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Ammonia-Nitrogen and Phosphate Removal in Leachate using Algae and Bacteria Mixture

Norhafezah Kasmuri and Muhammad Zaidi Misni

Abstract: ***Leachate is a liquid that has been produced by the waste especially organic waste. In an engineered sanitary landfill, biological treatment is a common practice to reduce the leachate contaminants. The effluent from treated leachate needs to comply with the standard set by the Department of Environment (DOE), Malaysia before it is being released into the river. The leachate contains a high number of contaminants such as ammonia-nitrogen, phosphorus, heavy metal, biochemical oxygen demand (BOD), chemical oxygen demand (COD) and suspended solids. This study focused on the possibility of treating leachate by using algae and bacteria in biological treatment in removing ammonia-nitrogen and phosphate in leachate obtained from Air Hitam Sanitary Landfill, Puchong. Initially, the characteristics of the leachate sample with in-situ and laboratory tests were analyzed to quantify the contaminants in the leachate. In this research, the concentration of leachate samples together with algae and bacteria was diluted in one (1) liter of distilled water varied between 10%, 30%, 50%, 80% and 100% (v/v) in each flask. Then, all the samples were aerated to ensure that the algae and bacteria were at the optimum condition to treat the contaminants in the leachate for all the respective flasks. The results of ammonia-nitrogen, phosphate, nitrite-nitrogen, and nitrate-nitrogen were taken for every 3 days for 15 days to determine the percentage of the removal due to the algae and bacteria uptake in the leachate for all the samples. After 15 days, the percentage of removal of the contaminants were analyzed using factorial design. It showed that 50% (v/v) of leachate concentration in the diluted flask exhibits the highest removal percentage of ammonia-nitrogen with 96.95% ammonia-nitrogen being removed from the leachate. For phosphorus, 10% (v/v) diluted leachate concentration marked highest which is 94.92% has been removed from the leachate. Finally, the regression equation was established to predict the rate of ammonia-nitrogen removal.***

Keywords: ***Algae, bacteria, factorial design, leachate.***

I. INTRODUCTION

In the last four decades, countries around the world have experienced economic growth, poverty reduction and improved welfare. This has led to the world population, including Malaysia that closely related to the generation of waste per capita. Waste generation has grown to a point where people consume at a faster rate than the earth can generate. This results in the waste generation that ends up in landfill site impose a negative effect on the health of the ecosystem.

Waste generation including municipal waste and industrial waste will end up in the landfill site and generate leachate. Landfill leachate has a variety of organic and inorganic constituents such as nitrogen, phosphorus, heavy metals, tannin and phenols [1] that are harmful to the environment if not treated well before being discharged. Without proper treatment of the leachate, the contaminants would pollute the surface water and groundwater resources [1]. The composition of the leachate varies depending on a few factors such as hydrogeology, amount of rainfall, age of the landfill, waste composition and degradation stage of the waste [2].

There are lots of technologies that have been used for leachate treatment such as physical or chemical treatment, biological treatment, and emerging technologies such as reverse osmosis (RO) and evaporation [3]. Biological leachate treatment is a proven technology for organics and ammonia removal in young and matured leachate. Leachate age can be classified in three stages as young (less than 5 years old), medium (5–10 years old), and mature (more than 10 years old) [4]. The anoxic or aerobic processes achieve nitrification and denitrification and reduce the oxygen demand for landfill leachate treatment [5-6]. Even though treatment by using membrane technology such as reverse osmosis (RO) is well known for its effectiveness in removing heavy metal and other substances, it also has some flaws. For example, RO is considered as an expensive technology and not suitable to treat wastewater with high total suspended solids (TSS) such as leachate. TSS will cause blockage in RO and make it less efficient [3].

