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GROWTH KINETICS OF *RHIZOPUS ARRHIZUS* IN SOLID STATE FERMENTATION OF TREATED CASSAVA

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SUMMARY

Growth kinetics of *Rhizopus arrhizus* MUCL 28168 were determined for different treatments of cassava during solid state fermentation. The best case gave a specific growth rate (μ) of 0. 24 h^{-1}, a yield calculated on a basis that oxygen consumption ($Y_{x/o}$) was 2.9 g biomass . g^{-1} O_2 consumed and the maintenance coefficient (m) was 0.004 g O_2 consumed . g^{-1} biomass . h^{-1}.

INTRODUCTION

The ability of certains strains of *Rhizopus* to produce amylases is well known (Scriban, 1984) but sometimes it is necessary to modify the nature of starch to allow better growth. However, according to some recent reports, raw cassava can be utilized as substrate (Nishise *et al*, 1988 ; Soccol *et al*, 1992). Cassava seems to be a good substrate for processes using *Rhizopus* strains with some amylolytic capacity.

Solid state fermentations (SSF) of cassava with other microorganisms have been reported by several authors. In those cases, the necessity of a previous treatment of the substrate was taken account in order to allow a better growth (Czajkowska and Ilnicka-Olejniczack, 1989 ; Raimbault and Alazard, 1980). To determine whether treatment of cassava brings some advantages when the strain *Rhizopus arrhizus* MUCL 28168 is used, several treatments were tested., with the milling of dry raw cassava pellets as reference.

A useful kinetic evaluation of SSF on a biotechnological basis is by the evaluation of such parameters as : specific growth rate (μ), yield based on oxygen consumption ($Y_{x/o}$) and the maintenance coefficient (m), as reported for other SSF processes (Rodriguez Leon *et al*, 1988 ; Sato *et al*, 1983). This paper describes the growth kinetics of *Rhizopus arrhizus* MUCL 28168 for cassava meal treated in various ways, as substrate.

MATERIALS AND METHODS

Microorganism. The strain *Rhizopus arrhizus* MUCL 28168 from the Université Catholique de Louvain (U.C.L., Louvain-La-Neuve, Belgique) was used. Dry biomass for such strain was estimated to be 33.0% on a protein content basis.

Cassava treatments. Five different treatments were considered :

I. A proportion of 1:3 dry raw cassava pellets to distilled water was mixed throughly and allowed to stand for 15 min to allow good rehydratation. The mixture was autoclaved at 120°C for 20 min, cooled and frozen for 12 h to permit the starch retrogradation. Afterwards it was dried at 65°C for 24 h,, milled and screened between 2-0.8 mm and moistened at 50% (mass H_2O/total mass) with salts solution (as described below) containing the spores.

II. Water was added to dry raw cassava pellets up to 40% (mass H_2O/total mass) with salts solution. The mixture was autoclaved at 120°C for 30 min. After cooling, the moisture was adjusted to 50% (mass H_2O/total mass) with the same solution and the spores of the microorganism.

III. Same as treatment II but the initial moisture was 30% (mass H_2O/total mass).

IV. The raw cassava pellets were hydrated with the same salts solution with a moisture of 30% (mass H_2O/total mass) and boiled at atmospheric pressure for 40 min. After cooling the moisture was corrected to 50% with the same solution with the spores.

V. Dry raw cassava pellets were hydrated with the same salts solution containing the spores to 50% (mass H_2O/total mass).

Medium and culture conditions. Salts solution was composed as follows : for 100 ml of distilled water, 5 g KH_2PO_4 ; 9.75 g $(NH_4)_2SO_4$; 2.4 g urea. The pH was adjusted to 4.5 with 5 N $NH_4(OH)$. 2×10^7 spores were inoculated for g of dry cassava pellet. SSF were developed in glass columns (4 cm diameter and 20 cm height) and placed in a water bath at 28°C (Raimbault and Alazard, 1980). Filtered and saturated air flow was 60 ml/min per column. CO_2 and O_2 were determinated in the exhausted air by gas chromatography Delsi model IGC (Saucedo-Castaneda, 1991).

Analytical procedures. Protein content was determined by the Folin phenol reagent with bovine serum albumin as standard (Lowry *et al*, 1951).

