Biosensor-based control of nitrification inhibitor in municipal wastewater treatment plants

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Abstract The effect of potassium cyanide (KCN) on nitrification processes in municipal wastewater treatment plants was studied by batch nitrification tests, which indicated that nitrification processes tend to be inhibited at a lower KCN concentration than the present discharge standard to sewerage. The experiment of the biosensor using nitrifying bacteria was also conducted for continuous monitoring of nitrification inhibitor in influent wastewater, and demonstrated that the biosensor can detect KCN at as low as EC10 of the abovementioned batch nitrification test. Moreover, to determine the effectiveness of application of the biosensor to avoid the impact of KCN due to an accidental spillage in a sewerage system, KCN was intentionally injected into the experimental models of activated sludge process equipped both with and without the biosensor. The model with the biosensor that could detect KCN could divert the wastewater including KCN to a refuge tank, which resulted in the avoidance of upset of the activated sludge process. On the other hand, the model without the biosensor was upset in the nitrification process due to KCN. Such differences demonstrate the effectiveness of the biosensor applied to countermeasures of an accidental spillage of toxic chemicals to avoid upset of nitrification in municipal wastewater treatment plants.
Keywords Biosensor; chemical spill; cyanide; nitrifying bacteria; wastewater treatment plant

Introduction

The fate of chemical compounds in the environment and their effect on the ecosystem has been of great concern since the 1980s. Sewage works collect domestic sewage and industrial wastewater, treat them and discharge into the water environment. Because domestic sewage and industrial wastewater contains various natural and artificial chemical compounds, an issue of chemical compounds in sewage works has been of great concern. In the Japanese legal system concerning the management of chemical compounds in sewerage, the Sewerage Law provides that the sewage works administrator can regulate wastewater from factories, in case of need, to protect the facilities from wastewater which injures them and prevents their function. However, at present, the discharge standards to sewerage are not determined in terms of protection of biological treatment function. The discharge standards to sewerage are determined in imitation of the Environmental Quality Standards for Water Pollution. However, the Environmental Quality Standards for Water Pollution provided by the Water Pollution Control Law were mainly determined in terms of the effect of chemical compounds on human health. Most sewage treatment plants in Japan have introduced the conventional activated sludge process and BOD and SS have been eliminated in the plant. Generally speaking, function removing BOD and SS is not sensitive to chemical substances, so the effect of chemical compounds to biological wastewater treatment function has never been seriously recognized. However, recent elimination of

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COD, nitrogen and the phosphorus in the discharge from sewage treatment plants is needed for the prevention of eutrofication, and advanced wastewater treatment processes are being installed. Generally, the biological nitrogen removal process, which consists of a nitrification de-nitrification process, is used for nitrogen removal in municipal wastewater treatment process. We found that some non-regulated chemical compounds affect the nitrification process and some regulated chemical compounds below regulation affect nitrification process. It is necessary to review the existing discharge standards to sewerage in terms of protection of the nitrification process, which is one of the functions of sewerage facilities. Meanwhile, according to a report by the Ministry of Land, Infrastructure and Transport of Japan, the number of chemical spills from a sewerage system caused by improper operation of pretreatment facilities for discharge to a sewerage system is increasing year by year. It is very difficult for a sewage works administrator to detect a chemical spill during daily monitoring and inspection. Toxic chemical compounds sometimes enter into biological treatment process, and affect the function and effluent water quality. It is indicated that the daily monitoring system (i.e. item and frequency) should be improved. However, it is actually impossible for a sewage works administrator to monitor a huge number of harmful chemical compounds with high frequency. Therefore, this situation requires an effective monitoring system. In our previous study, the bioassay method, which assesses toxicity overall, was applied to continuous water quality monitoring. The bioassay method uses nitrifying bacteria, which play an active part in the nitrification process in the wastewater treatment process (Tanaka *et al.*, 1998; Inui *et al.*, 2002). The bacteria are fixed into a toxicity biosensor that was developed by the Public Works Research Institute and Fuji Electric Co Ltd. Moreover, a toxicity biosensor is applied to influent wastewater quality monitoring on trials.

