Effect of mixing on enzymatic hydrolysis of cardboard waste: Saccharification yield and subsequent separation of the solid residue using a pressure filter

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Effect of mixing on enzymatic hydrolysis of cardboard waste: Saccharification yield and subsequent separation of the solid residue using a pressure filter

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Abstract
Cellulosic wastes, from sources such as low-quality cardboard and paper, are regarded as potential feedstocks for bioethanol production. One pathway from these cellulosic materials to ethanol is saccharification (hydrolysis) followed by fermentation. Saccharification is commonly performed using enzymes that are able to cleave the cellulosic structure to smaller units, preferably to glucose monomers. During the hydrolysis, mixing conditions have a considerable impact on the performance of the enzymes. Thus mixing conditions in the hydrolysis tank can also influence the downstream operations and, consequently, the overall economy of the bioethanol process. In this experimental study, four types of impeller, at different hydrolysis conditions were used. The effect of mixing on the glucose yield and on the filtration characteristics of the hydrolysate was evaluated. It was shown that not only the sugar yield depended on the mixing conditions: the effect on the solid-liquid separation step was even more significant.

Keywords: Mixing, Bioethanol, Enzymatic hydrolysis, Pressure filtration, Specific cake resistance

1. Introduction

In order to mitigate the looming problems caused by the accelerating use of fossil fuels, research and development activity for the production of ethanol from cellulosic biomasses is being increased (Abushammala and Hashaikeh, 2011; Balat et al., 2008). Bioethanol is one of the most viable candidate renewable fuels to be used as a substitute of gasoline in transportation (Talebnia et al, 2010; Várnai et al., 2010). In the best case, bioethanol could contribute to a reduction in carbon dioxide emissions. The overall effects of biofuels, however, should always be investigated in order to evaluate their sustainability (Soimakallio and Koponen, 2011). Real, net, benefits are most probably achieved when ethanol is produced from non-food raw materials such as biomass wastes, including agricultural, industrial, and municipal wastes and residues.

Corrugated cardboard is a common packaging material, available in large quantities all over the world. Cardboard waste typically contains at least 50 % cellulose, but the value

of such low-quality fiber lies mainly in its energy content. The physical structure of cardboard is quite favourable for bioethanol production, because the recalcitrant structure of wood is already disrupted in the pulping process. (Kádár et al., 2004; Yáñez et al., 2004; Yu et al., 2011)

Cellulose is a linear biopolymer, which consists of glucose units linked to each other by β-(1-4) linkages (Krässig, 1993). Other main components of cellulosic biomasses include hemicelluloses and lignin (Wickramasinghe and Grzenia, 2008). Starch is also found in many industrial biomass wastes, including cardboard. The polymeric structure of cellulosic biomasses can be converted to sugars, the most important of which is glucose. Conversion by enzymatic hydrolysis is regarded favourably, compared to the traditional acid hydrolysis, since fewer fermentation inhibitors are formed and milder conditions are required (Ingesson et al., 2001). Pretreatment of biomass (mechanical, chemical, thermal, etc.) is often necessary to facilitate the hydrolysis (Balat et al., 2008). The recalcitrance of lignocelluloses restricts the saccharification, principally due to low accessibility of crystalline cellulose and the barrier, formed by lignin and hemicelluloses, on the cellulose surface (Mooney et al., 1998; Zhang et al., 2007). From a technical point of view, it is not difficult to convert cellulose to bioethanol by means of enzymatic hydrolysis and fermentation. However, the main obstacles to the economic viability of the process are the huge demands of energy, chemicals, and enzymes (which require energy for their manufacture). Therefore it is important to pay attention to finding the best practicable conditions for each process step. In the present study, the impact of mixing on enzymatic hydrolysis and then subsequent solid-liquid (S/L) separation are investigated.

Mixing is one of the most important operations in the microbial conversion of cellulose to bioethanol. Adequate mixing under proper process conditions improves the rate of enzymatic hydrolysis (Ingesson et al., 2001; Samaniuk et al., 2011). Some earlier studies imply that intense mixing conditions may cause deactivation of the saccharifying enzymes, which leads to reduced conversion yields (Ingesson et al., 2001). On the other hand, a large enough surface area must be available for the enzymes as they attach the cellulosic surfaces (Taherzadeh and Karimi, 2008). Good mixing results at high solids contents have been obtained, for example, by utilizing pulping equipment designed for fibrous materials (Zhang et al., 2009), or a high-solids bioreactor designed by NREL (Roche et al., 2009). The impact of mixing of the slurry being hydrolyzed extends to downstream processes, in particular the solid-liquid separation. To the best of our knowledge, this is the first scientific paper showing the influence of mixing conditions in the hydrolysis stage on the subsequent S/L separation.