Landfill leachate has a high level of ammonia-nitrogen due to the large proportion of organic nitrogen in solid waste, such as proteins, amino acids or urea, which decomposed to ammonia-nitrogen and may not further decompose if under anaerobic conditions [1]. An excessive amount of phosphorus in the form of orthophosphates and nitrogen in landfill leachate will cause eutrophication in a freshwater system. This nutrient needs to be treated before discharges as it will deplete the level of oxygen in the water body and leads to the decay rate of aquatic animals. Algae are types of plant which can adapt to harsh environments due to their unicellular or simple multicellular structure [7]. They are also of easy availability, high growth and reliable yield of a per-unit-area, and not competing for land cultivation.

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* Correspondence Author
Norhafezah Kasmuri*, Faculty of Civil Engineering, University Technology MARA, 40450 Shah Alam, Selangor, Malaysia.
Muhammad Zaidi Misni, Faculty of Civil Engineering, University Technology MARA, 40450 Shah Alam, Selangor, Malaysia.

Algae, consist of two groups of micro-algae and macro-algae, are photosynthetic aquatic organisms. They have chlorophyll as the key photo-synthetic pigment to fix atmospheric carbon dioxide (CO_2) in the photosynthesis [8].

Micro-algae does not have stems, roots or leaves which make them advantageous as they are easy to handle and harvest in bioreactors [8]. On the other hand, the macro-algae have stems, roots or leaves. There are three important nutrients (carbon, nitrogen, and phosphorus) needed for the algae to grow. All these nutrients can be found abundance in different types of waste streams such as landfill leachates, composting filtrates coming from anaerobic composting sites, wastewater coming from household and municipal liquid wastes [9].

The colony of bacteria in biological treatment can reduced contaminants from landfill leachate. The existing nitrogen removal processes for examples; nitrification-denitrification on the process, nitritation - denitritation process, endogenous denitritation process, and Anammox process, are using bacteria in biological treatment [4].

This paper demonstrated the mixture of algae and bacteria in treating the ammonia-nitrogen and phosphate from leachate samples of Air Hitam Sanitary Landfill, Puchong. From the study, the regression equation in predicting the rate of ammonia-nitrogen removal was established.

II. MATERIAL AND METHODS

A. Collection of Leachate

Leachate was collected from Air Hitam Sanitary Landfill, Puchong, Selangor. The characteristic of the leachate initially was analyzed. In-situ and laboratory tests were conducted. The in-situ physical characteristic of the leachate measured temperature, pH value, turbidity, total dissolved solids (TDS) and dissolved oxygen (DO). The leachate sample was settled first and then was filtered using 2 µm filter paper to remove particulate matters in the sample.

The chemical characteristic of the leachate, which are Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Suspended Solid (TSS), Ammonia-nitrogen, Hardness, Zinc, Copper, Aluminium, Iron, Magnesium, Nitrate-nitrogen, Nitrite-nitrogen and Phosphorus content were conducted in the laboratory. Algae and bacteria inoculum were also obtained from Air Hitam Sanitary Landfill.

B. In-situ Testing

The in-situ test was conducted at the inlet pond for the leachate in Air Hitam Landfill, Puchong, Selangor. The in-situ test was performed using the HORIBA® probe to obtain the following parameters: temperature, pH value, turbidity, total dissolve solids (TDS) and dissolve oxygen (DO) of the sample.

C. Laboratory Testing

Laboratory test has been conducted to evaluate the Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Suspended Solid (TSS), Ammonia-nitrogen, Hardness, Zinc, Copper, Aluminum, Iron, Magnesium, Nitrate-nitrogen, Nitrite-nitrogen and Phosphorus. All the laboratory experiment was using standard method and was referred to DR 2800 Spectrophotometer: Procedures Manual, 2007 [10].

D. Experiment Setup

The leachate samples with a mixture of algae and bacteria have gone a series of dilution from 10%, 30%, 50%, 80% and 100% (v/v) leachate concentration in five flasks each contained one (1) liter distilled water, respectively [3]. Three replicates have been carried out in the experiment. To avoid any damages or alteration in properties, the samples and algae collected from the site were kept in the refrigerator or cold room at 4^oC of temperature.

