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Short Paper

DENITRIFICATION UNDER HIGH DISSOLVED OXYGEN BY A MEMBRANE-ATTACHED BIOFILM REACTOR

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ABSTRACT

In this study, the inhibition of a membrane-attached biofilm reactor (MFSB) by oxygen was investigated. Results show that the denitrification rate, under anoxic conditions, of the MFSB was close to that under conditions of DO (dissolved oxygen) as high as 7.7 mg/L in bulk phase. It appears that the inhibition of DO on denitrifying rates is not obvious for the MFSB. Under aerobic conditions, the denitrification rate of a conventional denitrifying reactor decreased to almost one half the rate under anoxic conditions. By preserving stability against DO inhibition, MFSB is a reasonable alternative for a stable denitrification system.

Key Words: membrane-feeding substrate bioreactor (MFSB), denitrification, oxygen inhibition, biofilm.

I. INTRODUCTION

It is generally accepted that denitrification must occur in an anoxic environment to prevent oxygen inhibition (Mitchell, 1974). As facultative anaerobes, the denitrifiers may change to aerobic respiration to obtain more energy in the presence of molecular oxygen (Sabaty *et al*., 1993). Under oxygen-short or anoxic conditions, they can switch to nitrate respiration immediately (Wilson and Bouwer, 1997; Hernandez and Rowe, 1987; Kawakami *et al*., 1985). In one word, oxygen decreases the denitrification rate even if denitrifiers possess aerobic denitrification ability (Patureau *et al*., 1996).

Since denitrification is inhibited by an increasing oxygen concentration (Kawakami *et al*., 1985; McKenney *et al*., 1994), a definite threshold value cannot be found. According to the literature, the startup of denitrification can be inhibited while the oxygen level is as low as 0.13 mg/L in a dispersed-well sludge reactor (Nelson and Knowles, 1978). At DO levels of 0.2 and 2 mg/L, the denitrification rates drop to one-half and 10% of the anoxic conditions, rate respectively (Focht and Chang, 1975). Recently, Wilson and Bouwer (1997) reported on the inhibition of denitrification at oxygen levels from 0.08 to 7.7 mg/L under different experimental conditions, showing that inhibition must take into account bioreactor characteristics, environmental factors, operation conditions and system configuration.

The main disadvantages of the conventional carbon feeding method, which adds carbon directly in the bulk phase, are the high residual COD (chemical oxygen demand) and the high level of suspended solids (SS). In our previous work, a new method of carbon addition for denitrification was developed. A carbon source (methanol) was fed from the lumen of a silicone tube to the biofilm formed on the surface of the same silicone tube (Chang and Tseng, 1998). This system was termed a membrane-feeding substrate bioreactor (MFSB). The main advantage of MFSB is that it can keep the bulk solution at lower COD levels throughout the denitrification process, despite its lower dentrification rate. In another earlier work, the

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denitrification rates of MFSBs remained almost unaffected even when the system DO was as high as 2 mg/L (Chang and Tseng, 1999). It is interesting to note that the inhibition of denitrification by oxygen may be prevented for both the biofilm barrier and carbon addition method.

In this study, the inhibition of MFSB denitrification by oxygen was investigated. In order to determine the oxygen threshold of inhibition, a batch and a continuous bench-scale bioreactor were operated at different DO concentrations. For a comprehensive comparison, another denitrification reactor using a conventional method of carbon addition also was observed.

II. MATERIALS AND METHODS

1. Synthetic Wastewater Composition

The mineral solution per liter of water contained $NaHCO_3$ (0.5 g), KH_2PO_4 (8.5 mg), K_2HPO_4 (21.75 mg), $Na_2HPO_4 \cdot 7H_2O$ (33.4 mg), $CaCl_2 \cdot 2H_2O$ (27.5 mg), $MgSO_4 \cdot 7H_2O$ (22.5 mg), and $FeCl_3 \cdot 6H_2O$ (0.25 mg). To prevent interference by nitrification under aerobic conditions, 20 mg NO_3^--N/L of sodium nitrate was applied as the sole nitrogen source both for growth and denitrification. The wastewater contained no ammonium during incubation and operation.

