**Simultaneous Saccharification, Filtration and Fermentation (SSFF): A novel
method for Bioethanol Production from Lignocellulosic Biomass**

Mofoluwake M. Ishola^{a,b*}, Arash Jahandideh^a, Behroz Haidarian^a, Tomas Brandberg^a,
Mohammad J. Taherzadeh^a

^aSchool of Engineering, University of Borås, Sweden

^bDepartment of Chemical and Polymer Engineering, Faculty of Engineering, Lagos
State University, Nigeria

*Corresponding author:

Tel: +46 33 435 4585

Fax: +46 33 435 4008

E-mail: mofoluwake.ishola@hb.se

Abstract

Simultaneous Saccharification, Filtration and Fermentation (SSFF) was developed for lignocellulosic ethanol production. In SSFF, pretreated lignocellulosic material is enzymatically hydrolyzed in a reactor, while the suspension is continuously pumped through a cross-flow membrane. The retentate goes back to the hydrolysis vessel, while a clear sugar-rich filtrate continuously perfuses through the fermentation vessel before it is pumped back to the hydrolysis vessel. The capacity and life span of the cross-flow filter module was examined for four weeks using enzymatically hydrolyzed slurry, initially with 14.4% suspended solids, without clogging or fouling. An ethanol yield of 85.0% of the theoretical yield was obtained in SSFF and a flocculating strain of *Saccharomyces cerevisiae* was successfully reused for 5 cultivations of SSFF.

Keywords:

Bioethanol, lignocellulosic biomass, hydrolysis, cross-flow filtration, fermentation, SSFF

1. Introduction

Bioethanol sticks out as the most important renewable fuel in the transportation sector in a global perspective, as its production reached 88.7 billion liters in 2011 (Renewable Fuels Association 2011). However, concerns regarding future supply of raw material for ethanol have triggered extensive research to broaden its raw material base, to include lignocellulosic materials. This technology is referred to as 2nd generation ethanol and has the potential to reduce greenhouse gas (GHG) emission with up to 86% in its wheel to well analysis (Wang et al. 2007). Lignocellulosic ethanol also requires a lower input of energy in its production process compared to starch derived ethanol (Farrell et al. 2006). Ethanol from lignocelluloses can be produced via (a) pretreatment to open up the crystalline structure of the biomass, (b) enzymatic hydrolysis of cellulose and hemicellulose, (c) fermentation of the sugars to ethanol, and (d) ethanol purification by e.g. distillation and dehydration. Lignocellulosic ethanol production is however associated with the recalcitrant structure of the lignocellulosic biomass. This necessitates the use of a process, which can optimize the hydrolysis and fermentation process.

The enzymatic hydrolysis and the fermentation can be carried out by separate hydrolysis and fermentation (SHF) or simultaneous saccharification and fermentation (SSF). SHF allows the fermentation and hydrolysis to be performed at separate conditions; hence the fermenting organism and the enzymes can be used at independent optimum temperature and pH. However, SHF results in enzyme inhibition by the hydrolysis products, i.e. as the hydrolysis progresses the sugar concentration in the hydrolysis bioreactor increases and this can reduce the efficiency of the cellulase enzymes used (Tengborg et al. 2001;

Söderström et al. 2005), which may reduce the efficiency of the hydrolysis. SSF is an alternative in which both the hydrolysis of the pretreated biomass and the fermentation to ethanol takes place in the same bioreactor. This approach requires a compromise between the optimum conditions of the enzymatic reaction and the fermenting organism. An advantage of SSF is that the glucose from the hydrolysis is immediately consumed by the fermenting organism and product inhibition of the enzymes during the hydrolysis is avoided (Wingren et al. 2003). However, a major drawback of the SSF is that the fermenting organism cannot be recirculated since it has been mixed with the biomass (Taherzadeh and Karimi 2007; Olofsson et al. 2008; Tomás-Pejó et al. 2008).

Simultaneous saccharification, filtration and fermentation (SSFF) seems to be an advantageous alternative to both SHF and SSF, since the problem of enzyme inhibition can be avoided. In addition, both the enzyme cocktail and the fermenting organism in SSFF can be used at their different optimal conditions. Furthermore, it would be possible to reuse the fermenting organism several times.

