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Characterization of *Raoultella planticola* Rs-2 microcapsule prepared with a blend of alginate and starch and its release behavior

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ABSTRACT

To judiciously use *Raoultella planticola* Rs-2 and develop its biodegradable and controlled-release formulations, Rs-2 was encapsulated with various combinations of sodium alginate (NaAlg) and starch. Sodium alginate, soluble starch, and $CaCl_2$ showed good biocompatibility with Rs-2 for preparing microcapsules. These microcapsules were spherical in shape and their particle size, embedding rate, swelling ratio of Rs-2 microcapsules and release numbers of viable Rs-2 cells increased with the increasing of starch and NaAlg concentrations. Meanwhile, the biodegradability of the microcapsules constantly increases when the wt% of starch increased, but decreased when the amount of NaAlg increased. In addition, the release mechanism of microcapsules was consistent with that of the Ritger–Peppas model, which involves the Case II diffusion mechanism. In summary, the desired properties of the microcapsules can be modulated by varying the starch and alginate amounts of capsule materials. This process has broad application prospects to meet the needs of agricultural production.

1. Introduction

Secondary salinization can be attributed to the long-term cultivation of a drip irrigation system under the mulch film model in Xinjiang, China. This process has resulted in substantial losses of plant yields. In recent years, a new bio-control approach has been developed to protect plants from salt stress in soil. This approach involves treating crops with plant growth promoting bacteria (PGPR) (Glick, 1995; Guo, Shi, & Wang, 2010; Markus, Helmut, & Wilfried, 2004; Nadeem, Zahir, Naveed, & Arshad, 2010; Yang, Kloepper, & Ryu, 2009). PGPR possess many advantageous properties, including nitrogen fixation, mineral dissolution, and synthesis of auxin, ethylene, cytokinin, vitamins, and other important plant hormones necessary for the rapid growth of crops and protection against salt stress. Cotton (Yao, Wu, Zheng, Kaleem, & Li, 2010), rice (Bal, Nayak, Das, & Adhya, 2013) and wheat (Schoebitz, Ceballos, & Ciampi, 2013) inoculated with PGPR have shown improvements in growth and salt tolerance. So far, many PGPR strain has been screened, identification and application in the world (Kloepper & Ryu, 2009; Wu, Yue, Lu, & Li, 2012).

In our previous screening studies, an indigenous PGPR strain Rs-2 with 1-aminocyclopropane-1-carboxylate acid (ACC) deaminase activity was isolated from salinized soil in a cotton field in Xinjiang Province (Wu, Yue, et al., 2012). This strain increases the germination rate of cotton seeds and promotes cotton seedling growth under salt stress conditions. Rs-2 strain can also secrete indole acetic acid (IAA), solubilize insoluble phosphate compounds and fix nitrogen. However, inoculation of cells into soil may be difficult because of competition from indigenous microflora, unfavorable physicochemical conditions, and fluctuation in pH and temperature (Wu, Zhao, Kaleem, & Li, 2011; Young, Rekha, Lai, & Aru, 2006).

Therefore, numerous scholars have focused on developing different techniques to improve the survival of the bacterial strain. Microencapsulation of bacterial cells is one of the newest and most efficient techniques. Microencapsulation via extrusion techniques has been employed for the protection of the cells to better survival in soil after inoculation (Cassidy, Mullineers, Lee, & Trevors, 1997). Schoebitz, Simonin, and Poncelet (2012) and Schoebitz, López, and Roldán (2013) found that encapsulation of rhizobacteria (*Azospirillum brasilense* and *Raoultella terrigena*) in alginate and starch enhances their survival during storage. In addition, microencapsulated cells could be released into the target soil in a slow and controllable manner that confers greater long-term effectiveness (Young et al., 2006).

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Sodium alginate (NaAlg) has been used extensively for the microencapsulation of bacteria because of its viscosifying and nontoxic properties, simplicity of handling, and gel-enhancing properties. Although this is a simple and fast way of obtaining encapsulating systems, the method presents a major limitation consisting in active compound loss during bead preparation. Besides, the presence of macrospores in the alginate matrix facilitates the diffusion of hydrophilic molecules (Cordoba, Deladino, & Martino, 2013; George & Abraham, 2006). The incorporation of a filler material into an alginate matrix is a good strategy for solving the above mentioned problem. Starch is commonly used as encapsulating material (Wu, Guo, Qin, Li, 2012) because it is abundant, inexpensive, renewable, and fully biodegradable (Konsonla & Liakopoulou-Kyriakides, 2006). Some authors attempted to provide solution by blending of NaAlg with cost-effective starch to develop a biodegradable and controlled-release bacterial fertilizer with the desired properties, such as regular-shape, appropriate release, biodegradability, lower production cost, that meet the various needs of agricultural applications (Chen, Xie, Zhuang, Chen, & Jing, 2007; Ishiaku, Pang, Lee, & Ishak, 2002).

Numerous microorganisms have been encapsulated with starch and alginate, but single survival rates or release properties have only been studied using composite wall materials. Wu, Wu, and Chang (2007) showed that the biodegradability of the capsules can be increased by adding starch to polycaprolactone. Using alginate and starch by internal gelation technology can be a suitable procedure for protecting the probiotic strain and can be more efficient than using alginate alone (Martin, Villoslada, Ruiz, & Morales, 2013). Cordoba et al. (2013) encapsulated yerba mate by using starch and NaAlg, which improved the encapsulation efficiency by 10% compared with the microcapsules with only NaAlg. Meanwhile, Roy, Bajpai, and Bajpai (2009) found that the swelling ratio of biopolymer microspheres increased when the wt% of starch increases from 20 to 57.3% with NaAlg. Therefore, the characteristics of Rs-2 microcapsules and the interactions of microcapsule properties with alginate and starch amounts must be understood to obtain the desired properties for potential application.

