Application of toxicity monitor using nitrifying bacteria biosensor to sewerage systems

T. Inui*, Y. Tanaka*, Y. Okayasu and H. Tanaka****

* Electric Equipment Technology Laboratory, Fuji Electric Corporate Research and Development, Ltd., 2-2-1, Nagasaka, Yokosuka City, 240-0194, Japan

** Water Environment Research Group, Public Works Research Institute, 1-6, Minamihara, Tsukuba City, 305-8516, Japan

Abstract Toxic substances may be included in wastewater influent and can damage biological processing of wastewater treatment, therefore continuous toxic-monitoring of wastewater influent is needed. This paper describes the potential toxic-monitoring applications of the toxicity monitor using a nitrifying bacteria biosensor to sewerage systems. The results of sensitivity tests show that aspects of wastewater do not affect the sensor sensitivity and confirm that the sensor can be applied to wastewater monitoring as it is. The monitor with a prototype of filtration system installed in a wastewater treatment plant is able to operate continuously for one month at least after the modification of filtration system and the optimization of operation conditions.

Keywords Biosensor; continuous monitoring; filtration system; nitrifying bacteria; toxicity; wastewater influent

Water Science and Technology **Vol 45 No 4–5 pp 271–278**

Introduction

Toxic substances flowing into a sewerage system can damage biological processing of wastewater treatment and, in particular, affect the nitrifying process required to remove nitrogen from wastewater. Current regulations require that plants susceptible to releases of toxic substances into the sewerage system install facilities to remove these substances from their wastewater before it is released into public sewerage systems. While these plants are inspected regularly by sewerage management personnel to check wastewater quality, they are not monitored continuously, and spill accidents may occur from other unidentifiable sources (i.e., illegal dumping) that cannot be monitored. These conditions call for a system capable of continuous monitoring of wastewater influent. We have developed a toxicity monitor using a nitrifying bacteria biosensor, and applied it to the toxic monitoring of purified water and river water (Tanaka *et al*., 1997). In this study, we investigate the potential toxin-monitoring applications of this monitor to sewerage systems.

Materials and methods

Nitrifying bacteria biosensor

The concept of the biosensor is shown in Figure 1. The biosensor consists of a dissolved oxygen (DO) electrode and an immobilized microbial membrane, in which pure cultured nitrifying bacteria, *Nitrosomonas europaea* (ATCC 25978), are contained (Tanaka *et al*., 1996). With constant feeding of standard ammonia solution with a sample solution, the output current of the electrode becomes stable at a level corresponding to the normal respiration rate of the bacteria. When the sample solution contains a chemical that inhibits the oxidation of ammonia, the assimilation is inhibited and the sensor current increases. Respiratory inhibition of nitrifying bacteria can be detected by comparing the output current of the standard ammonia solution with the output of an actual sample solution containing chemicals.