The aim of this work was to assess and present a new concept “Simultaneous saccharification, filtration and fermentation (SSFF)”, which thus circumvents the disadvantages of both SSF and SHF. With SSFF, pretreated lignocellulosic slurry is exposed to the enzymes and a sugar-rich filtrate is passed on to the fermentation by cross-flow filtration. Fermented liquid is pumped back to the hydrolysis vessel, while a culture of a flocculating strain of *Saccharomyces cerevisiae* is retained by settling (Fig. 1). In addition, this work includes a comparison of SSFF and SSF and also an assessment of a couple of potential weak spots of SSFF; the lifetime of a cross-flow

membrane in cellulose-rich slurry and the risk for infection in a yeast culture which is used for several batches.

2. Materials and Method and SSFF development

2.1. Lignocellulosic materials

Spruce chips, a softwood lignocellulosic biomass from Swedish forests were used. The chips were chemically pretreated with SO₂ impregnation and exposed to a pressure of 22 bars, 215°C for 5 min (SEKAB E-Technology, Sweden) and delivered as slurry, consisting of a liquid fraction and fine particles. It had pH 2.0, 15.9% suspended solids (SS) and 23.8% total solids (TS). The slurry was stored in a cold room of 5°C until use. The composition of the liquid fraction of the slurry was 22.8 g/L glucose, 25.3 g/L mannose, 13.7 g/L xylose, 5.8 g/L galactose, 4.7 g/L arabinose, 5.7 g/L acetic acid, 1.8 g/L HMF, and 2.0 g/L furfural. The solid fraction of the slurry had 46.5±0.1% cellulose, 46.1±0.3% Acid insoluble lignin (AIL), 5.01±0.1% Acid soluble lignin (ASL).

The theoretical ethanol yield from this lignocellulose was calculated based on 51% of the total fermentable carbohydrates with a conversion factor or 1.11 for cellulose in the solid fraction. Assuming 1.0 g/cm³ density for the solid fraction and taking the dilution effect of the fermenting vessel (initially without ethanol) into account, 36.6 g/L ethanol can in theory be reached in the SSFF experiment with 10% SS. The corresponding level for the experiment with 12% SS is 43.8 g/L.

2.2. Enzymes and Yeast strain

Cellic[®] CTec3 enzyme (Novozymes, Denmark) was used for the hydrolysis. A flocculating strain of yeast *Saccharomyces cerevisiae* CCUG 53310 (Culture Collection University of Gothenburg, Sweden) was used in the experiments. The yeast was maintained at 4°C on YPD agar plate containing 20 g/L agar (Scharlau), 20 g/L D-glucose (Scharlau), 10 g/L yeast extract (Scharlau) and 20 g/L peptone (Fluka). Prior to fermentation, 100 mL preculture in YPD medium containing 20 g/L D-glucose, 20 g/L peptone and 10 g/L yeast extract in 250 mL flask was provided. This was incubated in a shaking water bath (Grant OLS 200, Grant instrument ltd, UK) at 121 rpm and 30 °C for 24 h.

2.3. Simultaneous Saccharification Filtration and Fermentation (SSFF)

In SSFF, fermentation broth is circulated between the hydrolysis and fermentation vessels. Pretreated slurry is mixed with enzymes and pumped through a cross-flow membrane, so that a clear sugar-rich filtrate reaches the fermentation vessel.

Simultaneously, the volumes are kept in balance by pumping fermented liquid back to the hydrolysis vessel (Fig. 1). Retention of the fermenting culture is necessary and is facilitated by the use of a flocculating yeast strain. Through an internal settler (10 mL syringe of 1.5 cm internal diameter) in the fermentation reactor for sedimenting the culture (Fig. 1), the fermented liquid is pumped back to the hydrolysis reactor. The SSFF is composed of three parts of hydrolysis, filtration and fermentation (Fig. 1).

2.3.1. Hydrolysis

The hydrolysis was carried out using 2.5 L reactor (Infors AG107504, Minifors, Switzerland) with 10%, 12% SS and a total volume of 1690 mL, 1500 mL respectively.

