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Designing Prototype Micro-Technology for Sustainable Management of Natural Water Resource in Batu Pahat River, Johor

Regina LZL[1,2] and Teo SS[1,2*]

[1]*Department of Applied Sciences, UCSI University, No.1 Jalan Menara Gading, UCSI Heights, 56000 Cheras, Kuala Lumpur, W. P. Kuala Lumpur, Malaysia.*

[2]*Aquatic Science Student Association, Department of Applied Sciences, UCSI University, No.1 Jalan Menara Gading, UCSI Heights, 56000 Cheras, Kuala Lumpur, W. P. Kuala Lumpur, Malaysia.*

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***Corresponding author:** Teo Swee Sen, Department of Applied Sciences, UCSI University, No.1 Jalan Menara Gading, UCSI Heights, 56000 Cheras, Kuala Lumpur, W. P. Kuala Lumpur, Malaysia, Tel: +603-91018880; E-mail: teoss@ucsiuniversity.edu.my*

Abstract

Environmental management, wastes recycling, pollution prevention and wastewater treatment have become the critical issues and are included in the Sustainability Development Goals (SDGs). Rapid development in Malaysia has indirectly produced high level of wastes which inevitably ends up in the water bodies. Batu Pahat river are among the Malaysian rivers system that shows sign of sickness due to pollution cause by irresponsible dumping of domestic and industrial waste. Recently, potential usage of microorganism to reduce pollution in waste water/river water is highly discuss. Hence, the aim of this study is to evaluate the efficacy of new designed water filtration system which incorporate microorganism to improve the quality of natural water resource. The study was conducted to assess the physical and chemical water quality parameters of Batu Pahat River. A number of physiochemical water quality parameters were measured on-site. Water samples was tested for selected metal analysis which shown a minimum level in general in Batu Phat river. The result obtained shows that Effective Microorganism (EM) mud ball itself are not capable to reduce much of the concentration of COD as the COD increased from 170mg/L to 347mg/L where it increased 104% compare to the COD in the water system. The mixture of lotus pods and EM mud ball have the potential to reduce 15% of the COD concentration from 158mg/L to 135mg/L. Using lotus pod as a vessel capable in reduction of water minerals such as Na+, Ca^{2+} and K^{+} concentration, neutralized water pH to optimum level, increased DO level above 5 mg/L and enhanced the performance of microorganisms in the water system. The micro-technology system with lotus pods mixed with EM mud ball showed enhancement growth of microorganisms and better removal efficiency pollutants for sustainable management of natural water resource.

Keywords: Lotus Pod; EM mud ball; Sustainable Management; Wastewater treatments;

Introduction

All living organisms need water for growth, metabolism and reproduction. Natural water bodies such as rivers, lakes, ponds, wetlands, oceans and glaciers are important natural resources that involved in human consumption, domestic and industrial activities, agricultural practices, development as well as environmental activities. Rapid development and over-population has depleted the natural resources on earth and increased pollution level which threatened the health of environment. Water pollution was contributed by chemical contaminants and sewage wastes that flow into the water bodies which come from point sources and non-point sources. Point sources are defined as any single point where the pollutants discharged and easily detected. The examples are wastewater treatment plant, chemical wastes from industries and sewage wastes from farms. Non point sources refer to diffusible points from main sources which are difficult to detect where the contaminants come from. For instance, agricultural runoffs, storm water runoffs and urban runoffs. These contaminants disposed directly or indirectly into the water bodies can increase level of nutrients and bacteria in the water that can degrade the water quality of the water bodies and increase the disease outbreak.

