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Yeast cells immobilized in spherical gellan particles cross-linked with magnesium acetate

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ABSTRACT

In this paper we report on the production of microbioreactors using ionically cross-linked gellan containing immobilized yeast cells with potential application in glucose fermentation. Cross-linking was achieved through a novel extrusion process in capillary by ionotropic gelation under the action of magnesium acetate. Compared to commonly used methods, this provides a host of practical advantages. The particles were physico-chemically and morphologically characterized as their mechanical stability, behavior in aqueous media, and bio-catalytic activity are influenced by the amount of cross-linker used. This demonstrated their ability to be reused in a large number of fermentation cycles without losing their bio-catalytic activity. Our results are wholly comparable with the behavior of free yeast. We show that fermentation cycles can succeed either immediately or at variable intervals, ensuring high yields of glucose transformation, comparable-if not superior-to results currently obtained using free yeast.

1. Introduction

Yeast cell immobilization on different supports for obtaining ethylic alcohol through fermentation has been studied for over four decades, and continues to attract research interest worldwide. The advantages of using yeast cells immobilized over their traditional method – in free form – are numerous. A short list includes: (i) cell protection from destruction via mechanical application, environmental factors, or toxic metabolites such as those produced by ethylic alcohol, which preserves viability and activity for long time periods (Kandylis et al., 2012; Torresi et al., 2011); (ii) increased productivity of fermentation equipment by increasing the population density of the cells and the use of high substrate flow rates in the production process; (iii) reusing the biological catalyst (Tan et al., 2011); (iv) reducing contamination danger of the fermentation product by separating the biocatalyst at the end of the process through simple filtration; (v) the possibility of moving to continuous processes. This last method is preferred, due to its high efficiency, productivity, and lower operating costs (Kourkoutas et al., 2005).

Yeast cell in the food immobilization remains much explored industry as it is a key driver for wine and beer production which rely upon continuous fermentative processes. The techniques involved in microbial cells encapsulation – especially in the polymer supports – 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) and gel entrapment. Each of these techniques provides benefits and drawbacks. Choosing the optimal working technique depends upon the encapsulation support used, the immobilized microorganism, and the immobilized product properties. For microbial cells, inclusion in spherical polymeric particles with hydrogel character using the extrusion technique is the most often chosen due to mild processing conditions which allow optimal encapsulation while maintaining microorganisms viability (Park and Chang, 2000; Nedovic et al., 2005; Koyama and Seki, 2004).

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The most commonly used materials for immobilization are alginates (Fumi et al., 1987; Yoo et al., 1996; Corton et al., 2000; Idris and Wahidin, 2006), carrageenan (Rathore et al., 2013), ceramic or glass carriers (supports) (Goncalves et al., 1992), cellulosic materials (Bardi and Kountinas, 2004), γ-alumina (Loukatos et al., 2000), silica sol-gel films (Inama et al., 1993), polyacrylamide gels (Aykut et al., 1988) and combinations of various materials (Peinado et al., 2006).

The alginate gels are commonly used but show certain disadvantages such as low mechanical stability and reduced stability in the presence of chemical compounds, which can determine the diffusion of a large number of yeast cells along with their proliferation (Gerbsch and Buchholz, 1995; Maicas 2001; Callone et al., 2008; Brownlee et al., 2009).

Another approach is based on the construction of covalent polymer networks around biomolecules or cells which permanently remain trapped in the matrix (Gill and Ballesteros, 1998; Gill and Ballesteros, 2000-part 2; Gill and Ballesteros, 2000-part 1). Silica-based materials display some physical and chemical properties such as Si—O bond strength (425 J mol-1), which provides material stability. It is posible to obtain porous materials with suitable porosity for mass transport, the cells being well caught in the matrix.

Sol-gel immobilization requires several steps and the Si-O bond formation around the yeast cells or cell aggregates could be harmful due to the potential interactions between silica and biological agents. This could represent a negative aspect in the food industry. To prevent this drawback hydrogel particles of alginates with immobilized yeast cells have been prepared by ionic crosslinking, using various cations such as calcium and barium, subsequently coated with a layer of silica gel. Thus, the yeast cells are protected through the alginate matrix, and the layer of silicon on the surface of the particles provides stability and their ability to be used in continuous fermentation processes (Callone et al., 2008).

The low structural stability of the alginate particles with immobilized yeast cells motivated the research for using synthetic polymers as matrices for cell immobilization. Călinescu et al. (2012) prepared two supports for the immobilization of yeast cells using alginates and polyacrylamide. The polyacrylamide gel was widely used to immobilize several types of microbial cells (Dinu et al., 2007). The matrices with immobilized yeast cells prepared were tested in terms of fermentative activity and it was shown that polyacrylamide gel has a fermentation activity comparable with the alginates particles, but the polyacrylamide matrix has exhibited a better stability (Călinescu et al., 2012).

