Ammonia-nitrogen and Phosphate Reduction by Bio-Filter using Factorial Design

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Abstract. Untreated landfill leachate is known to have endangered the environment. As such new treatment must be sought to ensure its cost-effective and sustainable treatment. Thus this paper reports the effectiveness of bio-filter to remove pollutants. In this research, the reduction of nutrients concentration was evaluated in two conditions: using bio-filter and without bio-filter. Synthetic wastewater was used in the batch culture. It was conducted within 21 days in the initial mediums of 100 mg/L ammonia-nitrogen. The nitrification medium consisted of 100 mg/L of ammonia-nitrogen while the nitrite assay had none. The petri dish experiment was also conducted to observe the existence of any colony. The results showed 22% of ammonia-nitrogen reduction and 33% phosphate in the nitrification medium with the bio-filter. The outcome showed that the bio-filter was capable to reduce the concentration of pollutants by retaining the slow growing bacteria (AOB and NOB) on the plastic carrier surface. The factorial design was applied to study the effect of the initial ammonia-nitrogen concentration and duration on nitrite-nitrogen removal. Finally, a regression equation was produced to predict the rate of nitrite-nitrogen removal without conducting extended experiments and to reduce the number of trials experiment.

1. Introduction

The increase of population in urban areas has created industrial areas all over the world. This increase in turn leads to the surge of municipal and industrial solid waste [1]. To treat these municipal solid wastes (MSW), sanitary landfill is employed by city authorities in many countries despite the existence of many other treatment methods [2]. Sanitary landfill method continues to be used due to its relatively simple procedure and low operational cost [1]. Landfill leachate generated from landfills must be treated to meet the standard as it creates social and environmental issues [3]. The leachate contains various pollutants and high concentration of organic compound, nutrients, heavy metal, chemicals and inorganic salts [4].

Therefore, to reduce the nitrogen in order to improve the treatment of treated landfill leachate, wastewater requires a treatment process that involves biological oxidation which converts ammonia-nitrogen (NH_3-N) into nitrate-nitrogen (NO_3-N), which is called nitrification. Then, it is followed by the process of biological reduction known as de-nitrification which converts NO_3-N into nitrogen gas (N_2) [5]. The nitrification initiated from oxidation of NH_3-N to nitrite-nitrogen (NO_2-N) by several

genera of autotrophic nitrifying bacteria example; *Nitrosomonas* for AOB. Then, NO_2-N is oxidized to NO_3-N which much less toxic by several other genera of nitrifying bacteria, which is *Nitrobacter* as NOB. In addition, the importance of biological phosphorus removal is chosen because of the lower operation cost and environmental impacts [6].

At present, bio-filter packing medium has received considerable attention as an effective way to remove pollutants and ammonia nitrogen in landfill leachate. In the same vein, [7] studied the reduction of ammonia-nitrogen by bio-filters. The biological process of a bio-filter system to remove pollutants has been an alternative due to its low cost and maintenance compared to other treatment. The efficiency to remove pollutants is mainly from its physicochemical and microbiological properties. The bio-filter had been found to be practical in treating landfill leachate in previous reports [8]. Hence, the objective of the experiment was to reduce the ammonia-nitrogen and phosphate, and to produce nitrite-nitrogen and nitrate-nitrogen. This study was also to develop a regression equation for nitrite-nitrogen production for batch culture experiment as a tool to predict which in turn will limit the number of trial experiment in bio-filter system.

2. Material & Methods

This study employed three types of synthetic media. They were to be mixed to produce two types of media called nitrification and nitrite assay media [9]. The synthetic medium was employed instead of real as the later contained a variety of pollutants.

To note, this experiment took longer retention time as the nitrifying bacteria (AOB and NOB) are slow growers [9]. Two sets of flasks were prepared where each set contained two flasks. The two flasks were labeled Flask 1 and Flask 2. Flask 1 contained a bio-filter while Flask 2 did not. All flasks were injected with 20 mL of leachate for AOB and NOB. After the experiments, both retained media were used for agar plate experiments. The agar and nutrient agar plates were prepared to measure the presence of AOB and NOB colonies.

The formulations of media were chosen based on the previous study conducted by other researchers [7, 9]. Medium A was the formulation of ammonia-nitrogen solution to determine the ammonia-nitrogen reduction while Medium B was the formulation of nitrite-nitrogen solution, of which no ammonia-nitrogen was added. However, Medium C was the formulation of phosphate solution to reduce phosphate. Phosphate is also the nutrient requirement for autotrophic nitrifying bacteria to grow [6].

