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Comparison and evaluation of concurrent saccharification and anaerobic digestion of Napier grass after pretreatment by three microbial consortia

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HIGHLIGHTS

- Effect of microbial pretreatment on saccharide and methane production was evaluated.
- The delignification rates decomposed by microbial consortia were all above 30%.
- The saccharide and methane yields significantly increased after pretreatment.

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ABSTRACT

Napier grass is potentially a viable feedstock for biofuel production. The present study investigated biological pretreatment of Napier grass by three microbial consortia followed by saccharification and anaerobic digestion. The pretreatment efficiencies of three microbial consortia were compared in terms of degradation ability, saccharide and biogas yield. The lignocellulose loss rates of Napier grass varied largely. The biomass pretreated by the consortium WSD-5 gave 43.4% and 66.2% total sugar yield under low and moderate loadings of commercial enzyme mixtures, while the highest yield was 83.2% pretreated by the consortium MC1 under a high enzyme loading. The maximum methane yield of pretreated samples by the consortia MC1, WSD-5 and XDC-2 were 259, 279, 247 ml/g VS, respectively, which were 1.39, 1.49 and 1.32 times greater than the values of the untreated controls. This study showed that pretreatments by MC1, WSD-5 and XDC-2 were capable of significantly enhancing both the saccharide and methane yields from Napier grass.

1. Introduction

With the depletion of source and rising concern about the negative environmental impact of fossil fuel, renewable bioenergy has become a viable choice to meet the growing energy demand. As a second generation biofuel, lignocellulose is a promising energy source for its cost-effectiveness and regeneration ability. It can be hydrolyzed and fermented to produce ethanol, or digested into biogas in an anaerobic environment (Horn et al., 2011). Among the total amount of annual biofuel feedstock production of 342 million tons in China, grasses and shrubs are the second largest component, numbering at 45 million tons (Tian, 2010).

Napier grass (*Pennisetum purpureum*), also known as elephant grass, is a C4 perennial grass with high biomass. It grows fast and is highly adaptive, hence is suitable to be grown on marginal lands. The biomass production of Napier grass in China was 210 t/ha (O'Yang et al., 2013). Napier grass has a great potential to serve as a feedstock for biofuel production (Tsai, 2009). Napier grass is mainly composed of cellulose, hemicellulose and lignin. Due to the complex structure, the hydrolysis of lignocellulosic substrates is considered the rate limiting step during the conversion process. However, enzymatic hydrolysis of lignocelluloses without pretreatment is usually not efficient because of high structural complexity of the substrates.

Pretreatment is necessary to decompose lignin for processing cellulose and hemicellulose. Currently, the most widely used pretreatment methods fall into three major categories: physical, chemical and biological. However, the physical and chemical processes have shortcomings, such as infrastructural requirements and technological limitations. In addition, various chemicals such as cellulase inhibitors are involved in the pretreatment process, which could potentially jeopardize the conversion of cellulose and hemicellulose (Antizar Ladislao and Turrion Gomez, 2008). Although the biological pretreatment methods can avoid the

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problems of generating enzyme inhibitors and metabolite repression, there exist some other problems such as high cost and difficulty in large-scale applications (Mshandete et al., 2008; Soundar and Chandra, 1987). Previous studies about biological pretreatment were mainly focused on use of pure cultures, such as anaerobic bacteria, fungi, and actinomycetes, which were able to degrade lignocellulose (Desvaux et al., 2000; Xu and Goodell, 2001). However, pure cultures of microorganisms are difficult to maintain in an open system, and often require long pretreatment time. In fact, it has been approved that the complete decomposition of lignocellulose requires the combined actions of a diverse range of microorganisms (Slater and Lovatt, 1984). Several studies have demonstrated the efficiency of constructed microbial consortia for the rapid degradation of lignocellulose (Haruta et al., 2002; Wongwilaiwalin et al., 2010; Yang et al., 2011). To our knowledge, such microbial consortia have not been directly used in the pretreatment of Napier grass. In our previous studies, three microbial consortia MC1, WSD-5 and XDC-2 were developed. MC1 comprised of thermophilic bacteria was enriched from compost materials. The key cellulose-degrading bacteria of MC1 was an anaerobic cellulolytic bacteria, *Clostridium straminisolvens* CSK1 (Kato et al., 2004). WSD-5 originated from plant litter and soil was comprised of fungal and bacterial communities, which the most dominant fungi and bacteria were *Coprinus cinereus* and *Ochrobactrum* sp., respectively (Wang et al., 2011). XDC-2 was mainly composed of mesophilic bacteria in the genera *Clostridium*, *Bacteroides*, *Alcaligenes*, and *Pseudomonas* (Guo et al., 2010).

In the present work, a series of experiments were performed to determine the properties of pretreatment process and its residues of the three microbial consortia. This work can be divided into two phases. The first phase was pretreatment, which was to explore the ability of the three microbial consortia to decompose Napier grass. The second phase was the subsequent processing of the pretreated residues, which was the concurrent saccharification and anaerobic digestion of pretreated residues. In the saccharification process, three different enzyme loadings were used to test the saccharification conversion rate of residues pretreated by different microbial consortia during a pretreatment process of 21 days. The purpose of this part of the experiment was to understand how the enzyme loading influenced the saccharification of residues pretreated by different consortia. In the anaerobic digestion process, hydrolyte and substrate residues were both used for methane production to explore the effect of biological pretreatment by three microbial consortia on methane yield.

