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Integrated Hydrolyzation and Fermentation of Sugar Beet Pulp to Bioethanol

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Sugar beet pulp is an abundant industrial waste material that holds a great potential for bioethanol production owing to its high content of cellulose, hemicelluloses, and pectin. Its structural and chemical robustness limits the yield of fermentable sugars obtained by hydrolyzation and represents the main bottleneck for bioethanol production. Physical (ultrasound and thermal) pretreatment methods were tested and combined with enzymatic hydrolysis by cellulase and pectinase to evaluate the most efficient strategy. The optimized hydrolysis process was combined with a fermentation step using a *Saccharomyces cerevisiae* strain for ethanol production in a single-tank bioreactor. Optimal sugar beet pulp conversion was achieved at a concentration of 60 g/l (39% of dry weight) and a bioreactor stirrer speed of 960 rpm. The maximum ethanol yield was 0.1 g ethanol/g of dry weight (0.25 g ethanol/g total sugar content), the efficiency of ethanol production was 49%, and the productivity of the bioprocess was 0.29 g/l·h, respectively.

Keywords: Sugar beet pulp, bioethanol, ultrasound pretreatment, thermal pretreatment, enzymatic hydrolysis, *Saccharomyces cerevisiae*

Introduction

Sugar beet pulp is a lignocellulosic by-product of the table sugar industry. By 2005, the worldwide sugar beet production reached a total of 242 million metric tons per year and accounted for 35% of the global sugar production [12]. Each ton of sugar beet potentially yields 830 kg of sugar juice and 170 kg of wet sugar beet pulp after sucrose extraction. Sugar beet pulp is especially rich in pectin (38-62%), hemicelluloses (24-32%), and cellulose (22-30%), but has a low lignin content (~1%). Cellulose is a homopolymer of β-1,4-linked glucose residues, whereas hemicelluloses are heteropolymers of glucose and various pentoses, mainly arabinose and xylose. Sugar beet pectin is a heterogeneous polymer that is mainly composed of polygalactans, arabinan, rhamnose, and ferulic acid [2, 14, 19, 25]. Sugar beet pulp is considered a waste material and, therefore, it is mainly used as a cheap and readily available carbohydrate source for the production of renewable energy, especially liquid fuels.

In this context, a commonly followed strategy is the treatment of sugar beet pulp with cellulolytic and hemicellulolytic enzymes to hydrolyze the substrate into simple sugars that can be converted to ethanol by anaerobic fermentation [9, 10, 20, 28]. A major drawback for reasonable ethanol yield is the high chemical and physical integrity of sugar beet pulp. Consequently, the pretreatment is a crucial process operation that modifies the lignocellulose structure and enhances its accessibility for hydrolytic enzymes.

Depending on the source and composition of lignocellulosic raw materials, various physical, chemical, and biological pretreatment methods are available. Physical methods (milling, grinding, and steam-heating) in combination with chemical pretreatments (acid-base treatment, organic solvent extraction, and SO_2 pretreatment) are usually the methods

of choice. Disadvantages of physical and chemical pretreatments are their high energy and equipment costs and the accumulation of ecologically harmful solvents. Biological methods rely entirely on microbial or enzymatic pretreatment or combinations thereof [1].

Fermentation can be carried out by using different cultivation modes (batch, Fed-batch, repeated-batch, continuous with or without biomass recycling) in different bioreactor types (stirred tank bioreactor, packed bed, fluid bed, or different types of tubular and drum bioreactors) and with various microorganisams (*e.g.*, *Saccharomyces cerevisiae*, *Pachysolen tannophilus*, *Zymomonas mobilis*, *Escherichia coli*). The glucose released from cellulose can be readily converted to ethanol, but pentoses and galacturonan derived from hemicellulose and pectin cannot be metabolized by most of the available microorganisms [26]. An advantage of *Z. mobilis* and *E. coli* is their ability to produce ethanol also from pentoses; however, only moderate yields were obtained. On the other hand, *S. cerevisiae* is an extensively studied organism that is "generally regarded as safe" (GRAS) and can be used within the food and pharmaceutical industries [16]. A comprehensive approach for bioethanol production including hydrolysis and fermentation, termed simultaneous saccharification and fermentation (SSF), has been successfully applied recently. The advantage of SSF is the removal of glucose by fermentation, which avoids feedback inhibition by cellulolytic enzymes [14].

