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Optimization of an Empirical Model for Microorganism-Immobilized Media to Predict Nitrogen Removal Efficiency

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Abstract: The purpose of this study was to develop a model to predict the total nitrogen (T-N) concentration in treated wastewater effluent when microorganism-immobilized media are applied. The operational data for this study were obtained using synthetic wastewater and actual wastewater within a lab-scale reactor. The organic matter removal, nitrification, and denitrification rates were 81.8, 87, and 82.9%, respectively. These rates adequately satisfied the effluent water quality standard. The observed parameters from the lab-scale reactor operation were applied to develop the optimization model, and the model showed correlation coefficients as 0.9785 and 0.9811 for nitrification and denitrification efficiencies, respectively. The model predicted that T-N concentration could be reduced to <10 mg/L with the injection of the external carbon source. The predicted value for the T-N concentration was higher than the observed value from the lab-scale reactor, which operated under the same conditions. The model showed comparable values to the observed data, and the model seems to be useful for predicting related parameters in effluent water quality, with further development of the specifications required in the treatment facilities under various operating conditions.

Keywords: microorganism-immobilized media; nitrogen removal; experimental design method; optimization model

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1. Introduction

Although biological wastewater treatment is considered economical and sustainable, it continues to have a range of problems [1]. Its most prominent issue is the solid–liquid separation that requires subsequent treatment [2,3]. In addition, for the treatment of nitrogen and phosphorus of high load, a substantial amount of space and energy is required. However, despite these problems, the treatment of nitrogen and phosphorus is not stable [4]. Biofilm processes have emerged as an alternative solution to address the issues associated with traditional biological treatment, and various types of biofilters have been used, such as fixed and moving bed biofilms [5–8].

Biofilms are formed when bacteria or microorganisms attach to the surface of a support or media that contain binding sites for extracellular polymers [9]; various types of supports or media are used for biofilm formation. First-generation media are made of unmodified natural materials such as sand, gravel, diatomaceous earth, and pumice [10]. These attached-growth type materials undergo attachment naturally in the reaction tank. Second-generation media consist of synthetic resins obtained by processing materials such as plastic, ceramic, and cellulose-based materials [11]. As forms and materials have developed since the second generation, this is the most common type of media currently used. Third-generation media combine synthetic materials and microorganisms and may be referred to as the beginning of microorganism-immobilized media [12,13].

Microorganism-immobilized media possess four main characteristics: (1) the easy control of the biological process, (2) operation at high concentrations without the loss of microorganisms, (3) increased resistance to toxic compounds and the stability of the biocatalyst, and (4) high treatment efficiency [14–18].

GPS-X based on the Activated sludge (ASM) model [19] is used to derive the optimal conditions (e.g., hydraulic retention time (HRT), filling rate) [20,21]. However, it is difficult to simulate the configuration process for microorganism-immobilized media, with the kinetic coefficient also being different from the default value. Therefore, the optimization process through such simulation is difficult and requires a considerable amount of time to derive the optimum conditions through the lab-scale reactor. To address this problem, Wanner (2006) [22] published a model equation related to biofilm; however, the derivation of related factors was also a time-consuming process.

In this study, we applied microorganism-immobilized media to a wastewater treatment plant and analyzed the treatment efficiency on a lab scale and derived a process optimization model using an experimental design method (Box–Behnken method). The optimized model was suggested by applying the operational data of the wastewater treatment plant.

2. Materials and Methods

2.1. Microorganism-Immobilized Media

The microorganism-immobilized media used in this study were prepared through the crosslinking method; raw materials were mixed with polyvinyl alcohol (PVA) and polyethylene glycol (PEG), and the activated microorganisms were activated sludge with a mixed liquor volatile suspended solids (MLVSS) concentration of 56,000 mg/L. Fabrication methods and physicochemical properties for the present study were reported in our previous studies [18,23].

