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INVERTASE ACTIVITY OF INTACT *SACCHAROMYCES CEREVISIAE* CELLS CULTURED IN SUGARCANE MOLASSES BY BATCH FERMENTATION PROCESS

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ABSTRACT

Saccharomyces cerevisiae was cultured by batch fermentation in 2.6L of sugar cane molasses (supplemented with $(NH_4)_2SO_4$, (5.1g/L), $MgSO_4.7H_2O$ (75mg/L) and $Na_2HPO_4.12H_2O$ (2.4g/L); concentration of reducing sugars (RS) and total reducing sugars (TRS) 7g/L and 30g/L, respectively) inoculated with 0.6L of yeast suspension at agitation of 500 min^{-1} for 9h. The temperature (30°C, 35°C and 40°C), pH (4.0, 4.5, 5.0, 5.5, 6.0 and 6.5), and aeration (min^{-1}): (0, 0.5, 1.0, 2.0 and 3.0) were changed. Invertase was subjected to catabolite repression at RS concentration in the mash (expressed as glucose) over 4g/L. During fermentation the specific invertase activity of intact cells was enough for guaranteeing the amount of RS (the unique carbon source in the mash) needed for maintaining the yeast metabolism. The high invertase productivity (494gTRS/L.h) occurred at pH 5.5, aeration of 2.0 min^{-1} and 30°C.

KEYWORDS: Yeast, invertase, molasses, *Saccharomyces cerevisiae*, batch fermentation.

INTRODUCTION

Invertase (β-D-fructofuranosidase; E.C.3.2.1.26), which hydrolyzes the terminal nonreducing portion of β-fructofuranosides (sucrose, raffinose, for example), is an enzyme found inserted in the cell wall of yeasts – mainly *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis.* Particularly, sucrose (a nonreducing sugar) is hydrolyzed by invertase producing a mixture of reducing sugars which is sweeter than sucrose because of the high degree of sweetness of fructose. The name inversion applied to the hydrolysis of sucrose was derived from the observation of a change in the optical rotation of the solution, which in the

beginning was dextrorotatory (sucrose $[\alpha]_D = +66.5^o$) and after hydrolysis levorotatory ($[\alpha]_D = -39.5^o$: glucose $[\alpha]_D = +52.5^o$ plus fructose $[\alpha]_D = -92^o$). The invertase hydrolyzes the sucrose by breaking the glycosidic linkage from the fructosyl end of sucrose [C(2)-O].[1]

Invertase activity in aqueous yeast extract was originally detected by Berthelot in 1860. Since the beginning of the last century it was produced commercially. The primary use of invertase is the conversion of sucrose to fructose and glucose (invert sugar syrup). The major applications of invertase are in the production of non-crystallizable sugar syrup and in the conservation of blackstrap molasses during storage prior the use in ethanol production. As time goes by the use of invertase diversified, being important also in the manufacture of confections, syrups, desserts and artificial honey. In the confectionary industry, the enzyme is important for the production of fondants, chocolate coatings, and chocolate-coated candies with soft center. Moreover, invertase can also be a powerful anti-microbial and an anti-oxidant aids in the prevention of bacterial infections and gut fermentation due to oxidation of food nutrients; the invertase association to other enzymes has also been proved to help and heal colds, flu and other respiratory problems.[2] Recently, Rockland Immunochemicals Inc. (Limerick, PA 19468) claimed that ELISA kit containing goat-invertase antibody linked to horse radish peroxidase – still in testing phase – can be used for detecting infection in mammals by strains of genus *Candida*. A remarkable point is that invertase was used by Michaelis and Menten as a model enzyme for demonstrating their famous enzyme kinetic equation.[3]

S. cerevisiae, the main source of invertase, is a remarkable microorganism due to being a microbial species largely studied along the time, easily isolated and maintained in low expensive medium (minimal nutrient requirement: sugar, ammonium sulphate and sodium phosphate), growths quite well in industrial residues (blackstrap molasses, corn steep liquor, for instance), largely employed in ethanol production, and as microbial model in modern biotechnology.[4] Moreover, the cell wall localization of invertase enables the intact yeast cells to be a natural immobilized system, which would be used in the continuous hydrolysis of sucrose for attaining invert syrup.[5]

Fermentation is a process in which prokaryotic and eukaryotic cells are cultured under adequate growing conditions (temperature, aeration, pH, agitation, concentration of macro and micronutrients, amongst others) in order to attain a diversified variety of marketable

products.[6] This process can be carried out in batch, fed-batch, semi-continuous or continuous mode. Previously, it was observed that the specific invertase activity of intact yeast cells grown in blackstrap molasses through continuous mode either at transient or stationary regimen was not constant.[7] Thereby, the batch type was sorted out because it is naturally transient and the cells response to the environment variations (pH, temperature, aeration, etc.) is fast and easily measured.

