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Use of surface active additives in enzymatic hydrolysis of wheat straw lignocellulose

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Abstract

Monocot residues such as corn stover and straw are often not fully exploited and constitute a potential substrate for bioethanol production. However, a number of factors such as high enzyme loadings make large-scale utilization economically difficult. Addition of non-ionic surfactants and poly(ethylene glycol) to enzymatic hydrolysis of various lignocellulosic substrates has been found to increase the conversion of cellulose into soluble, fermentable sugars. We have shown that surfactants are able to increase cellulose conversion with up to 70%. This provides an opportunity of decreasing enzyme loading while retaining the same degree of hydrolysis. Investigations of five wheat straw substrates produced with different pretreatment methods revealed that surfactants have a more pronounced effect on acid and steam treated straw than, e.g. ammonia and hydrogen peroxide treated straw. Thus, lignin content is not directly proportional with the potential surfactant effect. Studies of adsorption of cellulases support the theory that the main mechanism behind the surfactant effect is prevention of unspecific adsorption of enzyme on the substrate lignin. This is believed to be due to hydrophobic interaction between lignin and the surfactant, causing steric repulsion of enzyme from the lignin surface. More research is needed to reveal which factors influence enzyme and surfactant adsorption.

Keywords: Cellulose; Cellulase; Hemicellulose; Surfactant; Adsorption; PEG

1. Introduction

For more than a decade lignocellulose has been recognized as a potential substrate for ethanol production [1]. Despite intensive research, several factors still prevent a large-scale utilization of lignocellulose for liquid fuel production. The main obstacle is the need of high enzyme concentrations in order to obtain a high rate of cellulose conversion into glucose along with long process times due to rapid decrease of the hydrolysis rate [1,2]. In addition, enzyme recycling is difficult as enzymes adsorb to residual lignocellulosic material. In order to make cellulose hydrolysis for ethanol production economically feasible it is important to identify methods to increase enzyme effectiveness.

It has been shown that addition of surfactants such as non-ionic detergents and protein significantly increases the enzymatic conversion of cellulose into soluble sugars [3–7]. Various mechanisms have been proposed and investigated for the positive effect of surfactant addition on the enzymatic hydrolysis of lignocellulose. Recent studies on steam-treated softwood substrate propose that the dominating mechanism responsible is the influence of surfactants on cellulase interaction with lignin surfaces [7]. Surfactant adsorption to lignin is believed to prevent unproductive binding of enzymes to lignin, thereby producing higher yields and better recycling of enzymes. This is in accordance with other results showing less adsorption of enzymes to lignocellulose during hydrolysis in the presence of a surfactant [3,6]. Added protein such as BSA is also believed to bind to lignin, preventing unproductive binding of cellulases [7,8].

Other mechanisms proposed include the surfactant being able to change the nature of the substrate, thereby increasing the available cellulose surface; in turn promoting reaction sites for cellulases to adsorb onto [6,9]. Surfactants may also have a stabilizing effect on the enzymes, effectively preventing enzyme denaturation during the hydrolysis. This possible binding of the

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surfactants to the tertiary structure of the enzyme-proteins is known from other enzymes [10].

The first step in the enzymatic hydrolysis where soluble cellulases convert solid cellulose into soluble sugars is the adsorption of the enzymes onto the cellulosic surface. It has been shown that the rate of adsorption is rapid compared to the actual hydrolytic activity of the enzymes, thus making the amount of adsorbed cellulase an important factor in the effectiveness of the reaction [11]. The pretreatment or processing of the lignocellulosic substrate has a significant effect on the rate and extent of cellulase adsorption [12].

Previous studies have focused on wood materials and especially softwood lignocellulose due to the regional abundance of it. However, corn stover and straw are agricultural residues, which are today not fully exploited and therefore interesting as raw material for bioethanol production. The lignin content of herbaceous materials is in general lower and has a different composition. These differences are likely to influence the interaction between substrate and enzymes and therefore also the effect of the surfactants.

The main focus of this study is to investigate if the conversion of straw cellulose into sugar can be increased with various surface active additives as effectively as is the case with, e.g. steam-treated spruce lignocellulose [7]. Furthermore, the relationship between the type of pretreatment process applied and the increase of hydrolysis caused by the surfactant is investigated. This was carried out by hydrolyzing five different types of pretreated wheat straw, using a commercial enzyme mixture. Hydrolysis was performed with various non-ionic surfactants added and with protein (BSA) for comparison. To our knowledge, this is the first time the relationship between the pretreatment type and the effect of various non-ionic surfactants on cellulose hydrolysis has been investigated. This relationship has helped shed more light on the mechanism of the surfactant effect. In order to clarify this mechanism and the important role of lignin further, the endoglucanase activity in the hydrolysis solutions was measured.

