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Direct alcoholic fermentation of starchy biomass using amylolytic yeast strains in batch and immobilized cell systems

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Summary. Direct alcoholic fermentation of dextrin or soluble starch with selected amylolytic yeasts was studied in both batch and immobilized cell systems. In batch fermentations, *Saccharomyces diastaticus* was capable of fermenting high dextrin concentrations much more efficiently than *Schwanniomyces castellii*. From 200 $g \cdot l^{-1}$ of dextrin *S. diastaticus* produced 77 $g \cdot l^{-1}$ of ethanol (75% conversion efficiency). The conversion efficiency decreased to 59% but a higher final ethanol concentration of 120 $g \cdot l^{-1}$ was obtained with a medium containing 400 $g \cdot l^{-1}$ of dextrin. With a mixed culture of *S. diastaticus* and *Schw. castellii* 136 $g \cdot l^{-1}$ of ethanol was produced from 400 $g \cdot l^{-1}$ of dextrin (67% conversion efficiency). *S. diastaticus* cells attached well to polyurethane foam cubes and a *S. diastaticus* immobilized cell reactor produced 69 $g \cdot l^{-1}$ of ethanol from 200 $g \cdot l^{-1}$ of dextrin, corresponding to an ethanol productivity of 7.6 $g \cdot l^{-1} \cdot h^{-1}$. The effluent from a two-stage immobilized cell reactor with *S. diastaticus* and *Endomycopsis fibuligera* contained 70 $g \cdot l^{-1}$ and 80 $g \cdot l^{-1}$ of ethanol using initial dextrin concentrations of 200 and 250 $g \cdot l^{-1}$ respectively. The corresponding values for ethanol productivity were 12.7 and 9.6 $g \cdot l^{-1} \cdot h^{-1}$. The productivity of the immobilized cell systems was higher than for the batch systems, but much lower than for glucose fermentation.

Introduction

The economy of an ethanol fermentation process of biomass is determined, in part, by the availability and cost of raw materials and the technology for their conversion into a liquid fermentation substrate. The cost of raw materials, including conversion into the fermentable aqueous medium, is the most important single factor in the process. Depending on the local conditions, this cost factor amounts to 50—70% of the total production cost of ethanol (Faust et al. 1983).

For ethanol production from starchy materials, at least two pre-fermentation steps are required, namely gelatinization of the starch by heating under acid conditions (cooking) and subsequent saccharification by amylolytic enzymes (Faust et al. 1983). Several attempts have been made to reduce or eliminate the cooking process by using fungal amylase preparations (Ueda and Koba 1980; Ueda et al. 1981; Matsuoka et al. 1982; Saha and Ueda 1983; Fujio et al. 1984) or by γ-irradiation (Han et al. 1983).

The expense represented by the use of commercial enzymes may be reduced by the fermentation with a starch-digesting yeast, e.g. *Saccharomyces diastaticus* (Laluce and Mattoon 1984) or *Schwanniomyces* spp. (Calleja et al. 1982; Frelot et al. 1982; Wilson et al. 1982; Dhawale and Ingledew 1983). In this study, selected amylolytic yeast strains were used to carry out direct fermentation of dextrin or soluble starch with both free suspended cells (batch cultures) and immobilized cells (continuous cultures). Attention was given to the different parameters leading to higher ethanol yields, regardless of the residual amount of non-fermentables left in the media.

Materials and methods

Microorganisms and culture media. Schwanniomyces castellii CBS 2863 was supplied by P. Galzy (Chaire de Génétique et de Microbiologie, Montpellier, France). *Endomycopsis fibuli-*

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gera IGC 3961-18 originated from C. Cabeça-Silva (Instituto Gulbenkian de Ciência, Oeiras, Portugal) and *Saccharomyces diastaticus* NCYC 625 was from the National Collection of Yeast Cultures, Nutfield, Great Britain). Stock cultures were maintained at 6° C on slants of a medium with soluble starch according to De Mot et al. (1984). The fermentation medium (F) contained yeast extract ($10\ g \cdot l^{-1}$), $(NH_4)_2SO_4$ ($1\ g \cdot l^{-1}$), KH_2PO_4 ($1\ g \cdot l^{-1}$), $MgSO_4$ ($0.5\ g \cdot l^{-1}$) and carbohydrates as indicated in the text. The pH was adjusted to 5.5 with sulphuric acid before sterilization. Maldex 15 (Amylum, Aalst, Belgium), a partial hydrolysate of starch with dextrose equivalent of 15 ± 1, was used as a dextrin substrate. Soluble starch was obtained from Difco (Detroit, USA). Batch fermentations were carried out using 500 ml Erlenmeyer flasks containing 150 ml of medium and closed with one-way valves. They were inoculated with 15 ml of a 24—48 hour yeast culture in the same medium as above.

