Bioresource Technology 102 (2011) 2890–2896

Contents lists available at ScienceDirect

Bioresource Technology

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

Fractal kinetic analysis of polymers/nonionic surfactants to eliminate lignin inhibition in enzymatic saccharification of cellulose

Zhilong Wang [a,*], Jian-He Xu [b], Hao Feng [c], Hanshi Qi [a]

[a] School of Pharmacy, Shanghai Jiao Tong University, Shanghai 200240, PR China
[b] State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, PR China
[c] Department of Food Science and Human Nutrition, University of Illinois at Urbana and Champaign, Urbana, IL-61801, USA

ARTICLE INFO

Article history:
Received 27 August 2010
Received in revised form 25 October 2010
Accepted 26 October 2010
Available online 3 November 2010

Keywords:
Cellulase
Cellulose
Lignin
Polymer/nonionic surfactant
Fractal kinetic analysis

ABSTRACT

The profile of enzymatic saccharification of Avicel in the presence and absence of lignin has been described with a fractal kinetic model (Wang and Feng, 2010), in which the retarded hydrolysis rate of enzymatic saccharification of cellulose has been represented with a fractal exponent. The lignin inhibition in the enzymatic saccharification of cellulose is indexed by the increase of fractal exponent, which can not be fully counterbalanced by high cellulase loading due to the high fractal exponent at high cellulase loading. On the contrary, fractal kinetic analysis indicates that an addition of some nonionic surfactant/polymers decrease the fractal exponent to the original values of enzymatic saccharification of Avicel without lignin and the corresponding toxicity of nonionic surfactants/polymers on the consecutive ethanol fermentation strain *Saccharomyces cerevisiae* is also examined.

1. Introduction

Lignocellulosic biomass has been recognized as a high potential feedstock for bio-ethanol production. The lignocellulose-based bio-ethanol industry is usually designed via four major consecutive steps: pretreatment, saccharification, fermentation, and separation. The enzymatic saccharification of cellulose is recognized as one of the most promising strategies as its low energy consumption, low waste disposal, and no equipment corrosion etc. However, the retarded hydrolysis rate, high cellulase loading, and little knowledge about the kinetics on enzymatic saccharification of lignocellulosic substrate remain as its main challenges (Zheng, 2010). It is necessary to understand the mechanism of heterogeneous enzymatic saccharification of cellulose and then develop techniques to make the lignocellulose-based bio-ethanol economically feasible.

Cellulose is degraded synergistically into glucose by at least three types of cellulase: endoglucanases, that randomly cleave β-1, 4-glycosidic bonds on the cellulose chains away from chain ends; cellobiohydrolases, that produce cellobiose by attacking cellulose from chain ends, in which Ce17A (cellobiohydrolase I) acts from the reducing ends while Ce16A (cellobiohydrolase II) acts from the non-reducing ends; β-glucosidases, that converts cellobiose to glucose. On the other hands, the different structural futures of substrate (surface character, reactivity, accessibility, and the presence of lignin etc.), such as the pretreated biomass with different pretreatment method under different conditions, make the enzymatic saccharification process even more complex (Gupta and Lee, 2009). Besides the complex cellulase and pretreated biomass, the most important future is that the cellulase must be adsorbed on the substrate surfaces and then diffused on the substrate surfaces to a reactive site before the heterogeneously enzymatic saccharification reaction is occurred (Zhang and Lynd, 2004). All of those lead to the kinetic model about enzymatic saccharification of cellulose is far from mature (Bansal et al., 2009). For an example, a classical Michaelis–Menten kinetic has been commonly utilized to analyze enzymatic saccharification of cellulose, in which Langmuir isothermal adsorption of cellulase onto the solid cellulose has to imbed due to the heterogeneous reaction. However, a partially irreversible binding cellulase to cellulose (Ma et al., 2008) does not comply with the assumptions of Langmuir isothermal model (Zhang and Lynd, 2004).

