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Phosphorus Removal in Pilot Plant Using Biofilm Filter Process from Farm Wastewater

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Abstract Various environmental conditions affecting total phosphorus removal from farm wastewater in a biofilm filter process were investigated using loess balls and *Chromobacterium* LEE-38 at a pilot plant. When *Chromobacterium* LEE-38 was used, the removal efficiency of total phosphorous was approximately 10- or 5-fold higher than that of *Acinetobacter* CHA-2-14 or *Acinetobacter* CHA-4-5, respectively. When a loess ball of 11~14 mm manufactured at a 960°C calcining temperature was used, the removal efficiency of total phosphorous was 90.0%. When 70% of the volume fraction was used, the maximum efficiency of total phosphorus removal was 93.1%. Notably, when the initial pH was in the range of 6.0 to 8.0, the maximum removal efficiency of total phosphorus was obtained after 30 days. When the operating temperature was in the range of 30 to 55°C, the maximum removal efficiencies of total phosphorus, 95.6 to 94.6%, were obtained. On the other hand, at operating temperatures below 20°C or above 40°C, the removal efficiency of total phosphorous decreased. Among the various processes, biofilm filter process A gave the highest removal efficiency of 96.4%. Pilot tests of total phosphorus removal using farm wastewater from the biofilm filter process A were carried out for 60 days under optimal conditions. When *Acinetobacter* sp. Lee-11 was used, the average removal efficiency in the *p*-adsorption area was only 32.5%, and the removal efficiencies of chemical oxygen demand (COD) and biological oxygen demand (BOD) were 56.7 and 62.5%, respectively. On the other hand, when *Chromobacterium* LEE-38 was used, the average removal efficiency was 95.1%, and the removal efficiencies of COD and BOD were 91.3 and 93.2%, respectively.

Keywords: phosphorus removal, farm wastewater, pilot plant, *Chromobacterium*

INTRODUCTION

Phosphorus is an irreplaceable constituent of all living organisms. Natural ore deposits containing phosphorus are limited, and they are being depleted rapidly [1]. Since the major current use of phosphorus is in fertilizers, phosphorus reuse and recycling are of great importance for sustaining long-term, profitable agricultural production. On the other hand, the increased input of phosphorus to lakes, bays, and other surface waters causes the growth of phytoplankton, referred to as algal bloom [2]. Hence, various chemical and physical-chemical treatment processes for the removal of phosphorus from water have been used for many years to solve these problemes. In these processes, iron, calcium, or aluminum salts are added to form sparingly soluble phosphate, which is then removed by settling. These methods, although very effective and reliable, have several disadvantages, including increasing costs of equipment, chemicals, sludge disposal, and various problems associated with sludge disposal. On the other hand, biological treatment methods, such as mainstream or side stream treatment, have become the focus of interest due to their advantages of being highly economical and very easy to operate. Moreover, it is unlikely that a secondary pollution source will be created by a wastewater disposal plant. However, such biological methods have their own associated problems, such as the need for treatment of the loading change and secondary treatment to remove the by-products of sludge [3-5].

We recently isolated a variety of bacteria and investigated its potential for use in the biological treatment of wastewater containing nitrogen and phosphorus compounds in soil [6,8]. The optimal growth temperature and pH for *Pseudomonas* WS were determined. The cell growth revealed an almost stationary phase after 18 h of culture and 99% of nitrate was degraded during this period [6]. We studied its ability to remove phosphorous using a loess ball and loess powder from artificial wastewater [7]. We also investigated factors affecting the denitrification in the wastewater treatment process using a loess ball and *Pseudomonas* DWC 17-8, and pilot tests of the effective denitrification using farm wastewater were carried out using the optimal wastewater treatment process [9]. We also investigated various environmental fac-

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tors in the effective removal of phosphorus from the practical wastewater. The efficiency of phosphorous removal from practical wastewater was higher than 95%. In contrast, the phosphorous removal efficiency from farm wastewater was very low [13]. Therefore, we isolated various strains from farm wastewater and obtained a *Chromobacterium* sp. mutant capable of removing high levels of phosphorous [10].

