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CHARACTERISTICS OF A WATER-PURIFICATION SYSTEM USING IMMOBILIZED PHOTOSYNTHETIC BACTERIA BEADS

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Abstract : The characteristics of nitrogen removal by the free cell and the immobilized cell of *R. capsulatus* were investigated. Denitrification by *R. capsulatus* cells resulted in reduction of ORP with the rapid depletion of DO and the increase of pH. Without accumulation of nitrite, the removal efficiencies of NO_3^--N for the free cell and the immobilized cell were 99.1 and 99.3%, respectively. During the three-month experiment of goldfish breeding equipped with a water-purification biofilter, the average values of pH and total cell numbers present in an aquarium were not significantly different between water-purification system and the control. The average concentrations of NH_4^+-N and PO_4^{2-}-P in water-purification system were relatively low, compared to that in the control. Goldfish died at 11th, 16th, 43rd, and 67th days in the control, while goldfish died at 10th, 20th, and 39th days in the water-purification system. On the days of goldfish's death, the total concentrations of nitrogenous compounds except for NO_2^--N were higher than those on the other days of the experiment, especially with the concentrations of NH_4^+-N ranging from 7.4 to 13.5 mg/L. The water-purification system also showed the less turbidity of water with more active movement of goldfish than the control. PVA gel beads showed almost the full denitrifying ability even after the long-term experiment. As a result, the water-purification system was effective to remove nitrogenous compounds with better survival of goldfish.

Key Words : Water purification, Photosynthetic bacteria, Immobilized cell, Nitrogen removal

INTRODUCTION

In closed aqua-environments such as fish breeding aquarium and ponds, nitrogenous compounds are major pollutants and occur in wastes from uneaten feed, feces, and bacteria.[1] Fish uses approximately 25% of the nitrogen assimilated from feed for biomass production, while the rest is released as ammonium-N, dissolved organic N, or feces.[2] Additional ammonium is released from microbial breakdown of uneaten feed and feces. The polluted environment sometimes brings about sudden death of fish, even foul odors. Hence, circulating filtration systems that remove organic materials, phosphorous and nitrogenous compounds have been developed and applied.[3-4] These systems are usually based on microbial activities under given environments, and more effective and compact equipment is required in an economic aspect.

Immobilized-cells processes have been receiving increasing attention in response to the need for the development of a more compact and an

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efficient system for purification of water, since the volumetric efficiency is greatly increased by cell immobilization.[5-8] The removal of nitrogenous compounds has been performed in bioreactors using immobilized denitrifying bacteria,[9-12] and purification of an aquarium for breeding carp could have been accomplished using a simple circulating filtration system packed with alginate-gel immobilized photosynthetic bacteria.[13] As widely recognized, entrapment of cells in a proper support matrix is an effective means for cell immobilization. However, the removal efficiency of the filtration system using the alginate gel beads decreased after two weeks with gradual decomposition of alginate gel. Compared with the alginate gel, the polyvinyl alcohol (PVA) gel was found to be quite stable over a one-month period with mechanical stability.[14,15] Cell immobilization using PVA has been reported to be successfully applied to the immobilization of denitrifying photosynthetic bacteria in the removal of nitrogenous compounds.[4,16]

It has been reported that photosynthetic bacteria can consume various types of organic substrates, nitrogenous and phosphorous compounds simultaneously, with a relatively high growth rate, and thus the photosynthetic bacteria have been used for water purification of fish breeding ponds.[17] Besides, photosynthetic bacteria have been used as a feed supplement and to prevent fish disease during breeding.[17] So far, there has been little research on characteristics of immobilized photosynthetic bacteria for application to a practical water-purification system. In this study, the characteristics of a water-purification system using the photosynthetic bacterium, *Rhodobacter capsulatus* immobilized in PVA beads were investigated by focusing on the long-term purification of aquarium water with test of the denitrifying ability of immobilized photosynthetic bacteria beads.

MATERIALS AND METHODS

Microorganism and Its Maintenance

The microorganism, *Rhodobacter capsulatus* used in this study was obtained from D-Environmental and Biotechnology Corporation (Youngin City, Kyongki-Do, Korea). The cell producing a red pigment was maintained on a solid agar plate which contained: 2 g/L of KNO_3; 1 g/L of malic acid; 2 g/L of casamino acid; 3 g/L of yeast extract; 1 mL/L of vitamin solution; 1 mL/L of mineral solution; and 20 g/L of agar. The vitamin solution contained: 0.2 g/L of nicotinic acid; 0.4 g/L of thiamine-HCl; 0.2 g/L of nicotinamide; and 0.008 g/L of biotin. The mineral solution contained: 3 g/L of $FeSO_4 \cdot 7H_2O$; 0.01 g/L of H_3BO_3; 0.01 g/L of $Na_2MoO_4 \cdot 2H_2O$; 0.02 g/L of $MnSO_4 \cdot H_2O$; 0.01 g/L of $CuSO_4 \cdot 5H_2O$; 0.01 g/L of $ZnSO_4$; and 0.5 g/L of ethylenediamine tetraacetic acid. The pH of the medium was adjusted to 7.2 before autoclaving, and the medium was sterilized at 121°C for 15 min. The photosynthetic bacterium was regularly checked under a microscope in order to eliminate any possibility of contamination, and transferred to a fresh agar plate every month.

Cell Immobilization

The photosynthetic bacteria were harvested in the late exponential phase of growth by centrifuge at 7,000 rpm for 15 min. The pellet was washed and resuspended in sterile distilled water (DW), and the resulting dense cell suspension was used for cell immobilization. The cell was immobilized in phosphorylated PVA gel beads according to the method of Chen et al.[18] A mixture containing concentrated cell of 200 mg/mL was thoroughly mixed with an equal volume of PVA (18% w/v; Kuraray PVA-HC, Kuraray Co. Ltd., Osaka, Japan). This cell-PVA mixture was dropped into a saturated solution of boric acid through the hole of a needle and gently stirred for 1 hr to form spherical beads. The formed labile beads were then transferred to a 0.5 M sodium phosphate solution for 1 hr for complete gelation by esterification of PVA with phosphate. The subsequent beads of 7 mm diameter were washed with sterile DW. The speci-

fic gravity of beads was approximated to 1.07.

