

EFFECT OF NON IONIC SURFACTANT ADDITION TO CELLULASE PERFORMANCE IN HIGH-SUBSTRATE-LOADING-HYDROLYSIS OF PALM OIL EFB AND WATER-HYACINTH

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ABSTRACT

Enzymatic hydrolysis with high substrate loading of palm oil (*Elaeis guineensis*) empty fruit bunch (EFB) and water-hyacinth (*Eichhornia crassipes*) were investigated as a prior part of ethanol production from lignocelluloses. Commercial surfactant Span 85 and Tween 20 were used as cellulase performance enhancer in hydrolysis process with substrate loading above 20% (w/w). Cellulase performances were compared based on hydrolysis conversion. Hydrolysis conversions of EFB using cellulase with concentration 10 and 15 FPU/g-substrate was 38.55% and 88.80% respectively. Addition 2% (v/v) of Tween 20 to EFB hydrolysis reaction with cellulase concentration 10 FPU/g-substrate gave the conversion 87.30%. This addition enhance the cellulase performance up to 226.5% or similar with the performance of cellulase 15 FPU/g substrate. Addition 2% (v/v) of Span 85 to the similar reaction only enhances cellulase performance to 174.7%. Hydrolysis conversion of boiling-pretreated water-hyacinth and autoclave-pretreated water-hyacinth using cellulase 15 FPU/g-substrate was 45.84% and 52.29% respectively. Addition 2% (v/v) of Tween 20 and Span 85 to boiling-pretreated water-hyacinth hydrolysis with cellulase concentration 15 FPU/g-substrate enhance cellulase performance of 128.9% and 153.5% respectively. Addition 1% (v/v) of Tween 20 and Span 85 to the similar reaction with cellulase concentration 10 FPU/g-substrate gave conversions 51.00% and 53.79% respectively, or similar with conversion of autoclave-pretreated water-hyacinth hydrolysis with 15 FPU/g-substrate.

Keywords: cellulase enzymatic hydrolysis; Tween 20; Span 85; bioethanol lignocellulose

ABSTRAK

Proses hidrolisis enzimatis dengan beban substrat tinggi menggunakan Tandan Kosong Kelapa Sawit (TKKS) dan eceng gondok telah dikaji sebagai tahap awal proses produksi etanol dari lignoselulosa. Surfaktan komersial Span 85 dan Tween 20 digunakan sebagai pemacu kinerja selulase dalam proses hidrolisis dengan beban substrat di atas 20% (w/w) ini dan kinerja diukur dari konversinya. Konversi hidrolisis pulp TKKS dengan konsentrasi selulase 10 dan 15 FPU/g-substrat berturut turut adalah 38,55% dan 88,80%. Penambahan 2% (v/v) Tween 20 pada hidrolisis TKKS dengan selulase 10 FPU/g-substrat memberikan konversi 87,30%. Artinya penambahan ini memacu kinerja selulase hingga 226,5% lebih tinggi atau setara dengan kinerja selulase 15 FPU/g-substrat. Penambahan 2% (v/v) Span 85 hanya memacu kinerja selulase hingga 174,7% lebih tinggi. Konversi hidrolisis eceng gondok yang sebelumnya dididihkan pada tekanan ruang (1 atm) dan pada tekanan autoclave (2 atm) menggunakan selulase 15 FPU/g-substrat berturut turut adalah 45,84% dan 52,29%. Penambahan 2% (v/v) Tween 20 dan Span 85 pada hidrolisis eceng gondok yang sebelumnya dididihkan pada tekanan ruang (1 atm) memacu kinerja selulase berturut turut 128,9% dan 153,5%. Penambahan 1% (v/v) Tween 20 dan Span 85 pada reaksi berbahan baku sama dan selulase 10 FPU/g-substrat berturut turut adalah 51,00% dan 53,79% atau setara dengan konversi hidrolisis eceng gondok yang sebelumnya dididihkan pada tekanan autoclave (2 atm) menggunakan selulase 15 FPU/g-substrat.

