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COMBINED ACTION OF AN ENZYME AND A METAL CATALYST ON THE CONVERSION OF D-GLUCOSE/D-FRUCTOSE MIXTURES INTO D-MANNITOL*

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ABSTRACT

A process involving both a bio- and a chemo-catalyst has been applied for the conversion of D-glucose/D-fructose mixtures into D-mannitol. Good yields (62–66%) were obtained by using D-glucose isomerase immobilised on silica in combination with a copper-on-silica catalyst (water, pH ~7, 70°, 50 kg/cm^2 of hydrogen, trace amounts of buffer, Mg(II), borate, and EDTA). Non-enzymic isomerisation and degradation reactions are negligible under these reaction conditions.

INTRODUCTION

Simultaneous action of a bio- and a chemo-catalyst is an attractive possibility for certain catalytic processes in solution. For example, a starting compound could be enzymically equilibrated with a product that could be irreversibly transformed by the chemo-catalyst into the final product. Problems associated with such double catalytic systems are the limited range of conditions for enzyme activity, the preferential conversion of the intermediate by the chemo-catalyst, and the possible mutual poisoning of the two catalysts.

We have reported[1,2] on the combined use of D-glucose isomerase and platinum in the preparation of the sugar substitute D-mannitol (46% yield) from D-glucose/D-fructose mixtures (*e.g.*, invert sugar or isoglucose). Ruddlesden and Stewart[3] used D-glucose isomerase together with ruthenium-loaded zeolite Y in a similar approach in order to protect the hydrogenation catalyst against poisoning by the enzyme. The zeolite pores were thought to be inaccessible to the inhibiting species and a 29% yield of D-mannitol was obtained from D-glucose using this system. Kearsly *et al.*[4] applied enzymic (alpha- and beta-amylase) hydrolysis and catalytic (Raney nickel) hydrogenation to maize starch with the aim of achieving more rapid and complete enzymic hydrolysis by catalytic hydrogenation of the D-

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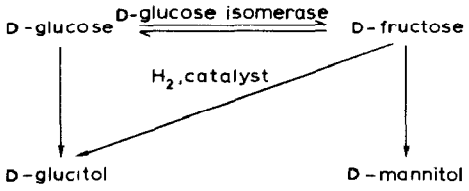

Fig. 1 Combi-process: simultaneous enzymic isomerisation and metal-catalysed hydrogenation of D-glucose/D-fructose mixtures.

glucose formed. However, the nickel catalyst inhibited the enzyme.

We now describe further investigations towards the development of an improved combi-production method for D-mannitol. Apart from its pleasant sweet taste and non-carious properties, D-mannitol is used in "sugar-free" chewing gums and pharmaceutical preparations due to its non-hygroscopicity. Up to now, D-mannitol has been obtained commercially in yields of <30% by the catalytic hydrogenation of D-glucose/D-fructose mixtures. The combinations of either base- or molybdic acid-catalysed isomerisation of D-glucose and hydrogenation have been proposed as possible alternative chemical combi-procedures. These and other possible alternatives have been reviewed[5].

The optimal formation of D-mannitol in a combi-process based on the enzymic interconversion of D-glucose and D-fructose and preferential hydrogenation of D-fructose, as depicted in Fig. 1, requires (*a*) selective hydrogenation of D-fructose to D-mannitol, (*b*) relatively fast D-glucose $\rightleftharpoons$ D-fructose interconversion in order to maintain the concentration of D-fructose at a maximum, and (*c*) minimum mutual interference and maximum activity of the two catalysts. The selective reduction of D-fructose and its conversion into D-mannitol can be effected with a copper-on-silica catalyst[6] which showed an initial selectivity of 0.9 for the hydrogenation of D-fructose in a 1:1 D-glucose/D-fructose mixture together with preferential (67–85%) formation of D-mannitol from D-fructose. Platinum, ruthenium, osmium, and iridium-on-carbon catalysts showed reasonable initial selectivities (0.8–0.9) but lower values (45–55%) for the selective formation of D-mannitol. For purposes of comparison, these catalysts have been included in the present investigation. The hydrogenation conditions are largely determined by the narrow range of conditions (50–70°, pH 6.5–8.0)[7] for the activity of the D-glucose isomerase. These moderate temperatures restrict the rates of hydrogenation. To meet the requirements (*b*) and (*c*), D-glucose isomerase was used in an immobilised form which possibly prevents serious interaction of the two catalysts. Three commercially available, immobilised D-glucose isomerases in combination with five different hydrogenation catalysts have been investigated.

