

Influência da fonte de carbono, taxas de agitação e aeração na produção de biomassa de levedura com potencial de uso no controle biológico

Influence of carbon source, agitation and aeration rates for production yeast biomass which potential of use for biological control

Influencia de la fuente de carbono, las tasas de agitación y aireación en la producción de biomasa de levadura con potencial para su uso en el control biológico

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Ana Paula Colares de Andrade

ORCID: <https://orcid.org/0000-0003-0554-4376>

Universidade Federal do Ceará, Brasil

E-mail: ana.colares@hotmail.com

Helder Levi da Silva

ORCID: <https://orcid.org/0000-0002-6326-9862>

Universidade Federal do Ceará, Brasil

E-mail: helderlevi@gmail.com

Gustavo Adolfo Saavedra Pinto

ORCID: <https://orcid.org/0000-0002-3672-8795>

Embrapa Agroindústria Tropical, Brasil

E-mail: Gustavo.saavedra@embrapa.br

Resumo

Os processos fermentativos são utilizados para promover melhores resultados quando se deseja aumentar a biomassa de organismos que podem ser utilizados no controle biológico. Assim, o objetivo deste trabalho foi ampliar a escala de produção de biomassa de leveduras com potencial de uso no controle biológico. Cinco linhagens de leveduras isoladas da microflora natural de frutas tropicais foram selecionadas e avaliadas quanto à influência da fonte de carbono, cultivo em shaker orbital e biorreator. Os testes realizados demonstraram que a utilização de açúcar invertido como fonte de carbono favoreceu a produção de material de biomassa quando comparado ao obtido com glicose. O cultivo da levedura em biorreator demonstrou um melhor desempenho, visto que houve um aumento de 24,12% (6,47 a 8,03 g.L⁻¹) na produção de biomassa de levedura quando comparado ao crescimento em shaker orbital, o que pode estar associado ao aumento da taxa de agitação e aeração.

Palavras-chave: biocontrole; biomassa microbiana; fungos; fermentação; bioprocesso industrial.

Abstract

The fermentation processes are used to promote better results when it is desired to increase the biomass of organisms that can be used in biological control. Thus, the aim of this work was to expand the scale of production of biomass of yeasts that have potential for biological control. A total of five strains of yeasts isolated from natural microflora of tropical fruits, a strain was selected and evaluated for the influence of the carbon source, cultivation orbital shaker and bioreactor. The tests performed demonstrate that the use of invert sugar as carbon source favored production of biomass material when compared to that obtained with glucose. The bioreactor cultivation of the yeast strain has enhanced performance, since it favors an increase of 24.12% (6.47 to 8.03 g.L⁻¹) in biomass production when compared to the fermentation orbital shaker and that may be associated with increased agitation and aeration rate.

Key-words: biocontrol; microbial biomass; fungi; fermentation; industrial bioprocess.

Resumen

Los procesos de fermentación se utilizan para promover mejores resultados cuando se desea aumentar la biomasa de organismos que pueden usarse en el control biológico. Por lo tanto, el objetivo de este trabajo fue ampliar la escala de producción de biomasa de levadura con potencial para su uso en el control biológico. Se seleccionaron cinco cepas de levadura aisladas de la microflora natural de frutas tropicales y se evaluó la influencia de la fuente de carbono, el cultivo en un agitador orbital y un biorreactor. Las pruebas realizadas mostraron que el uso de azúcar invertido como fuente de carbono favoreció la producción de material de biomasa en comparación con el obtenido con glucosa. El cultivo de levadura en un biorreactor mostró un mejor rendimiento, ya que hubo un aumento del 24,12% (6,47 a 8,03 g.L⁻¹) en la producción de biomasa de levadura en comparación con el crecimiento en un agitador orbital, que puede estar asociado con una mayor tasa de agitación y aireación.

Palabras clave: biocontrol; biomasa microbiana; hongos; fermentación; bioprocesos industriales.

1. Introduction

The international market has been attentive to quality and safety of agricultural products. Consumers and producers are both concerned that agricultural products are free from chemical residues. Other issues are the high cost of synthetic pesticides and the regular use of these components which contributes to the appearance of resistant strains of fungi (Gouvea, 2007).

In this context, biological control is a viable alternative to control pests and pathogens and also advantageous compared to chemical control, particularly with regard to environmental impact, cost, specificity and resistance development (Silveira et al., 2005; Schrank et al., 2001) Yeast can be used in biological control by presenting antagonistic effect on certain phytopathogenic micro-organisms (Bendo et al., 2009). The control of microbial growth occurs through different mechanisms (Janisiewicz et al., 2000; Droby et al., 2009; Chan et al., 2005). Moreover, other properties such as the lack of production of allergenic spores and antibiotics metabolites, such as the produced by bacterial antagonists, enhance the use of yeasts against some species of pathogenic fungi (Gouvea et al., 2007).