Process layouts encompassing separate hydrolysis and fermentation (SHF) vary greatly. S/L separations can be performed after pretreatment (Balat et al., 2008), after hydrolysis (Burke et al., 2011; Virkajärvi et al., 2009), after fermentation or after the distillation (Hahn-Hägerdal et al., 2006; Sassner et al., 2008). However, residual solids in the hydrolysate can have a negative influence on the fermentation and downstream separations (Burke et al., 2011). The main reason why the S/L separation in this study is performed immediately after the enzymatic hydrolysis is the poor controllability of the growth of the yeast cell mass and formation of side products during the fermentation, which could deteriorate the scientific value of the results. This study shows that proper

mixing in the hydrolysis stage improves the glucose yield as well as enabling effective S/L separation.

2. Materials and methods

2.1. Characterization of the feedstock

Shredded cardboard waste was selected as the model biomass for the experiments. Several impurities were observed in the material, e.g. plastic, staples of copper and aluminium, and inorganic particles (filler agents and coating pigments). The method of Black (1951) was utilized to determine the cellulose content of the oven-dry cardboard. The quantitative assay for lignin was conducted using a tailored liquid chromatographic method: HP Agilent 1050 device with Phenomenex Luna 3u C18(2) 100 x 2.0 mm column and 20 mM ammonium hydroxide and methanol (50/50 v-%) as eluents, pH 9, injection volume 5 µL. Detection of the analytes was performed using UV/VIS at 254 nm and electrospray ionization / mass spectrometer (ESI-MS). Relative to the dry weight, the proportions of cellulose and lignin were 63 w-% and 11.5 w-%, respectively. The ash content, 9.1 w-%, was determined according to ASTM D3516–89(2006) standard. Thus it was deduced that the hemicellulose content was about 15 w-%. The results presented later in Chapter 3.1 show that the raw material was likely to be moderately heterogeneous with respect to the cellulose content. Therefore it can be assumed that the approximate relative deviation of the results, caused by the heterogeneity, was smaller than 2.5 %.

The particle size of the original cardboard waste was reduced by dry milling. Over 10 kg of the material was milled for the experiments. Five vibrating sieves were used to divide the material into six fractions in order to determine an approximate particle size distribution of the material (Table 1).

Table 1. Particle size distribution of the raw material.

Particle size (µm)	Mass fraction (%)	Cumulative mass fraction (%)
< 125	18.5	18.5
125-250	22.3	40.8
250-500	15.2	56.0
500-1000	31.6	87.6
1000-1400	10.8	98.4
> 1400	1.6	100

2.2. Selection of impellers and design of experiments

The aim of the study was to compare the suitability of commonly-used impellers for mixing the slurry during enzymatic hydrolysis. Four different impellers were selected and an identical series of experiments were performed using each impeller.

The four different types of impellers: an anchor, a blade turbine (i.e. Rushton turbine), a propeller, and a double propeller are shown in Fig. 1A. Direction of rotation was clockwise in all experiments. The diameters of rotation of the impellers were 97 mm (anchor) and 70 mm (all other impellers).
Prior to creating the experimental plan, it was necessary to determine ranges for the following variables: 1) rotation speed of the impeller and 2) solid concentration of the slurry. Minimum and maximum rotation speeds for the impellers were first determined using an 8 w-% (total solids) slurry that was prepared in one mixing tank (V = 1.6 L). Enzymes were not used in these preliminary experiments. The minimum speed was just enough to keep the slurry (V = 1.1 L) surface in motion, whereas the maximum speed was the practical upper limit due to splashing and flooding. The range for the slurry solid content was selected based on knowledge gained from previous pressure filtration tests with a similar kind of slurry. Practical considerations limited the concentration of the slurry: reliable measurements of cake properties required relatively thick cake and thus solid concentration high enough, whereas a narrow feed pipe and valve in the filter equipment restricted the solid content.

Disposal of the solid residue is not favorable due to its relatively high energy content. More beneficial options for its utilization include, for instance, 1) recirculation to the bioethanol process, 2) combustion, and 3) biogas production.

Five tests were carried out with each impeller, according to 2^2 experimental design. Such a design comprises two levels (a minimum and maximum) for two variables, supplemented by the center point of the variable ranges. The experimental plan is presented in Table 2.

Table 2. The experimental plan.

