B−11 Factors affecting biodegradability of pharmaceuticals in wastewater by nitrifying activated sludge

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1. INTRODUCTION

The occurrence and fate of pharmaceutically active compounds (PhACs) in aquatic environment is required to develop the bioprocess capable of degrading these chemicals. Among the bioprocesses developed, nitrification process may play a key role in degradation of persistent organic micro-pollutants such as pharmaceuticals [1, 2]. Thus, to reinforce the hypothesis that the bacterial community in the nitrifying activated sludge plays a key role in pharmaceuticals degradation, a lab-scale study of selected PhACs degradation was performed. The objective of the study was to investigate the biodegradability of the selected PhACs by nitrifying activated sludge (NAS).

2. MATERIALS AND METHODS

(1) Chemicals and media

The composition of mineral-salts medium (MSM) was: Na_2CO_3 1514 mg/L; $MgSO_4.7H_2O$ 41.6 mg/L; $CaCl_2.2H_2O$ 50 mg/L; NaH_2PO_4 50.5 mg/L; $K_2HPO_4.3H_2O$ 75.6 mg/L. The stock MSM solution was diluted 25 times prior to using with other supplements such as ammonium, target compounds according to the experimental purposes.

The target compounds used in this study were 10 pharmaceutical compounds, clofibric acid (CA), gemfibrozil (GFZ), ibuprofen (IBP), fenoprofen (FEP), ketoprofen (KEP), naproxen (NPX), diclofenac (DCF), indomethacin (IDM), propyphenazone (PPZ) and carbamazepine (CBZ).

(2) Enrichment of nitrifying microorganisms

Activated sludge with low nitrifying activity of less than 5 mg NH_4-N/gMLSS.h was collected from a nitrification tank at Ariake Wastewater Treatment Plant in Tokyo, Japan. From this collection, activated sludge was enriched in fill-and-draw operation with a 2d-cycle in 2-L reactor at 30°C for more than two months. At the start of enrichment, activated sludge and the diluted MSM solution supplemented with ammonium-nitrogen was used as enrichment medium. The ammonium-nitrogen concentration was gradually increased from 100 to 300 mg/L during the enrichment periods depending on the growth of nitrifying activity of the sludge. Conventional activated sludge (CAS) was also used in the experiment for a comparison with NAS. It was taken from Shibaura Wastewater Treatment Plant.

(3) Batch degradation experiments of PhACs

Sterilized 300mL Erlenmeyer flasks were each filled with 100 mL of MSM supplemented with initial ammonium concentration of 100mg/L. Target compounds in methanol solution were then added to the flasks to achieve an initial concentration of 100 μg/L. The amount of nitrifying activated sludge in the flasks was adjusted to achieve an MLSS concentration of 1000 mg/L at the beginning of all batch tests. The pH of culture medium was maintained at approximately 7.5-8.0 using $NaHCO_3$ 30 g/L during cultivation. Flasks were cultivated on a temperature-controlled incubator at 30°C. Shaking ensured a sufficient supply of oxygen to keep the dissolved oxygen concentration higher than 3 mg/L. To access the PhACs degrading activities of nitrifying activated sludge in the absence of ammonia oxidation, 10 mg/L of allylthiourea (ATU) used as the AMO (the action of ammonia monooxygenase) inhibitor [2] with the ammonium-supplemented MSM. Adsorption experiments with fully inhibited biological activity of sludge were carried out to distinguish pure adsorption onto sludge from biodegradation. Inactivation of sludge was performed with the addition of NaN_3 at 0.2% weight to volume (w/v) into the ammonium-

supplemented MSM. During inoculation target compounds and NH_4-N, NO_2-N, NO_3-N, COD concentrations were measured. All batch experiments were implemented in triplicate.

(4) Analyses

The concentrations of target pharmaceutical substances such as CA, GFZ, IBP, FEP, KEP, NPX, DCF, and IDM were measured by the method using GC/MS after solid phase extraction and pentafluorobenzyl derivatization suggested by Sacher et al [3]. PPZ and CBZ, which require no derivatization, were quantified in the same chromatogram. Concentrations of NH_4-N, NO_3-N, and COD after heating with $K_2Cr_2O_7$ solution were determined using the colorimetric tests following the protocol of HACH method.