The aim of this research paper was to establish an integral and environmentally friendly bioprocess for bioethanol production from sugar beet pulp. Therefore, we compared physical and biological pretreatment methods in combination with bioethanol fermentation using *S. cerevisiae*. A three-step process was established, including (i) thermal or ultrasound pretreatment of sugar beet pulp, followed by (ii) enzymatic hydrolysis and (iii) anaerobic fermentation by *S. cerevisiae*.

Materials and Methods

Raw Material

Sugar beet pulp was purchased from Sladorana d.o.o. (Županja, Croatia). The moisture content in the fresh sugar beet pulp was 61%. Prior to all experimental procedures, sugar beet pulp was ground by a coffee mill (Moulinex; type-505) to obtain a final particle size of 0.3-0.5 mm.

Sugar Beet Pulp Ultrasound and Thermal Pretreatment

Sugar beet pulp pretreatment experiments were performed in 500 ml Erlenmeyer flasks filled with 200 ml of a 60 g/l sugar beet pulp slurry (23.4 g/l dry weight) in 50 mM citrate buffer, pH 4.8. Ultrasound pretreatment was performed with a SONOPLUS HD 2200 homogenizer in continuous mode by applying 50% of the maximum power (0.2 kW) for 5, 15, 25, 35, and 45 min. Thermal pretreatment was performed in a lab-scale autoclave by heating the sugar beet pulp slurry to 121°C for 20 min at a pressure of 1.2 bar.

Enzymatic Treatment of Sugar Beet Pulp

Commercially available cellulases (Sigma; cellulase from *Aspergillus niger*) and pectinases (Pascal Biotech; Endozym PR) were used for sugar beet pulp saccharification. Cellulase activity, expressed as filter paper units (FPU), was determined as the amount of enzyme that releases 1 μM glucose from Whatman No. 1 filter papers per minute at pH 5.0 and 50°C. One ferment depectinization unit (FDU) was defined as the amount of pectinase that depolymerizes 33.3 ml of a standard juice containing 0.4 g/l of pectin in 2 h at pH 3.5 and 55°C. Purchased enzyme concentrates

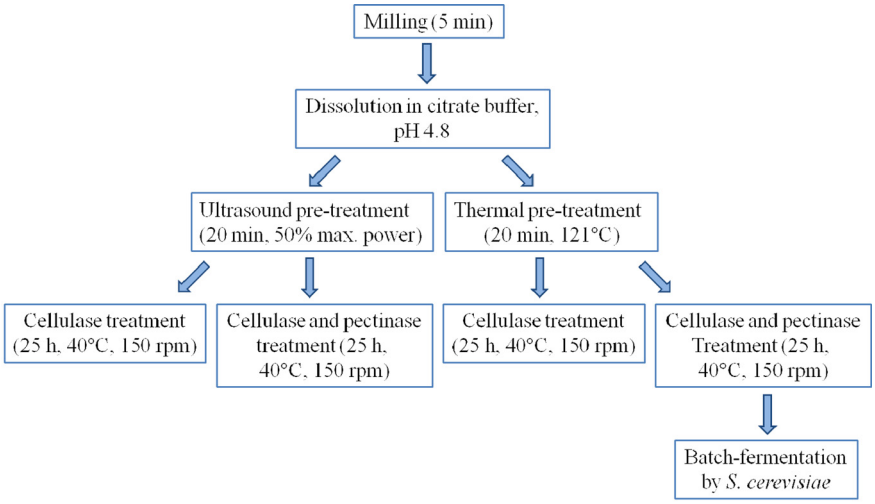

Fig. 1. Flow chart of sugar beet pulp pretreatment and treatment methods before bioethanol production.

had cellulase activity of 1.4 FPU/mg dry weight and pectinase activity of 3,000 FDU/ml, respectively. Enzyme hydrolysis was performed in 500 ml unbaffled Erlenmeyer flasks containing the pretreated sugar beet pulp slurries. The samples were incubated on a rotary shaker (150 rpm) at 40°C for 25 h. A flow chart of the sugar beet pulp pretreatment methods and hydrolyzation procedures before bioethanol production is presented in Fig. 1.