EXPERIMENTAL

Materials. — The catalysts 5% Ru/C and 5% Pt/C were obtained from

Drijfhout Amsterdam; the preparations of 10% Ir/C, 5% Os/C, and 20% Cu/silica are described elsewhere[6]. All the above-mentioned catalysts were used without prior treatment. Maxazyme GI-Immob., Sweetzyme Q, and Optisweet 22 were gifts of Gist Brocades B.V., NOVO Industri AS, and Miles Kali-Chemie K.G., respectively. Data for the enzyme preparations are given elsewhere[7]. All other chemicals were commercial products.

Apparatus and procedure. — The simultaneous enzymic isomerisation and catalytic hydrogenation experiments were carried out in a thermostatted Parr 450-mL Hastelloy B autoclave model 4562, equipped with a motor-driven impeller stirrer, a sampling device, and two needle valves.

The immobilised enzyme, the hydrogenation catalyst, and the additives were transferred to the aqueous solution of D-glucose or a 1:1 D-glucose/D-fructose mixture. The autoclave was sealed, flushed with hydrogen, thermostatted at the required temperature, and then pressurised with hydrogen to the required level. The reaction was started by switching on the stirrer. The conversion was followed by h.p.l.c. of samples withdrawn from the reaction mixture. The h.p.l.c. system consisted of a Waters M 6000 A pump, a Rheodyne 7125 injector, a Waters R 401 differential refractometer, and a column (30 cm × 7.0 mm i.d.) of Aminex A 7 8% cross-linked (Ca^{2+}) resin (7–11 μm) at 85°. The column was eluted with degassed and deionised H_2O at 0.6 mL/min. The procedure is described in more detail elsewhere[8].

RESULTS AND DISCUSSION

D-Glucose isomerase entrapped in cell material. — Maxazyme GI-Immob. and Sweetzyme Q, which consist of whole cells containing D-glucose isomerase immobilised in gelatin–glutardialdehyde and glutardialdehyde matrixes, respectively[7], were studied first. Ru/C, Os/C, Ir/C, and Pt/C were used in the hydrogenation of 1:1 D-glucose/D-fructose mixtures without enzyme (Table I) and in the presence of these enzyme systems (Tables II and III). The results clearly show a serious poisoning of the hydrogenation catalysts except for the combination of Maxazyme GI-Immob. and Ru/C.

TABLE I

CONVERSIONS AND PRODUCTS FOR THE HYDROGENATION OF 1:1 D-GLUCOSE/D-FRUCTOSE MIXTURES[a]

Catalyst	*Conversion (mole %)*	D-*Glucose (mole %)*	D-*Fructose (mole %)*	D-*Mannitol (mole %)*	D-*Glucitol (mole %)*
5% Ru/C	60	33	7	19	41
5% Os/C	45	40	15	19	26
10% Ir/C	20	48	32	10	10
5% Pt/C	37	45	19	19	18

[a]Conditions: D-glucose (30.0 g), D-fructose (30.0 g), catalyst (1.0 g), $MgSO_4$ (0.3 g), $CaCO_3$ (0.5 g), H_2O (250 mL), 60°, 20 kg/cm^2 H_2, pH 7.1–7.6, 25 h.

