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Optimization for Phosphorus Remove by Loess Ball Using *Chromobacterium*

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To investigate factors affecting the removal of phosphorus from the practical wastewater in the F-STEP PROCESS using a loess ball and *Chromobacterium* WS 2-14, first, the loess ball size and calcining temperature, initial pH, initial phosphorus concentration, working temperature, and aeration were studied. A 2~4 mm of loess ball made at 960℃ of calcining temperature was the most suitable one for the removal of phosphorus. When the initial pH was increased from 3.0 to 6.0, the removal efficiency of phosphorus was increased. Especially, at 6.0 of initial pH, the maximum removal efficiency of phosphorus was 88.7%. The maximum removal efficiency of phosphorous was gained, 1.8 mg/h when the initial concentration of phosphorous was 5.0 mg/l. When the operating temperature was 30℃, the maximum removal efficiency of phosphorus was obtained. In the case of aeration, when it was increased from 0.5 to 5.0 L/min, the removal efficiency of phosphorus was increased. On the other hand, above 7.0 L/min, the removal efficiency of phosphorus did not increased. Using the optimum operation conditions, pilot tests for the effective removal efficiency of phosphorus were carried out for 65 days. The average removal efficiency of phosphorus was 92.0%. The average removal efficiency of COD, BOD, and SS were 77.1, 74.2, and 86.4%, respectively. From the results, it can be concluded that F-STEP PROCESS using loess ball might be useful process for phosphorus removal.

Key words – Phosphorus removal, loess ball, biofilter system, *Chromobacterium*

The rapid development of human activity has resulted in large amounts of nutrients flowing from wastewater into rivers and lakes. Consequently, the water environment has deteriorated. To improve water quality, it is very important to remove nutrients such as nitrogen and phosphorus from wastewater. Nitrogen and phosphorus constitute a rlatively large part of the nutrient load of closed water bodies. Especially, Increased input of phosphorus to lakes, bays and other surface waters causes the growth of phytoplankton, which is called an algal bloom. Hence, considerable attention has been paid to the efficient removal of phosphorus from wastewater[9,14].

There is a chemical or physical method such as precipitation for phosphorus removal through the use of lime, alum, and ferrous or ferric sulfate. This method, although very effective and reliable, has several disadvantages. Increased equipment and chemical costs, together with sludge disposal costs and associated problems, are factors that must be considered seriously. On the other hand, the biological treatment method has been the target of interest with several merits. It is the most economical and easy method for its operation. Moreover, there is a low probability of creating a second pollution source from a wastewater disposal plant. However, it creates a few problems awaiting solutions. First, it makes it difficult to treat the loading change and further steps must be taken toward sludge treatment. A second treatment from the by-products of sludge is needed [1,7, and 16].

Nowadays, for solving these problems, the environmental material such as loess has been investigated. Particularly, the loess has been used for building materials, loess rooms, loess beds, ceramic materials, and medical fields, etc. for a long time. In addition, it has been used as a precaution and treatment for fish in fish cultivation. It also has been used for removing the red tide by applying the adsorption, ion exchange, and a cohesive reaction of loess[6,10, and 11].

Recently, we isolated various bacterials for biological treatment of wastewater containing nitrogen and phosphorus compounds in soil[2,4] and studied the capability of phosphorous removal from the artificial wastewater[3]. We also investigated factors affecting the denitrification in the F-STEP PROCESS using a loess ball as the bacterial support matrices[5].

In this study, in order to investigate factors affecting the removal of phosphorus in the F-STEP PROCESS using a loess ball and *Chromobacterium* WS 2-14, first, the loess ball size, initial pH, initial phosphorus concentration, working temperature, and aeration were studied by using the prac-

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tical wastewater. Second, using optimum conditions, pilot tests were also carried out in the F-STEP PROCESS.

Materials And Methods

Microorganism, Media, and Cultivation

The strain used in this study was *Chromobacterium* WS 2-14 isolated from wastewater treatment plant located in Kwang-ju[2]. The composition of the seed medium used was as follows (g/l): glucose 2; NH_4Cl, 1; NaCl, 2; Na_2SO4, 1, KCl, 0.1; Mg Cl_2, 0.01; $CaCl_2 2H_2O$, 0.01; $FeCl_3.6H20$, 0.01; and $NaHPO_4$, 0.22. All the media components were sterilized at 121℃ and 1.2 atm for 30 min. The pH of the media was adjusted to 6.8 before sterilization. One looful of *Chromobacterium* WS 2-14 was transferred to the slant medium and cultured at 30℃ for 2 days. Then, one looful of the slant culture of *Chromobacterium* WS 2-14 was inoculated into a 500 ml Erlenmeyer flask containing 100 ml of the seed medium and cultured at 30℃ for one day on a reciprocating shaker at 150 rpm. One % of the seed culture was inoculated into the practical wastewater and cultured at a range of 20 to 45℃.