Yeast Strain, Media, and Cultivation Conditions

Fermentation was performed with a wild-type strain of *Saccharomyces cerevisiae,* which was previously isolated by the Laboratory of Biochemical Engineering, Industrial Microbiology, Malting and Brewing Technology, University of Zagreb, Croatia. *S. cerevisiae* was subcultured at 30°C on a solid agar medium containing 20 g/l glucose, 1 g/l $(NH_4)_2HPO_4$, 1 g/l $(NH_4)_2SO_4$, 2 g/l yeast extract, and 20 g/l agar. Inoculum cultures were grown for 24 h at 30°C in 500 ml Erlenmeyer flasks filled with 200 ml of a liquid medium containing 20 g/l glucose, 3 g/l $(NH_4)_2HPO_4$, 3 g/l $(NH_4)_2SO_4$, and 6 g/l yeast extract.

Bioprocess Operational Conditions

Enzymatic hydrolysis and batch fermentations were performed in a 2 L bioreactor (Biostat MD, B. Braun, Germany) with a working volume of 1 L. First, 900 ml of the pretreated sugar beet pulp slurry (23.4 g/l dry weight) was added to the bioreactor and autoclaved for 20 min at 121°C. After cooling, enzymes were added and the temperature was maintained at 40°C. The stirrer speed was set to 960 rpm and the aeration rate adjusted to 0.5 l/min. After 22 h of enzymatic treatment, fermentation was started by addition of the yeast culture. The initial yeast concentration in the bioreactor was 17×10^5 CFU/ml. The batch fermentation was performed at 30°C with a stirrer speed of 960 rpm under anaerobic conditions, except for the initial 30 min where the aeration rate was set to 0.5 l/min. The pH was monitored but was allowed to float freely during the fermentation. The sugar yields from sugar beet pulp were calculated by Eq. (1):

$$Y_P = 100 \cdot \frac{m_p}{m_t} \quad (\%) \tag{1}$$

where m_p is the amount of released sugars (g) after pretreatment or/and enzyme hydrolysis, and m_t is the total sugar content (g) present in the dry matter of sugar beet pulp [26].

Determination of Viscosity, Settling Velocity, and Bioreactor Stirrer Speed

The apparent viscosities of various sugar beet pulp heterogenates in 50 mM citrate buffer, pH 4.8, were measured before and after treatment procedures with a Rheometric Scientific RM-180 rotational rheometer, using a cylinder-type head to determine the slurry viscosities at shear rates ranging from 750 to 1,291 1/s. Apparent viscosities were measured at the highest shear rate (1,291 1/s). The settling velocity (v_p) was determined in a graduated cylinder containing the filtrated solutions. All values given represent mean values from three independent repeats. The particle distribution was determined by image analysis, which was used to quantify the front of the fastest and slowest 10% of the particles as well as the median particle fraction. Three groups of different particle sizes ranging from 0.3 to 0.5 mm were analyzed. The densities of the filtrated sugar beet pulp solutions were determined pycnometrically at 30°C (ρ_f = 1,014 kg/m^3), and apparent densities (ρ_p) were calculated by Stoke's law (Eq. (2)) based on the given particle radii (r_p), assuming laminar fluid conditions (Re < 0.1) and standard gravity (g = 9.80665 m/s^2). The dynamic shape correction factor (x = 1.57) is given by Crowder *et al.* [7].

$$v_p = \frac{2(\rho_P - \rho_f) g r_P^2}{9 \eta x} \quad (m/s) \tag{2}$$

Archimedes numbers (Ar) and Reynolds numbers (Re) were calculated from the Kneule's correlation (Eqs. (3) and (4)) describing solid-liquid systems in stirred tank bioreactors equipped with four blade stirrers and four baffles [12]. The diameter of the blade stirrer (d) was 0.05 m.

