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Article

Inverted sugar syrup attained from sucrose hydrolysis using a membrane reactor

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Invertase, whether adsorbed on styrene-divinylbenzene copolymers or otherwise, was used for continuous sucrose hydrolysis using a cell-type membrane reactor (CTMR), coupled with an ultra (UF-100kDa), or a microfiltration (MF- pore diameter of 5 μm) membrane. In all tests, the pH (5.5), temperature (30 °C), reaction volume (10 mL) and agitation (100 rpm) were set constant; whereas, variable parameters were: feeding rate (0.4, 0.8 and 1.6 h^{-1}), inlet sucrose concentration (2.5, 6.5, 50 and 100 mM) and enzyme/resin ratio (1.64 mg or 3.28 mg of protein per 25, 50 or 100 mg of resin). The best result (yield of 100%, steady-state duration over 20h and specific reaction rate over 243 x 10^{-3} mmol/h.m_E) was obtained when insoluble invertase (1.64 mg protein/100 mg resin) was used to convert 50 mM or 100 mM of sucrose solution at 0.4 h^{-1} using a UF-CTMR.

Uniterms: Sucrose. Invert sugar. Membrane reactor.

Invertase, na forma adsorvida ou não em copolímeros de estireno-divinilbenzeno, foi usada para a hidrólise contínua de sacarose utilizando um reator com membrana (RM), acoplado a uma membrana de ultrafiltração (UF-100kDa), ou de microfiltração (MF - um diâmetro de poro de 5μm). Em todos os testes, o pH (5,5), a temperatura (30°C), o volume reacional (10mL) e a agitação (100 rpm) foram mantidas constantes; os parâmetros variados foram: a vazão de alimentação (0,4; 0,8 e 1,6 h^{-1}), a concentração de sacarose alimentada (2,5; 6,5; 50 e 100 mM) e a relação enzima/resina (1,64 mg ou 3,28 mg de proteína por 25, 50 ou 100 mg de resina). O melhor resultado (um rendimento de 100%, um período de estado estacionário acima de 20h e uma taxa de reação específica maior de 243 x 10^{-3} mmol/h.m_E) foi obtido quando a invertase insolúvel (1,64 mg de proteína/100 de mg resina) foi usado para converter 50 mM ou 100 mM de solução de sacarose a 0,4 h^{-1} usando UF-RM.

Unitermos: Sacarose. Açúcar invertido. Reator com membrana.

INTRODUCTION

There is increasing interest in the applications of enzymes. Due to their advantages compared with conventional chemical catalysts, enzymes´ selective and specific processes under very mild conditions allow the obtention of products of commercial interest with less toxic and pollutant effluents (Tomotani *et al.,* 2005). The advent of immobilization technique in the 1960s represented a great step forward in enzyme technology, leading to the re-utilization of the enzyme and the use of continuous reactors. Therefore, new and/or more purified products, such as, inverted sugar syrup, aspartame, high-fructose-corn syrup, L-amino acids, lactose-free whey, and 6-amino penicillanic acid have become commercially available (Godfrey, West, 1996). Among these products, the hydrolysis of sucrose to produce inverted syrup deserves special attention. It is used as a sweetener, carbon source in fermentation processes, and as a raw material to obtain chemicals, such as glucose, fructose, gluconic acid, sorbitol and oligosaccharides (Erzinger, Vitolo, 2006).

Sucrose hydrolysis can be carried out by using hydrochloric acid at 70-80°C or by using invertase (EC.3.2.1.26) at 30-45°C and pH 4.6. The enzymatic catalysis thus requires less energy than the acidic process. Another difference must be considered, i.e., the acid inverted syrup has several undesirable by-products (furfural and hydroxymethylfurfural) – cyclic derivatives from glucose and fructose when

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submitted to low pH and high temperature – which must be removed from the final product before its commercialization. In spite of the obligatory submission of the acid-attained syrup to a downstream procedure, the increase in the overall process cost is compensated by the less expensive hydrochloric acid used as a catalyst. Currently however, a concern over environmental spoilage as well as pollution has led us to the search for alternatives to minimize these detrimental outcomes. Thereby, the application of invertase becomes useful because it is available in the market, obtained from baking and/or brewer's yeasts – microorganisms fully accepted by the sanitation authorities all over the world and widely applied by the food industry – and used as an analytical tool (biosensors) and in confectionary (Said, Pietro, 2004).