2.1. Nitrification Medium

The formulation of Medium A included $(NH_4)_2\ SO_4$, 5 g and 1000 mL of distilled water. The solution was sterilized at 121°C for 15 minutes. The Medium C included a sterilized stock of Na_2HPO_4, 3.5 g, KH_2PO_4, 0.7 g, $MgSO_4.7H_2O$, 0.1 g, $FeCl_3.6H_2O$, 0.014 g, $CaCl_2.2H_2O$, 0.18 g and 1000 mL of distilled water. It was prepared separately from the basal medium. The stock solution of Medium C was added aseptically to give the final nitrification medium volume of 500 mL [7].

2.2. Nitrite Assay Medium

Medium B contained $NaNO_2$, 0.5 g, Na_2HPO_4, 13.5 g; $MgSO_4.7H_2O$, 0.1g, $FeCl_3.6H_2O$, 0.014 g, $CaCl_2.2H_2O$, 0.18 g and 1000 mL of distilled water and sterilized at 121°C for 15 minutes. A sterilized Medium C of Na_2HPO_4, 3.5 g, KH_2PO_4, 0.7 g, $MgSO_4.7H_2O$, 0.1 g, $FeCl_3.6H_2O$, 0.014 g, $CaCl_2.2H_2O$, 0.18 g and 1000 mL of distilled water was prepared separately from the basal medium. It was added aseptically to give the final nitrite assay volume of 500 mL [7]. The difference between nitrification and nitrite assay medium was their chemical measurements.

2.3. Batch Culture Experiment

Flask 1 contained bio-filter while Flask 2 did not. Ten (10) pieces of bio-filters were used and inserted in Flask 1 for both media. Then, 20 mL of leachate was injected into each flask. The flasks then were

placed under aeration to encourage the growth of bacteria. The samples were taken daily to record the concentrations of NH_3-N, NO_2-N, NO_3-N and PO_4^{3-} for 21 days.

2.4. Ammonia-agar plate

Ammonia-nitrogen agar was prepared using the formulation of 14 g of agar; 250 mL of Medium A and 250 mL of Medium C to reach a total volume of 500 mL. The solution was stirred using the glass rod until the agar dissolved completely. Afterwards, the solution was sterilized by autoclaving at 121°C for 15 minutes.

2.5. Nitrite-agar Plate

The formulation of the nitrite-agar plate was 14 g of agar, 250 mL of Medium B and 250 mL of Medium C. The solution was stirred by a glass rod to mix it well, then, autoclaved at 121°C for 15 minutes.

2.6. Nutrient-agar Plate

The nutrient agar plate contained 28 g nutrient agar and 1000 mL distilled water. Similarly, the solution was stirred, sterilized and autoclaved at 121°C for 15 minutes.

2.7. Factorial Design by Design of Experiment (DOE)

The relationship between the two factor variables and the response for the nitrite-nitrogen reduction was analyzed by a full general factorial design by the DOE [10]. The general factorial regression is used to predict the future percentage of nitrate-nitrogen removal. The multilevel factorial design used in this experiment was the one having at least one factor with more than two levels [10].

The two factors of the initial ammonia-nitrogen concentration and duration were denoted $\varkappa_1$ and $\varkappa_2$, respectively. The initial concentration consisted of two levels which were 0 and 100 mg/L, coded as -1 and 1. While the level of the duration that were referred to coded values -1, 0 and 1, used in this part of experiment were 8, 15 and 21 days. The values of each level mentioned were based on the results obtained from the batch experiment. Table 1 shows the summary of two factors with two levels of the initial ammonia-nitrogen concentration and three levels of the duration discussed earlier.

Table 1. Coded and Real Values for Multilevel Factorial Design of Initial Concentration and Time

Factor variables	Variables	Unit	Response		
			-1	0	1
Initial Ammonia Concentration	$\varkappa_1$	mg/L	0	-	100
Duration	$\varkappa_2$	days	0	18	21
Initial Ammonia Concentration	$\varkappa_1$	mg/L	0	-	100

The results showed the coefficient parameters which were estimated using factorial regression analysis of the software Minitab 17 [10]. Table 2 displays the standard order, run order and design matrix of the two factors with multilevel factorial designs. The standard order is sometimes known as the non-randomized order which contain the common arrangement of factorial design [10]. On the other hand, the run order is identified as the randomized order, which refers to the sequence of the experimental run. This dissimilarity is done to minimize the effect of unexplained variability in the observed response due to systematic errors [11]. In this research, the randomized order was set to avoid any systematic errors.

