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SPORULATION OF *Penicillium roqueforti* IN SOLID SUBSTRATE FERMENTATION

E. MAHEVA, G. DJELVEH, C. LARROCHE and J.B. GROS+

Laboratoire de Génie Chimique Biologique, Université de Clermont II, BP 45, 63170 AUBIERE, FRANCE.

SUMMARY

A study of the influence of temperature, aeration rate, and substrate water content on sporulation of *Penicillium roqueforti* on buckwheat seeds in a fixed bed reactor is described. Use of an experimental procedure based on a 2^3 factorial design allowed optimum to be determined as 23.5° C for temperature, 0.48 g/g dry matter for substrate water content and 4.42 VVH for aeration rate.

INTRODUCTION

In recent years, there has been renewed interest in solid state fermentation (Hesseltine, 1977 a, b ; Aidoo et al., 1982). This process implies growth of micro-organisms on substrates containing no or practically no free water, and so mainly involves the use of fungi.

Several new applications are under development (Aidoo et al.,1982) among which is the production of fungal spores. Their main utility lies in the fact that they possess a conversion activity 3 to 10 times that of the mycelium in terms of dry matter. Consequently, they may be useful as biochemical catalysts for the production of novel organic compounds (Ralph, 1976). In addition their physical form makes them a conveniently marketable material.

We report here the results of a study of the influence of 3 parameters (temperature, aeration rate, water content of the substrate) on the sporulation of *Penicillium roqueforti* . Cultures were performed

on finely divided substrates, i.e. under bulk conditions, this ensuring more extensive surface contact between mycelium and substrate than that commonly achieved in cultures in Roux bottles.

MATERIALS AND METHODS

- Microorganism

A strain of Penicillium roqueforti (Thom, 1930) was used. This was isolated from French blue cheeses by the firm Lactolabo, and was conserved by replicating on a gelosed czapek medium (Meyers and Knight, 1957).

- Preparation of the substrate

Dry cereal seeds, sorted so as to obtain an homogeneous sample, were soaked in water for the period necessary to obtain the required water content.

The maximum content was obtained by allowing the seeds to incubate for at least 24 hours. In the case of buckwheat this maximum water content was 0.481 g per gram of dry substrate.

The medium prepared in this way was steam sterilized for 15 mn at 120° C. The starch was gelified under these conditions, thus ensuring reliable growth of the amylolytic microorganism (Raimbault, 1981).

- Inoculation

From a culture grown on a Petri dish, aged 9 to 12 days, a suspension of spores was prepared containing 7 x 10^6 spores/ml in sterilized water. The inoculation level for the cultures was 10^5 spores/g dry substrate, i. e. 1 ml of solution for 70 g of substrate.

- Culture technique

A thermostated fixed-bed differential reactor containing about 70 g of substrate (figure 1) was used. It is assumed that the temperature within the substrate is uniform and equal to that of the water circulating in the jacket. The reactor was continuously supplied with air saturated with water and free of carbon dioxide by passing it through potassium hydroxide solution.

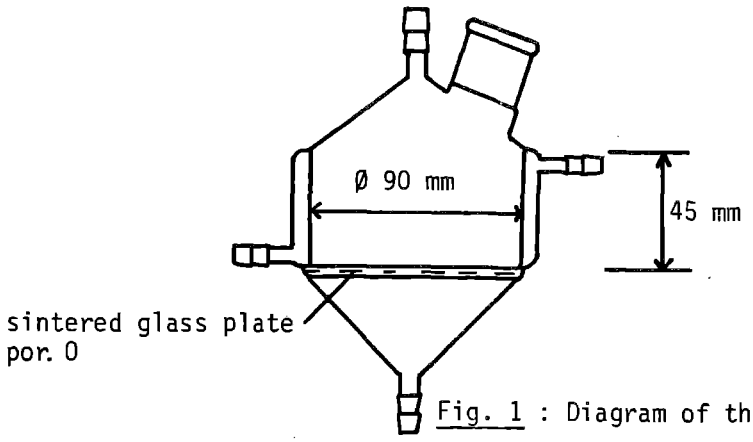

Fig. 1 : Diagram of the reactor

- Sporulation monitoring

One gram of sample was placed in 3 to 20 ml of 1 % Tween 80 solution. The extraction of external spores was carried out by vigorous agitation. Hematimetric counting (Malassez cell) under the microscope (enlargement 100 to 400) was performed on the supernatant fraction.

- Expression of the results

The data obtained during fermentation were expressed per unit mass of dry matter (DM).

RESULTS AND DISCUSSION

- Choice of substrate

A preliminary study on Petri dishes of the aptitude of various substrates to favor sporulation of *P. roqueforti* gave the following results (Table 1).

Substrate	Spores/g DM 10^8	Substrate	Spores/g DM 10^8
Wheat bran	18	Triticale	13
Oats	17	Wheat	12
Buckwheat	16	Rice	12
Sorghum	15	Barley	11
Maize	14	Millet	11
Hulled wheat	14	Potato	8
Rye	13		

Table 1 : Production in Petri dishes of spores of *P. roqueforti* on different solid starchy substrates obtained after 230 hours of fermentation at 25°C.

Inspection of these results shows that the use of complex cereal-type substrates gives fairly comparable yields. It seems that starchy substrates highly deficient in nitrogen-containing compounds, such as potato, give lower yields. In such cases mineral salts such as ammonium sulfate or potassium phosphate, and urea might have to be added, as suggested by Raimbault for Cassava meal. These mineral salts would also improve the buffering power of the medium.

