Biotechnology Letters Vol.3 No.6 263-268 (1981)

ETHANOL PRODUCTION FROM WHEY WITH IMMOBILIZED LIVING YEAST

Yu-Yen Linko, Hannele Jalanka and P. Linko*

Department of Chemistry, Helsinki University of Technology, SF-02150 Espoo 15, Finland

SUMMARY

Living *Kluyveromyces fragilis* yeast cells were succesfully entrapped in calcium alginate gel beads at cell loadings of 4 to 16 g yeast (0.8 to 3.2 g d.m.) per 1 g of sodium alginate. In batch systems, about 90 % conversion in 48 h was obtained both with free and immobilized yeast using demineralized whey of 5 to 10 % lactose content as substrate. In continuous packed-bed column operation nearly a constant 2 % product ethanol concentration could be maintained at 5 % substrate lactose level for at least one month.

INTRODUCTION

Total costs of biotechnical ethanol production are largely determined by the price of fermentable sugars employed (Kolot, 1980). Consequently, there has been an increasing interest in the utilization of waste materials and by-products as a cheap carbohydrate source. Whey, a by-product of dairy industry, appears to be an ideal raw-material both for technical and drinking ethanol production (Mann, 1980; Zoll, 1980). Approximately half of annual world whey production is currently wasted, (Zoll *et al.*, 1979), corresponding to about 2.5 million tons of lactose (Linko, 1981), a potential source for about 1 million tons of ethanol.

Most work on whey fermentation has involved drinking ethanol and alcoholic beverage production (Hesse, 1948; Holsinger *et al.*, 1974; Friend and Sahalani, 1979), and techniques for whey wine (Roland and Alm, 1975; Kosikowski and Wzorek, 1977; Gavel and Kosikowski, 1978a,b) and for vodka (Antonov *et al.*, 1977, 1978) production have been developed. Whey has also succesfully been used in brewing (Poznanski *et al.*, 1978), and in place of water in the fermentation of cereal grain (Cunningham *et al.*, 1975).

Liquid whey contains about 5 % of lactose resulting in about 2 % ethanol concentration in conventional batch fermentations, approximately equivalent to that obtained in the fermentation of sulfite spent liquor (Linko, 1980a). Unfortunately, relatively few yeasts are able to ferment lactose (Burgess and Kelly, 1979). Laham-Guillaume *et al.*, 1980) have recently reported 80 to 90 % ethanol yields from concentrated

whey of about 15 % lactose with *Candida pseudotropicalis* and *Kluyveromyces fragilis* yeasts. Several workers have employed demineralized whey UF-permeate as substrate (Mahmoud and Kosikowski, 1978; Moulin and Galzy, 1980; Philliskirk *et al.*, 1980), and the effect of enzymatic hydrolysis of whey lactose on ethanol production has also been investigated (O'Leary *et al.*, 1977a,b). The first commercial scale operation based on conventional fermentation techniques in Ireland produces high quality drinking alcohol from whey (Hansen, 1980), and one plant has been reported to be in the planning stage in the USA (Anon., 1980). Two other processes have been tested in large scale (Bernstein *et al.*, 1977; Reesen and Strube, 1978).

Owing to the low lactose content of whey, continuous processing based on immobilized living yeast reactor would appear to have special advantages (Linko, 1980b; Linko and Linko, 1981; Wada *et al.*, 1981). Large fermentation vessels and costly yeast separation with cell recycle can be avoided. However, few reports have been published on the application of heterogeneous biocatalysis in ethanol production from whey. Villet *et al.* (1979) determined kinetic parameters with *Kluyveromyces fragilis* yeast encapsulated in polyacrylamide gel. Linko (1980a) employed calcium alginate gel bead entrapped living *Saccharomyces cerevisiae* yeast blended with phenolformaldehyde resin adsorbed and crosslinked *Aspregillus niger* β-galactosidase as a two biocatalyst column reactor system in continuous conversion of deminerilized whey lactose (5 % w/v) to ethanol (about 2 % w/v) for extended periods at $\tau = 4$ h, 25 °C, pH 4.5. Hägerdal (1980) used alginate with covalently bound β-galactosidase for the entrapment of yeast cells, and obtained about 60 % conversion of lactose in whey UF-permeate at 30 °C, pH 4.5 for at least 20 d. The present paper describes in greater detail the application of immobilized living yeast technology for continuous ethanol production from whey lactose.

