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RESEARCH ARTICLE

Separate hydrolysis and fermentation (SHF) of pretreated sugar beet pulp (SBP) into ethanol

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Abstract

Environmentally friendly technologies are becoming more popular because of the increase in environmental pollution. One of them is the ethanol production process using renewable resources. One of these renewable resources is Sugar Beet Pulp (SBP) as a renewable, available and inexpensive raw material with high sugar content for ethanol fermentation. The process of converting biomass to ethanol consists mainly of three stages: pretreatment, enzymatic hydrolysis and fermentation. In this study, ethanol production from SBP was achieved through three steps: acid treatment, enzymatic hydrolysis of cellulose content in treated SBP into fermentable sugar and fermentation of fermentable sugar to ethanol. The weight of dried SBP after acid treatment was 34% of the original dried SBP. Two cellulase commercial enzymes named SternEnzymeC21032 and Cellic C Tec2 were used for hydrolysis of cellulose content in 10 and 15% solid load of the treated SBP. The Saccharomyces cerevisiae strains CY3079 and AH15 were used for the fermentation of the reduced sugar. The highest ethanol yields by S. cerevisiae CY3079 and AH15 were 5.61 and 5.58% of reduced sugar in hydrolyzed SBP with a 15% solid load. According to the results reported in this study, each ton of dried SBP gives 100 kg of ethanol. However, this level is relatively low, and more experiments are still needed to increase the productivity of this bioprocess.

Keywords: Sugar Beet pulp; Pretreatment; Hydrolysis; SHF; Fermentation.

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Introduction

The search for renewable sources of energy and fuels has been a research priority throughout the world to mitigate carbon dioxide emissions and the dependence on fossil fuels (Buckeridge et al. 2009). Lignocellulose is considered an attractive feedstock for the production of ethanol, because of its availability in large quantities and low cost (Cardona and Sanchez 2007). Sugar beet pulp is an abundant by-product from the sugar manufacturing industry that has high hemicelluloses and cellulose with low lignin contents (Curtin, 1983). Each ton of sugar beet mainly yields 830 kg of sugar juice and 170 kg of wet sugar beet pulp after sucrose extraction (Rezi´c 2013).
The pretreatment step is essential to improve microbial or enzyme access to cellulose fibers. There are many types of SBP pretreatment. Depending on the source and composition of lignocellulosic raw materials, various physical, chemical, and biological pretreatment methods are available (Rezi´c 2013). Chemical pretreatment involves the utilization of acid to disrupt the lignocellulosic structure. Studies were performed on SBP acid hydrolysis with sulfuric, hydrochloric, and phosphorus acids at 120˚C with a variation of hydrolyzing agent concentration from 1 to 5% (El- Tayeb et al. 2012). Pretreatment typically requires either concentrated acid at low temperatures (30-70% by volume and ~40° C) or lower concentrations and higher temperatures (<2% by volume with temperatures often >120° C) (Zheng et al. 2014), although H2SO4 is the most common acid used for pretreatment.
The hydrolysis of the cellulose fraction produces hexoses that could be fermented by Saccharomyces cerevisiae or Zymomonas mobilis (Ferrari et al. 1992). Enzymatic hydrolysis is affected by different factors including, cellulose degree of polymerization, cellulose crystallinity, biomass structure and available surface area (Qi et al. 2009). However, enzymatic hydrolysis processes are inhibited by high substrate and glucose concentrations.

For fermentation, the cell wall material needs to be degraded into fermentable monosaccharides. So, lignocellulosic feedstocks are often structurally modified by a pretreatment before the enzymatic release of fermentable sugar (Alvira 2010). Saccharomyces cerevisiae is the most widely used yeast for ethanol biosynthesis; it can ferment hexoses but is unable to ferment pentose sugars (Bai et al. 2008). In this study, we applied to dilute acid to pretreat sugar beet pulp and the resulting substrate was used for ethanol production after enzymatic hydrolysis.

Material and methods

Sample collection and analysis

SBP used in this study was obtained from Abu-Qurqas Sugar Factory. Beet pulp was washed with tap water and then dried at 100° C for 24h in MMM Medcenter, Venticell oven. The chemical composition of the beet pulp was analyzed for cellulose, hemicellulose, lignin, and ash according to Datta (1981).