In this study, we focus on potassium cyanide (KCN) because it has often been reported that cyanide compounds enter into sewerage and affect the wastewater treatment function. Moreover:

(1) the concentration of KCN which affects nitrification process in wastewater treatment plant, was investigated
(2) A nitrification inhibitor in the influent wastewater was continuously monitored using a toxicity biosensor
(3) KCN in the influent wastewater was detected
(4) influent wastewater containing KCN was led into an emergency tank and the biological wastewater treatment process was protected from nitrification inhibitor.

Understanding the concentration of potassium cyanide which affects the nitrification process by batch nitrification examination

Method. In order to estimate the concentration of potassium cyanide which affects the nitrification process, a modified batch nitrification rate examination (see Figure 1) was conducted. Detail of the method are as follows:

- reactors were prepared whose capacity is approximately ten times as the much as volume of the total sample. In each reactor, the air is continuously supplied through an air stone by an air pump
- Mixed liquor was collected from the aeration tank of a pilot plant which was set at Kohoku WWTP in Ibaraki prefecture, Japan. The pilot plant accepts actual raw wastewater and had been operated by conventional activated sludge method. The raw wastewater consists of mainly domestic wastewater because there are few factories in the sewered area
- 1 L of the mixed liquor was distributed into the reactor

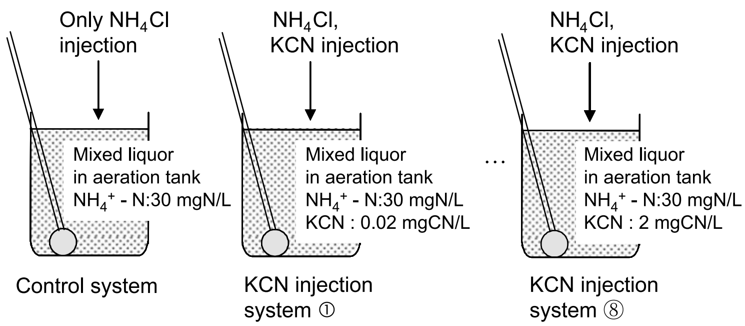

Figure 1 Estimation of effect of KCN to nitrification process using modified batch nitrification examination

- an ammonium, which is an essential substrate for nitrifying bacteria to grow, was added into the reactor. An initial concentration of ammonium was set at 30 mg/L as nitrogen
- the one of the reactors was made to be a control system. Moreover, a potassium cyanide solution was added into the other reactors and the initial concentration of potassium cyanide in the reactor ranged from 0.02 to 2 mg/L as cyanide
- a concentration of dissolved oxygen in the reactor was kept at more than 2 mg/L, and mixed liquor was grabbed every 2 h and filtered with glass fiber paper filter. A concentration of ammonium, nitrite and nitrate in filtrate was measured by Ion chromatography
- the nitrification rate is calculated from the nitrate production rate.
  Nitrification rate [mgN/L × hr] = Increase of nitrate concentration [mgN/L]/Time [h]
- the ratios of nitrification rate in the reactors to nitrification rate in the control system were calculated
- the dose–response relationship between concentration of KCN and nitrification rate was determined.

The examinations were conducted in a thermostatic room. The room temperature was controlled at 20 °C. The temperature in the mixed liquor in the reactors ranged from 19 to 21 °C.

Result and discussion

Figure 2 shows relationship between the concentration of KCN in the reactor and nitrification rate. Moreover, a regression curve determined by Probit analysis is shown in the figure. No-effect concentration was 0.05–0.1 mg CN/L and the EC50 value of KCN was calculated to be 0.31 mg CN/L. These values were lower than the discharge standards to sewerage (1 mg CN/L). The result indicates that even if a industrial wastewater from a factory meets the regulation, unless the industrial wastewater is well diluted, the cyanide compounds in the wastewater can affect the nitrification process in a wastewater treatment plant. In order to protect the function of sewerage, the existing discharge standards to sewerage would likely need to be reviewed. Furthermore, conditions (i.e. temperature, MLSS, pH, DO and so on) in the batch nitrification experiment are a little different from the conditions in an actual wastewater treatment plant. Therefore, there is the possibility that the result in the batch nitrification examination is different from the characteristics in an actual wastewater treatment process. So, examination using a large scale reactor must be conducted in order to completely reproduce the actual wastewater treatment process.

