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Recirculation of Process Streams in Fuel Ethanol Production from Softwood Based on Simultaneous Saccharification and Fermentation

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Abstract

The effect of process stream recirculation on ethanol production from steam- pretreated softwood based on simultaneous saccharification and fermentation (SSF) was investigated for two process configurations. In the first configuration, a part of the stillage stream after distillation was recycled and, in the second configuration, the liquid after SSF was recycled. The aim was to minimize the energy consumption in the distillation of the fermentation broth and in the evaporation of the stillage, as well as the use of fresh water. However, recirculation leads to an increased concentration of nonvolatiles in the first configuration, and of both volatiles and nonvolatiles in the second configuration. These substances might be inhibitory to the enzymes and the yeast in SSF. When 60% of the fresh water was replaced by stillage, the ethanol yield and the productivity were the same as for the configuration without recirculation. The ethanol production cost was reduced by 17%. In the second configuration, up to 40% of the fresh water could be replaced without affecting the final ethanol yield, although the initial ethanol productivity decreased. The ethanol production cost was reduced by 12%. At higher degrees of recirculation, fermentation was clearly inhibited, resulting in a decrease in ethanol yield while hydrolysis seemed unaffected.

Index Entries: Recirculation; ethanol; simultaneous saccharification and fermentation; inhibition; softwood.

Introduction

Ethanol obtained from biomass represents a sustainable substitute for fossil fuels. The commercialization of fuel ethanol is still a risky enterprise,

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since most economic calculations show that the estimated ethanol production cost would be higher than the price of gasoline *(1)*. Hence, the development of an economical process is an important and critical issue. Different process configurations have been reported *(2,3)* with the aim of increasing the ethanol yield and productivity, which, in turn, reduces production cost.

Simultaneous saccharification and fermentation (SSF) of softwood to ethanol was found, in a previous study, to be superior to separate hydrolysis and fermentation (SHF) in terms of ethanol yield, productivity, and production cost *(4)*. Different process configurations have been reported *(5–7)*, but a process including recirculation of process streams, based on SSF, has still not been proposed. Previous studies, on willow and softwood, based SHF were focused on the evaluation of the effect of the recycled stream on the ethanol yield *(8–10)*. The requirement of fresh water for the process can be reduced by internal recirculation of process streams. Less energy then will be required in the distillation of the fermentation broth and in the evaporation of the stillage stream from the distillation stage *(11)*. The drawback of the recirculation of process streams is the accumulation of dissolved substances that could be inhibitory to both yeast fermentation and enzymatic hydrolysis *(12)*. Some of these substances are present in the wood, and some are formed during pretreatment as degradation products of pentoses, hexoses, and lignin *(13–17)*. These substances might act as inhibitors both to the cellulases and to the yeast in SSF. Lignin-based compounds were identified as the most inhibitory substance *(15)*.

In a previous simulation study on ethanol production from steam-pretreated softwood, it was shown that the ethanol production cost could be reduced significantly by recirculation of either the liquid after SSF or the stillage stream after distillation *(10)*. However, in that investigation, it was assumed that the concentration of unfermentable dissolved substances (UDS), which accumulate during recirculation, had no effect on the yield or the productivity in SSF.

In the present study, the effect of various degrees of recirculation on ethanol yield and productivity was investigated for two different process configurations as presented in Fig. 1. In the first configuration, recirculation after distillation (RAD), part of the stillage stream was recycled in different amounts to the SSF reactor, which caused accumulation of the nonvolatile fraction, the UDS. In the second configuration, recirculation before distillation (RBD), the liquid stream after SSF was recycled in different amounts, resulting in the accumulation of both volatile and nonvolatile substances in the process.

