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Process Development for Effective Denitrification by Biofilter Using Loess Ball

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Abstract In order to investigate factors affecting the denitrification in the F-STEP PROCESS using a loess ball as support media and *Pseudomonas* DWC 17-8, calcining temperature, loess ball size, pH, nitrate concentration, working temperature, and inhibitor were studied in batch mode using synthetic sludge. A 5- 10 mm of loess ball (960°C of calcining temperature) was the most suitable for denitrification. When the initial pH was increased from 3.0 to 7.0, the removal efficiency of nitrate was increased. Specifically, at initial pH of 7.0, the maximum removal efficiency of nitrate was 5.0 mg/min. When the initial concentration of nitrate was increased from 100 to 400 mg/l, the removal efficiency of nitrate was proportional to the concentration of nitrate. The maximum removal efficiency of nitrate was 5.72 mg/min at 400 mg/l of initial concentration. When the operating temperature was increased from 10 to 30°C, the removal efficiency of nitrate was increased from 0.76 to 6.15 mg/min, and at above 40°C of operating temperature, it was decreased from 4.0 to 2.0 mg/min. Among the various inhibitors, higher than 10^{-1} M of sodium azide abolished this reaction completely. When the KCN concentration was above 10^{-1} M, the reaction was inhibited completely. In the case of 2,4-dinitrophenol and sodium sulphide, it was inhibited at above 10^{-2} M completely. For testing the various flow orders of the F-STEP PROCESS for effective denitrification using practical wastewater, continuous experiments under the optimum conditions were carried out for 60 days. Among the various processes, the PROCESS A gave the highest efficiencies of denitrification, nitrification, and total nitrogen (TN) removal with 86.5, 89.5, and 90%, respectively. For scale-up in the PROCESS A, real farm wastewater was used and pilot tests carried out for 90 days. The denitrification efficiency was 97.5%, which was increased by 12.7%. The efficiencies of TN removal and nitrification were 96.6 and 70.0%, respectively. The removal efficiency of chemical oxygen demand (COD) was 63.7%, which was increased by 20%.

Key words: Denitrification, loess ball, *Pseudomonas*, biofilter process

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 relatively large part of the nutrient load of closed water bodies. Specifically, nitrates stimulate eutrophication where pollution is caused in waterways by heavy algal growth, as they are both rate limiting nutrients for the process. Drinking water supplies with nitrate concentration greater than 10 mg/l is considered unsafe, particularly for infants. Nitrates may also lead to the possible formation of nitrosoamines, which are known carcinogens. In addition, it has been reported that nitrates have caused heart and behavioral problems in laboratory animals [21, 27,]. There are physical and chemical methods such as the ion-exchange process, reverse osmosis, electrodialysis, chemical precipitation, and distillation for nitrates removal using sewerage, groundwater, wastewater, soil, and river water. These methods only separate nitrates from one liquid phase to concentrate them in another, and further treatment of the concentrated nitrate solution is very expensive. Moreover, it is difficult to use the physical and chemical methods for *in situ* remediation [11, 16, 30]. 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 byproducts of sludge is needed [1, 10, 34]. Some researchers [4, 20, 37, 39] investigated the biological methods for treating the wastewater using inorganic particle, fiber,

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synthetic materials, and plastic, as support media of biofilm reactor. However, in spite of having several merits, it was difficult to use them, because of high prices and some environmental problems in the plants.

Nowadays, for solving these problems, environmental material such as loess has been investigated. Particularly, loess has a lot of features such as adsorption, ion exchange, and a cohesive reaction, and has been processed in many sites [9, 18, 19]. Recently, we isolated various bacterials for biological treatment of wastewater containing nitrogen and phosphorus compounds in soil [5, 6], and also studied the capability of phosphorous removal by using a loess ball in the artificial wastewater [7].

In this study, in order to develop the new process for effective denitrification in the F-STEP PROCESS using a loess ball as the bacterial support matrices and *Pseudomonas* DWC 17-8, the effect of a loess ball size, pH, nitrate concentration, working temperature, and inhibiter were first investigated at batch mode by using synthetic wastewater. Second, using optimum conditions, various flow orders of biofilter process systems using practical wastewater were investigated at continuous mode. Pilot tests using real farm wastewater were also carried out under the optimum F-STEP PROCESS.

