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Enzymatic Conversion of Sugar Beet Pulp: A Comparison of Simultaneous Saccharification and Fermentation and Separate Hydrolysis and Fermentation for Lactic Acid Production

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SUMMARY

This study compares the efficiency of lactic acid production by separate hydrolysis and fermentation (SHF) or simultaneous saccharification and fermentation (SSF) of sugar beet pulp, a byproduct of industrial sugar production. In experiments, sugar beet pulp was hydrolyzed using five commercial enzymes. A series of shake flask fermentations were conducted using five selected strains of lactic acid bacteria (LAB). The differences in the activities of the enzymes for degrading the principal sugar beet pulp components were reflected in the different yields of total reducing sugars. The highest yields after hydrolysis and the lowest quantities of insoluble residues were obtained using a mixture (1:1) of Viscozyme® and Ultraflo® Max. In the SHF process, only a portion of the soluble sugars released by the enzymes from the sugar beet pulp was assimilated by the LAB strains. In SSF, low enzyme loads led to reduction in the efficiency of sugar accumulation. The risk of carbon catabolic repression was reduced. Our results suggest that SSF has advantages over SHF, including lower processing costs and higher productivity. Lactic acid yield in SSF mode (approx. 30 g/L) was 80–90 % higher than that in SHF.

Key words: sugar beet pulp, enzymatic hydrolysis, lactic acid

INTRODUCTION

Organic waste, primarily lignocellulosic materials, is being generated in ever larger quantities, particularly by the food industry (*1*). To address environmental concerns over organic waste disposal, it is necessary to find cost-effective uses for these byproducts. One such byproduct, which is produced in large amounts, is sugar beet pulp. Sugar beet pulp remains after sucrose extraction from sliced beet roots. It is a valuable renewable source of polysaccharides and its bioconversion has great biotechnological potential (*2-4*). Lignocellulosic biomass has been increasingly used as a substrate in biotechnological processes, mainly for the production of biofuels and organic acids. The principal components of lignocellulosic materials are cellulose, hemicellulose and lignin (*1*).

Sugar beet pulp has until now been used mainly as an animal feed (*5*). However, it is increasingly converted into bioproducts such as yeast biomass (*6*) or biogas (*2*). This study, conducted at Lodz University of Technology, Poland, shows for the first time that sugar beet pulp is also a promising feedstock for lactic acid biosynthesis (*7,8*).

Biomass pretreatment is a crucial step in its hydrolysis, as it breaks down the crystalline structure of cellulose and decomposes lignin (*9,10*). The low lignin content of sugar beet pulp (approx. 2 %) means that cost-intensive treatments are not required to depolymerize the polysaccharide fractions. An increasingly popular method of biomass saccharification is enzymatic hydrolysis, either separated from (SHF) or coupled with (SSF) fermentation (*10-13*). In SHF processes, biomass hydrolysis and hydrolysate fermentation are two separate steps, which may be carried out in different vessels under conditions that are optimized separately (*11*). The disadvantage of this system is the inhibition of hydrolytic enzymes by reaction products (glucose, cellobiose or xylose), which reduces the yield of fermentable sugars from polysaccharides. Glucose and cellobiose inhibit the activities of the cellulases

(*10-15*). On the other hand, SSF is carried out in a single reactor, containing lignocellulosic substrates, hydrolytic enzymes and microorganisms. Fermentable sugars released by the enzymes from the substrates are rapidly consumed by the microorganisms conducting fermentation (*14,16*). Because of the utilization of sugars by the microorganisms, concentrations of glucose and other fermentable sugars are maintained at relatively low levels that do not cause enzyme inhibition (*14*). Simultaneous saccharification and fermentation is an attractive method, due to the lower initial investment required and shorter process time than for SHF (*12*). The most important consideration is the enzyme load. In this study, we therefore report on the optimization of enzyme dosing for effective saccharification and lactic acid fermentation of sugar beet pulp. The main aim of this study is to compare two modes of fermenting lactic acid from sugar beet pulp: separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF).

MATERIALS AND METHODS

LAB strains

Lactic acid fermentation was conducted using three collection strains from the Polish Collection of Microorganisms (IITD PAN, Wrocław, Poland): *Lactococcus lactis* PCM 2379, *Lactobacillus acidophilus* PCM 2510 and *Lactobacillus delbrueckii* PCM 490, as well as two environmental isolates: *Lactobacillus plantarum* R and *Lactobacillus plantarum* HII.

