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Immobilization of anaerobic sludge using chitosan crosslinked with lignosulfonate

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A new method for immobilization of anaerobic sludge in chitosan is described. It is based on the reaction of basic NH_2 groups of chitosan with the acidic sulfonic-groups of lignosulfonate to form sulfonilamide linkages. The new procedure features simplicity, low-cost and mild immobilization conditions. Batch tests of acetate consumption along with a continuous reactor operation confirmed the effectiveness of the immobilization technique for maintenance of long-term stability of the polymer.

Keywords: cell immobilization; chitosan; anaerobic sludge; lignosulfonate

Introduction

Recently there have been several reports on the successful immobilization of bacteria for potential application in wastewater treatment. The examples include immobilization of nitrifying, denitrifying and anaerobic sludges in carrageenan, alginate [7] and polyvinyl alcohol [4]. Sludge immobilization allows for better retention of slow-growing bacteria, provides high loading rates, and minimizes washout. In addition, the immobilization procedure protects microorganisms from severe concentration spikes of toxic compounds [3].

Each of the above-mentioned polymers has its own advantages and drawbacks. If these polymers were evaluated based on cost, toxicity of the immobilization procedure and long-term stability, none of them would be considered ideal. In addition, biological wastewater treatment is quite different from controlled cultivation with well-defined media: for example, the influent often contains various ions that may interact with the polymer.

In this work we developed a new procedure of sludge immobilization using chitosan crosslinked with lignosulfonate. The existing procedures of chitosan polymerization use either high pH or toxic compounds like glutaraldehyde or glyoxal [2]. The newly developed procedure features mild immobilization conditions and high stability of the chitosan-lignosulfonate bioparticles. The latter is of particular importance since wastewater treatment requires extensive and inexpensive reactor operation.

Materials and methods

Chemicals

Chitosan (high molecular weight) was purchased from Fluka Chemical Corp (Ronkonkoma, NY, USA). Lignosulfonate (lignosite 458) and Lignin (Indulin AT) were provided at no charge by Georgia-Pacific Corporation (Bellingham, WA, USA) and Paprican (Pointe-Claire, Quebec, Canada), respectively. All other chemicals were of reagent grade.

Microorganisms and media

Anaerobic sludge was obtained from an Upflow Anaerobic Sludge Bed (UASB) reactor treating wastewater from a food industry (Champlain Industries, Cornwall, Ont, Canada). Prior to immobilization, the sludge was homogenized for 60 s using a Kinematica CH-600 homogenizer (Kinematica GmbH, Lucerne, Switzerland). The stock nutrient and microelement solutions were as given in [8] with glacial acetic acid (200 g L^{-1}) as carbon source.

Immobilization procedure

The procedure of sludge immobilization in chitosan was as follows: thoroughly homogenized anaerobic sludge was mixed with a solution of chitosan in acetic acid (3%) to obtain a final chitosan concentration of 1.3–1.8% (w/v). Prior to immobilization, the pH of the chitosan solution was adjusted to 4.5 in order to avoid cell damage. The resulting mixture was added dropwise to a solution of lignosulfonate (3.5–14%) in water using a peristaltic pump and was stirred for 2–3 h to harden the beads. The pH of the solution was maintained at a constant value using Trizma baze (Sigma, St Louis, MO, USA) and a pH-controller (Cole Parmer, Niles, IL, USA). The resulting beads of 2–2.5 mm diameter were washed with distilled water and transferred into a nutrient solution containing 2–5 g L^{-1} of acetate.

Reactor setup and operation

Chitosan-immobilized sludge was cultivated in a 1.2-L Upflow Sludge Bed and Filter (USBF) reactor connected to a 0.5-L aeration column [8]. The reactor was operated at a hydraulic retention time of 20 h, a liquid upflow velocity of 2–6 m h^{-1} and a temperature of 25°C. The pH controller was capable of maintaining the pH at 7.3 ± 0.2 using a Trizma base solution.

Results and discussion

Figure 1 shows the chemical structure of chitosan crosslinked with lignosulfonate. It is hypothesized that the amino

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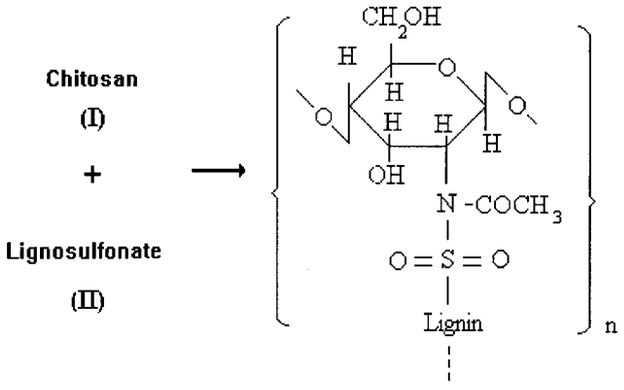

Figure 1 Proposed mechanism of the formation of chitosan crosslinked with lignosulfonate.

groups of chitosan (I) react with the acidic sulfonic-groups of lignosulfonate (II) to form sulfinylamide linkages. Lignosulfonate does not have a defined structure. It does however, contain a variety of other acidic groups, such as phenolics (—OH), and/or carboxylic groups which may also react with the basic NH_2 groups of chitosan to form amide linkages (—NH—C═O). Since initial attempts of polymerization with non-sulfonated lignin failed to give stable beads, we conclude that these alternative cross-linking mechanisms are not important in producing a stable polymer structure.