2.2. Evaluation of Modified Ludzack–Ettinger Process with Microorganism-Immobilized Media

To optimize the microorganism-immobilized media, a lab-scale reactor (effective capacity: 28 L) of the Modified Ludzack–Ettinger (MLE) method was manufactured and operated. The ratio of the anoxic tank and the aerobic tank was approximately 1:2.5, and separators were installed at the inlet and outlet of each reactor so that no part of the microorganism-immobilized media was lost. The reactor had an initial filling rate of 150%, aerobic dissolved oxygen (DO) of 3.5 mg/L, and the microorganism-immobilized media filling rate of approximately 15%, which were applied equally. The raw water properties and HRT of each mode were:

1. Mode I: Chemical oxygen demand (COD_{Cr}) 450 mg/L, NH_4^+-N 50 mg/L, NO_3^--N 40 mg/L, total phosphorous (T-P) 4.0 mg/L, alkalinity 350 mg/L as $CaCO_3$, aerobic HRT 12 h, anoxic HRT 4.8 h;
2. Mode II: COD_{Cr} 250 mg/L, NH_4^+-N 40 mg/L, NO_3^--N 0 mg/L, T-P 4.5 mg/L, alkalinity 250 mg/L as $CaCO_3$, aerobic HRT 8 h, anoxic HRT 3.2 h;
3. Mode III: COD_{Cr} 280~368 mg/L, NH_4^+-N 57~109 mg/L, NO_3^--N 24~30 mg/L, T-P 2.7~7.2 mg/L, alkalinity 280~355 mg/L as $CaCO_3$, aerobic HRT 6 h, anoxic HRT 2.4 h.

2.3. Experimental Design by Box–Behnken Method

An analysis was conducted to set the independent variable in advance and obtain an optimal value, and the experimental design method was used to find the optimal value with the least amount of time required [24–26]. To derive the optimum operating factors for each reactor, the experiment was designed with batch type media divided into anoxic and aerobic conditions. For the anoxic conditions, four independent variables were set; inflow NO_3^--N concentration, carbon to nitrogen (C/N) ratio, microorganism-immobilized media filling rate, and HRT). A total of 27 experiments designed by the Box–Behnken method were performed. The inflow NO_3^--N concentration and the C/N ratio were applied to the measured value and the experimental reaction value was measured after the reaction of NO_3^--N_{eff} concentration.

In the aerobic reactor, nitrification and organic matter removal occurred simultaneously, and as such, NH_4^+-N inflow and alkalinity were influencing the nitrification reaction, whilst COD_{Cr} inflow, HRT, and the filling rate were the independent variables. A total of 46 experiments designed by the Box–Behnken method were carried out in this reactor. The NH_4^+-N_{in}, COD_{Cr}, and alkalinity were measured, and the experimental response was measured by the NH_4^+-N_{eff} concentration. The reaction temperature proceeded at 18 °C as per the operation within a laboratory, and each level is shown in Table 1 below.

Table 1. Box–Behnken variables and levels.

Variable		Level		
		−1	**0**	**1**
Nitrification	NH_4^+-N Conc. (mg/L)	25	50	75
	COD_{Cr} (mg/L)	50	100	150
	NH_4^+-N/Alk.	4	7	10
	Filling rate (%)	5	12.5	20
	HRT (h)	4	7	10
Denitrification	NO_3^--N Conc. (mg/L)	40	80	120
	C/N ratio	2	5	8
	Filling rate (%)	5	12.5	20

2.4. Development of Treated Water Quality Prediction Model

The experiment was carried out, as detailed in Section 2.3, to predict treated water quality when microorganism-immobilized media were applied. A model with high reliability was developed through correlation and verification of the P and F values for significance and homogeneity of variances, respectively. Subsequently, the optimum conditions were derived, and the operational data of the wastewater treatment plant were used to confirm the treated water quality predictions and review the HRT.

The wastewater treatment plant data were analyzed using the operational data of the Seoul J Water Regeneration Center, used as the influent source of the lab-scale reactor. The following is a summary of the operational data required for the model:

- Operation factor: anoxic HRT 2.4 h, aerobic HRT 4.7 h;
- Summer influent data (1st): total nitrogen (TN) 36.0 mg/L, C/N ratio 4.42;
- Winter influent data (2nd): TN 39.0 mg/L, C/N ratio 3.87.

3. Results

3.1. Lab-Scale Reactor Operation Results

The organic matter behavior of the reactor inflow and effluent (Figure 1a) showed that the removal rate of total COD_{Cr} ($TCOD_{Cr}$) (Modes I and II) in synthetic wastewater was 74.5%, and that of sewage (Mode III) was 81.8%. Based on the soluble COD_{Cr} ($SCOD_{Cr}$) of the effluent (Figure 1b), it was observed to be approximately 20 mg/L from around 7 days after the start of operation, from when stable treatment water quality could be secured. The aerobic HRT of Mode II was 8 h; however, the aerobic HRT of Mode III was shortened to 6 h, equivalent to the aerobic HRT of the wastewater treatment plant. It did not change the $SCOD_{Cr}$ of the effluent, which enabled a further reduction of the HRT.