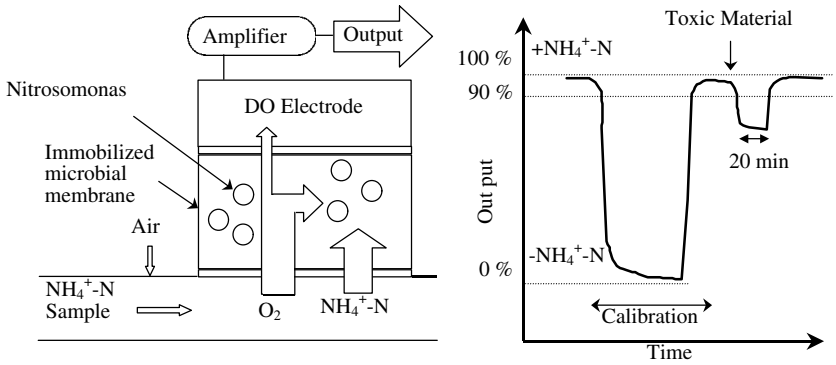

Figure 1 Schematic diagram of the biosensor principal

Toxicity monitor

Figure 2 shows the configuration of the toxicity monitor. The sample solution was injected at a rate of 3.5 ml/minute, and mixed with a feed solution (10 mM borate buffer solution of pH 9.0 containing nitrogen in an ammonia solution, flow rate 0.5 ml/minute) and air (500 ml/minute) from an air pump. Then, the sample solution saturated with oxygen is sent to the measuring part (isothermal box controlled at 30°C). Respiration rate is defined as 100% when the sample does not contain chemicals, and as 0% when the substrate is (ammonia-free) buffer solution. If the sensor output decrease less than 90% respiration rate, the monitor sends an alarm and orders the pump to take a sample for water quality analysis.

Sensitivity tests of the biosensor

We obtained the dose–response relationship of the biosensor for chlorophenols and heavy metal solutions. The monitor was set up in the laboratory. Ion-exchanged water was passed through the sensor as a control. During the tests, various solutions were passed through the device in place of ion-exchanged water. We set the exposure time of the solutions to 20 minutes and measured the decrease of the respiration rate at the end of the exposure. The effective concentration (EC) of the sample that causes 10% inhibition of the respiration rate is here defined as EC_{10}, which is determined from the dose–response curve. To determine how wastewater affects sensor sensitivity, we compared the results of two cases: one in which ion-exchanged water was used as the solvent of the substances (standard sample); and another in which filtrated wastewater was used as the solvent of the substances (wastewater sample). The wastewater was taken from the Kasumigaura Regional Sewerage System (Kohoku Wastewater Treatment Plant), which primarily treated domestic wastewater.

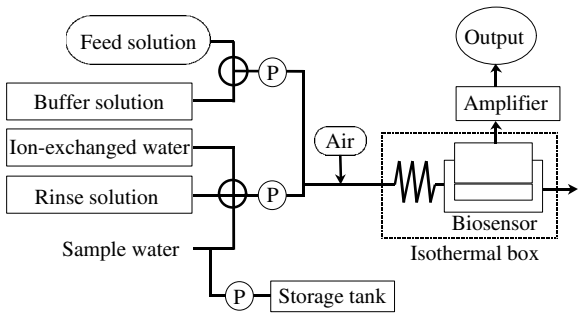

Figure 2 Schematic diagram of the toxicity monitor

Design of a filtration system

The greatest difficulty affecting water quality monitoring units used to continuously monitor wastewater in sewerage systems is the clogging that results from solid particles suspended in the wastewater. The toxicity monitor requires monthly maintenance to add necessary solutions and to change the immobilized microbial membrane. Our current goal is to also conduct the maintenance of the filtration system used to remove sand and suspended particles in the wastewater on a monthly basis for continuous monitoring. In this study, we devised a prototype filtration system (Figure 3) out of a 0.5 mm mesh screen (Toyo Screen S200/500-type ultra TN screen) and a filtration unit using a hollow fiber membrane that has 0.1 μm average pore size (Mitsubishi Rayon STNM424).

Screen. Sample wastewater flowed into upper tank of the screen, and then flowed over the mesh. Suspended solids were blocked on the surface of the mesh and filtrate flowed into the lower tank of the screen. Flow rate of the filtrate was affected by the clogging of the mesh. To prevent it, the surface was brushed manually once a week and the backside was always brushed automatically.

Hollow fiber membrane filtration unit. To prevent suspended particles from adhering to the surface of the hollow fiber membrane, we applied an upward cross current (0.7 m/minute) from the lower end of the membrane. The wastewater was filtrated for 10 minutes, with the suction rate set at 20 ml/min, then the membrane was cleaned by a reverse current of water (inner fiber membrane pressure 100 kPa) for 2 minutes. In addition, the membrane was continuously scrubbed with air from the lower end of the membrane. To prevent the bio-fouling of the filtrate tank, it was cleaned with water several times a day. Timers controlled the washing time and intervals and the primary condition was 1-minute washing every 24 hours.