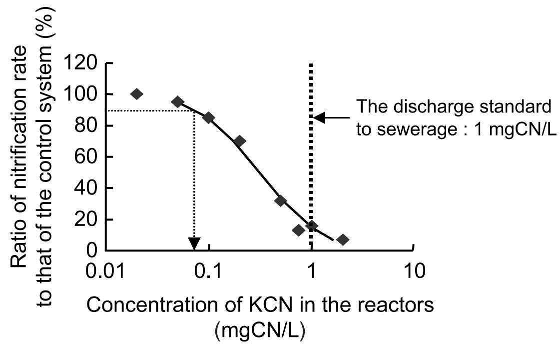

Figure 2 Estimation of effect of KCN to nitrification process using modified batch nitrification examination

Detection of KCN by biosensor and separation of KCN from a biological wastewater treatment process

Toxicity biosensor. A biosensor consists of two components: a bioreceptor and a transducer. The bioreceptor is a biomolecule that recognises the target analyte, whereas the transducer converts the recognition event into a measurable signal. The bioreceptor of a toxicity biosensor is an immobilised microbial membrane, in which pure-cultured nitrifying bacteria (*Nitrosomonas europaea*: ATCC25978) are contained. Respiration of the nitrifying bacteria is easily inhibited by various toxic substances. The transducer of a toxicity biosensor is a dissolved oxygen probe which is attached to the immobilised microbial membrane. The sample solution was injected at rate of 3.5 ml/min, and mixed with a feed solution and air from an air pump. Then, the sample solution is saturates with oxygen and is sent to the measuring part.

1. Zero calibration. In zero calibration, purified water is used as the sample solution. The respiration rate is defined as 0% when the feed is ammonia-free buffer solution.
2. Span calibration. In span calibration, purified water is used as the sample solution. The respiration rate is defined as 100% when the feed solution contains ammonia and buffer solution.
3. Assay the sample solution. With constant feeding of the feed solution (containing standard ammonia and buffer solution) with the 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 toxic 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. If the output decreases less than 90% of the respiration rate, the monitor issues a warning and orders the pump to take a sample for water quality analysis.

Detection of potassium cyanide by the biosensor. Response of the biosensor to KCN is as follows. The output value after 20 min exposure of 0.01, 0.05, 0.1 and 0.5 mg/L KCN is shown in Figure 3. Usually, the biosensor recognises inflow of nitrification inhibitor when the output value drops to less than 90%. Therefore, it seems that biosensor can detect 0.05–0.1 mg/L KCN. The detection limit by the biosensor was almost equal to the EC10 value in the batch nitrification examination, even though the exposure time in the biosensor was much shorter than that in the batch nitrification examination. Therefore, it was experimentally proved that the biosensor can detect the existence of a cyanide compound which can affect nitrification process.

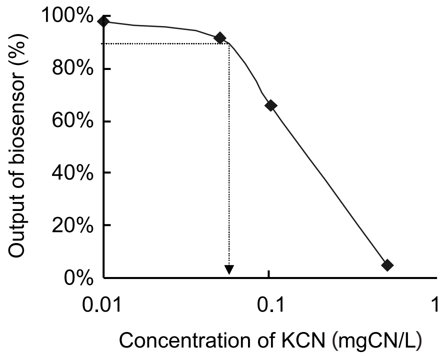

Figure 3 Response of the biosensor after 20 min KCN solution exposure

Detection of KCN by the biosensor and separation of wastewater containing KCN from a biological wastewater treatment process. If a nitrification inhibitor continuously flows into sewerage, the sewage administrator can relatively easily find the inflow of the nitrification inhibitor because the nitrification process is inhibited even if the operation condition is properly set up. On the other hand, in the case of a sudden inflow of nitrification inhibitor by an accidental spill and so on, it is very difficult to find the inflow and identify the source. Because the trend of concentration of nitrification inhibitor in influent depends upon the magnitude of the spill, hydraulic characteristics of catchment area and so on, it is very difficult to understand the mechanics. Very little information is available regarding the occurrence of inflow of nitrification inhibitor into a wastewater treatment plant. Therefore, no measure against a sudden inflow of nitrification inhibitor has been taken in any wastewater treatment plant. Now we are trying to apply a biosensor for continuous monitoring of an wastewater treatment plant influent and gather information about the inflow of nitrification inhibitor into a wastewater treatment plant. If actual/concrete information about the occurrence of inflow of nitrification inhibitor can be collected, a measure adapted to the occurrence will be devised. At present, one of the possible measures is that the biosensor detects nitrification inhibitor in wastewater, separates the wastewater from biological reactor and leads the wastewater to an emergency tank. In this study, a certain concentration of potassium cyanide has been injected for a certain period into a wastewater treatment plant model. It is examined whether a biosensor can detect the potassium cyanide and lead to an emergency tank or not.