The pH was adjusted to 5.0 and automatically controlled with adding 2 M NaOH, temperature set at 50°C, agitated at 500 rpm, 35 FPU/g SS enzyme load was used for 10% SS and 12 FPU/g SS was used for 12% SS. Enough antifoam (Silicone) was used in the experiments. Pre-hydrolysis was carried out for 24 h, followed by SSFF at 50°C. The pre-hydrolysis was a measure to reduce the viscosity of the slurry in order to avoid clogging of the filtration device. Backwash was applied on the concentrate pump at 1 h intervals for the first 24 h of integration to prevent clogging. For 10% SS, the experiments were carried out in duplicates and the average values are reported.

2.3.2. Filtration

A filter membrane module (MD 015 TP 2N, Microdyn-Nadir, Germany) made of polyethylene with a polypropylene housing was used in a cross-flow microfiltration, which is filtration through the micropores in the filter module in a perpendicular direction to the flow inside. The filter module had an inner diameter of 5.5 mm, pore size of 0.2 μm , an effective filtration area of 0.025 m^2 and a free flow area of 0.24 cm^2 . The membrane was hydrophilized with ethanol before use. A peristaltic pump (405U/L2 Watson-Marlow, Sweden) was used to pump the enzyme-slurry mixture (hereafter referred to as concentrate pump) from the hydrolysis reactor into the filter module at flow rate of 0.8 L/min. Another peristaltic pump (120 S/DM2 Watson-Marlow, Sweden) was used to pump the permeate out of the filter module (hereafter referred to as permeate pump) to the fermentation part initially at a flow rate of 1.1 mL/min and gradually increased up to 2.9 mL/min (which corresponds to a dilution rate of 0.2 - 0.6 h^{-1}) in order to balance efficient uptake in the fermentation vessel. The retentate was circulated back to the hydrolysis reactor. Periodic chemical backwash, in

reverse flow direction to the cross-flow microfiltration using 0.5 M NaOH was used on the membrane after each experimental run to regenerate the pores of the filter membrane module.

2.3.3. Fermentation

The fermentation was carried out in a 1.5 L bioreactor (Biostat[®] B plus 8843414 Satorius, Germany) with a working volume of 300 mL. Prior to SSFF, the flocculating yeast strain was aseptically transferred from the preculture to a sterile YPD medium and cultivated in 1 L medium up to 4 g/L yeast cells at pH of 5.0, 30 °C, agitated at 90 rpm and aerated with sterile air at 11.2 vvm. Afterwards, the volume was reduced to 300 mL while the stirrer was switched off and thus the flocculating yeast culture was concentrated. The whole SSFF process, consisting of the three integrated units, was carried out for 96 h.

2.4. Filtration Capacity Test

In order to evaluate the SSFF process, a filtration capacity test was performed on the filtration unit to determine what solid concentration can be pumped through and the lifetime of a cross-flow membrane. Tap water was initially used to examine the flow rate of water in the membrane and flow rate of water as permeate out of the membrane. Diluted pretreated slurry (5, 7.5, 10, 11, 12, 13 and 14%) was successfully pumped through the filtration device without clogging. Finally, slurry adjusted to 14.4% SS concentration was enzymatically prehydrolysed and used to evaluate how long the membrane can be used for effective filtration of the glucose until clogging or fouling occurs.

2.5. Fermentation Capacity Test

In order to evaluate the ability of the flocculating yeast strain to ferment glucose in the filtrate, a capacity test was performed on the fermentation part of SSFF set up using the liquid fraction of the spruce slurry as carbon source. Different dilution rates from 0.1-0.225 h⁻¹ was used, each of the dilution rate was observed for 24 h, and then samples were taken and analyzed for glucose, mannose, xylose, ethanol, glycerol and acetic acid. This is to see how well the glucose can be consumed and converted to ethanol at different dilution rates from the filtration unit, and how long the cell can be used for fermentation.

2.6. Simultaneous Saccharification and Fermentation (SSF)

In order to compare with SSFF, SSF of the same lignocellulosic slurry was performed. A commercial strain of yeast *Saccharomyces cerevisiae* (Ethanol Red, Fermentis, France) was used, and the SSF was carried out using a 2.5 L bioreactor (Minifors, Infors AG, Switzerland). The total volume in the reactor was 1690 mL consisting of 10% suspended solids. The pH of the slurry was adjusted to 5.0 using 2 M NaOH, stepwise addition of the neutralized slurry was used. Ammonium Sulfate (Scharlau) 3.2 g and Yeast extract (Scharlau) 1.2 g was used as nutrient source for the yeast, deionized water was used to adjust the total volume to 1690 mL and enough antifoam was added. The temperature was set at 35°C, 6.4 g of the yeast and 35 FPU/g SS enzyme was added and agitation was provided at 500 rpm. The experiments were run in duplicates and the average values reported. The SSF was conducted during 96 h and the samples was taken at 4 h intervals and analyzed for glucose, xylose, ethanol, glycerol and acetic acid.

