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ORIGINAL ARTICLE

Inhibition of the Algal Growth using TiO_2-embedded Expanded Polystyrene (EPS) balls in Lab-scale Outdoor Experiment

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ABSTRACT: TiO_2-embedded expanded polystyrene (TiEPS) balls with powdered TiO_2 particles embedded on the surface of EPS were developed, and the growth inhibition of *Chlorella ellipsoidea*, a green algae, was evaluated. The experiment was conducted using four reactors with various conditions of (A) natural sunlight, (B) natural sunlight + TiEPS balls, (C) dark, and (D) dark + TiEPS balls on the roof of the building during five days. Based on the analysis of cell number, cell morphology, concentrations of chlorophyll-*a* and phaeopigments, both surface reactions in heterogeneous photocatalysis and light shielding could inhibit the growth of *C. ellipsoidea*. The highly reactive hydroxyl radicals (OH·) from TiEPS balls degraded the lipid cell membrane through the peroxidation reaction with the light shielding, eventually resulting in cell inactivation. Although dominant inhibitory effects on the growth of *C. ellipsoidea* were ambiguous, TiEPS balls were feasible to prevent and inhibit the excessive growth of algae in eutrophic water body.

KEYWORDS: Algae, *Chlorella ellipsoidea*, Inhibitory effects, Light shielding, Photocatalysis, TiO_2-embedded expanded polystyrene balls

1. Introduction

Algae in freshwater is an important role as a primary producer in aquatic ecosystem. However, if algal blooms occur continuously due to overgrowth of blue green algae, algal blooms cause both aquatic ecosystem disturbance and aesthetic, physical adverse effects on human life (Paul 2008, Rabalais et al. 2009, Raven and Giordano 2014, Backer et al. 2015, Wells et al. 2015). Water eutrophication in lakes and rivers is widespread all over the world, and the occurrence of water eutrophication is a complex function of the various influencing factors (e.g., nutrient enrichment, hydrodynamics, water temperature, salinity, light irradiation, biodiversity, etc.) (Paul 2008, Rabalais et al. 2009, Raven and Giordano 2014, Backer et al. 2015, Wells et al. 2015). Also, climate change, abnormal high temperature, reduced precipitation, and increased sunlight result in the excessive algal blooms (Paul 2008, Rabalais et al. 2009, Raven and Giordano 2014, Backer et al. 2015, Wells et al. 2015). Therefore, various physical, chemical and biological algae countermeasure technologies have been developed (Paul 2008, Wells et al. 2015). However, due to the practical limitations of algae countermeasure applications in lakes and rivers, low-cost, efficient, and sustainable algal growth inhibition

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technologies have been required to reduce the occurrence of water eutrophication.

Recently, polymer-based photocatalyst composites have received increased attention using various polymer supports (e.g., polyaniline, polycarbonate, polyethylene, polystyrene, polyvinyl alcohol, polyvinyl chloride, etc.) with outstanding mechanical properties and compatibilities (Leong et al. 2014, Jang et al. 2016, Joo et al. 2013, 2016). These polymer-based photocatalyst composites can readily control both surface morphology and chemistry of composites through direct compounding, *in situ* synthesis, self-assembly, electrospinning, etc (Leong et al. 2014, Jang et al. 2016, Joo et al. 2013, 2016). Accordingly, self-floating photocatalyst/polymer composites utilizing both continuous illumination of solar energy and oxygenation in the air/water interface can be applied in contaminated lakes and wastewater reservoirs.

In this study, expanded polystyrene (EPS) is used as a practical and floating support for photocatalyst/polymer composites since EPS is an innocuous floating material with its thermal-softness and long durability (Ross and Evans 2003, Lee et al. 2010). Also, simple and cost-effective temperature-controlled melting method of EPS was developed (Joo et al. 2013, 2016), and modified as immobilization technique for mass production of TiO_2-embedded EPS (TiEPS) balls in this study. Finally, the photocatalytic activity of TiO_2-embedded EPS balls was evaluated using *Chlorella ellipsoidea* (*C. ellipsoidea*) as a probe algae under the natural solar light irradiation (285 - 660 nm & 0.02 - 0.14 mW/cm^2). From this study, the specific objectives were to develop the mass production procedures of TiO_2-embedded EPS (TiEPS) balls, and to evaluate the feasibility for usage of buoyant TiO_2-embedded EPS balls to inhibit the excessive growth of algae in lakes and rivers.