RESULTS AND DISCUSSION

Figure 1 shows the time course of oxygen uptake rate (OUR) for the SSF with different pretreated substrates (I-V). Each data was processed considering the OUR balance :

$$OUR = \frac{\Delta O_2}{\Delta t} = \frac{1}{Y_{x/o}} \frac{dX}{dt} + m X \qquad (1)$$

employing the analytical equation obtained from (1) (Sato *et al*, 1983) :

$$X_n = \left[Y_{x/o} \Delta t \left[1/2 (R_o + R_n) + \sum_{i=1}^{n-1} R_i \right] + (1 - a/2) X_o - a \sum_{i=1}^{n-1} X_i \right] / (1 + a/2) \qquad (2)$$

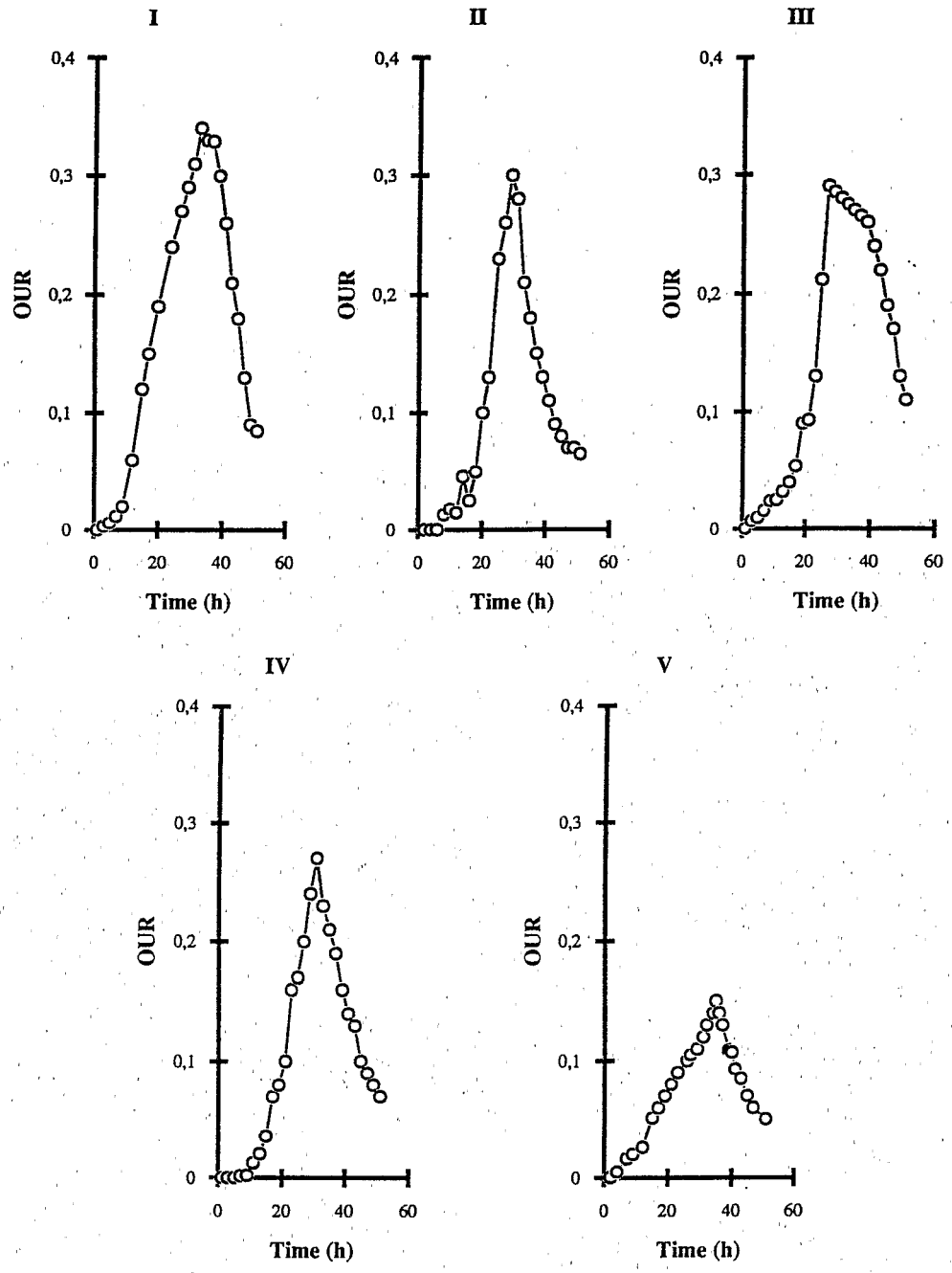

Figure 1. Time course of Oxygen Uptake Rate (OUR) for the different treatments of cassava during SSF

where : $a = m\ Y_{x/o}\ \Delta t$; R_i : OUR_i (g 0_2 consumed . h^{-1}) at time i ; X_i : biomass (g) at time i ; $Y_{x/o}$: biomass yield based in oxygen consumption (g biomass . g^{-1} 0_2 consumed); m : maintenance coefficient (g 0_2 consumed . g^{-1} biomass . h^{-1}) and Δt : time period (h).

