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Manganese Removal by Biofiltration Using Activated Carbon-barium Alginate-entrapped Cells: Morphology, Durability, Settling Velocity, and Treatment Efficiency

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Abstract

Occurrence of manganese in water supplies causes colored water and pipe rusting in water treatment and distribution systems. Moreover, consumption of manganese-contaminated water may lead to neurotoxicity in humans. Biofilters have the potential to alleviate the manganese issue through bio-oxidation, particle separation, and adsorption processes. Biofiltration performance can be enhanced by augmentation of the manganese-oxidizing bacterium entrapped in polymeric materials. This study aimed to investigate the potential of barium alginate-entrapped cells supplemented with powdered activated carbon (PAC) for manganese removal. *Streptomyces violarus* strain SBP1, an effective manganese-oxidizing bacterium, was selected. The experiments were divided into 2 parts, including 1) characterization of barium alginate bead and 2) manganese removal testing. Effect of PAC content (1, 5, and 10% w/v) in the entrapment material on bead morphology, bead durability, and settling velocity (relative to a filtration medium) was investigated. Micro-structural observation using a scanning electron microscope showed that the PAC was distributed through intra-porous structure of the beads. The PAC-supplemented barium alginate beads improved durability (up to three-time higher Young's modulus values). The PAC-supplemented barium alginate beads gave similar settling velocity compared to a filtration medium. The manganese removal efficiencies by the PAC-supplemented beads (no cells) ranged from 48 to 53%. Based on barium alginate bead characterization and manganese removal performance, the bead with 5% PAC content was selected for cell entrapment. The investigation revealed that the entrapped cells achieved faster manganese removal rate than free cell system. The findings from this study indicated high potential of the entrapped cells for use in future biofilter applications.

Keywords: Adsorption; Biotransformation; Immobilized cells; PAC; Metal; *Streptomyces violarus*

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Introduction

Biofiltration is a water treatment system involving particle separation, adsorption, and biotransformation processes. The biofilter, with attached microorganisms (forming a biofilm), can be used to efficiently treat organic- and metallic-contaminated water [1]. Among metallic contaminants, manganese is often found in the natural water sources of many countries [2]. Manganese in water can clog pipes and cause colored water. Long term consumption of manganese-containing water could lead to neurotoxicity in humans [3].

Generally, natural biofilm on a biofilter takes some time to form and then is easily detached and washed out during backwashing process [4]. This phenomenon leads to low cell numbers in the filter and causes unsatisfactory contaminant removal. To enhance biofilter performance, augmentation of the contaminant-removing microorganism could speed up cell growth in the biofilter. However, the cell augmentation still suffers from cell washed out of the filter media in which it periodically requires cell addition. Cell entrapment, a cell immobilization in polymeric materials, is one of promising techniques that could provide the specific microorganism for target contaminant and lessen cell washout of the biofilter [5]. The integration of cell augmentation and cell entrapment techniques could have high potential for improving contaminant removal by biofilters. However, while entrapped cell augmentation has been studied extensively on wastewater treatment and site remediation [6], no reports have been published on the use of the cell augmentation and cell entrapment techniques in water treatment.

Entrapment material used in water filtration systems must be non-toxic, environmentally friendly, durable, and high strength. Among entrapment materials, alginate, a widely used natural compound, matches most of properties and thus has potential for drinking water application [6]. Alginate can form polymeric spherical beads with divalent and trivalent cations, such as calcium (Ca^{2+}), barium (Ba^{2+}), and aluminum (Al^{3+}) [7]. Among them, barium alginate has proven to be a durable and less toxic entrapment material [8], and barium alginate (BA)-entrapped cells have been successfully applied to remove numerous organic contaminants, such as pesticides and antibiotics [9–12]. This indicates the potential of BA-entrapped cells for applying in biofilter.

For future utilization, the entrapped cells could be applied to accelerate manganese removal performance by mixing with a filter medium in a biofilter. During rapid sand filtration, the filter medium requires a backwashing (cleaning) process. Turbid in the filter is removed while the filter medium disperses and settles in the filter. However, BA is a lightweight material and could be washed out during backwashing process. Also, left BA would separate from filter medium after backwashing. Thus, modification of BA to increase the material weight (increase settling velocity against the backwashing velocity) is required. Previous works by Jittawattanarat et al. [13] had immobilized powder activated carbon (PAC) along with the cells to improve treatment ability of pentachlorophenol in wastewater activated sludge process. PAC is not only help in contaminant absorption but also provides the site of microorganism to grow. Since PAC is commonly used in water treatment, the addition of PAC was advantageous in part due to its ability to increase the settling velocity of BA.

The ultimate goal of this study was to investigate manganese removal in a biofiltration system using barium alginate-entrapped cells. Thus far, neither study reported feasibility of water treatment by biofiltration supplemented with entrapped cells nor related fundamental information. This work aimed to characterize barium alginate bead properties and investigate the effectiveness of manganese removal by the entrapped cells (Figure 1). A manganese-oxi-

dizing bacterium, *Streptomyces violarus* SBP1, was selected. To enhance material properties, PAC was added to the BA entrapment material. Since PAC might affect the entrapment material durability. Barium alginate-beads were characterized for morphology, durability, and settling velocity. Finally, manganese removal from water by the PAC-supplemented BA-entrapped cells was demonstrated in comparison with free cells. Findings from this study will be useful for future applications of entrapped cells in biofilter systems.

Materials and methods

1) Chemicals and materials

All chemicals were bought from Ajax Fine Chem (Australia), RCI Labscan (Thailand), Aquasorb (Thailand), and Sigma-Aldrich (Singapore) via local distributors in Khon Kaen, Thailand. The filter media (including sand and anthracite (Apza anthracite, Thailand)) and PAC (iodine number of 900 mg g^{-1} and sizes of 63 to 125 µm) were obtained from Inter Water Treatment Co., Ltd., Khon Kaen, Thailand.

