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Removal of nitrogen in wastewater by polyvinyl alcohol (PVA)-immobilization of effective microorganisms

Jintae Lee and Moo Hwan Cho†

School of Display & Chemical Engineering, Yeungnam University, Gyeongsan, Gyeongbuk 712-749, Korea
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Abstract−To remove nitrogen and carbon simultaneously from municipal wastewater, a mixture of effective microorganisms (EM) was immobilized in polyvinyl alcohol (PVA) hydrogel beads. The modified PVA beads with calcium alginate show characteristic pores and mechanical stability and flexibility. The EM-immobilized PVA system was established in a 3 L sequential batch reactor (SBR) with a synthetic wastewater and operated at an HRT of 12 h with COD loading rate of 0.5-2.4 g COD/L·d. In this system, intermittent aeration is more efficient than continuous aeration, and removal rates of COD and total nitrogen (T-N) were increased as the feed COD/N ratio was increased from 1.9 to 5.6. At an optimal condition, 73% of total nitrogen and 93% of COD were stably removed at a COD loading rate lower than 2.4 g COD/L·d and at 3 : 1 ratio of aerobic time to anoxic time.

Key words: Wastewater, Nitrogen Removal, Effective Microorganisms, PVA, Cell Immobilization

INTRODUCTION

Due to the accumulation of biological nutrients of nitrogen and phosphorous, cultural eutrophication in the fresh water and coastal marine environments is a serious environmental issue worldwide [1]. The traditional biological processes for nitrogen removal use suspended activated sludge with a combination of aerobic and anoxic reactors but usually require a large reactor volume, a long residence time, and often cause sludge bulking [2-4]. To solve the problems of low nitrification and operational instability, several new nitrogen removal processes have been recently developed: for example, the single reactor system for high ammonia removal over nitrate (SHARON), anaerobic ammonium oxidation (ANAMMOX), completely autotrophic nitrogen removal over nitrite (CANON) with ammonia-oxidizing bacteria and nitrite-oxidizing bacteria, and a combination of these processes and immobilization technique [2,5].

Application of cell immobilization offers not only a high cell density, but also the easy separation of treated effluent from solid sludge, and can eventually lead to a small reactor [6,7]. Also, the immobilized cells entrapped within the gel matrix may be protected from adverse environmental conditions, which could help maintain long-term operation and prevent sludge bulking phenomena [6,8]. As a supporting polymer matrix for cell immobilization, polyvinyl alcohol (PVA) is a good candidate due to its mechanical stability, no toxicity, and material cost, compared with other polymers such as polyacryl amide, κ-carrageenan, Ca-alginate, polyurethane, and agar [6,7,9]. Moreover, modified PVA beads with Ca-alginate would not float up to the surface of reactor by N_2 production because of high gas permeability [10] so that the immobilized PVA beads can be maintained in the reactor. To more efficiently utilize the PVA immobilization system, this study used a mixture of effective microorganisms (EM).

†To whom correspondence should be addressed.
E-mail: mhcho@ynu.ac.kr

EM consists of more than one hundred bacterial strains including lactobacillaceae, saccharomycetes, funguses, actinomyces, and photosynthetic bacteria without pathogenic bacteria [11]. The concept of EM was originally developed during 1970's at the University of Ryukyus, Okinawa, Japan for the inoculants of composting and soil enrichment [12]. A combination of beneficial microorganisms may positively influence any biological process such as decomposition of organic matter [12]. Hence, the EM technology has been employed in many agricultural, environmental, and even medical applications [11,12]. The application of EM has been expanded in diverse areas including wastewater treatment [13,14] and even as a health drink, called EM-X [11]. For example, it was reported that EM in the suspension form could be used in the sewage treatment process [14]. Also, it was hypothesized that EM in wastewater treatment is beneficial in reducing sludge volumes and that a group of non-pathogenic EM might reduce the population of pathogenic bacteria through competitive exclusion [13].

The objectives of this study are to construct efficient EM-immobilized PVA beads, to employ them in a sequential batch reactor (SBR), and to investigate the effects of important operating parameters in order to remove nitrogen and carbon simultaneously from a synthetic municipal wastewater. This study is the first application of EM-immobilization for wastewater treatment.