Materials and Methods

Raw Material

Fresh, chipped softwood was kindly provided by a sawmill in southern Sweden (Höörsågen AB), Höör. The softwood was rechipped and sieved to obtain a chip size of 2–10 mm. The chips were stored in a plastic

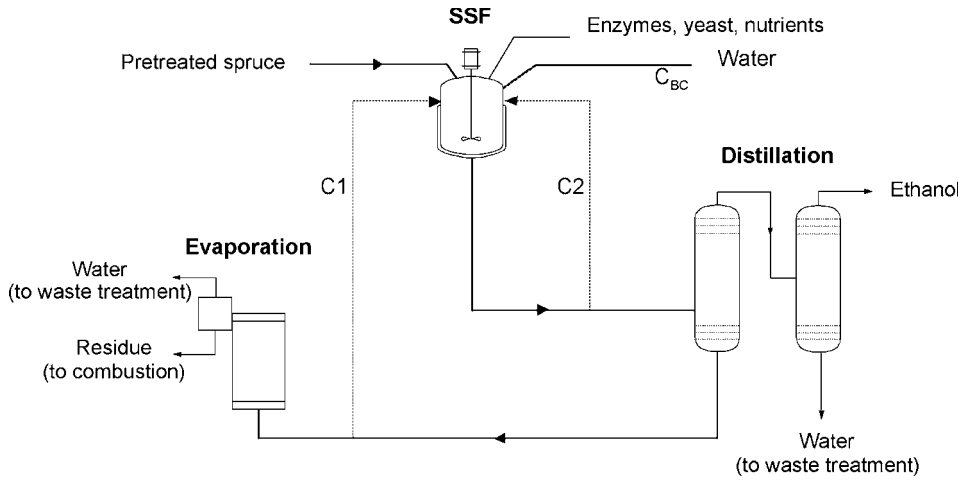

Fig. 1. Schematic flowsheet of ethanol production based on SSF with different recirculation configurations. C_{BC}, addition of fresh water; C1, recirculation of stillage after distillation; C2, recirculation of liquid before distillation.

Table 1
Composition of Spruce

Component	Dry Matter (%)
Glucan	42.5
Mannan	12.3
Xylan	5.4
Galactan	2.3
Arabinan	2
Lignin	26

bag at 4°C prior to use. The composition (see Table 1) was analyzed using the Hägglund method *(18)* followed by high-performance liquid chromotography (HLPC) analysis. The dry matter content was determined to be 52%.

Pretreatment

The wood chips were impregnated with sulfur dioxide (3% [w/w] moisture) for 20 min at room temperature. Impregnation was performed in a tightly closed plastic bag to allow penetration of the gas through the wood tissues. The amount of SO_2 absorbed was determined by weighing the plastic bag before and after impregnation, and was found to be 2.5% (w/w) moisture. The impregnated material was steam pretreated at 215°C for 5 min in a steam-pretreatment unit equipped with a 10-L reactor, which has been described previously *(19)*. Steam pretreatment was performed in six batches, and the amount of impregnated chips was 600 g dry wt in each batch. The whole slurry from all the batches was mixed and stored at 4°C for future use.

Preparation of the Liquid for Recirculation

SSF, with a 12.5-kg working weight, was performed in a 22-L fermentor (Bioengineering AG, Switzerland) to obtain a sufficient amount of process stream for recirculation. The slurry from the pretreatment step was diluted with fresh water to 5% dry matter (insoluble material). The operational parameters of this SSF run were the same as in the subsequent SSF runs, in which recirculation was evaluated (see the SSF section).

The liquid after SSF was filtered using a Büchner funnel and the solid residue was discarded. The filtrated liquid was concentrated in a Büchi Rotavapor R-153 (Switzerland) evaporation unit, where 88% of the liquid after SSF was evaporated. The residue after evaporation, the concentrated liquid, was collected and used in various amounts in the subsequent SSF runs to replace fresh water. This liquid was thus concentrated to a concentration factor (CF) of 8.3. The CF represents the ratio between the UDS in the liquid residue and in an SSF run with only fresh water, i.e., without recirculation of any process streams.