Table 1. Fermentation characteristics for the differents treatments.

Treatment	initial moisture (%)	initial dry mass (g)	final dry mass (g)	initial protein (%)	final protein (%)	Δ prot. (g)	biomass synthesized (g)
I	49.8	39.0	30.0	1.2	11.7	10.5	10.6
II	51.1	49.0	40.0	2.2	12.4	10.2	15.0
III	50.1	39.4	32.2	2.3	13.9	11.6	13.6
IV	54.0	46.0	39.0	2.4	11.9	9.5	14.1
V	50.6	45.0	37.9	1.7	10.9	9.2	12.5

Table 2. Biotechnological parameters corresponding teach treated substrate.

Treatment	μ_T	Yx/o	m	final biomass	predicted biomass	error	r	lag phase
	(h^{-1})	g biomass.g^{-1} O_2	g O_2.g^{-1} biomass.h^{-1}	(g)	(g)	(%)		(h)
I	0.131	3.06	0.0083	10.60	10.52	0.70	0.934	2
II	0.071	2.26	0.0051	15.03	14.76	1. 83	0.991	13
III	0.069	2.13	0.0104	13.61	13.67	0.40	0.987	10
IV	0.052	1.05	0.0314	14.05	7.99	43.01	0.978	17
V	0.049	3.45	0.0042	12.49	12.92	3.37	0.997	5

Table 3. Biotechnological parameters estimated considering two periods of time (1 : 0 - 26 h and 2 : 26 - 40 h)

Treatment	μ_1	μ_2	$Y_{x/o1}$	$Y_{x/o2}$	m_1	m_2
	(h^{-1})	(h^{-1})	g biomass . g^{-1} O_2	g biomass . g^{-1} O_2	0.237	g O_2 . g^{-1} biomass . h^{-1}
I	0.237	0.043	2.90	2.22	0.004	0.004
IV	0.117	0.051	1.72	1.96	0.035	0.024
V	0.005	0.003	3.41	2.22	0.001	0.002

Equation (2) was solved for each set of OUR data considering the parameters m and $Y_{x/o}$ as constant by a gradient method, minimizing the mean square deviation between the calculated amount of biomass and the experimental results, and considering data from initial point to maximun OUR and from maximun OUR to last point to determine the validity of those assumptions (Rodriguez Léon *et al*, 1988). In Table 1 we report the values obtained for each fermentation considering constant the parameters and the conditions in which each fermentation was developed. From Table 1 it could be seen that all fermentations develop similarly and the results of the

biomass synthesized and the OUR can bring the parameters that identify the processes. In other words, equation (2) must be solved for each case. Table 2 offers the results of such a procedure for each treatment.

The results reported in Table 2 suggest that treatment I is the best, and if we consider the error values it seems very fair to accept these results, except in the case of treatment IV. However we decided to consider the data of treatment I (the best results in Table 2), IV (the highest error) and V (the untreated raw cassava as reference) in order to see if the biotechnological parameters reported in Table 2 can be assumed as constant during the whole period of fermentation.

In Table 3, we reported the estimation of the biotechnological parameters considering two time: 0-26 h and 26-40 h. The error in these cases was not higher than 2% (including treatment IV) and the regression coefficient for the specific growth rate was always higher than 0.98. From these results we can conclude that again the preteatment I is the best of all the treatments tested. The Yx/o and m variations do not seem very important if we the whole data and the period between 0-26 h. However they must be considered if we are to compare the different treatments. The specific growth rate is the most variable parameter and shows that the process developed in the first 26 h.

For treatment V the results (Table 3) show that the specific growth rates (μ) was by far the worst and can be assumed constant for the whole period. An efficient result as the value of $Y_{X/O1}$ equal to 3.41 g biomass /g 0_2 consumed may not be sustained for a long time since the growth depends upon the available simple sugars and the nature of the production enzyme kinetics and starch hydrolysis.

Considering treatment I and IV is obvious that treatment helps the enzyme synthesis or starch hydrolysis or both. Treatment I gelatinizes the starch better (Raimbault, 1980) permiting best growth of *Rhizopus arrhizus* MUCL 28168.

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