Another limiting factor in converting lignocellulose into bioethanol is the lack of pentose-fermenting microorganism. The main sugar of monocot hemicelluloses is the pentose xylose, which often makes up a substantial part of the total sugar content of the cell wall. However, industrial yeast strains for this purpose are currently being produced [13]. Therefore, the effect of surfactants on xylan conversion was also investigated in the hydrolysis experiments.

2. Materials and methods

2.1. Substrates

Wheat (*Triticum aestivum*) was grown and harvested in Denmark in 2003. The straw was left to dry on the field and then pressed into big bales. The bales were stored dry at ambient temperature. Before use, the straw was cut into pieces up to 6–8 cm long by a forage harvester and stored in containers at ambient temperature. The dry matter (DM) content was approximately 90% (w/w).

Fresh, chipped spruce (*Picea abies*) free of bark was provided by a saw mill in southern Sweden. The chip size was 2–10 mm.

Table 1
Chemical composition of surface active additives

Surfactant/polymer	Composition
Berol (alcohol ethoxylate)	Berol ox 91-8: $CH_3–(CH_2)_{8–10}–O–(CH_2–CH_2–O)_8$; Berol 08: $CH_3–(CH_2)_{15–17}–O–(CH_2–CH_2–O)_{80}$
Poly(ethylene glycol) (PEG) (molecular masses: 2000, 4000 and 6000)	$HO–(CH_2–CH_2–O)_n–H$ ($n = 45$, 91 and 136)
Tween 80	Polyoxyethylene sorbitan monooleat
Bovine serum albumine (BSA)	

2.2. Surface active additives

The tested additives were: bovine serum albumine (BSA, Sigma–Aldrich, St. Louis, USA), poly(ethylene glycol): PEG 2000, PEG 4000 and PEG 6000 (Merck & Co., St. Paul, USA), Berol 08 and Berol ox 91-8 (Akzo Nobel, Stenungsund, Sweden). All surface active additives will be referred to as surfactants for the sake of convenience. Names and chemical compositions of the surface active additives used are listed in Table 1.

2.3. Pretreatment methods

Four different batches of pretreated straw were produced on the IBUS pilot plant at Fynsværket in Odense, Denmark [14]. In addition, one batch of pretreated straw and one batch of spruce were pretreated by steam explosion at Center for Chemistry and Chemical Engineering, Lund University, Sweden. The pretreatment conditions are summarized in Table 3.

Pretreatment on IBUS pilot plant. The straw was pretreated according to [14] using a feeding rate of 50 kg straw per h (= 45 kg DM h^{-1}), 250 l h^{-1} of counter-current water flow and a residence time of 6 min in the reactor. The reactor temperature was maintained at 190 or 195 °C (Table 3) by injection of steam. The pretreatment was performed using water or water with the addition of ammonia, sulfuric acid or hydrogen peroxide (Table 3). Pretreated straw had 23–26% DM. The pretreated straw was collected in plastic bags and stored at −20 °C until use.

Steam explosion pretreatment. The straw was treated with steam to reach a DM content of 59%. The straw was impregnated with SO_2 (2.7% (w/w)) for 1.5 h at room temperature in plastic bags. The amount of SO_2 absorbed was determined by weighing the plastic bags before and after impregnation. The impregnated material (750 g) was steam pretreated at 215 °C for 5 min in a steam pretreatment unit equipped with a 10 l reactor [15]. The material was stored at 4 °C. Before use the material was washed with two volumes of water to remove soluble sugars. The spruce was pretreated by similar means, but impregnated with 3% (w/w) SO_2 for 20 min.

2.4. Straw composition analysis

Dry matter (total dry matter including soluble and insoluble solids) was determined using a Sartorius MA 30 moisture analyzer at 105 °C (Sartorius AG, Goettingen, Germany). Samples were dried at 35 °C for 1–2 days and then cut and strained through a 1.5 mm sieve on a Retsch SM 2000 cutting mill (Retsch, Inc., Newtown, USA).

The composition of the straw was analyzed using two-step acid hydrolysis according to the procedure published by the National Renewable Energy Laboratory (NREL) [16]. The dried samples were treated with 3 ml of 72% H_2SO_4 and placed in a water bath with a temperature of 30 °C. The samples were diluted with 84 ml of Milli-Q water to give a H_2SO_4 concentration of 2.5%. The samples were autoclaved for 1 h at 121 °C. After cooling, 20 ml of the sample was neutralised with $CaCO_3$ to pH 5–6. Monosaccharide concentration was analyzed by HPLC.

Results are given as *glucan* (nearly all D-glucose originates from cellulose) and *hemicellulose*: D-arabinose and D-xylose in straw (mainly arabinoxylan) and

Table 2
Pretreatment conditions

Type	Temperature [°C]	Additive concentration [g (kg straw)$^{-1}$]	Residence time [min]
Water[a]	190	–	6
Alkaline[a]	195	NH_3, 25	6
Acid[a]	190	H_2SO_4, 35	6
Hydrogen peroxide[a]	190	H_2O_2, 25	6
Steam explosion[b]	215	SO_2, 18	5
Steam explosion spruce[b]	215	SO_2, 20	5

[a] Pretreated on the IBUS pilot plant.
[b] Pretreated at Lund University.

