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Research Article

Investigation on the Efficiency of Effective Microorganisms for Polluted Water Treatment

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Abstract: Water pollution, mainly caused by rapid industrialization and population growth, has been one of the major threats to the sustainability of living organisms. The urgency to preserve and restore the freshwater system has never been clearer, as only 1% of the world's total water supply is suitable for human consumption. Recently, biological treatment using Effective Microorganisms Activated Solution (EMAS) shows potential in reducing pollution in wastewater and river water bodies. Thus, this study aims to assess the water quality of water bodies in Malaysia, namely Kerayong River and Pandan Perdana Lake, and assess the effectiveness and optimum concentration of EMAS on polluted water. The results obtained showed that high levels of NO_2^-, NO_3^- and NH_3 were found in excess in Kerayong River, indicating pollution occurs whereas none of the tested parameters were detected in excess in Pandan Perdana Lake, hence showing it was not contaminated. The effectiveness of EMAS on synthetic wastewater was assessed through the measurement of few parameters which include Ca^{2+}, Na^+, K^+, NO_3^-, NH_4^+, pH and microbe concentration. It was found that in all concentrations of EMAS, ammonium ion concentration was effectively reduced, and microbe concentration was increased ($p < 0.05$). EMA (1 mL/L) and EMB (0.2 mL/L) samples had significantly lower nitrate concentration compared to the control samples. EMAS were not capable to reduce dissolved water minerals such as Ca^{2+}, Na^+ and K^+, but further contributes to the increase of dissolved minerals in the water. Whereas no significant effect of EMAS on pH of water samples was observed as all water samples fall within pH of 4.31 to 4.56 throughout 5 days. EMAS concentration of 1 mL/L was the optimum concentration for reducing ammonium and nitrate concentration.

***Keywords*:** effective microorganisms, water quality, polluted water treatment

1. Introduction

Water plays a vital role in human life as a mean of survival and to conduct human activities. Freshwater is a limited resource which can be obtained from natural water bodies such as rivers, lakes, wetlands etc [1]. However, rapid urbanization and population growth has increase water demand and led to water pollution. Human domestic, industrial, and agricultural activities have introduced a large number of contaminants into water bodies which leads to water degradation [2]. According to United Nations Development Programme (UNDP) [3], about 80% of the wastewater enters water bodies without adequate treatment. As a result, polluted water bodies not only threatened the health of human, animals and plants that depend on water for survival but also reduce the water availability for anthropogenic activities.

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In Malaysia, rivers are the main water resources as about 97% of the total water use originates from rivers [4]. However, most of the rivers in Malaysia are polluted and seem as drains to people. The data obtained from Department of Environment (DOE) shows that 54% of the rivers monitored was categorized as slightly polluted and polluted in 2017 [5]. These surface waters received pollutants from point sources and non-point sources along the river which then deteriorate water quality [6]. When these rivers become too polluted, they are not suitable to be consumed or used. Hence, it is important to reduce pollution and improve the water quality of these surface water bodies before it gets worse.

Nowadays, green technologies have been developed and widely used to provide solutions for these environmental problems [7]. One of the green technologies proposed is Effective Microorganisms (EM) technology. EM is an eco-friendly product which consists of more than 80 species of microorganisms which are employed for bioremediation [8]. The 5 main species of EM are photosynthetic bacteria (*Rhodopseudomonas palustris, Rhodobacter sphaeroides*), lactic acid bacteria (*Lactobacillus plantarum, Lactobacillus casei*), yeast (*Saccharomyces cerevisiae*), actinomycetes (*Streptomyces albus*) and fermenting fungi (*Aspergillus oryzae*) [9, 10]. Microorganisms are utilized to destroy or reduce the concentration of pollutants and contaminants in the polluted site. In this study, EM technology was chosen as it is cost cost-effective and environmental-friendly to the site of contamination [11]. Using EM, the microbes degrade organic matters and pollutant in water to less or non-toxic substances. The high concentration of contaminants serves as substrate and nutrients for microbes' growth and reproduction [12, 13]. Two types of EM technology show great potentials to be used in water bioremediation, which are EM Activated Solution (EMAS) and EM Mudball. Yet, there is an insufficient study in the efficiency of EM technology in water treatment. Besides that, the effect of EMAS on the ions in water bodies has not been studied before. There are also little studies conducted using different concentrations of EMAS for water treatment. Hence, this experiment is to evaluate the efficiency of EMAS in reducing the concentration of pollutants by using the naturally occurring microorganisms in EM.

In the preliminary study, Kerayong River and Pandan Perdana Lake were chosen as the study area due to little studies were conducted to evaluate the water quality of these two water bodies. Water samples were collected and tested from three different parts from each source located in Ampang, Selangor. This step was to evaluate the water quality of these water sources before testing with EMAS. The results of the test showed that Pandan Perdana Lake was not polluted. Hence, only Kerayong River was used as the targeted site in this study. However, due to Covid-19 pandemic, synthetic wastewater was prepared as a preventive measure to avoid exposure to potentially contaminated river water during water samples collection. Different concentrations of EMAS were added into synthetic wastewater in this study. The efficiency of EMAS for polluted water treatment was measured by water parameters which include potassium (K^+), sodium (Na^+), calcium (Ca^{2+}), nitrate (NO_3^-), pH, ammonium (NH_4^+) and microbe's concentration at OD_{600}. The objectives of this study are to assess the water quality of Pandan Perdana Lake, Ampang and Kerayong River, Ampang in Malaysia and to evaluate the efficiency of EMAS as well as to determine the optimum concentration of EMAS for polluted water treatment.

2. Materials and methods

2.1 *Equipment*

The list of equipment is shown in Table 1 with their respective manufacturer and country of origin.

