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Recycling of Process Streams in Ethanol Production from Softwoods Based on Enzymatic Hydrolysis

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ABSTRACT

In ethanol production from lignocellulose by enzymatic hydrolysis and fermentation, it is desirable to minimize addition of fresh-water and waste-water streams, which leads to an accumulation of substances in the process. This study shows that the amount of fresh water used and the amount of waste water thereby produced in the production of fuel ethanol from softwood, can be reduced to a large extent by recycling of either the stillage stream or part of the liquid stream from the fermenter. A reduction in fresh-water demand of more than 50%, from 3 kg/kg dry raw material to 1.5 kg/kg dry raw material was obtained without any negative effects on either hydrolysis or fermentation. A further decrease in the amount of fresh water, to one-fourth of what was used without recycling of process streams, resulted in a considerable decrease in the ethanol productivity and a slight decrease in the ethanol yield.

Index Entries: Ethanol production; recycling; softwood; inhibition; steam pretreatment.

INTRODUCTION

Ethanol can be produced from lignocellulosic materials by enzymatic hydrolysis and fermentation *(1–3)*. Before efficient hydrolysis can occur, the material must be pretreated to make the lignocellulose more susceptible to enzymatic attack *(4,5)*. Steam pretreatment at high temperatures is a method that is often utilized. The efficiency of the pretreatment can be enhanced by the addition of a catalyst, such as sulfur dioxide or sulfuric

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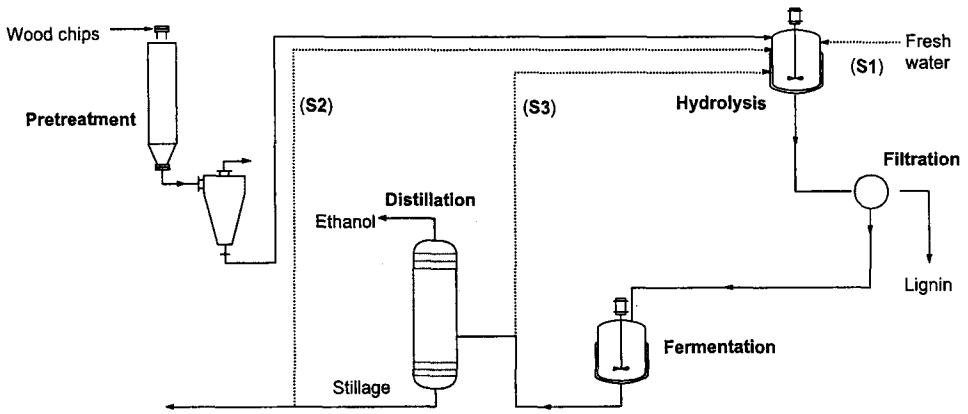

Fig. 1. Schematic flowsheet of ethanol production based on enzymatic hydrolysis.

acid *(6–8)*, which has been shown to be of special importance when softwoods are employed *(9–11)*. During pretreatment, different sugar- and lignin-degradation byproducts are formed that can be inhibitory to hydrolysis *(12–14)* and fermentation *(15,16)*.

In a process for production of ethanol from lignocellulosic materials, it is highly desirable to minimize the addition of fresh-water and wastewater streams. Recycling of process streams decreases the use of fresh water, and minimizes effluent volume. However, this leads to an accumulation of nonfermentable substances and inhibitors in the process *(17)*. Investigations to evaluate the effect of recycling have been performed in previous studies on willow and softwood in a bench-scale unit *(18,19)*. The most inhibiting substances were found to be nonvolatile, which is in agreement with the results from another study *(16)*.

The aim of the present study was to investigate the effects of recirculation of different process streams on hydrolysis and fermentation of a softwood material. Figure 1 shows a schematic flowsheet of the process, including the recirculation alternatives investigated. The fresh-water stream (S1) in the hydrolysis step can be replaced by part of either the stillage stream (S2) or the dilute-ethanol stream from the fermenter (S3). The latter alternative increases the ethanol concentration in the feed to distillation, which results in lower energy costs for distillation.

