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Stimulatory effect and adsorption behavior of rhamnolipids on lignocelluloses degradation system

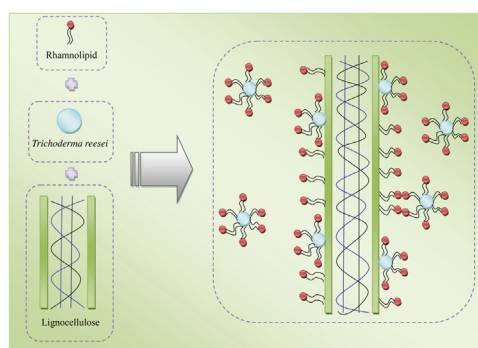
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HIGHLIGHTS

- Adding rhamnolipids could accelerate lignocellulose degrading process.
- Batch adsorption experiments investigated adsorption process of rhamnolipids in lignocellulose degrading systems.
- Adsorption kinetic models were built to estimate the adsorption pattern of rhamnolipids.
- Mechanisms of the stimulatory effect of rhamnolipids were analysed by SEM and FTIR.

GRAPHICAL ABSTRACT



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ABSTRACT

Di-rhamnolipid and mixed rhamnolipid were added to rice straw degrading system to investigate their mechanism of stimulatory effect. By batch adsorption experiments, it was shown that the equilibrium adsorption time of rhamnolipids on rice straw single system was the shortest (50 min). The adsorption capacity of *Trichoderma reesei* single system was the strongest, whose $Q_{e,exp}$ for di-rhamnolipid and mixed rhamnolipid was $10.57 \times 10^{-2} \text{ mg} \cdot \text{g}^{-1}$ and $8.13 \times 10^{-2} \text{ mg} \cdot \text{g}^{-1}$, respectively. The adsorption of rhamnolipids on consortia system was not the simple adduct of the two single systems. The adsorption of rhamnolipids on the three different systems might belong to chemisorptions. SEM and FTIR analyses were used to observe the morphology and to analyze the chemical functions in lignocellulosic biomass degradation with rhamnolipid. It was shown that after addition of rhamnolipids, the basic tissue in rice straw was severely destroyed and hydrogen bond was formed between biosurfactant and bacteria in lignocellulose degrading system.

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1. Introduction

Due to growing population, the demand of energy is increasing throughout the world (Gupta and Verma, 2015) and thus biofuels have attracted considerable attention. Biofuels are considered as promising renewable and sustainable alternatives to our limited fossil resources. Based on the conversion of sustainable non-food lignocellulosic biomass, bioethanol is one of the best options as a

feasible replacement of fossil fuel for transportation (Papa et al., 2015; Barros-Rios et al., 2016). Compared with gasoline, bioethanol has many advantages such as higher octane number, broader flammability limits, higher flame speeds and heats of vaporization, and less emission of greenhouse gas (Chen and Fu, 2016). However, in the conversion process of biomass to bioethanol, the degradation of lignocelluloses is still a major barrier to obtain overall high ethanol yield (Yu et al., 2016a,b). Thus, in order to increase bioethanol production yield, screening of high-efficiency cellulose degradation bacteria, pretreatment for breaking down the lignocellulosic structure, optimization of suitable enzymatic hydrolysis

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process, were investigated (Zhou and Runge, 2015; Fernandes et al., 2015; Yu et al., 2016a,b; Zhuang et al., 2016).

It has been shown that addition of surfactants (eg. Tween 20, Tween 80 and Triton 100) especially non-ionic detergents could significantly increase the enzymatic conversion of cellulose into soluble sugars (Wang et al., 2011). However, most of the surfactants are toxic and not biodegradable. Compared to synthetic chemical surfactants, biosurfactant (BS), which can be produced by microbes, have various advantages including low toxicity, high biodegradability, low critical micellar concentration (CMC), high surface activity and of eco-friendly nature (Zhu et al., 2012). The most studied representatives of BS are rhamnolipids due to their easily availability, remarkable surface activity and emulsifying properties (Zhao et al., 2016). They possess the highest potential for becoming the next generation of biosurfactants introduced on the market (Müller et al., 2012).

Rhamnolipids are secondary metabolites produced by strains of *Pseudomonas aeruginosa* (Abyaneh and Fazaalipoor, 2016). The polar head of these compounds consists of one or two rhamnose (Rha) units (linked by a α -1, 2-glycosidic bond) and their hydrophobic tail of one or two (very rarely three) β -hydroxy fatty acids linked to each other by an ester bond. More than fifty congeners have been described in the literature (Nott et al., 2013). Rhamnolipid have several potential industrial, agricultural and environmental applications. It could emulsify crude oil, lower oil-brine interfacial tension, and hence mobilize entrapped oil (Zhao et al., 2016). It could improve irrigation in the agricultural soil, especially under draught conditions (Renfro et al., 2014). It could also remediate PAH-contaminated soil (An et al., 2011), remove heavy metals from wastewater (Zeftawy and Mulligan, 2011) and promote the production of compost (Zhang and Sun, 2014). Recently, it was found that rhamnolipids were effective additives which could facilitate lignocellulose degrading process (Liu et al., 2012; Zhong et al., 2015). Many different explanations for their stimulatory effect were proposed, however, the mechanisms of stimulatory effect of rhamnolipids on lignocellulosic degradation system are distinct because of the various constituents in the system.