MATERIALS AND METHODS

Materials

Spray dried electrolytically demineralized whey powder (DEMI) and whey UF-permeate powder (UF-P) were obtained from Kuivamaito Oy (Lapinlahti, Finland), and used in suitable concentrations as substrates for ethanol fermentations. The percent substrate concentration in the following refers to the lactose content. All substrates were adjusted to pH 4.5 with HCl, and sterilized under pressure at 121 °C for 20 minutes.

All fermentations were carried out with *Kluyveromyces fragilis* Jorgensen B-I-5, obtained from Oy Alko Ab (Helsinki, Finland). The culture was maintained on whey agar slant [UF-P 150 g, dried corn steep liquor 5 g, $(NH_4)_2SO_4$ 3 g, $(NH_4)_2HPO_4$ 3 g, agar 20 g per liter; pH 4.5]. Active culture for inoculation was prepared in shake flasks at 27 °C for 20 h in the above medium except for agar, pH 5.0. Ten liters of nutrient solution [UF-P 830 g, dried corn steep liquor 50 g, $(NH_4)_2SO_4$ 50 g, $(NH_4)_2HPO_4$ 30 g per 10 l] in a 30 l fermentor was inocultaed with 1.5 % of yeast. After 2 h at 27 °C under aeration (1.5 vvm), 1.5 l of nutrient solution [lactose 600 g, $(NH_4)_2SO_4$ 20 g, $(NH_4)_2HPO_4$ 10 g] were added during a period of 10 h. The pH was automatically maintained at 5.0 by the addition of ammonia. Cells were harvested by centrifugation, washed with water, and used for immobilization.

Immobilization of Living Yeast Cells

Yeast cells (20.4 % d.m.) were entrapped in calcium alginate beads according to the method described by Linko *et al.* (1980), except that the final crosslinking with glutaraldehyde was omitted. Various quantities of wet yeast cells were suspended in 100 g of 8 % sodium alginate, and extruded through 0.6 mm diameter needles to 0.5 M $CaCl_2$ to form beads of an average diameter of 2.3 mm and a cell count of about 2.7×10^9 per gram.

Batch Fermentation

Batch fermentations with DEMI as substrate (5 and 10 % substrate lactose) were carried out in 250 ml Erlenmeyer flasks containing 170 ml of substrate solution and 3.9 g of free yeast or 10 g of immobilized biocatalyst, respectively. Flasks were incubated at 25°C under shaking (100 rpm).

Continuous Fermentation

Continuous fermentations were carried out in jacketed packed-bed glass columns of 2.8 cm diameter. The reactors were equipped with a stainless steel wire net at the bottom. In a typical run, 20 g (30 ml) of the immobilized biocatalyst beads were employed. Substrate (pH 4.5) was pumped to the bottom of the reactor, which was maintained at 25°C.

Ethanol Assay

Ethanol was determined enzymatically by Boehringer Mannheim GmbH Blood Alcohol Test Comination.

RESULTS AND DISCUSSION

Fig. 1 illustrates that in batch experiments with DEMI as substrate at 5 and 10 % substrate lactose levels immobilization did not affect adversly ethanol production by *K. fragilis*. At both lactose concentrations more than 90 % lactose utilization efficiency was obtained.

