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Physico-chemical characteristics and fermentative activity of the hydrogel particles based on polysaccharides mixture with yeast cells immobilized, obtained by ionotropic gelation

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ABSTRACT

The paper reports the preparation of particles, having a hydrogel behavior, with immobilized yeast cells from a mixture of polysaccharide based on gellan, i-carrageenan, and carboxymethyl cellulose sodium salt. These particles were ionically cross-linked, using the extrusion method, with magnesium acetate, calcium acetate and zinc acetate and were characterized, by FTIR and SEM, in terms of some physicochemical properties, morphology, stability in water, and water retention capacity. These properties are influenced by the composition of polysaccharide mixture, which determines the number of cross-linkable functional groups, and also by the type and the concentration of the cross-linking agent. Analysis, by scanning electron microscopy, reveals that the yeast cells are well immobilized and in high amounts in the polysaccharides matrix. This matrix maintains the cell viability at high levels in aqueous media. High fermentation yields were obtained and the specific productivity in ethanol was equal to 0.79 g ethanol/h × g yeast cells. Highest ethanol yield was obtained for the sample cross-linked with Mg^{2+}, and the lowest for the sample cross-linked with Zn^{2+}. Polysaccharides matrix provides structural stability to yeast cells and maintains the cells viability, at values higher than 82% even after 10 fermentation cycles, as well as their ability to proliferate. Moreover, the obtained particles are stable, can be readily recovered by filtration from the fermentation medium and can be reused for at least 10 fermentation cycles. The obtained bio-reactors could represent a new approach to solving the problem of producers in the alcoholic beverages industry concerning the deficit of mineral nutrients.

1. Introduction

Currently, there is a general interest regarding the increase of the ethanol production efficiency for alcoholic beverage industry but also for its use as biofuel. Production costs, environmental issues, fossil fuel depletion and rising gas prices have been identified as major factors that led to intensified research in order to find effective methods of obtaining ethanol by fermentation processes (Bai et al., 2004; Hill et al. 2006; Blieck et al., 2007; Bai et al., 2008; Silva et al. 2008). In this regard, the use of immobilized cells is a research area that has expanded due to an attractive preparation method and to the economic advantages that they present. Continuous processes are preferred as they have sub-

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stantially improved efficiency, higher productivity and lower operating costs (Kourkoutas et al., 2005). Also, production of ethanol, for use as biofuel, has enjoyed of a great interest in recent years. The production of fuel from ethanol is different from the beverage production due to the fact that it is not necessary a slow process for providing a quality final product; in this case, it is preferably to use a rapid and complete fermentation process in order to ensure a maximum profitability (Westman and Franzén, 2015).

Yeasts, especially *Saccharomyces* species, are preferred due to a large fermentation capacity, to their tolerance to ethanol and to other inhibitors, and to their ability of rapid growth under anaerobic conditions, which are characteristic of fermentative processes (Groboillot et al., 1994; Ivanova et al., 2011). The advantages of yeast cells immobilization on various supports are multiple: higher volumetric productivity as a result of the high cells density, increased tolerance to various stressors, such as higher ethanol concentrations, toxic metabolites produced by fermentation, high temperature and pH, improved mechanical and chemical stability, low risk of contamination and inhibition, maintenance of cell viability, lowering the production costs due to the fact that these micro-bio-reactors can be recovered easily from the fermentation medium and reused in other processes, and the possibility of using these systems in continuous fermentative processes (Brányik et al., 2001; Verbelen et al., 2006; Duarte et al., 2013).

Several techniques have been developed for the cells immobilization on a organic or inorganic support matrix by physical (adsorption) or chemical (covalent) binding, by flocculation (aggregation of the biomass) and by encapsulation in microcapsules or in more or less porous polymeric particles (Rathore et al., 2013). These techniques should be simple, cheap and it is essential that the immobilized cells to have an adequate fermentative activity, to be stable in the fermentation medium, and to maintain a high viability (Baptista et al., 2006). Methods commonly used to obtain systems with immobilized cells having applications in the food industry are: emulsification (Heng et al., 2003; Tan et al., 2011), extrusion (Wan et al., 1994), complex coacervation (John et al., 2011), spray drying (Luna-Solano et al., 2005), gel entrapment and radiation polymerization (Saraydın et al., 2002). Among them, the extrusion method is most often used due to the mild processing conditions that enable optimal encapsulation and also due to the fact that the cellular viability is not affected (Rathore et al., 2013). Some of the substrates used for immobilization are: alginates (Fumi et al., 1987; Corton et al., 2000; Idris and Wahidin, 2006; Yoo et al., 1996) glass or ceramic substrates (Goncalves et al., 1992; Santos et al., 2005), cellulosic materials (Credou and Berthelot, 2014), γ-alumina (Loukatos et al., 2000), silica sol–gel films (Inama et al., 1993), polyacrylamide gel (Aykut et al., 1988) and combinations of various materials (Peinado et al., 2006; Câmara et al., 2016). Choosing the proper media for immobilization is essential and the required characteristics are: good chemical and mechanical stability, absence of toxicity, biocompatibility, and high diffusion coefficient for both substrates and fermentation products (Baptista et al., 2006).