Recirculation

In all SSF runs, the slurry from the pretreatment step was diluted to 5% dry matter (insoluble material). In the recirculation runs, part of the fresh water was replaced either by the stillage stream or by liquid from SSF, as illustrated in Fig. 1. The recirculation of stillage was performed at five different recirculation degrees, RAD1–RAD5, corresponding to a CF of 1.2, 1.5, 1.8, 2.5, and 3.0, respectively. The addition of stillage was performed to simulate the ultimate concentrations of nonvolatiles obtained in a real continuous process. The recirculation of liquid after SSF (i.e., before distillation), was performed at five different recirculation degrees, RBD1–RBD5, corresponding to the same values of CF as when the stillage was recycled. Since the liquid after SSF contains both volatiles and nonvolatiles, the volatile components ethanol and acetic acid had to be added to the concentrated recirculation. The amounts of concentrated liquid, ethanol, and acetic acid added were calculated to give the concentrations of nonvolatiles and volatiles that would have been achieved at steady state in a continuous process.

Simultaneous Saccharification and Fermentation

The SSF experiments were performed in a 1-L fermentor (Belach AB, Stockholm, Sweden) with a working weight of 750 g under semisterile conditions. The slurry consisting of the pretreated material was diluted to 5% insoluble material using either fresh water (base case) or a combination of fresh water and recirculated liquid. The diluted slurry and the yeast nutrients were autoclaved at 120°C for 20 min. The enzyme preparations were added directly to the fermentor vessel. The enzyme preparations were Novozyme 188 with a β-glucosidase activity of 362 IU/g, and Celluclast 1.5L with a cellulase activity of 75 filter paper units (FPU) and a β-glucosi-

dase activity of 12 IU/g *(20,21)*. The enzyme preparations were kindly provided by Novo Industri A/S (Bagsvaerd, Denmark). The enzyme concentration of Novozyme 188 was 4% (w/w) fibrous material, which corresponds to a β-glucosidase activity of 28 IU/g of cellulose, and the amount of Celluclast 1.5L was 24% (w/w) fibrous material, which corresponds to a cellulase activity of 32 FPU/g of cellulose.

Normal baker's yeast (*Saccharomyces cerevisiae*) was inoculated at 0.5% of the total working weight, and the supplemented yeast media compositions were as follows: 0.5 g/L of $(NH_4)_2HPO_4$, 0.025 g/L of $MgSO_4 \cdot 7H_2O$, and 1.0 g/L of yeast extract. The initial pH value was adjusted to 4.9–5.1 using solid $Ca(OH)_2$, and during SSF the pH was maintained at 5.0 by the addition of 10% NaOH. All SSF experiments were performed at 37°C and pH 5.0 for 72 h. Antibiotics, (20,000 U/mL of penicillin and 20 mg/mL of streptomycin), were added to all SSF runs in order to avoid the risk of contamination *(22)*. This was done to ensure that changes in the ethanol yield were owing to inhibition by the recirculated liquid and not to competing microorganisms.

Analysis

Samples taken from the raw material analysis, liquid fraction of the pretreatment step, and SSF were analyzed using an HPLC instrument (Shimadzu, Kyoto, Japan) equipped with a refractive index detector (Shimadzu). Ethanol, glucose, furfural, hydroxymethylfurfural (HMF), acetic acid, and glycerol were analyzed using an Aminex HP-87H column (Bio-Rad, Hercules, CA) at 65°C, using 5 mM H_2SO_4 as eluent at a flow rate of 0.5 mL/min. Since mannose, xylose, and arabinose were eluted at the same time, these sugars were determined using a PL Hi-Plex Pb (Polymer, Shropshire, UK) column at 80°C, with ultrapure water as eluent at a flow rate of 0.5 mL/min. The dry matter content of the slurry obtained and the yeast were determined by drying the material at 105°C overnight.