Batu Pahat River was one of the polluted rivers which are currently in Class III at upstream and Class IV at middle stream based on Water Quality Index (WQI) [18]. According to Department of Environment (2015), the WQI of Batu Pahat River in 2014 and 2015 were 57 and 61 respectively which were in status of slightly polluted range. Batu Pahat River is originating from Sg. Simpang Kanan, Tongkang Pechah and then to the river mouth of Pantai Minyak Beku. The major role of Batu Pahat River was functioned as a pathway for irrigation, transportation, fisheries and water reservoir created by Bekok and Sembrong dams to store and supply water. Batu Pahat was one of the contributors to Johor's manufacturing industrial town such as textile, electronics, food processing, timber and plastic. It also contributes agricultural practices where Batu Pahat was the largest rubber, oil palm and cocoa plantation in Johor. Commercial

fisheries in Batu Pahat are well-known as it located near the river. Inappropriate or undeveloped wastewater treatment has become critical issues due to irresponsible and uncontrolled dumping waste and contaminants into the water bodies. Hence, a practical wastewater treatment has to be implemented to maintain and improve the water quality of Batu Pahat River.

Methods used to treat wastewater previously were Effective Microorganism technology using EM mud ball, aquatic macrophyte of water hyacinth, a phytoremediation agent and lotus plant as a tool in water bioremediation. These implications has the capabilities with their distinctive features in removing organic and inorganic pollutants including organic waste, heavy metals, sediments, water nutrients and microbial population. Yet, limitations of these technologies were observed due to insufficient study or no extension researches in long-term after application.

In this study, a new water filtration system was developed incorporated with microorganisms for wastewater treatment. The biological filtration system was added with lotus pod as filter to reduce the nutrients and sediment in the water and act as a media for microorganisms' growth. Water quality testing is carried out in-situ and at Batu Pahat River and ex-situ in laboratory using LAQUA Twin meter. Microbe identification is also carried out to determine the opportunistic bacteria present in Batu Pahat River by bacterial culture using nutrient agar and MacConkey agar. Gram-staining test and oxidase test are used to determine the culture bacteria colony are positive or negative bacteria and oxidase positive or negative. Identification of bacteria are tested using RapID TM ONE System and RapID TM NF PLUS System. The results are identified in microcode form and determine the bacteria species using Electronic Rapid™ Compendium identification (ERIC). The efficiency of lotus pod as filter was measured by testing the water parameters such as sodium (Na^+), calcium (Ca^{2+}), nitrate (NO_3^-), potassium (K^+), pH, ammonia (NH_3), Dissolved Oxygen (DO), Chemical Oxygen Demand (COD) and microbe concentration at OD_{600}. The objectives of this study were the evaluate the water quality of Batu Pahat River and to design and evaluate the efficiency of lotus pod as filter in micro technology system for polluted water treatment.

Methods

Water Quality Testing

Pre-test

Water samples and soil samples were collected every week for water quality testing from selected sites [Si Hai Long Wang temple (1.8431° N, 102.9243° E), Stadium Batu Pahat (1.8447° N, 102.9328° E) and Pine tree downstream (1.86° N, 102.94° E)]. The water samples were collected from the constantly flowing river water whereas the soil samples were collected from the bottom of the river.

Post-test

In-situ

The testing were carried out *in-situ* in Batu Pahat River at Si Hai Long Wang temple (1.8431° N, 102.9243° E) in mid of December using SERA Testing kit. It was tested for once a week for one month. The water parameters included NO_3-, pH, O_2 and NH_3. The analysis was triplicated for testing. These kits were using color chart for comparison with the mixed water sample with reagents and the concentration of each parameters were based on the color.

Ex-situ

Water samples of 40 mL from the Si Hai Long Wang temple were collected during mid of December by Grab sampling and brought back to Aquatic Science lab, UCSI University for analysis. The soil sample with a total of 50 g was also brought back by using soil tube for collection and sent to Bio Synergy lab for analysis.

a. Treatment MHL (mud ball, water hyacinth & lotus pod)

A total of 10 L of water sample was poured into 4 containers respectively. One of the containers act as control treatment (MHLC) where no vessel was added into the water sample while the other three beakers were added with one dried EM Mud ball (MHL1), water hyacinths (MHL2) and dried lotus pod (MHL3) respectively table 1. These MHL treatments were run for 3 weeks and the water parameters results of Na^+, NO_3-, Ca_2^+, K^+, pH and NH3 were taken once a week in triplicates. Meanwhile, the concentration of COD was taken from each sample once a week by using COD reactor. The results of COD concentration were measured using COD Vario photometer.