TABLE II

CONVERSIONS AND PRODUCTS FOR THE HYDROGENATION OF 1:1 D-GLUCOSE/D-FRUCTOSE MIXTURES IN THE PRESENCE OF SWEETZYME Q[a]

Catalyst	*Conversion (mole %)*	D-*Glucose (mole %)*	D-*Fructose (mole %)*	D-*Mannitol (mole %)*	D-*Glucitol (mole %)*
5% Ru/C	16	43	41	6	10
5% Os/C	13	45	42	6	7
10% Ir/C	4	48	48	2	2
5% Pt/C	9	44	45	5	6

[a]Conditions: D-glucose (30.0 g), D-fructose (30.0 g), Sweetzyme Q (3.0 g), catalyst (1.0 g), $MgSO_4$ (0.3 g), $CaCO_3$ (0.5 g), H_2O (250 mL), 60°, 20 kg/cm^2 H_2, pH 7.1–7.6, 25 h.

TABLE III

CONVERSIONS AND PRODUCTS FOR THE HYDROGENATION OF 1:1 D-GLUCOSE/D-FRUCTOSE MIXTURES IN THE PRESENCE OF MAXAZYME GI-IMMOB[a]

Catalyst	*Conversion (mole %)*	D-*Glucose (mole %)*	D-*Fructose (mole %)*	D-*Mannitol (mole %)*	D-*Glucitol (mole %)*
5% Ru/C	46	30	29	17	25
5% Os/C	19	41	40	9	10
10% Ir/C	8	46	46	4	4
5% Pt/C	9	44	45	5	6

[a]Conditions: D-glucose (30.0 g), D-fructose (30.0 g), Maxazyme GI-Immob. (10.0 g), catalyst (1.0 g), $MgSO_4$ (0 3 g), $CaCO_3$ (0.5 g), H_2O (250 mL), 60°, 20 kg/cm^2 H_2, pH 7 1–7 6, 25 h.

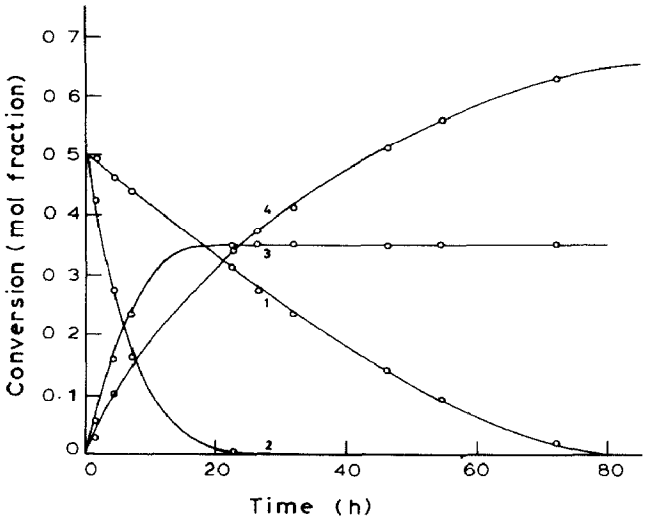

Fig. 2. Time course of the hydrogenation of 1:1 D-glucose/D-fructose over Cu/silica. Composition: D-glucose (30.0 g), D-fructose (30.0 g), 20% Cu/silica (5.0 g), $MgSO_4$ (0.3 g), $CaCO_3$ (0.5 g), $Na_2B_4O_7 \cdot 10$ H_2O (0.1 g), EDTA (0.05 g), H_2O (200 mL), 70°, 50 kg/cm^2 H_2, pH 7.1–7 6, 800 r.p m.; 1, D-glucose; 2, D-fructose; 3, D-mannitol; 4, D-glucitol.