Enzyme preparations

Sugar beet pulp hydrolysates were obtained using five commercial enzyme preparations: NS-22086, NS-22119, Viscozyme® and Ultraflo® Max (all from Novozymes, Bagsvžrd, Denmark) and Cellulosoft Ultra L (Novo Nordisk, Bagsvžrd, Denmark). Degradation of cellulose, pectin, xylan and sucrose by enzymes contained in the preparations was assayed at 50 °C and pH=5.0 using 0.4 % carboxymethylcellulose (CMC) (Merck, Kenilworth, NJ, USA), 0.5 % citrus pectin (Merck), 0.5 % birch xylan (Merck) and 1 % sucrose (Avantor Performance Materials Poland, Gliwice, Poland). Reducing sugars released from these substrates were quantified within 5 min using the alkaline 3',5'-dinitrosalicylic acid (DNS; Merck) reagent (*17*). Activity was expressed in international activity units (μmol of reducing sugars released in 1 min per 1 mL of enzyme preparation).

LAB enzyme profiles

Prior to testing, the bacterial biomass was suspended in 2 mL of distilled water, producing a very dense suspension (6 °McF, *i.e.* approx. $18 \cdot 10^8$ CFU/mL). Two drops of the bacterial suspensions were added to each of 20 cupules in an API ZYM strip (bioMérieux, Marcy-l'Étoile, France). The strip was placed in a chamber pre-moistened with 5 mL of distilled water. The chamber was incubated at 37 °C for 4 h. After incubation, one drop of each of the API reagents A and B was added to each of the cupules. The resulting colours were recorded as intensities which were read to give a semiquantitative notation (0 to 5) using a colour code supplied by the manufacturer. Each strain was tested at least twice to ensure the reproducibility of the results.

Preparation of inoculum

Enzymatic sugar beet pulp hydrolysates (from a sugar factory in Dobrzelin, Poland) were supplemented with (in g/L): yeast extract 4.0, beef extract 8.0 and peptone K 10.0 (all from BTL sp. z o.o., Łódź, Poland), ammonium citrate 2.0, dipotassium phosphate 2.0, sodium acetate 5.0, magnesium sulfate 0.2 and manganese sulfate 0.05 (all from Avantor Performance Materials Poland, Gliwice, Poland), sterilized for 15 min at 120 °C and used for the propagation of LAB strains. The LAB strains were grown in glass test tubes at 37 °C for 48 h. At least three cultivation passages were conducted prior to each SHF and SSF process.

Enzymatic hydrolysis of sugar beet pulp

Fresh sugar beet pulp was frozen and stored at -25 °C. Prior to enzymatic hydrolysis, the samples were thawed and suspended in plain warm water (50 °C) to achieve a mass per volume ratio on dry mass basis of approx. 10 %. Enzymatic sugar beet pulp hydrolysates were obtained in triplicate using each, and some mixtures, of the commercial enzyme preparations listed above. Sugar beet pulp saccharification was initiated by the addition of a suitable dose of enzyme preparation, and continued at 50 °C with agitation for up to 24 h. Samples of the hydrolysates were withdrawn at fixed time intervals and the mass fractions of reducing sugars was analyzed to monitor the progress of saccharification. At the end of hydrolysis, the enzymes were inactivated by heating at 80 °C for 10 min. The insoluble residues from hydrolysis were separated by filtration (filter paper MN 614 ¼, *d*=320 mm; Macherey-Nagel, Düren, Germany) and their dry mass was determined after drying at 105 °C to constant mass in analytical dryer (POL-EKO-Aparatura, Wodzisław Śląski, Poland).

Fermentation of sugar beet pulp hydrolysate

Fermentation was carried out in triplicate, in 100-mL Erlenmeyer flasks containing 50 mL of the liquid fraction of the sugar beet pulp enzymatic hydrolysates (after filtration) supplemented with (in g/L): yeast extract 4.0, beef extract 8.0 and peptone K 10.0 (all from BTL sp. z o.o), ammonium citrate 2.0, dipotassium phosphate 2.0, sodium acetate 5.0, magnesium sulphate 0.2 and manganese sulfate 0.05 (all from Avantor Performance Materials Poland). Sterile calcium carbonate ($CaCO_3$; Avantor Performance Materials Poland) suspension was added to stabilize the pH. The sterilized medium was inoculated with LAB monocultures and incubated at 37 °C for 48 h.

