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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*

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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 absence of free water (Lonsane *et al.* 1985). Consequently, pH control during fermentation is not possible (Mitchell & Lonsane 1992). Moreover, addition of acid/alkali to the medium for correcting pH poses problems of mixing with bulky solid substrate, changes in moisture content of the medium and contamination especially in tray-type bioreactors. An initial acidic pH, around 4·0, is therefore conventionally used in most cases without pH control during fermentation due to confinement of the SSF system to filamentous fungi and also to reduce chances of contamination at acidic pH (Mitchell & Lonsane 1992). In addition, buffers are employed for restricting the changes in pH but these are cost-intensive. A compromise in any of the above aspects leads to suboptimal process productivity. The SSF system has been extended to bacteria and yeasts in recent years (Ramesh & Lonsane 1989). Enhanced chances of contamination in many bioreactor types (Lonsane *et al.* 1985), however, pose constraints due to near neutral pH optima and higher moisture requirement of many yeasts and bacteria. For example the optimum initial pH for *Bacillus licheniformis* M27 in an SSF system is 7·0 (Ramesh 1989). Preliminary studies indicated near neutral optimal pH for growth of *Schwanniomyces castellii* in an unbuffered aerobic SSF process, a preculture for production of ethanol in an anaerobic SSF process. The aim of the studies was to select the simplest and most economical culture medium, without buffer, while attaining a high substrate utilization of about 90%.

* Corresponding author.

Materials and Methods

Schwanniomyces castellii CBS 2863 was obtained from Central Buereau Voor Schmmelculture, Delft, Holland. Methodology for its maintenance and inoculum preparation have been described elsewhere (Saucedo-Castañeda *et al.*

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

Attributes	pH of medium		Substrate utilization(%)
	Initial	Final	
(A) Initial pH of unbuffered urea (9·8 g/l) containing medium	5·0	5·3	29·5
	5·5	4·1	43·1
	6·0	3·6	59·3
	6·5	4·0	92·9
	7·0	4·6	92·1
(B) Buffering of urea (9·8 g/l) containing medium			
0·067 M phosphate buffer	5·5	5·6	93·7
0·025 M tartaric acid + 0·05 M phosphate buffer	5·5	6·0	81·4
0·05 M citric acid	5·5	7·5	76·1
0·05 M succinic acid	5·5	7·0	58·6
(C) Ratio of urea nitrogen to ammonium sulphate nitrogen in unbuffered medium*			
100 : 0	6·5	4·5	93·3
75 : 25	6·5	4·0	85·2
50 : 50	6·5	3·8	63·5
25 : 75	6·5	3·6	48·1
0 : 100	6·5	3·1	23·8

* Medium contained 9·8 g/l urea in case of 100 : 0 ratio and 21·7 g/l ammonium sulphate in case of 0 : 100 ratio.

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