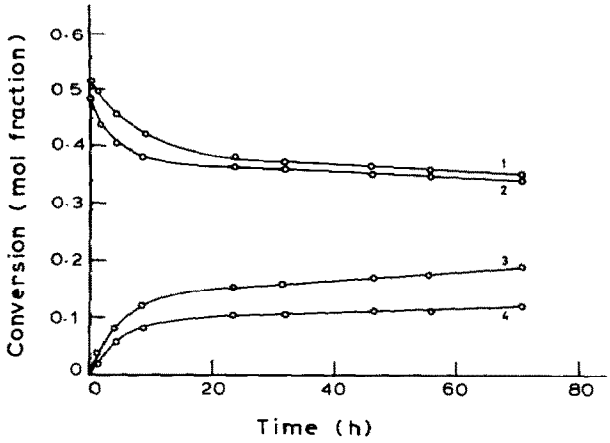

Fig. 3. The combi-process using Cu/silica and Sweetzyme Q. Composition: D-glucose (30.0 g), D-fructose (30.0 g), Sweetzyme Q (5.0 g), 20% Cu/silica (5.0 g), $MgSO_4$ (0.3 g), $CaCO_3$ (0.5 g), $Na_2B_4O_7 \cdot 10\ H_2O$ (0.1 g), EDTA (0.05 g), H_2O (200 mL), 70°, 50 kg/cm^2 H_2, pH 7.1–7.6, 800 r.p.m.; 1, D-glucose; 2, D-fructose; 3, D-mannitol; 4, D-glucitol.

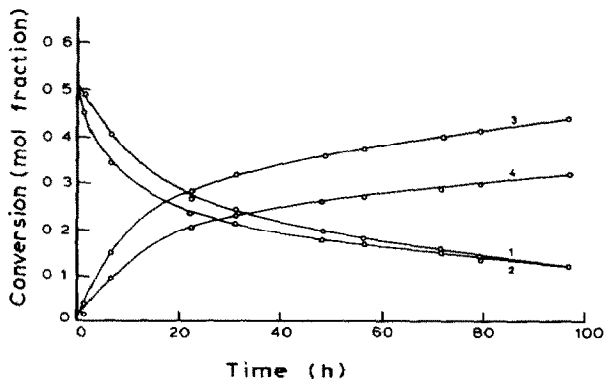

Fig. 4. The combi-process using Cu/silica and Maxazyme GI-Immob. Composition: D-glucose (30.0 g), D-fructose (30.0 g), Maxazyme GI-Immob. (15.0 g), 20% Cu/silica (5.0 g), $MgSO_4$ (0.3 g), $CaCO_3$ (0.5 g), $Na_2B_4O_7 \cdot 10\ H_2O$ (0.1 g), EDTA (0.05 g), H_2O (200 mL), 70°, 50 kg/cm^2 H_2, pH 7.1–7.6, 800 r.p.m.; 1, D-glucose; 2, D-fructose; 3, D-mannitol, 4, D-glucitol.

The poisoning effect of these enzyme systems is further illustrated by comparison of Fig. 2 with Figs. 3 and 4 for Cu/silica as the hydrogenation catalyst. This poisoning effect, which is more serious for Sweetzyme than for Maxazyme, is probably due to adsorption of fragments of the immobilised D-glucose isomerase system onto the hydrogenation catalyst.

In order to prevent such poisoning effects, the following parameters were investigated: (*a*) thorough washing of Maxazyme GI-Immob.[7], (*b*) extra cross-linking (with 5% glutardialdehyde), (*c*) the use of Maxazyme GI-Immob. which had functioned for 1050 h (its half-life) in the industrial high-D-fructose-corn-syrup process, (*d*) fixation in a metallic gauze during the hydrogenation reaction, (*e*) addition