Sample pretreatment

Rezende et al. (2011) method was used for SBP pretreatment with some modification. Each 100 g of SBP was suspended in 1L 1% H2SO4 and then autoclaved at 120° C for 40 min after that the substrate was filtered and washed with water and then dried at 70° C for 24 h. The resulting substrates were collected and analyzed to determine their contents of cellulose, hemicellulose, lignin and ash according to Datta (1981). The collected resulting substrates were used for further enzymatic hydrolysis.

Enzymatic hydrolysis

Two cellulase enzymes were used in this study, i.e., SternEnzymeC21032, 2.5 FPU/g (Germany) and Cellic CTec2, 100 FPU/ml (Novozymes, Denmark). Each 10 and 15% of pretreated SBP in 100 ml phosphate buffer at pH 5 hydrolyzed, 0.2 g SternEnzymeC21032, as well as 0.1 ml Cellic C Tec2 and a mixture of 0.1 g SternEnzymeC21032 and 0.05 ml Cellic C Tec2 per each g of dry pretreated SBP, were stirred at 45° C for 60 h. The hydrolysis yield can be calculated by the following equations:

Hydrolysis yield = glucose amount in 100 ml / (1.11) (f) (x)

Where F is the cellulose fraction percentage in one gram of substrate; X is the amount of substrate in 100 ml solution and 1.11 is the correlation factor due to the addition of water molecules into cellulose.

Fermentation

Preparation of starters

Two different Sacchromyces cerevisiae strains (CY3079 and AH15) were obtained from previous studies in our laboratories (Zohri et al. 2014). Fresh colonies of each strain were grown on a plate containing yeast extract and malt extract in agar medium (contains by g/l: glucose; 10, yeast extract; 3, malt extract; 3, peptones; 5 and agar; 15) for 48 h at 25° C. Yeast cultures were used to inoculate 100 ml of yeast extract malt extract broth medium (contains by g/l glucose; 10, yeast extract; 3, malt extract; 3 and peptone; 5) at 25° C for 48h at 125 rpm. The inoculum was inoculated in a ratio of 10% in the fermentation medium.

Fermentation process

After enzymatic hydrolysis, the solution was centrifuged and the clear liquid was used for fermentation after enrichment with 5 g/l peptones, 3 g/l yeast extract and 3 g/l malt extract. Fermentation was carried out using the two different yeast strains, separately, at 28° C and pH 5 for 72 h under anaerobic conditions.

Analytical Methods

Chemical analysis of SBP and determination of their cellulose, hemicellulose and lignin contents were achieved by the methods recorded in Rathin Datta (1981). Ash content was determined by burning the final residue in an ashing furnace (Vulcan 3-550). Sugar concentrations were measured by Miller (1959) method. pH values were measured by pH Meter 3540. Ethanol concentration was measured using the dichromate method (Zohri and Mostafa 2000).

Results and discussion

Pretreatment

Dilute acid pretreatment was used in this study for the pretreatment of SBP. Rezic et al. (2013) showed that thermochemical pretreatment had an effect on lignocellulosic substrates and favored the release of monosaccharides from cellulose and hemicellulose. Dilute acid hydrolysis treatment also caused disruption to the polymetric structure of the sugar beet pulp (Yucel and Aksu 2015).

The main chemical composition of the raw and pretreated SBP was determined. Figure 1 shows cellulose, hemicellulose, and lignin content in both raw and treated SBP. Cellulose represents around 25.59% of dry raw SBP where this presence after acid treatment raised to 83.22% (Figure 1). Nearly similar results were observed by several researchers. Chamy et al. (1994) pretreated SBP with 1.1 g H2SO4/g SBP, for 90 min at 80° C and 400 rpm. They observed that the cellulose content in pretreated SBP reached 86.3%. The chemical composition of SBP of the Refinery Factory at El-Beheira governorate, Egypt was investigated by El Tayeb et al. (2012). They reported that cellulose, hemicellulose and lignin were 26.3, 18.5 and 2.5% w/w, respectively.