The development of a fermentation process depends on the correct setting of basic factors such as type of microorganism, culture medium, how the process occurs and the steps for product recovery. Suitable operating conditions at bench scale favor obtaining high productivity and yield of the product of economic interest, which helps to expand production to an industrial scale. However, this step is directly related to the interaction of the factors mentioned, as well as to the possibility of reducing the operating costs.

The transfer of a process from bench scale to pilot scale using bioreactors allows obtaining better results, since the optimized conditions favor increased productivity to maximize or minimize, economically and technologically, important variables such as the yield of the process, the concentration of the product and costs (Gomes, 2010).

Thus, the present study objectified to expand the scale of production of biomass of yeasts that have potential for biological control., through changes in the variables: carbon source, agitation and aeration rates.

2. Methodology

Microorganisms

The microorganisms used in this study belong to the culture collection of Embrapa Semi-Arid (Petrolina, Pernambuco, Brazil) and were isolated from the natural microflora of mango, melon and grape. One yeast strain was selected from the five tested (L3A, L9, L10, L7K and LF), based in increased biomass production.

Activation of the micro-organism

The cultures maintained on potato-dextrose-agar (Difco) were activated in test tubes containing 5 mL of synthetic medium (10 g.L⁻¹ peptone, 20 g.L⁻¹ dextrose, supplemented with

1 % yeast extract, final pH adjusted to 5.6). The tubes were incubated in a BOD incubator (Tecnal, model TE-371) at 30 °C for 24 hours to obtain the inoculum. After the incubation period, an inoculum of 1 % (v/v) was used for tests in an orbital shaker (Tecnal, model TE 420) and in a bioreactor.

Tests on orbital shaker

Nutritional and environmental conditions were evaluated using fermentation processes performed in an orbital shaker (Tecnal, model TE 420), under the following conditions: 30 ± 1 °C at 150 rpm for 32 hours. A synthetic medium was used in this step, with 100 mL in 250 mL erlenmeyer flasks. All flasks were autoclaved at 121°C for 15 minutes.

Influence of synthetic culture medium

The influence of three different synthetic culture media on the biomass production of yeast was evaluated using Sabouraud broth supplemented with 1 % yeast extract and YM (Difco, 2009). Then, 100 mL of each medium were distributed into 250 mL Erlenmeyer flasks and sterilized at 121°C for 15 minutes. The fermentation test was performed at 30 ± 1 °C at 150 rpm for 32 hours.

Essays on bioreactor

The New Brunswinck (Scientific Co.) fermenter, model Bioflo/CelliGen® 115 with a 3L fermentation vat was used. At this stage, two turbines with six flat blades were used, also known as "Rushton" turbine, to improve the agitation of the reaction medium. Throughout the fermentation process, the pH was monitored using the electrode (Metler Toledo, model 405-DPAS-SC-K8S).

Culture medium was prepared directly in the vat, and pH was adjusted according to the manufacturer's specifications. At the end of the preparation of the medium, commercial soybean oil was added at a 0.30 % concentration as a foam prevention agent, and then the bioreactor containing the medium was autoclaved at 121 °C for 30 minutes. The simple batch fermentation occurred at 30 ± 1 °C for 24 hours at 300 rpm, with aeration rate of 1 vvm.

At first, the behavior of the lineage presenting higher biomass production on orbital shaker was evaluated. In another trial, the influence of the carbon source in biomass production was evaluated by tests that used glucose PA, sucrose PA, crystal sugar and inverted sugar. Once selected the carbon source, the trials were repeated in order to test the concentrations (10 g.L⁻¹ and 20 g.L⁻¹).

Determinations

Analytical determinations were performed in triplicate and at established time intervals for sampling: 1) Quantification of cells: biomass produced was determined by spectrophotometer (Varian Model Cary 50 Conc) at 600 nm using a dry weight standard curve previously established; and 2) Quantification of total reducing groups (TRGs): was determined by a colorimetric method using 3,5 dinitrosalicylic acid (DNS) (Miller, 1959); 3) pH control: the pH was monitored in a potentiometric way using a pH meter (Hanna Instruments, model HI 2221).

