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Enhanced hydrogen production and sugar accumulation from spent mushroom compost by *Clostridium thermocellum* supplemented with PEG8000 and JFC-E

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ABSTRACT

Clostridium thermocellum can degrade lignocellulosic materials to produce hydrogen, acetate, lactate and ethanol, and surfactants have been proven to have the ability of increasing the enzymatic hydrolysis of cellulosic substrates. Here, the effects of surfactants on the fermentation of spent mushroom compost by *C. thermocellum* were investigated and their underlying mechanisms were preliminarily studied. PEG8000 and JFC-E had different effects on the fermentation performance of spent mushroom compost. Under optimum conditions, PEG8000 increased the hydrogen production by 59.78%, compared to the control without surfactant, while JFC-E improved the accumulation of glucose and xylose rather than hydrogen yield. PEG8000 also had different impacts on the bioconversion of non-lignin substrates (Avicel, carboxyl methyl cellulose and filter paper). With added PEG8000, the hydrogen production was improved when using Avicel as the substrate, and with PEG8000, the yields of products and sugar accumulation were different from those without PEG8000, indicating that PEG8000 may increase the hydrogen yield by shifting the microbial metabolism.

Introduction

To address the energy crisis and environmental issues, exploring clean and renewable alternatives to fossil fuel is a matter of great urgency. The characteristics of hydrogen such as a high energy density (142 kJ/g) and water as its sole by-product of combustion make it a promising environmentally friendly energy carrier [1–3]. Biological hydrogen production methods include direct biophotolysis, indirect biophotolysis, biological water–gas shift reaction, photo-fermentation and dark-fermentation [4]. Fermentative hydrogen production processes can produce hydrogen from a variety of lignocellulosic materials, even agricultural wastes. The utilization of biomass residues is really economical and environmentally friendly [5].

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To date, the economic conversion of lignocellulosic biomass to fuels is still not very successful [6]. Some studies focused on enhancing the lignocellulose hydrolysis efficiency by additives, and surfactants (For convenience, surface active additives were also called surfactants.) have been proven to have the ability of increasing the enzymatic hydrolysis of cellulosic substrates [7–9]. A previous report showed that PEG6000 increased the hydrolysis of pretreated wheat straw [10]. Han and Chen [11] found that Tween 80, Triton X-100, PEG6000 and PEG10000 all could enhance the glucose production in steam-exploded corn stover hydrolysis, and among these surfactants, Tween 80 had a most obvious effect of 35.3% increase. Additionally, Tween 80 was also shown to improve the hydrolysis yield from 69 to 91% for steam exploded lodgepole pine [12].

Several mechanisms have been developed to explain the effect of surfactants on the biodegradation of lignocellulose. First, surfactants could affect the structure of the substrate, facilitating the digestion of lignocellulose. Kaar et al. [13] concluded from their study that Tween could disrupt the lignocellulose. Secondly, surfactants may enhance enzymatic hydrolysis by increasing enzyme stability and preventing denaturation of enzymes. PEG4000 was found to greatly improve the stability of Celluclast 1.5 L, a cellulose-degrading enzyme [14]. Furthermore, surfactants could affect the interactions between enzyme and substrate. Eriksson et al. [15] proposed that the dominating mechanism for surfactant effect on lignocellulose hydrolysis was that surfactant could adsorb to the lignin in the substrate, leading to the release of nonspecifically bound enzymes. Finally, surfactants may affect the microorganism. The presence of Triton X-100 could increase the positive electric charge of the cell surface, resulting in weakened mutual repulsion and better accessibility between the cell and substrate [16].

Spent mushroom compost (SMC), a kind of waste from edible fungi cultivation, contains lots of nutrients, such as cellulose, protein, trace elements, and so on. The nutrients in SMC have made it economic to reuse this waste as substrate, which is also helpful to solve the environmental problems caused by discarding or burning [17]. SMC is an efficient substrate for hydrogen fermentation [18].

