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High-solids ethanol fermentation with single-stage methane anaerobic digestion for maximizing bioenergy conversion from a C_4 grass (*Pennisetum purpureum*)

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HIGHLIGHTS

- High-solids SSF integrating single-stage anaerobic digestion was established.
- High titer ethanol of 62.6 g/L was achieved with a final substrate loading of 35%.
- Stable performance was achieved in semi-continuous CSTR at OLR of 6.0 g VS·L^{-1} d^{-1}.
- The overall production yield was: 121.6 g ethanol + 110.6 g methane/kg dry grass.

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ABSTRACT

High-solids simultaneous saccharification and fermentation integrating single-stage anaerobic digestion was designed for bioenergy conversion from steam-exploded *Pennisetum purpureum*, a perennial C_4 grass. Three scenarios for ethanol production, methane production and ethanol-methane co-production were investigated and compared in terms of production yield. A terminal ethanol titer of 62.6 ± 1.9 g/L with the highest ethanol yield of 90.9% was achieved when the fed-batch mode was carried out at a final substrate loading of 35% (w/v) dry matter content. The ethanol stillage was used for methane production in a semi-continuous CSTR system operated with different organic loading rates (OLRs) at a hydraulic retention time (HRT) of 30 days. The high performance was achieved at OLR of 6.0 g VS·L^{-1} d^{-1}, average yielding 358.7L CH_4 per kilogram volatile solid (VS) with a total VS removal rate of 73.8%. On the basis of mass balance, the overall production yield achieved in this study was: 121.6 g ethanol + 110.6 g methane per kilogram grass with a finally 89.6% of cellulose recovery + 62.5% hemicellulose recovery. These results indicated that the high-solids ethanol fermentation with single-stage methane anaerobic digestion of lignocellulosic biomass is a valid approach to maximize the holocellulose bioconversion and improve the bioenergy production.

1. Introduction

Lignocellulose-to-ethanol conversion is regarded as a feasible technology to supplement starch-based ethanol production due to the abundance and diverse of raw materials available. Perennial C_4 grasses are promising feedstocks for renewable bioenergy conversion, since they have high yield and extensive adaptability, can be cultivated in non-arable lands, avoiding the direct competition with food crops [1,2]. However, the main bottleneck in economical production of bioenergy from lignocellulose is the technological impediments of improving overall conversion efficiency and production yield [3].

High-titer cellulosic ethanol production through the efficient enzymatic saccharification and fermentation process of lignocellulose is considered to be a highly attractive strategy [4]. While high-solids simultaneous saccharification and fermentation (SSF) can increase ethanol concentration to facilitate downstream separation, it usually requires robust yeast strain with increased tolerance to ethanol and inhibitors [5,6]. In our previous study, we successfully performed fed-

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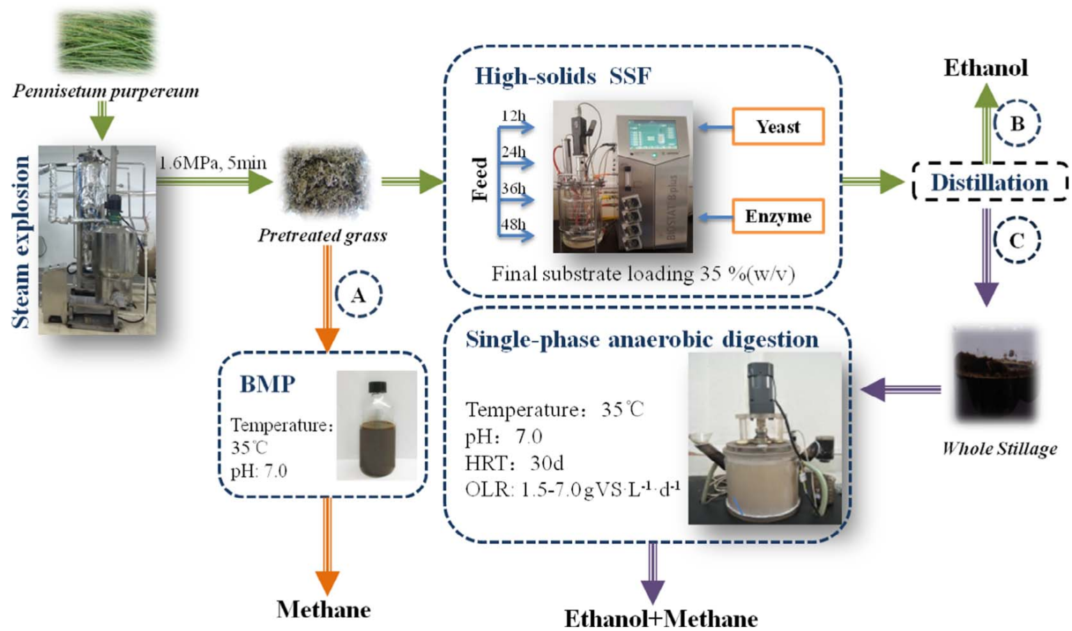