Results

Table 2 gives the composition of the steam-pretreated material. The recovery of the solid material was 60% based on the original dry wood, and the dry matter content of the pretreated material was 16.5%, which includes the dissolved substances, of which 11.5% was fibrous material (insoluble matter). The recovery of glucose and mannose in the liquid fraction was 18 and 62% of theoretical, respectively, whereas the recovery of glucan in the solid fraction was 69% of theoretical. The recovery of lignin was slightly above 100% in the solid fraction, which is probably owing to some condensation products *(23)* being included in the residue after the Hägglund analysis.

In the evaluation of SSF, the ethanol yield was expressed as a percentage of the theoretical yield based on the amount of fermentable substances (cellulose, glucose, and mannose) present during SSF. The

Table 2
Composition of Steam-Pretreated Spruce

Total dry wt	16%
Fibrous dry wt	11.5%
Solid fraction	
Cellulose	49% of dry matter
Lignin	48% of dry matter
Solid yield	60% of dry matter
Liquid fraction (g/L)	
Glucose	18.3
Mannose	18.3
Xylose	8
Arabinose	2.9
Galactose	3.7
HMF	2.5
Furfural	1.4
Acetic acid	3.6

productivity was calculated as an average of the ethanol production rate after the first 5 h (r_5h).

The sum of glucose and mannose concentrations in all SSF runs was approx 16 g/L at the start of SSF. In the base case, in which only fresh water was used and no stream was recirculated, the highest ethanol concentration was reached after about 72 h. The final ethanol yield was 88% of the theoretical and the productivity was 1.9 g/(L·h). Mannose and glucose were rapidly depleted. Furfural and HMF had been completely consumed by the end of SSF. The acetic acid concentration was increased slightly, from 2.3 to 2.7 g/L. No lactic acid formation was observed owing to the addition of antibiotics. These results are very similar to those obtained in the SSF runs performed in the 22-L fermentor at the same conditions.

Recirculation After Distillation

Table 3 shows the ethanol yield and productivity of SSF with recirculation of the stillage stream. The ethanol yield was almost the same as in the base case for runs RAD1–RAD4 (i.e., when up to 60% of the fresh water was replaced). The same was observed for the ethanol productivity.

In run RAD5, in which the highest amount of UDS was recycled, the ethanol yield decreased to 80% of theoretical and the productivity decreased to half that obtained in the base case. Figure 2 shows the ethanol, glucose, and mannose profiles in SSF for the base case and RAD5. The glucose and mannose consumption in RAD1–RAD4 was similar to that obtained in the base case, but it differed in the last case (RAD5). In run RAD5, fermentation was clearly inhibited compared with the base case. Ethanol productivity was affected by the presence of nonvolatile substances. Yeast was able to consume furfural completely in all the runs, according to HPLC analysis,

Table 3
Influence of Recirculation After Distillation
on Ethanol Yield and Productivity

Run	CF	Fresh water reduction (%)	Ethanol productivity (r_5h)	Ethanol yield in SSF
Base case	1	0	1.83	88
RAD1	1.2	24	1.9	87
RAD2	1.5	39	1.85	83
RAD3	1.8	49	1.87	88
RAD4	2.5	59	2	88
RAD5	3	68	1	80