The stabilization step of the microorganism-immobilized media was the injection of NH_4^+-N 40 ± 8.5 mg/L and NO_3^--N 45 ± 3 mg/L for 7 days. At this time, the average nitrification rate, denitrification rate, and T-N concentration were 85.7%, 92.7%, and 8.5 mg/L, respectively (Figure 1c,d). As the average T-N removal rate was 89%, we deduced that the microorganism-immobilized media had been sufficiently activated, thus conducting the Mode II operation. For the concentration range of NH_4^+-N, when the average inflow was 36 mg/L, it was nitrified more than 87%, and the resulting effluent was an average of 4.65 mg/L. After 20 days, the effluent NH_4^+-N concentration was less than 2 mg/L, demonstrating a very stable nitrification rate. The average effluent concentration of NO_3^--N was 6.40 mg/L, and the denitrification rate was 82.9%.

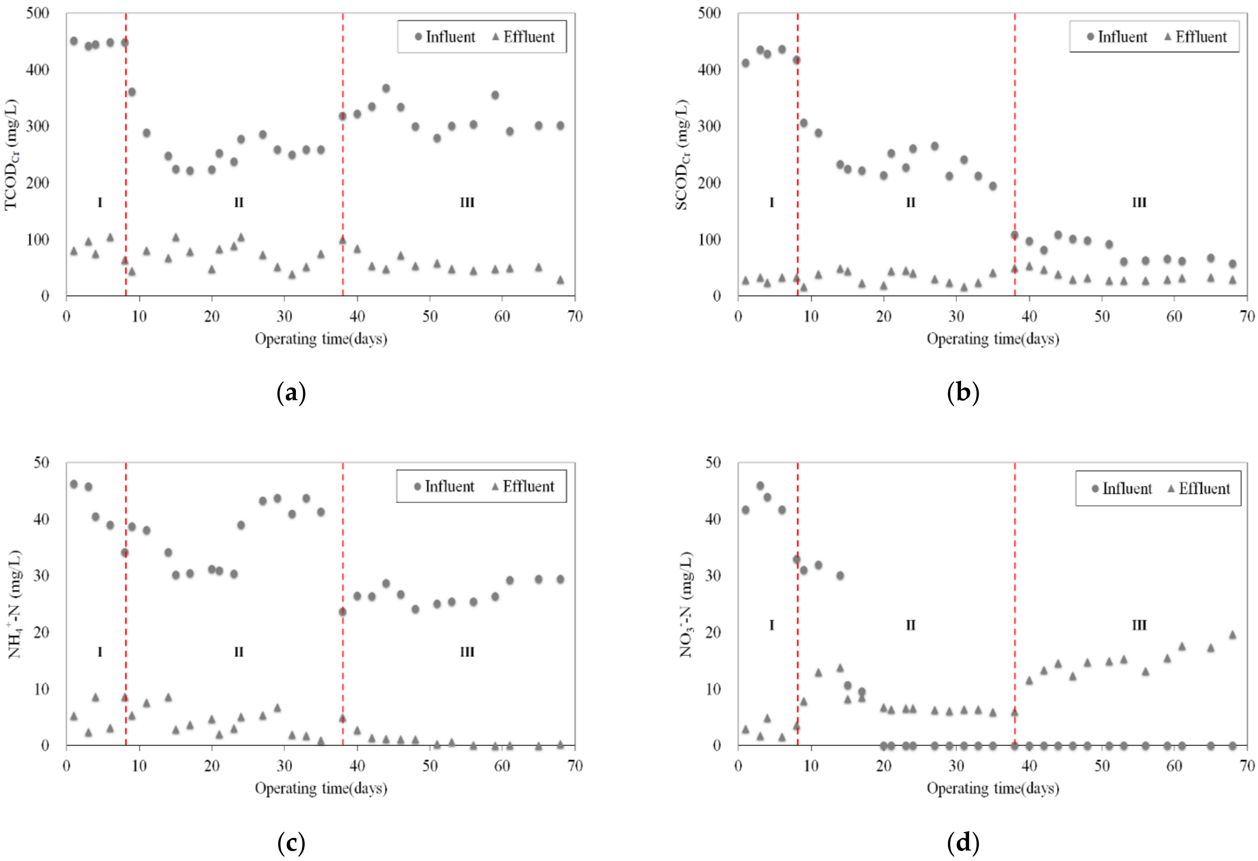


Figure 1. Performance of MLE Process with microorganism-immobilized media: (**a**) TCOD, (**b**) SCOD, (**c**) NH_4^+-N, (**d**) NO_3^--N.

The mode III effluent NH_4^+-N concentrations were below 1.5 mg/L, except for the initial four days of operation. However, the average effluent NO_3^--N concentration was 14.03 mg/L, indicating that the denitrification rate was very low. In Mode II, glucose was used for organic concentration, and in Mode III actual sewage was applied. The difference in the denitrification rate between Modes II and III may be due to the difference in readily biodegradable substrates.

Except for the initial two times of Mode II, the T-N effluent for approximately 70 d satisfied the 20 mg/L Water Quality Standards for effluent discharged from a wastewater treatment plant. For Mode III, where actual wastewater was used, the T-N concentration was constantly around 15 mg/L, and the NO_3^--N content was approximately 75% of the T-N in the effluent. To address this, external carbon sources should be added to secure high T-N removal.

3.2. Model Development by Box–Behnken Method

3.2.1. Anoxic Conditions