D-arabinose, D-xylose, D-mannose, D-galactose in spruce (mainly galactoglucomannan).

2.5. Hydrolysis experiments

The pretreated straw was dried at 35 °C for 1–2 days and then cut and strained through a 1.5 mm sieve on a Retsch SM 2000 cutting mill. The hydrolysis was performed using an enzyme mixture of Celluclast 1.5 l and Novozym 188 (weight ratio 5:1, from Novozymes A/S, Bagsvaerd, Denmark) with a filter paper activity of 74 FPU g^{-1}, as measured by the filter paper assay [17].

The hydrolysis was performed in 50 ml Falcon tubes (total reaction volume 40 g), at 5% DM (w/w) in a 50 mM sodium citrate buffer pH 4.80 and using an enzyme loading of 5 FPU (g DM)$^{-1}$. In the screening studies, the surfactant concentration was 0.05 g (g DM)$^{-1}$. In the concentration effect study, Berol 08 was tested at 0.005, 0.025, 0.05 and 0.10 g (g DM)$^{-1}$, and PEG 6000 was tested at 0.005, 0.01, 0.025 and 0.05 g (g DM)$^{-1}$. The test tubes where placed in a heated (50 °C), shaking water bath (80 rpm) for 24 h. All experiments were performed in triplicate. Samples for sugar analysis were boiled for 10 min to terminate the reaction and stored at −20 °C until analysis. Samples for determination of enzyme adsorption were frozen immediately after hydrolysis.

2.6. Sugar analysis by HPLC

Samples were filtered through a 0.45 μm filter and diluted appropriately by eluent (5 mM H_2SO_4). The content of monosaccharides (D-glucose, D-xylose and L-arabinose) was quantified on a Dionex Summit HPLC system (Dionex Corporation, Sunnyvale, USA) equipped with a Shimadzu refractive index detector (Shimadzu, Kyoto, Japan). The separation was performed in a Phenomenex Rezex RHM column (Torrance, USA) at 80 °C with 5 mM H_2SO_4 as eluent at a flow rate of 0.6 ml min^{-1}.

2.7. Determination of enzyme adsorption

Adsorption of enzyme onto the remaining solid material was determined by measuring residual endoglucanase activity in the liquid phase. Solids were removed by centrifugation for 10 min at 15,000 × *g*. Endoglucanase activity was measured using azo-carboxymethyl cellulose (Megazyme, Wicklow, Ireland) as substrate. The measurement was performed as described by [18]; except absorbance was measured at 590 nm. Standard curves were prepared using the same enzyme mixture of Celluclast and Novozym 188 as used in the hydrolysis experiments. Adsorption was calculated as the measured endoglucanase activity subtracted from the initial endoglucanase added.

2.8. Stabilization effect of surfactants

The direct effect of surfactants on enzyme stability was determined by preparing mixtures containing the same enzyme activity (250 FPU l^{-1}) and ratio between surfactant and FPU (0.01 g FPU^{-1}). The activity of the solutions (with and without surfactant) were measured by the filter paper assay [17] and by azo-carboxymethyl cellulose at $t = 0$ and 24 h. The solution was incubated at 50 °C for both the azo-carboxymethyl cellulose assay and the filter paper assay.

3. Results

3.1. Substrate composition

The lignin fraction of lignocellulose has been proved to be responsible for unspecific adsorption of cellulases [7]. However, the influence of the pretreatment method and conditions on this adsorption is less clear. To investigate this, five different types of pretreated wheat straw was produced. Four types were produced using a pilot scale pretreatment reactor [14] and one type using SO_2-catalyzed steam explosion [15]. In addition, spruce was pretreated using SO_2-catalyzed steam explosion. The conditions are summarized in Table 2. The composition of the resulting materials is shown in Table 3. The hemicellulose content in straw is based on content of xylose, arabinose, and in spruce on xylose, arabinose, mannose and galactose. The lignin content in the wheat straw pretreated in the pilot scale reactor varied only little, irrespective of the conditions applied. On average the lignin content in wheat straw was 22.6%. Steam exploded spruce contained significantly more lignin than pretreated straw (50.9%, see Table 3). Acid catalyzed pretreatment methods significantly lowered the xylan content and therefore also the total hemicellulose content.