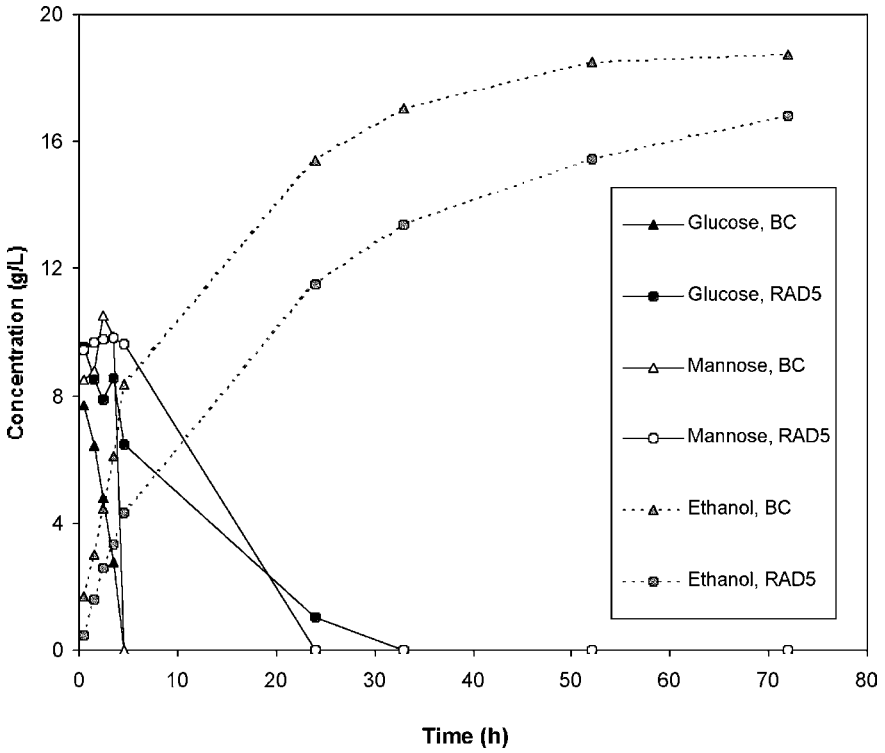

Fig. 2. Concentration of glucose, mannose, and ethanol as function of time in SSF for base case (BC) and RAD5.

which showed that no furfural was left at the end of SSF. The initial concentration of HMF was between 1 and 1.5 g/L in RAD1–RAD5 and decreased to very low concentrations. The highest final concentration, 0.42 g/L, was obtained in RAD5. The acetic acid concentration increased as degree of recycling was increased. The initial value was 2.24 g/L in the base case and reached 7.3 g/L in RAD5. Lactic acid formation was not observed in any run.

Table 4
Influence of Recirculation Before Distillation on Ethanol Yield and Productivity

Run	CF	Fresh water reduction (%)	Ethanol productivity (r_5h)	Ethanol yield in SSF	Ethanol (g/L)	Acetic acid (g/L)
Base case	1	0	1.83	88	0	—
RBD1	1.2	24	2	86	6	0.8
RBD2	1.5	39	1.5	85	12	1.6
RBD3	1.8	49	1.2	40	20	2
RBD4	2.5	59	0	0	28	2.2
RBD5	3	68	0	0	38	3

Recirculation Before Distillation

Table 4 shows the ethanol yield and the productivity during SSF with recirculation of the liquid stream after SSF. The ethanol yield was almost the same as in the base case for runs RBD1 and RBD2 (i.e., when up to 40% of the fresh water was replaced). However, there was a slight decrease in the productivity, which dropped to 1.5 and 1.2 g/(L·h) for RBD1 and RBD2, respectively. The productivity recovered after a lag phase of 24 h, after which it was about the same as that for the base case (data not shown).

In run RBD3, in which higher amounts of ethanol and acetic acid were recycled, the ethanol yield decreased to 40% of theoretical, and productivity was 1 g/(L·h). In runs RBD4 and RBD5, in which the highest amounts of ethanol and acetic acid were recirculated, no ethanol was produced.

Both glucose and mannose were completely consumed in RBD1 and RBD2, but the consumption was higher in RBD1. In run RBD3, the glucose concentration decreased to 4.5 g/L during the first 5 h but then increased again to 5.6 g/L after 24 h. Thereafter it continued to increase to 9.2 g/L, which was reached after 72 h. Mannose concentration remained at 4 g/L during the whole SSF process. In runs RBD4 and RBD5, the glucose concentration increased to even higher levels, 18 and 17 g/L, respectively, while the mannose concentration was maintained at its initial level (see Fig. 3).

HMF and furfural were completely consumed in RBD1 and RBD2, while the concentration decreased below 0.3 g/L in RBD3 and remained constant at its initial value in RBD4 and RBD5.