2) Bacterial preparation

The manganese-oxidizing bacterium, SBP1 (Genbank accession number MK212369), used in this study had been previously isolated from soil from Khon Kaen, Thailand [14]. A cultural medium consisted of 3.08 mg L^{-1} of $Mn_2SO_4 \cdot H_2O$, 1 mg L^{-1} of $Fe_2SO_4 \cdot H_2O$, 2,383 mg L^{-1} of HEPES buffer, 1,000 mg L^{-1} of peptone, and 250 mg L^{-1} of yeast extract [15]. To cultivate SBP1, the bacterium was maintained at 30±2 ºC and shaken in the cultural medium at 150 rpm for 1 d. Prior to the experiment, the SBP1 cells were centrifuged at 6,000 rpm at 4 °C for 15 min to obtain a concentrated SBP1 suspension. The concentrated SBP1 was used as free cells and in entrapped cell preparation.

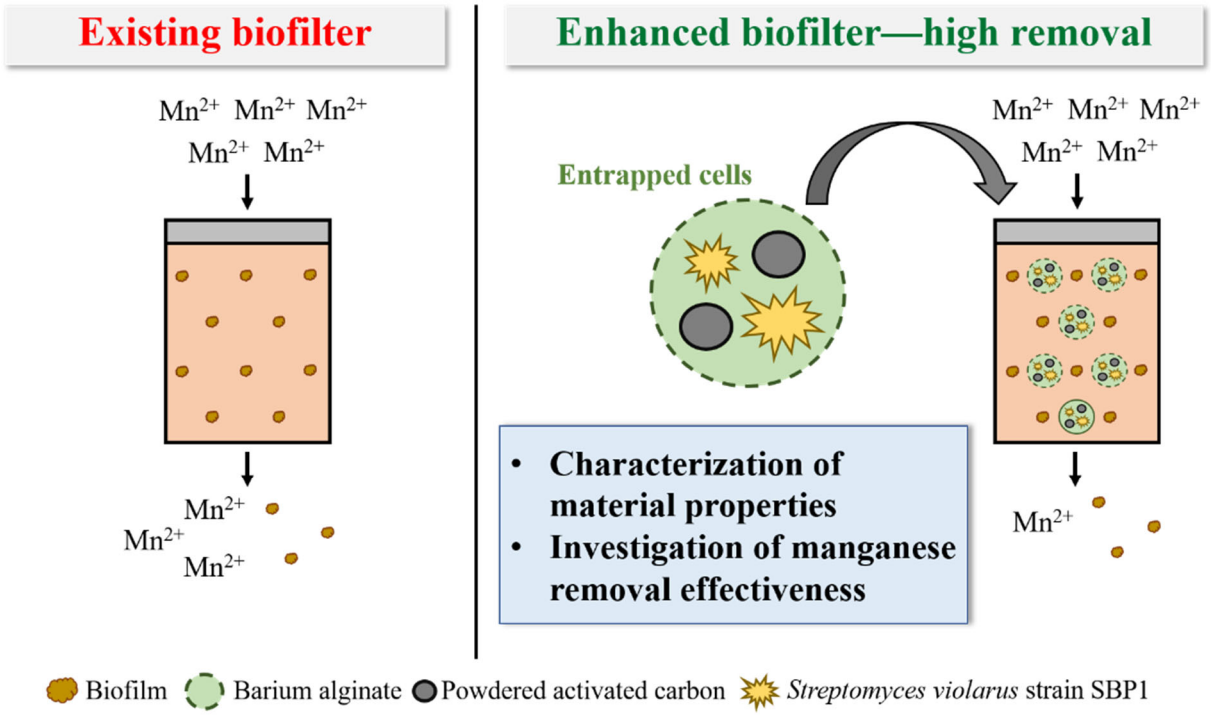

Figure 1 Schematic diagram of the study design.

3) Barium alginate and entrapped cell preparation

The entrapment materials applied in this study were BA and PAC-supplemented barium alginate (BAAC) with different PAC contents (1, 5, and 10% w/v). The barium alginate was prepared from sodium alginate (3% w/v) and barium chloride (3% w/v) solutions according to methods described by Taweetanawanit et al. [16]. The PAC-supplemented barium alginate was prepared by adding 1, 5, and 10% w/v PAC into the sodium alginate solution (samples designated BA1, BA5, and BA10, respectively).

The alginate and PAC-supplemented alginate solutions were prepared separately. Each solution was gently mixed by a magnetic stirrer at 50 ºC for 30 min and cooled at 4°C for 1 h. The cooled solutions were dropped into a barium chloride solution (3% w/v) by a peristaltic pump (Watson-Marlow, UK), and the spherical alginate beads were hardened in a barium chloride solution for 1 h. Then, the chemical residues in the beads were washed using reverse osmosis (RO) water (3 times) [16]. For the entrapped cells, the cooled alginate solution was mixed with the concentrated SBP1, and gel beads were formed using the method described above.

4) Entrapment material characterization

4.1) Material morphology

Bead sizes and morphology of the BA and BAAC samples were observed using Vernier Caliper and scanning electron microscopic (SEM) techniques. The BA and BAAC beads were fixed in a mixture of the glutaraldehyde (2.5% v/v) and barium chloride (3% w/v) solutions at 4 °C for 5 d. The fixed beads were then rinsed in the barium chloride solution for 20 m (3 times). Next, the rinsed beads were cut and dehydrated in serial ethanol solutions (30, 50, 70, 80, 90, and 99% v/v) for 20 m each. The dehydrated beads were dried in a critical point dryer (Quorum, K850, Canada), and the dried beads were placed on a stub and coated with gold (Cressington, 108auto, England). The beads were observed using SEM (LEO 1450VP, ZEISS, USA).

