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Nutrients Along with Calcium in Glucose Feed Enhance the Life of Alginate Entrapped Yeast Cells

Vasanthy Arasaratnam

Department of Biochemistry, Faculty of Medicine, University of Jaffna, Kokuvil, Sri Lanka

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Saccharomyces cerevisiae *cells were entrapped in 1% (w/w) alginate. The optimum flow rate and glucose concentration in the feed for maximum ethanol yield were 50 ml* h^{-1} *and 50 g* l^{-1} *respectively. Immobilized* S. cerevisiae *performed better with medium containing nutrients and 0·05* M $CaCl_2$ *(28 days) than with nutrients alone (10 days). Ethanol production decreased with an increase in alginate concentration. A packed bed reactor containing* S. cerevisiae *entrapped in 1·5% (w/w) alginate operated continuously for 40 days without problems and loss of productivity, when the glucose feed was supplemented with nutrients and calcium.*

INTRODUCTION

Many different techniques for immobilizing cells have been adopted for the last two decades.[1–4] Calcium alginate is the most widely used material among those reported for entrapments.[2,5] The popularity of alginate is due to the mild conditions and the single step procedure used for entrapment.[2] In addition, it provides a gentle environment for the entrapped cells and is permitted in food and pharmaceutical products.[5] Unfortunately, calcium alginate gel has a low stability in the presence of substances which have a high affinity for Ca^{2+} such as phosphate, citrate and lactate, and non-gel-inducing ions such as Na^+ and Mg^{2+}.[2,6]

Calcium alginate entrapped yeasts have been employed to produce ethanol from sugars in the absence of substances which disturb the gels.[1,6–8] In general, gel stabilizing substance such as $CaCl_2$ have been added to the sugar solution.

In this paper the continuous production of ethanol from glucose by calcium alginate entrapped *S. cerevisiae* is reported. The glucose feed was supplemented with nutrients for cell viability and calcium for bead stabilization. A packed bed reactor was checked for continuous operation under optimized conditions.

MATERIALS AND METHODS

Organism

Saccharomyces cerevisiae was obtained from Jästblagot, Sweden.

Cultivation of *S. cerevisiae*

Cells were grown before immobilization in a 10 litre fermentor containing nutrient medium having the following composition in g $litre^{-1}$ glucose

Corresponding author: V. Arasaratnam. Tel: 22481.

monohydrate 66·05, yeast extract 2·5, $NH_4H_2PO_4$ 0·25 and $MgSO_4 \cdot 7H_2O$ 0·025, at 35°C, 400 rev min^{-1} and pH 4·5. Cells were harvested in the late exponential phase of growth (18 h) and concentrated by centrifugation at 10000 rev min^{-1} in a refrigerated centrifuge for 10 min. Cells were washed three times with saline.

Immobilization of *S. cerevisiae*

A suspension of *S. cerevisiae* (5 g) in 15 ml sodium alginate (1%, w/w) was extruded through a syringe (0·7 mm diameter) into 500 ml 0·05 M $CaCl_2$. The beads were hardened in $CaCl_2$ solution for 2 h and then packed in a jacketed column (20 × 2 cm), which was maintained at 35°C.

Analytical methods

Residual glucose in the culture medium and the ethanol produced were determined by HPLC.[9]

RESULTS AND DISCUSSION

The effects of flow rate on ethanol production were studied by changing the flow rate of the nutrient medium from 5 to 100 ml h^{-1}. When the flow rate was increased from 5 to 50 ml h^{-1} (equivalent to a dilution rate of 0·04–0·4 h), glucose (60 g $litre^{-1}$) in the media was oxidized to ethanol (20 g $litre^{-1}$) (Fig. 1). The concentration of glucose in the effluent increased almost linearly at flow rates higher than 30 ml h^{-1}.

