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SOLID-STATE FERMENTATION OF SUGAR-BEET

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SUMMARY

Semi solid culture is adaptated to ethanol production from sugar-beet. This technique offers some advantages (reduction of liquid volumes and suppression of the diffusion step). With a tubular reactor, the ethanol concentration obtained reaches 8,3 %, and the yield is 0,407. The feasability of solid state fermentation on sugar-beet is demonstrated.

INTRODUCTION

Practical alcoholic fermentation processes are currently based on free cells in batch or continuous cultures, with or without biomass recycling. These kind of processes have technological limitations, which include the need to process large volumes of water, wort, and effluent. Several groups of researchers have adaptated the semi-solid culture to the alcoholic fermentation. The solid state fermentation (S.S.F) is applied to various substrates, and industrial productions are numerous : mushrooms, cheeses, citric acid, fermented food (koji, miso, saké, tempeh). Advantages of this system compared with the submerged culture include the smaller volumes involved, the lower risk of contamination, and the lower volume of effluent. These advantages are due to the combination of sugar extraction and fermentation in one step.

Alcoholic solid state fermentation has already been tested on various substrates : sorghum, sugar cane, grape rape, and sugar beets (Kargi et al, 1985; Er-el et al, 1981; Hang et al, 1986; Kirby et al, 1980). A horizontal fixed-bed was developped in 1980 by Rolz who is very active in this area.

In France, we are interested in using sugar beet as the substrate. Alcohol production from sugar beet in France is a traditional activity ($1{,}6.10^6$ hl/year), involving two main steps : sugar diffusion and semi-continuous fermentation. In a sugar beet distillery, the diffusion step uses a large amount of water (43 % of the total consumption), with 977 l per ton of processed sugar beet. In order to reduce the fermentor capacities and liquid volumes, a semi-solid system was tested by Kirby and Mardon (1980). This encouraging work is characterized by sugar-beet grinding before fermentation, and pressing at the end of the process.

The work presented here is based on the adaptation of the results of Rolz to sugar beet substrate. In the first part, we show the favourable action of S.S.F on sugar extraction and we determined the best conditions for sugar extraction in flasks. In the second part, we conduct a batch fermentation in a tubular reactor.

MATERIALS AND METHODS

Strain and media

The strain used is *Saccharomyces cerevisiae* 59 selected by De Miniac(1984). Precultures are performed with YPS medium (yeast extract: 10 g ; bactopeptone:20 g ; saccharose: 20 g ; water : qsp 1000 ml), over 24 hours at 29°C.

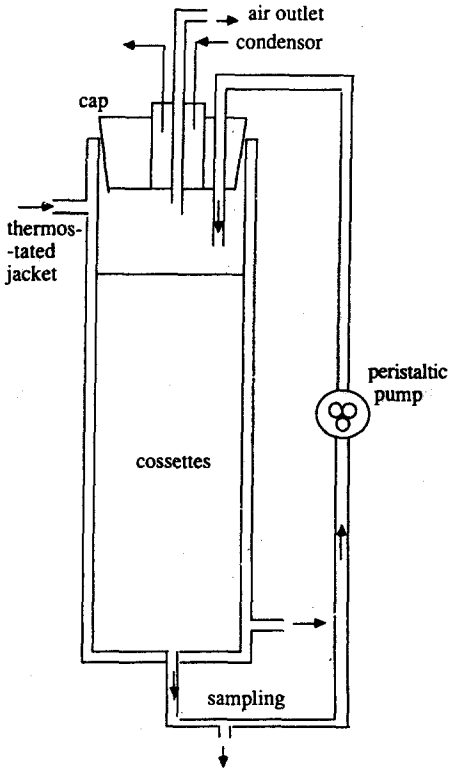
Substrate

Sugar beet cossettes used for the assays have the composition : Water: 72,5 %; pulp: 5 %; sugars: 21,5 %; ash: 1%.

Experimental procedure

Most of the assays are performed in 500 ml or 1000 ml flasks. Only one experiment in a tubular reactor is presented. (Figure 1).

Experimental conditions for each assay will be indicated with the results: cossettes weight, liquid volume, inoculum size, temperature, initial pH and incubation time. An important parameter is expressed as L/S : ratio of liquid volume to wet cossettes weight.



Column characteristics

Heigh : 48 cm
Heigh of cossettes : 42 cm
Diameter : 6 cm
Cossettes weight : 440 g (wet)
Liquid volume : 88.6 ml
L/S = 0.2
Flow : 160 ml/mn

Figure 1 Tubular reactor scheme

Analytical techniques

Determinations of sugar concentrations are carried out on the liquid phase, and on the cossettes. Washing, drying and grinding of the solid phase is necessary before suspending cossettes powder in distilled water. The samples are submitted to acid hydrolysis and the concentration of reducing sugars is determined by the Miller method (1959). Ethanol concentration is measured by gas chromatography (Intersmat 121 DFL). The inox column is filled with Chromosorb 101.