Test	Rotation speed (rpm)	Tip speed (m/s)	Solid concentration (w-%)
1	29^A, 197^B	0.15^A, 0.72^B	8
2	29^A, 197^B	0.15^A, 0.72^B	12
3	134^A, 348^B	0.68^A, 1.28^B	10
4	240^A, 495^B	1.22^A, 1.81^B	8
5	240^A, 495^B	1.22^A, 1.81^B	12

^{A}Anchor; ^{B}Other impellers

In addition to the variables (rotation speed and solid concentration) the corresponding tip speeds of the impellers are shown. Compared with Rushton turbine and the propellers, the dimensions of anchor are markedly larger and the mixing effect, especially near the surface, is stronger. Therefore, it was necessary to use reduced rotation speeds for the anchor to avoid flooding. In addition to the tests shown in Table 2, four experiments were performed without baffles in the mixing tanks to investigate the influence of shear stress on the conversion yield and subsequent filterability.

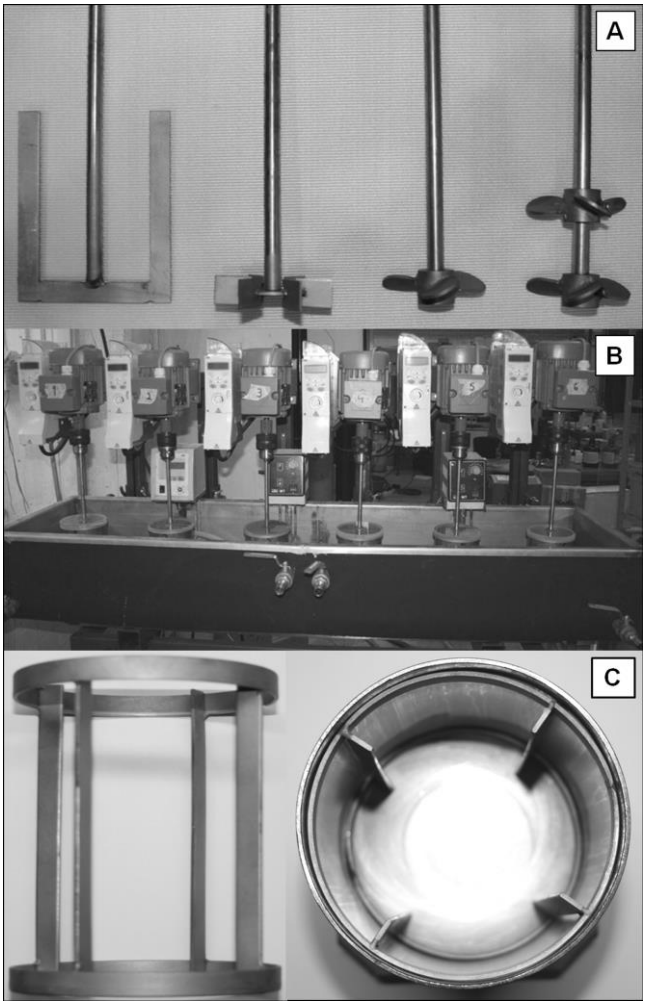

Fig. 1. The impellers left to right (A): Anchor, Rushton turbine, propeller, double propeller. The mixing device (B). The baffles and a mixing tank with the baffles (C).

2.3. Experimental set-up

The hydrolysis reactor was a specially designed mixing device (Fig. 1B), comprising six mixing tanks placed in a thermostatically controlled water bath. The tanks (height = 170 mm) were made of acid-proof steel to ensure negligible corrosion, as well as rapid temperature control due to high thermal conductivity. Each tank was equipped with four detachable baffles (Fig. 1C). The height of the baffles was 150 mm and the width of each baffle was 13 mm. The inner diameter of the tanks was 110 mm. The temperature in the tanks was controlled by three heating devices to an accuracy of 0.1 °C. The impellers were rotated by electric motors that were controlled by frequency converters (0 to 50 Hz, up to 1,000 rpm). Furthermore, the distance of the impellers from the tank bottom was adjustable. In this study, the distance was constant (15 mm).

2.4. Batch preparation

The enzymes used in the experiments were manufactured by Novozymes A/S, Denmark. Two kinds of saccharifying enzymes were used to convert the model biomass to sugars: a hemicellulase product, Cellic HTec, and a cellulase product, Cellic CTec2.

The slurry was prepared by mixing a calculated amount of the milled biomass with one liter of water at 40 °C. As already shown in the experimental plan, the final solids content was 8, 10, or 12 %. Dilute (20 w-%) sulfuric acid (J.T. Baker, 95-97 %, NL) was then added slowly until the pH was stabilized at 5.0. The hemicellulase dosage was 5 mL/kg biomass. To enhance the effectiveness of the hemicellulase, the slurry was heated up to 71 °C for 10 minutes. The cellulase (30 mL/kg cellulose) was added after rapidly cooling down to 46 °C. Slurry samples were taken at specific time intervals during the hydrolysis (72 h). The samples were cooled down to ambient temperature and centrifuged. Finally the supernatant was frozen for chemical assays.

