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Effect of Reduction in Yeast and Enzyme Concentrations in a Simultaneous-Saccharification-and-Fermentation–Based Bioethanol Process

Technical and Economic Evaluation

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Abstract

The ethanol production cost in a simultaneous saccharification and fermentation–based bioethanol process is influenced by the requirements for yeast production and for enzymes. The main objective of this study was to evaluate—technically and economically—the influence of these two factors on the production cost. A base case with 5 g/L of baker's yeast and an initial concentration of water-insoluble solids of 5% resulted in an experimental yield of 85%. When these data were implemented in Aspen Plus, yeast was assumed to be produced from sugars in the hydrolysate, reducing the overall ethanol yield to 69%. The ethanol production cost was 4.80 SEK/L (2.34 US$/gal). When adapted yeast was used at 2 g/L, an experimental yield of 74% was achieved and the estimated ethanol production cost was the same as in the base case. A 50% reduction in enzyme addition resulted in an increased production cost, to 5.06 SEK/L (2.47 US$/gal) owing to reduced ethanol yield.

Index Entries: Ethanol; economics; process; simultaneous saccharification and fermentation; yeast; enzymes.

Introduction

In many countries, bioethanol is already an alternative or a complement to gasoline. In Brazil, the raw material consists of sugarcane or sugarcane molasses *(1)*, both of which contain readily fermentable sugars. Starch-based crops such as corn and wheat are also utilized in full-scale processes mainly in the United States and Europe. In Sweden, the production of ethanol from agricultural materials is limited owing to the low availability and also to the

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limited market of byproducts such as cattle feed from plants using wheat as feedstock.

Lignocellulosic materials are an attractive feedstock in many countries because they are available in large quantities at a relatively low cost. Spruce is one such raw material. It has three main constituents: lignin, hemicellulose, and cellulose. Cellulose and hemicellulose are made up of hexosans or pentosans, which may be hydrolyzed to sugars and which can be fermented to ethanol. This process, however, is more of a challenge than production from sugarcane and starch-based crops. Because of the high crystallinity of the cellulose and the presence of lignin, the cellulose is recalcitrant to degradation. In the conversion of lignocellulosics into ethanol, a pretreatment step is therefore included with the purpose of softening the structure of the cellulose. Harsh conditions, i.e., high temperatures and sometimes the addition of acidic catalysts, are required. Although necessary, these harsh conditions often result in the formation of byproducts *(2,3)*, thus leading to a reduction in overall ethanol yield. In addition, the byproducts may act as inhibitors in subsequent hydrolysis and fermentation steps *(3)*.

In the enzymatic process,the pretreatment step is followed by either separate hydrolysis and fermentation (SHF) steps or these two steps carried out simultaneously in a process known as simultaneous saccharification and fermentation (SSF). The latter has been claimed to be superior to SHF, owing to a higher resulting ethanol yield and lower capital cost *(4,5)*. One major economic bottleneck in both the SSF and SHF processes is the cost of the enzymes, accounting for about 10–20% of the ethanol production cost *(4,6,7)*. In the SSF process, the cost of the yeast has also been identified as a bottleneck, accounting for 10% of the production cost *(4)*. It is common practice in ethanol production processes to recycle some of the yeast back to the fermentors, thereby maintaining a high cell density, which facilitates rapid conversion of the sugars into ethanol. Thus, the required usage of sugars for yeast growth can be minimized. This method can be applied in the SHF process. In the SSF process, however, the slurry leaving the SSF reactors contains solids other than yeast cells, i.e., nonhydrolyzed cellulose and lignin. Consequently, a selective separation of the yeast cells from the rest of the slurry is difficult. A high cell density is therefore not economically feasible because a large portion of sugars intended for ethanol production will be needed for yeast growth. In most laboratory experiments, yeast is not directly grown on this sugar fraction. Instead, yeast is acquired from a yeast-producing company, which results in an overestimation of ethanol yield in experimental work compared with what can be expected in a full-scale production plant.

The purpose of the present evaluation was to demonstrate the importance of being able to reduce the cell concentration and enzyme addition without a significant reduction in overall ethanol yield. Selected options were experimentally tested in a process development unit to

investigate the possibility of reducing yeast and enzyme concentrations. The results were the basis of the subsequent technoeconomic evaluation in which the impact of overall ethanol yield, residence time in SSF, and yeast and enzyme requirements on the overall ethanol production cost were determined.