4.2) Material durability

Durability and compression tests were carried out to evaluate whether material can withstand the environmental condition (pH) and the weight of filter media when the beads are in the biofilter, respectively. First, the prepared beads were shaken in pH-adjusted RO water (at pH 6, 7, and 8) to pre-screen them for bead durability. The beads were shaken at 150 rpm for 7 d, after which the intact (no damaged) bead (be not swollen, broken, tender, and crumbling) samples were selected.

A bead compression test was subsequently carried out on the entrapment material (BA, BA1, BA5, and BA10) using a universal testing machine (UTM) (EZ-LX, Shimadzu, Japan). Conditions were set to a compressive speed of 10 mm m^{-1}, a bead displacement of 50%, and a contact force of 0.05 N. The Young's modulus was calculated using Eq. 1 [17].

$$\text{Compression force} = \frac{4R^{1/2}}{3}\frac{E}{1-v^2}\left(\frac{H}{2}\right)^{3/2} \quad \text{(Eq. 1)}$$

Where compression force is in Pa, R is the initial radius of the spherical bead (m), E is Young's modulus, H is displacement (m), and v is its Poisson's ratio. Poisson's ratio was assumed to be 0.5 [17].

4.3) Settling velocity

In practice, the bead movement in the filtration unit is important. After filter backwashing, filter media and the beads start to settle. Ideally, the beads should settle down at a speed comparable to those of the filter media (sand and anthracite) to evenly distribute the beads throughout the filter depth. Therefore, this study investigated the settling velocity of the beads (BA, BA1, BA5, and BA10) and filter media (sand (SA) and anthracite (AN)). Effective size of SA

and AN media were 0.5 mm, respectively. The beads or filter media were dropped into water in an acrylic column and were observed for travelling distance along the column versus time. The settling velocity was calculated followed Eq. 2 [18].

$$\text{Settling velocity} = H/\Delta t \quad \text{(Eq. 2)}$$

Where settling velocity is the bead movement (m s^{-1}), H is column height (m), and Δt is the change in time (s).

5) Manganese removal investigation

5.1) Influence of PAC content in entrapment materials on manganese removal

The synthetic groundwater contained 0.10 mg L^{-1} of K_2HPO_4, 10 mg L^{-1} of Na_2SO_4, 8 mg L^{-1} of $NaHCO_3$, 3.08 mg L^{-1} of $Mn_2SO_4 \cdot H_2O$, 0.05 mg L^{-1} of $Fe_2SO_4 \cdot H_2O$, 0.67 mg L^{-1} of $CaCl_2 \cdot 2H_2O$, 20 mg L^{-1} of $MgSO_4 \cdot 7H_2O$, 2 mg L^{-1} of NH_4Cl, 1,000 mg L^{-1} of peptone, and 250 mg L^{-1} of yeast extract [19].

The entrapment materials (BA, BA1, BA5, and BA10) were added into the synthetic groundwater (ratio 1:10 by volume) with initial manganese concentration of 1 mg L^{-1}. The reactors were held at room temperature and shaken at 150 rpm for 48 h. Water samples were taken at 0, 6, 12, 24, 36, and 48 h and analyzed for manganese concentrations. Manganese removal was calculated by Eq. 3. The experimental method followed Therkiattikul and Siripattanakul-Ratpukdi [20]. It is noted that this experiment is aimed to study only influence of PAC content; therefore, no microbial cell is added.

5.2) Comparison of the effects of free cells, entrapped cells, and entrapment material on manganese removal

Based on the previous section, entrapment material with suitable characteristics and high manganese removal performance was selected. Manganese removals using the selected entrapment material, the entrapped cells, and the free cells were compared. The cell concentration (in the reactor) of the entrapped and free cells was 200 mg-MLSS L^{-1}. The prepared experimental conditions are shown in Table 1. The reactors (with initial manganese concentrations of 1 mg L^{-1}) were maintained at room temperature and shaken at 150 rpm for 48 h. Water samples were taken at 0, 6, 12, 24, 36, and 48 h and were analyzed for manganese concentrations. Manganese removal performance was calculated by Eq. 3. The experimental procedure described by Therdkiattikul and Siripattanakul-Ratpukdi [20]. Manganese removal kinetics was then estimated using chemical reaction kinetics.

$$\text{Removal performance} = \frac{Mn_i - Mn_r}{Mn_i} \times 100 \quad \text{(Eq. 3)}$$

Where removal performance is manganese removal (%), Mn_i is initial manganese concentration (mg L^{-1}), and Mn_r is remaining manganese concentration (mg L^{-1}).

Table 1 Prepared conditions of the free cells, entrapped cells, and entrapment material for the manganese removal experiment

Sample	Sample description	Microbial cells (mg-MLSS L^{-1})	Composition		
			Barium alginate (g)	PAC (g)	Water sample (mL)
FC	Free SBP1	200	0	0	300
EM	Barium alginate	0	0.9	0	300

Table 1 Prepared conditions of the free cells, entrapped cells, and entrapment material for the manganese removal experiment (*continued*)

Sample	Sample description	Microbial cells (mg-MLSS L^{-1})	Composition		
			Barium alginate (g)	PAC (g)	Water sample (mL)
EC	SBP1 immobilized in barium alginate	200	0.9	0	300
EM-AC	PAC-barium alginate	0	0.9	Selected condition from previous experiment	300
EC-AC	SBP1 immobilized in PAC-barium alginate	200	0.9		300

6) Analytical procedure

Manganese analysis was performed using nitric acid digestion standard method 3030E [21]. After filtering a 25-mL water sample using a nylon filter (0.22 μm, Agela Technologies, USA), the sample was digested on a hot plate under a fume hood until a sample volume of 5 mL was reached. The digested sample was then mixed with 10 mL concentrated nitric acid (RCI Labscan, Thailand) and subsequently boiled until a sample volume of 5 mL was again reached. The final 5-mL sample was adjusted to 25 mL by adding deionized water. The adjusted sample was analyzed using an atomic absorption spectrophotometer (AAnalyst 800, Perkin Elmer, Singapore), and the bacterial cells sample (referred to as MLSS) was measured using gravimetric standard method 2540D [21].