Kata Kunci: hidrolisis enzimatis; selulosa; Tween 20; Span 85; etanol lignoselulosa

INTRODUCTION

Utilization of lignocelluloses as potential raw materials in bio-energy processes has been studied extensively. Enzymatic hydrolysis of cellulose into

soluble sugars is one of central interest especially in the development of an effective cellulose-to-ethanol process for liquid fuel application. However, a rapid conversion of cellulose substrates by enzymatic hydrolysis is still difficult to obtain. The hydrolysis rate

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rapidly decreases during the time course of hydrolysis which leads to decreased yields and long process times [1-2]. High enzyme concentrations are needed to reach high cellulose conversion, and enzyme recycling is difficult due to adsorption of enzymes to residual lignocelluloses. The present study is focused on enzymatic conversion of palm oil (*Elaeis guineensis*) empty fruit bunch and water-hyacinth (*Eichhornia crassipes*). Both of raw materials were chosen due to their abundance.

The rapid growth rate of the invasive species, *Eichhornia crassipes* (*E. crassipes*), has caused problems in lakes and rivers through-out the world. It is one of the most efficient plant absorbers of nutrients and pollutants from natural and waste waters. It is possible that the harvested biomass of *E. crassipes* might be used to prevent further invasions. Traditional use of *E. crassipes* biomass has been limited to silage, organic compost, or biogas [3].

According to GAPKI, Indonesian palm oil production in 2011 had reached 23.5 million ton [4], which means also produced 25.9 million ton of palm oil empty fruit bunch. The availability of EFB is abundance and wide spread in Indonesia which makes EFB very potential for raw materials in bio-energy processes.

Enhancement of cellulose hydrolysis by adding surfactants to the hydrolysis mixture has been reported by several authors [5-7]. Ooshima et al. [5] compared amorphous cellulose with different types of crystalline celluloses (Avicel, tissue paper and reclaimed paper). They showed that the higher the crystallinity of the substrate, the more positive was the effect of the added surfactant. Different mechanisms have been proposed for the positive effect of surfactant addition to an enzymatic hydrolysis of cellulose. The surfactant could change the nature of the substrate, e.g. by increasing the available cellulose surface or by removing inhibitory lignin [7]. Surfactant effects on enzyme-substrate interaction have been proposed, e.g. adsorbed enzymes are prevented from inactivation by addition of surfactant which facilitates desorption of enzymes from substrate [8]. It was also found that there is a correlation between hydrophilic lipophilic balance (HLB) of the surfactant and increase in hydrolysis [9]. The effect of surfactant on enzymatic hydrolysis of corn stover was the object of intense research for use in an ethanol producing process [10-12]. The mechanism of surfactant effects in lignocellulose hydrolysis was extensively studied in steam Pretreated Spruce (SPS) and the important role of lignin in preventing an efficient conversion of lignocellulose was confirmed [13].

The objective of this work was to study the possibilities for increasing economic feasibility of water hyacinth and EFB utilization as raw material cellulose-to-ethanol process by developing their enzymatic

hydrolysis efficiencies. Surfactant addition in enzymatic hydrolysis was the main approach of this work to lower enzyme loading and to lower the condition of pretreatment process. The results will also useful for providing more data to explain the surfactant effect in cellulose – cellulase interaction.

EXPERIMENTAL SECTION

Materials

The EFB fiber was cut into 3-5 cm length and then it was digested using Kraft process (NaOH and Na₂S 22% as active alkali, with cooking liquor 1:5). Digestion was kept at 165 °C for 1.5 h. The soften EFB fiber was filtered and washed thoroughly until it reach normal pH. The soften EFB fiber was then fibrillated in a disc refiner 2 times. The resulted EFB has 9.3% lignin.

Water-hyacinth pulp was prepared by crushing it into fibers and then digested using 2M NaOH with cooking liquor 1:4. Digestion was kept in two different conditions, at 105 °C and 121 °C for 2 h. Pulping process in 105 °C then called boiled pulp and pulping process in 121 °C called autoclave pulp. The digestion results was filtered and washed thoroughly until it reaches normal pH.