3. RESULTS AND DISCUSSION

(1) Enrichment of nitrifying microorganisms

To have a sufficient quantity of nitrifying microorganisms to seed the batch experiments, enrichment of nitrifier-dominated populations in the sludge was performed in laboratory-scale SBR system for more than 2 months. The ammonium oxidizing activity of nitrifying sludge of this enrichment culture was estimated by the disappearance of ammonium over in the batch culture. Ammonium oxidizing activity gradually increased and achieved 30 mg NH_4-N/gMLVSS.h after 2 months enriched. During the batch degradation experiments, nitrifying activity of this sludge was maintained around 25 to 30 mg NH_4-N/gMLVSS.h. The volatile fraction of the nitrifying activated sludge was 70±10% of total suspended solids. This sludge system should be used for the following experimental purposes.

(2) Biodegradation of PhACs by CAS and NAS

Although the elimination of pharmaceuticals by conventional activated sludge (CAS) has been previously studied, those studies showed that removal efficiency of pharmaceuticals was generally not effective, especially for persistent pharmaceuticals such as CA, DCF, CBZ and PPZ, the removal efficiency was around 5-20%[4]. To illustrate whether there is any advantage of NAS system compared with CAS system in the PhACs degradation, a laboratory study was implemented with NAS and CAS systems under the same initial operating conditions. In this study, NAS system with nitrifying activity of 30 mg NH_4-N/gMLVSS obtained from enrichment process was used to ensure stable nitrification. A representative experiment of the PhACs degradation by NAS and CAS after 6 days of operation was shown in **Fig.1.**

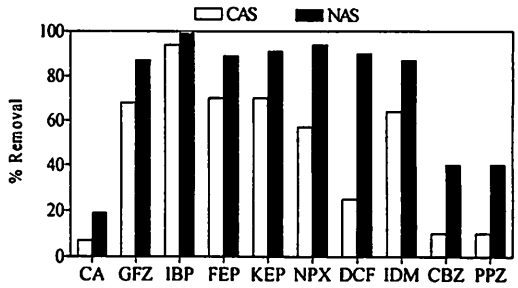

Fig.1. Degradation of the PhACs by CAS and NAS

From **Fig.1**, it can be concluded that IBP is easily removed by biological treatment processes. The removal efficiencies of IBP were in the range of 94 to 100 % by CAS and NAS, respectively. This indicates that IBP is considerably biodegradable. There was a very small difference between NAS and CAS in term of IBP elimination. In opposition to the case of IBP, the removal efficiencies of other target compounds such as CA, DCF, CBZ, and PPZ by CAS were very low. The persistence of these compounds in sewage treatment plants was also reported by some authors [4, 5]. In respect of CA, DCF, CBZ and PPZ did not show significant removal while NAS displayed considerable removal efficiencies. Obvious advantages of the NAS over CAS were recognized with respect to CA, GFZ, FEP, KEP, NPX, DCF, IDM, CBZ and PPZ. Especially, the reduction of CA, DCF and NPX by NAS compared with CAS. The results fit well the results as reported by [6]. **Fig.1** also showed that the overall removal efficiencies for 10 PhACs by NAS system varied from 18% (CA) to 99.8% (IBP), in which 5 of 10 compounds are removed at over 90% efficiency (IPB, KEP, FEP, NPX, DCF), and 3 of 10 compounds are removed at less than 50% efficiency (CA, CBZ, PPZ), probably due to their lower hydrophobicity ($\log K_{ow}<3$) and chemical structure as well. Overall, the results strengthen the conclusion that the elimination efficiency change as a function of the type of the PhACs and suggest that chemical characteristics play an important role in degrading each compounds in biological treatment.

(3) Biodegradability of PhACs by NAS in the presence of inhibitors

To reinforce the contributive role of nitrification process in degradation of PhACs by NAS, two kind of inhibitory were used to confirm if the removal of pharmaceuticals arisen from biological activity of sludge, particularly from activity of ammonium oxidizing bacteria (AOB) community. ATU and NaN_3 were used to inhibit ammonium oxidation activity and the complete biological activity of sludge, respectively.

