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Biodegradability and mechanical properties of polycaprolactone composites encapsulating phosphate-solubilizing bacterium *Bacillus* sp. PG01

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Abstract

This study examined the feasibility of using polycaprolactone (PCL) and its composites (with starch and/or clay) in encapsulating cells of phosphate-solubilizing bacteria (PSB) for the development of biodegradable and ''controlled-release'' bacterial fertilizer. The PSB used in this work was an indigenous *Bacillus* sp. PG01 isolate. The results show that the PG01 strain was able to degrade all the cell-loaded capsules made of PCL and PCL composites, resulting in a continual cell release. Morphology observation indicates that severe disruption of the capsule structure occurred after incubation for 15–20 days. The biodegradability of the capsules decreased in the order of PCL/starch (20 wt%) > PCL/starch (20 wt%)/cay (7 wt%) > PCL alone > PCL/clay (7 wt%). Similar trends were also observed for the decrease in tensile strength and elongation at break, suggesting strong connections between biodegradability and the mechanical properties. Addition of starch appeared to enhance the biodegradability of the capsules, whereas the clay-blended composites were less biodegradable. The amount and rate of cell release from cell-encapsulated PCL-based capsules were positively dependent on the biodegradability and on the decrease in the mechanical strength. Nevertheless, the pattern of cell release was quite similar for all types of capsules. The outcome of this work seems to suggest that by proper manipulation of composite compositions, the controlled release of the bacterial fertilizer (i.e., *Bacillus* sp. PG01 cells) might be achievable.

Keywords: Bacterial fertilizer; Biodegradable material; Clay; Composite; Controlled release; Phosphate-solubilizing bacteria; Polycaprolactone; Starch

1. Introduction

In light of an increasing environmental threat arising from extensive uses of plastics, the development of biodegradable and recyclable alternatives to conventional plastics is of urgent demand [1,2]. Among the commercialized biodegradable plastics, polycaprolactone (PCL) has received much attention due to its high flexibility and biodegradability as well as its hydrophobic nature [3]. However, the high production cost of PCL appears to limit its commercial applications. This limitation may be overcome by blending of PCL with cost-effective biodegradable natural biopolymers, such as starch, cellulose, and chitin, to create new materials with desired properties [4–15]. In particular, starch is most often used since it is abundant, inexpensive, renewable, and fully biodegradable [16]. Blending of PCL and starch could markedly reduce the cost and also enables flexibility in adjusting the biodegradability and mechanical properties of the hybrid products. However, since starch is fairly hydrophilic, the poor compatibility between the two phases could cause problems with blending of PCL and starch. Therefore, addition of a compatibilizer and/or a toughener is usually needed to enhance the compatibility of the two immiscible phases, resulting in better mechanical properties of the composite [17]. Literature shows that maleic anhydride-grafted-polyethylene [18] or clay [19] could be used for this purpose.

Excessive addition of chemically synthesized fertilizers for crop production has deteriorated the quality of farming lands due to the accumulation of heavy metals and insoluble phosphate complexes in the soil. This motivates the use of more environmentally friendly fertilizers for sustainable utilization of the farming lands. Phosphate-solubilizing bacteria (PSB) are one of the most popular choices of bacterial fertilizers [20,21], since they could solubilize and mobilize the insoluble phosphate compounds accumulated in soil by biotic acidification, chelation and exchange reactions [22]. In this way, the corps can utilize the soluble phosphate compounds released

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from the soil for growth without the need of supplying additional phosphate fertilizers. However, technical difficulty may arise when using bacterial cells as the fertilizer in practice. For instance, methods for effective inoculation of the bacterial fertilizers into the soil need to be established. In addition, feasible strategies need to be developed for maintaining viability and function of these bacterial fertilizers in the soil during the cultivation of corps. These difficulties may be resolved by encapsulating the PSB cells in biodegradable capsules, which could protect the cells, while allowing the release of cells to the soil in a controllable manner. As a result, the effectiveness and stability of the bacterial fertilizers could be markedly enhanced.

Apparently, the key issue to the development of the ‘‘controlled release’’ bacterial fertilizers is to develop and design suitable biodegradable materials for the controlled-release capsules. Therefore, this study was aimed to use PCL-based materials to create the capsules, which maybe potentially useful for the encapsulation of PSB. PCL was used as the core matrix material, which was blended with starch and clay to modify its physical properties, including biodegradability, permeability, and mechanical strength. In this work, PCL and various types of PCL composites were used to entrap cells of an indigenous PSB strain (namely, *Bacillus* sp. PG01). The PSB-loaded PCL composites were examined along with the incubation time for the change in their structure, mechanical properties, and biodegradability. The main objective of this work was to assess the feasibility of using the PCL-starch-type composites for the development of efficient ‘‘controlled-release’’ bacterial fertilizers for practical application.

2. Materials and methods

2.1. Microorganism and culture medium

Bacillus sp. PG01 is a phosphate-solubilizing bacterium isolated from farming soil in central Taiwan. The PG01 strain was grown in LB broth (Difco), consisting of $10\ g\ l^{-1}$ of tryptone, $5\ g\ l^{-1}$ of yeast extract, and $10\ g\ l^{-1}$ of NaCl. The cells were cultivated at 30 °C with an agitation rate of 200 rpm. The culture at its early stationary phase was collected for cell entrapment.