Polyoxyethylene-(20)-sorbitan monolaurate or also known as Tween 20 and ester sorbitan (mixture of 1,4-anhydrosorbitol, 1,5-anhydrosorbitol and 1,4,3,6-dianhydrosorbitol) or also known as Span 85 and Span 20 were obtained from Merck. Surfactant chemical structures were shown in Table.1. Cellulase and β -glucosidase from Novo Nordisk A/S, Bagesvaerd, Denmark was used in all enzymatic hydrolysis in this research. Cellulase activity was 70 FPU/mL and β -glucosidase activity of 404 IU/g.

Instrumentation

Kraft Process for EFB pretreatment was conducted in a wood digester which is custom made by Changhae Energering Co. Ltd. with total volume 2 L and working volume 0.5 L. Autoclave for water hyacinth pretreatment were fabricated by Cheng Yi Co. Ltd. all glass ware were produced by Pyrex Co. Ltd.

Procedure

Hydrolysis experiments

The reactions were initiated by preparing enzyme solution in 100 mL Erlenmeyer by mixing cellulase and β -glucosidase with volume ratio 5:1, added the observed surfactant, and diluted with sodium acetate buffer 50 mM at pH 4.8 mixing the enzyme solution until

Table 1. Surfactant chemical structure

Surfactant	Chemical structure
Span 20	
Tween 20	
Span 85	

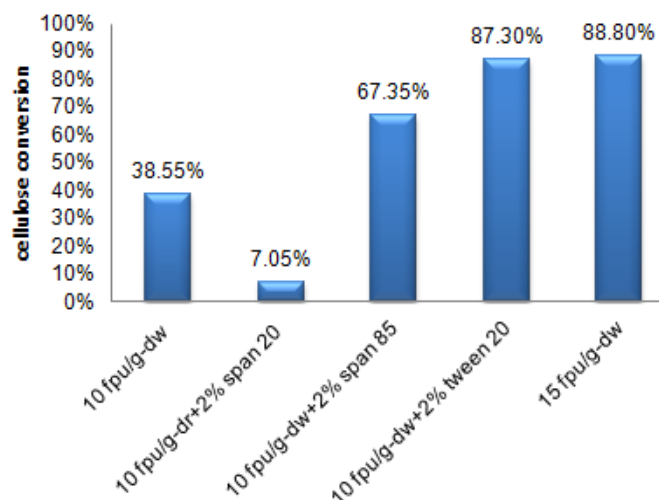
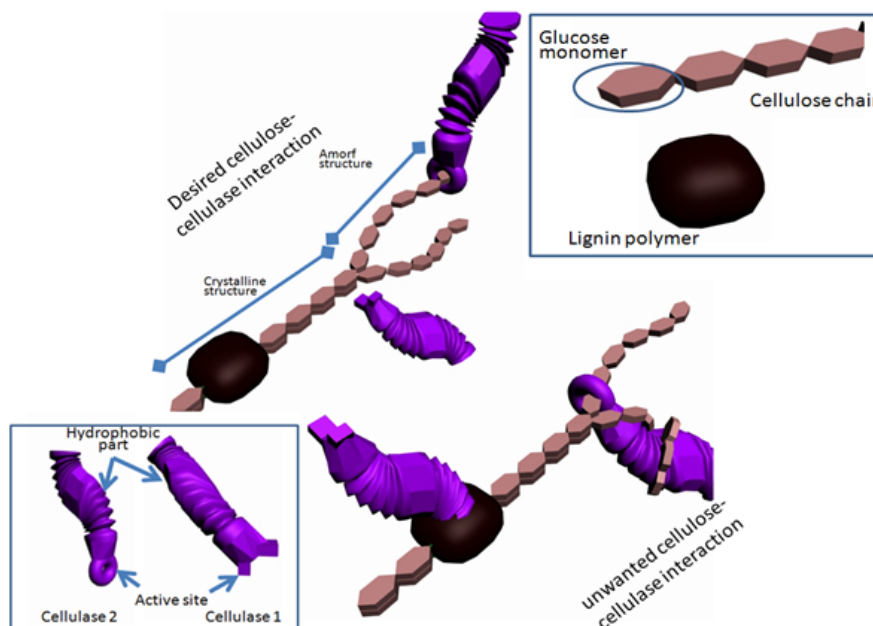
**Fig 1.** EFB pulp hydrolysis conversion