MATERIALS AND METHODS

Microorganism, Media, and Cultivation

The strain used in this study was *Pseudomonas* DWC 17-8 isolated from a wastewater treatment plant located in Kwang-ju [6]. The composition of the seed medium used was as follows (g/l): sodium citrate, 8.5; $MgSO_4$·$7H_2O$, 1.0; $FeCl_3$·6H20, 0.05; KH_2PO_4, 1.0; and $CaCl_2$·$2H_2O$, 0.2. All the media components were sterilized at 121°C and 1.2 atm for 20 min. The pH of the media was adjusted to 7.0 before sterilization. One loopful of *Pseudomonas* DWC 17-8 was transferred to the slant medium and cultured at 30°C for 2 days. Then, one loopful of the slant culture of *Pseudomonas* DWC 17-8 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. Five % of the seed culture was inoculated into the synthetic medium, practical wastewater, or farm wastewater, and cultured at 30°C for 5 days.

Materials

For nitrate removal from wastewater, various loess balls as the bacterial support matrices were used. The composition of the loess ball (Jeil Ceramic Industry, Chunnam, Korea) was as follows (%): Mg, 0.47; Al, 28.7; Si, 49.56; K, 2.91; Ti, 1.21; and Fe, 17.14. The calcining temperature of loess ball was 860 to 960°C and the holding time was 30 min. In order to carry out the optimization at batch mode using the loess ball, the synthetic sludge (mixture ratio of synthetic wastewater and sludge, 1:1) was used. The composition of the synthetic wastewater was as follows (mg/l): glucose, 187.5; NH_4Cl, 152.8; KH_2PO_4, 22.0; $NaHCO_3$, 250.0; $FeCl_3$, 0.225; HCl, 4.7; $MgSO_4$·$7H_2O$, 50.0; $CaCl_2$·$2H_2O$, 10.0. The synthetic wastewater containing 5% of seed culture was added to the anoxic reactor and circulated for 7 days. The retention time was 2.5 h. For effective denitrification, 400 mg/l of methanol as a carbon source was used and supplied continuously. The feeding rate was 1.0 ml/min. In order to test the flow order of the F-STEP PROCESS on the removal efficiency of nitrate, the practical wastewater (first treatment wastewater) received from a wastewater treatment plant located in Kwang-ju was used. The composition of practical wastewater was as follows (mg/l): total nitrogen (TN), 17.0-33.3: total phosphorous (TP), 2.0-4.5; chemical oxygen demand (COD), 15.0-42; and biological oxygen demand (BOD), 22.0-67.1. For pilots test using the F-STEP PROCESS A, farm wastewater received from a farming village (Chookchung-ri, Dogok-myeon, Hwasun-gun, Chunnam, Korea) was used.

F-STEP PROCESS System

As shown in Fig. 1, the F-STEP PROCESS was constructed 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. F-STEP PROCE A consisted of the following order:

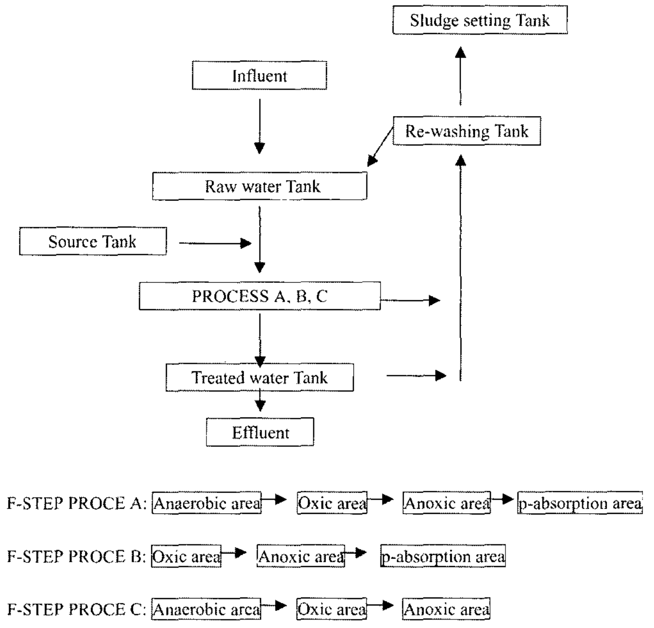

Fig. 1. Schematic diagram of the F-STEP PROCESSES.

