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Development of natural polymer beads for a variety of technological applications

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ABSTRACT

Alginate is a natural polymer extracted from various species of brown seaweed. Ca-alginate beads are used extensively in various industries such as in food processing to produce restructured foods, in biotechnology for immobilization of cells or enzymes and in environmental applications for removal of heavy metals. The simplicity of this polymer regarding to the ability to form gel beads in mild conditions and its biocompatibility and no-toxicity have stimulated Ca-alginate beads application in different areas. An evaluation of potential new industrial applications of Ca-alginate beads with different species entrapped is provided, based on the authors 5 years of experience in development of polymeric beads. In all evaluated applications Ca-alginate matrix could retain its properties after entrapping the species showing a different bead surface morphology to each case. On the other hand, the properties of the entrapped species showed less activity than when not entrapped. Due to the various benefits associated to the immobilized/encapsulated systems, such as reuse of species several times, protection to unstable compounds, etc. the development of polymeric bead with entrapped species may compensates these losses.

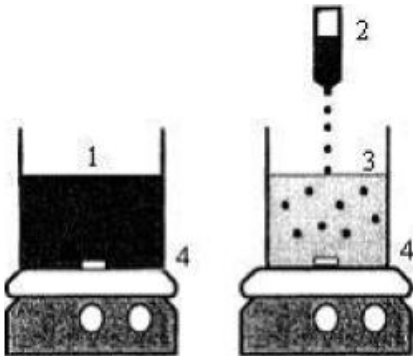
KEYWORDS

Potential industrial applications;
Polymer gel;
Ca-alginate entrapment;
Biopolymer.

INTRODUCTION

Alginate is a natural polymer extracted from various species of brown seaweed, generally greater than 130 kDa in size. It is composed of two acidic monomers: (1→4)-linked α-L-guluronate (G) and (1→4)-linked β-D-mannuronate (M). The residues are arranged in irregular blocks along a linear chain. The gel properties of alginate are largely due to cation bridges between adjacent molecules. Therefore, addition of divalent cations, (mainly Ca^{2+} and Mg^{2+} ions) facilitates gel formation. The binding of Ca^{2+} by alginate has been shown to be almost entirely due to the chelation of the cation by G-block regions of the polymer, this results in the formation of a three-dimensional gel network usually described by the "egg-box model"[1,2]. These properties were used to develop Ca-alginate beads with desired constituents for diverse purposes.

Entrapment is one of the simplest methods used for inclusion of species within polymeric matrices. The entrapped of compounds like drugs, cells, enzymes, flavours, among others, in Ca-alginate beads have been



(1) Na-Alginate+specie, (2) Syringe and needle, (3) $CaCl_2$ solution, (4) Magnetic stirrer

Figure 1 : Scheme of the specie immobilization/encapsulation by entrapment in Ca-alginate beads

extensively studied[3]. The simplicity of this polymer regarding to the ability to form gel beads in mild conditions and its biocompatibility and no-toxicity have propelled Ca-alginate beads application in different areas.

The entrapment of a specie or a mix of species in a polimeric matrix is interesting for different purposes. The traditional cell entrapment in Ca-alginate, for example, have been usually considered as an alternative for increasing the process overall productivity and for minimizing production costs mainly due to the possible cell reuse for several times[4,5].

The present study discusses and compares the results obtained by the development and application of different species entrapped in Ca-alginate gel beads for different purposes, based on the author's 5 years of experience: (1) yeast *Candida guillermondii* entrapment for biotechnological conversion of xylose-containing hydrolysate originating from hemicellulosic fraction of sugarcane bagasse, to xylitol; (2) rose bengal sensitizer entrapment for photo-oxidation of 1,5-dihydroxynaphthalene to juglone; (3) TiO_2 catalyst entrapment for photo-degradation of methylene blue dye; (4) anthocyanin pigments entrapment for their stabilization. All species studied were immobilized/encapsulated by entrapment in Ca-alginate beads by the same method.

EXPERIMENTAL

Specie preparation

Yeast *Candida guillermondii*

Candida guillermondii FTI 20037 entrapment procedure was done at the Biotechnology Department of the University of São Paulo (Lorena, SP, Brazil). An adequate volume of a cell suspension was added to a solution of sodium alginate (SG 1100, Saltgine, SKW Ltd., France), previously sterilized at 121°C for 15 min. The final concentration of cells was 3g/L (dry weight). A bioreactor, described by Sarrouh et al.[3], was loaded with 1.5L fermentation medium rich in xylose originating hemicellulosic fraction of sugarcane bagasse, containing 300g (20% of reactor) of entrapped cells in Ca-alginate beads and 1.2L of treated hydrolyzate supplemented with nutrients.