2.5. Separation of solid residues

In this study, the solid residues that remained after saccharification were separated from the hydrolysates by means of pressure filtration. The laboratory scale filter (Nutsche) was operated batchwise. A slurry sample of 200 g, at 30 °C, was poured into the filter chamber, which was then pressurized with nitrogen. The solids were separated from the liquid using gas pressures of 1, 3.5 and 6 bar. A filter cake formed on the filter medium was manually discharged after releasing the pressure out of the filter chamber. Regardless of the slurry quality, suspended solids were not detected in the filtrates. The proportion of solids in the filter cakes was about 30 %. A cellulosic disc (T1000, Pall Corporation) with a cut-off particle size of 24 µm was used as the filter medium and each experiment was performed with a fresh filter medium to make sure that the initial medium resistance would be practically constant. The thickness of the filter medium was 3.6 mm. The effective filtration area was 18.9 cm^2 and the filter was temperature controlled (using a water-jacket) at 30.0 °C.

2.6. Chemical and physical analyses

Samples collected from the hydrolysates were stored in a freezer until all of the experiments were completed. After thawing and agitating the samples, they were filtered through a syringe filter with a pore size of 0.2 µm. Determination of sugars (glucose and xylose) and acetic acid was conducted by High Performance Liquid Chromatography, or HPLC, (HP Agilent 1100 equipped with an applicable column Varian Metacarb 87H). The column temperature was 60.0 °C and 0.005 M sulfuric acid was used as the eluent. The pumping rate and the injection volume were 0.6 mL/min and 10 µL, respectively. Determination of dissolved solids in each sample was performed by drying in a heating chamber at 105 °C for at least 48 h. Moisture contents of the filter cakes were determined using the same procedure.

2.7. Basic equation of constant pressure filtration

Solid particles form a filter cake (a porous bed of solids) when the slurry is filtered. The liquid passes through this fixed array of particles that, together with the filter medium, cause a specific resistance. In cake filtration, Darcy's basic filtration equation is supplemented with the cake resistance R_c as described by Svarovsky (1981):

$$Q = \frac{A\Delta p}{\mu\left(R_m + R_c\right)} \tag{1}$$

where Q (m^3/s) is the flow rate of a filtrate, viscosity μ (Pa s), through a filter cake with a resistance R_c (1/m) and a filter medium with a resistance R_m (1/m). The driving pressure Δp (Pa) is used to push the filtrate through the solids (filtration area A (m^2)).

Specific resistances of filter cakes α are usually determined based on a more complete, reciprocal form of Eq. (1):

$$\frac{dt}{dV} = \frac{\alpha\mu c}{A^2\Delta p}V + \frac{\mu R}{A\Delta p} \tag{2}$$

where t is time, V is the filtrate volume and α (m/kg) is the specific resistance of the filter cake and c ($kg_{solids}/m^3_{filtrate}$) is the filtration concentration that is defined as the mass of solid material in the filter cake per unit volume of filtrate collected.

The specific cake resistance depends on the properties of the solid particles as well as on the properties of the liquid. Particle size and shape, including the distributions of those, are typically assumed to be the most important particle-related properties.

The degree of cake compressibility under pressure can be evaluated by determining the compressibility index n (Wakeman and Tarleton, 1999):

$$\alpha = \alpha_0\Delta p^n \tag{3}$$

where α_0 is the specific cake resistance at unit applied pressure.

3. Results and discussion

3.1. Effect of mixing on the conversion yield

Mixing was observed to have an important role in the enzymatic conversion of the biomass to sugars. Both the impeller type and the mixing conditions were shown to have an influence on the glucose yield. To briefly summarize the results, the yields of glucose were generally low (0.15 to 0.28), as expected due to the low enzyme dosage and lack of pretreatment. There was only a comparatively small variation between the parallel tests, i.e. runs 1 and 2. The median of the relative standard deviations between the duplicate runs was 2.5 %. In five tests, the relative standard deviations were smaller than 0.5 %.

The differences in the yields may be caused by the combined effect of the biomass heterogeneity and experimental error.

The principal task of the mixing system used in enzymatic hydrolysis is to ensure adequate mass and heat transfer to accelerate the reaction while avoiding shear forces that are too strong and could deactivate the enzymes. However, cellulosic biomasses have a tendency to swell by absorbing free water. This is a problem, especially in the initial phase of hydrolysis, as the slurry becomes difficult to mix. High rotation speeds are often required to keep the whole batch in motion if the impeller type is not suitable for the application. A comparison of the investigated impellers is presented in Fig. 2. The differences can be clearly seen first after 24 hours, by which time the quickest part of saccharification is over. Prior to paying attention to the figures that follow, it must be emphasized that selection of the impeller is of major importance regarding the conversion yield of cellulose to glucose.

