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Integrating sugar beet pulp storage, hydrolysis and fermentation for fuel ethanol production

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ABSTRACT

Sugar beet pulp (SBP) as received has a fairly high moisture content of 75–85%, which makes SBP storage a challenge. Ensilage was studied over 90 days and was found to effectively preserve SBP without lactic acid bacterium inoculation. Higher packing density yielded a slightly better silage quality. Ensilage improved sugar yield upon enzymatic hydrolysis of ensiled SBP washed with water. However, neither washing nor sterilization improved ethanol production from ensiled SBP using *Escherichia coli* KO11, suggesting ensiled SBP could be used directly in fermentation. The ethanol yield from ensiled SBP was nearly 50% higher than raw SBP. Fed-batch fermentation obtained approximately 30% higher ethanol yield than batch. Fed-batch could also be carried out at 12% solid loading with a 50% lower enzyme dosage compared to batch at the same solid loading, indicating opportunities to improve the economics of SBP conversion into liquid fuels.

1. Introduction

Rapid industrialization and population growth require environmentally sustainable energy sources. Bioethanol derived from plant biomass can contribute to a cleaner environment and help reduce US dependency on liquid fossil fuels. With the advancement of improved economics, rigorous sustainability analysis of lignocellulosic feedstocks and innovations in processing science, lignocellulosic bioethanol has become a more attractive fuel choice than earlier grain/starch based fuel ethanol [1]. Current commercial bioethanol production using crops such as sugar cane and corn as feedstocks are well-established. However, agriculture and fuel production compete for use of these crops [2]. As a result, utilization of more abundant, renewable, and inexpensive feedstocks such as lignocellulosic biomass could make bioethanol a more competitive alternative to fossil fuels [3]. Although lignocellulosic biomass constitutes the majority of renewable feedstocks, the complex structure of the cell wall makes degradation and subsequent processing of carbohydrates difficult.

Sugar beet pulp (SBP) is a valuable by-product from the manufacturing of beet sugar. Its carbohydrate (cellulose, hemicellulose, pectin, and others) contents have been reported to be as high as 85% (w/w, dry basis) and its lignin content as low as 1–2% (w/w, dry basis) [4,5]. It also contains 10–15% protein (w/w, dry basis). The US planted 1.2 million-acres of sugar beet crops and produced 29.5 million tons of sugar beet in 2009 [6], which resulted in more than 1.6 million dry tons of SBP after sucrose extraction [7]. Conventionally, SBP is dehydrated, pelletized and sold as a relatively low-value animal feed. The profitability of selling SBP as animal feed depends greatly on the economics of the energy and feed industries since SBP processing, including drying, pelletizing, and transporting, is energy-intensive [8]. In many parts of the world, utilization of SBP is an economically marginal part of beet sugar processing due to the low feed value and high drying cost [7]. In certain areas, dehydrating and pelletizing SBP contribute 30–40% of the overall energy cost of sugar beet processing [9]. Therefore, the beet sugar industry seeks to add value to SBP via a process that does not require drying. In light of this, converting SBP into fuel ethanol through biological pathways, including hydrolysis and fermentation, is an attractive option.

Storage is a major challenge in utilizing SBP for fuel ethanol production. Drying of SBP is common as it avoids carbohydrate loss due to microbial activity. However, in most countries this method is too expensive [10]. Furthermore, dry storage may not be advantageous when SBP is intended for conversion to biofuels and bio-based products since anaerobic digestion and fermentation are typically aqueous processes. For these reasons, it is worth investigating wet storage methods that minimize SBP carbohydrate loss while maintaining moisture content after sugar extraction.

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Ensilage is a storage technology applied to wet or partially dry biomass [11,12]. During ensilage, water soluble carbohydrates (WSCs) are rapidly fermented under anaerobic conditions into various organic acids, preferably lactic acid, to quickly and substantially lower the pH. Lactic acid bacterium (LAB) inoculants have often been used to control the fermentation pattern to avoid undesirable growth of spoilage microorganisms such as butyric acid-producing clostridia and enterobacteria [13–15]. The addition of molasses benefited the growth of LAB and improved the SBP silage quality with low dry matter loss [10]. Both the addition of acids and a pressing process were used to preserve SBP in ensilage to achieve high quality SBP with high lactic acid yield and low dry matter loss [16]. Ensilage has been used to preserve animal feed and may be applicable to preserve lignocellulosic biomass such as SBP for biofuel production. It was found that ensiling storage of maize improved biogas yield by 15% in anaerobic digestion compared to non-ensiled maize [17]. Moreover, Passoth et al. [18] reported that the ethanol yield from moist wheat grain was increased by 14% through airtight storage (ensilage), compared with the control obtained from traditionally dried stored grain. Previous research showed that the ensilage process significantly improved enzymatic hydrolysis of SBP for reducing sugar production [19]. However, little research has been done to examine post-ensiling treatments such as washing, sterilization, and chemical pretreatment prior to enzymatic hydrolysis and ethanol fermentation.