2.7. Analytical methods

Cellulose, hemicelluloses and lignin of the solid fraction of the slurry were determined according to NREL protocols (Sluiter et al. 2011). The slurry was first centrifuged at 4000×g for 5 min to separate the solid and the liquid fractions. The solid fraction was washed with 40 mL deionized water thrice to neutral pH and then freeze-dried (Labconco, USA) at -52°C until its moisture content was reduced to less than 10%. It was then hydrolyzed in two steps using 72% H₂SO₄ in a shaker water bath at 30°C for 60 min, followed by hydrolysis by 4% H₂SO₄ in an autoclave at 121°C for 60 min. Acid Soluble Lignin (ASL) was determined using a UV spectrophotometer (Libra S60, Biochrom, England) at 283 nm. Acid Insoluble Lignin (AIL) was gravimetrically determined as residual solid after hydrolysis corrected with ash content. The ash content was determined in the muffle furnace at 575°C overnight. Monomeric sugars contained in the hydrolysis liquid were determined by HPLC.

The sugars and metabolic products were analyzed using an HPLC (Walters 2695, Walters Corporation, Milford, USA). A hydrogen-based column (Aminex HPX-87H, Bio-Rad, Hercules, USA) at 60°C and 0.6 mL/min 5mM H₂SO₄ as eluent was used for the glucose, furans, carboxylic acids, ethanol, lactic acid and glycerol. Mannose, glucose, galactose, xylose and arabinose were analyzed using Aminex HPX-87P column (Bio-Rad) at 85°C and 0.6 mL/min ultrapure water as eluent. A UV absorbance detector (Walters 2487), operating at 210 nm wavelength was used in series with a refractive index (RI) detector (Walters 2414).

3. Results and Discussion

In this newly developed SSFF process (Fig. 1), the advantages of SSF and SHF are combined. The yeast cell and the enzymes were used at different optimal conditions in two different reactors. The glucose released during the hydrolysis passes through the filter membrane, and is assimilated by the yeast cells in the bioreactor to form ethanol, while the liquid is continuously recirculated between the hydrolysis and fermentation vessels. It results in low glucose concentration in the hydrolysis reactor, and continuous fermentation for the glucose released. SSFF provides the possibility to reuse the fermenting cells, which is crucial for a successful ethanol production process (Brandberg et al. 2007).

SSFF is also associated with some potential obstacles, which must be examined. The capacity and life span of the filter module in the filtration unit must be assessed as well as the long time fermentation performance of the fermentation unit. These hurdles have been assessed in this work and the limits and how the method can be successfully applied using a flocculating yeast strain were determined. Higher solid loading (12% SS) was also successfully applied on SSFF. A problem with clogging inside the filter module occurred during the first 24 h of integration, but this was solved by applying a backwash flow on the concentrate pump at an interval of 1 h during the first 24 h of integration.

3.1. Solid loading and durability of the filtration device

In examining the capacity of the filtration device, the polypropylene filter membrane module was found to be used comfortably for filtration of the pretreated spruce of different suspended solids (SS) up to 14% (Fig. 2a). It was also observed that the concentrate pump should be used at a flow rate at least 800 mL/min to facilitate the flow inside the filter membrane and prevent clogging. This is equivalent to 0.56 m/s of cross-flow velocity over the membrane, assuming plug flow. On the other hand, it was advantageous to use the permeate pump at a very low flow rate of 1-4 mL/min in order to reduce the rate of fouling in the membrane and to prolong the membrane life span.