Parameter calculations

Diffusion yield :

$$Y_D = \frac{100\,(S_o - S_1)}{S_o}$$

Consumption yield :

$$Y_S = \frac{100[S_o - (S_1 + S_L)]}{S_D}$$

Production yield : $Y_{P/S} = P/(S_O - (S_1 + S_L))$

where P = ethanol in liquid and solid phase (g)

S_O = initial sugars in cossettes (g)

S_1 = residual sugars in cossettes (g)

S_L = residual sugars in liquid (g)

S_D = diffused sugars (g)

$S_D = S_O - S_1$

RESULTS AND DISCUSSION

Influence of fermentative activity on sugar diffusion: Cossettes depletion is evaluated by two sets of assays carried out in flasks, for different values of L/S ratio. One set is conducted without inoculum, on a shaker at 60°C. The other set with flasks inoculated with $2.6.10^8$ cells/flask is incubated with gentle agitation at 30°C. The results are shown in Figure 2; from these we can conclude that yeast growth favours sugar extraction.

Influence of initial pH value: Depletion of cossettes and sugar concentration of the liquid medium are measured for different initial pH values. Results are reported in Figure 3. Optimal value determined is pH = 4.5.

Influence of inoculum size: Biomass load is expressed as number of cells/g cossettes (wet weight). Sugar content of cossettes and sugar consumption in the liquid are measured for various biomass load values. The results reported in Figure 4 show the drop of residual sugar concentration in the liquid, when the inoculum size is increased. On the other hand, the influence of inoculum size on cossettes depletion is well established up to 10^8 cells/g. When the inoculum size exceeds this value, cossettes depletion remains constant.

Ethanol formation: A set of experiments is carried out with 1000 ml flasks. Sugar and ethanol concentrations are measured over 48 hours. The values are

