## B029

## CONTINUOUS GLUCOAMYLASES PRODUCTION IN SOLID STATE FERMENTATION USING A COUNTER-CURRENT REACTOR

S.Roussos, G Gutiérrez, T Varzakas, M. Oliveira, J. Córdoba, J.D. Owens & D.L. Pyle Department of Food Science & Technology, The University of Reading, Reading RG6 6AP, U.K. FAX: +44 1734310080 g.gutierrez@afnovell.reading.ac.uk

Key words SSF, continuous-production, enzymes

Introduction. Advantages and drawbacks of solid substrate fermentation (SSF) have been described elsewhere (1). There has been a considerable amount of attention given to the physiology of the microorganisms involved and to the characteristics of the metabolites produced (2). Culture of filamentous fungi on solid supports has been applied to the production of biomass and primary and secondary metabolites. It has also been used for detoxification of a wide variety of materials Traditionally, SSF has been carried out as a batch process. Continuous production of biomass and metabolites has not been reported. The objective of this work is to describe the continuous production of glucoamylase by SSF in a counter-current reactor (CCR) adapted for this purpose.

Materials and Methods. Pre-germinated conidia of Aspergillus niger were used as inoculum and sugarcane bagasse, embedded with nutritive solution, was the solid support (Oriol et al: 2). A counter-current, reversible screw, stainless steel reactor, 3 I m long with 9 compartments was used (3) This reactor was fed at a rate of 0.5 kg/h for 70 h. The operating temperature was  $37^{\circ}$ C. A 20 h residence time of the solids was established by programming forward, backward and stationary periods of movement of the screw Residence Time Distribution (RTD) experiments were carried out by feeding one impulse of blue-coloured, humidified bagasse and measuring the concentration of the blue dve in the exit solids



Figure 1. RTD curve for the solids in the CCR

The solids containing pre-germinated conidia were fed to the first compartment of the CCR and the fermented bagasse was recovered at the end of the ninth compartment Samples of the material in each compartment of the reactor were collected for analysis every 5 h after the initial 24 h of operation. The temperature in each compartment was also measured. Glucoamylase activity, reducing sugars, pH and moisture content were measured as previously (2).

**Results and Discussion**. In Figure 1, the RTD graph for the bagasse is shown. A low dispersion of the solids was observed which resulted in a plug flow movement of the material along the reactor with minimal exchange between compartments. Hence, the mycelium in each compartment was in a similar physiological state. In the first compartment, only germinated conidia were present. In successive compartments increasing colonisation of the substrate was evident and by Compartment 5 the substrate was covered in dense mycelium. A marked increase in biomass occurred from Compartment 5 onwards. No mycelium damage or sporulation were observed.

Table1 Values of the parameters along the SSF

Parameter	Compartment								
	1	2	3	4	5	6	7	8	9
Temperature (°C)	30	35	37	39	40	38	39	39	36
pН	50	<b>5.0</b>	52	40	3.4	30	29	2.8	28
Water Content (%w.b)	74	77	72	73	71	72	71	66	ങ
Reducing Sugars (mg/g dry matter)	20	20	13	19	20	14	11	17	20
Amylases (IU/g dry matter)	0.4	07	04	06	2.6	3.2	4.2	3.8	37

In Table 1, the values of the measured parameters are shown. These values are similar to those reported for batch SSF using the same substrate and microorganism (2). However, moisture content dropped from about 74% in Compartment 1 to 63% in Compartment 9.

**Conclusions.** This study demonstrates that the continuous production of enzymes by SSF is feasible. Inoculation with pre-germinated conidia shortened the processing time and allowed control of the age of the mycelium in each compartment Aeration was accomplished by natural convection and moisture content should be controlled. This process can be applied to the continuous production of fungal biomass and metabolites in SSF.

## References.

1 Aido, K, Hendry, R, and Wood, G.B (1982) Solid substrate fermentation Adv Applied Microbiol 28, 201-237.

2 Oriol, E., Schettino, B., Viniegra, G and Raimbault, M. (1988) Solid state culture of *Aspergillus mger* on support J. Ferment. Technol, 66, 57-62

3 Gutiérrez, G., Robles, R., and Pyle, L. (1996) Performance of a counter current solid-liquid extractor for food processing Fronteras en Biotecnología y Bioingeniería. Galindo, E. SMBB, México. 245-255

Roussos Sevastianos, Gutierrez G., Varzakas T., Oliveira M J., Cordoba J., Owens J.D., Pyle D.L. (1997).

Continuous glucoamylases production in solid state fermentation using a counter-current reactor.

In : Memorias : 7. congreso nacional de biotecnologia y bioingenieria Mazatlan'97 y 2. simposio internacional sobre ingenieria de bioprocesos.

Mexico : ORSTOM ; UNAM, p. 375.

Biotecnologia y Bioingenieria Mazatlan'97 : Congreso Nacional; Ingenieria de Bioprocesos : Simposio Internacional, 7.; 2., Mexico (MEX), 1997.