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Augmentation of Self-Purification Capacity of Sewer Pipe by Immobilizing Microbes on the Pipe Surface

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Abstract

For the development of a sewer pipe that removes dissolved organic carbon and nitrogen, a rectangular channel with a fabricated porous ceramic bed was investigated. The experimental channel was 1 m long, 2.0×10^{-2} m wide, and 2.5×10^{-2} m deep, the total volume being 0.81 l. Blocks of the ceramic bed with 2.0×10^{-2} m^2 total surface area and 5.0×10^{-3} m thickness were installed along the channel bed for the immobilization of microorganisms. Synthetic wastewater was used as the model sewage. Removal of organic carbon and nitrogen was carried out with or without aeration. Dissolved oxygen and pH microelectrodes were employed to analyze structure of the biofilm, which played an important role in the removal of both organic carbon and nitrogen.

The results give evidence that the combination of aeration and biofilm development enhanced the simultaneous removal of organic carbon and nitrogen. Microelectrode study revealed that the biofilm was rough and heterogeneous in both vertical and horizontal directions and had an average thickness of 2.5-5 mm. The biofilm, consisting of an aerobic/anaerobic layer, was suggested to be responsible for the nitrification/denitrification process, while the aeration accelerated the removal of TOC, NH_4 or NO_2 from the model sewage. Sequential nitrification/denitrification proceeded in the biofilm even when aeration was carried out. This study suggests that the immobilization of microbes inside the sewer pipe may be effective for the simultaneous removal of organic carbon and nitrogen in the sewage line.

1. Introduction

Sewer pipe is simply considered to be a facility for transporting sewage from the source to the wastewater treatment plant. To meet the demand for reducing the sewage load on the wastewater treatment plant, the idea that the sewer pipe itself participates in the removal of organic load during transportation is proposed. Sewage qualities such as Biological Oxygen Demand (BOD) or Chemical Oxygen Demand (COD) change according to the flow of sewage through the sewer pipe have been reported [5,6]. For instance, 14% dissolved organic carbon (DOC) in the sewage is decreased through a 1.5 km flow in a sanitary gravity sewer pipe for 18 min, and it was estimated that the sewage liquid phase contributes 40% of the DOC removal while the sewage sediment contributes 60% of it [3,4]. By extending this observation, the increase of the self-purification capacity of the sewer pipe is expected. For the efficient biological conversion of sewage, the substrate to microbe concentration ratio is important [7]. Even though the concentration of the substrate in the sewage is relatively high, the concentration of microbes in the sewer pipe is not high as is. Therefore, for the rapid biological conversion of sewage organic substances, it is necessary to increase the microbial population in the pipe. Given such a background, the immobilization of microbes to the inner surface of the sewer pipe was proposed in the present study.

The removal of BOD or COD, and the oxidation of nitrogen substances generally occur under aerobic conditions, while anaerobic conditions are needed for denitrification. The formation of biofilm or the immobilization of microbes provides both aerobic and

anaerobic regions in an adjoining space [2,8]. Aerobic bacteria growing in the region near the aerobic bulk liquid consume dissolved oxygen (DO) and create an anaerobic region inside the biofilm or the immobilizing carrier. Therefore, the formation of the biofilm in the sewer pipe may contribute to both BOD and nitrogen removal from the sewage.

In the present study, porous ceramic material was installed for the immobilization of microbes. Oxygen was used as an electron acceptor. The integration of the immobilization technique and oxygen supply enables the removal of both dissolved organic carbon and nitrogen (self-purification) from the sewer pipe. Enhancement of self-purification capacity of sewer pipe may contribute to reduce the sewage load on the wastewater treatment plant.

2. Materials and methods

2.1. Operational conditions of the channel

The rectangular channel was 1 m long, 2.5×10^{-2} m deep and 2.0×10^{-2} m wide and had a total volume of 0.81 l. The configuration of the channel is depicted in Fig.1.A. Fifty blocks of fabricated porous ceramic with 2.0×10^{-2} m^2 total surface area and 5×10^{-3} m thickness were installed along the channel for immobilizing microorganisms. The porous ceramic bed has 71.5% void volume, and various pore diameters of less than 10 μm (16.8%), 10-20 μm (12.2%), 20-50 μm (44.2%), and 50-90 μm (26.8%), which were analyzed by the BET method. The channel was operated under the batch system with complete circulation of medium of 0.36 l. Reynolds number (*Re*) of the operational channel was calculated using Eq.(1).