Zieminski and Kowalska-Wentel (2017) found that cellulose and hemicellulose contributed to SBP were 29.50 and 27.51%, respectively. In our study,

hemicellulose represents 43.97 and 6.32% of raw and pretreated SBP, respectively (Figure 1). Chamy et al. (1994) pretreated SBP with dilute acid and found that the cellulose and hemicellulose in pretreated SBP were 86.3% and 7.8%, respectively.

Lignin exists at a very low ratio in SBP, 1.83% and 1.18% in raw and pretreated SBP, respectively (Figure 1). Grahovac et al. (2012) reported that SBP contains 20–25% cellulose, 25–36% hemicelluloses, 20–25% pectin, 10–15% protein, and 1–2% lignin content on a dry weight basis. Also, Chamy et al. (1994) reported that the composition of raw SBP in Chile was as follows: hemicellulose, cellulose and lignin, were 55±4.5, 21.5±2.1, and 2.2±1.0 respectively. Figure 2 shows the difference between SBP before and after acid pretreatment.

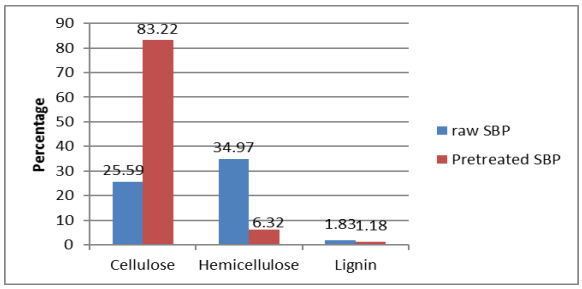

Figure 1. Cellulose, hemicellulose and lignin content in both raw and treated SBP.

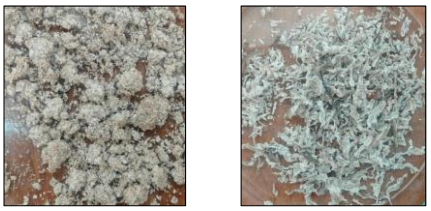

Figure 2. SBP: (a) before and (b) after acid pretreatment

Enzymatic hydrolysis

In this study, the SHF technique was used. Pretreated SBP at solids loading of 10 and 15% were hydrolyzed using two types of commercial enzymes named SternEnzymeC21032 and Cellic CTec2, in addition, to using a mixture of them for 60 h. Reducing sugar concentrations were measured every 12 h (Table 1). The highest reducing sugar yields from 10% solid load were 6.37% by using SternEnzymeC21032 and 7.88% by the second enzyme. While the reducing sugar yields from 15% solid load reached 9.54% by using SternEnzymeC21032 and 11.34% by the second enzyme. It is worthily mentioning that when using a mixture of the two enzymes, the reducing sugar yields were 8.01 and 11.72% from 10 and 15% solid load, respectively. The results showed that all experiments gave a high reducing sugar at 48 h. The hydrolysis yield of each experiment was calculated (Table 2). It is obvious that hydrolysis of 10% of substrate gave a higher yield than 15%. Also, using a mixture of the two enzymes under study gave a higher hydrolysis yield than using each enzyme, individually. The highest reducing sugar percentage was 11.72% with 84.50% hydrolysis yield followed by 11.34% with 81.76% from 15% substrate using the mixture of the two enzymes (SternEnzymeC21032, Cellic CTec2) and Cellic CTec2, respectively. Zheng et al. (2013) reported that acid pretreatment increased the enzymatic digestibility of SBP from 33% to 93%, where pretreatment takes place at 120° C, 0.66% acid concentration and 6% solid loading with 62% total reducing sugar yield. Borrion et al. (2007) examined a pre-hydrolysis of municipal solid waste using dilute acids (H2SO4, HNO3 and HCl at 1 and 4% for 180 min at 60° C). Then, they hydrolyzed the pre-hydrolyzed products using cellulases from T. reesei and T.viride. They reported that the highest glucose yield 73% was obtained from 1% H2SO4 followed by steam treatment at 121˚C and enzymatic hydrolysis with T. viride.

Table 1. Reducing sugar concentrations% of 10% and 15% solid load of pretreated SBP using SternEnzymeC21032 and Cellic CTec2, and a mixture of them for 60 h.