2. Membrane-Feeding Substrate Bioreactor (MFSB)

The MFSB was established and illustrated in our earlier work (Chang and Tseng, 1998). A silicone tube (1.5 mm [i.d] and 2.5 mm [o.d] by 6 m long) obtained from Fuji Systems Co. (Japan) was immersed and wrapped around the pillars in the reactor. The reactor was 1.5 liters and comprised a DO meter and a gas diffuser, both of which were connected to a DO controller. The gas diffuser supplied air at a flow rate of 530 mL/min. The diffuser on/off was controlled by a DO controller that was dependent upon the measured value from the DO meter. A magnetic stirrer mixed the contents of the reactor. The external carbon source, which supplied the methanol solution (30 g COD/L) by continuous recirculation from a 1-L container, was pumped into the silicone tube of the MFSB. The carbon stock solutions were replaced for each test run or once per day for a series of batch or continuous tests, respectively (Fig. 1).

3. Preparation of MFSB for Experiment

The bioreactors were inoculated with activated sludge from a municipal wastewater treatment plant. First, the excess activated sludge, nitrate and mineral solution were added and mixed well in the MFSB. Then, the external carbon source (methanol solution) was fed at a flow rate of 30 mL/min. The synthetic wastewater was replaced daily by a semi-batch method. Once the biofilm had formed visibly on the surface of the silicone tubes after 3 weeks development, the synthetic wastewater was fed continuously into the reactor at a flow rate of 6 mL/min until the startup of the batch tests. At that time, the biofilm thickness was approximately 800 μm. All incubation temperatures were controlled at 24 ± 2°C. Several experimental batch runs were conducted with different DO concentrations and external carbon addition methods, and the results are shown in Table 1. The continuous operation of the MFSB took place after the batch test was completed.

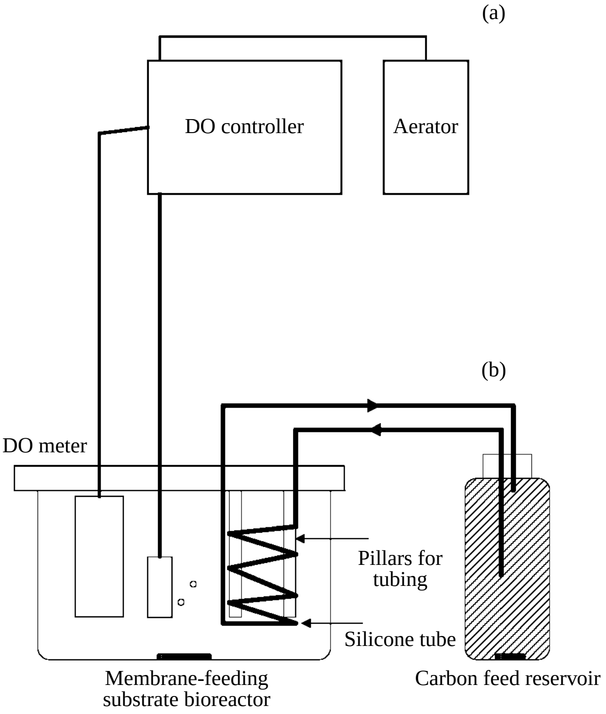

Fig. 1 Schematic diagram of a bench-scale system with (a) an MFSB reactor (b) equipped with a DO controller.

4. Control

In contrast to the carbon addition method of MFSB, adding carbon directly to the bulk phase of the reactor was employed as a control. In order to obtain the maximum denitrifying rate, excess methanol was added until the COD/nitrate-nitrogen ratio increased to 7. Except for the method of carbon addition, the biofilm incubation, biofilm carriers (silicone tube), DO controlling method and inoculation sludge of the control were similar to those of the MFSB.