influent area, anaerobic area, oxic area, anoxic area, p-absorption area, and effluent. F-STEP PROCE B consisted of the following order: influent area, oxic area, anoxic area, p-absorption area, and effluent. F-STEP PROCE C consisted of the following order: influent area, anaerobic area, oxic area, anoxic area, and effluent. 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: Loess balls (5- 10 mm, calcining temperature 960°C) were used for practical wastewater. The diameter and height of the anaerobic tank, the oxic tank, and the anoxic tank were 9 cm and 65 cm, respectively. The feed rate of the wastewater was 10 ml/min. In the case of the nitrification reactor, the rate of air was 2.0 l/min. For denitrification of farm wastewater, 5- 10 mm loess balls (calcining temperature 960°C) were used. 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 wastewater was 800 ml/min. In the case of the anoxic tank, the rate of methanol feed was 1.0 ml/min, and in the oxic tank, the rate of air was 130 l/min.

Analytical Methods

All samples were filtrated through a 0.22 micron filter before measurements. The nitrate concentration was quantified using the ultraviolet spectrophotometric method. In this case, all the volumes were reduced as necessary to allow the determination of nitrate concentration using smaller sample amounts. By dilution of samples, the nitrate concentration was brought to a detectable nitrate range and the effect of organic material was eliminated [38]. The nitrite concentrations were determined by its reaction with sulfanilamide in an acid solution to form a photodetectable diazo compound [14, 33]. The concentration of ammonia nitrogen was measured using the method, modified by Choi and Ki *et al.* [8, 22]. The total phosphorous concentration was measured according to DIN [15]. The concentrations of COD, BOD, and suspension solids (SS) were measured by a modified method [24].

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

Calcining temperature (°C)	Diameter (mm)	Removal efficiency of nitrate (relative, %)
860	2- 4	70
960	2- 4	80
960	5- 10	100
860	5- 10	90

RESULTS

Optimization of Environmental Conditions for Effective Denitrification

In order to investigate the effects of calcining temperature and size of loess ball, initial pH, initial nitrate concentration, working temperature, and inhibitors on denitrification, batch experiments using loess balls as the bacterial support matrices with synthetic sludge were carried out.

Table 1 shows the effects of calcining temperature and size of loess ball on denitrification in batch experiments, which were carried out for 30 min at room temperature. The initial concentration of nitrate-N was 200 mg/l. When 5- 10 mm of loess ball made at calcining temperature of 960°C was used, the denitrification efficiency was 100%, and in the case of calcining temperature of 860°C, it was 90%. These results indicate that the calcining temperature and size of loess ball for effective denitrification is an important factor. Therefore, 5- 10 mm of loess ball made at calcining temperature of 960°C was used in the following experiments. Generally, the optimum pH for effective denitrification was dependant upon wastewater state, feature, kinds of microorganisms or support media, nitrate concentration, and operating conditions. In order to investigate the effect of initial pH on nitrate and nitrite removal using synthetic sludge, batch experiments were performed (Table 2). The initial concentration of nitrate-N was 200 mg/l. The range of pH was from 3.0 to 11. When the initial pH was increased from 3.0 to 7.0, the removal efficiency of nitrate

Table 2. Effect of initial pH on nitrate and nitrite removal.

Initial pH	Removal time of nitrate (min)	Maximum concentration of detected nitrite (mg/l)	Removal efficiency of nitrate (mg/min)
3	150	70	1.33
4	130	50	1.54
5	80	30	2.5
6	45	20	4.45
7	40	10	5.0
8	50	8	4.0
9	60	7	3.34
10	70	6	2.86
11	80	6	2.5

*5- 10 mm of loess ball made at calcining temperature of 960°C was used.

Table 3. Effect of initial nitrate concentration on nitrate and nitrite removal.

Initial nitrate concentration (mg/l)	Removal time (min)	Maximum concentration of detected nitrite (mg/l)	Removal efficiency of nitrate (mg/min)
100	30	0	3.34
200	40	0	5.0
300	55	0	5.45
400	70	15	5.72
800	108	130	4.45
1,200	300	200	4.00

*Batch experiments, using 5- 10 mm of loess ball made at calcining temperature of 960°C were carried out in pH 7.0.

