SIMULTANEOUS ORGANIC CARBON REMOVAL AND NITRIFICATION BY BIOFILM FORMED ON OXYGEN ENRICHMENT MEMBRANE

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Key Words: Wastewater Treatment, Biofilm, Nitrification, Oxygen Enrichment Membrane, Nitrifier, Denitrifier, Silicone hollow fiber

Biofilm was formed on an oxygen enrichment-type support which consisted of a poly (dimethylsiloxane) hollow fiber membrane and a fibrous support woven around the fiber. Population distribution of nitrifiers and denitrifiers in the biofilm was investigated. Nitrifiers were dominant within the fibrous support while denitrifiers were dominant in the surface biofilm attached to the support. Simultaneous organic carbon removal and nitrification were carried out successfully in single-step treatment of domestic wastewater. The nitrification rate in the present biofilm was about 2.2 g/m^{2}d at an air pressure of 19.6 kPa or 29.4 kPa and was comparable to that in conventional biofilm processes designed especially for nitrification.

Introduction

Nitrogen compounds in surface water are one of the causes of eutrophication. They also cause an increase in the necessary chlorine dose in the disinfection process of potable water at water supply plants. Increases in chlorine dose increase the risk of formation of disinfection by-products. Some chlorination by-products are found to be mutagenic[14]. Nitrites can cause infant methemoglobinemia(blue baby syndrome)[20]. Nitrates may also lead to possible formation of nitrosamines which are known carcinogens[11].

Untreated or poorly-treated domestic wastewater discharged from sparsely populated areas is a major cause of carbonaceous and nitrogenous pollution of the aquatic environment. Treatment of such small-scale domestic wastewater has been increasingly significant not only for environmental protection but also for conservation of potable water resources. Therefore, nitrogen removal, as well as organic carbon removal, is required in small-scale wastewater treatment. Furthermore, both organic carbon and nitrogen removal processes should be combined compactly and efficiently in a small-scale treatment system.

Biological nitrogen removal is carried out with two successive processes; one is nitrification and the other is denitrification. Nitrification is considered to be the less reliable process so optimization of nitrification is essential for effective nitrogen removal. Nitrifiers compete with BOD oxidizers for oxygen. Nitrifiers are chemoautotrophs and their growth rates are very small compared with those of heterotrophic BOD oxidizers. Therefore, organic pollutants should be removed beforehand for successful nitrification. This requirement results in multi-stage processes in conventional attached growth systems. However, multi-stage processes seem to be disadvantageous in small-scale treatment. For single-stage simultaneous organic carbon removal and nitrification, the conditions in which nitrifiers can grow with less competition against BOD oxidizers should be realized.

Poly(dimethylsiloxane) (silicon) hollow fiber membrane can enrich the oxygen concentration in air and can be used for oxygen supply in aerobic bioprocesses[3, 6, 10]. In biofilm formed on oxygen enrichment membrane, oxygen permeated through the membrane is supplied from the bottom to the surface of the biofilm while BOD substances are supplied through the surface of the biofilm. Oxygen-rich and BOD substance-poor conditions, which are suitable for growth of nitrifiers, can be attained near the bottom region of the biofilm. Organic pollutant-rich conditions can be attained near the surface region. Nitrification will proceed near the bottom region without competition between nitrifiers and BOD oxidizers for oxygen, while BOD removal will proceed near the surface region. Thus, simultaneous BOD removal and nitrification are expected to proceed within biofilm formed on oxygen enrichment membrane in a single-stage process.