Analytical methods. The supernatants of the batch cultures and of the reactor effluents, obtained after centrifugation, were analyzed for ethanol (Amin and Verachtert 1982), total reducing sugars (Miller 1959), and total carbohydrate (Herbert et al. 1971). Total viable counts were determined according to Postgate (1969). Cell density in column effluents was estimated by measuring the optical density at 620 nm. Sugars were analyzed by descending paper chromatography using as solvent isopropanol:acetic acid:water (7:1:2). After 60 h, the papers were dried, and sprayed with a reagent prepared by mixing 2% (w/v) diphenylamine in acetone, 2% (w/v) aniline in acetone, and 85% (w/v) phosphoric acid in a ratio of 5:5:1 (McFarlane and Held 1953). Upon heating at 80° C for 10 min, the linear oligosaccharides turned blue whilst the branched oligosaccharides produced brown-yellowish spots. For thin layer chromatography, pre-coated plates (DC-Alufolien Kieselgel 60; Merck, FRG) were used. In addition to the solvent system for paper chromatography n-propanol:nitromethane:water (5:2:3), and n-propanol:1 M lactic acid:acetone (4:2:4) were used. The sugars were detected with the aniline-diphenylamine-phosphoric acid reagent. Amylolytic activities were estimated as previously described (De Mot et al. 1984). For batch cultures, the rate of fermentation was determined by measuring the production of CO_2, expressed as the weight loss of the fermentation flasks.

Immobilization of cells on polyurethane foam. Polyurethane is a porous reticulated foam with a porosity of 97% and a density of $30\ kg \cdot m^{-3}$. Type T_{40} was kindly supplied by H. Van der Plaetse (Recticel, Wetteren, Belgium). It contains 40 pores per inch with a mean pore diameter of 0.45 mm, and has a specific surface of $600\ m^2 \cdot m^{-3}$. Nearly 180 foam cubes ($1\ cm^3$) were fixed on 8 steel rods (400 mm and 1.5 mm in length and diameter respectively). These rods were arranged as shown in Fig. 1 and introduced inside a 500 ml double-walled glass column. The column bottom was connected to a sampling device to collect the biomass which was lost during the operation. This device was regularly replaced under aseptic conditions. The complete column was autoclaved and, after cooling, an active yeast culture (20 h) was poured into the column. It was allowed to stand for 8 h in order to initiate attachment of yeast cells to the porous support. Medium (F) containing $50\ g \cdot l^{-1}$ of dextrin (Maldex 15) was then circulated through the column at $100\ ml \cdot h^{-1}$, the medium in the feed vessel being replaced once a day with fresh medium. Attachment was considered adequate when the yeast cell count and amylolytic activity in the recycled medium reached constant values. Aeration of the influent substrate solution, using a 0.2 μm sterilizing filter, was sometimes employed to enhance the cell growth in the reactors.

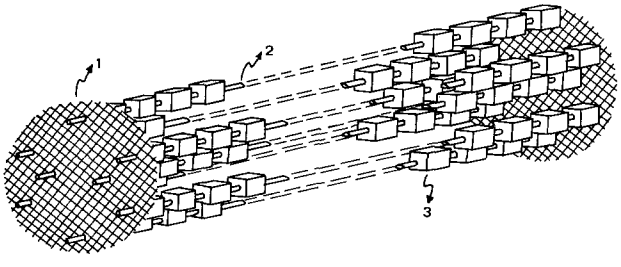

Fig. 1. Schematic representation of immobilized-cell reactor using polyurethane foam as support for entrapment of yeast cells: (1) stainless steel net; (2) stainless steel rod; (3) polyurethane foam cubes

Results

Characteristics of ethanol production from dextrin or soluble starch by Saccharomyces diastaticus, Schwanniomyces castellii or a mixed culture in batch systems

A screening of amylolytic yeasts for dextrin fermentation previously showed that good ethanol yields (about 70% conversion efficiency) were obtained with the glucoamylase producing species *Saccharomyces diastaticus* (De Mot et al. 1985). A strain of *Schwanniomyces castellii,* characterized by a lower efficiency (34%) but secreting both α-amylase and glucoamylase (Oteng-Gyang et al. 1981), was also selected for further characterization. The ethanol production from different concentrations of dextrin (Maldex 15) and soluble starch (Difco) with both species and with a mixed culture, was determined.