Table 1: Treatment MHL with their respective vessels

Treatment	Vessels	Amount
MHL_C	No vessel	-
MHL_1	EM Mud ball	10 g/L
MHL_2	Water hyacinth with 3 leaves (a stalk)	15 g/L
MHL_3	Lotus pod + EM Mud ball	15 g/L

b. Treatment ML (mud ball + lotus pod)

After three weeks treatment finished, another treatment were set-up by using Lotus pod mixed with EM Mud ball table 2. These treatments were run for three weeks where the results of Na_+, NO_3^-, Ca_2^+, K^+, DO and microbe absorbance were taken four times a week in triplicated. Statistical analysis was calculated using t-Test in Microsoft Excel.

Microbe Identification

Bacteria Culture

Preparation of bacteria culture started with sterilizing the laboratory bench and apparatus with 70% ethanol and set up the Bunsen burner on the bench to prevent contamination.

Table 2: Amount of EM Mud ball mixed with lotus pod in Treatment ML_C, ML_1, ML_2 and ML_3

Treatment	Lotus pod	EM Mud ball	Ratios
ML_C	0 g	0 g	0:00
ML_1	40 g	10 g	4:01
ML_2	40 g	20 g	2:01
ML_3	40 g	80 g	1:02

Cotton Swab Method

In this study, the first culture was streaked using cotton swab by touching the water samples from Si Hai Long Wang temple and swabbed on each pure nutrient agar and MacConkey agar. The streaking was in a zigzag pattern on each quadrant of the plate. Each water samples was streaked for three sets. After streaked, the petri dishes were parafilm and incubated for 24 hours to allow growth of bacteria.

Inoculation Loop Method

After first culture, the bacteria growth was replicated. An inoculation loop was first sterilized by passing through the flame of Bunsen burner. Then, the loop was cooled down and picked a colony from the first culture and streaked on the new pure nutrient agar and MacConkey agar respectively. The first streak was streaked in a zigzag pattern back and forth. The loop was re-sterilized after each streak.

Next, the second streak was streaked by turning the plate in 90^0 and dragging the end of first streak in three times continuing the zigzag motion. The procedure was repeated for third streak and fourth streak. After streaked, the petri dishes were parafilm and incubated for 24 hours. Culture of bacteria was repeated until obtaining a single pure colony.

Gram Staining and Oxidase Test

Gram staining was carried out to identify gram type of bacteria using crystal violet solution. In another hand, a single pure colony was picked up from nutrient agar and MacConkey agar respectively to spread over the filter paper with each drop of the reagent. This process is used as oxidase test.

RapID™ System Test – Gram-negative Bacteria (Thermo Scientific)

In this study, identification of gram negative bacteria was obtained from Gram staining method. After oxidase test, the bacteria species was identified by using RapID™ System Test including RapIDTM ONE System and RapIDTM NF plus System respectively based on oxidase results.

RapIDTM ONE System (Thermo Scientific)

RapIDTM ONE System consisted of RapID ONE Panels which have 18 reaction cavities respectively on a tray and reagents of RapID ONE Reagent, RapID Inoculation Fluid as well as RapID Spot In dole Reagent. The reagents were stored at 4^0C until use meanwhile the inoculation fluid can be stored at room temperature. Each reaction cavities contained respective dehydrated reactants which will react with the inoculation fluid containing pure bacteria colony.