Materials and Methods

Raw Material and Pretreatment

Freshly cut spruce chips (*Picea abies*) were milled in a hammer mill to a size between 2 and 10 mm. The wood chips were impregnated with gaseous SO_2 to a concentration of 2.6% (w/w water content in the chips) by letting SO_2 into a plastic bag filled with chips. The dry matter (DM) content of the wood chips prior to impregnation was 40%. The composition of the raw material (Table 1) was determined according to the analytical procedures of the National Renewable Energy Laboratory (Golden, CO) *(8–10)*.

After impregnation for at least 20 min, the material was transferred to a steam-pretreatment unit, which has been described elsewhere *(11)*. Steam pretreatment was carried out for 5 min at 215°C, after which the slurry was collected to determine the weight of the recovered slurry and the content of soluble solids and water-insoluble solids (WIS).

Enzymes and Fermenting Organism

Cellulose-hydrolyzing enzymes, Celluclast 1.5L with an activity of 65 FPU/g and a β-glucosidase activity of 17 IU/g, and Novozym 188 with a β-glucosidase activity of 376 IU/g (both kind gifts from Novozymes, Bagsvaerd, Denmark), were used in all experiments. The fermenting organism was either compressed baker's yeast, *Saccharomyces cerevisae* (Jästbolaget, Rotebro, Sweden), bought at a local supermarket, or adapted baker's yeast, which was cultivated on the sugar-containing pretreatment liquid using a fed-batch technique. Initially, an aerobic batch cultivation on glucose was carried out to produce cell mass. This was followed by a fed-batch phase during which the pretreatment liquid was added.

Batch Cultivation

Batch cultivation, with a working volume of 4 L, was run at 30°C under sterile conditions. The medium contained the following components: 21.0 g/L of glucose, 0.6 g/L of $(NH_4)_2SO_4$, 12.0 g/L of KH_2PO_4, 2.8 g/L of $MgSO_4$, 36 mL/L of trace metal solution, and 4.2 mL/L of vitamin solution. The pH was maintained at 5.0 by automatic addition of 10% NH3. The stirrer speed was kept at 650 rpm and the aeration was maintained at 5 L/min. The cultivation was started by adding 90 mL of inoculum.

Table 1
Composition of Raw Material

	Dry raw material (%)
Glucan	43.4
Xylan	6.6
Galactan	2.9
Mannan	15.1
Arabinan	1.7
Lignin	23.5

Fed-Batch Phase

After the ethanol produced during the glucose consumption phase was depleted (indicated by a sharp increase in dissolved oxygen tension), feeding of pretreatment liquid was started. Pretreatment liquid (3.9 L) enriched with 50 g of glucose was added during the fed-batch phase. The feed rate was initially set at 0.10 L/h and was increased linearly to 0.40 L/h at the end of the fed-batch phase, which lasted 16 h. The pH was maintained at 5.0 by automatic addition of 10% NH3. The stirrer speed was kept at 650 rpm and the aeration was maintained at 5 L/min.

Cell Harvest

The cells were harvested by centrifuging at 3000 rpm (around 1000 g) for 7 min using a Jouan C4.12 centrifuge. The harvested cells were mixed with sterile 0.9% NaCl solution in order to obtain a cell suspension with a cell mass concentration (dry weight) of 75 g/L.

Simultaneous Saccharification and Fermentation

SSF was performed in 30-L fermentor vessels from Bioengineering AG (Wald, Switzerland). All experiments were performed in duplicate. Table 2 provides a summary of the experimental parameters.

All SSF experiments were run using 5% WIS in a total weight of 20 kg. A reference base case (SSF-BC), which was used for comparison, was run using 5 g/L of baker's yeast. Solid $Ca(OH)_2$ was used to adjust the pH to 5.0 and a solution of NaOH (10%) to maintain the pH at 5.0. In one case, aqueous ammonia was used to neutralize the pretreatment slurry and to maintain pH during the SSF experiment (SSF-NH3). In this case, yeast was added to a concentration of 2 g/L. Two other experiments in which the yeast concentration was reduced to 2 g/L (DM) were also run: one using ordinary baker's yeast (SSF-2a) and one using the adapted yeast (SSF-2b). The WIS concentration was 5%. The Celluclast enzyme concentration was reduced in one case (SSF-EH50) to 50% of the base case. The concentration of Novozym 188 was not changed. Finally, in one case aeration was

Table 2
Summary of Experimental Conditions

Case	Conditions
SSF-BC	5 g/L of baker's yeast; 15 FPU/g of WIS
SSF-2a	2 g/L of baker's yeast; 15 FPU/g of WIS
SSF-2b	2 g/L of adapted yeast; 15 FPU/g of WIS
SSF-EH50	5 g/L of baker's yeast; 7.5 FPU/g of WIS
SSF-NH3	2 g/L of baker's yeast; 10% aqueous ammonia
SSF-Ae	2 g/L of baker's yeast; aeration during SSF

provided (SSF-Ae) and the yeast concentration was 2 g/L. An airflow of 0.27 L/min, equivalent to an air exchange rate of about 2 h, was employed.