Dispersion analysis was conducted based on the results of a batch experiment, to develop an optimized model of the microorganism-immobilized media for anoxic conditions. For the F-value, as it becomes larger it is more likely that the factor has a significant effect on the variability of the response or measurement variable. If the value is less than 0.05, the null hypothesis may be rejected. The null hypothesis in this study is that the coefficients of the independent variables included in the model are zero. Therefore, the F-value represents a significant probability, and a value less than 0.05 may be used to signify that the regression model is appropriate [27–29]. Based on the analysis of variance (ANOVA) results, all variables were deemed suitable, and the model equation was derived, as described in Equation (1):

$$Y = 0.4886\ A - 4.35\ B - 1.994\ C - 3.409\ D + 59.8, \quad (1)$$

where
A: NO_3^--Nin (mg/L);
B: C/N ratio;
C: Filling rate (%);
D: HRT (h).

The correlation coefficient (R) for the above model was 0.7877, the adjusted correction factor (adj-R) for the number of model terms was 0.7491, and the coefficient of determination (R^2) was 0.6205.

Variance analysis was performed again by including the variables in Equation (1). As a result of deriving the model equation, except for B * C, B * D, and C * D, which were larger than 0.05, the F-values representing the significance probability for each variable were expressed as Equation (2):

$$Y = 2.522\ A - 2.95\ B - 1.088\ C + 4.65\ D - 0.1192\ AB - 0.0494\ AC - 0.1289\ AD - 66.2 \quad (2)$$

The correlation coefficient (R) for this model was 0.9363, the adjusted correction factor (adj-R) for the number of model terms was 0.9128, and the coefficient of determination (R^2) was 0.8884.

Equations (1) and (2) were considered low applicability as first-order functions. Therefore, the second-order functions A^2, B^2, C^2, and D^2 were included to develop a highly applicable model. As per the previous method, model equations were derived except for variables A^2, B * C, B * D, and C * D, which were greater than 0.05 among the F-values representing the probability of each variable; the result is described in Equation (3):

$$Y = 0.786\ B^2 + 0.1332C^2 + 0.520\ D^2 - 0.1208\ AB - 0.04945\ AC - 0.1292\ AD + 2.544\ A - 5.74\ B - 2.243\ C - 1.65\ D - 11.0 \quad (3)$$

The correlation coefficient (R) for this model was 0.9785, and the adjusted correction factor (adj-R) for the number of model terms was calculated as 0.9651. This response model was considered to be highly suitable.