$$Ar = \frac{d^3 g}{\eta}(\rho_p - \rho_f) \tag{3}$$

$$Re = 0.82 Ar^{0.5} \varphi_v^{0.25} \tag{4}$$

where φ_v is the volume fraction of sugar beet pulp in the slurry. The stirrer speed (n) was calculated from Eq. (5).

$$Re = \frac{d^2 n \rho_f}{\eta} \tag{5}$$

Analytical Procedures

Concentrations of glucose, galactose, arabinose, galacturonic acid, ethanol, methanol, and glycerol were quantified by HPLC on a Supelcogel C-610H column using a refractive index detector (RID, Shimadzu 10 A VP; Kyoto, Japan). Analytes were separated at a flow rate of 0.5 ml/min with 0.1% H_3PO_4 as eluent at a constant temperature of 30°C. Prior to analysis, all samples were mixed with $ZnSO_4$ to a final concentration of 10% to induce protein precipitation. Solid debris was removed by centrifugation (4,500 rpm for 20 min). Before column application, sample solutions were passed through a 0.20 μm filter.

Viable cells were determined as colony forming units (CFU)/ml. Samples were continuously drawn from the stirred tank bioreactor and were appropriately diluted in sterile water and plated on YPD dishes. The number of colonies was counted after 30 h incubation at 30°C.

Results and Discussion

Mixing Properties of Sugar Beet Pulp in a Stirred Tank Bioreactor

In order to define the optimal operational conditions for bioethanol production from sugar beet pulp in a stirred tank bioreactor, hydrodynamic characteristics of the media were determined. The apparent densities of sugar beet

Table 1. Settling velocities and apparent densities of different sugar beet pulp particle sizes.

Particle size (mm)	Settling velocity (m/s)	Apparent densities (kg/m^3)
0.3	0.0021	1,112
0.4	0.0053	1,189
0.5	0.0110	1,221

pulp were found to exceed the density of sole citrate buffer solution (1,014 kg/m^3) by 98–207 kg/m^3 (Table 1). The particle density values were used to calculate the Archimedes numbers for the given impeller and bioreactor geometry, and the optimal impeller rotation speed was calculated for several sugar beet pulp concentrations and three particle populations. Stirrer speeds presented in Table 2 represent the minimum velocities for a homogeneous suspension of solid components in relation to particle size and concentration. A comparison between the Reynolds numbers indicates different flow patterns within each population of particles. Smaller particles require lower stirrer speed at lower sugar beet pulp concentrations, whereas higher stirrer speeds are required to maintain a uniform distribution of larger particles at higher sugar beet pulp concentrations. In solutions with a high percentage of solids, the particle settling velocity and viscosity change criterion for complete suspension of solid particles are difficult to control [8, 23]. The criterion for further research was to employ the highest possible sugar beet pulp concentration that can be reliably mixed in order to obtain homogeneous conditions inside the reactor. Therefore, a sugar beet pulp concentration of 23.4 g/l of dry weight and a stirrer speed of 960 rpm were chosen based on this experiment. The obtained results were additionally confirmed by further three-step bioethanol production experiments.

Table 2. Mixing properties of sugar beet pulp slurries.

Sugar beet pulp (g/l)	Particle size (mm)	Ar	Re	Minimal rotational speed (1/rpm)
40	0.3	1,010,345	2,021	731
	0.4	1,284,490	2,219	778
	0.5	1,814,890	2,351	803
60	0.3	479,236	1,781	899
	0.4	648,291	1,844	902
	0.5	863,452	1,978	960
80	0.3	318,176	1,675	1,001
	0.4	430,416	1,784	1,096
	0.5	573,266	1,914	1,181

Sugar Beet Pulp Pretreatment

In the following, the mass percentages of released glucose, galactose, sucrose, arabinose, and galacturonic acid units were calculated as a fraction of the total sugar mass of each sugar. The composition of sugar beet pulp, as measured by an acid-base hydrolysis experiment, was taken from [27] and [28]. Pretreatment of the sugar beet pulp by steam (1.2 bar, 121°C) and ultrasonic waves had a sugar releasing effect (Table 3). Thermal pretreatment favored the release of monosaccharides from cellulose and hemicelluloses, and notably high amounts of free glucose were detected after this procedure. Thermal treatment of sugar beet pulp has a strong physical effect on lignocellulosic substrates, which is caused by a sudden release of pressure leading to an explosive decompression and fraying of polymer fibers [24]. Sugar release might also be induced by weak organic acid hydrolysis [15]. However, after sugar beet pulp treatment with citrate buffer (pH 4.8), the concentration of released sugars was in the range of the sugar beet pulp aqueous solution. Previous studies pointed out that effective organic acid hydrolysis should be performed in combination with thermal treatment [15].