MATERIALS AND METHODS

The experimental set-up is shown schematically in Fig. 2. The study was performed in a bench-scale unit comprising the following units: a steam-pretreatment reactor, a hydrolysis reactor, a fermenter, a filter press, and an evaporator *(18)*. Five different experiments including hydrolysis, fermentation, and evaporation, (called the base case, R1, R2, R2E, and R3E) were run. In the base-case run, fresh water was used in the hydrolysis for

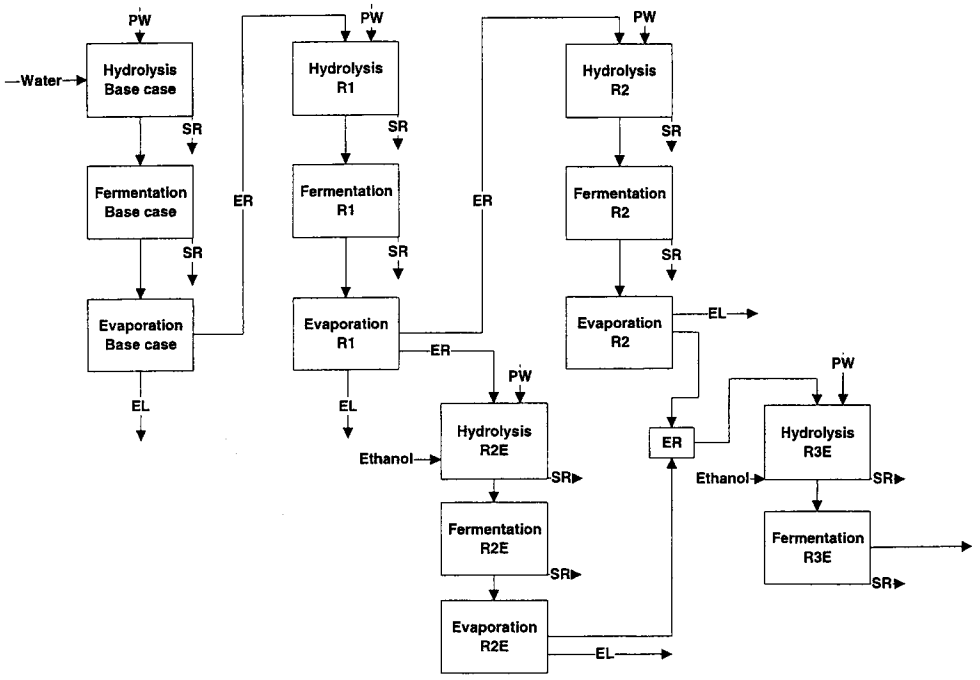

Fig. 2. Experimental procedure. Pretreated wood (PW); Evaporated liquid (EL); Evaporated Residue (ER); Solid Residue (SR).

dilution of the pretreated material. In runs R1 and R2, the fresh water in the hydrolysis stage was replaced by recycled liquid, produced by evaporation of the filtered fermentation broth from a preceding run, thus simulating recycling of the stillage stream (Fig. 1, stream S2). The same simulated recycling was performed in runs R2E and R3E, except that ethanol was added to simulate splitting of the process stream from the fermenter (Fig. 1, stream S3) to the distillation unit into two streams, one of which is recycled to the hydrolysis unit.

Raw Material

Chips of freshly cut spruce, free from bark were generously provided by a sawmill, Höörsågen AB (Höör, Sweden). The wood chips were rechipped and sieved and the fraction between 2 and 20 mm was used. The fractionated material had a dry matter content of 41.7%. The composition was determined according to the Hägglund method *(20)* (Table 1).

Steam Pretreatment

Prior to pretreatment, the wood chips were impregnated with sulfur dioxide (3.6% SO_2 wt/wt dry matter). The material was placed in a plastic bag, and SO_2 was supplied from a gas cylinder. The amount of SO_2 added

Table 1
Composition of Spruce

Composition	% Dry matter
Extractives	1.0
Galactan	1.8
Glucan	43.9
Mannan	12.0
Arabinan	1.1
Xylan	4.9
Lignin	28.1

to the bag was estimated by weighing the cylinder. The absorbed amount of SO_2 was determined by weighing the bag before and after SO_2 addition. After 20 min at room temperature, the treated material was steam pretreated at 215°C for 5 min. After steam pretreatment, a sample was collected, washed, and the dry matter content and the yield of fibrous material were determined. The liquid fraction was analyzed for glucose, mannose, furfural, 5-hydroxy-2-methylfurfural (HMF), and acetic acid. Steam pretreatment was performed in three separate batches because of the limited capacity of the steam-pretreatment equipment, which has a reactor volume of 10 L. The first batch was used in the base case run, the second in run R1, and the third in the following three runs.