MATERIALS AND METHODS

1. Chemicals and Microorganisms

PVA with a grade of 98% saponification and 2000 degree of polymerization was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals were reagent grade. A mixture of effective microorganisms (EM) was purchased from EM Research Organization Korea (Busan, Korea). The main species of EM include *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus fermentum*, *Lactobacillus delbrueckii*, *Streptococcus lactis*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Rhodopseudomonas*

palustris, *Rhodobacter sphaeroides*, *Candida utilis*, *Streptomyces albus*, *Streptomyces griseus*, *Aspergillus oryzae*, *Mucor hiemalis* [11,13]. However, names of all EM species are still confidential from the company. A mixture of EM was routinely cultured in 30 g/L molasses medium for several days, centrifuged, washed with distilled water to remove nutrients, and used for cell immobilization.

2. Formation of PVA Beads and EM-immobilization

EM cells were immobilized in PVA beads according to a modified way of a previous method [10] by using sodium phosphate monobasic solution to dissolve Ca-alginate component in the beads and to phosphorylate PVA beads. Briefly, a mixture of concentrated EM of 100 mg/mL was mixed thoroughly with an equal volume of PVA (11-15% w/v) including 1% sodium alginate. The EM-PVA mixture was dropped into a saturated boric acid solution including 1% $CaCl_2$ through a syringe needle and gently stirred for 4 h to form spherical beads. The formed beads were transferred to 0.5 M sodium phosphate monobasic solution for 1 h to dissolve Ca-alginate component in the beads and to phosphorylate the EM-immobilized PVA beads.

3. Operation of Sequential Batch Reactor (SBR)

The EM-immobilized PVA beads or activated sludge were acclimated in a synthetic wastewater for several days, and they were used in a bubble aeration type of sequential batch reactor (SBR) with a working volume of 3 L. Recently, an SBR was successfully used to remove nitrogen from municipal wastewater [15]. To compare the efficiency of the immobilization system and the activated sludge system, PVA beads immobilized with 1 g of EM cells or 1 g of the activated sludge (based on mixed liquor suspended solids) were tested in the SBR. The composition of the synthetic municipal wastewater was 225 mg/L glucose, 212 mg/L Na_2CO_3, 17.5 mg/L KCl, 59.4 mg/L NH_4Cl, 17.5 mg/L $CaCl_2$, 37.5 mg/L NaCl, 12.5 mg/L $MgSO_4$ and 35.1 mg/L KH_2PO_4, which corresponded to 235 mg/L COD, 16 mg/L T-N and 150 mg/L BOD [16]. Initially, 3 L synthetic wastewater was fed with EM-immobilized PVA beads (10% of reactor volume) into the reactor. The system was operated at an HRT of 12 h with the COD loading rate of 0.5-2.4 g COD/L·d, and the air was intermittently supplied at 3 L/min by an air compressor. A cyclic switch of aeration and non-aeration was automatically operated by a timer in order to investigate the effect of the ratio of aeration to non-aeration on the removal efficiency of COD and T-N. Reactor temperature was maintained at 30 °C with a thermostat bath through the reactor jacket. The initial pH of wastewater was about 7.0 and pH was maintained about 7.0 without any pH control. After one batch operation of 12 h, EM-immobilized beads were settled down quickly by stopping aeration to draw out treated effluent from the reactor and a new synthetic wastewater was filled into the reactor in order to treat the wastewater sequentially. During the batch operation, dissolved oxygen (DO) was measured continuously, and the samples drawn from the reactor were used to analyze COD, BOD, NH_4^+, NO_3^-, and T-N.

4. Analytical Methods

Physical properties, such as modulus of elasticity and elongation at break, of PVA beads were measured by a tensile-strength measurement instrument (Instron, Model 4464, Norwood, MA, USA). To observe the morphology of inside PVA beads and EM-immobilization in the PVA beads, a scanning electron microscope (S-4100, Hitachi, LTD, Japan) was used. The concentrations of COD, NH_4^+, NO_3^-, and T-N were measured by a water quality analyzer (Model DR/2500 Laboratory Spectrophotometer, Hach Co., USA). BOD was measured by a BOD analyzer (OxiTop, WTW, Weiheim, Germany).

