

COMPARISON OF VACUUM AND HIGH PRESSURE EVAPORATED WOOD HYDROLYZATE FOR ETHANOL PRODUCTION BY REPEATED FED-BATCH USING FLOCCULATING *SACCHAROMYCES CEREVISIAE*

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With the aim of increasing the sugars concentration in dilute-acid lignocellulosic hydrolyzate to more than 100 g/l for industrial applications, the hydrolyzate from spruce was concentrated about threefold by high-pressure or vacuum evaporations. It was then fermented by repeated fed-batch cultivation using flocculating *Saccharomyces cerevisiae* with no prior detoxification. The sugars and inhibitors concentrations in the hydrolyzates were compared after the evaporations and also fermentation. The evaporations were carried out either under vacuum (VEH) at 0.5 bar and 80°C or with 1.3 bar pressure (HPEH) at 107.5°C, which resulted in 153.3 and 164.6 g/l total sugars, respectively. No sugar decomposition occurred during either of the evaporations, while more than 96% of furfural and to a lesser extent formic and acetic acids disappeared from the hydrolyzates. However, HMF and levulinic acid remained in the hydrolyzates and were concentrated proportionally. The concentrated hydrolyzates were then fermented in a 4 l bioreactor with 12-22 g/l yeast and 0.14-0.22 h⁻¹ initial dilute rates (ID). More than 84% of the fermentable sugars present in the VEH were fermented by fed-batch cultivation using 12 g/l yeast and initial dilution rate (ID) of 0.22 h⁻¹, and resulted in 0.40±0.01 g/g ethanol from the fermentable sugars in one cycle of fermentation. Fermentation of HPEH was as successful as VEH and resulted in more than 86% of the sugar consumption under the corresponding conditions. By lowering the initial dilution rate to 0.14 h⁻¹, more than 97% of the total fermentable sugars were consumed, and ethanol yield was 0.44±0.01 g/g in one cycle of fermentation. The yeast was able to convert or assimilate HMF, levulinic, acetic, and formic acids by 96, 30, 43, and 74%, respectively.

Keywords: Fermentation; Flocculating yeast; Fed-batch; Vacuum evaporation; High-pressure evaporation; Ethanol

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INTRODUCTION

Production of ethanol from renewable resources including lignocelluloses has been motivated by environmental concerns and global warming. Cellulose and hemicellulose can be hydrolyzed by acids or enzymes to their monomeric sugars, followed by fermentation to ethanol by e.g. baker's yeast (Olsson and Hahn-Hägerdal

1993). The fermented solution is then distilled to separate ethanol from water and other soluble materials. Low concentrations of sugars in the hydrolyzate result in low ethanol concentration during the fermentation. Subsequently, it results in high energy consumption in the distillation process. On the other hand, a very high sugar concentration can result in high stress and inhibition of the microorganisms by either the sugars or ethanol.

The sugar concentrations in the lignocellulosic hydrolyzate are usually low (up to 60 g/l) due to the process limitations (Boussaid et al. 1999; Robinson et al. 2002). Fermentation of these hydrolyzates results in low ethanol concentration and high energy demand in the subsequent downstream processes. An ethanol concentration of 4% can be considered as minimum, assuming reasonable energy consumption in the downstream processes (Zacchi and Axelsson 1989). Thus, concentration of the hydrolyzates by e.g. evaporation can be helpful to increase the sugar concentrations. The evaporators are well established in different industries such as paper pulp industries for increasing the concentration of the black liquor, and in the ethanol industry for increasing the concentration of the stillage. However, if one-effect evaporation is used, more than one kg steam is necessary to evaporate one kg of water from the materials. Therefore, the evaporators are generally applied in multi-effect forms in order to reduce the steam consumption. These evaporators are usually run at different pressure levels (including vacuum and high pressures), in order to use the steam generated in one effect for evaporating in another effect. Evaporation processes for some different hydrolyzates at either vacuum or high-pressure conditions have been previously reported. For instance, ultra high pressure evaporation was previously applied by Lee et al. (2006) on starch hydrolyzates, while Robinson et al. (2003) reported vacuum evaporation on Douglas-fir hydrolyzate with delignification. However, no comparison between the two evaporation conditions on dilute-acid hydrolyzates and also without pretreatment was detected in the literature. It was therefore interesting to study whether both vacuum-evaporated hydrolyzate (VEH) and high-pressure-evaporated hydrolyzates (HPEH) can be successfully produced and fermented in ethanol production from lignocelluloses.

