

Modeling of Recycling Oxidic and Anoxic Treatment System for Swine Wastewater Using Neural Networks

Jung-Hye Choi¹, Jun-Il Sohn², Hyun-Sook Yang³, Young-Ryun Chung³, Minho Lee², and Sung-Cheol Koh^{1*}

¹Division of Civil and Environmental Engineering, Korea Maritime University, Pusan 606-791, Korea

²Department Sensor Engineering, Kyungpook National University, Taegu 702-701, Korea

³Department of Microbiology, Gyeongsang National University, Chinju 660-701, Korea

Abstract A recycling reactor system operated under sequential anoxic and oxidic conditions for the treatment of swine wastewater has been developed, in which piggery slurry is fermentatively and aerobically treated and then part of the effluent is recycled to the pigsty. This system significantly removes offensive smells (at both the pigsty and the treatment plant), BOD and others, and may be cost effective for small-scale farms. The most dominant heterotrophic were, in order, *Alcaligenes faecalis*, *Brevundimonas diminuta* and *Streptococcus* sp., while lactic acid bacteria were dominantly observed in the anoxic tank. We propose a novel monitoring system for a recycling piggery slurry treatment system through the use of neural networks. In this study, we tried to model the treatment process for each tank in the system (influent, fermentation, aeration, first sedimentation and fourth sedimentation tanks) based upon the population densities of the heterotrophic and lactic acid bacteria. Principal component analysis (PCA) was first applied to identify a relationship between input and output. The input would be microbial densities and the treatment parameters, such as population densities of heterotrophic and lactic acid bacteria, suspended solids (SS), COD, NH₄⁺-N, ortho-phosphorus (o-P), and total-phosphorus (T-P). Then multi-layer neural networks were employed to model the treatment process for each tank. PCA filtration of the input data as microbial densities was found to facilitate the modeling procedure for the system monitoring even with a relatively lower number of input. Neural networks independently trained for each treatment tank and their subsequent combined data analysis allowed a successful prediction of the treatment system for at least two days.

Keywords: piggery slurry, neural network, principal component analysis (PCA), heterotrophs, lactic acid bacteria (LAB), *Alcaligenes faecalis*

INTRODUCTION

Swine wastes may cause a serious deterioration of water quality, such as eutrophication and spread of pathogens in water bodies [1]. The daily volume of livestock wastewater in Korea reached 197,000 m³, while 50% of this volume was generated from dairy farms that are exempt from a legal pollution control. The amount of wastewater is relatively small compared with total wastewater including industrial and domestic wastewater (7% of the total), but contributes significantly to the pollution of the receiving waters because of its high organic nutrient concentration (>BOD 20,000 mg/L) [2]. According to the environmental protection law, the large size farms (more than 1,000 heads) are subjected to regulations for treatment facilities whereas small or middle size farms (less than 1,000

heads) are exempt from the regulations. The number of swine heads under the regulatory control, therefore, takes only 31% of the total number of heads [3].

Recently, a recycling reactor system operated under sequential oxidic and anoxic conditions for the treatment of swine wastewater has been developed, in which piggery slurry is fermentatively and aerobically treated and then its effluent recycled to the pigsty [4]. This system appears to significantly remove offensive smells (at both the pigsty and the treatment plant) and BOD, and turns out to be cost effective for relatively small size farms.

There are several treatment steps in the system. For its successful operation, it will be necessary to monitor microbial population density and treatment parameters. Modeling relationships among these variables will be useful in predicting treatment effects and managing the system. One of the best known models applied for wastewater treatment systems so far is the activated sludge model No. 1 (ASM 1) introduced by International Association for Water Quality (IAWQ) in 1987

*Corresponding author

Tel: +82-51-410-4418 Fax: +82-51-410-4415

e-mail: skoh@hanara.kmaritime.ac.kr

[5]. Application of the model to field treatment system, however, may have some limitations because there are many operational parameters and kinetic changes over time in the treatment system [6]. Neural network models that imitate the functions of the human brain have been successfully used for many engineering problems such as complex pattern classification and control of highly nonlinear dynamic systems [7-10]. Those models have the characteristic of allowing massive parallelism, many degrees of freedom, and adaptive learning. It was recently shown that the multi-layer neural network can approximate a function in L^p within an arbitrary accuracy [11], and can generalize a new data set that was not used in the learning process [12]. Recently, progress has been made in application of neural networks to control biological and chemical engineering processes. There has been, however, no report dealing with a neural network model for biological swine wastewater treatment systems.