Table 1 Summary of experimental conditions

Run #	Carbon feeding methods	DO in bulk phase	Denitrifaction rate (gN/m^2d)	Increasing Alk. rate ($gCaCO_3/m^2d$)	Alkalinity per one gram of nitrate denitrified
1	by silicone tube	0	2.8	10.2	3.7
2		1.0	2.4	8.9	3.9
3		4.0	2.8	9.0	3.3
4		7.7	2.2	9.2	4.1
5	conventional	0	6.9	22.4	3.3
6		1.0	3.3	11.7	3.6
7		2.0	3.5	13.8	4.0

5. Analysis

The electrode method was used for pH and DO measurements, which were taken using a pH meter (WTW, Germany) and DO meter (WTW, Germany), respectively. Nitrate and nitrite were measured with an ion chromatograph analyzer (Lab Alliance, U.S.A.). The procedures in Standard Method (APHA *et al.*, 1995) were followed for measuring COD and alkalinity.

III. RESULTS

1. Batch Results under Varied Bulk DO

To obtain reasonable comparisons, the same MFSB biofilm module was employed for the denitrification batch tests at different DO levels. All the batch results under different conditions are shown in Table 1. The denitrification rates were calculated from the nitrate variation via batch elapsed time. The initial rate method was applied to determine the initial denitrification rates by regressing the linear part (initial 3-5 consecutive data points) of the curve. Table 1 shows that the denitrification rates, within the same MFSB, under the anoxic condition (DO = 0 mg/L) were close to those under the condition of DO concentration increasing to 7.7 mg/L in the bulk phase (run 1-4, Table 1). It appears that the effect of DO on denitrifying rates was not obvious in the reactors in which carbon was fed by the silicone tubes.

The effect of oxygen on denitrification is apparent in the case of adding carbon by a conventional method, which is adding organic carbon directly to the bulk phase (run 5-7, Table 1). Under aerobic conditions, the denitrification rate decreases to almost one-half the rate under anoxic conditions. Although adding carbon to the bulk of the reactors produces a higher denitrifying rate than in an MFSB, the residual COD is a problem.

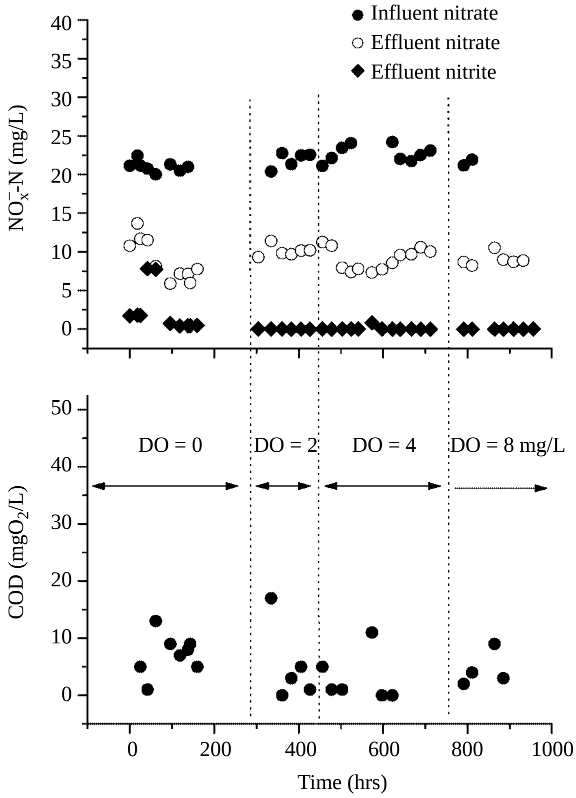

Fig. 2 Time dependent changes of the influent nitrate, effluent nitrate/nitrite and residual COD concentration under different controlled DO conditions.

