Biochemical Engineering Journal 118 (2017) 91–96

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Regular article

Interaction of silica with cellulase and minimization of its inhibitory effect on cellulose hydrolysis

Md. Mahabubur Rahman Talukder*, Hai Yuan Goh, Sze Min Puah

Institute of Chemical and Engineering Sciences, Singapore

ARTICLE INFO

Article history:
Received 30 June 2016
Received in revised form
10 November 2016
Accepted 19 November 2016
Available online 21 November 2016

Keywords:
Cellulase-silica interactions
Adsorption isotherm
Cellulose hydrolysis
Inhibition

ABSTRACT

Enzymatic hydrolysis of lignocellulosic biomass to sugar has attracted a lot of attention in recent years. Although lignocellulosic biomasses such as rice husk contain a substantial amount of amorphous silica, the role of silica in enzymatic hydrolysis of cellulose is poorly understood. In this study, the interaction of silica with a cellulase enzyme (CTec2) was studied by addressing the role of silica in cellulase adsorption and cellulose (avicel) hydrolysis. CTec2 adsorption on silica surface was found to be influenced by temperature, pH and contact time. The adsorption equilibrium data fitted the Langmuir isotherm model better than the Freundlich isotherm model. The maximum adsorption capacity (Q_m) of silica was found to be as high as 244 mg/g at a temperature of 50 °C and pH of 4.0, indicating strong interaction between silica and CTec2. This strong interaction inhibited avicel hydrolysis; consequently the maximum sugar yield was only 32%. The inhibitory effect of silica was almost completely minimized by introducing PEG1500, and the sugar yield reached 78%, similar to that (81%) obtained in the silica-free system. The results of this paper could, therefore, be useful in designing cellulase-catalyzed hydrolysis of silica containing cellulosic biomasses.

1. Introduction

Cellulose and hemicellulose, two major constituents of lignocellulosic biomass can be enzymatically hydrolysed to sugars, which can be subsequently converted to chemicals and fuels [1,2]. The third component, lignin, inhibits cellulose hydrolysis by physically blocking access of cellulase enzymes to cellulose and nonproductive adsorption of enzymes [3,4]. Different pretreatment methods have been applied to remove lignin and improve the accessibility of cellulase enzymes to cellulose. Complete removal of lignin, however, is cost prohibitive as it requires harsh pretreatment conditions, which increase cellulase crystallinity, reduces sugar yield and leads to formation of byproducts. In addition to lignin, a substantial quantity of silica is present in many lignocellulosic biomasses such as rice husk (or hull), the outmost layer of paddy. Silica content in rich husk is around 20 wt.% [5], similar to lignin content (~20 wt.%) in rich husk [6] and other lignocellulosic biomasses [7]. Veen et al. [8] observed a very strong interaction of silica with enzymes (lysozyme and α-lactalbumin). The adsorption of lysozyme and α-lactalbumin on silica has been found to reach the equilibrium within 10 and 400 s, respectively. This report indicates that silica can also adsorb cellulase enzymes by electrostatic interaction and/or hydrogen bonding. Like lignin, it can also block access of cellulase to cellulose due to steric hindrance. Hence, the presence of silica in the biomass can reduce free cellulase enzymes via adsorption and necessitate higher enzyme dosage, consequently increasing process cost. Although many evolving hypotheses and approaches are being explored to gain a better understanding of the nature of non-productive adsorption of cellulases on lignin, and lignocellulosic biomass conversion yields of 85–90% was attained [3,4,9–11], less attention has been focused on the role of silica in cellulase adsorption and cellulose hydrolysis. Takimoto et al. [12] immobilized cellulase via encapsulation with silica and found that the immobilized cellulase showed only 20% of the activity of free cellulase. Ivetic et al. [13] have immobilized β-glucosidase onto mesoporous silica and investigated the activity and recyclability of the immobilized enzyme using non-cellulosic substrate, 4-nitrophenyl-β-D-glucopyranoside. These previous studies, however, did not disclose the information that is useful to gain an insight of cellulase adsorption on silica, minimize such adsorption and improve cellulose hydrolysis. It is, therefore, crucial to understand the interaction of silica with cellulase enzymes to design an effi-