When the slurry had been loaded, the fermentor tanks were sterilized at 121°C for 20 min. Nutrients were then added to a final concentration of 1 g/L of yeast extract, 0.5 g/L of $(NH_4)_2HPO_4$, and 0.025 g/L of $MgSO_4$. The nutrient solution was autoclaved separately at 121°C for 20 min. The temperature was set to 37°C. Celluclast was added to the slurry at a concentration of 23% of the WIS content (corresponding to a filter paper activity of 15 FPU/g of WIS), except in SSF-EH50, in which the activity was 7.5 FPU/g of WIS. Novozym 188 was added to a concentration of 5% of the WIS content (23 IU/g).

Analysis

The liquid after the pretreatment step and the samples from SSF were analyzed with high-performance liquid chromatography (HPLC) equipment (Shimadzu, Kyoto, Japan) equipped with a refractive index detector. Glucose, mannose, arabinose, galactose, and xylose were separated using an Aminex HPX-87P column (Bio-Rad, Hercules, CA) at 85°C using water as eluant, at a flow rate of 0.5 mL/min. Glucose, ethanol, acetic acid, furfural, and hydroxymethylfurfural (HMF) were separated on an Aminex HPX-87H column (Bio-Rad) at 65°C using 5 mmol/L of H_2SO_4 as eluant, at a flow rate of 0.5 mL/min. All samples were filtered through 0.20-μm filters before HPLC analysis.

Technoeconomic Evaluation

The methodology used in the technoeconomic evaluation has been described elsewhere *(4,12)*. The proposed plant is assumed to be a grassroots plant utilizing 200,000 dry metric t of raw material annually. The process consists of feedstock handling followed by the steam-pretreatment step. After dilution, the slurry is fed to the SSF step, which is followed by traditional downstream processing involving distillation and evaporation. The solids phase, mainly lignin, is dried and part of it is incinerated for steam generation. The excess lignin, if any, is pelletized and sold as a solid fuel.

The plant was assumed to be located in the north of Sweden and has access to large amounts of cooling water. Capital costs were estimated using Icarus Process Evaluator (IPE) ver 12.0 from Aspen Technology (Cambridge, MA) and data from quotations as well as reports. The equipment costs obtained from IPE were validated by comparison with actual quotations from Swedish vendors, and good agreement was observed (±20%). The yearly capital cost was estimated by using an annualization factor of 0.103, corresponding to 6% interest and a 15 yr payoff time. Working capital was estimated as described by Peters and Timmerhaus *(13)*. Aspen Plus ver 11.1 from Aspen Technology was used to determine mass and energy flows in the process based on the results from the experiments described herein.

The size of the fermentors was assumed to be 1000 m^3 with an effective working volume of 800 m^3. They are equipped with top-mounted agitators with a power requirement of 16 kW each, and internal cooling coils are used for temperature control. The fermentors are made from stainless steel, grade SS304. The total noneffective fermentation time (i.e., time used for draining, cleaning, and filling) was assumed to be 12 h.

As stated, the starch-based processes use some of the sugars from the saccharification step for yeast production, and a future lignocellulosic process is not expected to operate differently. Thus, in this study sugars readily available in the hydrolysate from the steam-pretreatment step were utilized to produce the amount of yeast required for a desired cell concentration. It was assumed that both glucose and mannose could be utilized by the yeast in this process according to the following reaction:

$$C_6H_{12}O_6 + 0.621\ NH_3 + 2.171\ O_2 \rightarrow 3.655\ H_{1.76}CN_{0.17}O_{0.53} + 2.345\ CO_2 + 3.716\ H_2O$$

Thus, the yield coefficient for cells on hexoses is 0.5. In the experiments the yeast production was carried out in fed-batch mode. In the economic evaluation, however, it was simply assumed that the yeast is to be produced directly in the fermentors prior to SSF. The fractional conversions of the other reactions in the SSF step (i.e., hydrolysis of cellulose, formation of ethanol from fermentable sugars, and formation of byproduct) were in the simulations adjusted to match experimental data before yeast growth was considered. These fractional conversions were then assumed to be unaffected when yeast growth was accounted for.