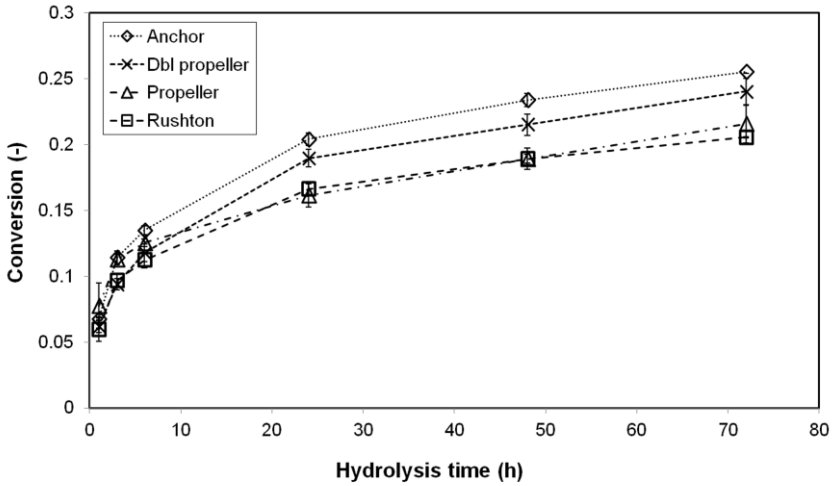

Fig. 2. Performance of the investigated impellers in enzymatic hydrolysis of the model biomass at 10 % solids content, medium rotation speed.

The conversions obtained at different rotation speeds (slow, fast) and slurry solids content (8 %, 12 %) are presented in Fig. 3 A-D, in which the effect of rotation speed on the conversion is shown in each figure. Comparison of Figs. 3A and 3B with Figs. 3C and 3D reveals the role of slurry solids content in the process. The contrasting performance of the impellers can also be evaluated from Fig. 3.

It was shown that the performance of anchor was the best with regard to the yield of enzymatic conversion (Fig. 3 A, 3C). The Rushton turbine was observed to decrease the performance of the enzymes, possibly due to its strong mechanical impact (Fig. 3A, 3C). The double propeller outperformed the propeller and the yields were highest when slow rotation speeds were used. When propellers are employed, it is recommended to install at least two of them on the same impeller shaft, provided that they are both submerged. It was also observed that the diameter of the impeller should preferably be over two thirds of the corresponding tank diameter, because the mixing should be comprehensive in the initial stage of hydrolysis as well. The yield was generally higher at 8 % solids content.

Two parallel control experiments were performed at 8 w-% and 12 w-% solid contents without mixing. The final yield (after 72 h) without mixing, as the average of two parallel tests, was 0.136 (8 w-%) and 0.118 (12 w-%). Both of these yields are lower than any of the yields obtained with mixing. A more general conclusion drawn from this is that there is an optimum mixing intensity for each biomass suspension, and it is very likely to be within the laminar flow regime.