Both enzymatic hydrolysis and fermentation are critical processes for bioconversion of SBP into fuel ethanol. Costly thermochemical pretreatment might not be needed for effective bioconversion of SBP due to the low lignin and high pectin contents. Pectin removal by pectinase hydrolysis improved cellulose hydrolysis [20,21]. Therefore, pectinase is usually used in addition to cellulase/β-glucosidase to hydrolyze SBP into monosaccharides and galacturonic acid for fermentation into fuel ethanol. However, conventional ethanol-fermenting yeasts and native strains such as *Saccharomyces cerevisiae* cannot metabolize both arabinose and galacturonic acid into ethanol [22]. Genetically engineered bacteria including *Escherichia coli* KO11, *Klebsiella oxytoca* P2 and *Erwinia chrysanthemi* EC 16 have been used to ferment hexoses, pentoses and galacturonic acid into ethanol [7]. *E. coli* KO11 was the most efficient at fermenting arabinose and galacturonic acid and yielded the highest ethanol concentration of 25.5 g/L followed by *K. oxytocaa* P2 and *E. chrysanthemi* EC 16. Rorick et al. [8] used both *E. coli* KO11 and *S. cerevisiae* (Type II -YSC2) in parallel and serial fermentation processes to convert SBP solids into ethanol. The highest ethanol yields for *E. coli* KO11 (0.144 g ethanol/g-dry SBP) were much higher than those for *S. cerevisiae* (0.092 g ethanol/g-dry SBP).

In this paper, ensilage was studied to stabilize SBP in 20-L containers. The effects of both packing density and LAB inoculation level (*Lactobacillus fermentum* NRRL B-4524) on the silage quality were investigated. Washing and sterilization of ensiled SBP were examined to determine if they improved or deteriorated reducing sugar yield upon enzymatic hydrolysis and ethanol yield from *E. coli* KO11 fermentation. In addition, size reduction, gas purging, solid loading level, and operation mode (fed-batch and batch) were studied to determine their effects on ethanol yield from SBP using *E. coli* KO11.

2. Materials and methods

2.1. Materials

SBP was obtained from Spreckels Sugar Company in Mendota, CA in 2007. The moisture content as-received was about 78% (wet basis). Fresh SBP was stored at −20 °C until use. *L. fermentum* NRRL B-4524 (LAB 137) was offered by the Department of Viticulture and Enology at University of California, Davis. The ethanologenic *E. coli* strain KO11 was purchased from the American Type Culture Collection (ATCC 55124).

2.2. Preparation of lactic acid bacterium inoculum and SBP ensilage set-up

Ensilage was performed at the 20-L scale. *L. fermentum* NRRL B-4524 was identified as the best LAB strain for SBP silage quality in previous studies at 50 and 1000 mL scales [19] and was further examined here. Ensilage of SBP without LAB inoculation was conducted as a negative control. The packing density of silage was selected as a variable in this study as it was found to significantly affect the ensilage quality in a previous study [23]. Three packing density levels, 0.48, 0.72, and 0.96 g/cm^3, were used. Three replicates were performed for each packing density for both LAB-inoculated silage and the control.

The LAB inoculum for ensiling SBP were prepared by thawing frozen *L. fermentum* stock and establishing a seed culture by adding 100 uL stock to 5 mL Lactobacillus deMan Rogosa Sharpe (MRS) medium. The seed culture was grown overnight at 28 °C with an agitation of 140 rpm. A 2.5 mL aliquot of seed culture was transferred to 100 mL fresh MRS medium in a 250-mL Erlenmeyer flask, which was incubated at 28 °C with 140 rpm agitation. Cells were harvested at an optical density (OD) (590 nm) of 0.5 by centrifuging the culture at 7700 g for 5 min at 4 °C. The cell pellet was washed twice in 1 M sodium phosphate buffer (pH = 7.0) to remove residual media. Washed cell pellets were resuspended in sterilized deionized (DI) water, adjusted to an OD value of 0.5, and kept cool on ice until used.

SBP was treated with either prepared LAB inoculum or sterilized DI water (for the control) using 1-L sprayers. The LAB inoculation level was 10^6 CFU/g-dry matter (DM). SBP was thoroughly mixed while spraying inoculum or water to achieve uniform inoculation. The final moisture content of inoculated SBP was 80%. The inoculated SBP (8 wet kg, equal to 1.6 dry kg) was packed into 30-L polyethylene bags with different density-specific working volumes. The bags were sealed using a thermal sealer and hung on a steel rack at ambient temperature (ca. 22 °C) for 90 days. Each ensilage bag was equipped with a one-way gas outlet valve, through which the produced gas within the bag was released to the ambient environment. After 90 days of ensilage, SBP silage was harvested and pH, organic acids, water soluble carbohydrates, ammonia, and ethanol levels were measured.

2.3. Effect of water washing on the enzymatic digestibility of ensiled SBP

Water washing was conducted by mixing deionized water with SBP silage to achieve liquid-to-solid ratios of 5:1, 10:1, 15:1, 20:1, 30:1, and 50:1 (g:g wet SBP silage). The mixtures were stirred with stir bars at 200 rpm for 1 h at ambient temperature, then filtered through glass fiber filter paper (Grade 934-AH, Whatman) with a Büchner funnel. The washed SBP silage was collected and stored in a refrigerator for enzymatic hydrolysis experiments. The unwashed SBP silage was also hydrolyzed as a control. Enzymatic hydrolysis was conducted with 6% solid loading at a 100-mL working volume in 250-mL flasks for 168 h. Hydrolyzates were withdrawn periodically for reducing sugar measurement.

2.4. Enzymatic hydrolysis

SBP was hydrolyzed using an enzyme mixture containing cellulase (Celluclast 1.5 L), β-glucosidase (Novozymes 188) and pectinase (Pectinex® Ultra SPL). All enzymes were purchased from