DMF Decomposition and Nitrogen Removal Performance by a Mesh-Filtration Bioreactor under Acidic Conditions

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Abstract

N,N-Dimethylformamide (DMF) is a water-miscible polar solvent that is used in a wide variety of chemical industries. In Japan, $0.668 \times 10^6 \text{ kg} \cdot \text{y}^{-1}$ of DMF is discharged to the sewerage system and is ranked highest in the PRTR (2006). In this work, a bench-scale mesh-filtration bioreactor was employed for aerobic biological treatment of DMF. Synthetic wastewater containing $1000 \text{ mg} \cdot \text{L}^{-1}$ of DMF was fed into the reactor at 5 or 2.5 days of hydraulic retention time (HRT) and at a volumetric loading rate of 0.125 or $0.25 \text{ kg} \cdot \text{DMF} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$; the pH of the reaction mixture was not controlled. Performances in removal of dissolved organic carbon (DOC) and nitrogen were examined. Although the pH of the reaction mixture decreased to around 3, DOC removal remained at more than 98%, nitrification was complete, and about 45% of total nitrogen was removed. The rates of DMF decomposition, nitrification, and denitrification were evaluated by batch experiments, with the result that for these three biological reactions the sludge in the reactor showed significant activities even at pH 3. These results suggest that the sludge contained acidophilic nitrifying bacteria.

Keywords:

Wastewater treatment, DMF, mesh-filtration bioreactor, nitrification, denitrification, acidic condition

1. Introduction

N,N-Dimethylformamide (DMF: $(\text{CH}_3)_2\text{NCHO}$) is a water-miscible polar solvent used in a wide variety of chemical industries, such as the synthetic polymer, organic chemical, pharmaceutical, and agrochemical industries. DMF is emitted to the environment directly or via some treatment processes at a rate of $14.136 \times 10^6 \text{ kg} \cdot \text{y}^{-1}$ in Japan (2006), ranking ninth in the Pollutant Release and Transfer Register (PRTR) [1]. $4.782 \times 10^6 \text{ kg} \cdot \text{y}^{-1}$ is discharged to the atmosphere, $0.292 \times 10^6 \text{ kg} \cdot \text{y}^{-1}$ to surface water, $8.687 \times 10^6 \text{ kg} \cdot \text{y}^{-1}$ to wastewater treatment processes and $0.668 \times 10^6 \text{ kg} \cdot \text{y}^{-1}$ to sewerage systems. The last discharged amount is ranked highest in the PRTR.

Waste DMF is treated primarily by using physicochemical processes such as distillation, absorption, and adsorption. Because DMF is water miscible, however, it is difficult to remove it effectively by these physicochemical processes, and considerable amounts of waste DMF are discharged in wastewater. Although the biodegradability of DMF is relatively low, effective biological treatments by activated sludge and biofilm processes have been reported [2-8], in many cases by feeding diluted DMF to the reactors. Bromley-Challenor et al. [7] examined the decomposition properties of DMF under aerobic, fermentative, and nitrate-reducing conditions and reported that it is not decomposed effectively under anaerobic conditions. Ghosal et al. [4] indicated two pathways for aerobic decomposition of DMF: (1)

decarbonylation followed by demethylation, and (2) two demethylation steps followed by decarbonylation. In the former pathway, dimethylamine and methylamine, which are produced as intermediate compounds, are toxic to biomass; this may account for the difficulties in treating wastewater with a high concentration of DMF.

The C/N weight ratio of DMF is 2.57, so it is important for biological DMF treatment processes to be able to remove nitrogen as well as organic carbon effectively. Funaishi et al. [8] reported that nitrogen could not be removed by an activated sludge system but was removed effectively by an anoxic–oxic process.