First, the prototype was installed in a pilot plant of the Public Works Research Institute inside the Kasumigaura Regional Sewerage System (Kohoku Wastewater Treatment Plant). The inner suction pressure of the hollow fiber membrane was continuously monitored by a pressure gauge. As might be expected, the pressure increased as particle accumulation gradually clogged the membrane. But, this pressure increase was not observed during 20 days continuous operation and filtration performed well.

Field test of the toxicity monitoring system

The toxicity monitoring system consists of the prototype filtration system and the toxicity monitor. The apparatus was installed in a wastewater treatment plant and operated for 3

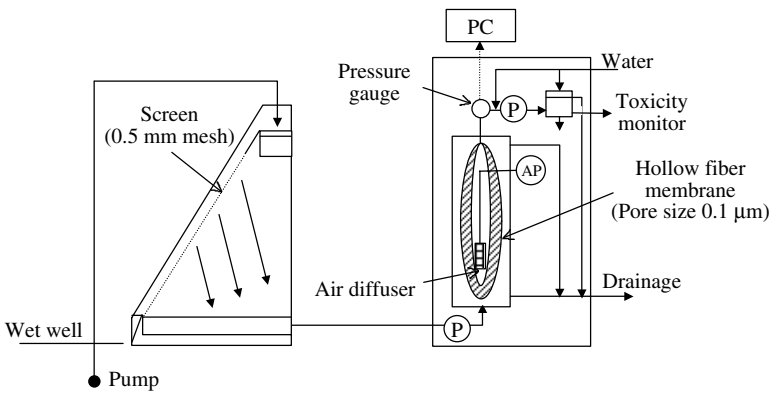

Figure 3 Schematic diagram of the prototype filtration system

months (from November, 2000 to February, 2001). The wastewater sample was pumped up from the wet well. We investigated the relationship between operation conditions and maintenance intervals of the filtration system. In order to check the filtration performance, water quality (SS, COD and pH) of the influent and filtrate was analyzed once a week. We also investigated the sensitivity to acetone used as the artificial toxic substance and the stability of the biosensor during continuous monitoring.

Results and discussion

Sensitivity of the biosensor to various substances

Chlorophenols. Figure 4 shows the response chart of the biosensor to 4-chlorophenol (4-CP) solution varying the concentration. The response to chlorophenols was sharp and the sensor output was recovered soon after the dosage. Figure 5 shows the dose–response curve of 4-CP. Table 1 lists the EC_{10} data to various chlorophenols. We found that there were no obvious differences between EC_{10} data for the standard samples and the wastewater ones.

Figure 6 shows the relationship between EC_{10} data and octanol–water partition coefficients (log P_{ow}). We found that EC_{10} had positive correlation with log P_{ow}, that is to say the sensitivity to hydrophilic substances was higher than that to hydrophobic substances. This result suggested that hydrophilic substances could easily permeate cell membranes of nitrifying bacteria and inhibit the oxidation of ammonia.

Heavy metals. Figure 7 shows the response chart of the biosensor to zinc. Figure 8 shows the dose-response curve of zinc. Table 2 lists the EC_{10} data to various substances. The time required for the output to fall is longer for metals than with chlorophenols and more time is needed for the recovery of sensor output after exposure to solutions containing heavy metals. These results suggested that the inhibition mechanism of heavy metals is different from that of chlorophenols. We also found that there were no obvious differences between EC_{10} data for the standard samples and the wastewater ones just as in the case of chlorophenols.