Hydrolysis

Enzymatic hydrolysis was performed at 40°C. The base case run, run R1, and R2 were performed in a stirred tank with a working volume of 20 L. Runs R2E and R3E were carried out in a 10-L fermenter (Bioengineering, Wald, Switzerland) to minimize the evaporation of ethanol. Liquid, either as tap water (base case run) or as nonvolatile evaporation residue from a previous run (runs R1, R2, R2E, and R3E) (*see* Fig. 2) was added to adjust the dry matter content to 7.5% (wt dry matter/wt total). The added liquid constituted approx one third of the total amount of liquid present in the hydrolysis. In hydrolysis runs R2E and R3E, ethanol was added to the liquid to concentrations of 2.3% (wt/wt liquid) and 4.4%, respectively. This corresponds to the ethanol concentration obtained when fermentation broth is recycled, resulting in concentration factors of 2.2 and 3.1, (Table 2).

The pH was preadjusted to 4.8 with solid calcium hydroxide and 10% (wt/wt) sodium hydroxide was then used to maintain the pH at 4.8 during hydrolysis. Novo Celluclast 2 L, 0.15 g/g fibrous material, supplemented with 0.03 g/g fibrous material of β-glucosidase in the form of Novozym 188 was added to perform the hydrolysis. Both enzyme preparations were

Table 2
Concentration Factors
for Nonvolatile Substances

Run	CF
Base case	1
R1	1.6
R2	2.2
R2E	2.2
R3E	3.1

kind gifts from Novo Industri A/S (Bagsvaerd, Denmark). The activity of Celluclast was 75 FPU/g *(21)*. The β-glucosidase activity in Celluclast was 12 IU/g *(22)*, and in Novozym it was 392 IU/g. Hydrolysis was allowed to proceed for 96 h and samples were withdrawn at regular intervals and analyzed for glucose, mannose, furfural, 5-hydroxy-2-methylfurfural (HMF), glycerol, and acetic acid. The solid residues after hydrolysis and after fermentation were separated from the liquid with a filter press unit, PF 0.1H2 (Larox OY, Helsinki, Finland). A pressure of 15 bar was applied to the slurry.

Fermentation

Fermentation of the filtered hydrolysates was performed in a Bioengineering NL22 fermenter with a working volume of 16 L, using compressed baker's yeast, *Saccharomyces cerevisiae*, from Jästbolaget AB (Rotebro, Sweden). The pH was not adjusted after hydrolysis but maintained at 4.8 during fermentation with 10% (wt/wt) sodium hydroxide. The hydrolysates were supplemented with nutrients to a final concentration of 0.5 g/L $(NH_4)_2HPO_4$ and 0.025 g/L $MgSO_4 \cdot 7H_2O$.

The hydrolysates were inoculated with yeast to 10 g dry weight/L (after inoculation) and incubated at 30°C. The broth was stirred at 300 rpm. Samples were withdrawn at regular intervals, and the fermentation was allowed to proceed until a glucose stick (Boehringer, Mannheim, Germany) was negative, or for a maximum of 25 h. The samples were analyzed for glucose, mannose, furfural, 5-hydroxy-2-methylfurfural (HMF), and acetic acid.

Evaporation

The filtered liquid from the fermentation stage was concentrated in an evaporation unit *(18)*. The evaporation residue was collected and used in the subsequent hydrolysis to replace water. The concentration factor (CF) was defined as the ratio between the amounts of nonvolatile solubles in the actual hydrolysis and in the hydrolysis in the base case run (Table 2). The CF was calculated by weighing both the fermentation

liquid prior to evaporation and the amount of evaporated liquid, and then compensating for the dilution of the recycled liquid in the next hydrolysis stage.

Analysis

The liquid fractions after pretreatment, hydrolysis, and fermentation were analysed on an HPLC (Shimadzu, Kyoto, Japan) equipped with a refractive index detector (Waters Millipore, Milford, CT). Glucose, ethanol, furfural, HMF, acetic acid, and glycerol were determined using an Aminex HPX-87H column (Bio-Rad, Hercules, CA) at 45°C, using 5 m*M* H_2SO_4 as eluant, at a flow rate of 0.6 mL/min. Mannose was determined using a Polymer Labs (Shropshire, UK) PL Hi-Plex Pb column at 80°C, using ultrapure water as eluant, at a flow rate of 0.4 mL/min. The dry-matter content of the washed steam-pretreated material was determined by drying the material at 105°C overnight. The dry weight of the yeast was determined by drying the samples in a microwave oven for 15 min.