The excellent mechanical properties of buckwheat (retention of structure, lack of agglomeration) along with its high sporulation yield led us to adopt this substrate for the main part of this study. This material allows a specific surface area of 750 cm^2/cm^3.

- Sporulation of P. roqueforti on buckwheat in a fixed reactor

An experimental procedure based on a 2^n factorial design as proposed by Box and Wilson (1951) and Himmelblau (1970) was used.

Three parameters were studied.

+ The temperature at which growth occurred (T)

It is known that the temperature inside a bulk substrate increases as fermentation proceeds. This increase is directly linked to the thickness of the substrate and the metabolic activity of the mircroorganism (Hayes, 1977).

The use of a differential reactor allows us to take the temperature of the water circulating around it as being that at which the fermentation takes place.

+ Aeration rate (F)

Aeration essentially plays two roles in solid state fermentation (Chahal, 1983).

- oxygen supply to the microorganism (for aerobic metabolism)
- removal of CO_2, water vapor and any secondary volatile metabolites.

Here again, the use of a differential reactor means we can assume that the oxygen present in the air supply is available to all the microorganism present.

+ Water content of the substrate (DM)

This parameter, which can be related to the activity of the water via a sorption isotherm (Multon, 1981). However, excess water causes the grains to stick together and so limits oxygen transfer and increases the risk of bacterial contamination.

Here, the pH remained constant at about 5, due to the buffering capacity of the substrate (Chahal, 1983).

The osmotic pressure remained constant since neither salts nor sugars were added.

The range of variation of each parameter was set as follows :

$22 < T < 28$ (°C) $\qquad 0.450 < F < 0.765$ (1/h)

$0.406 < S < 0.481$ (g/g DM)

The two extremes of the range of variation of S correspond to water activities of respectively 0.920 and 0. 955.

The following reduced variables were defined :

$$T_R = \frac{T - T_m}{\Delta T} \qquad F_R = \frac{F - F_m}{\Delta F} \qquad S_R = \frac{S - S_m}{\Delta S}$$

where $T_m = \frac{T_1 + T_2}{2}$, $\Delta T = \frac{T_2 - T_1}{2}$ etc...

The experimental range was thus represented as a cube centered at the origin (T_R, F_R and S_R vary from - 1 to + 1). (Figure 2).

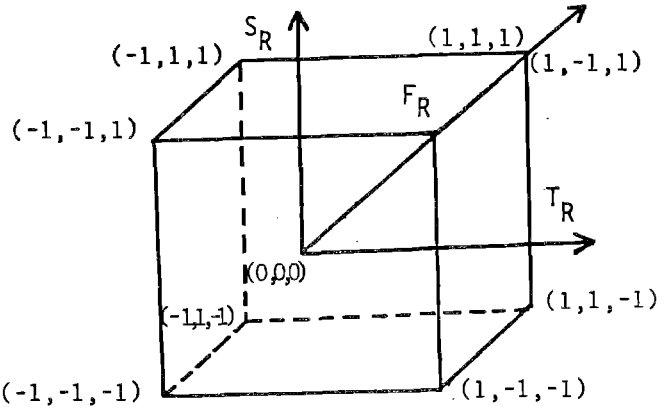

Fig. 2 : Schematic representation of the 2^3 factorial plan

Results are presented in table II.

Y represents the number of spores produced per gram of dry matter after 230 h.

- Optimum conditions for sporulation of P. roqueforti on buckwheat seeds

The experimental results concerning Y defined above fit the quadratic equation ;

$$Y = 6.47 - 1.307\ T_R + 1.354\ F_R + 1.130\ S_R - 1.239\ T_R^2 - 0167F_R^2 + 2.616\ S_R^2$$

Values of T_R , F_R and S_R were sought for which the first derivatives of Y were zero and their second derivatives negative, i.e. values corresponding to optimum conditions. Optimum values of T_R and F_R were obtained but S_R gave no maximum.

Optimum conditions for sporulation are thus:

$T_R = 0.527$, T = 23.42°C; $F_R = 4.17$, F = 1.263 l/h; $S_R = 1$, S = 0.481 g/gDM

Under these conditions, Y = 15.07×10^8 spores/g DM.

These results are presently used to study the behaviour of a 30 l drum fermentor.

Exp. (N°)	T (°C)	F (l/h)	S (gwater/g DM)	T_R	F_R	S_R	$Y(10^8)$ (spores/g DM)
1	22	0.450	0.406	-1	-1	-1	7.37
2	22	0.765	0.406	-1	+1	-1	7.43
3	22	0.450	0.481	-1	-1	+1	9.80
4	22	0.765	0.481	-1	+1	+1	12.56
5	28	0.450	0.406	+1	-1	-1	4.10
6	28	0.765	0.406	+1	+1	-1	7.30
7	28	0.450	0.481	+1	-1	+1	5.34
8	28	0.765	0.481	+1	+1	+1	8.75
9	25	0.608	0.444	0	0	0	6.80
10	25	0.608	0.444	0	0	0	5.91
11	25	0.608	0.444	0	0	0	6.70
12	22	0.400	0.481	-1	-1.32	+1	6.93
13	22	2	0.481	-1	+8.87	+1	9.51
14	20	0.765	0.481	-1.67	+1	+1	10.17
15	23.5	1	0.462	-0.5	+2.5	+0.5	15

TABLE II : Cultures performed at the extremes of the 2^3 factorial design cube (1 to 8), at the center of the cube (9 to 11), outside it(12 to 14), and close to the optimum (15).

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