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Solid-State Culture of *Aspergillus niger* on Support

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The growth kinetics of *Aspergillus niger* on a solid support *i.e.* sugarcane bagasse, impregnated with a liquid glucose medium, were investigated under different culture conditions. The water activity of the medium, the amount of spore inoculum and the support particle size were shown to be critical factors for mold growth. The elevated rates of growth observed with high substrate concentration media demonstrated the feasibility of the method for culturing filamentous fungi.

Solid-state cultures (SSC) of molds have traditionally been limited to polymerized substrates (starch, cellulose) able to retain water in their porous matrix, and few attempts have been made to grow filamentous fungi on solid inert materials impregnated with nutritive solutions. When substrate and support are separate and an absorbed liquid medium is used, it becomes possible:
1) to use monomeric carbohydrates immediately available for the microorganism
2) to avoid the degradation of the solid matrix during growth and thus ensure constant geometric conditions
3) to make easier comparisons with submerged cultures.

In 1935, Cahn[1] achieved citric acid production by *Aspergillus niger* cultured on a sucrose solution absorbed on sugarcane bagasse or sugarbeet pulp. This kind of culture has also been studied by Terui *et al.*[2] and more recently by Lakshminarayana *et al.*[3] Mineral or synthetic materials such as vermiculite[4] and polyurethan foam[5,6] have also been used as supports for growth and enzyme production by *Aspergillus* or *Penicillium* sp.

SSC on wood pulp already constituted a model for estimating growth of yeasts[7] but no work has dealt with the environmental factors affecting mold growth kinetics on solid supports. In the present work, an attempt was made to describe the growth of *A. niger* with a liquid medium containing glucose and salts absorbed onto a lignocellulosic support *i.e.* sugarcane bagasse. The effects of the moisture content and water activity of the medium, amount of spore inoculum and support particle size on the growth kinetics were investigated.

Materials and Methods

Microorganism *Aspergillus niger* var. *hennebergi* (N° 10) described by Raimbault & Alazard[8] was used.

Pretreatment of raw material Sugarcane bagasse, free of sugars, was obtained from a sugar factory in Zacatepec (Mexico). Unless otherwise specified the 30–50 mesh fraction was used and the bagasse, moistened to 50%, was sterilized in an autoclave at 1 bar for 30 mn prior to the culture.

Preparation of spore inoculum Spore inoculum was obtained as previously described,[9] unless otherwise specified fixing the inoculum size at 2×10^7 spores per gram of solid support.

Solid state culture The culture was achieved under non-aseptic conditions as previously described.[8] Column fermentor units containing 10 g of moistened medium were incubated in a 35°C water bath. The inlet air was bubbled twice in water and the air flow was set to 2 $l.h^{-1}$; two columns were removed at each

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sampling time. Composition of the salts mixture was: KH_2PO_4, 5 g; $(NH_4)_2SO_4$, 9.8 g; Urea, 2.4 g per 100 g of glucose independantly of the glucose concentration in the liquid medium. Salts and glucose were solubilized and sterilized separately at 120°C for 20 mn.

termined with a water activity detector Humidat IC II purchased from Novasina. 3 to 4 g of material were placed in a relative humidity captor thermostated at 30°C and the state of equilibrium was reached after 3 h.

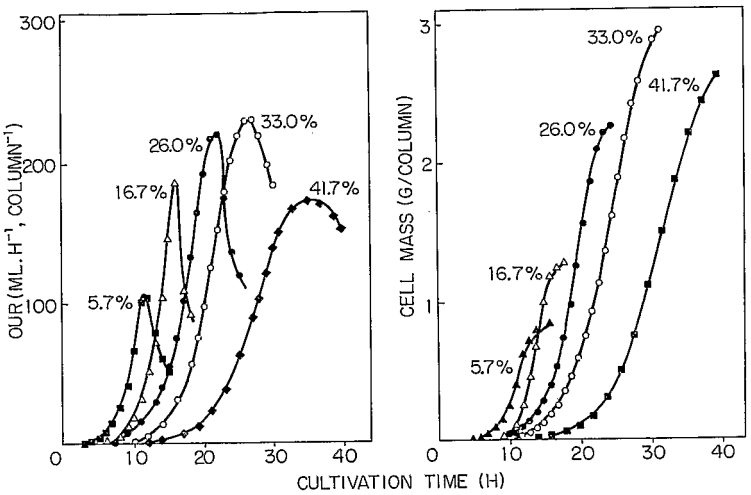

Fig. 1. Time course of OUR and cell mass calculated from OUR during growth of *A. niger* on bagasse with different glucose concentrations (% weight/volume) in the liquid phase. Culture conditions: bagasse/water (w/w) ratio, 0.36; inoculum, 2×10^7 spores/g of support; support particle size, 0.5 mm.

concentration in the liquid phase of 17% (w/v)) and increasing support/water ratios

medium.

SSC provided unique conditions for the

450 g·l^{-1}) in the liquid medium, condensation of water at the lower and upper parts of the column resulted in locally higher a_w, and gradients of growth were observed. Nevertheless, some average specific growth rates calculated here for low tonicity media (Table 1) are higher than those normally reported with fungi in submerged cultivations[14] (0.3 h^{-1}), showing that solid state culture conditions are more suitable than liquid ones for the growth of filamentous fungi.

