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Sorbitol Can Be Produced Not Only Chemically But Also Biotechnologically

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Abstract

Sorbitol, a polyol found in many fruits, is attracting increasing industrial interest as a sweetener, humectant, texturizer, and softener. It is principally produced by chemical means. The bacterium *Zymomonas mobilis* is able to produce sorbitol together with gluconic acid from fructose and glucose, respectively. This is possible in a one-step reaction via the enzyme glucose-fructose oxidoreductase, so far only known from *Z. mobilis*. The possibilities for the production of sorbitol by *Z. mobilis* are discussed also under the aspect of an industrial process and compared with the current chemical as well as other microbiologic processes. The production process by *Z. mobilis* shows economic possibilities for certain countries, such as Brazil, considering only the products sorbitol and ethanol as an important byproduct. For the other byproduct, gluconic acid, further studies for its partial substitution must be conducted.

Index Entries: Sorbitol; *Zymomonas mobilis*; glucose-fructose oxidoreductase; glucono-δ-lactone; gluconic acid.

Introduction

In 1984, the capability of the ethanol-producing bacterium *Zymomonas mobilis* to produce sorbitol from sucrose or mixtures of glucose and fructose was disclosed *(1–3)*, and Leigh et al. *(4)* reported that sorbitol formation is coupled with the dehydrogenation of glucose to form glucono-δ-lactone. Some possible pathways for sorbitol formation by *Z. mobilis* had been suggested by Viikari *(2,3)*, but the metabolic route was definitely elucidated by Zachariou and Scopes *(5)*, who showed that a sole enzyme,

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glucose-fructose oxidoreductase (GFOR) (EC 1.1.1.99), a tetrameric protein with tightly coupled NADP, was responsible for both the reduction of fructose to sorbitol and the dehydrogenation of glucose to glucono-δ-lactone. Additionally, they identified the presence of glucono-δ-lactonase (GL) (EC 3.1.1.17), an enzyme that hydrolyzes glucono-δ-lactone to gluconic acid.

Since then, different research groups have made efforts to define biotechnological processes for the production of sorbitol and gluconic acid or its salts at mild operation conditions. Interest in the subject also led to the publication of a number of articles including microbiologic, biochemical, and genetic aspects of the bioconversion of fructose and glucose to sorbitol and gluconic acid.

Our aim is to provide an overview of the production of sorbitol by *Z. mobilis* and to compare it with the current chemical as well as other microbiologic processes. Because of the industrial importance of sorbitol, the discussion also focuses on the properties, applications, and market for this compound.

Properties, Applications, and Industrial Production of Sorbitol

Sorbitol ($C_6H_{14}O_6$), also referred to as D-glucitol, is naturally found in many fruits as berries (except white grapes), cherries, plums, pears, and apples *(6,7)* (Table 1). This polyol has a relative sweetness compared to sucrose of ~60%, finding application in confectionery, chewing gum, candy, dessert, ice cream, and diabetic foods. Furthermore, it is used for a wide range of food products, not only as a sweetener but also as a humectant, texturizer, and softener. It is produced as an important precursor for the production of vitamin C. Other applications include pharmaceutical products, sorbose, propylene glycol, synthetic plasticizers, and alkyd resins, among others *(6,8,9)* (Table 2). The worldwide production of sorbitol has been estimated to be more than 500,000 t/yr, and the market is increasing *(9)*. More than 50% of this is used as 70% sorbitol solution, and about 25% is used for the synthesis of vitamin C.

The industrial production of sorbitol is traditionally performed by catalytic hydrogenation of D-glucose syrup at a concentration of about 50% (w/v). Several industrial processes were described in the past *(8–11)*. The basis for the actual processes was given by IG Farben AG *(12)*. Batch and continuous processes have been proposed and are used *(13–16)*. Worldwide, about 80% of sorbitol is produced by batch mode, performed under the following general conditions. The reaction is catalyzed by Raney nickel (3–6% [w/w], based on glucose), which is suspended in the glucose solution at pH 5.0–6.0. The temperature is in the range of 120–150°C and the pressure is about 70 bar. When a low-pressure system is used, the concentration of nickel may be up to 14% *(10)* and the pressure is below 50 bar. The average time of hydrogenation is 2–4 h. In continuous processes, hydrogenation is performed in the presence of fixed-bed nickel (2%, generally on Kieselguhr). At pH 6.0 a pressure of 180–200 bar and a tempera-