3. Results and Discussion

The strain L9 presented the highest biomass production after 96 hours of fermentation, but the strains L7K and L3A also showed a good performance. In addition, yield of total sugars were also the most significant for these three strains. In contrast, the performance of strains L10 and LF was inferior, under the same fermentation conditions, for biomass production and total sugars yield. Biomass production of LF reached only $1.50 \pm 0.01 \text{ g.L}^{-1}$ and was associated to the low yield of total sugars (around 7.44 %).

These data are corroborated by the low consumption of available sugars by the strain (32.18 %) after 32 hours. However, it is important to note that L10 strain although producing lower biomass (3.84 g.L^{-1}) consumed about 97.88 % of the available sugars at the end of the fermentation process. Therefore, it is necessary a more careful evaluation on the growth rate of the strain and the causes of the low biomass production with the consumption of the available sugars close to 100 %.

Table 1: Results of parameters for all strains investigated in orbital shaker

Strain	Biomass produced (g.L^{-1})	Sugar consumed (%)	pH	Yield $Y_{x/s}$
L9	$6.47 \pm 0.09^*$	97.53 ± 0.04	5.07 ± 0.00	0.34
L10	3.84 ± 0.11	97.88 ± 0.12	4.72 ± 0.04	0.18
L7K	5.64 ± 0.00	96.36 ± 0.05	4.81 ± 0.01	0.29
L3A	5.94 ± 0.02	70.11 ± 1.19	6.20 ± 0.02	0.30
LF	1.50 ± 0.01	32.18 ± 1.45	2.94 ± 0.00	0.07

* Standard deviation

The pH values were above 4.72 in all fermentations, except for the LF line that showed pH 2.94 after 96 hours of fermentation. It should be noticed that the higher pH values were related to the greater amounts of biomass produced. Rehm et al. (2005), demonstrated that the pH range that promotes yeasts growth is 2.5-7.0, with an optimum growth at 4.0-5.0.

This fact may explain the amount of biomass produced. Based on previous results, the line L9 showed the greatest production of yeast biomass, being chosen for the subsequent tests. This behavior can be due to the significant percentage of sugar consumed, indicating high conversion of substrate in product. Biochemical and molecular aspects of the adaptation of the yeast during its growth can cause stress to the cells of the micro-organism that induces intracellular changes and their response to the fermentation process (Torrado et al., 2005).

The effect of culture medium on biomass production can be observed in the Table 2 and Figure 1.

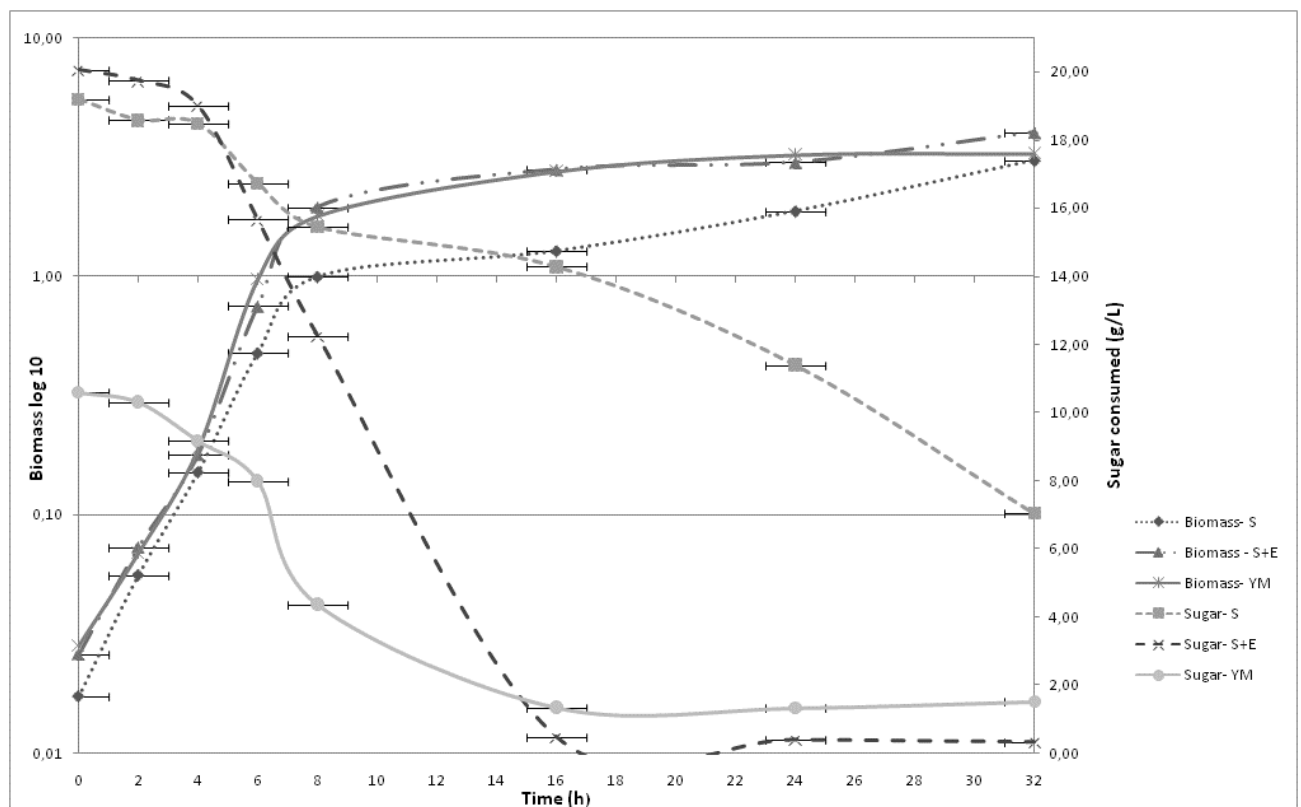
Table 2. Biomass production of yeast in different culture media

