Angel alcohol instant active dry yeast (a commercial strain of *Saccharomyces cerevisiae* for ethanol production was obtained from Hubei Angel Yeast Co. Ltd., China) was used throughout this study. The seed medium of yeast containing (g/L): glucose 20, yeast extract 8.5, CaCl₂ 0.06, MgSO₄ 0.1 and NH₄Cl 1.3. α -Amylase (20,000 IU/mL, Genencor Biotech Co. Ltd., Wuxi, China) and glucoamylase (150,000 IU/mL, Genencor Biotech Co. Ltd., Wuxi, China) were used for liquefaction and saccharification of cassava, respectively.

The sludge collected from Wujiang Alcohol Manufacturing Co. Ltd., Jiangsu Province of China, was used as the inoculum of the anaerobic sequencing batch reactor (ASBR) and up-flow anaerobic sludge blanket (UASB) for thermo- and mesophilic anaerobic digestion. The characteristics of thermophilic inoculum used was:

pH, 7.8; total suspended solids (TSS), 32.4 \pm 0.48 g L⁻¹; volatile suspended solids (VSS), 23.6 \pm 0.34 g L⁻¹; total solids (TS), 35.6 \pm 0.62 g L⁻¹; and volatile solids (VS), 24.4 \pm 0.25 g L⁻¹. The characteristics of mesophilic inoculum used was as follows: TSS, 26.8 \pm 0.32 g L⁻¹; VSS, 18.5 \pm 0.22 g L⁻¹; TS, 30.8 \pm 0.46 g L⁻¹; VS, 20.1 \pm 0.24 g L⁻¹; and pH, 8.2.

The cellulolytic microbial consortium was directionally constructed for the pretreatment of cassava fibres in our previous study (Zhang et al., 2011a). This consortium is composed of cellulolytic and non-cellulolytic microbes and owns high cellulose degradation ability. It can totally degrade filter paper within 40 h of incubation under static condition and the methane yield of cassava residues can be obviously increased after being pretreated by this consortium (Zhang et al., 2011a). The incubation condition of this consortium was as follows: tryptone 5 g/L, yeast extract 1 g/L, NaCl 5 g/L, CaCO₃ 3 g/L, filter paper 5 g/L and cassava residues 10 g/L, pH 7.5–8.0, temperature 55 °C.

2.2. Preparation of cassava medium and ethanol fermentation

Ethanol fermentation medium was prepared using cassava chips (Tianguang Fuel Ethanol Co. Ltd., Henan Province, China). First, cassava was milled and passed through 40 mesh screen. Followed by, cassava flour was mixed with tap water in the first batch or mesophilic effluent in the later recycling batches of ethanol fermentation at a ratio of 1:2.7 (w/w) and the pH was adjusted to 5.8. For liquefaction, α -amylase was added at a dosage of 10 IU/g-cassava, followed by heating of the mixture to 100 °C and maintaining for 60 min. The hydrolyzed solution was then cooled down to 60 °C and glucoamylase with a dosage of 130 IU/g-cassava was added in. Finally, 0.05% (w/v) of sterilized urea was added in the cooled saccharified broth (30 °C) and the pH was adjusted to 4.2–4.4. The inoculum volume and fermentation temperature was kept at 10% (v/v) and 30 °C respectively.

2.3. The pretreatment of cassava distillate with a constructed cellulolytic microbial consortium

The pretreatment process was performed in a 3 L of continuous stirred tank reactor (CSTR). Followed with the termination of distillation, the supernatant of cassava distillate after being cooled down to ambient temperature was directly digested in a 12 L of anaerobic sequencing batch reactor (ASBR), while the rest of cassava distillate containing 4–6% of solid content (i.e., cassava residues) was hydrolyzed by a cellulolytic microbial consortium (Zhang et al., 2011a) in the CSTR after being mixed with thermophilic effluent at a ratio of 1:2 (v/v), and then inoculated with 5% (v/v) of inoculum according to the previous study (Zhang et al., 2013).

Some literature indicated the biological hydrolysis of organic wastes under micro-aerobic conditions can enhance the hydrolysis rate and benefit the subsequent anaerobic process (Díaz et al., 2011; Lim and Wang, 2013; Mshandete et al., 2005). Therefore, in the present study, the hydrolysis of cassava residues by a cellulolytic microbial consortium was performed with addition of very small amounts of air through maintaining the ventilate velocity at 0.01 volume per volume per minute (vvm) during the pretreatment process (Zhang et al., 2013). The hydrolytic process was performed at 55 °C for 24 h with an agitation velocity of 50 rpm.

2.4. Thermo- and mesophilic anaerobic digestion

Thermophilic anaerobic digestion was performed in an ASBR with an effective working volume of 12 L. After accomplishment of an acclimatization of the inoculum to the substrates studied in the reactor, thermophilic digestion was started by directly feeding the

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