Kinetics of *Aspergillus niger* growth at high glucose concentrations in different types of the cultures

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ABSTRACT

Solid state fermentation (SSF) systems have been studied for several processes of biomass and metabolites production. Heterogeneity of substrate represents a serious problem in physiological studies to characterize solid state cultures. Thus, comparison of liquid, surface and solid state cultures is difficult to carry out. With the use of an inert support and the same culture medium, it is possible to evaluate the type of culture for growth and glucose consumption in different culture systems. In this work, amberlite IRA 900 and a minimal culture liquid medium were used to study growth of *Aspergillus niger*. This system allowed accurate evaluation of biomass and sugars. Glucose utilization and growth were less affected in solid state and surface cultures than in the submerged culture at high levels of glucose.

Keywords : Solid state fermentation, *Aspergillus niger*, kinetics, Amberlite IRA 900, minimum culture liquid medium, growth, high glucose concentrations, surface fermentation, liquid culture.

RESUME

Cinétiques d'*Aspergillus niger* cultivé selon différents procédés en présence des milieux hautement concentrés en glucose.

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La fermentation solide a été étudiée pour différents procédés de production de biomasse et de métabolites. L'hétérogénéité du substrat pose un sérieux problème pour l'étude physiologique et la caractérisation des cultures en milieu solide. Aussi, il est fastidieux de réaliser une étude comparative entre les différentes techniques de fermentation : liquide, de surface et solide. On peut cependant comparer la production de biomasse et la consommation du glucose, selon le type de fermentation, en utilisant un support inerte et un même milieu nutritif. Au cours de ces travaux, nous avons utilisé comme support l'Amberlite IRA 900 et un milieu nutritif minimal pour l'étude de la croissance d'*Aspergillus niger*. Ce dispositif permet de suivre l'évolution de la biomasse et des sucres. Les fortes concentrations initiales de glucose, affectent moins la consommation de glucose et la croissance en fermentation solide et de surface que dans le cas de la culture submergée.

Mots clés : Fermentation en milieu solide, *Aspergillus niger*, cinétiques, Amberlite 900, milieu de culture minimum, croissance, hautes concentrations de glucose, surface de fermentation, culture liquide.

INTRODUCTION

The potential of solid state fermentation (SSF) system is increasingly being recognized in recent years (Pandey, 1992, Lonsane *et al,* 1992). Even though, it has many advantages over submerged culture, it also has few limitations (Hesseltine, 1972; Shankaranand *et al,* 1992). One such limitation is the availability of reliable methods for growth characterization studies. The heterogeneity and complex nature of the materials commonly used interferred with the accurate determination of the process parameters. Thus, there are afew reports on growth kinetics of fungi in SSF system, based on the on-line monitoring of CO2 (Saucedo-Castañeda *et al,* 1992) or

by measuring some of the structural components, such as proteins, glucosamine and ergosterol contents (Desgranges *et al*, 1991).

Earlier studies on pectinase production in SSF system revealed that high initial carbon source concentration (up to 50%) was rapidly metabolized by *Aspergillus niger* CH4, without any inhibition in growth and pectinases production (Solís *et al*, 1993). Moreover, it was also observed that the glucose consumption rates were increased when the initial glucose concentration was high. In this work, attempts have been made to characterize growth and substrate utilization by *A. niger* in solid state, surface and submerged cultures. The results of growth kinetic parameters at varying initial glucose concentrations are presented here.

MATERIALS AND METHODS

MICROORGANISM

Aspergillus niger 10 was used in this study. It was inoculated on PDA slants, incubated for 4 days at 35°C and stored at 4°C.

MEDIUM COMPOSITION

The medium contained (g/l): KH_2PO_4, 2.47; $(NH_4)_2SO_4$, 6.6; $CaCl_2$, 0.48; $MgSO_4$ $7H_2O$, 0.38; NaCl, 0.23 and 1 ml of a mineral solution (Clutterbuck, 1994), Glucose concentrations from 50 to 200 g/Lwere used. The initial pH was 4.5 for the solid state cultures and 5.0 for the submerged and surface cultures.