2. Methods

2.1. Materials

Fresh Napier grass biomass was harvested from Guiping city in Guangxi Province, China, dried at 60 °C, manually divided into stem and leaf fractions and then milled. Napier grass biomass powders (leaf and stem in 1:1 weight ratio) were used as the sole carbon source for cultivation of microbial consortium.

Peptone cellulose solution medium (PCS) was used for cultivation of MC1, containing, peptone 2 g/L, yeast extract 1 g/L, $CaCO_3$ 2 g/L, NaCl 5 g/L; pH 7.0 ± 0.2. The modified medium based on PCS (MPCS) was used for cultivation XDC-2, to which additional $MgSO_4{\cdot}7H_2O$ and K_2HPO_4 were added at 0.35 g/L and 1 g/L, respectively.

Modified Mandels medium (MM) was used to cultivate WSD-5. MM contained $(NH_4)_2SO_4$ 1.4 g/L, $MgSO_4{\cdot}7H_2O$ 0.3 g/L, KH_2PO_4 2 g/L, peptone 2.5 g/L, $CaCO_3$ 2 g/L, $FeSO_4{\cdot}7H_2O$ 5 g/L, $MnSO_4$ 1.6 mg/L, $ZnCl_2$ 1.7 mg/L, and $CoCl_2$ 1.7 mg/L; and adjusted to pH 7.0.

2.2. Biological pretreatment experiments

The microbial consortia MC1, WSD-5 and XDC-2 were in 20% glycerol solution and were stored under −80 °C. PCR-DGGE analysis of successive subculture indicated that MC1, WSD-5 and XDC-2 were structurally stable over long-term restricted and directed cultivation (Guo et al., 2010; Wen et al., 2012; Yuan et al., 2012).

MC1 from frozen stock was inoculated into 100 ml of sterile PCS medium and allowed to grow statically at 50 °C for 3 days with the substrate of filter paper at a substrate loading rate of 2%. One hundred ml of PCS medium containing 3 g of untreated Napier grass was inoculated with 5% (v/v) of the 3-day-old MC1 culture and incubated in 150-ml flask under static conditions at 50 °C for 21 days. Aliquots of hydrolysates were sampled on days 3, 7, 13, 17, and 21. After centrifugation at 4 °C and 6000 rpm for 15 min, the supernatants were stored at −20 °C for further analysis.

MM (100 ml) was poured into a 500-ml flask. After inoculation of the frozen stock WSD-5 into the medium containing 2% filter paper as substrate, it was incubated at 30 °C and 150 rpm for 7 days. To 100 ml MM containing 3 g of untreated Napier grass was inoculated with 5% (v/v) of the 7-day-old WSD-5 culture, followed by incubation in a 500-ml flask at 30 °C and 150 rpm for 21 days. Aliquots of hydrolysate were sampled on days 3, 7, 13, 17, and 21. After centrifugation at 4 °C and 6000 rpm for 15 min, the supernatants were stored at −20 °C for further analysis.

XDC-2 from frozen stock was inoculated into 100 ml of MPCS medium that contained filter paper as the substrate at 2%, followed by static growth at 35 °C for 6 days. To 100 ml MPCS medium containing 3 g of untreated Napier grass was inoculated with 5% (v/v) of the 6-day-old XDC-2 culture and then incubated in a 150-ml flask under static conditions at 35 °C for 21 days. Aliquots of hydrolysates were taken on days 3, 7, 13, 17, and 21. After centrifugation at 4 °C and 6000 rpm for 15 min, the supernatants were stored at −20 °C for further analysis.

The Napier grass residues after 3, 7, 13, 17, and 21 days of biological pretreatment were thoroughly washed with distilled water to remove soluble substances and then dried in an oven at 60 °C for overnight. The dried, treated Napier grass residues were used for enzymatic hydrolysis. The data presented were the average of triplicate treatments.

2.3. Enzymatic hydrolysis

Cellulase (NS50013), β-glucosidase (NS50010) and xylanase (NS22002) were kindly donated by Novozymes Investment Co Ltd. (Beijing, China).

The enzymatic hydrolysis was conducted in 20 ml in a centrifuge tube (50 ml). The solid residues from different pretreatments were enzymatically hydrolyzed in 100 mM sodium citrate buffer (pH 4.8) at 2.5% solid loading at 50 °C for 48 h. The mixtures were autoclaved at 121 °C for 15 min prior to enzyme addition. Cellulase, β-glucosidase and xylanase (activity ratio 10:1:1) were examined in three loadings. The low enzyme loading contained cellulase (NS50013) 15 IU/g, β-glucosidase (NS50010) 1.5 IU/g and xylanase (NS22002) 1.5 IU/g of substrate (dry basis). The moderate enzyme loading contained cellulase 40 IU/g, β-glucosidase 4 IU/g and xylanase 4 IU/g of substrate (dry basis). The high enzyme loading contained cellulase 70 IU/g, β-glucosidase 7 IU/g and xylanase 7 IU/g of substrate (dry basis). After hydrolysis, the hydrolyte was centrifuged at 6000 rpm for 20 min at 4 °C, and the supernatants were stored at −20 °C for subsequent analysis. Substrate blanks without enzyme and enzyme blanks without substrate were run in parallel. The untreated Napier grass controls were run concurrently with all recovered samples to eliminate potential differences in enzymatic hydrolysis process.