Aeration and Glucose Concentration on *Saccharomyces boulardii* Probiotic Cultivation

Aeração e concentração de glicose no cultivo probiótico de *saccharomyces boulardii*

DOI:10.34117/bjdv5n10-027

Recebimento dos originais: 10/09/2019
Aceitação para publicação: 03/10/2019

**Tárcio Enrico Savoldi**  
Mestre em Engenharia Química pela Universidade Estadual do Oeste do Paraná  
Instituição: Universidade Estadual do Oeste Do Paraná  
Endereço: Rua da Faculdade, n° 645 - Jardim Santa Maria CEP: 85903-000 - Toledo PR  
E-mail: tarciosavoldi@gmail.com

**Thaís Lorana Savoldi**  
Doutora em Biotecnologia aplicada à Agricultura pela Universidade Paranaense.  
Instituição: Universidade Paranaense – Unipar.  
Endereço: Praça Mascarenhas de Moraes, 4282 - Centro - CEP: 87502-210 Umuarama – Paraná  
E-mail: thaislorana@hotmail.com

**Deisy Alessandra Drunkler**  
Doutora Doutora em Tecnologia de Alimentos pela Universidade Federal do Paraná  
Instituição: Universidade Tecnologica Federal do Paraná – UTFPR  
Endereço: Av. Brasil, 4232, Parque Independência, CEP 85884-000 Medianeira - Paraná – BR  
E-mail: deisyadrunkler@gmail.com

**Giane Andrea Linde Colauto**  
Doutora em Engenharia Química pela Universidade Estadual de Maringá.  
Instituição: Universidade Paranaense – Unipar.  
Endereço: Praça Mascarenhas de Moraes, 4282 - Centro - CEP: 87502-210 Umuarama – Paraná  
E-mail: gianilindi@prof.unipar.br

**Nelson Barros Colauto**  
Doutorado em Agronomia pela Universidade Estadual Paulista Júlio de Mesquita Filho  
Instituição: Universidade Paranaense - Unipar  
Endereço: Praça Mascarenhas de Moraes, 4282 - Centro - CEP: 87502-210 Umuarama – Paraná  
E-mail: nbc@prof.unipar.br

**Mônica Lady Fiorese**  
Doutora em Engenharia Química pela Universidade Federal de Santa Catarina  
Instituição: Universidade Estadual do Oeste do Paraná – Unioeste.
ABSTRACT

Saccharomyces boulardii viable cells have probiotic action. This study aimed to evaluate the effect of aeration on the production of S. boulardii viable cells in liquid cultivation medium with different initial glucose concentrations. Yeast has grown on yeast extract peptone dextrose medium with or without aeration (2 Lar min\(^{-1}\) to obtain 30 ± 1% of oxygen) and different initial glucose concentrations (20, 40 and 60 g L\(^{-1}\)). The viable cells were determined by serial dilution method and glucose concentration was determined by 3,5 dinitrosalicylic acid. The number of viable cells changed from 7.54 ± 0.04 to 7.77 ± 0.02 log of CFU mL\(^{-1}\) when glucose increased from 20 to 60 g L\(^{-1}\), respectively. The aeration of the cultivation medium increased the number of viable cells from 7.78 ± 0.04 to 8.27 ± 0.04 log of CFU mL\(^{-1}\) in the cultivation medium with 40 g L\(^{-1}\) glucose. The yeast biomass was ~3 times greater than 60 g L\(^{-1}\) glucose with aeration when compared to cultivation medium with 20 g L\(^{-1}\) glucose without aeration. Thus, the glucose increase and the cultivation medium aeration increment S. boulardii production and viability.