Although the acid can be replaced by invertase for the sucrose hydrolysis, it becomes costly to do so. Consequently, the use of immobilized invertase would be a viably practical alternative (Said, Pietro, 2004).

Among many methods for enzyme immobilization, adsorption on ionic resins (such as Dowex® resins) is one of the most simple and suitable procedures (Tomotani, Vitolo, 2004) - after the end of the reaction, both the enzyme and the support are easily recovered for further re-use.

Another approach would be using a continuous reactor such as a membrane reactor (MR). This device can be formed either by coupling the stirred tank in series with a membrane filtration cassette (bi-module MR) or by placing the membrane at the bottom of the stirred vessel (cell-type MR). It can operate with ultrafiltration or microfiltration membrane depending on the form of the enzyme employed, i.e., soluble or immobilized, respectively. The type of membrane to be chosen could also depend on the desired dilution rate to be used throughout the continuous reactor. The microfiltration membrane may be selected when a high dilution rate is preferred, as mentioned in previous work (Tomotani, Vitolo, 2006).

In this study, invertase was immobilized in styrene-divinylbenzene copolymers – a class of anionic resins, widely used in water desalinization and ion exchange chromatography (Tomotani, Vitolo, 2005) - for continuous sucrose hydrolysis. This procedure, which included both soluble and immobilized invertase, was carried out in a cell-type MR coupled with an ultra or microfiltration membrane.

MATERIAL AND METHODS

Material

Invertase (β-D-fructofuranosidase, EC.3.2.1.26), commercially known as Bioinvert®, was purchased from Quest International (São Paulo, SP, Brazil). One milliliter of original invertase solution corresponds to 120 units. One Unit (U) is defined as the amount of invertase activity that results in one milligram of total reducing sugars (TRS) per minute from 200mM sucrose at initial concentration at 37°C and pH 5.5 (Tomotani, Vitolo, 2005). The kinetic constants, V_{max} and K_M, for the soluble invertase were 0.0240 U/mL and 17.0 mM, respectively (Tomotani, Vitolo, 2006). The Dowex® 1X4:200 resin and BSA (Bovine Serum Albumin) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The 100kDa-UF-membrane (PLHK07610) and the MF-membrane (TMTP09030, pore diameter of 5 μm) made of regenerated cellulose were purchased from Millipore (Bedford, MA, USA). Other chemicals used were analytical grade.

Methods

Standard procedure for immobilization

Immobilizing invertase through adsorption on anionic-polystyrene beads (Dowex®-1X4:200 resin) was described elsewhere (Tomotani, Vitolo, 2004). An amount of Dowex®-1X4:200 resin (25 mg, 50 mg or 100 mg), previously equilibrated for 24h in deionized water (pH 5.5; 32°C and agitation of 100 rpm), was mixed with 3 mL of invertase solution containing 1.64 mg or 3.28 mg of protein, and then the system was left for 4h at pH 5.5, 32°C and agitation of 100 rpm. The resin-invertase complex was then centrifuged (2880g, 30min), rinsed three times with deionized water, and the final suspension stored at 4°C in deionized water. One milligram of resin-invertase complex (RIC) had a total activity of 3.6U. The activity of immobilized invertase was measured by mixing 108 mL of sucrose solution (120g/L in 0.010M acetate buffer, pH 5.5) with 12 mL of an aqueous suspension of RIC. Hydrolysis was carried out for 6 min at 37°C under agitation (100 rpm), as previously described (Vitolo *et al.*, 1995). One immobilized invertase unit was defined as the amount of total reducing sugars (mg) formed per minute under the test conditions. The kinetic constants, $(V_{max})_{app}$ and $(K_M)_{app}$, for the immobilized invertase were 0.0450 U/mL and 18.3 mM, respectively (Tomotani, Vitolo, 2006). Under the conditions cited, the immobilization yield was 100%.