The study aims to assess the feasibility of using alginate and inexpensive starch composites prepare Rs-2 microcapsules with the desired properties. At the same time, we can control the degradation and release properties by changing the addition amount of different components, and thus meeting microcapsules applications. The embedding rate, water content, particle size, swelling property and biodegradability of microcapsules were evaluated. In addition, the release behavior of Rs-2 microcapsules was measured by simulating time-course cell release data with a proposed mass transport model.

2. Materials and methods

2.1. Bacterial strains and culture medium

The strain Rs-2 used in this study was previously isolated from salinization soil in Xinjiang province of China (Wu, Guo, et al., 2012). Rs-2 were cultured in nutrient agar (NA) liquid medium (5 g beef extract, 10 g peptone, 5 g NaCl, 1000 ml H_2O, pH 7.0–7.2) with shaking at 200 rpm for 48 h at 30 °C. Cells were harvested at early stationary phase, and a concentration of 10^{13} cfu/ml was determined in the broth by counting the colony-forming units (cfu) present on NA agar plates after serial dilution for overnight incubation at 30 °C.

2.2. Biocompatibility

The concept of biocompatibility refers to the compatibility between of selected wall materials of capsule and strain Rs-2 by determining any growth and survival effects induced by the wall materials with Rs-2 cell. Ratner (2011) method was followed to measure biocompatibility. Certain amount of NaAlg (1%, supplied by Shantou Xilong chemical factory of Guangdong), soluble starch (1% maize resistant starch, supplied by Tianjin Sheng'ao chemical reagent Co., Ltd) and $CaCl_2$ (2%, obtained from Fuchen chemical reagents of Tianjin) were dissolved in NA mediums containing or without agar, respectively, and NA medium was used as the control. The same amount of original Rs-2 liquid broth was inoculated in different solid culture media containing agar to culture for overnight incubation at 30 °C. The viable cell number was determined using the plate count method. Similarly, 3% of the original Rs-2 liquid broth were inoculated into different liquid culture media without agar and then cultured with shaking at 200 rpm for 60 h at 30 °C. The growth process of Rs-2 in different liquid culture media was investigated by measuring the optical density value using an UV–vis spectrophotometer (Instruments Co. Ltd. Shanghai Yuanxi) of culture broth every 6 h.

2.3. Cell encapsulation

The cells were harvested by centrifugation when their growth reached the stationary phase. They were washed three times with saline solution (0.9%, NaCl) for 10 min to remove any nutrients carried from the NA broth. Encapsulation of bacteria within beads was carried out under sterile conditions. Wu et al. (2011) method was followed to measure encapsulate cell using the extrusion technique. The blend solutions from different compositions were prepared by varying starch and NaAlg content (Table 1). Collected Rs-2 cell was aseptically dispersed the blend solutions after sterilization at 121 °C for 15 min. These mixtures containing Rs-2 cells were extruded from a 10 ml sterile syringe (needle size 0.9 mm) from a 25 cm height into a $CaCl_2$ (2%) crosslinking agent solution (Fig. 1). Then after bead-formation reaction (about 2 h), the capsules were removed from the $CaCl_2$ solution and were cleaned with sterile water for 2–3 times, while the excess water was wiped with tissue paper and its quantity was measured immediately.

2.4. Drying of the microcapsules

A few samples of the wet microcapsules were collected and dried in electric oven blast (Industrial Co., Ltd., Medical Equipment Factory of Shanghai Boxun) maintained at 40 °C until constant weight was obtained. Then the dry microcapsules containing

Table 1
Diameter and yield of Rs-2 microcapsules prepared by varying amounts of NaAlg and starch.

Formulation	NaAlg (%, m/v)	Starch (%, m/v)	Wet microcapsules diameter (mm)	Dry microcapsules diameter (mm)	Yield (%)
AS0	1.0	0	2.21 ± 0.06	0.85 ± 0.02	91.2
AS1	1.0	2.0	2.26 ± 0.06	0.98 ± 0.03	90.4
AS2	1.0	4.0	2.83 ± 0.04	1.15 ± 0.03	87.0
AS3	1.0	6.0	3.44 ± 0.06	1.23 ± 0.06	84.8
AS4	0.5	4.0	2.67 ± 0.07	1.10 ± 0.05	85.5
AS5	0.5	6.0	3.10 ± 0.04	1.21 ± 0.04	81.0
AS6	0.5	8.0	3.23 ± 0.07	1.35 ± 0.04	73.2