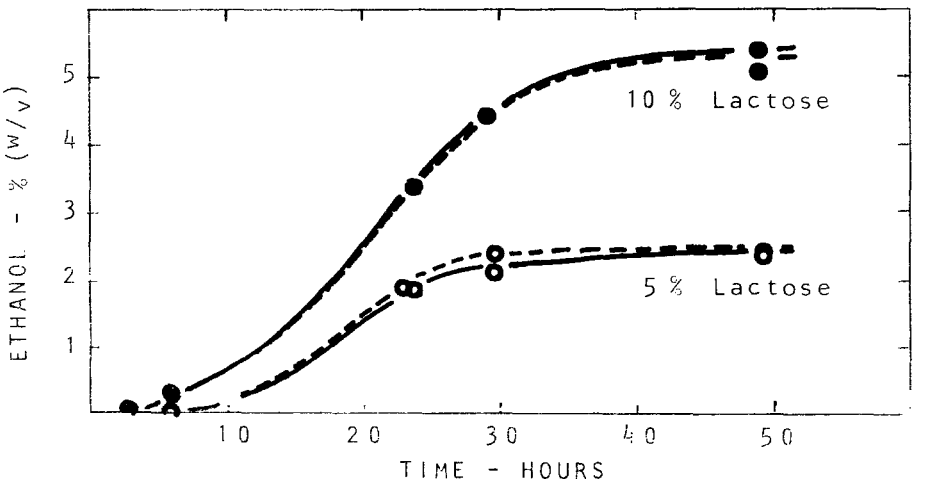

Fig. 1. Batch production of ethanol from DEMI with free (----) and immob-lized (——) *K. fragilis*.

The effect of initial cell loading on ethanol production with DEMI is shown in Fig. 2. In all cases both ethanol production and yeast cell count increased during the first 3 days of fermentation, with most marked increases at the two lowest cell loading levels of 16 and 32 g per 100 g of alginate solution. Most stable production was maintained

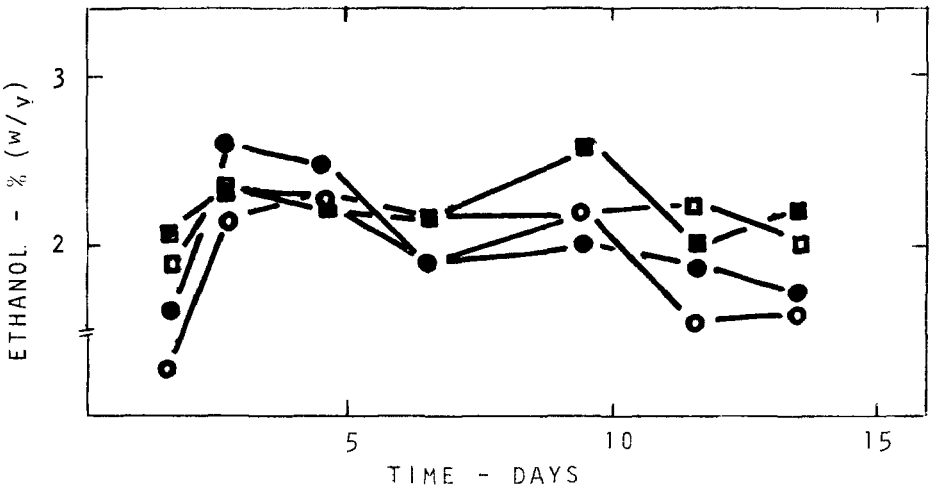

Fig. 2. The effect of initial cell loading on continuous ethanol production with immobilized *K. fragilis* (○ 16 g, ● 32 g, □ 64 g, ■ 128 g per 100 g of 8 % alginate. Substrate DEMI (5 % lactose), τ = 3.9 h.

