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## Chapter IV

### STEP CHANGE ANALYSIS

#### Observation of substrate concentration profile inside biofilm in step change analysis

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### 4.1 INTRODUCTION

In this chapter, step change in inlet substrate concentration represented by substrate was introduced into the completely mixed three-phase fluidized bed biofilm reactor. Substrates are total organic carbon (TOC), ammonium-ion ( $\text{NH}_4^+\text{-N}$ ), nitrite-ion ( $\text{NO}_2^-\text{-N}$ ), nitrate-ion ( $\text{NO}_3^-\text{-N}$ ) and dissolved oxygen (DO). Furthermore, oxygen concentrations inside biofilm at each times were measured by original fabricated oxygen microelectrode. The purpose is to study the conditions inside biofilm during dynamic response. The step changes of 2 times and quarter times larger cases were conducted.

### 4.2 STEP UP CHANGE OF SUBSTRATE CONCENTRATION

#### 4.2.1 MATERIALS AND METHODS

At start-up, the biofilm-attaching CB particles were washed to remove the excess sludge. The inlet TOC concentration of all the reactors was about  $400 \text{ g/m}^3$  at the flow rate of  $0.006 \text{ m}^3/\text{h}$ . This concentration was chosen because this concentration and flow rate was known to allow continuous concentration operation of reactors maintain a stable biofilm structure but avoid excessive production of suspended sludge. Concentration of substrates was measured until steady-state values were obtained. Once the steady state was attained, twice step change in inlet concentration was introduced into the system. The concentrations inside the reactor at the start of the step change analysis are summarized in Table 4.1. After the step-up change of inlet concentration, the substrate concentration, which was considered as the state variables, were measured until new steady-state level was attained.

**Table 4.1 Step-up change in substrate concentration  $\text{g/m}^3$**

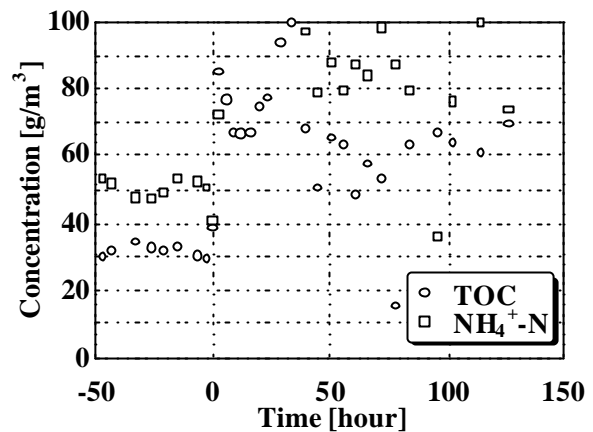
	TOC	$\text{NH}_4^+\text{-N}$
Before change	200	50
After change	400	100

## 4.2.2 RESULTS AND DISCUSSION

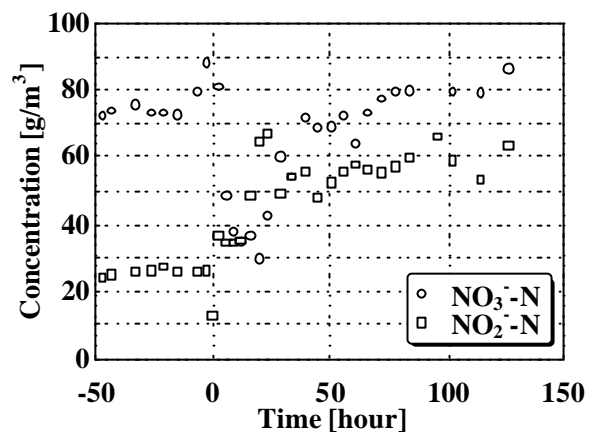
The results of water quality analysis are shown in Figure 4.1. When the inlet substrate concentration is suddenly doubled, the concentration of the substrate inside the reactor increases due to accumulation. TOC (Figure 4.1. (a)) was increased from 0 hour, was attained to the peak at 25 hours. The accumulated substrate is gradually consumed until the rate of increase due to accumulation equals substrate consumption by the biomass. Thus, the peak is generated. After, the peak, substrate consumption becomes greater than the accumulation, and consequently the concentration inside the reactor goes down until a new steady-state condition, the inflow of substrate into the reactor is balanced by the biomass substrate utilization and the outflow of substrate with the effluent. The steady state of TOC was approached after 25 hour. This phenomenon was most popular in the step change experiments.