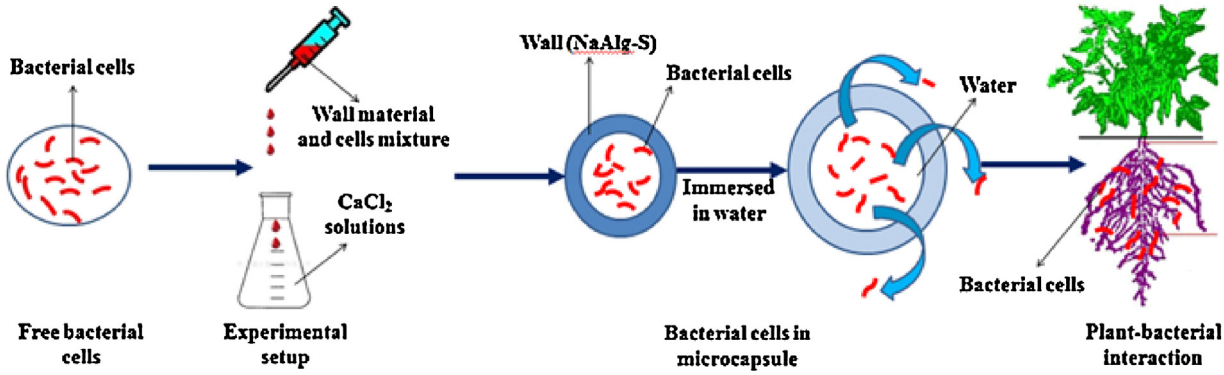

Fig. 1. Encapsulation of bacterial cells and release of bacteria from microcapsules.

Rs-2 were obtained for further experimentation. Finally, load-dried microcapsules were placed in a sterile EP tube. The EP tube was sealed to ensure that the capsules will not be contaminated.

2.5. Characterization

2.5.1. Determination of the microcapsules particle size

Ten different wet or dried wall material microcapsules diameter was measured by 150 mm vernier caliper under different conditions, and then averaged. All the experiments were carried out in triplicate.

$$D = \frac{D_1 + D_2 + \ldots + D_n}{n} \quad (2)$$

2.5.2. Encapsulation efficiency

Some bacteria were unembedded when the mixture contained Rs-2 was splashed into $CaCl_2$ solution. The number of the unembedded bacteria denoted as N_u could be determined by plate count method. Then the encapsulation efficiency of bacteria could be calculated as follows:

$$\text{Encapsulation efficiency}\,(\%) = \frac{N_0 - N_u}{N_0} \times 100 \quad (3)$$

where N_0 represents the initial colony-forming unit of Rs-2 per milliliter. The number of viable cells in dried beads (AS2) was measured and calculated to be 1.8×10^{14} cfu/g using methods reported by Wu, Guo, et al. (2012).

2.5.3. SEM analysis

The microcapsules were treated with glutaraldehyde followed by immersing in 50–100% acetone. Then the samples were dried at 50 °C for 48 h before coating with gold for SEM analysis. The morphology of the microcapsules was analyzed by using a SEM (JSM-6700F, Jeol, Tokyo, Japan).

2.5.4. Fourier transform infrared spectrophotometer (FTlR) analysis

The infrared spectra of the samples were recorded on a FTIR (TENSOR27, Bruker, Germany). The samples were completely dried and grounded to fine powder and blended with KBr.

2.5.5. Percentage yield

The quantity of all dry capsules obtained denoted as W_0, and the quantity of wall material used denoted as W_u. The percentage yield formed from each composition was calculated as given below in Eq. (4)

$$\text{Percentage yield}\,(\%) = \frac{W_0}{W_u} \times 100 \quad (4)$$

2.5.6. Swelling analysis

Dried microcapsules (0.2 g) were obtained and immersed in an excessive amount of physiological saline (0.9%) for 24 h and then sealed in a 10 ml EP tube. The microcapsules were removed from the immersion, and excess water was removed by wiping with tissue paper. The quantity was measured immediately and denoted as W_s. The weight of swelling microcapsules was measured by electronic balance (Beijing Sartorius Instrument Systems, Inc.). The swelling ratio of microcapsules was calculated as given below in Eq. (5).

$$\text{Swelling ratio}\,(\%) = \frac{W_s - W_0}{W_0} \times 100 \quad (5)$$

2.5.7. Release properties of encapsulated Rs-2 cells

The release properties can be described as the cumulative bacterial release from dried microcapsules prepared with various wall materials. 100 mg microcapsules were taken and immersed in 10 ml of sterile saline (0.9% NaCl) placed in 10 ml EP tube for 8 days at room temperature (Wu, 2008). Samples (0.1 ml) were removed at various intervals and the number of living cells in solution was determined respectively by the plate count method on nutrient agar (NA). All the experiments were carried out in triplicate.

2.5.8. Biodegradability analysis

The biodegradability of the microcapsules was studied by evaluating weight loss of samples at designed time in a soil environment. Six types of microcapsules were placed in very fine mesh (10–20 μm) nylon bags, and 100 mg of dried microcapsules per bag were buried 5 cm below the soil surface in the natural soil. The soil was maintained at room temperature for 30 days and kept water saturation by adding distilled water as necessary. In order to monitor the progress of degradation, the initial weight of dried microcapsules was defined as W_i, while the buried samples were dug out after various time periods, washed in distilled water, then weighed as W_t after drying them for 48 h to get constant weights. The % biodegradation was calculated by Eq. (5).

$$\text{Biodegradation}\,(\%) = \frac{W_i - W_t}{W_i} \times 100 \quad (5)$$

2.6. Release kinetics of Rs-2 in microcapsules

The release kinetics of different types of microcapsules was studied to understand the release pattern of Rs-2. The dried microcapsules containing Rs-2 were immersed in sterile physiological saline solution and the cumulative amount of viable bacteria at different time intervals (M_t) was measured by the plate count method. Experiments were performed at room temperature for cell release analysis.