was increased. In particular, when the initial pH was 7.0, the maximum removal efficiency of nitrate was 5.0 mg/ min. On the other hand, when the initial pH was above 8.0, it was decreased. In the case of nitrite concentration, it was increased when initial pH was low. For determining the optimal initial concentration of nitrate for effective removal of nitrate using synthetic sludge, the initial concentrations of 100, 200, 300, 400, 800, and 1,200 mg/l were used in batch mode (Table 3). The initial pH was 7.0. When the initial concentration of nitrate was below 300 m/l, the nitrite concentration was not detected. When the initial concentration of nitrate was increased, the time of nitrate removal was increased. However, when the initial concentration of nitrate was from 100 to 400 mg/l, the removal efficiency of nitrate was proportional to the concentration of nitrate, and then above 400 mg/l of nitrate, it was decreased. The maximum removal efficiency of nitrate was 5.72 mg/min when the initial concentration of nitrate was 400 mg/l. During denitrification at 400 mg/l of nitrate, the nitrite detection was begun. Specifically, when the initial concentration of nitrate was increased from 400 to 1,200 mg/l, the nitrite concentration was increased from 15 to 200 mg/l and then was decreased.

Table 4 shows the effect of operating temperature on nitrate and nitrite removal. The operating temperature was ranged from 10 to 50°C. The initial pH was 7.0 and the initial concentration of nitrate was 400 mg/l. When the operating temperature was increased from 10 to 30°C, the removal efficiency of nitrate was increased from 0.76 to 6.15 mg/min and the maximum concentration of detected nitrite were decreased from 120 to 30 mg/l. On the other hand, at operating temperature of above 40°C, the removal efficiency of nitrate was decreased from 4.0 to 2.0 mg/min and the maximum concentration of detected nitrite were increased from 30 to 100 mg/l. Therefore, the optimum temperature of 30°C for operation was used in the following experiments.

For investigating the effect of inhibitors on nitrite removal, batch experiments were carried out in the synthetic medium containing 1 to 10^{-6} M of inhibitor and 400 mg of synthetic medium in the pH 7.0 and working temperature of 30°C (Table 5). The reduction of N_2O to N_2 was strongly inhibited by sodium azid. Specifically, at the concentrations of above 10^{-1} M, this reaction was abolished completely. At the concentrations of less than 10^{-4} M of sodium azide, no inhibition was observed. When the KCN concentration was above 10^{-1} M, the reaction was inhibited completely and at the concentrations of less than 10^{-5} M of sodium azide, no inhibition was observed. In the case of 2,4-dinitrophenol and sodium sulphide, it was inhibited at above 10^{-2} M completely.

Comparison of Several F-STEP PROCESSES

Using practical wastewater adjusted to initial pH 7, continuous experiments for testing the various flow orders of the F-STEP PROCESS for effective denitrification using 5- 10 mm of loess ball made at calcining temperature of 960°C were carried out at operating temperature of 30°C for 60 days (Table 6). Methanol in the anoxic area was fed as the carbon source at the F-STEP PROCE A and B. In the case of F-STEP PROCE C, practical wastewater was fed as the carbon source.

When the PROCESS A was used, the TN concentrations ranged from 1.3 to 5.7 mg/l and the average efficiency of

Table 4. Effect of temperature on nitrate and nitrite removal.

Temperature (°C)	Removal time of nitrate (min)	Maximum concentration of detected nitrite (mg/l)	Removal efficiency of nitrate-N (mg/min)
10	400	120	0.76
20	120	180	3.34
30	65	30	6.15
40	100	100	4.0
50	600	500	0.67

*Batch experiments, using 5- 10 mm of loess ball made at calcining temperature of 960°C were carried out in the synthetic medium containing 400 mg of nitrate in pH 7.0.

Table 5. Effect of inhibitors on nitrite removal.

Concentration (M)	Inhibition (Relative, %)			
	NaN_3	KCN	DNP	Sulphide
1	100	100	100	100
10^{-1}	95	100	100	100
10^{-2}	71	85	93	93
10^{-3}	40	30	65	65
10^{-4}	0	6	11	11
10^{-5}	0	0	0	0
10^{-6}	0	0	0	0

*Batch experiments, using 5-10 mm of loess ball made at calcining temperature of 960°C, were carried out in the synthetic medium containing 400 mg of nitrate at pH 7.0 and working temperature of 30°C.

Table 6. Effect of order of system line on nitrification, denitrification, TN, COD, and BOD using practical wastewater in F-STEP PROCESSES.

Items	Process type		
	A	B	C
Nitrification (%)	89.5	84.5	86.2
Denitrification (%)	86.5	81.5	54.3
TN (mg/l)	1.3-5.7	0.7-5.0	4.8-8.5
Removal efficiency of TN	90	88.2	74.6
COD (mg/l)	2.1-14	8.7-23.0	7.5-14.7
Removal efficiency of COD (%)	76.5	57.9	78.2
BOD (mg/l)	1.8-26.3	9.0-20.9	8.1-15.3
Removal efficiency of BOD (%)	82.3	73.1	85.3

*The efficiencies of nitrification and denitrification were measured in the oxic area and anoxic area, respectively.
**COD, BOD, and TN were measured in the effluent area.