RapIDTM NF plus System (Thermo Scientific)

RapID™ NF plus System consisted of RapID NF plus Panels which have 10 reaction cavities respectively on a tray and the reagents included RapID NF plus Reagent, RapID Inoculation Fluid, RapID Nitrate a Reagent as well as RapID Spot In dole Reagent. The inoculation fluid was stored at room temperature whereas the rest of the reagents were stored at 4^0C until use. All these reagents have to be equilibrated to room temperature before used for testing. The 10 reaction cavities contained different dehydrated reactants in each cavity which react with the inoculation fluid containing bacteria species.

Lotus Pod Efficacy in Water Filtration System

Lotus pod was used as an empty vessel in the water filtration system for wastewater treatment in order to remove high concentration of water nutrients and organic waste in the water body. In this study, two treatments were using non-vessel treatment as control and lotus pod treatment respectively. The treatments were run for a week to measure the efficiency of vessel as compared to non-vessel in pollutant removal.

Treatment L $_{(Lotus\ pod)}$

A total of 1.75 L of green water was added into two fish tanks respectively. The green water was cultured in Aquatic Lab, UCSI University, which was used to represent water body with high level of water nutrients, algae and organic waste. In control treatment (LC) where no vessel was added to the water while lotus pod treatment (L1) was added with lotus pod in the filtration system as bio filter table 3.

Table 3: Treatment L with their respective vessels

Treatments	Vessels	Amount	Green Water Volume
L_C	No vessel	-	1.75 L
L_1	Lotus pod	10.59 g	1.75 L

These treatments were run for a week and test for the water quality by measuring water parameters of Na^+, Ca^{2+}, NO_3^-, K^+, NH3, pH, DO and microbe concentration. The measurements were triplicated and recorded daily for each parameter.

Colorimetric method – NH_3 Concentration

NH3 concentration was measured using manual colorimetric method by micro plate reader. It was tested for both Treatment LC and L1. 100 mg/L of NH_4^+ standard solution were used and prepared by serial dilution as shown in table 4. The total volume of standard solution prepared was 20 mL each as the reagent were NH3 Test (SERA kits) which required 10 mL of sample solution to be tested. After standard preparation, 6 drops of each reagent 1, reagent 2 and reagent 3 were added into each standard

Table 4: Preparation of standard solutions

Tubes	Volume of Standard	Volume of Deionized water	Total volume	Dilution factor
1	2.00 mL	18.00 mL	20.00 mL	1/10
2	10.00 mL (from Tube 1)	10.00 mL	20.00 mL	1/2
3	10.00 mL (from Tube 2)	10.00 mL	20.00 mL	1/4
4	10.00 mL (from Tube 3)	10.00 mL	20.00 mL	1/8
5	10.00 mL (from Tube 4)	10.00 mL	20.00 mL	1/16
6	10.00 mL (from Tube 5)	10.00 mL	20.00 mL	1/32
7	10.00 mL (from Tube 6)	10.00 mL	20.00 mL	1/64
8	10.00 mL (from Tube 7)	10.00 mL	20.00 mL	1/128
9	10.00 mL (from Tube 8)	10.00 mL	20.00 mL	1/256

accordingly. Then, master mix was prepared from water sample of each Treatment L by adding the reagents similar to standard.

After that, 200 µL of each standard and master mix were transferred using micropipette into the 96-well of microtiter plate accordingly. Then the microtiter plate was placed in the micro plate reader for absorbance reading at OD_{650} and read for three times.

Statistical Analysis

Statistical analysis was calculated using t-Test in Microsoft Excel.