Membrane bioreactors may be promising for the treatment of low degradable or refractory organic compounds, because this type of reactor can maintain a high concentration of bacteria even when their growth rate is low. In our previous work [9–13], we found that a mesh-filtration bioreactor (a reactor equipped with a mesh-filtration unit instead of a membrane) was able to maintain biomass at a very high concentration. In this process, stable filtration performance was obtained by intermittent filtration under low pH conditions [11–13]. Building on these results, in this study we employed a mesh-filtration bioreactor for DMF treatment and examined its dissolved organic carbon (DOC) and nitrogen removal performances. The pH of the reaction mixture was not controlled, because acidic conditions are preferable in the mesh filtration process [11–13]. DMF decomposition, nitrification, and denitrification rates were also examined by batch-type experiments using biomass obtained from the reactor.

2. Experimental

2.1 Reactor setup

The reactor consisted of a polyacrylate cylinder (working volume, 8 L) equipped with a mesh-filtration module (nylon mesh: opening size, 100 μm ; module size, 75 cm $\times$ 90 cm; effective mesh area, 135 cm²) at the bottom of the reactor (**Fig. 1**). The reactor was set up in the laboratory, and water temperature (which was around 20 °C) was not controlled. A diffuser was installed under the mesh module for air supply and for cleaning of the mesh surface. The reactor was aerated, except during the filtration period. Filtration was carried out by introducing a hydraulic pressure difference between the water level in the reactor and the effluent port. Because the initial filtrate from the mesh module contained suspended solids (SS) at a high concentration, the initial 1 min of filtrate was returned to the reactor. Thereafter, the filtrate was collected as effluent. After the filtration finished, aeration was restarted.

Fig. 1 Schematic figure of the mesh filtration bioreactor

2.2 Treatment of synthetic DMF Wastewater

Activated sludge was obtained from the domestic wastewater treatment facility at our institution (extended aeration process) and inoculated into the reactor after pre-cultivation with the synthetic DMF wastewater by fill and draw for more than 2 weeks. The synthetic wastewater (**Table 1**) was fed into the reactor by peristaltic pumping at a loading rate of 1.6 or 3.2 L·d⁻¹ for 20 h each day with a hydraulic retention time (HRT) of 5 or 2.5 days, respectively. Filtration was conducted twice or four times per day, and 0.8 L of the effluent was withdrawn during each mesh filtration operation. The time required to filter 0.8 L of wastewater was measured as the filtration time. Dissolved oxygen (DO) in the reactor was kept at 2–3 mg·L⁻¹ under aerated conditions. The experiments were conducted over 1 year. The experimental conditions are summarized in **Table 2**.

2.3 Rates of DMF decomposition, nitrification, and denitrification

The DMF decomposition rate of the biomass obtained from the reactor was measured. The biomass was collected by centrifugation (4650 g, 3 min) and washed twice with pure water. The batch-type experiments were conducted at pH 3, 5, and 7: the pH 7 solution was prepared with 0.01 M phosphate buffer; the pH 5 solution was prepared with 0.01 M phosphate buffer and H₂SO₄; and the pH 3 solution was adjusted with H₂SO₄ and NaOH. The initial DMF concentration was adjusted to 50 mg-C·L⁻¹.

Nitrification and denitrification rates of the biomass obtained from the reactor were also measured. Biomass obtained from the domestic wastewater treatment facility at our institution was used in a control experiment. The pH was adjusted to 3, 5, and 7. For measurement of the nitrification rate, NH₄Cl was used as the substrate. For measurement of the denitrification rate, NaNO₃ and methanol were used as substrates, and the weight ratio of initial methanol to NO₃-N was more than 5.