RESULTS AND DISCUSSION

1. Formation of EM-immobilized PVA Beads

To produce PVA beads, the previous method [10] was modified. Initially, different concentrations of PVA (11-15% (w/v)) were tested to form PVA beads. As a result, 13% PVA was chosen because beads were too fragile under 13% PVA while bead formation was difficult above 14% PVA because of high viscosity. Since calcium alginate is used to improve gas permeability of PVA beads [10], 1% sodium alginate was added to a PVA aqueous solution and the PVA beads were hardened in the solution of boric acid and 1% calcium chloride. The subsequent spherical beads in 3-7 mm diameter were washed with distilled water, and adapted with a synthetic wastewater before being used in the SBR reactor. As a result, most of EM-PVA beads were not floated during the nitrification (production of nitrogen) and no aeration, which facilitated removal of the treated effluent from the immobilized beads. The physical properties of our phosphorylated PVA beads were compared with those of other beads (Table 1). Our PVA beads were superior to other beads in terms of modulus elasticity, elongation break, and toughness so that the PVA beads are theoretically adequate for high shear stress in an aerated tank. Moreover, scanning electron micrographs clearly show porous structures inside PVA beads (Fig. 1(a) and 1(b)). Pore distribution and porosity of the PVA beads look similar to the result from the previous study [10,17]. Moreover, during the nitrogen removal in an SBR, a variety of effective microorganisms was attached on the inner surface of PVA beads (Fig. 1(c) and 1(d)).

2. Biological Activity of EM-immobilized PVA Beads Compared to Activated Sludge

Initially, the EM-immobilized PVA system was compared with

Table 1. Comparison of physical properties of PVA beads with those of other hydrogel beads. A tensile-strength measurement instrument (Instron, Model 4464, Norwood, MA, USA) was used to measure physical properties of modified PVA beads. Three independent experiments were performed, and one standard deviation is used

Hydrogel (w/w)	Modulus of elasticity [MPa]	Elongation at break [%]	Reference
Modified PVA (13%)	0.033±0.009	520±30	this work
PVA (7%)	0.03-0.04	100-240	[17]
Poly carbamoyl sulfonate (10%)	0.023	80-120	[17]
Agar (5%)	0.2	10	[22]
κ-Carrageenan (4%)	0.28	27-29	[22]
Ca-alginate (1%)	0.19	55-70	[22]

