Appl Microbiol Biotechnol (1991) 35:711-715
017575989100208X

Applied and Microbiology Biotechnology

Production of sorbitol and ethanol from Jerusalem artichokes by *Saccharomyces cerevisiae* ATCC 36859

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Received 28 February 1991/Accepted 24 May 1991

Summary. This study shows the possibile use of Jerusalem artichokes for the production of sorbitol and ethanol by *Saccharomyces cerevisiae* ATCC 36859. Ethanol was produced from the beginning of the process, while sorbitol production started after glucose had been entirely consumed from Jerusalem artichoke (J.a.) juice. The importance of yeast extract and inoculum concentrations on the production of sorbitol from the above raw material was demonstrated. With a low initial biomass concentration sorbitol was not produced in pure J.a. juice. When the juice was supplemented with 3% yeast extract, the concentration of sorbitol was 4.6%. The sorbitol, ethanol and biomass yields (gram of product produced per gram of sugars consumed) were 0.259, 0.160 and 0.071 at the end of the process respectively. Adding glucose to increase its concentration to about 9% in the J.a. juice with 3% yeast extract had a positive effect on the production of ethanol, while commencement of the production of sorbitol was delayed and its final concentration was less than 50% of its concentration in the medium without added glucose. The effect of glucose was much stronger when it was added during the process than when added at the beginning of the process.

Introduction

Sorbitol is a sweet polyol that is a suitable sugar substitute for diabetics. It is also useful in cosmetics, pharmaceuticals, foods and the chemical industry. It is produced industrially by glucose hydrogenation in the presence of a nickel catalyst at pressures of 40–50 atm and temperatures of 140–150° C followed by subsequent purification including exchange chromatography (Phillips 1963).

It is well known that sorbitol can be converted by microorganisms to other products (Fulmer and Underkofler 1947; Shaw 1956; Duvnjak and Tamburasev 1966; Barnett 1968), but less is known about the production of sorbitol by microorganisms. It has been reported that *Zymomonas mobilis* strains produce ethanol and some sorbitol (Viikari 1984a; Bringer-Meyer et al. 1985; Barrow et al. 1984; Doelle and Greenfield 1985; Viikari 1984b) when grown on sucrose or glucose-fructose mixtures. These two products were accompanied by levan (Viikari 1984a), acetaldehyde, gluconate and acetoin (Bringer-Meyer et al. 1985). The presence of glucose was crucial for sorbitol production from fructose. The production of sorbitol and gluconic acid by toluene-treated *Z. mobilis* cells has been investigated using equimolecular glucose-fructose mixtures in semi-batch and continuous processes with free and immobilized cells (Chun and Rogers 1988). *Candida boidinii* (*Kloeckera* sp.) no. 2201 produced sorbitol from glucose by initial isomerization of the substrate to fructose via xylose isomerase and subsequent reduction of fructose to sorbitol via sorbitol dehydrogenase (Vongsuvanlert and Tani 1988).

To the best of our knowledge, the production of sorbitol by *Saccharomyces cerevisiae* strains has not yet been reported. Recently it was noticed in our laboratory that *S. cerevisiae* ATCC 36859 produced some sorbitol during growth and ethanol production in a fructose medium. The strain preferentially uses glucose from glucose-fructose mixtures. This ability of the yeast has been exploited in the production of pure fructose syrups from raw materials containing the above sugars (Koren and Duvnjak 1989). Bearing in mind that Jerusalem artichokes (J.a.) contain a large amount of fructose and some glucose in the form of inulin, this commodity was used to study the production of sorbitol by *S. cerevisiae* in this work. Previously, J.a. has been extensively considered in studies on the production of ethanol and fructose (Margaritis and Bajpai 1982a, b; Duvnjak et al. 1981; Fleming and Groot Wassink 1979).

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Materials and methods

S. cerevisiae ATCC 36859 was used in this study (Lobo and Maitra 1977). In consumes fructose much more slowly than glucose from a fructose-glucose mixture. The strain was used in a previous work for the production of very enriched or pure fructose syrup (Koren and Duvnjak 1990).