Method

Figure 4 shows the schematics of an experimental model. A part of actual wastewater treatment plant influent of Kohoku STP in Ibaraki prefecture was continuously taken. The wastewater was then passed through a screen (Type S200/500 Ultra TN Screen manufactured by Toyo Screen Kogyo Co. Ltd.). Large solids, such as hair and toilet paper, were removed by the screen. The filtrate was led into both the biosensor and the experimental model. Two systems of an conventional activated sludge process model were operated. One system was not controlled by the biosensor and was designated as the system without control by the biosensor. The other system was controlled by the biosensor and was thus defined as the system with control by biosensor. In both the systems, inflow flow rate was 288 L/d (= 200 mL/min) and the volumes of the primary sedimentation tank, aeration tank and final sedimentation tank were 50, 100 and 50 L, respectively. The aeration tank was divided into ten units in order to simulate plug flow in an actual wastewater treatment plant. Hydraulic retention time of the primary sedimentation tank, the

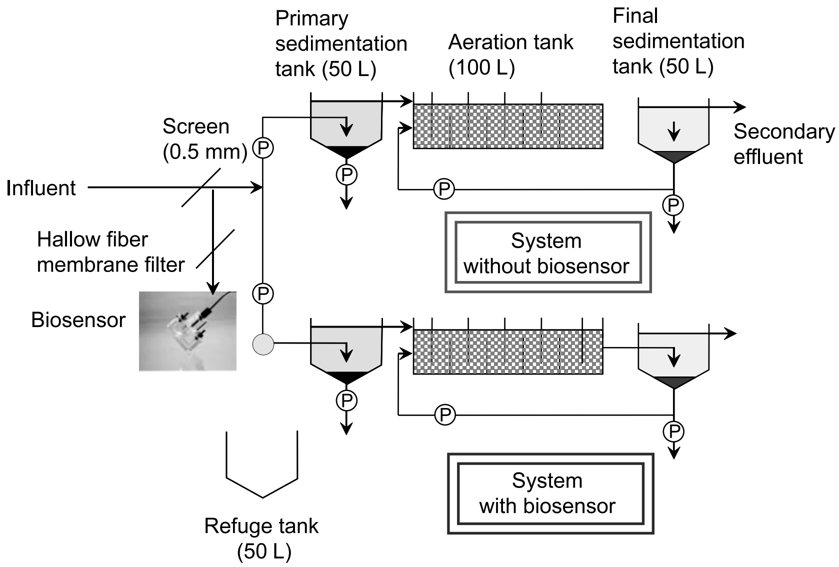

Figure 4 Experimental apparatus and procedures

aeration tank and the final sedimentation tank was set at 4.2, 8.3 and 4.2 h, respectively. The ratio of return sludge flow rate to influent flow rate was set at 30%, and 4 L of excess sludge a day was removed from both the systems and A-SRT became about 20 days. Nitrification was promoted in the aeration tank, and almost no ammonium was found in the final effluent. Initial activated sludge had been taken from an actual wastewater treatment plant operated by the conventional activated sludge process more than 2 months before. Then, the following examinations were conducted in a day when no effect by stormwater was observed. Influent was filtered by a hallow fiber membrane filter with 0.2 mm pore to avoid clogging the pipe in the biosensor. The concentration of potassium cyanide was set to be 2.23 mg CN/l in influent and the injection of KCN was continued for 2 h. 53.5 mg KCN was added to the model controlled by the biosensor. If KCN is completely the mixed in the model, concentration of KCN becomes 0.268 mgCN/L. The concentration is almost equal to the EC50 value in the batch nitrification experiment.

