B－11 **Kinetics of Soluble Organic Substrate Formation in Nitrification Processes**

H. M. Acuna (Department of Urban and Civil Eng., Ibaraki University)
H. Furumai (Department of Urban Eng., The University of Tokyo)

1. Introduction

Nitrification processes play a very important role in biological wastewater treatment. In the nitrification processes, growth of nitrifying bacteria, obtaining their energy from inorganic materials, serve as a potential organic carbon source for heterotrophs[1,2]. Besides their growth in mixed cultures, the nitrifiers produce soluble microbial products (SMP) that contribute to organic substrate for heterotrophs. The kinetic evaluation of the microbial products is very important to control the effluent quality from biological processes which receive industrial wastewater containing high ammonium. Furthermore, since nowadays the reuse of effluent from wastewater treatment plants is becoming a good solution to preserve water resources, it is important to determine SMP production because they can serve as possible precursors to trihalometanes and other disinfection products [2].

It has been found that nitrifying bacteria produce SMP that can support heterotrophic bacteria[3]. In other words, heterotrophic population can be maintained through the utilization of the nitrifier-produced SMP. The determination of formation kinetics parameters is a first step in order to explain the coexistence of both types of bacteria in biological processes. In this study, the SMP formation in nitrification processes and effects of the coexistence of heterotrophs, will be investigated by setting up the followings objectives:

1) To demonstrate the formation of organic substrate during stable nitrification process in a media containing a enriched culture of nitrifying bacteria.
2) To differentiate the SMP contribution from ammonium and nitrite oxidations.
3) To investigate the effect of coexistence of heterotrophs on SMP formation by nitrifying bacteria.

2. Material and Methods

Nitrifying bacteria were enriched in a batch reactor through fill and draw operation. For the enrichment, ammonium was used as single substrate. Sludge from a municipal treatment plant was used as inoculant. After the complete nitrification process was finished, all the biomass was recovered by filtrating it through a 0.45 μm menbrane filter. Trapped biomass on the filter was transferred into a new reactor by washing it with deionized water as shown in Figure 1. Then ammonium as substrate was fed again for a new cycle. The temperature was maintained between 20 - 25° C and the pH was in the range 6.5 - 8.0.

TABLE 1 shows the composition of the feed medium used for the enrichment of the nitrifying bacteria. After enrichment work of more than a month, experiments for the analysis of the organic carbon formation were performed. In the first experiment, recovered enriched culture was divided into two batch reactors of 300 ml volume each one. In one reactor (Run 1A), a concentration of 50 mgKNO_2-N/l was fed as sole substrate. The other reactor (Run 1B) was operated without substrate and it was used as a control reactor. When all the nitrite was consumed, the biomass from the two reactors was mixed and recovered by filtration. In the second experiment, two reactors were operated with addition of 50 mg NH_4Cl-N/l (Run 2A) and without substrate (Run 2B), respectively. In the batch type experiments (Run 1 and Run 2), samples were taken periodically, filtrated through Whatman GF/F filters for analysis.

TABLE 1 Media composition

NH_4-N	50 mg
$NaHCO_3$	600 mg
K_2HPO_4	21.75 mg
KH_2PO_4	8.5 mg
Na_2HPO_4 $12H_2O$	44.6 mg
$MgSO_4$ $7H_2O$	22.5 mg
$CaCl_2$	27.5 mg
$FeCl_3$ $6H_2O$	0.25 mg
Deionized water	1000 ml

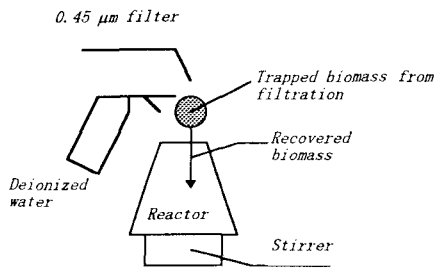

Figure 1. Experimental apparatus

The concentration of NH_4^+- N and NO_2^--N were determined by the colorimetric method . As for DOC, it was measured at the TOC-5000 (SHIMADZU) after purging the sample for 2 minutes with pure gas. To accomplish more accurate measurements of TOC analyzer, each sample was measured three times by the analyzer provided with High Sensitive catalyst. In order to avoid organic pollution of the samples, all vessels and vials were kept in contact with acid for one day before using them.