In this study, using *Chromobacterium* LEE-38 in a biofilm filter process containing a loess balls, various environmental conditions affecting phosphorus removal from farm wastewater were investigated. Under optimal conditions, feasibility tests of the effective removal of phosphorus from farm wastewater at the pilot plant scale were subsequently carried out.

MATERIALS AND METHODS

Strain, Media, and Culture

The composition of the seed medium used in this study was as follows (g/L): 2 glucose, 1 NH_4Cl, 2 NaCl, 1 Na_2SO_4, 0.1 KCl, 0.01 $MgCl_2$, 0.01 $CaCl_2 \cdot 2H_2O$, 0.01 $FeCl_3 \cdot 6H_2O$, and 0.22 $NaHPO_4$. All of the components of the media were sterilized at 121°C and 1.2 atm for 30 min. The pH of the media was adjusted to 7.0 before sterilization. One loop of *Chromobacterium* LEE-38 was transferred to the slant medium and cultured at 30°C for 2 days. Then one loop of the slant culture of *Chromobacterium* LEE-38 was inoculated into a 500 mL Erlenmeyer flask containing 100 mL of the seed medium and cultured at 30°C for one day on a reciprocating shaker at 150 rpm. One percent of the seed culture was inoculated into the farm wastewater and cultured at a temperature range of 20 to 45°C.

Loess Ball

Various loess balls were used as the bacterial support matrices for phosphorous removal from wastewater. The composition of the loess balls (Muan Ceramic Industry, Chunnam) was as follows (%): 0.51 Mg, 29.7 Al, 52.56 Si, 1.91 K, 1.21 Ti, and 17.14 Fe. Physical characteristics of media applied in this study were as follows: apparent porosity rate, 40.3~50.5%; specific surface area, 2.0~4.0 m^2/cm^2; and specific weight, 2.50~4.03. The calcining temperature of the loess balls was 860 to 960°C and holding time was 30 min.

Biofilm Filter Process

As shown in Fig. 1, biofilm filter processes for the pilot plant were composed of a raw water tank, an anaerobic tank, an oxic tank, an anoxic tank, a *p*-absorption tank, a treated water tank, a re-washing tank, a sludge-setting tank, and a methanol tank. The order of operation of the various biofilm filter processes and the carbon sources were as follows. Process A: an anaerobic area, an oxic area, an anoxic area, and a *p*-absorption area; glucose as carbon source. Process B: an oxic area, an anoxic area, and a *p*-absorption area; farm wastewater as carbon source. Process C: an anaerobic area, an oxic area, and an anoxic area; glucose as carbon source. Process D: an oxic area, and an anoxic area; farm wastewater carbon source. The reactors of the anaerobic tank, the oxic tank, the anoxic tank, and the *p*-absorption tank were packed with loess balls as the bacterial support matrices.

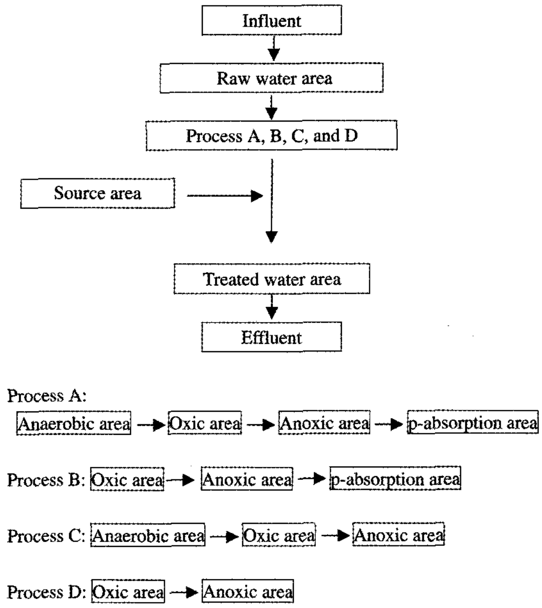

Fig. 1. Schematic diagram of the biofilter process for the pilot plant test of phosphorus removal.