Surprisingly, *Saccharomyces diastaticus* was capable of fermenting the higher dextrin concentrations more efficiently (Table 1). With 50 and $100\ g \cdot l^{-1}$ of dextrin nearly 50% of the theoretical yields were obtained whereas with 150 and $200\ g \cdot l^{-1}$ of dextrin 58 and 75% of the theoretical yields were achieved. With 300 and $400\ g \cdot l^{-1}$ of dextrin lower conversion efficiencies, 70 and 59%, respectively, were found but higher final ethanol concentrations, up to $120\ g \cdot l^{-1}$, were achieved. The maximum fermentation rate was observed during the second day from the beginning of the fermentation (Fig. 2). The dextrin fermentation by *S. diastaticus* was affected by increasing the temperature. With a medium containing $200\ g \cdot l^{-1}$ of dextrin, a normal ethanol production was only found at 30° C (Fig. 3). Complete inhibition of

Table 1. Ethanol production from different concentrations of dextrin or soluble starch using single or mixed cultures of *S. diastaticus* and *Schwanniomyces castellii*

Substrate ($g \cdot l^{-1}$)	*S. diastaticus*				*Schw. castelli*		*Mixed culture*			
	Dextrin		Sol. starch		Dextrin		Dextrin		Sol. starch	
	Ethanol ($g \cdot l^{-1}$)	E[a] (%)	Ethanol ($g \cdot l^{-1}$)	E (%)	Ethanol ($g \cdot l^{-1}$)	E (%)	Ethanol ($g \cdot l^{-1}$)	E (%)	Ethanol ($g \cdot l^{-1}$)	E (%)
50	12.6	49.3	ND[b]	ND	20.6	80.6	ND	ND	ND	ND
100	26.3	51.5	ND	ND	37.3	73.0	ND	ND	ND	ND
150	44.5	58.0	41.0	53.5	46.3	60.4	50.4	65.7	55.2	72.0
200	76.6	74.9	61.7	60.4	51.7	50.6	80.6	78.8	82.5	80.7
300	107.2	69.9	81.2	53.0	42.2	27.5	109.4	71.3	107.8	70.3
400	120.3	58.8	ND	ND	ND	ND	ND	ND	ND	ND
400	ND	ND	ND	ND	ND	ND	136.4[c]	66.7	ND	ND

[a] E = percentage of the theoretical efficiency of ethanol production (0.511 $g \cdot g^{-1}$ glucose)
[b] ND = not determined
[c] Fermentation with an inoculum with a high initial cell concentration of *S. diastaticus* (3.5 $g \cdot l^{-1}$ dry weight) and 5% (v/v) from an active *Schw. castellii* culture

amylase production and fermentation occurred at 42 °C.

Schwanniomyces castellii behaved differently. The results clearly showed that the efficiency of ethanol production was higher with the low dextrin concentration (Table 1). With 50 $g \cdot l^{-1}$ of dextrin, 80% of the theoretical value was obtained, whereas only 27% was found with 300 $g \cdot l^{-1}$. The fermentation rate decreased with increasing initial dextrin concentration (Fig. 2). Also, the maximum fermentation rate was delayed compared to *Saccharomyces diastaticus*.

With a mixed culture of *S. diastaticus* and *Schwanniomyces castellii* a slight increase in the efficiency of ethanol production was observed (Table 1). A maximum conversion efficiency of 79% was obtained with 200 $g \cdot l^{-1}$ of dextrin. More ethanol was produced with increased dextrin con-

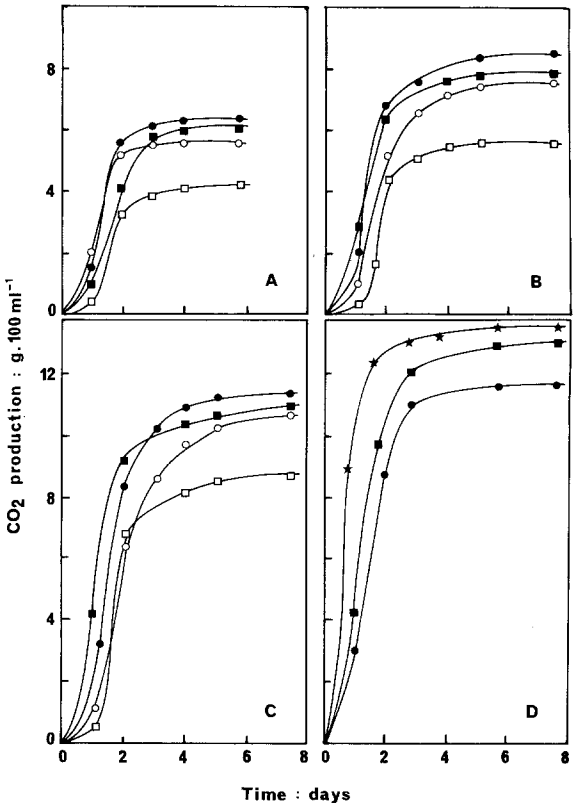

Fig. 2. Fermentation of dextrin (closed symbols) and soluble starch (open symbols) by *S. diastaticus* (■, □) or by a mixed culture *(S. diastaticus; Schw. castellii)* with a normal (●, ○) or with a heavy inoculum (★). Concentration ($g \cdot l^{-1}$) of substrate: (A) 150, (B) 200, (C) 300, (D) 400

