J. Microbiol. Biotechnol. (2001), **11**(1), 13–16

JOURNAL OF
MICROBIOLOGY
AND
BIOTECHNOLOGY

Glucoamylase Production in Batch and Fed-Batch Solid State Fermentation: Effect of Maltose or Starch Addition

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Received: May 24, 2000
Accepted: September 5, 2000

Abstract Maltose and soluble starch were used as secondary sources of carbon for glucoamylase production by *Aspergillus awamori* in solid state fermentation. During batch cultivation, maltose above 2.5% (w/w) repressed glucoamylase production, but, by adding either 2.5% (w/w) maltose or 1.25% (w/w) soluble starch to fed-batch cultivations, glucoamylase activity was increased by 15% and 170% over standard medium, respectively. The data showed that maltose is a weak inducer of glucoamylase production in solid state fermentation.

Key words: Maltose, solid state fermentation, glucoamylase, fed-batch culture

During solid state fermentation (SSF), microbial growth takes place on a moist solid surface in the absence of free water [3]. SSF has several advantages over submerged fermentation (Smf), and metabolic studies and automation of the technique as well as its industrial application have been carried out mainly in eastern countries [8, 15]. In developing countries, where the economic situation often makes it difficult to keep up with many biotechnological advances, SSF constitutes a unique opportunity for progress in the local production of bioactive compounds.

Glucoamylase is an extracellular enzyme, second world-wide to protease in production and industrial application. It can hydrolize starch into glucose and is used in the production of high fructose syrup [4]. Although liquid fermentation processes are being employed to generate most of the glucoamylase production, there has been a renewed interest in solid state fermentation in recent years [12, 13]. The increasing popularity of SSF relates to its several advantages over submerged cultures, especially the low energy requirement, smaller reactor volume and high productivity [5].

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When using discontinuous systems, nutrients are added right at the start of cultivation and productivity may be impaired by substrate repression. Fed-batch fermentation, where all or some of the nutrients are added during cultivation, aims at avoiding such reduction in yields [16]. With respect to fed-batch solid state systems, however, not much information is available in the literature, which makes it difficult to investigate scale-up strategies for such mode of cultivation [7].

Several studies have shown maltose and starch to be typical inducers of glucoamylase production during submerged fermentation [6, 9, 11, 13]; however, no investigation addresses their influence on solid state processes.

The present work was designed to determine the effect of maltose and soluble starch on batch and fed-batch solid state fermentation for glucoamylase production. Additionally, different feeding regimes for maltose and soluble starch were also investigated.

Materials and Methods

Microorganism

Aspergillus awamori NRRL 3112 was grown on potato dextrose agar (PDA) at 30°C for 5 days until complete sporulation. Spores were suspended in sterile water at 10^7 spores/ml and each cultivation medium was inoculated with 1.5 ml of this suspension.

Cultivation Medium

Wheat bran was used as the main source of carbon. The standard medium consisted of wheat bran (100 g) moistened with 55 ml of water and 45 ml of a saline solution containing KH_2PO_4 (0.2% w/v) and $MgSO_4$ (0.1% w/v). Urea (0.53 g) was used as a nitrogen source. The bran and salt solution were sterilized separately for 15 min at 121°C, mixed, and the pH was adjusted to 4.2 with 30 ml of 1.5 M

Table 1. Conditions of fed-batch cultivation.

Run	Carbon source	Final concentration (% w/w)	Feeding time (h)
1	maltose	2.00	24 and 38
2	maltose	2.50	24 and 38
3	maltose	5.00	24 and 38
4	maltose	7.50	24 and 38
5	maltose	7.50	12, 24, 38 and 48
6	soluble starch	0.75	24 and 38
7	soluble starch	1.25	24 and 38
8	soluble starch	2.50	24 and 38

H_2SO_4. The resulting medium had 63% moisture content. Maltose or soluble starch (Merck KGaA, Germany) were introduced as secondary sources of carbon.

For batch cultivation, the initial maltose concentration in the medium varied between 2.5, 7.5, and 15% (w/w) of dry matter. Fed-batch cultivation started as a standard batch culture and maltose was added to a final concentration of 2.0, 2.5, 5.0, and 7.5% (w/w) of dry matter in 2 (24 and 38 h) or 4 (14, 24, 38, and 48 h) feeds under aseptic conditions. When soluble starch was used, the feeding times were 24 and 38 h, established to a final starch concentrations of 0.75, 1.25, and 2.5% (w/w). Maltose or starch feed solutions were added by atomization and the mixture was homogenized by shaking. Table 1 shows the fed-batch conditions used in this work.

Culture Conditions

Cultures were grown in 250-ml Erlenmeyer flasks containing 20 g of medium; incubation was carried out at 30°C in a humidified atmosphere. At the desired time intervals, the flasks were removed and the contents dried at 38°C during 8 h. Each sample was assayed for total residual sugars, glucose, cell concentration, and enzyme activity. All experiments were done in replicate or triplicate.