3.2.2. Aerobic Conditions

Variance analysis was conducted based on the experimental results to develop an optimized model of the aerobic microorganism-immobilized media. To develop the model equation in the same manner as the anoxic conditions, Equations (4)–(6) were derived as follows:

$$Y = 0.5339\ A - 2.117\ C - 1.424\ D - 2.778\ E + 46.32, \quad (4)$$

$$Y = 2.208\ A + 1.922\ C + 0.555\ D + 0.96\ E - 0.0878\ AC - 0.03901\ AD - 0.0725\ AE - 36.8, \quad (5)$$

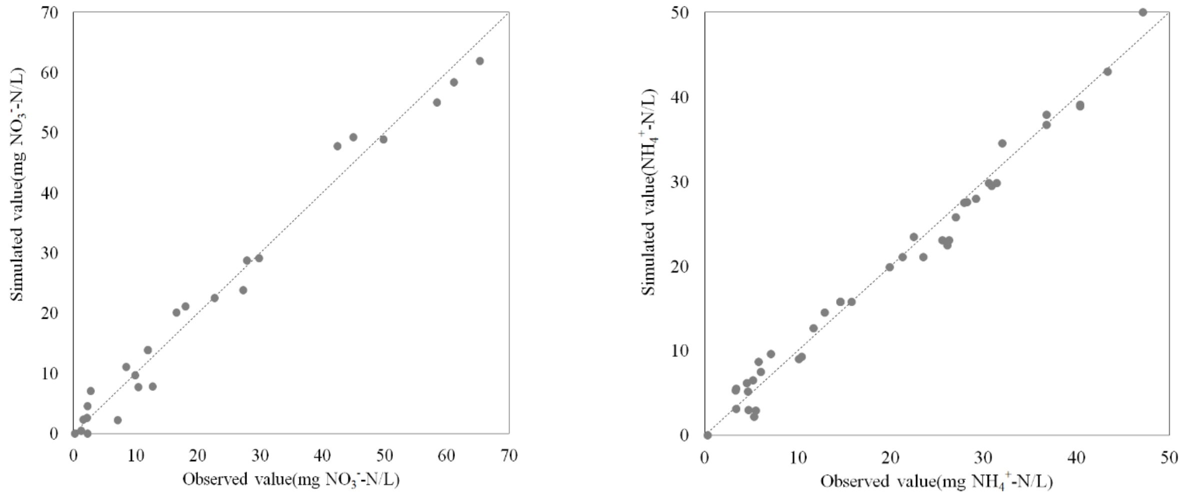
$$Y = 0.006696\ A^2 + 0.2599\ C^2 + 0.0493\ D^2 + 0.2552\ E^2 - 0.0420\ AC - 0.04269\ AD - 0.0728\ AE - 0.1025\ CD + 1.274\ A - 2.94\ C + 0.245\ D - 2.60\ E + 14.3, \quad (6)$$

where
A: NH_4^+-N_{in} (mg/L);
C: Alkalinity (mg/L as $CaCO_3$);
D: Filling rate (%);
E: HRT (h).

The estimated correlation coefficient (R) that demonstrates the suitability for the above model was 0.8767, 0.9569, and 0.9861, respectively. The adjusted coefficient (adj-R) based on the number of terms of the model was 0.8646, 9490, and 9811, respectively. Based on these results, Equation (6) was considered to be highly suitable.

3.2.3. Model Evaluation (Congruence, Significance)

Figure 2a presents the correlation between the predicted value and the measured value to determine how well the NO_3^--N from the predictive model of the microorganism-immobilized media simulates the measured value under anoxic conditions. The absolute mean error between the predicted value and measured value was 2.37 mg/L, the standard deviation of the error was 2.7, the correlation coefficient was 0.9896, and the coefficient of determination was 0.9793.



(**a**) (**b**)

Figure 2. Comparison of simulated and observed values for model validation under each condition: (**a**) anoxic condition, (**b**) aerobic condition.

Figure 2b presents the correlation between the predicted value and the measured value under aerobic conditions when the microorganism-immobilized media were applied. The absolute mean error between the model predicted value and the measured value was 1.42 mg/L, the standard deviation of the error was 1.4, the correlation coefficient was 0.9918, and the coefficient of determination was 0.9837. Based on these results, both previous models perform well in terms of simulating actual values.

The fit of the whole model was assessed using the normal probability plot, residual versus fits, histogram, and residual versus order. The normality, equal variance, and independence of the residuals were found to be satisfactory, indicating that the previously calculated model was not an abnormal model [30]. All independent variables of the model showed statistically significant results.