Membrane reactor

A 10mL cell-type MR (BIOENGINEERING® AG, Wald, Germany) was used in all tests. The reactor consisted of a 316-L stainless steel cylinder whose bottom has both an inlet and an outlet for the external water bath for temperature control. The diameter of the UF or MF-membrane used was 63mm. The reactor can be ster-

ilized (autoclave up to 134°C for 30 min) and can resist high temperatures (up to 150°C) and corrosion by most substances (except strong acids, pH <1.0; and alkalis, pH > 12.0). Moreover, it has a safety valve (set to nominal six-bar pressure limit) and can be coupled to a dosing pump, a pressure probe, a sterile filter, and a bubble trap (Tomotani, Vitolo, 2007).

Membrane-reactor tests

Ten milliliters of buffered solution (0.01M acetic acid/acetate buffer, pH 5.5) containing soluble or immobilized invertase, both at a total activity of 36U, was poured into the MR, whose bottom was fitted with a 100kDa-UF-membrane or 5 μm-MF-membrane. The reactor was fed continuously (feeding rates (D) of 0.4 h^{-1}, 0.8 h^{-1} or 1.6 h^{-1}) with a sucrose solution (2.5 mM, 50 mM or 100 mM). The reaction was carried out for 25h at 30°C and at an agitation of 100 rpm. The concentration of TRS as well as soluble protein was determined from the aliquot samples taken from the outlet solution. None of the tests performed presented protein leakage from the MR. The yield (Y) and the specific reaction rate (r) were calculated according to Eqs. (1) and (2), respectively. A total of thirteen tests were performed – each done in duplicate – using the operational conditions presented in Table 1.

$$Y\ (\%) = 0.95x[TRS] \div (S)_0 \qquad \text{(Eq.1)}$$

$$r\ (mmol/h.m_E) = (Q \times [TRS]) \div m_E \qquad \text{(Eq.2)}$$

Where: $[S]_0$ = inlet sucrose concentration (mM); [TRS] = outlet total reducing sugars concentration (mmol/L) ; Q = volumetric rate (L/h); m_E = weight of protein (mg).

Analytical techniques

- Protein determination

Protein was determined based on the difference between UV absorbance measured at 215 nm and at 225 nm, using 0.1 mg/mL bovine serum albumin solution as a standard (Segel, 1976). To establish the standard curve, the concentration of BSA was varied from 0.01 to 0.1 mg/mL.

- Measurement of total reducing sugars (TRS)

The TRS were measured by the conventional Somo-

TABLE I - Conditions under which all continuous experiments were conducted and their respective average conversion (Y), specific reaction rate (r) and steady-state duration (t_{st}) attained. The temperature (30°C) and agitation (100 rpm) were kept constant. In test 8 an MF-membrane (pore diameter of 5 μm) was used, whereas an UF-membrane (100 kDa) was used in all other tests.

TEST (n.)	PROTEIN (mg)	RESIN (mg)	SUCROSE (mM)	D (h^{-1})	r (mmol/h.m_E)x10^3	Y (%)	t_{st} (h)
1[a]	1.64	-	2.5	1.6	49	100	25
2[a]	3.28	-	2.5	1.6	24	100	25
3	1.64	25	2.5	1.6	-[b]	-	-
4	1.64	50	2.5	1.6	-	-	-
5	3.28	25	2.5	1.6	-	-	-
6	3.28	50	2.5	1.6	24	100	10
7	1.64	100	2.5	1.6	36	95	20
8	1.64	100	2.5	1.6	50	100	25
9[c]	1.64	100	6.5	0.4	32	100	10
			6.5	0.8	46	73	15
			6.5	1.6	114	90	20
10	1.64	100	50	0.4	243	100	22
11	1.64	100	50	0.8	439	90	23
12	1.64	100	50	1.6	585	60	25
13[d]	1.64	100	50	0.4	244	100	20
			100	0.4	488	100	20

[a] Tests in which the invertase was in soluble form. [b] The continuous process occurred under an unsteady-state. [c] An exploratory test for studying the effect of the dilution rate on r, Y and t_{st}. [d] An exploratory test for studying the stability of the steady-state against the increase of the inlet sucrose concentration.

gyi method, using a 0.2 mg/mL (w/v) glucose pA solution as a standard (Arruda, Vitolo, 1999). To establish the standard curve, the concentration of glucose was varied from 0.04 to 0.20 mg/mL.