2.2. Materials

Polycaprolactone (molar mass $80{,}000\ g\ mol^{-1}$) was supplied by Solvay (Warringto, England). Starch (27% amylose and 73% amylopectine) was purchased from Sigma Chemical Corporation (Steinheim, Germany). The clay (montorillonite) with a cation-exchange capacity of 119 mequiv. $100\ g^{-1}$ was obtained from Kunimine Inc. (Tokyo, Japan). Cetyl pyridium chloride (>99% purity, Sigma, Steinheim, Germany) was used as a cationic surfactant for the pretreatment of the clay.

2.3. Preparation of PCL/starch and PCL/clay/starch composites

The starch (Sigma, Steinheim, Germany) was dried in an oven at 105 °C for 24 h prior to blending. The starch was then blended with PCL (20 wt%) and the blends were prepared by the melt blending method using a BRABENDER ‘‘PLATOGRAPH’’ 200 Nm MIXER W50EHT instrument (Duisburg, Germany). A determined amount of PCL was put into the BRABENDER instrument with blade type rotor to melt it under the conditions that rotor speed and blending temperature were kept at 50 rpm and 80 °C, respectively. When the PCL had melted completely, pre-weighed amounts of the dried starch added into the MIXER to produce the blend for another 15 min. For preparation of PCL/starch/clay hybrids, the preparation of modified clays followed the method proposed by Liao and Wu [19], as the surface of clay was treated with cetyl pyridium chloride (CPC) to enhance the polymer/starch tensile strength at break [19]. Addition of CPC could enhance clay-polymer binding via increasing the dispersion of clay in the PCL matrix and causing exfoliation so that the mechanical property of the hybrid material can be improved. Similar behavior was also observed while preparing polystyrene-clay hybrids with CPC addition [23]. For the CPC pretreatment, a sample of 1 g sodium montmorillonite (clay) was dissolved in 50 ml of distilled water in a 100 ml breaker under vigorous stirring to form a uniformly dispersed solution. Then, 0.4 g of cetyl pyridium chloride were added to the solution at a clay to CPC ratio of 1:1 (wt%). The mass ratios of starch to PCL/10 wt% clay were chosen as 20/80. The hybrids of PCL/Starch/clay were also prepared by the melt blending method using the BRABENDER instrument as described earlier. After blending, the resulting products were pressed into 1 mm thin plates by a hot press and then were put into a dryer for cooling. Next, the thin plates were made into standard specimens for characterizations.

2.4. Encapsulation of Bacillus sp. PG01 in PCL and PCL composites

A fixed volume (1 ml) of *Bacillus* sp. PG01 culture containing 2.0 mg of cells (ca. 2.1×10^9 cells) was encapsulated by $60\ mm \times 35\ mm$ (0.05 ± 0.02 mm thick) capsules (Fig. 1a) made of PCL and its composites by using previously conditioned samples [24]. The cell-loaded capsules were immersed into sterile isotonic saline solution (0.85% NaCl) and incubated at 37 °C with an initial pH of 7.0. The structure, mechanical properties, and extent of degradation of the capsules were monitored at designated time intervals. The release of cells was also detected by measuring the cell concentration in the solution.

(a)
(b)

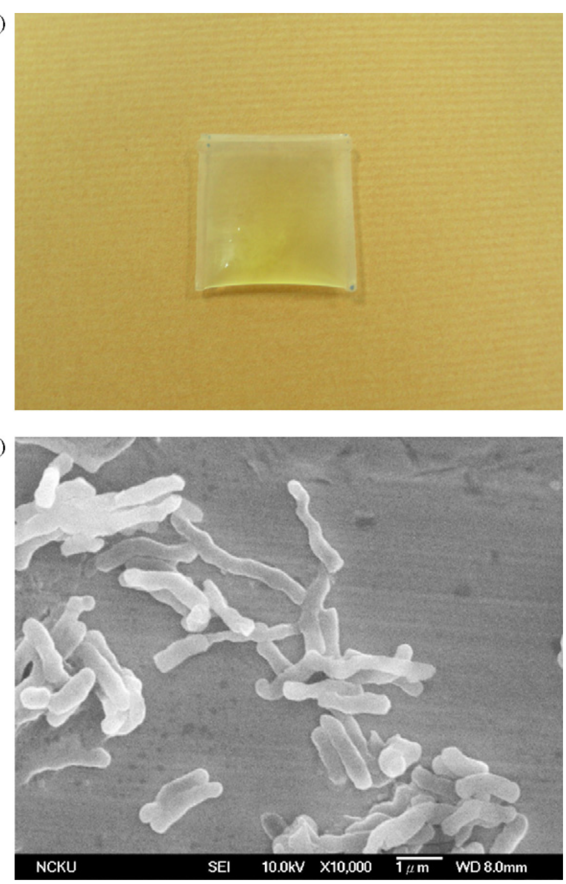

Fig. 1. (a) Photo of a PCL capsule and (b) SEM micrograph of *Bacillus* sp. PG01 cells.