RESULTS

All yields are expressed as g/100 g dry raw material, unless otherwise stated. The pretreated material was produced in three individual pretreatment runs, resulting in dry matter contents of 11.4, 10.9, and 12.2%, respectively. The yields of fibrous material after these three pretreatment runs were 59.9, 61.3, and 64.1 g/100 g, respectively. The yields of glucose and mannose in the liquids from pretreated material were approximately the same in all three pretreatment runs: 7 and 8 g/100 g, respectively.

The hydrolysis rates during the first 20 h were approximately the same for the first four runs, 0.63 g/l h, whereas run R3E exhibited a lower hydrolysis rate, 0.35 g/l h, (Fig. 3). The hydrolysis yield of glucose was between 13 and 16 g/100 g and resulted in an overall yield, i.e., for both pretreatment and hydrolysis, of 20–24 g/100 g. Since the pretreated material employed in the base case run, run R1, and runs R2, R2E, and R3E originated from three separate batches, it is difficult to draw any conclusions as to whether the increased recirculation or variations in pretreatment caused the decrease (Table 3). The amount of mannose obtained in the hydrolysis was approx 1 g/100 g.

The formation of ethanol in the fermentation for the five runs is shown in Fig. 4A. The productivity was calculated from the fermentation curves as the average ethanol production rate during five hours, r_{5h}, (Table 4). The productivities were approximately the same for the base case run and run R1, 3 g/L/h. In runs R2, R2E, and R3E they were considerably lower. For run R3E, r_{5h} was difficult to calculate because of the high initial ethanol concentration in the fermentation broth. However, the glucose consumption rates confirmed the trend of declining productivity (Fig. 4B).

Table 3
The Yield of Glucose and Mannose (in g/100 g)

Run	Glucose (in hydrolysis)	Glucose (overall)	Mannose (overall)
Base case	15.2	21.9	8.1
R1	16.1	23.6	8.4
R2	13.5	20.5	8.9
R2E	14.3	21.3	9.3
R3E	13.0	20.0	8.4

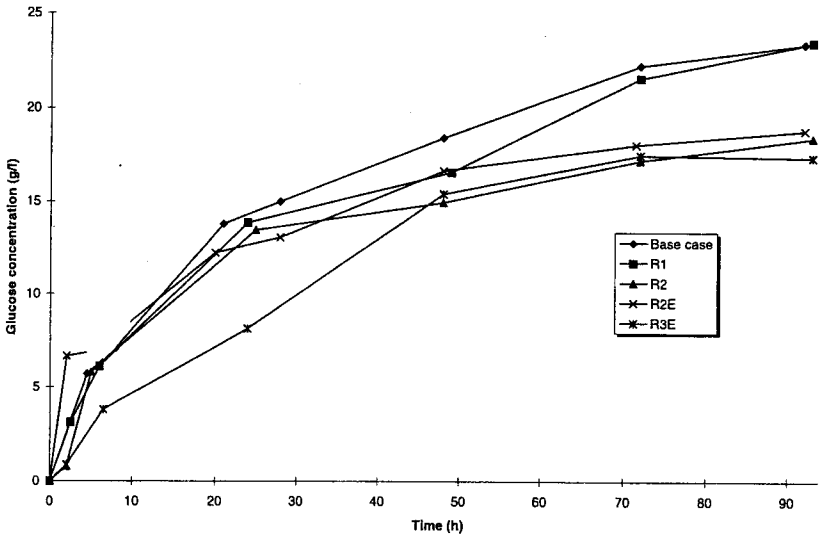

Fig. 3. Hydrolysis rates in the various runs.

The fermentation yield in the base case run was 85% of the theoretical yield, based on fermentable sugars in the hydrolysate (Table 4). The fermentation yields were somewhat lower in runs R1, R2, and R2E, and significantly reduced in run R3E. The overall ethanol yield was approx 13 g/100 g in the base case run and in run R1. In runs R2 and R2E, the overall yield was somewhat lower, approx 10–12 g/100 g, whereas run R3E resulted in only 7.6 g/100 g (Table 4).

The concentrations of HMF and furfural in the hydrolysates were all less than or equal to 2 g/L (Table 5). The acetic acid concentration increased because of the recirculation from 2.2 g/L in the base case fermentation to 7.1 g/L in fermentation R3E. A similar trend was observed for the glycerol concentration.