The potentiality of a new biofilm in which oxygen is supplied from the bottom of the biofilm was suggested by Timberlake *et al*[22]. But the carbon removal and nitrification efficiencies were poor for actual wastewater treatment[22]. No data that contribute to integrate a new biofilm concept into a wastewater treatment system have been available. The objective of the present study is to evaluate simultaneous organic carbon removal and nitrification by a biofilm formed on oxygen enrichment membrane to single-step treatment of small-scale domestic wastewater. A new biofilm support which consists of a silicone

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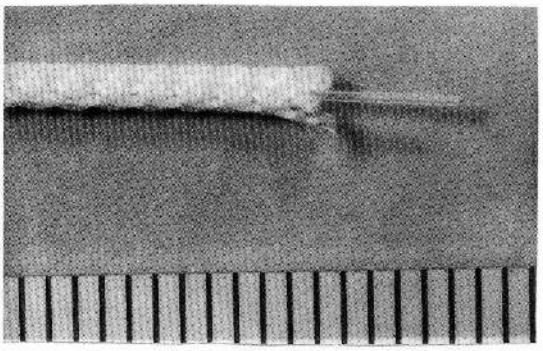

Fig. 1. Appearance of oxygen enrichment-type support

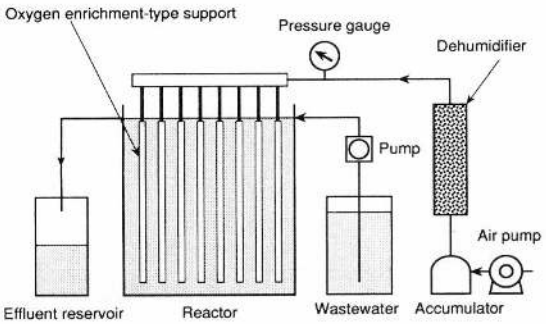

Fig. 2 Schematic diagram of experimental setup for measurement of microbial population in biofilm

hollow fiber and a fibrous support woven around the hollow fiber are used in the present study. Populations of nitrifiers and denitrifiers in the biofilm attached to and within the fibrous support were investigated. Advantages of the new biofilm was discussed in terms of spatial distribution of microorganisms in the biofilm. Continuous wastewater treatment was carried out and effects of air pressure on organic carbon removal and nitrification were investigated. Nitrification rates in the biofilm formed on oxygen enrichment membrane were compared with those in conventional attached growth systems.

1. Experimental

1.1 Oxygen enrichment-type support

Figure 1 shows the appearance of an oxygen enrichment type support (Nagayanagi Co., Ltd.). The support consists of a poly (dimethylsiloxane) (silicone) hollow fiber membrane and a fibrous support which is woven with super fine polyester fibers around the hollow fiber. The inner and outer diameters of the hollow fiber are 250μm and 400μm, respectively. The outer diameter of the fibrous support is about 1.7mm. The support can easily be modularized for practical use as will be described in section 1.

1.2 Population of nitrifier and denitrifier

Figure 2 shows the experimental setup used for investigation of spatial distributions of nitrifiers and denitrifiers. Eight pieces of the oxygen enrichment-type support (12 cm in length) were set in the reactor. The working volume was 225ml. The initial volume fraction of the supports was about 0.9% (v/v).

Table 1. Composition and characteristics of raw wastewater

Composition		
Glucose	13.0	kg/m^3
Peptone	13.0	kg/m^3
$(NH_4)_2SO_4$	11.8	kg/m^3
KH_2PO_4	1.7	kg/m^3
K_2SO_4	5.25	kg/m^3
$MgSO_4 \cdot 7H_2O$	2.75	kg/m^3
NaCl	2.25	kg/m^3
$NaHCO_3$	4.5	kg/m^3
$NaCO_3$	22.5	kg/m^3
Characteristics		
BOD	20.0	kg/m^3
TOC	11.4	kg/m^3
T-N	4.1	kg/m^3
T-P	0.52	kg/m^3

Nitrifier enriched activated sludge (MLSS was about 1 kg/m^3, volume V was 135ml) and activated sludge (MLSS was about 3 kg/m^3, V = 90ml) were introduced to the reactor. Batch wastewater treatment was carried out for 8 days without stopping oxygen supply to attach microorganisms to the supports. The composition and characteristics of the raw wastewater are summarized in **Table 1**[13]. Conditions of the batch treatment were as follows; 1 cycle = 24 h, BOD loading = 0.4 kg/m^{3}d, air pressure P at inlet of the supports = 19.6 kPa. The mixed liquor was replaced with water after 9 d and continuous treatment was carried out. Diluted raw wastewater was used as a synthetic domestic wastewater. Concentration of BOD, TOC and total nitrogen (T-N) of the influent wastewater were 200, 114 and 41 g/m^3, respectively. Hydraulic retention time of wastewater and volumetric BOD loading were 12 h and 0.4 kg/m^{3}d, respectively.

