

Comparison of economic indicators of the sucrose acid inversion or by enzymatic hydrolysis

Leissy Gómez-Brizuela¹, Jesús Luis-Orozco², Héctor L. Ramírez-Pérez¹, Mario Yll-Lavín², Santiago Díaz-Suarez², Georgina Michelena-Álvarez³, Julio C. Dustet-Mendoza⁴

¹ Centro de Estudios Biotecnológicos, Universidad de Matanzas
Carretera a Varadero Km 31/2, Matanzas, Cuba

² Facultad de Ciencias Técnicas, Universidad de Matanzas
Carretera a Varadero Km 31/2, Matanzas, Cuba

³ Instituto Cubano de investigaciones de los derivados de la Caña de Azúcar, ICIDCA
Vía Blanca 804 y Carretera Central, San Miguel del Padrón, La Habana, Cuba

⁴ Facultad de Ingeniería Química, Universidad Tecnológica de la Habana José Antonio Echevarría
Calle 114 # 11901 entre Ciclovía y Rotonda. Marianao, La Habana, Cuba
✉ gpesucuela.mtz@infomed.sld.cu

ABSTRACT

Glucose and fructose production is based in the inversion of sucrose. This work was aimed to obtain both compounds by enzymatic hydrolysis using immobilized invertase. The biocatalyst used was invertase-Chitosan:Chitin-carboxymethylcellulose which retained 50 % of the activity after 170 days of operation. The substitution of the acid inversion in the technological process to produce glucose and syrup by continuous enzymatic inversion was evaluated at the Basic Industrial Unit "Chiquitico Fabregat", in Villa Clara, Cuba. Parameters of design and operational conditions of the bed reactor were analyzed. The preliminary feasibility evaluation of the industrial enzymatic process showed its technical and economical superiority over the acid inversion system.

Keywords: sucrose, immobilized invertase, enzymatic hydrolysis, economic evaluation

Biotecnología Aplicada 2017;34:4401-4404

RESUMEN

Comparación de los indicadores económicos para la operación de inversión ácida de sacarosa o mediante hidrólisis enzimática. El proceso de producción de glucosa y fructosa se basa en la inversión de la sacarosa. El presente trabajo propone la obtención de los mismos mediante hidrólisis enzimática empleando invertasa inmovilizada. El biocatalizador utilizado es invertasa-quitosana: quitina-carboximetilcelulosa, el cual retiene un 50 % de la actividad al término de 170 días de operación. Se evaluó la sustitución de la etapa de inversión ácida por una inversión enzimática en continuo durante el proceso tecnológico de obtención de glucosa y sirope de fructosa, implementado en la Unidad Empresarial de Base Chiquitico Fabregat, de Villa Clara, Cuba. Se calcularon los parámetros de diseño y las condiciones de operación del reactor de lecho fijo propuesto. La valoración económica preliminar de la implementación industrial del proceso enzimático demostró la superioridad técnica y económica del mismo.

Palabras clave: Sacarosa, invertasa inmovilizada, hidrólisis enzimática, evaluación económica

Introduction

Inverted syrups are sucrose hydrolysis products of equivalent sweetness, which are composed of mixtures of sucrose, glucose and fructose (up to 50 % of the mixture). They are more soluble, hygroscopic, show lower freezing point, viscosity and are non-crystallizing, all these properties justifying their preferential and irreplaceable use as part of several products in the food industry.

These syrups are obtained by a technological process denominated sucrose inversion, which can be developed through acidification, ion exchange or enzymatic methods. Acidification has been extensively used, despite the formation of undesirable byproducts and the induced corrosion on the equipment, while ion exchange procedures generate high amounts of wastes and consume large quantities of chemical products for regenerating ion exchange resins. To circumvent these problems, enzymatic methods using invertase enzymes has been introduced, providing highly specific methods.