Time	10% Solid load			15% solid load		
	SternEnzymeC21032	Cellic CTec2	A mixture of SternEnzymeC21032 and Cellic CTec2	SternEnzymeC21032	Cellic CTec2	A mixture of SternEnzymeC21032 and Cellic CTec2
12	0.99 ±0.02	1.21±0.08	1.22±0.04	1.95 ± 0.08	2.03 ± 0.06	2.03 ± 0.01.01
24	2. 80 ±0.07	2.93±0.02	2.90±0.03	4.63± 0.06	5.72 ± 0.04	5.80 ± 0.03
36	5.01 ± 0.03	7.11±0.040.04	6.24±0.05	6.32 ± 0.04± 0.04	9.67 ± 0.01	9.52 ± 0.07
48	6.37 ± 0.05	7.88±0.04	8.01 ±0.05	9.54± 0.03	11.34 ± 0.02	11.72 ± 0.02 0.02
60	3.93 ± 0.04	3.88±0.02	4.19±0.02	7.90± 0.03	5.11±0.02	6.06± 0.06

Table 2. Reducing sugar concentration% and hydrolysis yield% after enzymatic hydrolysis of treated SBP at 10% and 15% solid load for 48 h.

	10% solid load			15% solid load		
	SternEnzymeC21032	Cellic CTec2	A mixture of SternEnzymeC21032 and Cellic CTec2	SternEnzymeC21032	Cellic CTec2	A mixture of SternEnzymeC21032 and Cellic CTec2
RS 48h after enzymatic hydrolysis	6.37±0.05	7.88±0.04	8.01±0.05	9.54±0.03	11.34±0.02	11.72±0.02
Hydrolysis yield%	68.96±0.54	85.31±0.43	86.72±0.54	68.78±0.22	81.76±0.15	84.50±0.15

Fermentation

After enzymatic hydrolysis of pretreated SBP, the reducing sugars resulting from each 10 and 15% solid load were fermented to give ethanol by Saccharomyces cerevisiae. Two strains of S. cerevisiae (CY3079 and AH15) were used. Tables 3 and 4 showed the kinetics of ethanol produced by the two yeast strains. The highest ethanol yields by S. cerevisiae CY3079 and AH15 were 5.61 and 5.58% (0.38 and0.38 g/g; 73.91 and 73.51% of the theoretical value) of reduced sugar in hydrolyzed SBP with 15% solid load (equal 30 g ethanol / 100 g of hydrolyzed SBP which have 7.81 g glucose).

Results clearly appeared that the highest ethanol yield was 44.26 g/l with 73.91% of the theoretical value obtained from the fermentation of hydrolyzed SBP with 15% solid load by CY3079 strain.

Our results are better than those recorded by Sutton and Peterson (2001) and Ber† owska et al. (2016). Sutton and Peterson (2001) used bioengineered ethanol genic K. oxytoca to produce ethanol from beet pulp and reported that it produced 5.4 g/l ethanol without fungal enzyme supplementation from pelleted pulp and 7.0 g/l ethanol from the pressed pulp. They found that by the inclusion of fungal enzymes (60 mg cellulase and 30 mg pectinase/ g DW SBP) increased ethanol production to 15.5 g/l ethanol using pelleted pulp, while fermentation of pressed pulp produced 18.3 g/l ethanol. While Bertowska et al. (2016) recorded that the highest ethanol concentration was 26.9±1.2 g/l with fermentation efficiency of 86.5±2.1% relative to the theoretical yield using the SSF technique of SBP suspended in 2% w/w sulfuric acid solution.

On the other side, Zheng et al. (2013) showed that the highest ethanol yield from acid pretreated SBP was 0.4 g ethanol/g dry matter in simultaneous scarification and fermentation (SSF). Rezi´c et al. (2013) reported that the maximum ethanol yield from 60 g/l of SBP substrate was 0.1 g ethanol/g of dry weight (0.25 g ethanol/ g total sugar content) with an ethanol fermentation efficiency of 49%.

Table 3. Fermentation Kinetics of ethanol yield from 10% and 15% hydrolyzed pretreated SBP using Stern enzyme C21032, Cellc CTec2 and a mixture of them by the CY3079 yeast strain at 28° C and a 5 pH for 72 h.