The experiment was carried out in the laboratory. The leachate samples and bacteria inoculum were exposed under the UV light. The diluted leachate sample was purged with air to ensure that the sample was constantly aerated, and algae are alive (see Fig.1). The initial ammonia-nitrogen content for each sample was determined and recorded using spectrophotometer HACH DR 2800 [10] as initial (Day 0) ammonia-nitrogen content. Then, the ammonia-nitrogen reading was repeated for every 3 consecutive days until the end of day 15.

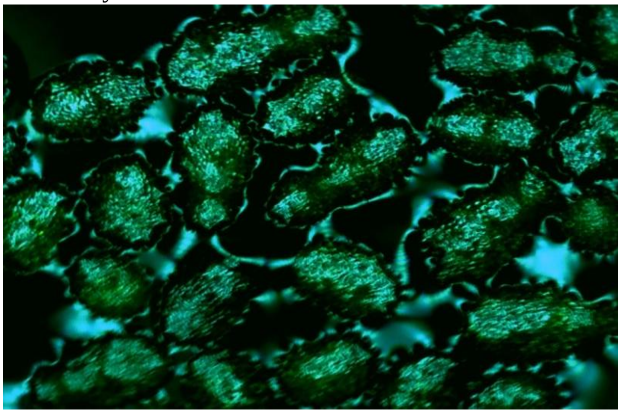

Fig. 1. Morphology of the algae

E. Factorial Design using Design of Experiment (DOE)

The relationship between the two-factor variables of Factor A (Day) and Factor B (Leachate Concentration (v/v)) with the response for the ammonia-nitrogen reduction was analyzed by a full general factorial design by the DOE from Minitab software [11]. The full factorial design by the DOE was used to predict the final concentration of ammonia-nitrogen removal. The multilevel factorial design used in this experiment was the one having at least one factor with more than two levels.

This experiment consisted of two factors to be used in the factorial design by Design of Experiment (DOE) using Minitab software [11]. The day (period of the experiment) is denoted as A and the leachate concentration is denoted as B. The A factors had 6 levels, which were day 0, 3, 6, 9, 12 and 15 while the B factor had 5 levels, which were 10%, 30%, 50%, 80% and 100% (v/v) leachate concentration of samples. Table-I shows the factors and levels of the parameters adopted for the factorial design of DOE.

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Table-I: Factors and levels of the parameter for the factorial design of DOE

Leachate concentration (v/v) (Factor B)	Day (Factor A)					
	Day 0	*Day 3*	*Day 6*	*Day 9*	*Day 12*	*Day 15*
	Response (mg/L)					
10%	68.0	24.0	10.0	5.0	2.2	3.5
30%	121.0	69.0	32.0	12.0	3.2	4.7
50%	174.0	115.0	58.0	14.0	3.5	5.3
80%	210.0	113.0	27.0	13.0	6.1	8.7
100%	221.0	178.0	33.0	11.0	7.9	9.3

III. RESULTS AND DISCUSSION

A. Characteristics of Leachate Sample determined in-situ

The leachate samples were tested based on in-situ at the inlet pond for the leachate in Air Hitam Landfill, Puchong, Selangor. Table-II shows the results of temperature, pH value, turbidity, total dissolve solids (TDS) and dissolve oxygen (DO) of the sample. The pH of leachate samples is 6.67 which between (6.0 to 9.0) the standard stated by DOE. However, the suspended solids (SS) is high (100 mg/L) than the standard of DOE (50 mg/L) [12].

B. Characteristics of Leachate Sample determined in a laboratory

The laboratory tests conducted in the Environmental Laboratory, Faculty of Civil Engineering, UiTM Shah Alam for leachate samples collected from Air Hitam Landfill, Puchong, Selangor. The results of Table II show the BOD, COD, ammonia-nitrogen, heavy metals (Zn, Al, Cu, Cr, Fe, Mg), nitrite-nitrogen, nitrate-nitrogen and phosphorus.