CULTURE CONDITIONS

Submerged cultures were carried out in 250 ml flasks incubated at 35°C on a rotatry shaker at 250 rpm. Surface cultures were carried out at 35°C in 100 mm Petri dishes with 30 ml of agar (1.5% w/v) medium. Solid state cultures were carried out at conditions reported previously (Raimbault and Alazard, 1980). Glass columns (11 x 150 mm) were used. Treated Amberlite IRA 900 (7 g) per column (Cordova, 1994),

impregnated with the inoculated culture medium (1 g amberlite per 1.5 ml of medium), was incubated in a water bath at 35°C with an air flow rate of 3 l/h.

ANALYSIS

Biomass

In the case of submerged culture, biomass was estimated by the dry weight method at 60°C after filtration (Whatman 41) and washing with 250 ml of distilled water. Culture medium and biomass were taken out the Petri dish, in the case of surface cultures. Agar medium was dissolved at 90°C in 250 ml of distilled water. Suspension was filtered (Whatman 41) and solids (biomass) washed with 250 ml of distilled hot water. Total biomass was determined by dry weight at 60°C. Mycelial biomass was estimated by the protein-dye binding method in solid state culture (Bradford, 1976), using biomass grown in surface cultures with the same glucose concentration as the standard. Before analysis, intracellular protein was released with 0.5 M phosphoric acid.

Glucose was measured using an enzymatic analyzer (YSI Model 2000). Moisture content in solid samples was determined, after drying at constant weight at 60°C. Data were analyzed by a logistic type model system (Okasaki *et al*, 1980).

RESULTS AND DISCUSSION

GROWTH AND BIOMASS PRODUCTION

The kinetics of growth of *A. niger* and related parameters, such as biomass production, and substrate consumption, during growth in submerged (SmF), surface (SF) and solid state (SSF) culture conditions at different initial glucose concentrations (50, 100 and 200 g/l) were studied (Fig. 1).

In SSF cultures, the growth began after 20 h incubation and reached a maximum at 40 h. The maximum biomass attained in SSF culture was about 30 g/Land it was independent of initial substrate concentration.

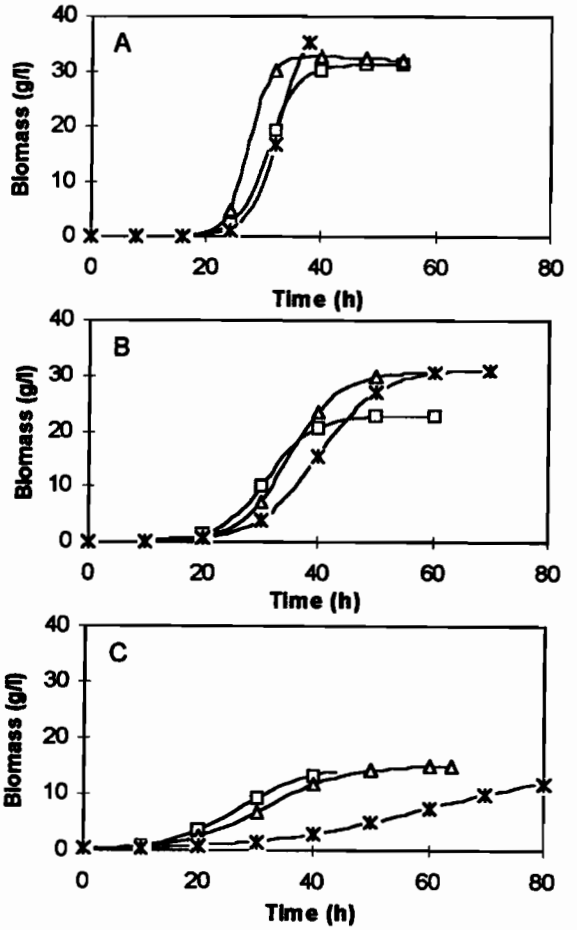

Fig. 1. Profiles of biomass production at different initial glucose concentrations (–, 50; Δ, 100 and X, 200 g/l). **A**: Solid state, **B**: Surface and **C**: Submerged cultures).