Materials

For phosphorous removal from practical wastewater, various loess balls as the bacterial support matrices were used. The compositions of the loess ball (Muan Ceramic Industry, Chunnam) were as follows (%): Mg, 0.51; Al, 29.7; Si, 52.56; K, 1.91; Ti, 1.21; and Fe, 17.14. 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, 2.5~4.03. The calcining temperature of loess ball was 860 to 960℃ and holding time was 30 min. The practical wastewater containing 1% of seed culture was added to the reactor and circulated. The practical wastewater (first treatment wastewater) received from a wastewater treatment plant located in Kwang-ju was used.

F-STEP PROCESS System

As shown in Fig 1, F-STEP PROCESS was constructed of a raw water tank, an anaerobic tank, an oxic tank, an anoxic tank, a treated water tank, a re-washing tank, and a sludge-setting tank containing 70% of volume fraction. The continuous system was consisted of the following order: influent area, anaerobic area, oxic area, anoxic area, and effluent. The diameter and height of the anaerobic tank, the oxic tank, and the anoxic tank were 0.3 m and 1.0 m, respectively. The feed rate of the wastewater was 800 ml/min. For the effective phosphorus removal using practical wastewater, pilot tests were carried out in the F-STEP PROCESS with 2~4 mm of loess ball made at 960℃ of calcining for 65 days at 30℃ of working temperature and 15 L/min of aeration. The diameter and height of the anaerobic tank, the oxic tank, and the anoxic tank containing 90% of volume fraction were 1.5 m and 4.0 m, respectively. The feed rate of the wastewater was 2000 ml/min. The practical wastewater was adjusted to initial pH 6.0.

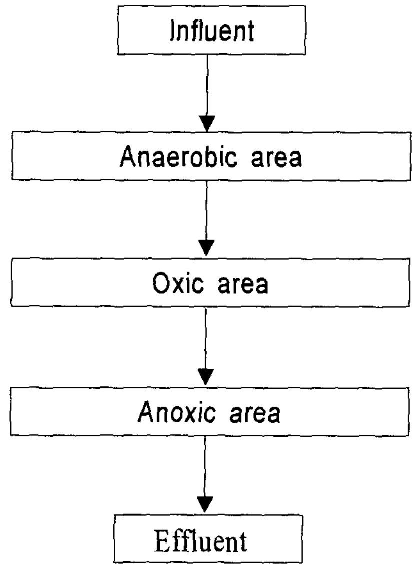

Fig. 1. Schematic diagram of F-STEP PROCESSES.

Analytical methods

All samples were filtrated through a 0.22 micro filter before measurements. The phosphorus concentration was measured according to DIN[8]. The concentrations of chemical oxygen demand (COD), biological oxygen demand (BOD), and suspension solid (SS) were measured by the modified method[12].

Results and Discussion

The nature of the support matrices used for immobilization of microorganisms has a significant effect on the reactor performance. For example, the support matrices must supply the enough space as the shelter of microorganisms against the shear stress by flowing water and be environment friendly in itself. A wide range of materials have been used as supports for immobilization of microorganisms at laboratory and pilot-scale, including inorganic materials such as glass beads, sand, sintered glass, synthetic fiber and polymers such as needle-punched polyester, polyurethane, polyvinylalchol bead[13,15]. However, there are some problems. For example, using these substances as support matrices for prolonged periods is difficult due to contamination and high cost. Nowadays, in order to solve such problems, the environmental materials such as the loess have been investigated.[6,10, and 11].

Recently, for biological treatment of wastewater containing nitrogen and phosphorus compounds in soil, we isolated *Nitrosomonas* WS, *Nitrobater* WS, *Pseudomonas* WS, and *Chromobacterium* WS[4]. Especially, 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[2]. We studied the capability of phosphorous removal by using a loess ball and loess powder at batch and continuous flow reactor using artificial wastewater[3]. We also investigated factors affecting the denitrification in the F-STEP PROCESS using a loess ball and *Pseudomonas* DWC 17-8 and pilot tests for effective denitrification using real farm wastewater were carried out under the optimum F-STEP PROCESS[5].

