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Evaluation of spent mushroom compost as a lignocellulosic substrate for hydrogen production by *Clostridium thermocellum*

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ABSTRACT

Spent mushroom compost (SMC), waste cultivation medium of mushroom, is a potential lignocellulosic substrate. The characteristics of *Clostridium thermocellum* growing on SMC were investigated to provide practical guidance for better utilizing the SMC. SMC could be utilized without any pretreatment by *C. thermocellum*, and grinding, washing and mild chemical pretreatment could improve the fermentation performance of SMC, and consequently improved the hydrogen production. Grinding, washing and NaOH pretreatment increased hydrogen production by 48.48%, 62.09% and 2.61 times, respectively. Compared with washing method, neutralizing the alkali pretreated SMC with waste acid had no significant effect on hydrogen production, while could save a lot of water. Besides, fermentation without sterilization and medium simplification were feasible for the fermentation of SMC. The present study shows that SMC is a feasible substrate for consolidated bioprocessing, but pretreatments are needed to degrade the SMC more effectively.

Introduction

As the biggest solid-state fermentation industry in the world, the annual yield of mushroom is enormous. It is estimated that producing 1 kg mushroom will generate 5 kg SMC [1], and at least 30 million tons of SMC is produced annually [2]. General disposal methods of SMC, such as combustion and landfill, could lead to serious environmental problems or public health problems [3]. As governments have taken more attention to environmental protection, the management of waste discharge becomes more and more strict, and the storage and disposal issues of SMC have limited the development of mushroom production industry. To manage this enormous waste, it is valuable to explore the potential commercial applications of SMC.

SMC is an inexpensive source of many valuable products, such as enzymes and polysaccharide [4]. Based on the ability

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to degrade many lignin-like structures, for example, polycyclic aromatic hydrocarbons and phenols, by the inherent enzymes, and the adsorption ability, SMC could be applied in bioremediation [4]. Besides, SMC could be used in cultivation matrix [5], fertilizer [6] and animal feed [2], since it contains lots of nutrients, such as cellulose, protein, trace elements, and so on. In fact, SMC is also a potential lignocellulosic substrate, in which cellulose is the main component ranging from 18% to 62% [7].

As a kind of renewable resource, lignocellulose is abundant and inexpensive worldwide, which could be used for producing a variety of products [8–10]. The utilization of lignocellulose is of great significance for sustainable development. Generally speaking, the utilization of lignocellulose via biological techniques contains four essential steps: pretreatment, enzymatic hydrolysis, fermentation and separation and purification of targeted products. However, consolidated bioprocessing (CBP), converting the lignocellulose into desired products in one step, has become a hot research topic, due to saving cost from operating and capital costs associated with cellulase production [11–13]. SMC has been applied in the production of butanol [14] and ethanol [15], while its utilization in CBP has not attracted sufficient attention.

Clostridium thermocellum, an anaerobic, cellulolytic thermophile with the ability to produce hydrogen, ethanol, carbon dioxide, acetic acid, formic acid and lactic acid, directly from cellulosic biomass [16], is promising for CBP. *C. thermocellum* has been used in the production of ethanol [17] and hydrogen [18,19] both alone and in co-culture with other thermophiles [20–22]. Producing biofuels and other chemicals from SMC by *C. thermocellum* is one of the feasible recycle approaches. Besides, compared with other management methods, degradation by microorganism such as *C. thermocellum* could truly achieve the reduction in volume and weight of SMC.

Accordingly, this study was conducted to investigate the characteristics of *C. thermocellum* growing on SMC, to evaluate the SMC as a lignocellulosic substrate for hydrogen production, so as to provide practical guidance for the SMC recycle.

Materials and methods

Materials

Flammulina velutipes SMC used in this study was obtained from Starway Bio-technology Co. Ltd, Guangdong province, China. The medium for the cultivation of *Flammulina velutipes* mainly consisted of cottonseed hull, wheat bran, corn powder, corncob, wood chip and gypsum. SMC was ground and sieved through a 100-mesh (0.15 mm) sieve, unless stated otherwise.

For the chemical pretreatment, SMC was ground and sieved through a 40-mesh (0.45 mm) sieve, then soaked in different solutions with a liquid to solid ratio of 25:1. After the chemical pretreatment, the suspension was filtrated, and the obtained residue was washed to near neutral pH with tap water, then dried at 50 °C and ground to through a 100-mesh sieve. For NaOH pretreatment, the SMC was soaked in sodium hydroxide solution (3%, w/v) and incubated in a water bath cauldron at 80 °C for 3 h [23]. For NaOH- H_2O_2 pretreatment, the SMC was soaked in mixed solution containing 1% (w/v) sodium hydroxide and 0.6% (w/v) hydrogen peroxide [24], and incubated at 60 °C for 6 h. The pretreatment of SMC using an NH_4OH–H_2O_2 solution was carried out according to Zhu et al. [25], with little modification. NH_4OH and H_2O_2 were mixed at a volume ratio of 2:1, then same volume of distilled water was added. The pretreatment was conducted at 60 °C for 6 h.

For the washing treatment, SMC was soaked in tap water and washed until near neutrality, then treated by air dryer at 50 °C, followed by grinding and sieving through a 100-mesh sieve, and the resulting SMC was called as WSMC.

Microorganism, media and culturing conditions

C. thermocellum DSM 1313 was purchased from Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany), and was employed in all fermentation experiments. *C. thermocellum* was grown anaerobically in DSMZ 122 medium [26] supplemented with 10 g/L Avicel pH-105 (FMC, USA) or 4% (w/v) SMC as carbon source. 120 mL serum bottles were used with a working volume of 50 mL and were sealed with a butyl rubber stopper and aluminum seals, then each bottle was purged and gassed three times with 100% nitrogen. All the media were autoclaved at 115 °C for 30 min unless stated otherwise. Inoculum was prepared with Avicel, and was transferred into the SMC medium with 10% (v/v) inoculum size by injection syringe. All of the fermentation was performed at 55 °C with rotary shaking at 150 rpm for 4 days.

Fermentation experimental design

For the grinding experiment, the SMC was ground and sieved through sieves with different aperture size (40- and 100-mesh), and SMC without grinding was set as control.

To investigate the effect of adjusting the initial pH value on the fermentation of SMC, sodium hydroxide solutions at different concentrations of 0.4, 0.6 and 0.8 mol/L were injected separately into the bottles with the volume of 1 mL before inoculation, and 1 mL distilled water was injected as control.

To investigate the effect of various methods for adjusting the pH value of alkali pretreated SMC on the fermentation, the SMC was treated by sodium hydroxide solution as described in Section Materials, except after alkali treatment, the SMC was simply washed 3–5 times with tap water, then the pH values of SMC were adjusted to near neutrality through various methods. For the washing method, SMC was washed with tap water until near neutrality; for the HCl and waste acid methods, the pH values were adjusted by concentrated hydrochloric acid and spent acid solution, respectively. To prepare the spent acid solution, black liquor from NaOH pretreatment was collected by filtration and centrifuged to remove residual solids. The supernatant fluid was adjusted to pH 3 with concentrated hydrochloric acid and incubated at 70 °C for 30 min. After centrifugation, the precipitate was crude lignin, and the supernatant was spent acid solution used for adjusting the pH value.

To investigate the feasibility of fermentation without sterilization, the SMC medium was inoculated directly without sterilization.