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Water and water activity in the solid state fermentation of cassava starch by *Aspergillus niger*

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Summary. During the solid state fermentation (SSF) of cassava starch by *Aspergillus niger* estimations were made of total water, consumed water and the residual water remaining in small quantities after 23 h. A theoretical calculation based on the Ross equation showed that the water activity (a_w) of the substrate decreased to 0.85 towards the end of the culture. Such low values were assumed to be inhibitory to growth. The a_w of the substrate was increased when sugarcane bagasse was used as a high water retention capacity support. Higher growth rates and substrate conversion to biomass were obtained with this system, confirming that water availability is a critical factor in the SSF of starch substrates.

Introduction

One of the differences between solid-state and submerged cultures is that in the former the moisture content of the substrate is low, resulting in a limitation of growth and metabolism of the microorganism. The concept of water availability in a substrate thus becomes very important. Water activity (a_w) is defined as the relative humidity of the gaseous atmosphere in equilibrium with the substrate.

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Abbreviations: A, B, Experimental constants; a_w, Water activity; H_2O_C, Consumed water; H_2O_R, Residual water; H_2O_T, Total water; IDW, Initial dry weight; IMC, Initial moisture content; OUR, Oxygen uptake rate; *S,* Substrate dry weight; Sc, Substrate conversion: consumed substrate/initial substrate; S_H, Amount of sugars hydrolysed; SSF, Solid state fermentation; *X,* Biomass dry weight; *W**, Amount of solids/g of water

The importance of water in SSF has been widely studied under quantitative aspects (Nishio et al. 1979; Raimbault and Alazard 1980; Hoe-Kim et al. 1985) but very few reports deal with the concept of a_w to explain growth of molds on solid substrates (Narahara et al. 1982).

Water content in SSF ranges from 30% to almost 80%, depending on the material, but the retention capacity of starchy substrates (rice, cassava) is fairly poor and generally does not exceed 1.0 to 1.2 g water/g of solids (50% to 55% moisture content). In the production of proteins from solid cassava flour by fungal culture (Raimbault and Alazard 1980), growth of *A. niger* stopped, leaving 30% of starch in the medium. We investigated water availability as the possible limiting factor for growth under SSF conditions.

Material and methods

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

Pretreatment of raw material. Cassava (*Manihot esculenta* var. *cubana*) flour was prepared from fresh cassava root which was cooked in an autoclave at 1 bar for 30 min and then cooled, frozen overnight and dried at 50° C in a convection oven. The gelatinized root was then milled, sieved and the 20–50 mesh fraction used. Sugarcane bagasse, free of sugars, was obtained from a sugar factory in Zacatepec (Mexico). The 20–50 mesh fraction was used and the bagasse moistened to 50% was sterilized in an autoclave at 1 bar for 30 mn previous to the culture.

Preparation of spore inoculum. Spore inoculum was obtained as described by Raimbault and Alazard (1980), fixing the inoculum size at 2×10^7 spores per gram of cooked cassava.

Solid state culture. The culture was achieved under non-aseptic conditions as described by Raimbault and Alazard (1980). Column fermentor units containing 20 g of moistened medium were incubated in a 35° C water bath. The inlet air was bub-

bled twice in water and the air flow was set to 4 l/h; two columns were removed at each sampling time. Composition of the solid substrate was: Cassava meal, 100 g; KH_2PO_4, 5 g; $(NH_4)_2SO_4$, 9.8 g; Urea, 2.4 g. Ph of the salt solution with spores was adjusted to 2.7 which resulted in a value of 4.5 for the solid substrate after inoculation.

Analysis. The exit air of one column reactor was dried by passing through a condensation and a Silicagel column. The oxygen content was then determined by pumping it through a paramagnetic Servomex Oxygen Analyzer (Taylor Instruments). For sample analysis, 4 g of sample were homogenized in 96 ml of water with an Ultra-Turrax. After dilution, the residual starch was hydrolysed with HCl 1 N for 3 h and the resulting reducing sugars were measured by the Miller (1959) method. Protein content of samples of the cassava cultivation was assayed by the method of Lowry et al. (1951). Protein content of the bagasse cultivation was estimated by hydrolysing with HCl 6 N for 20 mn at 120° C; this was neutralized and the aminoacids of the supernatant were determined by colorimetric reaction with ninhydrin (Snell 1956) to avoid interference from lignin. Protein was related to biomass assuming that 40% of the biomass is constituted by protein.

Determination of water activity. Water activity was determined with a water activity detector Humidat IC II purchased from Novasina. Prior to the measurements, 3 to 4 g of sample were cut if necessary and exposed for 10 min to an U.V. radiation of 254 nm wavelength and 300 W intensity to avoid any evolution of the sample during the measurement. The relative humidity captor was placed in a temperature regulation chamber at 30° C and the state of equilibrium was reached after 3 h.