The yeast was maintained on malt extract agar slants at 4° C. The medium for inoculum preparation was made from yeast extract (30 g), peptone (3.5), KH_2PO_4 (2 g), $MgSO_4$ (1 g), $(NH_4)_2SO_4$ (1 g), glucose (4 g) and distilled water (up to 1 l).

Either pure hydrolysed J.a. juice or juice supplemented with 3% (w/v) yeast extract and some glucose was used for sorbitol and ethanol production. Glucose was added, if desired, at the beginning of the process, or 12 h and 24 h after inoculation of media. The J.a. juice was prepared as described previously (Koren and Duvnjak 1989). Various batches of J.a. juice were used and some variations sometimes occurred in the initial glucose-fructose concentration.

All media were sterilized at 115° C for 15 min.

Yeast for inoculum and sorbitol production was grown in 500-ml erlenmeyer flasks containing 100 ml medium in a shaker at 33° C at a speed of 200 rpm. Yeast for inoculum was grown for 36 h then left overnight at 4° C to sediment. A large part of the clear upper phase was decanted and the rest used for inoculation of media for sorbitol production.

Biomass was measured by weighing after separation by centrifugation, washing and overnight drying at 105° C. Glucose, fructose and sorbitol were measured by a Waters high performance liquid chromatograph, Model 6000 (Milford, Mass., USA). A Sugar Pak (Milford, Mass.) column operated at 80° C, with deionized water as the mobile phase at a flow rate of 0.5 ml/min, was used. Ethanol concentration was determined using the alcohol dehydrogenase method (Bernt and Gutman 1977). Duplicate tests were carried out.

Results and discussion

In the beginning of this study, pure J.a. juice without any supplement was used for the growth of *S. cerevisiae* ATCC 36859. The yeast converted glucose and some fructose to ethanol and biomass; other products were not noticed in the broth (Fig. 1a). In this experiment the inoculum concentration was about 0.81% (w/v). When the concentration of inoculum was increased to 1.89% and 2.82%, in addition to ethanol, sorbitol appeared in the broth (Fig. 1b and c). A higher inoculum concentration gave a higher sorbitol concentration. Comparing the last two experiments it was noticed that sorbitol appeared earlier when the inoculum concentration was higher, but in both cases, there was a long lag between the consumption of glucose and sorbitol appearance. The amount of sorbitol produced in these two tests was relatively low.

The next experiment was carried out with a low inoculum concentration (0.6%) in J.a. juice supplemented with 3% (w/v) yeast extract. In this case the yeast also produced some sorbitol in addition to ethanol (Fig. 2). The sorbitol concentration was much higher than in unsupplemented J.a. juice, which did not contain yeast extract, and even in that fermented with a large amount of inoculum (Fig. 1a–c). Ethanol production started from the beginning of the process, while sorbitol appeared after glucose had been consumed entirely. These results and those from the two previous experiments show that

