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Effects of different pretreatment strategies on corn stalk acidogenic fermentation using a microbial consortium

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ABSTRACT

The effects of sulfuric acid, acetic acid, aqueous ammonia, sodium hydroxide, and steam explosion pretreatments of corn stalk on organic acid production by a microbial consortium, MC1, were determined. Steam explosion resulted in a substrate that was most favorable for microbial growth and organic acid productions. The total amounts of organic acids produced by MC1 on steam exploded, sodium hydroxide, sulfuric acid, acetic acid, and aqueous ammonia pretreated corn stalk were 2.99, 2.74, 1.96, 1.45, and 2.21 g/l, respectively after 3 days of fermentation at 50 °C. The most prominent organic products during fermentation of steam-exploded corn stalks were formic (0.86 g/l), acetic (0.59 g/l), propanoic (0.27 g/l), butanoic (0.62 g/l), and lactic acid (0.64 g/l) after 3 days of fermentation; ethanol (0.18 g/l), ethanediol (0.68 g/l), and glycerin (3.06 g/l) were also produced. These compounds would be suitable substrates for conversion to methane by anaerobic digestion.

1. Introduction

Corn stalk is a promising renewable feedstock for biological conversion to fuels and chemicals. Although microbial decomposition of such lignocellulosic materials has been studied extensively, most of these studies have focused on pure cultures of microorganisms (Lynd et al., 2002; Wong et al., 1988). Microorganisms in pure culture regularly demonstrate unsatisfactory lignocellulolytic activities (Kim et al., 2006; Koullas et al., 1992; Osborne and Dehority, 1989), and few of them are able to decompose natural lignocellulose with the complex composition and structure found in corn stalks.

MC1 is thermophilic cellulose degrading consortium (Haruta et al., 2002) that has not been fully characterized but is known to contain *Clostridium straminisolvens* CSK1, *Clostridium* sp. strain FG4b, *Pseudoxanthomonas* sp. strain M1–3, *Brevibacillus* sp. strain M1–5 and *Bordetella* sp. strain M1–6 (Kato et al., 2005). MC1 is capable of degrading rice straw (Haruta et al., 2002) and corn stalk (Peng et al., 2008) and of producing organic acids. The conversion of compounds present in such straws into organic acids is a prerequisite for biogas production (Kaparaju et al., 2009a,b; Weiland, 2010).

Since the conversion of lignocellulosic biomass such as straw and corn stalk is relatively recalcitrant to microbial degradation, appropriate pretreatment is a crucial prerequisite for bioconversion of lignocellulosic feedstock (Alvira et al., 2010; Chen et al., 2011; Himmel et al., 2007; Mosier et al., 2005). The current study measured biomass and fermentation products of MC1 cultured on corn stalk pretreated with acids, bases or by steam explosion to evaluate the potential for subsequent biogas production.

2. Methods

2.1. Lignocellulosic materials

Corn stalk (variety CAU 80, bred by the Centre for National Maize Improvement at China Agricultural University in Beijing, China), the lignocellulosic material, was obtained locally after corn harvest from experimental fields at China Agricultural University, Beijing, China, and dried at 80 °C. The corn stalks were cut into pieces approximately 3 cm in length for further use.

2.2. Pretreatment

2.2.1. Acid pretreatment

Fifty grams of dried corn stalks were soaked in 1 l H_2SO_4 (1 M) or acetic acid (1 M) under static condition at room temperature,

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resulting in 5% (w/v) dry straw solids loading. After 24 h, the corn stalks were collected and washed with tap water (approximately pH 6.0) and then with 1 N NaOH to reach a neutral pH before oven-drying to constant weight at 80 °C and milling to pass through 2 mm screens.

2.2.2. Alkaline pretreatment

Fifty grams of dried corn stalk was soaked in 1 l of 0.4 N of NaOH solution or $NH_3{\cdot}H_2O$ (1 M) under static condition at room temperature, resulting in 5% (w/v) dry straw solids loading. The stalk/alkali mixture was placed at room temperature. After 24 h, the corn stalks were collected and washed with tap water (approximately pH 8.0) and then with 1 N HCl to reach a neutral pH, oven-drying to constant weight at 80 °C and milling to pass through 2 mm screens.

2.2.3. Steam explosion pretreatment

The steam explosion pretreatment was carried out using laboratory scale equipment, consisting of a steam generator and a 1 l pressurized reactor. The reactor was filled with 20 g of feedstock per batch (the dried corn stalk was previously cut into 1 cm pieces) and was then heated to the desired temperatures to reach a pressure in the range of 0.65–0.75 MPa with saturated steam (165 °C), the corn stalks remained in the reactor with saturated steam for 30 s. After a predetermined cooking time (3 min), the valve was opened to release the pressure abruptly, and then the exploded samples were collected and washed with distilled water three times to remove the soluble substances generated in the explosion process. Samples were then oven-dried to constant weight at 80 °C and milled to pass through 2 mm screens.