In this research, for the efficient phosphorus removal from practical wastewater in the F-STEP PROCESS using loess balls as the bacterial support matrices and *Chromobacterium* WS 2-14, we first studied the various factors such as calcining temperature and size of loess ball, pH, and working temperature affecting the phosphorus removal at continuous mode. We previously investigated the effect of calcining temperature and size of loess balls for the effective denitrification using 860 or 960℃ of calcining temperature and 2~4 mm or 5~10 mm of loess ball using artificial wastewater at batch mod. When the high calcining temperature and large sized loess ball were used, the efficiency of denitrification was higher than that of low calcining temperature and small sized loess ball[5].

Table 1. shows the effects of calcining temperature and size of loess ball on phosphorus removal in continuous experiments, which were carried out for 5 days at room temperature. The initial concentration of phosphorous was 5 mg/l. When 2~4 mm of loess ball made at 960℃ of calcining temperature was used, the phosphorus removal efficiency was 85.4% and in the case of 760℃ of calcining temperature, it was 68.3%. On the other hand, when 5~10 mm of loess ball made at 960℃ of calcining temperature was used, the phosphorus removal efficiency was 81.5% and in the case of 760℃ of calcining temperature, it was 72%. These results indicate that the calcining temperature and size of loess ball for effective phosphorus removal is an important factor. Therefore, 2~4 mm of loess ball made at 960℃ of calcining temperature was used in the following experiments.

Table 1. Effect of calcining temperature and size of loess ball on phosphorus removal

Calcining temperature	Diameter (mm)	Removal efficiency of phosphorus (%)
760	2-4	68.3
960	2-4	85.4
960	5-10	81.5
760	5-10	72.8

Generally, the optimum pH for effective phosphorus removal was dependant upon wastewater state, feature, kinds of microorganisms or support media, phosphorus concentration, and operating conditions. In order to investigate the effect of initial pH of sludge on phosphorus removal, continuous experiments were performed for 5 days. The results are shown in Table 2. The initial concentration of phosphorus was 5 mg/l. The range of pH was from 3.0 to 11. When the initial pH was increased from 3.0 to 6.0, the removal efficiency of phosphorus was increased from 71.2 to 88.7%. Especially, when the initial pH was 6.0, the maximum removal efficiency of phosphorus gave 88.7%. On the other hand, when the initial pH was above 8.0, it was decreased.

For determining the optimal initial concentration of phosphorus for effective removal of phosphorus, the initial concentrations of 1.0, 3.0, 5.0, 7.0, 9.0, and 11.0 mg/l were used at batch mode. The results are shown in Table 3. The initial pH was 6.0. When the initial concentration of phos-

Table 2. Effect of initial pH on phosphorus removal

Initial pH	Removal efficiency of phosphorus (%)
3	71.2
4	80.5
5	84.3
6	88.7
7	86.4
8	85.7
9	72.7
10	63.6
11	54.9

Table 3. Effect of initial concentration of phosphorus on phosphorus removal

Initial phosphorus concentration (mg/l)	Removal efficiency of phosphorus (mg/h)
1.0	0.4
3.0	0.9
5.0	1.8
7.0	1.4
9.0	1.0
11.0	0.9
13.0	0.7

phorus was from 1.0 to 5.0 mg/l, the removal efficiency of phosphorus was proportional to the initial concentration of phosphorus and then above 7.0 mg/l of initial phosphorus, it was decreased. The maximum removal efficiency of phosphorus was 1.8 mg/h when the initial concentration of phosphorus was 5.0 mg/l.

Table 4. shows the effect of operating temperature on phosphorus removal at continuous mode for 5 days. The operating temperature was ranged from 20 to 45℃. The initial pH was 6.0 and the initial concentration of phosphorus was 5.0 mg/l. When the operating temperature was increased from 10 to 30℃, the removal efficiency of phosphorus was increased from 77.2 to 92.0%. On the other hand, at above 35℃ of operating temperature, the removal efficiency of phosphorus was decreased. Therefore, 30℃ of the optimum temperature of operation was used in the following experiments.

In order to investigate the effect of aeration on phosphorus remove, 0.5L/min, 1.5 L/min, 3.0 L/min, 5.0 L/min, and 7.0 L /min were carried out in continuous mode for 7 days at 30℃ in the oxic area. The results are shown in Table 5. When the aeration was increased from 0.5 to 3.0 L/min, the removal efficiency of phosphorus was increased from 80.7 to 95.5%. On the other hand, at above 5 L/min of aeration, the removal efficiency of phosphorus does not increased.