Fractal kinetic analysis provides a new point of view about heterogeneous chemical reaction, which believes that a reaction medium does not have to be a geometrical fractal in order to exhibit fractal kinetics (Kopelman, 1988). Fractal kinetic has been applied for complex reactions of intracellular environments with macromolecular crowding (Schnell and Turner, 2004). A simple fractal kinetic model has also been introduced to fit the data of enzymatic saccharification of cellulose (Valjamae et al., 2003). Xu et al. incorporates the fractal exponent of fractal kinetics into

* Corresponding author. Tel.: +86 21 34205437; fax: +86 21 34204746.
E-mail address: zlwang@sjtu.edu.cn (Z. Wang).

0960-8524/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2010.10.132

Michaelis–Menten kinetic equation to analyze the enzymatic saccharification of cellulose and the effect of overcrowding cellulase on the cellulose surfaces has been studied (Xu and Ding, 2007). This fractal kinetic model is also applied to analyze the kinetics of enzymatic saccharification of Avicel (Bommarius et al., 2008). A new fractal kinetic model has been developed to describe the complex kinetics of enzymatic saccharification of cellulose in our previous work. The fractal kinetic model has two parameters, rate constant and fractal exponent. The rate constant is corresponded to the initial rate with a unit of cellulose loading, which is a function of cellulase loading. At the same time, it is also related to the structural futures of cellulose, such as surface character, reactivity, accessibility etc. The fractal exponent represents the time effect of the retarded saccharification rate, which is the intrinsic character of heterogeneously enzymatic saccharification of cellulose. The effect of cellulase loading, temperature, pretreated conditions of biomass, and addition of polymer polyethylene glycerol (PEG) and nonionic surfactants has been evaluated with the fractal kinetic model (Wang and Feng, 2010). However, the model fails to distinguish the apparent fractal parameters, such as causing with cellulase inactivity, product inhibition, lignin inhibition etc., from the intrinsic fractal parameters.

The containing lignin in pretreated biomass is regarded as a main obstacle in the enzymatic saccharification process (Guo et al., 2009; Börjesson et al., 2007; Wyman et al., 2005; Kumar and Wyman, 2009), in which high cellulase loading has to apply to fulfill the demand of high glucose yield (Wu and Ju, 1998; Gusakov et al., 2007). On the other hand, there are a lot of experimental facts about improvement of the enzymatic saccharification of lignocellulose efficiency by addition of surfactants/polymers into the reactive media (Park et al., 1992; Wu and Ju, 1998; Börjesson et al., 2007; Mizutani et al., 2002; Zheng et al., 2008; Ouyang et al., 2010; Eriksson et al., 2002). The positive effect of nonionic surfactants/polymers may be related to one or several of following causes: improvement of cellulase stability, alteration of cellulose structure, and protection of the non-productive adsorption of cellulase onto lignin (Zheng et al., 2008). However, as the above mechanisms are based on specific reaction systems, including substrate/cellulase, reaction condition, concentration range, and the character of nonionic surfactant/polymer etc., there are few mechanisms covering all the observed phenomena. In other words, the exact mechanisms about the effect of nonionic surfactant/polymer on enzymatic saccharification of lignocellulose remain unclear (Zheng, 2010).

In present work, the fractal kinetic model was applied as a tool to analyze the effect of nonionic surfactants/polymers on the enzymatic saccharification of cellulose (Avicel) in the presence and absence of lignin. The fractal kinetic analysis indicated that lignin inhibition was mainly indexed by a high fractal exponent. A high cellulase loading only led to a limited rate constant increase while an addition of nonionic surfactants/polymers decreased the fractal exponent effectively. Based on the fractal kinetic parameters, a robust polymers/nonionic surfactants for elimination of the lignin inhibition had been screened and the potential of polymers/nonionic surfactants for enzymatic saccharification of lignocellulose was further evaluated.