Enzymes are generally soluble molecules, difficult to reuse and of low stability, and together with the high cost for its production, all these make difficult

their application at industrial scale [1-3]. One strategy further improving its use considers enzyme immobilization in inert matrices bioreactors [4, 5].

In Cuba, sucrose inversion has been traditionally made by acidification with a nationally developed methodology operating under discontinuous regime. It normally renders glucose and fructose-rich syrups from refined sucrose, but consuming phosphoric acid at high temperatures (85-90°C), further generating other stained compounds carrying ashes and undesired byproducts [6, 7].

Therefore, in this work it was economically estimated the implementation of an enzymatic sucrose inversion system, employing an invertase-chitosan biocatalyst immobilized onto a chitin-carboxymethylcellulose support. The biocatalyst was produced at the laboratories of the University of Matanzas, Cuba, for the hydrolysis of sucrose into glucose and fructose. The proposed system operates under continuous regime without adding chemical reagents, devoid of corrosive effects as the ones demonstrated by the acid hydrolysis and rendering high quality syrups at economically competitive costs.

1. Datta S, Christena R, Rani Y, Rajaram S. Enzyme immobilization: an overview on techniques and support materials. *3Biotech*. 2013;3(1):1-9.

2. Bolivar JM, Eisl I, Nidetzky B. Advanced characterization of immobilized enzymes as heterogeneous biocatalysts. *Catalysis Today*. 2015;259:66-80.

3. Carvalho F, Paradiso P, Saramago B, Ferraria AM, Botelho do Rego AM, Fernandes P. An integrated approach for the detailed characterization of an immobilized enzyme. *J Mol Catal B: Enzymatic*. 2016;125:64-74.

4. Aguilera J, Estévez C. La Biotecnología Industrial: una realidad hoy, una necesidad mañana. *Biotech Magazine*. Publicaciones M K M. 2014 [cited: 2014 May]. Available from: <http://www.mkm-pi.com/biotech/la-biotecnologia-industrial-una-realidad-hoy-una-necesidad-mañana/>

5. Patel AK, Singhania, RR, Pandey A. Novel enzymatic processes applied to the food industry. *Curr Op Food Sci*. 2016;7:64-72.

6. Duarte E. Siropes invertidos. Pasado y presente. *Revista ATAC*. 1997:47.

Materials and methods

System design

Sucrose was enzymatically hydrolyzed by using an invertase-chitosan:chitin-carboxymethylcellulose (INV-QSA:Chit-CMC) biocatalyst, which was synthesized following the methodology described by Gómez *et al.* [8]. The traditional sucrose acid hydrolysis process for glucose production (acidic glucose) was used as reference process for operation modification.

The price of the INV-QSA:Chit-CMC biocatalyst was estimated assuming the 80 % cost of raw materials as established by Fernández *et al.* [9], in addition to other costs (equipment, power, water, labor workforce salary, maintenance of facilities and depreciation). The costs for the acquisition of raw materials were based on criteria from the Import Division of the Ministry of Higher Education of Cuba [10]. Revenues were set as 10 %.

Biocatalyst half-life and periods corresponding to conversion intervals were calculated from the mathematical processing of the equation ruling the operation kinetics of invertase immobilized onto the chitin-carboxymethylcellulose support (Figure 1).

Design parameters and operation conditions are described in table 1, and the operational process flow chart in figure 2A shows the operating process for the biocatalytic reactor during the industrial process producing glucose and fructose syrup.

Technological process for production of glucose and fructose syrup by acid hydrolysis

The acid hydrolysis process to obtain glucose and fructose syrup is composed of seven steps (Figure 2B). At start, refined sugar is dissolved into a dissolver tank provided with mechanical air stirring and steam heating, in which inversion is further done by adding phosphoric acid under operational control until finished. The inverted solution is further pumped into the cooling crystallizer for the first crystallization up to a capacity of 90 %, where cooling by air bubbling takes place. When temperature reaches 35 °C, the solution is pumped into the operational crystallizers where they get seeded by a specific seeding material on each one. When the mass becomes exhausted (15-20), it is purged through a filter press, generating the fructose enriched syrup which is pumped into the storage tank.