The ethanol yield remained almost constant at about 0·36 g ethanol per g glucose, while the ethanol productivity reached a maximum of 10·5 g $litre^{-1}$ h^{-1} at a dilution rate of 0·6 h^{-1} (Fig. 2). Bajpai and Magaritis[8] have reported that maximal ethanol production by calcium alginate entrapped *Kluyveromyces marxianus* was obtained at a dilution rate of 0·5 h^{-1}.[8]

Preliminary studies showed that the optimal glucose concentration for continuous fermentation was 50 g $litre^{-1}$ (Fig. 3). An increase in glucose concentration to 500 g $litre^{-1}$ resulted in a decrease in the yield of ethanol to 0·148 g per g glucose. McGhee *et al.*[1] have reported that calcium alginate entrapped *S. cerevisiae* completely converted, 1, 3 and 5% glucose to ethanol but were less efficient (80%) in converting 20% (w/w) glucose. In this study when 200 g $litre^{-1}$ glucose was used the efficiency dropped to 50·8%.

The entrapped cells could only be used in continuous fermentation for 9 days if the feed was supplemented with nutrients with no added calcium. The addition of calcium to feed supplemented with nutrients increased the useful life of the reactor to 30 days. In the control experiment without calcium, cell multiplication disrupted the alginate beads leading to a leakage of the yeast into the elute. These results suggest that problems of disruption of alginate beads due to cell multiplication might be controlled by adding calcium to the glucose feed. The avoidance of magnesium

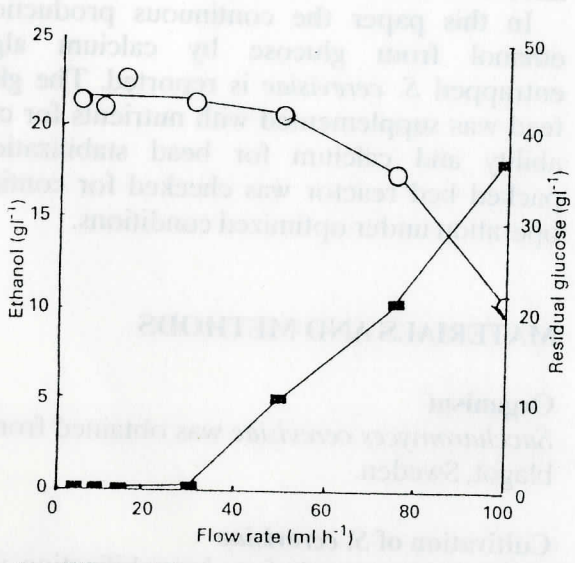

Fig. 1. Effect of flow rate on the continuous fermentation of glucose (60 g $litre^{-1}$) to ethanol by *S. cerevisiae* entrapped in calcium alginate ((○); ethanol; (■) residual glucose). Conditions as described in the text.

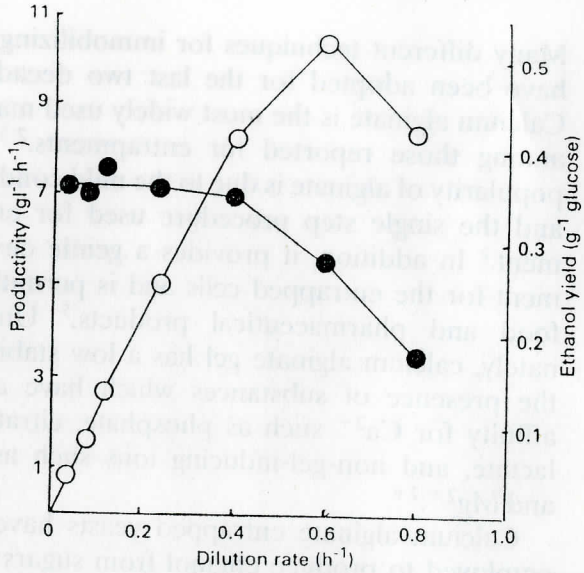

Fig. 2. Changes in ethanol productivity and ethanol yield (g g^{-1}) glucose as a function of dilution rate ((○); ethanol productivity; (●); ethanol yield (g g^{-1}) glucose). Conditions as described in the text.