The growth of *A. niger* in SF culture also began after 20 h incubation and reached a maximum (20 g/l) at 40 h in the medium containing 50 g/Linitial glucose concentration, but reached a maximum (30 g/l) at 50 to 60 h, in the case of media 100-200 g/Linitial glucose concentration. A slow growth pattern could be observed in the SF culture at 200 g/Linitial glucose concentration. The biomass production in SmF culture was low (below 20 g/l), although growth began after 20 h incubation, as in the case of SF and SSF cultures. The growth pattern of *A. niger* in SmF culture at 50 and 100 g/Linitial glucose concentrations was almost similar, but at 200

g/Lglucose concentration, a considerable lag period (30-40 h) was detected. Furthermore, the strain grew very slowly in SmF culture at 200 g/Lglucose concentration. Thus, the SSF and SF cultures attained equal amount of biomass (about 30 g/L) within 40 h, while it took more than 40 h in the SmF culture. The low biomass obtained in the SmF culture (15 g/L) at more than 60 h was due to the slow growth of the culture.

GLUCOSE CONSUMPTION

The glucose consumption profile of the *A. niger* grown under SSF, SF and SmF culture conditions at three different glucose concentrations is shown in Fig. 2. Maximum amount of glucose was consumed during the vegetative growth period (20 - 60 h) of *A. niger* in SSF, SF and SmF cultures at 50 and 100 g/L of initial glucose concentration.

After 60 h growth, the substrate was completely consumed in SSF cultures at all the three different initial glucose concentrations. Considerable amount of residual glucose was present in SF cultures at 200 g/L initial glucose level and in SmF cultures at 100 and 200 g/Linitial glucose concentrations. In SmF culture, at 200 g/Linitial glucose concentration, the glucose consumption was rather low and only 50 g/Lof glucose was consumed, thereby leaving three - fourths of the substrate unutilized. The poor growth of *A. niger* in SmF culture at 200 g/Lwas due to the effect of the high substrate concentration.

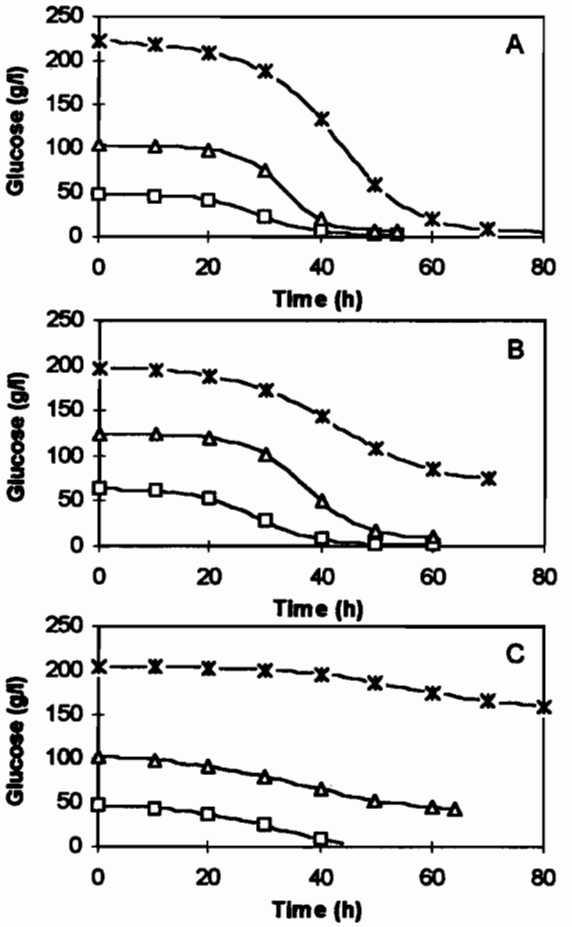

Fig. 2. Profiles of glucose consumption at different initial glucose concentrations (o, 50; Δ 100, and X 200 g/l). **A**: Solid state, **B**: Surface and **C**: Submerged cultures)

KINETIC PARAMETERS AND YIELDS

The calculated specific growth rate, biomass yields and substrate conversions for the three types of cultivation of *A. niger* at different initial glucose concentrations are shown in Table 1. The specific growth rate (μ) of *A. niger* was generally higher in SSF than in SF and SmF cultures. It was interesting to note that the strain showed three-fold higher μ in SSF, than in SmF culture at 50 g/Linitial glucose

concentration. The growth of *A. niger* was limited in the SmF culture rather than the SSF and SF cultures. One of the limiting factors responsible for the growth in SmF culture could be the low availability of oxygen, since the solubility of oxygen is rather low in the liquid culture. But in SF and SSF cultures, the oxygen was readily available and there was no problem of solubility of gases.