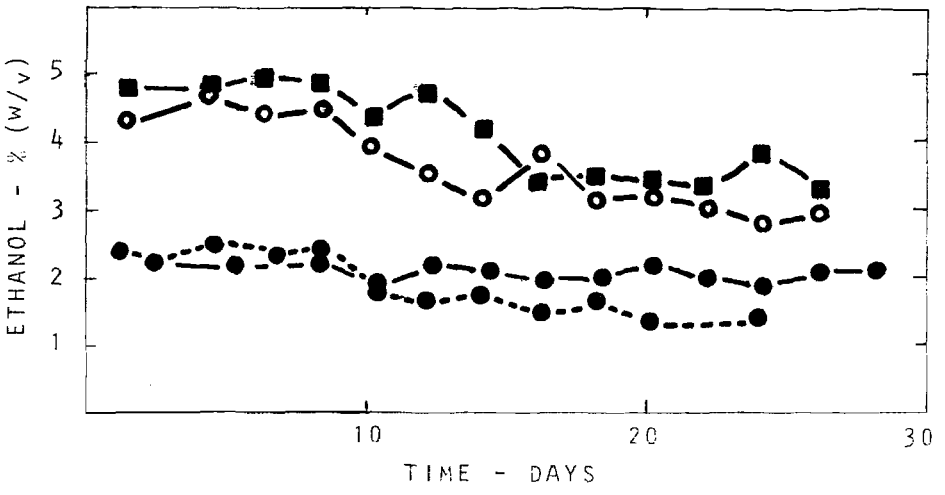

Fig. 3. The effect of lactose concentration (● 5 %, τ = 3.9 h, ○ 10 %, τ = 8.3 h, ■ 15 %, τ = 15 h) on continuous ethanol production from DEMI (——) and UF-P (----) with immobilized *K. fragilis* (64 g yeast per 100 g 8 % alginate)

at 64 and 128 g of yeast per 100 g of alginate, with about 80 % lactose utilization efficiency. Approximately 10^7 cells per ml of effluent were released during continuous processing, with a relatively stable yeast population of 2.7×10^9 g^{-1} in the biocatalyst bed.

Fig. 3 shows that with DEMI at 5 % substrate lactose level at τ = 3.9 h ethanol production remained nearly constant at about 2 % (w/v) for at least 31 d, with about 80 to 90 % of lactose utilized. An increase in lactose concentration to 10 %, at τ = 8.3 h, product ethanol concentration remained at about 4.5 % for 9 d, followed by a gradual decline to about 3 % in 27 d. A further increase in lactose concentration to 15 % did not result in an increased ethanol production, even at nearly doubled residence time, and the lactose utilization efficiency decreased from a maximum of about 60 % in the beginning to approximately 40 % after 27 d. This behaviour was explained, at least in part, with the inability of the *K. fragilis* strain to tolerate higher ethanol levels under the experimental conditions employed. It is also possible that lactose hydrolysis rate is a limiting factor under these conditions. In batch experiments, O'Leary *et al.* (1977b) obtained the highest ethanol concentration of 3.72 % with UF-P at 10 % total solids (~8 % lactose), and of 2.3 % at 20 % total solids (~16 % lactose), using *K. fragilis* NRRL 1109. They explained this inability to obtain more than about 4 % of ethanol with whey UF-P by the inhibition of yeast β-galactosidase by ethanol, thus reducing the organism's ability to utilize lactose. On the other hand, Moulin and Galzy (1980) obtained ~9.5 % (w/v) ethanol with UF-P at 20 % substrate lactose in 90 h batch fermentation both with *K. fragilis* CBS 397 and *C. pseudotropicalis* IP 513.

Fig. 3 also illustrates that somewhat more stable ethanol production level could be maintained with DEMI than with UF-P as substrate. The average composition of DEMI and UF-P used is shown in Table I. The mineral salt content of ~9 % of UF-P is approximately 9 times as high as that of DEMI, and is likely to be one reason for the somewhat decreased stability after about 10 d of continuous processing. The effect of salt content, and the possible adaptation of *K. fragilis* to higher salt levels was not investigated in detail. It should be noted, however, that although DEMI is likely to be too costly substrate in actual industrial practice, approximately 50 % demineralization of UF-P is considered economically feasible.

Table I. Average composition of DEMI and UF-P (%)

Substrate	Lactose	Protein	Ash
DEMI	80...84	14	1
UF-P	82...86	1	9

REFERENCES

Anon. (1980) *Biosources Digest* 2(8), 3.

Antonov, S. F., Kreshenin, P. F., Boganov, V. N., Khramtsov, A. G. & Eremin, C. E. (1977) *Brit. Pat.* 1,491,405.

Antonov, S. F., Kreshenin, P. F., Boganov, V. N., Khramtsov, A. G. & Eremin, C. E. (1978) *U.S. Pat.* 4,086,368.

Bernstein, S., Tzeng, C. H. & Sisson, D. (1977) *Biotechnol. Bioeng. Symp.* 7, 1-9.

Burgess, K. J. & Kelly, J. (1978) *Irish J. Fd Sci. Technol.* 3(1), 1-9.