7) Statistical analysis

Statistical analyses of Young's modulus, settling velocity, and manganese removal data are presented using averages (± standard deviation (SD)). The statistical software used was STATA (14, StataCorp, USA). A one-way analysis of variance (ANOVA) (at p-value < 0.05) was applied, and the Duncan's multiple range test was carried out for multiple comparisons.

Results and discussion

3.1) Material morphological observation

The barium alginate beads were sphere-shaped. The beads without and with PAC (BA, BA1, BA5, and BA10 beads) had quite similar diameters, including 4.02±0.08, 4.05±0.07, 4.06± 0.06, and 4.37±0.18 mm, respectively. Based on the SEM investigation, BA bead surfaces were smooth (Figure 2a), whereas the beads with PAC displayed rough surfaces due to the distribution of PAC over the surface (Figure 2b). The cross-sectional view of the beads made the porous structure of BA clearly visible (Figure 2c). This indicated that barium and alginate cross-linking promoted a dense net structure in the outer (surface) and inner layers of the bead [22].

On the other hand, Figure 2d shows the PAC infused to the barium and alginate net. Previous studies have reported that PAC does not directly react to the entrapment material [23]. The incorporation of PAC to barium alginate beads should somehow affect mechanical properties (strength and durability) of the BAAC beads in which will be discussed in the following section.

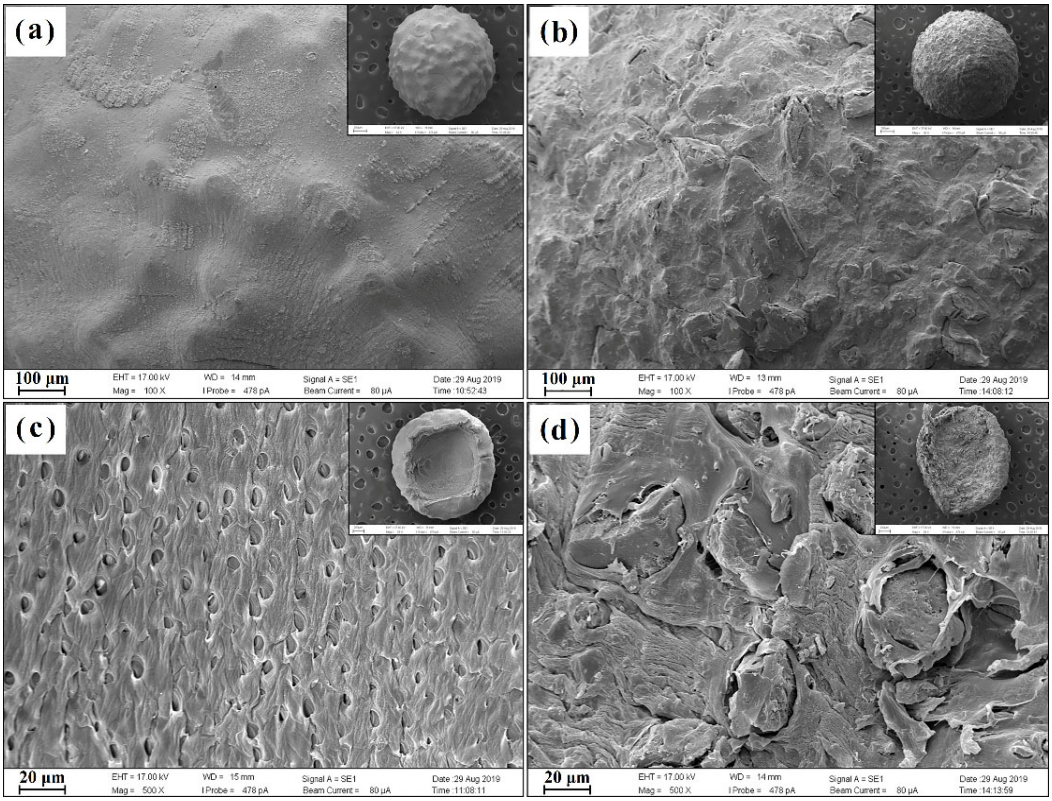

Figure 2 Bead morphology: (a) and (b) surface views of BA and BAAC beads, respectively, at a resolution of 100X and (c) and (d) cross-sectional views of BA and BAAC beads, respectively, at a resolution of 500X.

2) Material durability test

The pre-screening test revealed no damaged beads (swollen, broken, tender, or crumbling) subsequent to their being shaken in RO water. Figure 3 shows the compression test results for BA, BA1, BA5, and BA10 prior to the experiment and subsequent to shaking in RO water at pHs of 6, 7, and 8. All beads (BA, BA1, BA5, and BA10) prior to experimentation showed higher Young's modulus values of 0.389, 0.413, 0.773, and 1.230 MPa, respectively (Figure 3). It was clear that the PAC increased the bead strength. This was likely due to lower water content in the hydrogel with PAC [24–25].

After the beads were shaken in RO water at pHs 6 to 8, their strength as represented by Young's modulus values was slightly lower. Statistical analysis revealed that the strengths of BA and BA1 were not significantly different while those of BA5 and BA10 significantly decreased after shaking (Figure 3). The experiments were performed under pHs of 6 to 8 in order to simulate typical pH values in the environment. Beside that of BA10, it was found that the tested pHs (6-8) had no apparent effect on bead strength.