RESULTS AND DISCUSSION

In a previous study (Tomotani, Vitolo, 2004), it was shown that the Dowex-1X4-200 resin adsorbed invertase thoroughly. In the present study, resin-invertase complexes (RIC) were prepared by combining different amounts of invertase (1.64 mg and 3.28 mg, in terms of protein) and resin (25, 50 and 100 mg), in order to verify their catalytic performance in a membrane reactor. The main results are presented in Table 1 and Figures 1 and 2.

In Table 1, it can be seen that the reaction rate varied from $24x10^{-3}$ mmol/h.m_E (tests 2 and 6) to $585x10^{-3}$mmol/h.

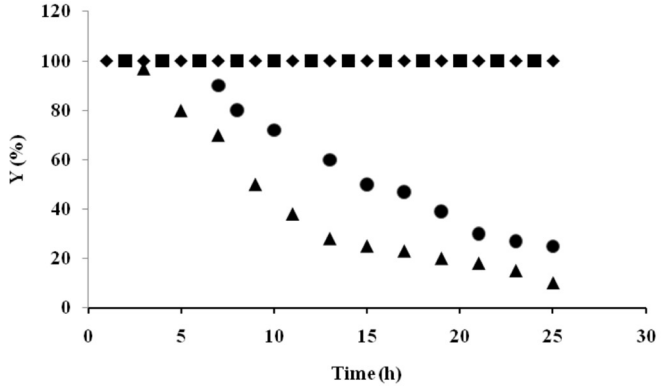

FIGURE 1 - Variation in conversion during continuous sucrose hydrolysis catalyzed by soluble invertase [test 1 (■)] and immobilized invertase [test 3 (●); test 4 (▲) and test 8 (◆)]. In all tests, the protein loading was equal to 1.64 mg/mL.

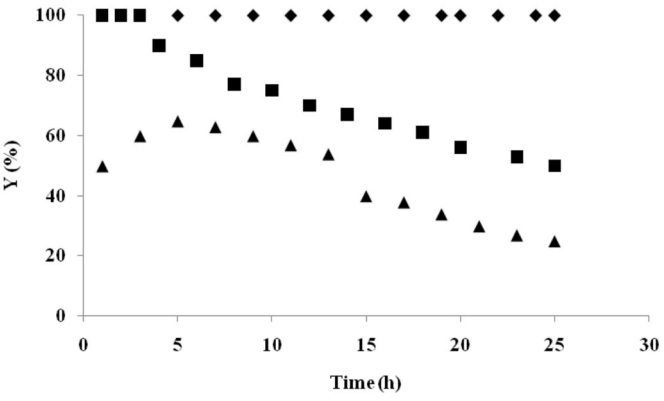

FIGURE 2 - Variation in conversion during continuous sucrose hydrolysis catalyzed by soluble invertase [test 2 (◆)] and immobilized invertase [test 5 (▲) and test 6 (■)]. In all tests, the protein loading was equal to 3.28 mg/mL.

m_E (test 12), and that unsteady-state regimes occurred in tests 3, 4 (Figure 1) and test 5 (Figure 2). The yield of sucrose hydrolysis was near 100% (Table 1) for tests in which the reactor was fed with 2.5 mM sucrose solution and the steady-state remained for at least 20 h (tests 1, 2, 7 and 8). Out of tests 3-8, carried out with insoluble invertase, only 7 and 8 (RIC consisting of enzyme/resin ratio of 1.64 mg of protein/100mg of resin) presented the best results in terms of the specific reaction rate, the yield and the steady-state duration. Tests 7 and 8 also differed on the type of membrane coupled to the reactor, i.e., a 100-kDa-UF-membrane (test 7) and a 5µm-MF-membrane (test 8). Focusing on the values of specific reaction rates of both tests (Table 1), test 7 presented a reaction rate about 28% lower than that of test 8. This is in accordance with previous findings of Tomotani, Vitolo (2007), in which the specific reaction rate for the test carried out with UF-membrane (reaction conditions: 0.0164 mg protein/mg of resin; 30°C, pH 5.5, 100rpm, 2.5mM sucrose and $1.6h^{-1}$) was approximately 15% lower than that of the test done with MF-membrane under the same conditions. The hindrance to the outlet flux caused by the MF-membrane was probably lower than that caused by the UF-membrane. However, this rate difference could also result from the fact that different membranes have different back-pressures, insofar as these experiments were pump-driven. In the literature, the importance of this result in the scaling-up of the process has been reported where in contrast to the case of UF-membrane, by using the MF-membrane, high outlet flux can be attained with low or zero pressurization of the reactor (Tomotani, Vitolo, 2006).