Pilot Plant

The pilot plant was built at a wastewater treatment site located in Chookchung-ri, Dogok-myeon, Hwasun-gun, Chunnam, Korea. The pilot plant, which used a biofilm filter process, was designed for the removal of phosphorous from farm wastewater (Fig. 2). The pilot plants comprised a raw water area, an anaerobic area, an oxic area, an anoxic area, a *p*-adsorption area, a treated water area, a re-washing tank, and a sludge-settling area. The continuous operation was performed in the following order: influent area, anaerobic area, oxic area, anoxic area, *p*-adsorption area, and effluent. The diameter and height of the anaerobic, oxic, and anoxic tanks were 2.0 and 4.5 m, respectively. The feed rate of the wastewater was 1,000 mL/min. For effective phosphorus removal using farm wastewater, pilot tests of the biofilm filtering process were carried out with a loess ball of 11~14 mm manufactured at a calcining temperature of 950°C for 60 days, at a working temperature of 30 to 35°C, and at 150.0 L/min of aeration. The farm wastewater was adjusted to an initial pH of between 6.0 and 8.0.

Analysis Methods

All samples were filtrated through a 0.22 microfilter

Fig. 2. Pilot plant for phosphorus treatment from farm wastewater.

before measurements were taken. The total phosphorus concentration was measured according to DIN [11]. The cell concentration of *Chromobacterium* LEE-38 was measured by the packed cell volume method, in order to solve the problem of insoluble components that do not dissolve completely [12]. The concentrations of chemical oxygen demand (COD) and biological oxygen demand (BOD) were measured using a modified method [14-16].

RESULTS

Comparison of Various Strains on the Removal of Phosphorus

In order to carry out effective phosphorous removal using a loess ball in this study, various strains were investigated in the batch mode. The results are shown in Table 1. The cultures using farm wastewater containing 15 mg/L of phosphorus were carried out at 30°C. The growth of *Acinetobacter* sp. CHA-2-14 was lower than those of other strains after 24 h of culture. However, the growth of *Chromobacterium* LEE-38 was similar to that of *Acinetobacter* sp. CHA-4-15 (data not shown). Notably, when *Chromobacterium* LEE-38 was used, the total phosphorous removal efficiency was 100%, which was about 10.0- or 5.0-fold higher than that of *Acinetobacter* CHA-2-14 or *Acinetobacter* CHA-4-5, respectively.

Optimization of Environmental Conditions for the Effective Removal of Phosphorous

In order to investigate the effects of calcining temperature, size and volume fraction of the loess ball, and the initial pH of wastewater, and the working temperature on the removal of phosphorus using farm wastewater, continuous experiments in the biofilm filter process with loess balls as the bacterial support matrices were carried out. Table 2 shows the effects of calcining temperature and loess ball size on total phosphorus removal in continuous experiments from farm wastewater; these experiments were carried out for 30 days at room temperature using 60% of the volume fraction and an initial pH of 6.0. When loess balls of 11~14 mm manufactured at a calcining temperature of 960°C were used, the total phosphorus removal efficiency was 90.0%, and at a calcining temperature of 860°C, it was 82.2%. On the other hand, when loess balls of 15~20 mm manufactured at a calcining temperature of 960°C were used, the total phosphorus removal efficiency was 88.9%, and at a calcining temperature of 860°C, it was 86.8%. Therefore loess balls of 11~14 mm manufactured at a calcining temperature of 960°C were used in the following experiments.

To determine the optimal volume fraction of loess balls on total phosphorus removal in the biofilm filter process, 50, 60, 70, 80, and 90% of volume fractions were used for 30 days with an initial pH of 6.0~7.0 and an operating temperature of 30°C. The results are shown in Table 3. When 50 and 60% of volume fractions were used, the efficiencies of total phosphorus removal for 20 days were 90.8 and 91.8, respectively. However, these values decreased after 30 days. On the other hand, when 70% of the volume fraction was used, the maximum efficiency of total phosphorus removal was 93.1%. In the case of levels higher than 80 and 90% of volume fraction, the efficiency did not increase.