The BOD and ammonia-nitrogen concentration exceed the limit of DOE standards, which is 85 mg/L compared to 20 mg/L and 470 mg/L higher than 5 mg/L, respectively. The results of heavy metals including Zn, Cu, Cr and Mg are higher than the allowable limit stated by DOE [12]. As for that, these contaminants need to be treated before it can be released to the river.

C. The removal of ammonia-nitrogen

Fig. 2 shows the percentage of removal of the ammonia-nitrogen from five different leachate concentration of samples. It shows that leachate with 50% (v/v) concentration exhibit the highest percentage of ammonia-nitrogen removal recorded 96.95% was successfully removed. In this case, the nitrifying bacteria in the leachate samples consumed the ammonia-nitrogen in the nitrification process under aerobic condition [13]. By referring to the nitrogen cycle, the ammonia-nitrogen are converted to the nitrite-nitrogen by nitrification bacteria in this flask [14].

However, the leachate sample with 10% (v/v) concentration showed the lowest ammonia-nitrogen removal (94.85%). This is due to the high dilution rate in the leachate sample compared to the four other flasks. Whereas, the colony of bacteria in 10% (v/v) leachate sample was less than other flasks. Herein, limited nitrifying bacteria can only oxidize a small amount of ammonia-nitrogen in the leachate sample.

Table-II: Leachate characteristics from Air Hitam Landfills, Puchong

Parameter	*Unit*	*Raw Leachate*	*D.O.E Standard*
pH	-	6.67	6.00-9.00
Temperature	0^C	29.30	40
Turbidity	NTU	560	-
TDS	mg/L	670	-
DO	mg/L	7.98	-
BOD	mg/L	85	20
COD	mg/L	250	400
SS	mg/L	100	50
NH_3-N	mg/L	470	5
$CaCO_3$ (Mg)	mg/L	4.2	-
$CaCO_3$ (Ca)	mg/L	91	-
Zn	mg/L	65	2.0
Al	mg/L	37	-
Cu	mg/L	26	0.20
Cr	mg/L	1.3	0.20
Fe	mg/L	2.0	5.0
Mg	mg/L	4.2	0.20
Nitrite-nitrogen	mg/L	2.0	-
Nitrate-nitrogen	mg/L	0.5	-
Phosphorus	mg/L	22	-

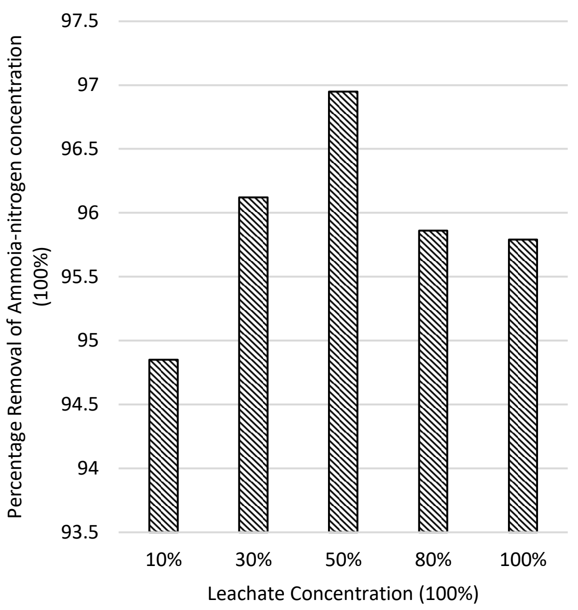

Fig. 2. The percentage removal of ammonia-nitrogen in the leachate with different concentration

D. Statistical analysis by full factorial design in Design of Experiment

Two factors were used in the Design of Experiment (DOE) using full factorial design for this research. There was a period of experiment and the leachate concentration denoted as A and B respectively. The period of the experiment had six-levels start with Day 0, 3, 6, 9, 12 and 15. While the leachate concentration had five-level start with 10%, 30%, 50%, 80% and 100% (v/v) concentration. The combination of these factors and levels outlined a total of 30 runs of the experimental matrix as shown in Table-III.