Rose bengal sensitizer

Rose Bengal sodium salt solution (Sigma Aldrich, Ireland) entrapment procedure was done in School of Chemistry at Dublin City University (Dublin, Ireland). An adequate volume of a Rose Bengal was added to a solution of sodium alginate (SG 1100 Saltgine, SKW Ltd, France). The final concentration of rose bengal was 5g/L (dry weight). A photoreactor, described by Santos et al.[2], was loaded with a 100mL solution containing 0.16 g of 1,5-dihydroxynaphthalene dissolved in 2-methyl-2-butanol (t-amyl-alcohol) fresh or/and recycled and the required amount of entrapped rose bengal in Ca-alginate beads (5, 10 and 20 % of photoreactor). A chromatography column was prepared to purify the product (juglone) using a 3:1 mix of cyclohexane/ethyl acetate.

TiO_2 catalyst

TiO_2 (Titanium (IV) oxide, anatase powder, 99.8% (metal basis), Sigma Aldrich, Ireland) entrapment procedure was done in School of Chemistry at Dublin City University (Dublin, Ireland). An adequate volume of a TiO_2 solution was added to a solution of sodium alginate (SG 1100 Saltgine, SKW Ltd, France). The final concentration of TiO_2 was 2.5g/L (dry weight). A photoreactor, described by Albarelli et al.[6], was loaded with a 200mL solution of methylene blue dye (used to simulate textile processing dyes) at concentration of 20μM/L (Methylene Blue Hydrate, Fluka Chemika, Ireland) and the required amount of TiO_2 entrapped in Ca-alginate beads (10, 20 and 40% of photoreactor).

Anthocyanin pigments

Anthocyanin Pigments entrapment procedure was

done at the School of Chemical Engineering at University of Campinas (Campinas, SP, Brazil) using anthocyanin pigments extracted from jabuticaba (Myrciaria cauliflora) peels in Faculty of Food Engineering at University of Campinas (Campinas, SP, Brazil). An adequate volume of anthocyanin solution was added to a solution of sodium alginate (SG 1100 Saltgine, SKW Ltd, France). The final concentration of anthocyanins was 10μg/L (dry weight).

Figure 2 : Digital picture of Ca-alginate beads

Specie entrapment in polymeric alginate beads

All species were immobilized/encapsulated by entrapment in calcium alginate beads by dripping an adequate volume of a solution 2% (w/v) of sodium alginate and the specie solution, using a 19-G needle (1.5 inch) and a 10mL syringe. Figure 1 shows a scheme of the entrapment method. The beads were maintained in the $CaCl_2$ solution at 4°C for overnight. They were then washed with distilled water and dried. The beads containing *Candida guillermondii*, rose bengal and TiO_2 after drying procedure were introduced into the reactor (bioreactor or photoreactor).

Analytical determinations

Biotechnological process application

Xylose, glucose, acetic acid, ethanol, xylitol and arabitol concentrations were measured by HPLC model LC-10-AD (Shimadzu, Tokyo, Japan), equipped with an Aminex HPX-87H (300×7.8 mm) column (Bio-Rad, Hercules, CA, USA) and a refractive index RID 6 A detector. Samples were previously filtered through a Sep Pak C18 filter and injected in the chromatograph under the following conditions: injection volume of 20μL, column temperature of 45°C, 0.01 mol/L H_2SO_4 as the mobile phase used at a flow rate of 0.6mL/min.

Free and immobilized cell concentrations were determined by absorbance at 600nm using a spectrophotometer, model DU 640B (Beckman Coulter, Fullerton, CA, USA) and correlated with the cell dry weight through a corresponding calibration curve. The liquid phase of the samples taken during the fermentation runs was centrifuged (2000×g, 15 min) and the cells were resuspended in water for determination of the free cell concentration. Ca-alginate beads (0.2g) taken during the fermentation runs and previously dried with an absorbent paper were dissolvent in 2% (w/v) potassium citrate under agitation. The resulting suspension was centrifuged (2000×g, 15 min) and the cells were resuspended in water for determination of the immobilized cell concentrations.

Photochemistry application

The photo-induced conversion of 1,5-dihydroxynaphthalene to juglone was followed with thin layer chromatography (TLC) analysis using silica plates or/and ^1H NMR analysis. The spectra of the products were recorded on a 300 MHz Bruker (Billerica, MA, USA) DRX-300.

Photo-oxidation yields were quantified by gravimetry, weighing the products generated using an analytical balance.

Scanning electron microscopy (SEM) of the bead surface was performed with a Hitachi (Tokyo, Japan) S-3000N SEM. An acceleration voltage of 5kV was employed.

Wastewater treatment by photochemical process application

Absorbance measurements were performed using a Varian UV-Vis spectrophotometer with variable wavelength detector (Cary 50 UV-Vis, Palo Alto, CA, USA) to detect the photocatalytic degradation of methylene blue dye.

