Available online at www.sciencedirect.com
ScienceDirect
Bioresource Technology 99 (2008) 1274–1279
BIORESOURCE TECHNOLOGY

Nitrate and phosphate removal by chitosan immobilized *Scenedesmus*

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Received 26 August 2005; received in revised form 8 February 2007; accepted 10 February 2007
Available online 24 May 2007

Abstract

The effect of chitosan immobilization of *Scenedesmus* spp. cells on its viability, growth and nitrate and phosphate uptake was investigated. *Scenedesmus* sp. (strains 1 and 2) and *Scenedesmus obliquus* immobilized in chitosan beads showed high viability after the immobilization process. Immobilized *Scenedesmus* sp. strain 1 had a higher growth rate than its free living counterpart. Nitrate and phosphate uptake by immobilized cells of *Scenedesmus* sp. (strain 1), freely suspended cells and blank chitosan beads (without cells) were evaluated. Immobilized cells accomplished a 70% nitrate and 94% phosphate removal within 12 h of incubation while free-living cells removed 20% nitrate and 30% phosphate within 36 h of treatment. Blank chitosan beads were responsible for up to 20% nitrate and 60% phosphate uptake at the end of the experiment. Chitosan is a suitable matrix for immobilization of microalgae, particularly *Scenedesmus* sp., but this system should be improved before its application for water quality control.

Keywords: Microalgae; Immobilization; *Scenedesmus*; Chitosan; Nutrient removal

1. Introduction

Eutrophication has become a major problem in water quality management. Microalgal wastewater treatment systems have a high efficiency level at removing nutrients however, these systems also have disadvantages, such as costly biomass harvesting and the need for large culturing areas (Oswald, 1988). Microalgae immobilization in polymeric matrices has become an attractive alternative that facilitates the biomass recovering and provides a greater operational flexibility (Mallick and Rai, 1993). Moreover, immobilized-microalgae systems prevent biomass from being washed out and grazed by herbivores, and allow hyperconcentrated cultures (Chevalier and de la Noüe, 1985a). A constant metabolic activity over long time periods for immobilized microalgae has also been reported (Chen, 2001).

In addition to wastewater treatment, immobilized algae systems have several applications, which include metal removal, stock culture management and, production of useful chemicals (Cohen, 2001; Robinson et al., 1986). The immobilization matrix could be a synthetic polymer (e.g. acrylamide, polyurethane), or a natural polymer (alginate, carrageenan, agar, collagen); but it must fulfill various requirements, such as photo-transparency, non-toxicity, retention of cellular viability, and stability in the culture medium (Mallick, 2002).

Lau et al. (1997) pointed out that there was a considerable removal contribution from carrageenan matrices in addition to microalgal assimilatory processes. The anionic groups in carrageenan gel adsorbed ions and concentrated them near gel entrapped cells. Hence, the sort of polymer used for immobilization has a considerable effect on nutrient efficiency removal. Recently, most studies regarding nutrient uptake by immobilized microalgae have used alginate as immobilization matrix; there have been however only minimal testing conducted on others biopolymers of interest, such as chitosan (Aguilar-May, 2006). Chitosan is obtained mainly from chitin, the second most abundant

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doi:10.1016/j.biortech.2007.02.043

organic compound in nature as it forms the exoskeleton of crustacean and other organisms (Yan-Tan and Lee, 2002). Often, it is obtained from shrimp shells, a waste by-product in shrimp food industry.

Interaction between immobilized algae (or any organism) and the matrix, may include effects on cellular physiology. These effects could be either synergistic, antagonistic, or neutral for algal cells (Robinson et al., 1986). Since chitosan immobilization has not been investigated as extensively as that of alginate, we considered it necessary to evaluate immobilized cells viability in addition to their growth and nutrient removal. Furthermore, the chitosan immobilization method used in this work includes very drastic changes of pH, an important factor for cellular physiology. In the present work, we examine the effect of chitosan immobilization of *Scenedesmus* spp. cells on its viability, growth and nitrate and phosphate uptake. A comparison with that of freely suspended cells was also done.