Table 1
Content of Sorbitol in Fruit

Apples	0.20–1.01 g/100 g
Pears	1.20–2.80 g/100 g
Plums	0.60–2.01 g/100 g
Peaches	0.50–1.25 g/100 g
Sweet cherries	1.40 g/100 g
Black grapes	0.20 g/100 g
White grapes	No sorbitol
Berries (strawberries, blackberries, raspberries)	Traces

Table 2
Applications of D-Sorbitol

Food industry
Sugar substitute for diabetic foods (chocolates, dragees, chewing gum, tablets, and so on)
Reduced-calorie sweetener
Softener and moisture stabilizer (marzipan, cakes, aromatic substances, mayonnaise, sauerkraut, ice cream, tobacco products, and so on)
Cosmetics
Water stabilization (3–5%) and softening (10–20%) (creams, emulsions, lotions, gels, toothpastes, and so on)
Pharmaceuticals
Similar use as in cosmetics; spray-dried sorbitol also used for uniform binding of solid agents (tablets, granules, antibiotics)
Medical
Pyrogen-free sorbitol used for infusions (10–20% solution) (acceleration of diuresis in osmotherapy, stimulation of cholokinesis, serves as laxative)
Technical
Moisture stabilizer and softener (prevents embrittlement of paper, used in finishing of textiles and leather, stabilizes moisture in polyurethane foams; about 25% of worldwide production used for synthesis of ascorbic acid [vitamin C])

ture of 140–170°C are applied. The raw sorbitol solution is cooled and the catalyst eliminated by precipitation and filtration. Purification of the sorbitol solution is performed by ion-exchange chromatography and activated charcoal filter. The 70% sorbitol solution, which is commercially the most common form, is obtained by evaporation of water in vacuum.

Z. mobilis: General Aspects

Originally, *Z. mobilis* was isolated from fermenting plant juices from agave, palms, and sugarcane as well as from spoiled cider and beer. Swings and De Ley *(17)* published an extended review on the biology of *Z. mobilis*, which is a Gram-negative bacterium of rod-shaped cells with rounded ends *(18–20)*.