2. Materials and Methods

2.1 Manufacturing method of TiO_2-embedded EPS balls

Commercial low density uniform EPS balls were purchased, and the diameter of the EPS balls used was 3 cm. In this study, powdered nanoscale P25 TiO_2 (Evonik Degussa, Germany) was used. Glycerin was purchased (Daejung Chemicals & Metals Co., Korea), and used as a dispersing medium for TiO_2. The suspension of TiO_2 particles was heated to 140-150°C, which is the temperature at thermal denaturation of the polymer occurs, using a digital temperature controller and a heating pan equipped with a magnetic stirrer. EPS balls were repeatedly immersed, so that the TiO_2 particles were evenly embedded in the EPS balls surface. Then, the surface of the EPS balls was completely freeze-dried using liquid nitrogen to enhance the embedding of TiO_2 particles on the surface of EPS balls. Finally, the TiO_2-embedded EPS (TiEPS) balls were washed with distilled water at 700 W for 2 minutes using an ultrasonic washing machine (POWER SONIC 420, Hwasin Tech, Korea) to remove excess TiO_2 particles that were not rigorously embedded to the surface of the EPS balls. After washing, the TiEPS balls were allowed to air dry at room temperature. The detail mass production processes of TiEPS balls are displayed in Fig. 1.

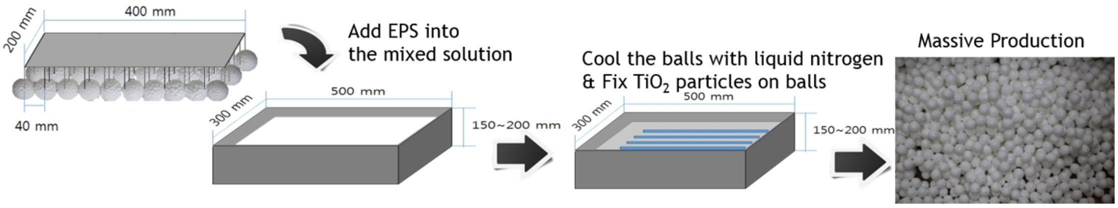

Fig. 1. Mass production procedures of TiO_2-embedded expanded polystyrene (TiEPS) balls developed in this study.

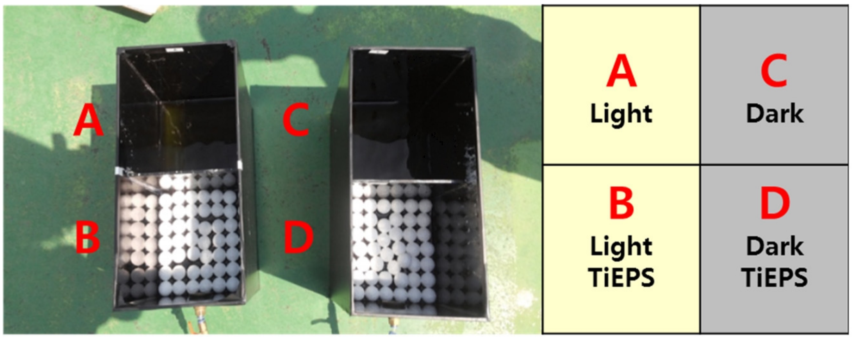

Fig. 2. Pictorial view of lab-scale outdoor inhibition experiment using TiO_2-embedded expanded polystyrene (TiEPS) balls against the growth of *Chlorella ellipsoidea* with different conditions.

2.2 Experiment on inhibition of algal growth using TiO_2–embedded EPS balls

Chlorella ellipsoidea, which was used to verify the inhibitory effect of TiEPS balls on algal growth, was obtained from KRIBB (Korea Research Institute of Bioscience and Biotechnology) and cultured using BG-11 medium (MB Cell Co., USA). The culture conditions in the lab were as follows: light intensity of 1,800 lux, temperature of 25°C, light cycle of 12 hr (light)/12 hr (dark), and cultivation for 7 days before the outdoor experiment. The initial concentration of *C. ellipsoidea* was set at 4 L with $1x10^6$ cells/mL of the algal concentration. The outdoor experiment was performed using an acrylic reactor, the height of the water was 10 cm from the bottom, and total 49 TiEPS balls were inserted in each reactors of (B) and (D). The outdoor experiment was conducted using four reactors with various conditions of (A) natural sunlight, (B) natural sunlight + TiEPS balls, (C) dark, and (D) dark + TiEPS balls on the roof of the building during five days (see Fig. 2).

Both supernatant and mixture solution were sampled at certain time, and the changes in both cell number of *C. ellipsoidea* and concentration of chlorophyll-*a* (Chl-*a*) and phaeopigments were observed. The number of *C. ellipsoidea* was counted using an optical microscope (DN-10A, Samwon Scientific Ltd., Korea) using a hemocyte counter (Marienfeld, Germany) and the concentrations of chlorophyll-*a* and phaeopigments were measured using ICES Techniques [ICES Techniques in Marine Envirionmental Sciences (No. 30 Chlorophyll-a: Measurement by Spectroscopy ISSN 0903-2606)] (Aminot and Rey 2000, Bartram and Rees 2000).