Results and Discussion

From the pre-test results of three selected site which were Si Hai Long Wang temple (1.8431° N, 102.9243° E), Stadium Batu Pahat (1.8447° N, 102.9328° E) and Pine tree downstream (1.86° N, 102.94° E) in the end of November 2018, the measurement of water parameters using SERA Test kits showed that water from Si Hai Long Wang temple was the most polluted and contaminated among the others (data not presented). This was due to the Batu Pahat River was branched and disseminated as drainage basin to Stadium Batu Pahat and Pine tree downstream in small quantity of water. As the water from main river flow to the drainage basin in Stadium Batu Pahat and Pine tree downstream, the water volume was disseminated and depended on the raining season. The reason was that the water in the drainage basin was sometime get dry and able to see the bed when the weather was hot and dry as the drainage depth was shallow. Hence, the water sample from Stadium Batu Pahat and Pine tree downstream was not collected. Besides, water from Si Hai Long Wang temple was come from the main river of Batu Pahat which was more corresponds to the actual situation of Batu Pahat River.

IN-SITU

Based on the results from table 5 below, the in-situ water quality testing by measuring the water parameters in Si Hai Long Wang temple with coordinate of 1.8431° N, 102.9243° E using SERA Test kits to analyse the concentration of water parameters on the spot. The analysis results showed no significant changes from week 1 to week 4. The results showed were mean ± standard deviation (SD).

Table 5: Concentration of NO_3^-, pH, O_2 and NH_3 taken in-situ

Weeks	Parameters			
	NO_3-	p^H	O_2	NH_3
1	0.00 ± 0.00	4.67 ± 5.00	6.00 ± 0.00	1.00
2	0.00 ± 0.00	5.83 ± 0.29	4.00 ± 0.00	0.5
3	0.00 ± 0.00	4.50 ± 0.00	4.00 ± 0.00	1.00
4	0.00 ± 0.00	4.67 ± 0.29	4.33 ± 0.58	1.00

The data of the table was mean ± SD. The analysis was taken during December of 15, 22 and 26 as well as 3 of January. It was taken once a week. The time during analysis was approximately 12 noon with a sunny weather. In the mid of December, the season was raining season.

Natural occurring NO_3^- was present in low concentration in water body which was less than 1 mg/L. The water quality testing using SERA Test kits showed that NO_3^- concentration was 0 mg/L and yellow solution for all 4 weeks. The NO_3^- concentration was low might be due to the water was polluted with nitrogen-rich organic matter. High organic matter present in the water reduced the DO level in the water body as decomposition happens which directly slow down the conversion of NH3 to NO_3^- as nitrification required O_2 [14]. Low concentration of NO_3^- does not harm to aquatic organisms, however, excess NO_3^- which more than 50 mg/L cause significant effect on human health.

NH_3 in the water body was more toxic to aquatic organisms especially fish even in very low concentration compared to NO_3-. Natural NH_3 in water body are toxic to aquatic organisms

and indicated contamination if the concentration was higher than natural level which was more than 0.03 mg/L. The safe concentration of NH_3 was in the range of 0.02 to 0.4 mg/L. From the analysis test, the NH_3 levels from selected site were 0.5 mg/L and 1 mg/L which were more than 0.03 mg/L. The color observations of the solution were light green and green.

The river water from selected site was considered in polluted range as the NH_3 concentration was more than 0.03 mg/L. The NH_3 concentration was high because of the excretion of nitrogenous waste products from fish in the water, agricultural runoff, sewage effluent and irresponsible dumping of trash from the selected site into Batu Pahat River. As many events carried out throughout the whole year at selected site temple, the unwanted rubbish and waste were directly thrown into the river water which eventually increased the NH_3 level in the water. Besides, high NH_3 might due to high water temperature and pH level [23].

Water measurement for pH level was considered slightly acidic which were around 4.5 to 5.8. This level was not suitable for a freshwater ecosystem as acidic water indicated there were high organic pollutants that acidify the water. The optimum water pH of a river system was 7.4. The colors of the solution after added reagents were orange, light orange and dark yellow for 4 weeks. Moreover, high level of NH_3 can reduce the pH of water. Acidic water were caused by high water temperature, chemical contaminants discharged, sewage effluents and fossil fuel emissions such as carbon dioxide especially for Chinese temple practices that burn joss stick and fake money. Acidic water might affected the tolerance of fish species living in the water column and stressful to them which can physically damage their body system [19].