In order to deeper understand the release profile of the cumulative viable bacteria from the microcapsule prepared different materials, first order release kinetics (Benita, 1984), Higuchi release kinetics (Ravi, Kotreka, & Saha, 2008) and Ritger–Peppas release kinetics models (Ritger & Peppas, 1987) were fitted to Rs-2 release from different type microcapsules, respectively.

First order release kinetics model is expressed as Eq. (6)

$$\ln(M_\infty - M_t) = -K_1 t + C_1 \quad (6)$$

Higuchi release kinetics model Eq. (7)

$$M_t = K_H t^{1/2} + C_H \quad (7)$$

Ritger–Peppas release kinetics model can be calculated from the following Eq. (8)

$$\frac{M_t}{M_\infty} = Kt^n \quad (8)$$

where M_∞ is the total cumulative compound release, generally as 100%; M_t is the cumulative drug release percentage at the release time t; K_1, K_H and K are release rate constants related with the features of the bacterial-microcapsules system; C_1, C_H is a constant. In Eq. (8), n is the diffusion exponent indicative of mechanism of drug release.

Higuchi model, a commonly used drug release model is based on Fick diffusion theorem. This model is based on skeleton spreading medium as the main controlled-release behavior in the form of water-soluble drugs.

Ritger–Peppas model involves the addition of drug release and dissolution and uses a simple semi-empirical index equation. When $0.45 < n < 0.89$ for non-Fick release, namely diffusion and dissolution coexist; when $n < 0.45$, for Fick diffusion; and $n > 0.89$ indicates Case II release, dissolution mechanism for the skeleton.

2.7. Statistical analysis and control

The results were expressed as the mean and standard deviation of triplicate studies. Statistical differences were tested with one-way ANOVA with Turkey's multiple comparison tests (SPSS 19.0, Chicago, Il, USA). Any p-values less than 0.05 were considered to be significant. The NaAlg beads without starch were taken as the control.

3. Results and discussion

3.1. Biocompatibility

The effects of NaAlg, soluble starch, and $CaCl_2$ on the growth of Rs-2 were investigated. The colonies of the same Rs-2 broth were not significantly different among the different culture medium plates (Fig. 2). These findings suggest that NaAlg, soluble starch, and $CaCl_2$ have no effect on the growth of Rs-2.

The growth curve patterns of Rs-2 were markedly similar for all the culture broths and were at a stationary phase after 24 h (Fig. 2). And, the growth of Rs-2 in complex medium with 1% NaAlg or 1% starch was slightly better ($p \leq 0.05$) than that in the control NA culture medium. This observation also indicates that NaAlg and starch may be utilized as carbon sources to improve the growth of the strain. In addition, $CaCl_2$ did not show any adverse effect on the growth of Rs-2. Biocompatibility test results have shown that NaAlg (1%) and starch (1%) can be used as microcapsule wall materials for Rs-2.

3.2. Morphological analysis of the Rs-2 microcapsules

The morphological features of beads were studied by recording their SEM images as shown in Fig. 3. The images were recorded at different magnifications to gain clear insights into the surface topography of the biopolymer beads, as shown in photographs (Fig. 3). The features were similar for all the capsules. The microspheres were nearly spherical, wrinkled and the surface shows minor cracks and small clusters of biopolymeric blends at low magnification (Fig. 3a). However, the surfaces of the native beads showed irregularly shaped bundles of polymer blends at high magnification (Fig. 3b). After drying, some beads exhibited depressions and small cracks that may facilitate bacterial release upon water absorption and bead swelling. Wu et al. (2011) have observed similar results.

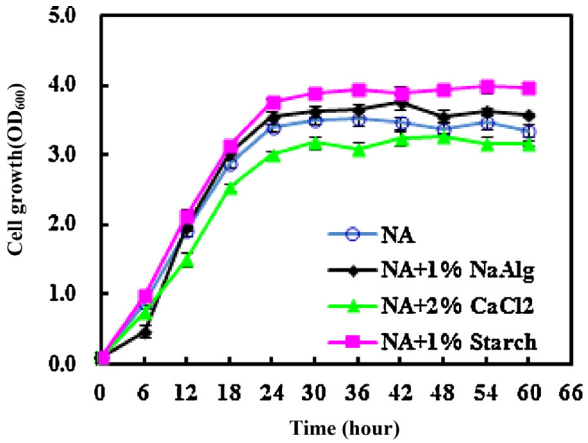

Fig. 2. Effects of different wall materials on the growth of Rs-2 (the culture conditions: 3% of the inculcated amount, shaking speed 170 rpm at 30 °C for 60 h).

3.3. FTIR analysis

FTIR spectra of pure alginate, starch and starch–alginate crosslinked beads were recorded in KBr pellets and are presented in Fig. 3c, respectively. In all the three cases the strong and broad absorption band has been observed on 3600–3200 cm^{-1} due to —OH stretching which related with water absorbed and swelling ratio. And 2700–1800 cm^{-1} for azide group present in all spectra indicates the stable nature of NaAlg and starch. This reaction did occur between NaAlg and starch, demonstrated by the absence of corresponding peaks in the NaAlg–S spectrum at 2700–1800 cm^{-1}. As indicated above, the appearance of peaks near 1600 cm^{-1} was observed for all the three cases. The evidences for the crosslink between NaAlg and starch come from the peaks observed at 1420 cm^{-1} due to the carboxylate anion of NaAlg–S and 870 cm^{-1} and 775 cm^{-1} due to —OH bonding of absorbed water. The discernible weaker shoulder near 1020 cm^{-1} on NaAlg–S reveals the C—O and C—O—C stretching vibrations, which are the characteristic of the natural polysaccharide. These peaks provide evidence of the exits of —OH of NaAlg and starch. This result was also reported by Singh, Sharma, and Gupta (2009), who assigned an absorption peak in the FTIR spectrum of starch–alginate beads.