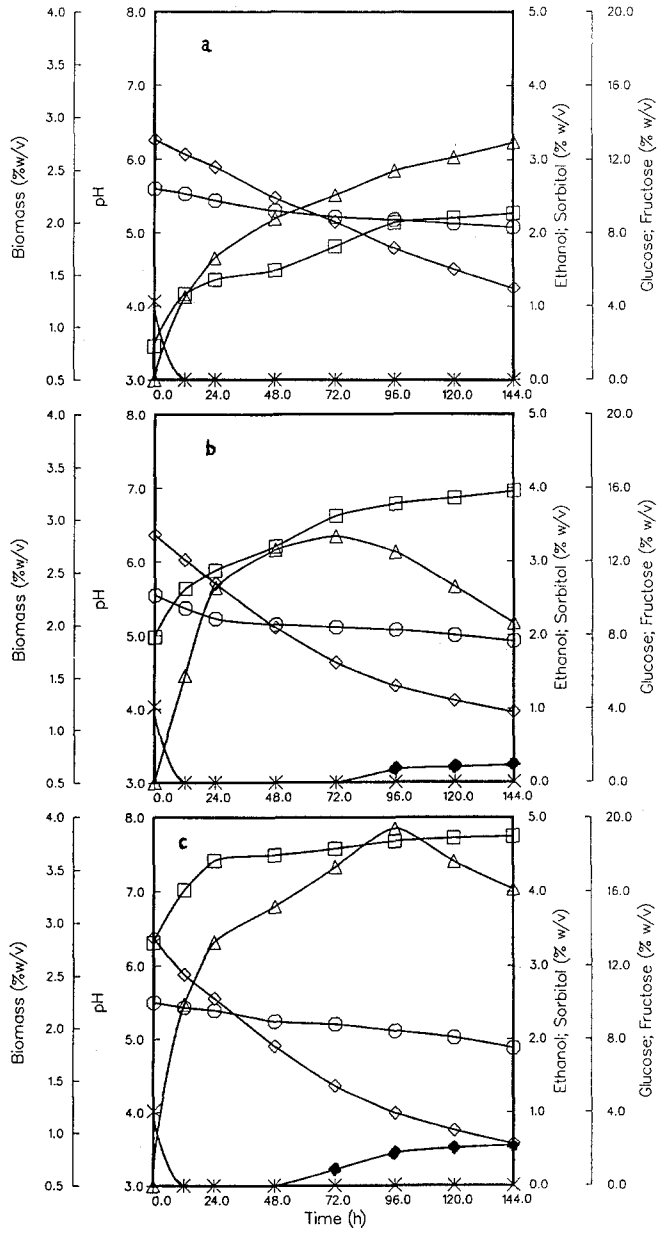

Fig. 1a–c. Growth of *Saccharomyces cerevisiae* ATCC 36859 and production of ethanol and sorbitol in pure Jerusalem artichoke (J.a.) juice inoculated with various amounts of inoculum. **a** 0.82%. **b** 1.89%. **c** 2.82%: □, biomass; ×, glucose; ◇, fructose; △, ethanol; ◆, sorbitol; ○, pH

the latter product was produced only from fructose. In this process, the final sorbitol and ethanol concentrations were about 4.6% (w/v), and 2.8% (w/v) respectively.

Sorbitol, ethanol and biomass yields, and overall efficiency are shown in Table 1 for three different times of this process. Although sorbitol was formed only from fructose, and biomass and ethanol from fructose and glucose, the sum of the total amounts of both sugars consumed was taken for the calculation of these yields because it was difficult to dissociate the quantity of each of these sugars used for the production of a particular product. Bearing in mind that ethanol and

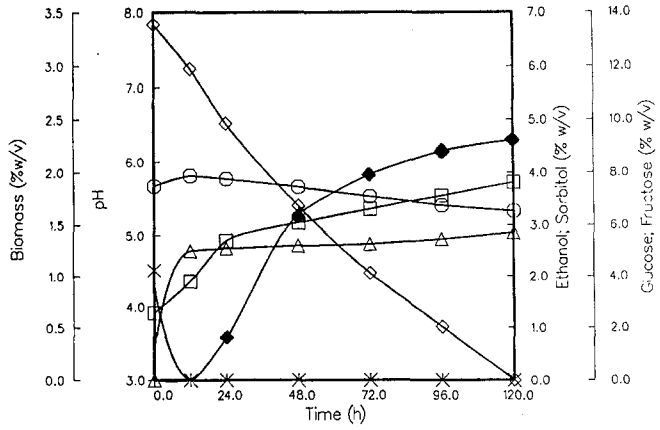

Fig. 2. Production of sorbitol and ethanol in J.a. juice supplemented with 3% yeast extract: □, biomass; ×, glucose; ◇, fructose; △, ethanol; ◆, sorbitol; ○, pH

Table 1. Sorbitol, ethanol and biomass yields, and overall efficiency of the process (Fig. 2) carried out in Jerusalem artichoke (J.a.) juice supplemented with 3% yeast extract

Time (h)	Sorbitol yield (Y_S/sub)	Ethanol yield (Y_E/sub)	Biomass yield (Y_B/sub)	Overall efficiency
48	0.289 (0.289)	0.235 (0.461)	0.080 (0.160)	0.910
72	0.290 (0.290)	0.193 (0.378)	0.074 (0.148)	0.816
120	0.259 (0.259)	0.160 (0.314)	0.071 (0.142)	0.715

Yield is the amount (g) of product (sorbitol, Y_S, ethanol, Y_E, biomass, Y_B) produced per amount (g) of carbohydrate (glucose + fructose, sub) consumed. Numbers in brackets denote the amounts of carbohydrates required to produce the corresponding amount of products. For this conversion the following coefficients were used: 1 g sorbitol or 0.51 g ethanol or 0.5 g biomass produced per 1 g of carbohydrates used. Overall efficiency is the sum of the numbers in brackets

biomass were also produced, the sorbitol yield can be considered relatively high in the J.a. juice supplemented with 3% yeast extract.