Table 1 Composition of the synthetic wastewater

Substance	Concentration (mg·L ⁻¹)
DMF	1000
Na ₂ HPO ₄	176
KCl	5
CaCl ₂	1.8
MgSO ₄	7
Alkalinity	100

Table 2 Operation conditions

Phase	1	2
Period (d)	0-157	158-419
HRT (d)	5	2.5
Filtration (times·d ⁻¹)	2	4
Synthetic wastewater		
DMF (mg·L ⁻¹)	1000	
COD _{Cr} (mg·L ⁻¹)	986	
TOC (mg·L ⁻¹)	493	
T-N (mg·L ⁻¹)	192	
pH (—)	8.14	
Volumetric Loading Rate		
COD _{Cr} (kg·m ⁻³ ·d ⁻¹)	0.125	0.250
TOC (kg·m ⁻³ ·d ⁻¹)	0.062	0.125
T-N (kg·m ⁻³ ·d ⁻¹)	0.024	0.048

2.4 Analytical methods

All effluent samples were filtered with a cellulose acetate membrane filter (0.45 μm). DOC was determined by using a TOC analyzer (TOC-5000, Shimadzu, Kyoto, Japan). Ammonia and nitrite were analyzed by phenate method [15] and by colorimetric method [16], respectively. Nitrate was analyzed by an ion chromatograph equipped with a column of Tosoh TSKgel IC-Anion PW_{XL}. Kjeldahl nitrogen was analyzed [16], and organic nitrogen was evaluated from the difference between Kjeldahl nitrogen and ammoniac nitrogen.

2.5 Respiration activity of the biomass

The metabolically active cells in the sludge were evaluated on the basis of the respiration activity detected by the 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) staining method. The biomass was obtained from the reactor on the 407th day. CTC is converted to fluorescent CTC-formazan precipitates when reduced by biological redox reactions such as respiratory electron transport. We used the CTC staining method as modified by Yoshida and Hiraishi [14]. The sludge samples were sonicated in an ice bath for 90

s with a 2-s intermittent burst (20 kHz; output power, 50 W) and immediately used for the CTC staining process. The activated sludge was harvested by centrifugation (8230 g, 5 min), washed three times with 50 mM 3-morpholinepropanesulfonic acid (MOPS) buffer (pH 6.5), and resuspended in 50 mM MOPS buffer. The reaction mixture containing 870 μL of sludge, 120 μL of 50 mM CTC, and 10 μL of the substrate mixture (0.05% for peptone and yeast extract and 1 mM for glucose and succinate) was incubated in a water bath at 30 °C for 4 h with gentle shaking. Five microliters of the cell suspension was spotted on a slide glass and fixed by drying at 30 °C for 5 min. The fixed cells were counterstained for 5 min with 10 μL of SYBR green I solution: the dye solution used was a 10^{-4} -fold dilution of the purchased solution (Molecular Probes, Eugene, OR, USA). Then, the slide glass was rinsed with cooled MilliQ water to remove the excess dye and dried at room temperature in the dark. A drop of Citiflour (Citiflour, Ltd., London, UK) was placed on top of each sample. The samples were observed under an Olympus BX-50 epifluorescence microscope equipped with a Flovel FD-120M digital CCD camera (Flovel Co., Tokyo, Japan). The number of positive cells per a view area was counted, and 11 view areas were measured.

3. Results and discussion

3.1 Filtration performance

The concentration of mixed liquor suspended solids (MLSS) was initially about 4000 $\text{mg}\cdot\text{L}^{-1}$, and it was increased to 5000 $\text{mg}\cdot\text{L}^{-1}$ at HRT = 5 d (Phase 1). The HRT was then changed to 2.5 d (Phase 2), and the MLSS was increased to 11 000 $\text{mg}\cdot\text{L}^{-1}$. The mesh-filtration bioreactor was able to maintain a high concentration of biomass. Profiles of MLSS, mixed liquor volatile suspended solids (MLVSS), filtration time, effluent SS, and pH are shown in Fig. 2.

When the loading rate was low (Phase 1), filtration occurred very quickly (filtration time: about 10 min), and the effluent SS also remained very low (about 6 $\text{mg}\cdot\text{L}^{-1}$). These results indicate that the cake layer that formed on the mesh surface could effectively block sludge particles.