For the effective phosphorus removal using practical wastewater, pilot tests were carried out. The results are shown in Fig. 2. For effective phosphorus removal in the oxic area, the glucose was also used as the carbon source. The concentrations of phosphorus in the influent area ranged from 4.8 to 5.3 mg/l. In the anaerobic area, the phosphorus concentrations were ranged from 3.8 to 4.3 mg/l and the average removal efficiency was 19.3%. In the oxic area, the phosphorus concentrations were ranged from 1.4 to 0.72 mg/l and the average removal efficiency was 79.3%. In the

Table 4. Effect of operating temperature on phosphorus remove

Operating temperature (℃)	Removal efficiency of phosphorus (%)
20	77.2
25	85.4
30	92.0
35	90.7
40	82.1
45	62.0

Table 5. Effect of aeration on phosphorus remove.

Aeration (L/min)	Removal efficiency of phosphorus (%)
0.5	80.7
1.5	88.8
3.0	95.5
5.0	95.1
7.0	95.3

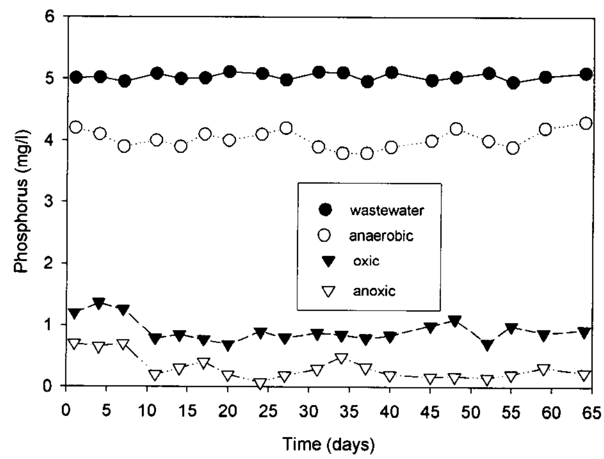

Fig. 2. Pilot test on the concentration of phosphorus removal.

anoxic area, the phosphorus concentrations were ranged from 0.2 to 2.0 mg/l and the average removal efficiency was 92.0%.

Fig. 3 shows the removal efficiencies of COD, BOD, and SS using practical wastewater in pilot. The concentrations of COD, BOD, and SS in the influent were ranged from 23.2 to 28.0, 29.2 to 24.0, and 35.5 to 28.2 mg/l, respectively. The COD concentrations in the final effluent area ranged from 3.6 to 9.1 mg/l, and the average removal efficiency was 77.1%. The concentrations of BOD in the final effluent area ranged from 4.0 to 9.5 mg/l and the average removal efficiency was 74.2%. In the case of SS, it ranged from 28.5 to 35.2 mg/l in the influence area and in the final effluent area, 1.5 to 3.2 mg/l. The average removal efficiency of SS