This study was carried out to investigate the mechanistic basis of the recycling treatment system in terms of population dynamics and activity (treatment effect) of the indigenous heterotrophic bacteria, by establishing a non-linear model emulator using multi-level neural networks. The eventual goal of this study will be to construct a real time monitoring system of the recycling treatment for swine waste using a multi-layer neural network with an error back propagation learning algorithm. The multi-layer neural networks contribute to modeling the complex relationship between the various population densities of microorganisms and treatment effects of the recycling system for piggery slurry.

MATERIAL AND METHODS

System Overview

A scheme for the recycling treatment system at a bench scale is shown in Fig. 1. Piggery slurry and treated effluent used as washing water were collected in Tank 1, and this influent then flows into the fermentation tank (Tank 2; 15 L). There is a channel between Tank 2 and an aeration tank (Tank 3; 15 L) so that the fermented wastewater can be transported into Tank 3 where oxidative treatment occurs under aerobic conditions (7.8 vvm air). The treated water then goes through a sedimentation process in Tanks 6 and 7, and finally is stored in Tank 8. A portion of the effluent was recycled and used to wash the pigsty. The wastewater used in this study was sampled from a mixing and storage tank at a Kimhae piggery slurry treatment plant and had a COD of 4,000 (mg/L), BOD of 7,000 (mg/L), T-N of 2,100 (mg/L), and T-P of 172 (mg/L). The influent consisted of piggery slurry (33%, v/v), effluent (57%) and tap water (10%) and was supplied every 4 days. Glucose was added to the formulated influent to make a C/N ratio of 100:15 [13] and a microbial agent (YC2000, Yoonchang Agricultural Management, Inc., Cheju, Korea) was also added up to 1 % (w/v). The hy-

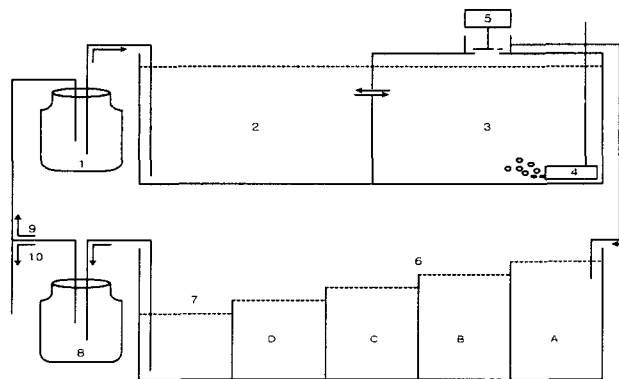


Fig. 1. Schematic diagram of the recycling treatment system for piggery slurry; (1) Influent tank, (2) Fermentation tank, (3) Aeration tank, (4) Blower, (5) antifoaming device, (6) Sedimentation tanks (A, B, C and D), (7) Reservoir, (8) Storage tank, (9) Recycling flow, (10) For land application.

draulic retention time of the system was 4 days and was operated for 47 days.

Isolation and Identification of Microorganisms

Heterotrophic bacteria potentially involved in the piggery slurry treatment within the system were isolated using selective media [14]. To isolate and grow lactic acid bacteria (LAB), MRS medium was used. The ingredients of the medium were, per liter: Bacto proteose peptone NO.3 5 g, yeast extract 2.5 g, dextrose 10 g, Tween 80 g, $(\text{NH}_4)_2\text{HC}_6\text{H}_5\text{O}_7$ 1 g, $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$ 4.14 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.1 g, $\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$ 0.04 g, $\text{Na}_2\text{PO}_4 \cdot 12\text{H}_2\text{O}$ 2.5 g, Beef extract 10 g; pH 6.5. After autoclaving, a trace amount of bromophenol blue was added as an indicator. LAB were grown at least 2 weeks before identification and counting were performed. Other heterotrophs were grown on TSA (Difco Trypticase Soy Agar) for at least 1 week, and then identified and counted.