Next, some biofilm samples were get from the fluidized reactor at -2, 0, 3, 6, 9, 12, 20, 24, 29, 40, 51, 102 hours. The distribution of oxygen concentration inside a biofilm was measurement by microelectrode for each biofilm sample. The results of oxygen distribution are shown in Figure 4.2. The distribution of oxygen concentration inside the biofilm (DOCB) was increased until 6 hour. The DOCB wasn't changed from at 6 hours to at 20 hours. This term was the condition of most low oxygen concentration.

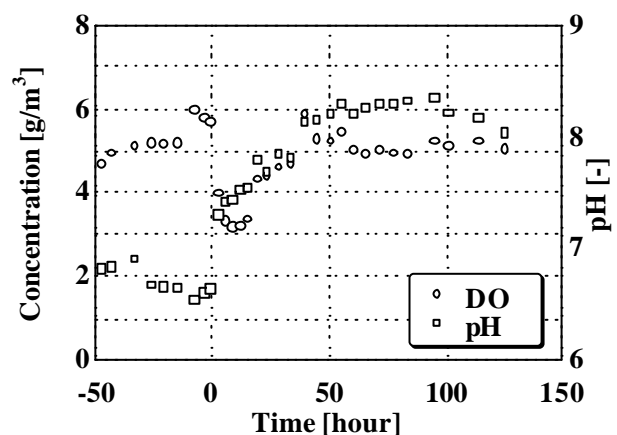
Time courses of the penetration depth of oxygen concentration inside the biofilm (PDOCB) and the oxygen concentration in the bulk (ODB) are shown in Figure 4.3. The PDOCB was like two peas in a pod with the ODB.



(a) TOC and  $\text{NH}_4^+\text{-N}$



(b)  $\text{NO}_x^-\text{-N}$



(c) DO and pH

**Figure 4.1. Time course of substrate concentration and pH in fluidized bed biofilm reactor at step-up change analysis**

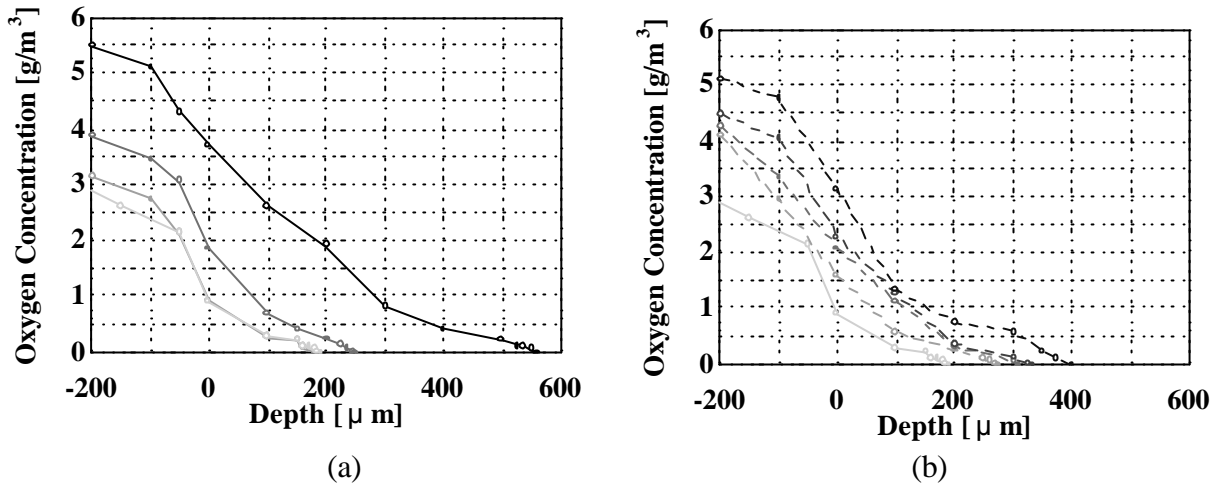


Figure 4.2. Oxygen concentration profile inside biofilm at step-up change analysis

### 4.2.3 CONCLUSIONS

It could be successful that the distribution of oxygen concentration inside the biofilm by oxygen microelectrodes in the step change analysis. As a result, the distribution of oxygen concentration inside the biofilm was influenced by the oxygen concentration in the bulk.