Simultaneous saccharification and fermentation

SSF processes were carried out in triplicate, in 100-mL Erlenmeyer flasks containing 5.7 g of sugar beet pulp dry mass (from the sugar factory in Dobrzelin, Poland), in the form of wet pulp containing approx. 80 % moisture, 25 g of supplement solution and 1 g of $CaCO_3$. The samples were replenished with water to achieve a final water content of 50 mL per each sample. The supplement solution consisting of (in g/L): yeast extract 4.0, beef extract 8.0 and peptone K 10.0 (all from BTL sp. z o.o), ammonium citrate 2.0, dipotassium phosphate 2.0, sodium acetate 5.0, magnesium sulfate 0.2 and manganese sulfate 0.05 (all from Avantor Performance Materials Poland) was added. The medium was sterilized for 15 min at 120 °C. The process was initiated by the addition of five different doses of two commercial multi-enzyme preparations, Ultraflo Max and Viscozyme from Novozymes (Novozymes). Pre-hydrolysis was conducted at 37 °C for 10 h. Then, the medium was inoculated with LAB monocultures and incubated for 48 h at 37 °C. Further incubation at 80 °C was conducted for 15 min to deactivate the enzymes and microbial cells, and to improve the solubility of the calcium lactate (*7*). To evaluate the efficiency of hydrolysis, the mass of the obtained hydrolysate was measured after separation of the solid fraction for 10 min at 3000×*g* (centrifuge model 5805 R; Eppendorf AG, Hamburg, Germany).

Analysis of hydrolysates and culture media

The concentration of lactate ions was measured spectrophotometrically using a D-/L-Lactic Acid (K-DLATE) Assay Kit (Megazyme, Bray, Ireland). To improve the solubility of the calcium lactate, the samples were heated at 80 °C for 15 min.

The monosaccharide profile of the sugar beet pulp hydrolysates was analyzed using UV-spectrophotometer (Thermo Scientific Multiskan GO; Thermo Fisher Scientific, Munich, Germany) and Megazyme Kits: D-xylose assay kit (K-XYLOSE) for xylose, D-glucuronic acid and D-galacturonic acid assay kit (K-URONIC) for galacturonic acid determination, raffinose/D-galactose assay kit (K-RAFGA) for raffinose, D-mannose, D-fructose and D-glucose assay kit (K-MANGL) for glucose, mannose and fructose, L-arabinose and D-galactose assay kit (K-ARGA) for arabinose and L-rhamnose assay kit (K-RHAMNOSE) for rhamnose. The assays were conducted according to the manufacturer's instructions.

Statistical analysis

Both the biological experiments and analytical tests were conducted in triplicate. The results were analyzed statistically to find the standard deviation in Origin v. 8.5.1 (OrginLab Corporation, Northampton, MA, USA) (*18*), which is indicated in the graphs as error bars.

RESULTS AND DISCUSSION

The aim of biomass enzymatic degradation is to break down polymeric substances into easily fermentable sugars (*19*). Sugar beet pulp has been shown to provide good yields of carbohydrates during hydrolysis. However, high levels of enzymes or chemicals are usually required for its depolymerization (*20*). Therefore, we decided to compare the enzyme doses required in SHF with those needed for SSF.

Selection of enzyme preparation

Many researchers have reported the use of commercial enzyme preparations (Multieffect XL, Celluclast, Novozym 342, Novozymes 188, SP 584, Cellulase, Novozym 431, Viscozyme L, Pectinex Ultra SPL, Celustar XL and Cellulyve Tr 300G+AN 6000) for the saccharification of sugar beet pulp (*21-25*).

In our study, the enzymes were evaluated based on a comparison of the effects of five commercial preparations. All of the preparations showed enzyme activity for depolymerization of the principal sugar beet pulp components, such as cellulose, hemicelluloses and pectin (**Table 1**). Viscozyme and NS-22119 showed the highest pectinolytic enzyme activity, approx. 400 and 350 U/mL respectively, while the highest cellulolytic activities were obtained with Cellulosoft and NS-22086 (approx. 70 and 60 U/mL, respectively). The latter was characterized by higher xylanase activity (approx. 210 U/mL) than the other four preparations. With the exception of Cellulosoft, the preparations also exhibited the activity for invertase. All of the preparations contained reducing sugars, including glucose. This was considered when calculating the yield of sugar beet pulp enzymatic saccharification.

The differences in the activities of the enzymes for degrading the principal sugar beet pulp components were reflected by the different yields of total reducing sugars and glucose that were released from the sugar beet pulp over 24 h of hydrolysis at 50 °C (**Table 2**).

