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ETHANOL PRODUCTION BY COUPLED SACCHARIFICATION AND FERMENATION OF SUGAR CANE BAGASSE

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Summary: As initial studies showed that enzymatic saccharification of sugar cane bagasse in columns with recycling of eluate was slightly more efficient than in agitated flasks, ethanol production by fermentation of the eluates with fast-decanting yeast and recycling of the fermentate through the bagasse columns was studied. The alcohol yield from these coupled columns after 24 or 48 h was more than 10% more than that in a simultaneous saccharification and fermentation in agitated flasks at 40°.

INTRODUCTION

Mandels et al. (1971) carried out experiments on enzymatic hydrolysis of cellulosic materials in columns, and more recently Buchholz et al.(1980) studied such procedures in more detail. Some advantages include the possibility of working with a smaller liquid:solid volume ratio, avaoiding the need to agitate thick slurries, etc. On the other hand Tagaki et al.(1977) showed that alcohol production from cellulosic materials via enzymatic hydrolysis is more effective when saccharification and fermentation are simultaneous, and at a temperature intermediate betweenthe optima for both processes. This is due to removal of the glucose formed in the hydrolysis, which is an inhibitor of the saccharification.

Here we report on the production of alcohol in a system that uses two columns, one for saccharification of cellulose and the other for glucose removal by fermentation and recycling.

MATERIALS AND METHODS

Sugar cane bagasse from local sugar factories was dried and hammer milled. After screening, the material retained between 40 and 60 mesh was used as untreated bagasse. For most studies it was pretreated with 1% NaOH at 10 ml per gram of bagasse at 80° for 3 h, washed to neutrality, and dried.

The enzyme used was Trichoderma viride cellulase (Miles Lab. USA), with a filter paper activity of 0.069 units per mg determined according to Mandels et al.(1976). The Nelson-Somogyi method was used for determination of reducing sugars (RS)(Spiro,1960)

The determination of ethanol was by the alcohol dehydrogenase method (Boehringer Manheim, Germany) or by a modified dichromate method described in the appendix to this paper.

For ethanol production, Saccharomyces cerevisiae C 1 from our collection, a fast-decanting strain, was used. Yeast cells were grown at 35° in 500 ml agitated flasks with 150 ml of medium containing, in $g.l^{-1}$: $(NH_4)_2SO_4$ 4.0, KH_2PO_4 2.0, $MgSO_4.7H_2O$ 0.5, yeast extract 1.5, glucose 20.0, Na lactate (70%) 8 ml; pH was 4.5. Cells ere harvested at 24 h, centrifuged and suspended in 0.8% Na Cl solution. Three ml of suspension, containing about 67 mg dry weight, were used as inoculum for each run.

Saccharification experiments were carried out on 1.0g bagasse in flasks, agitated at 150 r.p.m., or in columns, 1.5 cm diameter eluted at 15ml.h^{-1}, at 50° in 0.05M citric acid/citrate buffer pH 4.8 containing 2 mg.l^{-1} of tetracycline to prevent contamination (Reese and Mandels, 1980).

In the coupled system, saccharification was performed in columns with 2.0g of pretreated bagasse at 50°. Fermentation was carried out in a decanter tube as indicated in figure 2. The liquid recycled in the system, at a flow rate of 32ml.h^{-1}, was 40 ml of a medium containing, in g.l^{-1}: $(NH_4)_2SO_4$ 4.0, KH_2PO_4 o.4, yeast extract 1.0, malt extract 1.0, citric acid 2.0; the pH was 5.0 and 5 FP units of enzyme was added together with 2mg.l^{-1} of tetracycline as preservative (previous tests showed no effect on alcohol production).