Keywords: yeast, fungus, fermentation, cultivation, biomass, CFU

RESUMO

As células viáveis de Saccharomyces boulardii têm ação probiótica. Este estudo teve como objetivo avaliar o efeito da aeração na produção de células viáveis de S. boulardii em meio de cultivo líquido com diferentes concentrações iniciais de glicose. A levedura cresceu em meio de peptona dextrose com extrato de levedura com ou sem aeração (2 Lar min\(^{-1}\) para obter 30 ± 1% de oxigênio) e diferentes concentrações iniciais de glicose (20, 40 e 60 g L\(^{-1}\)). As células viáveis foram determinadas pelo método de diluição em série e a concentração de glicose foi determinada por 3,5 ácido dinitrosalicílico. O número de células viáveis mudou de 7,54 ± 0,04 para 7,77 ± 0,02 log de UFC UFC-1 quando a glicose aumentou de 20 para 60 g L\(^{-1}\), respectivamente. A aeração do meio de cultivo aumentou o número de células viáveis de 7,78 ± 0,04 para 8,27 ± 0,04 log de UFC UFC-1 no meio de cultivo com 40 g L\(^{-1}\) de glicose. A biomassa de levedura foi ~3 vezes maior que 60 g L\(^{-1}\) de glicose com aeração quando comparada ao meio de cultivo com 20 g L\(^{-1}\) de glicose sem aeração. Assim, o aumento da glicose e a aeração do meio de cultivo aumentam a produção e a viabilidade de S. boulardii.

Palavras-chave: levedura, fungo, fermentação, cultivo, biomassa, UFC

1 INTRODUCTION

Probiotic microorganisms are non-pathogenic bacteria and yeasts that can provide several benefits to their hosts (De Vrese, 2008). Among these microorganisms, yeasts, more specifically Saccharomyces boulardii (current name Saccharomyces cerevisiae), can act against pathogens such as Clostridium difficile, Vibrio cholerae, Salmonella enterica, Shigella.
spp and *Escherichia coli* (Czerucka & Rampal, 2002) and diarrhea associated with antibiotics (Mcfarland et al., 1993). The probiotic activity of this yeast is aggregated to the capacity to hinder the colonization of the intestinal mucosa by pathogenic agents, improve responses to the immunologic system, inhibit the action of pro-carcinogenic enzymes, and induce the action of enzymes that promote the absorption of benefic compounds to the host (Vandenplas, 2008). Moreover, this yeast is resistant to antibiotics, multiplies itself rapidly, and is easily eliminated by the host (Czerucka et al., 2007).

For yeast cultivation, the carbon source, which composes almost 50% of the cells, is a nutrition demand and is fundamental in the synthesis of primary cell components of the cell metabolism with direct effect on the amount of biomass (Bailey & Ollis, 1986; Halasz & Lasztity, 1991; Quintero, 1993). Cultivation conditions such as aeration of the cultivation medium are essential to a quick and efficient cell development due to the increase of the energetically oxidative metabolic pathway (Alferone, 2004).

Several strategies to improve dried yeast biomass production have been studied about glucose (Trigueiros et al., 2016) or aeration in the cultivation medium (Muller et al., 2007). However, there have been no reports on the relationship between viable yeast biomass produced in liquid cultivation with different initial glucose concentrations and aeration conditions. The production of viable cells is important to guarantee probiotic action. This action occurs with the fixation of viable cells to the intestine. Thus, it is paramount that the probiotics presents a great concentration of viable cells that go through the stomach barrier and reach the host’s intestine (Saad, 2006; Cook et al., 2012). Therefore, this study aimed to evaluate the effect of aeration on the production of *S. boulardii* viable cells in liquid cultivation medium with different initial glucose concentrations.