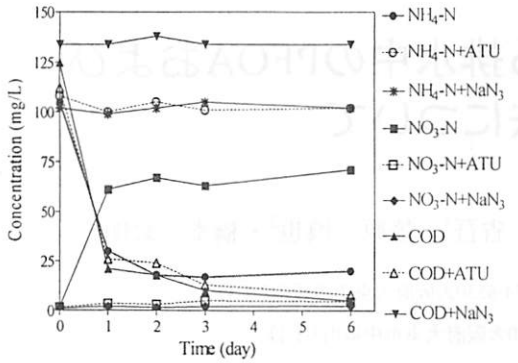

Fig.2. Change in NH_4-N, NO_3-N and COD concentrations during PhACs degradation by NAS with/without inhibitors

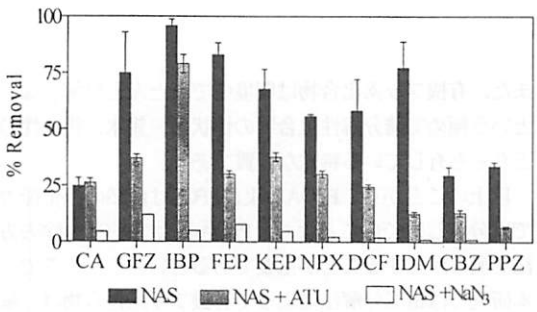

Fig.3. Removal of the PhACs by NAS with/without inhibitors

As shown in **Fig.2**, when only NAS and the selected PhACs are added into MSM, NH_4-N gradually decreases during the PhACs degradation period of 6 days, NO_2-N temporarily increases and then decreases via NO_2-N oxidation, and the total nitrogen concentrations are nearly constant. In contrast, when NAS and the selected PhACs, and ATU are added into MSM, the decrease of ammonium concentrations was not significant with negligible generation of nitrite or nitrate, however, COD concentration significantly decreased from 102 mg/L to 8 mg/L during the pharmaceuticals degradation period. This indicates that ATU effectively inhibited ammonium oxidation. However, ATU is only an inhibitor of ammonia monooxygenase in AOB, thus a large amount of IBP biodegraded and some selected PhACs such as GFZ, KEP, FEP, NPX, IDM and DCF partially biodegraded during the degradation period may be because of the activity of heterotrophs (**Fig.3**). In the case of NaN_3 added medium, the changes in pharmaceuticals concentrations was not significant. Sodium azide inactivates almost all of the biological activity of microorganisms, thus the decline of pharmaceuticals in this experiment was caused by pure adsorption onto sludge flocs and excluded the elimination possibility by biological activity. Concerning the quantitative contribution of heterotrophic activity to the removal of pharmaceuticals, the difference in removal efficiency of PhACs between the both cases of inhibition can be considered as the degradation by heterotrophic activity. The degradation of IBP in NAS was mainly dependent on the activity of heterotrophic microorganisms. The more organic compounds oxidation that took place, the more IBP was removed. The differences between the results of the experiments with/without inhibitors can demonstrate that the degree of biodegradation of tested PhACs by nitrification process. The current data agree well with the results as reported by Batt et al [1] who found that a significantly higher biodegradation of iopromide and thimethoprim was observed in the bioreactor where activity of nitrifying bacteria was not inhibited, relative to the bioreactor where the activity of nitrifying of bacteria was inhibited by ATU. In conclusion, these results provide strong evidence that nitrifying bacteria communities play an important role in enhancing the biodegradation of PhACs.

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日本語要旨

無機塩のみを基質として与えることによって独立栄養細菌の比率を高めた活性汚泥を用いて，10種類の医薬品の分解特性を調べた。2ヶ月の馴養により30 mg NH_4-N/gMLVSS・hのアンモニア性窒素硝化活性を持った活性汚泥を得た。通常の活性汚泥と比較して，この硝化汚泥では，高い医薬品分解活性を持っていることがわかった。また，硝化阻害剤ATUを添加することによって，医薬品の分解は，大きく影響を受けた。