Glucose cakes are dissolved into the dissolver tank and then they are pumped into the cooling crystallizer for the second crystallization process. This time the solution is cooled by air bubbling and, after reaching 40 °C, it is pumped into the operational crystallizers for specific seeding. Then, when the mass is exhausted after seven days, it is purged by discontinuous centrifugation. The mother liquor corresponding to glucose-rich syrup is returned to the first crystallizer.

Glucose obtained is then packed, weighed and stored at dry and fresh conditions.

Comparatively, the acid hydrolysis process requires a dissolver with agitation and a pump, while the enzymatic hydrolysis process includes an additional dissolver with agitation and the enzyme-containing column.

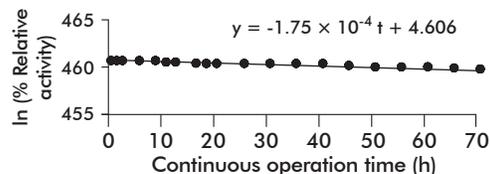


Figure 1. Operational kinetics of invertase immobilized in a chitin-carboxymethylcellulose support, in a packed reactor with 50 mmol/L sodium acetate buffer, pH 5.0, at 30 °C.

Table 1. Design parameters and operation conditions for continuous operation of the fixed-bed reactor packed with the INV-CSA:Chit-CMC biocatalyst

Parameter	Value
Reactor size (m ³)	0.8
Reactor height (m)	2
Reactor diameter (m)	0.75
Support mass (kg)	106
Enzyme mass (kg)	0.9
Catalyst specific activity (U/mg)	1820
Feeding flow (m ³ /h)	2
Feeding substrate concentration (kg/m ³)	342
Average inversion	0.75
Temperature (m)	30
pH	4.5

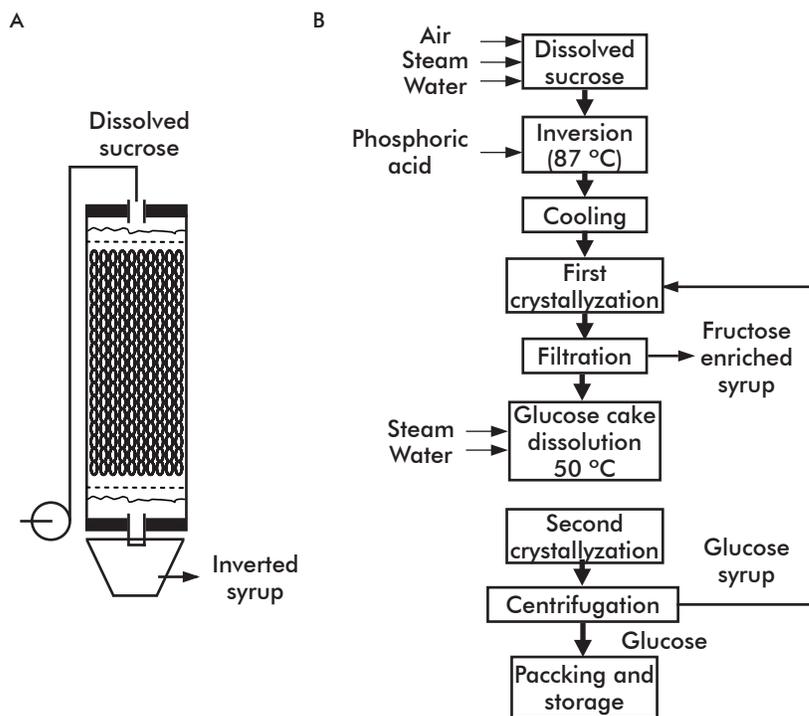


Figure 2. Steps of the technological process for the production of glucose and fructose syrup. A) Diagram of the enzymatic continuous reactor at laboratory scale. B) Acid hydrolysis.