Cunningham, M. L., Friend, B. A. & Shahani, K. M. (1975) *J. Dairy Sci.* 62, Suppl. 1, 60.

Friend, B. N. & Shahani, K. M. (1978) *New Zealand J. Dairy Sco. Technol.* 14, 149-152.

Gavel, J. & Kosikowski, F. V. (1978a) *J. Fd Sci.* 43, 1031-1032.

Gavel, J. & Kosikowski, F. V. (1978b) *J. Fd Sci.* 43, 1717-1718.

Hansen, R. C. (1980) *North Eur. Dairy J.* 46(1-2), 10-17.

Hesse, A. (1948) *Z. Lebensm.-Unters. u. -Forsch.* 89, 499-508.

Holsinger, V. H., Posati, L. P. & Devilloiss, E. D. (1978) *J. Dairy Sci.* 57, 849-859.

Hägerdal, B. (1980) *Proc. 6th Int'l Fermentation Symp.*, 20.-25. July, 1980, London, Ontario, Canada. Pergamon Press, New York (in press).

Kolat, F. B. (1980) *Process Biochem.* 15(7), 2-8.

Kosikowski, F. V. & Wzorek, W. C. (1977) *J. Dairy Sci.* 60, 1982-1986.

Latham-Guillaume, M., Moulin, G. & Galzy, P. (1978) *Lait* 59, 489-498.

Linko, P. (1980a) In *Food Process Engineering*, Vol. 2, *Enzyme Engineering in Food Processing* (P. Linko & J. Larinkari, eds), Applied Science Publ., London, 27-39.

Linko, P. (1980b) *Proc. 6th Int'l Fermentation Symp.*, 20.-25. July, 1980, London, Ontario, Canada. Pergamon Press, New York (in press).

Linko, P. (1981) *Proc. Int'l Symp. Nutritive Sweeteners*, 30. March - 1. April, 1981, Weybridge, England. Applied Science Publ., London (in press).

Linko, Y.-Y. & Linko, P. (1981) *Biotechnol. Lett.* 3(1), 21-26.

Linko, Y.-Y. & Linko, P. (1980) In *Food Process Engineering*, Vol. 2, *Enzyme Engineering in Food Processing* (P. Linko & J. Larinkari, eds), Applied Svience Publ., London, 81-91.

Mann, J. (1980) *Dairy Ind. Int'l* 45(3), 47-48.

Mahmoud, M. K. & Kosikowski, F. V. (1978) *J. Dairy Sci.* 61, Suppl. 1, 114-115.

Moulin, G., Boza, H. & Galzy, P. (1980) *Biotechnol. Bioeng.* 22, 2375-2381.

O'Leary, V. S., Green, R., Sullivan, B. C. & Holsinger, V. H. (1977a) *Biotechnol. Bioeng.* 19, 1019-1035.

O'Leary, V. S., Sutton, C., Bencivengo, H., Sullivan, B. C. & Holsinger, V. H., (1977b) *Biotechnol. Bioeng.* 19, 1689-1702.

Philliskirk, G. & Yates, H. J. (1978) *Brit. Pat.* 1,504,618.

Poznanski, S., Leman, J., Bednarski, V., Smelich, V., Kowalewska, J., Chadkowski, M. & Wieliczka, R. (1978) *Nahrung* 22, 275-285.

Reesen, L. & Strube, R. (1978) *Process Biochem.* 13(11), 21-24.

Roland, J. F. & Alm, W. L. (1975) *Biotechnol. Bioeng.* 22, 1935-1948.

Wada, M., Kato, J. & Chibata, I. (1981) *Eur. J. Appl. Microbio. Biotechnol.* 11, 67-71.

Viller, R., Dillon, J. & Manderson, G. (1979) *Sun 2, Proc. Int'l Sol. Energy Soc. Silver Jubilee Congr.*, 78-82.

Zoll, R. R., Kuipers, A., Muller, C. C. & Marshall, K. R. (1979) *New Zealand J. Dairy Sci. Technol.* 14, 79-83.

Zoll, R. R. (1980) *Dairy Ind. Int'l* 45(4), 30-39.