Kinetic Parameters	10% hydrolysed pretreated SBP			15% hydrolysed pretreated SBP		
	SternEnzymeC21032	Cellic CTec2	A mixture of SternenzymeC21032 and Cellic CTec2	SternEnzymeC21032	Cellic CTec2	A mixture of SternenzymeC21032 and Cellic CTec2
g/l	25.17 ±0.24	32.35±0.40	33.30±0.40	36.93±0.08	43.08±0.47	44.26±0.240.24
V\V%	3.19±0.03	4.1 ±0.05	4.22 ±0.05	4.68±0.01	5.46 ±0.06	5.61 ±0.03
% of theoretical	77.33 ±0.74	80.32±0.98	81.35±0.97	75.75±0.16	74.34±0.81	73.91±0.39
$Y_{g/gIS}$ *	0.40±0.00	0.41± 0.00	0.42 ±0.00	0.39±0.00	0.38 ±0.00	0.38±0.00
$Y_{g/gIsb}$ **	0.25±0.00	0.32 ± 0.00	0.33±0 .00	0.25 ±0.00	0.29 ± 0.00	0.30 ±0.00
$Y_{g/g\ beet\ pulp}$ ***	0.09±0.00	0.11 ± 0.00	0.11 ±0.00	0.08 ±0.00	0.10 ± 0.00	0.10 ±0.00

*Y g/gIS: Ethanol yield (gram) / initial sugar (gram)
Y g/gIsb : Ethanol yield (gram)/ solid load of substrate (gram)
*** Yg/g beet pulp: Ethanol yield (gram)/ initial beet pulp (gram)

Table 4. Fermentation Kinetics of ethanol yield from 10% and 15% hydrolyzed pretreated SBP using Stern Enzyme C21032, Cellic C Tec2 and a mixture of them by the AH15 yeast strain at 28° C and 5 pH for 72 h.

Kinetic Parameters	10% hydrolysed pretreated SBP			15% hydrolysed pretreated SBP		
	SternEnzymeC21032	Cellic CTec2	A mixture of SternenzymeC21032 and Cellic CTec2	SternEnzymeC21032	Cellic CTec2	A mixture of SternenzymeC21032 and Cellic CTec2
g/l	25.48±0.24	31.32±0.40	32.59±0.40	37.08±0.08	42.53 ± 0.48	44.03±0.240.24
V\V%	3.23±0.03	3.97±0.05	4.13 ±0.05	4.70±0.01	5.39 ±0.06	5.58±0.03
%of theoretical	78.29 ±0.73	77.79±0.98	79.62±0.97	76.07 ±0.16	73.38±0.82	73.51±0.39
Y g/gIS *	0.40±0.00	0.40 ± 0.00	0.41 ±0.00	0.39±0.00	0.38 ±0.00	0.38±0.00
$Y_{g/gIsb}$ **	0.25±0.00	0.31 ± 0.00	0.33±0.00	0.25 ±0.00	0.28 ± 0.00	0.29 ±0.00
$Y_{g/g\ beet\ pulp}$ ***	0.09±0.00	0.11 ± 0.00	0.11 ±0.00	0.08 ±0.00	0.10 ± 0.00	0.10 ±0.00

*Y g/gIS: Ethanol yield (gram) / initial sugar (gram)
**Y g/gIsb **: Ethanol yield (gram)/ solid load of substrate (gram)
***Yg/g beet pulp: Ethanol yield (gram)/ initial beet pulp (gram)

Conclusions

From our results, it can conclude that the SBP, as an inexpensive byproduct of sugar beet production, provided alternative raw materials for bio-ethanol production. The diluted acid treatment used in this study is effective and highly decreased hemicellulose with increasing cellulose content to 83.22% in the treated SBP. The highest reducing sugar percentage reached 11.72% with 84.50% hydrolysis yield from 15% substrate using the mixture of the two enzymes (SternenzymeC21032, Cellic CTec2). The highest ethanol yield was 44.26 g/l with 73.91% of the theoretical value which was obtained from the fermentation of hydrolyzed SBP with 15% solid load by CY3079 strain. This ethanol yield equals 30 g ethanol / 100 g of hydrolyzed SBP which have 7.81 g glucose). According to the results recorded in this study, each ton of dried SBP gives 100 kg of ETHANOL. This level is relatively low, and more experiments are still needed to increase the productivity of this bioprocess.

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