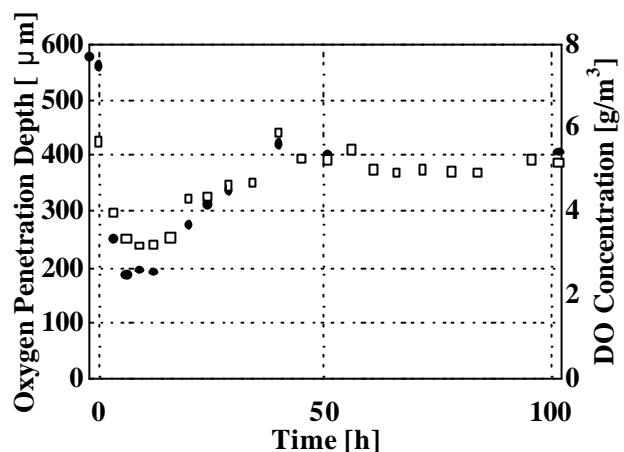


Figure 4.3. Relationship between oxygen penetration depth and DO concentration in bulk solution at step-up analysis

## 4.3 STEP DOWN CHANGE OF SUBSTRATE CONCENTRATION

### 4.3.1 MATERIALS AND METHODS

The experiments to down inlet substrate concentration were conducted using biofilm sample which have no nitrifying activation by oxygen rate controlling. In this case, the recovery of nitrifying activities could be monitored. The substrate concentration and microprofile inside biofilm were determined. Two reactors were run, 1) RUN1: biofilm thickness is 120 μm, package rate is 20 % and 2) RUN2: biofilm thickness is 1200 μm,

package rate is 20%.

A complete-mixing three-phase fluidized bed reactor was used for continuous nutrient oxidation. After the steady state was confirmed, the inlet substrate concentrations were stepped down.