Scanning electron microscopy (SEM) of the bead surface was performed with a Hitachi (Tokyo, Japan) S-3000N SEM. An acceleration voltage of 5 kV was employed.

Stabilization of natural pigments application

Absorbance measurements were performed using a Varian UV-Vis spectrophotometer with variable wavelength detector (Cary 1G UV-Vis, Palo Alto, CA, USA) to detect encapsulation efficiency of anthocyanin pigments.

RESULTS AND DISCUSSION

When in contact with $CaCl_2$ solution, alginate forms a polymeric matrix capable of retaining its shape under stress and highly hydrophilic, been constituted of 99% of water[2]. By the dripping method adopted calcium alginate beads were formed with diameter varying form 2,8 to 3,2 mm (Figure 2). Independently of the specie entrapped no significant difference related to the diameter beads was observed. But, some differences in terms of bead shape were observed (Figure 3). Probably, the irregular shape is related to the manual dripping procedure. It could be avoid controlling the rate of solution dripping using a automatic nozzle spray for bead formation.

Figure 4 shows the surface of Ca-alginate beads after entrapment of different species, different surface aspects can be noticed (Figure 4A and 4B). Possibly, due the low molecular weight of TiO_2 (79.88g/mol), it could easily diffuse to the surface of the alginate bead showing a different surface morphology (Figure 4B). In contrast rose bengal has a high molecular weight (1017.64 g/mol) so its diffusion on polymeric matrix was restricted.

The Ca-Alginate bead system containing different species entrapped was analyzed in different processes regarding to its efficiency and well fit to the process conditions and necessities. The following pages show the results and discussion of each application.

Yeast *Candida guillermondii* entrapment

Yeast *Candida guillermondii* entrapment for biotechnological conversion of xylose-containing hydrolysate originating from hemicellulosic fraction of sugarcane bagasse, to xylitol, was evaluated.

Using a Fluidized Bed Reactor (FBR) a good xylose-to-xylitol bioconversion was achieved. A high xylitol concentration and immobilization efficiency was obtained.

In comparison with other immobilization support, such as zeolite and porous glass in a FBR system, higher aeration rates to improve the oxygen transfer into the immobilized cells have to be used. Due to this high aeration rate, a high concentration of total cells was obtained, which resulted in a decrease in the total fermentation time and an increase in the xylitol production rate.

The system exhibited a promising biotechnological alternative for xylitol production due to the following advantages: low cost of alginate as immobilization support, better rates of mass transfer and oxygen transfer in comparison with other systems, and the absence of beads abrasion found in Stirred Tank Reactor (STR) resulting from direct contact with the agitation turbines, which contributes to the increase in cell leakage from the alginate beads, according to previous works developed by other researchers[7].

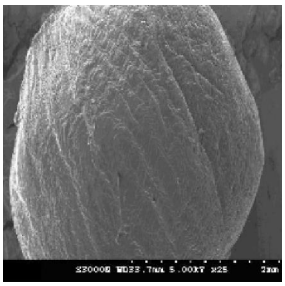
Increase in xylitol yield could be obtained by recycling the immobilized cells through a repeated-batch fermentation system, due to a possible cell adaptation to the toxic compounds present in the hydrolyzate medium[8].

Potentially, the use of polymeric alginate beads is more frequently applied to cell and enzime entrapment, then the application of this tool to other species and consequently other applications should be interesting.

Rose bengal sensitizer entrapment

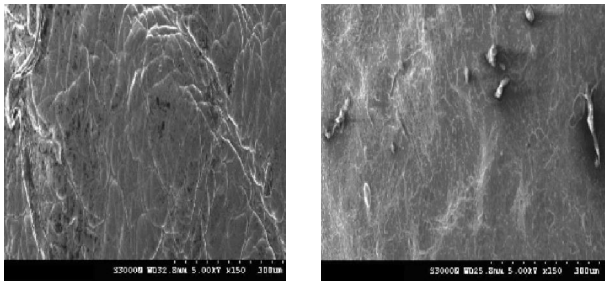
Rose bengal (RB) sensitizer entrapment for photooxidation of 1,5-dihydroxynaphthalene to juglone was evaluated.

To verify which concentration of $CaCl_2$ in the preparation of the beads should be used to prevent leaching



(A) (B)

Figure 3 : SEM image of bead shape (A) entrapped rose bengal, (B) entrapped TiO_2



(A) (B)

Figure 4 : SEM image of bead surface (A) entrapped rose bengal, (B) entrapped TiO_2

when in contact with t-amyl-alcohol, experiments using 2, 10 and 20% w/v of $CaCl_2$ solution were carried out. Probably the exchange of sodium ions from the Na-alginate with calcium and consequent gelation and cross-linking of the Ca-alginate were intensified by the increase in $CaCl_2$ concentration. Consequently this intensification resulted in the formation of a stronger gel preventing leaching.