3.2. Effect of pretreatment and surfactant on hydrolysis

The surfactants and pretreatments were compared by hydrolyzing a 5% substrate solution containing 0.05 g (g DM)$^{-1}$ of surfactant for 24 h using an enzyme loading of 5 FPU

Table 3
Composition of materials used in hydrolysis experiments

Pre-treatment	Klason lignin	Ash	Glucan	Hemicellulose[a]
Untreated straw	17.7	7.0	34.8	25.2
Water, straw	19.6	2.5	54.3	18.8
H_2O_2, straw	24.0	2.6	54.0	19.2
H_2SO_4, straw	22.7	5.8	56.8	8.2
NH_3, straw	23.0	1.8	50.6	20.0
Steam explosion, straw	23.6	6.3	56.7	8.5
Untreated spruce	27.0	0.1	43.0	19.7
Steam explosion, spruce	45.0	0.1	48.0	∼0

All values are in percent of total content on a dry matter basis.
[a] The hemicellulose fraction includes a number of polymers. In straw the main hemicellulose polymer is arabinoxylan. In spruce the main hemicellulose polymer is galactoglucomannan.

$(g\,DM)^{-1}$. The cellulose and xylan conversion was measured by quantifying the amount of released glucose and xylose, respectively, by HPLC. Although the hemicellulose in straw is present as arabinoxylan, only xylose was used as an estimate of the hydrolysis of the hemicellulose as the arabinose content was only 1–2%. Thus, in the following, xylan conversion refers to the release of xylose from arabinoxylan. Seven different surfactants were initially tested for their ability to enhance enzymatic hydrolysis of the pretreated wheat straw. Three types of PEG with an average molecular mass of 2000, 4000 and 6000 Da were tested. The results revealed a trend of slightly higher cellulose conversion with increasing molecular weight, which has also been observed when used with spruce lignocellulose [19]. Therefore, PEG 6000 was chosen for further studies. The results of Berol ox-91-8 and Berol 08 were not statistically different (not shown), and Berol 08 was selected for further studies.

Hydrolysis without the addition of surfactant resulted in glucose concentrations ranging between 5.0 and 15.7 g/l, depending on pretreatment. The acid pretreatment resulted in the lowest conversion of cellulose whereas the highest conversion was obtained using the steam-exploded straw. The degree of cellulose conversion or hydrolysis, defined as amount of glucose released relative to the maximum theoretical, was 36% for the water pretreated wheat straw but only 16% for the acid pretreated wheat straw (Fig. 1A). For the steam-exploded straw the cellulose conversion was 51%.

With surfactants added, the glucose concentration increased in all experiments, although not all of them being a statistically significant increase. Interestingly, the increase in cellulose conversion of acid treated straw was substantially higher than any of the other pretreatments, ranging from 58% (BSA) to 70% (Berol 08) (Fig. 1B). This increase brings the cellulose hydrolysis of the acid treated straw to the same level as for the other pretreatments. For the other types of substrate, the effect was in the range of 3–23% improvement in cellulose hydrolysis.

The xylan conversion varied between 36 and 60% of the theoretically possible, depending mainly on pretreatment method (Fig. 2A). Interestingly, the xylan conversion of the steam exploded and acid pretreated straw was comparable to the other pretreatment methods despite containing less than half the xylan (Table 3). The effect of surfactants on the hydrolysis of the xylan was not as pronounced as seen with glucose with improvements in the order of 0–10%, except for steam exploded straw where the increase in xylan conversion was 11–17% (Fig. 2B).

3.3. Surfactant concentration

The correlation between amount of surfactant added and effect on hydrolysis was investigated for Berol 08 and PEG 6000 on water and acid pretreated wheat straw (Fig. 3). For both substrates and both surfactants, the effect of increasing the surfactant concentration on the cellulose hydrolysis leveled off above 0.025 g $(g\,DM)^{-1}$. The optimum ratio between surfactant and substrate was approximately 0.05 g $(g\,DM)^{-1}$. Although the effect of Berol 08 on cellulose hydrolysis was higher on acid pretreated wheat straw compared to water pretreated straw