The durability of the membrane was examined by successful filtration of enzymatically treated slurry with an initial SS content of 14.4% during 28 days (Fig. 2b). For this purpose, the prehydrolysed slurry was pumped through the filtration unit continuously with concentrate flow of 800 mL/min and an initial permeate flow rate of 3.7 mL/min was applied. The permeate flow rate was observed to decrease sharply within the first 5 days followed by a fluctuation around 2 mL/min and eventual leveling out without clogging or fouling of the filter module. This is probably an indication that SSFF can be applied for continuous process also on industrial scale, and a cross-flow filtration can be applied with this kind of substrate for extended periods of time without regeneration.

3.2. Repeated batches of SSFF and dilution effect on the fermentation unit

The real potential of SSFF can in principle only be assessed in a series of experiments, where the dilution effect of the first experiment is eliminated and the potential of the fermenting culture is assessed in several consecutive batches. Such a series was carried out (Table 1), no washout of the fermenting culture was observed and judging from the

absence of residual glucose from batch 2 and onwards, the fermentation was efficient. In addition, no fouling of the membrane was observed in this series of the experiments. The apparent difficulty to reproduce the level of ethanol at the end of each experiment might be attributed to evaporation of ethanol and to an infection, which got foothold in the second batch. Infections frequently occur in processes with continuous fermentation and SSFF may not be an exception, this is why the problem must be addressed in future work.

Fig. 3 shows the results of sugar consumption and production of metabolites at different dilution rates as an evaluation of the fermentation capacity of cells with sugar supply from the filtration unit. The highest ethanol concentration (33.9 g/L) during the fermentation capacity test was obtained at a dilution rate of 0.125 h^{-1} . Higher dilution rates resulted in accumulation of glucose in the bioreactor. In an industrial context, the dilution rate should be regulated in relation to glucose uptake capability range of the fermenting organism for a good ethanol yield and productivity.

3.3. SSFF evaluation and comparison with SSF

3.3.1 SSFF base case evaluation

SSFF was successfully examined with 10% SS pretreated spruce and 35 FPU/g SS enzyme dosage. The result (Fig. 4) shows that $31.1 \pm 1.2 \text{ g/L}$ ethanol was observed in the hydrolysis reactor after 96 h processing with SSFF, corresponding to 85.0% of the theoretical level. This corresponds well with the reported values in literatures (Survase et al. 2011; Mohsenzadeh et al. 2012). The glucose concentration after 24 h prehydrolysis was $49.4 \pm 1.0 \text{ g/L}$, suggesting that 64% of the cellulose in the solid

fraction was hydrolyzed. More glucose was released during the continuation of the experiment, but all fermentable sugars were nearly depleted after 96 h of SSFF. Lactic acid concentration was almost constant until 76 h and then increased, indicating a bacterial infection. The level of acetic acid increased from 3 g/L to 5 g/L, while the glycerol concentration remained below 2.2 g/L (data not shown).

3.3.2. Traditional SSF versus SSFF

SSF and SSFF were carried out with the same amount of slurry and the respective results are compared in Fig. 5. If measurements at 68 h and 72 h of fermentation are considered, a nearly identical amount of ethanol production is observed, on average corresponding to 84.2% and 85.3% of the theoretical yield for SSFF and SSF, respectively. This observation indicates that SSFF produces almost the same amount of ethanol as SSF with the same input of enzymes and carbohydrates. Furthermore, the amount of xylose at the end of SSF was higher than in SSFF which implies that more sugars was consumed during SSFF than SSF (Table 2), which is most likely due to lactic acid formation in SSFF. Glycerol formation in SSFF was also lower compared to SSF, possibly due to the different chemical environment or the use of a different fermenting strain. It can also be speculated that the physical stress of solid particles in SSF has an impact. Similar ethanol yields can be interpreted as an advantage for SSFF over SSF, since it is not necessary to supply new yeast for each batch with SSFF. There is presumably an increased risk of infection with SSFF as compared to SSF, which is typical for continuous fermentation systems. An economic evaluation, where for

instance the increased energy costs are assessed and compared to the apparent benefits of SSFF was beyond the scope of this study and need to be investigated in the future.