3.4. Characterization of the Rs-2 microcapsules

3.4.1. Effect of varying composition proportions on size and yield

Table 1 shows the particle size and yield of Rs-2 microcapsules prepared by different NaAlg and starch complexes. The microcapsules formed were spherical with particle sizes ranging from 2.21 ± 0.06 mm to 3.23 ± 0.07 mm when wet and from 0.85 ± 0.02 mm to 1.35 ± 0.04 mm after drying. The diameter of the beads prepared with NaAlg and starch was larger than that of the beads prepared with NaAlg alone. Furthermore, the diameter of beads increased as the concentration of the NaAlg and starch in the

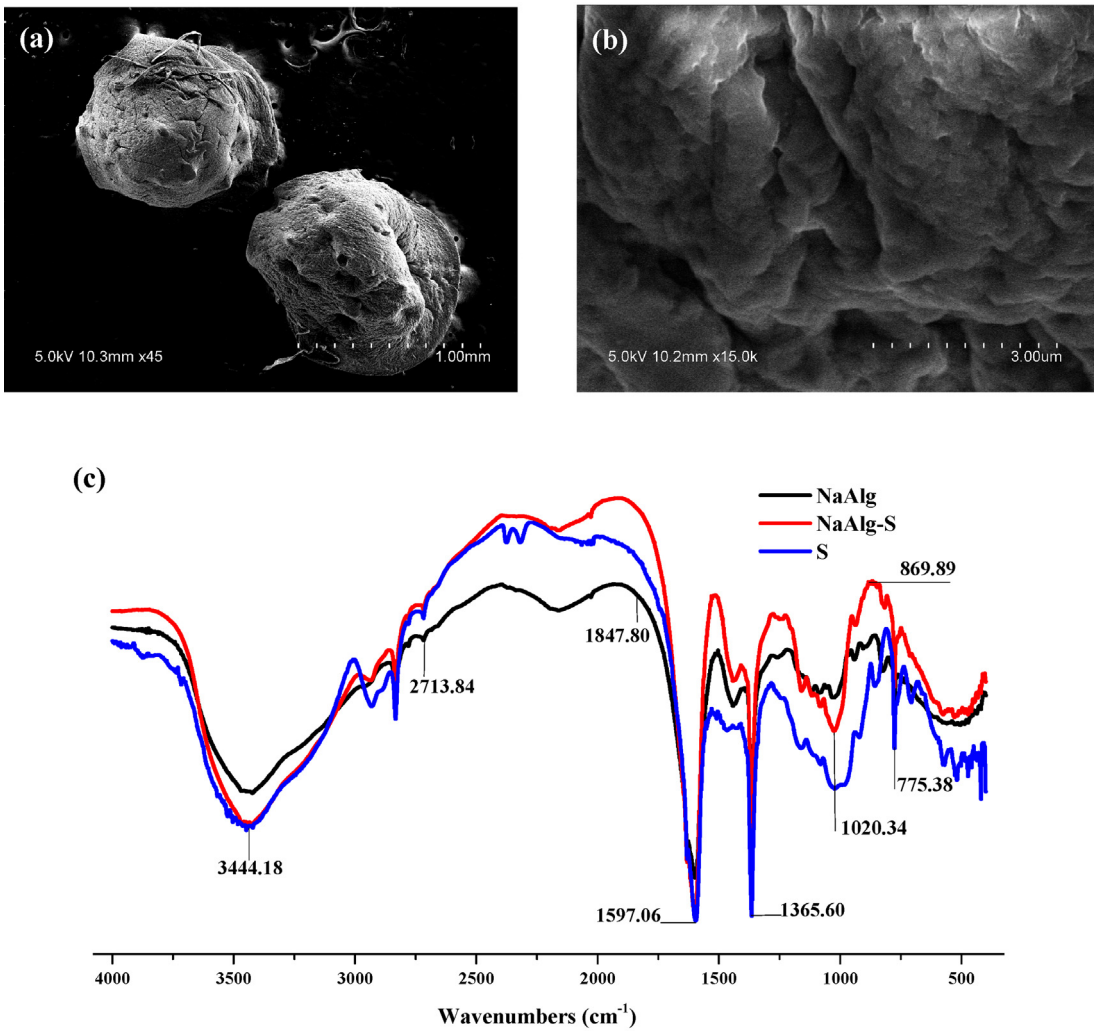

Fig. 3. External morphology (SEM) and FTIR spectra of NaAlg, starch and NaAlg–S microcapsules (NaAlg content: 1.0% (m/v) and starch content: 4% (m/v)), (a) 5.0 kV, 10.3 mm, 45×, (b) 5.0 kV, 10.2 mm, 15.0k×. and (c) FTIR spectra.

formulations increased. The particle size of the capsules was positively correlated with the concentration of the NaAlg and starch used. When the liquid drop fell into the gelling bath to form a bead, the bead size was found to be smaller than the liquid drop. This phenomenon has been attributed to the syneresis effect during the gelation process, and the bead size can be mainly influenced by the shrinkage (Chan, Lee, Ravindra, & Poncelet, 2009). The shrinkage of beads significantly reduced with increasing concentrations of starch and NaAlg with hydrophilic nature (Chan et al., 2011). This result suggests that adding NaAlg and starch can effectively enhance the particle size of beads. This observation is consistent with the results of previous works (Wu, 2008).

