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Continuous production of fructose syrup and ethanol from hydrolysed Jerusalem artichoke juice

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SUMMARY

The results from this study showed that Jerusalem artichoke juice can be used for the production of very enriched fructose syrup by selective conversion of glucose to ethanol in a continuous process using immobilized cells of *Saccharomyces cerevisiae* ATCC 36859. The product contained up to 99% of the total carbohydrates as fructose compared to 76% in the feed. Using Jerusalem artichoke juice supplemented with some glucose a product was obtained with 7.5% w/v ethanol which made ethanol recovery economically favourable. It was found that some fructose was consumed in these continuous processes; the glucose/fructose conversion rate ratio was regulated by the glucose concentration in the product stream.

INTRODUCTION

Fructose syrups are currently manufactured by the inversion of sucrose or by the isomerization of glucose from corn starch [9]. Normally such syrup contains 50% glucose, 42% fructose and 8% other saccharides and is known as 42% High Fructose Corn Syrup (HFCS). Since fructose is the sweetest naturally occurring carbohydrate [7,15], the sweetness of this syrup can be increased by increasing its fructose content. This increase would mean that smaller amounts of HFCS would be needed to produce the same sweetness resulting in fewer calories in the product. Other syrups containing 55 and 90% fructose (55 and 90% HFCS) have been made but due to the high production costs associated with separating these two sugars only 55% fructose syrups are produced commercially [5].

Fructose can also be obtained by the hydrolysis of polyfructans. These polyfructans or inulin molecules are made of multiple fructose units terminated by a glucose unit [10] and serve as a food reserve in plants of the Compositae family. Previous studies have investigated the production of fructose by chemically or enzymatically hydrolysing polyfructans from Jerusalem artichoke tubers [2,8,11,20]. Fructose syrups produced in this manner normally yield a product containing 80% of the total carbohydrate as fructose [10].

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An alternative method that has been suggested to produce a syrup containing a high fructose content is to selectively convert glucose to a substance easily separated from fructose. In this way the difficult glucose–fructose separation step is eliminated. Reusser et al. [16] proposed utilization of *Tricholoma nudum* for hydrolysis of sucrose and then conversion of the glucose to biomass. When the sucrose concentration was 8.0% w/v only 75% of the fructose was present in the final product. Ueng et al. [18] reported that two mycelial fungal systems, *Mucor* sp. M105 and *Fusarium* sp. F5 selectively utilize the glucose from glucose–fructose mixtures to produce ethanol. Both showed significant fructose consumption when both sugars are present in the medium. The bacterium *Zymomonas mobilis* hydrolyses sucrose to glucose and fructose and then ferments the glucose to ethanol [1]. When sufficient glucose and fructose are present in the medium, sorbitol is also produced [13,19]. A mutant of *Saccharomyces cerevisiae* has also been shown to possess selective fermentation capabilities [14]. Preliminary work carried out [6,12] has shown the mutant (ATCC 36859) to be very selective with no sorbitol being produced.

The objectives of this work are: (a) to study the production of a high fructose syrup from juice extracted from Jerusalem artichokes and from juice supplemented with some glucose in a continuous process using *Saccharomyces cerevisiae* ATCC 36859 for the conversion of glucose to ethanol; and (b) to study the performance of the immobilized cell reactor and the relationship between the ratio of the glucose to fructose consumption rate and the glucose concentration in the outlet stream.

MATERIALS AND METHODS

The microorganism *S. cerevisiae* ATCC 36859, which was obtained by mutation [14], was maintained on malt extract agar slants. The medium for the preparation of the inocula consisted of: glucose (10 g), yeast extract (30 g), peptone (3.5 g) KH_2PO_4 (2 g), $MgSO_4$ (1 g), $(NH_4)_2SO_4$ (1 g) and distilled water (up to 1 l). Growth was carried out at 33 °C for 30 h. This time was sufficient to ensure that the cells were in the exponential growth phase.