TN removal was 90%. The efficiencies of nitrification and denitrification were 89.5 and 86.5%, respectively. The concentrations of COD and BOD ranged from 2.1 to 14 and 1.8 to 26.3 mg/l and the average removal efficiencies of COD and BOD were 76.5 and 82.3%, respectively. In the case of the PROCESS B, the TN concentrations ranged from 0.7 to 5.0 mg/l and the average efficiency of TN was 88.2%. The efficiencies of nitrification and denitrification were 84.5 and 81.5%, respectively. The concentrations of COD and BOD ranged from 8.7 to 23 and 9.0 to 20.9 mg/l and the average removal efficiencies of COD and BOD were 57.9 and 73.1%, respectively. Using the PROCESS C, the TN concentrations ranged from 4.8 to 8.5 mg/l and the average removal efficiency was 54.3%. The efficiencies of nitrification and denitrification were 86.2 and 54.3%, respectively. The concentrations of COD and BOD ranged

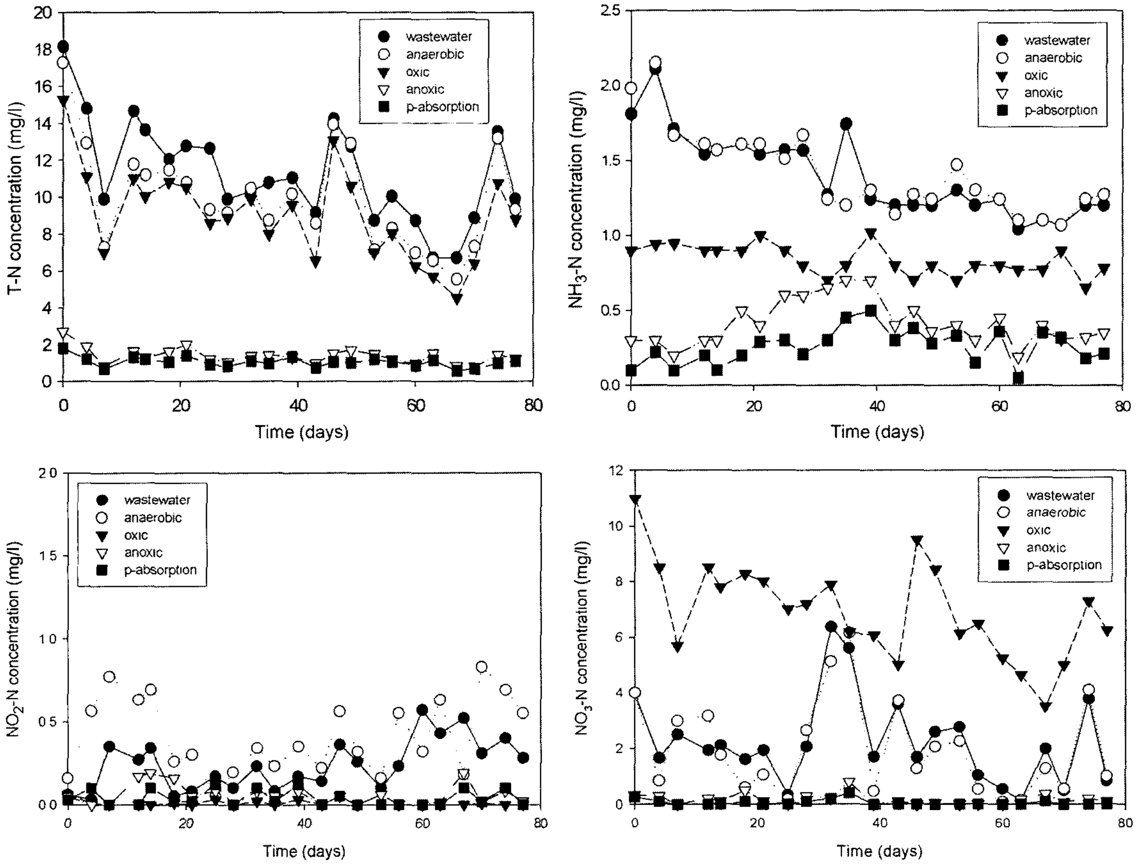

Fig. 2. Pilot test on the concentrations of TN, ammonia nitrogen, nitrate nitrogen, and nitrite nitrogen.

from 7.5 to 14.7 and 8.1 to 15.3 mg/l and the average removal rates of COD and BOD were 78.2 and 85.3%, respectively.