Denitrifying Ability of Free and Immobilized Cells

The denitrifying ability of photosynthetic bacterium, *Rhodobacter capsulatus* was examined in a tightly sealed four-neck flask of 300mL in duplicate. The dissolved oxygen (DO) probe with a thermometric sensor, the oxidation-reduction potential (ORP) probe and the pH probe were inserted in three necks of the flask, respectively. A screw cap with the septum was equipped on the other neck of the flask for gas analysis. A sampling port was set up at the same neck in which the pH probe was inserted. To test denitrifying ability of the photosynthetic bacterium, the cells harvested at the end of the exponential growth phase were inoculated under an aseptic condition with approximately 2.44 g (wet weight basis) of the cells into the culture medium containing 0.3 g KNO_3, 0.07 g malic acid, 0.14 g casamino acid, 0.21 g yeast extract, 1 mL vitamin solution and 1 mL mineral solution per liter of DW (initial pH 7.2). The characteristics of the synthetic medium are presented in Table 1. Except for the space that DO, ORP and pH probes occupied, the remaining space in the flask was filled full with the culture medium in order to provide an anaerobic condition inside at the beginning (working volume, 290 mL). For the experiments of immobilized cells, gel beads were suspended in the four-neck flask with 246.5 mL of the same culture medium. The packing ratio of beads was 15% (equivalent volume of 43.5 mL), and the amount of cells immobilized in the beads was the same as that of free cells. The flask prepared in this way was executed in a hot-stirring bath system (Eyela, Japan) and maintained at 30±0.2°C. The Variomag Tele-system (H+P Labortechnik AG, Germany) was equipped under the bath system in order to get adequate mixing inside the flask.

During denitrification reaction in the four-neck flask, the values of DO, pH and ORP were obtained by real-time measurement. The gas

Table 1. Characteristics of the synthetic medium

Constituents	Concentration (mg/L)
COD_{Cr}	450
BOD_5	335
TKN	37
NH_4^+-N	0
NO_3^--N	45
NO_2^--N	0
TN	81
TP	4

produced by the photosynthetic bacterium was sampled through the septum by the use of a Hamilton gastight syringe for gas analysis. At the same time, liquid broth was sampled from the flask by a peristaltic pump using Tygon tubing and the concentrations of nitrate, nitrite and ammonium ions were analyzed. Except for sampling time, the sampling tubing whose one end was immersed in liquid broth inside the flask was clamped and the other end was immersed in 95% ethyl alcohol always. From the second sampling, approximately 5 mL- broth that was remained in the tubing after sampling was wasted in order to obtain the broth present in the flask. The ability of denitrification by the photosynthetic bacterium was verified by measuring both N_2 gas production and nitrate reduction.

Water-purification System

Figure 1 shows the schematic diagram of a water-purification system used for this experiment. Goldfish breeding was performed using 11.7 L- mixed water of DW and tap water (ratio of 3:1) in an acryl aquarium equipped with a separate circulated filter column (packed-ed type biofilter), and air was supplied at 1 L/min. The filter column (with diameter of 10 cm and height of 30 cm) was held by a stand, and water was filled up to 70% (v/v) of the column. The rest space remained empty for gas collection. In the filter column, 5.67 g (wet weight basis) of gel beads were packed with packing ratio of 30% per the total volume of the column. A peristaltic pump (with a flow rate of 20 mL/min) circulated the water purified by

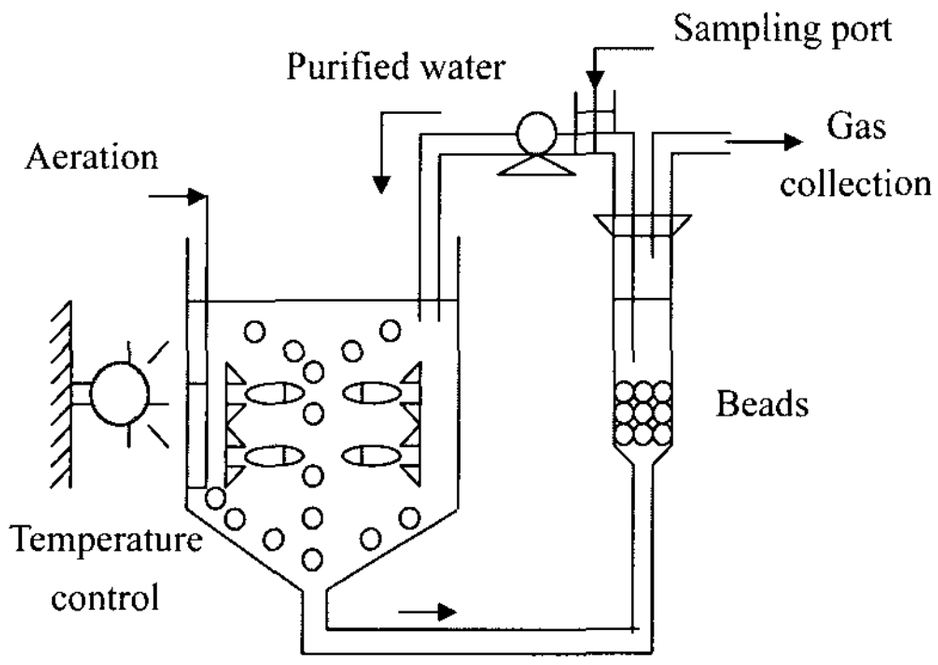

Figure 1. A water-purification system using immobilized photosynthetic bacteria.