Results and discussion

Water activity (a_w) of the substrate

The two components of the initial substrate medium were the cassava flour and the mixture of salts. We thus determined the sorption isotherm of each ingredient which could be summarized with good approximation (Correlation >0.995) by equation (1).

$$a_w = A(W^*) + B \qquad (1)$$

where W^* is the amount of solids/g of water, A and B are constants.

Figure 1 shows that, as expected, the mixture of salts had a greater influence than the flour on the a_w of the medium, the gradient being 10 times greater for the first ingredient. The water activity of a mixture may be estimated through the Ross (1975) equation:

$$a_w = (a_w^0)_1 (a_w^0)_2 (a_w^0)_3 \ldots \qquad (2)$$

where $(a_w^0)^n$ is the *aw* of each component of the mixture.

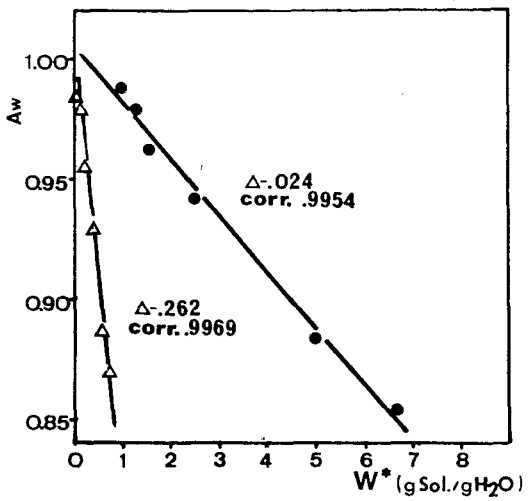

Fig. 1. Sorption isotherms of water for cassava flour (—●—) and for the salts mixture (— Δ —) as linearized from equation (1)

The initial moisture content of the substrate was 50%; that is, 1.0 g of water/g of solids and the composition of the solid substrate was 0.85 g of flour and 0.15 g of salts. The calculated value gave an a_w of 0.944 for the initial substrate while the measured one was 0.937. It is important to notice that the initial a_w of the substrate was low mainly because of the mixture of salts and that it can be satisfactorily approximated by using the Ross equation.

Growth of A. niger

Figure 2 shows the growth of *Aspergillus niger* on cassava starch with a 50% initial water content.

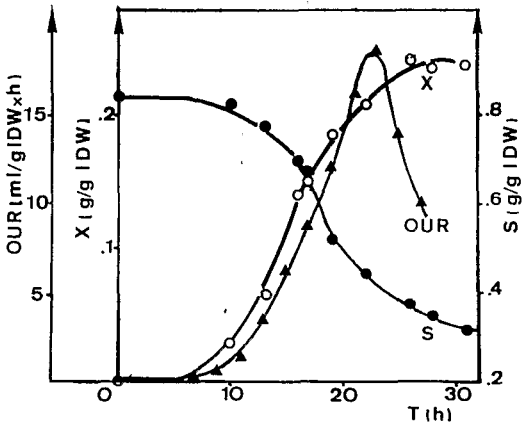

Fig. 2. Growth kinetics of *A. niger* on cassava starch; X=biomass, S=substrate and OUR=oxygen uptake rate

The maximum biomass was reached after about 25 h and as may be seen substrate consumption was not total.

Figure 2 also shows the oxygen uptake rate (OUR); although the maximum respiratory activity took place 3 h prior to maximum biomass production, the analysis of the gas evolution in the exhaust air was a useful tool for estimation of growth (Okazaki et al. 1980; Narahara et al. 1982).

Evolution of the water content during growth

During growth, the amount of total water (H_2O_T) in the medium is the only accessible by measure. The residual water (H_2O_R) in the substrate is given by:

$$H_2O_R = H_2O_T - H_2O_C \quad (3)$$

where H_2O_C is the amount of water consumed which may be estimated by the following equation:

$$H_2O_C = 0.1\, S_H + (1/0.33)X \quad (4)$$

where S_H is the amount of sugars hydrolysed
X is the biomass dry weight
0.33 is the stochiometric biomass yield for water ($g X/g H_2O$) as determined by Raimbault (1980).

Figure 3 shows the time course of total water as measured during growth and the time course of consumed water and residual water in the medium as calculated from the data reported in Fig. 2.

Total water increased slightly as growth proceeded passing from 1.00 to 1.12 g water/g IDW. The device used in these experiments allowed a good regulation of the relative humidity of the circulating air which did not produce a drying of the substrate, contrary to what has been reported for other systems (Narahara et al. 1982).