Previously, it was shown that exposure to ultrasonic waves can be potentially used for cellulose and pectin pretreatment [32]. In view of our data, ultrasound pretreatment of sugar beet pulp had a much weaker sugar releasing effect than thermal treatment, releasing only low amounts of soluble monosaccharides (Table 3). Nevertheless, the liberation of high amounts of sucrose indicates a significant effect of ultrasonic treatment on sucrose extraction. Previously, it was hypothesized that the formation and collapse of air bubbles resulting from the application of an ultrasound field causes cavitation effects that break up the sugar beet

Table 3. Content of different sugars and galacturonic acid after thermal and ultrasound pretreatment of sugar beet pulp (39 g/l dry weight) calculated as a fraction of total sugars.

Pretreatment	Glucose (%)	Galactose (%)	Sucrose (%)	Arabinose (%)	Galacturonic acid (%)
Thermal	16.18	27.23	28.13	9.99	16.25
Ultrasound	n.d.	9.12	64.33	n.d.	n.d.

n.d., not detectable.

pulp surface, thus contributing to sucrose release [32]. Different ultrasound residence times (5–45 min) were examined but did not affect the release of sugars other than sucrose. As a result, the residence time of sugar beet pulp in the ultrasonic field was restricted to 5 min in all further experiments.

Enzymatic Treatment of Sugar Beet Pulp

The pretreated sugar beet pulp preparations were incubated with cellulases or a combination of cellulases and pectinases for the hydrolysis step. Enzyme concentrates were added to the sugar beet pulp slurries to give final activities of 1.12 FPU and 2 FDU per mg of dry weight sugar beet pulp. The amounts of liberated sugars after enzymatic treatment of all fractions are summarized in Table 4. Generally, a combination of cellulase and pectinase showed a much higher saccharification efficiency when compared with the treatment with cellulase alone. The strongest effect of pectinase addition was observed with galactose, resulting in the release of 91.3% of total galactose from sugar beet pulp. Likewise, cellulose degradation into glucose monomers and release of arabinose from hemicellulose increased as a result of the pectinase addition (the exception is sucrose). The effect of positive synergism when cellulolytic activity is coupled with pectinase was also described by Spagnuolo *et al*. [26]. In addition, our data support the idea that pectin limits the rate of enzymatic hydrolysis by acting as a physical barrier, thus restricting the access of hydrolytic enzymes to the digestible parts of the substrate [5].

The type of pretreatment clearly affected the sugar release by hydrolytic enzymes, and total yields of monosaccharides were generally higher after thermal pretreatment of sugar beet pulp. Most notably, yields of galactose, arabinose, and galacturonic acid were increased in the range of 10–30% compared with the ultrasonic pretreatment. Glucose liberation, however, was only slightly affected by the pretreatment method and reached maximum yields of 21.6–29.6% for both pretreatment methods.