the immobilized photosynthetic bacteria in the filter column back to aquarium. To prevent the decrease in water levels of aquarium and column from sampling and water evaporation, the mixed water of 250 mL was supplemented for the deficiency when sampling was carried out. Seven goldfish of approximately 5 cm in length were bred in this aquarium and were fed with three granules of a commercial feed (Sera Goldy Royal, Germany) per fish per day. When any goldfish died, the same number of new goldfish was supplemented into the aquarium tank to maintain the same density of breeding. The temperature of aquarium was controlled at 30±3°C by the use of incandescent bulbs. The number of viable cells that were present in the aquarium tank and the filter column were measured, and the cell size, motility and morphology were determined microscopically. Two aquariums in the same type were conducted in duplicate for three months: one was control and the other was packed with 5.67 g respective gel beads. When the experiment was terminated, the denitrifying ability of sludge produced in the filter column and PVA beads used during the experimental period was examined in 50 mL-syringes that served as the reaction vessel, together with the denitrifying ability of free cell as the control. The syringe was prepared as follows: Tygon tubing was put on each syringe needle and clamped at its end, and then the syringes were autoclaved. Sludge, PVA beads and free cells were suspended in the sterile syringes with the same amounts of cells. The denitrifying ability was tested against various types of water (aquarium water, column water and the culture medium) with the volume of 30 mL under an aseptic condition. The aquarium water and the column water were those left in aquarium and filter column after the long-term experiment, respectively, and the culture medium was the same one as that used in the previous tests of denitrifying ability. The syringes prepared in this way were incubated in a shaking incubator at 30°C and 150 rpm. The gas produced in the syringe during incubation was sampled through the Tygon tubing and analyzed by gas chromatography (GC).

Analytical Methods

The concentrations of nitrite, nitrate, phosphorus and ammonium ions were estimated by ion chromatography (Metrohm 792 Basic IC, Switzerland). The columns used in these analyses were Metrosep Supp 5-150 (150×4.0 mm) and Metrosep C2-150 (150×4.0 mm) for anion and cation, respectively. Chemical oxygen demand (COD), total nitrogen (TN) and total phosphorus (TP) concentrations were analyzed by the Water-quality Analyzer (Humas Co., Ltd, Korea). The 5 days biological oxygen demand (BOD_5) and total Kjeldahl nitrogen (TKN) was analyzed by the OxiDirect BOD-System (Lovibond, Germany) and by the 2100 Kjeltec system (Foss, Sweden), respectively. With a proper dilution, the numbers of viable cells sampled from the aquarium water and the column water were measured by counting colonies formed on the plate of the culture medium containing 2.0% (w/v) agar. The dry-cell weight (DCW) was determined by weighing the cell pellet after being dried in an oven at 100°C for 12 hrs. The cell pellet was prepared by centrifuging a 20 mL sample of broth culture at 5,000 rpm for 10 min and then by decanting the supernatant after washing twice with distilled water.

For determination of nitrogen, 20 $\mu\ell$ samples (injection volume) were taken by a Hamilton gastight syringe for GC/TCD (Perkin Elmer

Instruments, USA) analysis. The carrier gas was helium at a flow rate of 20 mL/min. The column used was a 'molecular sieve 5A' (stainless steel, mesh 80/100, 6 ft×1/8 in). The column and detector temperatures were 70 and 120°C, respectively. The amount of nitrogen was calculated by applying the ideal gas law. All measurements were performed in two replicates.

Statistical Analyses

Statistical analyses were done with measurements obtained from this study. Since the sample observations were not arranged in a frequency distribution, the standard deviations were calculated by the following procedures: each deviation was squared, the sum of the squares was divided by (n-1), one less than the sample size (n), (this resulted in the sample variance) and finally extraction of the square root recovered the original scale of measurement. Comparisons of means were performed by the Tukey method[19] using the SAS program, since all sample sizes were equal. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Nitrogen Removal by Free Cell

The characteristic of nitrogen removal by the free cell of *R. capsulatus* was investigated in a 300 mL four-neck flask with 6 g (wet weight basis) of cells grown at late-log phase. The results are presented in Figure 2. As shown in Figure 2(a), pH increased gradually for first three hours and then reduced slowly and finally to 6.85. DO decreased abruptly and almost used