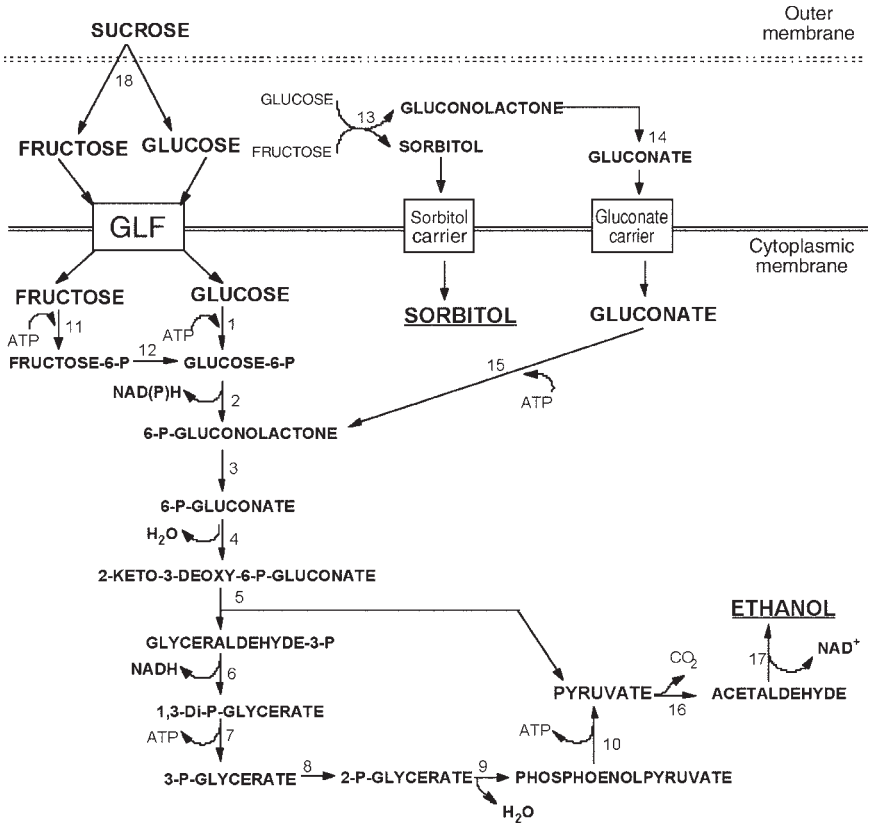

Fig. 1. Mechanism of sorbitol, gluconate, and ethanol formation from glucose and fructose in *Z. mobilis*. (From ref. *30*.) GLF, glucose facilitator (common uniport system for hexoses). 1, Glucokinase; 2, glucose-6-P-dehydrogenase; 3, 6-P-gluconolactonase; 4, 6-P-gluconate dehydratase; 5, KDPG aldolase; 6, glyceraldehyde-P-dehydrogenase; 7, phosphoglycerate kinase; 8, phosphoglycerate mutase; 9, enolase; 10, pyruvate kinase; 11, fructokinase; 12, glucose-6-P-isomerase; 13, GFOR; 14, gluconolactonase; 15, gluconate kinase; 16, pyruvate decarboxylase; 17, alcohol dehydrogenase; 18, levan sucrase.

Much attention has been paid to strains from *Zymomonas* because of its quite unusual physiologic and biochemical properties (for general overviews, see refs. *17*, *19*, and *21–31*; for special aspects, see refs. *5* and *32–38*).

Among these properties is that *Zymomonas* utilizes the Entner-Doudoroff pathway anaerobically *(39–42)* to degrade 1 mol of glucose to almost 2 mol each of ethanol and CO_2 *(43,44)* (Fig. 1). During this process, only 1 mol of adenosine triphosphate (ATP) is gained, and a small percentage (2–5%) of the glucose is converted into cell mass *(17)*. The molar conversion of glucose to ethanol and CO_2 may vary by the chosen culture conditions *(40,45,46)*. In the same way as glucose, *Zymomonas* is able to degrade fructose to ethanol, CO_2, and cell mass *(17,27)*. Besides the two hexoses, only the disaccharide sucrose can be utilized as carbon source. Sucrose can be hydrolyzed to glucose and fructose and further metabolized in the cell and/or polymerized to levan *(25,31,47–54)*.

As already mentioned, the main products of fermentation with glucose and fructose under anaerobic conditions are ethanol and CO_2, whereas under aerobic conditions acetaldehyde *(55–57)* and some acetate *(55,58,59)* are also produced. When supplied under anaerobic conditions with fructose and glucose as carbon source, depending on the fermentation conditions, sorbitol and ethanol and/or gluconic acid are the products, respectively *(1–3,60,61)*.

Production of Sorbitol Via GFOR

Leigh et al. *(4)* were the first to give a correct description of the pathway that produces sorbitol and gluconic acid in *Zymomonas*. Two years later, Zachariou and Scopes *(5)* showed that the oxidation of glucose to glucono-δ-lactone and the reduction of fructose to sorbitol was catalyzed by a single enzyme—GFOR. This was possible through tightly bound NADP as a cofactor that permitted the redox reaction through this hydrogen carrier. GFOR is a tetramer *(5,62)* whose reaction obeys a ping-pong mechanism *(63)*. Its structure, formation, and location in the bacterium has been elucidated *(62,64–69)*. The physiologic function of GFOR seems to be the regulation of osmotic stress of the cell when grown on high glucose concentrations *(35)*. The enzyme produces sorbitol, which works as a compatible solute as its accumulation is linked to high glucose concentrations outside the cell. From the periplasm, sorbitol is transported by a special carrier into the cytoplasm *(30,35)*.

To get high GFOR activities, *Zymomonas* has to be grown with glucose as the sole carbon source *(5)*. Both glucose and fructose enter the periplasm via a glucose facilitator, but at fructose concentrations >500 m*M* a special fructose facilitator seems to act *(70)*. Outside the cell, GFOR is quite unstable when used for the production of sorbitol and gluconic acid *(71–74)*, but thiol reagents and urea are able to protect the enzyme activity *(71,73–76)*. Because of its tightly bound NADP, the enzyme may be used for the production of aldonolactones other than gluconolactone *(5,77)*.

Biotechnological Production of Sorbitol

In a conventional batch fermentation of glucose and fructose (equimolar summed concentration of 150 g/L) by *Z. mobilis*, ethanol is the main product and sorbitol accumulation corresponds to 11% of the consumed substrate *(3)*. During the process, formed gluconic acid is metabolized via the Entner-Doudoroff pathway (Fig. 1), principally to CO_2 and ethanol *(37)*.