Two pieces of the support were cut into 10 cm lengthes and removed from the reactor at 8, 28, 62 and 82 d. Biomass grown at the surface of the support was mechanically scraped by passing the support through a hole, 1.7 mm in diameter, opened through an acrylic resin plate. Biomass grown inside the fibrous support was detached with use of an ultrasonic disperser. Each portion of biomass was suspended in a saline solution and dispersed with the ultrasonic disperser. The population of nitrifiers (ammonia oxidizers) and denitrifiers was evaluated by the MPN (most probable number) method[4].

1.3 Wastewater treatment

Figure 3 shows the experimental setup used for continuous wastewater treatment. Two sheet-type modules (Nagayanagi Co. Ltd.) were used. The module consists of 25 pieces of the oxygen enrichment-type supports connected in parallel as a rectangular sheet (c.a. 30cm in length, 5 cm in width and 1.7 mm in thickness). The working volume was 1630 ml. Diluted raw wastewater was used as a synthetic domestic wastewater. BOD, TOC and T-N concentrations of the influent wastewater were 200, 114 and 41 g/m^3, respectively. Hydraulic retention time of the wastewater was 12 h. Volumetric BOD loading, BOD and TOC loadings based on the surface area of the module were

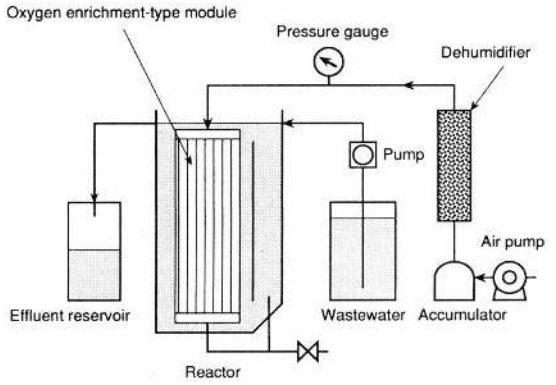

Fig. 3 Schematic diagram of experimental setup used for wastewater treatment

0.40 kg/m^{3}d, 11.6 g/m^{2}d and 6.6 g/m^{2}d, respectively. The surface BOD loading was comparable to that in a rotating biological contactor for treatment of domestic wastewater[9]. The volume fraction of the modules' volume to the working volume was about 2.1%. Air pressure was monitored at the inlet of the modules and varied from 19.6 to 39.2 kPa. Air was introduced to the reactor through a glass pipe (2.6 mm in inner diameter) for liquid mixing by an air-lift function in the modules.

1.4 Analysis

The amount of organic pollutants was evaluated as total organic carbon (TOC). TOC was measured with a TOC analyzer (TOC500, Shimadzu Corp.). Total nitrogen (T-N) and ammoniacal nitrogen (NH_4-N) were determined according to the standard methods[7]. Nitrites (NO_2-N) and nitrates (NO_3-N) were measured with an ion chromatograph (column; Shim-pack IC-A1, Shimadzu Corp., mobile phase; 0.2 M phosphate buffer at pH 7.0). Dissolved oxygen (DO) concentration and pH were monitored with a DO meter and a pH meter, respectively.

2. Results and Discussion

2.1 Population of nitrifiers and denitrifiers

Figure 4 shows the changes in effluent TOC, T-N, pH and DO during the treatment. DO concentration began to decrease at about 29 d. Two pieces of the support were cut and removed at 8, 28, 62 and 82 d. The decrease in DO concentration is due to the decrease in number of the hollow fibers through which oxygen is supplied. Effluent TOC concentration in the continuous treatment period was about 10 g/m^3. TOC and T-N concentrations at the end of the batch treatment period (0-8 d) were higher than those in the continuous period because of accumulation of TOC and T-N.