A cell slurry was added to an equal volume of a 4% Na-alginate solution. The mixture was then added dropwise to 0.2 M $CaCl_2$ by extruding it through a 3-mm glass tube. Upon contact with the $CaCl_2$, spherical beads (3 mm diameter) of Ca-alginate were formed and left to harden in solution for 2 h.

The bioreactor was a borosilicate glass tube (3.1 cm I.D.) with a plexiglass jacket. The beads were confined to a 11.2-cm (84 ml) section in the reactor by metal screens. About 75% of the space was occupied by the beads and the voids between them at the begining of the test. Although the beads settled to the bottom of the reactor after they were placed in the reactor they floated to the top within 1–2 h. After this time the production of CO_2 was very vigorous, resulting in the creation of pockets of gas within the reactor. This led to channeling and to the expansion of the bed so that it occupied the total reactor volume. This phenomenon was also observed by McGhee et al. [21]. It is expected that during this initial period of adjustment, some growth was occurring which resulted in an increase in the bead volume as was noted by Lee et al. [16]. Since changes in the bed volume were observed throughout the experiment due to these phenomena, the volume between the screens was used as the basis for dilution rate calculations. All tests were carried out at 33 °C.

A positive displacement pump (Watson-Marlow) introduced the feed at the bottom of the reactor. Hydrolysed Jerusalem artichoke juice was as the feed since it contains glucose and fructose and nutrients that support growth and ethanol production by the yeast cells. The procedure used to hydrolyse the juice was described previously [6]. It was supplemented with 10 mM of $CaCl_2$ to improve the stability of the beads. In some experiments the glucose concentration was increased by adding glucose to the hydrolysed juice. The media were sterilized at 115 °C for 15 min prior to their use.

The ethanol concentration was determined enzymatically using alcohol dehydrogenase [3]. Fructose and glucose concentrations were quantitated with a Waters high-performance liquid chromatograph. A Sugar Pak I column was operated at 80 °C with deionized water flowing at 0.5 ml/min.

RESULTS AND DISCUSSION

A continuous system with immobilized yeast cells was used to study the production of high fructose syrup from hydrolysed Jerusalem artichoke juice. The performance of the immobilized cell reactor at different flow rates using Jerusalem artichoke juice feed is shown in Fig. 1. The beads contained 47 g biomass per litre of alginate material. At a dilution rate of 0.106 h^{-1} the glucose concentration was reduced to 0.10 from 4.55% w/v producing ethanol in a concentration of 2.67% w/v. The ethanol concentration was higher than it was theoretically possible to produce from the glucose used because some fructose was also consumed, reducing its concentration to 13.27% w/v from 14.44% w/v. The ethanol yield, when the total amount of carbohydrate consumed was used as the basis for the calculation is 89% of the theoretical value. At this dilution rate, the carbohydrate content of the product was 99.25% fructose and only 0.75% glucose. When the dilution rate was increased to 0.254 h^{-1} the fructose concentration approached its inlet value. At this dilution rate the product contains 96% of the total sugars as fructose and 4% as glucose. Tests were also carried out at other dilution rates; the whole process lasted for 420 h, the steady state product concentrations are provided in Table 1. The results show that even at the highest dilution rate (0.625 h^{-1}), the product contained 93% of the total sugars as fructose and only 7% as glucose. Comparing this carbohydrate composition with that in the feed (77.2% fructose and 22.8% glucose) it can be concluded that a substantial increase in the fructose/glucose ratio can be obtained from Jerusalem artichoke juice applying this technology. Therefore, the sugar mixtures produced from these treated juices would contain much less glucose than those which would be obtained from untreated ones.