**Table 4.2 Step-down change in substrate concentration g/m<sup>3</sup>**

	TOC	NH <sub>4</sub> <sup>+</sup> -N
Before change	400	100
After change	40	10

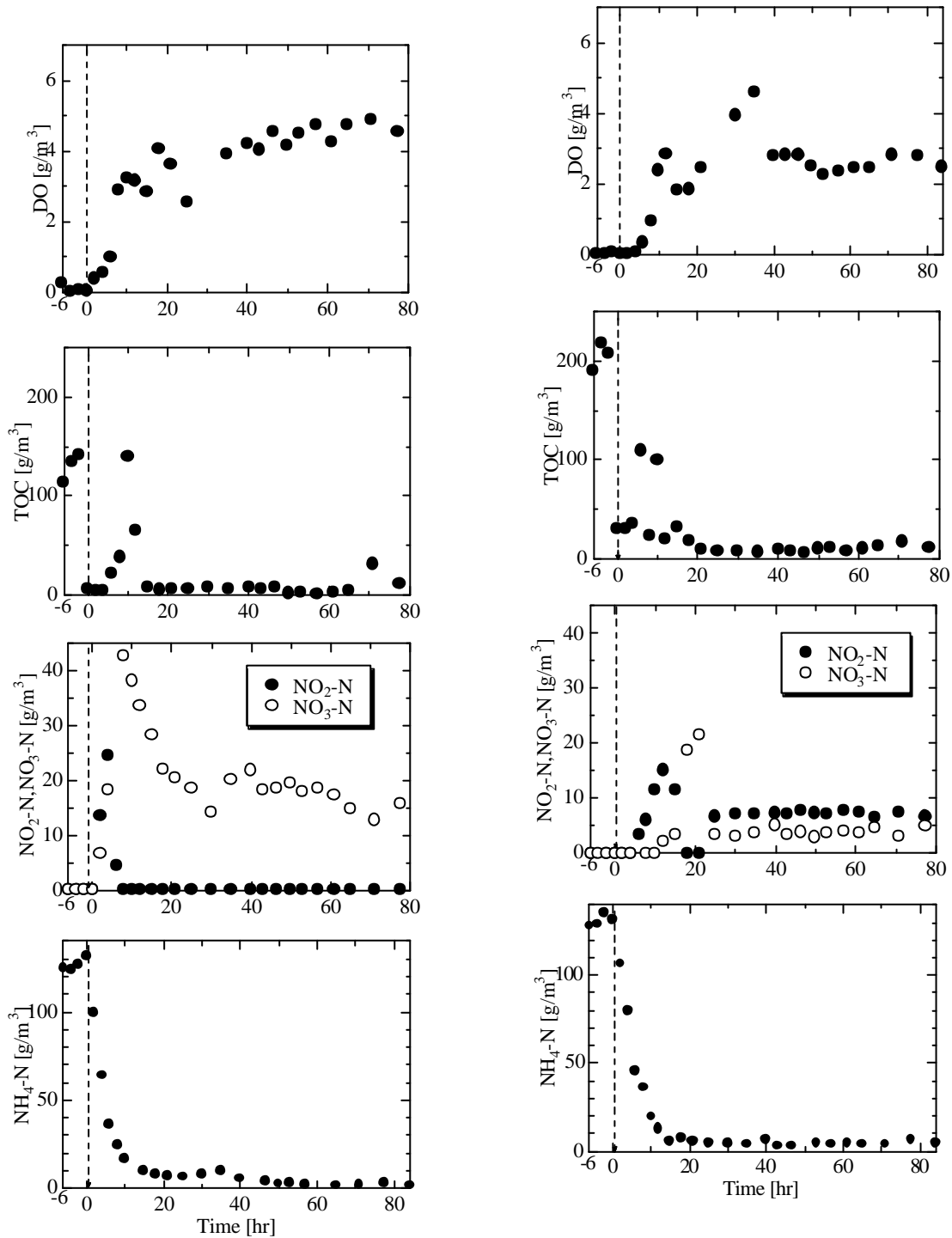
### 4.3.2 RESULTS AND DISCUSSION

Figure 4.4 shows the time courses of water quality and Figure 4.5 shows the distribution of oxygen concentration inside the biofilm. First, in the case of RUN1 (biofilm thickness = 120 μm), the biofilm had a nitrifying activity after the step change. Therefore, they had nothings before the step change. Because of the oxygen was penetrated inside the biofilm after step change, the oxygen concentration was nearly zero before the step change. The biofilms were released from oxygen rate controlling to decrease the substrate concentrations in the bulk solution at the 2.5 hours after. Consequently, oxygen existed inside biofilm and penetrated until 30 μm from biofilm-bulk interface into the biofilm. At the time, we suggested that the ammonia-oxidizing bacteria were existed in the region between 0 to 30 μm from the surface to the bottom of biofilm because nitrite-ion existed in the bulk. Figure 4.6 shows the image of FISH analysis. As a result, NSO190 probes (targeting an ammonia-oxidizing bacteria) were mainly reacted in the area between 0 to 30 μm in the biofilm. We found that ammonia-oxidizing bacteria inside biofilm can have activity, if very small quantity of oxygen penetrates into the biofilm.

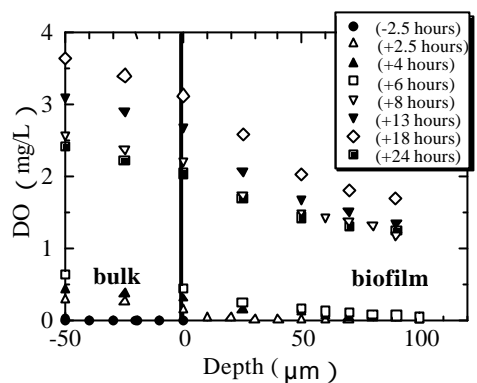
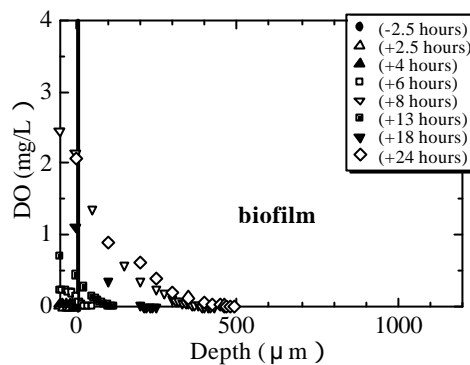
Oxygen completely penetrates until particle which is the bottom part of biofilm at 4 and 6 hours after. In this case, oxygen concentration inside the biofilm was low. Therefore, two factors are suggested because nitrite-ion was not oxidized. First reason is the necessity of higher oxygen concentration is for nitrite-oxidization bacteria, second reason is the delay of responds for nitrite-oxidization bacteria forward an environmental change.

Furthermore, oxygen concentration was over 1 g/m<sup>3</sup> in the bottom area of biofilm at 8 hour. A whole biofilm condition was completely aerobic and nitrification smoothly proceeded.

Next, nitrification could not conducted by oxygen controlling before step change in the case that the biofilm thickness is 1200 μm. The oxygen concentration in the bulk was nearly zero. After step change, also oxygen was not penetrated into the biofilm at 4 hour.

(a) RUN1 - Biofilm thickness: 120  $\mu\text{m}$ (b) RUN2 - Biofilm thickness: 1200  $\mu\text{m}$ 

**Figure 4.4** Time course of wastewater concentration in fluidized bed biofilm reactor at step-down change analysis

(A) Biofilm thickness: 120  $\mu\text{m}$ (B) Biofilm thickness: 1200  $\mu\text{m}$ **Figure 4.5 Concentration profile of DO inside biofilm**

### 4.3.3 CONCLUSION

It could be successful that the distribution of oxygen concentration inside the biofilm by oxygen microelectrodes in the step change analysis. As a result, the distribution of oxygen concentration inside the biofilm was influenced by the oxygen concentration in the bulk. Oxygen completely penetrates until particle which is the bottom part of biofilm.