Aside from the large amount of biomass that needs to be incorporated, the supports chosen for the yeast cells immobilization must have other important features, such as mechanical and chemical stability, should be non-toxic and biocompatible with the cells, and have a high diffusion coefficient for both substrates (Baptista et al., 2006).

The polysaccharides remain the main materials used in microbial cell immobilization due to their biocompatibility and lack of toxicity. Gellan is the most recent polysaccharide to find broad use as a gelification agent in the food industry (Morris et al., 2012; Hasheminya and Dehghannya, 2013). It is a linear anionic biopolymer with repeating tetrasaccharide sequences which are made of two β-D-glucose residues: one of β-D-glucuronic acid and one of α-L-rhamnose in the ratio 2:1:1 (O'Neill et al., 1983). It is a biodegradable polymer-free of toxicity-and has a stable pH value ranging from 2 to 10 (Moslemy et al., 2003). There are, however, few studies dedicated to obtaining hydrogels based on ionically cross-linked gellan, designed to include yeast cells. Tan et al. (2011) obtained $CaCl_2$ cross-linked gellan microparticles containing encapsulated yeast cells (microbioreactors) through an emulsifying method and demonstrated the possibility of using the microbioreactos in multiple fermentative cycles. The microbioreactors proved to be stable, easy to recover by filtration from the fermentation medium, and can be reutilized for at least 10 fermentation cycles with a relatively high ethanol yield. Ethanol production in the first three fermentation cycles was comparable to that obtained with a single free yeast fermentation (Tan et al., 2011). This biocatalyst holds genuine promise for use in sparkling wine production technology; it is composed of gellan spherical particles in which yeast cells have been immobilized, (as proposed by Mantaluta et al., 2012). The particles were obtained by extruding the gel formed through a capillary in a cross-linking bath that contained $CaCl_2$ solution with a concentration of 2% (used as ionic cross-linking agent).

The yeast, especially *Saccharomyces* species, is usually selected due to its features: production of ethanol, high capacity of fermentation, tolerance to ethanol and other inhibitors, ability of rapid growth under anaerobic conditions which are characteristic to the fermentation equipments widely used in the food industry (Ivanova et al., 2011).

The yeast cells require a wide range of metals for their growth and metabolism (Walker, 1994; Youatt, 1993; Udeh et al., 2014). The bioavailability of metal ions in the fermentation medium is an important factor that affects yeast cell physiology and ethanol production in fermentation processes (Udeh et al., 2014).

In the present work, we obtain hydrogel – like spherical particles containing yeast cells, based on ionically cross-linked gellan – ionotropic gelation – through an extrusion process using magnesium acetate as the cross-linking agent. As far as we can tell, the scientific literature makes no mention of obtaining such particles with immobilized yeast cells. We have chosen this specific ionic cross-linker due to the advantages $Mg(CH_3COO)_2$ has over $CaCl_2$.

CH_3COO^- ion, as opposed to the Cl^- ion, promotes a shift in equilibrium of the ionic chain-reaction towards forming the network, which leads to more mechanically stable structures.

For concentrations under 0.7% (mass) the acetate ion, does not affect the cell's viability, in direct contrast to Cl^- ion's behavior.

The benefits of using magnesium ions for cell growth and fermentation processes are manifold. The magnesium ion, most abundant in the yeast cells, is an important divalent metal ion that is involved in a wide range of metabolic processes (Elin, 1994; Hartwig, 2001; Cyert and Philpott, 2013). This ion constitutes about 0.3% of the entire quantity of dry yeast and activates over 300 enzymes (Rees and Stewart, 1997; Walker et al., 2006; Bose et al., 2011). Maintaining the cell integrity, which includes a structural stabilization of the nucleic acids, polynucleotides, chromosomes, polysaccharides and lipids, was attributed to the magnesium ion (Birch and Walker, 2000). Magnesium is actively absorbed by the yeast cells from the fermentation medium to perform physiologically essential roles (Udeh et al., 2014). It is required for the activation of some glycolytic enzymes such as glucokinase, glucose-6-phosphate dehydrogenase, phosphoglycerate kinase and enolase, and its role in cell growth and cell protection during fermentation processes is well documented (Birch and Walker, 2000). Magnesium ion deficiencies lead to a sluggish metabolism in the yeast cells and a may slow or stop the fermentation processes (Udeh and Kgatla, 2013; Udeh et al., 2014).

Finally, we note a key positive attribute: the cross-linker lacks toxicity.

The micro-bioreactors obtained preserve the cell viability for a long period of time (over 87.93% after 48 h of storage in double distilled water) and protects yeast cells from the toxic metabolites produced by ethanol (the cell viability is over 88% after a period of 84 h of storage in an alcoholic solution with a concentration of 100 g ethanol/l).

The particles we obtained, characterized from the physico-chemical and morphological points of view, are mechanically stable (form, size), and possess the capacity for being reutilized in mul-