Fig. 2 Profiles of MLSS, MLVSS, filtration time, effluent SS and pH

When the loading rate increased during Phase 2, the filtration time increased to more than 20 min. At around the 170th day, the filtration time spiked, and the effluent SS and pH also increased abruptly. At this time the aeration had stopped because the blower malfunctioned. The blower was replaced, and aeration was restarted. Since the pH in the reactor decreased again within 3 days, the feeding of synthetic wastewater was restarted. As a result, both the filtration time and the effluent SS recovered.

The filtration time was also long during days 330–340. At this time, the effluent pH decreased to 2.5, and deflocculation of the sludge was observed. The deflocculation may have been induced by strongly acidic conditions, because the filtration performance recovered after the pH was increased to 3 by the addition of a small amount of NaHCO_3 to the synthetic wastewater. The filtration time was also long during days 370–410. At this time deflocculation was also observed, but the reasons were not clear.

3.2 DMF decomposition performance

We examined the profile of dissolved organic carbon (DOC) in the effluent (**Fig. 3**). During the initial few days, high concentrations of DOC ($45\text{--}65\text{ mg-C}\cdot\text{L}^{-1}$) were observed, but DOC remained lower than $10\text{ mg-C}\cdot\text{L}^{-1}$ during the experimental period (average DOC = $6\text{ mg-C}\cdot\text{L}^{-1}$). The DOC concentration of the synthetic wastewater was $493\text{ mg-C}\cdot\text{L}^{-1}$, and the DOC removal rate was more than 98%. These results indicate that the fed DMF had been almost completely mineralized and that reaction intermediates, such as *N*-methylamide, formamide, dimethylamine, and methylamine, did not accumulate. The apparent overall DMF decomposition rate was evaluated as $4.06\text{ mg-C}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ in Phase 1 and $8.12\text{ mg-C}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ in Phase 2, where the effluent DOC was assumed to be caused by residual DMF.

The DMF decomposition rates were also examined by batch experiments, conducted at pH 3, 5, or 7, with the sludge obtained from the reactor on the 419th day. In these experiments, the DMF decomposition rate was evaluated by the rate of decrease of DOC. The DMF decomposition rate was approximated as a first-order reaction (**Fig. 4**). The relationship between the reaction rate constant ($\text{mg-C}\cdot\text{L}^{-1}\cdot(\text{g}^{-1}\cdot\text{VSS}\cdot\text{L}^{-1})\cdot\text{h}^{-1}$) and pH is illustrated in **Fig. 5**. The highest decomposition rate was obtained at pH 3, and the rate decreased as the pH was increased.

Fig. 3 Profile of the effluent DOC

Fig. 4 DMF decomposition rates

Fig.5 Effect of pH on the rate constant of DMF decomposition

The DMF decomposition rate was simulated by using the reaction rate constant pH 3 shown in **Fig. 5**. The calculation was conducted under the following conditions: the reaction rate constant = $0.123\text{ (g-VSS L}^{-1})^{-1}\cdot\text{h}^{-1}$, MLVSS = $11\text{ g}\cdot\text{L}^{-1}$ and DOC = $5\text{ mg-C}\cdot\text{L}^{-1}$. The simulated value of $6.78\text{ mg-C}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ was similar to the apparent overall DMF decomposition rate ($8.12\text{ mg-C}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$) in the reactor. However, it should be noted that the microbial metabolites of DMF may contribute to the effluent DOC from the reactor. When the DMF concentration in the effluent is lower than $5\text{ mg-C}\cdot\text{L}^{-1}$, the simulated reaction rate may decrease

significantly.

The respiration activity of the biomass in the reactor was monitored by double staining with CTC and SYBR green. The proportion of cells stained by both CTC and SYBR green relative to those stained by SYBR green alone was $33.0\% \pm 6.0\%$, indicating significant aerobic respiration activity, although the value was lower than that for common activated sludge ($44.2\% \pm 2.3\%$) [14]. Some DMF-decomposing bacteria have been isolated [5, 6], but these bacteria were cultivated at near neutral pH.