Residual water decreased and reached 0.152 g/g IDW, meaning that theoretically there would be no limitation of water during growth, nevertheless the availability of that water has to be considered.

Changes in a_w during growth

The a_w measured during growth (Fig. 4) shows a regular increase after 10 h and the a_w became almost equal to 1.00 after 22 h. This is, however, the result of two phenomena: an increase in the water-content of the mycellium and a decrease in the residual water in the substrate. The growth pattern of *A. niger* on cassava starch has been illustrated by electron micrographs (Aufeuvre et Raimbault 1982). The hyphae gradually invaded the surface of the starch granule which was totally covered by the mycelium after 25 h. It may thus be presumed that if a_w were measured with a device which did not register water loss, as the microorganism colonized the substrate, the mea-

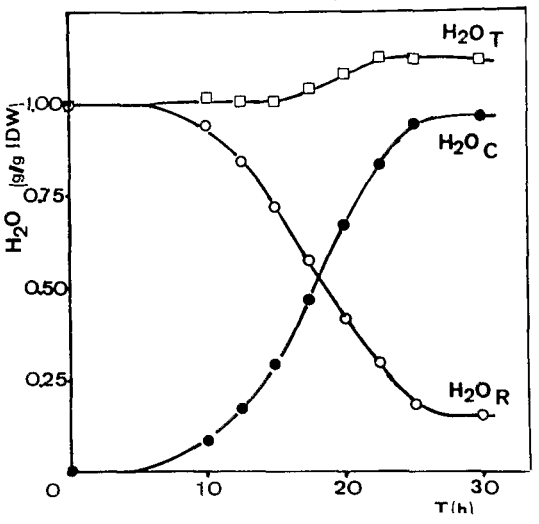

Fig. 3. Evolution of water during growth of *A. niger* on cassava starch. (H_2O_T = total water, H_2O_C = consumed water, H_2O_R = residual water)

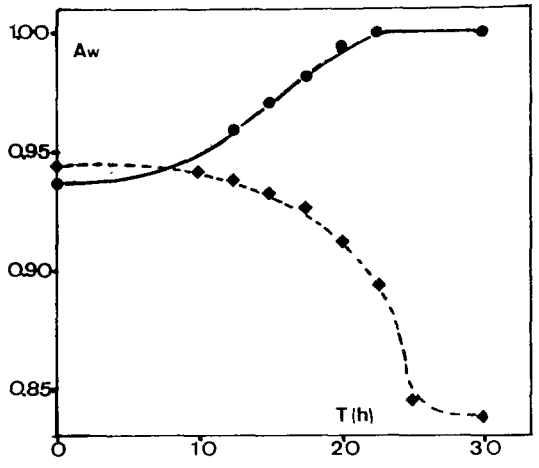

Fig. 4. Evolution of the measured a_w of the medium (—●—) and of the calculated a_w of the substrate (—◆—) during growth of *A. niger* on cassava starch

sured a_w would gradually become that of the mycelium, which was almost equal to 1.00.

Consequently, the a_w of the residual substrate was only accessible by calculation. Assuming that the rate of salt uptake was the same as the rate of carbohydrate consumption, the consumption of the salts during growth might be deduced. By inserting the values for residual water (Fig. 3) and for the two ingredients of the medium into the Ross equation (2) it was possible to estimate the a_w of the remaining substrate throughout the fermentation time. As can be seen in Fig. 4, the calculated value of the a_w of the substrate decreases during growth until it falls below 0.90 after 21 h. Such low a_w values are known to inhibit growth; Pitt and Hocking (1977) reported drastic reductions (10 times) in the growth rate of *A. flavus* when decreasing the a_w of an agar plate medium from almost 1.00 to 0.9.

Reduced a_w is supposed to result in lower mass transfers and little water availability for the microorganism and may thus be held responsible for the incomplete conversion of substrate to biomass.

This hypothesis might be verified by working with a higher initial a_w. This could be achieved by reducing the amount of salts or by changing the composition of the salt mixture (sulphate ions for instance are known to produce drastic decreases in the a_w of solutions). Another alternative was investigated by Sato et al. (1982) who introduced a support of high water absorption capacity (wood pulp) to enhance the production of amyloglucosidases and spores by *A. oryzae* on wheat bran. Extending this idea to the SSF of cassava, sugarcane bagasse which is widely available in tropical areas, was chosen for use as a lignocellulosic support since it has, a high water-absorption capacity.