### 2 MATERIALS AND METHODS

#### 2.1 MICROORGANISM

Saccharomyces boulardii Seguela, Bastide & Massot, current name Saccharomyces cerevisiae (Desm.) Meyen 1838, CCT 4308, reference UFPEDA 1176 from the Tropical Culture Collection (Fundação André Tosello, Campinas, SP, Brazil) was utilized. Lyophilized yeast was transferred to an Erlenmeyer flask (250 mL) with 100 mL of yeast extract peptone dextrose (YEPD) cultivation medium prepared with 5 g L-1 yeast extract (Himedia®), 10 g L-1 meat peptone (Himedia®) and 20 g L-1 glucose (Merck®) and autoclaved at 121 °C for...
15 min (Casal et al., 2004). The liquid cultivation medium was kept at 30 ± 2 ºC for 24 h with agitation at 100 rpm. Next, a 10 mL aliquot was transferred to 90 mL YEPD in a new tube and incubated as previously described, and the procedure was repeated. The cultivation medium grown with yeast was utilized as inoculum for subsequent experiments. Part of that was transferred to an agar slant test tube with YEDP and 20 g L-1 agar (Himedia®), incubated at 30 ± 2 ºC for 48 h and stored at 4 ºC.

2.2 YEAST BIOMASS CULTIVATION

The yeast cultivation was carried out in liquid cultivation medium containing 5 g L-1 yeast extract, 10 g L-1 meat peptone and glucose at the concentrations of 20, 40 and 60 g L-1, coded when without aeration as SA20G, SA40G and SA60G, respectively, and coded when with aeration as AE20G, AE40G and AE60G, respectively. In the treatments with aeration, the external air was inserted in the cultivation medium by an air compressor (2 L min-1 flow) connected to a sterile filtrating membrane (0.22 μm pore size) to obtain 30 ± 1% of oxygen. The amount of dissolved oxygen in the cultivation medium was verified by an oxygen meter (Instrutherm®). In each storing flask (500 mL), previously autoclaved at 121 ºC for 15 min, 270 mL of cultivation medium, inoculated with 30 mL of YEPD grown with yeast, was added and incubated at 30 ± 2 ºC for 24 h with agitation at 100 rpm, with or without aeration. Each flask had a metal lid with a vent made of a silicon tube with cotton and another to withdraw samples that were autoclaved at 121 ºC for 15 min. The treatments with aeration had a third silicon tube to insert air to the cultivation medium. The yeast biomass growth was analyzed by spectrophotometry (600 nm absorbance) every 2 h withdrawing 3 mL aliquot of each cultivation medium. The aliquots were analyzed in triplicate, and the arithmetic average and standard deviation were calculated.

2.3 DETERMINATION OF GLUCOSE INTAKE

Glucose intake was determined by 3-5 dinitrosalicylic (DNS) acid method from the standard glucose curve of 3 g L-1 (Miller, 1959).
2.4 DETERMINATION OF YEAST BIOMASS CONCENTRATION

After 24 h of microbial growth, 40 mL aliquot of the cultivation medium containing yeast wasfiltered by a cellulose acetate membrane (0.45 μm pore size). The membrane was previously dried at 90 °C, without air circulation, until reaching constant mass. The membrane with yeast biomass was filtered and dried at 90 °C, without air circulation, until constant mass, and the dry yeast biomass concentration was determined according to Equation 1.

\[ X = \frac{m_f - m_m}{V} \]  

Equation (1)

where \( X \) is the dry yeast biomass concentration in the cultivation medium (g L\(^{-1}\)), \( m_f \) is the mass of filtrating mass with dry yeast (g), \( m_m \) is the dry filtrating membrane mass (g) and \( V \) is the cultivation medium aliquot volume with filtered yeast (L).

2.5 DETERMINATION OF YEAST VIABLE CELLS

The number of yeast viable cells was determined by serial dilution method, with transfer of 1 mL of cultivation medium with the yeast to 9 mL 0.1% peptone water (Himedia\(^{\circledR}\)) at zero and 12 h after yeast inoculation. For each dilution, an aliquot of 0.1 mL was spread on the cultivation medium surface, dried in laminar flow chamber, and incubated at 30 ± 2 °C for 48 h to count colony-forming units (CFU) according to the spread plate method. Potato dextrose agar (PDA, Himedia\(^{\circledR}\)) was utilized as cultivation medium with addition of 10% tartar acid solution just before pouring PDA on a 90-mm-diameter Petri dish (Beuchat et al., 1990). All analyses were done in triplicate, the arithmetic average and standard deviation were calculated, and the significance compared by Scott-Knott test (\( p \leq 0.05 \)).