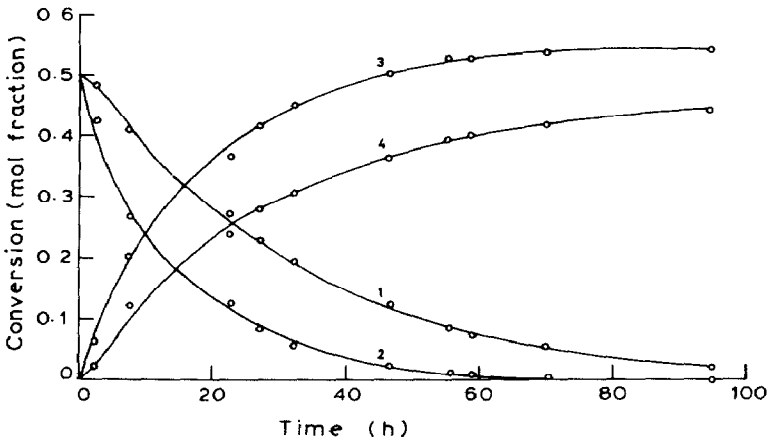

Fig. 5. The combi-process using Cu/silica and Optisweet 22. Composition: D-glucose (30.0 g), D-fructose (30.0 g), Optisweet 22 (3.0 g), 20% Cu/silica (5.0 g), $MgSO_4$ (0.3 g), $CaCO_3$ (0.5 g), $Na_2B_4O_7 \cdot 10\ H_2O$ (0.1 g), H_2O (200 mL), 70°, 50 kg/cm^2 H_2, pH 7.1–7.6, 800 r.p.m., 1, D-glucose; 2, D-fructose; 3, D-mannitol; 4, D-glucitol.

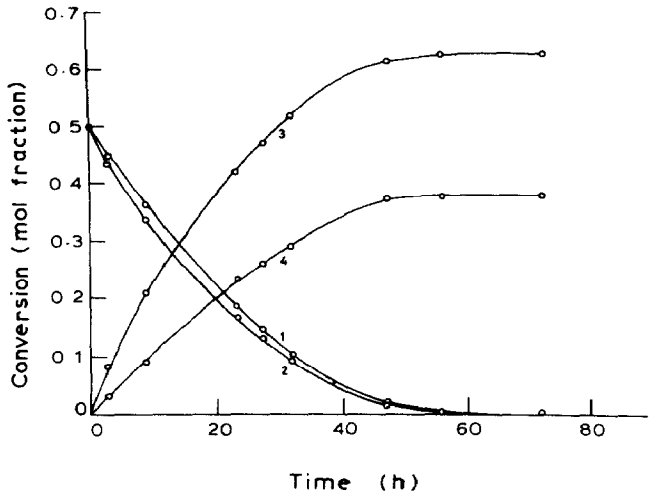

Fig. 6. Improvement of the activity of Optisweet 22 by EDTA in the combi-process using Cu/silica (*cf.* Fig. 5). Composition: D-glucose (30.0 g), D-fructose (30.0 g), Optisweet 22 (3.0 g), 20% Cu/silica (5.0 g), $MgSO_4$ (0.3 g), $CaCO_3$ (0.5 g), $Na_2B_4O_7 \cdot 10\ H_2O$ (0.1 g), EDTA (0.05 g), H_2O (200 mL), 70°, 50 kg/cm^2 H_2, pH 7.1–7.6, 800 r.p.m.; 1, D-glucose; 2, D-fructose; 3, D-mannitol; 4, D-glucitol.

of silica or activated carbon during the hydrogenation reactions in order to adsorb the mobile fragments, and (*f*) some combinations of the above-mentioned precautions. The Maxazyme GI-Immob. preparations thus obtained were still sufficiently active, but still poisoned the hydrogenation catalysts.

D-*Glucose isomerase immobilised on silica.* — Optisweet 22 consists of a purified D-glucose isomerase anchored onto a silica support. This enzyme system is