Clostridium thermocellum is a thermophilic anaerobe, which can degrade lignocellulosic materials to products such as acetate, hydrogen, lactate and ethanol by expressing a suite of cellulolytic enzymes that are exported from the cell and assembled into a complex structure on the surface of the cell called cellulosome [16,19–22]. In the present study, *C. thermocellum* was used to degrade SMC, and the effects of surfactants (PEG8000 and JFC-E) on SMC fermentation were investigated. In order to understand the mechanism underlying the effect of surfactant, non-lignin substrates were also biodegraded with PEG8000.

Material and methods

Sources of chemicals and substrates

SMC used in this study was obtained from Starway Biotechnology Co. Ltd, Guangdong province, China. SMC was soaked in water and washed until neutrality, and then treated by air dryer at 50 °C, followed by grinding and sieving the SMC through a 200-mesh sieve. The composition of the SMC was analyzed mainly according to the procedures published by the National Renewable Energy Laboratory [23], and the major components of SMC were 32.37 ± 0.47% cellulose, 25.21 ± 0.66% hemicellulose and 25.88 ± 0.40% lignin, on dry weight basis.

PEG8000 was purchased from Aladdin Industrial Corporation, Shanghai, China. Isooctyl alcohol polyoxyethylene ether (JFC-E) was purchased from Lanshanlusen Chemical Co. Ltd, Linyi city, Shandong province, China. Carboxyl methyl cellulose (CMC) was purchased from Tianjinzhiyuan Chemical Reagen Co. Ltd, China. Avicel was purchased from FMC BioPolymer, USA. Filter paper was obtained from Whatman International Ltd, England.

Microorganism, media and inoculum preparation

C. thermocellum ATCC 27405 was donated by Professor Lynd (Dartmouth College, USA), and was employed in all fermentation experiments. *C. thermocellum* used as inoculum was grown in 100 mL serum bottle (working volume 50 mL) with modified MTC medium [24] composed of the following constituents (per liter): 2 g tripotassium citrate, 1.25 g citric acid monohydrate, 1.0 g sodium sulphate, 1.0 g potassium dihydrogen phosphate, 2.5 g sodium bicarbonate, 5.0 g urea, 1.5 g ammonium chloride, 1 g yeast extract, 1.0 g magnesium chloride hexahydrate, 0.2 g calcium chloride dihydrate, 0.1 g ferrous chloride tetrahydrate, 1.0 g L-cysteine hydrochloride monohydrate, 20 mg pyridoxamine dihydrochloride, 4 mg p-aminobenzoic acid, 2 mg D-biotin, 2 mg vitamin B12, and 4 mg thiamine, with 3 g/L microcrystalline cellulose added into the modified MTC medium as the carbon source. For all fermentation experiments, the concentration of urea was 20 g/L in the modified MTC medium, and the carbon source was 6% (w/v) SMC. All the serum bottles were sealed with a butyl rubber stopper and aluminum seals, and then each bottle was purged and gassed with 100% nitrogen. For a seed culture, *C. thermocellum* was repeatedly transferred when grown to mid-log phase at 55 °C with rotary shaking at 150 rpm (C24KC refrigerated incubator shaker, Edison, New Jersey, USA).

Experimental design

Surfactant addition concentration experiment

All of the experiments were carried out in 100 mL serum bottles with 10% (v/v) inoculum size and all the fermentation was performed at 55 °C with rotary shaking at 150 rpm for 10 days. The working volume before adding surfactant was 50 mL. On the 5th day of fermentation, surfactants at different concentrations of 0.2%, 0.4%, 0.6%, 0.8% and 1.0% (w/v, the same as below) were injected separately into the bottles with the volume of 1 mL, and meanwhile, 1 mL of distilled water was injected as control.

Surfactant addition time experiment

With the optimal concentration of 0.4%, surfactants were injected into the bottles either on the day before inoculation, the 1st, 3rd, 5th, and 7th day after inoculation or at the time of inoculation. The control was performed without surfactants.