A number of by-products are formed during acid hydrolysis of lignocelluloses (Luo et al. 2002). These by-products may include furans, e.g. furfural and hydroxymethylfurfural (HMF), carboxylic acids such as acetic, levulinic and formic acids, and several phenolic compounds, which inhibit the fermentation process (Chung and Lee 1985; Larsson et al. 1999; Palmqvist et al. 1999; Sanchez and Bautista 1988; Taherzadeh et al. 1997b). The evaporation at high pressure or vacuum might affect the chemical composition of the hydrolyzates in terms of the sugars' or inhibitors' concentrations.

The objective of the current work was to investigate and compare the effect of vacuum and high-pressure evaporation on the sugars and inhibitor enrichment of dilute-acid hydrolyzates prepared from spruce chips, as well as the possibility of fermenting the hydrolyzates after the evaporation. The goal was to obtain more than 100 g/l fermentable sugars in the hydrolyzate for industrial purposes. Furthermore, a flocculating strain of *Saccharomyces cerevisiae* CCUG 53310 was exploited to facilitate separation of the cells from the fermentation broth by sedimentation.

EXPERIMENTAL

Hydrolysis of Lignocelluloses and its Evaporation

Swedish forest wood chips originating mainly from spruce were used as the lignocellulosic materials. The composition of the wood chips (per dry weight) was 41.6% glucan, 11.5% mannan, 4.7% xylan, 2.0% galactan, 5.4% extractives, and 25.7% lignin, as previously reported (e.g. Taherzadeh et al. 1997a). The chips were impregnated with sulfuric acid and hydrolyzed in a two-stage dilute-acid continuous hydrolysis reactor at SEKAB E-Technology (Sweden). After initial treatment with steam, the wood chips were exposed to 9 bar and 180°C for 5 minutes and to 22 bar and 220°C for approximately 6 minutes in two continuous (serial) reactors.

After the first stage of hydrolysis, liquid was squeezed out by compression in the transportation screws between the first and the second reactor. The resulting hydrolyzates from the first and the second steps were mixed, and the remaining solid material was removed by a filter press. The hydrolyzate was stored at 8°C until the evaporation. High pressure was maintained by injection of steam, and addition of sulfuric acid kept the pH at 1.7 and 1.8 in the two reactors. The liquid fraction was to a large extent separated between the two reactors. The composition of this hydrolyzate is presented in Table 1.

The hydrolyzate was then concentrated by about threefold from its initial concentration either under vacuum (VEH) at 80°C and 0.5 bar, or under high pressure (HPEH) at 107.5°C and 1.3 bar. During the evaporation, the pH was kept above 2.1 and the temperature under 120°C, in order to avoid decomposition of sugars. The composition of these two concentrated hydrolyzates is presented in Table 1.

Table 1. Composition of the Dilute-Acid Hydrolyzate, Originally and after Evaporation at 80°C (VEH) or 107.5°C (HPEH) to about Threefold Concentration in Comparison to Expected Composition after the Evaporation

Component in hydrolyzate	Original hydrolyzate (g/l)	Expected* composition (g/l) after evaporation	Vacuum evaporation (VEH) (g/l)	High-pressure evaporation (HPEH) (g/l)
Glucose	19.67	60.98	61.53	64.83
Galactose	3.42	10.60	10.90	11.33
Mannose	15.66	48.55	47.08	52.85
Total fermentable sugars	38.75	120.13	119.51	129.01
Xylose	8.74	27.09	27.07	28.71
Arabinose	2.11	6.54	6.71	6.88
Total sugars	49.6	153.8	153.3	164.6
HMF	1.46	4.53	4.54	4.93
Furfural	1.00	3.10	0.03	0.10
Formic acid	0.67	2.08	0.89	1.34
Levulinic acid	0.69	2.14	2.13	1.46
Acetic acid	2.99	9.27	3.90	3.91
Lignin	2.15	6.67	6.80	8.20
Sulfate	1.70	5.27	5.80	6.00