Analytical Determinations

Total residual sugars (TRS) and glucose. Total Residual Sugars in the medium were assayed by acid hydrolysis with 1.5 M H_2SO_4 during 15 min at 121°C. Total reducing sugars and glucose were quantified by the glucose-oxidase method [2]. Results were expressed as milligrams of glucose per gram of dry matter (mg/g dm).

Cell growth. The growth of *Aspergillus awamori* was estimated according to the oxygen uptake rate (OUR) method described by Sato *et al.* [14] and adapted to our conditions of cultivation [10]. Moisture of the medium was determined according to AOAC [1].

Glucoamylase activity. Each sample (1 g) was added to 15 ml of acetate buffer (pH 4.2) and shaken for 30 min at 200 rpm. This suspension was filtered and the extract obtained was assayed for enzyme activity. One glucoamylase activity unit (U) was defined as the amount of enzyme that releases 1 g glucose in 60 min in the presence of a 4% (w/v) soluble starch solution, at 60°C and pH 4.2.

Results and Discussion

Batch Cultivation

To determine the effect of initial maltose concentration on glucoamylase activity, *Aspergillus awamori* was first grown in a batch system. The standard medium, containing wheat bran as the main source of carbon, was supplemented with maltose at initial concentrations of 2.5, 7.5, or 15% (w/w). The maximum enzyme activities in all the experiments were observed at about 36 h of cultivation. The final glucoamylase activity was 21 U/g dry matter (dm) in standard medium and 32, 29, or 27 U/g dm in media containing 2.5, 7.5, or 15% (w/w) maltose, respectively. The profile of glucoamylase formation did not change; however, at initial maltose concentrations greater than 2.5%, glucose accumulation in the medium caused enzyme repression by-product. In order to analyze this further, glucose was added to the standard medium at levels that ranged between 0 and 200 mg/g dm. The results showed that glucoamylase activity decreased as glucose concentrations increased (Fig. 1). The reduction was according to the equation $A=15.2e^{-0.0086[glu]}$ ($r=0.998$), where A and [glu] correspond, respectively, to enzyme activity (U/g dm) and glucose concentration (mg/g dm).

Since the best results under batch cultivation were obtained at 2.5% maltose, kinetics experiments were run using this initial sugar concentration. The data are shown in Fig. 2 and refer to cell growth, substrate consumption, and glucoamylase activity. Glucoamylase production and cell growth occurred in association. Substrate utilization followed cell growth linearly when the total residual sugars (TRS) were between 80 and 300 mg/g dm. The corresponding time interval was from 0 to 24 h, and the

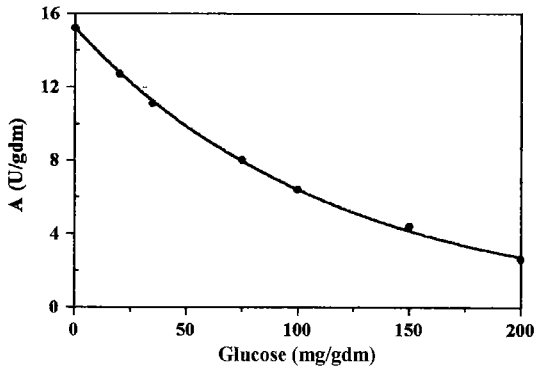

Fig. 1. Effect of initial glucose concentration on glucoamylase activity.
"gdm" indicates gram of dry matter.

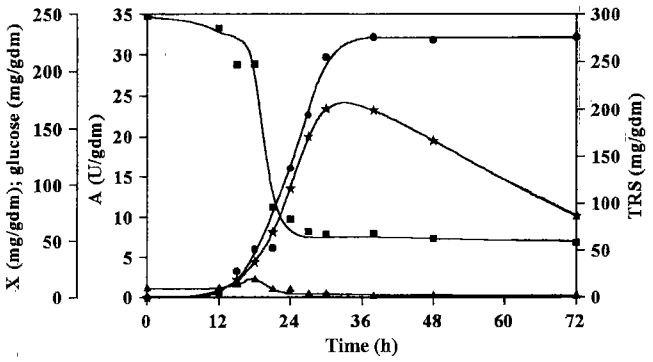

Fig. 2. Time course of batch solid state fermentation with wheat bran and 2.5% (w/w) maltose by *Aspergillus awamori*.
A, glucoamylase activity (●); X, Biomass (★); TRS, total residual sugars (■) and glucose (▲); g dm, gram of dry matter.

