Biodegradability and mechanical properties of polycaprolactone composites encapsulating phosphate-solubilizing bacterium \textit{Bacillus} sp. PG01

Ken-Jer Wu\textsuperscript{a,b}, Chin-San Wu\textsuperscript{b}, Jo-Shu Chang\textsuperscript{a,*}

\textsuperscript{a}Department of Chemical Engineering, National Cheng Kung University, Tainan 701, Taiwan
\textsuperscript{b}Department of Biochemical Engineering, Kao Yuan University, Kaohsiung 821, Taiwan

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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 \textit{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%)/clay (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., \textit{Bacillus} sp. PG01 cells) might be achievable.

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...
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 crops. 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 (Warrington, England). Starch (27% amylose and 73% amylopectine) was purchased from Sigma Chemical Corporation (Steinheim, Germany). The clay (montmorillonite) 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 beaker 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 × 10⁹ cells) was encapsulated by 60 mm × 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.

![Fig. 1. (a) Photo of a PCL capsule and (b) SEM micrograph of *Bacillus* *sp.* PG01 cells.](image)
2.5. SEM analysis

The morphology of PSB-treated PCL composites was observed with a scanning electron microscope (SEM; Hitachi Microscopy Model S-1400, Tokyo, Japan). The samples were first treated with glutaraldehyde and then were immersed in 50–100% acetone solutions. After drying at 50 °C for 48 h, the samples were coated with gold for SEM analysis.

2.6. Cell release experiments and the measurement of cell concentration

Cell-free (control) and cell-loaded capsules were suspended in saline solution (0.85% NaCl) and the cell concentration in the solution was monitored with respect to time. The cell concentration was determined by counting the colony-forming unit (CFU) present on LB agar plates after serial dilution and overnight incubation at 30 °C.

2.7. Analysis of mechanical property and biodegradation

Following the ASTM D638 method, the Instron mechanical tester (Model LLOYD, LR5K type) was used to measure tensile strength and elongation at break of the capsules entrapping PSB cells. After incubation, the composites were pressed into 1 mm-thick plates using a hot press at 100 °C, and were then placed into a desiccator for cooling. The cooled plates were then made into standard samples for characterization. For each sample, a mean value was obtained from five measurements taken at a crosshead speed of 20 mm/min. Weight loss of the composite was monitored with respective time using vacuum-dried (40 °C, 24 h) samples.

3. Results and discussion

3.1. Biodegradability of PCL and its composites by Bacillus sp. PG01

Pure strain of Bacillus sp. PG01 is in rod-like shape with the size of 1.8–3 μm × 0.5–1 μm (Fig. 1b). Time-course changes in the morphology of PCL matrix loaded with Bacillus sp. PG01 cells are indicated in Fig. 2. After incubation for 5 days, cell growth on the surface of PCL matrix was observed (Fig. 2b). After 20 days, disruption of PCL matrix structure became obvious (Fig. 2d and e), most likely due to biodegradation by Bacillus sp. PG01. The surface morphology of cell-free PCL matrix (control) remained unchanged (Fig. 2f), indicating that the alternation in morphology changes for cell-loaded PCL capsules was indeed due to bacterial activity. The biodegradation is further confirmed by an increasing weight loss of the PCL matrix along with the incubation time (Fig. 3), showing a nearly 30% weight loss in PCL after incubation for 60 days. The weight loss of PCL is considered to be due to biodegradation, as PCL is a polyester compound known to be biodegradable. Very little information is available in the literature regarding bacterial degradation of PCL [25], whereas it has been reported that PCL can be degraded by a variety of enzymes, such as lysozyme [26–28]. Our results show that Bacillus sp. PG01 seemed to be very effective in degrading PCL.

When PCL was blended with 20 wt% of starch, the SEM micrographs show that the PCL/starch composite seemed to be
degraded more easily by the loaded PSB cells. When compared to the PCL matrix, cells grew more abundantly on the surface of PCL/starch composite at day 5 and day 10 (Fig. 4b and c) and the extent of destruction was also higher for PCL/starch composite at day 20 and day 40 (Fig. 4d and e). Biodegradation created larger pores on the PCL/starch composite and the cells were coated on the surface to form biofilm. Fig. 3 also shows that weight loss of PCL/starch composite was much faster than that of PCL alone. For the PCL/starch composite, over 60% weight loss was reached in 60 days (Fig. 3). These results clearly indicate that addition of starch enhanced the biodegradability of the composite, and may thereby facilitate release of the entrapped cells into the environment. Furthermore, comparison of morphology (Fig. 5) of PCL/starch and PCL/starch/clay composites indicates that the latter is less biodegradable. Based on the weight loss results indicated in Fig. 3, the ranking of biodegradability
decreased in the order of PCL/starch > PCL/starch/clay > PCL > PCL/clay. This suggests that addition of clay decrease the biodegradability. This may be attributed to the recalcitrant nature of clay, which contains SiO₂ and other inorganic components that are quite difficult to degrade biologically. Thus, the biodegradability played a major role in affecting the cell release. In other words, the release efficiency of the bacterial fertilizer can be controlled by addition of biodegradable supplements (e.g., starch) or non-biodegradable materials (e.g., clay) into the core PCL matrix (Fig. 5).