Considering the low yields achieved for sorbitol in a conventional fermentation process, Chun and Rogers *(78)* proposed an interesting approach. Previously grown and concentrated cells of *Z. mobilis* strain ZM4 (ATCC 31821), which had been permeabilized with 10% (v/v) toluene, were used in this process. The reason for the permeabilization of cells was to release the essential soluble cofactors necessary for the conversion of gluconic acid into ethanol and other catabolic products via the Entner-

Doudoroff pathway. With free toluene-treated cells, sorbitol and gluconic acid concentrations of 290 and 283 g/L, respectively, were achieved in a 16-h batch process. Yields for both products close to 95% were obtained. In batch mode, the use of toluene-treated cells immobilized in Ca-alginate beads allowed similar results although some loss of enzyme activity occurred when the beads were reutilized. Chun and Rogers *(78)* also described a 125-h continuous operation with immobilized cells in which sorbitol and gluconic acid concentrations of 80–85 g/L were sustained with productivities of 7.6 and 7.2 g/(L·h), respectively. Scopes et al. *(79)* patented this production process.

Ichikawa et al. *(80)* reported that high concentrations of sorbitol and gluconic acid could be achieved, with minimal ethanol production, when dried cells of *Z. mobilis* were used in the bioconversion of fructose and glucose into these products. According to them, in drying cells, the enzymes responsible for converting the substrates into ethanol would be inactivated, whereas GFOR and GL would retain their activities.

Rehr et al. *(81)* used intact glucose-grown *Z. mobilis* ATCC 29191 cells to perform the bioconversion of equimolar solutions of glucose and fructose up to 600 g/L and measured final concentrations of sorbitol and gluconic acid of "only" 240 and 210 g/L, respectively. In further experiments, seven different detergents were evaluated as permeabilizing agents, and cetyltrimethylammonium bromide (CTAB) was selected. According to Rehr et al. *(81)*, the use of any permeabilizing agent, including toluene, presented no significant effect on GFOR activity in *Z. mobilis* cells, and the treatment with 0.1% (w/v) CTAB for 10 min was sufficient to stop ethanol production. With free CTAB-treated cells, in batch mode, yields of 98 to 99% with specific productivities of 1.8–2.1 g/(g·h) for sorbitol and gluconic acid, respectively, were achieved. Immobilization of permeabilized cells in κ-carrageenan beads, followed by hardening with polyethyleneimine and glutaraldehyde, led to some loss of enzyme activity, owing to diffusional problems or enzymatic inactivation of GFOR during the immobilization procedure, resulting in decreasing specific productivities (1.4–1.8 g/[g·h] for sorbitol and gluconic acid, respectively). Rehr et al. *(81)* described a two-stage continuous process with κ-carrageenan immobilized cells in which yields >98% for both products were measured. Rehr and Sahm *(82,83)* later patented both the application of CTAB-treated immobilized cells of *Z. mobilis* in the bioproduction of sorbitol and gluconic acid and the method for cell immobilization.

Bringer-Meyer and Sahm *(84)* patented a process involving an alternative method for cell permeabilization. *Z. mobilis* cells, which had been permeabilized by freezing at –20°C and thawing at room temperature, were used in the bioconversion process, resulting in yields close to 100% for both sorbitol and gluconic acid.

Different methods of cell immobilization were evaluated for the long-term activity of the biocatalyst for the production of sorbitol and gluconic acid. According to Jang et al. *(85)*, good results in bioconversion were found

when CTAB-permeabilized *Z. mobilis* cells were treated with glutaraldehyde before immobilization in κ-carrageenan. Using an improved immobilization method, the rigidity of immobilized beads was increased twofold by adding glycerol and propylene glycol to κ-carrageenan prior to immobilization of CTAB-permeabilized cells. During a 72-h semibatch process, the loss of enzyme activity was <10% *(86)*.

