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Synthesis of Microaglae-Capturing Magnetic Microcapsule Using $CaCO_3$ Microparticles and Layer-by-Layer Coating

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Abstract Microalgae produce not only lipids for biodiesel production but also valuable biochemicals which are often accumulated under cellular stress mediated by certain chemicals. While the microcarriers for the application of drug delivery systems for animal cells are widely studied, their applications into microalgal research or biorefinery are rarely investigated. Here we develope dual-functional magnetic microcapsules which work not only as flocculants for microalgal harvesting but also potentially as microcarriers for the controlled release of target chemicals stimulating microalgae to enhance the accumulation of valuable chemicals. Magnetic microcapsules are synthesized by layer-by-layer(LbL) coating of PSS-PDDA on Fe_3O_4 nanoparticle-embedded $CaCO_3$ microparticles followed by removing $CaCO_3$ sacrificial templates. The positively charged magnetic microcapsules flocculate microalgae by electrostatic interaction which are sequentially collected by the magnetophoretic separation. The microcapsules with a polycationic outer layer provide efficient binding sites for negatively charged microalgae and by that means are further utilized as a chemical-delivery and flocculation system for microalgal research and biorefineries.

Key words microcapsule, microalgae, calcium carbonate, layer-by-layer, flocculation.

1. Introduction

Polymeric capsules ranging from nanometer to micrometer scale have been receiving wide attention as drug delivery carriers.[1)] The polymeric delivery systems have been developed in a way to be controlled for releasing chemicals and targeting to the defined sites by some triggers such as magnetic field or pH changes.[2-5)] Layer-by-layer(LbL) technique, which is based on alternating adsorption of oppositely charged polyelectrolytes, has become an attractive route to produce various types of polymeric capsules with controllable permeability.[2,6-9)]

Microalgae are looked upon as one of the most promising source for biodiesel production, since they gain several advantages over the conventional oil crops by having a high biomass productivity per area and high lipid content.[10-12)] Harvesting of microalgae is an energy-consuming process which takes up 20-30 % of the total cost of microalgal biodiesel production.[10)] The high cost originates from the low concentration, normally under 2 g/L, and the small size of cell body with negatively charged surface, which lead to good dispersion of microalgae. The most widely utilized microalgae harvesting techniques are centrifugation, filtration, and electrolysis-based technologies, which are incompatible with low cost microalgal biorefinery, unfortunately.[13-15)] The flocculation is considered to be the most effective and economical method for large-scale microalgae harvesting.[16)] Recently, magnetic-nanoparticle-based flocculants have drawn attentions in this field with the advantages being the recyclability and the ease of their recovery from microalgal flocs causing no contamination of both medium and

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biomass.[17-20)]

Moreover, microalgae produce high value biochemicals such as β-carotene and astaxanthin.[21-23)] It has been known that the accumulation of targeted compounds is involved with cellular stress normally such as nitrogen depletion and/or strong light.[24)] Recently, certain chemicals such as azide and lactone antibiotic have been reported to trigger the induction of the target compounds.[25,26)] For better understanding of the mechanism, microcapsule system can be used as a fascinating tool, which would enable controlled release of such chemicals as well as, on-site observation of the local environmental cell changes in close proximity to source of chemicals. While the microcapsules have been widely studied for the application of *in-vivo* or *in-vitro* researches of animal cells, little has been reported on the microalgal research.

The purpose of this study is to develop dual-functional magnetic microcapsules which work not only as flocculants for microalgal harvesting but also as microcarriers for the controlled release of target chemicals stimulating microalgae to enhance the accumulation of valuable chemicals. Herein, we report microalgae-capturing magnetic microcapsules prepared by LbL coating of poly(sodium 4-styrenesulfonate) (PSS) and poly(diallyldimethylammonium chloride) (PDDA) and by using Fe_3O_4 nanoparticle-embedded $CaCO_3$ microparticles as sacrificial templates. We show that the microcapsules with the cationic polymer-coated surface become efficient binding sites for negatively charged microalgae. Furthermore, by labeling fluorescent marker on the capsules, the clear geographical information of microcapsules and microalgae could be obtained by fluorescent microscopic observation.