The percentage yield of beads varied from 73.2% to 91.2% with different contents of starch and NaAlg. In addition, the percentage yield of microcapsules increased with increasing NaAlg content during the preparation of microcapsules. The consistent effect of NaAlg on the improvement of the percentage yield of beads was reported in previous studies (Wu et al., 2011). With the addition of NaAlg to the formulation, more and more alginate and calcium ions were available for the cross-linking of other alginate and calcium ions, which increased the percentage yield of the microcapsules. Singh et al. (2009) also showed that the percentage yield of starch–alginate–Ca^{2+} beads increases with increasing alginate amount and crosslinker concentration. However, the yield of beads decreased with increasing starch amount, which was possibly due to the increased leaching of starch from the blends during the synthesis of beads. And the reason may be interpreted as starch has shown interaction with alginate only through hydrogen bonding which were limited in particular formulations when the amount of alginate was constant. When all the available interactions have been used, no functional moiety was left in alginate to bind the more starch in the formulation which the increased leaching of starch from the blends. Conversely, Singh et al. (2009) reported that the yield of beads is not affected by increasing starch content.

3.4.2. *Effect of varying composition proportions on encapsulation efficiency*

The unembedded bacteria could be determined by measuring the concentration of $CaCl_2$ solution after specific time intervals during the production of microcapsules. The unembedded bacteria decreased from 10^8 to 10^7 (cfu/mL) with the addition of starch to the NaAlg (Fig. 4a). The embedding rate that was calculated using Eq. (3) was more than or almost 100% because of the enormous initial amount of Rs-2 (10^{13} cfu/mL). Wu et al. (2011) reported similar findings using *Klebsiella oxytoca* Rs-5 calcium–alginate microcapsules. In their study, the embedded rate of the treated

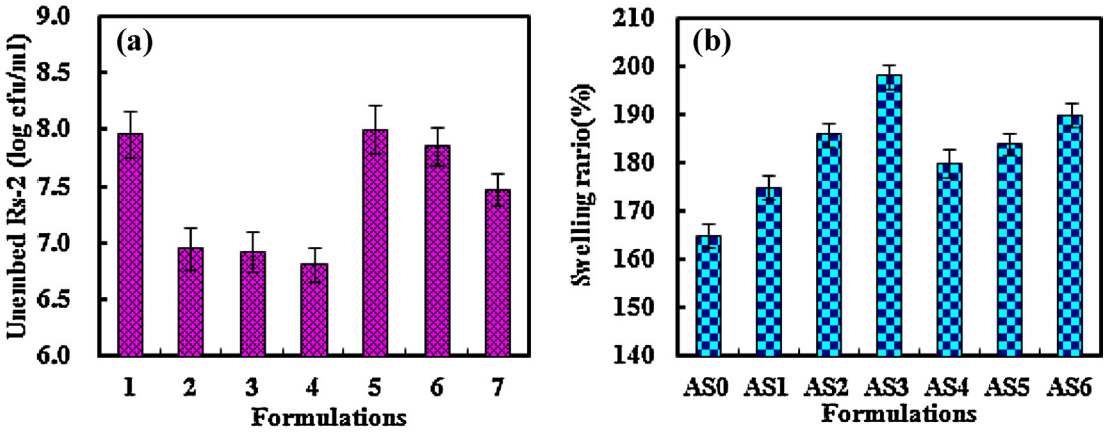

Fig. 4. Effect of varying concentrations of NaAlg and starch on unembedded number and swelling ratio of capsules: (a) unembedded number and (b) swelling ratio.

microcapsules reached 99.99% when >1% NaAlg was used. The number of microcapsulates in the unembedded bacteria decreased with increase of starch amount in the blends with the same NaAlg concentration.

Furthermore, the encapsulation efficiency of the microcapsules prepared by 2%, 4%, and 6% starch and 1% NaAlg significantly increased compared with that of the microcapsules prepared using 1% NaAlg alone. This result can be attributed to the satisfactory biocompatibility of the NaAlg–starch complex with the Rs-2 cells. Thus, the complex showed satisfactory protective effect on Rs-2. Previous studies (Sultana et al., 2000) revealed that starch can improve the entrapment capacity of Rs-2 beads prepared using NaAlg and starch. Thus, the inclusion of NaAlg and starch can greatly enhance encapsulation efficiency with minimal additional cost. Martin et al. (2013) also indicated that microencapsulation in alginate beads with starch was more appropriate for microencapsulation of *Lactobacillus fermentum* CECT5716 than the same technique without starch.

3.4.3. Effect of varying composition proportions on swelling property

The swelling ratio of each formulation (SA0–SA7) was studied after 24 h. The effects of different NaAlg and starch proportions on the swelling ratio of beads were determined (Fig. 4b). The swelling ratio of the beads significantly varied ($p \leq 0.05$) from 175% to 198%. This result suggests that the beads have different structures and properties. The Rs-2 beads prepared with different NaAlg and starch proportions showed increased swelling as the amounts of starch and alginate increased.