Preliminary economic estimation

The economic evaluation was done in two steps, the first evaluating the feasibility of the base case and the second estimating the economic impact of the time for enzyme replacement.

7. Albertini A, Cadena P, Silva J, Nascimento G, Reis A, Freire V, et al. Performance of invertase immobilized on glass-ceramic supports in batch bioreactor. *Chem Eng J.* 2012;187:341-50.

Premises were:

1. A working year was assumed of 170 days, corresponding to the half-life of the biocatalyzer (enzymatic glucose). During the operation of the enzymatic reactor in the traditional acid hydrolysis (acid glucose) process, it is operated for 85 production cycles at the top capacity of the plant (15 cycles per month).

2. The sucrose hydrolysis step was the only one considered.

The estimates for raw materials, products and auxiliary services of the sucrose hydrolysis process and the marketing prices corresponding to those established at the acid glucose plant are listed in table 2.

The preliminary economic feasibility of the process was analyzed following the indicators recommended by Peter and Timmerhaus [11].

Amortized costs were estimated for the acid hydrolysis following the parameters proposed by Turton and Bailie [12], and data for the enzymatic reactor was used as defined by the reactor design. Production volume and production value in both processes are determined considering glucose and glucose syrup production.

Results and discussion

The preliminary economic estimation of using the INV-QSA:Chit-CMC during the enzymatic hydrolysis of sucrose considered only the inversion step. In fact, it is proposed to replace acid hydrolysis by the enzymatic hydrolysis. Regarding the equipment, the discontinuous reactor used in the acid hydrolysis is substituted by a fixed-bed reactor packed with the INV-QSA:Chit-CMC biocatalyst, this last supporting the continuous operation. The projection followed the design proposed by Martínez [13] for a reactor using immobilized invertase as fixed-bed.

As shown in table 3, the production cost of enzymatic glucose exceeds in 33 % the value for the traditional acid hydrolysis process, reaching 1 528 390.06 CUP (Cuban peso). This is caused mainly by the increased volume of sucrose being processed, with a higher consumption of raw materials, the use of two dissolvers and a higher power demand for the process, together with increased salary costs. Nevertheless, the entire value of the product obtained is 2.4 fold the value of the acid glucose product, despite the increased cost of the enzymatic glucose process, also derived from the continuous production regime that generates a higher production volume in a given time unit. The operational/monetary unit (CUP) balance is less than 1.0 for both processes, being lower for the enzymatic hydrolysis (0.51) as compared to the acid hydrolysis (0.82), and derived from the increased production volume.

In a second step, there was analyzed the influence of two other factors on the operational cost/monetary unit (CUP) balance: enzyme turnover and support increased costs. The analysis was carried out foreseeing errors when estimating the cost of the support, assuming increase values in the range 40-100 % (Table 4). An operation cycle comprised 170 days corresponding to an operating year, with time intervals in days corresponding to changes for conversion over time. As demonstrated by values obtained (Table 4), there was seen a tendency for the production to decrease on

Table 2. Price estimates for raw materials, products and auxiliary services of the sucrose hydrolysis process*

Materials and auxiliary services	Price (CUP)
Phosphoric acid (1000 kg)	2053.90
Water (m ³)	0.10
Refined sugar (1000 kg)	516.15
Electricity (kW)	0.09
Biocatalyst (kg)	11.70
Steam (1000 kg)	4.00
Fructose (1000 kg)	849.70
Glucose (1000 kg)	621.35

* Prices were estimated following the recommendations by Kalk [9].