The aim of this work was determining the effect of pH, aeration and temperature on the invertase activity of intact *S. cerevisiae* cells grown in blackstrap molasses by batch fermentation process.

MATERIAL AND METHODS

Material

Blackstrap molasses was purchased from COPERSUCAR (São Paulo, Brazil) and cultures media purchased from DIFCO (Leeuwarden, The Netherlands). The used culture of *Saccharomyces cerevisiae* was isolated and purified from compressed yeast of Fleischmann-Royal Inc. (São Paulo, Brazil). All analytical reagents (P.A. degree) were purchased from traditional suppliers.

Methods

Blackstrap Molasses Pre-treatment

Blackstrap molasses was diluted with water at proportion of 1:1, followed by addition of $NaH_2PO_4.H_2O$ (2.25 g/L). The mixture was left successively at 100^oC/15min and 120^oC/15 min. After that, the mixture was left resting for 48h at room temperature, being the clarified medium separated by decantation.

The clarified medium was diluted with water at sucrose concentration near 30 g/L and the pH adjusted to 7.0 with 4.0M of NaOH. After that the medium was autoclaved at 120^oC for 30min. At the end, the clarified and sterilized medium was supplemented with sterile solutions containing 2.4 g/L of $Na_2HPO_4.12H_2O$, 0.075 g/L of $MgSO_4.7H_2O$ and 5.1 g/L of $(NH_4)_2SO_4$.

Inoculum Preparation

The stock culture of *Saccharomyces cerevisiae* was maintained on slant tubes (medium composition: agar nutrient Difco 23.0 g; glucose 1.0 g; distilled and sterilized water, 1.0 L) at 4^{o}C.

The inoculum was prepared as follows. A loopful of the stock culture was transferred to slant tubes (medium composition: glucose, 10.0 g; peptone, 5.0 g; yeast extract, 3.0 g; agar, 15.0 g; distilled and sterilized water, 1.0 L) maintained at 33^{o}C for 24h. After that, the yeast was transferred to test tubes containing 10.0mL liquid medium (composition as cited above, but with no agar) and incubated at 30^{o}C for 48h. Two tubes were then used to inoculate 200mL molasses medium in a 1-L Erlenmeyer flask, which was then incubated at 30^{o}C for 22h in a NBS Gyratory Shaker (frequency = 200 min^{-1}; stroke = 3 cm).

Batch Fermentation

Three Erlenmeyer flasks (600mL yeast culture) were used to inoculate a 5-L fermenter (New Brunswick Scientific Co.) containing 2.4L sterilized molasses medium. In tests conducted under aerobiosis the air was bubbled into the medium for 30 min before the inoculation.

In all the experiments the following parameters were fixed: agitation = 500 min^{-1}; initial volume of inoculated and supplemented medium = 3.0L; volume of inoculum = 600mL; dimethylpolisiloxane (antifoam added when needed); total duration = 9h. The parameters varied were pH (4.0, 4.5, 5.0, 5.5, 6.0 and 6.5), temperature (30^{o}C, 35^{o}C and 40^{o}C) and aeration (min^{-1}): 0, 0.5, 1.0, 2.0 and 3.0. Each test was identified according to Table 1.

Table 1: Identification of tests conducted at each varied parameter (pH, temperature and aeration).