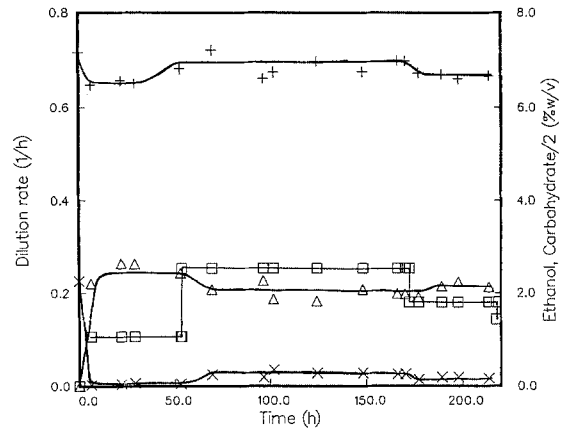

Fig. 1. Ethanol and fructose syrup production from hydrolysed Jerusalem artichoke juice in an immobilized cell reactor. (×) glucose, (+) fructose, (△) ethanol and (□) dilution rate.

TABLE 1

Product concentrations from an immobilized cell reactor using hydrolysed Jerusalem artichoke juice

Dilution rate (1/h)	Feed[a]	Ethanol (% w/v)	Glucose (% w/v)	Fructose (% w/v)
0.106	A	2.57	0.10	13.27
0.143	A	2.23	0.25	13.18
0.180	A	2.17	0.36	13.32
0.254	A	2.01	0.53	13.84
0.329	B	1.84	0.56	13.55
0.477	B	1.62	0.79	13.59
0.625	B	1.46	1.04	13.82

[a] A, 4.55% w/v glucose, 14.44% w/v fructose; B, 4.11% w/v glucose, 13.90% w/v fructose.

In the following set of tests, a study of the performance of the reactor at higher glucose concentrations was carried out. Attention was paid to the productivity of the reactor and to the effect of the glucose concentration in the outlet stream on the ratio of glucose to fructose consumption rates. Hydrolysed Jerusalem artichoke juice supplemented with some glucose was used as the feed medium.

Bearing in mind the objectives of this part (which differ from those in the preceeding section), in some tests the glucose concentration in the product stream was sometimes higher than in Jerusalem artichoke juice which was not supplemented with glucose. Hydrolysed Jerusalem artichoke juice has been chosen here only as a cheap food grade high fructose raw material. It is also used instead of a synthetic glucose–fructose medium as a source of nutrients other than carbohydrates for the yeast.

The tests of the performance of the reactor at different dilution rates were carried out with hydrolysed Jerusalem artichoke juice supplemented with glucose to concentrations of 8.5 and 12.9% w/v. The results from these tests concerning the relationship between the dilution rates and the ethanol, glucose and fructose concentrations in the outlet stream are shown in Tables 2 and 3. The ethanol concentration in the product was higher than that obtained in juice which was not supplemented with glucose. With an increase in the dilution rate and the glucose and total carbohydrate concentrations in the feed, increasing amounts of glucose were passing unconverted through the reactor (Fig. 2). The results also show (Fig. 3) that the reactor's productivity at lower dilution rates increased with an increase in the glucose concentration in the feed. At a moderate dilution rate of 0.254 h^{-1}, the productivity started levelling off due to substrate inhibition when the reactor was fed with the medium containing 12.9% w/v glucose and in which the total carbohydrate concentration was above 26% w/v. When the glucose and total carbohydrate concentration were lower

TABLE 2

Product concentrations from an immobilized cell reactor using hydrolysed Jerusalem artichoke juice supplemented with glucose for a total of 8.49% w/v glucose[a]

Dilution rate (1/h)	Ethanol (% w/v)	Glucose (% w/v)	Fructose (% w/v)
0.106	3.80	0.53	12.16
0.143	3.71	1.15	12.71
0.180	3.59	1.44	13.08
0.254	3.22	2.24	13.61

[a] Feed contains 8.49% w/v glucose, 14.10% w/v fructose.