Bead durability was determined according to bead damage, strength, and observed changes after shaking. Overall, BA5 and BA10 had high strength, but BA10 was more sensitive in a wide range of environmental pH values resulting in significant lower Young's modulus values from the tests under higher pH environment (Figure 3).

This fundamental information is important for bead usage in the biofilter in the future. For practice, the beads strongly contact other beads and backwashing water. This may result in the bead damage. In this study, no damaged bead was reported. This revealed that the BAAC bead was durable and potential for real world application. In addition, the compression test data obtained from this study can be further used for the biofilter design later on.

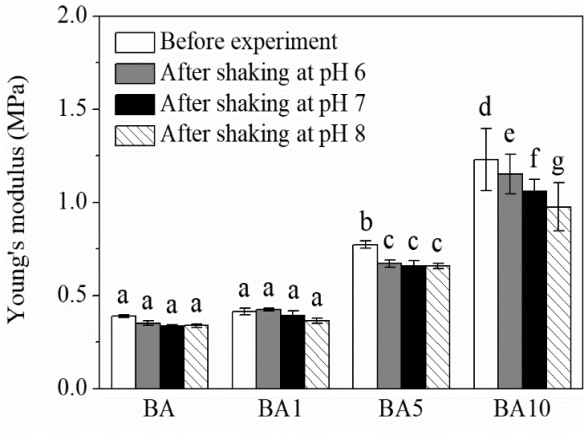

Figure 3 Young's modulus of the beads at 50% displacement (error bars represent the standard deviation and the different letters indicate statistical significance ($p < 0.05$)).

3) Settling velocity test

The settling velocities of SA, AN, BA, and BAAC with various activated carbon contents are shown in Figure 4. Between the filter media, SA and AN, SA has settling velocity twice higher than AN because SA has higher specific gravity compare to AN (2.65 versus 1.5) [26]. Among the alginate beads, those with higher PAC contents had higher settling velocities (Figure 4). This was due to increasing of beads density by PAC [24]. Based on statistical analysis, BA5 had a similar settling velocity to AN. Settling velocity of the beads is important parameter when applying the beads in biofilter. Too small settling velocity of the bead indicate that the bead is light and might be easily washed out of the biofilter during backwash. On the other hand, high settling velocity of the bead suggested that the beads might be falling at the bottom of filter bed causing the beads to receive the pressure of filter media and eventually rupture. Having the bead settling velocities same as filter media is favorable to keep the beads in filter bed (settling velocity > backwash velocity). Also, it is likely that the beads would settle and mixed well within the filter media (AN) layer. Comparing with recommended dual filter media backwash rate (0.017 m s^{-1}) [26], all BA with and without PAC had settling velocities greater than the design criteria (> 0.05 m s^{-1}). However, based on discussion above BA5 appears to be appropriate to use with AN media.

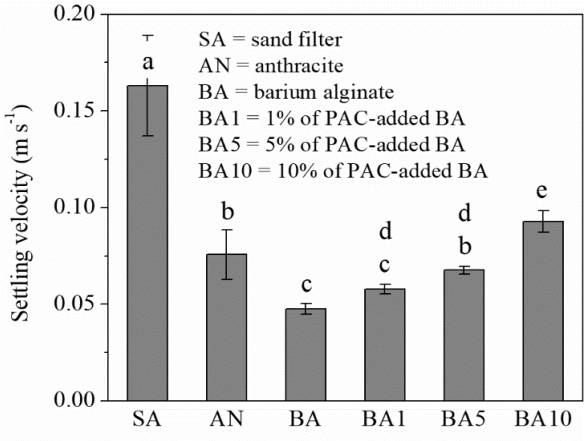

Figure 4 Comparison of barium alginate bead and filter medium settling velocities (error bars represent the standard deviation and the different letters indicate statistical significance ($p < 0.05$)).

4) Manganese removal under different PAC percentage conditions in entrapment materials

Manganese removal by BA, BA1, BA5, and BA10 beads was carried out. All removal tests reached equilibrium at 24 h (Figure 5a). Manganese removal efficiencies at 48 h of BA, BA1, BA5, and BA10 were 47.77±0.98, 48.19±0.68, 51.65± 1.98, and 53.41±2.47 %, respectively. As can be seen in Figure 5b, the BA and BA1 beads gave the similar significant level of manganese removal while both the BA5 and BA10 beads provide another significant level of manganese removal.

At equilibrium, all beads had reached steady state without achieving complete removal of manganese from the synthetic groundwater because of low tested manganese concentration. Among the tested beads, while metal adsorption by alginate has been investigated in previous research [23], the present study well supported the hypothesis that an increased PAC percentage can promote manganese removal [24, 27–28]. BA5 was durable, had a settling velocity comparable to the filter media, and demon-

strated high manganese adsorption. Therefore, BA5 was selected for further experimentation for entrapped cells.