Due to the difficulty of precisely determining cell mass concentration in SSC, some uncertainty still remains concerning the value of mO_2. Watson[15] reported 10-fold increases of the maintenance coefficient for the growth of yeasts when adding 1.0 M NaCl to the cultivation medium. In the experiments mentioned above, values of final cell mass as determined by amino-acid analysis are in good agreement with those calculated from the OUR (Table 1–3), assuming a constant mO_2 coefficient *i.e.* 0.07 g O_2·gX^{-1}·h^{-1}. Discrepancy between the values was only observed for the higher tonicity solution; this is a point needing further investigation.

Spore inoculum size The support/water ratio and the substrate concentration in the liquid phase were maintained constant and the spore inoculum size was changed from 8×10^5 to 1×10^9 spores per g of support *i.e.* 2.7×10^5 and 3.4×10^8 per ml of liquid medium. The evolution of the OUR and of cell mass as calculated from the OUR are represented in Fig. 3. Contrary to what has been reported in the literature with high density spore inocula in submerged cultures,[16] no inhibition of growth was observed on solid support. When the culture time was plotted against the decimal logarithm of the inoculum (Fig. 4), a linear relation was observed. These results confirmed those obtained previously by Oriol *et al.*[17] during microcalorimetric studies under similar cultivation conditions, accounting for an enhancement of the germination process when the amount of spore inoculum was increased.

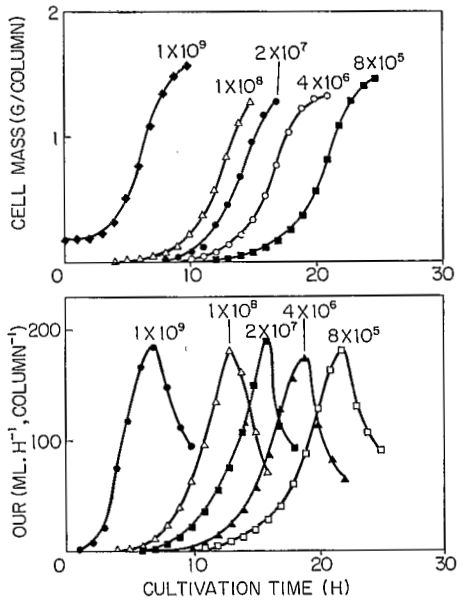

Fig. 3. Time course of OUR and cell mass calculated from OUR during growth of *A. niger* on bagasse with different amounts of spore inoculum (spores/g of support).
Culture conditions: bagasse/water (w/w) ratio, 0.36; glucose concentration in the liquid phase 16.8% (w/v); support particle size, 0.5 mm.

For the culture containing 1×10^9 spores/g of support, even if ungerminated spores remained, substrate (16% w/v in the liquid phase) was almost exhausted as soon as 11 h after inoculation. As may be seen in

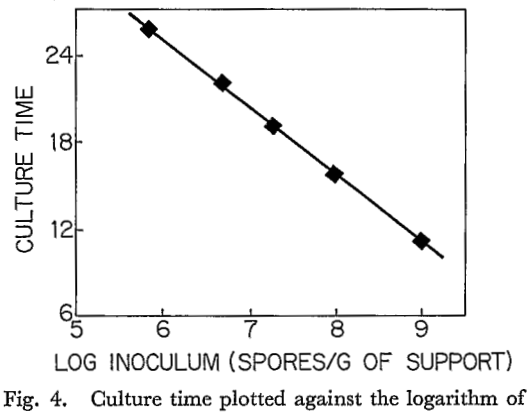

Fig. 4. Culture time plotted against the logarithm of the amount of spore inoculum.

Table 2. Effect of the amount of spore inoculum on the parameters of growth of *A. niger* on support with an absorbed liquid medium containing 168 $g \cdot l^{-1}$ (w/v) of glucose.

Spore inoculum	Final cell mass[a]	Final cell mass[b]	Substrate	Specific	Time

Table 3. Effect of the support particle size on the parameters of growth of *A. niger* on support with an absorbed liquid medium containing 280 g·l^{-1} (w/v) of glucose.

Support particle size (mm)	Initial density (kg·l^{-1})	Final cell mass [a] (g/column)	Final cell mass [b] (g/column)	Substrate consumption (g/column)	Specific growth rate (h^{-1})	Time (h)
2.50	.230	2.16	2.05	4.75	0.323	27
1.43	.295	2.66	2.60	4.95	0.330	26
0.79	.295	2.52	2.40	5.42	0.348	26
0.51	.295	2.76	2.60	4.76	0.400	25
0.10	.485	2.53	2.33	4.58	0.375	25

[a] calculated from the amino-acid content
[b] calculated from the OUR

et al.[18]

Conclusion

The culture method involving a synthetic liquid medium absorbed on a solid support was demonstrated to be suitable for the growth of filamentous fungi, and it allowed the utilization of high-concentration substrate solutions. The water activity of the liquid phase, the support particle size and the amount of spore inoculum were found to be critical factors for the growth of molds, the latter accounting for different spore germination patterns between solid and submerged cultures. This kind of cultivation method might broaden the use of solid state cultures for producing fungal metabolites with low cost technology.

Acknowledgments

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