TABLE 3

Product concentrations from an immobilized cell reactor using hydrolysed Jerusalem artichoke juice supplemented with glucose for a total of 12.92% w/v glucose[a]

Dilution rate (1/h)	Ethanol (% w/v)	Glucose (% w/v)	Fructose (% w/v)
0.106	4.86	2.84	12.68
0.143	3.91	4.73	–
0.180	3.57	5.63	12.97
0.254	2.65	7.33	13.20

[a] Feed contains 12.92% w/v glucose, 13.40% w/v fructose.

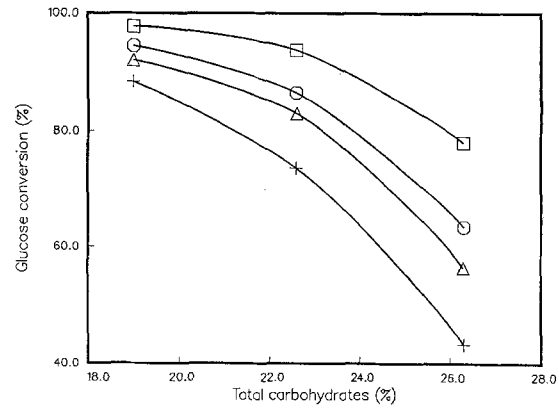

Fig. 2. Relationship between glucose conversion and total initial carbohydrate concentration at different dilution rates. (□) 0.106, (○) 0.143, (△) 0.180 and (+) 0.254 h^{-1} (glucose and fructose contents for the three total carbohydrate concentrations are shown in Tables 1, 2 and 3).

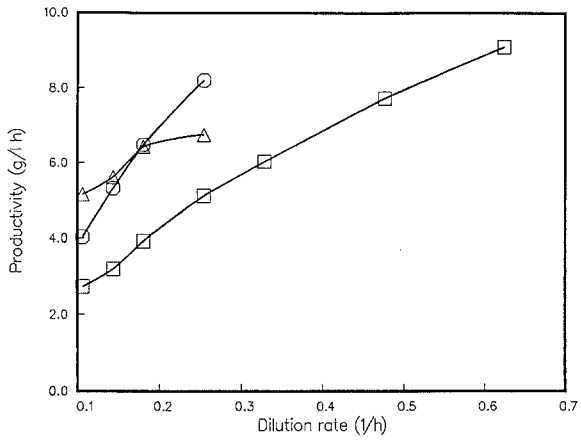

Fig. 3. Effect of dilution rate on ethanol productivities at different glucose concentrations. (□) 4.55% w/v glucose + 14.44% w/v fructose, (○) 8.49% w/v glucose + 14.10% w/v fructose and (△) 12.92% w/v glucose + 13.40% w/v fructose.

in the feed, the reactor's productivity continued increasing even at higher dilution rates (Fig. 3). The results indicate that greater than 90% of the fructose in the feed can be recovered in the product from this process (Tables 2 and 3) whereas only 50% could be recovered using molds [18]. As well, the product did not contain sorbitol, whereas similar processes tested with bacteria contained up to 3.5% w/v [17] and 6% w/v [4] of the by-product.

In all these experiments it was noticed that the ratio of glucose to fructose consumption rates was strongly dependent on the outlet glucose concentration. When the concentration of glucose in the product stream was reduced nearly to zero, glucose was consumed about four times as fast as fructose (Fig. 4). The rate of fructose consumption was about 20 times lower than that of glucose when the glucose concentration was 6% w/v in the outlet stream. A least squares analysis of the data indicates that the relationship between the ratio of glucose to fructose consumption rates and the outlet glucose concentration can be given by the following empirical equation:

$$Y = 3.66 + 2.82(X)$$

where Y is the ratio of glucose to fructose consumption rate and X is the outlet glucose concentration. The line representing this equation is drawn on Fig. 4. Using this relationship one can determine the amount of fructose that will be consumed from a glucose–fructose mixture by the yeast for a given outlet glucose concentration which is the result of the operating conditions of the reactor.