*Expected composition by evaporation to 1/3 of original volume with no degradation and evaporation of the components

Yeast Strain and Medium

A flocculating yeast strain of *Saccharomyces cerevisiae* isolated from an ethanol plant (Domsjö Fabriker AB, Örnsköldsvik, Sweden) and registered at the culture collection in the University of Göteborg (Sweden) as CCUG 53310 was used in all experiments. The strain was maintained on agar plates made from 10 g/l yeast extract, 20 g/l soy peptone, and 20 g/l agar, with 20 g/l D-glucose as additional carbon source. The yeast was inoculated in 300-ml flasks and incubated in a shaker bath at 30°C with 170 rpm agitation for 24 h aerobic cultivation. The liquid volume was 100 ml. The culture had 3.42 g/l (NH₄)₂SO₄, 22 g/l glucose and 3 g/l yeast extract. The yeast that was flocculated and settled was aseptically separated from the culture and transferred to the bioreactors.

Cultivation in Bioreactor

The cultivations were carried out in a bioreactor (Belach BR 0.4, Sweden) with a total volume of 4 l. In order to prepare enough yeast biomass for the cultivations, 700 ml medium containing 50 g/l beet molasses (autoclaved separately) and 7.5 g/l (NH₄)₂SO₄, 3.5 g/l KH₂PO₄, 0.75 g/l MgSO₄·7H₂O, and 0.36 mg/l biotin was inoculated and cultivated for 24 h. The cultivation was then continued in fed-batch mode, by addition of 850 ml of diluted molasses (38 g/l) within 24 h. Then, the cells were settled and a part of the medium was removed to obtain 700 ml in the bioreactor. This procedure resulted in 12 g/l yeast in 700 ml culture in the bioreactor. In some experiments, more molasses (another 850 ml with 38 g/l molasses) for a longer time (totally 72 h) was added in order to provide approximately double the amount of yeast (22 g/l) in the remaining 700 ml in the bioreactor (Fig. 1). The aeration rate was 2 l/min with an agitation rate of 500 rpm at 30°C.

The fermentations of the hydrolyzates were carried out by a repeated fed-batch process, with the initial volume of 700 ml containing 12 or 22 g/l flocculating yeast. It was filled up to 3.5 l within 18 or 27 h with constant feed rates, followed by 3 h fermentation with no feeding (Fig. 1). Then, the stirrer was turned off to settle the yeast flocculi before withdrawing 2.8 l culture medium. The remaining 700 ml in the reactor was then aerated for 2 h to prepare the cells for a new cycle. These cycles were repeated until the end of the experiments again within 18 or 27 h. The experiments according to feeding profile I (Fig. 1) were carried out with both VEH and HPEH, three times each. Experiments with feeding profile II and III (Fig. 1) were carried out with HPEH, two times for each feeding profile.

Analytical Method

The metabolites were quantified using either HPLC or GC. Formic acid, acetic acid, levulinic acid, and ethanol concentrations were determined by HPLC, using an ion-exchange column (Aminex HPX-87H, Bio-Rad, USA) with a refractive index (RI) detector at 60°C, using 5 mM sulfuric acid as the eluent at flow rate 0.6 ml/min. Furfural and HMF were detected by a UV detector. Sugars were quantified by HPLC, using Aminex HPX-87P column (Bio-Rad) at 80°C and pure water as the eluent. Glycerol was derivatized to silylester and then analyzed using GC-MS. Sulfate was measured with

titration, using the standard method SCAN-N 6:85. Dissolved lignin was measured with a spectrophotometer at 286.5 nm after dilution. Indulin AT lignin was used as standard.