Contour plots (Figure 3) were drawn using the above model, where the hold value was:

- Denitrification: NO_3^--Nin—50 mg/L, HRT—5 h, C/N ratio—5, filling rate—15%;
- Nitrification: NH_4^+-Nin—50 mg/L, HRT—9 h, alkalinity—9 mg as $CaCO_3$/L, filling rate—15%.

The contour plots of the denitrification media were analyzed comprehensively when the microorganism-immobilized media were applied to the anoxic conditions (C/N ratio 4.57 or more and maintaining a charge rate of 20%). The analysis showed that the NO_3^--N removal rate per hour was approximately 18 mg/L. This was a lower rate than the 23.85 mg/L observed in a previous study. In addition, when microorganism-immobilized media were applied to aerobic conditions, the alkalinity should be maintained at a minimum theoretical value (NH_4^+-N 1 mg/L per 7.14 mg/L as $CaCO_3$). When the filling rate was 20%, it was found that approximately 7 h was required for nitrate of NH_4^+-N 50 mg/L; this is approximately twice as long as in the anoxic conditions.

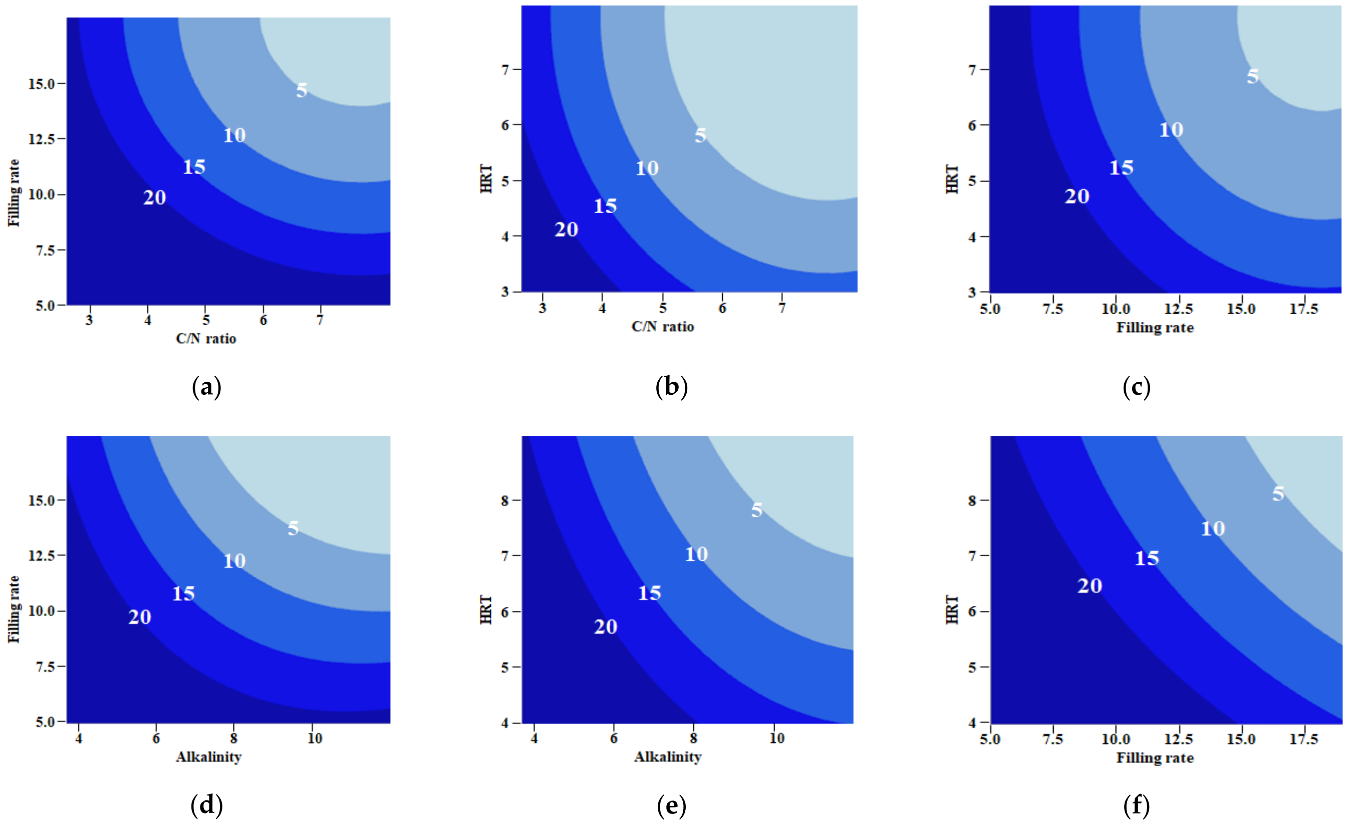


Figure 3. Contour plots for denitrification: (**a**) filling rate—C/N ratio, (**b**) HRT—C/N ratio, (**c**) HRT—filling rate and nitrification, (**d**) filling rate—alkalinity, (**e**) HRT—alkalinity, (**f**) HRT—filling rate.

3.3. Model Validation versus Lab Test Results

The operational results of the lab-scale reactor were compared with the predicted values of the model to verify the applicability of the deduction model to the continuous reactor (Figure 4).

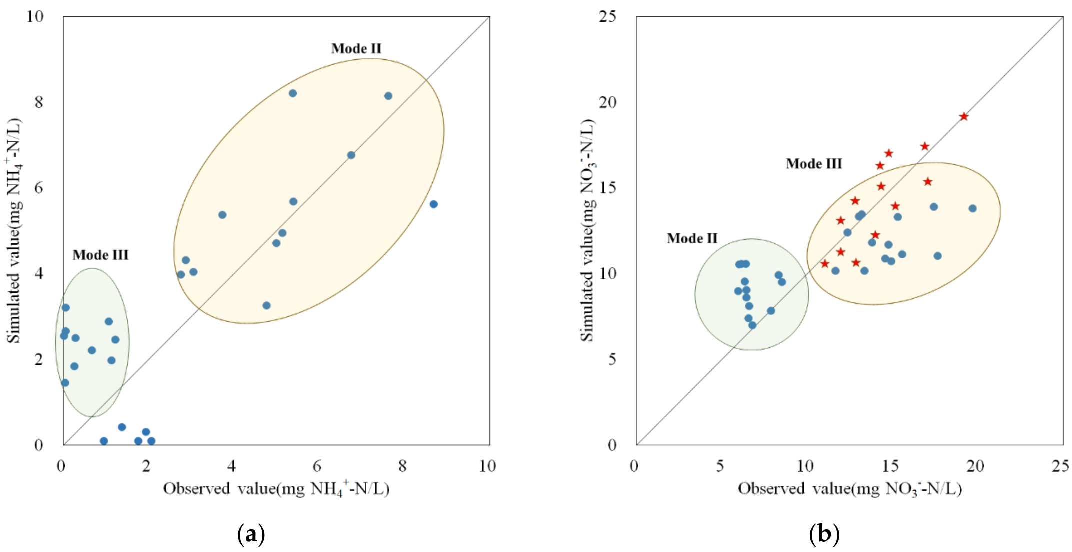


Figure 4. Comparison of estimation and lab-scale results for model validation under each condition: (**a**) anoxic condition, (**b**) aerobic condition.

Figure 4a presents the correlation between the predicted value and the measured value to determine how well the effluent NH_4^+-N prediction model matches the measured value in aerobic conditions.

The mean error between the predicted value and the measured value of the model was −0.6 mg/L. The absolute mean error was 1.5, the standard deviation of the error was 1.4, and the correlation coefficient was 0.7758. The correlation was relatively low compared with the batch test; however, the absolute mean error had the same value, indicating that the model's prediction for the continuous reactor was accurate. For the wastewater treated with the synthetic wastewater mode, the absolute mean error was 1.3 and the correlation coefficient was 0.8267. For Mode III, the absolute mean error with real wastewater was 1.5 and the correlation coefficient was 0.6171. The correlation of mode III shows that the predicted values were all higher than the measured values. Based on these results, a safety factor should be considered when applying a model that predicts treated water quality, providing a highly reliable model.

Figure 4b shows the correlation between the predicted value and the measured value based on the predicted effluent NO_3^--N concentration in anoxic conditions. The mean error was 0.4 mg/L, the absolute mean error was 2.6, the standard deviation of error was 3.0, and the correlation was 0.7636.