Most of the previous investigations were focused on the stimulatory effect and adsorption behavior among rhamnolipids, lignocelluloses and cellulase. Many attentions were paid on the adsorption behavior between rhamnolipids and microbes (Sotirova et al., 2009). However, the effects of surfactants on the biodegradation are dependent on the interactions not only between surfactants and degrading microbes, but also between surfactants and lignocelluloses (Zhao et al., 2011). The adsorption behavior among rhamnolipids, lignocelluloses and microbes were rarely observed. Based on our previous study (Zhang and Cai, 2008; Zhang et al., 2009a,b), di-rhamnolipid and mixed rhamnolipid were chosen as detergents to investigate their stimulatory effects and adsorption behaviors on lignocellulose degradation system, which could help us to insight the mechanism of stimulatory effect of rhamnolipid biosurfactant on lignocellulosic biomass degradation. The morphology due to influence of rhamnolipid in biomass degradation was observed under scanning electron microscope, while Fourier Transform Infrared Spectrometer (FTIR) was used to analyze the chemical functions in lignocellulosic biomass degradation with rhamnolipid.

2. Materials and methods

2.1. Materials

2.1.1. Rice straw

Rice straw, harvested in early Oct. 2014, was obtained from Wujing Town, Shanghai, China. It was washed 4–5 times with

tap water to remove extraneous matters, and then cut to 3 cm. The chopped rice straw was dried in oven at 70 °C until constant weight. Then, it was smashed by pulverizer below 40 meshes, and stored at room temperature till further use. The initial composition of rice straw was determined to be 36.1% cellulose, 24.7% hemicellulose, 16.4% lignin and 22.8% ashes.

2.1.2. Rhamnolipids

Mono-rhamnolipid (C₂₆H₄₈O₉) and di-rhamnolipid (C₃₂H₅₈O₁₃) were purchased from Zijin Biotechnology Ltd., Huzhou City. The mixed rhamnolipid was prepared by mono-rhamnolipid and di-rhamnolipid with a proportion of 1:1. Compared to mono-rhamnolipid, di-rhamnolipid possesses lower CMC concentration, better bioavailability and stronger stimulatory effect on lignocelluloses degradation system. Thus, it was targeted as objective in this research. Furthermore, mixed rhamnolipids were also chosen to be investigated considering its extremely lower cost compared to the price of pure mono-rhamnolipid and di-rhamnolipid.

2.1.3. Microorganism

Trichoderma reesei was bought from China Center of Industrial Culture Collection (CICC). Potato Dextrose Agar medium (PDA) was chosen as solid medium for its growth. The ingredients of liquid medium were 5 g·L⁻¹ potato extract, 20 g·L⁻¹ glucose, 0.1 g·L⁻¹ agar and trace chloramphenicol. *Trichoderma reesei* was formed to globular after 2–3 days growth in liquid medium, thus the quantity of its spores was used to ensure the accurate inoculum quantity. In order to collect the inoculum, the fermentation broth was centrifuged at 8000 r·min⁻¹ after 60 h growth. Finally, the inoculum was washed by phosphate buffer for 3–5 times and then washed by sterile water for twice.

2.2. Methods

2.2.1. Determination of CMC

CMC is a crucial parameter for surfactants. Properties of many systems remain unchanged above the CMC value since additional surfactant forms micelles rather than increasing the surfactant aqueous activity. The CMC values of rhamnolipids were determined by the ring method.

2.2.2. Building the lignocelluloses degradation consortia system

Based on our previous study, 2% NaOH pretreatment is an effective pretreatment method for rice straw hydrolysis (Zhang and Cai, 2008). The smashed rice straw was first pretreated by 2% NaOH at 85 °C for 1 h before enzymatic hydrolysis. Then, the pretreated rice straw was in-situ degraded by *Trichoderma reesei* at 30 °C for 60 h (achieve stationary phase) in a shaking bed of 200 r·min⁻¹. Thus, the lignocelluloses and *Trichoderma reesei* co-degradation system (consortia system) was built.

2.2.3. Optimization of lignocelluloses degradation consortia system

Orthogonal experiments were carried out to obtain the optimal lignocellulose degrading conditions, including temperature, initial pH value and the adding concentration of rhamnolipids (shown in Table S1, supplementary material). The optimal lignocellulose degrading conditions were adding 10 mg/L di-rhamnolipid at 30 °C when initial pH was 7.5, whereas adding 15 mg/L mixed rhamnolipid at 30 °C when initial pH was 5.5. All of the subsequent experiments were carried out in the optimal conditions to ensure the maximum production of reducing sugars.

Furthermore, in consideration of the adding concentration of rhamnolipids is an important factor for both lignocellulose degradation and their adsorption behavior, the adding concentration of rhamnolipids was further optimized by single factor experiment based on the result of orthogonal experiments.

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