2. Experimental Procedure

Fig. 1 shows the procedure of preparing magnetic microcapsules. First, 31 mg of Fe_3O_4 nanoparticles, which is calculated to be 3 wt% in the final $CaCO_3$ precipitates, was dispersed in 2 mL of PSS (Mw ~70,000, Sigma-Aldrich) stock solution with the concenctration of 2 mg PSS in 1 mL of 0.5 M NaCl solution. This step would provide the Fe_3O_4 particles affinity with $CaCO_3$ matrix, since calcium ions(Ca^{2+}) are known to readily adhere to the sulfonate groups(-SO^{3-}) of PSS. Second, Fe_3O_4-$CaCO_3$ composites were prepared using co-precipitation method by adding 50 mL of 0.2 M sodium carbonate (Na_2CO_3, ≥ 99.5 %, Sigma-Aldrich) solution into 48 ml of 0.2 M calcium chloride ($CaCl_2 \cdot 2H_2O$, ≥ 99.0 %, Sigma-Aldrich) containing magnetic nanoparticles (Fe_3O_4, < 50 nm, Sigma-Aldrich) followed by stirring at 1000 rpm using magnetic stirrer for 30 min. Light brownish precipitates were formed where the color comes from Fe_3O_4 particles. Third, PSS and PDDA (Mw 100,000~200,000, Sigma-Aldrich) in equal concentration (2.0 mg in 1 mL of 0.5 M NaCl solution) were used for LbL coating forming the$(PSS/PDDA)_3$ shell on Fe_3O_4-$CaCO_3$ core. $CaCO_3$ crystals were introduced into PSS and PDDA solutions sequentially with gentle shaking for 15 minutes. Depositions of PSS and PDDA were repeated three times each, resulting in the adsorption of six layers onto the $CaCO_3$ surface. After each adsorption, the particles were centrifuged and washed repeatedly at least three times with distilled water. For the fifth layer of deposition, PSS was labeled with fluorescent dye, dihydrorhodamine 123 (DHR123, > 95 %, Sigma-Aldrich), to visualize polymer shell under fluorescence microscope. Finally, the microcapsules were obtained by dissolving the $CaCO_3$ cores using 0.2 M EDTA solution at pH 7. The flocculation test was performed by mixing the microcapsules with microalgal cells (*Chlorella sp*. KR-1, cultured at the Korea Institute of Energy Research).[27)]

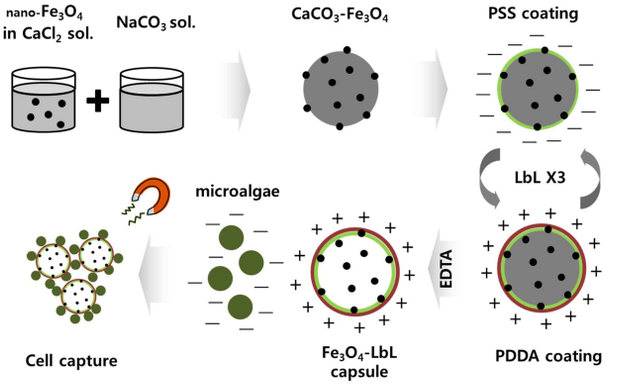

Fig. 1. Preparation of magnetic microcapsule.

The optical density(OD) measurement was carried out at 660 nm by using UV-VIS spectrophotometer (Optizen 2120 UV, Mecasys Co., Korea). Zeta-potential measurements were performed in water using a Zetasizer (ZS90, Malvern). Microalgal cells, $CaCO_3$ crystals, polymer microcapsules and their combinations were observed in bright-field, cross-polarization and fluorescence modes of optical microscopy (Microscope Axio Imager A2, Carl Zeiss). For the fluorescence images, a long-pass filter (Excitation filter: 450-490 nm band pass; BS FT: 510 nm; EM LP: 515 nm) was used. The morphology of the $CaCO_3$ crystals and polymer microcapsules with elemental mapping was investigated by field-emission scanning electron microscopy (FE-SEM, S-4800, Hitachi) equipped with energy-dispersive X-ray(EDX) spectroscopy.