According to the literature, cellulose degradation is more efficient when both pectinases and cellulases are used. Micard *et al.* (*21*) reported that the degree of hydrolysis could be increased significantly by applying pectinolytic mixture SP 584, followed by cellulosic Celluclast. Therefore, because of the relatively large amounts of insoluble residues that remained after sugar beet pulp hydrolysis using a single enzyme preparation (at least 40 % of dry mass of Viscozyme and NS-22119), in our study mixtures of Viscozyme and either Ultraflo Max or Cellulosoft were also tested. The highest yields on dry mass basis of total reducing sugars (approx. 18.9 %) and glucose (approx. 8.6 %) and the lowest mass fractions of insoluble residues (less than 35 %) were obtained using a mixture (1:1) of Viscozyme and Ultraflo Max (**Table 2**). The relationship between the dose of this mixture and the results of sugar beet pulp hydrolysis was therefore further investigated (**Table 3**). When 0.1 mL of enzyme mixture was added per each gram of dry mass, this led to liquefaction of 88.9 % of the sugar beet pup. A three times lower dose gave slightly worse results (85.6 % liquefaction), whereas a six times lower dose liquefied only 65.1 % of the dry biomass.

Table 1. Activities of tested enzymes evaluated *via* degradation of cellulose, pectin, xylan and sucrose at 50 °C and pH=5.0

Enzyme	Activity/(U/mL)				
	Viscozyme®	Ultraflo® Max	Cellulosoft Ultra L	NS-22086	NS-22119
Cellulase	13.9±1.1	27.8±3.1	72.3±8.2	62.1±5.9	10.8±1.2
Xylanase	25.1±2.3	127.4±11.8	46.7±4.1	214.2±24.5	17.7±2.0
Pectinase	412.2±39.4	24.2±2.6	11.6±1.3	135.4±12.8	353.8±30.4
Invertase	84.5±7.9	2.1±0.3	0.4±0.1	0.9±0.1	92.5±9.1

Results are presented as mean value±standard deviation

Table 2. Efficiency on dry mass basis of sugar beet pulp hydrolysis by enzyme preparations in the first step in the SHF mode

Enzyme preparation	*w*/%		
	Reducing sugars	Glucose	Insoluble residue
Cellulosoft Ultra L	2.5	1.1	77.9
NS-22086	1.0	0.7	90.1
NS-22119	11.9	3.5	43.2
Ultraflo Max®	0.9	0.8	88.1
Viscozyme®	16.8	4.2	40.3
Viscozyme®+Cellulosoft (1:1)	19.0	5.4	34.8
Viscozyme®+Ultraflo Max® (1:1)	18.9	8.6	35.2

V(enzyme)=0.1 mL, *m*(SBP)/*V*(solution)=10 %, SHF=separate hydrolysis and fermentation

Table 3. Effect of Viscozyme® and Ultraflo® Max doses on the efficiency of sugar beet pulp liquefaction and saccharification for further fermentation process (SHF mode)

V(enzyme)/mL*	Reducing sugars		Glucose		Insoluble residue
	m/g	*w*/%	*m*/g	*w*/%	*w*/%
0.2	12.6	38.5	6.5	21.2	10.6
0.1	10.8	37.4	5.6	17.9	12.5
0.03	8.4	27.3	4.1	14.1	15.3
0.017	5.7	19.1	2.5	9.2	32.3
0.008	4.9	15.8	2.1	7.2	36.5
0.003	2.4	7.8	1.1	3.4	61.3
0.001	1.6	4.8	0.5	2.2	67.7
0.00075	0.8	2.8	0.3	1.3	75.4
0.0005	0.4	1.4	0.2	0.5	83.1

*Expressed per 1 g of sugar beet pulp dry mass. Each reaction mixture contained 30.0 g of sugar beet pulp dry mass corresponding to a substrate mass per volume ratio of 10 %. SHF=separate hydrolysis and fermentation

Separate hydrolysis and fermentation

The main components of sugar beet pulp biomass are cellulose, hemicellulose and pectin. Generally, treatment of this substrate using a mixture of cellulase and pectinase improves saccharification efficiency compared to the treatment with cellulase alone (*22*). The sugar profiles of sugar beet pulp hydrolysates obtained after 24 h using a mixture of Viscozyme and Ultraflo Max in six different doses (0.1, 0.03, 0.017, 0.012, 0.008 and 0.003 mL per 1 g of sugar beet pulp) are shown in **Table 4**. For enzyme doses of 0.1 mL/g the following concentrations of tested carbohydrates were determined (in g/L): raffinose approx. 22, arabinose and galactose approx. 12 and 14 respectively, glucose approx. 11, fructose approx. 6, xylose approx. 1, mannose approx. 1 and galacturonic acid approx. 1.5. A one-third reduction of the enzyme doses resulted in a 50 % decrease in glucose content and a 20–30 % decrease of fructose, mannose, arabinose, galactose and raffinose. Further lowering of the Viscozyme and Ultraflo Max loads caused a decrease in hydrolysis efficiency. The most significant reductions were observed of raffinose, galactose, arabinose and glucose. Glucose is released mainly from cellulose, while the hemicelluloses and pectin in sugar beet pulp provide sources for arabinose and galactose. Xylose and mannose are components of sugar beet pulp hemicelluloses, while galacturonic acid is a building block of pectin (*25*). Pectinolytic activity has the main effect on galactose release. Likewise, the addition of

