[redacted]

Yeasts - like fungi - are able to support fairly wide ranges of osmotic pressure and water activity. Growth and fermentative activity have been reported at aw's lower than 0.7[6]. It is one of the major reasons for regarding yeasts as microorganisms with promising potential in SSF culture conditions. SSF processes have gained renewed interest[19]and yeasts have been studied in the past ten years for various applications, such as protein enrichment of agricultural wastes (sweet potato[28], potato and cassava flour[21]); ethanol production from apple pomace[13], pinneaple waste[16], sweet sorghum[7] or rice and maize wastes[23]. Among the great diversity of yeasts studied, few papers report the use of Candida strains. Nevertheless, a study employing C. lipolytica on rice wastes medium and complemented with amylolytic enzyme showed the ability of the yeast to grow in solid state fermentation conditions and displayed the potential of respirometry to estimate microbial growth[22]. Another paper reported the use of a Candida utilis strain to ferment ryegrass straw to increase its in vitro digestibility, protein content and crude fat content[12]. The fermentation, achieved under semisolid conditions (70% moisture), showed the ability of the yeast to grow on pre-hydrolyzed straw and to support drastic conditions (temperature varying from 20°C to 40°C, and oxygen partial pressure close to zero)[12].

To solve the problem of gaseous ethanol containing streams, the biotransformation of ethanol in the gas phase into ethyl acetate is currently under study. As the first step, the feasability of growing Candida utilis is approached. The growth kinetics of the yeast in solid state fermentation using three different supports : natural without any culture medium (wheat bran), sugar cane bagasse and synthetic polymer (Amberlite), both complemented with an appropriate culture medium have been followed with cell count, dextrose consumption rate and respirometry.

MATERIALS AND METHODS

Microorganism and culture media.

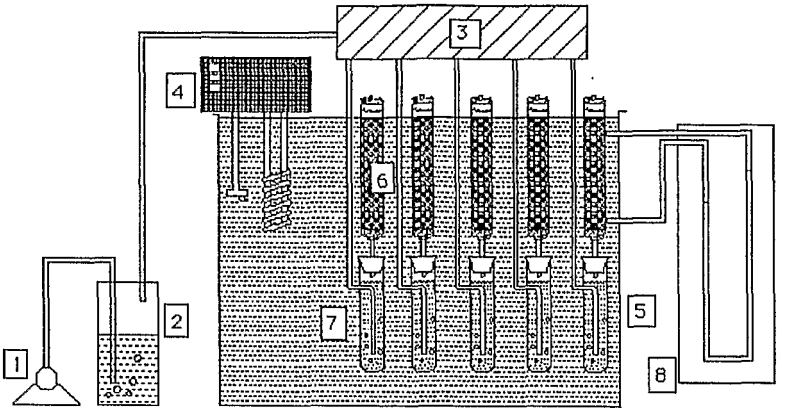
Candida utilis ATCC 9950 (CDBB L245) was maintained on slants of potato dextrose agar medium (PDA) at 6°C. To prepare the inoculum, it was grown in 50 ml of dextrose (20 g/l) and malt extract (20 g/l) medium in 150 ml erlenmeyer flask with shaking at 200 rpm at 30°C. The yeast was then grown on the prepared support complemented with the minimal salts medium of Thomas and Dawson[25].

Substrate and supports preparation.

- Wheat bran was milled and sieved through -20+40 mesh screens to obtain particles of 0.42 to 0.82 mm in diameter. It was then autoclaved at 121°C for 15 min and its pH adjusted to 6.

Fermentation system.

A schematic diagram of the experimental set up is shown in Figure 1.



1. Air supply, 2. Humidifier, 3. Air collector, 4. Temperature controller
5. Water bath, 6. Column fermentor, 7. Humidifiers, 8. Pressure drop gauge.

Figure 1 : Experimental set up for the Solid-State columns fermentors.

The columns were filled with aproximatively 12g of dry matter and fed with pre-humidified air. For each experimental sample, a column was removed from the water bath and total residual sugars, cell number, cell viability, pH and moisture content were determined. One column was maintained throughout the fermentation to assay exit gas composition.