3 RESULTS AND DISCUSSION

The produced biomass and glucose concentration in the cultivation medium during 24 h of cultivation are in Figure 1. Most glucose was consumed until 8 h of cultivation. However, the cultivation media with greater initial glucose concentrations (40 and 60 g L\(^{-1}\)) presented depletion of this carbon source at 10 and 12 h, respectively. For cultivation medium with 20 or 40 g L\(^{-1}\) glucose, aeration did not affect time for the depletion of this carbon source. On the other hand, for cultivation medium with 60 g L\(^{-1}\) glucose, aeration anticipated glucose depletion time in 2 h. Probably the greater glucose concentration avoided this confounding.
variable and allowed that aeration had its effect isolated. Coincidentally, the beginning of the stationary phase of biomass production was between 10 and 12 h of cultivation, soon after glucose depletion (Fig. 1).
Figure 1. Concentration of Saccharomyces boulardii produced in liquid medium of yeast extract (5 g L\(^{-1}\)), meat peptone (10 g L\(^{-1}\)) added with different glucose concentrations, with or without aeration. (a) SA20G = 20 g L\(^{-1}\) glucose without aeration; (b) SA40G = 40 g L\(^{-1}\) glucose without aeration; (c) SA60G = 60 g L\(^{-1}\) glucose without aeration; (d) AE20G = 20 g L\(^{-1}\) glucose with aeration; (e) AE40G = 40 g L\(^{-1}\) glucose with aeration; (f) AE60G = 60 g L\(^{-1}\) glucose with aeration.

Regarding biomass production, the glucose increase in the cultivation medium promoted a raise of biomass production in all treatments. Biomass production, after 24 h, changed from 2.37 ± 0.27 g L\(^{-1}\) in the cultivation medium with 20 g L\(^{-1}\) glucose to 4.63 ± 0.21 g L\(^{-1}\) glucose in the cultivation medium with 60 g L\(^{-1}\) glucose, respectively.

Aeration also promoted biomass production increase in our study. The average of yeast biomass cultivated without aeration was 1.85 ± 0.49 g L\(^{-1}\), and with aeration it was 3.31 ± 0.93 g L\(^{-1}\). However, the greatest biomass productions were obtained with a combination of high glucose concentration and aeration. Yeast production was approximately 3 times greater in AE60G compared to SA20G in 10 h cultivation (Fig. 1).

After 24 h cultivation, the produced biomass was removed, dried and quantified. The biomass concentration values obtained for non-aerated cultivations were 2.37 ± 0.27 g L\(^{-1}\) for SA20G, 3.42 ± 0.13 g L\(^{-1}\) for SA40G, and 4.63 ± 0.21 g L\(^{-1}\) for SA60G. For aerated cultivations, the values for dry biomass production were 4.91 ± 0.88 g L\(^{-1}\) for AE20G, 5.84 ± 0.56 g L\(^{-1}\) for AE40G and 6.56 ± 0.27 g L\(^{-1}\) for AE60G. Thus, these concentrations were 52 and 75% greater than those estimated by optical density (Fig. 1). Despite the differences between real and estimated values, the tendency was kept, that is, the dry biomass was greater in the treatments with greater glucose concentrations and aeration. AE60G produced greater dry yeast biomass concentration at the end of the cultivation (6.56 ± 0.27 g L\(^{-1}\)).

