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Biochemical Engineering Journal 37 (2007) 294–297

Biochemical Engineering Journal

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Production of bacterial α-amylase by *B. amyloliquefaciens* under solid substrate fermentation

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Received 3 February 2006; received in revised form 8 May 2007; accepted 18 May 2007

Abstract

Production of α-amylase by *Bacillus amyloliquefaciens*, under solid substrate fermentation (SSF) was investigated in shaken-culture. The maximum α-amylase activity was obtained under the following optimized conditions: corn gluten meal (CGM) 30 g/l, yeast extract (YE) 10 g/l, agitation rate 150 rpm and fermentation temperature 33 °C. The results showed that α-amylase production in a medium with CGM was five times higher than that in the medium contained starch and other components. The temperature of fermentation was found to be most crucial factor in α-amylase production.
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Keywords: Solid substrate fermentation; Corn gluten meal; *B. amyloliquefaciens*; α-amylase

1. Introduction

α-Amylases are extracellular enzymes that randomly cleave the α-1,4 glucosidic bonding of linear amylose and branching amylopectin. They are the most important group of enzymes produced commercially. Bacterial α-amylases have several applications in many food and textile processes [1–3].

α-Amylase can be produced by different species of microorganisms using both submerged fermentation (SMF) and solid substrate fermentation (SSF). Most of the production has been carried out using SMF; however, SSF systems appear promising due to the natural potential and advantages they offer [4]. SSF is generally characterized by the growth of microorganism on and/or within particles of a solid substrate in the presence of varying amounts of water. The solid substrate acts as a source of carbon, nitrogen, minerals and growth factors, and has a capacity to absorb water, necessary for microbial growth. As the microorganisms in SSF are growing under conditions similar to their natural habitats, they may be able to produce certain enzymes and metabolites more efficiently than in submerged fermentation [5–7].

SSF has many advantages over SMF, including superior productivity, simple technique, low capital investment, low energy requirement and less water output, better product recovery and lack of foam build up and reported to be the most appropriate process for developing countries. A further advantage of SSF is that cheap and easily available substrates, such as agriculture and food industry by-products [8]. Crude or partially purified enzymes produced by SSF have industrial applications (e.g. pectinases used for fruit juice clarification, α-amylase for saccharification of starch) [9].

Inexpensive agriculture and agro-industrial residues represented one of the most energy-rich sources on the planet can be used as a substrate in SSF. These residues are in fact, one of the best reservoirs of fixed carbon in nature [10]. In the SSF, the solid substrate not only supplies the nutrients to the culture, but also serves as an anchorage for the microbial cells [11].

The composition and concentration of media and fermentation conditions greatly affect the growth and production of extracellular enzymes from microorganisms. Cost and availability are important considerations, and therefore the selection of an appropriate solid substrate plays an important role in the development of efficient SSF processes [12]. On preliminary cost analysis, a net savings of about 60 and 50% on fermentation medium cost and the expenditure on down-stream processing, respectively, as compared to the presently employed SMF technique was evident [13].

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doi:10.1016/j.bej.2007.05.009

Corn gluten meal (CGM), a by-product of corn wet milling, generated large quantities from starch industrial practice is relatively cheap substrate. Traditionally, CGM has been used for animal feed, and, therefore, it is desirable to find new uses for CGM [14]. It contains rich proteins (≥60%) and vitamin and other minerals.

It is known that SSF is mainly confined to processes involving fungi and not suitable for bacterial cultures because of higher water activity requirements. However, successful bacterial growth and production α-amylase by using the SSF technique is known in many natural fermentations [11]. The genus *Bacillus* is major source of industrial enzymes and *B. amyloliquefaciens* one of the most widely used species for the bulk production of α-amylase [15].

The main objective of this study was to investigate into α-amylase production by *B. amyloliquefaciens* under SSF condition with CGM.

2. Materials and methods

2.1. Strain and medium

The *B. amyloliquefaciens* NRRL B-645 was obtained from Agricultural Research Service Culture Collection in USA. The strain was maintained on agar slopes at 4 °C. A standard inoculum medium containing (g/l) glucose 15, peptone 2.5, yeast extract (YE) 2.0, NaCl 1.5, KH_2PO_4 0.5, $MgSO_4$ 0.5 and $CaCl_2$ 0.1 was inoculated into 250 ml. Erlenmayer flask which was then kept at 37 °C and 150 rpm for 18 h. The initial pH of the medium was adjusted to 7.0. 1% (v/v) inoculum was transferred into 250 ml Erlenmayer flasks containing 50 ml production medium.