Analytical procedures

Sugar determination : In the case of wheat bran, 2 g were heated for 2.5 h with 200 ml H_2O and 20 ml of HCl with reflux. The hydrolyzate was then refrigerated, neutralized with NaOH and discolored with the Carrez solution[20]. Sugar measurements were made with the phenol sulfuric method[10]. Reducing sugars in bagasse and Amberlite were measured by the DNS method[18] after washing 1 g of sample in 25 ml of water, homogenizing with Ultra turax, centrifugating and collecting the solids-free phase.

Cell number was determined by dispersing the samples in distilled water with 9 g/l NaCl, homogenizing with blender (1 g in 25 ml), adding methylene blue solution to determine the proportion of viable cells and counting in a Neubauer chamber. In liquid culture, it was found that 10^7 cells were equivalent to 0.092 mg of biomass (correlation coefficient = 0.993).

pH was determined by mixing 1 g of sample with 25 ml of distilled water and homogenizing for 5 mn.

Water content of the medium was determined by drying the sample in an oven at 95°C during 24 hours to a constant weight.

Carbon dioxide evolution in the exit gas of the column was measured with a gas chromatograph (Gow Mac, USA) equipped with thermal conductivity detector and a concentric column CTR1 (Alltech, USA). Carbon dioxide production rate values (CDPR) were then calculated with the following equation :

$$\mathbf{CDPR} = (\%\ CO_{2\ \text{produced}} \times F) / (100 \times W)$$

where F = Input air flow (ml/h) and W = Initial dry matter loaded in the column (g)
This value is expressed as ml/g DM.h. At the atmospheric pressure of Mexico (580 mm Hg)

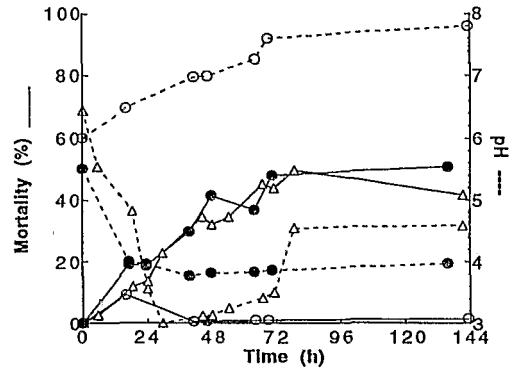


Figure 3 : Evolution of cells mortality and pH vs. time with three different supports. (Δ Amberlite, o Wheat bran, • Bagasse)

Mortality curves for Amberlite and bagasse were very similar, increasing slowly during the first 3 days to reach 50% while cell mortality remained close to zero for wheat bran throughout the fermentation (figure 3). This difference can be explained by the important drop in pH observed for both Amberlite and bagasse within the first 24 hours -probably due to the production of organic acids - and then remaining close to 4 for bagasse and 3 for Amberlite during two days. This showed the relatively poor buffering properties of these two supports. This can be explained also by the rapid dextrose consumption in both cases (figure 4). On the contrary, pH increased from 6 to nearly 8 for wheat bran probably because of the partial proteolysis of the bran. It may be observed in each case, that in spite of a previous buffering step of the solid substrates, pH regulation might become an important problem.

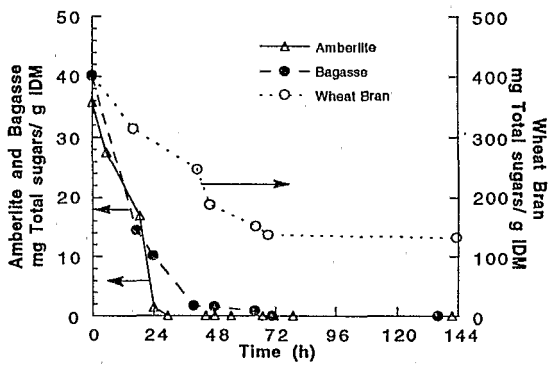


Figure 4 : Total sugar evolution vs. time for the three supports.