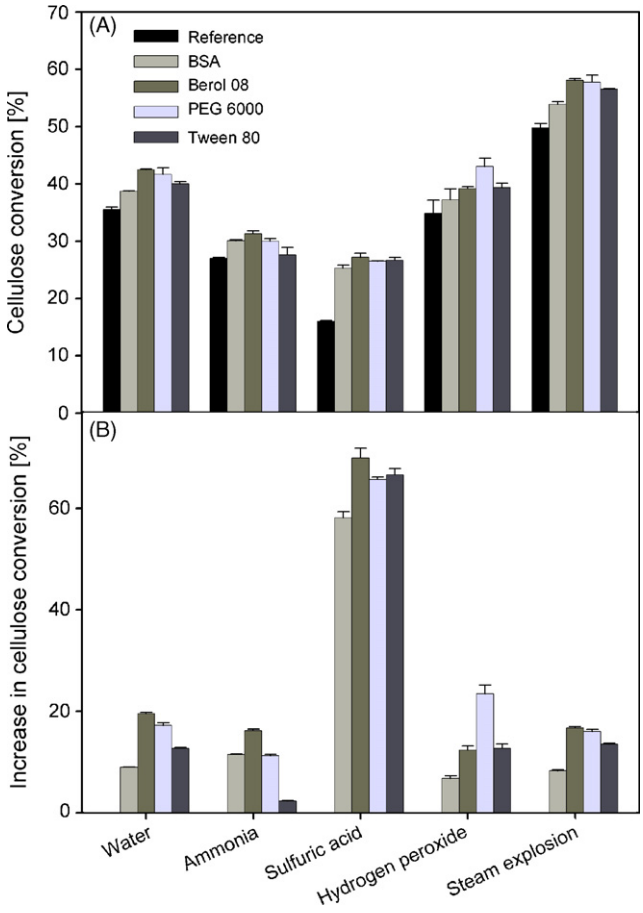

Fig. 1. (A) Cellulose conversion of straw after 24 h of hydrolysis in percentage of theoretical maximum as a function of pretreatment method. Substrate concentration was 5% (w/w). Reference was without addition of 0.05 g/g DM surfactant. Results are averages of triplicates. (B) Results from (A) calculated as percent increase in cellulose conversion compared to the reference. Results are averages of triplicates.

(Fig. 1B), the optimum concentration for both substrates (Fig. 3) was 0.025–0.05 g $(g\,DM)^{-1}$. At lower concentrations, PEG 6000 had a slightly higher effect on hydrolysis of water pretreated straw compared to Berol 08, but above 0.025 g $(g\,DM)^{-1}$ the difference was negligible.

No significant effect of surfactant concentration was seen on xylan hydrolysis (data not shown).

3.4. Enzyme activity in solution

It has been suggested that the surfactant effect is due to hydrophobic interaction between the surfactant and lignin on the lignocellulose, thereby either releasing unspecifically bound enzyme or preventing unproductive enzyme adsorption [4,7]. The effect of substrate and surfactants on the adsorption of enzyme was studied by measuring the endoglucanase activity remaining free in solution after the hydrolysis. Endoglucanases may become deactivated during hydrolysis for other reasons than adsorption. However, due to the stability of the enzymes, remaining endoglucanase activity is used as a measure of adsorption of cellulases.

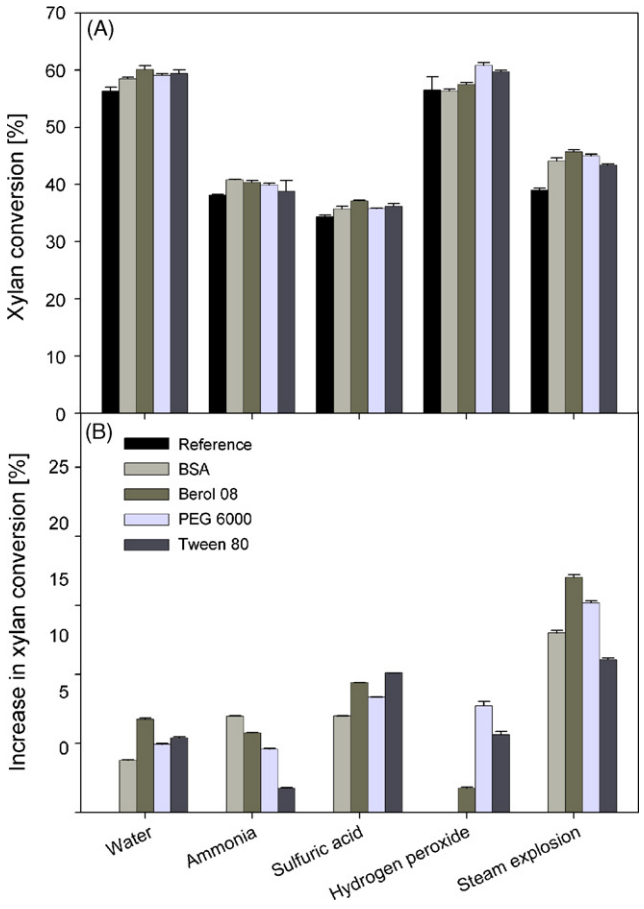

Fig. 2. (A) Xylan conversion of straw after 24 h of hydrolysis in percentage of theoretical maximum as a function of pretreatment method. Substrate concentration was 5% (w/w). Reference was without addition of 0.05 g/g DM surfactant. Results are averages of triplicates. (B) Results from (A) calculated as percent increase in xylan conversion compared to the reference.