3. Result and Discussion

The composite particles were synthesized with 5-10 μm in size and $CaCO_3$ was formed in crystalline phase

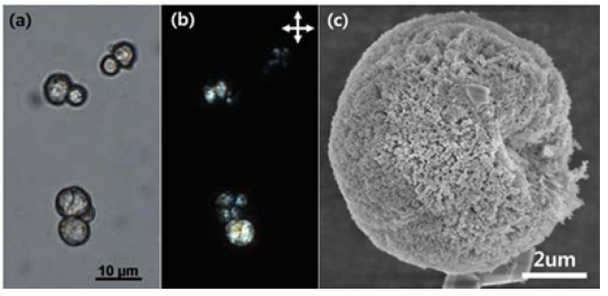

Fig. 2. (a) Optical, (b) cross polarization microscope and (c) SEM images of Fe_3O_4-$CaCO_3$ microparticles.

as shown in optical microscope images with cross polarization in Fig. 2. SEM image shows the typical porous morphology of vaterite phase of $CaCO_3$. High porosity of vaterite can be a beneficial property as a sacrificial template with the facile integration of foreign molecules.[28,29] Furthermore, the biocompatibility and the mild decomposition condition allow $CaCO_3$ as an attractive sacrificial template in the preparation of drug delivery carriers.[30,31]

Fig. 3 shows that magnetic microcapsules were successfully prepared after removal of $CaCO_3$ crystalline cores from polymer coated Fe_3O_4-$CaCO_3$ microparticles. The presence of crystalline phase is confirmed by the polarization light microscope while the chemical elements are visualized by SEM-EDX spectroscopy mapping. In the cross polarization microscope images, the crystalline core structure(bright part in Fig. 3aII) is not visible in Fig. 3bII after EDTA treatment whereas the existence of polymer shell was detected by fluorescent green signal. The EDTA treatment could selectively dissolve $CaCO_3$ while Fe_3O_4 nanoparticles remaining in the polymer shell as shown by SEM image and EDX spectroscopy mapping(Fig. 3(d)). Removing $CaCO_3$ crystal in the polymer

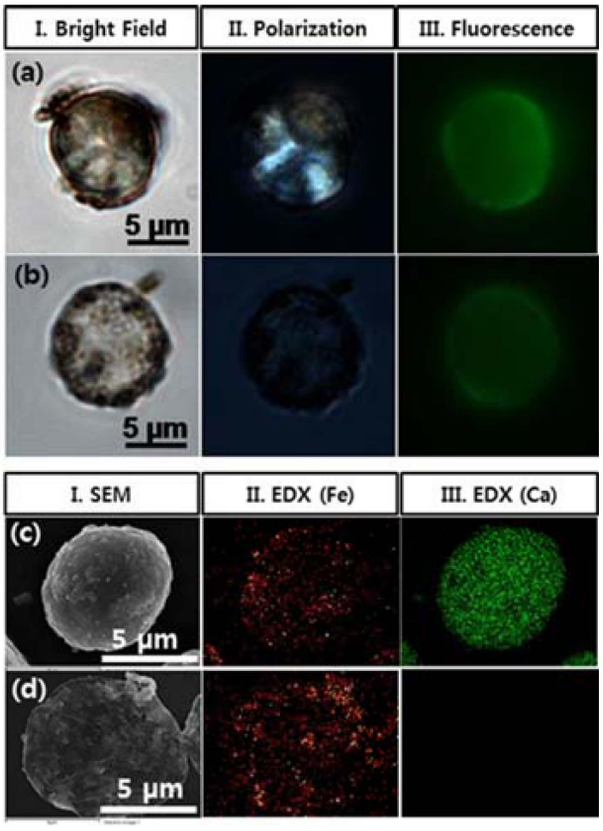

Fig. 3. (a, b) Optical/polarization/fluorescent microscope and (c, d) SEM and EDX spectroscopy mapping images of (a, c) before and (b, d) after removal of $CaCO_3$ core structures.

shell by the EDTA treatment is such a mild process that even the microalgae embedded in calcite could be intact after the gentle dissolvation of $CaCO_3$ using EDTA as reported by Kim et al.[32] Regarding the thickness of polymer shell, it was reported that multiple layers of polyelectrolyte coating are required to obtain mechamically