Results and discussion

Figure 5 shows the response of biosensor after the injection of potassium cyanide. It took 15 min before the biosensor began to respond to the inflow of potassium cyanide. After

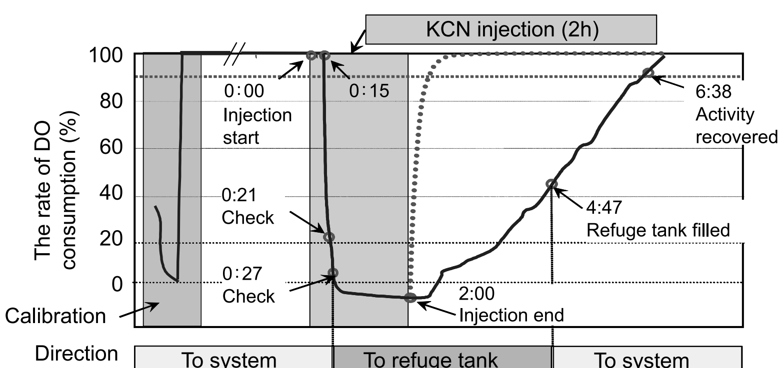

Figure 5 Response of the biosensor to KCN inflow

15 min, the activity of nitrifying bacteria declined and the rate of dissolved oxygen consumption decreased and the dissolved oxygen value increased. The biosensor checks the dissolved oxygen value every 6 min. If the rate of dissolved oxygen consumption becomes less than 90% compared to the span calibration, the biosensor regards this condition as an inflow of nitrification inhibitor. In order to avoid false operation, the biosensor issues a warning when it detected an consecutive nitrification inhibitor. Therefore, the biosensor first found the inflow of potassium cyanide after 21 min, and issued a warning after 27 min. At the same time, the direction of influent flow was automatically changed by an electrical signal from the biosensor and influent containing potassium cyanide was led into a vacant tank. After the injection of potassium cyanide, while the concentration of KCN in influent drastically decreased, the output of the biosensor did not recover drastically. There is a possibility that the KCN remained inside the pipe and continued to affect nitrifying bacteria. On the other hand, another explanation is that nitrifying bacteria was seriously damaged and took a long time to recover the activity. Even though the inflow of nitrification inhibitor finished, the output of the biosensor was not recovered. This situation needs to be improved. The trend of dissolved nitrogen compounds in secondary effluent is shown in Figure 6. Before the injection of potassium cyanide, nitrification was promoted in the activated sludge process and a major form of dissolved nitrogen compounds in secondary effluent was nitrate in both the systems. In the system without control by the biosensor, influent containing potassium cyanide flowed into the system and potassium cyanide is spread in the primary sedimentation tank, the aeration tank and the final sedimentation tank by convection and diffusion. The formation of dissolved nitrogen compounds in the secondary effluent began to change after 4 h, ratios of nitrite and ammonium increased. The change of trend seemed to be caused by nitrification inhibition. Meanwhile, no great change of organic compounds removal was observed. On the other hand, in the system with control by the biosensor, influent containing potassium cyanide had flowed into the biological wastewater treatment system for 27 min until the direction of influent flow was changed. From 27 min to 4 h 47 min later, the influent containing potassium cyanide had flowed into the emergency tank, and the biological wastewater treatment process had been protected from nitrification inhibitor. While the influent was led into the emergency tank, overflow from the final sedimentation tank was stopped and activated sludge circulated between the aeration tank and the final sedimentation tank. After the emergency tank was filled with the influent, the direction of influent flow was automatically changed and influent flowed into the biological wastewater treatment system again. After that, overflow from the secondary sedimentation tank was restarted. The major formation of dissolved nitrogen compounds in the secondary effluent was nitrate and the nitrification function was maintained. We found that monitoring of