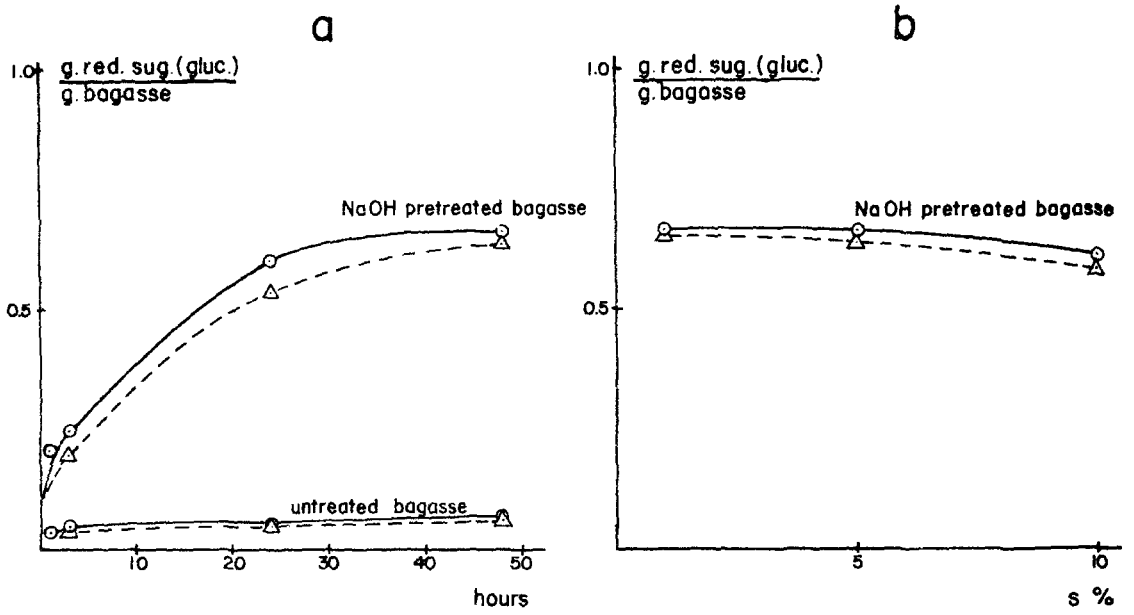
The simultaneous saccharification and fermentation processes were carried out in shake flasks at 40° using the same amounts of substrate, enzyme, medium and yeast as in the coupled system.

RESULTS AND DISCUSSION

Experiments were carried out to compare saccharification in columns with recycling eluate with that in shaken flasks. The recycling operation in columns was aimed at obtaining a more concentrated sugar solution and at ensuring a better contact between the substrate and dissolved enzymes. In figure 1a can be seen the time course of reducing sugars (RS) production by both systems, sugar production in the columns being always somewhat greater. Figure 1b shows the effect of varying the liquid volume on RS production at a fixed enzyme/substrate ratio E:S. A tenfold volume reduction (i.e. a tenfold increase of substrate and enzyme concentration) resulted in only a 10%, or less, decrease of RS production. The effect of changing the E:S ratio, for a bagasse concentration of 5%, is shown in figure 1c. With untreated bagasse there was a slight increase in saccharification, but with NaOH pretreated bagasse there was a sharp increase in RS yield up to about 10FP units/g bagasse, followed by a further but less significant increase. For all these experiments, results in agitated flasks or in columns were practically the same.

Figures 1a and 1c also show the well known beneficial effect of the alkali pretreatment (Toyama and Ogawa, 1972). Similar effects, somewhat less pronounced but cheaper (at least in our country) are obtained using $Ca(OH)_2$ instead of NaOH (Ellenrieder and Castillo, unpublished).

In cellulose saccharification glucose has an inhibitory effect, as already noted. In the saccharification column, glucose is removed in the effluent liquid, and if it is removed in a separate unit before recycling, glucose inhibition should be almost eliminated and the action of the cellulase complex greatly facilitated. Figure 2 shows how a system of this kind was set up, coupling the saccharification unit with a fermenting unit which contains the yeast cells.