Fig 2. Desired and unwanted cellulose–cellulase interaction. (cellulase deactivation caused by irreversible interaction between cellulose chain and cellulase). Irreversible cellulose-cellulase interaction caused by cellulase entangled or trapped in cellulose crystalline structure (Kaar & Holtzapfle) or caused by irreversible interaction between hydrophobic part of cellulase with lignin (Eriksson-Börjesson-Tjerneld)

the volume reach 50 mL. Solid substrate hydrolysis experiments then begun by adding 20% (w/w) of the observed substrate. The experiments were performed in room temperature (26–28 °C) for 48 h. The hydrolysis was terminated by filtering the mixture through filter paper. Conversion was calculated from the resulted sugar which was measured based on reducing end using Luff Schoorl method according to SNI 01-2892-1992.

The cellulase was varied 10 and 15 FPU/g substrate in dry weight. Surfactant addition was varied 1

and 2% (v/v) for water-hyacinth pulp hydrolysis experiment but kept constant at 2% in EFB pulp hydrolysis. Negative control for EFB pulp hydrolysis experiments were conducted by using cellulase with concentration 10 and 15 FPU/g substrate in dry weight without any surfactant addition. Negative control for water hyacinth hydrolysis experiments were conducted by using boiling-pretreated water-hyacinth and autoclave-pretreated water-hyacinth using cellulase 15 FPU/g substrate in dry weight.

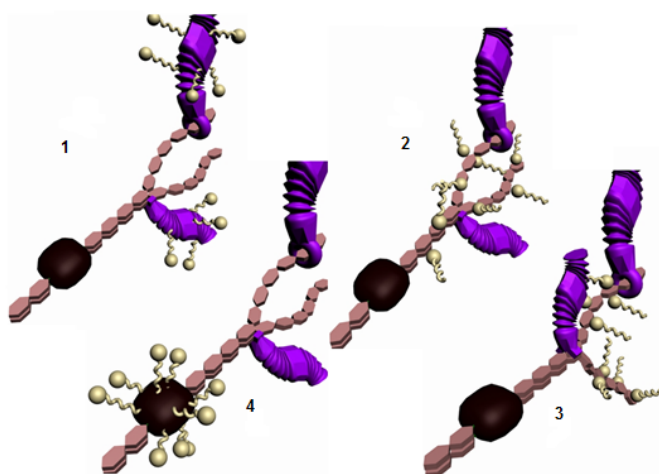


Fig 3. Prior theories for explaining surfactant effect as cellulase performance enhancer. (1) Kaar & Holtzapfle: surfactant “protect” cellulase and increase its stability. (2) Kaar & Holtzapfle: positively affect enzyme–substrate interaction leading to more effective conversion of cellulose (3) Kaar & Holtzapfle: surfactants could cause substrate structural changes and make it more accessible for enzymatic hydrolysis (4) Eriksson-Börjesson-Tjerneld: hydrophobic part of the surfactant binds through hydrophobic interactions to lignin on the lignocellulose fibers and the hydrophilic head group of the surfactant prevents unproductive binding of cellulases to lignin

RESULT AND DISCUSSION

EFB Hydrolysis and Surfactant Effect

Addition of three tested nonionic surfactant was kept constant at 2% (v/v) in all EFB pulp hydrolysis. This can be considered as surfactant screening. As shown in Fig. 1, Tween 20 was the best cellulase performance enhancer which increases conversion 226.5% compares to the hydrolysis with same amount of enzyme with no surfactant addition. Span 85 also works as cellulase performance enhancer which increases conversion 174.7% but Span 20 cannot be considered as cellulase performance enhancer.