On the other hand, some problems were previewed for the industrial bioproduction of sorbitol. Among these problems is the relatively high cost of substrates, particularly fructose, compared to the value of products. In this sense, Ro and Kim *(87)* studied the bioconversion of sucrose to sorbitol and gluconic acid using toluene-treated *Z. mobilis* and invertase coimmobilized in both chitin and calcium alginate. The optimal substrate concentration for the coimmobilized enzymes was determined to be 200 g/L. With that sucrose concentration, in a batch run at pH 6.0 and 45°C, the process was completed in 22 h with a yield of 93% for both sorbitol and gluconic acid. In a continuous operation with a recycle packed-bed reactor (RPBR), maximum productivities of 5.1 g/(L·h) and conversion yields of 92% for both products were observed. According to Ro and Kim *(87)*, the coimmobilized enzymatic system retained its stability for 250 h in the RPBR. When applying toluene-permeabilized cells of *Z. mobilis* with coimmobilized inulinase in calcium alginate beads, the bioconversion of a mixture of glucose/inulin to gluconic acid and sorbitol could only achieve a yield of 44.4%, although maximum productivities were 23.4 and 26.0 g/(L·h) *(88)*.

Most of the work reported involved permeabilized *Z. mobilis* cells. However, the use of nonpermeabilized *Z. mobilis* cells in this process was also proposed *(61,89,90)*, considering that sorbitol and gluconic acid have a relatively low market price, and, therefore, any reduction in the production costs is desirable. With free-untreated *Z. mobilis* ATCC 29191, Silveira et al. *(61)* studied the influence of the initial concentration of glucose and fructose on the batch bioproduction of gluconic acid and sorbitol. The use of up to 650 g/L of an equimolar mixture of the substrates resulted in a nearly complete bioconversion of these to sorbitol and gluconic acid, without ethanol formation, and yields of >91% for both products, in 8 h of operation (Table 3) (Fig. 2). According to Silveira et al. *(61)*, the enhancing yields of bioconversion with increasing S_0 are linked to the concentrations of both substrates and products present in the medium through two effects: (1) loss of cell viability owing to the high osmotic pressure; and (2) sequential inhibition of the normal metabolism of the microorganism by substrates and products, resulting in preferential utilization of substrates via the GFOR/GL system. A further aspect of the economic viability of producing sorbitol was the analysis for cheap media *(91)*, in which the use of corn steep liquor (CSL) played a crucial role in obtaining high activities of GFOR for the subsequent bioconversion (Table 4). Using 25 g/L of CSL instead of 5 g/L of yeast extract, the costs for the medium can be reduced between 25 and 50%. The results of some studies on the bioproduction of sorbitol

Table 3
Yields and Productivities for Gluconic Acid and Sorbitol With Different Initial Glucose Plus Fructose Concentrations[a]

	Glucose + fructose (g/L)						
	100	300	400	500	600	650	750
Gluconic acid yield (%)	7.5	40	58	80	83	91	90
Sorbitol yield (%)	35	83	74	79	86	91	92
Specific gluconic acid productivity (g/[g·h])	0.24	1.3	1.5	2.2	1.6	1.6	1.3
Specific sorbitol productivity (g/[g·h])	0.80	1.7	1.6	1.8	1.5	1.5	1.3

[a]Strain: *Z. mobilis* ATCC 29191; medium: 2.0 g/L of $(NH_4)_2SO_4$, 1.0 g/L of $MgSO_4$, 3.5 g/L of KH_2PO_4, 0.01 g/L of $FeSO_4$, 0.2 g/L of Na-citrate, 30.0 g/L of cell mass; temperature: 39°C. Calculations were made when one of the substrates was completely depleted; the specific productivity is referred to as grams of product obtained per gram of cell mass per hour.