Fig. 1. Schematic diagram of processes used for experiments (A: Batch biochemical methane potential assay; B: High-solids ethanol fermentation; C: Combined process for coproduction of ethanol and methane).

batch SSF operation to convert lignocellulosic materials by using adapted strain *Saccharomyces cerevisiae* Y5 without detoxification [7].

Efficient conversion of bio-ethanol from lignocellulosic biomass remains a challenge despite much progress for integration and optimization has been made in many areas of biomass biorefinery. For example, modifying higher pretreatment severity usually achieves the better cellulose accessible and sugar release, however it would also bring about the loss of hemicellulose and the generation of fermentation inhibitors [8]. The cellulosic ethanol production in most reported studies thus only from low-solids loading (< 20% dry matter content (w/v)) due to the difficulties in the fermentation of inhibitor containing pretreatment substrate [9–11]. Various detoxification techniques, such as ion exchange resin, washing, overliming, etc., have been commonly applied before SSF, which adds an extra process step in practice and can result in sugar losses [12–14]. When using ordinary industrial *Saccharomyces cerevisiae* for ethanol production, only hexoses will be fermented, while the pentoses and other carbon sources could not be utilized [15]. As a result, few studies have demonstrated efficient overall conversion (> 70%) (based on holocellulose: cellulose + hemicellulose) [9,16–19]. Therefore, a large proportion of biomass waste (i.e., ethanol stillage) will likely be produced as a by-product in the lignocellulose-based ethanol fermentation. Disposal of stillage can reduce carbon footprint while increase energy recovery efficiency of ethanol production.

Considering that it is of great practical importance to maximize both substrate utilization rate and bioenergy production yield in any biomass-to-bioenergy conversion system, anaerobic digestion (AD) of stillage as a substrate would be a promising option [20–22]. AD rests on converting carbohydrate, proteins and lipid to biogas in presence of a community of anaerobic microorganisms [23,24]. Although biogas digestion using stillage as sole source has been studied in previous researches by adopting batch mode, there are few publications on semi-continuous single-stage anaerobic digestion of whole stillage in reactors [25–28]. It is well known that the core requirements of AD include improving process efficiency with a continuously high and sustainable organic loading rate (OLR), increasing volatile solids (VS) degradation with shortening the fermentation time [29,30]. Single-stage anaerobic digestion system remains the preferred option for majority of wastes because in which synergistic reactions of microorganisms community on hydrolysis, acidogenesis or fermentation, acetogenesis and methanogenesis take place corporately in single reactor [31]. Continuous stirred tank reactors (CSTRs) have been studied as a suitable type of reactor for treating high strength wastes which have high total solids and volatile solids, and it is simple to operate and provides good contact between substrate and microorganisms [32].

The main objective of the present work is to investigate the effectiveness of a new processing: "high-solids ethanol fermentation with single-stage methane digestion" to overcome the technological barriers of improving the overall yield and conversion efficiency for bioenergy production. During the cellulosic ethanol fermentation stage, we focused on in situ high-titer ethanol production from pretreated lignocellulose at high-solids loading by operating fed-batch SSF mode; the subsequent biogas conversion stage was developed on the basis of high ethanol yielding and carried out by using a semi-continuous CSTR system of single-stage anaerobic digestion. Three scenarios for ethanol production, methane production and ethanol-methane coproduction, using a perennial C_4 grass, *Pennisetum purpureum* as raw material, were studied and compared in terms of production yield. Mass balance data were provided to demonstrate the viability of this new process for practical applications.

2. Materials and methods

2.1. Experiment design scheme

The whole experiment was performed following the schematic process flow diagram shown in Fig. 1.

2.2. Materials

The *Pennisetum purpureum* used in this study was provided by Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences. Steam explosion pretreatment was carried out by Institute of Process Engineering, Chinese Academy of Sciences. The feedstock was chopped to 2–3 cm size and steam heated at a pressure of 1.6 MPa and a temperature of 195 °C for 5 min. The feedstock was thereafter discharged through restricted orifices and exploded at atmospheric pressure into a pulp. The pretreatment material was dried to reduce moisture content to 10% at