It has also been found that sorbitol was produced from fructose by *Z. mobilis* by both free cells (Barrow et al. 1984) and cell-free extract (Leigh et al. 1984). In the case of sorbitol production by *Z. mobilis* cells or cell-free extract, the presence of glucose was crucial although sorbitol was produced from fructose. This shows differences in the enzymatic systems involved in the production of sorbitol by *Z. mobilis* and *S. cerevisiae*.

To increase the ethanol concentration, the J.a. juice was supplemented with glucose and the inoculum and yeast extract concentrations were kept at the same level as in the previous test. The addition of glucose increased ethanol production, and at the end its concentration was about 6.2% (w/v) (Table 2a). This modification of the medium composition had a tremendously negative effect on the production of sorbitol, the concentration of which fell to 2.2%.

The process shown in Fig. 2 lasted for 120 h and the concentrations of biomass and ethanol were increasing up to that time. The production of sorbitol practically finished in 72 h in the latter process (Table 2a) although the broth still contained a relatively large amount of fructose at that time, and the yeast continued to use it for growth and ethanol production. This shows that the

Table 2. Effect of inoculum concentration on sorbitol and ethanol production in J.a. juice supplemented with glucose and 3% yeast extract

Conditions	Time (h)	Glucose (g/l)	Fructose (g/l)	Sorbitol (g/l)	Ethanol (g/l)	Biomass (g/l)
a	0	9.11	13.88	0.00	0.00	0.63
	12	2.12	12.84	0.00	2.83	1.00
	24	0.00	10.91	0.21	3.45	1.39
	48	0.00	7.60	1.35	4.78	1.54
	72	0.00	5.03	2.19	6.11	1.64
	96	0.00	2.42	2.23	6.18	1.77
	120	0.00	0.84	2.09	6.23	1.88
b	0	9.21	14.03	0.00	0.00	1.19
	12	0.00	12.03	0.00	3.88	1.50
	24	0.00	9.33	0.41	4.80	1.72
	48	0.00	6.09	1.93	5.89	2.12
	72	0.00	3.13	2.14	6.33	2.43
	96	0.00	0.00	2.05	6.74	2.50
	120	0.00	0.00	1.92	6.48	2.52
c	0	9.04	13.74	0.00	0.00	2.71
	12	0.00	10.93	0.90	3.95	2.96
	24	0.00	8.61	1.93	4.45	3.12
	48	0.00	4.29	1.84	5.77	3.25
	72	0.00	0.00	1.84	6.08	3.35
	96	0.00	0.00	1.79	5.86	3.37

a, inoculum concentration, 0.63 (g/l); b, inoculum concentration 1.89 times higher than in a; c, inoculum concentration 4.30 times higher than in a

Table 3. Sorbitol, ethanol and biomass yields, and overall efficiency of the processes (Table 2) with various inoculum concentrations

Process and initial biomass conc (g/l)	Time (h)	Sorbitol yield (Y_S/sub)	Ethanol yield (Y_E/sub)	Biomass yield (Y_B/sub)	Overall efficiency
a (0.63)	48	0.088 (0.088)	0.311 (0.610)	0.059 (0.118)	0.816
	72	0.122 (0.122)	0.340 (0.667)	0.056 (0.112)	0.901
	96	0.108 (0.108)	0.300 (0.588)	0.055 (0.110)	0.806
	120	0.094 (0.094)	0.281 (0.551)	0.056 (0.112)	0.757
b (1.19)	48	0.112 (0.112)	0.343 (0.672)	0.054 (0.108)	0.868
	72	0.106 (0.106)	0.315 (0.618)	0.062 (0.124)	0.848
	96	0.088 (0.088)	0.290 (0.569)	0.056 (0.112)	0.769
c (2.71)	24	0.136 (0.136)	0.314 (0.616)	0.029 (0.058)	0.810
	48	0.099 (0.099)	0.312 (0.612)	0.029 (0.058)	0.769
	72	0.081 (0.081)	0.267 (0.523)	0.029 (0.058)	0.662
	96	0.078 (0.078)	0.257 (0.504)	0.029 (0.058)	0.640