2. Methods

2.1. Materials

Accellerase 1500, which was a commercial cellulase of Genencor, was used in our study. It is an enzyme complex containing multiple enzyme activities with endoglucanase activity 2200–2800 CMC U/g (CMC: carboxymethylcellulose) and β-glucosidase activity 525–775 *p*NPG U/g (*p*NPG: *p*-nitrophenol glucoside). The high β-glucosidase activity ensures almost complete conversion of cellobiose to glucose. Avicel PH 101, a kind of crystal cellulose, was purchased from Sigma. Lignin (alkali) was purchased from Shanghai Chemical Agent Co. Ltd.

Nonionic surfactants, including Span 20, Tween 80 (Shanghai Chemical Agent Co. Ltd., China), Brij 30, Brij 92v, Triton X-45, Triton X-114, Triton X-100 and Tergitol TMN-3 (Fluka), and Polymers, including PEG 4000, PEG 10000, and PPG 2000 (Shanghai Chemical Agent Co. Ltd., China), triblock copolymer L61, L62, L64 (Zhejiang Huangma Chemical Co. Ltd., Zhejiang, China), and F68 (Fluka), were used without further purification. Their basic properties were listed in Table 1. The other chemicals were of agent grade or better.

2.2. Enzymatic saccharification process

A batch enzymatic saccharification of cellulose was conducted in an aqueous solution or in 2 g/100 ml of nonionic surfactants/polymers aqueous solution, respectively. The reaction was carried out in a 50-ml screw-capped flask with 10 ml total working volume. The reaction system was composited of Avicel loading 5 g/100 ml with or without solid lignin in a 50 mM citrate buffer (pH 4.8). Before the enzymatic saccharification process, the substrate, nonionic surfactant and buffer solution were preheated in a back-and-forth incubator shaking at 100 rpm at 50 °C for 30 min to allow the substrate completely dispersible, and then a certain cellulase (Accellerase 1500) was loaded to initiate the reaction. During the enzymatic hydrolysis process, aliquots of hydrolysis broth were pipetted from each flask and subjected to centrifuge. Then 0.2 ml of the clear supernatant was diluted with distilled water to 10 ml, which was utilized as sample for reducing sugar analysis. All experiments were duplicated.

2.3. Determination of nonionic surfactant/polymer toxicity

Dried baker's yeast (*Saccharomyces cerevisiae*) was obtained from Angel yeast Co. Ltd. (Wuhan, China). A nutrient medium was consisted of 2 g of glucose, 2 g of peptone, and 1 g of yeast extract in every 100 ml of tap water. For an activation culture, 0.1 g of the dried yeast was added into a 250 ml flask containing 20 ml of the nutrient medium, which was shaken at 200 rpm in a stirring shaker under 30 °C for 48 h. The activated culture broth was diluted and transferred onto plates (with the same composition as the nutrient medium except containing 1.5 g of agar in every 100 ml of tap water) and cultured at 30 °C for 1 day. The pure colonies were isolated from the plate and transferred to slants for pure culture and further utilization.

In a biomass culture, 20 ml of the nutrient medium inoculated with the purified *S. cerevisiae* was filled in a 250-ml flask and shaken at 200 rpm under 30 °C for one day. After the biomass culture, the culture broth was subjected to a centrifugation at 12,000g at 4 °C for 10 min for harvest cells. The harvest cells was washed with a 0.02 M (pH 7.0) phosphate buffer solution and centrifuged at 12,000g at 4 °C for 10 min and repeated three times. Then the biomass was determined by wet cell weight and used immediately for next relative activity analysis.

The relative biomass activity in the presence of nonionic surfactant or other organic compounds is determined by glucose degradation rate (Dai et al., 2010). The basic procedure was as follows: an aqueous solution with the biomass (wet cells) 2 g/100 ml and glucose 5 g/100 ml in the presence or absence of 0.2 g/100 ml polymer/nonionic surfactant was prepared, which was filled in a 50-ml flask and shaken at 100 rpm at 30 °C for about 10 h. At every 1.5–2 h, 0.2 ml of sample was drawn for the glucose concentration analysis. The time course of glucose degradation was linear under our experimental condition and the glucose degradation rate