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Effect of Constant Glucose Feeding on the Production of Exopolysaccharides by *Tremella fuciformis* Spores

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Abstract A fed-batch culture system with constant feeding (glucose 80 g L^{-1}, 0.25 ml min^{-1}) was used to study the influence of glucose on cell dry weight and exopolysaccharides production from submerged *Tremella fuciformis* spores in a 5-L stirred-tank bioreactor. The results showed that high levels of cell mass (9.80 g L^{-1}) and exopolysaccharides production (3.12 g L^{-1}) in fed-batch fermentation were obtained after 1 h of feeding, where the specific growth rate (μ) and exopolysaccharides yield on substrate consumed (YP/S) were 0.267 d^{-1} and 0.14 g g^{-1}. Unlike batch fermentation, maximal cell mass and exopolysaccharides production merely reached 7.11 and 2.08 g L^{-1}; the specific growth rate (μ) and exopolysaccharides yield on substrate consumed (YP/S) were 0.194 d^{-1} and 0.093 g g^{-1}, respectively. It is concluded that the synthesis of exopolysaccharides can be promoted effectively when feeding glucose at a late exponential phase.

Keywords Cell dry weight · Constant glucose feeding · Exopolysaccharides · *Tremella fuciformis* spores

Introduction

Recently, the production of exopolysaccharides from mushrooms have received special attention due to their various physiological activities [1–3]. The basidiomycete *Tremella fuciformis*, which produces single yeast-like conidia derived from mycelium, has been reported to have beneficial pharmacological activities, including immunostimulating, antitumor, and hypoglycemic activity [4, 5]. As a result of its perceived health benefits,

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T. fuciformis has gained wide popularity as an effective health food and has become one of the valuable mushrooms. To date, there are many reports regarding the culture condition of *T. fuciformis* spores, characterization of polysaccharides, and their biological activities produced from the extract of these spores [4, 6–8].

Many studies have tried to improve cell biomass and exopolysaccharide production by optimizing culture media using statistical experimental methods including response surface methodology and Taguchi parameter design methodology [6, 9]. Nevertheless, to the best of our knowledge, no attempt has been made to improve cell dry weight and exopolysaccharides production from *T. fuciformis* spores by supplementing certain materials, such as glucose, minerals, ATP, and phosphorus to cultures. Fed-batch fermentation is a production technique in between batch and continuous fermentation. A proper feed rate, with the right component constitution, is required during the process engineering. So, in order to obtain bioactive exopolysaccharides, submerged culture of *T. fuciformis* spores by fed-batch fermentation deserves investigating.

In the present study, a fed-batch culture system with constant feeding (glucose 80 g L^{-1}, 0.25 ml min^{-1}) was used to study the influence of glucose on cell dry weight and exopolysaccharides production by *T. fuciformis* spores in a 5-L stirred-tank bioreactor. Detailed growth yield information was also obtained. The results showed that the synthesis of exopolysaccharides can be promoted effectively when feeding glucose at a late exponential phase. It is hoped that this information can be used for the development of specific fermentation processes using *T. fuciformis* spores or strains and species with similar physiology.

Materials and Methods

Microorganism and Media

T. fuciformis ACCC50546 was purchased from the Agricultural Culture Collection of China (ACCC, Beijing) and the stock culture was maintained on potato dextrose agar (PDA) slants. After 10 days, yeast-like conidia were isolated from mycelia, incubated on PDA slant at 25 °C for 7 days, then stored at 8 °C and subcultured once a month.

Inoculum Preparation and Flask Cultures

T. fuciformis spores were initially grown on PDA medium in a tube, and then transferred to the seed culture medium with a sterilized inoculating loop. The seed culture was grown in a 250-ml flask containing 50 ml of fermentation medium (g L^{-1}) 15 glucose, 2 yeast extract, 2 peptone, 0.5 $MgSO_4 \cdot 7H_2O$, 1 K_2HPO_4, and 0.46 KH_2PO_4 at 25 °C on a rotary shaker incubator at 150 rpm for 3 days.

Bioreactor Fermentation

The fermentation medium was inoculated with 2% (*v*/*v*) of the seed culture and then cultivated at 25 °C in a 5-L stirred-tank reactor (Biotech, Shanghai, China) equipped with pH and dissolved oxygen electrodes. Unless otherwise specified, fermentations were performed under the following conditions: temperature, 25 °C; aeration rate, 2 vvm; agitation speed, 250 rpm; initial pH 8; working volume, 3 L. The seed culture was

transferred to the fermentation medium and was cultivated for 7 days. All experiments were performed at least in duplicate.