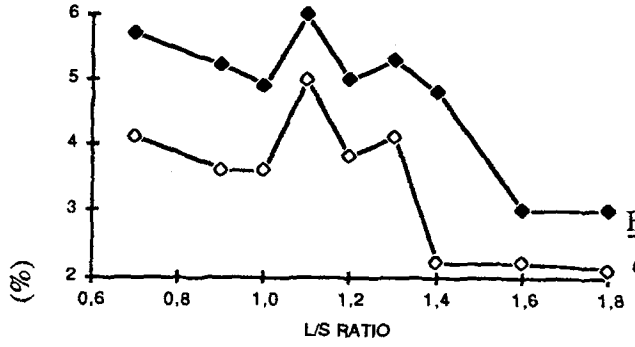


Figure 2 Cossettes depletion during diffusion and fermentation-diffusion

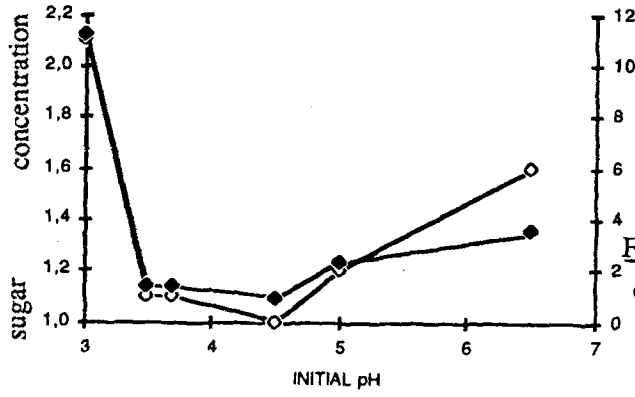


Figure 3 Influence of initial pH value on the residual sugar concentration

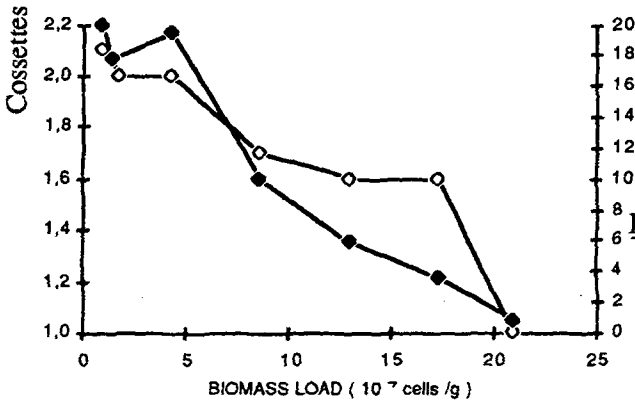


Figure 4 Influence of the inoculum size on the residual sugar concentration

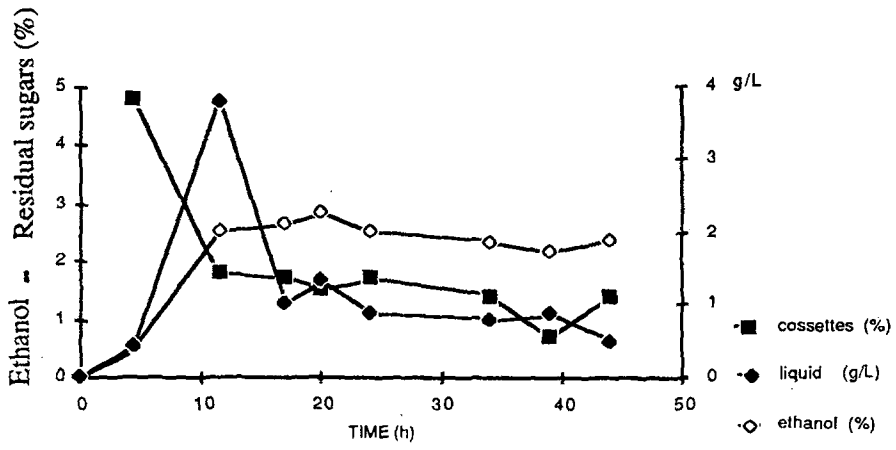


Figure 5 Sugar and ethanol concentration during a batch culture carried out in flasks

reported on the curves of Figure 5. The maximal value of ethanol concentration does not exceed 2.86 % being reduced afterwards, due to evaporation.

In 10 hours the cossettes depletion is almost achieved. The sugar diffusion slowly continues from 10 to 48 hours.

At 10 hours the sugar concentration in the liquid phase is maximum. From 10 to 48 hours, we observe the drop of sugar concentration resulting of yeasts consumption.

From these experiments, we can evaluate the yield values :

Y_D (24 h) = 92 %
Y_C (24 h) = 99.3 %
$Y_{P/S}$ (24 h) = 0.28

where S_0 = 12.9
S_1 = (1.72 x 60) / 100
S_L = (0.88 x 84) / 100
P = [(2.52 x 84) / 100] + [(2.52 x 48) / 100]

These low values may be explained by :
- aeration in the flasks,
- too high L/S ration,
- uncontrolled evaporation.

Fermentation-Diffusion in a tubular reactor : In order to favour fermentative metabolism, we have considered the use of a tubular reactor with L/S = 0.2.

The ethanol concentration obtained in the liquid and inside the cossettes reaches the maximum value of 8,3%. After 28 hours of fermentation-diffusion, the sugar concentration values are as follows:

Cossettes	Liquid medium	
residual sugar concentration (%)	sugar concentration (g/l)	ethanol concentration(%w/v)
0.99	3.87	8.3

From these measurements we can write the process assessment:

The initial cossettes (440 g wet weight) contain 94.6 g of sugars from which 0.29 g remain in the solid phase and 0.34 g in the liquid phase.The sugars diffused are consumed by yeasts which produce 7.39 g of ethanol in the liquid phase, since 29.2 g are entrapped in the cossettes and have to be pressed.

From these results, we can calculate the yield values :

Y_D (28 h) = 95.4 %
Y_S (28 h) = 99.6 %
$Y_{P/S}$ (28 h) = 0.407

where S_0 = 94.6
S_1 = (0.95 x 440) / 100
S_L = (3.87 x 89) / 100
P = [(8.3 x 89) / 100] + [(8.3 x 352) / 100]

Diffusion yield is better than when the experiments are conducted in flasks, whereas the consumption yield is almost the same. Fermentative metabolism is increased with a final ethanol concentration of 8.3 %. But $Y_{P/S}$ values remain low.

CONCLUSION

This study, realised on a laboratory scale was undertaken to demonstrate the feasability of the SSF on sugar beet cossettes.

Concerning sugar diffusion out of the cossettes, the results could be improved. Sugar content, at the end of the culture, reaches 0.99 %. This value has to drop to 0.6 %. With sugar cane, a residual sugar content of 0.2% was obtained (Rolz, 1980). We have shown the positive influence of fermentative yeast activity on sugar transfer to the liquid phase.

Sugar consumption during batch fermentation in the tubular reactor is estimated by the residual sugar concentration in the liquid phase. The value obtained has to be reduced by a gentle agitation of the column.

During this experiment $Y_{P/S}$ reaches 0.407 which is comparable to the 0.3 and 0.4 achieved by Rolz. The traditional value of 0.48 cannot be reached. It may be explained by yeast growth which cannot be nor measured or controlled. In fact, sugar beet juice gives all the necessary nutrients for yeast growth. The theoretical production can be calculated = 0.083kg Ethanol/kg sugar beet. The 8 % ethanol concentration obtained in the column is also attractive.

But the disadvantages presented by this system must be pointed out. Continuous work with S.S.F needs the development of an endless screw reactor, the yeast biomass must be recycled by centrifugation, filtration or sedimentation with flocculent cells, and pressing cossettes to extract the ethanol solution is an obligate step.
However, this system presents several technological advantages : suppression of the diffusion step, reduction of reactor capacity, and ease of use.

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