Non-ionic surfactants have been shown to be the most effective surfactants in improving cellulose conversion. Park et al. showed a correlation between hydrophilic lipophilic balance (HLB) of the surfactant and increase in hydrolysis [9]. Our results on EFB agree well with these results. Tween 20 and Span 85 as nonionic surfactants increased the hydrolysis and Tween 20 which has HLB value 16.7 gave higher conversion enhancement compares to Span 85 that only has 1.8 HLB value. Similar results were also obtained by Erickson et al. [13] in enzymatic hydrolysis of steam pretreated spruce (SPS). Adsorption of cellulase into

substrate was also measured in that experiment and it was showed that the concentration of free unbound enzyme was approximately doubled upon addition of Tween 20.

Kaar et. al. suggested that cellulase deactivation could be caused by damage of cellulose protein structure or irreversible interaction between cellulose chain and cellulase as illustrated in Fig. 2. Cellulase categorized into two components based on how it works. Cellulase 1 cuts cellulose polymer chain in the middle and Cellulase 2 attach to cellulose chain and cuts the chain from the reducing ends into cellobiose. Both cellulase could trapped in the cellulose chain and deactivated.

Three different explanations of surfactant effect on cellulose hydrolysis proposed by Kaar et al. in earlier studies were based on the surfactant function to prevent deactivation [10-12]. Those explanations were illustrated in Fig. 3. First explanation is that surfactants may increase enzyme stability and prevent denaturation of enzymes during hydrolysis as illustrated in Fig. 3.1. Two factors that mostly concerned in enzyme denaturation were thermal deactivation and shear deactivation. All hydrolysis in this study were conducted in room temperature then thermal deactivation can be neglected. Shear denaturation is deactivation by shear forces due to agitation of the hydrolysis mixture. All hydrolysis in this study were conducted in high substrate loading which caused reaction system in viscous condition and prevent vigorous agitation. Then this explanation cannot be used for significant enhancement of cellulase performance by addition of Tween 20 and Span 85.

The second explanation was surfactants could positively affect enzyme–substrate interaction leading to more effective conversion of cellulose and the third explanation was that surfactants could cause substrate structural changes and make it more accessible for enzymatic hydrolysis as illustrated in Fig. 3.2 and 3.3 However, Erickson et al. disagree with both of this explanation based on their finding that there is no significant enhancement in hydrolysis conversion of pure cellulose by surfactant addition [13].

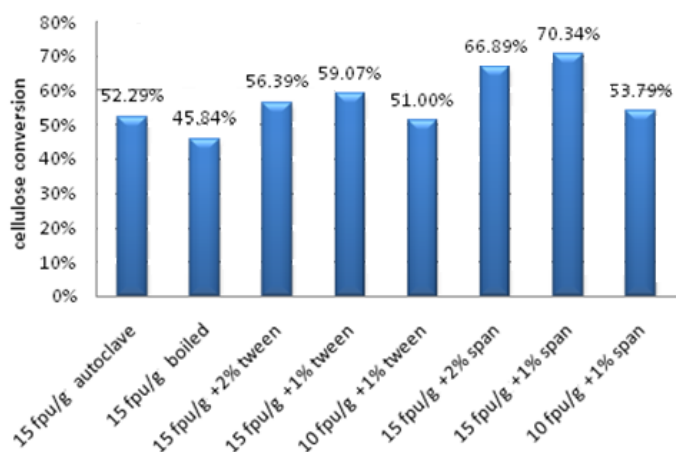
Erickson proposed explanation for surfactant effect on lignocellulose hydrolysis is that the hydrophobic part of the surfactant binds through hydrophobic interactions to lignin on the lignocellulose fibers and the hydrophilic head group of the surfactant prevents unproductive binding of cellulases to lignin.

