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USE OF MICROCALORIMETRY FOR MONITORING THE SOLID STATE CULTURE OF ASPERGILLUS NIGER

Oriol, E.*, Contreras, R.[1], Raimbault, M.

ORSTOM, 213 rue Lafayette 75480 Paris-Cedex 10, FRANCE.

[1] Centro Nacional de Investigacion cientifica, P.O. Box 6880, Cd. Habana, CUBA.

*To whom all correspondance should be addressed: Present address: Département de Génie Biochimique et Alimentaire INSA, Av. de Ranguell 31077 Toulouse-Cedex, FRANCE.

SUMMARY

A model solid state culture of Aspergillus niger was monitored by microcalorimetry and a transient phase between germination and apical growth was characterized by an exothermic phenomenon whose intensity could be related to culture conditions.

INTRODUCTION

Microcalorimetry has been widely used for characterization and physiological studies of bacteria and yeasts (Belaich, 1980; Lamprecht, 1980) but very few attempts have been made concerning growth and metabolism of filamentous fungi. The critical conditions of agitation and aeration required to ensure mold growth have been difficult to provide in the microcalorimetry devices employed until now. Solid state culture (SSC) conditions have been demonstrated to be adequate in static reaction vessels (Cannel & Moo-Young, 1980) and SSC might thus be a useful model to study the growth pattern of fungi (Contreras et al., 1986).
To provide a good oxygen transfer in the solid medium, a great interface between the liquid film and oxygen was necessary. This situation can be achieved by using a liquid synthetical medium inoculated with spores, absorbed on a porous matrix of low density. A lignocellulosic material, i.e. sugarcane bagasse has been chosen as a suitable support material for the growth of Aspergillus niger, a strain with no cellulolytic action.

MATERIAL AND METHODS

Microorganism. Aspergillus niger var. hennebergi (N° 10) described by Raimbault & Alazard (1980) was used.

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Preparation of spore inoculum. Spore inoculum was obtained as described by Raimbault & Alazard (1980) and was used at 3 x 10^5 spores / reaction vessel unless otherwise specified.
Cultivation conditions: Sugarcane bagasse, free of sugars, was obtained from a sugar factory in Zacatepec (Mexico). The 20-50 mesh fraction was used.
Composition of the medium was: KH_2PO_4, 5 mg; $(NH_4)_2SO_4$, 9.8 mg; Urea, 2.4 mg for 100 mg of glucose independantly of the glucose concentration in the solution.
15 mg of support were introduced in a 3 ml reaction vessel and sterilized apart from the liquid medium in an autoclave at 1 bar for 20 mn. The pH of the salts solution containing spores was adjusted to 2.7. The support was then moistened with 40 μl of the liquid glucose-salts medium inoculated with spores. A saturating oxygen flow was passed in the vessel for 1 mn before it was hermetically sealed.
Microcalorimetry apparatus: A 4 channel LKB Bioactivity Monitor was used. Attenuation was fixed to 1000-3000 microWatts and power-time curves (thermograms) were registered continuously. All experiments were duplicated and good reproductibility was observed.
Determination of water activity Initial Water activity of the medium (Aw) defined as the relative humidity of the gaseous atmosphere in equilibrium with the medium was determined with a water activity detector (Humidat IC II purchased from Novasina^r). Samples (3 to 4 g) were introduced in a relative humidity captor placed in a temperature regulation chamber at 30°C; the state of equilibrium was reached after 3 h.

RESULTS

Influence of the liquid phase glucose concentration on the power-time curves

Given standard inoculum conditions, four glucose concentrations ranging from 30 to 220 g/l were used and the power-time curves (full line) are given in Fig. 1. The system enabled the detection of an activity as soon as 1 h after inoculation. Other monitoring devices such as exit gas analysis are not sensitive enough to give any signal during this period. The germination time increased with the glucose concentration and for the three higher glucose concentrations two peaks were observed while for the lower one, one only peak was detected. That first peak has not previously been reported in microcalorimetry experiments dealing with spore germination of Fusarium roseum (Ljungholm et al., 1980).
When an ideal curve (fig 1 : dashed lines) was drawn under the first peak, it became possible to measure the area of this peak. An increase in the glucose concentration of the liquid phase produced a decrease in the initial Aw of the medium which could be correlated with the heat evolved in the first peak. as can be seen in Figure 2. For the three higher glucose concentrations, the heat corresponding to the first peak is about 5% the total heat produced during growth.