In order to investigate the effect of the initial pH of farm wastewater on total phosphorus removal, continuous experiments were performed for 30 days using 70% of the volume fraction and an operating temperature of 30°C. The pH ranged from 4.0 to 9.0, and the results of this investigation are shown in Table 4. When the initial pH was increased from 4.0 to 6.0, the total phosphorus removal efficiency increased from 65.4 to 91.6%. Notably, when the initial pH ranged from 6.0 to 8.0, the maximum total phosphorus removal efficiency was obtained after 30

Table 1. Comparison of various strains on phosphorus removal

Strains	Removal efficiency of total phosphorous (relative, %)
Chromobacterium LEE-38	100
[a]*Acinetobacter* sp. CHA-2-14	9.2
[b]*Acinetobacter* sp. CHA-4-15	20.0

[a]*Acinetobacter* sp. CHA-2-14 was isolated from solid.
[b]*Acinetobacter* sp. CHA-2-5 was isolated from practical wastewater.

Table 2. Effect of loess ball calcining temperature and size on phosphorus removal

Calcining temperature	Diameter (mm)	Removal efficiency of total phosphorous (%) Culture time (days) 10	20	30
860	11-14	96.8	89.3	82.2
960	11-14	98.2	93.5	90.0
860	15-50	95.3	84.1	86.8
960	15-20	98.9	91.9	88.9

Table 3. Effect of volume fraction on phosphorous removal

Volume fraction (%)	Removal efficiency of total phosphorous		
	Culture time (days)		
	10	20	30
50	99.1	90.2	83.6
60	98.9	91.8	86.2
70	99.0	94.1	93.1
80	98.7	94.9	93.0
90	99.1	95.2	92.5

Table 4. Effect of initial pH on phosphorus removal

Initial pH	Removal efficiency of total phosphorous		
	Culture time (days)		
	10	20	30
4	80.9	75.2	65.4
5	90.2	89.2	80.9
6	92.3	99.1	91.6
7	95.8	98.2	92.1
8	97.2	98.3	93.5
9	75.6	70.9	78.6

days with 70% of the volume fraction and at an operating temperature 30°C. However, when the initial pH was above 9.0, the removal efficiency decreased.

Table 5 shows the effect of operating temperature on phosphorus removal at a continuous mode for 30 days using 70% of the volume fraction and an initial pH of 6.0~8.0. The operating temperature ranged from 15 to 45°C. When the operating temperature was increased from 15 to 25°C, the total phosphorus removal efficiency increased from 36.9 to 89.6%. Conversely, at an operating temperature above 40°C, the total phosphorus removal efficiency was decreased. Therefore, an optimum temperature of operation of 30 to 35°C was used in the following experiments.

Comparison of Several Systems of Biofilm Filter Processes on the Removal Concentrations of Phosphorous, COD, and BOD

Using farm wastewater, continuous experiments were carried out to test the various flow orders of the biofilm filter processes for effective removal of total phosphorus under optimal conditions for 45 days. The results are shown Table 6. When biofilm filter process A was used, the average efficiency of total phosphorus removal was 96.4%. The average removal efficiencies of COD and BOD were 92.5 and 93.8% respectively. In the case of biofilm filtering process B, the average efficiency of total phosphorus removal was 89.4%. The average removal efficiencies of COD and BOD were 89.9 and 90.4% respectively. Using biofilm filter process C, the average removal efficiency of total phosphorus was 93.4%. The average removal efficiencies of COD and BOD were 90.5 and 93.7%, respectively. Using biofilm filter process D, the average removal efficiency of total phosphorus was 89.7%. The average removal efficiencies of COD and BOD were 88.1 and 89.7%, respectively.