3. Results and Discussions

Figure 2 shows nitrite consumption and nitrate production during a stable nitrification process in Run 1A (nitrite as sole substrate). A very rapid consumption of nitrite was found, indicating a high activity of *nitrobacter* in the reactor. Figure 3 presents the change of DOC observed during nitrite oxidation. In both cases, an initial consumption of organic carbon was found. This initial concentration of carbon could be due to a slight contamination of carbon incorporated to the media during the filtration process for washing the biomass. Once this organic carbon was consumed, increase of DOC was detected. As there is a very little organic substrate in the added medium, the DOC increase was due to SMP formation from nitrification and biomass decay. It is known that SMP is categorized into two groups, which are called UAP (Utilization Associated Products) and BAP (Biomass Associated Products). The formation rates of UAP and BAP are expressed by the following equations[2]:

$r_{UAP} = k_1 * r_{su}$ where k_1: UAP formation rate constant, r_{su} : substrate consumption rate,
$r_{BAP} = k_2 * X$ where k_2 : BAP formation rate constant , X: biomass concentration

When nitrite was fed into the media (Run 1A) , the DOC increased from 0.98mg/l at 3 hr. to 1.86mg/l at 23 hr. When no substrate was fed (Run 1B), there was also a slight increment in the DOC. BAP formation seemed to contribute to the DOC increment.

Figure 4 illustrates the amount of total inorganic nitrogen in the Run 2A with addition of ammonium. The fractional change of nitrogen components indicates the ammonium consumption, nitrite production and consumption and nitrate accumulation in sequence. Figure 5 shows the DOC change in the Run 2A and 2B. DOC increase was detected in Run 2A after 8 hrs. No apparent formation was observed in Run 2B.

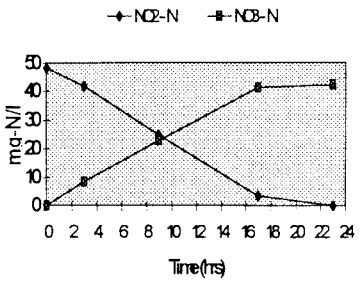

Figure 2. Changes of nitrite and nitrate with time in Run 1A. Nitrite consumption rate= 2.67 mgNO2/l-hr

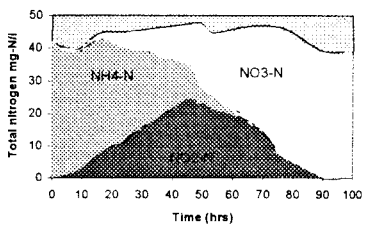

Figure 4. Total inorganic nitrogen in Run 2A.

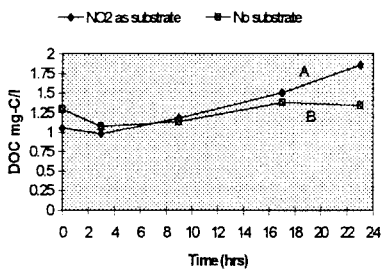

Figure 3. DOC formation with time in Run 1A and 1B. MLVSS=52 mg/l, both reactors.

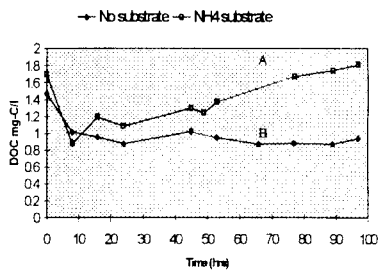

Figure 5. DOC formation with time in Run 2A and 2B. MLVSS=14 mg/l, both reactors.

In the system of the batch experiments, change of SMP is expressed as follows:

$$d[SMP_C]/dt = r_{UAP,ns} + r_{UAP,nb} + r_{BAP,ns} + r_{BAP,nb} + r_{BAP,het} - r_{SMP}$$

where $[SMP_C]$: SMP concentration in terms of DOC, r_{SMP}: degradation rate of SMP by heterotrophs
subscript ns= NH_4 oxidizers, nb= NO_2 oxidizers, het= heterotrophs.

Since there is no activity of NH_4 oxidation in Run 1A, UAP from NH_4 oxidation was not considered. BAP from heterotrophs was neither considered, because the bacterial population of the enrichment culture was composed mainly by nitrifiers. Thereby, the change of DOC was simplified as follows;

$$d[SMP_C]/dt = r_{UAP,nb} + r_{BAP,ns} + r_{BAP,nb} - r_{SMP}$$

Since an increase of nitrifiers was negligible in Run 1A, BAP formation seemed similar for both cases. In addition, assuming that heterotrophs activity in both reactors was the same, the r_{SMP} must be the same. Therefore, the difference between the DOC concentrations of Run 1A and 1B represents the UAP formation from nitrite oxidation. The UAP formation rate by nitrite oxidation was estimated at 0.027 mg-C l^{-1} hr^{-1}. Considering the nitrite consumption rate of 2.67 mgNO_2-N l^{-1} hr^{-1}, the UAP formation rate constant (k_1) for NO_2 oxidation was estimated at 0.010 mg-C mg-N^{-1}.