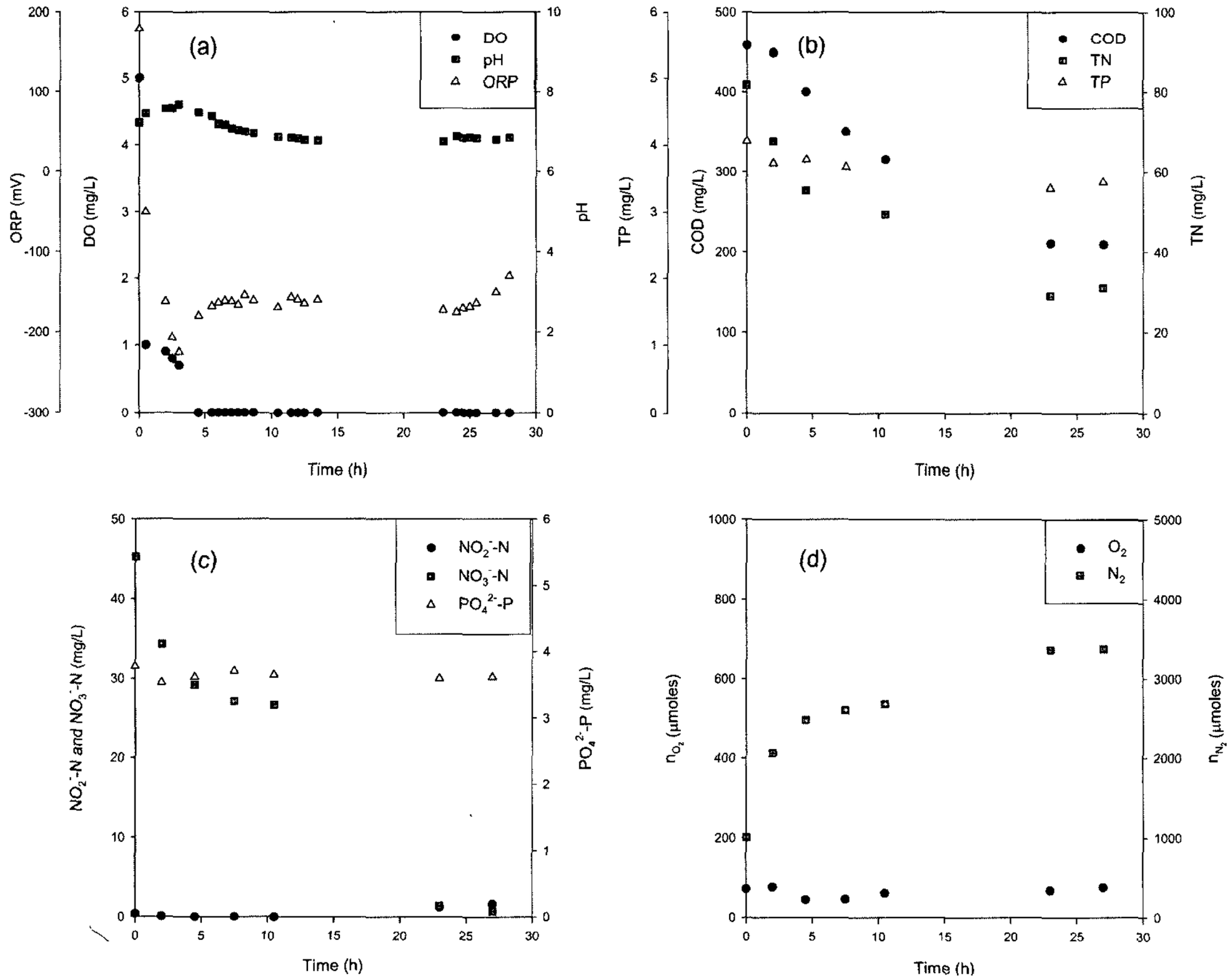

Figure 2. Changes of various parameters in nitrate removal by free cell photosynthetic bacteria. (a) DO, pH and ORP; (b) COD, TN, and TP; (c) NO_2^--N, NO_3^--N and PO_4^{2-}-P; and (d) O_2 and N_2.

up within 4.5 hrs. ORP that showed 180 mV at the beginning reduced to -225 mV within 3 hrs, then increased to -150 mV, and finally stayed at approximately -130 mV. These results suggested that denitrification by the free cell of *R. capsulatus* resulted in reduction of ORP with the rapid depletion of DO and the increase of pH.

COD_{Cr} decreased gradually and its removal for 10.5 hrs was 143 mg/L (Figure 2(b)). It removed further up to 249 mg/L after 23 hrs. The removal efficiency of COD_{Cr} was 54.4%. It has been known that microorganisms use the carbon source as an electron donor in denitrification for energy production and cell synthesis.[20] During the experiment, the increase of DCW was 0.21 g. It is reasonable because the specific growth rate of *R. capsulatus* found to be 0.04 hr^{-1} (data not shown). Therefore, the carbon source was used for energy production, not much for cell synthesis in this experiment. The concentration of TN removed was 52.6 mg/L, and the small amount (0.7 mg/L) of TP removed. In Figure 2(c), changes of concentrations of NO_2^--N, NO_3^--N and PO_4^{2-}-P were seen. Nitrite was almost not accumulated in this experiment, and 44.6 mg/L of NO_3^--N was removed with the maximum removal rate of 5.5 mg/L/hr. From these results, the organic nitrogen utilized in this experiment was calculated to be approximately 8 mg/L. This much difference was also obtained by the measurement TKN for initial and final concentrations. Consequently, the removal efficiency of TN was 64.9% with low cell growth and that of NO_3^--N was 99.1% with active denitrification. The removal of PO_4^{2-}-P was measured to be very small (0.2 mg/L). Probably, it was due to low production of biomass, since it has known that the biomass contains 2 to 3% P in its dry weight normally under an anaerobic condition.[20] The N_2 gas produced on this occasion was 2,365 μmoles (Figure 2(d)).

Nitrogen Removal by Immobilized Cell

The characteristic of nitrogen removal by the immobilized cell of *R. capsulatus* was also investigated in a 300mL four-neck flask with the same amounts of cells that used in the experiment of free cell. The results are presented in Figure 3. As shown in Figure 3(a), pH increased gradually for first 3 hrs, then fluctuated a little bit and reduced finally to 7.0. However, the profile of pH was almost the same as that obtained in the experiment of free cell. DO decreased abruptly and used up almost within 3.5 hrs, which was similar to the DO profile of free cell. ORP showed 177 mV at the beginning, and reduced to -204 mV up to 12.5 hrs. After then, ORP increased to -135 mV and finally stayed at this value. These results suggested that denitrification by the immobilized cell of *R. capsulatus* resulted in reduction of ORP with the rapid depletion of DO and the increase of pH. However, the decrease rate of ORP was lower, compared to that of free cell. Probably, this difference resulted from the diffusion problem in immobilized cell system.[21]

As seen in Figure 3(b), COD_{Cr} decreased gradually and its removal for 10 hrs was 277 mg/L and then no further removal was shown. The removal efficiency of COD_{Cr} was 59.3 %, which was almost the same as that of the free *R. capsulatus* cell. The concentration of TN removed was 45.2 mg/L, and the removal of TP was measured to be very small (0.7 mg/L). Changes of concentrations of NO_2^--N, NO_3^--N and PO_4^{2-}-P were seen in Figure 3(c). Nitrite was almost not accumulated in this experiment. The suppression of denitrification by PVA immobilization of cells has been reported, which results in accumulation of nitrite.[22] However, that kind of phenomenon was not found in this study. The NO_3^--N was removed by 44.7 mg/L with the maximum removal rate of 5.7 mg/L/hr. Therefore, the calculated utilization of organic nitrogen was only 0.5 mg/L. This result suggested that the cells immobilized inside of PVA gel did not utilize the organic nitrogen for their cell synthesis and thus biomass production was lower than the free *R. capsulatus* cell. The removal of TN was 54.9% and that of NO_3^--N was 99.3%. The removal of PO_4^{2-}-P was mea-