Table 4. The composition on dry mass basis of the sugar beet pulp hydrolysates after hydrolysis conducted with different enzyme doses

	(*V*(enzyme)/*m*(substrate))/(mL/g)					
	0.1	0.03	0.017	0.012	0.008	0.003
Carbohydrate	*γ*/(g/L)					
Glucose	10.2±1.05	4.5±0.36	2.6±0.19	2.2±0.19	1.1±0.12	0.5±0.06
Fructose	5.7±0.62	4.5±0.52	3.8±0.33	3.5±0.31	0.1±0.02	0.0±0.00
Mannose	1.1±0.21	0.7±0.06	0.5±0.04	0.2±0.01	0.1±0.01	0.1±0.01
Arabinose	11.9±1.42	9.8±0.82	8.8±0.52	5.5±0.63	4.6±0.44	2.8±0.42
Galactose	14.5±1.22	10.5±1.00	10.2±1.27	5.5±0.33	2.5±0.22	2.6±0.23
Raffinose	22.1±1.78	15.2±1.68	14.3±1.44	8.1±0.78	0.8±0.08	0.7±0.08
Rhamnose	0.5±0.06	0.5±0.06	0.5±0.07	0.4±0.06	0.4±0.06	0.4±0.05
Xylose	1.0±0.09	1.0±0.08	1.0±0.09	0.9±0.07	0.8±0.07	0.8±0.07
Galacturonic acid	1.5±0.17	1.3±0.14	1.2±0.15	0.8±0.07	0.7±0.07	0.5±0.07

Results are presented as mean value±standard deviation

a preparation containing pectin results in degradation of cellulose and increases the amount of arabinose released from hemicelluloses (*25*).

Pectinase was therefore used as a sugar feedstock for lactic acid fermentation conducted with selected LAB strains. The efficiency of the process is presented in **Fig. 1**. The strains produced 12–14 g/L of lactic acid.

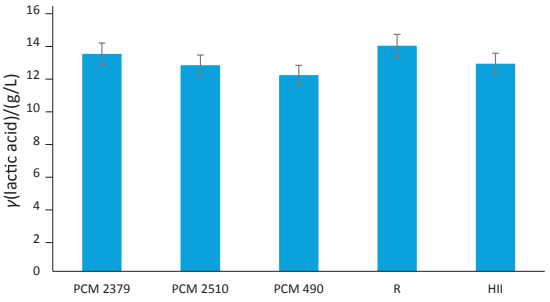

Fig. 1. Efficiency of lactic acid fermentation (g/L) in the separate hydrolyses and fermentation (SHF) system (by 0.1 mL of enzymes per g of sugar beet pulp dry mass) with *Lactococcus lactis* PCM 2379, *Lactobacillus acidophilus* PCM 2510, *Lactobacillus delbrueckii* PCM 490, *Lactobacillus plantarum* R and *Lactobacillus plantarum* HII

In SHF, lignocelluloses are hydrolyzed to monomers and then fermented. The major advantage of this solution is that it is possible to carry out the depolymerization and biological utilization of carbohydrates under conditions optimal for each process (*26,27*). Analysis of the sugar profiles of the residues of fermentation media showed that only a portion of the soluble sugars released by the enzymes from the sugar beet pulp was assimilated by the applied strains of LAB in SHF processes (**Table 5**). However, the sugar utilization profiles and acidification dynamics were found to be strain-dependent (*5*). In all cases, the pools of glucose, fructose and mannose were metabolized almost completely, whereas galactose, arabinose, xylose and raffinose concentrations were stable or increased. The higher content of non-utilized carbohydrates after fermentation confirms the progress of sugar beet pulp hydrolysis. In the presence of organic acids, due to the lower pH, pectin degradation occurred (*28*). Pectin derivatives were not metabolized by the tested strains, which may be explained by catabolite repression. This regulation effect occurs when the cells are exposed to more than one carbon source. Catabolite repression is usually related to glucose, which is utilized preferentially (*28-30*).