The hydrophilic nature of the starch may have led to these results because of the number of interactions of —OH groups present in the starch that interacted with water and bring about an increase in swelling ratio of the beads (Olivas and Barbosa-Canovas, 2008; Rhim, 2004). Beside, the observed increases in swelling ratio could be attributed to the fact that due to anionic nature of alginate its increasing amounts in the beads will cause enhanced repulsion among the molecular chains and this results in an increase in swelling ratio, similar to the result of dynamics of controlled release of chlorpyrifos studied by Roy et al. (2009) and their results clearly showed that the swelling ratio constantly increases when the wt% of alginate increases from 20 to 57.3.

3.4.4. Effect of varying composition proportions on biodegradability

The effect of NaAlg and starch on the biodegradability of beads was studied by varying their amounts in the range of 0.5–1.0% and 0–8% (m/v), respectively. This biodegradation was further confirmed by the increasing weight loss of the beads matrix with longer incubation times in the nature and open conditions (Fig. 5a).

Weight loss also accelerated when a higher concentration level of starch composite was used than when a lower concentration was used. Weight loss may have been caused by microorganism biodegradation. Microorganisms are present in the degradation system because starch is the main carbon biodegradable source. The degradation rate of the encapsulation matrix will have a direct relationship with the biological activity of soil microorganisms (Schoebitz, López, et al., 2013).

Carbohydrates, such as starches, their low viscosity at high solids contents and good solubility, weaken the intermolecular forces between the starch and alginate macromolecules, enhance the dimensional stability of the beads, and thus facilitate the release of the entrapped cells into the environment (George & Abraham, 2006; Gouin, 2004).

Hence, biodegradability may participate in the cell release. The release of the bacterial fertilizer can be controlled by the addition of biodegradable supplements (e.g., starch) into the core matrix, with higher levels of supplement leading to more rapid release. The result reported in the present study is similar to that of Wu (2008) who conducted a controlled-release evaluation of bacterial fertilizer from an acrylic acid grafted polybutylene succinate (PBSU-g-AA)/starch matrix and found that *Bacillus* sp. PG01 is effective in degrading PBSU.

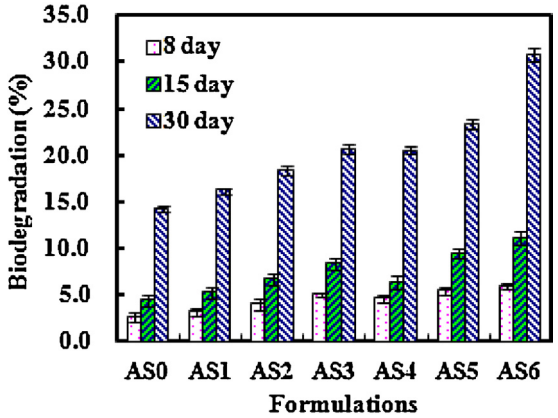

Fig. 5. Effect of concentrations of NaAlg and starch on the biodegradation of Rs-2 microcapsules.

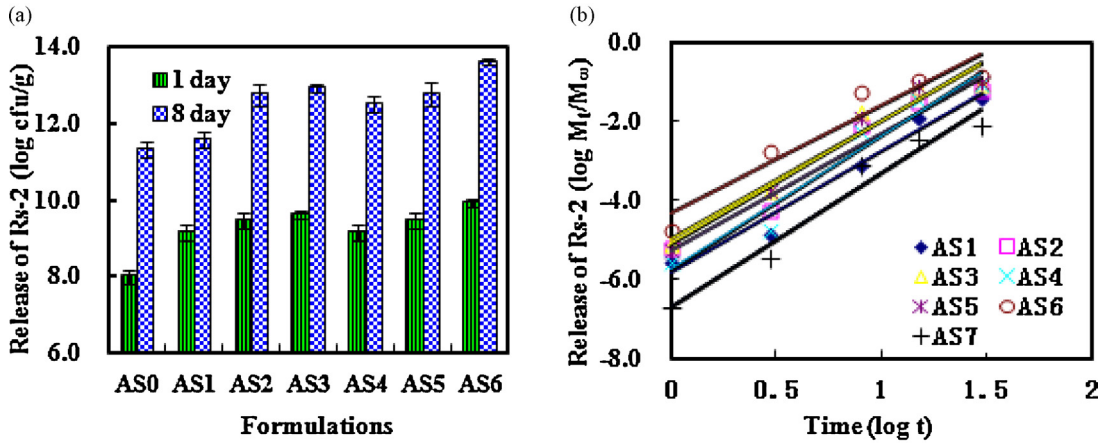

Fig. 6. Release properties and kinetics of Rs-2 from microcapsules prepared with varying NaAlg and starch concentrations, (a) release property and (b) release kinetics fitting with Ritger–Peppas release kinetics model.

NaAlg is an anionic polymer, and an increase in NaAlg wt% in the beads can increase the number of anionic-charged centers. Thus increasing number of polar chains cause greater intermolecular interactions between the alginate and starch macromolecular due to hydrogen bonding and weak electrostatic interactions (Roy et al., 2009). This obviously holds the macromolecular chains so tightly that the water molecules exert less effect on the surface and thus results in decreasing % biodegradation erosion. Moreover, greater interaction between Ca^{2+} and NaAlg molecules also enhances integrity of the beads, therefore produces less degradation (Roy et al., 2009). In addition, the biodegradability was confirmed by increasing weight loss in the microcapsules with progressing incubation time.

As a result, therefore the beads surfaces loses integrity and due to interaction between polymer chains and water molecules the % erosion increases (shown in FTIR). Thus, the beads with 8.0% (m/v) starch and 0.5% (m/v) alginate showed nearly 30% weight loss after 30 d.