$$Re = \frac{De.\ \upsilon\ .\ \rho}{\mu} \quad (1)$$

where υ is linear velocity (m sec^{-1}), ρ is density of water (kg m^{-3}), μ is viscosity of water (kg m^{-1} sec^{-1}), and *De* is equivalent diameter (m) which was calculated using Eq.(2) for the rectangular channel:

$$De = \frac{4(W\,H)}{(2H + W)} \quad (2)$$

where *W* is the width of the channel (m), and *H* is the flow depth of the channel (m). Synthetic wastewater with the following composition (per liter): 250 mg glucose, 74.4 mg $(NH_4)_2SO_4$, 12.6 mg KH_2PO_4, 9.5 mg $MgSO_4$, 1.2 mg $CaCl_2.2H_2O$ and 0.1 mg $FeCl_3.6H_2O$ was used and circulated using a peristaltic pump. For biofilm development, activated sludge obtained from a domestic wastewater treatment facility was used as the seed culture. Half of the circulating medium was exchanged with fresh synthetic wastewater daily.

The dissolved oxygen (DO) concentration of the medium was monitored continuously using a DO sensor (OBS Digital DO controller, Tokyo, Japan). Total organic carbon (TOC) in the medium was analyzed using a TOC analyzer (Shimadzu TOC-5000A, Shimadzu Co., Ltd., Tokyo, Japan), and nitrogen compounds were analyzed by ion chromatography (Shimadzu CTO-10A, Shimadzu Co., Ltd., Tokyo, Japan).

2.2. Oxygen transfer coefficient of the system

To analyze the oxygen supply rate through the wall of the channel, the channel was completely filled with water and covered with a plastic sheet (non-open channel), and the

DO concentration change in the water was analyzed. Oxygen supply rate through the free surface of the water was analyzed by measuring DO concentration change of the circulating water without covering the channel (open channel). Oxygen supply rate through the sparger under an air supply of 3.6 l h^{-1} was also analyzed. The measurements were conducted using running water without immobilizing microorganisms. The linear velocity of water was adjusted to 5.3 m min^{-1}, which gave a laminar flow condition of *Re*=1550. Aeration of nitrogen gas in the medium realized a DO=0 condition. Then, the rise of the DO concentration was monitored to examine the oxygen supply rate under each condition.

2.3. Oxygen consumption rate by immobilized microorganisms

DO concentration decrease in the non-open channel condition was used for the measurement of the activity of immobilized microorganisms during the acclimation period. Under this condition, the O_2 supply to the medium was assumed to be zero. Therefore, the decrease of DO concentration of the medium was due to exhaustion of O_2 by the immobilized microorganisms. The activity of the immobilized microorganisms was measured weekly during the acclimation period of 2 months.

2.4. Total O_2 consumption

Total O_2 consumption rate was calculated from the slope of DO concentration decreasing from the saturated condition after circulation of the medium for 20 minutes. The non-open channel flow condition was used in this measurement to eliminate DO supply through the

free surface. The measurement was conducted after biofilm grew on the porous material. Oxygen consumption rate was calculated using Eq.(3).

$$\frac{dC_{O2}}{dt} = K_L a\,(C_{O2}^{*} - C_{O2}) - r_{O2} \qquad (3)$$

where C_{O2}^{*} is saturated DO concentration, C_{O2} is DO concentration at time t, and r_{O2} is O_2 consumption rate by immobilized microorganisms (mg l^{-1} min^{-1}). When the O_2 supply through the free surface is limited, Eq.(3) can be changed into Eq.(4), in which the DO concentration decrease from the saturated condition expresses total O_2 consumption of the immobilized microorganisms.

$$\frac{dC_{O2}}{dt} = - r_{O2} \qquad (4)$$

2.5. DO concentration and pH profile in biofilm

DO concentration and pH profiles in the biofilm grown on the surface of the porous ceramic material were analyzed using microelectrodes [1]. The schematic of DO and pH microelectrode measurement is depicted in Fig.1.B. One block (2×2 cm) of fabricated porous ceramic was stripped off from the reactor after 8-week incubation and used for the experiment. DO and pH microelectrodes have 100 μm and 200 μm tip diameters, respectively. For the measurement of DO concentration and pH profiles in the biofilm, the microelectrode was moved by a micromanipulator (Shimadzu MMS-20) with a step motor that allowed a descent of 50 μm to the biofilm. Air was continuously introduced into the test vessel (Volume: 0.35 L) through the sparger. Three kinds of medium were used in this measurement. The first medium contained $(NH_4)_2SO_4$ (37.2 mg l^{-1}), equivalent to NH_4-N