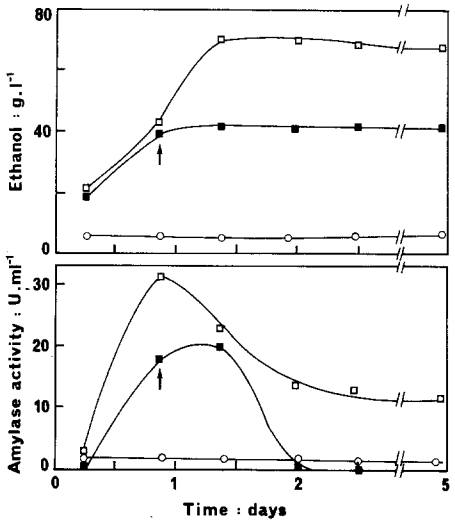

Fig. 3. Effect of temperature on ethanol production and amylase secretion during batch fermentation of dextrin (200 $g \cdot l^{-1}$) with *S. diastaticus*. Incubation: 30 °C (□), 42 °C (○), and 30 °C for 20 h with shift (arrow) to 42 °C (■)

centrations but the conversion efficiency decreased. So, from 400 $g \cdot l^{-1}$ of dextrin a final ethanol concentration of 136 $g \cdot l^{-1}$ was attained (67% conversion efficiency) with a mixed inoculum of 5% (v/v) of a *Schwanniomyces castellii* culture and a heavy *Saccharomyces diastaticus* cell suspension (3.5 $g \cdot l^{-1}$ dry weight). More than 75% of the carbohydrate was converted during the early fermentation period (24 h) (Fig. 2).

S. bayanus (Amin and Verachtert 1982) was used as a control to test the ability of a non-amylolytic yeast to ferment Maldex 15. Only traces of ethanol were detected (results not shown).

The results of experiments with soluble starch (Difco) are also listed in Table 1. Similar results were obtained with single and mixed cultures, but the values of both conversion efficiency and final ethanol concentration were lower. This was also observed for several other amylolytic yeast strains (Amin 1984).

Using thin layer chromatography and paper chromatography, the cell-free supernatants of the fermentation media were analyzed for residual sugars. In the soluble starch and dextrin media inoculated with *S. diastaticus,* glucose, maltose, maltotriose, isomaltose, small oligosaccharides with a degree of polymerization (DP) up to 6, and unresolved, higher oligosaccharides (DP>6) were found at the beginning of the fermentation. At the end of the fermentation only the unresolved oligosaccharides were detected. Similar carbohydrate patterns were obtained with the mixed culture. However, in the *Schwanniomyces castellii* medium the residual sugar profile did not change significantly throughout fermentation. The whole range of sugars, from glucose to the higher unresolved oligosaccharides, was present.

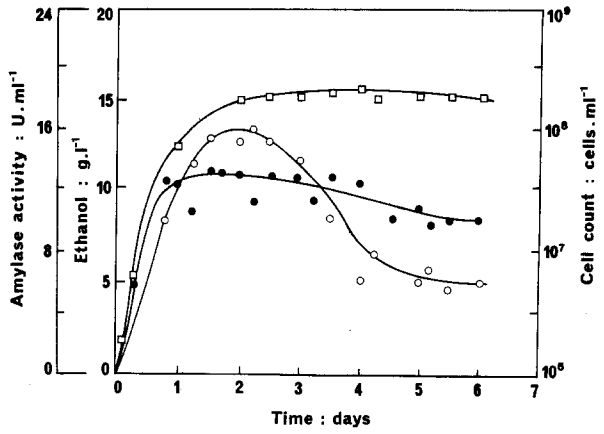

Fig. 4. Behaviour of a reactor with *S. diastaticus,* fed with 50 $g \cdot l^{-1}$ of dextrin at 100 $ml \cdot h^{-1}$, during the attachment period. Concentration of ethanol (□), cell count (●), and amylase activity (○) in the effluent

Ability of different yeast strains to adsorb to polyurethane foam cubes

Polyurethane foam was chosen as the substrate for immobilization. Its good porosity might make it more accessible towards polysaccharides than gel systems. The immobilization of *Saccharomyces diastaticus,* the yeast being most efficient in batch systems, was compared with the behaviour of *Schwanniomyces castellii* and a derepressed mu-