3.3 Nitrification and denitrification properties

Because the nitrogen content of DMF is high ($C/N=2.57$), the synthetic wastewater ($1000 \text{ mg-DMF}\cdot\text{L}^{-1}$) contained $191.8 \text{ mg}\cdot\text{L}^{-1}$ of nitrogen, so evaluation of total nitrogen (T-N) removal by biological DMF removal processes is also important. We examined the profiles of the nitrogen components (Fig. 6). Organic nitrogen (Org-N) was present in Phase 1 but disappeared during part of Phase 2. $\text{NH}_4\text{-N}$ was also observed in Phase 1 but then disappeared until the latter half of Phase 2. $\text{NO}_2\text{-N}$ was observed at only trace levels throughout the experimental period, and most nitrogen was $\text{NO}_3\text{-N}$ during Phase 2. Considering that the pH in the reactor was kept at around 3, these results were surprising and unusual. Although T-N removal fluctuated, the average T-N removal during Phase 2 was 45%. These results suggest that denitrification also occurred despite the acidic conditions. The T-N removal amount may correspond to the denitrification ratio, and the limited denitrification may have been caused by an insufficiency in the amount of hydrogen donor owing to the low C/N ratio of the DMF and the acidic condition of the reaction mixture.

The effects of pH on nitrification and denitrification rates were examined in batch-type tests. For the tests, sludge obtained from the reactor (S-1) and activated sludge obtained from the domestic wastewater treatment facility at our institution (S-2) were used. The nitrification and denitrification rates were measured at pH 3, 5, and 7 (Figs. 7 and 8). In the case of the S-2 sludge, both nitrification and denitrification activities were highest at pH 7 and were not observed at pH 3; these are the results that have been observed commonly. On the other hand, in the case of the S-1 sludge, nitrification activity was observed even at pH 3 and was higher at pH 5 than that in the S-2 sludge, although the nitrification activity was lower than that in the S-2 sludge at pH 7. The highest denitrification activity in the S-1 sludge was observed at pH 3, although a large fluctuation was observed. Considering that the denitrification rate in the S-1 sludge at pH 3 was higher than the nitrification rate at pH 7, the low rate of T-N removal in the reactor (Fig. 6) may have been caused by insufficiency in the amount of hydrogen donor.

Fig. 6 Profiles of nitrogen components

Fig. 7 Effect of pH on nitrification rate

S-1: the sludge obtained from the reactor
S-2: the sludge obtained from a domestic wastewater treatment facility

Fig. 8 Effect of pH on denitrification rate

S-1: the sludge obtained from the reactor

S-2: the sludge obtained from a domestic wastewater treatment facility

These results suggest that the nitrifying and denitrifying bacteria in the reactor were acidophilic. Although bacteria having activity under acidic conditions are rare, Tarre et al. [15] isolated acidophilic nitrifying bacteria from a soil and observed their nitrifying activity at pH 3.2. However, acidophilic nitrifying and denitrifying bacteria have not been isolated from activated sludges in wastewater treatment processes. The pH of the reaction mixture in our reactor decreased to a very low value (pH 3) owing to the low alkalinity of the synthetic wastewater. The mesh-filtration device effectively prevented wash-out of biomass from the reactor, which retained a high concentration of biomass. Because of the long-term operation of the reactor, the unique bacterial consortium described above may have become acclimated. It will be important in future work to reveal the characteristics of the biomass in the reactor.

4. Conclusion

DMF treatment was conducted in a mesh- filtration bioreactor without pH control. Although the pH of the reaction mixture decreased to a very low level (pH 3), the DMF was effectively mineralized, and complete nitrification and partial denitrification (45%) were also observed. The sludge in the reactor showed higher activities of DMF decomposition, nitrification and denitrification at pH 3 than activated sludge, suggesting that acidophilic heterotrophic and nitrifying bacteria had become acclimated in the reactor. Characterization of the unique bacteria in the reactor remains a subject for future investigation.

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