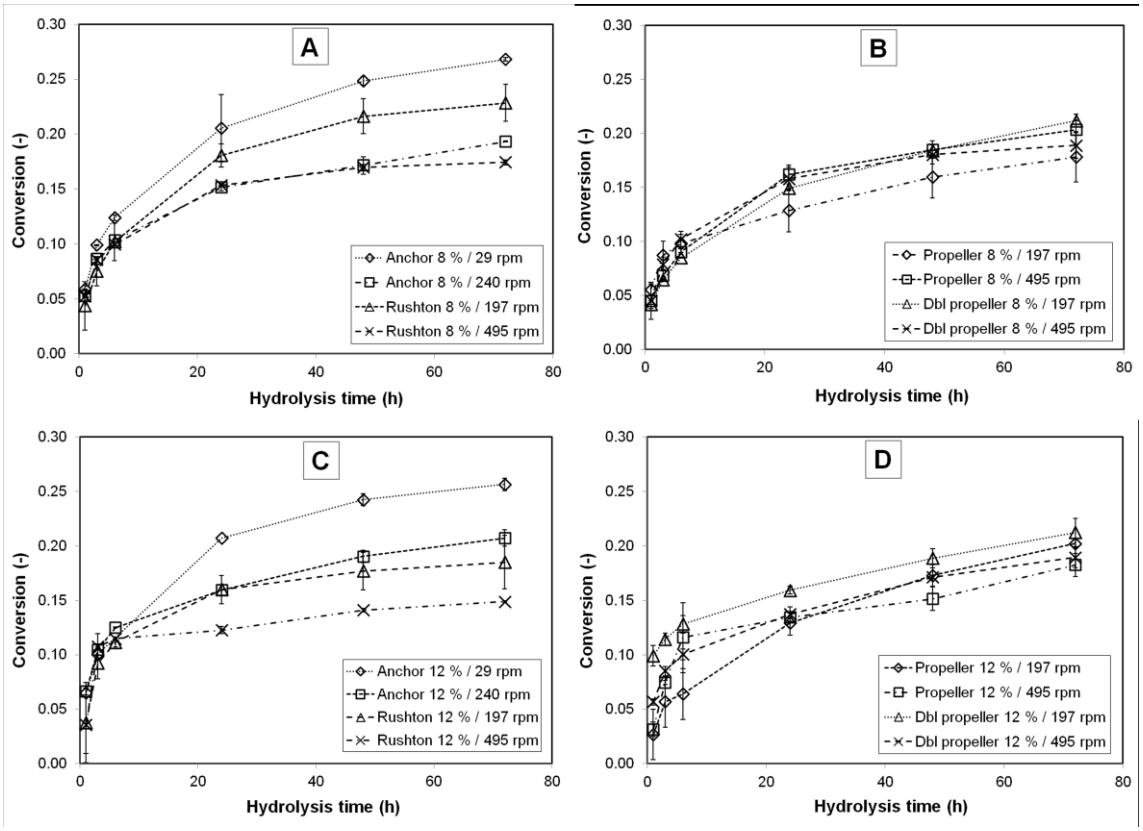

Fig. 3. Effect of rotation speed of the impeller and solids content of the slurry on the enzymatic conversion. Solids content of 8 % (A, B) and 12 % (C, D).

In this study, it has been shown that there is no reason to waste energy in order to overcome the recalcitrance of cellulose by strong mixing during enzymatic conversion. Reduction of the particle size of the raw material to submicron scale has been shown to be beneficial for the glucose yield, provided it is carried out as a preliminary treatment prior to the start of the hydrolysis (Yeh et al., 2010). The high energy consumption, however, would be the main obstacle to utilizing preliminary grinding in an industrial scale. Therefore, thermally assisted pretreatment techniques, such as steam explosion, are considered more useful.

Baffles are commonly installed into the stirred tanks to prevent the formation of a large vortex around the impeller axis and to make mixing more complete. In the case of enzymatic hydrolysis, however, the baffles can cause unnecessarily strong shear forces to occur, which leads to declined yields (up to 10 %) as shown in Fig. 4. The influence of baffles on the yield was investigated using two of the impellers, the double propeller and the Rushton turbine. The adverse effect of baffles on the conversion is clear, especially at 8 % solids content. This is probably because of the better ability of the lower solids (8 %)

slurry to flow. Although the shear stress in the 12 % slurry is increased by baffles, the change compared to that of the 8 % slurry (with and without baffles) is relatively small, as is the effect on yield.

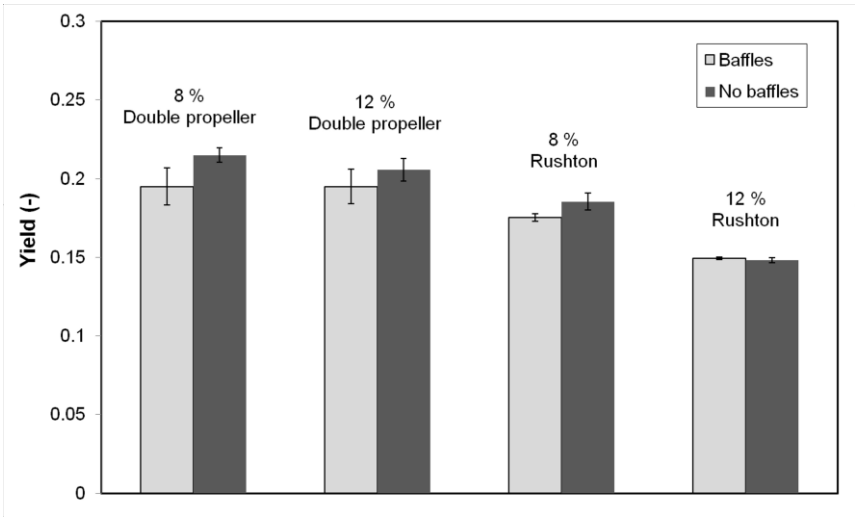

Fig. 4. Glucose yields obtained at different slurry solids content (8 and 12 %) by double propeller and Rushton turbine with and without baffles. The rotation speed was constant, 495 rpm.

Flow regulation with baffles did not improve the enzymatic hydrolysis. On the contrary, the baffles probably had a slightly negative influence on the saccharification. The differences, however, are so small that further experiments should be performed to confirm the statistical significance of these results.