Table 2. Experimental Design Matrix by Factorial Design

Standard Order[a]	Run Order[b]	Initial Concentration of Ammonia-nitrogen		Duration	
		Coded values	Real values (mg/L)	Coded values	Real values (days)
4	1	1	100	-1	1
6	2	1	100	1	21
5	3	1	100	0	18
2	4	-1	0	0	18
3	5	-1	0	1	21
1	6	-1	0	-1	1

[a] Nonrandomized order
[b] Randomized order

3. Results and Discussion

3.1. Statistical Analysis by Factorial Design

The factorial design in this experiment consisted of two factors. They were the initial ammonia-nitrogen concentration and the duration, denoted as $\varkappa_1$ and $\varkappa_2$, respectively. The two levels of the initial ammonia-nitrogen concentrations were 0 and 100 mg/L while the three durations were 0, 18 and 21 days. The combination of these factors and the levels outlined a total of 6 runs experimental matrix, shown in column 2 in Table 3. Coded (±1) and real levels of two factors are given on columns 3 - 6 in Table 3. The last column shows the percentage nitrite-nitrogen removal obtained in each run. The objective of using factorial design was to predict future removal of nutrients and to investigate the effect of each factor on a response. The response is recognized in the amount of the nitrite-nitrogen being removed as presented in Table 3.

Factorial Design Analysis Figure 1 shows the study of the factors affecting the nitrite-nitrogen removal. The mean of the response variable for various levels of each factor is represented by the points in the plot. The initial ammonia-nitrogen concentration of 100 mg/L has a high percentage of nitrite-nitrogen removal. The duration of 21 days also has a high percentage of nitrite-nitrogen removal which indicated that when the duration is increases the percentage of removal also tend to be increased. The horizontal reference line drawn at the grand mean of the response data shows the mean processing time for all runs. In this study, the time interval has a significant effect on the nitrite-nitrogen removal. The mean of the nitrite-nitrogen removal increases in polynomial manner by increasing the duration as well as increasing the concentration (Figure 1). The two mentioned effects contributed to enhance the nitrite-nitrogen removal by the bio-filter.

Table 3. Two Factors with Two Levels of Initial Concentrations and Three Levels of Duration in Factorial Design

Standard Order[a]	Run Order[b]	Factors				Response
		Initial Ammonia -nitrogen Concentration ($\varkappa_1$)		Duration ($\varkappa_2$)		
		Coded values	Real Values (mg/L)	Coded values	Real Values (Days)	% Nitrite-nitrogen Removal
4	1	1	100	-1	0	0
6	2	1	100	1	21	37.5
5	3	1	100	0	18	25
2	4	-1	0	0	18	8
3	5	-1	0	1	21	24
1	6	-1	0	-1	0	0

[a] Nonrandomized order [b] Randomized order

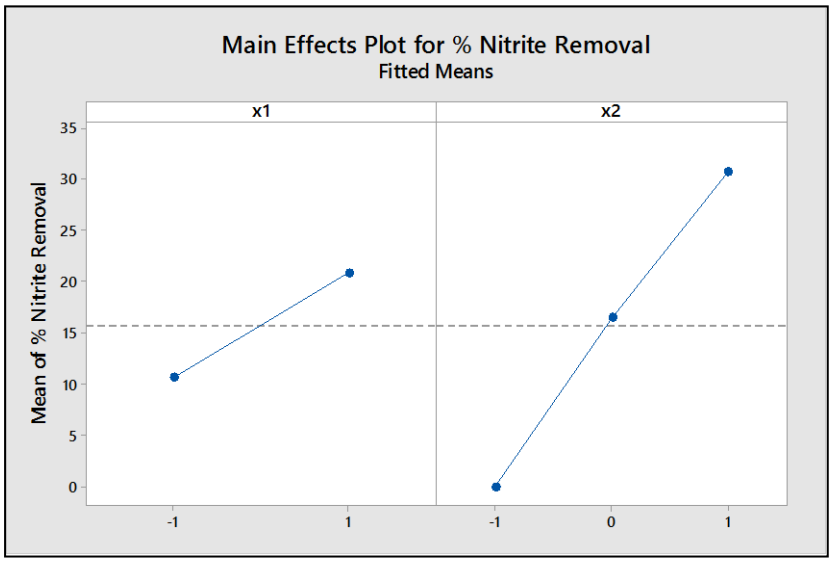

Figure 1. Main Effects Plot of the Nitrite Percentage Removal from the Minitab 17' Software

The Minitab 17's software developed the regression equations by the main effect plot (Minitab, 2017). Other studies reported that the main effects of operation time and current density have significant effect on the nitrite-nitrogen removal. The regression model which explains the removal efficiency is optimized to find the maximum level of removal [12]. Meanwhile in this research, the duration of time is the significance factor for the regression model for percentage removal of nitrite-nitrogen in developing the regression equation.