Table 5. Effect of temperature on phosphorous removal

Temperature (°C)	Removal efficiency of total phosphorous		
	Culture time (days)		
	10	20	30
15	39.5	40.3	36.9
20	69.2	68.9	59.7
25	90.3	89.3	89.6
30	97.6	95.8	95.6
35	98.5	95.3	94.6
40	89.6	92.1	89.5
45	86.3	87.5	78.1

Table 6. Effect of the order of the system on the removal of phosphorus, COD, and BOD in various biofilm filter processes

Items	Order of biofilm filter process			
	A	B	C	D
Removal efficiency of total phosphorus (%)	96.4	89.4.	93.4	89.7
Removal efficiency of COD (%)	92.5	89.9	90.5	88.1
Removal efficiency of BOD (%)	93.8	90.4	93.7	89.7

Pilot Plant Test for the Phosphorus Removal in a Biofilm Filter Process

In order to compare *Chromobacterium* LEE-38, the phosphorous mutant isolated from farm wastewater and *Acinetobacter* sp. Lee-11 also isolated from farm wastewater, pilot tests on total phosphorus removal in biofilm filter process A using farm wastewater were carried out for 60 days. The results are shown in Fig. 3. For effective phosphorus removal in the oxic area, glucose was also used as the carbon source. The concentration of total phosphorus in the influent area ranged from 7.2 to 8.9 mg/L. When *Acinetobacter* sp. Lee-11 was used, the total phosphorus concentration ranged from 6.3 to 7.8 mg/L and the average removal efficiency was 12.5% in the anaerobic area. In the oxic area, the total phosphorus concentration ranged from 5.3 to 6.6 mg/L and the average removal efficiency was 25.6%. In the anoxic area, the total phosphorus concentration ranged from 5.0 to 6.2 mg/L and the average removal efficiency was 30.2%. In the *p*-adsorption area, the average removal efficiency was only 32.0%. On the other hand, when *Chromobacterium* LEE-38 was used, the total phosphorus concentration ranged from 5.4 to 6.6 mg/L and the average removal efficiency was 26.2% in the anaerobic area. In the oxic area, the total phosphorus concentrations ranged from

