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Non-ionic surfactants do not consistently improve the enzymatic hydrolysis of pure cellulose

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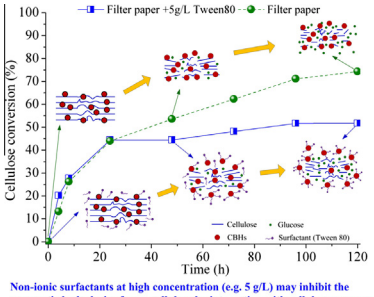
HIGHLIGHTS

- Improvement of pure cellulose hydrolysis by non-ionic surfactants is limited.
- The beneficial action of non-ionic surfactants depends on various conditions.
- Higher surfactant concentration inhibits enzymatic hydrolysis of pure cellulose.
- Surfactant–enzyme interaction might inhibit the productive adsorption of cellulases.

GRAPHICAL ABSTRACT



Non-ionic surfactants at high concentration (e.g. 5 g/L) may inhibit the enzymatic hydrolysis of pure cellulose by interacting with cellulase enzymes

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ABSTRACT

Non-ionic surfactants have been frequently reported to improve the enzymatic hydrolysis of pretreated lignocellulosic biomass and pure cellulose. However, how the hydrolysis condition, substrate structure and cellulase formulation affect the beneficial action of surfactants has not been well elucidated. In this work, it was found that the enzymatic hydrolysis of pure cellulose was not consistently improved by surfactants. Contrarily, high surfactant concentration, e.g. 5 g/L, which greatly improved the hydrolysis of dilute acid pretreated substrates, actually showed notable inhibition to pure cellulose conversion in the late phase of hydrolysis. Under an optimal hydrolysis condition, the improvement by surfactant was limited, but under harsh conditions surfactant indeed could enhance cellulose conversion. It was proposed that non-ionic surfactants could interact with substrates and cellulases to impact the adsorption behaviors of cellulases. Therefore, the beneficial action of surfactants on pure cellulose hydrolysis is influenced by hydrolysis condition, cellulose structural features and cellulase formulation.

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1. Introduction

Demand for ethanol as a clean and renewable second-generation biofuel has increased in the past few years. More than 40 million tons of lignocellulosic materials, including agricultural and forestry residues, waste paper, and energy crops, are produced every year, but much of them is just thrown away (Sanderson, 2011). Biological conversion of this discarded lignocellulose to ethanol via enzymatic hydrolysis attracts widespread interest due to the significant environmental and economic advantages (Zhu and Zhuang, 2012). However, the main limitation for this process is the high production cost of releasing sugar from biomass. Previous researchers have found that the addition of surfactants, especially non-ionic surfactants, could significantly enhance the cellulose hydrolysis thus reducing the enzyme loading (Tu and Saddler, 2010). However, inhibitory effects were observed with some amphoteric, anionic and cationic surfactants addition (Eriksson et al., 2002; Hemmatinejad et al., 2002). The usually used non-ionic surfactants are Tween and PEG series with

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concentrations in the range of trace to 20 g/L (Cui et al., 2011; Eriksson et al., 2002; Kim et al., 2007; Borjesson et al., 2007; Sipos et al., 2011; Wu and Ju, 1998). The mechanism of the positive effect of non-ionic surfactant on the enzymatic hydrolysis of pretreated lignocellulosic biomass is generally believed to be the prevention of the non-productive adsorption of cellulases onto the lignin fraction, which increases the amount of free enzyme that would be beneficial for the hydrolysis of cellulose substrate (Alkasrawi et al., 2003; Jorgensen et al., 2007; Sipos et al., 2011).

However, lignocelluloses are different from pure cellulose in terms of surface hydrophobic properties, the primary driving force to bind cellulase, due to the presence of lignin (Nakagame et al., 2011). The effects of surfactants on the hydrolysis of pure cellulose thus may be different. Some studies have been reported on the effects of non-ionic surfactants on the hydrolysis of pure cellulose, and the results consistently indicate that non-ionic surfactant can improve pure cellulose hydrolysis by different extents (Eriksson et al., 2002; Borjesson et al., 2007; Mizutani et al., 2002; Ouyang et al., 2010; Yang et al., 2011; Kim et al., 2007; Helle et al., 1993) (see Supplemental data Table S1). For example, Kim et al. (2007) found that the enzymatic digestibility of α-cellulose and filter paper increased from 59.4% and 69.2% to 81.3% and 91.2%, respectively, by addition of 0.5% Tween 80 at the enzymatic hydrolysis stage. The conversion of high purity cellulose powders (Sigmacell 100) could be increased from 7% to 43% when 4 g/L Tween 80 was added (Helle et al., 1993). However, the effects of surfactants on the enzymatic hydrolysis of pure cellulose are dependent on not only surfactant type, but also hydrolysis conditions. For example, shaking speed was found to affect the action of surfactant on pure cellulose hydrolysis (Yang et al., 2011). In the experiments, it was found that Tween 20 and 80 at concentration of 5 g/L actually showed inhibitive action in late phase of enzymatic hydrolysis of pure cellulose (filter paper (FP) and micro-crystal cellulose (MCC)). It indicates that non-ionic surfactants do not exclusively increase cellulose hydrolysis but depending on several factors such as substrate structure, surfactant type and concentration and hydrolysis conditions. Similar conclusions were obtained by Wang et al. (2013) and Lou et al. (2014) when lignosulfonate (a type of anionic surfactant) was added to the enzymatic hydrolysis of pure cellulose. The enhancing effect of lignosulfonate varied with its properties, loading and hydrolysis pH. Inhibitive effect on cellulose saccharification was also observed using lignosulfonate with large molecular weight and low degree of sulfonation (Lou et al., 2014). In the present work, several non-ionic surfactants were tested under different operational conditions (such as shaking speed, pH, substrate concentration, cellulose crystallinity etc.) in order to figure out the effects of operational parameters on the improving action of surfactant for enzymatic hydrolysis of pure cellulose.