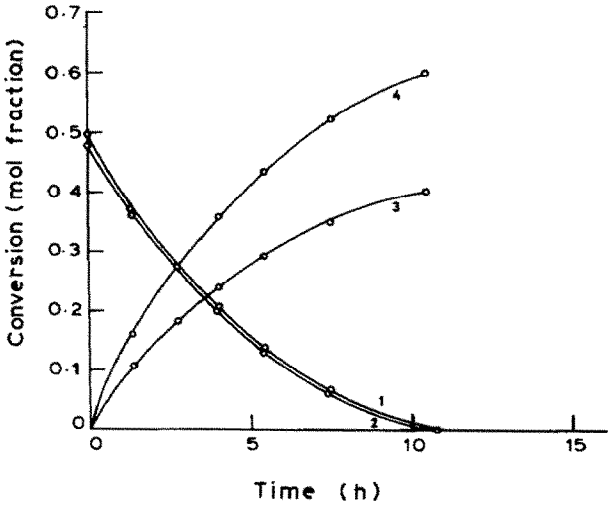

Fig. 7. The combi-process using Optisweet 22 and Ru/C. Composition: D-glucose (30.0 g), D-fructose (30.0 g), Optisweet 22 (3.0 g), 5% Ru/C (1.0 g), $MgSO_4$ (0.3 g), $CaCO_3$ (0.5 g), H_2O (200 mL), 70°, 50 kg/cm^2 H_2, pH 7.1–7.6, 800 r.p.m.; 1, D-glucose; 2, D-fructose; 3, D-mannitol; 4, D-glucitol.

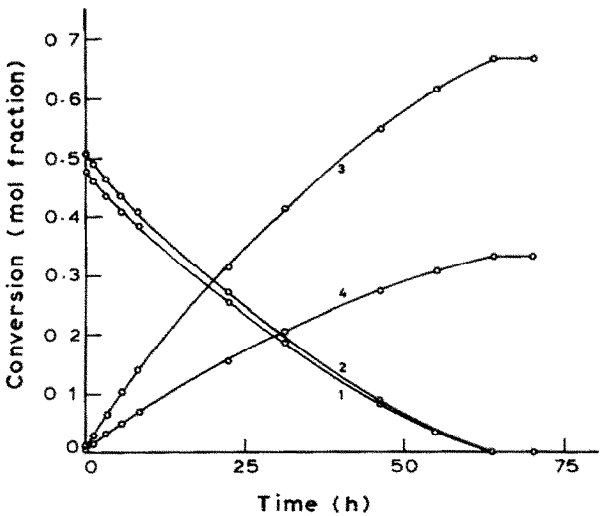

Fig. 8. The combi-process using Optisweet 22 and Cu/silica in the presence of $MgHPO_4$. Composition: D-glucose (30.0 g), D-fructose (30.0 g), Optisweet 22 (3.0 g), 20% Cu/silica (5.0 g), $MgHPO_4 \cdot 3\ H_2O$ (1.0 g), $Na_2B_4O_7 \cdot 10\ H_2O$ (0.1 g), EDTA (0.05 g), H_2O (200 mL), 70°, 50 kg/cm^2 H_2, pH 7.1–7.6, 800 r.p.m.; 1, D-glucose; 2, D-fructose; 3, D-mannitol; 4, D-glucitol.

more sensitive to species present in the hydrogenation mixture than the cellular enzyme preparations. For example[7], traces of copper ions inhibit Optisweet 22, as shown from the slow D-glucose/D-fructose interconversion during the Cu-catalysed hydrogenation (Fig. 5). This inhibitory effect can be counteracted easily by the addition of EDTA (equimolar with the copper ions), and Fig. 6 clearly

demonstrates the high activity of Optisweet 22 to maintain the D-glucose/D-fructose equilibrium, thus resulting in a 62% yield of D-mannitol. EDTA has no influence on the rate of hydrogenation and there was no further extraction of cations from the hydrogenation catalysts under the conditions used.

Comparison of Figs. 3 (Sweetzyme Q) and 4 (Maxazyme GI-Immob.) with Fig. 6 (Optisweet 22) shows that the poisoning effect exerted by Optisweet 22 is less serious.

A relatively fast procedure, but which yielded less D-mannitol, was the combination of Optisweet 22 and Ru/C. Complete hydrogenation of a 1:1 D-glucose/D-fructose mixture occurred within 10 h, yielding 40% of D-mannitol (Fig. 7). The addition of EDTA was not necessary, since ruthenium ions tend to activate immobilised D-glucose isomerases[7].