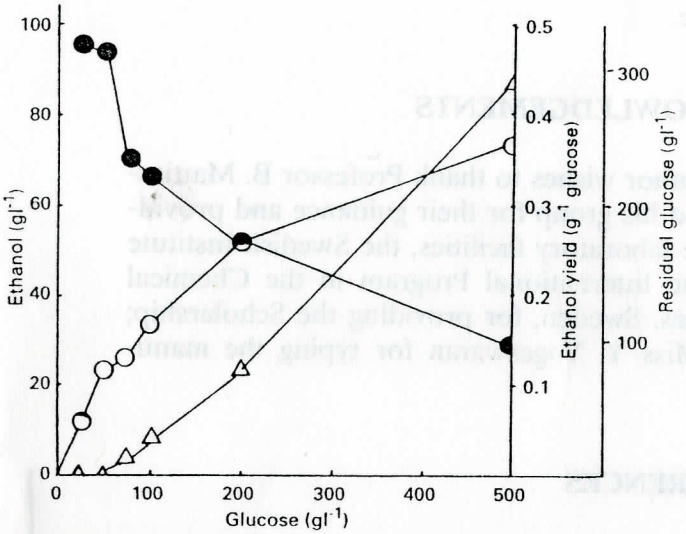

Fig. 3. Effect of glucose concentration on continuous fermentation (flow rate 50 ml h^{-1}) to ethanol by *S. cerevisiae* entrapped in calcium alginate ((○); ethanol (●); yield of ethanol (△); residual glucose). Conditions as described in the text.

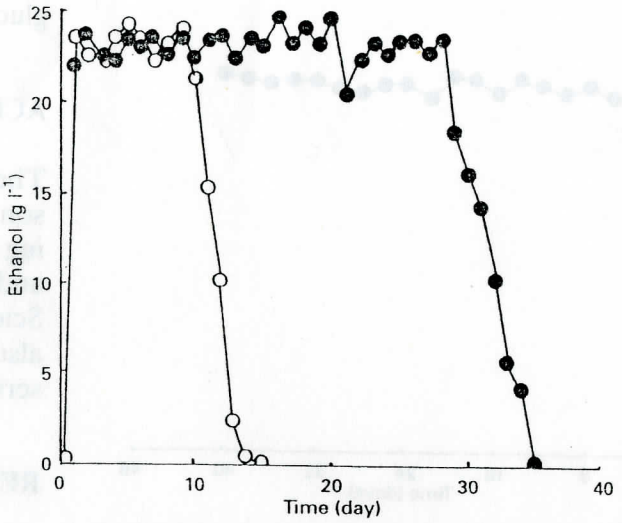

Fig. 4. Effect of calcium on the continuous fermentation (flow rate 50 ml h^{-1}) of glucose (50 g $litre^{-1}$) supplemented with nutrients by *S. cerevisiae* entrapped in alginate (1%, w/w) (medium without (○) and with (●) $CaCl_2$). Conditions as described in the text.

and phosphate in the medium is not possible as they are essential for the metabolism, maintenance of cell viability and cell wall integrity.[10]

In this study, instead of using glucose solution supplemented with $CaCl_2$ as previously reported,[6,7] the glucose feed with calcium (0·05 M) was supplemented with nutrients to strengthen the calcium alginate beads. The results (Fig. 4) indicate that the added calcium did not alter the productivity of the ethanol.

To evaluate the effect of alginate concentration on the performance of entrapped *S. cerevisiae* a batch experiment was carried out. Entrapped cells (15 ml) were inoculated into 200 ml (final volume) of nutrient medium containing 50 g $litre^{-1}$ glucose and 0·05 M $CaCl_2$. A decrease (11·5%) in the efficiency of ethanol production was observed when the alginate concentration was increased from 1% to 1·5% (Fig. 4). An increase in alginate concentration from 0·5 to 2·5% (w/w) resulted in a decrease in ethanol production of 41% (Fig. 5). Similar results were reported by other groups.[6,8] In contrast, with 0·5 and 0·75% (w/w) alginate, the beads formed were fragile and easily disrupted during the batch process. With an increase in alginate concentration the beads became more stable with respect to the retention of *S. cerevisiae*.