In Fig. 5, the difference between the DOC concentration of Run 2A and 2B corresponds to overall UAP formation from ammonium and nitrite oxidation. Although it is difficult to differentiate UAP formation by NH_4 and NO_2 oxidations accurately, the UAP formation rate for NH_4 oxidation was approximately estimated using data on increasing DOC from 8 hr. to 45 hr. Ammonium oxidation process was predominant in the period. Considering the corresponding NH_4 oxidation rate (0.79 mgNH_4-N l^{-1} hr^{-1}), the UAP formation rate constant for NH_4 oxidation was estimated at 0.014 mg-C mg-N^{-1}. The constant value was greater than that of the UAP formation rate for nitrite oxidation. Similarly the UAP formation rate constant for NO_2-N oxidation was estimated using data from 53 hr. to 89 hr. in Run 2. The value of 0.017 mg-C mg-N^{-1} was newly estimated which was larger than that obtained in Run 1. Since the initial VSS concentration (14mg/l) was much less than in the Run 1 (VSS=52mg/l), growth of nitrifiers might produce more BAP in Run 2A than the nitrifiers in Run 2B as control one. The values of kinetic constant for UAP formation might be overestimated in Run 2, because DOC increased composed of UAP and additional BAP. Unfortunately, in these batch experiments we could not consider quantitative effects of heterotrophs which must have coexisted with nitrifiers. In the further analysis, the SMP degradation and amount of heterotrophs should be incorporated in the kinetic analysis of SMP formation by nitrification.

The estimated values here were compared with observed values[4] for pure cultures and with provisional values[3] obtained from theoretical consideration, which are shown in TABLE 2. The reported values are estimated based on COD instead of DOC. Although it is difficult to compare them directly, they are almost in the same order assuming that the value of COD/C conversion factor is 2.67 for SMP.The value was based on COD/C ratio in bacterial composition ($C_5H_7O_2N$).

TABLE 2 Comparison of UAP formation rate constant

Reference	for NH_4 oxidation	for NO_2 oxidation
Rittmann et al. (1994)	0.03 mgCOD/mgN	0.025 mgCOD/mgN
Furumai and Rittmann (1992)	0.11 mgCOD/mgN	0.03 mgCOD/mgN
This study	0.014 mgC/mgN	0.010 mgC/mgN
(converted values)	(0.037 mgCOD/mgN)	(0.027 mgCOD/mgN)

4. Conclusions

Batch tests were carried out using enrichment culture of nitrifiers. The changes of DOC were measured in the tests with and without addition of substrates for nitrifiers which contained only inorganic medium. The DOC changes demonstrated that there was a soluble organic carbon formation as long the nitrification process took place. A preliminary kinetics analysis for UAP formation was made in terms of ammonium and nitrite oxidations. The UAP formation rate constants were approximately estimated at 0.014 mg-C mg-N^{-1} and 0.010 mg-C mg-N^{-1} for ammonium and nitrite oxidations, respectively.

References

1) Pan., P. and W.W. Umbreit. (1972) Growth of mixed cultures of autotrophic and heterotrophic organisms. *Can. J. Microbiol.* 18: 153-156. 2) Rittmann, B.E. et al. (1986) A critical evaluation of microbial product formation in biological processes. *Wat. Sci. & Tech.*, 19, 517-528. 3) Furumai H. and B.E. Rittmann (1994) Interpretation of Bacterial Activities in Nitrification Filters by a Biofilm Model Considering the Kinetics of Soluble Microbial Products. *Wat. Sci. & Tech.*, 30, No.11, 147-156. 4) Rittmann, B.E., Regan, J. M. and Stahl, D. A. (1994) Nitrification as a source of soluble organic substrate in biological treatment. Proc. of IAWQ Conference at Budapest. 5) Furumai, H. and Rittmann, B.E. (1992) Advanced modeling of mixed populations of heterotrophs and nitrifiers considering the formation of exchange of soluble microbial products. *Wat. Sci. & Tech.*, 26, No.3/4, 493-502.