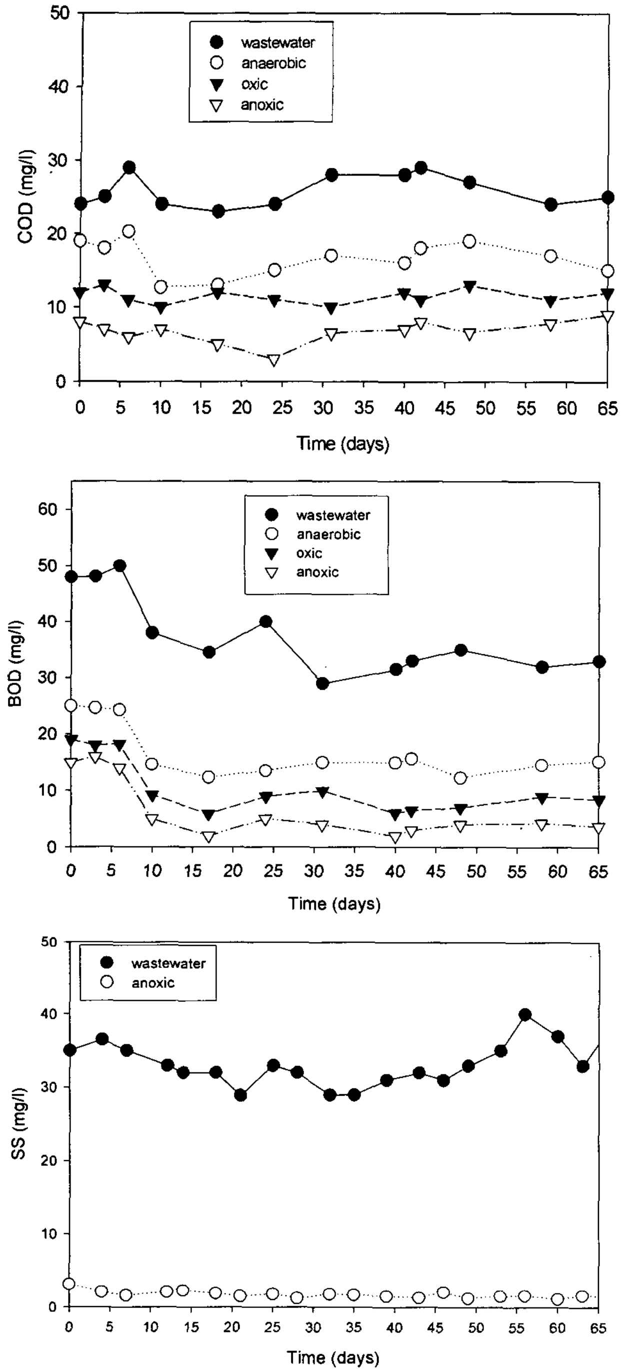

Fig. 3. Pilot test on the concentration of COD, BOD, and SS.

was 86.3%. From the F-STEP PROCESS system using loess balls, the wastewater treatment showed a lower concentration of nutrient salts than the standard of terminal disposal plant of sewage (the special counter plan area) and wastewater treatment facilities (sanitary sewage and wastewater treatment facilities of industrial and rural areas). Therefore, the F-STEP PROCESS would be applied for biological treatment of wastewater containing nitrate and phosphorous in the future. We are screening mutants that are resistant to high concentrations of phosphorous. Moreover, we are currently investigating the various farm wastewaters for effective phosphorous and nitrate removal in the F-STEP PROCESS using loess balls in the industrial fields.

Conclusions

This study investigated various factors affecting the removal of phosphorus in the F-STEP PROCESS with a loess ball as the bacterial support matrices and *Chromobacterium* WS 2-14 by using the practical wastewater. A 2-4 mm of loess ball made from loess at 960℃ of calcining temperature was the best one for the removal of phosphorus. These results indicate that the calcining temperature and size of loess ball for effective phosphorous removal from wastewater is an important factor. At 6.0 of initial pH, the maximum removal efficiency of phosphorus was obtained. When the initial concentration of phosphorous was from 1.0 to 5.0 mg/l, the removal efficiency of phosphorous was proportional to the initial concentration of phosphorous. When the operating temperature was 30℃, the maximum removal efficiency of phosphorus was showed, however, at above 40℃ or below 35℃, it was decreased. In the case of aeration, when it was increased from 0.5 to 3.0 L/min, the removal efficiency of phosphorus was increased, however, above 5.0 L/min of aeration, the removal efficiency of phosphorus did not increased.

Using the optimum operation conditions, pilot tests for the effective removal efficiency of phosphorus were carried out for 65 days. The average removal efficiency of phosphorus was 92.0%. It is expected that F-STEP PROCESS system using the loess ball made from loess can be applied economical as a multipurpose supports used in the removal of phosphorus as well as nitrogen in wastewater.

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초록 : *Chromobacterium*을 이용한 황토볼에 의한 인산 제거를 위한 최적화

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담체인 황토볼와 *Chromobacterium* WS 2-14을 이용하여 F-STEP공정(혐기 → 호기 → 무산소)에서 실제폐수에 존재하는 인산을 효율적으로 제거하기위해 최적 황토볼 크기 및 소성 온도, 폐수의 초기pH, 폐수의 초기 인산염 농도, 운전온도, 그리고 통기를 검토 했다. 최적 조건은 다음과 같다: 황토볼 크기 및 소성 온도, 2~4 mm, 960℃ ; 실폐수의 초기pH, 6.0; 실폐수의 초기 인산 농도, 5.0 mg/l. 운전온도, 30℃; 그리고 통기, 5.0 L/min등이 얻어졌다. 그리고 최적 운전조건을 이용해서 pilot test을 65일 동안 진행 했다. 인산 평균 제거율은 92.0%였고. 또한 최종 유출수에서 COD와 BOD의 평균 제거율은 각각 77.1와 74.2%였으며, SS의 경우는 평균 제거율이, 86.4%였다. 이상의 결과로부터 황토볼을 담체로 이용한 **Biofilter System**은 실제폐수에 존재하는 인산 제거 가능성을 암시했다.