The outer surface of the supports was almost covered with biofilm at 15 d. No significant change in appearance was observed thereafter. From visual inspection, the thickness of the biofilm formed on the surface was of the order of 1 mm.

Figure 5 shows changes in effluent concentration of

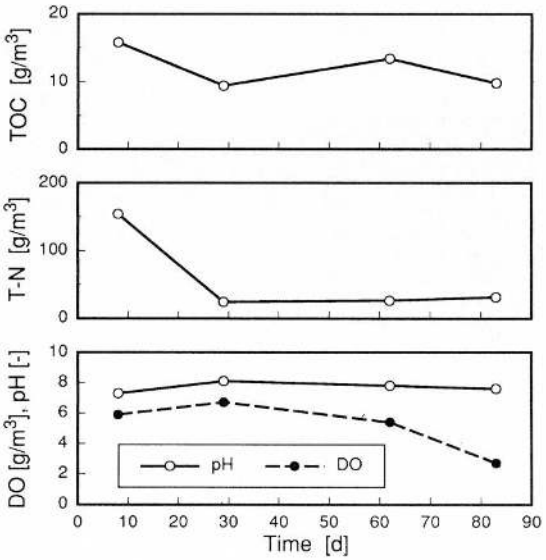

Fig. 4 Changes in TOC, T-N, DO concentrations and pH during treatment for measurement of microbial populations

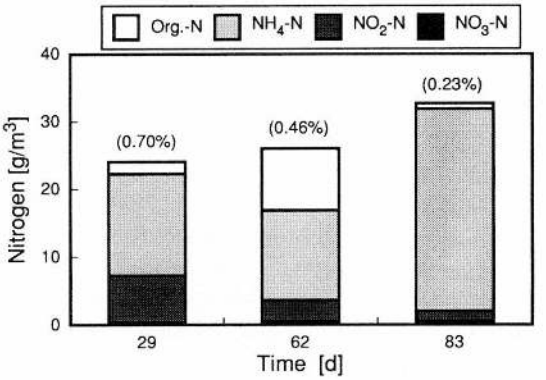

Fig. 5 Changes in concentration of nitrogen compounds during treatment for measurement of microbial populations. Values in parenthesis show volume fraction of the oxygen enrichment-type support

nitrogen compounds. T-N concentration at 29 d was about 25 g/m^3 and increased slightly with time. Effluent T-N concentration was smaller than influent concentration. This means nitrogen was partially removed during the treatment. Nitrogen removal is considered to be due to assimilation by heterotrophs such as BOD oxidizers and denitrifiers. Partial denitrification by some of the denitrifiers in the biofilm and sedimented sludge may contribute to nitrogen removal[18]. Concentration of oxidized nitrogens (nitrites and nitrates, NO_X-N) at 29 d was about 8 g/m^3. NO_X-N concentration decreased gradually with time. Formation rates of NO_X-N per unit length of the support were about 0.048, 0.036 and 0.038 mg/dcm at 29, 62 and 83 d, respectively. T-N removal rates per unit length of the support were about 0.11, 0.15 and 0.16 mg/dcm at 29, 62 and 83 d, respectively. Nitrification and nitrogen removal activities were almost the same after 62 d, suggesting that biofilm activity was almost in the steady state. The