Glucose Feeding Strategy and Analytical Methods

Constant feeding strategy was employed after exhaustion of glucose in the batch medium. Glucose at 80 g L^{-1} was fed at 0.25 ml min^{-1}. In batch and fed-batch experiments, samples taken at 12-h intervals were centrifuged at 10,000×*g* for 15 min. The dry weight of spore biomass was measured after repeated washing of the cell pellets with distilled water and drying overnight at 90 °C. The filtrate from membrane filtration was analyzed quantitatively for residual glucose concentration using D-Glucose Assay Kit (Equl-Megazyme, Ireland) according to the manufacturer's instruction.

Estimation of Fermentation Kinetics

The specific growth rate, μ (d^{-1}), was calculated from the equation: $\mu=(1/X)(dX/dt)$; where X is the cell concentration (g l^{-1}) at time t (d). The specific consumption rate of substrate, $Q_{S/X}$ (g g^{-1} d^{-1}) was estimated by the equation: $(dS/dt)(1/X)$; where S is the glucose concentration (g l^{-1}) at time t (d). The specific production rate of exopolysaccharides, $P_{P/X}$ (g g^{-1} d^{-1}) was estimated by the equation: $(dP/dt)(1/X)$; where P is the concentration of exopolysaccharides (g l^{-1}) at time t (d). The yield of exopolysaccharides on glucose, $Y_{P/S}$ (g g^{-1}) was estimated by the equation: $(dP/dt)/(dS/dt)$.

Isolation and Estimation of Exopolysaccharide

The culture broth was collected at various intervals from the fermentor and centrifuged at 10,000×*g* for 15 min, and the resulting supernatant was filtered through a membrane filter. The resulting culture filtrate was mixed with four times its volume of absolute ethanol and stirred vigorously. Precipitation of exopolysaccharides proceeded at 4 °C for 24 h and the precipitate collected by centrifugation at 10,000×*g* for 10 min, discarding the supernatant. The precipitate of exopolysaccharide was lyophilized in vacua and the weight of it was also estimated. All experiments were performed in triplicate to ensure the reproducibility; the data presented in the "Results" section represents the mean of three independent experiments.

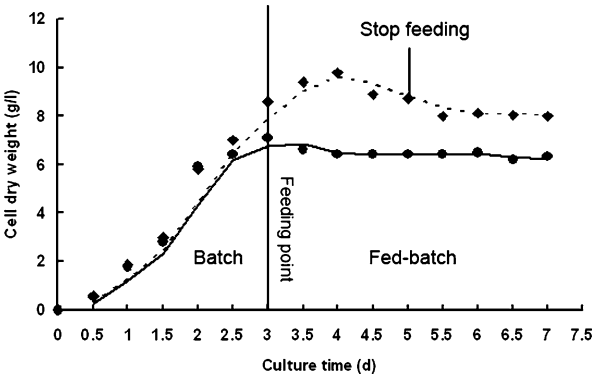

Fig. 1 Time course profiles of reducing glucose during submerged culture of *T. fuciformis* spores in a 5-L stirred-tank bioreactor in batch (*filled circle*) and fed-batch (*filled diamond*) fermentation. The constant glucose feeding was initiated after the consumption of glucose in the batch medium. At day 5, glucose feeding was stopped

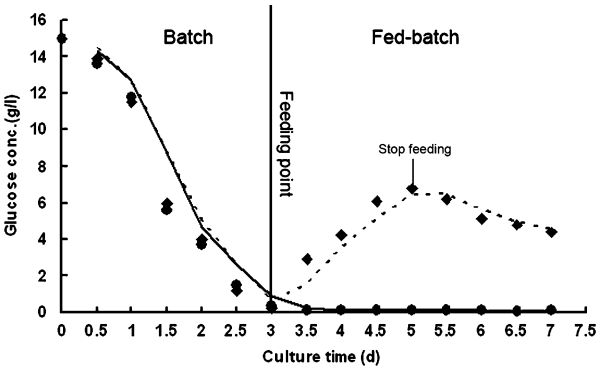

Fig. 2 Time profiles of cell dry weight during submerged culture of *T. fuciformis* spores in a 5-L stirred-tank bioreactor in batch (*filled circle*) and fed-batch (*filled diamond*) fermentation. The constant glucose feeding was initiated after three days. At day 5, glucose feeding was stopped