Table 1. List of equipment, their respective manufacturer and country of origin

Equipment	Manufacturer	Country of Origin
SERA Test Kit	Sera	Germany
pH meter	Mettler Toledo	USA
Horiba LAQUAtwin Meter	Horiba, Ltd	Japan
Spectrophotometer	AHS Laboratory Supplies	Malaysia

2.2 *Study area*

Kerayong River is one of the main tributaries of Klang River with 12.5 km length and 52 km^2 catchment area, originates from Pandan Indah and joins Klang River near Pantai Dalam Komuter station [14, 15]. Kerayong river is in a highly urbanized area with residential, industrial, and commercial area nearby. Hence, residents and industrial owners pollute the river by disposing wastes into the river. Besides that, there are numerous restaurants and shops along the river that also contribute wastes which are believed to cause water pollution in Kerayong River [16]. Based on data from DOE, Kerayong River is categorized as polluted with a Water Quality Index (WQI) value of 53 and 52 in 2016 and 2017, respectively. Kerayong River falls in Class V, which is the worst category based on its biochemical oxygen demand and ammoniacal nitrogen level. The coverage area of Kerayong River is shown in Figure 1.

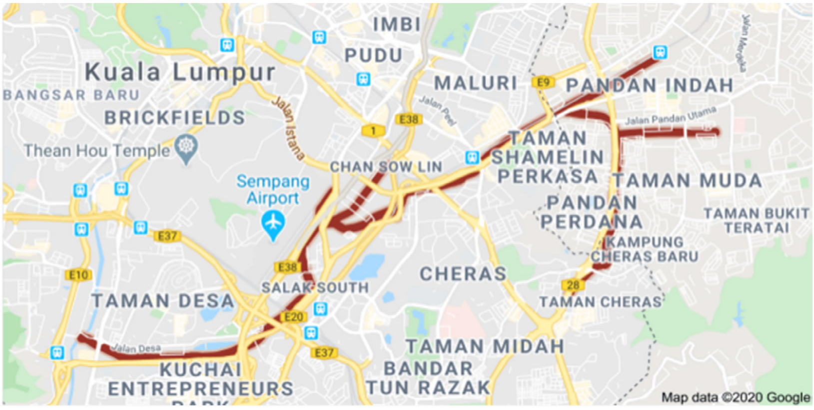

Figure 1. Kerayong River coverage area obtained from Google Maps

Another study area is Pandan Perdana Lake which located at the border of Cheras and Ampang, Selangor. Pandan Perdana Lake has an area of approximately 36,140 m². It acts as a public recreation park and fishing pond in the residence area. There are also some restaurants and bars located beside the lake, hence it has risks of getting polluted by human activities. Figure 2 shows the location of Pandan Perdana Lake in Google Maps.

Figure 2. Location of Pandan Perdana Lake in Google Maps

2.3 *Sampling*

Water samples were collected from 3 different parts of the river and lake twice a week for 3 consecutive weeks.

Sampling was carried out in the month of February 2020 at noon without rain. The average temperature was 34°C with a standard deviation of ±2°C based on data from AccuWeather database. The sampling was conducted in February due to it is the dry season in Malaysia where little rain occurs during this period whereas the other months especially October to December are the raining period in Malaysia. Hence, water quality will be affected by heavy rainfall. The water samples for lake originated from Pandan Perdana Lake, Ampang which the lake water does not have a runoff flow and stays relatively stagnant. Water samples were collected from selected sites [Fishing site (3.1180° N, 101.7435° E), Bench site (3.1176° N, 101.7445° E) and Hut Shelter site (3.1169° N, 101.7444° E)]. Whereas the water samples for river originated from Kerayong River, Ampang which flows through Kuala Lumpur and Selangor. The constantly flowing river water was sampled from 3 selected sampling sites along the river [Jalan 2 (3.1100° N, 101.7458° E), Persiaran Majlis Perbandaran Ampang Jaya (MPAJ) (3.1271° N, 101.7515° E) and Cempaka Light Rail Transit (LRT) Station (3.1378° N, 101.7529° E)]. All the sampling sites fall in the UTM zone of 47 N. Table 2 shows the description of water sampling sites and the reason for the sampling. Figure 3 shows the sampling sites for Kerayong River and Pandan Perdana Lake, Ampang on Google Maps. The water samples collected were brought back to Biotechnology Laboratory, UCSI University for analysis. The water parameters tested are pH, NO_2^-, NO_3^- and NH_3 using SERA Test Kit.

Table 2. Description of sampling sites

Water Sources	Site Code	Sampling Site Name	Geographical Coordinate		Identified Anthropogenic Activities	Reasons for Sampling
			Latitude/Longitude	UTM Zone		
Pandan Perdana Lake, Ampang	L1	Fishing site	3.1180° N, 101.7435° E	47N	Fishing	To evaluate the water quality of the lake which might be polluted by fishing activities
	L2	Bench site	3.1176° N, 101.7445° E		-	To provide sampling replicates for the similar lake
	L3	Hut Shelter site	3.1169° N, 101.7444° E		-	To provide sampling replicates for the similar lake
Kerayong River, Ampang	R1	Jalan 2	3.1100° N, 101.7458° E		Industrial and municipal wastewater discharges	To evaluate the water quality of the river which is polluted by nearby residential area and industries
	R2	Persiaran Majlis Perbandaran Ampang Jaya (MPAJ)	3.1271° N, 101.7515° E		Commercial and municipal wastewater discharges	To evaluate the water quality of the river which is polluted by nearby residential and commercial businesses such as dumping of excess food and waste into the river
	R3	Cempaka Light Rail Transit (LRT) Station	3.1378° N, 101.7529° E		Municipal wastewater discharges	To evaluate the water quality of the river which is polluted by nearby residential area and people who use public transport

Figure 3. Study area showing sampling sites in Google Maps

2.4 *Activation of Effective Microorganisms (EM)*

The Effective Microorganisms solution (EM·1) was activated to produce EMAS prior to usage in the experiment. EMAS was activated using EM·1 solution, molasses and tap water in the ratio of 1:1:20. Both EM·1 solution and molasses were purchased from EMRO Malaysia Sdn. Bhd. The mixture was added into a plastic bottle and about 10% of air space was left inside the plastic bottle to allow pent-up gas formation. The initial pH of the solution was measured using a calibrated pH meter. The EMAS solution was then kept at room temperature for 10 days to allow fermentation to occur prior to usage in the experiment. The cap was loosened daily after 2 days from initial fermentation to release the pent-up gas. The quality of EMAS solution was checked after 10 days using a calibrated pH meter in which a pH lower than 3.6 and a sweet-sour smell indicate the solution was ready to be used [17].