Since *S. cerevisiae* entrapped in 1·5% (w/w) alginate showed only a 11·5% reduction in the efficiency of ethanol production compared with

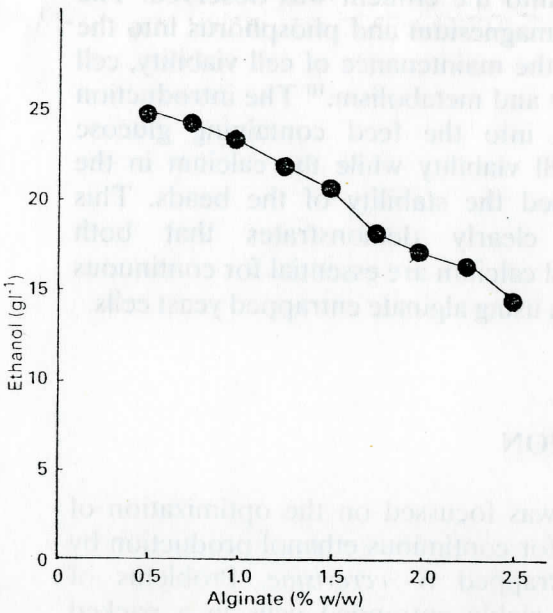

Fig. 5. Ethanol production by calcium alginate entrapped *S. cerevisiae* as a function of alginate concentration. Conditions as described in the text.

1% (w/w) alginate and considering both the reactor life and stability of the beads, cells which were entrapped in 1·5% (w/w) calcium alginate were selected for a glucose fermentation trial in continuous mode. It was possible to continue the fermentation for a long period, although the trial

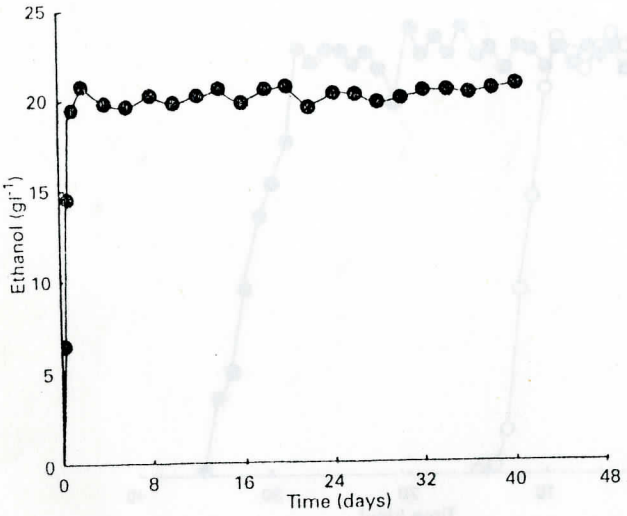

Fig. 6. Continuous fermentation (flow rate 50 ml h^{-1}) of glucose (50 g $litre^{-1}$) to ethanol in a nutrient medium containing 0·05 M $CaCl_2$ using *S. cerevisiae* entrapped in alginate (1·5%, w/w). Conditions as described in the text.

was terminated on the 40th day (Fig. 6). Although the disruption of beads was not apparent, leakage of the cells into the effluent was observed. The inclusion of magnesium and phosphorus into the feed helped the maintenance of cell viability, cell wall integrity and metabolism.[10] The introduction of nutrients into the feed containing glucose increased cell viability while the calcium in the feed increased the stability of the beads. This experiment clearly demonstrates that both nutrients and calcium are essential for continuous fermentation using alginate entrapped yeast cells.

CONCLUSION

This study was focussed on the optimization of parameters for continuous ethanol production by alginate entrapped *S. cerevisiae*. Problems of maintaining viable entrapped cells in a packed bed column have been overcome to some extent by using nutrients in feed media in addition to glucose.

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