Several studies addressed the enzymatic hydrolysis of sugar beet pulp. The preparation and pretreatment of sugar beet pulp as well as the used enzymes and loading vary from case to case, which strongly influences the obtained monosaccharide composition. Micard *et al*. [19] used commercial cellulases and pectinase complexes (Multieffect XL, Celluclast, Novozym 342, SP 584, Cellulase, Cellulyve Tr 300G+ AN 6000) in different combinations. Cellulose hydrolysis was found to be more complete when adding pectinases and cellulases sequentially. The degree of hydrolysis could be increased to 41.6% when applying the pectinolytic mixture SP 584, followed by treatment with Celluclast. Other enzymes used in the investigation resulted in yields of 0.1% and 19.3% for the individual sugars. Kühnel *et al*. [17] examined the effects of mild acid hydrothermal pretreatment and employed enzymes from the fungus *Chrysosporium lucknowense* for sugar beet hydrolysis. Reported yields were as high as 91% for glucose, 2% for galactose, and 24% for arabinose, based on the total amount of individual sugars present in sugar beet pulp. However, the pretreatment procedure included a treatment with mild sulfuric acid in a pressurized reactor operated from 120°C to 170°C (corresponding to the steam pressure range of approximately 1.8–3.5 bar). The main difference to our approach is the pretreatment procedure before enzymatic hydrolyzation. Advantages of our procedure are its simplicity, low energy demand, and a lower enzyme dosage. However, a lower glucose content was observed in our research compared with the presented procedure [17]. For galactose and arabinose, similar or higher yields were observed in our research.

Apparent viscosities of treated and untreated sugar beet pulp fractions indicated efficient hydrolysis of sugar beet pulp (Table 5). The increase in viscosity results from the release of polymeric components. Highest viscosities were measured after cellulase treatment as a result of pectin liberation. Release of pectin increases the viscosity, contributes to the inefficient mixing behavior, and promotes medium heterogeneity, which makes the measurement of sugar concentrations imprecise after cellulase addition. Additionally, the high medium viscosity promotes arabinose

Table 4. Content of different sugars and galacturonic acid after combination of ultrasound, thermal, and enzyme treatment of sugar beet pulp (39 g/l dry weight), calculated as a fraction of total sugars.

Pretreatment	Glucose (%)		Galactose (%)		Sucrose (%)		Arabinose (%)		Galacturonic acid (%)	
	Cell	Cell/Pec	Cell	Cell/Pec	Cell	Cell/Pec	Cell	Cell/Pec	Cell	Cell/Pec
Ultrasound	21.6	48.1	48.3	77.2	30.1	9.4	n.d.	22.2	5.3	34.7
Thermal	29.6	59.6	75.1	91.3	10.3	5.1	1.7	37.5	24.2	53.9

Cell, cellulase treatment.

Cell/Pec, cellulase and pectinase treatment.

n.d., not detectable.

Table 5. Apparent viscosity of untreated and pretreated sugar beet pulps at the highest stirring rate of 1,290 1/s.

Sugar beet pulp (g/l)	Apparent viscosity (mPa s)						
	Untreated	Ultrasound	Thermal	Ultrasound		Thermal	
	No enzymes	No enzymes	No enzymes	Cell	Cell/Pec	Cell	Cell/Pec
40	7.9	8.9	10.8	11.6	11.4	14.5	12.3
60	8.7	10.0	12.1	13.1	12.3	16.3	13.8
80	9.5	11.5	14.2	18.7	17.4	25.2	20.1

Cell, cellulase treatment.
Cell/Pec, cellulase and pectinase treatment.

and sucrose incorporation in the viscous polymer matrix and decreases the fraction of free arabinose and sucrose. This is the reason why after thermal pretreatment of sugar beet pulp, the arabinose content was found to be higher than the arabinose content detected after combining thermal and enzyme treatment [26]. Incubation with both cellulase and pectinase reduced viscosities owing to pectin degradation [22]. Apparent viscosities after hydrolysis were always higher for thermally pretreated fractions. Based on our findings, a combination of thermal and enzymatic treatment is the most suitable method for sugar beet pulp pretreatment and was selected for further experiments.

Three-Step Bioethanol Production in a Stirred Tank Bioreactor

Bioethanol production from sugar beet pulp was accomplished in a stirred tank bioreactor in three consecutive steps: (i) thermal treatment of sugar beet pulp slurry at 121°C for 20 min, (ii) enzymatic treatment with pectinases and cellulases for 22 h, and (iii) fermentation with *S. cerevisiae*. Sugar beet pulp concentrations above 60 g/l (23.4 g/l dry weight) resulted in a considerable collapse of the mixing properties owing to the elevated viscosity (Table 5) and complicated the withdrawal of representative samples. As a consequence, the bioprocess was performed with a sugar beet pulp concentration of 60 g/l (23.4 g/l dry weight).