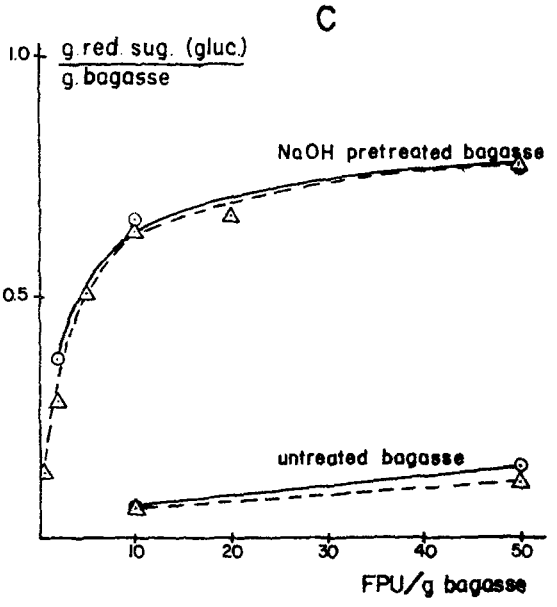


Fig. 1. Reducing sugar production in columns and agitated flasks.

-o- columns
-Δ- agitated flasks.
50°C, citric citrate buffer pH 4.8

a. Time course of the process. Substrate concentration (s): 5%, enzyme activity: 10 FPU/g bagasse.

b. Effect of the volume of liquid (Enzyme and Sustrate conc.) for a fixed enzyme bagasse ratio E/S: 10 FPU/g bagasse.

c. Effect of the E/S ratio. Substrate concentration: 5%

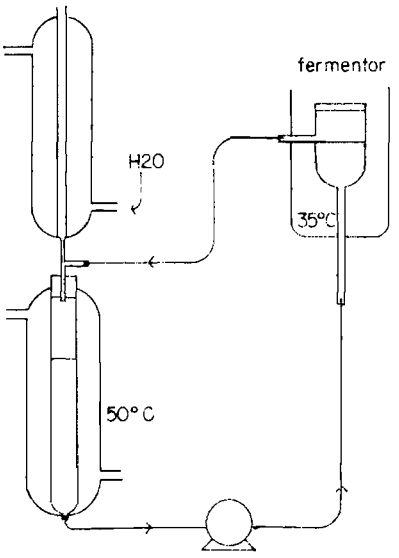


Fig. 2. Coupled saccharification and fermentation system for alcohol production.

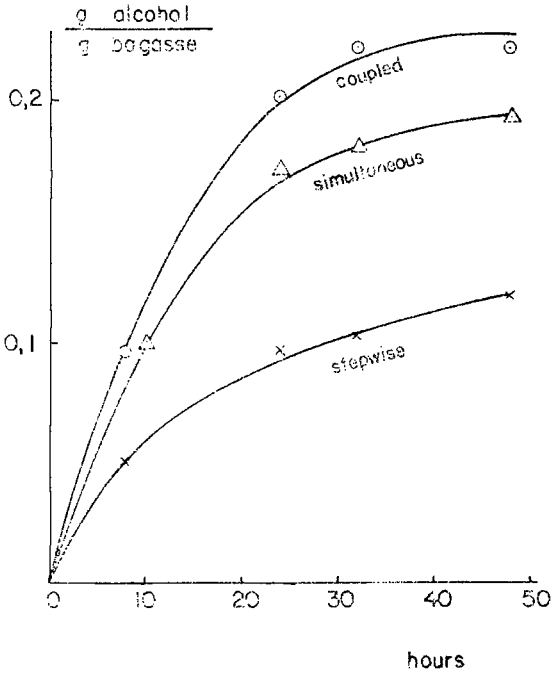


Fig. 3. Time course of alcohol production for the three systems.