Undoubtedly, the data presented in Table I – particularly tests 3-8, which differed on the enzyme/resin ratio – led to the conclusion that a compromise between the amounts of enzyme and resin must be established (in this case, 1.64 mg of protein/100mg of resin), considering an optimized sucrose hydrolysis using a membrane reactor. By using a certain enzyme/resin ratio, the continuous process (through a membrane reactor, in the present case) may or may not occur in a consistent steady-state regime (Figure 1). In addition, the effect of enzyme to substrate ratio on the specific reaction rate (r) and on the duration of the steady-state regime can also be ascertained by comparing tests 6 (3.28 mg of protein to 2.5mM sucrose) and 7 (1.64 mg of protein to 2.5mM sucrose) (Table I).

As shown in Table I, in tests 3, 4 and 7, the enzyme/resin ratio was 1.64mg of protein to 25mg, 50mg and 100mg of resin, respectively. However, test 7 presented the highest yield (95%) and reaction rate ($36x10^{-3}$mmol/h.m_E) as well as a t_{st} of 20h (Table I and Figure 1). No enzyme leakage from the carrier was detected for any of the enzyme/resin proportions studied (data not shown).

It can be concluded that a higher amount of the resin (in this case 100mg) offered the most adequate surface area to accommodate the enzyme molecules.

Specifically in the case of invertase – an enzyme which presents a quaternary structure level constituted by two inter-twisted glycoprotein chains held together by weak chemical bonds – the molecules, which are natural dimmers, can aggregate forming more complex structures (tetramers, hexamers and octamers) (Reddy, MacColl, Maley, 1990). It was also demonstrated that the aggregates are in equilibrium in solution (dimmers ⇆ tetramers ⇆ hexamers ⇆ octamers), their catalytic activity and the overall negative charge grow as the degree of aggregation increases (Reddy, MacColl, Maley, 1990). Thereby, a high amount of resin implies more particles available to attach high molecular weight aggregates (mainly, hexamers and octamers), resulting in more active resin/enzyme complexes. Conversely, high enzyme/resin ratio (over 0.0164 mg of protein/mg of resin) would lead the octamers to imbricate on the surface of resin beads, resulting in a less active invertase-DOWEX complex.

The effects of substrate concentration and feeding rate (D) on sucrose hydrolysis by invertase conducted in a membrane reactor were therefore focused on the best selected enzyme/resin ratio.

In Figure 3, the feeding rate significantly affects the yield of sucrose conversion, which oscillated between 70% and 100% as D varied from 0.4 h^{-1} to 1.6 h^{-1}. The steady-state was always observed at different yield (Y) values. Such a response from a continuous reactor (particularly in a membrane reactor) is a clear indication of its suitability for sucrose hydrolysis. A similar pattern was also observed in tests 10-12, in which 50 mM sucrose solution was hydrolyzed under the same D values (Table 1). However,