Temperature (^{o}C)	pH	Aeration (min^{-1})				
		0	0.5	1.0	2.0	3.0
30	4.0	V	II	III	I	IV
	4.5	VII	IX	VIII	VI	X
	5.0	XI	XII	XIII	XIV	XV
	5.5	XVI	XVII	XVIII	XIX	XX
	6.0	XXVI	-	XXV	XXIV	-
	6.5	XXI	-	XXII	XXIII	-
35	5.5	-	-	XXVII	-	-
40	5.5	-	-	XXVIII	-	-

The fermentation was followed through 10mL-samples taken each hour. Five milliliters were filtered through a Millipore® membrane (pore diameter = 0.45μm), being the filtrate used for measuring the concentration of reducing sugars (S) and total reducing sugars (S') and the cake used for measuring the dried cell mass. The other 5mL were centrifuged (3000xg/10min), being the supernatant discharged and the precipitate washed twice with 10mL of distilled water. The washed cake was suspended in 25 mL of water and stored at 4°C until the determination of invertase activity.

Analytical Techniques

Measurement of cell concentration (dry matter)

Dry matter cell concentrations were measured by filtering a 5mL sample through a 0.45μm-Millipore® membrane; after washing with 50mL of distilled water, the material was dried in a stove at 105°C for 2h.

Measurement of reducing sugars

The reducing sugars (S) were measured (always in triplicate) by introducing in Folin-Wu test tube 1mL of sample (filtrate adequately diluted) and 1mL of alkaline Somogy's solution. The tubes were left in a boiling water bath for 10min. After cooling 2mL was added of cupric-molibdenium Somogy's solution and the color developed read at 540nm.[8] The reducing sugars concentration was expressed as grams of glucose per Liter (g/L). The glucose standard curve was made by using amount of glucose varied from 20 mg/L to 200 mg/L.

Measurement of Total Reducing Sugars

The total reducing sugars (S') were measured by introducing 5mL of diluted filtrate in a 100mL-volumetric glass flask containing 25mL of 4M HCl. The flask was left in water-bath at 70°C for 15min. After cooling, 1mL of hydrolyzate was mixed in a Folin-Wu tube with 1mL of Somogyi's alkaline solution. The procedure followed as described above. The total reducing sugars concentration was expressed as grams of glucose per Liter.

Measurement of invertase activity

Invertase activity measurements (in duplicate) were carried out at 37°C in a mixture of 1.5mL 0.01M acetate-acetic acid buffer (pH 4.6), 2.5 mL 0.3M sucrose solution and 0.5 mL cell suspension (adequately diluted to assure that less than 2.0% of the sucrose present in the solution would be hydrolyzed). After 3 min, the hydrolysis was stopped by adding 1.0 mL of

the Somogyi's alkaline solution, quickly followed by immersion in a boiling water bath for 10 min. The reducing sugars concentration was determined as described above.

The calculation of invertase activity was made by using the equation:

$$v = (2fV_1/m.t).[(y - y_o - a)/b] \quad \text{(Eq. 1)}$$

Where v = specific invertase activity (gRS/g cell.h); f = dilution factor; V_1 = volume of the sample (mL); m = dry cell mass (g); t = reaction time (h); y = absorbance read at 540nm; y_o = initial absorbance read at 540nm; a = inclination of the standard curve; b = linear coefficient of the standard curve.

One invertase unit (U) was defined as the amount of enzyme catalyzing the formation of 1g of reducing sugars per hour at pH 4.6 and 37^{o}C.

RESULTS AND DISCUSSION

In all tests samples were taken at each hour in which the cell concentration (X), invertase activity (v), reducing sugars (S) and total reducing sugars (S') concentrations were measured. Due to the impossibility of presenting and handling the full data of each experiment realized (twenty eight in all), the experimental data of tests VIII, XIV, XVIII and XX were presented in Table 2 as an example. Results from test XVIII were chosen for handling the data as follows. The data collected were plotted in graphics correlating each measured parameter with the fermentation time (Figure 1). From the curves presented in figure 1 the specific rate of reducing sugars (μ_S) and total reducing sugars ($\mu_{S'}$) consumption due to the cell growing as well as the specific rate of cell growth (μ) through equations 2-4 were calculated (Table 3). The derivatives were calculated by using the Le Duy-Zajic's method.[9]

$$\mu_S = (1/X).[(dS/dt)_c] \quad \text{(Eq. 2)}$$

$$\mu_{S'} = (1/X).[(dS'/dt)_c] \quad \text{(Eq. 3)}$$

$$\mu = (1/X).(dX/dt)_c \quad \text{(Eq. 4)}$$

Where X = cell concentration in the medium (g/L); $(dS/dt)_c$ = rate of reducing sugars consumption due to cell growth (g/L.h); $(dS'/dt)_c$ = rate of total reducing sugars consumption due to cell growth (g/L.h); $(dX/dt)_c$ = cell growing rate (g/L.h); μ_S = specific rate of reducing sugars consumption (h^{-1}); $\mu_{S'}$ = specific rate of total reducing sugars consumption (h^{-1}) and μ = specific rate of cell growing (h^{-1}).