Alginate-based matrices obtained by the ionotropic gelation, even if frequently used, have some drawbacks: limited diffusion of nutrients, of metabolites and of oxygen as a consequence of the gel matrix and of the high density of cells immobilized into the particles (Nedovic et al., 2005) Moreover, alginate gels have a low mechanical and chemical stability which can lead to a diffusion of a large number of cells in the same time with their proliferation (Maicas, 2001; Idris and Wahidin, 2006; Călinescu et al., 2012; Nedović et al., 2015). These properties may be improved by grafting synthetic polymers on alginate or by the incorporation, in gels, of inorganic materials such as silica, sand and alumina (Rosevear, 1984; Hong et al., 2016). The polyacrylamide gel was widely used to immobilize several types of microbial cells (Călinescu et al., 2012; Dinu et al., 2007) and the fermentative activity of these systems with immobilized yeast cells was shown to be comparable to those based on alginates, nevertheless having a higher stability (Călinescu et al., 2012). The systems based on polyacrylamide may be obtained by polymerization using gamma radiation (Deshpande et al., 1987; Ahmed, 2015), which, in comparison with the chemically initiated polymerization, has the advantage that it can occur at low temperatures, down to −75 °C. These conditions prevent the inactivation caused by temperature and samples can be frozen in any form: particles, tubes or membranes. This technique has been also extended to the cell immobilization in gelatin (El-Hadedy et al., 2014).

Hydrogel particles based on alginates were prepared by ionic cross-linking using Ca^{2+}, Ba^{2+}, and then covered with a layer of silica gel. Thus, the yeast cells are protected by the alginate-based matrix and the silica layer provides stability and the possibility to use these systems in continuous fermentation processes. The strength of the Si—O bond (425 J mol^{-1}) which provides a higher stability to the material (Callone et al., 2008).

Besides alginates, the possibility of using other polysaccharides, as matrices for cell immobilization, was investigated in the literature.

Gellan, an anionic biopolymer (O'Neill et al., 1983; Grasdalen and Smidsrod, 1987; Morris et al., 2012) allowed the preparation of a biocatalyst in the form of spherical particles with real prospects of being used for sparkling wine production technology. The particles have been formed by extruding the polysaccharide solution, containing a suspension of yeast cells, through a capillary in a bath with a solution of 2% $CaCl_2$, as ionic cross-linking agent (Mantaluta et al., 2012). Tan et al. (2011), have immobilized yeast cells using gellan as support by a emulsification method and have stated that the obtained micro-bio-reactors can be reused. The production of ethanol during the first three fermentation cycles was comparable to that obtained in the presence of free yeast. Moreover, these authors have indicated that the micro-bio-reactors are stable and readily recovered from the fermentation medium, by filtration, and that they can be reused for at least ten fermentation cycles with a relatively high yield of ethanol (Tan et al., 2011).

Carboxymethyl cellulose sodium salt (CMCNa) is a water soluble derivative of cellulose (Sannino et al., 2009). His mixture with a graft copolymer based on *N*-vinyl-2-pyrrolidone (PVP) was used to immobilize the baker's yeast *Saccharomyces cerevisiae*, by the extrusion method, using $AlCl_3$ as a cross-linking agent. The obtained spherical particles were tested in the fermentation process for 27 h and allowed the preparation of ethanol at a concentration of 57 g/L. Furthermore, it was indicated that the yield of ethanol increases when the particles cross-linking degree decreases (Yiğitoğlu et al., 2007; Gokgoz and Yiğitoğlu, 2011).

Carrageenan is a polygalactan rich in sulfate esters, characterized by a low solubilization temperature and low gel strength (Necas and Bartosikova, 2013). K-carrageenan has been successfully used as a matrix for the yeast cells immobilization allowing the preparation of ethanol with a high yield. Hettwer and Wang (1982) have added hydroxyapatite or tricalcium phosphate to the gel matrix based on k-carrageenan. Tricalcium phosphate maintains the pH and the cell viability at an optimal level, increases the gel density and the ethanol productivity (Wang and Hettwer, 1982). Particles of k-carrageenan with immobilized yeast cells were obtained, by the extrusion method using KCl as cross-linking agent, by Martinez et al. (2016) and Godia et al. (1987). K-carageenan particles were also obtained by the emulsification technique, which involves the dispersion of the polysaccharide aqueous solution in a non-miscible organic phase, followed by the gelation and the separation of microparticles. Since the k-carrageenan particles are not very stable it can be coated with a layer of chitosan in order to reduce the pore size leading thus to an increase of the gel mechanical and chemical stability (Raymond et al., 2004).

The yeast cells require a wide range of metal ions for their proliferation and metabolism and these ions have a significant impact on important parameters, such as: rate conversion of sugar into ethanol, the final yield in ethanol, growing and multiplying yeast, cell viability, stressors and yeast flocculation behavior (Walker, 1994; Cyert and Philpott, 2013). Cations of magnesium and zinc are mostly used as they act as cofactors for several important glycolytic enzymes and also as modulators of the stressors for yeast (Rautio et al., 2007; Gibson, 2011), such as: rapid fluctuations in temperature, high osmotic pressure, increased concentration in ethanol and lack of nutrients that adversely affects the metabolism of the yeast cells leading to an ineffective fermentation process (Ding et al., 2009; Zhao and Bai, 2009). Different studies have pointed out that the yeast cells tolerance to stressors is dependent on the nutritional composition of the fermenta-