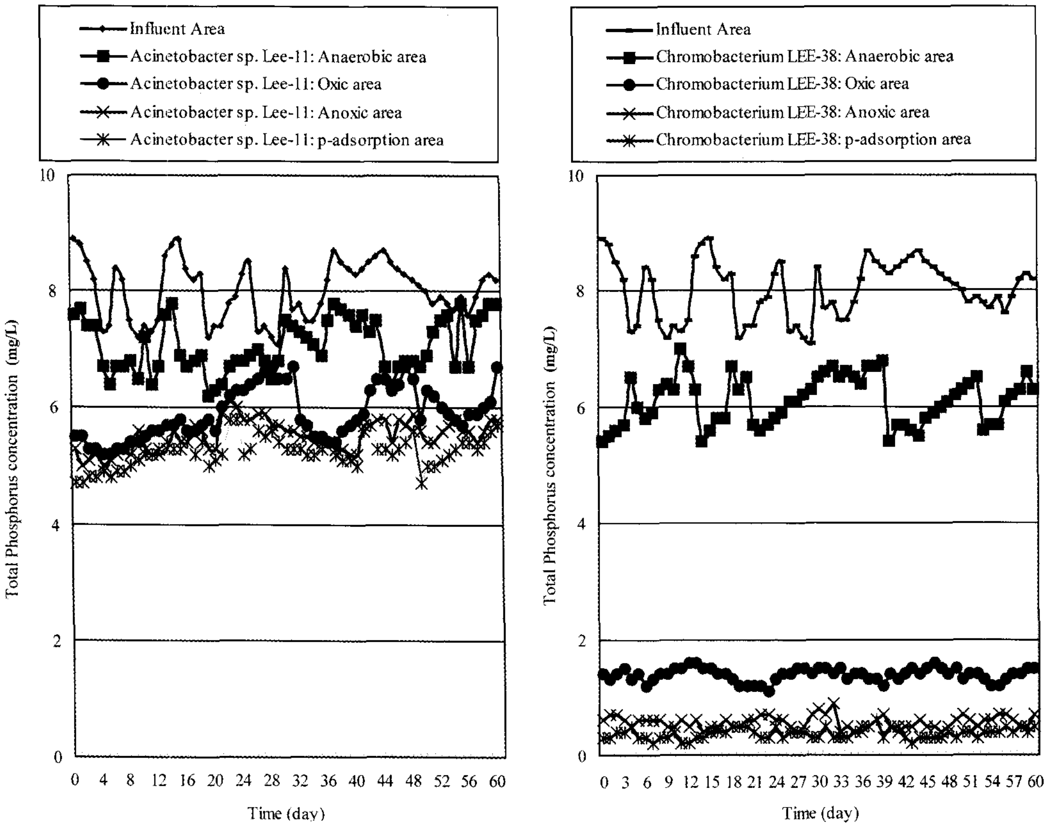

Fig. 3. Pilot test of the concentration of phosphorus removal.

1.2 to 1.5 mg/L and the average removal efficiency was 83.5%. In the anoxic area, the total phosphorus concentration ranged from 0.5 to 0.6 mg/L and the average removal efficiency was 93.4%. In the *p*-adsorption area, the average removal efficiency was 95.3%.

Fig. 4 shows the removal efficiencies of COD and BOD from farm wastewater in the pilot plant. The concentrations of COD and BOD in the influent ranged from 32.6 to 35.4 mg/L and 38.2 to 40.2 mg/L, respectively. When *Acinetobacter* sp. Lee-11 was used, the average removal efficiencies of COD and BOD were 56.7 and 62.5%, respectively. Conversely, in the case of *Chromobacterium* LEE-38, the COD and BOD concentrations ranged from 3.1 to 3.9 mg/L and 2.3 to 2.8 mg/L, respectively, in the final effluent area. The average removal efficiencies of COD and BOD were 91.3 and 93.2%, respectively.

DISCUSSION

The removal of phosphorous from wastewater is an important process that is needed to protect lakes and other natural waters from cultural eutrophication. Therefore, we isolated various bacteria from soil and investigated their potential for phosphorous removal by using a loess balls and loess powder in a batch and continuous flow reactor using artificial wastewater [3,4]. Using practical wastewater, we also researched the various environmental factors affecting the activated sludge process of wastewater treatment. The removal efficiency of phosphorous from practical wastewater was over 92.0% [6]. However, in the case of farm wastewater, the phosphorous removal efficiency was only 35%. Therefore, we isolated *Chromobacterium* sp., *Psedomonas* sp., and *Acinetobacter* sp. in order to test their ability to effectively remove the total phosphorous from farm wastewater received from a farming village. Among the various bacteria, *Chromobacterium* sp. was found to be the best strain for effectively removing total phosphorous from farm wastewater. We also obtained the *Chromobacterium* LEE-38 mutant, which is capable of removing high levels of total phosphorous after *N*-methyl-*N*-nitro-*N*-nitrosogua-nidne mutagenesis. The *Chromobacterium* LEE-38 mutant removed about 10 times more total phosphorous than that of its parent strain [11].

The ability of various strains to effectively remove the total phosphorus was investigated using a loess ball in batch mode with farm wastewater. The growth of *Chromobacterium* LEE-38 was similar to that of *Acinetobacter* sp. CHA-4-15. On the other hand, when *Chromobacterium* LEE-38 was used, the total phosphorous removal efficiency was about 10- or 5-fold higher than that of *Acinetobacter* CHA-2-14 or *Acinetobacter* CHA-4-5, respectively. We also previously investigated the effect of the calcining temperature and loess ball size on effective phosphorous removal using various calcining temperatures and various sizes of loess balls from artificial waste-