Table 1. Kinetic parameters and yields of *Aspergillus niger* in different type of cultures with different initial glucose concentrations (g / l)

	Solid state culture			Surface culture			Submerged culture		
Glucose	50	100	200	50	100	200	50	100	200
μ	0.323	0.313	0.238	0.222	0.232	0.170	0.091	0.083	0.043
Yx/s	0.680	0.357	0.199	0.366	0.272	0.254	0.312	0.25	0.234
%C	91	93	98	96	96	64	95	58	20

The effect of substrate concentration increase on the growth of *A.niger* in three different culture conditions revealed that the growth rate was less affected in SSF culture. It was seen that μ was almost similar (0.32 and 0.31 h^{-1}) at 50 and 100 g/L initial glucose concentrations in SSF culture. At 200 g/Lglucose concentration, it was reduced to 0.24 h^{-1}. A similar trend of the effect of substrate concentrations on the specific growth rate of *A. niger* in SF and SmF cultures could also be observed. The growth rate was not affected significantly with the increase in glucose concentration from 50 to 100 g/Lin SF culture (0.22 and 0.23 h^{-1}) and in SmF cultures (0.091 and 0.083 h^{-1}), although the magnitude of the specific growth rate was rather low in these cultures, as compared to that in the SSF culture. The specific growth rate of *A. niger* at 200 g/Lwas significantly affected in all three cultures, although the culture grew considerably well in SSF culture with a specific growth rate of 0.24 h^{-1}, as compared to the poor specific growth rate in SF (0.17 h^{-1}) and SmF (0.04 h^{-1}) cultures.

Analysis of biomass yield (Yx/s) of *A. niger* in different culture methods revealed that it was generally high in SSF culture. At 50 g/Linitial glucose concentration, it was 0.68 g/g in SSF, 0.37 g/g in SF and 0.31 g/g in SmF cultures. However, an increase in initial glucose concentration severely affected this yield parameter in SSF culture, more than in SF and SmF cultures. For example, increase in concentration of glucose from 50 to 100 g/L resulted in an approximately two-fold reduction of biomass yield (0.68 g/g to 0.35 g/g) in SSF culture. But in other culture methods, although the biomass yield was reduced, the reduction was not as similar

as the SSF culture (0.37 g/g to 0.27 g/g in SF and 0.31 g/g to 0.25 g/g in SmF cultures). Increase in initial glucose concentration to 200 g/L further affected the biomass yield in SSF culture (0.17 g/g). But in SF and SmF cultures, the biomass yield was not further affected significantly at 200 g/Lglucose concentration (0.25 g/g in SF and 0.23 g/g in SmF cultures).

Estimation of the residual substrate in the three cultures of *A. niger* at different glucose concentrations revealed that, in SSF culture all substrate was completely utilized within 70 h, independent of the initial substrate concentrations. At 50 and 100 g/L initial glucose concentrations, the substrate was completely utilized within 40 h growth in SSF culture. In contrast, considerable amount of substrate remained unutilized within 70 h in SF culture grown at 200 g/L initial glucose level, despite the complete utilization of substrate by *A. niger* grown at 100 g/L of initial glucose concentration. However, in the SmF culture, complete utilization of substrate occurred only in the culture with 50 g/L of initial substrate concentration, but about 50% of the substrate remained unutilized, when the culture was grown at 100 g/L of initial glucose concentration. More than 80% of the substrate remained unutilized, if the culture was grown at 200 g/L initial glucose concentration.

These results suggested that inhibition of growth by increasing the substrate concentration in SmF culture, is evident from the reduction in growth rate, biomass yield and residual glucose concentration. This type of effect, despit its existence in SF cultures at 200 g/L initial glucose concentration, was not prominent at 100 g/L initial glucose concentration. On the contrary, the SSF cultures were less affected by the increase in initial glucose concentration. Surprisingly, in the SSF cultures at 50 - 200 g/L initial glucose concentrations, all the substrate was utilized. Significant reduction in growth rate and biomass yield was observed in the SSF culture grown at a initial glucose concentration of 200 g/L, thereby suggesting the possible physiological and metabolic change on the organism for utilizing the substrate to produce fermentation products rather than biomass. Thus, the present study suggests the difference in the pattern of fungal growth and substrate utilization in SSF, SF and SmF cultures as well as the possible differences in their physiology and metabolism.

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