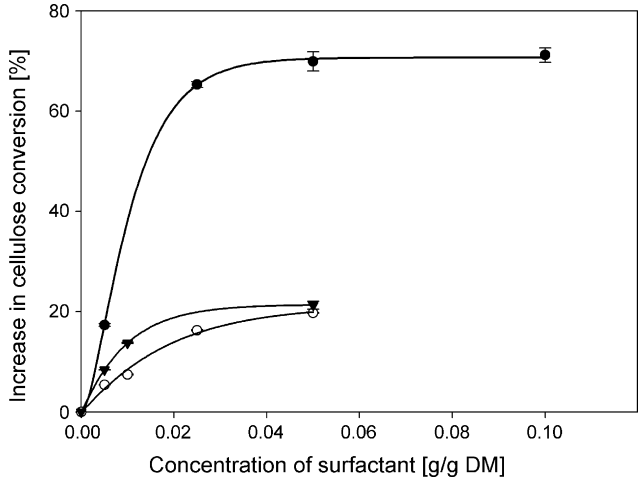

Fig. 3. Effect of surfactant concentration (Berol 08 and PEG 6000) on cellulose conversion of water and acid pretreated wheat straw. (●) Sulfuric acid treated straw and Berol 08; (◯) water treated straw, PEG 6000; (▼) water treated straw, Berol 08. Results are averages of triplicates.

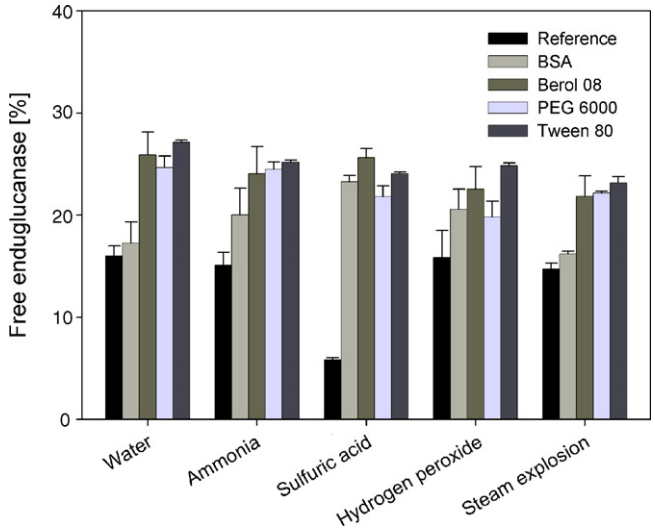

Fig. 4. Endoglucanase activity free in solution after 24 h hydrolysis of wheat straw (5%, w/w) depending on pretreatment and surfactant type, respectively. The enzyme activity was calculated as percentage of initially added activity. Reference was without addition of 0.05 g/g DM surfactant. Results are averages of triplicates.

The endoglucanase activity in the solution after hydrolysis of straw pretreated by water, ammonia, hydrogen peroxide and steam explosion without addition of surfactants were all around 15% of the activity initially added, except for the solution of the acid pretreated straw without surfactant which was only 6% (Fig. 4). Assuming that the endoglucanase activity can be used to estimate adsorption of cellulases, the results reveal that 85–94% of the cellulase enzymes are adsorbed onto the substrate after 24 h of hydrolysis.

The addition of surfactant increased the endoglucanase activity in solution by a minimum of 25% with the exception of BSA, which was less efficient (endoglucanase activity between 20 and 26% for all substrates, Fig. 4). The increase in endoglucanase activity by addition of surfactants correlated well with the concurrent improvement observed in the hydrolysis of cellulose (Fig. 1A and B). Interestingly, the addition of all surfactants, including BSA, increased the low enzyme activity measured in the acid pretreated straw solution to a point where the activity was equal to that of the other pretreatment types with surfactant added (Fig. 4). The endoglucanase activity in the solution of the acid treated straw was between 2.7 and 3.4 times higher than the reference without surfactant.

3.5. Spruce hydrolysis

Surfactants have previously been reported to improve the cellulose hydrolysis of steam pretreated spruce significantly [7]. In order to compare the effect of surfactants on materials from different origins, hydrolysis studies were performed on spruce pretreated by steam explosion under similar conditions as used with wheat straw (Fig. 5A). Conversion of steam exploded spruce cellulose was close to 80% with surfactant addition. This is equivalent to an improvement of the conversion from 59%