(ammonia-N) 10 mg l^{-1}, as a sole nitrogen source and other components used for synthetic wastewater. This medium was used for the nitrification test. The second medium contained $NaNO_2$ (15.0 mg l^{-1}), equivalent to NO_2-N (nitrite-N) 10 mg l^{-1}, as a sole nitrogen source and other components used for synthetic wastewater. This medium was used for the denitrification test. The third medium was circulating medium in the reactor for one day. No detectable carbon and nitrogen sources were found in the third medium (data not shown). Five different points of the biofilm on the porous ceramic bed were randomly selected for the measurements of DO concentration and pH profiles. The cross-sectional image of the biofilm was visualized using a microscope connected to a digital image processor (Olympus DP 11).

Nitrification was evaluated based on the conversion of NH_4-N into NO_2-N or NO_3-N using the nitrification test medium. Denitrification activity was evaluated by using the denitrification test medium. The effects of aeration on both nitrification and denitrification tests were investigated.

3. Results and discussion

3.1. Activity of immobilized microorganisms

The activity of immobilized microorganisms, expressed by the O_2 consumption rate during the acclimation period of eight weeks, is depicted in Fig.2. During the first 4 weeks, microbial growth in thickness direction of the biofilm on the porous ceramic bed surface was observed. The thickness of the biofilm reached 2.5-5 mm after four weeks incubation

period and the biofilm covered the 2×10^{-2} m^2 surface of the porous bed. Oxygen consumption rate was estimated from the slope of the DO concentration decrease and reached 2.3×10^{-1} mg l^{-1} min^{-1} after an eight-week incubation period, after which the value remained almost constant.

3.2. Average volumetric O_2 transfer coefficient of the system

O_2 supply from the channel wall was assumed to be zero when the channel was operated under the non-open channel flow condition (Fig.3). When the channel was operated under the open channel flow condition, O_2 supply rate from the free surface of the medium was estimated to be 2.25 mg l^{-1} $min^{-1}m^{-2}$ surface area of the porous bed. When oxygen was supplied through the sparger in the buffer tank (Fig.1.A) and the free surface of the medium, the O_2 supply rate reached 8.0 mg l^{-1} min^{-1} m^{-2} surface area of the porous bed, which was 3.6 times higher than that of free surface only. This value was about 69 % of the O_2 consumption rate (11.5 mg l^{-1} min^{-1} m^{-2}) by the immobilized microorganisms in the channel. Since the O_2 consumption rate by the immobilized microorganisms was higher than the O_2 supply rate through the sparger and the free surface of the medium, an aerobic-anaerobic condition was realized in the biofilm when oxygen was supplied through free surface of the medium and the sparger.

3.3. Biofilm structure

The structure of the biofilm grown on the surface of the porous ceramic bed was

analyzed using a microscope connected to a digital image processor (Fig.4). From the cross-sectional view, the biofilm was found to be composed of four layers: the bulk water phase, the heterogeneous nondense layer that was very rough and mainly composed of filamentous microorganisms, the dense layer and the porous bed. The thickness of the biofilm (B+C layers in the Fig.4) ranged from 2.5-5 mm after a four-week incubation period.

An oxygen microelectrode mounted on a micromanipulator was used to analyze the vertical profile of DO concentration in the biofilm after an eight-week incubation period by using three different kinds of medium (Fig.5). Five sampling points for the measurements of DO concentration in the three kinds of medium are not identical. Thickness of the film was almost same as that of 4-week incubation period. Non-uniform DO concentration profiles in the biofilm were observed. DO analysis by the microelectrode was initiated from the bulk liquid and the position of the tip of microelectrode was monitored on the TV screen. The depth zero in the Figure 5 and 6 was defined as the point in which tip of the microelectrode went into the biofilm. When fresh nitrification or denitrification medium was supplied to the test vessel, a rapid decrease in DO concentration was observed in the region close to the biofilm surface (Figs.5.A and B). The observed difference in the starting point of the DO concentration decrease depending on the measurement position of the test piece may indicate the heterogeneity of the biofilm surface or the existence of a possible water channel in the biofilm. However, DO concentration in the biofilm at the five test points were almost zero at the middle of the biofilm, which was the region where aerobic

bacteria were active in assimilating carbon sources and consumed oxygen. On the other hand, when the circulating medium obtained from the channel was used in the test vessel, a gradual decrease in DO concentration was observed up to the middle of the biofilm (Fig.5.C). However, a rapid decrease in DO concentration was observed in the dense layer. Oxygen consumption in the dense layer was considered to be due to the endogenous respiration of the microbes in this layer. These data demonstrated that the DO concentration profiles in the biofilm were heterogeneous in both vertical and horizontal directions. On average, an anaerobic region was created on the porous bed surface while medium, either nutritionally rich or poor, was circulated in the channel.