To verify the existence of sulfonilamide linkages IR-analysis was carried out between 2.5–20 μm. The presence of the sulfonilamide linkages was confirmed by comparing the IR-spectrum of the starting materials (I and II) with that of the crosslinked polymer. The library of IR spectra (p. 1175 in Ref. [1]) suggests a characteristic band for sulfonilamide compounds at $\lambda = 3.4$ μm. This band was clearly observed in the IR-spectrum of the crosslinked polymer.

Examination of a bead using light microscopy revealed formation of a polymer capsule (Figure 2). This is probably attributable to the high molecular weight of lignosulfonate which ensures that it cannot penetrate inside the chitosan beads. The strength of the polymer film was found to be dependent on two major factors: the concentration of lignosulfonate and the pH of the hardening solution. Since the mixture of sludge and chitosan had a pH of 4.5, its injection into the lignosulfonate solution decreased the pH. Consequently, a pH-controller was installed to maintain a constant pH.

A number of immobilization tests were carried out at pH values between 6 and 8 and concentrations of lignosulfonate between 3.5 and 14%. The chitosan : sludge ratio in these experiments was 3 : 1. To test the stability of immobilized sludge, the beads were placed in 120-ml serum bottles containing Trizma buffer (pH 7.5) and agitated at 100 rpm. The beads were visually inspected on a daily basis over 5 days. Overall, stronger beads were obtained using 10% (w/v) lignosulfonate solution (or greater) and a pH between 7.2 and 7.7. Outside of these limits the beads did not retain their integrity.

To verify survival of bacterial populations after the immobilization, batch activity tests of acetate consumption were performed according to the procedure described in [8]. The specific rate of acetate consumption by immobilized sludge was found to be 146 ± 12 mg acetate (g VSS)$^{-1}$ day^{-1}. This value is in agreement with the value of 189.6 ± 47 mg acetate (g VSS)$^{-1}$ day^{-1} that was determined for the sludge before immobilization.

While the immobilization tests demonstrated good short-term stability of the polymer, the long-term bead stability may be affected by bacterial metabolism and biogas production. In particular, natural sludges contain a wide variety of microorganisms which may biodegrade various polymers [5]. Thus, the long-term stability of the chitosan-immobilized sludge was determined using a continuously operated modified USBF reactor. Modifications were made to provide oxygen supply for aerobic bacteria as described in [8]. The reactor was loaded with 300 g of immobilized sludge and operated over a 5-week period. In this reactor aerobic and anaerobic metabolisms co-existed due to the natural oxygen gradient across the bead radius [6]. The acetate loading rate in the reactor was maintained at a level of 2.0 g L^{-1} day^{-1}. Intensive methane production inversely related to the aeration rate was observed (Figure 3). Visual inspection of the beads over the 5-week period of reactor operation showed that the beads remained intact. In

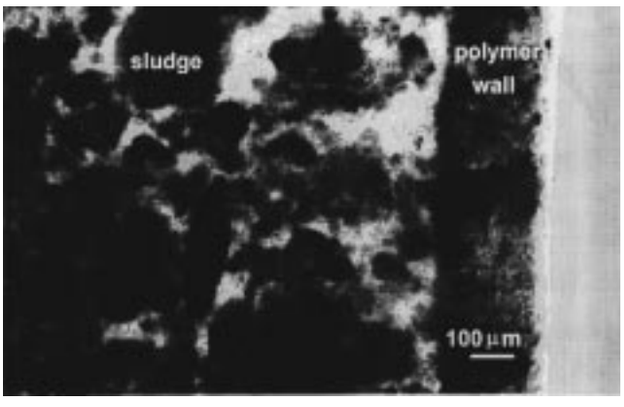

Figure 2 Section of a chitosan-lignosulfonate bead with a polymer wall.

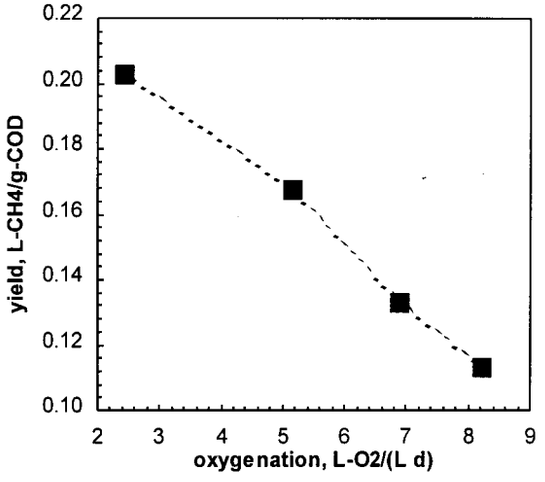

Figure 3 Methanogenic activity of immobilized sludge as a function of oxygenation rate.

addition, stability of chitosan-lignosulfonate beads was not affected during storage in solutions of phosphate and bicarbonate buffers [8] for 6 months at 4°C.

Conclusions

A new method for sludge immobilization using a chitosan-lignosulfonate support was developed. The method has the following advantages: (i) simple and low-cost immobilization procedure; (ii) mild immobilization conditions; (iii) high stability of bioparticles. When compared to the procedure of sludge immobilization in polyvinyl alcohol that requires 10–15% (w/v) concentration of the polymer, the newly developed method uses only a 1–1.5% (w/v) solution of chitosan.

Acknowledgements

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