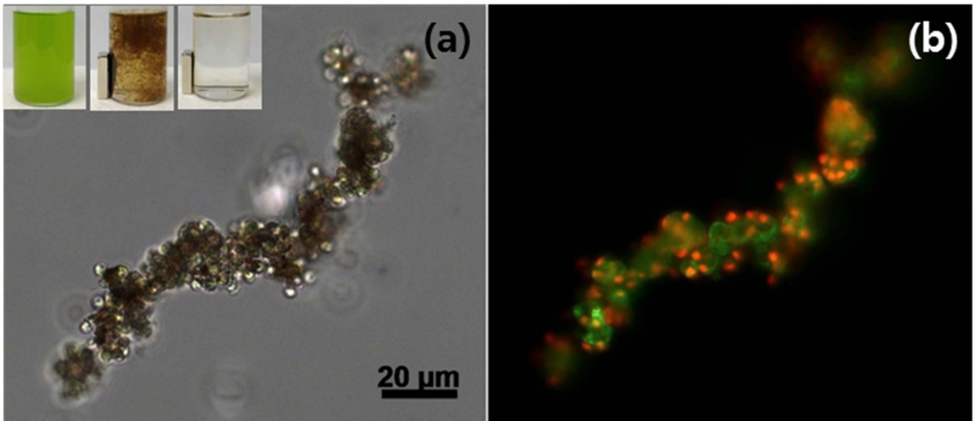

Fig. 4. (a) Optical and (b) fluorescent microscope images of microalgae-magnetic microcapsule flocculate(inset, photos of before and after injection of microcapsule dispersion into microalgal culture followed by magnetophoretic separation).

stable microcapsules and the thickness of polymer shell proportionally increased with the number of coating reaching to 24 nm by 11 times of alternating adsorption of oppositely charged polyelectrolytes(PAH/PSS).[33]

The zeta potential measurements indicate that the surface potential of the magnetic microcapsules with PDDA outer coating is +31 mV at pH 6.5. Since the zeta potential value of microalgae, *Chlorella* sp. KR-1, is negative(−18 mV) at pH 6.5 of culture medium, the electrostatic interaction between the microcapsule and microalgae could be occurred. As shown in the inset of Fig. 4, microalgae were flocculated with magnetic microcapsules by simple mixing of the two dispersions and further could be magnetophoretically separated by applying external magnetic field. Over 99 % of microalgae were found to be attached on microcapsules according to the OD measurement and the equation, harvesting or separation efficiency $[\%] = (1 - OD_f/OD_i) \times 100$, where OD_i and OD_f are the initial OD and the OD of the supernatant after magnetophoretic separation, respectively.[17,34]

Fig. 4 shows the microscope image of the electrostatically agglomerated heterogeneous elements. The fluorescent microscope images in Fig. 4(b) obtained by superimposing green fluorescence from magnetic microcapsules and red autofluorescence from microalgae provide clear information of geographical arrangement of two attached elements. The magnetic microcapsules can be further applied as microcarriers for the controlled release of chemicals such as azide and lactone antibiotic triggering the induction of the targeted valuable compounds of microalgae.[24-26]

4. Conclusion

We synthesized magnetic microcapsules by PSS-PDDA alternate coating on Fe_3O_4-bearing $CaCO_3$ microparticles followed by removing $CaCO_3$ sacrificial templates. Microalgae were electrostatically attached on microcapsule by taking advantage of positive surface charge of polyelectrolyte coating of PDDA and negatively charged microalgae. The developed magnetic microcapsules with a surface charge would be useful for the practical applications as microcarriers inducing targeted valuable components of microalgae and the microalgal research by *in-situ* monitoring of chemical response in micro-environment.