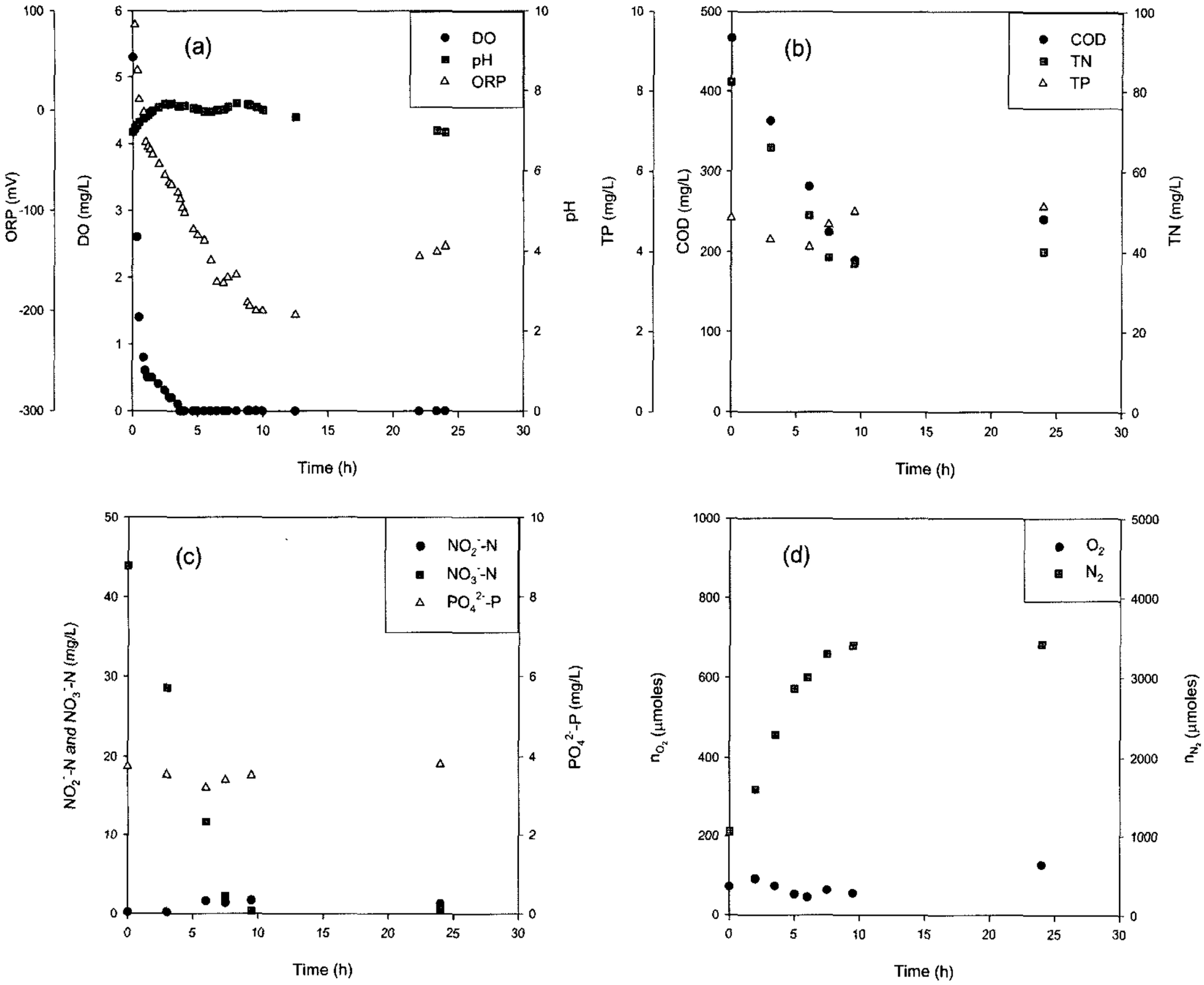

Figure 3. Changes of various parameters in nitrate removal by immobilized photosynthetic bacteria. (a) DO, pH and ORP; (b) COD, TN, and TP; (c) NO_2^--N, NO_3^--N and PO_4^{2-}-P; and (d) O_2 and N_2.

sured to be very small (0.5 mg/L), and the N_2 gas produced on this occasion was 2,370 μmoles (Figure 2(d)).

Application of the Water-purification System

To test the removal ability of nitrogenous compounds by a water-purification system, the long-term experiment was observed for three months. After two weeks of experiment, sludge started to appear apparently in the column in which immobilized PVA beads were packed. Around the gel beads, organic matter such as feces of goldfish and microorganisms in the water was accumulated gradually like sediment mud. The accumulation of sludge on the gel beads probably influenced the diffusion of substrate into the beads and the diffusion of product out of the beads as well. The organic matter was probably decomposed to volatile fatty acids or other organic acids by microorganisms present in the aquarium.[22] The amount of the sludge produced for three months was 2.41 g (dry weight base). Attachment of bacteria on the glass wall of aquarium was also observed after three weeks of experiment and it was thickened slowly as the experiment proceeded.