3.4. Improvement of SSFF with lower enzyme dosage and higher suspended solid

The SSFF process was also examined under conditions closer to an industrial reality by reducing the enzyme load to 12 FPU/g SS and increasing the SS concentration to 12%. The ethanol yield in as compared to the theoretical level was significantly lower, 69.0% after 72 hours of SSFF. Ethanol corresponding 30.2 g/L was produced in the system, but if the dilution effect of SSFF is compensated for the figure would rise to 37 g/L, close to the critical level for industrial implementation (Gnansounou and Dauriat 2010). It was also confirmed that this higher SS level could be handled from a practical point of view, without clogging of the cross-flow filtration device or fouling of the membrane. Higher load of solids and reduced enzyme concentration are two important considerations for the economic feasibility of the process (Modenbach and Nokes 2012).

3.5. Carbohydrate composition of the Spruce Slurry after SSFF

The compositional analysis of the solid fraction of the 12% SS, low enzyme dosage SSFF experiment during the progress of SSFF (Table 3) shows that the slurry is dominated by cellulose and lignin and the hemicellulose fraction had been completely hydrolysed during the pretreatment. It was also observed that after 96 h SSFF, the cellulose fraction was not completely hydrolyzed, which in theory gives room for further optimization of the hydrolysis. The 24 h prehydrolysis was mainly introduced in the present study as a measure to reduce the risk for clogging and the prehydrolysis time should be reduced or eliminated in future applications for higher productivity. Acid

insoluble lignin was almost constant as well as acid soluble lignin throughout the process which shows that a more purified lignin can be obtained from SSFF process.

4.0. Conclusion

A novel method of lignocellulosic ethanol production called Simultaneous Saccharification, Filtration and Fermentation (SSFF) was developed. SSFF allows the fermenting organism and the enzymes to be used at their optimal conditions; the fermenting cultures can also be reused for several cultivations. An ethanol yield of 85.0% of the theoretical yield was obtained in SSFF and the filter module can be used for filtration for 28 days continuously without clogging or fouling with an initial SS of 14.4%. By optimization of the SSFF process, it could potentially replace the conventional methods of lignocellulosic ethanol production.

Acknowledgement

This work was financially supported by the Swedish Energy Agency. The authors are grateful to SEKAB E-Technology (Sweden) for supplying the spruce slurry as well as Novozymes (Denmark) for supplying the enzymes.

References

- 1) Brandberg, T., Karimi, K., Taherzadeh, M. J., Franzen, C. J. and Gustafsson, L. (2007). "Continuous fermentation of wheat-supplemented lignocellulose hydrolysate with different types of cell retention." *Biotechnol. Bioeng.* 98(1): 80-90.

- 2) Farrell, A. E., Plevin, R. J., Turner, B. T., Jones, A. D., O'Hare, M. and Kammen, D. M. (2006). "Ethanol Can Contribute to Energy and Environmental Goals." *Science* 311(5760): 506-508.
- 3) Gnansounou, E. and Dauriat, A. (2010). "Techno-economic analysis of lignocellulosic ethanol: A review." *Bioresour. Technol.* 101(13): 4980-4991.
- 4) Modenbach, A. A. and Nokes, S. E. (2012). "The use of high-solids loadings in biomass pretreatment-a review." *Biotechnol. Bioeng.* 109(6): 1430-1442.
- 5) Mohsenzadeh, A., Jeihanipour, A., Karimi, K. and Taherzadeh, M. J. (2012). "Alkali pretreatment of softwood spruce and hardwood birch by NaOH/thiourea, NaOH/urea, NaOH/urea/thiourea, and NaOH/PEG to improve ethanol and biogas production." *J. Chem. Technol. Biotechnol.* 87(8): 1209-1214.
- 6) Olofsson, K., Bertilsson, M. and Liden, G. (2008). "A short review on SSF - an interesting process option for ethanol production from lignocellulosic feedstocks." *Biotechnol. Biofuels* 1(1): 7.
- 7) Renewable Fuels Association (2011). "Global Ethanol Production to reach 88.7 billion litres in 2011." <http://www.ethanolrfa.org/news/entry/global-ethanol-production-to-reach-88.7-billion-litres-in-2011/> accessed on 4 November 2012.
- 8) Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D. and Crocker, D. (2011). Determination of Structural Carbohydrates and Lignin in Biomass Laboratory Analytical Procedure, NREL/TP-510-42628.
- 9) Survase, S. A., Sklavounos, E., Jurgens, G., van Heiningen, A. and Granström, T. (2011). "Continuous acetone–butanol–ethanol fermentation using SO₂–ethanol–water spent liquor from spruce." *Bioresour. Technol.* 102(23): 10996-11002.