A mathematical expression similar to the Monod expression with substrate inhibition was used for the correlation between the residence time and the concentration of ethanol in the SSF:

$$Y = \frac{R \cdot K_1}{K_2 + R + (R \cdot K_3)^2} \quad (1)$$

in which R is the residence time (h) and Y is the concentration of ethanol in the liquid (g/L). Fitting of the parameters K_1, K_2, and K_3 was performed in MATLAB using a nonlinear least-squares data-fitting function. This expression was then used in the evaluation of the ethanol cost as a function of residence time.

Results and Discussion

Experimental Cases

After pretreatment a slurry was collected. The solids content (including soluble solids and WIS) of the slurry was 21%. The WIS content was determined to be 16%. The slurry contained hydrolyzed sugars originating mainly from the hemicellulose fraction of the spruce wood. The glucose concentration was 39 g/L and the mannose concentration was about 15 g/L, the former being rather high, indicating that the pretreatment was on the severe side. In the base case, SSF-BC, the final ethanol concentration reached slightly above 25 g/L after about 30 h, after which the concentration leveled off (Fig. 1). This corresponds to an overall ethanol yield of about 85% based on the glucan and mannan available in the raw material. When only half the enzyme was added to the reactor (SSF-EH50), the maximum concentration reached was about 23 g/L. The time course was more or less the same as in SSF-BC. If the yeast was allowed to adapt to the harsh environment while growing, as in case SSF-2b, the maximum ethanol concentration was almost reached after 20 h of hydrolysis and fermentation. In this case, the concentration of ethanol was about 22 g/L. Finally, when aqueous ammonia (SSF-NH3) was used to set and maintain the pH, slightly less than 20 g/L of ethanol was obtained. However, the time required to reach the maximum concentration was 72 h. In contrast to the other three cases, the concentration had not leveled off when the experiment was interrupted, which indicates that it might be possible to attain concentrations higher than 20 g/L. When 2 g/L of yeast was employed (SSF-2a) the result was less predictable, with concentrations varying from 5 to almost 20 g/L (data not shown).

When aeration was provided (SSF-Ae), the yeast did not ferment well (data not shown). The rationale for this experiment was the hypothesis that the yeast needs some oxygen to be able to survive and even grow. However, there was no major difference between SSF-2a and SSF-Ae.

The concentration of inhibitors may have a considerable impact on the outcome of SSF, both in terms of yield and in the productivity in the conversion of the lignocellulose into ethanol. Several potential inhibitors have been identified, which may have a significant effect on, above all, the fermentation part of SSF *(3,14,15)*. Enzymatic hydrolysis is also affected by various inhibitors *(16)*. Some of these are degradation products from the lignin fraction of the wood, such as phenolic or aromatic substances *(17,18)*. It has also been shown that furfural and HMF inhibit yeast. This

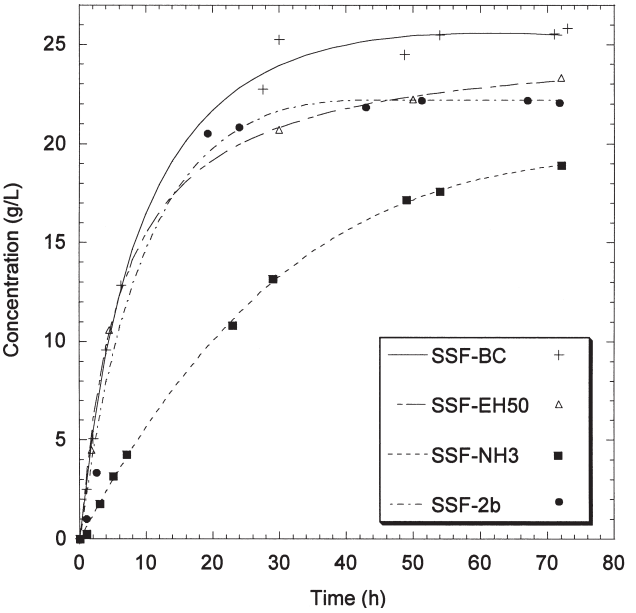

Fig. 1. Concentration of ethanol in SSF as function of residence time. The lines indicate fitted expressions and the symbols the experimental results.