3. Results and Discussion

3.1 Cell number and morphology of *C. ellipsoidea*

In case of natural sunlight only during the outdoor experiment, the growth of *C. elliipsoidea* increased along the Monod growth curve, and no inhibition of the growth was observed. Therefore, it is considered that there is no significant growth inhibitory factor of *C. ellipsoidea* under the natural sunlight irradiation. However, the number of cells in the cases of (B), (C) and (D) were not significantly different up to 80 hours, displaying that the growth inhibition effect of *C. ellipsoidea* was observed.

Based on the comparison of cell number of *C. elliipsoidea* between mixture solution (Fig. 3(a)) and supernatant (Fig. 3(b)), the cell numbers increased during the sunlight irradiation, and decreased during the night time in case of (A) in the supernatant, whereas the cell numbers increased in the mixture solution regardless of the sunlight irradiation, indicating that cells of *C. elliipsoidea* migrate vertically during the outdoor experiment.

Considering the cell numbers decreased faster in cases of (B), (C) and (D) in both mixture and super-

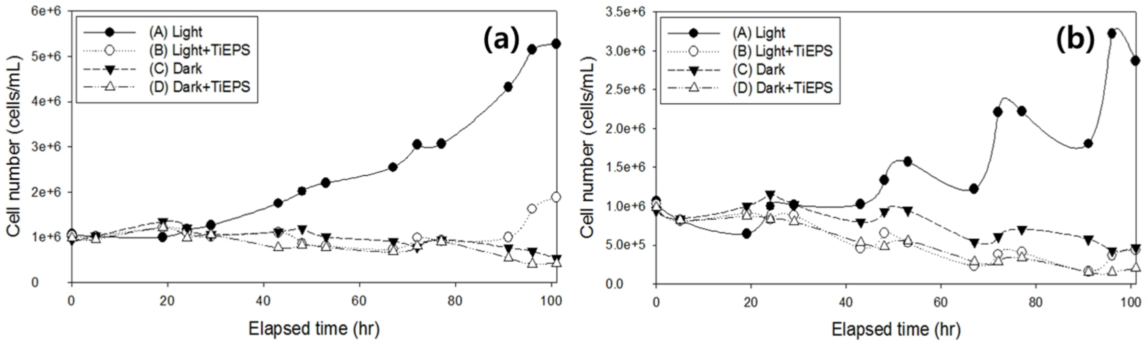

Fig. 3. Changes in cell number of *Chlorella ellipsoidea* in lab-scale inhibition experiment using TiO_2-embedded expanded polystyrene (TiEPS) balls with different conditions; number of *Chlorella ellipsoidea* in mixture solution (a) and in supernatant (b).

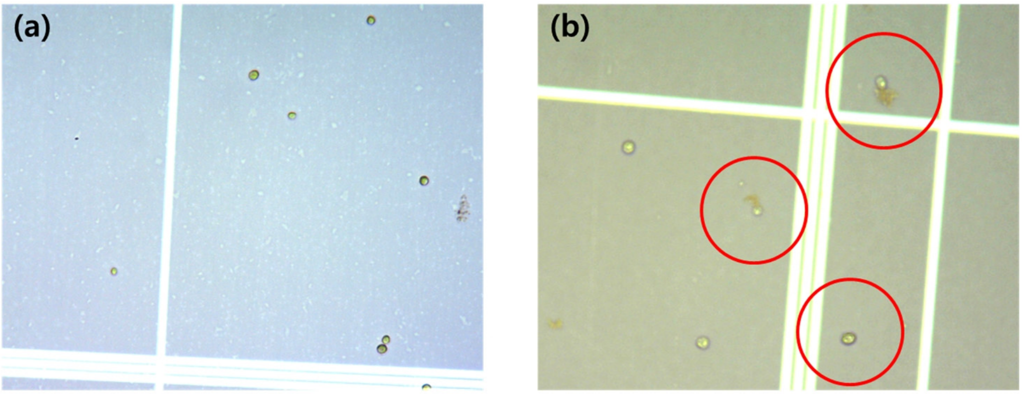

Fig. 4. Comparison of cell shape and morphology in the lab-scale inhibition experiment against the growth of *Chlorella ellipsoidea*. Light only (a), and light with TiO_2-embedded expanded polystyrene (TiEPS) balls (b). (Note: red circles indicate the distorted and ruptured cells)

natant, both surface reactions in heterogeneous photocatalysis and light shielding could inhibit the growth of *C. elliipsoidea*. Although dominant inhibitory effects on the growth of *C. elliipsoidea* were ambiguous, TiEPS balls were found to be effective to inhibit the excessive growth of algae in the stagnant water body.