Sabouroud broth					
Time (hours)	Biomass (g.L⁻¹)	Sugar (g.L⁻¹)	pH	Sugar Consumed (%)	Yield Y_{x/s}
0	0.02±0.0008	19.19±0.3380	5.32±0.0044	0.00±0.0000	0.00
2	0.06±0.0048	18.58±0.3010	5.40±0.0044	3.10±2.9443	0.10
4	0.15±0.0015	18.48±0.1720	5.28±0.0089	3.66±1.8757	0.15
6	0.48±0.0050	16.73±0.0344	5.05±0.0044	12.76±1.6461	0.17
8	0.99±0.0104	15.44±0.1161	4.86±0.0000	19.49±1.3012	0.21
16	1.27±0.0106	14.28±0.1299	4.66±0.0044	25.57±0.6811	0.24
24	1.87±0.0106	11.39±0.0576	4.36±0.0000	40.58±1.2575	0.25
32	3.06±0.0213	7.05±0.0138	4.18±0.0044	63.45±0.0714	0.25
Sabouraud broth supplemented with 1% de extract of yeast					
Time (hours)	Biomass (g.L⁻¹)	Sugar (g.L⁻¹)	pH	Sugar Consumed (%)	Yield Y_{x/s}
0	0.03 ±0.0003	20.06±0.2520	5.62±0.0200	0.00±0.0000	0.00
2	0.07±0.0005	19.75±0.0654	5.51±0.0111	1.52±1.4383	0.05
4	0.18±0.0032	19.00±0.0499	5.53±0.0000	5.22±1.4186	0.06
6	0.75±0.0050	15.66±0.0559	5.31±0.0089	21.90±1.2487	0.10
8	1.95±0.0121	12.26±0.0757	5.05±0.0000	38.86±0.9858	0.17
16	2.80±0.0078	0.48±0.0021	4.92±0.0000	97.58±0.0199	0.19
24	3.00±0.0305	0.40±0.0007	5.03±0.0156	97.99±0.0267	0.20
32	4.01±0.0163	0.34±0.0172	5.09±0.0044	98.32±0.0862	0.20
YM broth					
Time (hours)	Biomass (g.L⁻¹)	Sugar (g.L⁻¹)	pH	Sugar Consumed (%)	Yield Y_{x/s}
0	0.03±0.0004	10.58±0.0860	5.91±0.0244	0.00±0.0000	0.00
2	0.07±0.0009	10.30±0.0705	5.84±0.0044	2.63±0.1418	0.00
4	0.19±0.0011	9.17±0.0258	5.66±0.0000	13.33±0.5547	0.06
6	0.97±0.0023	7.99±0.1032	5.11±0.0044	24.49±0.4872	0.16
8	1.79±0.0053	4.37±0.0353	4.64±0.0000	58.73±0.2701	0.25
16	2.75±0.0422	1.35±0.0048	4.81±0.0000	87.28±0.0699	0.34
24	3.23±0.0231	1.33±0.0138	4.96±0.0000	87.41±0.1575	0.35
32	3.26±0.0121	1.52±0.0303	5.00±0.0000	85.83±0.2826	0.36

Data showed that the supplementation of culture medium with a nitrogen source contributed to the increase of biomass in the trials using Sabouraud broth supplemented with 1% yeast extract and YM broth, compared to the growth on Sabouraud broth.

