Letters in Applied Microbiology 1992, **15**, 164–167

Importance of medium pH in solid state fermentation for growth of *Schwanniomyces castellii*

G. SAUCEDO-CASTAÑEDA, B.K. LONSANE*†, J.M. NAVARRO‡, S. ROUSSOS§ & M. RAIMBAULT† *Department of Biotechnology, Autonomous Metropolitan University, Iztapalapa Campus, AP 55-535, C.P. 9340, Mexico City, Mexico,* †*Fermentation Technology and Bioengineering Discipline, Central Food Technological Research Institute, Mysore 570 013, India,* ‡*Laboratoire de Génie Microbiologique, Université des Sciences et Techniques du Languedoc, Place E. Bataillon, 34060 Montpellier, France and* §*ORSTOM, Centre de Montpellier, Biotechnology Unit, 911 Avenue Agropolis, BP 5045, 34032 Montpellier Cedex 1, France*

FS/139: received 30 October 1991 and accepted 18 June 1992

SAUCEDO-CASTAÑEDA, G., LONSANE, B.K., NAVARRO, J.M., ROUSSOS, S. & RAIMBAULT, M. 1992. Importance of medium pH in solid state fermentation for growth of *Schwanniomyces castellii*. *Letters in Applied Microbiology* **15**, 164–167.

Utilization of soluble starch by *Schwanniomyces castellii* in a solid state fermentation system was highest in unbuffered medium when initial and final pH of the medium were 6·5–7·0 and 4·0–4·6, respectively. An economic strategy involving the use of urea as a sole nitrogen source in medium with initial pH of 6·5 allowed maximum substrate utilization in the absence of buffer and without any contamination in column fermenter.

One of the serious limitations of a solid state fermentation (SSF) system is an inability to measure pH of moist solid medium due to

& Lonsane 1989). Enhanced chances of contamination in many bioreactor types (Lonsane *et al.* 1985) however pose constraints due to

1992). All the experiments were performed in triplicate and mean values are reported as the % variation less than ±2%. The liquid medium used for impregnating inert pith bagasse contained (g/l): soluble starch (Prolabo) as carbon substrate, 100; peptone (Merck, pancreatically digested casein), 1; yeast extract, 1; KH_2PO_4, 5; NaCl, 1; $MgSO_4$. H_2O, 2; and distilled water, 1000. Urea (to obtain 4·6 g total N_2/l) was incorporated and the pH was adjusted to desired levels using 5 N HCl. After autoclaving at 121°C for 15 min, cooling to about 30°C and adding 2 ml of vitamin-mineral solution (Roussos 1987), the liquid medium was inoculated with 5×10^7 cells/ml. Sugar cane pith bagasse of 0·3–0·8 mm particle size was washed with distilled water, autoclaved and dried to obtain inert solids for impregnation with inoculated liquid medium at a ratio of 14 : 86 on weight basis (Saucedo-Castañeda *et al.* 1992). The resulting medium in 60 g moist weight quantity was charged in a column fermenter of 20 cm length × 4 cm diameter, for fermentation at 28 ± 1°C for 30 h under aeration at a rate of 1·4 ml humidified air/min/g wet material. Other fermenter operation details were as described elsewhere (Saucedo-Castañeda *et al.* 1992). Similar experiments were also performed by buffering the medium with four different buffers and using different ratios of urea : ammonium sulphate as nitrogen sources. Moist solid fermented medium (4 g) was vortexed for 5 min in a sterilized beaker with 36 ml of sterile distilled water (containing a drop of Tween 80) and filtered through a 50 μm sieve to separate bagasse. The filtrate was used to count yeast cells in haemocytometer, to determine dry cell mass after drying at 95°C for 24 h and also for measuring pH. CO_2 concentration in exhaust air from the column fermenter was estimated with a gas chromatograph (ICG 11, Delsi, France), fitted with a thermal conductivity detector and coupled to a personal computer provided with an integration programme (Chroma, Societe Biosysteme, France).

Results and Discussion

Data presented in Table 1 shows that the substrate utilization in urea containing unbuffered medium was maximum when the initial pH was 6·5–7·0. The final cell counts at these initial optimum pH values were $1·5 \times 10^{10}$ per g initial dry matter (IDM). The substrate utilization was reduced drastically to 59·3% even when the initial pH was 6·0. The analysis of the effect of various physico-chemical parameters by blocked factorial design 2^{k-p} (De Meo *et al.*

Table 1. Effect of initial pH and different strategies for minimizing pH changes on substrate utilization by *Schwanniomyces castellii* in an aerobic solid state fermentation system

MITCHELL, D.A. & LONSANE, B.K. 1992 Definition, types and characteristics. In *Solid Substrate Cultivation* ed. Doelle, H.W., Mitchell, D.A. & Rolz, C.E. Essex: Elsevier Science Publishers (in press).

RAMESH, M.V. 1989 Production of heat stable bacterial alpha-amylase. Ph.D. Thesis, University of Mysore, Mysore, India, pp. 291.

RAMESH, M.V. & LONSANE, B.K. 1989 Solid state fermentation for production of higher titres of thermostable alpha-amylase with two peaks for pH optima by *Bacillus licheniformis* M27. *Biotechnology Letters* **11**, 149–152.

ROSSI, J. & CLEMENTI, F. 1985 Protein production by *Schwanniomyces castellii* on starchy substrate, in liquid and solid cultivation. *Journal of Food Technology* **20**, 319–330.

ROUSSOS, S. 1987 Croissance de *Trichoderma harzianum* par fermentation en milieu solide: physiologie, sporulation et production de cellulases. These Doct., Université de Provence, Marseille, France, pp. 193.

SAUCEDO-CASTAÑEDA, G. 1991 Controle du metabolisme de *Schwanniomyces castellii* cultive sur support solide. These Doct., Université de Sciences et Techniques du Languedoc, Montpellier, France, pp. 180.

SAUCEDO-CASTAÑEDA, G., LONSANE, B.K., KRISHNAIAH, M.M., NAVARRO, J.M., ROUSSOS, S. & RAIMBAULT, M. 1992 Maintenance of heat and water balances as a scale-up criterion for the production of ethanol by *Schwanniomyces castellii* in a solid state fermentation system. *Process Biochemistry* (in press).

YANG, S.S. 1988 Protein enrichment of sweet potato residue with amylolytic yeast by solid state fermentation. *Biotechnology and Bioengineering* **32**, 886–890.