The bacterial communities in the system were analyzed based on their isolation, identification and determining the colony forming unit number (population density) of each dominant populations in each medium. Identification of the bacteria was done using the selective media and differences in their physiological and biochemical characteristics as described by Smibert *et al.* [15] and Holt *et al.* [16]. Utilization of sugar, amino acids and organic acids by Gram negative bacteria were tested using an API Kit (Bio Merieux SA, France) according to the manufactures protocol.

Analytical Methods for Piggery Slurry from the Treatment System

Monitoring parameters, such as SS, T-N, $\text{NH}_4^+\text{-N}$, T-P, o-P and COD, were measured following the Standard Methods for the Examination of Water and Wastewater

[17]: COD by closed reflux, titrimetric method, T-P and *ortho*-P by ascorbic acid method, suspended solids by total suspended solids cride method, and $\text{NH}_4^+\text{-N}$ by indol phenol method.

Modeling of the Treatment System Using Neural Networks

For an optimal treatment of piggery slurry, it is important to understand the physiological characteristics of microorganisms and their relationships, but may be difficult to identify the relationship by a linear analytical method. The relationship between the population densities of the microorganisms and the treatment efficiency shows a nonlinear dynamic characteristic. We used a multi-layer neural network with an error back propagation algorithm to model the complex relationship in the recycling system. Since the multi-layer neural network is able to approximate an arbitrary nonlinear function with sufficient input and output data, the modeling of the recycling piggery slurry treatment system can be accomplished using the neural network for complex dynamic systems. For modeling of the recycling system, we considered a cause and effect relation in each tank. As independent parameters in each tank, the population densities of the microorganisms, MRS type 1 and TSA types 1, 2, 3, were considered because those could significantly affect the treatment efficiency for piggery slurry. Also, COD, total-P, *ortho*-P, SS and $\text{NH}_4^+\text{-N}$ were considered as treatment parameters in each tank. Thus, we designed a multi-layer neural network in which the input nodes consisted of 4 independent parameters in any given tank and 5 treatment results from the previous tank, and the output nodes were generated as the 5 treatment results in the given tank.

To model the recycling system, there are two ways to use the neural network. One is to use a single neural network for modeling the characteristics of all the tanks in the recycling system shown in Fig. 1. The other is to use the neural network to model the characteristics of each tank followed by the serial connection of these neural networks according to the tank each modeled, allowing the a monitoring of the entire recycling system. In this study, it was difficult to model the overall characteristic of all tanks by a single neural network because each tank in the treatment system has different role and characteristics. Thus, we used a serial neural network that models the characteristic of each tank, and the overall model of whole system was obtained by the connection of each neural network. Fig. 2 shows a proposed modeling protocol for the recycling system. We used principal component analysis (PCA) as a pre-processor of the neural network, which allowed the reduction of the input to each neural network to 3 principal values from 9 independent parameters. Target data of the neural network was the COD, total-P, *ortho*-P, SS and $\text{NH}_4^+\text{-N}$ in the current tank.

To accomplish a successful modeling, the connectivity within the neural networks was adjusted to best predict the measured values obtained at the following

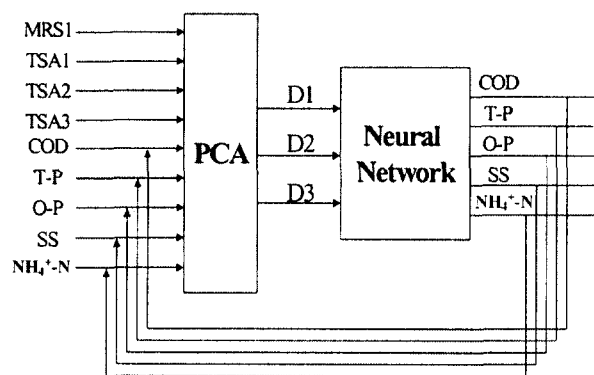


Fig. 2. A schematic diagram describing training strategy for the neural networks in this study.

treatment step using the values for SS, T-N, $\text{NH}_4^+\text{-N}$, T-P, *o*-P and COD as input variables.