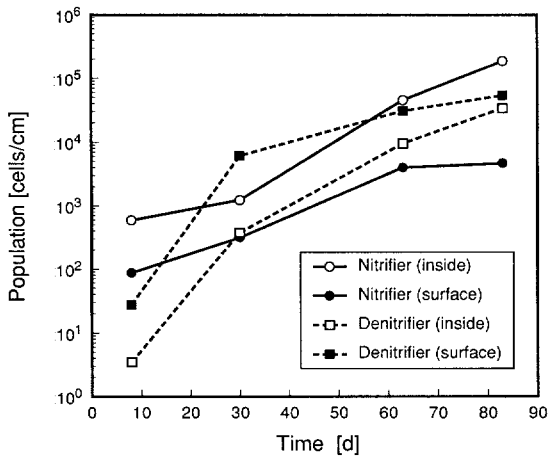

Fig. 6 Distribution of nitrifiers and denitrifiers in biofilm formed on oxygen enrichment-type support

volume fractions of the supports were about 0.70, 0.46 and 0.23% at 29, 62 and 83 d, respectively. The decrease in NO_X-N concentration and the increase in T-N concentration with time are attributed to a decrease in biomass.

From the results above, nitrification was confirmed to proceed simultaneously with organic carbon removal in the biofilm formed on the oxygen enrichment-type support.

Figure 6 shows the populations of nitrifiers and denitrifiers per unit length of the support. The number of nitrifiers inside the fibrous support was much larger than that in the biofilm grown at the surface of the support. The number of nitrifiers at 8 d reached the order of 10^5cells/cm and was comparable to that in a tertial submerged biological filter[17]. On the contrary, the growth of denitrifiers both within the support and in the surface biofilm leveled off after 62 d. The number of denitrifiers inside the support was smaller than in the surface biofilm. These results are attributed to the fact that an oxygen-rich and organic substance-poor condition was attained within the fibrous support while an organic substance-rich condition was attained in the biofilm grown on the surface of the support. As for spatial distribution of the microorganisms, it should be noted that nitrifiers were dominant within the support (see open symbols) while denitrifiers were dominant in the surface biofilm (see solid symbols). Nitrification in the present biofilm is expected to be less sensitive to competition with heterotrophs. It can be pointed out that concentration profiles of oxygen and organic pollutants, i.e., spatial distributions of nitrifiers and denitrifiers, are suitable for simultaneous nitrification and organic carbon removal in a single-step treatment system with a biofilm formed on an oxygen enrichment membrane.

2.2 Wastewater treatment

1) Organic carbon removal **Figure 7** shows the results of continuous treatment of synthetic domestic wastewater. The treatment was started with pressure P = 19.6 kPa. The air pressure was increased to 29.4 and 39.2 kPa at 19 and 57 d, respectively. The module surface was almost covered with biofilm at 10 d. No remarkable change in appearance

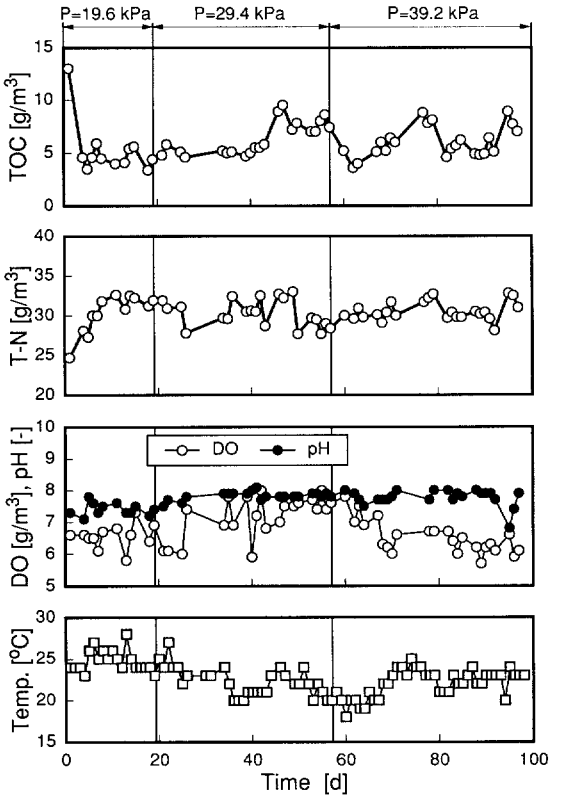

Fig. 7 Continuous wastewater treatment by biofilm formed on oxygen enrichment-type support