Table 2: Variation of specific invertase activity (v; gTRS/gcell.h), cell (X; g/L), reducing sugars (S; g/L) and total reducing sugars (S'; g/L) concentrations during 9h-batch fermentations.

Test	Variables	Fermentation time (h)									
		0	1	2	3	4	5	6	7	8	9
VIII	X	0.47	0.57	0.74	0.85	1.20	1.67	2.19	2.98	3.53	3.87
	S	7.40	9.00	10.5	10.9	11.9	10.5	8.20	2.30	0.63	0.56
	S'	25.3	23.4	22.6	20.9	20.1	16.8	12.1	4.70	1.10	0.90
	V	12.3	10.7	8.94	7.44	4.98	3.36	4.20	24.9	87.6	94.8
XIV	X	0.70	0.82	1.20	1.63	2.50	3.16	4.27	4.96	4.99	5.00
	S	10.6	12.9	13.0	12.7	10.8	6.30	1.80	0.74	0.69	0.50
	S'	24.0	23.2	21.0	19.4	15.0	9.20	2.60	0.72	0.52	0.40
	V	11.8	13.3	7.92	5.70	3.3	6.18	44.5	78.0	94.2	101
XVIII	X	0.60	0.65	0.70	0.87	1.30	1.88	2.73	3.71	4.01	4.08
	S	10.3	11.2	12.3	12.9	11.6	9.50	6.00	2.01	0.71	0.54
	S'	23.1	21.4	20.2	19.4	18.5	16.1	9.60	3.50	1.00	0.70
	V	7.62	9.48	7.38	6.30	4.14	3.00	4.32	39.5	100	102
XX	X	0.77	0.88	1.16	1.65	2.29	3.01	3.60	4.56	4.66	4.68
	S	15.7	16.1	15.4	13.3	10.1	5.90	1.80	0.78	0.60	0.55
	S'	24.3	22.2	20.8	17.5	13.1	7.70	1.00	0.55	0.38	0.30
	V	15.8	14.2	9.96	8.40	4.44	3.90	64.8	78.6	84.0	87.0

Focusing on test XVIII (Table 2) it is possible to observe that as the reducing sugars (S) and total reducing sugars (S') decreased sharply during fermentation the cell concentration and specific invertase activity increased 6.8 and 10.8 times, respectively. The highest increase of specific invertase activity than biomass would be due to the insertion of several enzyme molecules inside the cell wall. Moreover, during the process the specific invertase activity oscillated from 9.48 to 3.0 to 102gTRS/gcell.h as the cell mass increased continuously (from 0.6 to 4.08 g/L). Probably, this result could be related to the catabolite repression effect, commonly observed to enzymes located in the cell walls.[10] It must be enhanced that the invertase inserted into the cell wall acts as immobilized enzyme, enabling the use of intact yeast cells in the production of invert sugar syrup from sucrose hydrolysis carried out in continuous process.[11]

From Figure 1 it is observed that v decreases until t = 6h, following a sharp increase up to the end of the fermentation. Between 6h and 7h from the beginning of the fermentation S and S' were below 4g/L, indicating that at this concentration the reducing sugars (glucose and fructose) did not exert repression on invertase biosynthesis (catabolite repression). Besides, the sharp augment of v could be envisaged as an attempt by the yeast metabolism to over hydrolyze remaining sucrose (represented by total reducing sugars of the medium) in order to

provide more fermentable sugars for yeast growing. Obviously, the fermentation ends when S and S' are zeroed.