Table 4
Ethanol Yield in Fermentation, Overall Yield, and Initial Productivity

Run	Productivity, r_{5h} (g/L/h)	Yield of ethanol in fermentation (% of theoretical)	Overall yield of ethanol (g/100 g)
Base case	3.0	85.3	13.1
R1	2.9	79.9	13.0
R2	0.9	66.1	9.9
R2E	1.4	74.8	11.7
R3E	0.6	52.7	7.6

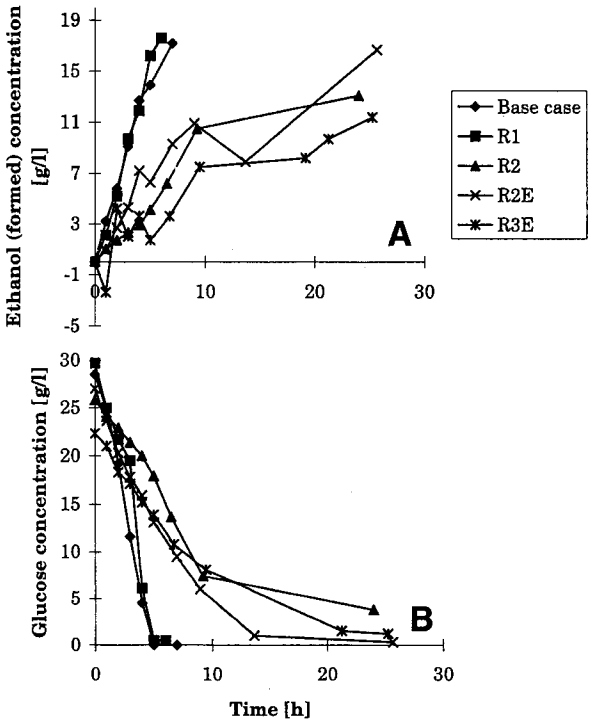

Fig. 4. **(A)** Ethanol formation rates; **(B)** Glucose consumption rates in the various runs.

DISCUSSION

In the present study, the influence of increasing concentration of soluble compounds on hydrolysis and fermentation, when fresh water is replaced by process liquids, has been investigated. However, in the fermentation it was only possible to evaluate the effect of inhibitors on ethanol production, and not on cell growth, because of the high initial cell concentration.

Table 5
The Concentrations of Byproducts in the Hydrolysate

Run	HMF (g/l)	Furfural (g/l)	Acetic acid (g/l)	Glycerol (g/l)
Base case	1.2	0.8	2.2	0.2
R1	1.7	1.0	4.3	2.0
R2	1.4	0.6	4.6	2.6
R2E	1.4	0.7	4.3	2.4
R3E	2.0	0.8	7.1	4.1

Table 6
Amount of Water Used and Required Dry-Matter Content After Pretreatment

	Amount of fresh water used (kg/kg dry raw material)		
Run	In pretreatment (as steam)	In hydrolysis	Required dry matter content after pretreatment (%)
Base case	1.4	1.6	12
R1	1.4	–	12
R2	0.8	–	16.5
R2E	0.8	–	16.5
R3E	0.3	–	23

The base case requires the addition of fresh water corresponding to 3 kg/kg dry raw material, which is added as 1.4 kg/kg steam in the pretreatment stage (flash vapor excluded) and 1.6 kg/kg water in the hydrolysis stage. This is based on a raw material having a moisture content of 50%, a fiber yield after pretreatment of 60%, and an initial dry-matter content in the hydrolysis stage of 7.5%. The amount of fresh water can be reduced by replacing the fresh water in hydrolysis with recycled process liquid. This was simulated experimentally in runs R1, R2, R2E, and R3E. In runs R1 and R2, recycling of the stillage stream from the distillation unit, and in runs R2E and R3E recycling of the outlet stream from the fermenter were simulated (Fig. 1). By replacing all the fresh water used in hydrolysis (run R1) the remaining water requirement will be 1.4 kg/kg for steam pretreatment (Table 6). This corresponds to a concentration of nonvolatile substances 1.6 times higher than in the base case run (*see* Table 2).

To reduce the amount of fresh water even further, a drier material after pretreatment is required (*see* Table 6). This can be accomplished in an alternative type of pretreatment equipment, using indirect heating, or by using a drier raw material. Runs R2, R2E, and R3E (which correspond to a higher dry matter content after pretreatment) were simulated by con-

centrating the recycled process liquid further, to estimate the effect of the increased inhibitor concentrations. The procedure used in the present experimental investigation assumes that the same amount of inhibitors is formed independent of the dry-matter content in the pretreatment step.