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Reference

1. J. Kost and R. Langer, Adv. Drug Delivery Rev., **46**, 125 (2001).
2. C. Déjugnat and G. B. Sukhorukov, Langmuir, **20**, 7265 (2004).
3. N. Gaponik, I. L. Radtchenko, G. B. Sukhorukov and A. L. Rogach, Langmuir, **20**, 1449 (2004).
4. M. F. Bédard, B. G. De Geest, A. G. Skirtach, H. Möhwald and G. B. Sukhorukov, Adv. Colloid Interface Sci., **158**, 2 (2010).
5. B. Zebli, A. S. Susha, G. B. Sukhorukov, A. L. Rogach and W. J. Parak, Langmuir, **21**, 4262 (2005).
6. A. A. Antipov and G. B. Sukhorukov, Adv. Colloid Interface Sci., **111**, 49 (2004).
7. A. A. Antipov, D. Shchukin, Y. Fedutik, A. I. Petrov, G. B. Sukhorukov and H. Möhwald, Colloids Surf., A, **224**, 175 (2003).
8. S. A. Sukhishvili, Curr. Opin. Colloid Interface Sci., **10**, 37 (2005).
9. B. G. De Geest, N. N. Sanders, G. B. Sukhorukov, J. Demeester and S. C. De Smedt, Chem. Soc. Rev., **36**, 636 (2007).
10. T. M. Mata, A. A. Martins and N. S. Caetano, Renew. Sust. Energ. Rev., **14**, 217 (2010).
11. R. H. Wijffels, M. J. Barbosa and M. H. Eppink, Biofuels, Bioprod. Biorefin., **4**, 287 (2010).
12. H.-W. Yen, I.-C. Hu, C.-Y. Chen, S.-H. Ho, D.-J. Lee and J.-S. Chang, Bioresour. Technol., **135**, 166 (2013).
13. S.-A. Choi, J.-S. Lee, Y.-K. Oh, M.-J. Jeong, S. W. Kim and J.-Y. Park, Algal Res., **3**, 44 (2014).
14. T. Hwang, S.-J. Park, Y.-K. Oh, N. Rashid and J. I. Han, Bioresour. Technol., **139**, 379 (2013).
15. J. Kim, B. G. Ryu, K. Kim, B. K. Kim, J. I. Han and J. W. Yang, Bioresour. Technol., **123**, 164 (2012).
16. D. Vandamme, I. Foubert and K. Muylaert, Trends Biotechnol., **31**, 233 (2013).
17. K. Lee, S. Y. Lee, J.-G. Na, S. G. Jeon, R. Praveenkumar, D.-M. Kim, W.-S. Chang and Y.-K. Oh, Bioresour. Technol., **149**, 575 (2013).
18. K. Lee, S. Y. Lee, R. Praveenkumar, B. Kim, J. Y. Seo, S. G. Jeon, J.-G. Na, J,-Y. Park, D.-M. Kim and Y.-K. Oh, Bioresour. Technol., **167**, 284 (2014).
19. Y.-C. Lee, K. Lee and Y.-K. Oh, Bioresour. Technol., **184**, 63 (2015).
20. J. Y. Seo, R. Praveenkumar, B. Kim, J.-C. Seo, J.-Y. Park, J.-G. Na, S. G. Jeon, S. B. Park, K. Lee and Y.-K. Oh, Green Chem., **18**, 3981 (2016).
21. M. A. Borowitzka, J. Appl. Phycol., **25**, 743 (2013).
22. H. Danxiang, L. Yantao and H. Qiang, Algae, **28**, 131 (2013).
23. O. Pignolet, S. Jubeau, C. Vaca-Garcia and P. Michaud, J. Ind. Microbiol. Biotechnol., **40**, 781 (2013).
24. G. Markou and E. Nerantzis, Biotechnol. Adv., **31**, 1532 (2013).
25. T. R. Zalogin and U. Pick, Algal Res., **3**, 8 (2014).
26. S. Kim, H. Kim, D. Ko, Y. Yamaoka, M. Otsuru, M. Kawai-Yamada, T. Ishikawa, H.-M. Oh, I. Nishida, Y. Li-

Beisson and Y. Lee, Plos One, **8**, e81978 (2013).
27. K. D. Sung, J. S. Lee, C. S. Shin and S. C. Park, Korean J. Chem. Eng., **15**, 449-450 (1998).
28. N. Feoktistova, J. Rose, V. Z. Prokopović, A. S. Vikulina, A. Skirtach and D. Volodkin, Langmuir, **32**, 4229 (2016).
29. X. Yan, J. Li and H. Möhwald, Adv. Mater., **24**, 2663 (2013).
30. A. Yashchenok, B. Parakhonskiy, S. Donatan, D. Kohler, A. Skirtach and H. Möhwald, J. Mater. Chem. B, **1**, 1223 (2013).
31. S. Schmidt and D. Volodkin, J. Mater. Chem. B, **1**, 1210 (2013).
32. M. Kim, M. G. Choi, H. W. Ra, S. B. Park, Y.-J. Kim and K. Lee, Materials, **11**, 296 (2018).
33. J. Heuvingh, M. Zappa and A. Fery, Langmuir, **21**, 3165 (2005).
34. J. Y. Seo, K. Lee, S. Y. Lee, S. G. Jeon, J.-G. Na, Y.-K. Oh and S. B. Park, Bioresour. Technol., **152**, 562 (2014).