was observed thereafter. From visual inspection, the thickness of the biofilm formed on the module was of the order of 1 mm. Temperature and pH were about 24 °C and 7.8, respectively during the treatment. DO concentrations at P = 19.6, 29.4 and 39.2 kPa were about 6.6, 7.2, 6.3 g/m^3, respectively. Effluent TOC concentration was about 5 g/m^3 at P = 19.6 kPa, and about 6.5 g/m^3 at P = 29.4 or 39.2 kPa. The dependency of TOC removal efficiency on air pressure was not clear in the present stage because air passed through the module was returned to the tank for air-lift function purposes. To clarify the effect of air pressure on TOC removal, further study is required in terms of oxygen balance within the biofilm.

Average TOC removal rate during all periods was of the order of about 6.3 g/m^{2}d. The TOC removal rate in the present biofilm was comparable to that in conventional biofilms under the same range of organic loading[2, 15, 16]. The TOC removal efficiency was above 95% in the present biofilm process. The results show that good organic carbon removal is possible with the present biofilm system.

2) Nitrification **Figure 8** show the changes in concentration of nitrogen compounds during single-step treatment of domestic wastewater. NO_X-N concentration increased to about 28 g/m^3 at 8 d but decreased slightly to about 20 g/m^3 at 77 d. NH_4-N concentration at P = 19.6 and 29.4 kPa was only about 3g/m^3. Nitrification efficiency at P =19.6 and 29.4 kPa, defined by the ratio of NO_X-N to T-N, was about 90%. The ratio decreased to about 64% at 77 d and thereafter. In a rotating biological contactor, nitrifi-

Table 2. Nitrification rates in attached growth systems

process or configuration	nitrogen loading [g/m²d]	nitrification rate [g/m²d]	organic loading or influent concentration	ref.
Submerged biological filter				
1-stage	0.6	0.65	1 g-COD/m²d	2
1-stage	0.9	0.5	1 g-BOD/m²d	19
2-stage (1st stage)	-	1.32	-	16
2-stage (2nd stage)	-	1.58	-	16
2-stage	2-5	1-3.5	BOD 5-18 g/m³	19
4-compartment	1.7-13.3	1-4.5	COD 6.3-50 g/m³	1
Rotating biological contactor				
4-stage	0.88-2.4	0.3	COD 80-210 g/m³	21
4-stage	1-2.3	0.2-0.6	COD 240-690 g/m³	21
4-stage + supplemental aeration	0.88-2.4	0.83-1.4	COD 80-210 g/m³	21
4-stage + supplemental aeration	1-2.3	1.0	COD 240-690 g/m³	21
Trickling filter				
1-stage	0.6	0.65	1 g-COD/m²d	2
tertiary stage	-	2.1-2.9	BOD < 10 g/m³	15
Attached-growth circulating reactor				
1-stage	0.3-0.91	0.3-0.5	3.6-10.2 g-COD/m²d	9
Biofilm formed on oxygen enrichment membrane				
1-stage P =19.6 kPa	2.4	2.2	11.6 g-BOD/m²d	
P =29.4 kPa	2.4	2.2	BOD 200 g/m³	
P =39.2 kPa	2.4	1.7		

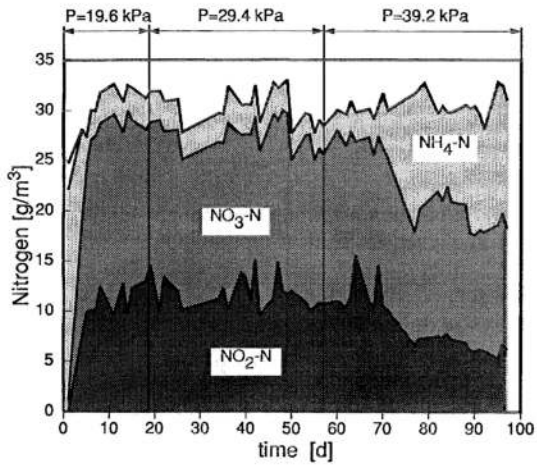

Fig. 8 Changes in concentration of nitrogen compounds during wastewater treatment