RESULTS AND DISCUSSION

Population Dynamics of Microorganisms and Their Roles

The most dominant heterotrophic bacteria in the treatment system were 4 aerobic bacteria and 3 lactic acid bacteria (LAB). The identified organisms were TSA-1 (*Brevundimonas diminuta*), TSA-2 (*Abiotrophia defectiva*), TSA-3 (*Alcaligenes faecalis*) and MRS-3 (*Streptococcus* sp). One of the most dominant aerobes was identified as *Alcaligenes faecalis* TSA3 whose differential characteristics are shown in Table 1. Population dynamics of the representative aerobic bacterium, *Alcaligenes faecalis* (TSA3), during the 47-day running period is shown for each tank in Fig. 3. Interestingly, *Alcaligenes faecalis* TSA3 was a predominant species among aerobes in the aeration tank ($10^7\sim 10^8$ c.f.u./mL) but was also observed in the influent and fermentation tanks. Thus, the strain appeared to survive and grow under anoxic (non-aerated) condition. Unpublished data in our laboratories have shown that ammonium ions (NH_4^+) supplied as $(\text{NH}_4)_2\text{SO}_4$ in the minimal salts medium (citrate as a sole carbon source) can be utilized as a sole nitrogen source for the growth of *Alcaligenes faecalis* TSA3. This indicates a direct utilization of NH_4^+ by a heterotroph and hence removal of nitrogen from the system by circumventing the nitrification process, which uses large amounts of energy and oxygen. It, therefore, appears that the ammonium uptake and utilization could contribute to the nitrogen removal in the treatment system (particularly in the aeration tank). A reported species of *Alcaligenes faecalis* could oxidize ammonia and produce NO_2^- under aerobic conditions and also denitrify nitrate ions via NO and N_2O gases under anoxic conditions [18-20]. Population dynamics of the predominant lactic acid bacterium (MRS1) during the 47-day running period is shown for

Table 1. Differential characteristics of a Gram-negative bacterial species TSA-3 isolated from the recycling treatment system compared with a known species of *Alcaligenes faecalis*

Characteristic	<i>Alcaligenes faecalis</i> **	TSA 3
Gram staining	-	-
Cell shape	Rod, coccid rod, or cocci	Rod
Oxidase	+	+
Catalase	+	+
Anaerobic growth	-	-
*Acid from :		
D-Glucose	-	-
Mannitol	-	-
Inositol	-	-
Salicin	-	-
D-Melezitose	-	-
L-Fucose	-	-
D-Sorbose	-	-
L-Arabinose	-	-
D-Ribose	-	-
D-Sucrose	-	-
Rhamnose	-	-
Maltose	-	-
*Utilization of :		
Valerate	+	+
Citrate	+	+
2-Hydroxybutyrate	-	-
3-Hydroxybutyrate	+	+
4-Hydroxybenzoate	-	-
Itaconate	-	-
Suberate	-	-
Malonate	+	+
Acetate	+	+
DL-Lactate	+	+
5-Ketogluconate	-	-
3-Hydroxybenzoate	-	-
Glycogen	-	-
*Decomposition of		
Histidine	+	+
L-Proline	+	+
L-alanine	+	+
L-Serine	-	-

Symbols: +, 90% or more positive; -, 0~10% positive

*Tested using API identification program (ID 32 GN: Bio Merieux SA, France)

**Data from Bergey's Manual of Determinative Bacteriology (9th ed.)

each tank in Fig. 4. The population of the strain MRS-type1 was more dominant in the influent and fermentation tanks than in the aeration and sedimentation tanks, indicating its facultative anaerobic characteristics. The overall population density was in the range of 10^4 - 10^7 c.f.u./mL.

The COD removal during the extent of the experiment is shown, for each tank, in Fig. 5 with an overall treatment efficiency of about 54%. The COD removal may be mostly accomplished by biological oxidation or absorption (or uptake) of organic compounds derived

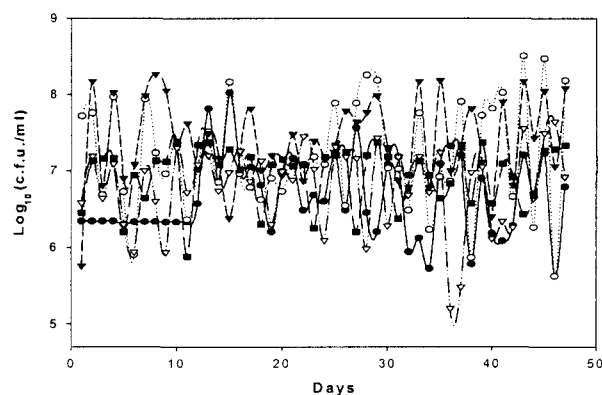


Fig. 3. Population dynamics of a heterotrophic bacterium (*Alcaligenes faecalis*, TSA3) in the recycling treatment system (●- Influent tank; ○- Fermentation tank; ▼- Aeration tank; ▽- Effluent tank A; ■- Effluent tank D).