3.2. Filtration and cake characteristics

Selection of the most suitable impeller for the enzymatic hydrolysis is of crucial importance, since the mixing conditions also affect the downstream processes. The purpose of this study was not to design novel impellers but to compare the performance of the most common types. It was observed that pressure filtration of the hydrolyzed slurry is a good option for separating the residual solids from the hydrolysate containing the dissolved sugars. The average specific cake resistance is a common measure of the filterability of the slurry. In the case of biomasses and other compressible materials, the average specific cake resistance can increase as the filtration pressure is increased.

A comparison of filter cakes from suspensions produced using different mixing conditions (impellers and speeds) is presented in Fig. 5. Differences in the average specific cake resistances α_{av} were found to be larger when faster rotational speeds of impellers were used. The absolute values of α_{av} were larger as well. Although an anchor impeller is not mechanically as rough as the other impellers employed, it was shown to cause considerably higher specific cake resistances at 12 % slurry solids content (Fig. 5A and B). The likely reason for this is that the higher conversion decreases the fiber length (Lu et al., 2011), changes its morphology (Clarke et al., 2011) and increases the

proportion of lignin in the solids. Hence it can be concluded that conversion of the biomass polymers to sugar oligomers and monomers has the most significant influence on the filtration of slurries with a high solids content. However, the combined effect of a relatively high conversion yield and mechanically intense conditions were probably responsible for the high specific cake resistances obtained with the propellers (Fig. 5B and C). As Fig. 5 shows, the specific cake resistances resulting from mixing conditions cannot easily be predicted. That is because the operation of each mixer is differently influenced by the conditions, i.e. the mixers have different pumping properties, besides their varying impacts on fibers and enzymes resulting from shear. The role of lignin in the filtration process is not absolutely clear, because its proportion varies as a function of the conversion yield, but the filtration resistance of lignin cakes has been reported to be high by Johansson (2005).

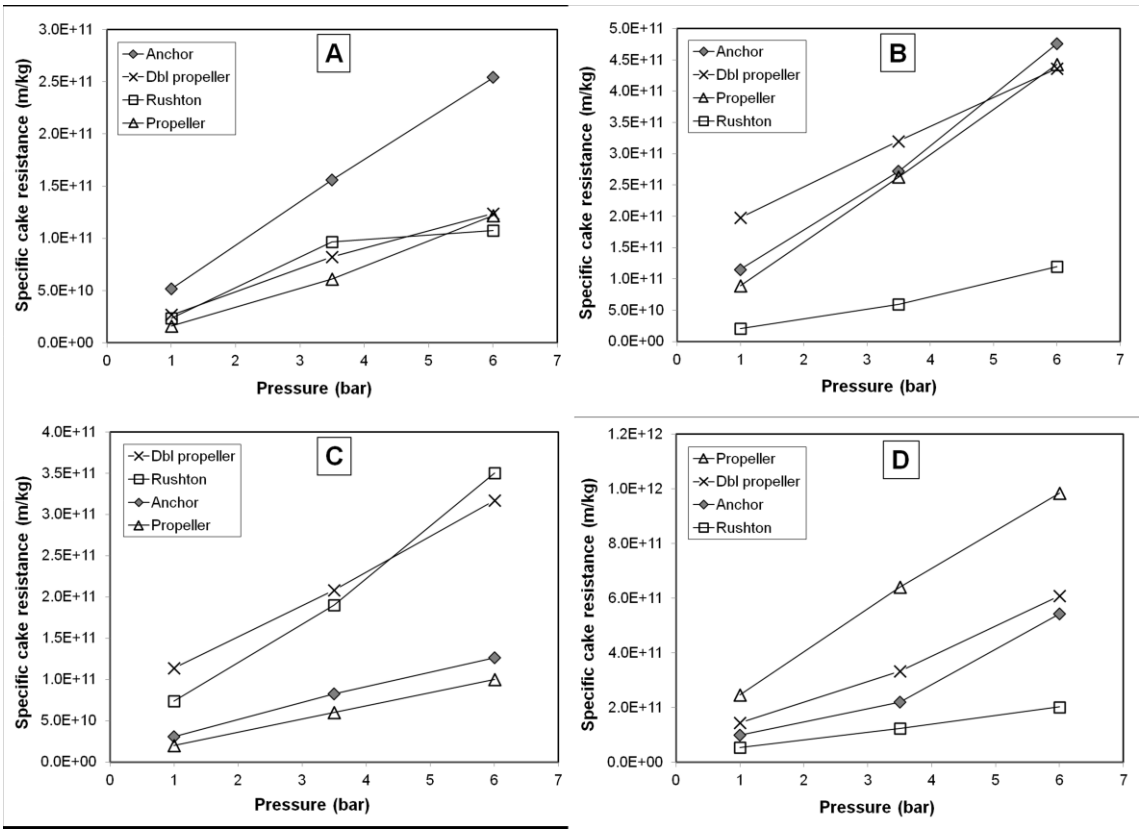

Fig. 5. Average specific cake resistances against applied filtration pressure. Slurry solids contents of 12 w-% (A, B) and 8 w-% (C, D). Slow (A, C) and fast (B, D) rotation speed of the impellers.