2. Methods

2.1. Substrates and enzymes

Filter paper (Hangzhou Special Paper Industry Co., Ltd., China) and microcrystalline cellulose (Sinopharm Chemical Reagent Co., Ltd., China) were used as pure cellulose substrates. Three commercial cellulase formulations were used in the hydrolysis experiments, which were kindly provided by Novozymes A/S (Denmark, Cellulase I), Erbslöh Geisenheim AG (Germany, Cellulase II) and Shandong University (China, Cellulase III).

2.2. Surfactants

Five non-ionic surfactants, Tween 20, Tween 80, Triton X-100, Triton X-114 and PEG 4000 were used. Tween 20 was purchased from Sinopharm Chemical Reagent Co., Ltd. China. Tween 80, Triton X-100 and PEG 4000 were purchased from Xilong Chemical Co., Ltd., China, and Triton X-114 from Beijing Biodee Biotechnology Co., Ltd., China.

2.3. Hydrolysis experiments

Filter paper was cut into about 0.5 × 0.5 cm size. Enzymatic hydrolysis was performed in 50 mL flasks at 50 °C, pH 4.8 (in 0.05 M sodium acetate buffer) with total working volume of 10 mL. The experiment was carried out with substrate concentrations of 5% (w/v) and enzyme loading of 5 FPU/g substrate. Flasks without surfactant addition were used as the control. To study the concentration of surfactant, experiments with various concentrations (ranging from critical micelle concentration (CMC) to 5 g/L) were conducted. All the flasks were placed in an air-bath shaker at a constant shaking speed of 150 rpm. At regular intervals, 100 μL aliquots were withdrawn from each flask and centrifuged at 14,000 rpm for 5 min. The supernatant was diluted and then analyzed for monomer sugars. Experiments were performed in duplicate and results were represented as the mean values. Similarly, to investigate the effects of operational condition, substrate concentrations (5–20%), pH (4.0–6.0), shaking speed (100–200 rpm), and cellulose crystallinity (8–80%) were considered as variables.

2.4. Enzyme adsorption

Enzyme adsorption on cellulose matrix was performed at substrate concentration of 5% (w/v) at pH 4.8, 25 °C and 150 rpm. After adsorption for 1 h, the protein concentration of supernatant was measured.

2.5. Tween 80 adsorption

Tween 80 adsorption on cellulose matrix was performed at substrate concentration of 5% (w/v) at pH 4.8, 25 °C and 150 rpm. After adsorption for 30 min and 1 h, Tween 80 concentration of supernatant was measured at 235 nm using Microplate spectrophotometer (Multiskan GO, Thermo Scientific, MA, USA).

2.6. Analytical methods

Monomer sugars were measured by HPLC (high-performance liquid chromatography, Shimadzu, Kyoto, Japan) using an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) with 5 mM H_2SO_4 as mobile phase at a flow rate of 0.8 mL/min at 65 °C. The cellulose conversion was calculated as follows:

$$\text{Cellulosic conversion } (\%) = \frac{\text{Glucose produced (g)} \times 0.9}{\text{Initial cellulose substrate (g)}} \times 100 \quad (1)$$

Filter paper activity (FPA) was measured according to Adney and Baker (2008). Measurement method of Exo-β-1,4-glucanase activity with Avicel as substrate was similar to that of FPA (Dashtban et al., 2010). β-Glucosidase activity and endo-β-1,4-glucanase (CMCase) activity were measured according to Ghose (Ghose, 1987) using cellobiose and carboxymethylcellulose (CMCell) as substrates, respectively. Protein content of cellulase was measured by Coomassie Brilliant Blue dye method with BSA as a standard according to Bradford (Bradford, 1976).

The crystallinity of cellulose was measured by XRD (X-ray diffraction). The diffracted intensity was measured in a 2θ range between 5° and 40°, at a speed of 5 °/min. Crystallinity index (*CrI*) of samples were calculated by the following equation:

$$CrI = (I_{002} - I_{am})/I_{002} \times 100\% \quad (2)$$

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