can often be seen as a lag phase during which no ethanol fermentation occurs. If the concentration is not too high, the overall yield may still be the same, but the productivity becomes lower *(3)*. Figure 2 shows the rapid depletion of furfural in SSF-BC and SSF-2b. This can be explained by the high concentration of yeast in SSF-BC, where the furfural is quickly depleted (converted into furfuryl alcohol). Also in SSF-2b a rapid decrease in furfural can be noted. This is explained by the ability of the adapted yeast to withstand the stress of the harsh environment. The furfural is more or less consumed in SSF-EH50 and SSF-NH3; however, this requires a somewhat longer time during which the yeast does not perform well.

The effect of inhibitors is basically the same when HMF is considered. The adapted yeast metabolizes the HMF very rapidly, while in cases SSF-EH-50 and SSF-NH3, about 25% of the starting concentration remains after 72 h (data not shown).

Simulation Cases

Only the most promising experiments were included in the technoeconomic evaluation and compared with the base case. These were the case employing a 50% enzyme reduction and the case using the adapted yeast. All other experimental runs showed such a large decrease in ethanol yield that it was obvious that they would have a significantly higher production cost than the base case.

The simulated recovery of WIS after the pretreatment step was 54% and the concentration 24%, the latter being higher than in the experiments,

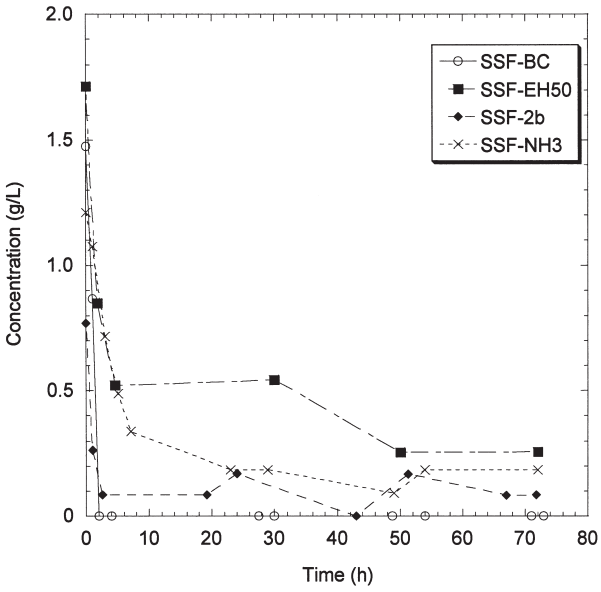

Fig. 2. Concentration of furfural as function of residence time in various SSF cases.

in which it was about 16%. The main reason for this is that the small-scale laboratory equipment was operated in batch mode, which resulted in a high heat loss. The collecting vessel was also cooled in the experiments, which caused condensation of steam produced during flashing. These effects will not be seen in a full-scale continuous process. After the addition of fresh water to dilute the slurry to 5% WIS, the total volumetric flow rate to the SSF step is 274 m^3/h.

For the proposed process with an intake of 200,000 t of dry raw material annually, the experimental results for the SSF-BC case yields an ethanol production of 6990 kg/h at a residence time of 72 h (without yeast production). This corresponds to a very high overall ethanol yield, 85%, and requires that essentially all the cellulose from the pretreatment step to be hydrolyzed to glucose and that 95% of these released sugars together with the glucose and mannose from the pretreatment step be converted into ethanol. In the simulations, it was assumed that 50% of the glucose and mannose not converted into ethanol produced glycerol and that the rest remained unfermented.

Equation 1 was used in Aspen Plus to determine the yield at different residence times in the SSF step. The parameters fitted to Eq. 1 can be seen in Table 3. Figure 3 shows the ethanol production cost as a function of residence time in the SSF step for SSF-BC, as well as for SSF-2b and SSF-EH50. The calculations are based on the assumption that the yeast required in the process is produced from sugars in the hydrolysate, which lowers the ethanol yield. The base case, SSF-BC, has a minimum ethanol production cost of 4.80 SEK/L [Swedish kroner per liter corresponding to 2.34 US$/gal (US dollar per gallon) at an exchange rate of 7.75 SEK/US$] at a

Table 3
Parameters in Monod Equation[a]

	SSF-BC	SSF-EH50	SSF-NH3	SSF-2b
K_1	10.7245	6.3032	92.8376	14.8552
K_2	34.5407	25.2079	58.8744	37.7259
K_3	0.0534	0.000	0.1067	0.0908

[a]Valid to 72 h; adapted yeast valid to 40 h.