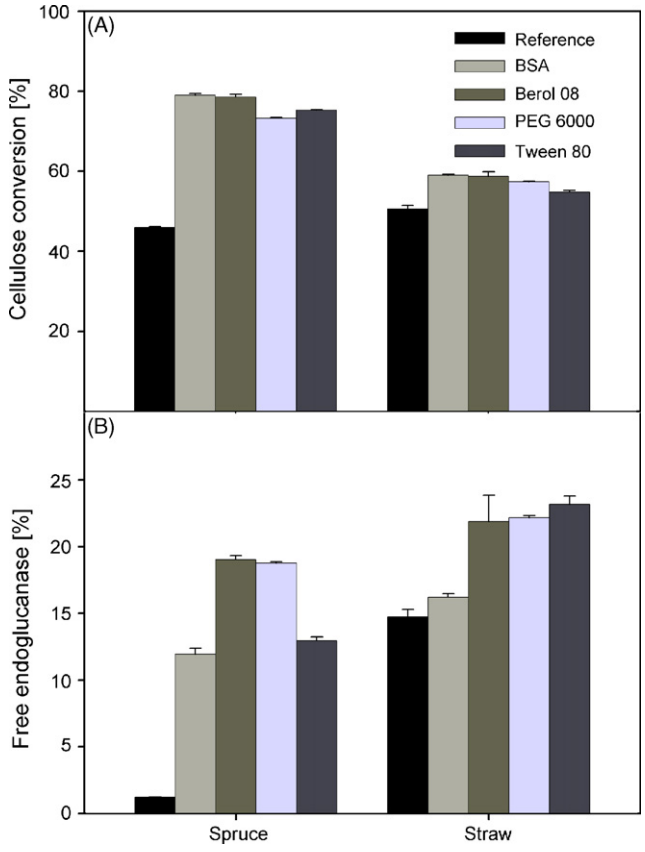

Fig. 5. (A) Effect of surfactants (0.05 g/g DM) on hydrolysis of wheat straw and spruce, respectively (substrate concentration 5% (w/w), 24 h hydrolysis). Both raw materials were pretreated by SO_2-catalyzed steam explosion. Results are averages of triplicates. (B) Percentage of added endoglucanase found free in solution of the hydrolyzed substrates in (A).

(Tween 80) to 72% (Berol 08). In contrast, hydrolysis of cellulose was only improved by 8–17% using the steam exploded wheat straw as substrate (cellulose conversion approximately 60%, see Fig. 5A).

3.6. Stabilizing effect of surfactants

As poly(ethylene glycols) and other surfactants have been reported to have a stabilizing effect on some enzymes [10], it was investigated if this was also the case with the tested surfactants and cellulases. The endoglucanase activity was measured on azo-carboxymethyl before and after 24 h incubation using the same enzyme mixture as used previously for the hydrolysis. Activity was measured on solutions containing no surfactant, PEG 6000, BSA and Berol 08, respectively. The decrease in enzyme activity in solutions with surfactant added was found to be slightly less (1.7–5.2%, data not shown) than the reference (5.4% decrease). However, the difference was not statistically significant. The FPU assay (measuring the overall cellulase activity) did not confirm the possible stabilizing effect as it showed no difference between the solutions with and without surfactant (data not shown).

4. Discussion

4.1. Combined effect of surfactants and pretreatment

Lignocellulose is a highly complex structure with a whole range of characteristics that influence and limit the hydrolysis of carbohydrate polymers into fermentable sugars [20]. As lignin is generally believed to be one of the most limiting factors of enzymatic hydrolysis of lignocellulose [7,21,22], it was interesting to investigate the relationship between lignin and convertibility of the pretreated materials.

Even though only the lignin content of the water pretreated wheat straw was slightly lower (19.6%) than that of the other pretreated wheat straw (22.7–24.0%, see Table 3), the cellulose conversion varied from 16 to 51%. This strongly suggests that although lignin content has been proven to be an important factor for degradability [7,21], other factors are perhaps equally important. The various pretreatments had a more differentiated effect on the xylan content, ranging from 7.8 to 18.4%. This is likely due to hemicelluloses being dissolved in some pretreatments, such as acid catalyzed pretreatment.

Surfactants were found to increase the cellulose hydrolysis significantly. Interestingly, the added surfactants had the most pronounced effect on the straw treated with sulfuric acid (increase in cellulose conversion more than 60%). Without surfactant, the acid treated straw showed lowest conversion. It is possible that the acid pretreatment makes the lignin more receptive to cellulose adsorption through a change of surface properties, e.g. increased hydrophobicity or hydrogen bonding capacity. It is also possible that the treatment dissolves hemicelluloses associated with or covering lignin, thereby increasing the accessibility of lignin and hence the adsorption. The low xylan content (7.5%) supports the last theory. However, the steam explosion pretreated straw has similarly low xylan content (7.8%) yet the increase is more modest. This could be due to the cellulose hydrolysis of the steam exploded straw already being closer to the theoretical maximum. More research is needed in order to establish a clear relationship between effect of surfactants and substrate characteristics.

Unlike previous studies, which have focused on materials with little hemicellulose content [7,23], the effect of surfactants on xylan hydrolysis was also studied. Xylan conversion was determined as xylose released from arabinoxylan, the main hemicelluloses in straw. Steam exploded and acid pretreated straw had low xylan content, yet the xylan conversion was still more than 30%. Addition of surfactant also had an effect on xylan hydrolysis, although not as pronounced as seen with cellulose. It is not known if this is due to the properties of the xylanases (lower tendency to unspecific adsorption) or perhaps the smaller content of xylan in the material compared to cellulose. When pentose-sugar-fermenting microorganisms become industrially available, the utilization of xylan will be an important factor in lignocellulose hydrolysis and add to the effectiveness of bioethanol production.