3.1.1. Factorial Design Analysis Factorial Regression Analysis: Percentage of Nitrite-nitrogen Removal Versus $\varkappa_1$ and $\varkappa_2$ The summary report presented in Figure 2 demonstrates that the model is statistically significant with the duration, $\varkappa_1$ at the 95% confidence level since p-value were less than alpha (α) of 0.10. The R^2 is high showing the data good fit.

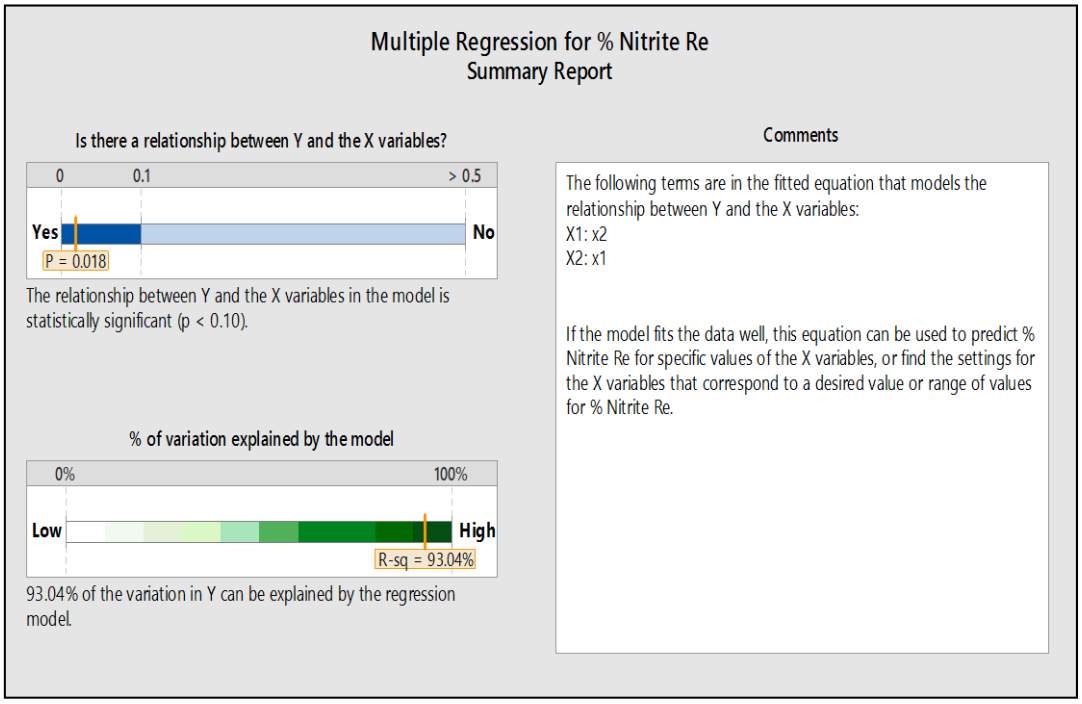

Figure 2. Summary Report from the Minitab 17' Software

The positive correlation R = 0.96 indicates that when $\varkappa_1$ increases, the removal of nitrite-nitrogen increases. The R^2 obtained in the experiment was 0.9304, which indicates a good agreement between the response (% nitrite-nitrogen removal) and the factor variables (duration). The factor (duration) with their square interactions (P=0.018 < 0.10) was significant at the 95% confidence level.

The factorial regression model obtained from the Minitab 17' software, as shown in Equations 1 and 2, can be used to predict the long term removal of nitrite-nitrogen without conducting any extended experiment. This in turn can reduce the number of experimental trials traditionally required to assess multiple parameters and their interactions.

For $\varkappa_1$ = 0 mg/L,

$$\% \text{ Nitrite-nitrogen Removal} = 10.67 + 15.37\ \varkappa_2 \qquad (1)$$

For $\varkappa_2$ = 100 mg/L,

$$\% \text{ Nitrite-nitrogen Removal} = 20.83 + 15.37\ \varkappa_2 \qquad (2)$$

where: $\varkappa_1$: initial ammonia concentration
$\varkappa_2$: duration

4. Conclusion

In conclusion, the bio-filters are potential as reliable supporting media for microbial population to grow in order to remove pollutants in leachates. It is also an economical means to develop more advanced biological treatment system. Not only beneficial in terms of cost, bio-filter is also sustainable. In this case, the duration taken to reduce pollutants is found to have a positive influence on the response and it is classified significantly able to remove the nitrite-nitrogen. The regression equation to remove nitrite-nitrogen can be studied deeper by future researchers by including more parameters to enhance the regression equation. This may help reduce reliance on the use of sanitary landfills to treat municipal solid wastes, leachates and pollutants.

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