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Kinetics of biodegradation of free gossypol by *Candida tropicalis* in solid-state fermentation

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Abstract

In the present work experiments were carried out to study the effect of free gossypol on the growth of *Candida tropicalis* ZAU-1, evaluate its ability in biodegrading free gossypol, analyze the time course of solid-state fermentation, and model the microbial growth by determining the kinetics of dry matter weight loss, total carbohydrate concentration and the free gossypol content during solid-state fermentation. Results showed that the biomass in inorganic salts glucose medium were unaffected by free gossypol at 500 and 1000 mg/l levels, compared with the control group ($p > 0.05$); degradation of free gossypol reached 95.12% and 94.12%, respectively. A logistic equation ($R^2 = 0.9922$), describing the growth model of *C. tropicalis* ZAU-1 was obtained, with the maximum values of u_m and X_m at 0.0970 h^{-1} and 21.8631% of dry matter weight loss, respectively. A good-fit curvilinear regression model was achieved to describe the change pattern of total carbohydrate concentration ($R^2 = 0.9910$), and the biodegradation pattern of free gossypol ($R^2 = 0.9825$). These models could be used to predict the fermentation course by *C. tropicalis* ZAU-1 under solid-state fermentation.

Keywords: Solid-state fermentation; Biodegradation; Modeling; Free gossypol; Kinetics; Microbial growth

1. Introduction

Free gossypol (FG) is a yellow coloring pigment existing in cotton plant. Its toxicity is a major concern for use of cottonseed meal as an animal feed [1]. Gossypol intake has been reported to cause the decrease of animal growth and feed conversion, and depression of fertility in bulls and reduction of viability of gametes in cattle [2,3]. Standards of the maximum FG level for different animal feeds have been prescribed in China. For example, the maximum levels in feeds for broiler and growing chicken, egg laying chicken and growing pig are 100, 20 and 60 mg/kg, respectively [4]. In China, a large amount of cottonseed meal is produced in the oil industry and can be used as a protein source in monogastric feed industry. A primary technical objective in the processing of cottonseed meal used for feeding animals is detoxification of free gossypol and improvement of nutritional value in feed industry.

During the oil recovery process, minimization of heat and processing time is vital to maintain protein quality and acceptable free gossypol content in the meal, otherwise the protein nutritive quality will be severely impaired due to lysine decrease. Actually, it is very difficult to decrease free gossypol level without affecting protein quality. Some processes such as the liquid cyclone process [5] and the application of acetone [6] can remove gossypol, but now they are not in commercial use. Detoxification by inactivation of the binding sites of gossypol is another method, but iron sulfate appeared to be responsible for the binding of free gossypol, it did not block absorption of free or bound gossypol [7,8]. It is vital to develop a new approach for degrading free gossypol and maximally blocking the gossypol absorption into animal body. Although gossypol concentrations did not change during bovine rumen fluid anaerobically fermented with cottonseed meal [9], some research found that a few microorganisms are capable of degrading free gossypol, including *Candida tropicalis*, *Torulopsis candida*, *Aspergillus flavus* and *Aspergillus niger*. Cottonseed meal detoxicated by microorganisms not only reached safe criteria, but also highly enhanced the content of protein and amino acids [10–12].

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The optimal process for biodegradation of free gossypol should be solid-state fermentation (SSF). SSF is used commercially in Asia to produce industrial products including enzymes as well as microbial biomass, and is an attractive process to produce valuable products due to its lower capital investment and lower operating expenses [13]. In order to monitor the process of free gossypol biodegradation, analysis of kinetics of fermentation is very important. Although some microorganisms have been proven to have the ability of biodegrading free gossypol, the kinetics of biodegradation of free gossypol under solid-state fermentation has not been reported. The objectives of this study were to investigate the ability of *C. tropicalis* ZAU-1 in degrading free gossypol by analyzing the fermentative course, and modeling the microbial growth, changes of total carbohydrate concentration and free gossypol content during solid-state fermentation so as to provide practical guidelines to get more efficient and economical fermented production.

2. Material and methods

2.1. Microorganism

C. tropicalis ZAU-1 [11] cells from stock were incubated on potato-dextrose agar (PDA) at 30 °C for 10 days. It was maintained on PDA slants at 4 °C and subcultured every 2 months.