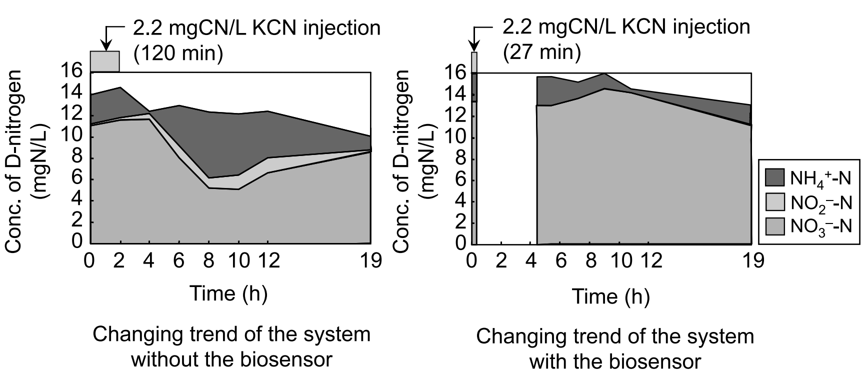

Figure 6 Changing trend of nitrogen compounds in secondary effluent

nitrification inhibitor in wastewater treatment plant influent by the biosensor and control using an emergency tank is effective to protect biological wastewater treatment process. There are problems to be solved. One of the problems is how to deal with the time lag between inflow of nitrification inhibitor and response to the inflow in the biosensor. One possible solution is that the biosensor is put upstream. Once the nitrifying bacteria of the biosensor is exposed to a high concentration of nitrification inhibitor, the bacteria loses its reversibility. Therefore, the relationship between concentration of nitrification inhibitor and characteristics of recovery of output needs to be understood. Moreover, another problem is how to design the emergency tank and operate it. The method for design and operation needs to be decided based on information of magnitude of spill accident.

Disposal of wastewater containing nitrification inhibitor

When wastewater a containing nitrification inhibitor is disposed of, at first, the nitrification inhibitor needs to be identified by analyses. Then, proper measure against the identified nitrification inhibitor are chosen. One of the possible measures is dilution and treatment in the activated sludge treatment process. In that case, the contaminated wastewater must be diluted in order to not affect the nitrification process, and in order that effluent quality meets effluent quality standards. In this study, dilution and treatment in the activated sludge treatment process was adopted and the effect on the nitrification process was estimated by the following procedure.

Method

In this study, the nitrification inhibitor contained in the wastewater in the emergency tank is already identified as potassium cyanide. Concentration of potassium cyanide is approximately 2.23 mg/L as cyanide. Because the effluent quality standard regarding cyanide compounds is set at 1 mg/L, the wastewater should be diluted more than 2.23 times with influent wastewater containing no cyanide compound. Moreover, in order to estimate the effect on the nitrification process by returning the wastewater separated into the emergency tank, a modified batch nitrification rate examination was conducted (see Figure 7). A concrete procedure is as follows:

- A reactors were prepared whose capacity is approximately ten times the volume of the total sample. In each reactor, the air is continuously supplied through an air stone by an air pump
- the wastewater treatment plant influent and return sludge are taken from an actual wastewater treatment plant which was not polluted by nitrification inhibitor. In this study, the influent was taken from Kohoku WWTP of Ibaraki prefecture and the return sludge was taken from a pilot plant operated by a conventional activated sludge process established in the Kohoku wastewater treatment plant
- the one of the reactors was made to be a control system. Influent and return sludge (55:25) were poured into the reactor

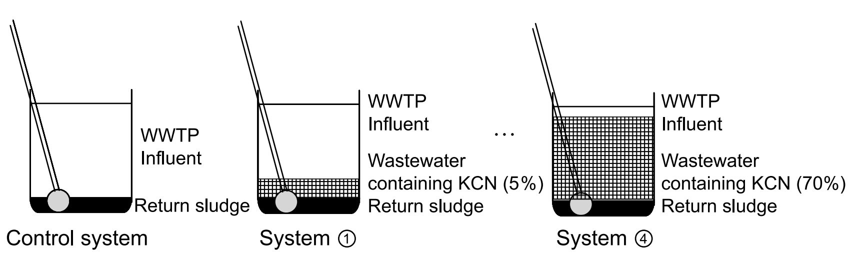

Figure 7 Estimation of effect of wastewater containing KCN to nitrification process using modified batch nitrification examination

- a mixture of influent, wastewater containing KCN and return sludge were poured into the other four reactors. Ratios of wastewater containing KCN were set at 5, 10, 20 and 70%
- a concentration of dissolved oxygen in the reactor is kept at more than 2 mg/L, and mixed liquor was grabbed every 2 h and filtered with a glass fiber paper filter. A Concentration of ammonium, nitrite and nitrate in filtrate was measured by ion chromatography
- the nitrification rate is calculated from nitrate production rate
  Nitrification rate [mgN/L × hr] = Increase of nitrate concentration [mgN/L]/Time [h]
- ratios of nitrification rate in the reactors to nitrification rate in the control system were calculated
- the dose-response relationship between ratio of wastewater containing KCN and nitrification rate was determined.