According to Malta (2006), in a study of the propagation of yeast, using yeast extract as a substitute for a conventional nitrogen source, such as ammonium sulfate, it was observed an increase of 2.2 times the cell mass and a yield of 0.22 g/g. Some studies show that nitrogen is an essential element for living organisms and yeast cells can use a wide variety of nitrogenous compounds such as ammonia, amino acids and peptides that induce high growth rates (Terschure et al., 2000; Nitschke et al., 2001).

Figure 1. Relationship between biomass and sugar consumed in different culture media



Consumption of sugars in Sabouraud broth supplemented with 1 % yeast extract was 98.33 % and 85.66 % in YM broth. However, although the sugar consumption was lower in the YM broth, the initial concentration in this medium was 10 g.L⁻¹ compared to the initial concentration of Sabouraud broth supplemented with 1 % yeast extract, which was 20 g.L⁻¹.

Considering the costs of acquiring the culture media, it appears that the YM broth is cheaper (\$242,00) compared to the broth Sabourad (\$302,00). Although the production of yeast biomass in YM medium has been lower than in the Sabouraud medium supplemented with 1 % yeast extract, it is inferred that this yield may be considered relevant, since a culture

medium and cheaply which has in its composition a low concentration of the carbon source, the amount of biomass produced was significant and similar to that of the culture medium which had a high biomass production. According to Reifenberg (1997), the concentration of the carbon source in the medium plays a regulatory role in the metabolism of yeast and contributes as a global regulator of the cell growth mechanism.

The pilot-scale test in a bioreactor was used to evaluate the behavior and performance of the line previously selected in the trials performed in the orbital shaker, selected due to the highest biomass production, the most relevant parameter in this study.

Table 3: Comparative results for the parameters investigated in trials orbital shaker and bioreactor for strain L9

Condition	Biomass produced (g.L ⁻¹)	Sugar consumed (%)	pH	Yield Y _{R/S}
Shaker	6.47±0.09*	97.53±0.04	5.07±0.00	0.35±1.88
Bioreactor	8.03±0.11	90.78±0.31	6.89±0.04	0.56±1.18

*Standart deviation

In general, assays done in the bioreactor enhanced the performance of the line L9. An increase in biomass production of 24.12 % was observed compared to the fermentation in the orbital shaker. A reduction of 6.91 % in the percentage of sugar consumed was observed, while the percentage of yield on total sugar and consumed sugar increased 50.15 % and 61.31 %, respectively.

Probably the conditions determined for the bioreactor, such as increased agitation from 150 rpm to 300 rpm and forced aeration, resulted in increased biomass production and, consequently, improved yields. Neves (2003), reported that agitation and aeration are essential for the development of better conditions for the growth of the microorganism in the fermentation processes.

According to Silva et al. (2001), oxygen is important to the whole metabolism, since acts in the generation of energy in the mitochondrial respiratory chain and is essential for obtaining a significant specific growth rate.

As occurred in the trials on orbital shaker, a direct relationship between pH and biomass production was observed, ie, the highest biomass volume (8.03 g.L⁻¹) was associated with a highest pH value (6.89).

The effect of carbon sources on the biomass production of the line L9 is shown in Figures 2 and 3. The highest biomass production (6.9 g.L⁻¹) at the end of 48 hours of fermentation was recorded when glucose was used as carbon source in the fermentation

medium. According to De Leon et al. (2002), yeast are microorganisms able to growth under aerobic and anaerobic conditions and may use a wide variety of compounds as a carbon source, but glucose is the preferred source for the fermentative metabolism.

Figure 2 - Comparison of different carbon sources on the production of yeast biomass