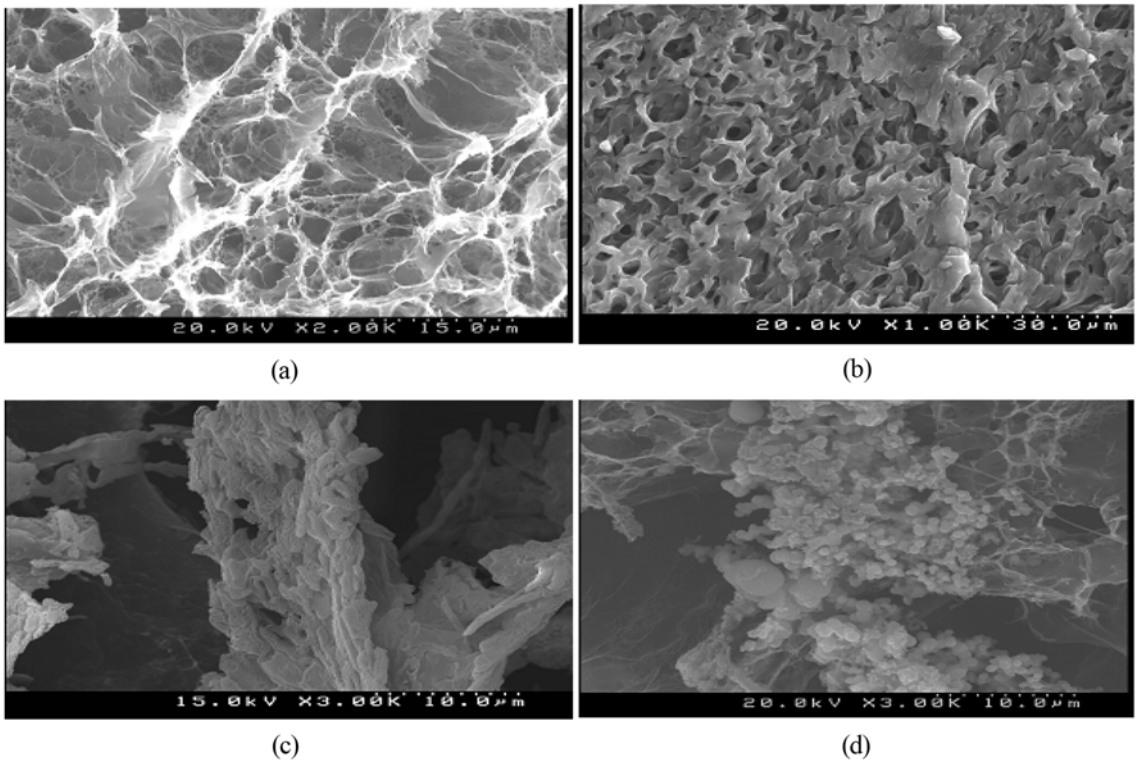

Fig. 1. Pore structure of PVA beads and EM-immobilization. Scanning electron micrographs of inside PVA beads ((a) and (b)) and EM-immobilized cells in PVA beads ((c) and (d)).

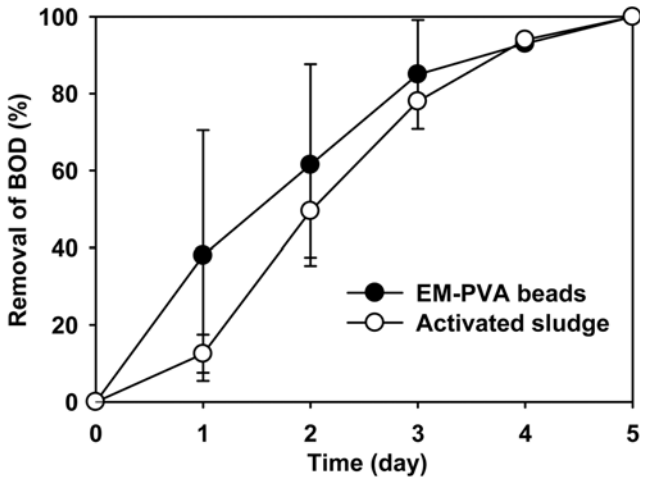

Fig. 2. Comparison of biological activity of EM-immobilized PVA system and activated sludge system. The EM-immobilized PVA beads and activated sludge were acclimated in the synthetic wastewater, and PVA beads contained 1 g EM cells or the activated sludge (1 g) was added into a BOD analyzer with a synthetic wastewater (165 ml). Two independent cultures were performed, and one standard deviation is shown.

a conventional system with activated sludge taken from a wastewater treatment plant (Daegu, Korea) in terms of BOD removal rate. The same amount (1 g) of EM cells immobilized in the PVA beads or the activated sludge was added into a BOD analyzer with the synthetic wastewater (165 ml). The initial BOD removal rate with the EM-immobilized beads was more or less faster than that with the activated sludge (Fig. 2). Hence, the biological activity of EM-immobilized PVA beads was confirmed and EM-immobilized cells are more efficient than activated sludge.

3. Effect of Organic Loading Rate and Feed COD/N Ratio on the Performance of EM-immobilized PVA System

Since nutrient loading was a major parameter for eutrophication [1], the effect of organic loading rate was investigated on the removal efficiencies of carbon (COD) and total nitrogen (T-N). The loading rate was varied from 0.5 to 2.4 g COD/L·d by changing the concentration of synthetic wastewater at the fixed HRT of 12 h and at the fixed aeration cycles of 1 h aeration and 1 h no aeration. It appears that the optimal loading rate for COD and nitrogen removal was 0.9 g COD/L·d under the conditions (Fig. 3(a)). Since influent COD/N ratio in municipal wastewater is often varied and the ratio is a critical factor during denitrification [18], different ratios of COD/N were tested. As the feed COD/N ratio was increased from 1.9 to 5.6, COD removal rate was increased from 78% to 92%, and T-N removal rate was gradually increased from 33% to 61% at 1 : 1 ratio of aeration time to anoxic time (Fig. 3(b)). This result is consistent with previous studies for nitrogen removal in an SBR [4,18], which indicates that an appropriate control of carbon concentration can enhance nitrogen removal.