Results

In this work, batch and fed-batch fermentation in a 5-L bioreactor were performed to explore the effect of constant glucose feeding on cell growth and exopolysaccharide production. The difference in growth rates during the initial phase of cultivation was negligible. Substantial differences were seen at the late exponential phase of growth from the beginning of feeding. The time profiles were shown in Fig. 1. In fed-batch fermentation, the spores exhibited higher cell viability and grew to a higher cell density, compared to batch fermentation. In two parallel experiments, complete sugar depletion was observed after 3 days (Fig. 2). At this point in time, glucose feeding started. With addition of glucose, a critical influence of glucose feeding on cell growth and exopolysaccharides production was observed as shown in Figs. 1 and 3. High levels of cell mass (9.80 g L^{-1}) and exopolysaccharides production (3.12 g L^{-1}) in fed-batch fermentation were obtained after 1 h of feeding, where the specific growth rate (μ) and exopolysaccharides yield on glucose consumed (*Y*P/S) were 0. 267 d^{-1} and 0.14 g g^{-1}. Unlike batch fermentation, maximal cell mass and exopolysaccharides production merely reached 7.11 and 2.08 g L^{-1}; the specific growth rate (μ) and exopolysaccharides yield on substrate consumed (*Y*P/S) were 0.194 d^{-1} and 0.093 g g^{-1}, respectively. At the same time, we also investigated dissolved oxygen (DO) levels during batch and fed-batch fermentation (Fig. 4). The DO levels at all growth phases were declined from 100% at the beginning of fermentation to around 0~10% at 2~3 days. In batch fermentation, the DO level increased rapidly as the growth entered a stationary phase. But in fed-batch fermentation, extremely low levels of dissolved oxygen

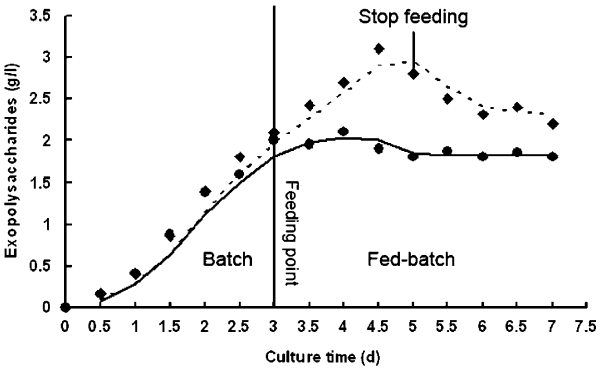

Fig. 3 Typical time profiles of exopolysaccharides production during submerged culture of *T. fuciformis* spores in a 5-L stirred-tank bioreactor in batch (*filled circle*) and fed-batch (*filled diamond*) fermentation. The constant glucose feeding was initiated after the consumption of glucose in the batch medium. At day 5, glucose feeding was stopped

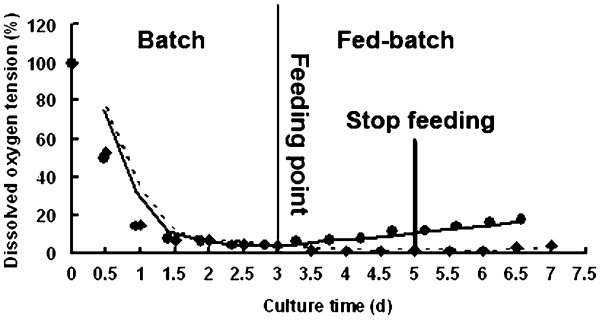

Fig. 4 Time profiles of dissolved oxygen during submerged culture of *T. fuciformis* spores in a 5-L stirred-tank bioreactor in batch (*filled circle*) and fed-batch (*filled diamond*) fermentation. The constant glucose feeding was initiated after the consumption of glucose in the batch medium. At day 5, glucose feeding was stopped

(DO) were indicated from the feeding point of the fermentation, which may be attributed to the second growth phenomenon of *T. fuciformis* spores.

Discussion

To date, many investigators have studied the production of mushroom exopolysaccharides by submerged cultures [10, 11]. Nevertheless, relatively few authors have used feeding strategy in fermentation process. The constant glucose feeding was proved to be a useful technique for enhancing cell growth and exopolysaccharide production in submerged culture of *T. fuciformis* spores. The strategy established in this study may be worth attempting with other mushroom fermentation processes for enhanced production of mushroom polysaccharides, particularly those with industrial potential. In fact, there are factors that most affect the production of exopolysaccharides by *T. fuciformis* spores [6] such as initial concentrations of carbon and nitrogen sources, initial pH, shear rates [12], dissolved oxygen, and aeration. Moreover, sporular morphological forms (e.g. ovoid, elongated, and double yeast forms) also significantly affected exopolysaccharides production; it is noteworthy to mention that the increased population of elongated yeast probably contributed to an increased EPS production [6].

In conclusion, fed-batch fermentation with constant glucose feeding resulted in higher yields of cell dry weight (9.80 g L^{-1}) and exopolysaccharides (3.12 g L^{-1}). This work will give us an understanding that will allow us to devise process manipulations that will lead to higher exopolysaccharide productivities in submerged culture of *T. fuciformis* spores.

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