Table-III: Leachate characteristics from Air Hitam Landfills, Puchong

Standard Order	Run Order	Factors		Response
		Duration A (Day)	*Leachate concentration/ B (%)*	*Ammonia-nitrogen concentration (mg/L)*
1	1	0	10	68.0
2	2	0	30	121.0
3	3	0	50	174.0
4	4	0	80	210.0
5	5	0	100	221.0
6	6	3	10	24.0
7	7	3	30	69.0
8	8	3	50	115.0
9	9	3	80	113.0
10	10	3	100	178.0
11	11	6	10	10.0
12	12	6	30	32.0
13	13	6	50	58.0
14	14	6	80	27.0
15	15	6	100	33.0
16	16	9	10	5.0
17	17	9	30	12.0
18	18	9	50	14.0
19	19	9	80	13.0
20	20	9	100	11.0
21	21	12	10	2.2
22	22	12	30	3.2
23	23	12	50	3.5
24	24	12	80	6.1
25	25	12	100	7.9
26	26	15	10	3.5
27	27	15	30	4.7
28	28	15	50	5.3
29	29	15	80	8.7
30	30	15	100	9.3

Fig. 3 shows the Factorial Design Analysis and the factors that affect the ammonia-nitrogen removal from the leachate sample. The "A" graph shows the mean of the ammonia-nitrogen concentration within the period of experiment starting with Day 0 until Day 15. While "B" graph shows the mean of ammonia-nitrogen for each leachate concentration starting with 10% to 100% leachate concentration. Each point in the graph represents the mean of the response (ammonia-nitrogen concentration) variable for the various levels of each factor (day and leachate concentration).

It appears that the ammonia-nitrogen concentration is high at the beginning of the experiment and reduced towards the end of the period of the experiment. This shows that ammonia-nitrogen are successfully removed by the mixed of the algae and bacteria. The 100% leachate concentration shows that it has the highest content of ammonia-nitrogen before the treatment. This due to the 100% leachate sample was not diluted from the original sample collected from Air Hitam Sanitary Landfill.

The horizontal reference line drawn at the grand mean of the response data shown in Fig. 3 represents the mean processing time for all runs. In this study, the time interval had a significant effect on the ammonia-nitrogen removal. The mean of the ammonia-nitrogen concentration reduced as the duration of the experiment is extended. The two mentioned effects contributed to the ammonia-nitrogen removal efficiency by mixed of algae and bacteria. The previous researcher used ammonia-nitrogen concentration and duration as the two main factors that affect the ammonia-nitrogen removal [14-15].

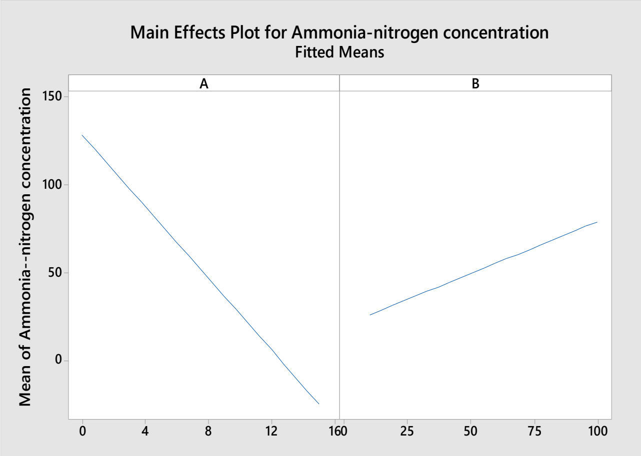

Fig. 3. Main effects plot of the ammonia-nitrogen concentration from Minitab 18 software

E. Regression analysis from the factorial design of DOE

Regression equation of the factorial design was developed using Minitab 18 [11]. The p-value for both factors shows it is less than 0.1 which was significant at the 95% confidence level. This shows that both factors were important in determining the final concentration of the ammonia-nitrogen in the leachate sample. The correlation coefficient, R-value obtained from the regression analysis developed in Minitab 18 at the end of the experiment is 0.705.