The increase in glucose concentration from 20 to 60 g L\(^{-1}\) increased the number of viable cells from 7.54 ± 0.04 to 7.77 ± 0.02 log of CFU mL\(^{-1}\), respectively (Table 1). However, again the concentration of high glucose concentration and aeration promoted the greatest values in the number of viable cells. The maximum value of viable cells was 8.30 ± 0.06 log of CFU mL\(^{-1}\) in the culture medium with aeration and 60 g L\(^{-1}\) glucose (Table 1). Our results showed that glucose induced biomass production and increased yeast viability until cell growth inhibition by the substrate. This inhibition condition by the substrate has already been reported (Borzani, 2006).
Table 1. Colony-forming units (CFU) of yeast produced in cultivation medium with different initial glucose concentrations with or without aeration for 12 h

| Treatment code* | Glucose concentration (g L\(^{-1}\)) | Cultivation aeration | Yeast (log of CFU mL\(^{-1}\)) |
|----------------|--------------------------------------|----------------------|-------------------------------|
| SA20G          | 20                                   | without              | 7.54 ± 0.04\(^c\)            |
| SA40G          | 40                                   | without              | 7.78 ± 0.04\(^b\)            |
| SA60G          | 60                                   | without              | 7.77 ± 0.02\(^b\)            |
| AE20G          | 20                                   | with                 | 8.32 ± 0.06\(^a\)            |
| AE40G          | 40                                   | with                 | 8.27 ± 0.04\(^a\)            |
| AE60G          | 60                                   | with                 | 8.30 ± 0.06\(^a\)            |

In our study there was an increase in the production and cell viability of S. boulardii when grown in medium with 30% of dissolved oxygen. Jouhten and co-workers (2008) observed that when the dissolved oxygen in S. cerevisiae cultivation increases from 0 to 21%, the biomass production was incremented around 5 fold. In our study 30% of dissolved oxygen promoted (in average) an increase of 70% in the biomass production and 7% in cell viability.

Under low or no aeration conditions only glycolysis takes place and as a result there is a smaller energy production by yeasts. For a greater energy production, the complete respiratory cycle and total glucose oxidation need to occur, which demands the presence of oxygen (Fiechter, 1981). Moreover, glycolysis releases secondary compounds such as organic acids and alcohol that alter pH of the medium and reduce cell viability (Hopp & Hansford, 1984).

4 CONCLUSION

The aeration and glucose concentration increment in the cultivation medium increase S. boulardii biomass production and cell viability. The stationary phase of the cell growth occurs between 10 and 12 h of cultivation. The maximum production occurs at 10 h of cultivation in the culture medium with 30% of dissolved oxygen and 60 g L\(^{-1}\) glucose.
ACKNOWLEDGEMENT

The authors thank the State University of West Paraná (UNIOESTE) and Paranaense University (UNIPAR).

REFERENCES

Alferone, S., Cameleyre, X., Benbadis, L., Bideaux, C., UribeKarrea, J.L., Goma, G., Molina-Jouve, C. & Guillouet, S.E. (2004): Aeration strategy: a need for very high ethanol performance in Saccharomyces cerevisiae fed-batch process. *Appl. Microbiol. Biotechnol.*, 63, 537–542. https://doi.org/10.1007/s00253-003-1393-5

Bailey, J. E., & Ollis, D. F. (1986): Biochemical engineering fundamentals, McGraw-Hill, New York, 984 p.

Beuchat, L. R., Nail, B. V., Brackett, R. E. & Fox, T. L. (1990): Evaluation of a culture film (Petrifilm™ YM) method for enumerating yeasts and molds in selected dairy and high-acid foods. *J. Food Prot.*, 53, 869–874. https://doi.org/10.4315/0362-028X-53.10.869

Borzani, W. (2006): Batch ethanol fermentation: the correlation between the fermentation efficiency and the biomass initial concentration depends on what is considered as produced ethanol. *Braz. J. Microbiol.*, 37, 87–89. https://doi.org/10.1590/S1517-83822006000100016