The examinations were conducted in a thermostatic room. The room temperature was controlled at 20 °C. Temperature in the mixed liquor in the reactors ranged from 19 to 21 °C.

Result and discussion

Figure 8 shows the relationship between the ratio of the contaminated wastewater and nitrification rate. Moreover, a regression curve determined by Probit analysis is shown in the figure. No observed effect ratio of the contaminated wastewater was approximately 6.4%. The result indicated that the contaminated wastewater can be treated in the activated sludge treatment process if the ratio of the contaminated wastewater to influent is set at less than 6.4%. When the contaminated wastewater is treated, the amount of influent increases, and the hydraulic retention time in the treatment process becomes shorter. Because drastic shortening of HRT makes the effluent quality worse, the flow amount of the contaminated wastewater is limited. In this study, the ratio of the flow of the contaminated wastewater to that of influent was set at 5%, and the contaminated wastewater was treated. The returning of the contaminated wastewater was started 1 week after the injection of KCN. It seemed that the cyanide potassium which entered into the system controlled by the biosensor had already been washed out because cyanide potassium is a hydrophilic substance. Figure 9 shows the trend of forms of dissolved nitrogen compounds in the secondary effluent after the return. We found that the ratio of nitrate was maintained at more than 80% and the effluent quality did not worsen, and the contaminated wastewater could be treated.

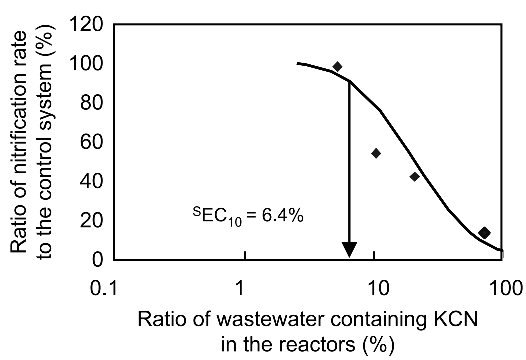

Figure 8 Estimation of effect of wastewater containing KCN to nitrification process

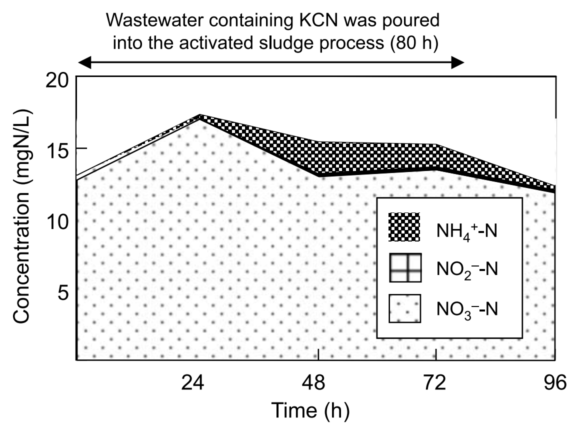

Figure 9 Changing trend of dissolved nitrogen compounds in secondary effluent

Summary and conclusion

(1) The effect of cyanide compounds to the nitrification process in a wastewater treatment plant was studied by modified batch nitrification examination. The result indicates that even if the concentration of the cyanide compound is below the standard it affects the nitrification process.

(2) A biosensor using nitrifying bacteria was applied for continuous monitoring of nitrification inhibitor in wastewater treatment plant influent. A biosensor could detect potassium cyanide at the same concentration as NOEC in the batch nitrification examination.

(3) In the model of the conventional activated sludge process, potassium cyanide, which is a nitrification inhibitor, was intentionally injected in the influent, and detected by a toxicity biosensor. Moreover, wastewater treatment plant influent containing potassium cyanide was automatically led into an emergency tank and separated from the biological wastewater treatment process. This control was effective function of biological treatment. On the other hand, there were problems to solve. A quick response by the biosensor seems to be required and how to design and operate an emergency tank should be decided.

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