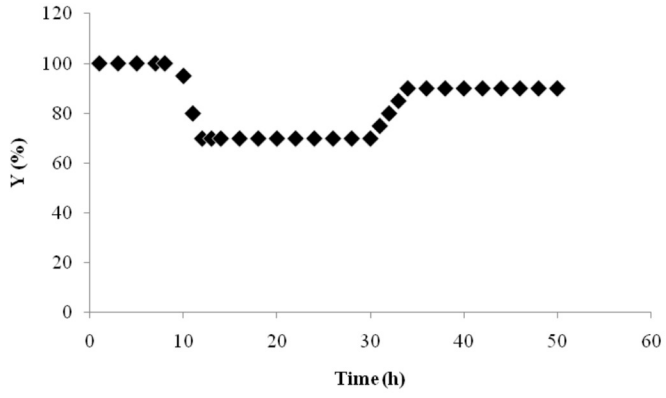

FIGURE 3 - Variation in conversion during continuous sucrose hydrolysis catalyzed by immobilized invertase for test 9: D = 0.4 h^{-1} (time interval: 0-10h); D = 0.8 h^{-1} (time interval: 11-30h) and D = 1.6 h^{-1} (time interval: 31-50h).

when the reactor was fed with a 50 mM sucrose solution, the yield diminished as the dilution rate increased. This corroborates the data published by Tomotani, Vitolo (2007), which stated that in a cell-type-MR fed with sucrose solution at concentrations over 12.5mM, the yield and feeding rate were inversely correlated. This is a plausible behavior considering that a high D implies low residence time, reducing the contact time between the enzyme and substrate. This critical situation occurs when the inlet sucrose concentration is increased.

Regarding the specific reaction rate (r), Table 1 (tests 9-12) shows that under fixed sucrose concentration (test 9: 6.5 mM, and tests 10-12: 50 mM) its value always increased with D. For these tests, a four-fold increase in D (from 0.4 h^{-1} to 1.6 h^{-1}) led to an approximate 3.5- (test 9) and 2.4- (tests 10-12) fold augmentation in the specific reaction rate. The high feeding rate leading to a high specific reaction rate or vice-versa might be due to the fact that the interaction invertase/sucrose was diminished by better removal of the products. This relationship could be indirectly explained by comparing tests 7 (Y = 95% and r = $36x10^{-3}$mmol/h.m_E) and 8 (y = 100% and r = $50x10^{-3}$ mmol/h.m_E), in which the only difference between them was the type of the membrane used (Table 1). The MF-membrane (test 8) has far larger pores than the UF-membrane (test 7). Therefore the microfiltration membrane might cause less constraint on the mass flux throughout the reactor, leading to the high conversion yield (100%) and increase in specific reaction rate (around 28%). Moreover, the improvement of the flux throughout the membrane reactor could also explain the high stability and equal duration (25h) of the steady-state observed in test 1 (soluble invertase and UF-membrane) and test 8 (insoluble invertase and MF-membrane) (Table 1).

According to the previous argument, both feeding rate and inlet substrate concentration are important parameters to optimize the operation of the membrane reactor. Other factors which must be observed, i.e., temperature and pH have been studied previously (Tomotani, Vitolo, 2007). Further evaluations of the membrane reactor at highest values of feeding rate and sucrose concentration must be assessed, if a future industrial application is to be considered.

The following points represented the intrinsic limitation of the membrane reactor used. The small operational volume of the reactor restricts the use of D over 1.6 h^{-1}. The narrowness of the Teflon tubes may reduce the flow of sucrose solution when its concentration increases due to its viscosity (sucrose solution over 100 mM).

To clarify the perspective of the scale-up process, an exploratory assay was designed (test 13), in which the

inlet concentration of the sucrose solution was changed from 50mM to 100mM (Figure 4). The feeding rate was set at 0.4 h^{-1} (Table 1). Figure 4 shows a conversion yield of 100% and a steady-state for 20h. This occurred when the reactor was fed consecutively with a 50mM and a 100mM sucrose solution. Although the results matched those attained in tests 7, 8 and 10 (Table 1), the reaction rate increased about 50% as the inlet sucrose concentration was varied from 50mM to 100mM (Table 1). This result showed that the current process allowed conversion of forty times more sucrose under the same operational conditions (except for the type of membrane) than that previously described (Tomotani, Vitolo, 2007). These results are promising with regard to potential for scaling up the process. The engineering required to attain scale-up already exists (Said, Pietro, 2004). The products resulting from sucrose hydrolysis have a significant market appeal, especially for sugarcane rich countries (Das Neves, Vitolo, 2007).