2.5 *Preparation of different concentration of EMAS*

Different concentrations of EMAS were prepared by diluting EMAS with distilled water. A total of 50 mL of 5 different concentrations of EMAS were prepared in 5 different falcon tubes by adding EMAS into distilled water according to the volumes shown in Table 3.

Table 3. Compositions of different concentrations of EMAS

EMAS labels	Volume of EMAS (mL)	Volume of distilled water (mL)
EM Control	0	50.00
EMA	5.00	45.00
EMB	1.00	49.00
EMC	0.50	49.50
EMD	0.25	49.75

2.6 *Preparation of synthetic wastewater*

Due to Covid-19 pandemic, water samples were not collected from the sampling sites to avoid exposure to river water that is potentially contaminated with coronavirus. Hence, synthetic wastewater was prepared in this study. The synthetic wastewater was composed according to Saeed and Sun [18] with slight amendments as follows: (g in 1 L distilled water): 2.2-$C_6H_{12}O_6$, 0.05-0.22 NH_4Cl, 0.01-0.20 KH_2PO_4, 0.11-0.34 $CaCl_2 \cdot 2H_2O$, 0.32-0.92 $MgSO_4 \cdot 7H_2O$, 0.15-0.44 $NaHCO_3$, 0.12-0.80 NaCl and 0.30-$NaNO_3$.

2.7 *EMAS treatment*

The synthetic wastewater sample and distilled water were divided into aliquots of samples with a volume of 40 mL each in falcon tubes. Distilled water acts as a control to compare the effect of EMAS on synthetic wastewater sample. Then 0.4 mL of EMAS with different concentrations were added accordingly into 40 mL of synthetic wastewater and distilled water. All the water samples with and without EMAS had triplicates and labelled respectively to obtain an average value. Table 4 shows concentrations of different treatments.

The treatment ran for 5 days and water parameters: Ca^{2+}, K^+, NO_3^- and Na^+ were measured daily using Horiba LAQUAtwin meter. Meanwhile, pH of the water samples was measured using pH meter and microbial concentration was measured using spectrophotometer at OD_{600} [19]. All the parameters were measured in triplicates to obtain the mean value.

Table 4. Concentrations of EMAS for different treatments

Treatment	EMAS Concentration (mL/L)
EM Control	0.00
EMA	1.00
EMB	0.20
EMC	0.10
EMD	0.05

NH_4^+ concentration was also measured on day 1 and day 5 using manual colorimetric method by spectrophotometer. 1000 mg/L of NH_4^+ standard solution was prepared by dissolving 4.7190 g of $(NH_4)_2SO_4$ in 1 L of distilled water [20]. Different NH_4^+ concentrations were prepared by serial dilution as shown in Table 5. Then 10 mL of each sample were added into the vial, followed by adding 6 drops of each reagent 1, 2 and 3 of SERA NH_4^+/NH_3 Test kit accordingly. Distilled water with each reagent was also added which act as blank. After 5 minutes, 1 mL of the standard and samples were transferred to cuvette to measure their absorbance at OD_{640} for three times using spectrophotometer [21].

Table 5. Preparation of standard solutions

Tube	Volume of Standard	Volume of Distilled Water	Concentration
1	10 mL	90 mL	100 mg/L
2	40 mL (from Tube 1)	10 mL	80 mg/L
3	25 mL (from Tube 2)	25 mL	40 mg/L
4	25 mL (from Tube 3)	25 mL	20 mg/L
5	25 mL (from Tube 4)	25 mL	10 mg/L
6	25 mL (from Tube 5)	25 mL	5 mg/L
7	25 mL (from Tube 6)	25 mL	2.5 mg/L
8	0 mL	50 mL	0 mg/L

2.8 *Statistical analysis*

Statistical analysis was calculated using t-Test in Microsoft Excel to determine the significant differences between control treatment and EMAS treatment.

3. Results and discussion

The average values for water parameters tested at 6 sampling sites were shown in Table 6.

The water measurements of pH level for both lake and river were in the range of 7.42 to 7.58. The colour observed were light green, light blue-green and blue-green colour for the 3 weeks. The optimum pH range for the freshwater system is at a pH of 6.8 to 7.6 [22] whereas the pH range appropriate for aquatic life is from 6.5 to 9.0 [23]. Hence, water samples from all the sampling sites fall within the normal pH range.

Table 6. Concentration of pH, NO_2^-, NO_3^- and NH_3 for different sites

Sampling Site		pH		NO_2^-		NO_3^-		NH_3	
		Average	σ	Average	σ	Average	σ	Average	σ
Pandan Perdana Lake	Fishing site	7.58	0.20	0.00	0.00	0.00	0.00	0.00	0.00
	Bench site	7.42	0.20	0.00	0.00	0.00	0.00	0.00	0.00
	Hut Shelter site	7.42	0.20	0.00	0.00	0.00	0.00	0.00	0.00
Kerayong River	Jalan 2	7.42	0.20	0.50	0.77	7.50	9.87	0.15	0.04
	Persiaran MPAJ	7.58	0.20	0.50	0.00	10.00	0.00	0.23	0.15
	Cempaka LRT Station	7.50	0.00	0.33	0.41	7.50	9.87	0.17	0.00

The data of the table was in average and σ represents standard deviation. The unit for NO_2^-, NO_3^- and NH_3 was in mg/L.