2.2.4. Without pretreatment

The corn stalks were not pretreated with any chemicals before drying to constant weight at 80 °C and milling to pass through 2 mm screens.

2.3. Cultivation with consortium MC1

MC1 from frozen stock was inoculated into 125 ml of sterile peptone cellulose solution (PCS) pH 7.0 ± 0.2 containing (g/l), peptone, 5; yeast extract, 1; $CaCO_3$, 2; NaCl, 5; printer paper, and allowed to grow statically at 50 °C for 3 days. Two-hundred-fifty ml of PCS medium containing 10 g of treated or untreated corn stalk instead of printer paper was inoculated with 5% (v/v) of the 3-day-old MC1 culture and incubated in 500 ml flask under static conditions at 50 °C for 15 days. Samples were taken on days 0, 1, 3, 6, 9, 12, and 15 for analysis. Unless otherwise specified, the data presented are the average of three replications.

2.4. Measurements of microbial growth and culture pH

MC1 growth was based on bacterial protein evaluation as previously described (Bensadoun and Weinstein, 1976; Brown et al., 1989). The pH was determined using a HORIBA Compact pH meter (Model B-212, Japan).

2.5. Determination of lignocellulose component and weight loss of corn stalk

The cultures were subjected to centrifugation at 12,000*g* for 10 min, and the pellets were washed with acetic acid/nitric acid reagent followed by a water rinse to remove non-cellulosic materials (Updegraff, 1969). Uninoculated medium served as the control. The weight loss of residual substrates was determined as described by Peng et al. (2008). Residual corn stalk materials were passed through 1 mm screens, and a 0.5-g sample was transferred into a filter bag (F57, ANKOM Technology, USA). The components of residual lignocellulosic materials were analyzed according to Goering and Van Soest (1970) using a fiber analyser (Model ANKOM220, USA) as described by Guo et al. (2010).

2.6. GC–MS analysis of volatile products

On days 0, 1, 3, 6, 9, 12, and 15, 0.5 ml of fermentation broth was centrifuged at 12,000*g* for 10 min, and the supernatant was filtered using an aperture of 0.22 μm. The filtrate was analyzed by GC–MS (model QP-2010, Shimadzu, Japan) on line with capillary column CP-Chirasil-Dex CB (25 × 0.25 mm). Conditions were as follows: initial temperature of 60 °C, 1 min; linear ramp up to 100 °C at 7 °C/min and to 195 °C at 18 °C/min for 3 min; injector temperature: 190 °C; ion source temperature: 200 °C; carrier gas: He (60 kPa); rate of flow: 34 ml/min; splitter ratio: 1/20; voltage of detector: 0.7 kv; sample volume: 1 μl.

Qualitative identification of the resulting peaks was accomplished using the NIST database (Wang et al., 2006). Furthermore, dilutions of the corresponding compounds were injected as standards to quantify the compounds and confirm the peak positions.

3. Results and discussion

3.1. Effect of pretreatments on solubilization and composition of corn stalk

The percent solubilization of corn stalks under different pretreatments is shown in Fig. 1. Steam explosion pretreatment and sodium hydroxide pretreatment produced greater solubilization of corn stalk than the other pretreatments, and alkali pretreatment (sodium hydroxide or aqueous ammonia pretreatment) gave greater solubilization than acid pretreatment (sulfuric acid or acetic acid); no significant difference ($P = 0.178$) in solubilization between sodium hydroxide pretreatment and steam explosion pretreatment was detected.

The compositional changes in under different pretreatments are summarized in Table 1. The corn stalk mainly lost hemicellulose after acid treatment, and the relative contents of cellulose and lignin increased. The corn stalk mainly lost lignin after alkaline treatment, in addition, the hemicelluloses content decreased, while the relative content of cellulose increased noticeably. Hemicellulose decreased after steam explosion, and the relative cellulose and lignin content increased.

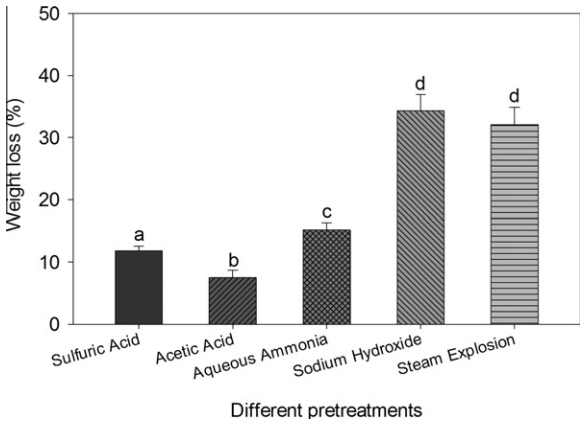

Fig. 1. Solubilization of corn stalk by different pretreatments. Solubilization is expressed as the percentage of raw material dissolved by pretreatment on the basis of dry weight. Bars represent the standard deviation on the set of values. Values (means of three replicates) not sharing common letters are significantly different (one-way ANOVA with Student–Newman–Keuls method, $P < 0.05$).