Cell dry weights were determined using direct measurements of duplicate 8-ml samples, which were centrifuged, washed with distilled water, and dried for 24 h at 105°C in an oven.

The vitality of the non-settling yeast at the end of each fed-batch cycle was measured. For this purpose, the flocculated yeasts were settled for 5 min, and the samples were withdrawn from the supernatant. The vitality, i.e. the fraction of metabolically active cells in this supernatant, was measured using Methylene blue staining. The vital cells remained unstained and were examined by light microscopy.

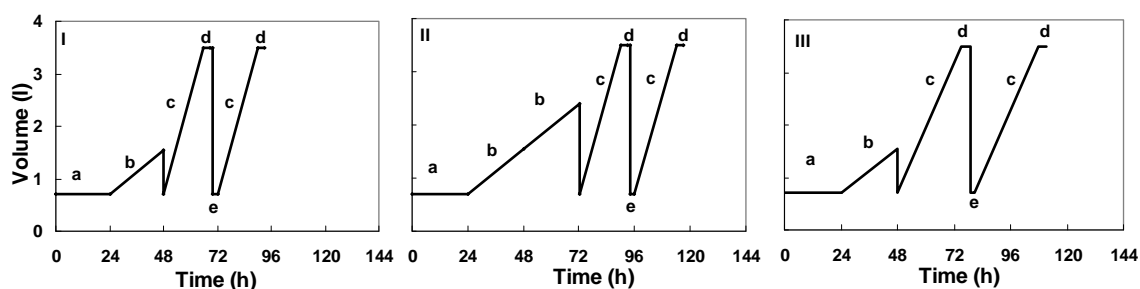


Fig. 1. Feeding patterns in the fed-batch cultivations. The different phases are (a) batch cultivation with 50 g/l sugars (molasses) for biomass preparation, (b) more biomass preparation by feeding 38 g/l sugar solution (molasses) for 24 (I, III) or 48 h (II), (c) cultivation of the hydrolyzates in fed-batch with ID 0.22 (I, II) or 0.14 h⁻¹ (III), (d) cultivation in 3 h batch at the end of each fed-batch cycle, (e) 2 h aeration of the yeasts prior to the next fed-batch cycle.

Calculations

A biomass composition of CH_{1.8}O_{0.5}CH_{1.8}N_{0.2} was used in the carbon balance calculations. The metabolite and biomass yields were calculated from the determined concentrations at the end of the exponential growth phase. The carbon balance of the experiments was calculated based on the measured yields of ethanol, glycerol, biomass, and calculated carbon dioxide.

All the experiments were either duplicated or triplicated, and the average standard deviation of all the repeated results was 4.62%.

RESULTS AND DISCUSSION

Concentrating the Hydrolyzate

In this study, concentrating of the spruce lignocellulosic hydrolyzate was carried out by two methods of evaporation, high pressure and vacuum. The results were compared in order to illustrate the possibility and differences of using these methods for concentrating the hydrolyzate. Both of the evaporated hydrolyzates in this work were then fermented without any prior detoxification by a flocculating strain of *S. cerevisiae*. The goal was to fulfill the two concerns of industrial production of ethanol, increasing the concentration of sugars in hydrolyzate, and eliminating the need for detoxification.

The total initial concentration of the sugars in the dilute-acid hydrolyzate was 45.3 g/l (Table 1). In order to increase this concentration for an industrial fermentation, the hydrolyzate was evaporated under vacuum (VEH) and high pressure (HPEH) at 80.0°C and 107.5°C, respectively. During these evaporations, the volume of the hydrolyzate was reduced to about 1/3 of the original volume. No foaming, incrustation, precipitation, or odor was observed during the evaporations. The evaporations resulted in increasing the sugars' concentrations 3.6 times by VEH and 3.4 times by HPEH (Table 1).