The factorial design regression model equation obtained from Minitab 18 software [11] is shown in Equation (1). Equation (1) can be used to predict the final concentration of the ammonia-nitrogen without conducting any extended experiment. This is crucial since the Department of Environmental (DOE), Malaysia [12] required that ammonia-nitrogen concentration should be less than 5 mg/L before it can be discharged to the river. This, in turn, can reduce the number of experiments trials traditionally required to assess multiple parameters and their interaction.

$$\text{Ammonia-nitrogen removal} = 96.7 + 0.587B - 10.18A \qquad (1)$$

where;

A is day or duration of the experiment
B is leachate concentration in %

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A three-dimensional surface plot and a two-dimensional contour plot are illustrated in Fig. 4 and Fig. 5, respectively, to provide better visualization of the statistically significant factors derived from the statistical analysis. The effects and interactions of the period of treatment (days) and concentration of the leachate on the removal of ammonia-nitrogen are illustrated in both figures. The period of treatment (days) always had a positive effect on the removal of ammonia-nitrogen.

Since the samples in the batch test were maintained in room temperature, the maximum ammonia-nitrogen removal was achieved at the longest period of treatment. The effect of leachate concentration was dominant at a shorter period of leachate treatment. The ammonia-nitrogen removal efficiency increased with a longer period of treatment. With an extended period of treatment, the ammonia-nitrogen removal efficiency of leachate concentration of 50 % to 100 % (v/v) relatively close. The optimum period of treatment and leachate concentration for ammonia removal was found to be in 15 days and a leachate sample concentration of 50% (v/v).

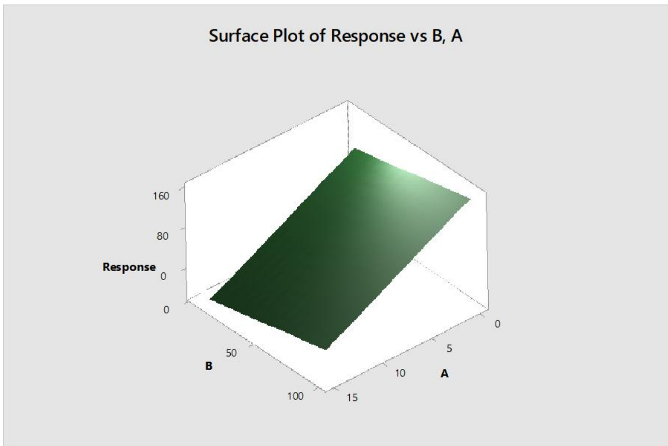

Fig. 4. Three-dimensional surface plot of Response versus Factor A (period of the experiment (day)) and Factor B (leachate concentration)

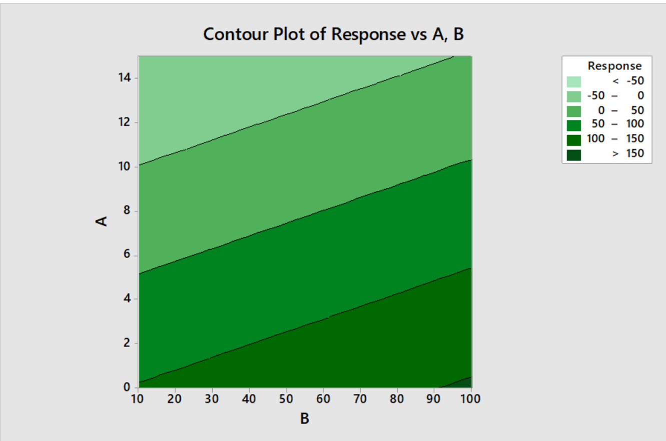

Fig. 5. Two-dimensional contour plot of Response versus Factor A (period of experiment in (day)) and Factor B (leachate concentration)

F. The percentage removal of phosphorus

Fig. 6 shows the percentage of removal of the phosphorus from five (5) different leachate concentration of samples. It shows that leachate with 10% concentration marked the highest percentage of removal of ammonia-nitrogen recorded 94.92% phosphorus was successfully removed.