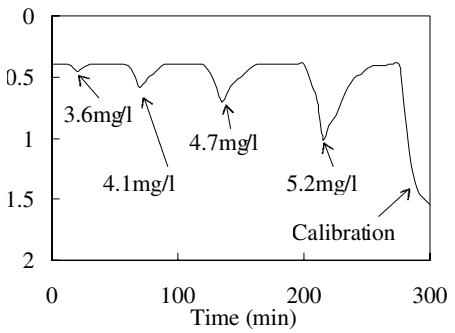

Figure 4 Response chart of the biosensor to 4-CP (standard sample)

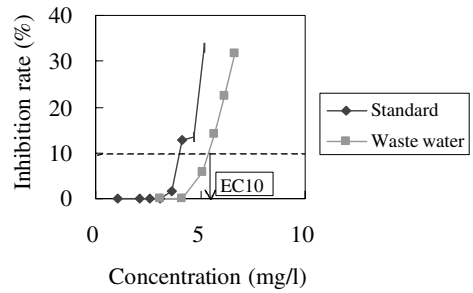

Figure 5 Dose–response curve of 4-CP

Table 1 The sensitivity of the biosensor to chlorophenols. (DCP: dichlorophenol, TCP: trichlorophenol)

Substrate	Standard EC_{10}(mg/l)	Wastewater EC_{10}(mg/l)	Substrate	Standard EC_{10}(mg/l)	Wastewater EC_{10}(mg/l)
Phenol	2.2	2.9	2,5-DCP	7.6	9.1
2-CP	1.3	0.9	2,6-DCP	59	90
3-CP	1.4				
	2.8	3,4-DCP	32	37	
4-CP	4.1	5.4	3,5-DCP	28	33
2,3-DCP	15	12	2,3,4-TCP	280	220
2,4-DCP	40	31	2,4,5-TCP	327	262

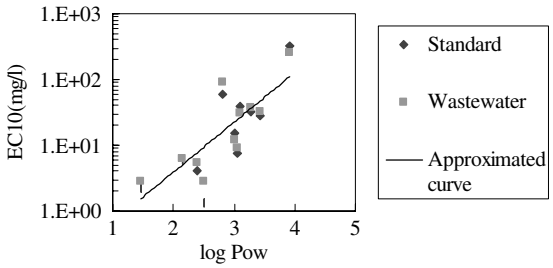

Figure 6 Correlation between EC_{10} and log P_{ow}

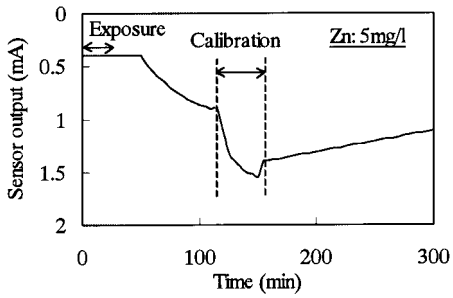

Figure 7 Response chart of the biosensor to Zn

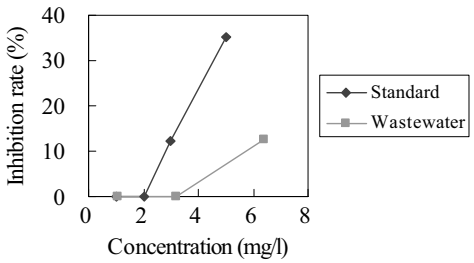

Figure 8 Dose–response curve of Zn

Table 2 The sensitivity of the biosensor to heavy metals

Metals	Substrates	Standard	Wastewater	Response time
		EC_{10}(mg/l)	EC_{10}(mg/l)	(min)
Zn	$ZnCl_2$	2–3	3.2–6.4	45–50
Ni	$(CH3COO)_2Ni$	0.1–1.0	0.1–0.2	20
Cd	$CdSO_4$	1.0–3.0	0.5–1.0	20
Cu	$CuSO_4$	3.0–5.0	0.9–2.8	95

Field test results of the toxicity monitoring system

Filtration system. The screen had no mechanical problems during the test. The decrease of filtrate flow rate by the surface clogging was observed at the weekly maintenance, but it was enough for the hollow fiber membrane filtration unit. Table 3 shows the water quality analysis data. There were some cases when SS and COD of the filtrate were higher than those of the influent. It was caused by the suspended solids retention in the filtrate tank, therefore the structure of the filtrate tank needs to be modified to reduce the load on the hollow fiber membrane.