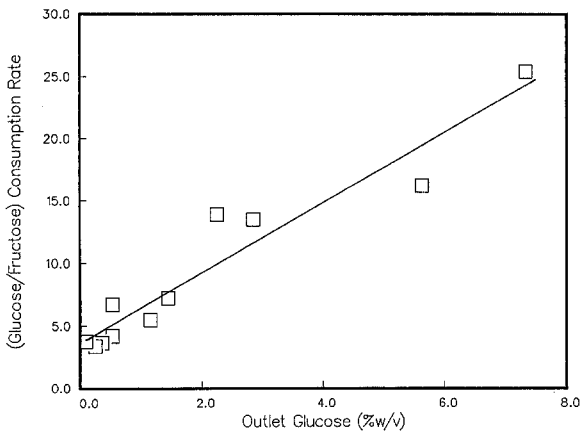

Fig. 4. Effect of the outlet glucose concentration on the glucose/fructose consumption rate ratio.

In batch experiments practically no fructose was consumed because the glucose concentration was low only at the end of the experiment when it was stopped [6]. In addition, the biomass concentrations that were used were low, resulting in a relatively low glucose rate and an even lower fructose rate which was not significant enough to be observed in batch experiments.

The immobilized cell system was operated for more than 1100 h without aeration using hydrolysed Jerusalem artichoke juice with and without added glucose. During this time the activity of the cells remained relatively constant. Some loss in activity was observed after 1150 h of operation; 145 h after this period the ethanol productivity dropped to 4.6 from 5.5 g/l · h. This decline in activity may be due to the fact that the yeast cells were in contact with a solution containing little or no oxygen and some ethanol for a long period of time. For the most part the beads remained spherical throughout the experiment. A small amount of cellular material was observed in the product stream, indicating some cell leakage from the alginate beads. In addition, due to bead disruption, some alginate and yeast cells accumulated at the bottom of the reactor.

To increase the economic viability of the process it is necessary to increase the glucose conversion rate and the outlet concentration of ethanol which is a valuable coproduct in this process. To attain these objectives, the cell concentration in the beads was increased to 102 g/l (approx. two times higher than the previous beads). Jerusalem artichoke juice (supplemented with glucose) which contained 15.7% w/v glucose and 11.9% w/v fructose was used as the feed in this process. The results showed (Table 4) that pure fructose syrup was produced with 7.5% w/v ethanol at a dilution rate of 0.086 h^{-1}. About 17% of the fructose was consumed in the process because of the low dilution rate. Increasing the dilution rate to 0.113 h^{-1} the amount of fructose consumed was reduced to 6% and the concentration of ethanol in the product dropped to 6.7% w/v. The experiments with the higher bead cell concentration exhibited the same type of relationship between the glucose–fructose consumption

TABLE 4

Performance of the reactor with a high concentration of yeast cells in beads[a]

Dilution rate (1/h)	Ethanol (% w/v)	Glucose (% w/v)	Fructose (% w/v)
0.086	7.56	0.00	9.86
0.113	6.70	0.00	11.16
0.130	5.65	3.61	11.26

[a] Feed consists of Jerusalem artichoke juice supplemented with glucose (15.7% w/v glucose and 11.9% w/v fructose).

ratio. This last test showed that Jerusalem artichoke juice supplemented only with glucose can be used for the production of a product containing fructose and a relatively high concentration of ethanol in a continuous process using *S. cerevisiae* ATCC 36859.

CONCLUSION

From the results it can be concluded that Jerusalem artichoke juice can be used for the production of very enriched fructose syrup by converting the glucose to ethanol in a continuous process using *Saccharomyces cerevisiae* ATCC 36859. The viability of the process was increased by using Jerusalem artichoke juice supplemented with some glucose which resulted in a higher ethanol concentration in the reactor's outlet stream.

In the continuous process some fructose was consumed with glucose. It was demonstrated that the ratio of glucose to fructose consumption rates was regulated by the outlet glucose concentration. An empirical linear expression was obtained which correlated these two parameters.

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