This inorganic phosphate in the leachate samples concentration has been used by the algae as the nutrient source [16]. This phosphate plays a critical role in cell development and is a key component of molecules that store energy, such as ATP (adenosine triphosphate), DNA and lipids (fats and oils). Insufficient phosphorus will result in a decreased of the plant such as algae [16].

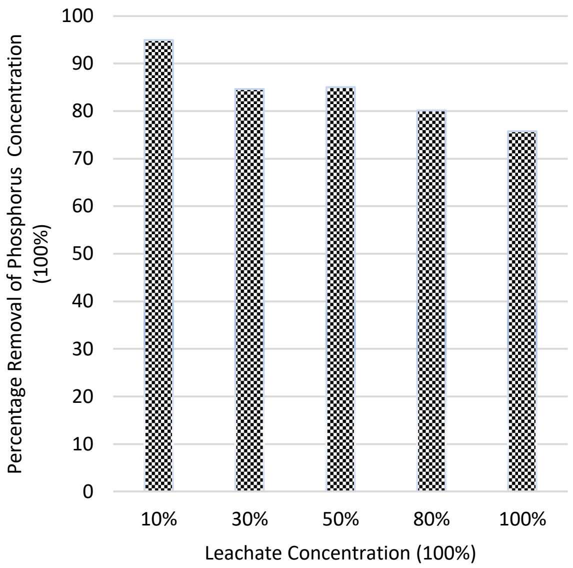

Fig. 6. Phosphorus percentage removal for 15 days of leachate with different concentration

IV. CONCLUSION

In conclusion, the algae and bacteria culture can be potentially used as a medium to remove pollutants from leachates especially ammonia-nitrogen and phosphorus. It is also an economical way to treat the leachate comparing to more advanced biological treatment system. The algae and bacteria culture is a sustainable source and can adapt themselves to the leachate conditions. In this study, the duration is taken to reduce the pollutants and the leachate concentration have a positive influence on the response. From the results obtained in the experiment and DOE from Minitab software, the optimum period of treatment and leachate concentration for ammonia-nitrogen removal was in 15 days and leachate sample concentration of 50% (v/v). It is recommended that the regression equation established to remove ammonia-nitrogen can be further investigated by future researchers to include more parameters to enhance the equation. This may reduce reliance on the use of sanitary landfills to treat municipal solid wastes, leachates and pollutants.

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AUTHORS PROFILE

Norhafezah Kasmuri has obtained her Degree in Civil and Environmental Engineering from Universiti Kebangsaan Malaysia in 2001. She has received her Master of Science in Water Resources Engineering and Management (WAREM) from Universitat Stuttgart, Germany in 2004. She has completed her study of Doctor of Philosophy in Chemical Engineering from Swansea University, Wales, the United Kingdom in 2014. She has been serving UiTM since August 2014 as a senior lecturer at the Faculty of Civil Engineering. Her research interests are interdisciplinary includes water resources engineering and management, water and wastewater treatment, water reclamation and reuse, environmental engineering and sustainability management, biochemical and bioprocess engineering.

Muhammad Zaidi bin Misni has obtained his Bachelor of Engineering (Hons) Chemical in Universiti Teknologi MARA, Shah Alam in 2017. He also did receive his Master of Science in Environmental Engineering in Universiti Teknologi MARA, Shah Alam. His has completed his course in integrated project design focusing on drawing equipment using AutoCAD, performing thermodynamic analysis of the process and study the chemical process and sustainability of the process towards human and environment. The other course was Fluid Flow, which he has experienced in design and drawing mixing tank based on properties of the liquid and analyzed various factor affecting the fluid flow pattern in the mixing tank.