Comparison of the five tested surfactants revealed that no individual surfactant seems to be particularly well suited to a certain type of pretreatment. Regarding cellulose conversion,

Berol 08 and PEG 6000 have a tendency to perform the best, whereas all the used surfactants performed equally well on xylan hydrolysis. PEG 6000 had a tendency to outperform PEG 2000 and PEG 4000 with shorter chain lengths.

4.2. Mechanism of surfactant effect

The positive effect of surfactants on enzymatic hydrolysis has been reported a number of times. Various explanations to the surfactant effect have been proposed including increase of enzyme stability and increasing accessibility of the substrate. However, the most recent research suggests that prevention of unproductive enzyme adsorption to lignin is the major mechanism behind the surfactant effect [7]. The mechanism has been explained by hydrophobic sites on lignin being occupied by surfactant. The hydrophilic portions of the surfactant will in turn protrude into the aqueous solution and cause steric repulsion of enzyme from the lignin surface. It has also been shown that surfactant is able to displace already adsorbed enzyme [7].

The endoglucanase activities measured in the substrate solutions have been used to calculate adsorption of the enzyme mixtures, assuming that non-adsorbed enzyme is still active in the solution. The enzyme mixtures used for the hydrolysis have been found to be highly resistant to degradation and inactivation over a period of several days. Hence, the enzyme activity measured in solution can be correlated to the degree of adsorption.

Although not identical, there is a clear connection between the cellulose conversion of pretreated straw (Fig. 1A) and the endoglucanase activity in solution (Fig. 4). In both cases the acid pretreated substrate is below that of the other substrates. Similarly, with surfactants added, both the cellulose conversion and the free endoglucanase activity of the acid treated straw experiment increase significantly. Although the variation is higher, the increase in enzyme activity due to addition of surfactants is also comparable to the increase in cellulose conversion with surfactants. Likewise, the measured enzyme adsorption on spruce corresponds well with the improvement in cellulose conversion when surfactants were added. Consequently, there seems to be a clear relationship between cellulose conversion of pretreated straw lignocellulose and enzyme adsorption. Furthermore, the higher surfactant effect on spruce substrate compared to straw may be explained by the higher lignin content of spruce (51% compared to 20–24%, Table 3). These relationships strongly support the current theory on the dominating effect of surfactants being due to steric hindrance of enzyme interaction with lignin surfaces.

The correlation between surfactant concentration and increase in cellulose conversion showed that the effect leveled off at concentrations above approximately 0.025 g $(g\,DM)^{-1}$. An explanation as to why the leveling off and optimum concentration were equal for different substrates may be that all possible binding sites on lignin are occupied by surfactant when it reaches a certain concentration, irrespective of the ability of the substrate to unspecifically bind enzymes. Thus, there may be a number of potential sites on the lignocellulose that may either adsorb enzyme or surfactant. When these sites are all associated with surfactant, further addition will not increase hydrolysis.

However, this does not explain why the optimum surfactant concentration is equal for the two substrates tested, irrespective of the varying increase in cellulose conversion. In other words, it seems that the type of material or pretreatment does not have any influence on the amount of adsorption sites, yet the lignin content and adsorption can be correlated to the degree of conversion.

It is possible that the lignin interaction discussed above is not the only mechanism responsible for the positive surfactant effect. It has been suggested that surfactants have a stabilizing effect on some enzymes [10]. The experiments performed indicated that this mechanism was not responsible for the increased enzyme performance. However, the experiments were carried out without addition of substrate. It is possible that surfactants may have a stabilizing effect on an enzyme/substrate complex.

5. Conclusions

We have shown that addition of surface active additives, such as non-ionic surfactants and PEG, increased enzymatic conversion of pretreated straw lignocellulose for bioethanol purposes. The degree of surfactant effect varied depending on type of pretreatment. Although the surfactant effect was not as high as seen with spruce lignocellulose, it is most likely possible to lower the enzyme loading by adding, e.g. PEG 6000, while retaining the same degree of cellulose conversion. However, due to the lack of industrial scale prices of surfactants and enzyme, it has not been possible to perform economic calculations on the feasibility of surfactant addition.

Surfactants were also found to increase xylan conversion moderately.

Enzyme adsorption was measured and could be correlated to cellulose conversion of pretreated straw substrates with and without surfactant added. The results strongly support the prevalent theory that the main mechanism of the surfactants is prevention of unproductive enzyme adsorption with lignin surfaces.

The optimum surfactant concentration was found to be similar, irrespective of pretreatment type. Furthermore, as seen with the acid pretreated wheat straw substrate where surfactant addition improved conversion dramatically, lignin content and surfactant effect is not always directly proportional. Further research is needed to fully understand the factors influencing surfactant and enzyme adsorption.

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