4. Investigation of Aeration Cycle for Nitrogen Removal in SBR

Generally, ammonium nitrogen is first oxidized to nitrate in aerobic state by autotrophic microorganisms, and nitrate is further reduced to nitrogen gas in anoxic state by heterotrophic bacteria [19]. Hence, the effect of aerobic/anoxic periods has been considered as an im-

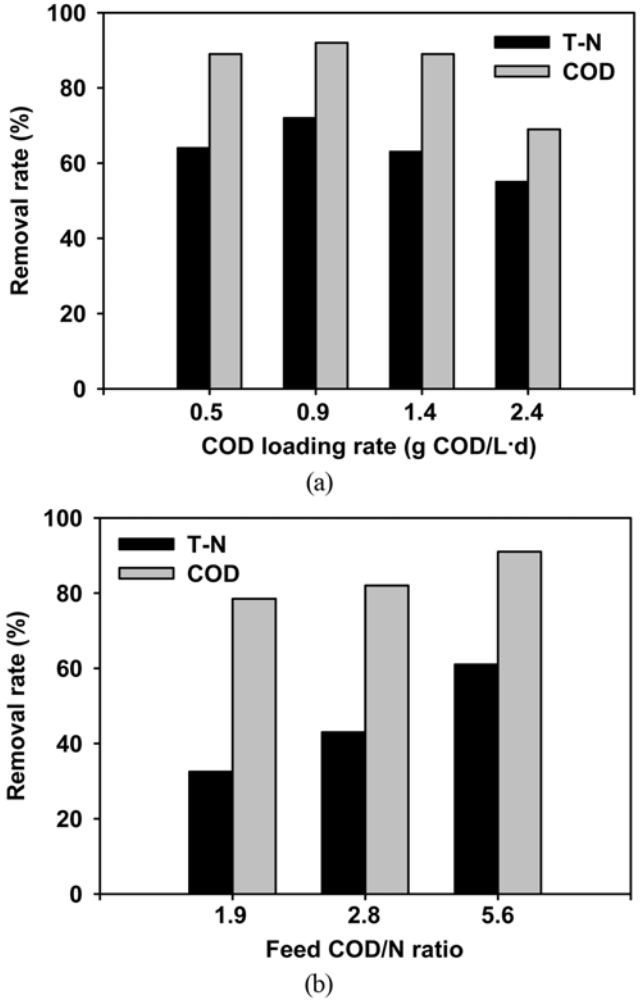

Fig. 3. Effect of organic loading rate (a) and feed COD/N ratio (b) on the performance of EM-immobilized PVA system. Air was intermittently supplied at a ratio of aerobic (3 L/min) to anoxic (1 hr : 1 hr).

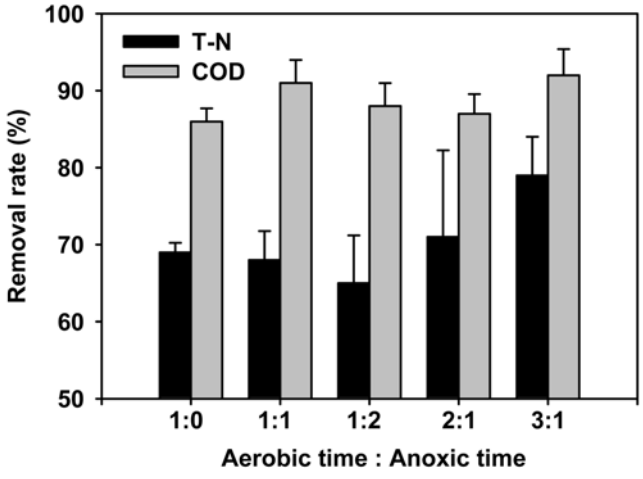

Fig. 4. Effect of aeration time ratio on the removal efficiencies of COD and T-N. Initial COD was 235 mg/L and initial total nitrogen (T-N) was16 mg/L in the influent. Each data point was averaged from five repeated batch experiments, and one standard deviation is shown.

portant parameter to enhance the efficiency of nitrogen removal [19-21]. In the EM-immobilized PVA system, high ratio (3 : 1) of aeration time to anoxic time increased the removal efficiency of total nitrogen (Fig. 4), while in the activated sludge system, low ratio of aeration time to anoxic time increased nitrogen removal [20,21]. The different results are possibly due to oxygen diffusion and oxygen gradient in the immobilized cells. Most EM-immobilized cells grow as a form of biofilm on inner pores of PVA so that more aeration may be required than the suspended activated sludge cells.