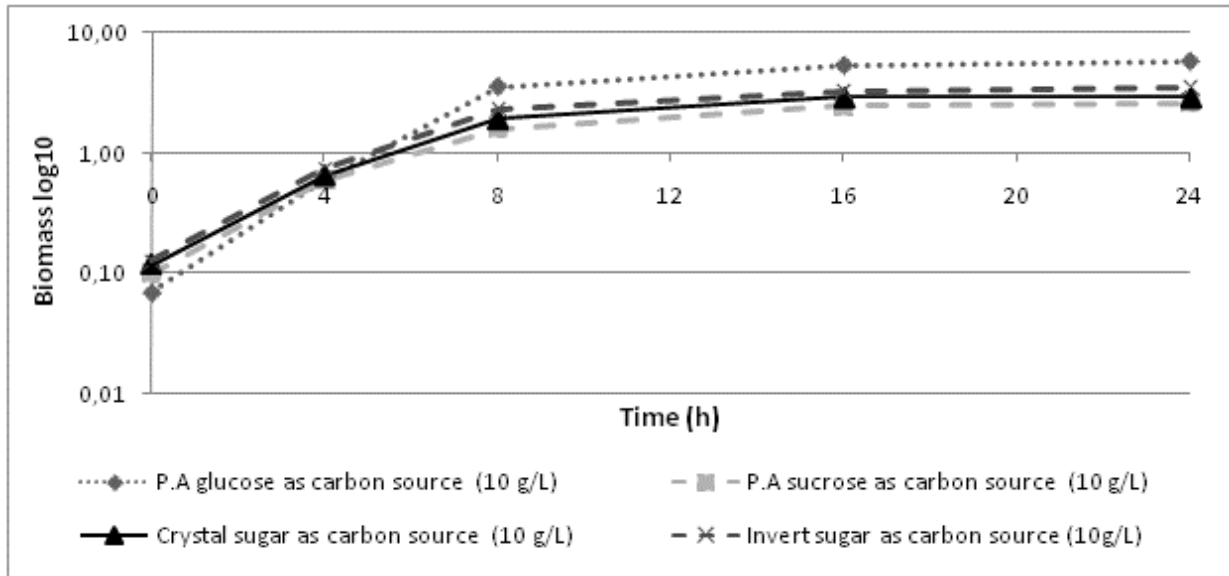
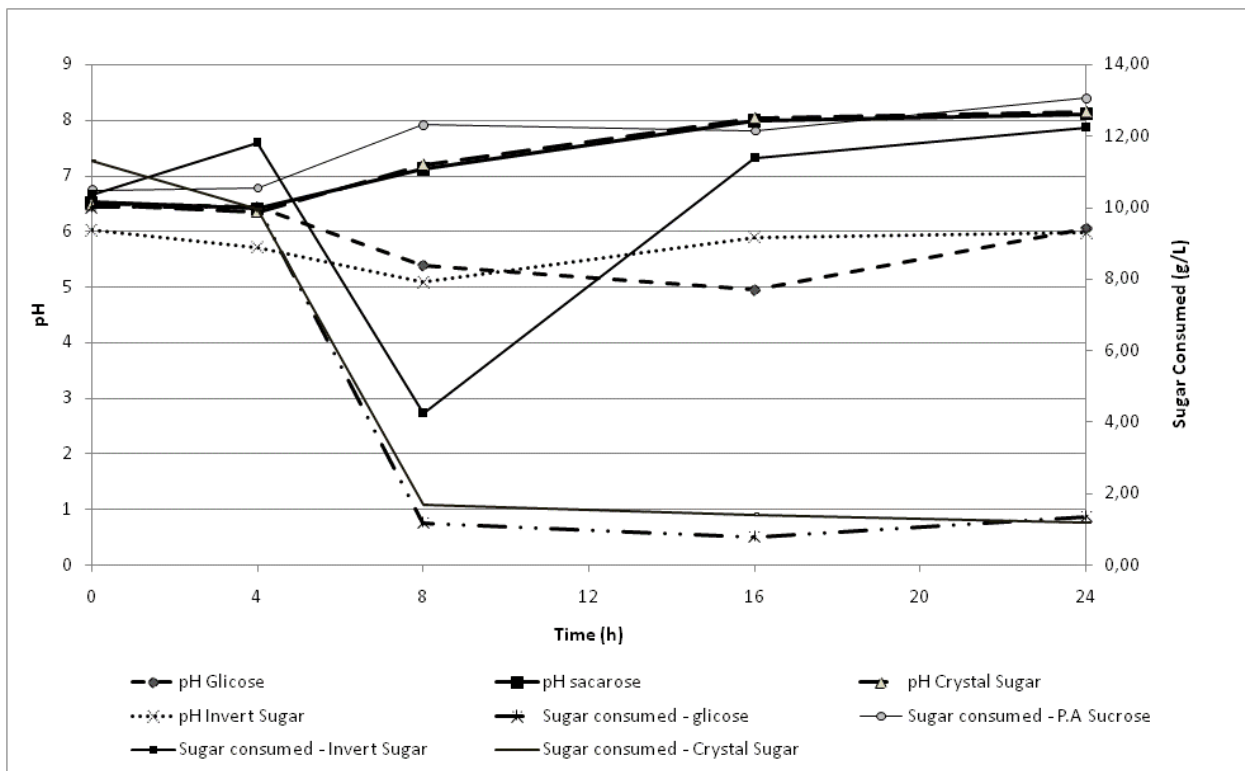


Figure 3 - Relationship between pH and sugar consumed in the production of yeast biomass in different carbon sources



In the tests employing crystal sugar and sucrose, biomass produced did not exceed 2.92 and 2.63 g.L⁻¹, respectively, after 24 of fermentation. Kurzman and Fell (1998), reported that some yeasts do not have the ability to ferment sucrose, using only the glucose present in the culture medium. This could explain the low biomass production observed in this experiment.