During hydrolysis, the release of sugars may be halted due to feedback inhibition, which requires more enzyme loads (*19*). Therefore, we considered the SSF mode with lower enzyme doses. In this process, enzymes work with fermenting microorganisms to convert the released components into bioproducts (*31*). Regarding the activities of enzymes correlated with carbohydrate catabolism, α-mannosidase and α-fucosidase were not detected, while α- and β-galactosidase, α- and β-glucosidase and β-glucuronidase activities were found in all tested strains. On the other hand, four strains showed higher galactosidase and glucosidase activities than β-glucuronidase activity, which could broaden the potential use of the tested LAB strains as lactic acid producers from plant material rich in polymeric components. The obtained data confirm the results of Iorizzo *et al.* (*32*) for these enzyme activities in LAB of plant origin.

The following amounts on dry mass basis of Viscozyme and Ultraflo Max were used in our experiments: 0.03, 0.017, 0.012, 0.008 and 0.003 mL/g. The efficiency of the process was measured in terms of the mass of liquid released from the hydrolyzed biomass suspension (*33*) (**Table 6**). The efficiency of hydrolysis depended on the dose of the enzyme mixture. Satisfactory results were achieved with a dosage of 0.03 mL/g of the tested enzymes. Further reducing the enzyme load lowered the efficiency of hydrolysis as follows: 10–15 % for processes conducted with enzyme doses of 0.017 and 0.012 mL/g and 30–50 % with enzyme doses of 0.008 and 0.003 mL/g.

Despite decreases in the level of biomass liquefaction at lower enzyme doses, the productivity of lactic acid fermentation remained at similar levels with the two tested strains. The amount of lactic acid biosynthesized in SSF was double (approx. 30 g/L) that produced during SHF (**Fig. 2**).

A significant reduction in sugar release rates (especially with 10-fold smaller doses: 0.003 mL/g) did not radically compromise lactic acid productivity. A concentration of 15 g of product per L of the fermentation medium was measured

Table 5. Carbohydrate profiles in sugar beet pulp hydrolysates after fermentation by LAB in SHF mode

LAB strain	γ/(g/L)								
	Glucose	Fructose	Mannose	Arabinose	Galactose	Rhamnose	Raffinose	Xylose	Galacturonic acid
L. lactis PCM 2379	1.1±1.0	0.13±0.03	0.0±0.0	15.46±0.06	18.1±1.2	1.7±0.1	21.0±2.3	2.4±0.2	2.0±0.2
Lb. acidophilus PCM 2510	2.2±0.2	0.39±0.03	0.0±0.0	13.6±0.3	17.9±0.7	2.2±0.4	19.9±0.4	2.58±0.05	2.3±0.5
Lb. delbrueckii PCM 490	1.7±0.3	0.22±0.04	0.0±0.0	12.6±0.4	13.3±1.3	2.5±0.3	17.4±0.6	2.82±0.06	2.0±0.4
Lb. plantarum R	1.5±0.4	0.23±0.05	0.01±0.00	14.0±0.7	16.2±1.3	2.0±0.2	20.6±0.4	2.4±0.1	2.4±0.5
Lb. plantarum HII	2.0±0.2	0.02±0.00	0.0±0.0	16.0±0.6	14.7±1.1	2.0±0.4	20.8±1.5	2.5±0.1	2.3±0.5

Results are presented as mean value±standard deviation, LAB=lactic acid bacteria, SHF=separate hydrolysis and fermentation

Table 6. Efficiency on dry mass basis of sugar beet pulp hydrolysis measured as the amount of liquid fraction obtained in SSF process

LAB strain	(*V*(enzyme)/*m*(substrate))/(mL/g)				
	0.03	0.017	0.012	0.008	0.003
	m(hydrolysate)/g				
L. lactis PCM 2379	45.5±0.6	36.8±0.4	33.1±1.5	28.6±1.4	22.5±1.5
Lb. acidophilus PCM 2510	44.3±0.7	39.5±0.4	35.1±0.4	25.6±0.6	24.4±1.8
Lb. plantarum HII	46.2±1.0	32.4±0.6	32.4±1.6	31.4±0.6	27.7±2.5
Lb. delbrueckii PCM 490	44.6±0.3	43.7±0.4	31.7±1.3	28.5±1.3	18.5±1.2
Lb. plantarum R	44.8±1.2	39.6±1.2	33.8±0.7	25.4±1.0	25.7±0.8

Results are presented as mean value±standard deviation, SSF=simultaneous saccharification and fermentation