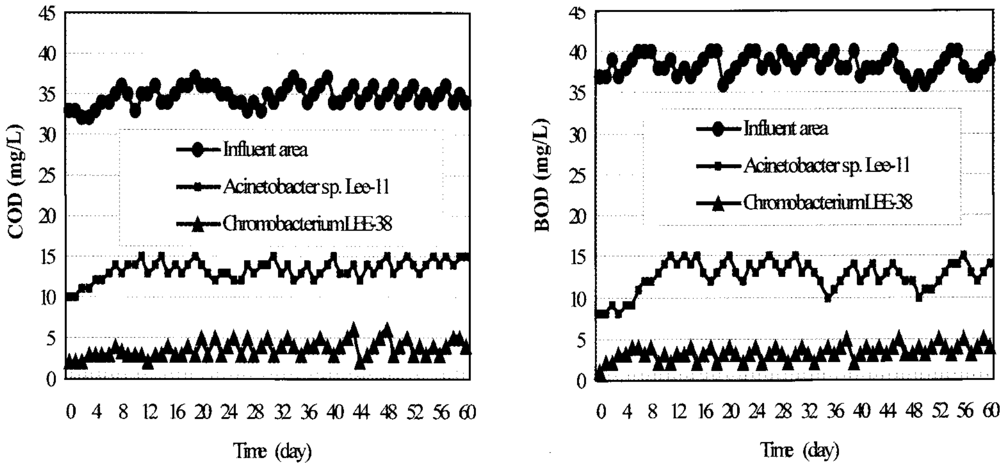

Fig. 4. Pilot test of the COD and BOD concentrations.

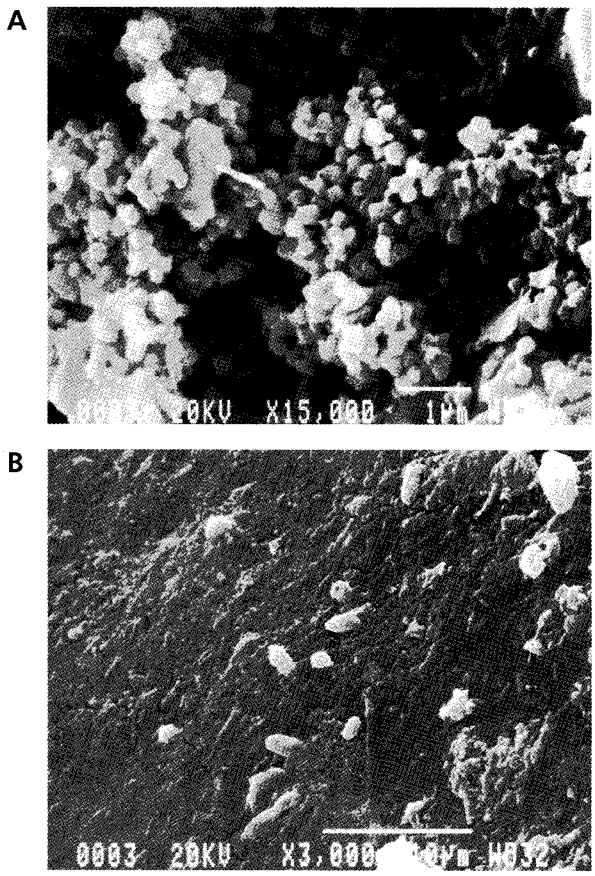

Fig. 5. SEM of microorganisms immobilized on the surface of a loess ball using farm wastewater. (A) *Chromobacterium* LEE-38, (B) *Acinetobacter* sp. Lee-11.