The highest consumption of total sugars (91.52 %) occurred in trials using inverted sugar (20 g L⁻¹). The use of sucrose resulted in the lowest consumption of total sugars (19.55 %), whereas the presence of glucose caused a consumption of 58.72 %. It was observed that the high consumption of total sugars at the end of fermentation (24 hours) did not cause a high yield of biomass ($Y_{R/S}$: 0.21 g/g) in tests with inverted sugar (20 g.L⁻¹). Similar results were observed in tests with sucrose and crystal sugar ($Y_{R/S}$: 0.25 and 0,28 g/g, respectively) at the same time period, for a lower consumption of total sugars.

The use of glucose caused total sugars consumption of 58.72 % and 91.78 % for the trials with 10g.L⁻¹ and 20g.L⁻¹ glucose, respectively. The results for biomass production at both concentrations were similar (5.82 and 5.34) suggesting good conversion of carbohydrate into biomass. Taccari et al. (2012), optimized the conditions to produce yeast biomass using a glucose concentration of 20 g.L⁻¹ and observed a biomass production of 5 g.L⁻¹. In another study, Moares and Bianchi (2013), reported that the biomass produced in 24 hours of fermentation by yeasts isolated from grape skins, using glucose at a concentration of 20 g.L⁻¹ ranged from 3.19 to 3.8 g.L⁻¹.

The results reported for the tests using inverted sugar at 10 and 20 g.L⁻¹, showed biomass production of 3.53 g.L⁻¹ and 3.94 g.L⁻¹, respectively. Despite the low biomass yield compared to that obtained by using glucose, a significant consumption of sugars occurred (87.53 g.L⁻¹ and 91.52 g.L⁻¹).

Although glucose PA has shown significant results for the biomass produced, it should be remembered that the high cost of this carbohydrate favors a expensive scale-up process, which suggests the use of inverted sugar as a viable alternative to reduce the operating cost of the process of yeast biomass production. The hydrolysis of the crystal sugar, although causing a higher initial cost, produces an equimolar mixture of glucose and fructose, which are sources more absorbable by the microorganism, which favors the increase of biomass.

The pH is a significant factor for industrial fermentations because of its importance in the control of bacterial contamination and also its effect on yeast growth, fermentation rate

and formation of byproducts (2009). Yeasts growth under pH between 3.0 and 8.0. However, pH values ranging from 5.0 to 6.0 are optimal for a high biomass production. The best results were observed using pH in this range, and glucose as carbon source.

4. Conclusion

The use of invert sugar as a carbon source showed a significant biomass production compared to that obtained with glucose, which enables the technique of yeast biomass production with potential to be used in biological control.

In addition, it is observed that it is possible to have a process to produce yeast biomass with a reduced cost and good yield in biomass. However, the relevance of the results is influenced by the choice of carbon source and the increase in agitation and aeration rates.

Future experiments should be carried out in order to observe the potential use of yeasts in the biological control of fungi in food.

References

- Bendo, M. I.; Viecelli, C. A. (2009). Controle biológico de *Rhizopus nigricans* em pós-colheita de morango pela utilização da levedura *Saccharomyces cerevisiae* em leite in natura. *Cascavel*, v.2, n.3, p.23-35.
- Chan, Z.; Tian, S. (2005). Interaction of antagonistic yeasts against post-harvest pathogens of apple fruit and possible mode of action. *Postharvest Biology Technology*, v. 36, n. 2, p. 215-223.
- De Leon, C. A. P.; Bayon, M. M. (2002). Selenium incorporation into *Saccharomyces cerevisiae* cells: A study of different incorporations methods. *Journal Applied of Microbiolgy*, v. 92, n.4, p. 602-610.
- Difco & BBL Manual. (2009). *Manual of Microbiological Culture Media*. Second Edition. Disponível em: http://www.bd.com/ds/technicalCenter/misc/difcobbllmanual_2nded_lowres.pdf. Acesso em: 12.05.2018
- Droby, S.; Wisniewski, M.; Macarisin, D.; Wilson, C. (2009). Twenty years of postharvest biocontrol research: is it time for a new paradigm? *Postharvest Biology Technology*, v. 52, n. 2, p.137-145.