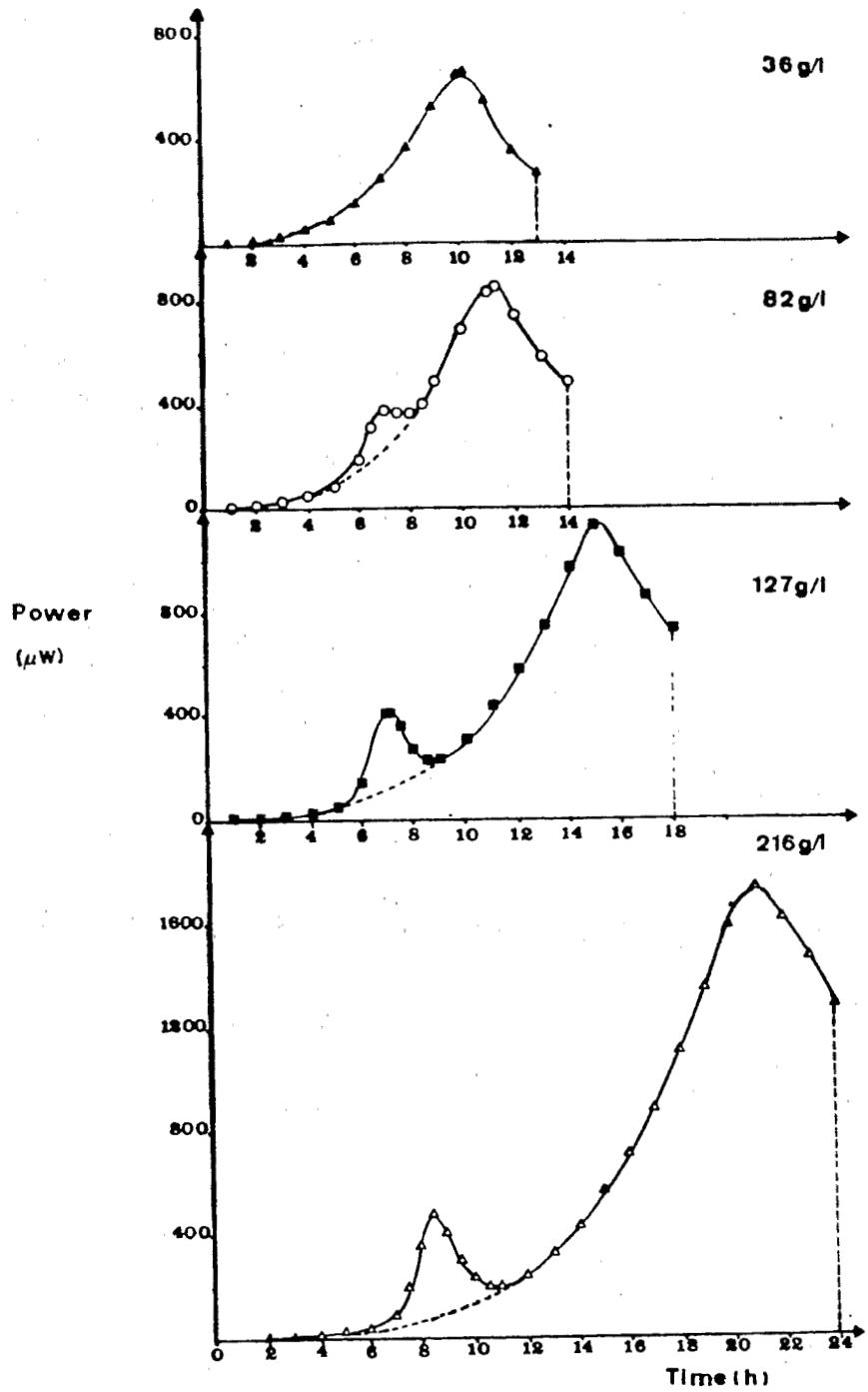

Figure 1: Power-time curves observed during growth of A.niger on bagasse with different glucose concentrations in the absorbed liquid medium.

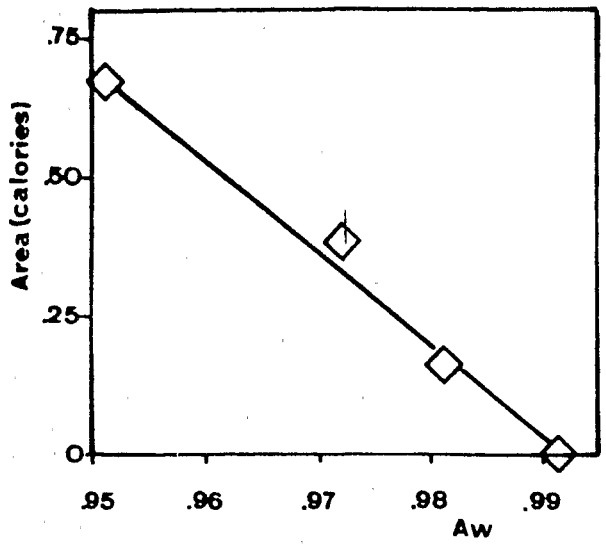

Figure 2: Heat evolved in the first peak as a function of the water activity (Aw) of the medium.