The average values for parameters of the system performance were compared between the control and the water-purification system. The results are presented in Table 2. From each sample, nine different types of colonies appeared on the agar plate after two days incubation at 30°C with the dilution of 10^6. The total cell numbers in the control and water-purification system were not significantly different except for that in column water of the control. Since water

Table 2. Comparison of water quality between the control and the water-purification system using immobilized *R. capsulatus* during the long-term experiment[1]

Measurement	Control		With immobilized *R. capsulatus*	
	Aquarium water	Column water	Aquarium water	Column water
Number of cells (CFU/mL)	$[(1.8\pm2.5)\times10^6]^b$	$[(8.1\pm19.9)\times10^6]^a$	$[(1.3\pm1.6)\times10^6]^b$	$[(2.3\pm2.1)\times10^6]^b$
pH	7.93 ± 0.39^a	7.87 ± 0.65^a	7.94 ± 0.54^a	7.86 ± 0.53^a
PO_4^{2-}-P (mg/L)	6.83 ± 4.56^{ab}	7.09 ± 4.88^a	3.45 ± 2.92^b	4.35 ± 2.34^{ab}
NH_4^+-N (mg/L)	4.63 ± 3.69^{ab}	6.02 ± 3.28^a	3.23 ± 3.67^b	4.03 ± 3.47^{ab}
NO_2^--N (mg/L)	0.27 ± 0.08^a	0.26 ± 0.09^a	0.24 ± 0.08^a	0.30 ± 0.10^a
NO_3^--N (mg/L)	1.88 ± 0.98^b	2.52 ± 1.08^{ab}	2.70 ± 0.92^a	2.84 ± 0.75^a

[1]Means with different superscript are significantly different ($P < 0.05$). Values represent mean±S.D. of two replicates.

including waste solids in the bottom of the aquarium had to be circulated into the column by the peristaltic pump, sludge was accumulated gradually in the column as the experiment proceeded. Thus, this resulted in higher number of cells in the column, and the phenomenon was distinct in the control. However, the phenomenon was not distinct in water-purification system probably due to the activity of photosynthetic bacteria.[22)]

The pHs of the both systems changed in a range of 7 to 9 during the experiment, but the average value of pH in each sample was not significantly different. The small variance of pH could be resulted from the effect of dilution by supplement of water after sampling. The concentration of PO_4^{2-}-P in each system was little high for approximately two weeks, but it reduced after then. The average concentration of PO_4^{2-}-P in water-purification system was relatively low, compared to that in the control. This reflected that photosynthetic bacteria could utilize some phosphorous compounds together with carbon and nitrogenous compounds simultaneously.[17)] The concentrations of nitrogenous compounds fluctuated during the long-term experiment. The concentration of NH_4^+-N was higher than those of NO_2^--N and NO_3^--N in all cases, since the majority of nitrogenous compounds released from microbial breakdown of uneaten feed and feces was ammonium-N.[23)] The concentration of NH_4^+-N in the water-purification system was somewhat reduced and maintained low after 55 days of the experiment, and the average concentration of NH_4^+-N in the water-purification system was relatively lower than that in the control. This result suggested that ammonium-N in aquarium was degraded by the immobilized photosynthetic bacteria. In all cases of the experiments, the concentrations of NO_2^--N were observed to be very low, while the concentrations of NO_3^--N were a little high. From these results, it was presumed that the metabolism of ammonium-N conversion to N_2 by *R. capsulatus* used in this study was fast, and the occurrence of NO_3^--N could happen due to natural nitrification by microorganisms present in the aquarium. It has reported that PVA could affect the rate of nitrate respiration due to the partial inhibition of nitrite reductase and this resulted in accumulation of nitrite as an intermediate product of nitrogen reduction.[22)] However, the inhibition of nitrite reductase by PVA was not found with *R. capsulatus*.

Although the accumulation of sludge on the gel beads probably caused the diffusion problem, the gel beads somewhat actively removed nitrogenous compounds during the long-term experiment. In Figure 4 and 5, changes of concentrations of the nitrogenous compounds in the both systems were plotted. It was observed that goldfish died at 11^{th}, 16^{th}, 43^{rd}, and 67^{th} days in the control, while goldfish died at 10^{th}, 20^{th}, and 39^{th} days in the water-purification system. Coincidentally, the total concentrations of nitrogenous compounds on the days of goldfish's death were