Discussion

Computer simulations have shown that the recirculation of process streams leads to the accumulation of inhibitors *(11)*. These inhibitors, which are generated in the pretreatment step, have been shown to be inhibitory to both yeast and enzymes. *(17)*. In the present study, SO_2-impregnated spruce was steam pretreated at 215°C for 5 min corresponding to a severity of 3.5 *(19)*. At this severity, the fermentability of the liquid from pretreatment has previously been found to be high *(19)*. As a result, the energy demand and

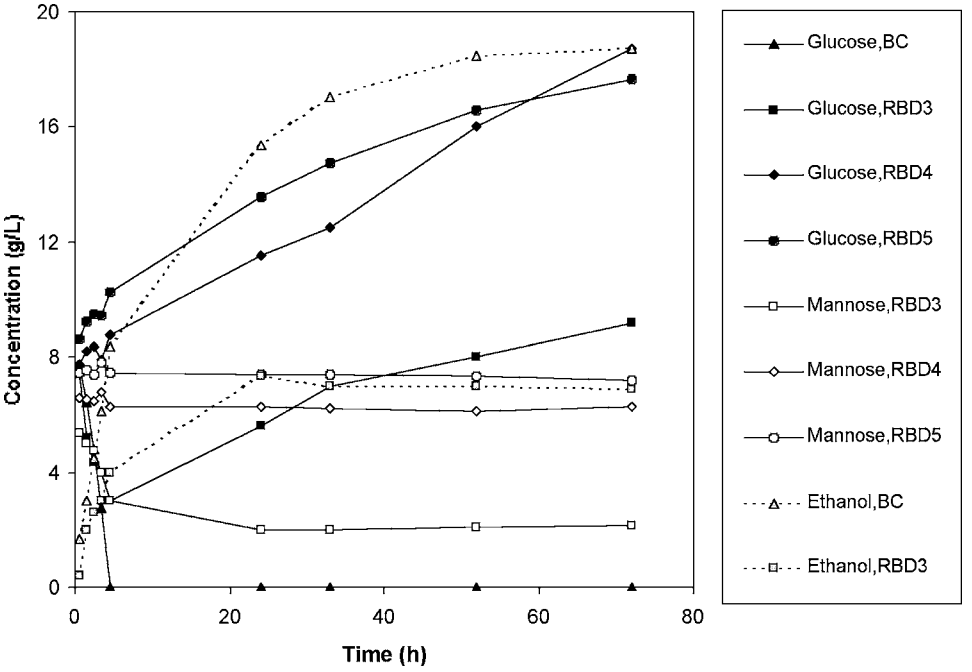

Fig. 3. Concentration of glucose, mannose, and ethanol as function of time in SSF for base case (BC), RBD3, RBD4, and RBD5.

the fresh water consumption may be reduced by recirculation of part of the stream leaving the fermentor vessel. Figure 4 shows the calculated production cost for various process configurations with recirculation of process streams. The degree of recirculation is expressed in terms of the CF during SSF. The corresponding reduction in fresh water demand is also given. The production cost is based on a plant size of 200,000 metric t of dry raw material/yr with continuous operation of 8000 h/yr *(10)*. The ethanol yield and productivity in SSF were assumed to be unaffected by the recirculation of the process streams.

Recirculation After Distillation

Recycling had no influence on the ethanol yield or productivity of RAD1–RAD4 (Table 3). This may be owing to the use of SO_2 in the pretreatment step, resulting in only moderate production of nonvolatile inhibitors, and to the tolerance of the yeast to the inhibitors present in the recirculated stream.