Ammonia is toxic and harmful to aquatic life even at a very low concentration. A level higher than the general range which is less than 0.05 mg/L of ammonia indicates contamination of the water body [24]. Based on the results shown in Table 6, no ammonia was observed in all sampling sites of Pandan Perdana Lake. The colour of the solutions after adding SERA Test Kit reagents was yellow. Whereas the water quality test using SERA Test Kit showed 0.15 mg/L, 0.23 mg/L and 0.17 mg/L of ammonia respectively in the 3 sampling sites of Kerayong River. The colour of the solution after adding reagents was dark green. This indicates that water from Kerayong river was considered as polluted as all the ammonia levels were higher than the normal range. High concentration of ammonia might be due to the wastes containing ammonia from industrial and municipal waste effluents as it flows along industrial and commercial area. Besides that, human immoral behavior of littering in and around the river could also lead to ammonia pollution as garbages were commonly seen in the water of Kerayong River. Ammonia exists in water in two forms: ionized ammonium and unionized ammonia form [25]. The toxicity and concentration of ammonia were also affected by pH and temperature of the water. As the pH increases, the unionized ammonia increases which is more toxic to aquatic life [26].

Nitrite exists in the natural environment at a low concentration as an intermediate in the nitrogen cycle. High level of nitrites of more than 0.10 mg/L is toxic to the freshwater system and indicates poor water quality [24]. From the results shown in Table 6, the nitrite concentration in all 3 sampling sites of Kerayong River ranges from 0.33-0.50 mg/L, which has exceeded the safe nitrate level. The colours observed from SERA Test Kit were light yellow, dark yellow and light orange. Excess amounts of nitrites may be due to high concentration of ammonia which was detected in the river that may convert to nitrites. This process naturally occurs in the environment by specialized bacteria known as ammonia-oxidizing bacteria (AOB) such as *Nitrosomonas sp*. and *Nitrosospira sp.* [27, 28]. When aquatic organisms take up a high level of nitrites, nitrite oxidizes the haemoglobin which results in the inability of blood to transfer oxygen to the cell, leading to death [29, 30].

Nitrates occur naturally in water and are essential nutrients for plants growth. The natural level of nitrates in water bodies is typically low, which is less than 1 mg/mL [31]. However, excess amounts of nitrates may cause eutrophication, lowering the concentration of dissolved oxygen in water, hence killing aquatic animals [32]. Based on the results obtained, water samples from all 3 sampling sites of Kerayong River showed excess nitrates level ranged from 7.5-10 mg/mL. The colours of solutions after adding reagent were yellow, light orange and dark orange. High nitrates concentration was due to a high amount of organic matter in water bodies which decomposed into ammonia and oxidized to nitrate by nitrifying bacteria [33].

Based on the results shown in Table 6, Pandan Perdana Lake was considered as not polluted as all the samples tested showed that the concentration of NO_2^-, NO_3^- and NH_3 were zero. This might be due to Pandan Perdana Lake is a recreation lake where there are no industrial and agricultural activities nearby the lake. Hence, there are not many pollutants introduced into the lake water. Whereas for Kerayong River, the measurement of water parameters showed that water samples tested from Persiaran MPAJ were the most polluted and contaminated among the other sampling sites. This might be due to Persiaran MPAJ located in the commercial and residential area. Numerous restaurants and public sectors such as hospital are located around this area, hence contributing more pollutants and wastes into the river compared to the other two sampling sites. However, no significant differences were observed between the conditions of pH, NO_2^-, NO_3^- and NH_3 for each sampling sites in Kerayong River. This might be due to all sampling sites were polluted by a similar level of anthropogenic activities. From the results shown in Table 6, it is observed that the parameters of NO_2^- and NO_3^- for both Jalan 2 and Cempaka LRT Stations showed very high deviations for each sample compared to the average of the samples. Whereas water parameters result from Persiaran MPAJ had higher consistency compared to the other sampling sites. Besides that, as Cempaka LRT Station was in the upstream of Kerayong River, hence the water volume was low and river depth was shallow especially during the hot and dry season.

3.1 *EMAS treatment*

Laboratory analysis for water parameters of Na^+, NO_3^-, Ca^{2+}, K^+, pH and microbe concentration before and after EMAS treatment were shown in Table 7 and Table 8 below.

Table 7. Concentration of ions, microbes present and the pH of synthetic wastewater samples before treatment

Composition	Before treatment	
	Average	σ
Na^+ (ppm)	356.67	5.77
K^+ (ppm)	39.33	4.51
Ca^{2+} (ppm)	50.00	1.73
NO_3^- (ppm)	93.00	8.89
pH	4.38	0
Microbe	0.34	0.01
NH_4^+ (mg/L)	11.76	0.02

The data of the table was in average and σ represents standard deviation.
The unit for Na^+, K^+, Ca^{2+} and NO_3^- were in parts per million (ppm) whereas NH_4^+ was in milligram per litre (mg/L).

Table 8. Concentration (ppm) of ions, microbes present and the pH of synthetic wastewater samples after treatment