The production medium contained CGM and tap water only. However, in order to determine the effect of different concentrations of medium constituents and process conditions on the production of α-amylase, enzyme production medium based on that described by Anderson et al. was also used [16]. The pH of medium was initially adjusted to 7 and allowed to follow its natural course throughout the fermentation.

2.2. Enzyme analysis

The fermented broth was taken after 30 h and centrifuged at 7000 rpm for 15 min, and then supernatant was used for estimation of enzyme activity. The activity was measured by decrease in iodine color reaction showing dextrinization of starch. The activity was measured against the control in which no enzyme was added. The detail of the method is given elsewhere [17]. One unit of enzyme activity is defined 0.0284 optical density reduction of blue color intensity of starch iodine solution at 37 °C [18].

3. Results and discussions

In SSF, the selection of a suitable substrate for a fermentation process is a critical factor [19]. The change of α-amylase production with incubation time, in which medium contained CGM and tap water only, is shown in Fig. 1. The results showed that

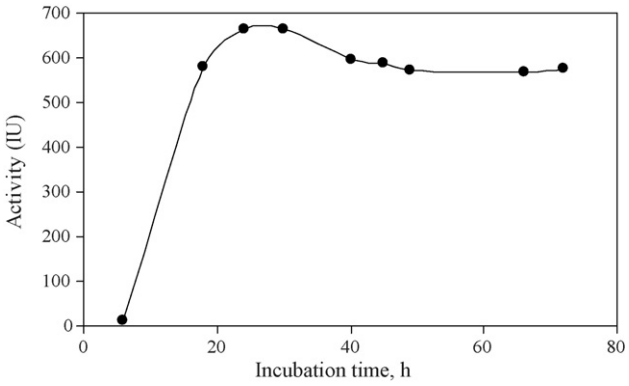

Fig. 1. Time course α-amylase production by *B. amyloliquefaciens* on CGM (Initial pH: 7.0, CGM concentration: 10 g/l, fermentation temperature: 37 °C, agitation rate: 150 rpm).

the *B. amyloliquefaciens* utilized the CGM effectively, producing α-amylase. The physico-chemical parameters and amount of media components apparently influenced the production of the enzyme. The results reported here are the averages of three values. The production of α-amylase reached a peak of 663 IU at 24 h. The production of enzyme relatively decreased after 30 h for this basal medium. In order to follow the profile of enzyme production, the fermentation was run for a long time of period (72 h).

In this study, two different sizes of CGM (50 and 100 mesh) were used. Commercially produced CGM has very small particles, so it cannot be ground more. The results show that there is no difference in the values of enzyme and time to reach maximum enzyme production in both experiments. Therefore, it was decided that the natural size of CGM was sufficient for α-amylase production.

The Fig. 2 shows the effect of nitrogen source (peptone and YE) in fermentation medium on α-amylase production. In all concentrations, the higher enzyme activity was obtained when YE was used as a nitrogen source. The figure also shows that 10 g/L of YE is the optimum for maximum α-amylase production. However, the activity decreased higher and lower concentrations of YE. Bajpai and Santos also reported similar results in their works [20,21].

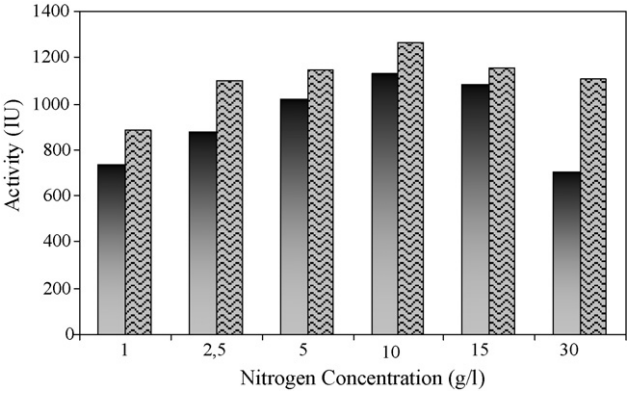

Fig. 2. Effect of the YE and peptone concentration on α-amylase production. (Initial pH: 7.0, CGM concentration: 10 g/l, fermentation temperature: 37 °C, agitation rate: 150 rpm).

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