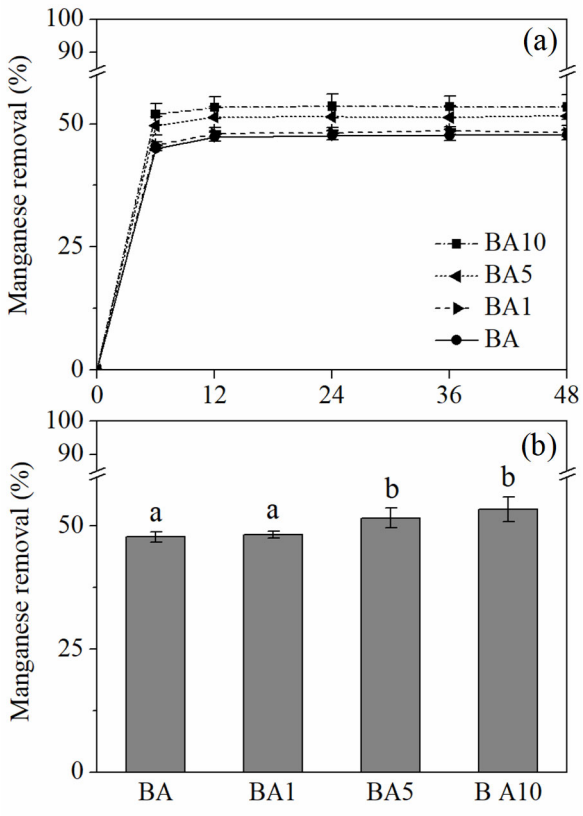

Figure 5 Manganese removal: (a) manganese removal performance of entrapment materials, and (b) the average percentage of manganese removal at 48 h (error bars represent the standard deviation and the different letters indicate statistical significance ($p < 0.05$)).

5) Manganese removal by free cells, entrapped cells, and entrapment material

Manganese removal by the free cells, entrapped cells (EC and EC-AC), and entrapment materials (EM and EM-AC) is shown in Figure 6. Manganese removal by entrapment materials (EM and EM-AC) was quick and reached stable at 12 h, while the manganese removal by the entrapped cells (EC and EC-AC) continuously increased. Manganese removal by FC had the slowest rate but continual increase, plateauing at 48 h. At test completion (48h), manganese removal by FC, EM, EC, EM-AC, and EC-AC was 58.05±1.50, 46.03±0.87, 54.67±1.40, 51.32± 1.46, and 57.91±1.65 %, respectively.

The findings on manganese removal by FC confirmed that SBP1 effectively transformed dissolved manganese (Mn^{2+}) to insoluble manganese (Mn^{3+} and Mn^{4+}) in water [14]. The entrapped cell samples (EC and EC-AC) resulted in a consistently higher performance than the entrapment materials (EM and EM-AC). This indicated that the SBP1 was entrapped and effectively removed manganese even in the entrapment material [20]. This finding is consistent with findings from previous works investigating removal of Hg^{2+}, Cd^{2+}, Zn^{2+}, and Ni^{2+} by entrapped cells [29–30]. A previous work reported the BA-entrapped cell leaching during the application [31]. But the BA-entrapped cell system maintained high contaminant removal efficiency since the microbial cells could grow inside the entrapment material. It is noted that PAC (applied in this work) might influence bacterial attachment in the BAAC-entrapped cells. Activated carbon was well-known supporting material because of surface area, roughness, and charge [32]. The BAAC-entrapped cells likely retained cells better than the BA-entrapped cells. In this study, the single batch experiment was performed; therefore, the cell leaching did not obviously expect and monitor. In the future, the cell leaching should be monitored during the long-term test.

For removal mechanism, manganese is an important trace element playing an important role in environment [33]. Dissolved manganese was removed by bacterial cells via either biosorption or biotransformation (manganese transformation to be insoluble forms) during metabolism process [34–35]. As mentioned previously, SBP1 mainly removed manganese via biological transformation. The biochemical reaction kinetics including zero, first, and second order kinetic equations were evaluated. The result from this study appeared to be second order kinetic mechanism and were then calculated by Eq. 4.

$$\frac{1}{Mn_r} = kt + \frac{1}{Mn_i} \quad \text{(Eq. 4)}$$

Where Mn_i is manganese initial concentration (mg L^{-1}), Mn_r is manganese remaining concentration (mg L^{-1}), t is time (d), and k is rate constant (d^{-1}).

Table 2 shows the manganese removal kinetic results and efficiencies. The manganese removal kinetics followed second order mechanism indicated that not only manganese concentration played a role in the treatment. This could imply that the manganese removal in this study was influenced by factors. The manganese transformation depended on microbial cell and manganese concentrations [36]. This also correlated to a previous work describing microbial transformation of xenobiotics at low concentrations [37]. The contaminant diffusion into porous material might influence manganese removal as well. Adsorption reaction was faster than the biological reaction. However, after 48 h it is likely that the biological transformation would play the main role in manganese removal. The entrapped cells resulted in rapid and consistent manganese removal due to the integration of adsorption and bio-oxidation effects. In practice, the entrapment material could lessen environment stresses on the microbial cells [12, 38].

Entrapped cells were successfully applied in wastewater treatment and site remediation [10–11, 39–40]. In overall of this study, the potential of the entrapped cells in terms of material characteristics and contaminant removal for water treatment system was proved. The entrapped cells could directly add into the existing filter by mixing with filter medium. The entrapped cell-added filter did not require extra configurations and chemicals.

The reusability of the bead is important for the water treatment process. This study preliminarily indicated the potential to apply the entrapped cells in the water treatment system by the bead durability test. In the future, the pilot application of the entrapped cell-added biofilter is required to confirm the system performance and reusability.

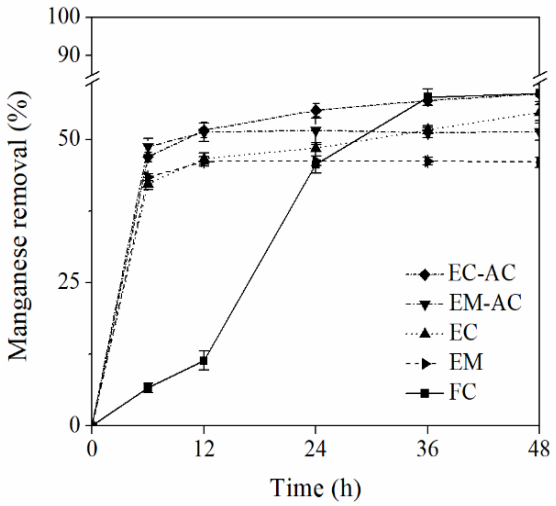

Figure 6 Manganese removal experiments by the free cells, entrapped cells, and entrapment materials

Table 2 Manganese removal kinetics and efficiency rates

Sample	Kinetic equation[a]	R^2	Manganese removal efficiency (%)
FC	y=0.954x+0.626	0.97	58.05
EM	y=1.547x+1.004	0.83	46.03
EC	y=1.551x+0.974	0.87	54.67
EM-AC	y=1.893x+1.029	0.82	51.32
EC-AC	y=1.894x+0.992	0.87	57.91

Remark: [a] Kinetic equation (as shown in Eq. 4), where x is time (t) in d, and y is 1/manganese remaining concentration (1/ Mn_r) in L mg^{-1}.