In run RAD5, the ethanol yield dropped by 10%, undoubtedly as a result of the increase in the amount of nonvolatile inhibitors by a factor of 3 (CF = 3). These inhibitors probably affected the enzymatic hydrolysis more than the yeast fermentation. Although the ethanol productivity decreased to 50% of that in the base case, the sugars (glucose and mannose) had been completely consumed by the end of run RAD5. The decreased ethanol

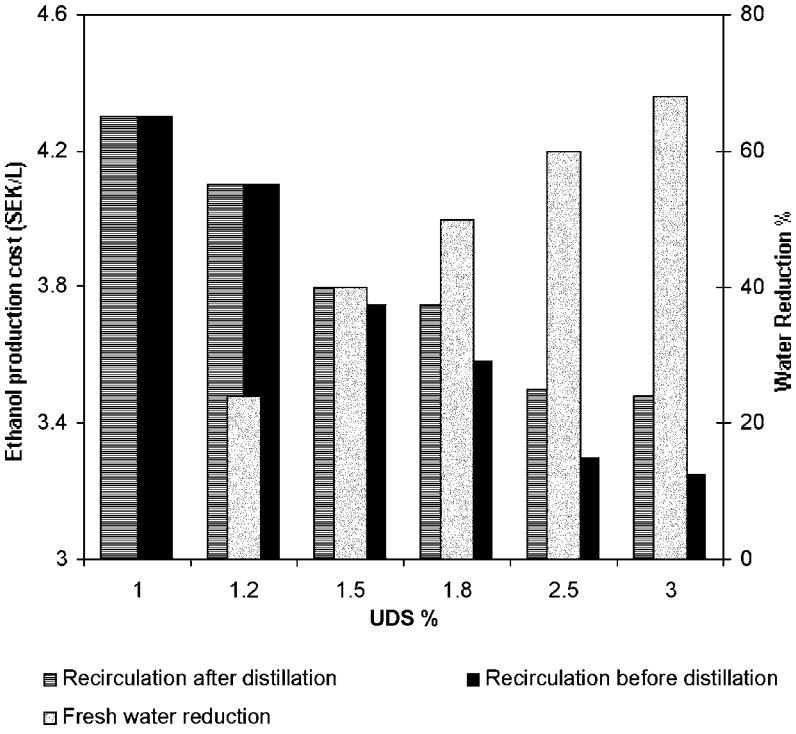

Fig. 4. Ethanol production cost in Swedish krona (SEK) as function of UDS and water reduction.

productivity was probably owing to the adaptation of the yeast to the increased amount of soluble substances in the fermentation media. Palmqvist et al. *(12)* showed that the nonvolatile inhibitors generated by steam pretreatment of willow decreased cellulose conversion by one-third when the concentration was increased by a factor of five compared with the base case using fresh water. This indicates that the decrease in ethanol yield in RAD5 was probably owing to less efficient cellulose conversion. In SSF employing the RAD4 alternative, the fresh water can be reduced by 60% compared with the base case, which, in turn, will reduce the cost of ethanol production by 17% from the initial cost based on the base case (see Fig. 4).

In previous investigations, the possibility of adapting *S. cerevisiae* to cope with the hydrolysate inhibitors has been demonstrated *(24,25)*. It was also shown that the viability of the cells was not adversely affected after adaptation to the inhibitors present in oak hydrolysate *(25)*. These investigations make the RAD5 alternative interesting (despite the observed inhibition) because the ethanol production cost may be reduced by 20%.

Recirculation Before Distillation

The recirculation of the stream before distillation (i.e., the outlet from SSF), results in an increase in both nonvolatile and volatile compounds.

This means that for the same CF, the RBD alternative will lead to a higher concentration of potential inhibitors (i.e. the volatile components).

In RBD1 and RBD2, there was no decrease in the ethanol yield compared with the base case. The initial concentrations of ethanol were 6 and 12 g/L and of acetic acid were 0.8 and 1.6 g/L, in RBD1 and RBD2, respectively. These acid concentrations are well below the toxicity level of the aliphatic acids in general, which is about 12 g/L *(26)* at pH 4.5. However, in RBD1 and RBD2, a relatively low productivity was obtained compared with the RAD1 and RAD2 runs, which was probably owing to the required adaptation of the yeast to the increased initial concentration of ethanol and acetic acid.