Water-hyacinth Hydrolysis

Hydrolysis conversion of boiling-pretreated water-hyacinth and autoclave-pretreated water-hyacinth using

Table 2. Compositions of lignocelluloses basic component

	Water hyacinth (%)	Palm oil EFB (%)
Cellulose	19.7± 0.13	18.20 ± 0.012
Hemicelluloses	27.1± 0.43	48.70 ± 0.027
Lignin	Not determined	3.50 ± 0.004
Source	[14]	[15]

**Fig 4.** Water hyacinth pulp hydrolysis conversion

cellulase 15 FPU/g-substrate was 45.84% and 52.29% respectively, as shown in Fig. 4. Pretreatment in 121 °C increased the conversion 114.1% compares to the one that pretreated in 105 °C. All surfactant addition was applied to water hyacinth boiled in 105 °C. Addition of Tween 20 increased the conversion 123.02–128.9% which is higher than substrate boiled in 121 °C. Surfactant addition increase enzymatic hydrolysis conversion better than elevating pretreatment temperature. These results will be valuable in searching efficient bioethanol production process from lignocelluloses. Hydrolysis conversion using 10 FPU/g-substrate with addition of 1% Tween 20 actually similar with the conversion of substrate boiled in 125 °C using 15 FPU/g substrate.

High temperature and pressure could disrupt the cellulose fiber and reduce cellulose crystallinity in water hyacinth pulp. the structure of the resulted pulp become more accessible for enzymatic hydrolysis. This explanation can be applied for the results of higher hydrolysis conversion by elevating pretreatment temperature. However, the cellulose structural change caused by thermal is not similar with the changes caused by surfactant since surfactant did not reduce cellulose crystallinity.

The difference of conversion enhancement between water hyacinth and palm oil EFB were suggested to be closely related with the difference compositions of lignocelluloses basic component between both substrates. As shown in Table.2 water

hyacinth have higher amount of hemicelluloses which is naturally in non-crystalline form. This study suggested that surfactant also enhance cellulase performance in hydrolysis of non-crystalline cellulose substrate. And therefore, the mechanism of surfactant as cellulase performance enhancer will be different with previously proposed. Role of lignin as reported in Erickson et al. [13] cannot be applied in water hyacinth since its lignin content is very low and can be neglected after pretreatment.

Substrate structural changes caused by surfactant were questioned in explaining surfactant role as cellulase performance enhancer. High enzyme adsorption due to trapped enzyme within cellulose crystalline structure and caused irreversible enzyme-substrate interaction will be a logical consequence of that explanation. Erickson et al. whom questioned the proposed explanation based on their finding in pure cellulose enzymatic hydrolysis. However, this explanation suggested being more appropriate in water hyacinth hydrolysis since hemicelluloses is in amorphous form.

CONCLUSION

Addition of non-ionic surfactant increased hydrolysis conversion of EFB pulp and water hyacinth pulp. An important effect of surfactant addition in hydrolysis process is the possibility to lower the enzyme loading and lower pretreatment process condition. With addition of Tween 20 at 2% of reaction mixture in EFB hydrolysis was possible to lower the enzyme loading by 33% and at the same time retains cellulose conversion. In case of water hyacinth, addition of 1% of Tween 20 or Span 85 was possible not only to lower enzyme loading by 33% but also to lower pretreatment process condition from 125 °C, 2 atm to 105 °C, 1 atm.

Surfactant effects on enzymatic cellulose hydrolysis have been studied earlier; however, no detailed mechanism has been presented. EFB hydrolysis results agrees with the previously proposed mechanism that surfactant adsorption to lignin in substrate prevents unproductive binding of enzymes to lignin. Our result in water hyacinth hydrolysis indicates that surfactant also enhance cellulase performance in hydrolysis of non-crystalline cellulose substrate.

Substrate structural changes caused by surfactant were being more appropriate mechanism in this case. However, more extensive study were required for explaining how the structural changes and why the new structure made the substrate easier to be hydrolyzed by enzyme.

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