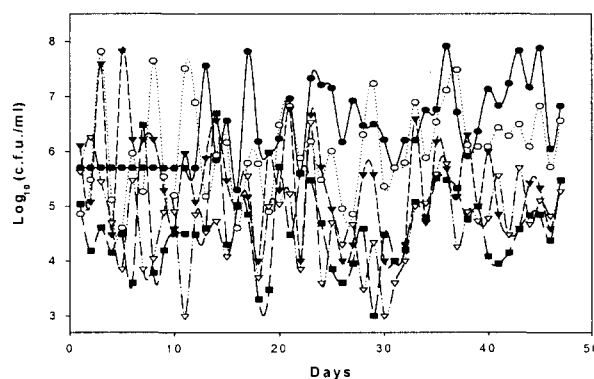


Fig. 4. Population dynamics of a putative lactic acid bacterium (MRS1) in the recycling treatment system (●- Influent tank; ○- Fermentation tank; ▼- Aeration tank; ▽- Effluent tank A; ■- Effluent tank D).

from the livestock feeds since livestock wastewater contains generally little xenobiotic compounds.

The phosphorus removal effect was also obvious in the aeration and sedimentation tanks. The possible mechanism for the phosphorus removal would be an uptake of phosphorus by cells under aerobic conditions and a subsequent sedimentation of the cells. Surplus phosphorus that is taken up may be transformed to poly-phosphorus as a storage material within the cells [21]. A discharge of phosphorus is known to occur under anaerobic conditions [22,23].

The best removal effect of suspended solids was observed in the aeration tank. This seems to be due to the transport hole between the fermentation and aeration tanks, which only passed the treated water, not the sedimented solids.

Principal Component Analysis

It was rather difficult to obtain enough training data

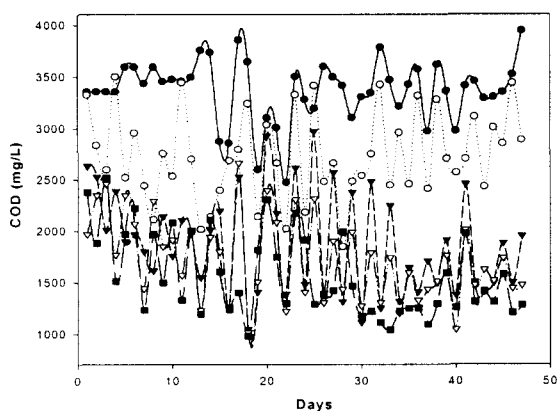


Fig. 5. Dynamics of chemical oxygen demand (COD) removal in the recycling treatment system (●- Influent tank; ○- Fermentation tank; ▼-Aeration tank; ▽- Effluent tank A; ■ - Effluent tank D).

because the biological processes of the microorganisms usually take a long time to reach a stable treatment efficiency. Moreover, the input and output dimensions of the neural networks was 9 and 5, respectively. Training data measured over 47 days, was not enough to figure out the complex correlation between the input and output in each tank, and it was also hard to formulate a generalization. Moreover, there were some noises in data due to measuring error or unstable bioprocesses. In order to remove the noisy data and also reduce the input and output dimensions, we first used the principal component analysis (PCA) method to analyze the training data. PCA projects high dimensional data onto low dimensional coordinates that consist of the principal component axes. In other words, PCA finds a few orthogonal coordinates that can best express the variations in the high dimensional data and then represents the high dimensional data on the new orthogonal coordinates with low dimensional equivalents. To find new orthogonal coordinates for the measured data, we should calculate eigen-values from the correlation matrix of the measured data, and then select a few eigen-values after ordering them according to their magnitudes. Finally, the new orthogonal coordinates become eigen-vectors for the selected eigen-values. Plotting the values to be represented by eigen-vectors on planes makes it easy to understand the correlation between each high dimensional data set.