Treatment	Composition	Duration (Days)									
		Day 1		Day 2		Day 3		Day 4		Day 5	
		Average	σ	Average	σ	Average	σ	Average	σ	Average	σ
EM Control	Na^+ (ppm)	364.22	23.09	374.44	0.00	367.67	11.55	369.56	6.94	379.56	19.53
	K^+ (ppm)	37.44	0.19	38.78	0.84	35.44	3.06	42.11	0.69	43.11	1.26
	Ca^{2+} (ppm)	45.89	8.28	69.56	2.46	75.78	3.20	83.89	0.77	91.56	7.46
	NO_3^- (ppm)	78.89	8.40	116.67	8.82	128.00	3.33	134.22	0.00	145.67	5.09
	pH	4.36	0.04	4.50	0.04	4.44	0.00	4.34	0.05	4.32	0.02
	Microbe	0.34	0.01	0.34	0.03	0.36	0.03	0.31	0.03	0.34	0.07
EMA	Na^+ (ppm)	314.78	24.83	376.56	10.00	372.11	5.77	404.22	0.00	398.56	1.92
	K^+ (ppm)	36.33	0.88	40.11	1.07	43.44	2.36	51.33	2.31	51.56	2.59
	Ca^{2+} (ppm)	48.56	1.26	64.44	0.69	63.78	1.02	81.78	2.08	93.33	9.64
	NO_3^- (ppm)	60.78	9.28	89.67	6.38	88.56	5.70	93.44	8.08	96.44	8.82
	pH	4.38	0.07	4.56	0.11	4.48	0.11	4.43	0.16	4.54	0.43
	Microbe	0.38	0.08	0.55	0.04	0.63	0.04	0.63	0.03	0.70	0.01
EMB	Na^+ (ppm)	349.33	21.87	366.89	13.47	381.00	5.77	401.67	5.77	394.33	15.28
	K^+ (ppm)	36.22	1.35	40.89	0.69	44.89	0.69	53.33	0.58	51.89	3.17
	Ca^{2+} (ppm)	53.44	2.34	67.33	0.33	65.78	0.00	84.67	0.58	92.56	1.68
	NO_3^- (ppm)	63.78	8.82	95.33	5.87	111.78	0.00	115.44	5.77	110.00	0.00
	pH	4.38	0.04	4.47	0.03	4.35	0.01	4.33	0.02	4.40	0.03
	Microbe	0.36	0.02	0.38	0.01	0.57	0.03	0.56	0.03	0.62	0.06
EMC	Na^+ (ppm)	397.78	43.46	370.56	0.00	374.33	5.77	402.33	5.77	394.00	5.77
	K^+ (ppm)	36.67	0.00	38.67	2.31	45.11	1.54	53.00	0.00	47.33	3.06
	Ca^{2+} (ppm)	55.44	4.17	68.00	1.33	74.22	0.38	84.00	1.00	85.33	3.79
	NO_3^- (ppm)	61.78	9.21	96.67	14.82	110.56	10.00	124.11	1.92	118.00	5.77
	pH	4.36	0.01	4.50	0.02	4.35	0.00	4.31	0.01	4.34	0.01
	Microbe	0.37	0.03	0.37	0.04	0.52	0.02	0.57	0.05	0.64	0.04
EMD	Na^+ (ppm)	327.78	3.85	374.67	1.92	369.89	13.47	402.33	5.77	387.67	17.32
	K^+ (ppm)	35.33	0.00	39.56	0.38	43.00	1.53	51.78	0.38	51.67	1.15
	Ca^{2+} (ppm)	57.89	2.36	65.56	0.19	71.33	1.53	83.78	0.19	93.78	3.27
	NO_3^- (ppm)	77.44	7.32	105.67	3.33	120.56	5.77	119.11	5.77	136.33	5.09
	pH	4.41	0.03	4.51	0.01	4.40	0.01	4.33	0.02	4.37	0.04
	Microbe	0.37	0.03	0.41	0.04	0.50	0.04	0.58	0.01	0.60	0.03

The data of the table was in average and σ represents standard deviation. The unit for Na^+, K^+, Ca^{2+} and NO_3^- were in parts per million (ppm).

Table 9 shows the ammonium concentration in milligram per litre (mg/L) on day 1 and day 5 for synthetic wastewater samples treated with Effective Microorganisms Activated Solution (EMAS) and samples which were not treated were act as control.

Table 9. Concentrations of ammonium (NH_4^+ in mg/L) present in the synthetic wastewater samples

Treatment	Composition	Duration (Days)			
		Day 1		Day 5	
		Average	σ	Average	σ
EM Control		11.73	0.05	11.85	0.03
EMA		11.56	0.06	11.01	0.05
EMB	NH_4^+ (mg/L)	11.46	0.15	10.98	0.02
EMC		11.69	0.02	11.43	0.04
EMD		11.76	0.03	11.42	0.04

The data of the table was in average and σ represents standard deviation. The unit for NH_4^+ was in milligram per litre (mg/L).

3.2 *Minerals ions*

Based on data in Table 8, only samples from Treatment EMC showed a reduction in sodium ion concentration of 1% after 5 days. Whereas, control, EMA, EMB and EMD treatment samples all had an increase in the sodium content with the percentage of 4.2%, 27%, 13% and 18% respectively. Although Treatment EMC showed a very low reduction of 1% in sodium content, all other EM treated samples had a higher increment in sodium concentration. Figure 4 presented the changes of sodium ions for all treatments from day 1 to day 5.

An overall increase in potassium ion was observed in both the control and EMAS-treated samples as shown in Figure 5. However, the control sample had the lowest increment of 15% from an initial concentration of 37.44 ppm to a final concentration of 43.11 ppm after 5 days compared to treated water samples. Whereas, among all EMAS treatment, Treatment EMD showed the highest increase in potassium content from an initial reading of 35.33 ppm to a final reading of 51.67 ppm with an overall increment of 46% after 5 days. All the treated water samples had potassium concentration exceeded that of the control sample. Hence, it can be concluded that EMAS is not effective in reducing potassium ions concentration in water samples, but further contribute to the increase of potassium ions.

Based on the graph shown in Figure 6, it is observed that calcium ions in all water samples with and without EMAS treatment increased from day 1 to day 5. In terms of calcium concentration, the control sample has significantly increased by 100% which is doubled of the initial amount of calcium content. Whereas EMA, EMB, EMC and EMD treatments showed an increment of 92%, 73%, 54% and 62% respectively over the duration of 5 days. Although calcium concentration of all EM treatments had lower increment compared to the control sample, however, there is still an overall increase in calcium content with EMAS treatments.