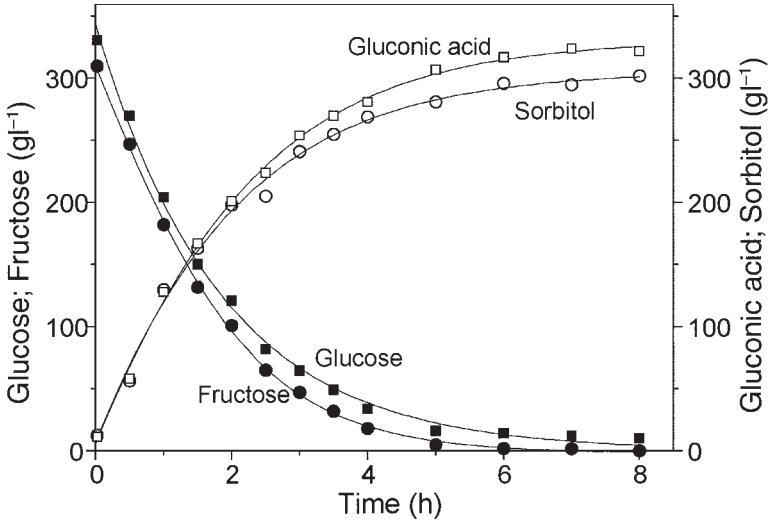

Fig. 2. Time course of typical bioconversion run with initial glucose plus fructose concentrations of 650 g/L. Strain: *Z. mobilis* ATCC 29191; medium: 2.0 g/L of $(NH_4)_2SO_4$, 1.0 g/L of $MgSO_4$, 3.5 g/L of KH_2PO_4, 0.01 g/L of $FeSO_4$, 0.2 g/L of Na-citrate, 30.0 g/L of cell mass; temperature: 39°C.

Table 4
Production of GFOR[a]

	Yeast extract (g/L)	CSL (g/L)			
	5	5	15	25	100
Process time (h)[b]	11.7	11.7	11.8	11.7	13.5
Maximum specific growth rate (h^{-1})	0.35	0.34	0.34	0.33	0.28
Specific GFOR/GL (U/g)	13.1	10.6	11.0	13.2	11.0
Cell yield (g/g)	0.032	0.023	0.027	0.031	0.027
GFOR/GL yield (U/g)	0.42	0.25	0.29	0.41	0.29
Ethanol yield (%)[c]	94	95	92	93	94
GFOR/GL productivity (U/[L·h])	5.4	3.2	3.7	5.3	3.3
Ethanol productivity (g/[L·h])	6.2	6.2	5.7	6.0	5.3

[a]General results of runs with *Z. mobilis* ATCC 29191 in media (1.0 g/L of $[NH_4]_2SO_4$, 0.5 g/L of $MgSO_4$, 1.0 g/L of KH_2PO_4, 150.0 g/L of glucose) containing different CSL concentrations or 5 g/L of yeast extract.

[b]Time necessary for the complete consumption of the carbon source.

[c]Calculated in relation to the theoretical maximum.

and gluconic acid by GFOR/GL present in *Z. mobilis* cells are summarized in Table 5.

Attempts have been made to develop a functional process for the biotechnological production of sorbitol and gluconic acid by cell-free GFOR/GL from *Z. mobilis* using continuous ultrafiltration membrane reactors

Table 5
Experimental Studies on Bioproduction of Sorbitol and Gluconic Acid Using *Z. mobilis* Cells

Biocatalyst	Bioreactor	Initial or feed substrate (g/L)[a]	Sorbitol yield (%)	Gluconic acid yield (%)	Sorbitol productivity (g/[g·h])	Gluconic acid productivity (g/[g·h])	Process time	Ref.
Toluene-permeabilized cells Ca-alginate immobilized (strain ATCC 31821)	Batch	300 g/L glucose 300 g/L fructose	94.3	92.7	—	—	16 h	*78*
Toluene-permeabilized cells Ca-alginate immobilized (strain ATCC 31821)	Continuous stirred-tank reactor	100 g/L glucose 100 g/L fructose (D = 0.085 h^{-1})	—	—	0.24	0.26	125 h	*78*
Toluene-permeabilized cells Ca-alginate coimmobilized with invertase (strain ATCC 31821)	RPBR	200 g/L sucrose (D = 0.053 h^{-1})	92	92	0.40	0.39	250 h	*87*
Cells permeabilized by freezing and thawing (strain ATCC 29191)	Batch	234 g/L glucose 234 g/L fructose	100	99	1.1	1.1	5 h	*84*
CTAB-permeabilized cells (strain ATCC 29191)	Batch	300 g/L glucose 300 g/L fructose	99	98	1.8	2.1	—	*81*
CTAB-permeabilized cells κ-Carrageenan immobilized (strain ATCC 29191)	Continuous fluidized-bed reactor	100 g/L glucose 100 g/L fructose (D_{1st} = 0.055 h^{-1})	—	—	0.32	0.33	75 d	*81*
CTAB-permeabilized cells κ-Carrageenan immobilized (strain ATCC 29191)	Continuous two-stage fluidized-bed reactor	100 g/L glucose 100 g/L fructose (D_{1st} = 0.075 h^{-1}) (D_{2nd} = 0.050 h^{-1})	>98.0	>98.0	0.19	0.21	—	*81*
Untreated cells (strain Z1-81)	Batch	300 g/L glucose 300 g/L fructose	92.1	91.0	1.0	1.1	11 h	*90*
Untreated cells (strain ATCC 29191)	Batch	325 g/L glucose 325 g/L fructose	91.0	91.0	1.5	1.6	8 h	*61*

[a]D = dilution rate in continuous processing.