After eight weeks of operation, pH change in the biofilm was analyzed using a pH microelectrode. Sampling points for the measurements of pH concentration are not identical to those of DO measurements. And five sampling points for the measurements of pH concentration in the three kinds of medium are not identical. Even though the pH of the bulk medium was controlled to 7.0, the pH decreased to below 5.0 near the biofilm surface when nitrification medium was applied to the test vessel. A slight increase in pH was observed in the dense layer. Since the DO concentration in the heterogeneous layer did not reach zero, the production of nitric acid or nitrous acid by nitrifying bacteria in this layer might decrease the pH value when NH_4-N was used as the nitrogen source (Fig.6.A). When NO_2-N was used as the nitrogen source, the pH decreased to 5.0-5.6 at the middle of the biofilm, and increased slightly in the dense layer (Fig.6.B). The pH decrease in the heterogeneous layer was considered to be due to excretion and diffusion of organic acids as

a result of anaerobic respiration. In both tests, the consumption of nitric acid or nitrous acid by denitrification in the anaerobic region on the porous bed surface might produce the slight increase in pH. When the circulating medium that was circulated in the channel for one day was applied, the pH remained almost constant in the layer above the middle of the biofilm. Then, the pH slightly decreased up to the surface of the dense layer (Fig.6.C). This phenomenon suggested that the dense biofilm was still underwent endogenous respiration even though the circulating medium was applied.

3.4. Effect of O_2 supply rate on the removal of organic carbon and nitrogen

The effects of O_2 supply rate on the removal of organic carbon and nitrogen are depicted in Fig.7. Since the linear velocity of the circulating medium in the rectangular reactor was 5.3 m min^{-1}, 180 min circulation experiment might demonstrate the change of water qualities equivalent in the 954 m (5.3 m $min^{-1} \times 180$ min) long sewer pipe. As was mentioned in 3.2, oxygen supply rate through the sparger and the free surface of the medium was 3.6 times higher than that of free surface only. In the nitrification test, when air was introduced through the sparger in the buffer tank (Fig.7.A), the DO value of the circulating medium was decreased to 2.0 mg l^{-1} within the first 15 minutes, and then increased gradually to 6.0 mg l^{-1} when organic carbon in the medium was almost completely consumed at 60 minutes of the incubation period. TOC was reduced to 5 mg l^{-1} at 75 minutes of the incubation period. However, when no aeration was applied, the DO concentration decreased to 0 mg l^{-1} within 30 minutes and an anaerobic condition was

maintained until 120 minutes of the incubation period. Then the DO concentration gradually increased to 4.0 mg l^{-1} during the rest of the incubation period (Fig.7.B). In the nitrification test, when aeration was applied, 56% of the initial NH_4-N was removed within 180 minutes. On the other hand, the value was about 38% when air was not supplied through the sparger. This finding indicated that oxygen supply might be the rate-limiting step for NH_4 removal in the nitrification test. NO_2 and NO_3 concentrations in the nitrification test were low, indicating that the conversion of NO_2 and NO_3 to nitrogen gas by denitrification might be rapid compared with that by nitrification. The slight increase in pH in the dense layer of the biofilm close to the porous bed surface suggested the conversion of NO_2 or NO_3 into N_2 gas in the dense layer (Figs.6.A and B). The nitrification reaction decelerated with decreasing O_2 and the subsequent denitrification was expected to be decelerating in the dense layer. Since the denitrification rate was higher than the nitrification rate, no intermediate product of NO_2 could be detected in the medium.

In the denitrification test, DO concentration in the medium with aeration was maintained above 1 mg l^{-1} over the 90-minute incubation period, and this was followed by a gradual increase with decreasing TOC (Fig.7.C). DO concentration in the medium without aeration decreased to zero in 15 minutes, and remained almost zero for more than 180 minutes. Oxygen supply through the sparger enhanced both TOC and NO_2 decrease. NO_3 concentration in the medium reached 1.5 mg l^{-1} and gradually increased according to the increase in DO concentration in the medium when air was supplied through the sparger. On the other hand, NO_3 concentration was below 1.0 mg l^{-1} throughout the test period when

aeration was not applied. Increasing oxygen supply to the medium was considered to promote the oxidation of NO_2 to produce NO_3, followed by the denitrification of NO_3 to produce nitrogen gas. The possible conversion of NO_2 or NO_3 to N_2 in the denitrification tests with or without aeration was also confirmed by the slight increase in pH value in the dense layer near the porous bed surface (Fig.6.B).