water. When a low calcining temperature and small-sized loess balls were used, the phosphorous removal at batch mode was higher than that observed when a high calcining temperature and large-sized loess ball were used [4]. However, in the case of denitrification using *Pseudomonas* sp., when the large-sized loess balls was used at the same temperature, the denitrification rate was higher than that seen with the small-sized loess balls. Conversely, when the calcining temperature was high and a loess ball of the same size was used, the denitrification rate was also higher than that of a low calcining temperature [5]. In the case of total phosphorus removal from farm wastewater; when a loess ball of 11~14 mm manufactured at a calcining temperature of 960°C was used, the total phosphorus removal efficiency was 90.0%, On the other hand, when a loess ball of 15~20 mm manufactured at a calcining temperature of 960°C was used, the total phosphorus removal efficiency decreased. In the case of practical wastewater, a loess ball of 2~4 mm manufactured at a calcining temperature of 960°C was the most suitable for the removal of phosphorus [6]. These results indicate that the total phosphorus treatment efficiencies were strongly affected by the calcining temperature and loess balls size, as well as the features of the wastewater.

For determining the optimal volume fraction on total phosphorus removal in the biofilm filter process, various volume fractions were used. When 50 and 60% of volume fractions were used, the efficiencies of total phosphorus removal for 20 days increased. However, they decreased after 30 days. On the other hand, when 70% of the volume fraction was used, the maximum efficiency of total phosphorus removal was obtained. When the initial pH of farm wastewater increased from 4.0 to 6.0, the removal efficiency of total phosphorus increased from 65.4 to 91.6%. Notably, when the initial pH ranged from 6.0 to 8.0, the maximum removal efficiency of total phosphorus was obtained. When the operating temperature was increased from 15 to 25°C, the removal efficiency of total phosphorus increased from 36.9 to 89.6%. Conversely, at an operating temperature above 40°C, the total phosphorus removal efficiency decreased.

Using farm wastewater, we carried out continuous experiments to test the various systems of biofilm filter processes for effective removal of total phosphorus using a loess ball of 11~14 mm manufactured at a calcining temperature of 960°C, 70% of the volume fraction, a farm wastewater pH of 7.0 to 8.0, and an operating temperature of 30 to 35°C. Among the various systems of biofilm filter processes, when the process A was used, the average removal efficiencies of total phosphorus, COD, and BOD were higher than those of processes B, C, and D. These results indicated that this process is good for removing a lot of organic materials, nitrogen, and phosphate from the wastewater when the efficiency of process treatment was low or the concentration of BOD and COD was high. In the case of process B, the average efficiency of total phosphorus removal was similar to those of process B and C. These results indicated that this process is good for removing the wastewater where there is a lot of nitrogen or phosphate [9]. In order to compare the total phosphorus removal by *Chromobacterium* LEE-38 and by *Acinetobacter* sp. Lee-11, pilot tests in biofilm filter process A using farm wastewater were carried out under optimal conditions. When *Chromobacterium* LEE-38 was used, the average removal efficiency of total phosphorus was 95.3% in the *p*-adsorption area, which was about 3.0-fold higher than that of *Acinetobacter* sp. Lee-11 and the average removal efficiencies of COD and BOD were 91.3 and 93.2%, respectively.

Fig. 5 shows microorganisms attached to the surface of the loess balls during total phosphorus removal in a biofilm filter process using farm wastewater. As shown in Fig. 5, when *Chromobacterium* LEE-38 was used, it was confirmed that many microorganisms were attached. These results indicated that the total phosphorus removal efficiency of farm wastewater depends on the immobilization capacity of the loess balls in the biofilm filter process. The concentration of *Chromobacterium* LEE-38 was 28.5 g/m^2, which was approximately 10.5-fold higher than that of *Acinetobacter* sp. Lee-11 at the flask level (data not shown).

In conclusion, the phosphorous treatment capacity of the biofilm filter process system using loess balls and *Chromobacterium* LEE-38, from farm wastewater was similar to those of the standard rule of the special counter plan area and the sanitary sewage and wastewater treatment facilities of industrial and rural areas. Therefore, in the future, a biofilm filter process using a loess ball and *Chromobacterium* LEE-38 could be applied to biological treatment of farm wastewaters containing high concentrations of phosphorous. We are currently investigating various farm wastewaters for effective phosphorous and nitrate removal in the biofilm filter process using loess balls with various microorganisms in agricultural and industrial fields.

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