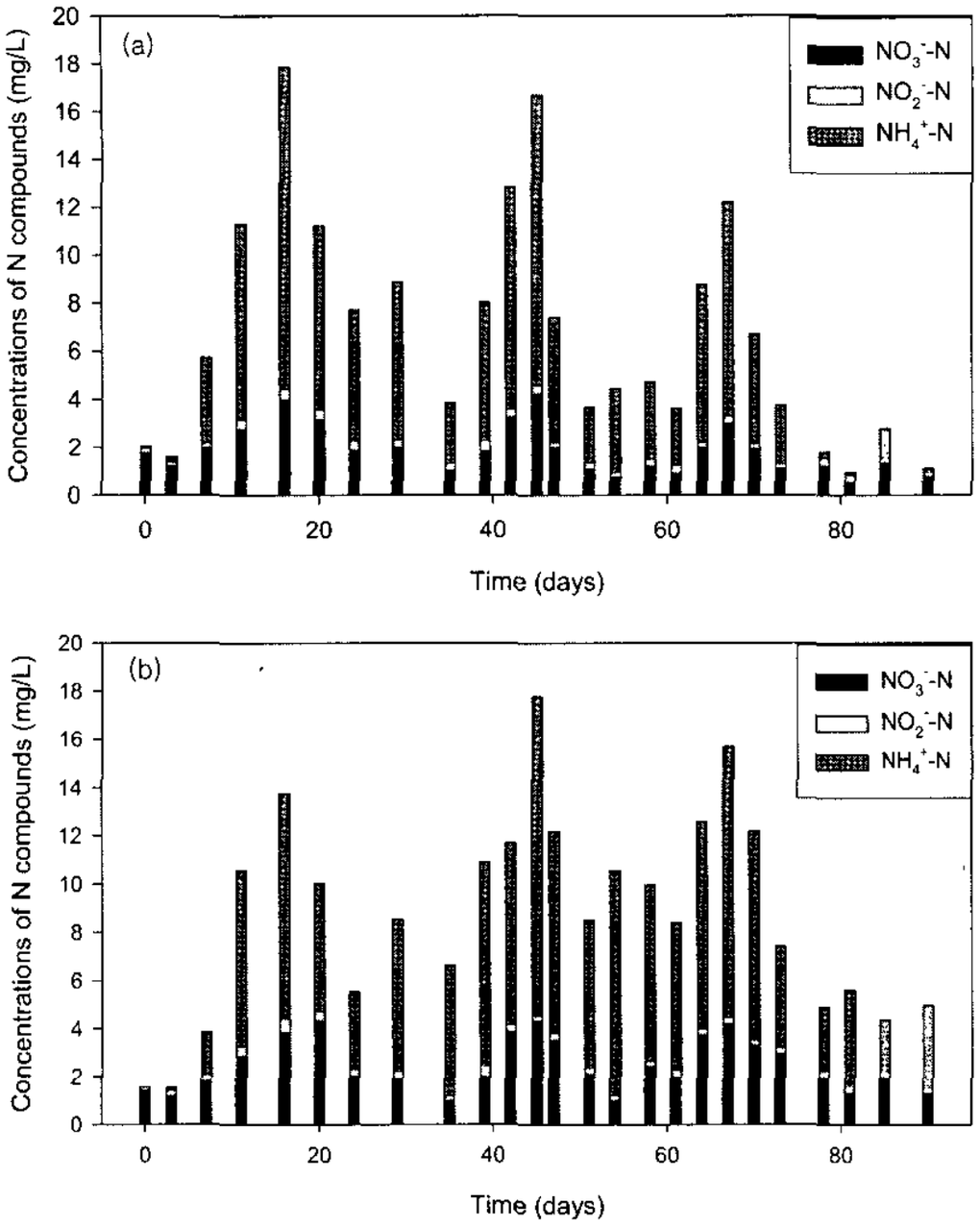

Figure 4. Changes of concentrations of nitrogenous compounds in aquarium water (a) and in column water (b) in the control during the long-term experiment.

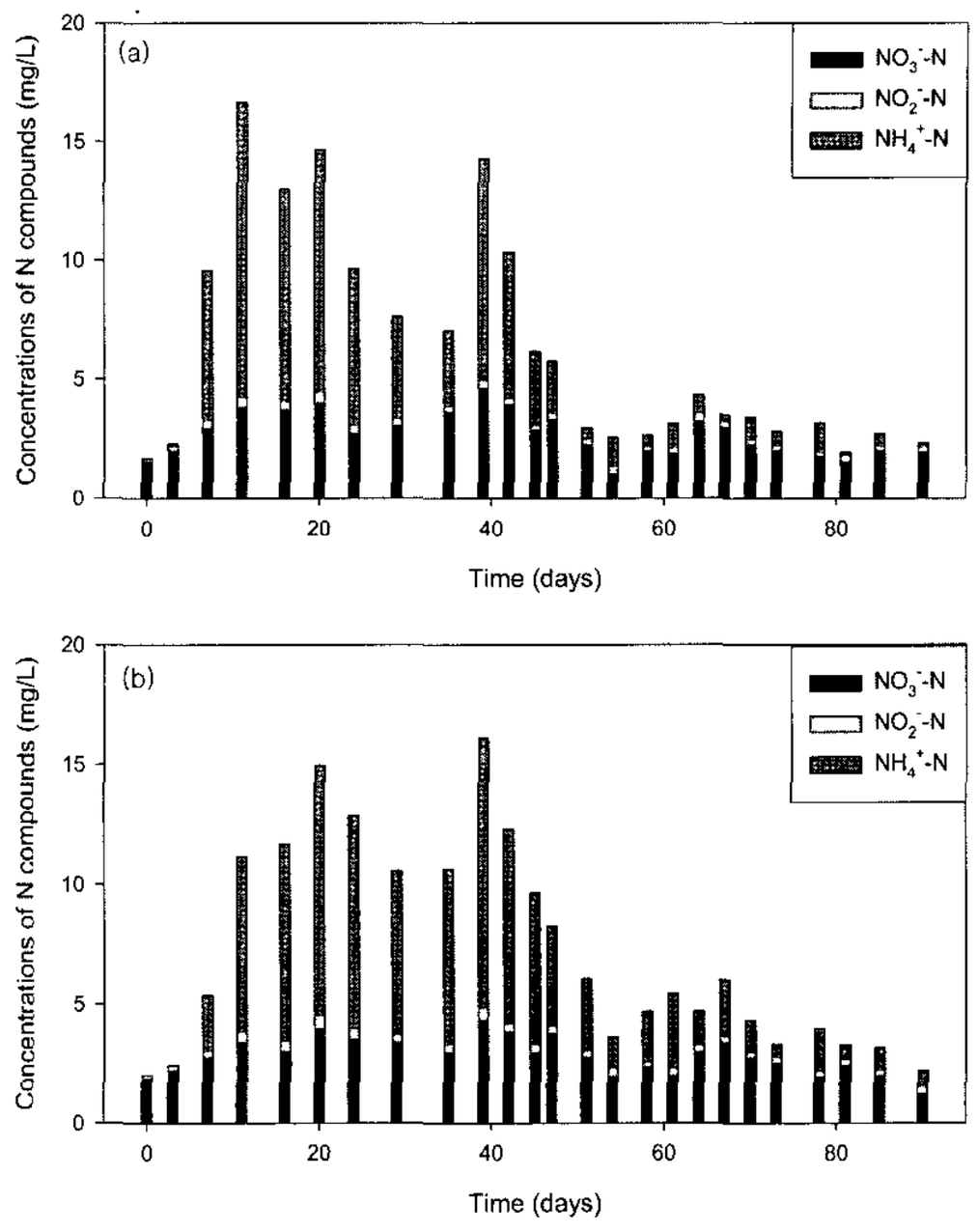

Figure 5. Changes of concentrations of nitrogenous compounds in aquarium water (a) and in column water (b) in the water-purification system during the long-term experiment.