Hollow fiber membrane filtration unit. Figure 9 shows the suction pressure trend of the hollow fiber membrane. At initial operation there was no increase of the suction pressure for 4 weeks, but it reached to the upper limit (70 kPa) after 5 weeks. It was found that the reason was the decrease in the air scrubbing effect by clogging of the air diffuser. After the modification of the air diffuser position, a second operation was conducted and a suction pressure increase had not been observed for 6 weeks. Ordinarily, the filtrate flow rate did not change. The removal ratio of SS was 100% and that of COD (Table 3) was more than 90% during the field test. These results confirmed that the filtration performance was excellent.

Despite the good filtration performance, a sticky deposit was observed in the filtrate tank after a week of continuous operation. The analysis of the deposit and inner surface of

Table 3 Water quality analysis data of the influent and the filtrate. (100) = 100% removal ratio

	SS (mg/l)			COD (mg/l)			pH		
	Influent	Screen filtrate	Membrane filtrate	Influent	Screen filtrate	Membrane filtrate	Influent	Screen filtrate	Membrane filtrate
12/04/00	210	510 (33)	17 (99)	130	240 (–)	13.6 (90)	8.9	8.1	8.5
12/11/00	610	440 (–)	ND (100)	220	210 (5)	11 (95)	6.1	–	–
12/18/00	410	720 (–)	3.2 (99)	189	270 (–)	11 (94)	7.0	7.7	7.4
12/25/00	284	296 (–)	ND (100)	156	168 (–)	8.7 (94)	7.0	–	–
01/04/01	620	820 (–)	12 (98)	350	350 (–)	17.1 (95)	7.4	8.1	7.7
01/09/01	900	950 (–)	ND (100)	470	460 (2)	6.6 (99)	7.1	7.0	7.1
01/15/01	480	710 (–)	ND (100)	210	240 (–)	8.9 (96)	8.7	7.2	7.2
01/22/01	430	270 (38)	ND (100)	240	170 (29)	6.7 (97)	8.5	8.3	7.3
01/29/01	270	12 (96)	ND (100)	140	140 (0)	5.4 (96)	8.6	–	–
02/05/01	560	290 (48)	ND (100)	180	150 (17)	10 (94)	8.8	8.7	7.8
02/19/01	430	1100 (–)	ND (100)	190	190 (–)	11 (94)	8.5	7.9	7.8

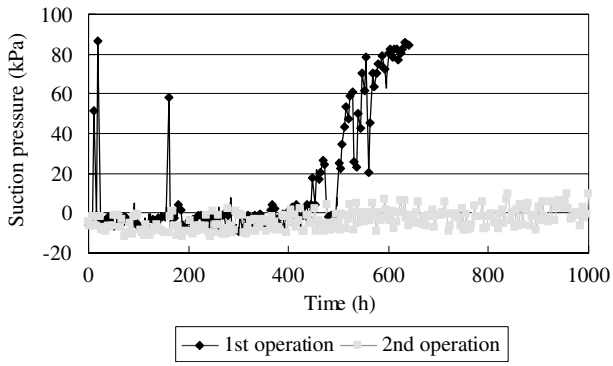

Figure 9 Suction pressure trend of the hollow fiber membrane

the hollow fiber membrane manifested the fact that microbial contamination occurred in the filtrate lines of the filtration unit. Figure 10 shows the SEM image of the deposit. Microbial scale of the filtrate lines flowed into the biosensor and affected the sensor output as described below. In order to contain the microbial contamination, we optimized the operation conditions of the filtrate tank washing. Table 4 shows the results of the optimization. It was found that No.3 condition was enough to retain the monthly maintenance of the biosensor. The cause of the microbial contamination might be propagation through the atmosphere, or a structural problem of the filtration unit.