Gomes, T. M. C. (2010). Produção de Hidromel: efeito das condições de fermentação. *Dissertação de Mestrado*. Escola Superior Agrária de Bragança. Bragança, p.76.

Gouvea, A. (2007). Controle em campo e pós-colheita de doenças e metabolismo do morangueiro após tratamento com *Saccharomyces cerevisiae*. *Tese de Doutorado*. Universidade Federal do Paraná. Curitiba.

Janisiewicz, W. J.; Tworkoski, T.J.; Sharer, C. (2000). Characterizing the mechanism of biological control of postharvest diseases on fruits with a simple method to study competition for nutrients. *Phytopathology*, v. 90, n.11, p. 1196-1200.

Kurzman, C. P.; Fell, J. W. (1998). *The yeasts: a taxonomic study*. 4. Ed. New York: Elsevier, 1074 p.

Malta, H. L. (2006). Estudos de parâmetros de propagação de fermento (*Saccharomyces cerevisiae*) para produção de cachaça de alambique. *Dissertação de Mestrado*. Universidade Federal de Minas Gerais. Belo Horizonte – MG.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, v, 31, p. 426.

Moraes, D. C.; Bianchi, V. L. D. (2013). Avaliação da produção de etanol a partir de glicose e xilose através de batelada alimentada por leveduras isoladas da casca de uvas (*vitis spp*). *Analytica*, v. 62, p. 38.

Neves, L. C. M. (2003). Obtenção da enzima glicose 6-fosfato desidrogenase utilizando *Saccharomyces cerevisiae* W303-181. *Dissertação de Mestrado*. Faculdade de Ciências Farmacêuticas. Universidade de São Paulo. São Paulo.

Nitschke, M.; Rodrigues, V.; Schinatto, L. F. (2001). Formulação de meios de cultivo a base de soro de leite para produção de goma xantana por *Xanthomonas campestris* C7L, *Ciência e Tecnologia de Alimentos*, v. 21, p. 82-85.

Rehm, H.J.; Reed, G.; Pühler, A.; Stadler, P. (1995). *Biotechnology: a multi volume comprehensive treatise*. 2. Ed., vol. 9. Enzymes, Biomass, food and feed. ed. By G. Reed and T.W Nagodawithana. Cap. 5. Production of Microbial Biomass.

Reifenberg, E.; Boles, E.; Ciriacy, M. (1997). Kinetic characterization of individual hexose transporters of *Saccharomyces cerevisiae* and their regulations to the triggering mechanism of glucose repression. *European Journal of Biochemistry*, v.245, n. 2, p.324-333.

Schrank, A.; Franceschini, M.; Guimarães, A.P.; Camassola, M.; Frazzon, A. P.; Baratto, C, M.; Kogler, V.; Silva, M, V.; Dutra, V.; Nakazonto, L.; Castro, L.; Santi, L.;Vainstein, M. H. (2001). Biotecnologia aplicada ao controle biológico - O entomopatógeno *Metarhizium anisopliae* – *Biotecnologia, Ciência e Desenvolvimento*, nº 23.

Silva, D, P.; Pessoa JR, A.; Roberto, I. C.; Vitolo, M. (2001). Effect of agitation and aeration on production of hexokinase by *Sacharomyces cerevisiae*. *Applied Microbiology Biothechnology*, v. 91, n.3, p. 605-613.

Silveira, N.S.S.; Michereff, S.J.M.; Silva, I.L.S.S.; Oliveira, S.M.A. (2005). Doenças fúngicas pós-colheita em frutas tropicais: patogênese e controle. *Revista Caatinga*, v.18, n.4, p.283-299.

Souza, C. S. (2009). Avaliação da produção de etanol em temperaturas elevadas por uma linhagem de *S. cerevisiae*. Tese de Doutorado. Programa de Pós-Graduação Interunidades em Biotecnologia (USP). Instituto Butantan (IPT), São Paulo.

Taccari, M.; Canonico, L.; Comitini, F.; Mannazzu, I.; Ciani, M. (2012). Screening of yeasts for growth on crude glycerol and optimization of biomass production. *Bioresource Technology*, v.110, p.488-495.

Porcentagem de contribuição de cada autor no manuscrito

Ana Paula Colares de Andrade – 60%

Helder Levi da Silva – 30%

Gustavo Adolfo Saavedra Pinto – 10%