Concentrations of glucose, galactose, sucrose, arabinose, galacturonic acid, and glycerol were measured throughout the enzymatic treatment and are presented in Fig. 2. Concentrations of ethanol, methanol, and acetic acid were below the detection limits. Amounts of released galacturonic acid, galactose, and arabinose increased steadily throughout the enzymatic treatment, indicating efficient pectin and hemicellulose hydrolysis. Hemicellulose decomposition results in an increase of the mean pore size of the substrate, which improves the accessibility of cellulose fibers for cellulolytic and hemicellulolytic enzymes [4]. The steady increase of glucose concentration in the supernatants indicates efficient cellulose hydrolysis. The increasing galactose and arabinose concentrations indicate the hydrolysis of hemicellulose and pectin [16].

The degree of acetylation in hemicellulose was previously reported to be a limiting factor in biorefinery operations, since acetyl groups may hamper the polysaccharide breakdown [5]. In below our experiments, the concentration of acetic acid was under the detection limit throughout the pretreatment process. Thus, acetylation appears to be of no relevance in sugar beet pulp pretreatment.

Bioethanol production was started by addition of *S. cerevisiae* culture without deactivation of enzymes. Changes in the concentration of soluble sugars, ethanol, methanol, acetic acid, and glycerol are presented in Fig. 3. The glucose concentration still increased within the first 2 h of fermentation owing to action of glucosidases during the lag-phase period of the yeast. After that, glucose, sucrose, and galactose concentrations rapidly decreased (Fig. 3), whereas the ethanol concentration increased simultaneously. In addition to alcoholic fermentation, glycerol formation was also observed, which represents a further strategy of NADH reoxidation in order to maintain the intracellular redox balance and compensate for oxidative biosynthetic

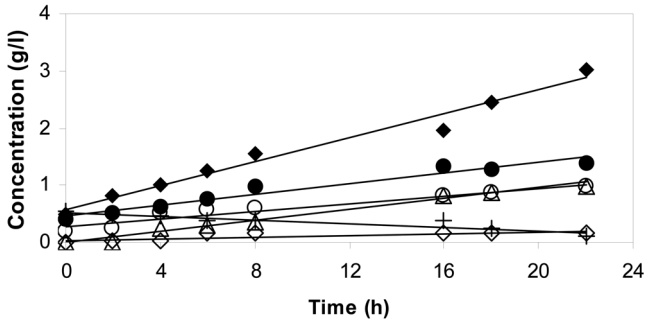

Fig. 2. Concentration of glucose (◆), sucrose (+), galactose (○), arabinose (△), galacturonic acid (●), and glycerol (◇) during enzymatic pretreatment of autoclaved sugar beet pulp in a stirred tank bioreactor.

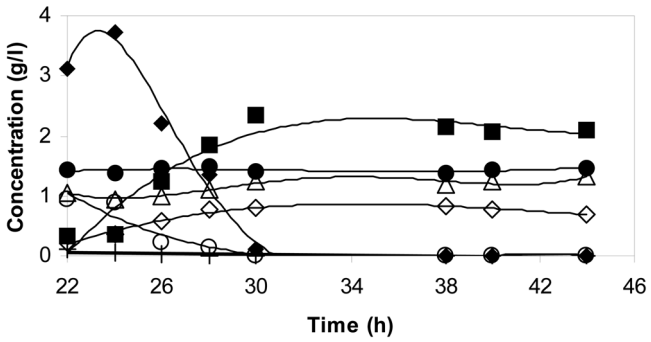

Fig. 3. Concentrations of glucose (◆), sucrose (+), galactose (○), arabinose (△), galacturonic acid (●), ethanol (■), and glycerol (◇) during batch fermentation of pretreated sugar beet pulp slurry in a stirred tank bioreactor.

reactions under anaerobic growth conditions [3, 6]. After 8 h of fermentation, the ethanol concentration reached a constant level of 2.35 g/l. Wild-type *S. cerevisiae* strains readily ferment glucose and fructose as well as the disaccharides sucrose and maltose *via* the Embden-Meyerhof pathway, whereas galactose is fermented *via* a combined action of the Leloir pathway and glycolysis [31]. Arabinose and galacturonic acid were not metabolized by the yeast and remained at a constant level throughout the fermentation (Fig. 3). Owing to aeration in the first 0.5 h of the fermentation the cell number increased slightly from 17×10^5 to 22×10^5 CFU/ml, but then remained constant until the end of the fermentation.