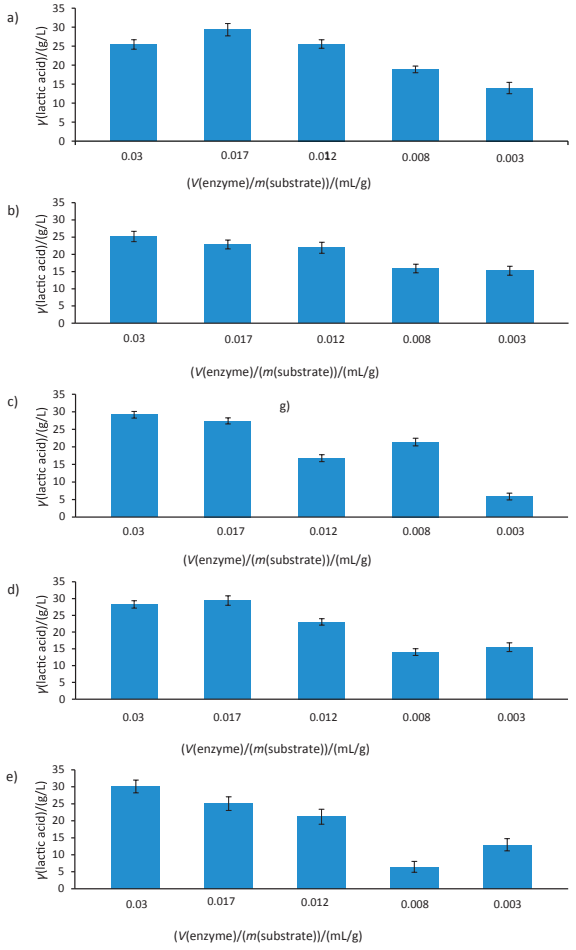

Fig. 2. Productivity of lactic acid (g/L) in the simultaneous saccharification and fermentation (SSF) processes conducted with different enzyme loads using: a) *Lactococcuss lactis* PCM 2379, b) *Lactobacillus acidophilus* PCM 2510, c) *Lactobacillus delbrueckii* PCM 490, d) *Lactobacillus plantarum* R, and e) *Lactobacillus plantarum* HII

with the lowest load of enzyme mixture; the same as in the case of SHF processes.

Analysis of the carbohydrate profiles of media obtained after SSF with low enzyme loads (**Table 7**) revealed reductions in the efficiency with which unfermented sugars were released. Total utilization was observed with glucose, fructose and mannose. The entire pool of these released saccharides cannot be determined, because they were metabolized immediately, preventing feedback inhibition. It can be concluded that under such conditions, in SSF mode, no inhibition of celleulases (present in both formulations) occurred. Low levels of glucose also reduce the risk of carbon catabolic repression, which may enable the use of saccharides such as arabinose and galactose (*33*). According to Ishola *et al.* (*31*), lowering the glucose content in the fermentation medium improves xylose uptake, thereby facilitating simultaneous sugar utilization. Öhgren *et al.* (*1*) also report the reduction of glucose inhibition during enzymatic hydrolysis and the detoxifying effect of SSF. These authors conclude that SSF is a superior process configuration to SHF.

The use of more than one carbon source may explain the relatively high efficiency of the fermentation processes. Research into lactic acid production using renewable materials as the carbon source has been conducted by several authors (*15,19,34-36*). The product yields of lactic acid reported in these studies range from 2.3–166 g/L. Substrates such as rice and wheat bran, wheat straw, cassava bagasse, molasses and wood hydrolysate showed the highest productivities, while the lowest amounts of lactic acid were produced from hydrolyzed xylan and wheat straw hemicellulose. Similar results to those in our study have been reported by Cui *et al.* (*35*), Tanaka *et al.* (*37*) and Yáñez *et al.* (*38*), who fermented corn stover, defatted rice bran and pretreated cardboard

Table 7. Carbohydrate profiles on dry mass basis of sugar beet pulp hydrolysates after fermentation by LAB in SSF process