3.4.5. *Effect of varying composition proportions on release rate of Rs-2*

The release of bacteria from the beads prepared with NaAlg and starch is shown in Fig. 6a, which clearly reveals that the release amount constantly increases ($p \leq 0.05$) with increasing starch concentration in the formulations. An increase in the released amount becomes even more significant with extended duration. The increase in the fractions of starch, nonionic polymer, and hydrophilic polymer in the beads increased the swelling of the composite, which may facilitate the release of the entrapped cells. In addition, the release rate of bacteria increases with increasing NaAlg concentration in the beads, which can be explained by the different swelling ability of alginate as discussed previously. Moreover, the volume fraction of polymer also increases with increasing alginate amount, which results in the beneficial diffusion of the bacteria from the swollen beads. The release of encapsulated Rs-2 was positively associated with the swelling and biodegradation properties of the capsules. These results are consistent with the conclusions of Roy et al. (2009) and Cordoba et al. (2013). Therefore, the release rate and amount of cells may be adjusted and controlled by simply altering the addition of different concentrations of NaAlg and starch materials.

3.5. *Release dynamics*

As shown in Fig. 6b, the cell-release pattern was similar for all the capsules, whereas the amount and rate of release were significant different ($p \leq 0.05$). This result may be due to the different starch and alginate contents loaded in the microcapsules. With the addition of starch, the release rate of microcapsules shows the characteristics of reservoir systems, which have an initially high release rate, indicating the so-called "burst" effect (Morita and Karube, 1995). This result can be attributed to the capacity of hydrophilic starch to increase the swelling of the microcapsules. In addition, the NaAlg–starch composite wall material may reduce the density membrane structure of microcapsules, which increases the outer membrane permeability and is conducive to the rapid release of bacteria. The addition of starch positively affected the amount of cells released.

First-order, Higuchi, and Ritger–Peppas release kinetics models were fitted to experimental data to analyze the release mechanism. The kinetic parameters obtained are shown in Table 2. The goodness of fit was evaluated using the corrected coefficient of correlation (R^2) and the residual analysis. The mechanistic aspects of the release process in swelling-controlled bacterial release can be explained well using the Ritger–Peppas model, in which the release exponent "n" varies in accordance with the release mechanism. Considering that all the values for the diffusion exponent "n" were >0.89, the main Case II release mechanism with skeleton

Table 2
Diffusion constants and mechanism involved in the release process of Rs-2 from beads prepared with varying wall material compositions.

Samples	First order release model			Higuchi release model			Ritger–Peppas release model		
	R^2	K_1	C_1	R^2	K_H	C_H	R^2	$K \times 10^3$	n
AS0	0.770	0.038	3.621	0.847	7.129	51.790	0.958	1.235	3.390
AS1	0.905	0.048	3.547	0.932	6.731	57.056	0.968	2.921	3.066
AS2	0.805	0.048	3.368	0.855	6.283	61.675	0.956	3.207	2.971
AS3	0.741	0.051	3.251	0.814	6.165	64.12	0.948	6.035	2.975
AS4	0.852	0.056	3.472	0.875	7.211	57.885	0.951	5.111	3.363
AS5	0.770	0.055	3.288	0.829	6.459	63.018	0.959	6.933	3.105
AS6	0.679	0.052	3.031	0.733	5.451	69.317	0.913	13.365	2.719

corrosion mechanism. The release of Rs-2 occurs as it diffuses out when the beads swell by absorbing water. Singh et al. (2009) studied the release dynamics of thiram fungicide from starch–alginate beads and reported a similar diffusion mechanism.

With the expansion of the microcapsules, the calcium alginate matrix was also partially dissolved. At the same time, the microcapsule wall materials interacted with the solution, and the cell itself can also degrade starch. All these factors caused the increase in the dissolution of microcapsules (Martin et al., 2013; Wu et al., 2007). In addition, the release rate constant K of various microcapsule component values increased with increasing starch content. This result suggests that the release rate increases with increasing starch content. The results were consistent with the release property results obtained above.

4. Conclusions

This study describes the effects of the addition amount of starch and NaAlg on the characterization of NaAlg–S beads and release property. Studies have shown that NaAlg, soluble starch, and $CaCl_2$ have good biocompatibility with Rs-2 and that they can be used as wall materials for Rs-2 microcapsules. Furthermore, the diameter of the microcapsules decreased with increasing NaAlg and starch concentrations. The percentage yield of beads increased with increasing NaAlg content, whereas yield decreased with increasing starch content. This result suggests that the embedded bacteria in the microcapsules gradually increased with increasing NaAlg and starch concentrations.

In addition, the starch–alginate beads prepared with different NaAlg and starch concentrations showed increased swelling when the concentrations of the components were increased. The biodegradability increased when the wt% of starch increased. The cell-release pattern was similar for all the capsules, whereas the amount and rate of release were different. Moreover, the release mechanism of the Rs-2 NaAlg–starch microcapsules was consistent with that of the Ritger–Peppas model, which mainly involves the Case II diffusion mechanism by relaxation-controlled transport.

Changing the content of NaAlg and starch was found to modulate the particle size, embedding rate and swelling ratio of Rs-2 microcapsules, which are desirable property for processing, handling and consumer usage. In conclusion, extrusion technique with alginate and starch seems to be a suitable procedure for protecting Rs-2 cell and could provided insights into the promising application of using controlled-release bacterial fertilizers in farmlands.

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