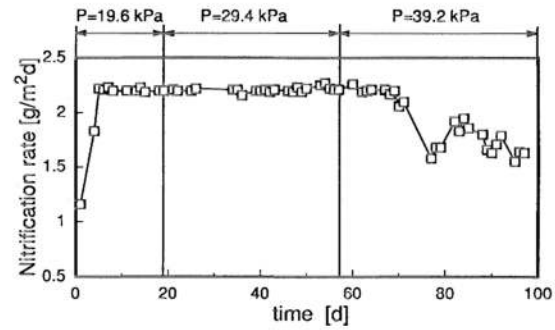

Fig. 9 Change in nitrification rate

cation efficiency was reported to be about 90% at a BOD loading of 2 g/m²d, but decreased sharply to about 50 % at a BOD loading of 8 g/m²d[9]. A nitrification efficiency of about 90% was obtained in the present biofilm at a BOD loading of 11.6 g/m²d. Nitrification efficiency in the present biofilm was found to be higher than that in a rotating biological contactor.

The decrease in nitrification efficiency may be attributed to assimilation of NH_4-N by heterotrophs and to hindered transportation of NH_4-N to the nitrifiers by the dense cells of heterotrophs[5].

Nitrification proceeded successfully in the present biofilm at P = 19.6 and 29.4 kPa. The nitrification rate was evaluated from the apparent removal rate of NH_4-N. **Figure 9** shows the change in the nitrification rate in the present biofilm during the single-step treatment of domestic wastewater. The nitrification rate reached a plateau at about 8 d and was quite stable up to about 70 d. The average nitrification rates at 19.6, 29.4 and 39.2 kPa were about 2.2, 2.2 and 1.7 g/m²d, respectively. Typical nitrification rates, defined by the apparent removal rate of NH_4-N, in conventional biofilm processes are summarized in **Table 2**. Nitrification rates in conventional biofilm processes used for organic carbon removal are reported to be about 0.5g/m²d. Higher nitrification rates were reported in multi-stage submerged biological filters[1, 16, 19] and a tertiary nitrifying trickling filter[15]. These biofilm processes[15, 16, 19] designed especially for nitrification have nitrification rates ranging from 1 to 3 g/m²d. The nitrification rate depends on organic loading, namely on competition between nitrifiers and BOD oxidizers for oxygen. In a conventional biofilm, nitrification proceeded with BOD concentration of below 10 g/m³, namely just after BOD substances were almost exhausted[9, 12]. On the contrary, nitrification rates in the present single-stage biofilm process for treatment of domestic wastewater were comparable to those in multi-stage nitrifying biofilm processes[15, 16, 19]. That is, higher nitrification rates were obtained in the present biofilm used for organic carbon removal with a BOD loading of 11.6 g/m²d and an influent BOD concentration of 200 g/m³. The higher nitrification rates in the present biofilm are attributed to the fact that the nitrifiers in the bottom region could grow with little competition from BOD oxidizers.

It can be concluded that the present biofilm process is suitable for single-stage simultaneous organic carbon removal and nitrification in the treatment of small-scale domestic wastewater. The nitrification rate and TOC removal rate were hardly affected with air pressure ranging from 19.6 to 29.4 kPa. Lower pressure is preferred from an economic view point. For practical application of the biofilm formed on an oxygen enrichment membrane, further investigation on the effects of operational conditions such as air pressure and loading on long term treatment performance are necessary.

Conclusion

The distribution of nitrifiers and denitrifiers in the biofilm was found to be suitable for simultaneous organic carbon removal and nitrification. Advantages of the new biofilm were verified experimentally in terms of spatial distribution of microorganisms in the biofilm.

Nitrification proceeded successfully and its rate was comparable to that of conventional nitrifying biofilms. It was concluded that simultaneous organic carbon removal and nitrification can be carried out successfully by a single-stage treatment with the new biofilm process.

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