The concentration of sugars in the hydrolyzate increased to 153.3 g/l in VEH and to 164.6 g/l in HPEH, however because *S. cerevisiae* is able to utilize just hexoses, we will discuss only the fermentable sugars in the rest of this paper. The concentration of fermentable hexoses (glucose, galactose, and mannose) increased to 119.5 g/l and 129.0 g/l by vacuum and high-pressure evaporations, respectively. Furthermore, the ratio between xylose and glucose in the hydrolyzates remained constant at 0.44 g/g after either of the evaporations. These results might indicate an absence of decomposition of the carbohydrates.

The evaporation resulted in decreased concentrations of some inhibitors (Table 1). In general, VEH was more successful in removing the inhibitors than HPEH. Furfural was removed by 97 and 90 % of the original concentration with VEH and HPEH, respectively. Formic and acetic acids were also partially evaporated. If no evaporation of acetic acid had occurred, one would have expected more than 9.0 g/l of the acid in the concentrated hydrolyzates, while it was only present at 3.9 g/l in the hydrolyzates (Table 1). On the other hand, the evaporations had negligible effect on HMF and levulinic acid, and their concentrations were increased almost by the same proportion as the sugars in the hydrolyzates (Table 1). An increased concentration of dissolved lignin in VEH was almost proportional to the increases in sugar concentration. However, a higher concentration of lignin in HPEH was observed (Table 1).

The evaporations resulted in decreasing the pH of the hydrolyzates from 2.4 to 2.1 by both VEH and HPEH. This might correspond to the total increase in sulfate and carboxylic acids in the hydrolyzates. The total concentration of the acids in the feed can be calculated as 0.11 M after the evaporations (Table 1).

Fermentation of the Concentrated Hydrolyzates

Fermentability of the concentrated hydrolyzates, VEH and HPEH, was investigated using two different concentrations of *S. cerevisiae* (12 or 22 g/l) in a repeated fed-batch mode of operation with initial dilution rates (ID) of 0.14 and 0.22 h⁻¹. Each experiment was performed twice for checking the reproducibility of results and each number reported in Table 2 is the average result of two tests. The fed-batch process was repeated two times, where the fermented hydrolyzates were withdrawn from the bioreactor to the initial level of 0.7 l after settling the yeast, and fed in again to the maximum volume of 3.5 l (Fig. 1). The most important results of two-cycle fed-batch cultivations are summarized in Table 2.

All of the experiments were successful, meaning that the cells were able to consume sugars and produce ethanol. The ethanol yield ranged from 0.40 to 0.44 g/g of consumed sugars after a cycle of 21 h cultivation. However, the consumption of the

sugars depended on how the hydrolyzates had been concentrated, the initial yeast concentration in the bioreactor, and also the dilution rate used. Glycerol and the yeast biomass were the main by-products in these experiments (Table 2).

Fermentation of VEH in the first cycle of the fed-batch process with 12 g/l initial yeast concentration and ID 0.22 h⁻¹ resulted in the consumption of 263±36 g fermentable sugars in the hydrolyzate during 18 h feeding period. This corresponds to consumption of 76±6% of the total available fermentable sugars. The yeasts in the subsequent 3 h batch – where no feeding occurred – were able to assimilate 34.6±0.2 g more sugars, which was equal to 9.6±0.7% of added sugars and produced ethanol. Consequently, the ethanol concentration reached 32.8±2.8 g/l, and 15.2±0.08% of the total fermentable sugars remained in the culture within a total of 21 h fermentation in one cycle of fed-batch operation.

Table 2. Degree of Sugar Consumption, Inhibitors Conversion, and Ethanol, Glycerol and Biomass Yields from the VEH or HPEH, with Different Yeast Cell Concentrations and Dilution Rates in Fed-batch Cultivation of the Hydrolyzates