At higher degree of recirculation (RBD3) decreased the ethanol yield to 40% of theoretical. According to Maiorella et al. *(27)*, ethanol inhibition of *S. cerevisiae* starts at 25 g/L and is complete at 95 g/L. In RBD3, an initial concentration of 20 g/L of ethanol resulted in clear inhibition, compared with RAD3, in which no ethanol was initially present. The final concentration of ethanol was 27 g/L. It might be difficult to attribute the low yield to a certain group of inhibitors, such as externally added volatile compounds or recirculated nonvolatile substances, since ethanol is usually not inhibitory at these low concentrations, and especially since the toxicity of internally formed ethanol during yeast fermentation is higher than that of the externally added ethanol *(28)*.

The limited glucose consumption in RBD3 up to 24 h, indicates the onset of yeast inhibition owing to the increased formation of ethanol. Increased ethanol concentration resulting from fermentation of mannose and glucose leads to an increase in the total amount of inhibitors during SSF. The enzymatic hydrolysis continued to some extent, indicating that the inhibition of the yeast was more severe than that of the enzymes.

In RBD4 and RBD5, there was no ethanol production at all and the yeast was completely inhibited. This means that a concentration of 28 g/L of ethanol and 2 g/L of acetic acid, in addition to the nonvolatile components corresponding to CF 2.5, make the liquid totally inhibiting. This is in contrast to RAD4, the main difference being the ethanol concentration, which was not inhibitory.

Hydrolysis resulted in glucose concentrations of 18 and 17 g/L, corresponding to yields of 58 and 61% of the theoretical (based on cellulose and initial glucose) in RBD4 and RBD5, respectively. The decrease in glucose yield in RBD4 and RBD5 is probably a result of end-product inhibition rather than inhibition caused by the volatile components, since the glucose yield was nearly the same in both runs. It can thus be concluded that the cumulative effect of the inhibitors was less on the enzymatic hydrolysis than on the yeast.

Furfural and HMF were totally consumed in RBD1 and RBD2, but only partially consumed in RBD3, whereas in RBD4 and RBD5 they remained at the initial concentration. However, previous studies have shown that these components do not cause any significant inhibition at

concentrations of about 1 to 2 g/L *(26)*. It is probable that neither furfural nor HMF had any significant role in enzymatic hydrolysis or yeast fermentation in the present study.

Conclusions

The results of SSF were influenced to a higher degree by the amount of liquid recirculated before distillation than by recirculation of the stream after distillation. This was caused by the increase in the concentration of volatile components, mainly ethanol, in RBD. A concentration as low as 28 g/L of ethanol significantly reduced the amount of nonvolatile components that could be recirculated, without causing total inhibition.

Our study shows that it is possible to recirculate process streams without affecting ethanol yield. The degree of recirculation is limited by either the amount of liquid present in the pretreated material (i.e., by the steam in the pretreatment step), or the influence of the inhibitors that accumulate in the process streams.

Our study also showed that it was possible to reduce the amount of fresh water required by 60% when the stillage stream was recycled without influencing the ethanol yield. This resulted in an estimated reduction in the cost of ethanol production of 17%, mainly owing to a decrease in energy demand in the evaporation unit.

When the liquid after SSF was recycled, it was only possible to reduce the amount of fresh water by up to 40% without affecting the ethanol yield. In this case, the cost of ethanol production was reduced by 12% owing to a reduced energy demand in both distillation and evaporation.

A further increase in the fraction of recirculated process streams could probably be achieved either by using yeast that is adapted to the inhibitors or by detoxifying the recirculated streams. Detoxification will, however, introduce another process step, which adds complexity to and increases the costs of the process.

Future studies will focus on recirculation of various combinations of process streams, including evaporation condensates, and detoxification of the liquid prior to recirculation.

Acknowledgment

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