Conclusion

PAC-supplemented barium alginate beads can be applied effectively as an entrapment material for manganese removal in a biofiltration system. Morphological observation showed that the PAC was distributed over the bead pore.

The PAC-supplemented barium alginate bead had high strength (up to 1.23 MPa) and durability. In addition, PAC increased the entrapment material weight, leading to a settling velocity comparable to that of the filter medium. The cells entrapped in barium alginate with 5% PAC resulted in the highest manganese removal rate (58%), indicating the potential for PAC-barium alginate-entrapped cells to be applied in biofilters. Further study on the influence of operational conditions should be conducted before the cells are used in practice.

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References

[1] Zhu, I.X., Getting, T., Bruce, D. Review of biologically active filters in drinking water applications. Journal - American Water Works Association, 2010, 102(12), 67–77.

[2] Patil, D.S., Chavan, S.M., Oubagaranadin, J.U.K. A review of technologies for manganese removal from wastewaters. Journal of Environmental Chemical Engineering, 2016, 4(1), 468–487.

[3] Cai, Y., Li, D., Liang, Y., Luo, Y., Zeng, H., Zhang, J. Effective start-up biofiltration method for Fe, Mn, and ammonia removal and bacterial community analysis. Bioresource Technology, 2015, 176, 149–155.

[4] Bai, Y., Zhang, J., Li, Y.-F., Gao, Y.-N., Li, Y. Biomass and microbial activity in a biofilter during backwashing. Journal of Zhejiang University: Science, 2005, 6B (5), 427–432.

[5] Kourkoutas, Y., Bekatorou, A., Banat, I.M., Marchant, R., Koutinas, A.A. Immobilization technologies and support materials suitable in alcohol beverages production: a review. Food Microbiology, 2004, 21(4), 377–397.

[6] Siripattanakul, S., Khan, E. Fundamentals and applications of entrapped cell bioaugmentation for contaminant removal. *In:* Shah, V. Emerging Environmental Technologies. Volume II. Springer: Dordrecht, 2010, 147–169.

[7] Brus, J., Urbanova, M., Czernek, J., Pavelkova, M., Kubova, K., Vysloužil, J., Kulich, P. Structure and dynamics of alginate gels cross-linked by polyvalent ions probed via solid state NMR Spectroscopy. Biomacromolecules, 2017, 18(8), 2478–2488.

[8] Bajpai, S.K., Sharma, S. Investigation of swelling/degradation behaviour of alginate beads crosslinked with Ca^{2+} and Ba^{2+} ions. Reactive and Functional Polymers, 2004, 59(2), 129–140.

[9] Siripattanakul, S., Wirojanagud, W., McEvoy, J.M., Casey, F.X.M., Khan, E. A feasibility study of immobilized and free mixed culture bioaugmentation for treating atrazine in infiltrate. Journal of Hazardous Materials, 2009, 168(2–3), 1373–1379.

[10] Khalid, S., Han, J.-I., Hashmi, I., Hasnain, G., Ahmed, M.A., Khan, S.J., Arshad, M.

Strengthening calcium alginate microspheres using polysulfone and its performance evaluation: Preparation, characterization and application for enhanced biodegradation of chlorpyrifos. Science of the Total Environment, 2018, 631–632, 1046–1058.

[11] Ruan, B., Wu, P., Chen, M., Lai, X., Chen, L., Yu, L., …, Liu, Z. Immobilization of *Sphingomonas* sp. GY2B in polyvinyl alcohol-alginate-kaolin beads for efficient degradation of phenol against unfavorable environmental factors. Ecotoxicology and Environmental Safety, 2018, 162, 103–111.

[12] Taweetanawanit, P., Ratpukdi, T., Siripattanakul-Ratpukdi, S. Performance and kinetics of triclocarban removal by entrapped *Pseudomonas fluorescens* strain MC46. Bioresource Technology, 2019, 274, 113–119.

[13] Jittawattanarat, R., Kostarelos, K., Khan, E. Immobilized-cell-augmented activated sludge process for treating wastewater containing hazardous compounds. Water Environment Research, 2007, 79 (5), 461–471.

[14] Youngwilai, A., Kidkhunthod, P., Jearanaikoon, N., Chaiprapa, J., Supanchaiyamat, N., Hunt, A.J., …, Siripattanakul-Ratpukdi, S. Simultaneous manganese adsorption and biotransformation by *Streptomyces violarus* strain SBP1 cell-immobilized biochar. Science of the Total Environment, 2020, 713, 136708.

[15] Cerrato, J.M., Falkinham, J.O., Dietrich, A.M., Knocke, W.R., McKinney, C.W., Pruden, A. Manganese-oxidizing and -reducing microorganisms isolated from biofilms in chlorinated drinking water systems. Water Research, 2010, 44(13), 3935–3945.

[16] Taweetanawanit, P., Radpukdee, T., Giao, N.T., Siripattanakul-Ratpukdi, S. Mechanical and chemical stabilities of barium alginate gel: influence of chemical concentrations. Key Engineering Materials, 2017, 718, 62–66.