Cook, M. T., Tzortzis, G., Charalampopoulos, D. & Khutoriankiy, V. V. (2012): Microencapsulation of probiotics for gastrointestinal delivery. *J. Control. Release*, 162, 56–67. https://doi.org/10.1016/j.jconrel.2012.06.003

Czerucka, D. & Rampal, P. (2002): Experimental effects of Saccharomyces boulardii on diarrheal pathogens. *Microbes Infect.*, 4, 733–739. https://doi.org/10.1016/S1286-4579(02)01592-7

Czerucka, D.; Piche, T. & Rampal, P. (2007): Review article: yeast as probiotics – Saccharomyces boulardii. *Aliment. Pharmacol. Ther.*, 26, 767–778. https://doi.org/10.1111/j.1365-2036.2007.03442.x

De Vrese, M & Schrezenmeir, J. (2008): Probiotics, prebiotics, synbiotics. *Adv. Biochem. Eng. Biotechnol.*, 111, 1–66. https://doi.org/10.1007/10_2008_097

Fiechter, A. & Seghezzi, W. (1992) Regulation of glucose metabolism in growing yeast cells. *J. Biotechnol.*, 27, 27–45. https://doi.org/10.1016/0168-1656(92)90028-8
Guslandi, M., Mezzi, G., Sorghi, M. & Testoni, P.A. (2000): Saccharomyces boulardii in maintenance treatment of Crohn’s disease. *Dig. Dis. Sci.*, 45, 1462–1464. https://doi.org/10.1023/A:1005588911207

Halasz, A. & Lásztity, R. (1991): Use of yeast biomass in food production, CRC Press, Boca Raton, 352 p.

Hoppe, G.K. & Hansford, G.S. (1984): The effect of micro-aerobic conditions on continuous ethanol production by *Saccharomyces cerevisiae*. *Biotechnol. Lett.*, 6, 681–686. https://doi.org/10.1007/BF00133837

Jouhten, P., Rintala, E., Huuskonen, A., Tammien, A., Toivari, M., Wiebe, M., Ruohonen, L., Penttilä, M. & Maaheimo, H. (2008): Oxygen dependence of metabolic fluxes and energy generation of *Saccharomyces cerevisiae* CEN.PK113-1A. *BMC Syst. Biol.*, 2(60), 1–19. https://doi.org/10.1186/1752-0509-2-60

Mcfarland, L.V. & Bernasconi, P. (1993): Saccharomyces boulardii: a review of an innovative bio therapeutic agent. *Microb. Ecol. Health Dis.*, 6, 157–171. https://doi.org/10.3109/08910609309141323

Miller, G. L. (1959): Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Anal. Chem.*, 31, 426–428. https://doi.org/10.1021/ac60147a030

Muller, J.L., Protti, K.L., Machado, M.S. da, Lacerda, L.L.V. de, Bresolin, T.M.B. & Podlech, P.S. (2007): Comparison of *Saccharomyces boulardii* growth in an air-lift fermentor and in a shaker. *Food Sci. Technol.*, 27, 688–693. https://doi.org/10.1590/S0101-20612007000400003

Quintero, R. (1993): Biochemical engineering: theory and applications. Alambra Mexicana, México, 332 p.

Saad, S.M.I. (2006): Probiotics and prebiotics: the state of the art. *Braz. J. Pharm. Sci.*, 42, 1–16.

Trigueros, D.E.G., Fiorese, M.L., Kroumov, A.D., Hinterholz, C.L., Nadai, B.L. & Assunção, G.M. (2016): Medium optimization and kinetics modeling for the fermentation of hydrolyzed cheese whey permeate as a substrate for *Saccharomyces cerevisiae* var. boulardii. *Biochem. Eng. J.*, 110, 71–83. https://doi.org/10.1016/j.bej.2016.02.014

Vandenplas Y. (2009): Saccharomyces boulardii in childhood. *Eur. J. Pediatr.*, 168, 253–265. https://doi.org/10.1007/s00431-008-0879-7