2.2. Materials

Cottonseed meal, wheat bran, rice bran, rice wine spent grain, glucose, molasses, sugar $(NH_4)_2SO_4$, $MgSO_4{\cdot}7H_2O$, KH_2PO_4, $ZnSO_4{\cdot}7H_2O$, $MnSO_4{\cdot}H_2O$, $CuSO_4{\cdot}5H_2O$, $CoCl_2{\cdot}6H_2O$ and other chemical reagents were obtained locally, and free gossypol (2,2′-bis(8-formyl-1,6,7-trihydroxy-5-isopropyl-3-methylnaphthalene) was purchased from Sigma (St. Louis, MO, USA).

2.3. Media

2.3.1. Potato-dextrose agar (PDA)

The medium contained 100 ml potato extract, 2 g glucose and 2 g agar, and was autoclaved at 110 °C for 20 min.

2.3.2. Inoculum medium

The inoculum medium used for the cultures was as follows: malt extract, 12 Brix; pH, 6.0. The medium was autoclaved at 110 °C for 20 min.

2.3.3. Inorganic salts glucose medium

The medium contained 2% glucose, 0.5% $(NH_4)_2SO_4$, 0.1% KH_2PO_4, 0.05% $MgSO_4{\cdot}7H_2O$, 0.5% peptone, 0.1% yeast extract, 0.2% $ZnSO_4{\cdot}7H_2O$, 0.06% $MnSO_4{\cdot}H_2O$, 0.04% $CuSO_4{\cdot}5H_2O$ and 0.02% $CoCl_2{\cdot}6H_2O$. The medium was autoclaved at 110 °C for 20 min except glucose. Free gossypol and glucose were added after the liquid cooling down to room temperature.

2.3.4. Solid medium

The solid medium contained cottonseed meal 60%, wheat bran 20%, rice bran 18%, rice wine spent grain 2%, molasses 1%, $MgSO_4{\cdot}7H_2O$ 0.5% and KH_2PO_4 1.5% with the moisture content of 55% (w/w) and initial pH 6 (adjusted with 1N HCl or 1N NaOH). The medium was autoclaved at 110 °C for 20 min.

2.4. Inoculum preparation

The cultures maintained on PDA was reactivated on the surface of PDA at 30 °C for 48 h. Inocula were prepared by transferring 2 ml suspension from 48 h old slant culture, into 250 ml Erlenmeyer flasks containing 50 ml of sterile inoculum medium which was malt extraction liquid. The medium was incubated on a rotary shaker at 210 r/min at 30 °C for 24 h.

2.5. Cultivation methods

2.5.1. Culture in inorganic salts glucose medium

The inoculum was transferred into flasks containing inorganic salt glucose medium with different levels of free gossypol. Inoculum level was 1×10^7 cell/ml medium. Fermentation was performed in 250 ml flasks with a working volume of 25 ml. The strain of *C. tropicalis* ZAU-1 grew aerobically in a rotary shaker (210 r/min) at 30 °C for 72 h. The experiments were carried out in triplicate three times.

2.5.2. Culture on solid medium

The inoculum was prepared the same as that for fermentation in inorganic salts glucose medium. The inoculum was transferred into flasks containing solid medium. The solid substrate fermentation was carried out in 20 g of solid medium in 500 ml flasks, with an inoculum concentration of 1×10^8 cell/g substrate, and cultivation was maintained at 30 °C for 72 h. At each incubation time, three flasks containing fermented substrate were removed from the incubation, and then samples were separately freeze-dried for about 45 h. The samples were then weighed, milled in a mortar and stored at −20 °C for later analysis. Fermentation was carried out three times, each in triplicate.

2.6. Analytical methods

2.6.1. Assay of dry cell weight in inorganic salts glucose medium

To determine dry cell weight, culture broth was first centrifuged at 5000 rpm for 20 min, washed with 10 ml distilled water, recentrifuged, and then dried to constant weight at 105 °C, and dry cell weight was recorded. All measurements were carried out in triplicate.

2.6.2. Measurement of dry matter weight loss of solid substrate

Direct determination of biomass in solid medium is very difficult because it is impossible to separate the organism from the substrate. Terebiznik and Pilosof [14] found that dry matter weight loss was highly correlated with the biomass, and the biomass in solid-state fermentation system can be estimated by