Toxicity monitor. Figure 11 shows the sensor output trend during continuous monitoring for 1 month. There was no problem with the monitor performance and the sensitivity to 1.0% V/V of acetone solution was 16% inhibition rate after 2 weeks operation. Then, the system alarms occurred several times because of the decrease of the voltage span at the calibration. After 4 weeks operation, the sensitivity to 1.0% V/V of acetone solution decreased to 0% inhibition rate. The microbial scale adhesion to the surface of the immobilized microbial membrane was observed and it caused the inhibition of DO diffusion to the electrode through the immobilized microbial membrane. The result of the optimization as mentioned above will solve this problem.

Conclusions

In this study, we investigated the application of the toxicity monitor using a nitrifying bacteria biosensor to continuous monitoring of the wastewater influent. The results of sensitivity tests show that aspects of wastewater do not affect the sensor sensitivity and

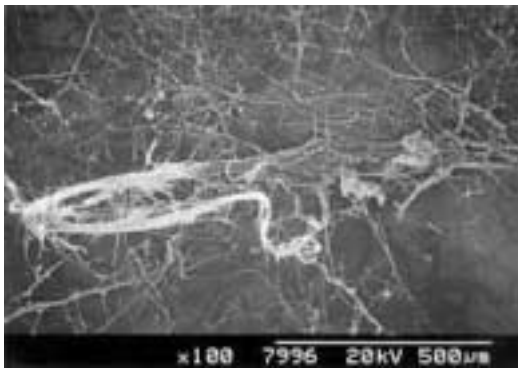

Figure 10 The SEM image of the deposit

Table 4 Operation conditions of the filtrate tank washing

	Washing intervals	Washing time	Occurrence of the microbial scale
No.1	24-h	1-min	1 week
No.2	12-h	2-min	2 to 4 week
No.3	2-h	2-min	None during 4 week

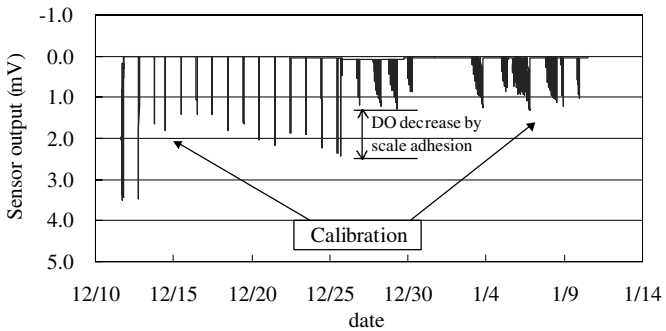

Figure 11 Sensor output trend during continuous monitoring for 1 month

confirm that the sensor can be applied to wastewater monitoring as it is. It was also found that the mechanism of the nitrification inhibition for chlorophenols differed from zinc for heavy metals. The monitor with a prototype of the filtration system installed in a wastewater treatment plant is able to operate continuously for one month at least after the modification of the filtration system and the optimization of operation conditions. This monitoring system is expected to become an important tool for the protection of the biological processing of wastewater treatment.

Acknowledgements

In the field test at the wastewater treatment plant, work was supported by M. Mizuno (Aichi Building Technology Research Center) and R. Hattori (Aichi Water and Greenery Public Corporation).

References

Tanaka, H., Minamiyama, M., Toyoda, T., Komori, K., Tanaka, Y. and Taguchi, K. (1997). Monitoring of the toxicity in water using nitrifying bacteria. *Proceedings of the 7th Japanese-German Workshop on Wastewater and Sludge Treatment*, 1–12, Public Works Research Institute.

Tanaka, Y., Taguchi, K., Ohoto, T., Hosikawa, T., Sasaki, H., Mitumori, H., Saito, Y. and Kashimura, O. (1996). Development of continuous automatic apparatus for acute toxicants using biosensor. *EICA*, **1**, 250–253.

Tanaka, H., Minamiyama, M., Toyoda, T., Komori, K., Tanaka, Y. and Taguchi, K. (1998). Development of a toxicity monitor using bacteria, *Proceedings of WEFTEC '98 Water Environment Federation*, Vol.4, 165–180.