In this study, we used three axes as orthogonal coordinates. These axes were obtained by analysis of the PCA analysis to remove the data through one-to-many mapping. Fig. 6 shows the PCA results for the measured data in the aeration tank. The X-axis denotes the mapping result according to the first eigen-vector, and Y-axis denotes the result with the second eigen-vector. The number of each point in Fig. 6 indicates the day. Fig. 7 showed the PCA results for target data measured in sedimentation Tank A, and each axis was same with

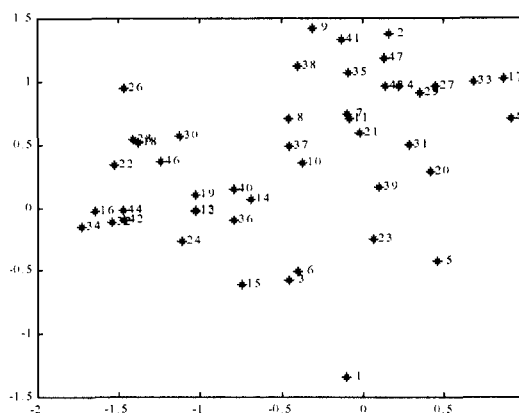


Fig. 6. Principal component analysis of the input from the aeration tank data during the 47 days' running period.

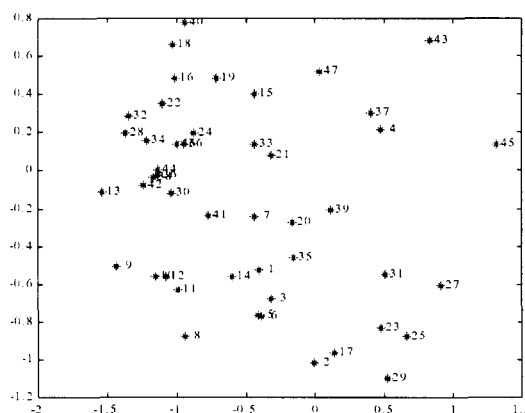


Fig. 7. Principal component analysis of the output from the aeration tank data during the 47 days' running period.

that of Fig. 6. As shown in Figs. 6 and 7, there were several data with one-to-many mapping property so that we removed these values in the training process.

Computational Results

Among 47 training data, we reversed the 6th, 11th, 16th, 21st, 26th, 31st, 36th, 41st, 46th, 47th data sets for the training phase, which were randomly selected and used as test data to evaluate the generalization performance of the neural network. We used one hidden layer with 30 nodes that were determined by an *ad hoc* method. The weighted values were adjusted by the error back-propagation algorithm.

Through computational experiment we confirmed that the neural network successfully imitated each tank of the treatment system and approximated the target values of the input pattern well. Table 2 shows the predicted values for T-P, o-P and SS, for the 46th and 47th

Table 2. Predicted results of T-P, *ortho*-P and SS by the trained neural networks on the 46th and 47th days*

Day	Tank	Values	T-P (mg/L)	<i>ortho</i> -P (mg/L)	SS (mg/L)
46th	Influent	M	36.00	30.43	0.560
		P	35.95	31.01	0.563
	Fermentation	M	28.00	27.31	0.305
		P	27.70	27.20	0.320
	Aeration	M	22.00	22.00	0.185
		P	21.80	18.10	0.130
	Sediment 1	M	22.00	21.00	0.175
		P	24.10	23.10	0.220
Sediment 4	M	20.00	23.00	0.180	
	P	34.10	38.90	0.210	
47th	Influent	M	49.00	53.84	0.880
		P	28.50	33.80	0.172
	Fermentation	M	36.00	43.85	0.395
		P	39.10	31.50	0.183
	Aeration	M	24.00	39.00	0.275
		P	22.00	24.70	0.269
	Sediment 1	M	22.00	36.00	0.295
		P	21.40	23.50	0.130
Sediment 4	M	25.00	39.00	0.230	
	P	21.60	27.10	0.155	

* M measured values; P predicted values by neural networks.

days, by neural networks that were serially connected to model the recycling system. In the Table, for each tank, the upper rows represented the measured values and lower rows were the results predicted by the neural networks. Fig. 8 show the estimation of the COD and $\text{NH}_4^+\text{-N}$ values, respectively. The numbers on the X-axis represent the tanks from 46th day's influent tank to 47th day's sedimentation tank D. The numbers at the X-axis indicate influent tank (1, 6), fermentation tank (2, 7), aeration tank (3, 8), sedimentation tank A (4, 9), and sedimentation tank D (5, 10). As shown in Fig. 8 and Table 2, the proposed neural networks could successfully model the treatment according to the population densities of the microorganisms.