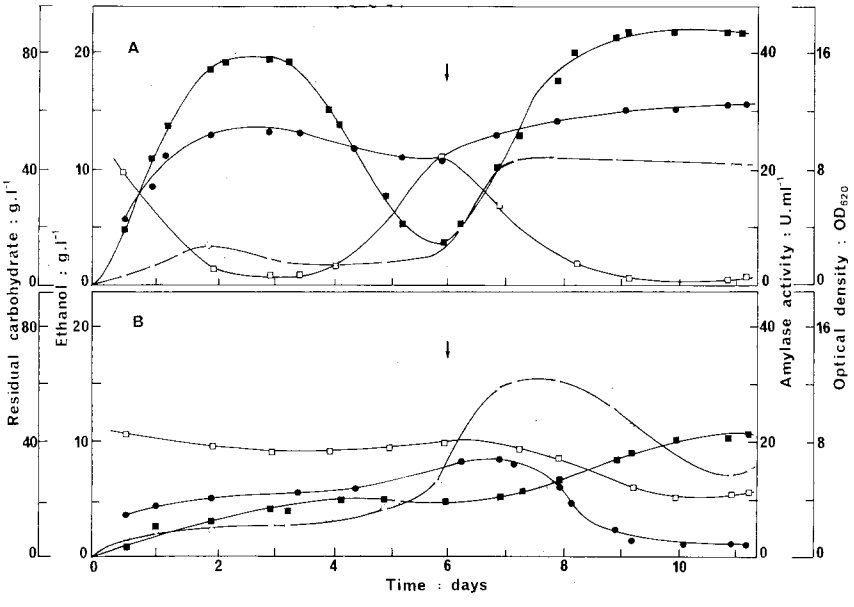

Fig. 5. Behaviour of a reactor with *Schw. castellii* (A) or *E. fibuligera* (B), fed with 50 $g \cdot l^{-1}$ of dextrin at 100 $ml \cdot h^{-1}$, during the attachment period. Aeration was applied after 6 days (arrow). Concentration of ethanol (■), optical density (●), amylase activity (○), and residual carbohydrate (□) in the effluent

tant strain of *Endomycopsis fibuligera*. The latter yeast, which ferments dextrin and soluble starch with low efficiency (< 10%) (Amin 1984), was selected for its good amylase secretion (Cabeça-Silva 1982). During the attachment period, in the presence of medium F with 50 $g \cdot l^{-1}$ of dextrin, these yeasts behaved differently.

Saccharomyces diastaticus readily adsorbed to the foam cubes, as indicated in Fig. 4. A constant level of 15 $g \cdot l^{-1}$ of ethanol (conversion efficiency of 59%) was reached after two days. Amylolytic activity and cell counts in the effluent reached constant values after about 4 days.

With *Schwanniomyces castellii* more than 18 $g \cdot l^{-1}$ of ethanol was obtained after a few days, but its concentration dropped rapidly to less than 5 $g \cdot l^{-1}$ after 6 days (Fig. 5). Residual carbohydrates accumulated in the effluent. The cell concentration in the effluent remained high but amylolytic activity was low. In batch cultures it was found that aeration had a positive effect on the production of ethanol and the secretion of amylolytic activity. With the reactor, aeration of the inlet medium induced an increase of ethanol concentration up to 21 $g \cdot l^{-1}$ (conversion efficiency of 82%), an increase of amylolytic activity and a decrease of residual carbohydrates. However, cell density in the effluent strongly increased. In fact most cells were released from the support and the results must therefore be attributed to the activity of non-adsorbed cells.

With *E. figuligera* less than 5 $g \cdot l^{-1}$ of ethanol was formed during the first days (Fig. 5). More residual carbohydrates, lower cell density and comparable amylolytic activity were registered compared to *Schwanniomyces castellii*. On aeration the ethanol concentration increased to 13 $g \cdot l^{-1}$. Residual carbohydrates and cell density decreased, whereas the amylolytic activity showed a significant increase. Thus, aeration enhanced the attachment of *E. fibuligera*. As with the bacterium *Zymomonas* (Amin and Verachtert 1982) an improved attachment might be due to a shift in morphology, as long filamentous forms developed from normal budding cells during aeration.

Characteristics of an immobilized Saccharomyces diastaticus reactor

The *S. diastaticus* reactor was selected for further study for its better constant ethanol production and good retention of the yeast cells. After the attachment period (first week), a medium with a higher dextrin concentration was pumped into the reactor and after reaching a steady state a new feed rate was selected as shown in Fig. 6. Ethanol production and residual sugars increased by increasing the initial dextrin concentration in the feed line. Lowering the feed rate then resulted in more ethanol and less residual carbohydrates. A further increase in dextrin concentration again increased the ethanol concentration and the residual sugars. Further lowering of the feed rate resulted in still more ethanol and a lowering of the residual carbohydrates. At the final steady state, with a feed rate of 55 $ml \cdot h^{-1}$ and a dextrin concentration of 200 $g \cdot l^{-1}$, an ethanol concentration of around 69 $g \cdot l^{-1}$ was maintained. Ethanol productivities (gram of ethanol produced per hour and per liter of reactor volume) at points 1, 2, 3 and 4 were 3, 5.4, 5.3 and 9.4 respectively. At the final steady state it was 7.6 (Fig. 6). Nearly constant values for both pH and amylolytic activity of the effluent were maintained.