- 10) Söderström, J., Galbe, M. and Zacchi, G. (2005). "Separate versus Simultaneous Saccharification and Fermentation of Two-Step Steam Pretreated Softwood for Ethanol Production." *J. Wood Chem. Technol.* 25(3): 187-202.
- 11) Taherzadeh, M. J. and Karimi, K. (2007). "Enzyme-based hydrolysis processes for ethanol from lignocellulosic materials: a review." *BioResources* 2(4): 707-738.
- 12) Tengborg, C., Galbe, M. and Zacchi, G. (2001). "Reduced inhibition of enzymatic hydrolysis of steam-pretreated softwood." *Enzyme Microb. Technol.* 28(9-10): 835-844.
- 13) Tomás-Pejó, E., Oliva, J. M., Ballesteros, M. and Olsson, L. (2008). "Comparison of SHF and SSF processes from steam-exploded wheat straw for ethanol production by xylose-fermenting and robust glucose-fermenting *Saccharomyces cerevisiae* strains." *Biotechnol. Bioeng.* 100(6): 1122-1131.
- 14) Wang, M., Wu, M. and Huo, H. (2007). "Life-cycle energy and greenhouse gas emission impacts of different corn ethanol plant types." *Environ. Res. Lett.* 2: 13.
- 15) Wingren, A., Galbe, M. and Zacchi, G. (2003). "Techno-Economic Evaluation of Producing Ethanol from Softwood: Comparison of SSF and SHF and Identification of Bottlenecks." *Biotechnol. Progr.* 19(4): 1109-1117.

Figure Legends

Fig. 1. Schematic representation of SSFF integrated process.

Fig. 2. Flow rates of (a) 5, 7.5, 10, 11, 12, 13 and 14% SS of the slurry through the filter membrane module and (b) permeate through the membrane module during a 4-weeks experiment.

Fig. 3. Fermentation capacity test at different dilution rates. The symbols represent ethanol (♦), acetic acid (x), glycerol (◇) and glucose (●), mannose (■) and xylose (▲).

Fig. 4. Glucose and metabolites concentrations in the hydrolysis reactor during SSFF base case with 10% SS and 35 FPU/g SS enzyme loading. Prehydrolysis without any fermentation is carried during the first 24 hours. Ethanol is represented by (●), glucose (♦), lactic acid (▲) and glycerol (+).

Fig. 5. Comparison of ethanol produced per supplied amount of slurry (15.9% SS) in SSFF (○) and SSF (●).

Table Legends

Table 1: Fermentation ability evaluation of the yeast strain used in several sequential batches of SSFF.

Table 2: Yield of ethanol, glycerol, lactic acid and residual concentrations of glucose and xylose per added g slurry (with 15.9% SS) after 76 h of SSFF and SSF respectively.

Table 3: Characterization of the raw slurry, during and after SSFF process.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Table 1.

SSFF Batches	Cultivation 1	Cultivation 2	Cultivation 3	Cultivation 4	Cultivation 5
Time (h)	76	78	96	96	92
Residual Glucose (g/L)	3.5	0.1	0.5	ND	ND
Ethanol (g/L)	28.3	31.3	34.6	28.7	27.5
Lactic acid (g/L)	ND	4.3	8.5	15.7	12.2

Table 2

Metabolites & Residual Sugars (g/kg slurry)	SSFF	SSF
Ethanol	51.3 ± 2.3	55.1 ± 1.4
Glycerol	2.7 ± 0.2	9.8 ± 0.1
Lactic acid	9.6 ± 2.0	ND
Glucose	1.5 ± 0.3	ND
Xylose	3.7 ± 0.9	9.5 ± 0.2

Table 3.

Slurry	Cellulose (%)	ASL (%)	AIL (%)	Total lignin (%)
Initial Slurry	46.7±0.1	5.0±0.0	46.0±0.3	51.3±0.3
Prehydrolysis (0 h)	46.7±0.4	4.8±0.1	45.6±0.3	50.4±0.4
Prehydrolysis (24 h)	39.0±1.0	4.1±0.0	54.7±0.0	58.9±0.0
SSFF (96 h)	24.1±1.1	2.8±0.0	71.0±0.0	73.9±0.1