5. Optimal Operation of EM-immobilized PVA System

At an optimal condition of 3 : 1 hour ratio of aerobic/anoxic period and 12 h HRT, repeated batchwise SBR operation was performed seven times for the removal of nitrogen and carbon simultaneously from the synthetic wastewater (Fig. 5). The result demonstrates that our EM-immobilized PVA system can be stably operated over the time and total nitrogen removal was 73±8% and COD removal was 93±1%. The overall results are comparable with a recent report for nitrogen removal; PVA immobilized beads with activated sludge

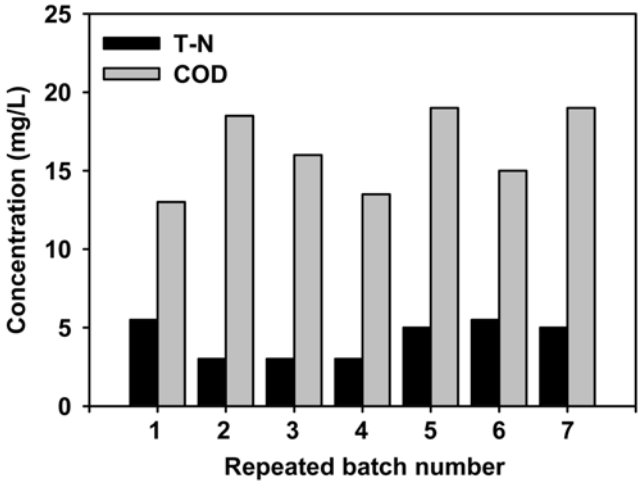

Fig. 5. Profiles of COD and T-N concentrations during seven repeated operations of immobilized EM-PVA system with 3 : 1 hour ratio of aerobic to anoxic. Initial COD was 235 mg/L and initial total nitrogen (T-N) was 16 mg/L in the influent.

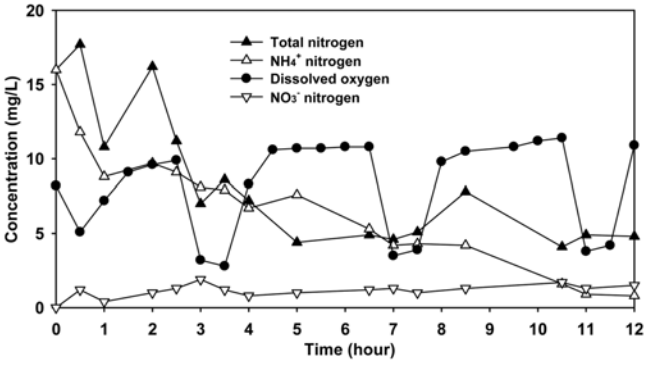

Fig. 6. Profiles of nitrogen and DO concentrations during a batch operation with 3 : 1 hour ratio of aerobic to anoxic. Initial COD was 235 mg/L and initial total nitrogen (T-N) was 16 mg/L in the influent.

led to 80% nitrogen removal from 25-75 mg/L for 48 h [6]. Additionally, profiles of various nitrogen (NH_4 and NO_3) and DO concentrations were observed during a batch operation. It was confirmed that DO concentration was clearly influenced by the 3 : 1 hour ratio of aerobic/anoxic and that concentrations of total nitrogen and NH_4 were gradually decreased for the 12 h-batch operation (Fig. 6).

CONCLUSIONS

We combined a cell immobilization technique and EM application in an SBR to improve the nitrogen removal from municipal wastewater. To our knowledge, this is the first report of EM-immobilization system for wastewater treatment. The preliminary results in this study suggest another sustainable application for nitrogen removal in wastewater process. At optimal conditions, the EM-immobilized PVA system led to 70% total nitrogen removal and 90% COD removal at a COD loading rate lower than 2.4 g COD/L·d for 12 h. Because of the complexity of multiple EM species, the exact mechanism of EM technology has to be scientifically investigated yet. Also, further studies of a long-term operation in terms of sludge reduction by EM-immobilization and researches of scaled-up/modified components are also necessary.

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