Figure 4 shows that for Amberlite and bagasse, all initial dextrose was consumed, in 24 and 48 hours, respectively, and for wheat bran residual sugars reached a final value of 130 mg/g IDM (67% of initial sugars consumed) after 72 hours of fermentation. These different sugar consumption rates may be explained by the different availability of carbohydrates in each substrate.

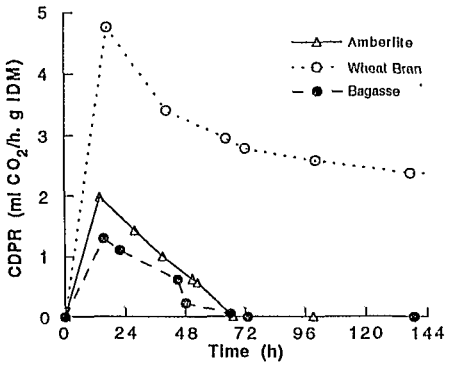


Figure 5: Evolution of carbon dioxide production rate vs. time for the 3 supports.

As may be expected, the highest carbon dioxide production rate (CDPR) - close to 5 ml/h . g IDM- was reached with wheat bran corresponding to the highest biomass production and highest sugar consumption (figure 5). CDPR maxima coincide in the three cases : around 20 hours, at the end of the exponential growth phase. Nevertheless, a major difference is observed between Amberlite and bagasse in one hand and wheat bran in the other hand : while in the two first cases, CDPR decreased to zero in the same manner as dextrose consumption, for

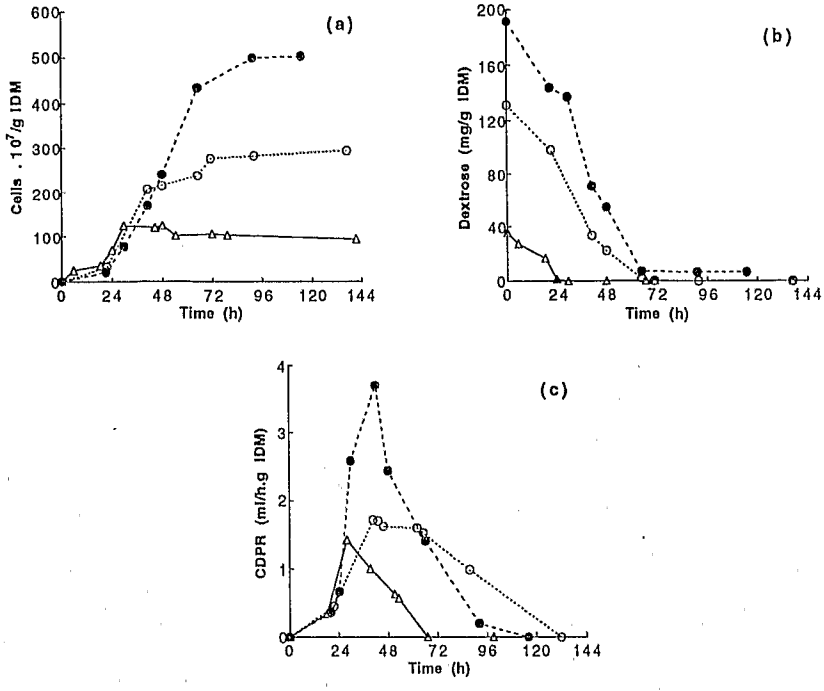


Figure 6 : Candida utilis growth on Amberlite with 3 different initial dextrose concentrations (Δ--Δ 40mg/g IDM, o...o135mg/g IDM, ●--● 200mg/g IDM). Cell growth (a); Dextrose consumption (b); Carbon dioxide production rate (c).

The yield values were found to be between 0.2 and 0.285 for Amberlite and cane bagasse while a lower value was found on wheat bran. Experiments performed in liquid fermentation with the dextrose- mineral medium showed similar yields. The values obtained are lower than those reported for the growth of C. utilis for biomass production but they reflect the effect of ethanol accumulation [25]. The maximum CDPR for Amberlite at 200 mg/g IDM is similar to those reported for A. niger on the same support[5].

CONCLUSION

The ability of Candida utilis to grow on solid substrate (wheat bran) or impregnated solid

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