Based on 10 mg/L of the lab-scale reactor operation results (*x*-axis), Mode II was distributed on the left and Mode III on the right. A comparison between the predicted value and the operational result value for Mode III shows that the latter was higher than the former. This may be attributed to the different types of wastewater used in each mode. The synthetic wastewater used in Mode II consists of a carbon source composed of organic substances that are easily biodegradable. In contrast, Mode III is not an organic material that is 100% easily biodegradable as actual sewage was applied. According to the International Water Association [31] and W. Gujer [32], in general non-biodegradable COD (NBDCOD) content accounts for an estimated 20% of sewage. Conversely, 80% of incoming organic matter (COD_{Cr}) in Mode III was assumed to be biodegradable organic matter. As a result of the model applying these contents, it was predicted as "★". The absolute mean error was 2.3, the standard deviation of the error was 2.8, and the correlation coefficient was 0.8607. The future application of this model should input the appropriate C/N ratio of biodegradable organic matter.

3.4. Sewage Treatment Plant Water Quality Prediction through Model Application

The results obtained by inputting the operational data into the model are shown in Table 2. The concentration of influent NH_4^+-N was applied as the influent T-N concentration, and the influent NO_3^--N concentration was applied as the value obtained by subtracting the effluent NH_4^+-N concentration from the influent T-N concentration (i.e., $T\text{-}N_{in}–NH_4^+\text{-}N_{out}$).

Table 2. Comparison of operational data and simulated values for wastewater treatment plant.

Classification		Operating Factor					Effluent T-N (mg/L)	Remarks
		MLSS (mg/L)	Filling Rate (%)		HRT (h)			
			Anoxic	Aerobic	Anoxic	Aerobic		
1st data	Observed	2500	-	-	2.4	4.7	14.0	
	Simulated	-	15	20	2.4	4.7	9.75 **8.43**	 C/N ratio: 5.0
2nd data	Observed	2850	-	-	2.4	4.7	14.3	
	Simulated	-	15	20	2.4	4.7	13.56 **9.84**	 C/N ratio: 5.0

The MLE process effluent TN concentration in the primary operational data was 14.0 mg/L; however, it was 9.75 mg/L in the model predictions when the microorganism-immobilized media were applied. In addition, the application of the second set of operational data confirmed that the effluent TN concentration decreased by approximately 1 mg/L, from 14.3 to 13.56 mg/L. This demonstrates that when microorganism-immobilized media are applied to the same structure it may be possible to achieve a higher nitrogen removal rate than the MLE process using conventional activated sludge.

As the influent C/N ratio of the second set of operational data was lower than the influent C/N ratio of the primary set of operational data, the predicted change in the effluent water quality was considered to be small. Therefore, the effluent T-N concentration was predicted when the external carbon source was injected to achieve a C/N ratio of 5.0. As a result, the effluent TN concentrations were 8.43 and 9.84 mg/L, respectively. This indicates that the effluent T-N concentration may be maintained below 10 mg/L if an external carbon source is injected and the C/N ratio is high.

The predicted runoff quality from the wastewater treatment plant using microorganism-immobilized media demonstrates that a higher treatment efficiency may be achieved compared to that using activated sludge. Using this model, it is possible to estimate the HRT required to obtain the same water quality as the existing wastewater treatment plant. If the microorganism-immobilized media are applied, the HRT will be reduced, enabling the size reduction of the treatment plant. To reliably and accurately predict the effluent quality of the process with this model using microorganism-immobilized media, it is necessary to input biodegradable fractions through organic matter (COD) and nitrogen (T-N).

4. Conclusions

A lab-scale reactor was used to test the efficiency of microorganism-immobilized media, which could replace activated sludge. The results showed that this technology achieved an organic matter removal efficiency, nitrification rate, and denitrification rate of more than 80, 95, and 45%, respectively. If the post-denitrification tank is installed, it is expected to achieve a higher removal efficiency than the conventional activated sludge process. The model derived through the experimental design showed a high correlation and seems to be a considered application to the real wastewater treatment plant. The model reliability could be improved with the following considerations:

- In the case of the aerobic model, the NH4+-N predicted value was estimated at 1.5 mg/L higher than the measured value. When the model is used to predict the HRT (size) of the reactor, this should be considered to avoid overdesigning.
- In the case of applying an anoxic model, it is possible to obtain more accurate predictions by applying readily biodegradable organic matter through the COD fraction by inputting the C/N ratio among the variables.

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