The nitrification/denitrification tests and the analysis of biofilm structure by DO and pH microelectrodes revealed that the formation of biofilm, which consisted of the aerobic/anaerobic region, and enhancement of oxygen supply promoted the conversion and removal of both organic carbon and nitrogen in the synthetic sewage.

4. Conclusions

To endow sewer pipes with the self-purification capacity, porous ceramic support was installed in the model sewer pipe. During the eight-week acclimation period, a biofilm with a 2.5- to 5-mm-thick biofilm was formed on the ceramic sheet. The biofilm consisted of four layers: the bulk water phase, the heterogeneous nondense layer, the dense layer and the porous bed. DO concentration and pH profiles measured by the microelectrodes revealed the structure of the biofilm. The heterogeneous nondense layer was aerobic, where both DO and TOC concentrations decreased because of biological oxidation. The pH decrease in this layer arose from the nitrification or the acid fermentation. On the other hand, the dense layer was anaerobic regardless of the medium condition responsible for the denitrification of NO_2 and NO_3. Increasing of oxygen supply through the air sparger

promoted the conversion of both NH_4-N and NO_2-N to nitrogen. These observations could be used to promote the degradation of pollutants, especially carbon- and nitrogen-containing materials, during transport in the sewer.

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KEYWORDS

Immobilization, nitrification, denitrification, microelectrode technique

5. List of symbols

C_{O2}	dissolved O_2 concentration (mg l^{-1})
C_{O2}*	saturated dissolved O_2 concentration (mg l^{-1})
K_La	volumetric O_2 transfer coefficient (min^{-1})
r_{O2}	O_2 consumption rate (mg l^{-1} min^{-1})
De	equivalent diameter (m)
W	width (m)
H	height (m)
Re	Reynolds number

Figure legends

Figure 1. A. Schematic diagram of rectangular channel. B. Schematic diagram of DO and pH microelectrode measurement,

1. Peristaltic pump, 2. Rectangular reactor, 3. Buffer tank, 4. Air sparger, 5. Effluent port, 6. DO sensor, 7. Recorder, 8. Micro volt ammeter, 9. TV screen monitor, 10. Micromanipulator, 11. Microscope, 12. Block sample with biofilm, 13. DO or pH microelectrode, 14. Air sparger, 15. Reference electrode for pH, 16. Test vessel. --- is the line for pH microelectrode measurement, — is the line for DO microelectrode measurement. The Ag/AgCl electrode was used as a reference electrode for pH measurement.

Figure 2. Oxygen consumption rate of immobilized microorganisms grown on the porous ceramic bed during acclimation period

A. DO decrease ●:0 week, ○:1st week, ▼:2nd week, ▽: 4th week, ■: 8th week. B. O_2 consumption rate

The experiment was performed under the non-open channel flow condition. O_2 supply through the free surface was estimated to be zero.

Figure 3. Oxygen supply by aeration or through free surface

●: non-open channel, volume of the circulation medium was 0.81 l, ○: open channel, volume of the circulation medium was 0.36 l, ▼: open channel with aeration.

Figure 4. Cross-sectional view of the biofilm grown on porous ceramic bed

A. Bulk phase, B. Aerobic layer composed of heterogeneous rough biofilm, C. Anaerobic

layer composed of dense biofilm, D. Porous ceramic bed.

Figure 5. DO concentration profile in the biofilm

A. Nitrification medium, B. Denitrification medium, C. Circulating medium medium (medium obtained after one-day circulation). ●: sampling point 1, ○: sampling point 2, ▼: sampling point 3, ▽: sampling point 4, ■: sampling point 5.

Figure 6. pH profile in the biofilm

A. Nitrification medium, B. Denitrification medium, C. Circulating medium (medium obtained after one-day circulation). ●: sampling point 1, ○: sampling point 2, ▼: sampling point 3, ▽: sampling point 4, ■: sampling point 5.

Figure 7. Effect of aeration on nitrification/denitrification and organic carbon removal

●: DO, ▽: NO_2, ■: NO_3, ◇: NH_4, ○: TOC. Nitrification: NH_4-N as N source, Denitrification: NO_2-N as N source. The experiment was performed under the open-channel flow condition with total medium volume of 0.36 l and *Re* =1550 (laminar flow).