* Corresponding author. Present address: Department of Bioprocess Engineering Centre, Institute of Chemical and Engineering Sciences, 1 Pesek Road, Jurong Island 627833, Singapore.
E-mail addresses: talukder@ices.a-star.edu.sg, talukderbuet89@gmail.com (Md.M.R. Talukder).

http://dx.doi.org/10.1016/j.bej.2016.11.016
1369-703X/

cient and cost effective process for hydrolysis of silica containing cellulosic biomasses such as rice husk.

In this paper, the interaction of silica with a cellulase enzyme (CTec2) was studied by addressing the role of amorphous silica on the adsorption of CTec2 cellulase enzyme and the hydrolysis of water insoluble crystalline cellulose (avicel). Amorphous silica was chosen because silica in cellulosic biomass exists as amorphous form [5]. The adsorption equilibrium data were used to fit the Langmuir and the Freundlich adsorption isotherm models. The Langmuir isotherm model, which fitted the adsorption data better, was applied to understand the nature of the adsorption, the affinity/capability of silica to adsorb CTec2 and the binding strength between them. The present work first revealed that CTec2 cellulase enzymes have high affinities toward silica; and the adsorption of the enzymes on silica is nonproductive and endothermic in nature. A simple method that effectively reduces such nonproductive adsorption and simultaneously enhances CTec2 cellulase enzymes activities to cellulose hydrolysis was also developed.

2. Materials and methods

2.1. Materials

Amorphous silica fume (purity 99.8%) was purchased from Sigma-Aldrich (Singapore) and had a surface area of 175–225 m^2/g. Commercial cellulase enzyme cocktail (Cellic CTec2) was generously provided by Novozym (Denmark). CTec2 contained about 94 mg/ml of protein and its cellulase activity was found to be 137 filter paper unit (FPU) per ml according to National renewable energy lab (NREL) method [14], similar to those (120–150 FPU/ml) reported in the literature [3,15,16]. Cellulose (Avicel PH-101) was purchased from Fluka. Polyethylene glycol of nominal molecular weight of 1500 (PEG1500), Bradford reagent, bovine serum albumin, sodium acetate and acetic acid were used as received from Sigma-Aldrich (Singapore).

2.2. Methods

2.2.1. Cellulase enzyme (CTec2) adsorption on silica

The adsorption of cellulase enzymes (CTec2) on amorphous silica was performed inside a 2.0 ml eppendorf tube containing enzymes (0.20–0.94 mg/ml) and amorphous silica (0.5 mg/ml), 50 mM acetate buffer (pH 3.6–5.5) at different temperatures (30–50 °C) and contact times (1–5 min). A typical experimental procedure is described below. CTec2 was diluted to a desired enzyme concentration by mixing with 50 mM acetate buffer of a desired pH. The diluted CTec2 and amorphous silica suspension (1 mg/ml) were preheated at a set temperature for 15 min. The preheated diluted CTec2 (0.5 ml) was then mixed with the preheated silica suspension (0.5 ml) inside an eppendorf tube at 200 rpm using shaker incubator. After the specified mixing (contact) time, the samples were taken out of the incubator and immediately centrifuged at 10,000 rpm using a mini-spin eppendorf centrifuge (F-45-12-11). The protein content in the supernatant was determined according to the Bradford method as described by the supplier. The supernatant (0.1 ml) was mixed with Bradford reagent (3 ml) by gently turning the tube upside down and back again. The sample was then allowed to stand for 10 min at room temperature and read at 540 nm using ultraviolet spectrophotometer. Various concentrations of bovine serum albumin were used as standard to prepare calibration curve. The amount of enzyme adsorbed on silica was calculated by subtracting the enzyme content in the supernatant of the sample from that of the control. The samples without of silica were used as controls.

