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Study of Freeze-dried Chitosan Beads Encapsulating Live Lactic Acid Bacteria for Removal of Reactive Dyes ⋆

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Abstract

Water pollution by the discharge of dye residue has been polluting the planet for decades. Chitosan is one of the natural abundant biomasses that show excellent adsorption toward colorants. Nevertheless, sophisticated chemical modifications of chitosan are inevitable to enhance physical and chemical stabilities in practical applications. In the present study, simple freeze-drying has been adopted to chitosan (CHI) beads showing good stability at low levels of pH. The results show complete decolourisation of Reactive Blue 19 (RB19) within 2 hours at pH 3 and temperature of 37 °C. The encapsulation of live bacterium *Lactobacillus casei* (*L. casei*) into chitosan beads for dyestuff removal is also evaluated. Kinetics studies show a faster initial rate of adsorption by *L. casei*-chitosan beads at pH 3 and temperature of 37 °C. The adsorptions partially follow the intraparticle diffusion mechanism.

Keywords: Chitosan Beads; Freeze-drying, *Lactobacillus Casei*; Reactive Blue 19; Removal

1 Introduction

Water pollution is one of the key issues in the world. Besides organic pollutants and heavy metal ions, textile dyes are one of the pollutants that are causing serious environmental problems. A few ppm scale concentration of dyes already expresses high colour intensity which alters the light penetration to the aqua ecosystem [1]. The pollution spreads to agriculture and humans in developing countries without proper water treatment facilities. Since decades ago, research has been focusing on the use of environmentally friendly and cost effective biomasses to remove polluting colourants in water [2]. Among numerous biomasses (plant origins, industrial wastes, fruits peels and pits, etc.), chitosan has shown an excellent adsorption capacity to synthetic dyes, giving 10^2 to even 10^3 mg g^{-1} of removal [3]. However, since chitosan dissolves in acidic medium,

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chemical modifications are usually required to maintain its chemical and physical stabilities during the adsorption process. The modifying partners are usually bi-functional and reactive toward the —NH_2 group in chitosan, such as glutaraldehyde, epichlorohydrin, etc. The modification processes are sometimes complicated and might involve toxic chemicals.

Another popular type of adsorbent is microbial biomass [4]. The application of bacteria and fungi in the removal of water pollutants has been intensively studied for years. The microbial cells adsorb synthetic dyes by the functional groups on the cell wall and chemical modifications can even enhance the adsorption capacity. Although excellent biosorption capacity can be achieved, the separation of microorganisms after water treatment in industrial plants remains problematic as clogging of parts might occur [5]. Moreover, the harsh bulk environments are threatening if it is necessary for the microorganisms to be alive during the water treatment process.

In the present study, an environmentally safe ionotropic crosslinking agent, sodium triphosphate penta-basic (NaTPP), has been used to form chitosan beads followed by a simple freeze-drying process. Freeze-drying the chitosan beads stabilizes the viability of the encapsulated bacterium (*Lactobacillus casei*) during storage or before any adsorption process. Immobilization of the microorganisms by polymeric supports is one of the most effective methods to solve the problems in the industry while maintaining satisfactory adsorption capacity toward pollutants.

2 Experimental

2.1 Chemicals

Chitosan (practical grade, from shrimp shells, deacetylation $\geq 75\%$), sodium triphosphate penta-basic ($\geq 98\%$), potassium bromide (KBr, FTIR grade), and Remazol Brilliant Blue R (Reactive Blue 19, dye content 50%) were obtained from Sigma-Aldrich LLC. Glacial acetic acid was obtained from VWR International LLC. Fig. 1 shows the molecular structure of Reactive Blue 19.

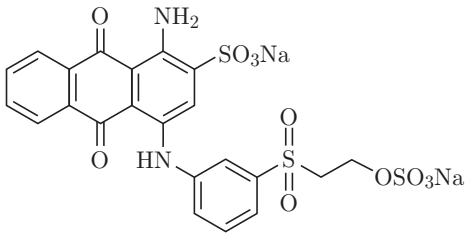

Fig. 1: Molecular structure of Reactive Blue 19 (RB19)

2.2 Microorganism and Culturing

Lactobacillus casei (*L. casei*, ATCC 393) was obtained from ATCC Co. The culture medium (de Man, Rogosa and Sharpe (MRS)) broth and agar were obtained from BD Co. The bacterium was rehydrated and inoculated in accordance with standard procedures. Cultured *L. casei* was harvested after inoculation of 5 days at a temperature of 37 °C followed by 10 minutes of centrifugation at 6000 rpm under a temperature of 4 °C. The bacterium pellets were subjected