3.2. Time-course analysis of mechanical properties

Due to the biodegradation activity of Bacillus sp. PG01, the tensile strength at break of all the cell-loaded capsules decreased along with the incubation time (Fig. 6). After incubation for 40 days, the decrease in tensile strength became less significant. The percentage decrease of tensile strength (at 40 days) was in the order of PCL/starch (55–60%) > PCL/starch/clay (45–50%) > PCL alone (35–40%) > PCL/clay (20–25%). This trend seemed to be consistent with the trend for biodegradability indicated in Fig. 3. Comparison of PCL with PCL/starch and PCL/clay with PCL/starch/clay also shows that blending with starch appeared to lead to a more significant decrease in tensile strength, suggesting substantial mechanical incompatibility of the two phases. Moreover, degradation of starch by the bacterium may also be responsible for the decrease in tensile strength. In contrast, addition of clay could maintain higher tensile strength during incubation. This is consistent with our previous finding, showing that clay addition could enhance tensile strength of polymer composites [19]. The positive effect of clay blending on improving tensile strength may be due to the bio-inert property of clay as well as enhanced clay dispersion and exfoliation behavior with the addition of cationic surfactant (i.e., CPC).

Time-course profiles of elongation at break of PCL and its composites (Fig. 7) show a similar trend to that for tensile strength. The only difference was that addition of clay had a less significant effect on maintaining the elongation at break. Like the results for tensile strength, the material with higher biodegradability seemed to exhibit a lower elongation at break. Thus, the decrease in this mechanical property could be due primarily to the biodegradative destruction of those materials. The addition of biodegradable supplements (such as, starch) may lead to poor adhesion and dispersivity between PCL and starch, resulting in higher biodegradability and weaker mechanical strength of the PCL/starch composite. In contrast, addition of non-biodegradable and nano-scale clay supplement could slightly improve elongation at break of the PCL composites (Fig. 7).

3.3. Release profile of Bacillus sp. PG01 cells encapsulated by PCL and its blends

The foregoing results indicate significant bacterial destruction of the cell-entrapped capsules during the course of...
incubation. Thus, it is of interest to observe the time-course profile of cell release. After incubation for 60 days, the final cell concentration in the saline solution was \(2.3 \times 10^7\) to \(6.0 \times 10^7\) CFU/ml (Fig. 8), indicating that cells were released into the solution and remain viable. The cell-release pattern was similar for all the capsules, whereas the amount and rate of cell release were different. As depicted in Fig. 8, the cell concentration reached a peak at day 10, and was then decreasing for the next 10 days. However, the cell concentration started to increase after day 20 (especially for the composite blending with starch) and kept increasing from day 20 to day 60. It is thought that the early increase in cell concentration was due to the release of cells from the leaks of the capsules. After that, cells released to the saline solution may become inactive or lysed, resulting in a decrease in viable cell concentration. As for the next increase in cell concentration (day 20 to day 60), there are at least two possible causes. As the SEM micrographs (Figs. 2, 4 and 5) show that after 20 days, the structure of all the capsules was severely damaged, leading to the release of cells as well as the residual starch and fragments of PCL matrix into the solution. Therefore, in addition to cell release from the capsules, the second increase in cell concentration may also be contributed by the cell growth by utilizing starch and/or depolymerized PCL fragment as the substrates. Since starch is more readily biodegradable, the solution incubating starch-containing capsules reached a higher final cell concentration with a faster increase in cell concentration at day 20 to day 60. It is evident that addition of starch had positive effects on the amount of cell release, while addition of clay reduced the release of cells, probably due to the lower biodegradability and smaller pore size of the clay-blended composites. This suggests that the rate and amount of cell release might be adjustable by altering the composition of composite materials.

4. Conclusions

This study demonstrated that PCL and its composites could be used to encapsulate cells of an indigenous phosphate-solubilizing bacterium (Bacillus sp. PG01), forming a “controlled release” bacterial fertilizer. The PG01 strain was able to degrade the capsule materials, causing the cell release. The biodegradability and mechanical properties of the capsules depended on the type of materials used, as addition of starch made the capsules more biodegradable, whereas the capsules became more recalcitrant when blending with clay. This suggests that the release efficiency of bacterial fertilizers (i.e., Bacillus sp. PG01 cells) might be controllable via proper formulation of composite capsules.

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