CONCLUSION

We have proposed a novel monitoring system for a piggery slurry recycling treatment system. Multi-layer neural networks combined with PCA successfully modeled the tank characteristics. It was possible to train the neural network with the given training data by reducing the number of input dimensions with a minimal loss of information and to remove excess noise with the

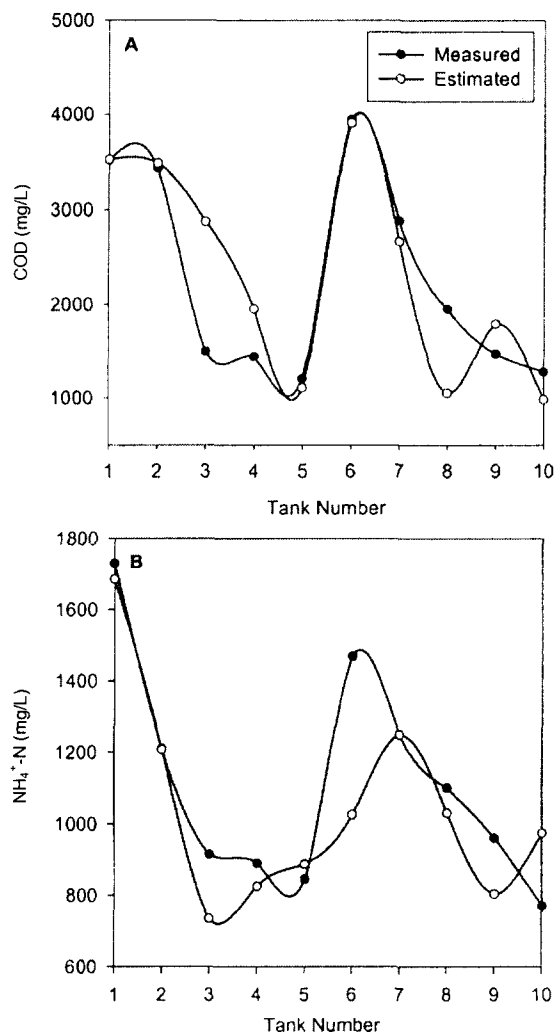


Fig. 8. Prediction for various treatments COD (A) and $\text{NH}_4^+\text{-N}$ (B) for 2 days (Days 46 and 47) by the neural networks model (●- measured data; ○- predicted data). The numbers at the X-axis indicate influent tank (1, 6), fermentation tank (2, 7), aeration tank (3, 8), sedimentation tank A (4, 9), and sedimentation tank D (5, 10).

one-to-many mapping property. The proposed model may be useful in developing a reverse neural network model that could be used to determine optimal microbial densities critical for a desired quality level of treated wastewater.

Acknowledgements This work was supported by the Korea Research Foundation (Grant # 1998-024-G-00041).

REFERENCES

[1] Shin, H. S., J. K. Koo, J. O. Kim, H. K. Shin, and Y. K. Jeong (1990) Management alternatives of livestock