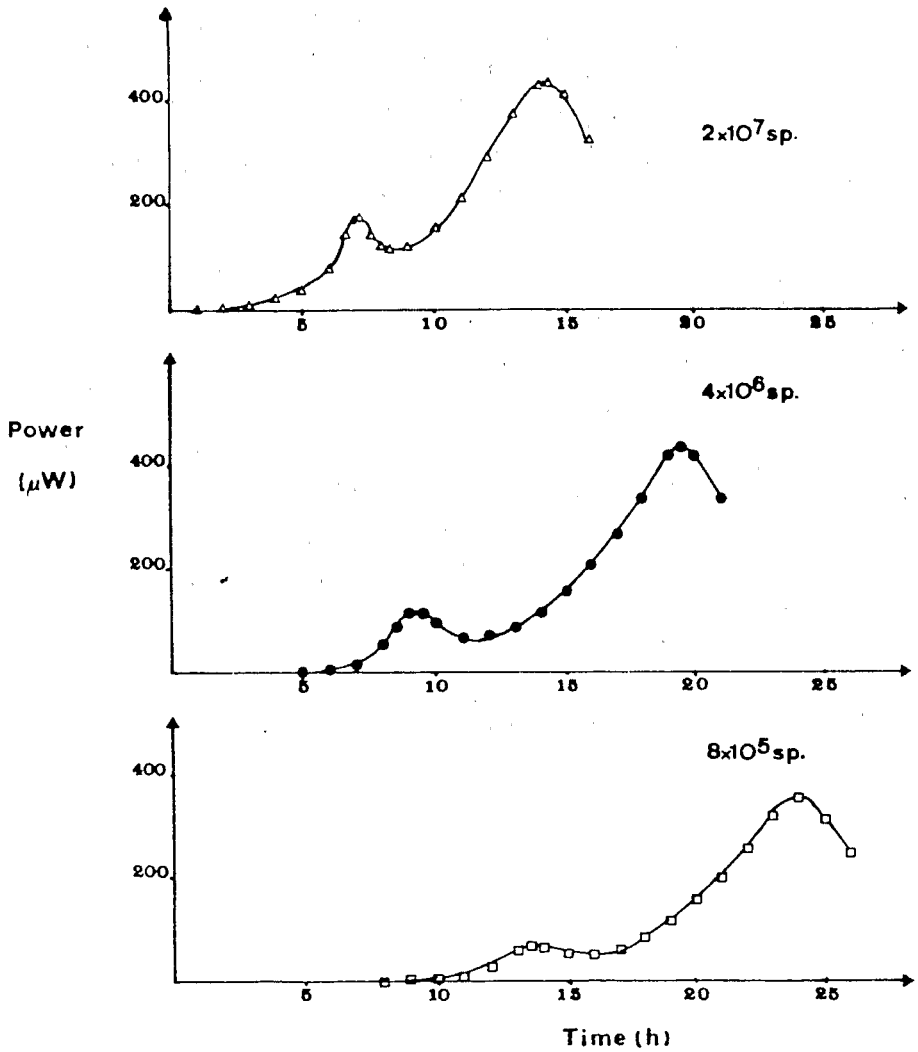

Figure 3: Power-time curves observed during growth of A.niger on bagasse with different amounts of spore inoculum per g of support.

A microscopic examination made at the maximum height of the first peak showed germinated spores in an homogeneous phase with a germinating tube approximately five times larger than the spore.

Influence of the amount of spore inoculum on the power-time curves

Given standard glucose concentration in the liquid phase (89 g/l), three trials were run with decreasing amounts of spores in the reaction vessel. As may be seen in Fig 3, the delayed appearance of the first peak as well as its height were affected by the size of the inoculum. This might signify that under SSC conditions, the germination process occurs faster when the inoculum is more concentrated, perhaps due to the presence of an auto-activator of germination (Sussman, 1976).
Furthermore, the cultivation time increased drastically when the inoculum was small, passing from 16 to 26 h for a 25-fold decrease of the number of spores.

DISCUSSION

The sensitivity of the apparatus allowed the detection of a signal corresponding to a transitional phase between germination and apical growth. Studies achieved in column incubators showed that this signal was not associated with respiration or biomass production. As the intensity of the peak seemed to be dependent on the water activity and thus the osmotical pressure of the medium and on the number of spores, two hypothesis might be established:
- A large part of the spore germination is endogenous (Sussman, 1965) and the signal should thus be associated with a structural change such as a rearranging of the permease transport system at the beginning of the exogenous phase.
- An accumulation of macromolecules (polyols) inside the germinating spore accompanied with a water expulsion for adapting to the osmotic conditions of the medium.

As biomass is always difficult to determine precisely in SSC, the utilization of the support model demonstrated that the microcalorimetry method is feasible for monitoring the growth of molds and for describing its variations to culture conditions.

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