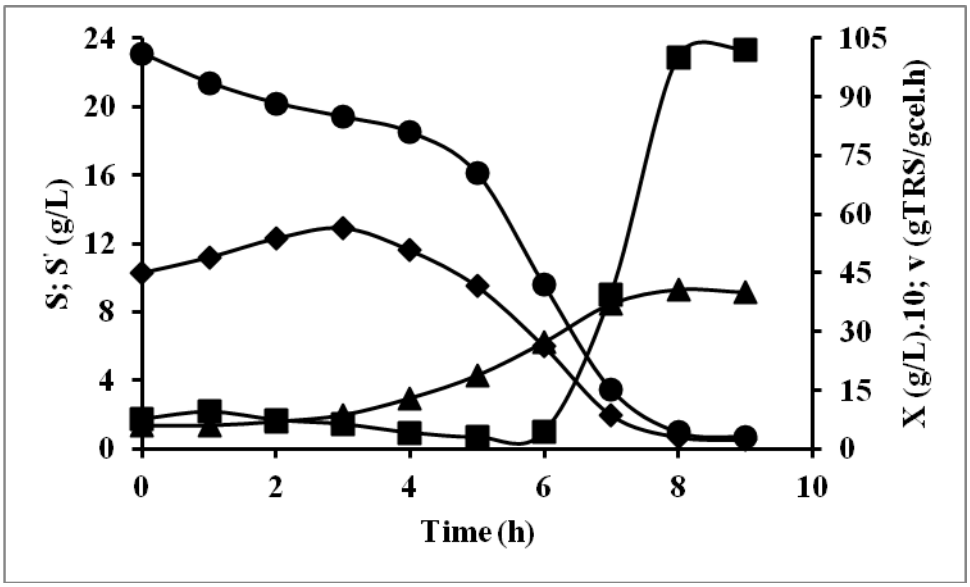

Figure 1: Variation against time of cell concentration (▲), reducing sugars concentration (◆), total reducing sugars (●) and specific invertase activity (■) for test XVIII (pH 5.5; 30°C and aeration of 1.0 L/L.h).

Table 3: Specific invertase activity (v), specific growth rate (μ), specific reducing sugars (μ_S) and total reducing sugars ($\mu_{S'}$) consumption rates related to test XVIII (30°C, pH 5.5 and aeration of 1.0 L/L.h).

t (h)	v (gTRS/gcell.h)	μ (h^{-1})	μ_S (h^{-1})	$\mu_{S'}$ (h^{-1})	v/μ	v/μ_S	v/$\mu_{S'}$	μ_S/μ	$\mu_{S'}$/μ
0	7.62	0.038	3.46	5.00	201	2.20	1.52	91.1	132
1	9.48	0.088	1.77	3.82	108	5.36	2.48	20.1	43.4
2	7.38	0.150	1.83	2.74	49	4.03	2.69	12.2	18.3
3	6.3	0.490	1.47	0.50	13	4.30	15.8	3.73	1.02
4	4.14	0.390	1.64	0.42	11	2.52	14.8	4.21	1.10
5	3.00	0.360	2.22	0.43	8.3	1.35	6.98	6.17	1.19
6	4.32	0.370	2.76	1.32	12	1.57	3.27	7.46	3.57
7	39.54	0.140	1.18	0.45	282	33.5	88.0	8.43	3.21
8	100	0.050	0.16	0.099	2000	625	1030	3.2	1.98
9	102	0.024	0.15	0.053	4250	680	1887	6.3	2.21

Considering that the ratios v/μ, v/μ_S and v/$\mu_{S'}$ were always higher than the unit, then the specific invertase activity was not a limiting factor for the disposal of reducing sugars as carbon source for yeast metabolism (Table 3). Besides, μ_S and $\mu_{S'}$ were higher than μ indicating that the amount of reducing sugars present in the medium never became a limiting

factor to yeast growing. As μ_S **and** μ_S, were always higher than μ so the reducing sugars present in the medium were used not only for cell growing, but also for synthesizing metabolites in general such as trehalose, glycogen and ATP.[12]

In Table 4 the values of generation time (t_g) and invertase productivity (P) related to all the tests realized were presented. The generation time was calculated through the equation below,

$$t_g = Ln2 \div \mu' \qquad \text{(Eq. 5)}$$

Where t_g = generation time (h); μ' = average of specific growth rate during exponential growing phase (h^{-1}).