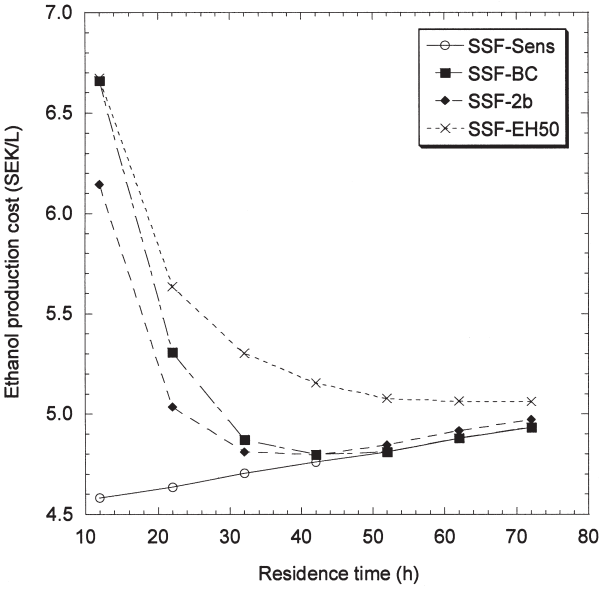

Fig. 3. Ethanol production cost as function of residence time in SSF.

residence time of about 42 h, and increases to 4.93 SEK/L at 72 h. This increase in production cost is owing to the facts that the number of fermentors increases with residence time, and that the yield reaches its maximum at about 50 h. Thus, there is no reason for a longer residence time than 50 h. At 42 h, the total ethanol production is 5620 kg/h, compared with 6990 kg/h for the experimental yield of 85%. The reduction in ethanol production is owing to the required yeast cell production.

A sensitivity study (SSF-Sens) was conducted to investigate the effect of a shorter residence time while maintaining the ethanol yield achieved in SSF-BC. From Fig. 3 it can be seen that if the residence time is reduced from 72 h to an extremely short time of 12 h, the production cost decreases by 0.35 SEK/L. The decrease in ethanol production cost with the residence time in SSF is mainly owing to the reduction in capital cost. Whether the highest ethanol yield can be reached in 12 h can be questioned. However, the SSF-2b case has a very high productivity despite the fact that the yeast concentration

Table 4
Various Costs Used in Economic Analysis and Calculated Costs for Base Case at Residence Time of 42 h

	Flow rate(kg/h)	Cost (SEK/kg)	Cost (SEK/L of 100% EtOH)
Raw material			
Wood (DM)	25,000	0.42	1.46
Chemicals			
SO_2	250	1.5	0.05
CaO	400	0.7	0.04
Defoamer	13	20	0.04
NaOH (50%)	415	2.0	0.12
$(NH_4)_2HPO_4$	137	1.5	0.03
$MgSO_4 \cdot 7H_2O$	7	4.4	0.00
Enzymes	$205 \cdot 10^6$ FPU/h	21 SEK/10^6 FPU	0.62
By-product income			
Solid fuel (DM)	1097	0.79	–0.12
CO_2	6016	0.03	–0.03
Utilities			
Electricity	4.41 MWh/h	250 SEK/MWh	0.16
Cooling water	2896 m^3/h	0.14 SEK/m^3	0.06
Process water	236 m^3/h	1.40 SEK/m^3	0.05
Other costs			
Labor[a]		500,000 SEK/employee	0.22
Insurance	Annually 1% of fixed capital		0.15
Maintenance	Annually 2% of fixed capital		0.31
Capital			
Fixed capital	Annually 10.3% of fixed capital		1.58
Working capital	Annually 6% of working capital		0.07
Total (SEK/L)			4.80

[a]Twenty-five employees assumed.

is low. If the yeast can be further adapted, it might be possible to enhance the productivity and thereby shorten the residence time in the SSF step.