Growth of A. niger on mixed media

Studies on water absorption in diffent mixtures of cassava and bagasse showed that with 20% dry wt. of bagasse, moisture content could be reduced at the outset to 70% without water drainage. Initial moisture contents ranging from 42% to 70% were used, resulting in initial water activities from 0.94 to 0.98. As shown in Table 1, good agreement was shown between the calculated values and the measured ones.

The oxygen uptake rate (OUR) was used as a growth parameter (Fig. 5). It was integrated by the method of Sato et al. (1983) and allowed the average specific growth rate to be calculated and then plotted against the initial a_w of the medium (Fig. 6). An increase in the a_w of the substrate caused an increase in the specific growth rate and shortened the spore germination time. With a 0.98 a_w, cultivation took only 20 h and resulted in a high specific growth rate (0.45).

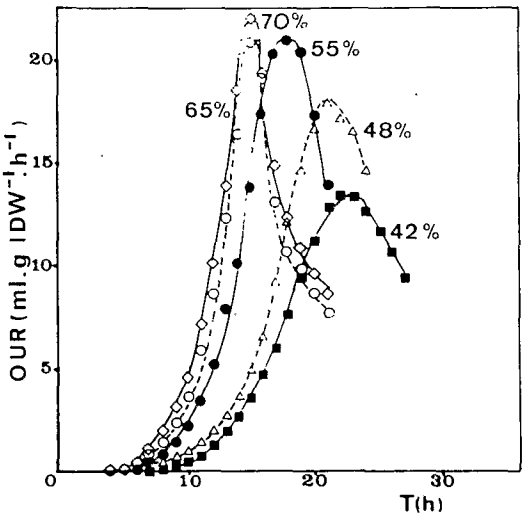

Fig. 5. Evolution of the oxygen uptake rate during growth of *A. niger* on mixed medium with different initial water contents

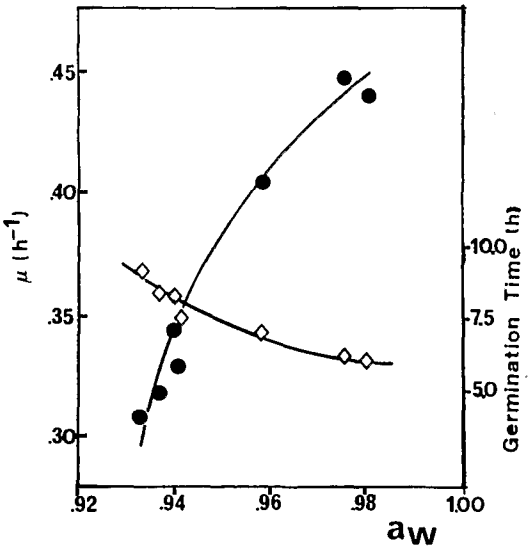

Fig. 6. Evolution of the specific growth rate (—●—) and of the germination time (—◇—) as a function of the initial water activity of the medium

Table 1. Parameters of growth of A. niger on cassava starch and bagasse with differents initial water contents

IMC %	Initial a_w	Initial a_w *	Incubation time (h) **	X (g/g IDW)	Substrate Consumption (g/g IDW)	Sc*** (%)
42	0.933	0.937	28	0.123	0.233	33.2
48	0.940	0.950	26	0.172	0.344	48.9
55	0.958	0.963	23	0.251	0.417	59.3
65	0.975	0.977	20	0.324	0.477	68.0
70	0.980	0.982	20	0.339	0.558	79.5

* Calculated values
** Time of maximum biomass as calculated from the OUR
** Substrate conversion: consumed substrate/initial substrate
IMC Initial moisture content
IDW Initial dry weight

As may be seen in Table 1, higher a_w also enhanced the substrate conversion to fungal biomass. A calculation of the evolution of the a_w, made on the same basis as previously described showed that it remained above 0.98 for the entire cultivation period. The support thus acted as a reservoir of water and helped to maintain high water availability throughout the cultivation.

Conclusion

The limitation of growth by water availability, as predicted from theoretical calculations of the a_w of the substrate during growth, was confirmed experimentally. The effect of a_w was observed on the growth rate of the mold as well as on its substrate utilization. Nevertheless, even though 70% to 80% starch conversion could be achieved, sugar still remained in the medium and therefore other critical factors such as intraparticle mass transfer (Moo-Young et al. 1983) or fungal packing density (Laukevics et al. 1985) need to be investigated.

From the practical standpoint, the introduction of a support enabled greater biomass contents to be obtained in a short time but at the expense of the volumetric productivity. In addition, the resulting animal feeds, with a more than 20% fiber content, might not be tolerated well by some monogastric animals.

As a model for studying the influence of the a_w on growth, the introduction of a support proved to be demonstrative and pointed out that optimization of SSF might be expected from increasing and controlling the a_w of the substrate during the process.

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