Scale-Up for Effective Denitrification Using Farm Wastewater in F-STEP PROCESS A

For effective denitrification using real farm wastewater, pilot tests were carried out in the F-STEP PROCESS A for 3 months (Fig. 2). For effective denitrification in the anoxic area, the methanol was used as the carbon source. In the final effluent area, the TN concentrations ranged from 0.6 to 1.8 mg/l and the average removal efficiency was 96.6%. The concentrations of ammonia nitrogen in the influent area ranged from 1.1 to 1.8 mg/l, and in the final effluent area, it was 0.1 to 0.5 mg/l. The concentrations of nitrate nitrogen in the influent area ranged from 0.1 to 6.4 mg/l, and in the final effluent area, 0.0 to 0.3 mg/l. In the case of nitrite nitrogen, the concentrations in the influent area ranged from 0.1 to 0.5 mg/l, and in the final effluent area, 0.0 to 0.1 mg/l. The efficiencies of nitrification in the oxic tank and denitrification in the anoxic tank were 70.0 and 97.5%, respectively.

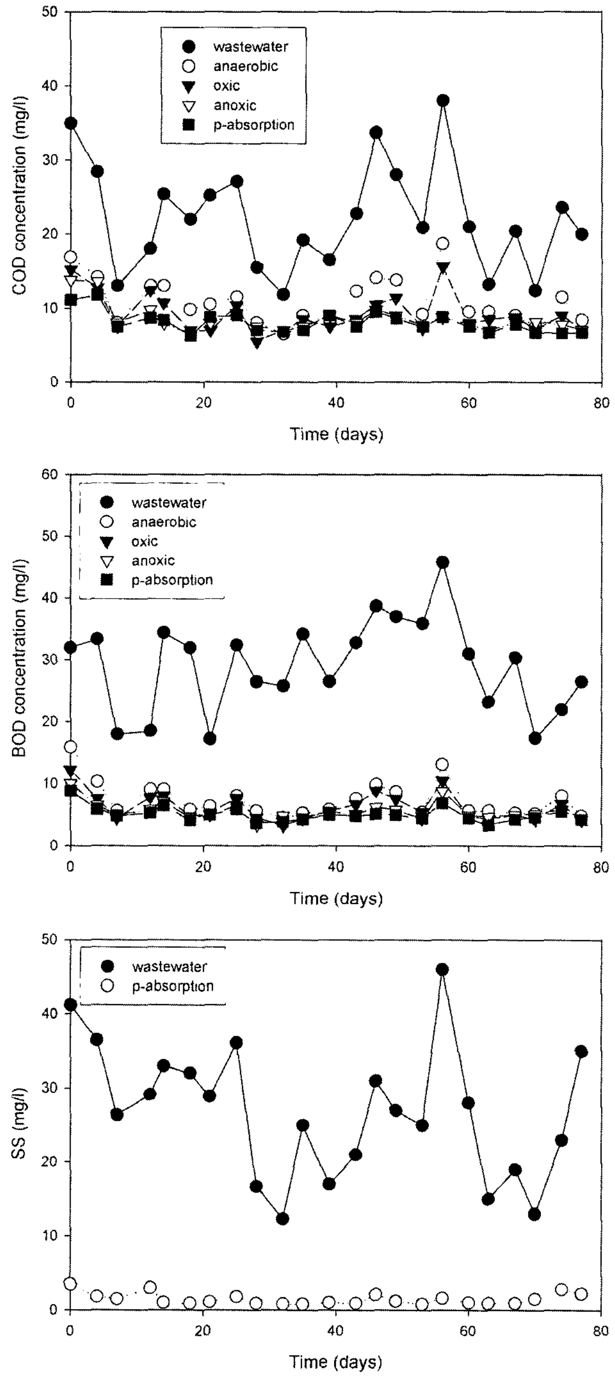

Fig. 3. Pilot test on the concentration of COD, BOD, and SS.