Table 4 shows a breakdown of costs for the base case with the lowest production cost, 4.80 SEK/L, at a 42-h residence time. The raw material cost and the capital cost are the largest contributors to the overall production cost, as has been found in other studies *(19,20)*. The cost of the enzymes was estimated to be 0.62 SEK/L. The high energy demand in the downstream processing steps requires that the main part of the solids remaining after drying be incinerated for steam production. The fixed

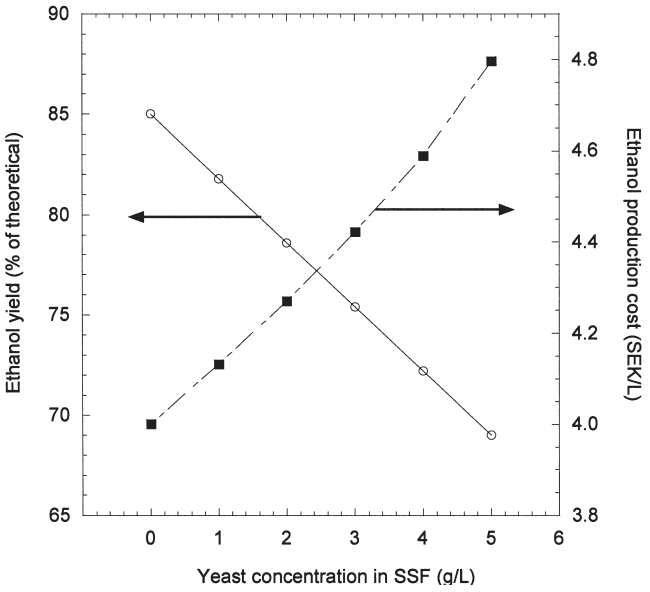

Fig. 4. Ethanol production cost as function of yeast concentration in SSF step.

capital investment was estimated to be 874 million SEK, of which the SSF step constitutes 16%. Working capital was estimated to be 56 million SEK.

The use of adapted yeast at 2 g of cells/L results in the same ethanol production cost as in the base case, 4.80 SEK/L at a residence time of 42 h (see Fig. 3). Although the experimental yield is much higher in SSF-BC (85%) compared with the experimental yield in SSF-2b (74%), the amount of sugar needed for yeast growth in SSF-BC is so high that the overall ethanol yield becomes almost the same. If a cell concentration of 2 g/L in laboratory-scale experiments could be used while still maintaining an ethanol yield of 85%, the estimated full-scale production cost would be 4.27 SEK/L (see Fig. 4).

A reduction in enzyme concentration (SSF-EH50) results in a final ethanol cost of 5.06 SEK/L at a residence time of 72 h (Fig. 3). In this case, the ethanol yield does not reach its maximum after 72 h, owing to the lower hydrolysis rate. The minimum ethanol cost is not reached after 72 h. However, a slight decrease in the cost with longer residence time is still possible. The cost of the enzymes is, however, very uncertain and a sensitivity analysis was conducted to investigate the effect of a change in this cost. The sensitivity analysis was based on the lowest base case cost (4.80 SEK/L) and the lowest cost in the case with reduced enzyme addition (5.06 SEK/L). Breakeven occurs at an enzyme cost of 42 SEK/million FPU when the corresponding ethanol cost is 5.40 SEK/L, which is 0.60 SEK/L higher than in SSF-BC (Fig. 5).

It is apparent that the overall ethanol yield and thus the ethanol production are significantly reduced when yeast production from sugars available in the hydrolysate is accounted for. Another sensitivity study was carried out to investigate the effect of reducing the yeast concentration in the

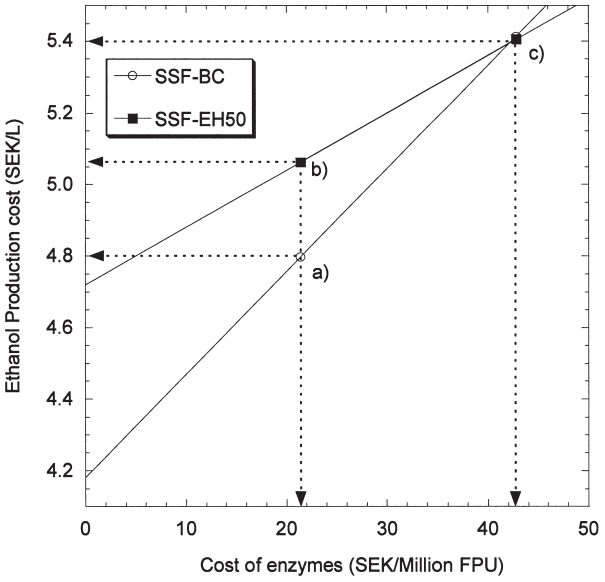

Fig. 5. Ethanol production cost as function of enzyme cost: a) and b) indicate the lowest cost in the SSF-BC case and SSF-EH50 case, respectively; c) indicates the breakeven cost.