- wastes for water resources conservation. *J. Korea Solid Waste Eng. Soc.* 7: 45-57.
- [2] National Institute of Environmental Research (1986) A Study of the Current Status Livestock Waste Discharge and its Environmental Impact. National Institute of Environmental Research, Seoul, Korea.
- [3] Office of Environment (Korea) (1988) Environmental Protection Act and Wastes Management Act.
- [4] Choi, J. H., Y. R. Chung, and S. C. Koh (1999) Microbial Treatment of Swine Wastewater by Recycling Reactor System with Sequential Oxidic and Anoxic Conditions, Abstract of Korea Environ. Eng. Soc. May 7-8. Seoul, Korea
- [5] Henze, M., C. P. L. Grady, W. Gujer, G. V. R. Marais, and T. Matsuo (1987) Activated sludge model No. 1. Scientific and Technical Reports No. 1. IAWPRC, London.
- [6] Lee, D. S. (2000) Modeling for industrial wastewater treatment process using hybrid neural networks. Abstract of the Korea Soc. for Biotechnol. Bioeng. April 8. Taejeon, Korea.
- [7] Barto, A. G., R. S. Sutton, and C. W. Anderson (1983) Neuronlike adaptive elements that can solve difficult learning control problems, *IEEE Trans. Systems. Man. Cybernetics.* 13: 834-846.
- [8] Lee, M. H., S. Y. Lee, and C. H. Park (1992) Neural controller of nonlinear dynamic systems using higher order neural networks. *Electronics Lett.* 28: 276-277.
- [9] Morgan, D., and C. Scofield (1991) *Neural Networks and Speech Recognition*, Kluwer Academic Publisher.
- [10] Weigend, A. S. and N. A. Gershenfeld (1994) *Time Series Prediction: Forecasting the Future and Understanding the Past*, Addison-Wesley Publishing Co., NY, USA.
- [11] Hornik, K. (1991) Approximation capabilities of multilayer feed forward networks. *Neural Networks* 4: 251-257.
- [12] Baum, E. B. and D. Hausser (1989) What size net gives valid generalization? *Neural Informa. Process. Systems* 1: 81-90.
- [13] Liao, C. M., T. Maekawa, H. C. Chiang, and C. F. Wu (1993) Removal of nitrogen and phosphorus from swine wastewater by intermittent aeration processes. *J. Environ. Sci. Health* B28: 335-374.
- [14] Krieg, N. R. and P. Gerhardt (1994) Solid, liquid/solid, and semisolid culture In: *Methods for General and Molecular Bacteriology*, Am. Soc. Microbiol. pp. 216-223. Washington, DC, USA.
- [15] Smibert, R. M. and N. R. Krieg (1981) General characterization, pp. 409-433. In: P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. R. Krieg, and G. B. Phillips (ed.). *Manual of Methods for General Bacteriology*. Am. Soc. Microbiol., Washington, DC, USA.
- [16] Holt, J. G., N. R. Krieg, P. H. A. Sneath, J. T. Staley, and S. T. Williams (1994) *Bergey's Manual of Determinative Bacteriology*. 9th ed. Wilkins Co., Baltimore, MD, USA.
- [17] American Public Health Association, American Water Works Association and Water Environment Federation (1992) *Standard Methods for the Examination of Water and Wastewater*, 18th ed., Washington, DC, USA.
- [18] Anderson, I. C., M. Poth, J. Homstead, and D. Burdige (1993) A comparison of NO and N₂O production by the autotrophic nitrifier *Nitrosomonas europaea* and the heterotrophic nitrifier *Alcaligenes faecalis*. *Appl. Environ. Microbiol.* 59: 3525-3533.
- [19] Otte, S., N. G. Grobden, L. A. Robertson, M. S. M. Jetten, and J. G. Kuenen (1996) Nitrous oxide production by *Alcaligenes faecalis* under transient and dynamic aerobic and anaerobic conditions. *Appl. Environ. Microbiol.* 62: 2421-2426.
- [20] Papen, H., R. Berg, I. Hinkel, B. Thoene, and H. Rennenberg (1989) Heterotrophic nitrification by *Alcaligenes faecalis*: NO₂⁻, NO₃⁻, N₂O, and NO production in exponentially growing cultures. *Appl. Environ. Microbiol.* 55(8): 2068-2072.
- [21] Hiraishi A., Y. Ueda, and J. Ishihara (1998) Quinone profiling of bacterial communities in natural and synthetic sewage activated sludge for enhanced phosphate removal. *Appl. Environ. Microbiol.* 64: 992-998.
- [22] Bond, P. L., R. Erhart, M. Wagner, J. Keller, and L. L. Blackall (1999) Identification of some of the major groups of bacteria in efficient and non-efficient biological phosphorus removal activated sludge systems. *Appl. Environ. Microbiol.* 65: 4077-4084.
- [23] Rimmerman, M. W. (1984) Biological phosphorus removal in wastewater treatment. *Microbiol Sci.* 1: 149-152.

[Received May 23, 2000; accepted October 3, 2000]