[17] Wang, C.X., Cowen, C., Zhang, Z., Thomas, C.R. High-speed compression of single alginate microspheres. Chemical Engineering Science, 2005, 60(23), 6649–6657.

[18] Wang, Z., Belovich, J.M. A simple apparatus for measuring cell settling velocity. Biotechnology Progress, 2010, 26(5), 1361–1366.

[19] Ito, A., Miura, J.I., Ishikawa, N., Umita, T. Biological oxidation of arsenite in synthetic groundwater using immobilised bacteria. Water Research, 2012, 46(15), 4825–4831.

[20] Therdkiattikul, N., Siripattanakul-Satpukdi, S. Manganese removal from groundwater using powdered activated carbon-added barium alginate entrapped cells. UBU Engineering Journal, 2020, 13(2), 77–87. (in Thai)

[21] American Public Health Association (APHA), American Water Works Association (AWWA), Water Environment Federation (WEF). Standard methods for the examination of water and wastewater. 23rd edition. Washington, DC: APHA, 2017.

[22] Mørch, Y.A., Qi, M., Gundersen, P.O.M., Formo, K., Lacik, I., Skjåk-Bræk, G., …, Strand, B.L. Binding and leakage of barium in alginate microbeads. Journal of Biomedical Materials Research - Part A, 2012, 100 A(11), 2939–2947.

[23] Wang, B., Wan, Y., Zheng, Y., Lee, X., Liu, T., Yu, Z., …, Gao, B. Alginate-based composites for environmental applications: a critical review. Critical Reviews in Environmental Science and Technology, 2019, 49(4), 318–356.

[24] Kim, T.Y., Jin, H.J., Park, S.S., Kim, S.J., Cho, S.Y. Adsorption equilibrium of copper ion and phenol by powdered activated carbon, alginate bead and alginate-activated carbon bead. Journal of Industrial and

Engineering Chemistry, 2008, 14(6), 714–719.

[25] Tomović, N.S., Trifković, K.T., Rakin, M.P., Rakin, M.B., Bugarski, B.M. Influence of compression speed and deformation percentage on mechanical properties of calcium alginate particles. Chemical Industry and Chemical Engineering Quarterly, 2015, 21(3), 411–417.

[26] Davis, M.L. Water and wastewater engineering: Design principles and practice. New York: McGraw-Hill, 2010.

[27] Choi, J.W., Yang, K.S., Kim, D.J., Lee, C.E. Adsorption of zinc and toluene by alginate complex impregnated with zeolite and activated carbon. Current Applied Physics, 2009, 9(3), 694–697.

[28] Hassan, A.F., Abdel-Mohsen, A.M., Elhadidy, H. Adsorption of arsenic by activated carbon, calcium alginate and their composite beads. International Journal of Biological Macromolecules, 2014, 68, 125–130.

[29] Arica, M.Y., Bayramoğlu, G., Yilmaz, M., Bektaş, S., Genç, O. Biosorption of Hg^{2+}, Cd^{2+}, and Zn^{2+} by Ca-alginate and immobilized wood-rotting fungus *Funalia trogii*. Journal of Hazardous Materials, 2004, 109(1–3), 191–199.

[30] Bayramoğlu, G., Yakup Arica, M. Construction a hybrid biosorbent using *Scenedesmus quadricauda* and Ca-alginate for biosorption of Cu(II), Zn(II) and Ni(II): kinetics and equilibrium studies. Bioresource Technology, 2009, 100(1), 186–193.

[31] Sonsuphab, K., Ratpukdi, T., Siripattanakul-Ratpukdi, S. Influence of infiltration rates during profenofos pesticide removal by attached and entrapped bacterial cells. Desalination and Water Treatment, 2018, 120, 311–322.

[32] Basu, O.D., Dhawan, S., Black, K. Applications of biofiltration in drinking water treatment - A review. Journal of Chemical Technology and Biotechnology, 2016, 91(3), 585–595.

[33] Tebo. B.M., Johnson, H.A., McCarthy, J.K., Templeton, A.S. Geomicrobiology of manganese(II) oxidation. Trends in Microbiology, 2005, 13(9), 421–428.

[34] Nealson, K.H. The manganese-oxidizing bacteria. *In:* Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, KH., Stackebrandt, E. The Prokaryotes. New York: Springer, 2005.

[35] Tobiason, J.E., Bazilio, A., Goodwill, J., Mai, X., Nguyen, C. Manganese removal from drinking water sources. Current Pollution Reports, 2016, 2(3), 168–177.

[36] Schmidt, S.K., Simkins, S., Alexander, M. Models for the kinetics of biodegradation of organic compounds not supporting growth. Applied and Environmental Microbiology, 1985, 50(2), 323–331.

[37] Paris, D.F., Steen, W.C., Baughman, G.L., Barnett Jr., J.T. Second-order model to predict microbial degradation of organic compounds in natural waters. Applied and Environmental Microbiology, 1981, 41(3), 603–609.

[38] Siripattanakul, S., Wirojanagud, W., McEvoy, J., Khan, E. Effect of cell-to-matrix ratio in polyvinyl alcohol immobilized pure and mixed cultures on atrazine degradation. Water, Air, and Soil Pollution: Focus, 2008, 8(3–4), 257–266.

[39] Cruz, I., Bashan, Y., Hernàndez-Carmona, G., de-Bashan, L.E. Biological deterioration of alginate beads containing immobilized microalgae and bacteria during tertiary wastewater treatment. Applied Microbiology and Biotechnology, 2013, 97(22), 9847–9858.

[40] Solé, A., Matamoros, V. Removal of endocrine disrupting compounds from wastewater by microalgae co-immobilized in alginate beads. Chemosphere, 2016, 164, 516–523.