Table 3. Economic indicators for the conversion of sucrose by acid hydrolysis or enzymatic hydrolysis with the aid of an invertase-chitosan:chitin-carboxymethyl-cellulose biocatalyst

Indicator	Enzymatic glucose	Acid glucose
Production cost (CUP)	1 528 390.06	1 022 786.84
Production volume (ton/d)	24.50	20.00
Operation time (d)	170.00	170.00
Production value (CUP)	3 061 255.05	1 250 392.50
Operational cost/monetary unit (CUP)	0.51	0.82

day 13 of operation, while increasing the operational cost/monetary unit (CUP) balance. Additionally, there were two options for improving yields, leading the operation to proceed until days 26 or 40. This provides valuable information as to when it is more feasible to change the biocatalyst used as fixed-bed, from the economic and technical points of views.

In fact, the lowest estimation of the operational cost/monetary unit (CUP) balance is attained at day 26, providing the highest amount of glucose produced in the entire 170-days period, with a conversion yield equivalent to that of the acid hydrolysis of sucrose. Nevertheless, the process operating for 26 days would require more steps to replace the exhausted biocatalyst than leaving the process to continue until day 40. At this time, the operational cost/monetary unit (CUP) balance and conversion yield are similar to that on day 26 and it is not detrimental for the subsequent steps of the process for glucose production.

The other options can be considered according to the conditions and the availability of the support, or attending to the properties and quality of the final

Table 4. Behavior of the operational cost/monetary unit (CUP) balance of the production of enzymatic glucose considering an increase in the 40-100 % range for the price of the support and at different times for biocatalyst replacement

Days	Inversion	Glucose mass/170 days cycle (ton)	Operational cost/monetary unit (CUP) ratio for the process at different support costs		
			Estimates	40 % cost increase	100 % cost increase
00-13	100-95	2516.80	0.3970	0.4000	0.4020
14-26	95-90	2554.02	0.3950	0.3960	0.3970
27-40	90-85	2510.91	0.4090	0.4100	0.4110
41-55	85-80	2429.25	0.4190	0.4192	0.4200
56-71	80-75	2431.95	0.4230	0.4235	0.4240
72-88	75-70	2309.70	0.4470	0.4479	0.4484
89-106	70-65	2264.10	0.4566	0.4569	0.4574
107-125	65-60	2093.00	0.4940	0.4943	0.4950
126-147	60-55	2072.36	0.4990	0.4992	0.5000
148-170	55-50	2016.00	0.5140	0.5150	0.5160

8. Gómez L, Ramírez HL, Villalonga R. Immobilization of chitosan-invertase neoglycoconjugate on carboxymethylcellulose-modified chitin. *Prep Biochem Biotechnol.* 2006;36:259-71.

9. Fernández E, Gonzales G, Mayo O. Ingeniería económica para ingenieros químicos. La Habana: Facultad de Ingeniería Química, CUJAE; 2002.

10. AZCUBA. Contrato marco de suministros, noviembre de 2014. Matanzas: Azcuba; 2014.

11. Peters MS, Timmerhaus KD. Plant design and economics for chemical engineers. 4th Ed. Singapore: McGraw-Hill; 1991.

12. Turton R, Bailie WB. Analysis, synthesis and design of chemical processes. New York: Prentice Hall; 2009.

13. Martínez CL. Tesis en opción al título de Ingeniero Químico. Matanzas: Universidad de Matanzas; 2014.

product required at the end of the hydrolysis. Noteworthy, the cost of the support does not put at risk the application of the enzymatic hydrolysis, since the operational cost/monetary unit (CUP) balance is still lower than that of the acid hydrolysis even doubling the price of the support, for all the analyzed combinations.

In summary, the preliminary economic analysis evidences the feasibility of the proposed enzymatic conversion process proposed, which is superior in both its technical and economic aspects to the acid hydrolysis

process for sucrose conversion. This also validates the introduction of the INV-QSA:Chit-CMC biocatalyst as part of an industrial scale process.

Acknowledgements

The authors thank to Eng. Jorge Luis Cárdenas Morales, who is the Technical Director of the Basic Industrial Unit (UEB) Refinery “Chiquitico Fabregat”, in Villa Clara, Cuba, for providing the data for the economic estimations.

Received in May, 2017.

Accepted in October, 2017.