(72,74,92). According to Gollhofer et al. *(72)*, the use of a cell-free enzymatic system could lead to increasing productivity of both products because higher enzyme concentrations could be employed without mass transfer limitations. On the other hand, GFOR maintained good stability in *Z. mobilis* cells, whereas a cell-free enzyme system is strongly subjected to inactivation owing to its own catalytic action. As such, besides the evaluation of process yields and productivities, those reports *(72,74,92)* also dealt with the operational instability of GFOR and approaches to overcome this problem.

With a continuous stirred tank reactor coupled to a 10-kDa cutoff ultrafiltration membrane, Nidetzky et al. *(74)* obtained a long-term operational stability of GFOR (over 250 h) by controlling the pH with weak bases as tris (hydroxymethyl) aminomethane or imidazole, adding dithiothreitol to prevent loss of activity, and using lower temperatures (<30°C) to avoid thermal inactivation. With 15 kU of GFOR/L at a dilution rate of 0.06 h^{-1}, a sorbitol productivity of 4.37 g/(L·h) and a conversion rate close to 40% were achieved. Silva-Martinez et al. *(92)* used a single-stage loop reactor with tangential ultrafiltration at a dilution rate of 0.04 h^{-1}, 5 kU of GFOR/L, and a 3 *M* substrate concentration. In this case the conversion yield was superior to 85%.

Finally, it is important to observe the purification steps to obtain the final products—sorbitol and gluconic acid. Technically, the final purification was possible by the precipitation of gluconic acid with methanol. Sorbitol remained in solution. Methanol could be recovered and gluconic acid achieved purities of >99% (unpublished results).

Other Microorganisms That Are Able to Produce Sorbitol

Besides *Z. mobilis*, only a few other systems have been described that might be able to produce sorbitol. Tani and Vongsuvanlert *(93)* and Vongsuvanlert and Tani *(94)* described sorbitol production by the methanol yeast *Candida boidinii*. They got good results, but only for rather small working volumes. No further publications have appeared on this process. The presented data are insufficient to show whether this process is suitable for large-scale production of a rather cheap product such as sorbitol.

Duvnjak et al. *(95,96)* reported on the production of sorbitol together with ethanol using a mutant of *Saccharomyces cerevisiae* (ATCC 36859) and juice from Jerusalem artichokes as carbon source. A critical point of this process is that Jerusalem artichokes are not a cheap source for obtaining the juice. Consequently, they are not suitable for economic production of fructose. Additionally, high concentrations of yeast extract were used, which is expensive and thus an important negative cost factor for economic production of sorbitol. Furthermore, the described strain may consume sorbitol when grown on fructose, and the separation of ethanol and sorbitol in order to obtain the end products was not shown. Since then no further publications have appeared.

Hiroyuki and Izumori *(97)* identified a strain from *Candida famata* that was able to reduce sorbose to sorbitol and iditol at a ratio of 3:2, respectively. However, also in this case no further studies have been conducted.

From the aforementioned data it can be concluded that only *Z. mobilis* offers conditions for industrial biotechnological production of sorbitol.

Perspectives for Biotechnological Industrial Production of Sorbitol and Gluconic Acid or Other Byproducts

With the aim of utilizing the enzymes GFOR and GL from *Z. mobilis* in an industrial-scale production of sorbitol and gluconic acid, some important aspects must be considered for each strategy proposed. The use of permeabilized and immobilized cells is advantageous when considering the simplicity of the bioreactor required for the continuous processing. On the other hand, a very long-term stability of the system must be ensured to avoid the need for frequent production of the biocatalyst.

The method with untreated *Z. mobilis* requires cell cultivation in each run to produce the enzymes and, therefore, the cost of this phase is critical for the overall economic balance. However, this cost could be compensated for by the ethanol produced provided that a low-cost medium is available. In this sense, Silveira et al. *(91)* described the possibility of producing sorbitol, gluconic acid, and ethanol by a two-step operation using CSL as a cheap source of vitamins and nitrogen in the first step of the process. In this case, the bioconversion process should be carried out in batch mode.