higher than those on the other days of the experiment. This implied that the total concentration of nitrogenous compounds influenced the death of goldfish. In the control, the total concentrations of nitrogenous compounds were measured to be 11.3, 17.9, 16.7 and 12.2 mg/L in aquarium water and 10.6, 13.8, 17.8 and 15.7 mg/L in column water on 11^{th}, 16^{th}, 43^{rd}, and 67^{th} days, respectively. In the water-purification system, the total concentrations of nitrogenous compounds were measured to be 16.7, 14.8, and 14.3 mg/L in aquarium water and 11.2, 15.0, 17.8 and 16.2 mg/L in column water on 10^{th}, 20^{th}, and 39^{th} days, respectively. Especially in all the cases, the concentrations of NH_4^+-N on the days of goldfish's death were found to be in a range of 7.4 to 13.5 mg/L, which were significantly higher than the average values reported in Table 2. This implied that high concentrations of NH_4^+-N could cause the death of goldfish.[24] After 80 days of the experiment, approximately half of the beads floated up by 5 cm, and this made beads (packed in the column) separate into two groups. This phenomenon took place because N_2 gas produced inside the beads could not squeezed out,[25] probably due to diffusion problem by the accumulation of sludge on the beads. The result suggested that the denitrification by immobilized *R. capsulatus* took place inside PVA beads. During the long-term experiment, the water-purification system also showed the less turbidity of water with more active movement of goldfish than the control. As a result, the better survival of goldfish could be shown in the water-purification system.

Denitrifying Ability of Sludge and PVA Beads

After the long-term experiment for an application of the water-purification system, the denitrifying ability of sludge produced in column water and PVA beads used during the experimental period was examined on three different types of water, together with the denitrifying

ability of free cell as the control. The result is tabulated in Table 3. PVA beads on column water produced a bubble with a diameter of 6.0 mm after 3 days incubation at 30°C, whereas few very small bubbles were evolved on aquarium water. The volume of gas produced on the culture medium by the PVA beads was 1.0 mL on average, and it was found from the GC analysis that the composition of N_2 in the gas was in a range of 93-98%. The different results were obtained among three different types of water, since the concentration of NO_3^--N was present differently in each type of water. As reported in Table 3, the denitrifying ability of PVA beads was almost the same as that of free cell. Besides, the theoretical maximum volume of N_2 gas is estimated to be 1.11 mL from stoichiometric equation for conversion of NO_3^- to N_2. Therefore, this result indicates that the denitrifying ability of the PVA beads was able to be durable for three months without any significant loss of activity. This is not surprising because PVA gel has been reported to be successfully applied to the immobilization of denitrifying photosynthetic bacteria in the removal of nitrogenous compounds due to its mechanical stability.[4,14-16] Meanwhile, a small bubble with a diameter of 2.0 mm was evolved on the culture medium by the sludge. This suggested that natural purification could take place in aquarium more or less. Consequently, it is proved that the PVA beads packed in the water-purification system had been working on the removal of nitrogenous compounds during the long-term experiment, which resulted in lower concentrations of nitrogenous compounds in aquarium water than those of control.

CONCLUSIONS

The characteristics of nitrogen removal by the free cell and the immobilized cell of *R. capsulatus* were investigated. Denitrification by *R. capsulatus* cells resulted in reduction of ORP with the rapid depletion of DO and the increase of pH. The removal efficiencies of COD_{Cr} for the free cell and the immobilized cell were 54.4 and 59.3 %, respectively. Without accumulation of nitrite, the removal efficiencies of TN and NO_3^--N for the free cell and the immobilized cell were 64.9 and 54.9 % (TN) and 99.1 and 99.3% (NO_3^--N), respectively. The removal of P was very small with low production of biomass.

Goldfish breeding was performed in an aquarium equipped with a water-purification biofilter for three months. After two weeks of experiment, organic matter was accumulated gradually around the PVA beads. Nine different cells were present in the system, but the total cell numbers in the control and water-purification system were not significantly different except for that in column water of the

Table 3. Results of gas volume evolved in the syringe after 3 days incubation at 30°C[1]

Source	Type of water used in the experiment		
	Aquarium water[5]	Column water[5]	Culture medium
Sludge[2]	Not detectable	Few very small bubbles	A small bubble (D= 2.0±0 mm)
PVA beads[3]	Few very small bubbles	A bubble (D= 6.0±0.1 mm)	1.0±0.2 mL
Free cell[4]	Few very small bubbles	A bubble (D= 6.0±0.1 mm)	1.1±0.1 mL

[1]Values represent mean±S.D. of two replicates.
[2]produced in the column during the experimental period, with the same wet weight as that of cells used in the experiment of PVA beads.
[3]used in the column during the experimental period.
[4]suspended photosynthetic bacterium *Rhodobacter capsulatum*, with the same amount of cell as that used in the experiment of PVA beads.
[5]Water in aquarium and column that were left after the long-term experiment, respectively.

control. The pHs of the both systems changed in a range of 7 to 9 during the experiment, but the average value of pH in each sample was not significantly different. The average concentrations of NH_4^+-N and PO_4^{2-}-P in water-purification system were relatively low, compared to that in the control. The concentrations of NO_2^--N were observed to be very low, while the concentrations of NO_3^--N were a little high. Goldfish died at 11th, 16th, 43rd, and 67th days in the control, while goldfish died at 10th, 20th, and 39th days in the water-purification system. Coincidentally, the total concentrations of nitrogenous compounds on the days of goldfish's death were higher than those on the other days of the experiment, especially with the concentrations of NH_4^+-N ranging from 7.4 to 13.5 mg/L. The water-purification system also showed the less turbidity of water with more active movement of goldfish than the control. As a result, the water-purification system was effective to remove nitrogenous compounds with better survival of goldfish.

Even after the long-term experiment, the PVA gel beads showed almost the full denitrifying ability with the composition of 93-98% N_2. Therefore, the denitrifying ability of the PVA beads was able to be durable for three months without any significant loss of activity. Consequently, it is proved that the PVA beads packed in the water-purification system had been working on the removal of nitrogenous compounds during the long-term experiment, which resulted in lower concentrations of nitrogenous compounds in aquarium water than those of control.

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