2. Methods

2.1. Organisms and culture conditions

Monospecific and non-axenic cultures of two strains of *Scenedesmus* sp. and *Scenedesmus obliquus* isolated from an hypertrophic environment (in Ensenada, México), were obtained from the Centro de Investigación Científica y de Educación Superior de Ensenada, México. The microalgae were cultivated in medium "f" (Guillard and Ryther, 1962) and kept at 32 ± 1 °C with a constant light intensity of 43 μmol m^{-2} s^{-1} in climatic chamber (VWR Scientific, model 2015).

2.2. Immobilization of algal cells

Chitosan flakes for immobilization were obtained from shrimp shells, and provided by the "Laboratorio de Ingeniería de Biopolímeros del Centro de Investigación en Alimentación y Desarrollo" (CIAD), Hermosillo, México. The chitosan solution was prepared by mixing chitosan flakes with 1% acetic acid and its pH was adjusted to 4. The cells of each strain were harvested in their exponential phase of growth by centrifugation at 2465*g* for 10 min, resuspended and mixed with chitosan solution to give a final concentration of 2% and a desired cell density which is specified in the following section.

Chitosan beads were obtained by dropping the chitosan–algal mixture into a solution of 0.1 N NaOH (pH 12) using a burette (Rodríguez-Sánchez and Rha, 1981). Because of the high viscosity of chitosan solution, the burette top was connected to an air pump (Hagen Optima, Mansfield, MA, USA) to increase dripping rate. The chitosan–cells mixture was allowed to drip for 2 min, and then beads were subsequently left in agitation 3 more minutes, and picked up with a mesh. These beads were washed off several times with sterile distilled water until their pH reached values between 7 and 8. To reduce the contact time of cells with NaOH solution (pH 12), this procedure of dropping (for 2 min), agitation (3 min), and washing, was repeated until the chitosan–cells mixture was completed. Blank beads (without microalgae) were made for control treatments.

2.3. Viability

Chitosan immobilized microalgae *Scenedesmus* sp. strains 1 and 2, and *S. obliquus* were cultivated monospecifically in batch using assay tubes with 10 mL of "f" medium and maintained under the same culture conditions as described in Section 2.1. A sample of 10 beads of each triplicate was taken. The viability was assessed qualitatively by observation of algal colonies, its abundance and color, intracellular content and refringency of chitosan immobilized cells, using a stereoscopic microscope (ZEISS Stemi 2000-C, Thornwood, NY, USA) and a light microscope (Nikon Labophot-2, Japan). Absence of colonies, no intracellular content and chlorotic pigmentation were judged as low viability features. If we encountered a high percentage of these features (>30% of beads), the experiment would be repeated changing critical variables of the immobilization process, such as contact time with sodium hydroxide.

2.4. Growth assessment

Biomass increments of the immobilized microalgae *Scenedesmus* sp. strains 1 and 2, and *S. obliquus* in chitosan beads were compared to those of their free cell counterparts. Chitosan–algal beads were prepared as described in Section 2.2. The cellular density of each strain used for immobilization was 1×10^6 cells mL^{-1} in each replicate. The same cellular density was used in the control treatment of free cells. All treatments (blank beads, immobilized and free cells), were cultured by triplicate in 250 mL Erlenmeyer flasks with 100 mL of medium "f" and 250 beads were placed in each flask. The cultures were maintained under the same culture conditions (as in Section 2.1). Every day a sample of three beads from each replicate was collected and dissolved with 1% acetic acid (pH 4.0). Samples from free cell cultures were fixed with lugol. The solutions of re-dissolved chitosan–algal beads were sonicated at 60 MHz for 1 min to dislodge microalgal colonies for counting by hematocytometer.

2.5. Nutrients removal

Entrapment of *Scenedesmus* sp. strain 1 cells was made with a cellular density of 5.85×10^6 cells mL^{-1} of chitosan solution, and as described in Section 2.2. Chitosan beads were stored without nutrients by 3–5 days at room temperature (25 °C) until their utilization. The chitosan–algal beads, freely suspended cells and blank chitosan beads without cells were cultured in 1 L Erlenmeyer flasks containing 500 mL of medium "2f" with 44 mg L^{-1} nitrate and 6 mg L^{-1} phosphate. A fourth treatment with only