The effect of increasing the temperature of the immobilized system from 30° C up to 42° C was studied. The results in Fig. 7 indicate that an in-

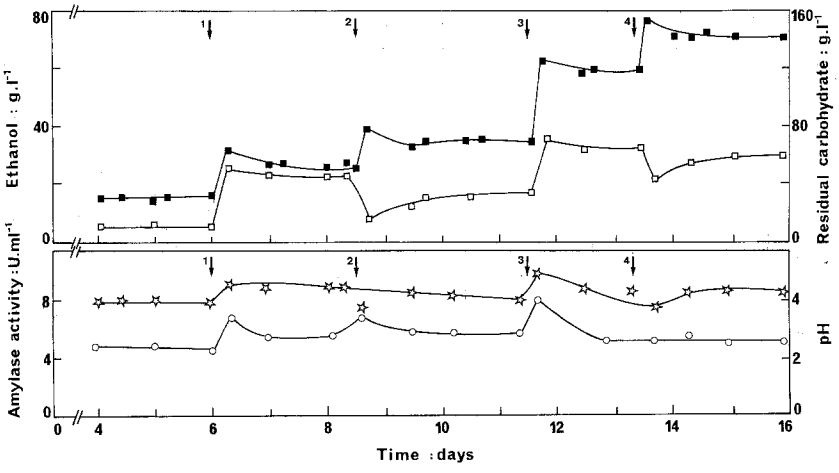

Fig. 6. Effect of dextrin concentration and feed rate on the performance of a *S. diastaticus* immobilized cell reactor. The initial dextrin concentration of the influent (50 $g \cdot l^{-1}$) was changed to 100 $g \cdot l^{-1}$ (1) and 200 $g \cdot l^{-1}$ (3). The initial feed rate (100 $ml \cdot h^{-1}$) was decreased to 80 $ml \cdot h^{-1}$ (2) and 55 $ml \cdot h^{-1}$ (4). Concentration of ethanol (■), amylase activity (○), residual carbohydrate (□), and pH (*) of the effluent

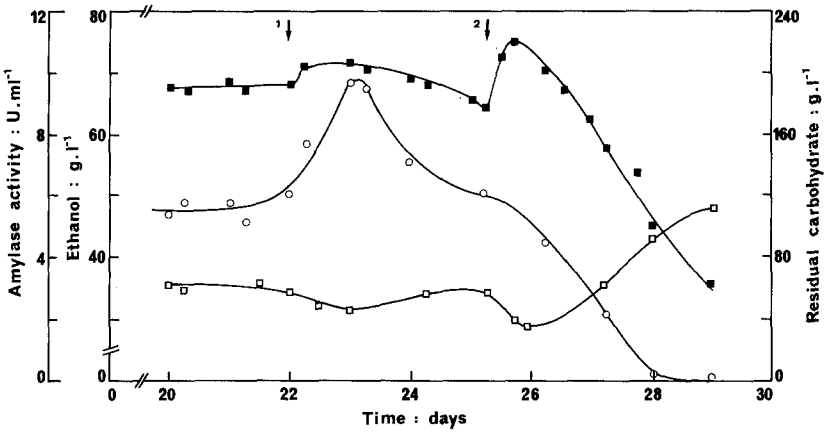

Fig. 7. Effect of temperature on ethanol production and amylase secretion of a *S. diastaticus* immobilized cell reactor. The initial temperature (30° C) was increased to 37° C (1) and 42° C (2). Concentration of ethanol (■), amylase activity (○), and residual carbohydrate (□) in the effluent

crease from 30° C to 37° C initially resulted in a higher amylolytic activity in the effluent, a nearly constant ethanol production and constant residual sugars concentration. However, after 2 days at 37° C the amylolytic activity decreased rapidly to the original value. When the temperature was raised to 42° C, the ethanol production and the amylolytic activity dropped to very low values and residual sugars accumulated. A similar effect of temperature on dextrin fermentation was obtained with the batch cultures.

Two-stage immobilized cell reactor with S. diastaticus and E. fibuligera

As *S. diastaticus* produces no α-amylase, it was attempted to obtain higher dextrin conversion than with the *S. diastaticus* reactor by the use of an additional yeast also producing α-amylase. *E. fibuligera* was preferred to *Schwanniomyces castellii* because of its better attachment to the polyurethane foam.

Reactors with immobilized cells of *S. diastaticus* and *E. fibuligera* were connected to form a two-stage immobilized reactor (Fig. 8). The fermentation was carried out using two set-ups: the ES system and the SE system. In the ES system the medium with 200 $g \cdot l^{-1}$ of dextrin was first fed to an *E. fibuligera* reactor and the effluent was fed to a *Saccharomyces diastaticus* reactor. With the SE system the medium was first fed to the *S. diastaticus* reactor, and then to the *E. fibuligera* reactor. For both systems the feed rate of the first reactor was 180 $ml \cdot h^{-1}$. Table 2 shows the results for both two-stage reactors.