Table 4: Productivity (P; gTRS/L.h), generation time (t_g; h) and average specific growth rate (μ'; h^{-1}) for all the tests realized. The aeration (ϕ) was expressed as min^{-1}.

TEST	pH	ϕ	P	*μ'	t_g	TEST	pH	ϕ	P	*μ'	t_g
I	4.0	2.0	43.8	0.25	2.7	XV	5.0	3.0	347	0.40	1.8
II	4.0	0.5	51.6	0.18	4.0	XVI	5.5	0	238	0.21	3.3
III	4.0	1.0	158	0.25	2.6	XVII	5.5	0.5	390	0.35	2.0
IV	4.0	3.0	335	0.24	2.7	XVIII	5.5	1.0	412	0.40	1.7
V	4.0	0	167	0.26	2.5	XIX	5.5	2.0	434	0.40	1.8
VI	4.5	2.0	233	0.28	2.5	XX	5.5	3.0	393	0.30	2.3
VII	4.5	0	25.8	0.40	1.7	XXI	6.5	0	113	0.24	2.9
VIII	4.5	1.0	361	0.32	2.2	XXII	6.5	1.0	278	0.32	2.2
IX	4.5	0.5	289	0.30	2.4	XXIII	6.5	2.0	143	0.25	2.8
X	4.5	3.0	215	0.33	2.1	XXIV	6.0	2.0	227	0.34	2.0
XI	5.0	0	169	0.37	1.9	XXV	6.0	1.0	224	0.35	2.0
XII	5.0	0.5	349	0.32	2.2	XXVI	6.0	0	121	0.24	2.9
XIII	5.0	1.0	71.4	0.31	2.3	XXVII[a]	5.5	1.0	233	0.31	2.3
XIV	5.0	2.0	492	0.32	2.2	XXVIII[b]	5.5	1.0	97.8	0.26	2.7

*Average specific growth rate during the exponential growing phase occurred between 3h and 6h of fermentation. [a] Test carried out at 35°C; [b] Test carried out at 40°C.

From Table 4 it can be seen that both productivity and generation time varied largely as pH was changed from 4.0 to 6.5 and aeration from zero to 3.0 min^{-1}. The highest productivity (492 gTRS/L.h) and the lowest generation time (1.7h) occurred in tests VII and XVIII, respectively. Moreover, comparing tests XVIII, XXVII and XXVIII conducted at 30°C, 35°C and 40°C, respectively, but at fixed pH (5.5) and aeration (1.0 min^{-1}) the values of productivity were, respectively, 412, 233 and 97.8 gTRS/L.h, whereas t_g were 1.7h, 2.3h and 2.7h, respectively. As *S. cerevisiae* is a mesophyle microorganism temperature over 35°C

affects significantly the metabolism by interfering indistinctly on the activity of anabolic and catabolic intracellular enzymes.[13]

Regarding pH and aeration (envisaged as dissolved oxygen) it is possible to say that both have distinct mechanism of action inside the cell.

The dissolved oxygen acts inside the mitochondria at the level of respiratory chain stimulating the synthesis of intermediate metabolites.

pH has an unspecific action both outside (at cell wall formation) and inside (on enzyme activities) the cell. During yeast budding, in which cell wall mannan is formed (a polymer constituted by monomers of mannose and manuronic acid linked through N-glycosidic bonds) and invertase molecules are inserted simultaneously. Both are subject to the interference of hydrogen ions (H_3O^+) concentration either on the polysaccharide elongation (N-glycosidic bonds formation) or invertase insertion (linkage to mannan) and conformation (secondary and tertiary structures disturbed by inappropriate ionization of chemical groups belonging to lateral chains of amino acids constituting the enzyme primary structure).

Particularly to invertase biosynthesis, besides the generalized effects of pH, temperature and dissolved oxygen the glucose concentration in the cytoplasm must also be taken in account. This is due to the fact that glucose can repress or not events occurring at DNA transcription, RNA translation, ribosome stability and invertase transportation through cytoplasmic membrane to the cell wall.