As reported elsewhere[6], an improvement of the selectivity in the formation of D-mannitol from D-fructose by hydrogenation over Cu/silica is obtained by the addition of catalytic amounts of borates. This effect was suppressed when $CaCO_3$ was used as buffer. Therefore, magnesium hydrogenphosphate was used as both buffering and enzyme-stabilising agent in the presence of small amounts of borates. There was an increase in the selective formation of D-mannitol (67–72%) from

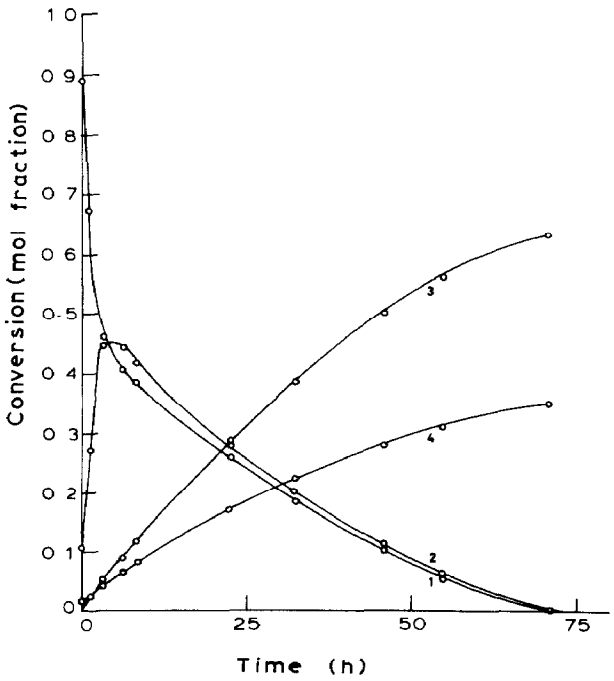

Fig. 9. Conversion of D-glucose into D-mannitol by the combi-process. Composition: D-glucose (60.0 g), Optisweet 22 (3.0 g), 20% Cu/silica (5.0 g), $MgHPO_4 \cdot 3\,H_2O$ (1.0 g), $Na_2B_4O_7 \cdot 10\,H_2O$ (0.1 g), EDTA (0.05 g), H_2O (200 mL), 70°, 50 kg/cm^2 H_2, pH 7.1–7.6, 800 r.p.m.; 1, D-glucose; 2, D-fructose; 3, D-mannitol; 4, D-glucitol.

D-fructose, whereas the initial selectivity for the hydrogenation of D-fructose in 1:1 D-glucose/D-fructose mixtures remained the same (0.92). The activity of the Cu/silica catalyst, however, is ~50% less in the presence of magnesium hydrogenphosphate. Optisweet 22 was able to maintain the D-glucose/D-fructose equilibrium during the hydrogenation reaction (Fig. 8) and a 66% yield of D-mannitol was obtained.

D-*Glucose as starting material.* — Fig. 9 shows that, in the presence of magnesium hydrogenphosphate and borate, D-glucose can be used as starting material in the combi-process. The yield of D-mannitol (64%) was about as high as that obtained using a 1:1 D-glucose/D-fructose mixture as starting material.

It is concluded that D-glucose isomerase immobilised on silica (Optisweet 22) in combination with a Cu/silica hydrogenation catalyst can be used effectively in a combi-process. High yields of D-mannitol (62–66%) have been obtained with D-glucose or 1:1 D-glucose/D-fructose mixtures as starting materials. Small amounts of either $CaCO_3$ and $MgSO_4$ or $MgHPO_4$ must be present in order to maintain sufficient enzyme activity [*i.e.*, pH ~7 and Mg(II) required by the enzyme], and the enzyme must be protected against traces of Cu^{2+} by the addition of EDTA. This combi-process is a great improvement on others reported in the literature for the production of D-mannitol.

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