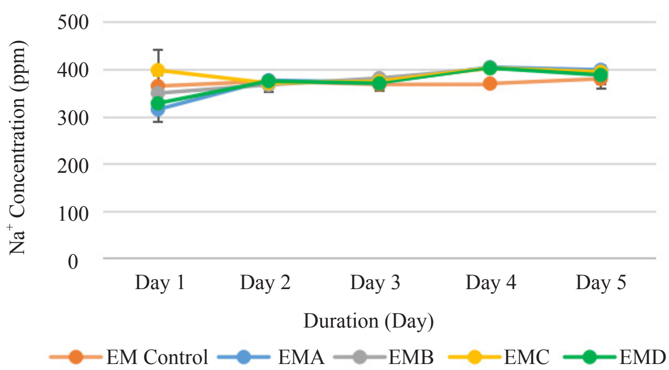

Figure 4. Na^+ concentration (ppm) of water samples against duration (days)

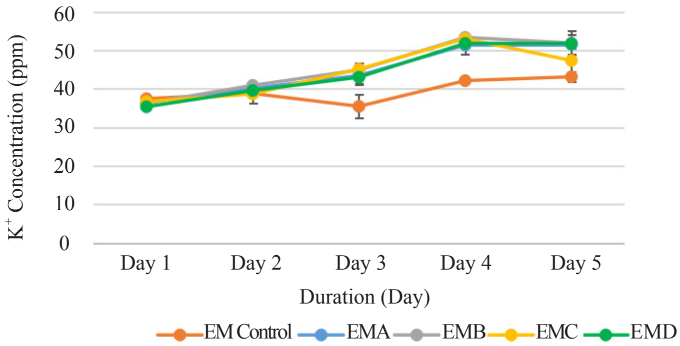

Figure 5. K^+ concentration (ppm) of water samples against duration (days)

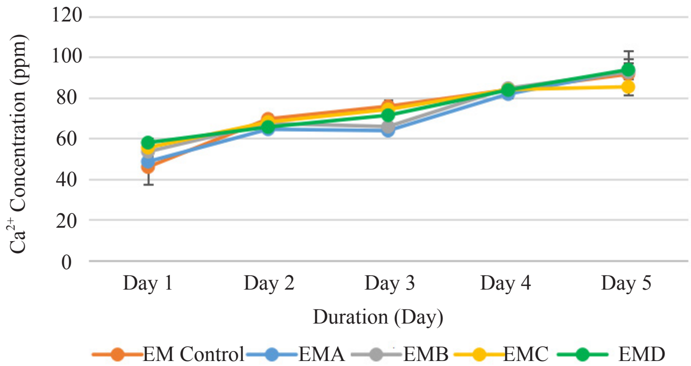

Figure 6. Ca^{2+} concentration (ppm) of water samples against duration (days)

3.3 *Conversion of NH_4^+ to NO_3^-*

Based on the graph plotted in Figure 7, it is observed that ammonium concentration for control samples slightly increased from 11.73 mg/L on day 1 to 11.85 mg/L on day 5. Whereas all samples with treatments showed a reduction of ammonium concentration on day 5 where Treatment EMA had the highest reduction of 4.8% after 5 days of treatment. Meanwhile, the percentage of reduction for Treatment EMB, EMC and EMD were 4.2%, 2.2% and 2.9% respectively. The reduction of ammonium ion in water samples might be due to the presence of photosynthetic bacteria such as *Rhodopseudomonas sp.* and *Rhodobacter sp.* in Effective Microorganisms. These photosynthetic bacteria are also known as purple non-sulfur bacteria which are ubiquitous in fresh and marine water, soil, wastewater and activated sludge [34]. It is reported that the addition of photosynthetic bacteria in water can remove ammonium [35] and reduce levels of nitrogen-containing inorganic substances [36], hence improves the water quality.

The nitrate concentrations increased in all the treatments throughout the 5 days as shown in Figure 8. EM control sample had an initial concentration of 78.89 ppm and increased to a final concentration of 145.67 ppm, which showed an increment of 85%. From the data shown in Table 8, the percentage increase for Treatment EMA, Treatment EMB, Treatment EMC and Treatment EMD were 59%, 73%, 91% and 76% respectively. The increase of nitrate concentration might be due to the conversion of ammonium to nitrate as a result of nitrification catalyzed by nitrifying bacteria present in water samples and photosynthetic bacteria in EM [37]. However, all water samples with EMAS treatment showed lower nitrate concentrations as compared to the control sample. The lower concentration of nitrate ions in treated samples might be due to the presence of denitrifying bacteria which are capable to reduce nitrates to dinitrogen or nitrous oxide as a pathway to eliminate nitrogen from water systems [38]. Examples of such bacteria include *Rhizobium* sp., *Rhodopseudomonas* sp., *Rhodobacter* sp., *Escherichia* sp. and other more species that can secrete nitrate reductase enzyme [39]. Few of the listed bacteria such as *Rhodobacter sphaeroides* and *Rhodopseudomonas palustris* are commonly found in EM [40]. It is then said that these bacteria present in EMAS convert nitrates to nitrogen and returning it to the atmosphere [38-40]. This shows that EMAS has the capability to slow down the increase of nitrates in water samples.