An overview on the interaction between pH and aeration with the invertase productivity by *S. cerevisiae* can also be extracted from Table 4. At pH 5.5 and aeration interval of 0.5 min^{-1} and 3.0 min^{-1} the invertase productivity changed between 390 and 434 gTRS/L.h, thereby a variation of 10%. However, the high productivity (494 gTRS/L.h) occurred at pH 5.0 and aeration of 2.0 min^{-1}, though at this pH and aeration of 1.0 min^{-1} the productivity (71.4 gTRS/L.h) was 86% lower than at 2.0 min^{-1}. It is clear that a compromise between pH and aeration must be set when the aim is to obtain invertase by yeast *S. cerevisiae* cultivated in batch fermentation. In the present case, the decision must involve choosing pH 5.0, in which the productivity variation is about 10% under an aeration interval (0.5-3.0 min^{-1}), or pH 5.5 and fixed aeration of 2.0 min^{-1} in order to attain productivity of 17.6% high as compared with the average productivity (407 gTRS/L.h) in pH 5.0.

CONCLUSION

The data presented led to conclude that invertase biosynthesis is subjected to catabolite repression by glucose at concentration above 4 g/L in the mash. Though specific invertase activity started increasing after 5-6h from the beginning of fermentation it was not a limiting factor for the disposal of reducing sugars as carbon source to yeast metabolism. Moreover, pH, aeration and temperature affected significantly both productivity and generation time. Regarding the hydrogen ion concentration it is important to highlight that at pH 5.5 and aeration of 2.0 min^{-1} the productivity (494 gTRS/L.h) was 17.6% higher than average productivity of 407 gTRS/L.h at pH 5.0 and aeration interval of 0.5 to 3.0 min^{-1}.

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REFERENCES

1. D'addezio F, Yoriyaz EJ, Cantarella M, Vitolo M. Sucrose hydrolysis by invertase using a membrane reactor: effect of membrane cut-off on enzyme performance. Brazilian Journal of Pharmaceutical Sciences, 2014; 50: 257-259.
2. Kulshrestha S, Tyagi P, Sindhi V, Yadavilli KS. Invertase and its applications – a brief review. Journal of Pharmacy Research, 2013; 7: 792-797.
3. Vitolo M. Enzymes: the catalytic proteins. In Vitolo M (ed). Pharmaceutical Biotechnology, São Paulo; Blücher, 2015; 203-228.
4. Burgess SM, Powers T, Mell JC. Budding yeast *Saccharomyces cerevisiae* as model genetic organism. Wiley Online Library, 2017, Nov 20. Doi: 10.1002/9780470015902.a0000821.pub2.
5. Rocha-Filho JA, Vitolo M. Guide for practical classes in enzyme and fermentation biotechnology. São Paulo; Blücher, 2017.
6. Carvalho JCM, Matsudo MC, Bezerra RP, Sato S. Fermentation technology. In Vitolo M (ed). Pharmaceutical Biotechnology, São Paulo: Blücher, 2015; 103-156.
7. Vitolo M, Vairo MLR, Borzani W. Invertase activity of intact cells of *Saccharomyces cerevisiae* growing on sugar-cane molasses. Steady-state culture tests. Biotechnology and Bioengineering, 1985; 27(1): 1229-1235.
8. Somogyi M. Notes on sugar determination. Journal Biological Chemistry, 1952; 195(1): 19-23.

9. Hiss H. Kinetic of fermentative processes. In Schmidell W, Lima AU, Aquarone E, Borzani W (eds). Industrial Biotechnology, São Paulo: Blücher, 2001; 114-120.
10. Kayikci O, Nielsen J. Glucose repression in *Saccharomyces cerevisiae*. FEMS Yeast Research, 2015 Jul 23. Doi: 10.1093/femsyr/fov068.
11. Safarik I, Sabtkova Z, Safarikova M. Invert sugar formation with *Saccharomyces cerevisiae* cells encapsulated in magnetically responsive alginate microparticles. Journal of Magnetism and Magnetic Materials, 2009; 321: 1478-1481.
12. Nwaka S, Holzer H. Molecular biology of trehalose and the trehalases in the yeast *Saccharomyces cerevisiae*. Progress in Nucleic Acid Research and Molecular Biology, 1998; 58: 197-237.
13. Postmus J, Canelas AB, Bouwman J, Smits GJ. Quantitative analysis of the high temperature-induced glycolytic flux increase in *Saccharomyces cerevisiae* reveals dominant metabolic regulation. The Journal of Biological Chemistry, 2008; 283(35): 23524-23532.