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IMMOBILIZATION OF SACCHAROMYCES CEREVISIAE FOR BEER PRODUCTION: FERMENTATIVE CYCLE AND SENSORY ANALYSIS

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RESUMO

1.Introdution Beer sector is one of the areas with the greatest tradition in the fermentation process. Beer industries have been searcher for innovation in production to improving industrial performance as continuous fermentation and immobilized yeasts (Es, Vieira & Amaral,2015). Yeast immobilization is possible to use fermentation of wine (Moreno-Garcia et al.,2018) production and, beer (Naydenova et al.,2014). Technique is physically confining cells in restricted space and biological activities in continuous or discontinuous operations (Moreno-Garcia et al.,2018). This process promotes higher fermentation rates due cell density, increases and keeps specifics metabolites, like aromatics, improves the control and stability of cells, allows regeneration and reuse of biocatalyst, eases the separation of the biocatalyst from reaction medium (Oliveira et al.,2011) and protects cells against shear forces. Technique can change yeast's morphology, growth, and physiology, affecting the formation of bioflavors (Nedovic et al.,2015), changing the product's taste and impeding traditional character's training. Also, solid immobilization matrix can interfere on flavor profile, and studies presented decrease or increase of the concentration of higher alcohols (Djordjevic et al.,2016), increase production of esters (Djordjevic et al.,2015), changes in concentration of aldehyde (Naydenova et al.,2014) and increase production of volatile compounds (Pereira et al.,2014). This project studies the efficiency of batch fermentation of beer using yeasts immobilized, evaluating the viability of the encapsulated cell for the reuse of the microorganism in different cycles and sensory evaluation. **2. Materials and Methods** 2.1. Wort The wort production occurred by the infusion method of ground Pilsen malt (250g/L) in water, keeping the temperature between 65 to 67°C. Hop Tradittion (5.20% alpha-acid) were added on boiling stage. It was filtered and cooled to 20°C for inoculation. 2.2. Inoculum and Cell immobilization The inoculum was obtain add 0.575g/L yeast ($3x10^8$cells/ml) in 10%(v/v) of wort, at 150rpm for 30 minutes for 24 hours at 19±0.5°C. The gel used in the immobilization was carried out add alginate (1.5%(w/v) in water at 80°C (Santos, Oliveira & Maugeri,2007). After homogenization, inoculum (75%(v/v) was added in the gel solution with slow agitation, and dropped into reticulation solution of calcium chloride (0.2M). Diameter's spheres 3.63mm±0.24. Spheres storage at 19±0.5°C until fermentation. 2.3. Fermentaion The fermentation with immobilized yeast was developed in two steps. 1. Test tubes closed with cotton in volume of 20mL. 2. Studies of fermentation cycles carried out in polypropylene tanks (10L), 19±0.5°C, temperature controller. 2.4. Analysis The techniques the analysis shown at Table 1. Table 1. Analysis *Parameters Tecnique Substrate* Spectrometry (Miller,1959) *Soluble solids* Refractometer *pH* pHmeter *Ethanol* Ebulliometer *Cell viability* Neubauer chamber *Sensory analysis* Triangular test (p<0,05) **3. Results and Discussions** Fermentation1, immobilized yeasts shown 69 hours less than fermentation free yeast (Figure 1). Ethanol production, consumption of substrate, and good productivity. Figure 1. Fermentacion1 Yeasts immobilized maintained in the wort for 48 hours more than free yeasts, causing increase cell concentration and of cell density. Similarity between substrate consumption and alcohol formation in fermentations with free and immobilized yeasts (Figures2,3,4), causing similarity of efficiency, productivity, and substrate-product conversion (Table 2). Figure 2. Fermentacion2 Figure 3. Fermentacion3 Figure 4. Fermentacion4 Table 2. Ethanol production rate (PP), substrate-to-product conversion coefficient ($Y_{P/S}$) and Process yield (R%). ***PP(g/L.h) $Y_{P/S}$ R (%) Fermentação1*** **Livre/imob.** 0.202/0.454 0.431/0.433 84.382/84.712 ***Fermentação2*** **Livre/imob.** 0.564/0.579 0.434/0.438 84.981/85.754 ***Fermentação3*** **Livre/imob.** 0.527/0.522 0.431/0.436 84.421/85.290 ***Fermentação4*** **Livre/imob.** 0.568/0.556 0.439/0.449 85.953/87.791 There is decrease cell viability of yeasts immobilized in Fermentation4, approximately 44%. The deterioration in the structure of the spheres could be a possible cause (Figure 5). Figure 5. Deteriorated spheres The rupture the spheres is related with mechanical and electrostatic interaction between cell and alginate matrix and CO_2 formation and/or chemical interaction of the matrix with components present in reaction medium, such as non-gelling ions and chelating compounds (Pajic-Lijakovic et al.,2015). Also, electrostatic interaction between alginate and cell membrane cause deterioration on spheres by repulsion and production of CO_2 influence structural changes, generating internal pressure and rupture of structure spheres (Rafiee et al.2014). Among 100 tasters, 55 identified the distinct sample among the three. The results shown significant sensory differences. Chemical and biological processes during the primary fermentation of immobilized cells limit cellular metabolism, causing sensory changes to the final product. **4. Conclusion** Immobilization technique allowed the reuse of biocatalysts in four fermentation cycles with a good yield of 84.71%, 85.75%, 85.29% and 87.79%. After the four cycles, the structure of spheres was damaged. Sensory analyse of beer with immobilized and free yeast shown a significant difference. **5. References** Djordjevic,R.;Gibson,I.I.;Vunduk,K.J.;Nikicevic,N.;Nedovick,V. 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PALAVRAS-CHAVE: reutilization, cell viability, fermentative profile, triangular test, calcium alginate, beer

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