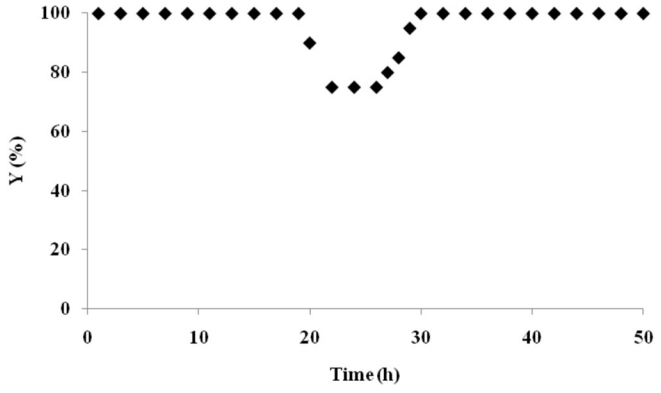

FIGURE 4 - Variation in conversion during continuous sucrose hydrolysis catalyzed by immobilized invertase for test 13: [Sucrose] = 50 mM (time interval: 0-20h) and [Sucrose] = 100 mM (time interval: 21-50h).

CONCLUSIONS

The most adequate proportion (w/w) of invertase (expressed as mg of protein) and resin was 1.64 mg of protein/100 mg of resin. The feeding rate was an important parameter to operate the MR at the steady-state and must be adjusted for each inlet sucrose concentration used. The best conditions for sucrose hydrolysis (50 – 100mM) were pH 5.5, 30°C, D = 0.4 h^{-1} using a UF-membrane-CTMR. It should be emphasized that process scaling up can be envisaged, insofar as at least up to 100 mM sucrose solution were shown to be efficiently hydrolyzed using invertase.

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REFERENCES

ARRUDA, L.M.O.; VITOLO, M. Characterization of invertase entrapped into calcium alginate beads. *Appl. Biochem. Biotechnol.,* v.81, p.23-33, 1999.

DAS NEVES, L.C.M.; VITOLO, M. Use of glucose oxidase in a membrane reactor for gluconic acid production. *Appl. Biochem. Biotechnol.,* v.136-140, p.161-170, 2007.

ERZINGER, G.S.; VITOLO, M. Zymomonas mobilis as catalyst for the biotechnological production of sorbitol and gluconic acid. *Appl. Biochem. Biotechnol.,* v.129-132, p.787-794, 2006.

GODFREY, T.; WEST, S. *Industrial enzymology*. 2. ed. London: MacMillan, 1996. p.64.

REDDY, A.V.; MACCOLL, R.; MALEY, F. Effect of oligossacharides and chloride on the oligomeric structures of external, internal and deglycosylated invertase. *Biochemistry,* v.29, p.2482-2487, 1990.

SAID, S.; PIETRO, R.C.L.R. *Enzimas como agentes biotecnológicos.* Ribeirão Preto: Legis Summa Publisher, 2004. p.412.

SEGEL, I.H. *Biochemical calculations*. 2.ed. New York: John Wiley & Sons Inc., 1976. p.232.

TOMOTANI, E.J.; DAS NEVES, L.C.M.; VITOLO, M. Oxidation of glucose to gluconic acid by glucose in a membrane bioreactor. *Appl. Biochem. Biotechnol.,* v.121-124, p.149-162, 2005.

TOMOTANI, E.J.; VITOLO, M. Catalytic performance of invertase immobilized by adsorption on anionic exchange resin. *Process Biochem.*, v.41, p.1325-1331, 2006.

TOMOTANI, E.J.; VITOLO, M. Production of high-fructose syrup using immobilized invertase in a membrane reactor. *J. Food Eng.,* v.80, p.662-667, 2007.

TOMOTANI, E.J.; VITOLO, M. Screening of Dowex® anion exchange resins for invertase immobilization. *Appl. Biochem. Biotechnol.,* v.113-116, p.145-159, 2004.

VITOLO, M.; DURANTI, M.A.; PELLEGRIM, M.B. Effect of pH, aeration and sucrose feeding on the invertase activity of intact Saccharomyces-cerevisiae cells grown in sugarcane blackstrap molasses. *J. Ind. Microbiol.*, v. 15, p.75-79, 1995.

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