Evaporation condition	VEH	HPEH	HPEH	HPEH
Yeast (g/l)	12	12	22	12
Fed-batch ID (h ⁻¹)	0.22	0.22	0.22	0.14
Consumption of fermentable sugars* (%)				
Glucose	92.6 (±2.5)	97.6 (±0.6)	95.9 (±0.1)	92.9 (±6.0)
Mannose	64.3 (±12.0)	74.3 (±3.0)	74.7 (±2.0)	94.5 (±0.6)
Galactose	60.2 (±5.0)	16.1 (±4.0)	33.3 (±4.0)	47.9 (±14.0)
Conversion of the inhibitors* (%)				
HMF	95.3 (±0.8)	97.2 (±1.1)	90.4 (±5.7)	95.5 (±1.3)
Levulinic acid	26.9 (±26.8)	13.3 (±2.8)	30.0 (±10.6)	16.3 (±3.7)
Acetic acid	-1.5 (±4.0)	12.7 (±3.0)	23.9 (±9.0)	22.0 (±2.3)
Formic acid	46.5 (±19.0)	46.1 (±17.0)	69.7 (±0.5)	74.5 (±4.7)
Yields* (g/g sugars consumed)				
Y _{biomass/S}	0.015 (±0.004)	0.015 (±0.005)	0.019 (±0.012)	0.050 (±0.010)
Y _{glycerol/S}	0.025 (±0.003)	0.030 (±0.004)	0.030 (±0.012)	0.030 (±0.003)
Y _{ethanol/S}	0.410 (±0.012)	0.404 (±0.027)	0.411 (±0.004)	0.401 (±0.009)

*The results are for the entire experiment after two sequential fed-batch cycles. The data in the two first columns (the comparison between VEH and HPEH) were repeated three times each, while the two following experiments were repeated two times. The values in parentheses are calculated to cover the maximum and minimum measured data.

Fermentation of HPEH was as successful as that of VEH by the fed-batch cultivation at the identical conditions. When 12 g/l initial yeast concentration and ID 0.22 h⁻¹ were used, on average 86% of the total fermentable sugars were consumed and 35.3±2.1 g/l ethanol was produced within the period of 21 h cultivation. However, at the end of this period 13.2±0.04% of fermentable sugars remained in the bioreactor. Therefore, in order to increase sugar consumption, the biomass was increased, and the experiment was repeated with 22 g/l initial yeast concentration. This was an improvement

based on one cycle (21 h) compared to the previous results, where 93.6% of sugars were consumed and the ethanol concentration reached 39.9 ± 0.0 g/l. In the second cycle, the cells were not as active as in the first one. The sugar consumption was measured at the end of two cycles too. A total of 82% of the sugars was consumed by 22 g/l yeast, which can be compared with 81% sugar consumption by 12 g/l yeast at identical conditions.

On the other hand, better results were obtained by decreasing the feeding rate compared to increasing the yeast concentration. The yeast culture with an initial concentration of 12 g/l at ID 0.14 h^{-1} was able to consume 97 ± 2 % of the total fermentable sugars in 30 h and produced 42.8 ± 1.5 g/l ethanol from HPEH. The corresponding ethanol concentration with 0.22 h^{-1} ID was 34.1 ± 3.5 g/l.

The biomass yield after one cycle of fermentation with VEH and HPEH was 0.025 and 0.022 g/g, respectively, which was increased to 0.05 g/g by decreasing the ID from 0.22 to 0.14 h^{-1} . However, the biomass yield was decreased further by continuing the second cycle of fermentation (cf. Table 2).

The performance of the yeast was not as good in the second cycle of the fed-batch process as in the first one. In fermentations with 12 g/l yeast and 0.22 h^{-1} ID, the sugar consumption of the second cycle reached 71%, which was less than the corresponding sugar consumption in the first cycle (86%). Furthermore, the cells in the third cycle, carried out in experiment with VEH and ID 0.22 h^{-1} and 12 g/l biomass were not as active as in the previous cycles, and ethanol production decreased drastically (Fig. 2). The ethanol, biomass, and glycerol productions in different cycles are presented in Fig. 2.