SSF step while maintaining the overall experimental yield that was reached in SSF-BC at a 42-h residence time. In the simulations, the yeast concentration was varied from 1 to 5 g/L and the effect on the overall ethanol yield as well as ethanol production cost was determined. The reduction in yield was 3.2 percentage points for every gram of yeast needed per liter (Fig. 4). Thus, the overall reduction in yield was 16.0 percentage points at 5 g/L, and the estimated production cost increased from 4.00 (no cell production) to 4.80 SEK/L. The yeast production itself increases the cost by 0.80 SEK/L, or 20%, compared with a process without yeast. This does not include possible increases in capital cost owing to the in-house production. In a previous study *(4)*, the cost of the yeast was calculated to be 0.5 SEK/L for the same conditions as in our study. However, in that study the yeast was not produced in-house but was assumed to be purchased from a yeast-producing company.

The ethanol production costs reported in our evaluation are higher than those reported previously *(4,12)*, in which a WIS concentration of about 8.5% was evaluated. Because a higher concentration of WIS results in a less dilute ethanol stream to be processed downstream, the energy requirement will be lower (assuming that the ethanol yield does not change). A higher concentration of WIS at the start of SSF will also affect the reduction in overall ethanol yield owing to cell growth. Ethanol yield as a function of the initial concentration of WIS in the SSF step can be seen in Fig. 6. At 10% WIS, only half the amount of sugar required for yeast production is required compared with a case at 5% WIS.

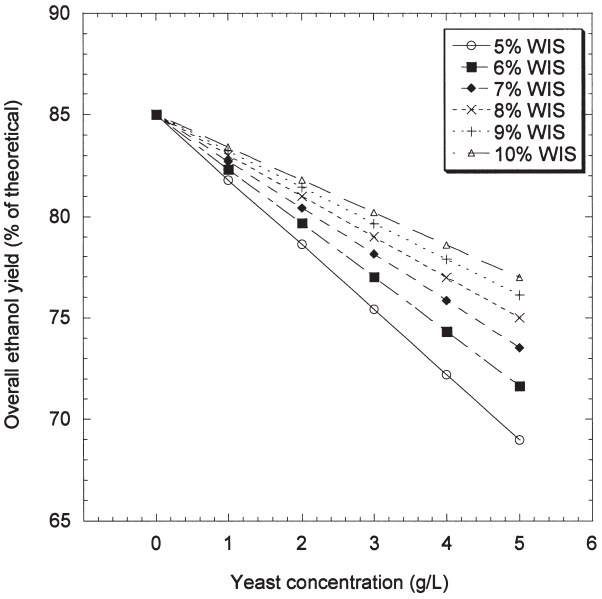

Fig. 6. Ethanol yield as function of yeast concentration in SSF for various initial concentrations of WIS.

A comparison can be made with a commercial full-scale ethanol plant using wheat or corn as raw material. If the ethanol production for such a plant is the same as in the SSF-BC, the volumetric flow rate to the fermentation step would be 70 m^3/h compared with 274 m^3/h for SSF-BC. This is due to the much higher ethanol concentration (8–10 % [w/w] in the starch-based process *(21)*. Thus, if the cell concentrations are the same, the actual amount of sugar needed for yeast production in the starch-based process is one-fourth of the amount required in the ethanol plant evaluated in this study.

Conclusion

For the hypothetical base case process employing 5 g/L of yeast, in which no sugars are utilized for yeast production, the estimated ethanol production cost was 4.00 SEK/L (1.95 US $/gal). However, in a real process the yeast must be produced from the available sugars, which will ultimately reduce the ethanol yield. This will be reflected in a higher production cost of 4.80 SEK/L (2.34 US $/gal). Adapted yeast resulted in an experimental overall yield of 74%, and the estimated ethanol production cost was the same as in the base case. When the addition of enzymes was reduced by 50%, the ethanol production cost increased to 5.06 SEK/L (2,47 US $/gal).

The results indicate that a lower ethanol production cost could be expected when adapted yeast is used at concentrations between 2 and 5 g/L. It is also important to investigate the impact of WIS concentrations higher

than 5%. This will affect not only the cost of the downstream processing but also the amount of yeast needed. Although the ethanol production cost was found to be higher when the enzyme loading was decreased, further evaluation is required.

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