Theoretically, the alkalinity increased by 3.57 g (as $CaCO_3$) for each gram of nitrate reduced. The increased alkalinity (as g $CaCO_3$) per gram of nitrate denitrified ranged from 3.3 to 4.1, and this corresponds to different DO levels in the bulk phase in this study (Table 1, column 6). This suggested that the alkalinity variance was mainly caused by the reduction of nitrate. Thus, the MFSB can ensure a reasonable denitrification rate even under the higher DO condition.

2. Continuous Operation Results of the MFSB

In order to demonstrate the stability and reliability of denitrification in an MFSB under aerobic conditions, a continuous experiment was conducted after the batch test. Fig. 2 shows the variations of the influent/effluent nitrate, nitrite and the residual COD against time under different bulk DO concentrations. It appears that the denitrification rates were not affected by the DO in the bulk phase. Nitrite build-up, which often occurs in aerobic or carbon-limited conditions (Wilderer *et al*., 1987; McKenney *et al*., 1994), was not found in this situation. The residual COD concentrations also could be controlled at an acceptable level (< 20 mg/L), which is similar to the level under anoxic conditions. All of the results show that the MFSB could maintain denitrification rates and keep low residual COD at high oxygen concentrations.

IV. DISCUSSION

Compared to a bioreactor using a conventional carbon feeding method, the MFSB maintained almost constant denitrification rates under different DO levels. Many studies have reported that the occurrence of denitrification under aerobic conditions is due to the existence of anoxic microenvironments (Hernandez and Rowe, 1987; Wilson and Bouwer, 1997; Robertson and Kuenen, 1984; Christensen *et al*., 1989).

Although micro-anoxic niches exist close to the substratum of a conventional fixed-film biofilm, the denitrification rates are limited because of an inadequate carbon supply. The carbon is mostly taken up by denitrifiers, which shift to aerobic respiration when exposed directly to oxygen, on the surface of the biofilm (Kotlar *et al*., 1996). Besides oxygen depletion, carbon diffusion across the oxic surface layer to the anoxic zone also significantly affects the integral denitrification rates (Christensen *et al*., 1989). Furthermore, air stripping of methanol, which is often used as an external carbon source for denitrification, may also hinder denitrification under aerated conditions. Air stripping is often applied to remove excess residual external carbon in the bulk phase consequent to the denitrifying progress (Reising and Schroeder, 1996). These adverse effects of oxygen on denitrification apparently do not occur in the MFSB.

The alkalinity for each gram of nitrate-nitrogen denitrified, termed Alk/Den ratio, summarizes the distribution of nitrate used in this system (Table 1). Theoretically, Alk/Den ratios should be equal to or lower than 3.57 due to the part of the nitrate contributed to biomass assimilation, but aeration may have interfered with those values. The stripping of carbon dioxide under aerobic conditions may have decreased acidity and thereby increased whole Alk/Den ratios (Runs 4 and 7, Table 1). In Run 7, the air flow rate was close to that in Run 4, but the DO in the bulk phase could not be raised due to the presence of abundant suspended microbes, induced by aerobic/heterotrophic conditions. In addition, a higher growth rate under aerobic conditions may have accelerated nitrate assimilation and diminished the integral Alk/Den ratios. However, the Alk/Den values in this study fell within a range close to the theoretical value in denitrification, demonstrating that nitrate decayed mainly via denitrification instead of nitrate assimilation.

To achieve complete nitrification, excess air is added to the aerobic tank in a two-stage BNR system. Thus, the inlet for the anoxic tank usually contains residual DO. In contrast, the DO level of the inlet for the denitrifying unit may vary from 0.14 to 4.4 mg/L in a single-sludge BNR system (Oh and Silverstein, 1999). The advantages of the MFSB include: (1) no need to do post-treatment; (2) high stability with different DO levels; (3) lower residual COD level in the treated water during denitrification periods; and (4) no need to add excessive carbon. Compared to aerobic denitrification, the MFSB provides a useful alternative to a stable BNR system.

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