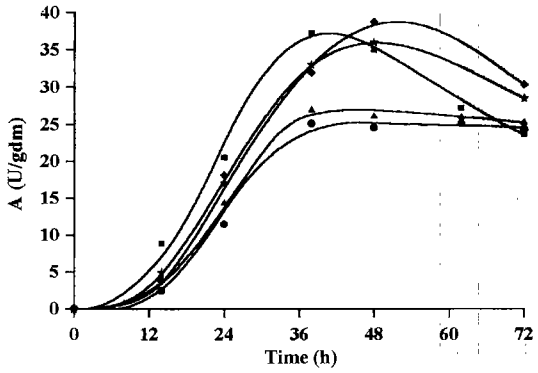

Fig. 3. Effect of different feeding conditions and maltose concentrations in glucoamylase activity during fed-batch solid state fermentation with *Aspergillus awamori*.
(▲) run 1; (■) run 2; (◆) run 3; (★) run 4; (●) run 5; g dm, gram of dry matter.

biomass yield coefficient ($Y_{X/S}$) was 76% (w/w). The enzyme yield coefficient ($Y_{A/S}$) was 0.144 U/mg.

The importance of maltose as a glucoamylase inducer in submerged fermentation is well-known. Ilori *et al.* [6] and MacKenzie *et al.* [9] reported the induction of glucoamylase when saccharides such as starch, dextrin, maltose, glycerol, glucose, mannitol, and sucrose were used as carbon sources by *Lactobacillus brevis* or *Aspergillus awamori* in Smf. The greatest enzyme yields were obtained with media containing soluble starch and maltose. However, maltose did not show a similar effect in SSF, possibly due to the limited availability of water in solid media, which leads to a much higher build-up of produced glucose than those generated in submerged systems. Therefore, maltose appears to act as a glucoamylase inducer at low initial concentrations only.

Fed-Batch Cultivation

The effect of maltose on glucoamylase activity in a fed-batch system was analyzed next. Figure 3 shows the pattern of glucoamylase production by *Aspergillus awamori* under the feeding regimes tested. The serial addition of maltose over time in the fed-batch system led to a lesser accumulation of glucose in the solid medium and hence lower repression of glucoamylase production. Nonetheless, the highest enzyme yields were recorded in media with 2.5% added maltose for both the batch and fed-batch systems, with maximum enzyme activities of 32 U/g dm and 38 U/g dm, respectively. Enzyme activities in run 5 indicated that maltose addition prior to 24 h of cultivation, when the fungus had not reached exponential growth, caused a 40% reduction in glucoamylase production. Maltose addition after 24 h of cultivation (runs 1, 2, 3, and 4) did not cause the repression observed in run 5.

Figure 4 shows the kinetics for run 2, where 2.5% maltose was added at two feeding times (24 and 38 h). Cell growth and substrate consumption patterns were similar to those obtained with batch cultivation. The glucose and Total Residual Sugars (TRS) curves indicate that glucose accumulations were distributed over feeding times. Glucoamylase yield increased by 15% only.

Glucoamylase is an inducible enzyme among fungi of the genus *Aspergillus*. Soluble starch is a well-known inducer in submerged cultures and, in this study, it was used as a secondary source of carbon in three fed-batch solid state fermentations (runs 6, 7, and 8). The maximum enzyme yield (59 U/g dm) was observed at 48 h in medium fed with 1.25% soluble starch (run 7). This value was 170% and 85% higher than the yields obtained with standard and 2.5% maltose batch media, respectively. Such results indicate that maltose is a weak inducer of glucoamylase production in solid state fermentation, possibly because its short chain can be readily hydrolyzed and leads to a fast accumulation of glucose around the cell.

This is the first study showing the effect of maltose on glucoamylase synthesis during solid state fermentation. Complementary studies should be designed to minimize the effect of homogenization (shaking) in the fed-batch

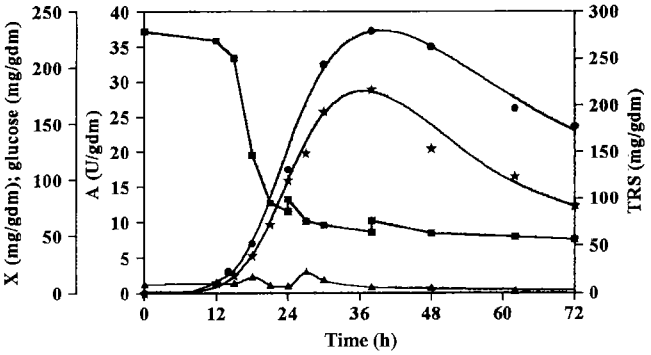

Fig. 4. Time course of fed-batch solid state fermentation with wheat bran and two feeds of 2.5% (w/w) maltose (24 and 38 h).
A, glucoamylase activity (●); X, biomass (★); TRS, total residual sugars (■) and glucose (▲); g dm, gram of dry matter.

mode and thus further improve the advantages of solid state fermentation for producing bioactive compounds.

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