Figure 3 shows the removal rates of COD, BOD, and SS. The concentrations of COD and BOD in the influent ranged from 11.8 to 38.0 and 45.0 to 17.2 mg/l, respectively. The COD concentrations in the final effluent area ranged from 6.3 to 11.8 mg/l, and the average removal efficiency was 63.7%. The concentrations of BOD in the final effluent area ranged from 3.3 to 8.8 mg/l and the average removal efficiency was 82.7%. In the case of SS, its concentrations in the influence ranged from 12.3 to 46.0 mg/l and in the final effluent area, 0.8 to 3.5 mg/l. The average removal efficiency of SS was 94.5%.

DISCUSSION

In the bioreactor, providing stable microorganisms is one of the most important things for improving the denitrification efficiencies by microorganisms. In the industry, in order to maintain the high cell concentration in the bioreactor, immobilization methods have been used. There are two kinds of immobilization methods using support surface and the inside of support. Agar, alginate, chitosan, and polyvinyl alcohol as support for effective immobilization have been used for a long time [29, 31]. However, there are some problems. For example, using these substances as a support for prolonged periods is difficult due to contamination. Nowadays, in order to solve such problems, environmental materials such as loess have been investigated. Particularly, 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 [9, 18, 19]. Recently, for biological treatment of wastewater containing nitrogen in soil, we isolated *Pseudomonas* DWC. Specifically, the optimal growth temperature and pH for *Pseudomonas* DWC 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].

In this research, for the efficient denitrification from wastewater in the F-STEP PROCESS using loess balls as the bacterial support matrices and *Pseudomonas* DWC 17-8, we first studied the various factors such as calcining temperature and size of loess ball, nitrate concentration, pH, temperature, and inhibitor affecting the denitrification at batch mode. We previously investigated the effect of calcining temperature and size of loess balls for the effective phosphorus removal using calcining temperature of 860 or 960°C and loess ball of 2-4 mm or 5-10 mm. When the low calcining temperature and small sized loess ball were used, the efficiency of phosphorus removal was higher than that of high calcining temperature and large sized loess ball [7]. However, in the case of denitrification, when the large sized loess ball was used at the same temperature, the denitrification rate was higher than that of small sized loess ball. On the other hand, when the calcining temperature was high at the same sized loess ball, it was also higher than that of low calcining temperature.

The denitrification related to pH in pure culture as well as in natural system was investigated. The low pH was reported to be the only factor that prevented the occurrence of denitrification [23]. Thus, it appears that both decreasing pH and increasing O_2 concentration tend to decrease the overall denitrification [17]. Wijler and Delwiche [36] reported that at above pH 7.0, N_2O was produced but was subsequently reabsorbed and reduced to nitrogen gas. Below pH 7.0, some N_2O was formed, but there was little absorption with further denitrification; this reaction is differently affected by acid conditions. The NO was produced at low levels between pH 6.0 and 7.0 under more acidic conditions. In the case of *P. aeruginosa*, the denitrification was carried out in a pH range of 5.8 to 9.2, and the optimum range was to 7.0 to 8.2 [13]. However, using *Ochrobactrum anthropi* SY 509 under nongrowing condition, the optimum pH of nitrate reductase was 7.0 for denitrification under aerobic condition, but the optimum pHs of nitrate and nitrite reductase were shifted to 10.0 and 9.0, respectively, under anaerobic condition. In particular, in the alkaline pH, the denitrification rate of nitrate was increased up to about 2 fold over that of a neutral pH [21]. Jeong *et al.* [18] reported that adsorption of Pb (II), Cu (II), Cr (III), and Zn (III) ions by domestic loess minerals was affected by pH changes, Specifically, the adsorptivity of Cu (II) and Zn (II) was 99% in the range of pH 3 to 7, except below pH 2 and above pH 8 [18]. We previously reported that the pH changes of artificial wastewater had an influence on the removal of phosphorus at 4 ppm with pH 4 and 2.5 ppm with pH 10 in 10 h. This also showed that the removal was more effective in acidic condition than in alkalinic conditions. Loess balls removed more than 80% of phosphorous in a continuous flow reactor using artificial wastewater [7]. In the case of denitrification using synthetic wastewater, when the initial pH was increased from 3.0 to 7.0, the removal rate of nitrate was increased. Specifically, at initial pH of 7.0, the maximum removal efficiency of nitrate were 5.0 mg/min