The final ethanol yield was 0.10 g ethanol/g sugar beet pulp dry weight. In previous studies, final ethanol yields of 0.04 to 0.06 g ethanol/g sugar beet pulp dry weight were reported. The low yields reflect the recalcitrant nature of sugar beet pulp. A crucial parameter that has to be taken into account is the moisture content and storage time of the employed substrate. Prolonged storage of sugar beet pulp was reported to decrease the efficiency of enzymatic degradation, which directly affects the total ethanol yield [19]. Therefore, in our study, wet sugar beet pulp was employed in order to obtain a higher final ethanol yield. Previously, bioethanol production from lignocellulosic raw materials employing *S. cerevisiae* was also investigated by Talebnia and Taherzadeh [29], Tang *et al*. [30], and Kim *et al*. [14]. Some of the referred bioprocesses were performed with recombinant *S. cerevisiae* that was engineered to metabolize pentose sugars [14], and for other processes, encapsulating or flocculating *S. cerevisiae* strains were used in continuous bioprocesses [29, 30]. Strategies to further increase the ethanol yield from sugar beet pulp may also include the use of genetically engineered bacteria with a broader substrate range, as was successfully demonstrated by Sutton and Peterson [28]. Different bioreactor constructions, alternative bioprocess designs, as well as fine-tuning of pretreatment methods bear additional potential for improvement in ethanol productivity from lignocellulosic raw material. Table 6 summarizes recent approaches of bioethanol production from lignocellulosic raw materials. All presented processes [11, 21, 29], except the concentrated sulfuric acid pretreatment bioprocess [30], had higher productivities and ethanol yields. However, our bioprocess was conducted by wild-type *S. cerevisiae* and a "weak" thermal pretreatment process. The processes listed in Table 6 used "strong" thermal pretreatment (*e.g.*, 12 bar steam, SO_2 steam explosion) and genetically modified *S. cerevisiae* strains. With respect to environmentally friendly bioprocess operations, our approach represents a promising alternative

Table 6. Comparison of lignocellulose hydrolysation and bioethanol production processes from different lignocellulosic raw materials using the yeast *S. cervisiae*.

S. No.	Raw material	Type of hydrolysis	Strain type	Fermentation conditions	Bioethanol yield (g/g)	Ref.
1	Lodgepole pine	SO_2-catalyzed steam explosion, cellulase, β-glucosidase	*S. cervisiae* T1	37°C, pH 5.0, 30 h SSF	0.24	[11]
2	Corn stove	SO_2-catalyzed steam explosion, cellulase, β-glucosidase	Baker's yeast	30°C, pH 5.5, 144 h SSF	0.29	[21]
3	Spruce forest	0.5% H_2SO_4 at 12 bar for 7–10 min	Immobilized *S. cerevisiae* CBS 8066	30°C, pH 5.0, continuous fermentation 0.5 l/h	0.4	[29]
4	Coniferous trees	Concentrated sulfuric acid	*S. cerevisiae* KF-7	35°C, pH 4.5, continuous fermentation 0.3 l/h	0.05	[30]
5	Sugar beet pulp	Thermal pretreated enzyme hydrolysis (cellulase, pectinase)	Wild-type *S. cerevisiae*	30°C, pH 4.8, batch fermentation, 60 g/l SBP	0.10	This study

to conventional lignocellulose pre-treatments like acid-base treatment or the use of organic solvents. Therefore, overall, an advantage of our bioethanol production process is the promotion of a low energetic pretreatment process integrated with sustainable wild-type *S. cerevisiae* for bioethanol production. Additionally, we were able to implement all process steps – from pretreatment to fermentation – in a single bioreactor without the need of additional unit operations.

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