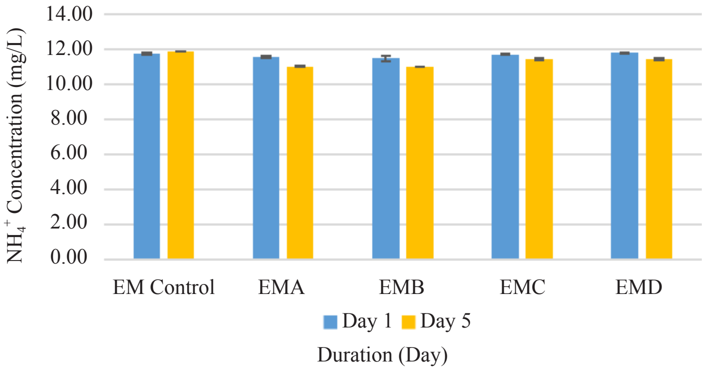

Figure 7. NH_4^+ concentration (mg/L) of water samples against duration (days)

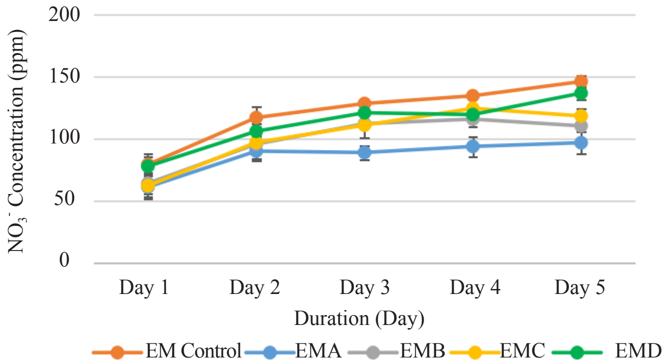

Figure 8. NO_3^- concentration (ppm) of water samples against duration (days)

3.4 *pH of synthetic wastewater samples*

As presented in Figure 9, all water samples fluctuate between a narrow pH range of 4.31 to 4.56 from day 1 to day 5, which were in the acidic form. The pH of all samples did not undergo significant changes after 5 days, which showed that different concentrations of EMAS had no significant effect on the pH of water samples. Introduction of EMAS was not able to increase the pH to the optimum pH level of the river system which is 6.5 to 9.0 which is a higher pH [23]. Majority of the aquatic life prefer pH between optimum range as pH levels out of the range may stress aquatic systems and reduce the survival rate [41]. However, EMAS may act as a buffer as the results showed no significant changes of pH throughout the treatment. Hence, it may prevent the fluctuation of water pH that can affect the water quality and the health status of aquatic life [41].

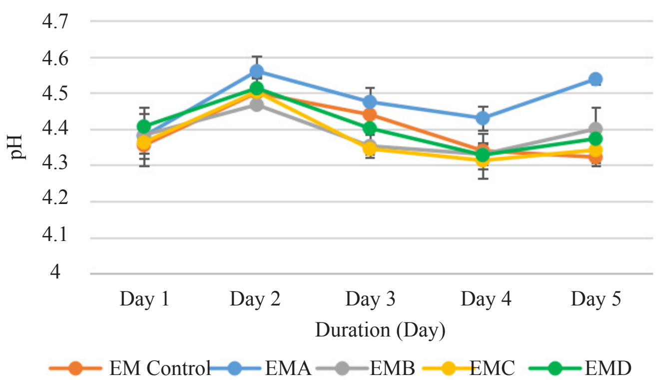

Figure 9. pH of water samples against duration (days)

3.5 *Microbial growth enhancement*

All water samples with EMAS treatment showed increased microbe concentration after 5 days. There is a major contrast in the trend between the control sample and treated samples as the control sample showed stable trendline as seen in Figure 10. The percentage of increment for treatment EMA, EMB, EMC and EMD were 84%, 72%, 73% and 62% respectively after 5 days. Among all treatments, it is clearly shown that EMA treatment showed the highest increment and has reached the microbe concentration of 0.70 on day 5. This is due to the higher amount of EMAS (1 mL/L) inoculated into water samples compared to other treatments, resulting in higher multiplication rate of the microbial population. EMAS contains various types of microorganisms such as photosynthetic bacteria, lactic acid bacteria, yeast, etc [42]. Examples of bacteria expected to be available in EMAS are *Rhodopseudomonas palustris, Rhodobacter sphaeroides, Lactobacillus plantarum, Saccharomyces cerevisiae*, etc [9]. These beneficial microorganisms take up organic pollutants in water as their food source for growth and reproduction [12, 13]. As the amounts of minerals and nutrients in the water increases, more microorganisms are produced which leads to an increased level of microbe concentration.

Based on the microbe growth curve, it can be said that the lag phase occurred until day 2 as not much increase in microbe biomass was observed in treatment EMB, EMC and EMD. The lag phase is the process where the bacteria adapt to the new environment and condition, increase in cell size but not replicating, hence little increase in biomass. Whereas it is observed that until day 5 the microbes were still in the exponential phase where the microorganisms grow and replicate rapidly [37]. Hence, EM is believed to be sustainable in water samples during the treatment period.

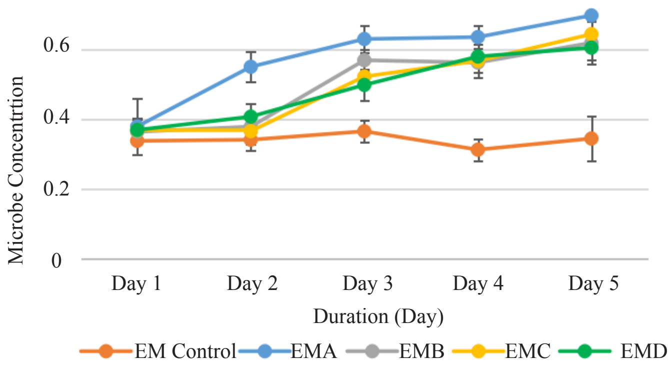

Figure 10. Microbe concentration of water samples against duration (days)

3.6 *Statistical analysis*

The significant difference among means between Treatment EMAS and control treatment were calculated using t-Test using Excel as shown in Table 10. A p-value of less than 0.05 (typically ≤ 0.05) is said that statistically significant difference was observed between Treatment EMAS and control treatment, meanwhile, a p-value > 0.05 is vice-versa.