The performance of the coupled saccharification and fermentation system (CSF) is shown in figure 3. Alcohol production is greater than in the simultaneous saccharification and fermentation system (SSF) (Takagi et al. 1977) carried out in agitated flasks; the optimum temperature for the SSF system was 40^0, as reported by Takagi et al. Figure 3 also shows ethanol production from a previously-hydrolysed bagasse sample, which gives appreciably lower yields than are obtained by the other two systems.

The higher ethanol yields in the CSF system are probably due to the fact that each process, saccharification and fermentation, can be run at its optimum temperature, that is, 50^o and 35^o respectively.

Besides the greater ethanol production, the CSF system retains the other advantages noted for the saccharification columns; aeration of thick suspensions is not necessary, liquid to solid ratio can be small, and the system can be appropriate for medium scale operation.

REFERENCES

Buchholz, K., Puls, J., Gödelmann, B. and Dietrichs, H.H. (1980): Process Biochem. Dec/Jan 1980/81, 37-43.

Mandels, M., Kostick, J. and Parizek, R. (1971): J. Polymer Sci. C, 36, 445-459

Mandels, M., Andreotti, R. and Roche, C. (1976): Biotech. Bioeng. Symp. 6, 21-23

Reese, E. T. and Mandels, M. (1980): Biotech. Bioeng. 23, 323-335

Spiro, R.G. (1960): J. Biol. Chem. 235, 2860

Takagi, M., Abe, S., Suzuki, S., Emert, G.H. and Yata, N. (1977): Proc. Int. Bioconversion Symp. IIT Delhi, 551-571

Toyama, N. and Ogawa, K. (1972): Proc. IV IFS Fermentation Technology Today, 743-757.

APPENDIX

SIMPLIFICATION OF THE DICHROMATE METHOD FOR ETHANOL DETERMINATION
(S. Blanco and G. Ellenrieder)

An old procedure for the determination of ethanol in those systems in which it is the only volatile compound capable of reducing potassium dichromate is the method of Widmark (1922; Lundquist, 1959). It was used for large-scale determinations in blood and urine and can also be used for alcohol and acetic acid fermentations. The Widmark method uses titration with potassium iodide and sodium thiosulphate for the determination of the excess dichromate, and is laborious and time-consuming.

In acid solution, dichromate has an absorption maximum at 350 nm which is eliminated on total reduction. However this was not used for any quantitative determinations of the non-reduced dichromate, probably because it is known that the system does not obey Beer's law in acid solutions, because of the dichromate-chromate equilibrium (Körtum, 1936; Davies and Prue, 1955). To overcome this, Kolthoff et al. (1969) suggested the use of an empirical calibration curve.

We have found that de-ermination of alcohol from standard curves is very simple. Making an appropriate dilution, the plot of absorbance decrease against amount of alcohol is linear in the range from 0.05 to 0.4 mg of ethanol.

The analysis was carried out using modified Widmark flasks, consisting of 25 ml tubes instead of 100 ml conical flasks. One ml of dichromate reagent (2.0 g potassium dichromate dissolved in 10 ml water plus 790 ml concentrated sulphuric acid) was added to the tubes with a syringe pipette and controlled by weighing.

The samples, containing 0.05 to 0.5 mg of ethanol, were added to the cups of the Widmark tubes, which were stopped up and allowed to stand for 2 h at 60°. The stoppers were then removed and 20 ml of water added to each tube.

The absorbance at 350 nm was measured with a Beckman Model 25 spectrophotometer, and blanks and standards were measured in the same way. The standard deviation for ten measurements of samples of 0.2 mg of ethanol was 0.0053 mg.

References.

Davies, W. G. and Prue, J. E. (1955): Trans Farad. Soc. 31, 1045

Kolthoff, T. M., Sandell, E. B., Meehan, E. J. and Bruckenstein, S (1969): Quantitative Chemical Analysis p. 1089

Körtum, G. (1936): Z. phys. Chem. (Leipzig) B33, 243

Lundquist, F. (1959): Methods of Biochemical Analysis 7, 232

Widmark, E. M. P. (1922): Biochem. Z. 131, 473.