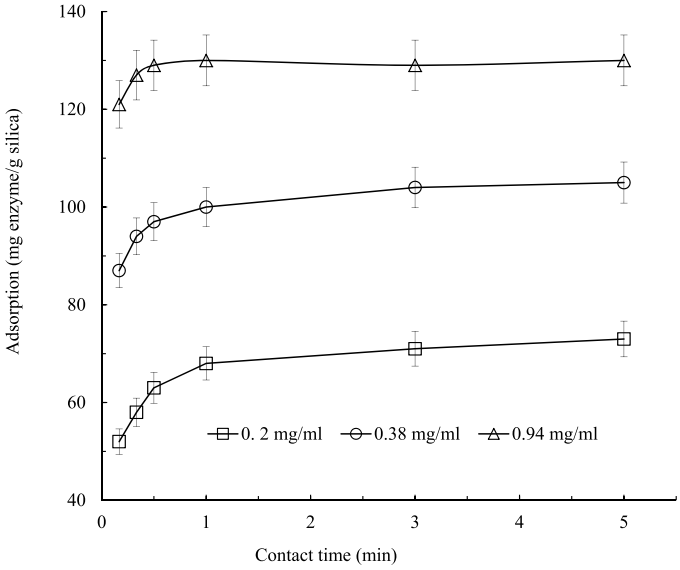

Fig. 1. Effect of contact time on adsorption of CTec2 cellulase enzymes on silica. Adsorption conditions: CTec2 0.20–0.94 mg/ml, amorphous silica 0.5 mg/ml, pH 5.0, shaking speed 200 rpm and temperature 40 °C.

2.2.2. Cellulose hydrolysis

Cellulose (avicel) hydrolysis was carried out inside a 2.0 ml eppendorf tube at a fixed avicel concentration of 50 mg/ml, CTec2 enzyme concentration of 11 FPU/g avicel, temperature of 50 °C and 50 mM acetate buffer pH of 5.0 in the presence and absence of silica (10 mg/ml). PEG1500 concentration was varied in the range of 1–8 mg/ml. A typical experimental procedure for avicel hydrolysis is described below. 50 mg of avicel, 10 mg of silica and 0.5 ml of 50 mM acetate buffer (pH 5.0) with or without PEG1500 were placed into an eppendorf tube and mixed for 15 min at 50 °C and 250 rpm before starting the reaction. The reaction was started by adding 0.5 ml of 125-time diluted CTec 2 cellulase enzyme of 0.76 mg/ml (1.1 FPU/ml) into the tubes. The tubes were periodically taken out and centrifuged for 5 min at 10000 rpm using a mini-spin eppendorf centrifuge (F-45-12-11). The supernatant was analyzed for reducing sugars according to the dinitrosalicyclic (DNS) colorimetric method [14]. The color intensity was measured at 540 nm by ultraviolet spectrophotometer and the concentration of reducing sugar was calculated using glucose standard. The reducing sugar yield was calculated as follows

$$Sugar\ yield(\%) = \frac{Sugar\ concentration\ \left(\frac{mg}{ml}\right)}{Avicel\ concentration\ \left(\frac{mg}{ml}\right) \times 1.11} \times 100$$

2.3. Statistics and reproducibility

All data are average of 3–6 batches of experiments under identical conditions and reproducible within ±8%. Error bars of chart represent 4–8% of the mean value of the data.

3. Results and discussion

3.1. Adsorption of CTec2 cellulase enzymes on amorphous silica

The adsorption of CTec2 cellulase enzymes on silica was investigated for various enzymes concentrations, pH, temperatures and contact (mixing) times. Fig. 1 shows the effect of the contact time on the adsorption at 40 °C and pH 5.0. The amount of enzyme adsorbed per gram silica increased with increasing contact time and reached equilibrium within 5 min for all enzyme concentra-