With purified or cell-free enzyme, a reduction in reactor size and the possibility of applying larger enzyme concentrations could lead to improved productivity, provided that GFOR is sufficiently stable under process conditions. On the other hand, the need for the addition of enzyme stabilizers, the use of more expensive bases to control the pH, and the use of a ultrafiltration membrane—all with well-known problems associated with this kind of separation system—should be carefully taken into account considering that sorbitol and gluconic acid are bulk products with large volumes produced at relatively low commercial prices.

A further essential matter is the development of a feasible method for the separation and purification of sorbitol and gluconic acid or gluconate. Few attempts have been reported in the literature. Chun and Rogers *(78)* reported that separation of the products was achieved using a basic anion-exchange resin and a solution of $Na_2B_4O_7/H_3BO_3$ as eluent. However, they considered this method suitable only for the laboratory scale, because for industrial production the costs would be too high. Silveira et al. *(89)* proposed a method for recovering sorbitol and sodium gluconate by selective precipitation with organic solvents such as methanol and ethanol. Recently, Ferraz et al. *(98)* reported the use of an electrodialysis system, coupled to the bioreactor, to remove gluconic acid from the medium simultaneously with its production. Although good results were described for these methods in laboratory scale, a cheap and efficient method must be developed and optimized for an industrial process.

Besides the technical details to be solved or improved for the industrial use of *Z. mobilis* enzymes in the bioproduction of sorbitol and gluconic acid, the low price of products compared with substrates cost is another critical aspect that could make the economic application of this technology impractical *(91)*. However, the commercial feasibility of this process could be attained in some countries or specific regions of the world. For instance, whereas in Europe 1 t of sorbitol is sold at an average price of US $1500 for the pure powder and US $450 for the 70% (w/w) solution, in the Mercosul region, in South America, most of the sorbitol consumed is imported and its price in the local market varies from US $900 to US $1000 for 70% (w/v) solution. However, in Mercosul, both glucose syrup (70% [w/v]) and high-fructose syrup (55% [w/v] fructose and 40% [w/v] glucose) are produced and cost approx US $430/t and US $620/t, respectively. Therefore, considering both these values and the normal yields of the bioconversion process, a favorable economic balance can be achieved *(91)*.

In 1995–1997, the Companhia Lorenz (Blumenau, Brazil) contacted the Centro de Desenvolvimento Biotecnológico (CDB) (Joinville, Brazil), because it was interested in the biotechnological production process for sorbitol developed by this research center. Companhia Lorenz calculated the process for a production volume of at least 5000/t/yr and considered it economically feasible, even when taking only the sorbitol and the byproduct ethanol into account.

Another aspect is the size of the market for gluconic acid and its salts, which is almost 10 times smaller than for sorbitol. Because equimolar amounts of sorbitol and gluconic acid are formed through the action of GFOR and GL on the substrates, the commercial balance for this process is inadequate, even considering the different gluconates that can be produced depending on the alkali used to control the pH. A possible alternative for this problem is the production of different organic acids and respective salts, which, combined with gluconic acid and gluconates, could compose a group of products with a market equivalent to sorbitol. That possibility was first demonstrated in the work of Satory et al. *(77)* in which different aldose sugars—e.g., D-xylose, D-galactose, D-maltose, and D-lactose—were converted into the corresponding aldonic acid through the action of GFOR. Recently, Concatto et al. *(99)* and Carra et al. *(100)* showed that it is possible to obtain good quantities of lactobionic acid using a 0.7*M* fructose/lactose solution in a biotransformation process with permeabilized cells of *Z. mobilis*. Lactobionic acid has a good potential market, because it can be used for medical purposes *(101–103)*, production of detergents *(104)*, and cosmetics *(105)*. The possible production of idonic acid or galactonic acid through this process should be investigated, because these substances can be used for the production of vitamin C *(106)*.

Conclusion

The chemical process for sorbitol production is for most countries favorable, because the costs for it are lower when compared with a biotech-

nological process. Regarding the biotechnological process, only the process using *Z. mobilis* can really be considered.

Considering the calculations made by Companhia Lorenz for the production process developed by Silveira et al. *(89)* at CDB, a biotechnological production of sorbitol is economically possible in at least some countries. Special emphasis should be given to evaluating the possibilities of byproducts other than gluconic acid that may be of interest for industrial purposes.

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