LAB strain	(*V*(enzyme)/ *m*(substrate))/ (mL/g)	*γ*/(g/L)								
		Glucose	Fructose	Mannose	Arabinose	Galactose	Rhamnose	Raffinose	Xylose	Galacturonic acid
L. lactis PCM 2379	0.03	0.0±0.0	0.0±0.0	0.0±0.0	15.2±0.9	16.0±1.2	0.52±0.07	13.1±0.8	3.6±0.5	14.9±0.3
	0.017	0.0±0.0	0.0±0.0	0.0±0.0	4.9±0.3	5.4±0.5	0.39±0.05	4.7±0.2	2.11±0.07	12.2±0.2
	0.012	0.0±0.0	0.0±0.0	0.0±0.0	1.6±0.5	1.9±0.9	0.5±0.2	1.2±0.2	1.4±0.1	11.3±0.6
Lb. acidophilus PCM 2510	0.03	0.0±0.0	0.0±0.0	0.0±0.0	13.9±1.3	14.2±1.4	1.29±0.06	15.4±1.1	1.96±0.05	14.1±1.0
	0.017	0.0±0.0	0.0±0.0	0.0±0.0	5.5±0.4	5.5±0.6	0.83±0.03	6.1±0.8	1.7±0.4	13.2±0.4
	0.012	0.0±0.0	0.0±0.0	0.0±0.0	2.0±0.4	1.6±0.5	0.69±0.03	0.58±0.08	1.11±0.09	11.1±0.3
Lb. delbrueckii PCM 490	0.03	0.0±0.0	0.0±0.0	0.0±0.0	12.8±0.8	15.0±0.5	1.51±0.09	15.2±1.6	2.8±0.2	14.8±1.0
	0.017	0.0±0.0	0.0±0.0	0.0±0.0	5.2±0.3	5.0±0.2	1.1±0.2	5.5±0.9	1.4±0.4	11.1±0.9
	0.012	0.0±0.0	0.0±0.0	0.0±0.0	3.1±0.9	2.4±0.6	0.87±0.03	2.7±0.9	1.09±0.09	9.0±0.9
Lb. plantarum R	0.03	0.0±0.0	0.0±0.0	0.0±0.0	9.9±0.8	11.5±1.8	0.6±0.2	6.2±0.6	2.1±0.7	15.1±0.8
	0.017	0.0±0.0	0.0±0.0	0.0±0.0	4.8±0.6	5.7±0.3	0.58±0.15	3.0±0.5	1.6±0.5	14.0±1.1
	0.012	0.0±0.0	0.0±0.0	0.0±0.0	3.4±0.5	3.0±0.4	0.46±0.06	2.0±0.3	1.3±0.2	10.5±0.3
Lb. plantarum HII	0.03	0.0±0.0	0.0±0.0	0.0±0.0	8.9±0.6	6.3±0.8	1.4±0.2	15.8±1.0	2.6±0.3	14.4±0.7
	0.017	0.0±0.0	0.0±0.0	0.0±0.0	6.4±0.5	0.9±0.1	1.4±0.2	9.3±0.2	2.2±0.7	11.6±0.9
	0.012	0.0±0.0	0.0±0.0	0.0±0.0	0.7±0.1	0.3±0.1	0.81±0.07	0.35±0.06	1.5±0.3	8.8±0.2

Results are presented as mean value±standard deviation, LAB=lactic acid bacteria, SSF=simultaneous saccharification and fermentation

using *Lb. rhamnosus* and *Lb. brevis*, *Lb. delbrueckii* IFO 3202 and *Lb. coryniformis* ATCC 25600, respectively (*15*).

The risk of microbial contamination is much lower in SSF than in SHF and fewer vessels are used, resulting in lower costs. The difference between the optimum conditions for enzymatic hydrolysis and those for fermentation could cause problems (*39*). However, a comparison of SHF and SSF conducted by Rana *et al.* (*40*) showed that SSF was more efficient than SHF, despite using a lower reaction temperature, which is suboptimal for enzyme hydrolysis. The lower temperatures and shorter operating time required for SSF processes result in energy savings, which is a significant advantage of the system (*16,41,42*). However, some disadvantages may occur during scale-up. The separate hydrolysis of sugar beet pulp was successfully implemented in a 3 m^3 stirred reactor at a sugar factory in Dobrzelin (*7,43*), but with smaller enzyme doses such parameters as the presence of a dispersed gas phase and turbulent flow may be more significant for the efficiency of the process.

CONCLUSION

Two variants of laboratory scale enzymatic hydrolysis and fermentation of sugar beet pulp were studied: simultaneous saccharification and fermentation (SSF) and separate hydrolysis and fermentation (SHF). However, previous comparative studies of these two modes focused on ethanol fermentation processes. The present study suggests that there may be some analogies with lactic acid fermentation. The results indicate that SSF has advantages over SHF due to the lower enzyme loads required and the higher concentrations of produced lactic acid. Using 50 % lower enzyme doses, SSF produced 80–90 % more lactic acid than the SHF process. The initial conditions of the process appear to have been more important when bacterial strains were used for lactic acid fermentation than yeast. In particular, the efficiency of prehydrolysis (with different enzyme doses) gave varying of fermentable sugars, and was the deciding parameter for fermentation yield.

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