Using the best preparation of the Ca-alginate beads (20% solution of $CaCl_2$) the effect of different concentration of beads (5, 10 and 20% of photoreactor) in the photo-oxidation reactor was evaluated.

The results suggest that the use of 5% of beads is the best proportion tested. Using this proportion of beads in the system configuration a good mixed was observed leading to better mass transfer during the photoreaction. It is known that the alginate gels imitate a semi-permeable membrane through which low molecular weight molecules can diffuse[9], so that better mixing could lead to better diffusion of raw material into the pores of the beads. When using 10 and 20% of beads the agitation was not sufficient to provide a good mixing to the system, and ended up stressing the beads, resulting in some degradation and consequently leaching of RB into the solution. In addition, the bubbled of air through the solution besides the mechanical stirring can also have contributed to the RB leaching. As a result a good photo-oxidation performance could be observed using 10 and 20% of beads due to the released RB suspended in the solution.

The experiment using suspended RB showed better results than the immobilized system. This was expected, immobilization of catalysts on supports often reduces the efficiency of the photocatalytic process as a consequence of a reduction in active surface area[10]. However, immobilized systems have advantages such as the possibility of scale up and/or continuous operation reduced costs and reduced waste[5].

To our knowledge, there are only few papers evaluating the use of sensitizer immobilization. In general, the use of immobilized sensitizer may facilitate easy removal of the sensitizer from the reaction, simplifying the recovery and purification processes.

TiO_2 catalyst entrapment

TiO_2 catalyst entrapment for photo-degradation of methylene blue (MB) dye was evaluated.

The effect of different proportions of Ca-alginate beads (10, 20 and 40% of photoreactor) on the photo-degradation was verified.

The results suggest that the best proportion studied is when 10% of the reactor volume is filled with beads. Also, it was observed that using this proportion of beads the system configuration was well mixed. When increasing the amount of bead inside the photoreactor it was observed that the mechanical agitation was not sufficient to mix the system well. All though no leaching was verified comparing with the photo-oxidation experiments using RB entrapped in Ca-alginate.

One more time, it was demonstrated that suspended species provided better results than those obtained with immobilized species. Even though, the immobilized system has several advantages as the possibility of scaling up the UV/TiO_2 process using an environmentally safe photocatalyst for processing of wastewater. The use of immobilized TiO_2 can be economically attractive because it eliminates the need of a post-process filtration step to remove the powder photocatalyst from treated water to disposal, enables the simple reuse of photocatalyst and facilitates the photoreactor configuration as a consequence of the easier light penetration at the immobilized system.

In order to study the recycling of TiO_2-gel beads for continued use, experiments using the same beads 3 times were carried out using a bead proportion of 10% of beads. An improvement on the degradation rate was observed during the reuses. A possible explanation is that, in the first use, the dye could not penetrate the beads' pores easily, on account of MB relatively large molecular size[11]. Hence, the degradation occurred mainly because of the MB reaction with TiO_2 near the bead surface.

It is known that using others species immobilized in Ca-alginate beads they can be recycled much more 3 times. Kumar and Chandrasekaran[12] using immobilized cells in Ca-alginate beads reported that they could be used up to 20 cycles in a packed bead bioreactor until its efficiency decreases. Using TiO_2 alginate beads in the photoreactor, the number of cycles without lost in efficiency should be smaller due the degradation caused by the mechanical stirring.

Anthocyanin pigments entrapment

Anthocyanin pigments entrapment for their stabilization was preliminary evaluated.

Anthocyanins extracted from vegetable sources are highly unstable and easily susceptible to degradation whose their color stability is strongly affected by pH, temperature, anthocyanin concentration and structure, oxygen, light, enzymes, among others[13-15].

Anthocyanin pigment encapsulation in polymeric alginate beads could help maintain its stability and improve shelf lives and effects.

The anthocyanin encapsulation efficiency was approximately 98%. This result demonstrates that encapsulation in Ca-alginate beads may be a good choice to protect and consequently stabilize these compounds.

The use of alginate beads containing such organic compounds is relatively new. Indeed, this polymeric matrix seems to be a promising alternative to stabilize this pigment and other unstable compounds extracted from nature. Further work will be done evaluating the entrapment of other compounds.

CONCLUSIONS

In all applications showed in this study, Ca-alginate matrix could retain its properties after entrapping the species. An analysis of the bead surface morphology has shown that surface characteristics are dependent of the entrapped specie. In all cases the entrapment of the species had decreased their activity reducing the efficiency of any process that use these polymeric beads. A mass loss of the active compound during entrapment process was observed in all applications evaluated. In spite of these negative points the various benefits related to the immobilized/encapsulated systems not only compensate them but also may enable the use of some species in new industrial processes and/or become more economic attractive to some processes.

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