In fact the saccharification itself causes reduction of fiber dimensions and broken cellulosic fibers should be easier for the enzymes to digest. On the contrary, rough mixing at a tip speed of 1.8 m/s (able to reduce the fiber dimensions as well) is a major cause for low yields. That could explain why the Rushton turbine, unlike the other impellers, produced lower specific cake resistances at high rotation speeds. The enzyme dose was constant in this study. However, it has been previously observed that a more complete conversion, as a result of higher enzyme dosage, generally increases the specific cake

resistance. Generally, the specific cake resistance increased with lower slurry solids content.

It was shown that the use of baffles decreases the yield of enzymatic hydrolysis. In this case the benefits of mixing without baffles were especially notable, because improved yields were obtained simultaneously with reduced specific cake resistances (Fig. 6A, B). This effect is particularly notable with the double propeller impeller. The effect of baffles on specific cake resistances, after employing the Rushton turbine in the hydrolysis, was almost negligible both at 8 % and 12 % solids content. One reason for this is likely to be the radial flow pattern of the impeller. The high rotation speed (495 rpm) was the reason for the low conversion yield, which consequently was the explanation for the low specific cake resistances. Even though high rotation speed may facilitate the mass transfer and the availability of cellulose to the enzymes, deactivation and desorption of enzymes also occur, thus reducing the obtainable yield.
Filtration of the hydrolysates at three different pressures enabled the evaluation of the cake compressibility. The determined compressibility indices n as defined by Eq. (3) ranged from 0.7 to 1.1, which means that the cakes were moderately ($0.5 < n < 1$) or highly ($n > 1$) compressible (Oja, 1996). There was no clear correlation between the experimental variables and n.

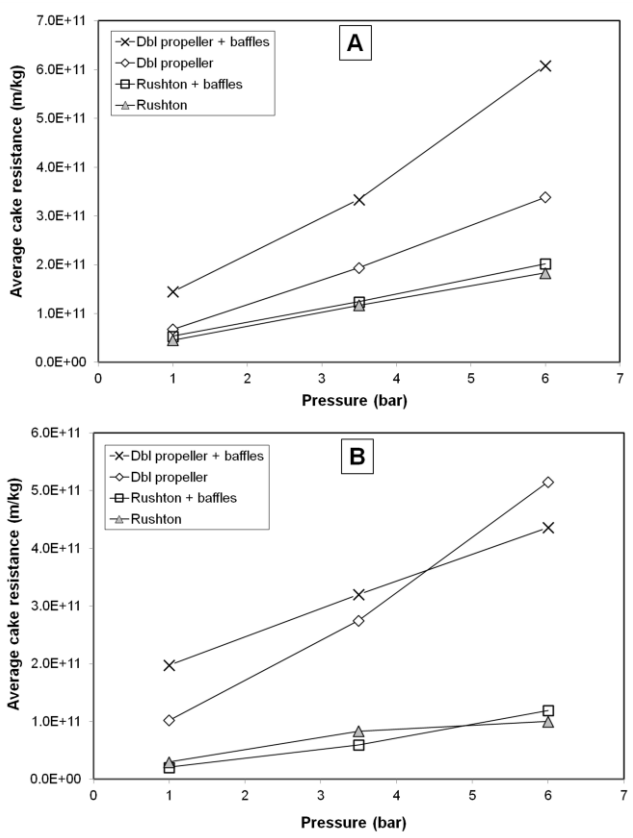

Fig. 6. Influence of baffles installed in the hydrolysis tank on average specific cake resistances at different filtration pressures. Rotation speed of the impellers (double propeller and Rushton turbine) 495 rpm, slurry solid contents 8 w-% (A) and 12 w-% (B).

4. Conclusions

The objectives of this study were to investigate the effect of mixing conditions on the yield of enzymatic hydrolysis and the subsequent S/L separation. The results show that mixing conditions (impeller-type, baffles and speed) do indeed have a significant effect. Gentle mixing conditions proved most suitable for enzymatic hydrolysis yield, under the conditions studied. Experiments performed with a laboratory-scale pressure filter showed that the filterability is also affected by mixing conditions, as a result of conversion yield (which affects particle size and properties) and shearing of the suspension (which affects particle size). In general, high intensity mixing should be avoided.

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