For the calculation of the yields, overall efficiency and meaning of the numbers in brackets see the footnotes of Table 1

relatively high ethanol concentration affected the production of sorbitol, while biomass growth continued.

Two additional experiments were carried out with inoculum concentrations about 1.8 (Table 2b) and 4.2 (Table 2c) times higher than in the test shown in Table 2a. In these cases, sorbitol was produced earlier than in the previous process and its concentration decreased slightly with increase in the size of inoculum. Maximum ethanol concentrations also were attained earlier (Table 3b and c).

Yields of sorbitol, ethanol and biomass in the process from Table 2 are shown in Table 3. The yields were shown for different periods of each of the three processes because ethanol, sorbitol and biomass concentrations did not attain their maxima at the same time. As mentioned for the yields shown in Table 1, in this case the total amount of sugars consumed was also taken for the calculation of these yields and overall efficiency. Comparison of the results from Tables 1 and 3 indicates that the addition of glucose to the J.a. juice did not affect the overall efficiency, whereas the sorbitol yields were decreased by more than 50%.

To evaluate the effect of the time of glucose addition on the production of sorbitol from J.a. juice, in the following two experiments glucose was added after growth and production of ethanol had started. Figure 3 shows the result of adding glucose 12 h after the beginning of growth. This caused a delay in the commencement of sorbitol production (Fig. 3), and its maximum concentration was much lower than when approximately the same amount of the sugar was added at the beginning of the process (Table 2a). Bearing in mind that a certain amount of fructose had not been consumed, the ethanol concentration was also affected. In an other experiment (not shown in this paper) the initial stage of the experiment in Fig. 3 was repeated and approximately the same amount of glucose that was added 12 h after the beginning of growth was also added after 24 h. This new glucose addition caused a decrease in sorbitol concentration of about 18.5% and an increase in ethanol concentration of about 17.3% compared to the final results shown in Fig. 3.

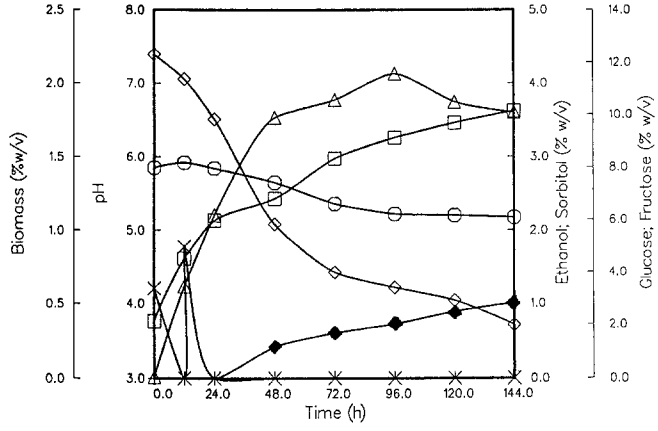

Fig. 3. Effect of addition of glucose 12 h after the beginning of the process on the production of sorbitol: □, biomass; ×, glucose; ◇, fructose; △, ethanol; ◆, sorbitol; ○, pH

From the above results it can be concluded that a considerable amount of sorbitol can be produced from J.a. juice supplemented with yeast extract by the yeast *S. cerevisiae* ATCC 36859. Production started after glucose had been consumed. Under the experimental conditions, production of sorbitol in pure J.a. juice was noticed only when the juice was inoculated with a large inoculum. The addition of glucose to juice supplemented with yeast extract caused a shift and decrease in sorbitol production. The effect of glucose on the production of sorbitol was much more severe when it was added during the process than at the beginning. An increase in the size of inoculum caused earlier sorbitol appearance in both pure J.a. juice and in that supplemented with yeast extract.

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