Table 10. Comparison of significant difference between Treatment EMAS and control based on t-test

Treatment Na^+		Parameters						
		K^+	Ca^{2+}	NO_3^-	pH	Microbe	NH_4^+	
EMA	One-Tailed Test	0.02	0.03	0.48	0.00	0.23	0.02	0.00
EMB		0.05	0.01	0.31	0.01	0.12	0.04	0.00
EMC		0.29	0.05	0.18	0.16	0.11	0.00	0.00
EMD		0.02	0.01	0.25	0.19	0.50	0.00	0.00
p-value					0.05			

Based on the results, Na^+ and K^+ concentrations had a significant difference between Treatment EMAS and control treatment as the p-value were less than 0.05. It is found that the Na^+ and K^+ concentrations were much higher in Treatment EMAS samples as compared to control treatment sample. This has shown that EMAS introduced more minerals in the water samples rather than reducing them. Whereas there is no significant difference observed in Ca^{2+} concentration between Treatment EMAS and Treatment EMAS Control as p-value were more than 0.05. All treatments had increased the concentrations of calcium ions in water samples. Hence, EM is said to increase the salinity and dissolved ions in the water samples. This result agrees with Regina and Teo [21] who observed that EM promotes excessive nutrients and minerals in the water after treating river water with EM Mudball. Iriti et al. [43] also has reported that plants with EM treatment showed an increment in dissolved ions content such as sodium, potassium, and calcium in their experiment of treating the soil with EM. EM has the capability to improve the availability and solubility of mineral nutrients [44] which explains the increase of sodium, potassium, and calcium after EMAS treatment. Besides that, soluble inorganic compounds are commonly produced because of the biodegradation of organic matter [45].

No significant difference was observed on pH between control treatment and Treatment EMAS as the p-value was more than 0.05. However, the treated and control sample were in acidic form. This might be due to the organic acids secretion from EM that suppresses harmful microbes from the decomposition of organic matter [46].

For NH_4^+ concentrations, statistically significant differences were found in all treatments when compared to the Control Treatment, which p-values were lower than 0.05. The ammonium concentrations in EMAS-treated samples were significantly decreased as compared to the control sample. This showed that Treatment EMAS had the capability to convert ammonium ions to non-toxic form of nitrate ions. However, the efficiency of ammonium removal using EM was considered lower than the study done by de-Bashan et al. [47] which eliminated 100% of ammonium using coimmobilization of microalgae *Chlorella vulgaris* and microalgae growth-promoting bacterium *Azospirillum brasilense* under semi-continuous synthetic wastewater culture conditions after 48 hours.

According to the results shown, only Treatment EMA and EMB showed significant differences in NO_3^- concentration when compared to control treatment. It can be said that more denitrifying bacteria were found in higher concentrations of EMAS, hence reducing nitrates content in water [48]. However, the nitrate ions increased in both control treatment and Treatment EMAS. The increase of nitrate concentrations can be explained by the nitrification process where ammonia nitrogen was oxidized to nitrite then to nitrate. This same result was also observed in the work of El Moussaoui et al. [37] which treated synthetic urban wastewater using activated sludge pilot plant.

The p-values of microbe were less than 0.05, which indicate significant differences between Treatment EMAS and control treatment. All treatments with different concentrations of EMAS showed a significant increment in microbe concentration as EMAS which contains various beneficial microorganisms were introduced into water bodies. These microbes degrade contaminants in water and transform hazardous compound to a less harmful or harmless one through natural processes [11].

4. Conclusion

The overall objective of this study is to evaluate the water quality of Kerayong River and Pandan Perdana Lake, Ampang, Malaysia. It also aims to evaluate the efficiency of EM for wastewater treatment as well as the optimum concentration of EM required to reduce water minerals, nitrates, and ammonium in water. The water quality of Kerayong River was considered as polluted as high levels of nitrates, nitrites and ammonia were detected at all three selected sampling sites. These water parameters that exceeded the acceptable range and were not suitable for most of the aquatic life. Persiaran MPAJ was the most polluted site among the other sampling sites which might be due to its location where most of the activities were concentrated at the township. Whereas no pollution was observed in Pandan Perdana Lake as all the water parameters were in the safe range. The pH for both Kerayong River and Pandan Perdana Lake fall within the normal range for the freshwater system.

In this study, different concentrations of Effective Microorganisms Activated Solution (EMAS) were used as a treatment for synthetic wastewater. It is found that all EMAS concentrations were able to reduce ammonium concentration significantly in water samples, whereas only Treatment EMA and EMB showed a significant reduction of nitrates content in water. This could be due to the presence of nitrifying and denitrifying bacteria such as *Rhodobacter* *sphaeroides* and *Rhodopseudomonas palustris* that were found in EMAS [40]. These bacteria have the capability to convert ammonium to nitrate and reduce nitrate to nitrogen gas which returns to the atmosphere [38], hence reducing the concentrations of these nutrients compared to the control treatment. EMA (1 mL/L) was considered as the optimum concentration for reducing ammonium with the highest percentage of ammonium removal being 4.8% after 5 days. EMA also had the lowest nitrate concentration on day 5 which is 49.27 ppm lower than the control treatment.

This study also showed that water minerals such as sodium, potassium and calcium ions have increased in all different concentrations of EMAS-treated samples. This indicates that EMAS increases the minerals dissolved in water. The potential reason for this could be due to the biodegradation of organic matters which released soluble inorganic compounds in water [45]. Besides that, it may also be due to EM which is commonly used as biofertilizer, was able to convert organic matter to nutrients and minerals in a soluble form that is usable by plants.

From this study, EMAS is observed to be no significant effect on the pH of synthetic wastewater samples. All the wastewater samples were in the acidic range of 4.31 to 4.56, which were out of the normal range for a healthy freshwater system. However, EMAS can act as a buffering agent to prevent the fluctuation of pH in the water. Moreover, there has been a noticeable improvement of the microbial population in all concentrations of EMAS-treated samples compared to that of the control sample. EMAS has the capability to enhance microbial growth by utilizing the nutrients in the synthetic wastewater.

Acknowledgements

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Conflict of interest

We declare that we have no conflict of interest.

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