The hydrolysis yield was not influenced by recycling, whereas the rate decreased in run R3E. In fermentation, neither the yield nor the productivity was influenced when the fresh water was replaced by process liquid in run R1 (Tables 3 and 4). However, when the amount of fresh water was further decreased the yield, as well as the productivity, decreased.

The concentrations of HMF and furfural were approximately the same in all the runs, 2 g/L and 1 g/L, respectively (Table 5). Furfural was not recycled since it is rather volatile and was removed in the preceding evaporation stage. HMF is nonvolatile and should therefore increase, but analyses of the HMF concentrations before and after fermentation showed that the yeast consumed HMF. This has also been shown previously *(23)*. At these low concentrations, furfural or HMF do not cause any significant inhibition *(15,24)*. Acetic acid is distributed between the volatile and nonvolatile fractions and increases with increasing degree of recycling. In runs R1, R2, and R2E, the acetic acid concentrations were about the same. Various references on the toxicity of acetic acid can be found in the literature. However, the exact inhibiting concentration of acetic acid is difficult to determine *(25,26)*.

The presence of ethanol in run R2E did not affect the yield or the productivity in the fermentation step compared with run R2 in which the same liquid was used except for the addition of ethanol. This is in accordance with another study showing that inhibition starts at 25 g/L and is total at 95 g/L *(26)*. Neither was the presence of ethanol in run R3E the major cause of the decreased yield and productivity, since the ethanol concentration in this case was also low, approx 50 g/L. However, it is not possible to draw any conclusions regarding the synergistic action of ethanol, acetic acid, and other byproducts present during fermentation. It is more likely that the decrease in productivity and yield in fermentation runs R2, R3, and R3E originated from the increased concentration of nonvolatile lignin-degradation products formed at the high temperatures employed during steam pretreatment *(27)*.

Mannose was not completely consumed in any of the fermentation runs, so the initial concentration in the fermentation broth increased from 12 to 16 g/L when process liquids were recycled. The concentration of mannose remaining after fermentation was in the range 2–5 g/L. The affinity of *S. cerevisiae* for mannose is one tenth that for glucose, and mannose will not be utilized until the glucose concentration has reached a sufficiently low level *(28)*. Thus, the ethanol yields might be improved by increasing the fermentation time.

The low overall yield of ethanol (13 g/100 g DM) was mainly caused by inefficient hydrolysis. The yields of fermentable sugars after hydrolysis

were lower than expected, also in the absence of recycled liquid. This is most likely caused by inhibitors already present in the pretreated material. In laboratory scale experiments, the hydrolysis-rate and yield increased by approx 50% when the fibrous material was washed and the hydrolysis was performed in buffer solution (data not shown). The inhibitory effect of the liquid was also reduced when the enzyme load was doubled, and the glucose yield increased by approx 25%. A similar result has been obtained in a study in which eucalyptus was used as raw material *(29)*. This suggests that the inhibition may be caused by product inhibition by the sugar-rich liquid from the steam-pretreated material. This is also supported in a study on simultaneous saccharification and fermentation (SSF) of softwoods in which higher yields and productivities have been achieved (data not shown). The relatively low yield may also be caused by difficulty in stirring the material properly. At 7.5% dry-matter content, the mixture is rather viscous, thus hampering mass transfer in the tank. In a previous study, where mixed softwoods were hydrolyzed at 5% dry-matter content, a higher yield was obtained *(19)*. In general, softwoods are more recalcitrant to hydrolysis than hardwoods *(4,6)*.

CONCLUSIONS

This study shows that the amount of fresh-water used, and thus the amount of waste-water produced, in the production of fuel ethanol from softwood, can be reduced to a large extent by recycling of either the stillage stream or part of the liquid stream from the fermenter. A reduction in fresh-water requirement by more than 50% from 3 kg/kg dry raw material was obtained without any negative effects on either hydrolysis or fermentation. Recycling of the liquid stream from fermentation increases the ethanol concentration in the fermentation broth. No negative effects on fermentation were observed with an ethanol concentration in hydrolysis of 2.3 wt% (run R2E). The ethanol concentration in the feed to the distillation unit increased from 1.8 to 3.4 wt% compared with the case of no recycling. This will reduce the energy demand in distillation by 42% *(30)*. A further decrease in the amount of fresh water, to one fourth of what was used without recycling of process streams, resulted in a considerable decrease in the ethanol productivity and a slight decrease in the ethanol yield.

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