With the ES system amylolytic activity was always higher in the first stage but the ethanol concentration was low. On the other hand, the ethanol concentration in the effluent of the second stage was much higher. The maximum ethanol concentration after 4 days was 57 $g \cdot l^{-1}$ (Table 2) with an ethanol productivity of 10.3 $g \cdot l^{-1} \cdot h^{-1}$ (Table 3).

Better results were achieved with the SE system. A high ethanol concentration was obtained in the first stage and still higher values in the second stage. The amylolytic activities at both stages remained lower than in the ES system. However, the ethanol concentration after the second stage

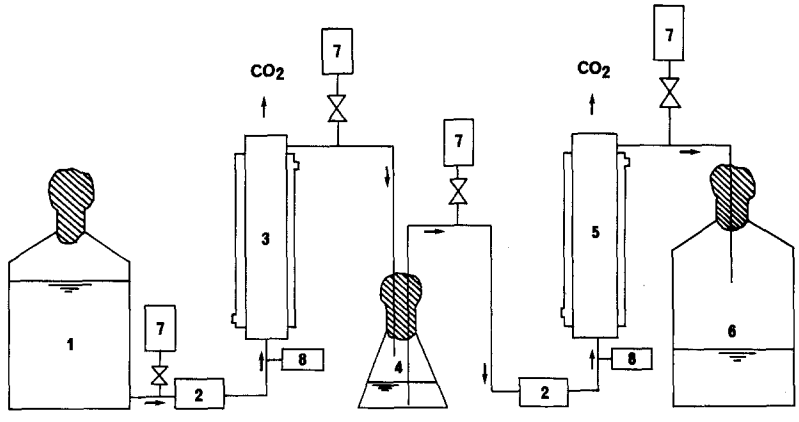

Fig. 8. Schematic representation of the two-stage immobilized reactor for ethanol production from starchy materials: (1) feed medium vessel, (2) peristaltic pump, (3) (5) immobilized reactor, (4) overflow device, (6) flask for effluent collection, (7) sampling port, (8) device for collecting excess biomass

Table 2. Ethanol and amylase production in two-stage immobilized reactors

Fermentation time (h)	Reactor stage	ES system[a] (200 g·l^{-1} dextrin)		SE system[a] (200 g·l^{-1} dextrin)		SE system[b] (250 g·l^{-1} dextrin)	
		Ethanol (g·l^{-1})	Amylase activity (U·ml^{-1})	Ethanol (g·l^{-1})	Amylase activity (U·ml^{-1})	Ethanol (g·l^{-1})	Amylase activity (U·ml^{-1})
24	1	11.5	15.6	53.7	3.7	65.7	2.9
	2	50.4	6.1	64.5	7.5	69.8	6.4
48	1	13.7	17.5	55.2	3.4	64.9	3.0
	2	55.0	5.7	67.4	8.6	77.8	7.8
72	1	14.5	20.0	56.4	3.8	66.4	3.5
	2	56.5	7.4	68.8	9.2	80.2	6.5
96	1	16.4	19.5	57.5	3.6	67.0	4.0
	2	57.0	7.9	70.4	9.0	79.8	7.3

[a] Feed rate = 180 ml·h^{-1}
[b] Feed rate = 120 ml·h^{-1}

attained 70.4 g·l^{-1} (Table 2) and the productivity was increased to 12.7 g·l^{-1}·h^{-1} (Table 3). When the initial dextrin concentration was increased to 250 g·l^{-1}, the ethanol concentration reached 79.8 g·l^{-1} (Table 2), but the productivity dropped to a value of 9.6 g·l^{-1}·h^{-1} (Table 3), although a lower feed rate (120 ml·h^{-1}) was used.

The difference in behaviour of the two systems might be explained as a higher carrying over of *S. diastaticus* cells to the *E. fibuligera* reactor in the SE system, compared with the carrying over of *E. fibuligera* cells to the *S. diastaticus* reactor in the ES system. *S. diastaticus* cells did not adsorb to the solid support as well as did the *E. fibuligera* cells. The result would be a better combined activity of both yeasts in the last stage of the SE system. A co-immobilization of both yeasts in one column was not attempted.