As shown in Fig. 4, cell morphology was clear and healthy in the case of (A), whereas cell contents were eluted out of the cell, and the circular shape of cell was distorted in the case of (B). This might be attributed to the attack of oxygen species ($OH\cdot$, $O_2\cdot-$, and H_2O_2) generated by TiO_2 particles on the surface of TiEPS balls against the algal cell membrane. Further study is warranted to elucidate more dominant effects on the algal growth inhibition using intact vs. TiEPS balls.

3.2 Chlorophyll-*a* and Phaeopigments

Chlorophyll-*a* (Chl-*a*) is an indirect indicator of the distribution of algae, and phaeopigments are indirect indicator of algae death (Aminot and Rey 2000, Bartram and Rees 2000). As displayed in Fig. 5(a), Chl-*a* concentrations increased with time in case of light only whereas Chl-*a* concentrations decreased with time in case of (B), (C), and (D). Similar to the results of cell numbers, TiEPS balls were also found to be effective to inhibit the excessive growth of algae based on the Chl-*a*.

As displayed in Fig. 5(b), phaeopigment concentrations increased with time in cases of (A) and (B), whereas phaeopigment concentrations decreased with time in cases of (C) and (D). These results suggest that

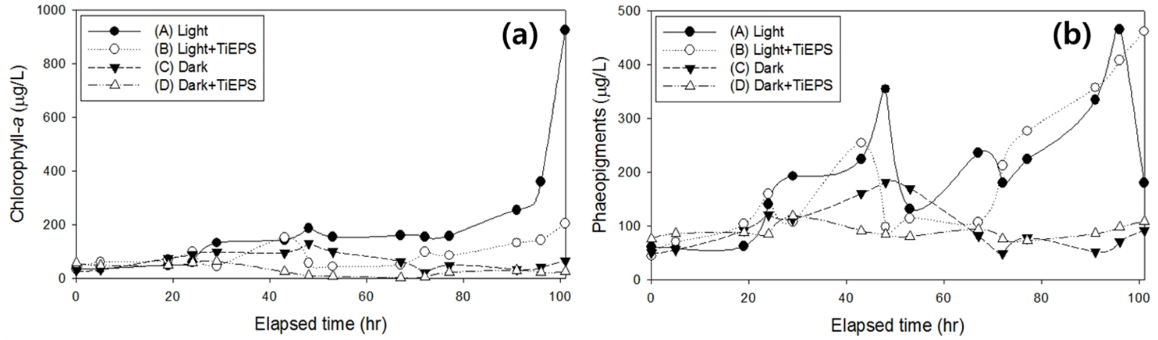

Fig. 5. Changes in chlorophyll-*a* and phaeopigments during the lab-scale inhibition experiment using TiO_2-embedded expanded polystyrene (TiEPS) balls against the growth of *Chlorella ellipsoidea*; chlorophyll-*a* (a), and phaeopigments (b).

both algal growth and death occurred simultaneously in cases of (A) and (B), whereas both algal growth and death did not occur in cases of (C) and (D). Consequently, synergistic effects of both photocatalysic reactions and light shielding using TiEPS balls were significant in case of (B).

These photocatalytic degradation of *C. elliipsoidea* using TiEPS balls can be explained by the reaction of oxygen species ($OH\cdot$, $O_2\cdot$-, and H_2O_2) released from the irradiated TiO_2 particles on the surface of TiEPS balls. Similar results have been reported that highly reactive hydroxyl radicals ($OH\cdot$) can attack the lipid cell membrane through the peroxidation reaction and inhibit the self-protection mechanisms, eventually resulting in cell inactivation (Li et al. 2008, Altin and Sokmen 2015). Therefore, TiEPS balls were feasible to prevent and inhibit the excessive growth of algae in eutrophic water body.

4. Conclusions

Simple and cost-effective temperature-controlled melting and liquid nitrogen-based cooling method of expanded polystyrene (EPS) was developed as immobilization technique for mass production of TiO_2-embedded EPS (TiEPS) balls. Then, the photocatalytic activity of TiEPS balls was evaluated using *Chlorella ellipsoidea* (*C. ellipsoidea*) as a probe algae under the natural solar light irradiation (285 - 660 nm & 0.02 - 0.14 mW/cm^2). Since the photocatalytic reactions occurred around the water surface, highly reactive transitory species effectively inhibit the growth of *C. elliipsoidea* on the water surface, thus, *C. elliipsoidea* might migrate to the bottom of reactors. Also, both surface reactions in heterogeneous photocatalysis and light shielding could inhibit the growth of *C. elliipsoidea*. Although dominant inhibitory effects on the growth of *C. elliipsoidea* were ambiguous, TiEPS balls were found to be effective to inhibit the excessive growth of algae in the stagnant water body. Further study is warranted to evaluate the inhibitory effects of harmful algae growth in eutrophic rivers and lakes as a mesocosm field study.

Acknowledgments

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