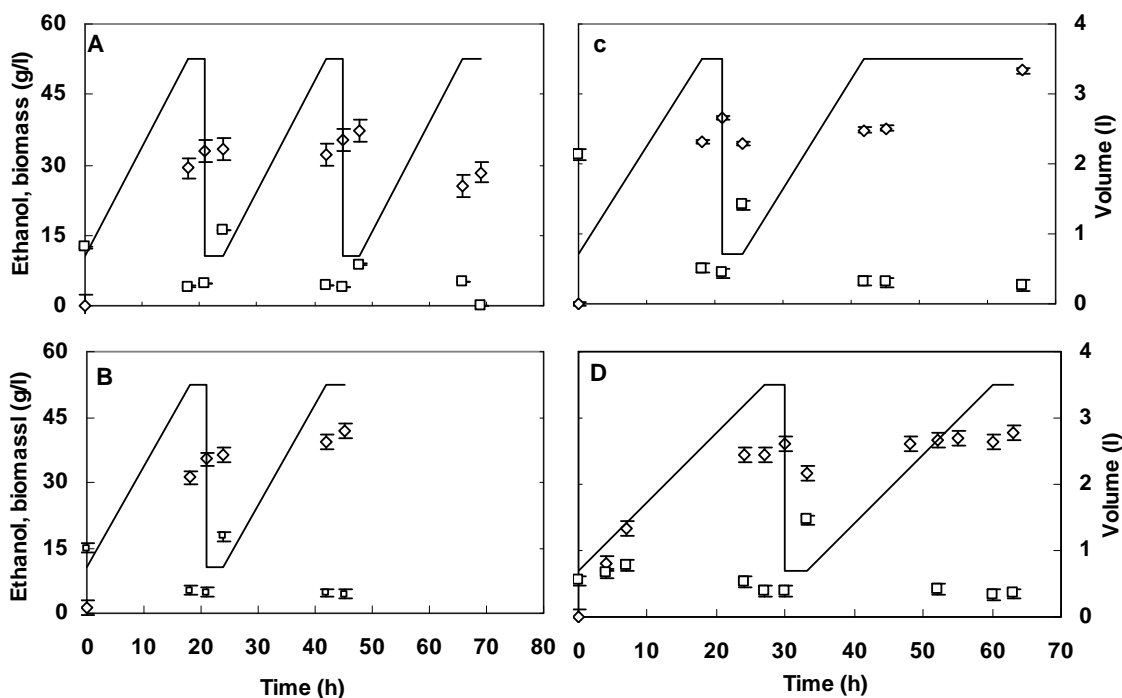


Fig. 2. Trends of medium volume (-), ethanol (\diamond) and biomass (\square), in fed-batch cultivation of (A) VEH with 12 g/l biomass and ID 0.22 h^{-1} , (B) HPEH with 12 g/l biomass and ID 0.22 h^{-1} , (C) HPEH with 22 g/l biomass and ID 0.22 h^{-1} , and (D) HPEH with 12 g/l biomass and ID 0.14 h^{-1} .

The yeast was able to convert or assimilate the inhibitors during the fed-batch cultivations (Table 2). More than 90% of HMF was converted by the yeast through the cultivations, which is enough for the cells to tolerate this inhibitor (Taherzadeh et al. 2000). Formic and levulinic acids were also converted or consumed, but not as much as HMF. Acetic acid present in HPEH was partially assimilated, but production of the acid was observed during the cultivation of VEH (Table 2).

Settling and Vitality of the Yeast

After completing the fed-batch cycles, the cells were settled in 5 min, where the supernatant and settled yeast were clearly separated into two phases. However, part of the yeast remained in the supernatants, where its concentration was dependent on the cultivation conditions. Increasing the initial yeast concentration from 12 to 22 g/l with both VEH and HPEH resulted in increases in the remaining cells in the supernatant from 8.3% to 20.94% of the total yeast in the culture, which corresponds to dry weights of 1.0 g/l to 4.6 g/l, respectively. Lowering the ID from 0.22 to 0.14 h⁻¹ resulted in decreasing non-settled yeasts in the supernatant from 8.3% to 6.2%; this was equal to 0.75 g/l vital. The cells in the supernatants were on average 76% vital.