Discussion

Out of a large number of amylolytic yeast species, *S. diastaticus* and *Schw. castellii* strains were selected (Amin 1984; De Mot et al. 1985), and compared for ethanol production from high concentrations of dextrin and soluble starch using batch cultures. *S. diastaticus* was superior with respect to fermentation of higher initial concentrations of dextrin or soluble starch. High concentrations of ethanol (> 100 g·l^{-1}) were produced. The high ethanol tolerance of *S. diastaticus* has been reported previously (Duvnjak and Kosaric 1981; De Mot et al. 1985). The polysaccharide substrates were converted incompletely to ethanol, which was confirmed by chromatographic analysis of the fermented media. These findings are consistent with the properties of the amylolytic system of *S. diastaticus*. This yeast species secretes multiple forms of glucoamylase (Erratt 1980; Tamaki 1980; Yamashita et al. 1984) but no α-amylase nor debranching activity (Searle and Tubb 1981; Sills and Stewart 1982). Laluce and Mattoon (1984) reported the almost complete conversion of amylase-treated crude manioc starch. However, their preparation was more extensively depolymerized than our dextrin preparation (Maldex 15). The authors did not exclude the presence of debranching activity in the amylase preparation used (Takatherm). The alcoholic fermentation of dextrin by *S. diastaticus* was sensitive to temperature variations. This phenomenon probably is a result of

Table 3. Comparison between different immobilized cell systems for ethanol production from dextrin

Fermentation characteristics	Single reactor (*S. diastaticus*)			ES system	SE system	
Dextrin (g·l^{-1})	50	100	200	200	200	250
Ethanol (g·l^{-1})	15.0	33.2	68.9	57.0	70.4	79.8
Residual carbohydrate (g·l^{-1})	14.0	26.3	53.2	76.5	47.3	76.1
Ethanol productivity (g·l^{-1}·h^{-1})	3.0	5.3	7.6	10.3	12.7	9.6
Amylase activity (U·ml^{-1})	4.7	5.1	5.4	7.9	9.0	7.3

several effects, including changes in the rate of ethanol production, the level of amylase production (Estrela et al. 1982; Calleja et al. 1984), the rate of enzymatic hydrolysis of the polysaccharide substrate, and probably the combined effect of ethanol and heat on the cell's metabolism.

As reported previously by several authors (Calleja et al. 1982; Wilson et al. 1982), only low concentrations of dextrin or soluble starch were fermented well by *Schwanniomyces castellii*. The lower ethanol tolerance of this yeast species (Wilson et al. 1982; De Mot et al. 1985) probably is the major factor limiting efficient fermentation of high concentrations of dextrin or soluble starch, as *Schwanniomyces* species produce all the enzymes necessary for extensive starch degradation, namely glucoamylase with debranching activity and α-amylase (Oteng-Gyang et al. 1981; Wilson and Ingledew 1982; Sills et al. 1984; Simões-Mendes 1984). Residual sugars from the fermentation media included glucose, indicating that the conversion to ethanol, rather than starch hydrolysis to fermentable sugar, was affected.

The conversion efficiency of the batch fermentation with *Saccharomyces diastaticus* was improved by using a mixed culture with *Schwanniomyces castellii,* thus supporting the idea that the *Saccharomyces diastaticus* amylolytic system was limiting fermentation and could be supplemented by using an additional, α-amylase producing yeast. Wilson et al. (1982) described the associative fermentation of starch, using *Schwanniomyces alluvius* for its amylolytic activity and *Saccharomyces uvarum* for ethanol production. Dostálek and Häggström (1983) used a mixed culture of *E. fibuligera* and *Zymomonas mobilis* for alcoholic fermentation of soluble starch. In their system, glucose production from starch was the rate-limiting reaction.

With respect to the immobilized systems, the different behaviour of several yeasts was noted, not only with respect to ethanol production but also with regard to oxygen requirement and attachment of the cells to the support. With *S. diastaticus* and *E. fibuligera,* reactors were run for two months. The productivity of the *S. diastaticus* system was higher than in batch system but much lower than for glucose fermentation with yeasts such as *S. bayanus* (Amin et al. 1983; Amin et al. 1984).

Using a two-stage system some improvement was achieved, especially when a *S. diastaticus* reactor was used before an *E. fibuligera* reactor (SE system). The latter yeast produces glucoamylase with debranching activity and α-amylase (Kol'tsova and Sadova 1970; Sukhumavasi et al. 1976; Kato et al. 1976; Gracheva et al. 1977). With a medium containing 250 $g \cdot l^{-1}$ of dextrin an ethanol concentration of 80 $g \cdot l^{-1}$ was obtained, which is certainly a concentration favourable for further distillation. The productivity was 9.6 $g \cdot l^{-1} \cdot h^{-1}$ (Table 3). Compared to a batch reactor of 100 m^3 producing from 15% (w/v) glucose about 7,000 kg of ethanol in 48 h (Amin et al. 1983), the two-stage system with 5 m^3 reactors would produce 2,400 kg of ethanol from 25% (w/v) dextrin in 48 h. Using a dextrin concentration of 20% (w/v) around 3,000 kg of ethanol would be produced in 48 h with less residual sugars in the effluent but with a lower ethanol concentration (70 $g \cdot l^{-1}$). The residual sugars are not necessarily lost. By aeration of the effluents a further conversion of these sugars to biomass could be achieved and possibly the amylases could be recovered.

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