Many researchers have investigated the effect of temperature on denitrification. Nommik [28] reported that the rate of denitrification to be insignificant at 3°C, but significant at 12-13°C. The efficiency of denitrification was increased with increasing temperature up to 70°C and then decreased with further temperature increase: at 85°C the denitrification ceased. Bremner and Shaw [3] also reported that the denitrification increased in the temperature range of 2 to 25°C, beyond which the process was not significantly affected by increasing temperature up to 70°C, where the process was inhibited. Balmelle *et al.* [2] investigated the influence of temperature on the nitrification process using *Nitrobacter.* The *Nitrobacter* was active over a range of temperature between 10 to 20°C. Under these conditions, nitrite buildup remained low, which can be explained by the fact that the effect of *Nitrobacter* activation by temperature prevails over its inhibition by free ammonia. On the other hand, at above 20 to 25°C, a slowing of nitrating activity was observed with an activation of the nitrating activity, which passes through a maximum at 25°C [2]. Welander *et al.* [35] suggested the suspended-carrier biofilm process to be a useful alternative for the denitrification at low temperature using *Psychrotrophic* microorganisms, since the denitrification rate showed only a rather weak dependence on the temperature, the rate at 3°C being approximately 55% of that at 15°C. The maximum rate of denitrification obtained at 15°C was 2.7 g N/m^2 carrier/d. The maximum rate of denitrification at 3°C for 8 days was found to be constant [35]. In our case, when the operating temperature was increased up to 30°C, the removal rate of nitrate was increased and the maximum concentration of detected nitrite was decreased from 120 to 30 mg/l. On the other hand, at operating temperature of above 40°C, the removal rate of nitrate was decreased and the maximum concentration of detected nitrite was increased.

The reductase involved in denitrification is susceptible to inhibition by a variety of compounds. The mechanism of reaction is not clear for any of the inhibitors, many of which exert metabolic effects on specific reductases involved in denitrification. David and Weintraub [12] reported that the nitrite reduction was inhibited by cyanide in the culture of *Pseudomonas aeruginosa.* Myers [26] also reported that increasing sodium sulphide concentration depressed production of nitrogenous gases, indicating inhibition of the denitrification process. There was no gaseous N detected at the highest sodium sulphide concentration (40 mg/g). At the highest sodium sulphide concentration, however, over 80% of the nitrate remained unchanged [26]. Matsubara and Mori [25] also reported that the inhibition concentration of sodium azide for denitrification was 5×10^{-1} M. When the KCN concentration was above 10^{-3} M, the reaction was inhibited completely. In the case of 2,4-dinitrophenol, it was inhibited at above 10^{-4} M completely [25]. In our case, there were also some inhibitions during the denitrification. Therefore, we investigated the effects of inhibitors such as sodium azide, KCN, sodium sulphide, and 2,4-dinitrophenol (DNP). At the concentrations of above 10^{-1} M of sodium sulphide, this reaction was abolished completely. When the KCN concentration was above 10^{-1} M, the reaction was inhibited completely. In the case of 2,4-dinitrophenol, it was inhibited at above 10^{-2} M completely. Generally, the inhibition concentrations for denitrification were 2-3 fold higher than those of Matsubara and Mori [25].

Using the optimum results, the comparison of process design for the effective denitrification using practical wastewater in the F-STEP PROCESS was carried out. Generally, when the PROCESS A was used, nitrification, denitrification, and the average removal efficiencies of TN and TP were higher than those of PROCESS B or C. These results indicated that this process is good for removing wastewater having a lot of organic materials, nitrogen, and phosphate when the efficiency of process treatment was low. In the case of PROCESS B, the average efficiencies of denitrification and TP were higher than that of PROCESS C. These results indicated that this process is good for removing wastewater having a lot of nitrogen or phosphate. Using PROCESS C, the average efficiencies of BOD and COD were higher than that of PROCESS B or C. These results indicated that this process is good for removing wastewater with high concentrations of BOD and COD.

Pilot scaleup for effective denitrification using real farm wastewater was carried out using 5-10 mm of loess ball made at calcining temperature of 960°C for 3 months at working temperature of 30°C. The real farm wastewater was adjusted to initial pH 7. The removal efficiency of TN was 96.6%. In the case of efficiency of denitrification, it was 97.5%, which was increased by 12.7%. However, the efficiency of nitrification was decreased by 22%. The removal efficiency of COD was 63.7, which was increased by 20%, and in the case of BOD, it was 82.7%.

Through 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 A would be applied for biological treatment of wastewater containing nitrate in the future. We are screening mutants that are resistant to high concentrations of nitrate. Moreover, we are currently investigating the various farm wastewaters for effective denitrification in the F-STEP PROCESS using loess balls in the industrial fields.

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