Interpretation of the Results

The results of this work show a successful increase of the sugars' concentration in lignocellulosic hydrolyzates obtained from spruce wood chips followed by successful fermentation by repeated fed-batch operation using a flocculating strain of *S. cerevisiae* without prior detoxification of hydrolyzates. Both evaporations under vacuum or even high-pressure resulted in high-sugar and fermentable hydrolyzates with no sign of carbohydrates degradation in both evaporation processes. These results may be interesting industrially, since higher sugar concentrations in the hydrolyzates lead to less energy consumption in the distillation and downstream processes, while fermentation can be carried out successfully with no prior detoxification. Furthermore, the successful evaporation of the hydrolyzates under vacuum and pressure can be incorporated into a process design utilizing multi-effect evaporators, in which the hydrolyzate can be evaporated with low consumption of energy.

The evaporations under either vacuum or pressure used in this work did not decompose the sugars, but were able to remove the volatile inhibitors partially or completely. Therefore, either high pressure or vacuum evaporations can be applied for concentrating hydrolyzates in an industrial scale. As long as the evaporation is performed at pH higher than 2.1 and temperature lower than 120°C, the risk of sugars' decomposition is negligible. The boiling points of the inhibitors reported in this work at normal pressure are 161.7, 118.1, 100.8, and 245°C for furfural, acetic acid, formic acid and levulinic acid, while HMF is not volatile and boils at ca. 115°C at 1 mbar. Comparing these boiling points with the results presented in Table 1 indicates that volatility is important, but probably not the only factor that governs decreasing the concentration of the inhibitors, since e.g. more furfural (less volatile) than formic acid (more volatile) disappeared from the hydrolyzate during evaporations. This point needs more investigations, but degradation of furfural to e.g. formic acid during the evaporations could be a hypothesis to be considered. Furfural is a strong inhibitor for

baker's yeast (Azhar et al. 1981). Carboxylic acids might inhibit or enhance fermentation, depending on the cultivation conditions. The presence of acetic acid in the cultivation media can result in higher ethanol yield from sugars, while more than 5 g/l of undissociated molecules of this acid can severely inhibit the fermentation.

During cultivation of flocculating yeasts in toxic dilute-acid hydrolyzates, some yeast cells lose their vitality as well as their ability to flocculate. This means that the viability and vitality are high with the flocculated cells, but probably not with the non-flocculated cells. In this work the cells in the supernatants that had a ratio of 1g/l out of total 12 g/l and 4.6g/l out of total 22 g/l biomass had 76% vitality, and thus they were still able to produce ethanol.

CONCLUSIONS

1. Evaporation of lignocellulosic hydrolyzates from spruce with low sugar concentration and subsequent fed-batch cultivation by flocculating yeast may help to fulfill the demands for industrial production of ethanol from lignocellulosic materials without the need for detoxification.
2. In this work we succeeded in increasing the total- and fermentable sugar concentrations in the lignocellulosic hydrolyzate up to 164 and 129 g/l, respectively, with successful subsequent fermentation.
3. The evaporation increases the sugar concentrations in inverse proportionality to the volume. It does not decompose the sugars in either VEH or HPEH in controlled condition, and removes part of the toxic components.
4. The results showed no significant difference between the vacuum and high-pressure evaporation of hydrolyzates, so either of these methods may be used in an industrial process of ethanol production.
5. The flocculating yeasts tolerate the remaining inhibitors in fed-batch operation, and they are able to consume most of the fermentable sugars and produce ethanol in high concentration if the dilution rate is kept low (0.14 h^{-1}).

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NOMENCLATURE

HPEH	High pressure evaporated hydrolyzate
HMF	Hydroxymethylfurfural
ID	Initial dilution rate in fed-batch cultivation
VEH	Vacuum evaporated hydrolyzate

$Y_{\text{biomass/s}}$	Yield of biomass from the consumed sugars
$Y_{\text{glycerol/s}}$	Yield of glycerol from the consumed sugars
$Y_{\text{ethanol/s}}$	Yield of ethanol from the consumed sugars

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