For O_2 level, the results were between 4 mg/L to 6 mg/L. The color of solution was yellowish-brown to brown after reagent was added into the water samples. High inputs NH_3 concentration in the water reduced the level of O_2 as oxidation of nitrogen-rich organic waste reduced oxygen level available in the water column [31]. High organic matter in the water body can increase the competition of oxygen uptake between aerobic bacteria for decomposition and fish for physical voluntarily activities as well as metabolism [6]. Low oxygen level in the water are stressful to fish as most freshwater fish required at least 5 mg/L and more for excellent growth and reproduction performance.

EX-SITU

The analysis for water samples for Treatment MHL and ML were measured the water parameters using LAQUA Twin meters and COD reagent to test. The soil samples were brought to Bio Synergy for analysis.

Treatment MHL

Laboratory analysis for water parameters of Na^+, pH, NO_3^-, Ca^{2+}, K^+ and NH_3 in Treatment MHL was shown in table 6 below.

Table 6: Concentration of water parameters in Treatment MHL

Weeks Na^+		Parameters					
		pH	NO_3-	Ca^{2+}	K^+	NH_3	
Before treatment							
No vessel		1433 ± 57.74	6.38 ± 0.04	113 ± 5.77	67 ± 1.53	51 ± 0.58	5
After treatment							
1	MHL_c	1667 ± 57.74	6.77 ± 0.01	153 ± 3.61	103 ± 6.43	67 ± 2.52	5
	MHL_1	2000 ± 0.00	7.66 ± 0.03	266 ± 5.29	167 ± 5.77	190 ± 10.00	0
	MHL_2	1767 ± 57.74	6.45 ± 0.02	197 ± 7.02	110 ± 10.00	68 ± 1.53	0
	MHL_3	1600 ± 0.00	7.32 ± 0.03	217 ± 15.28	139 ± 1.16	203 ± 5.77	5
2	MHL_C	2200 ± 0.00	6.50 ± 0.06	203 ± 5.77	140 ± 1.53	111 ± 3.06	5
	MHL_1	1800 ± 0.00	8.95 ± 0.05	260 ± 2.00	121 ± 4.16	161 ± 6.56	0
	MHL_2	1200 ± 0.00	5.95 ± 0.05	140 ± 2.52	85 ± 5.86	37 ± 3.056	0
	MHL_3	1400 ± 0.00	7.67 ± 0.02	269 ± 1.73	140 ± 1.53	210 ± 3.00	5
3	MHL_C	3600 ± 0.00	5.44 ± 0.07	315 ± 5.69	252 ± 2.65	232 ± 5.69	10
	MHL_1	1967 ± 57.74	8.75 ± 0.09	251 ± 4.58	191 ± 3.06	205 ± 5.00	0
	MHL_2	1500 ± 0.00	6.27 ± 0.03	162 ± 4.36	169 ± 2.65	66 ± 3.61	0
	MHL_3	1500 ± 0.00	7.74 ± 0.08	222 ± 4.73	220 ± 3.51	180 ± 3.00	0

The data in the table was mean ± SD. The unit for water parameters of Na^+, NO_3^-, Ca^{2+}, K^+ and NH_3 was milligram per liter (mg/L). Treatment of MHL indicated addition of EM Mud ball (M), water hyacinth (H) and lotus pod (L); MHL_C = control treatment, MHL_1 = EM Mud ball treatment, MHL_2 = water hyacinth treatment and MHL_3 = lotus pod treatment.

The results were taken for 3 weeks as the presence of mosquitoes' larvae and the health condition of aquatic plants easily wilt as it growed under natural sunlight. The data showed were mean ± SD.

Minerals Deposited Increased

Based on table 6, the water minerals dissolved increased for all the treatments in these 3 weeks. Water parameter of Na+ for Treatment MHLC was dramatically increased 151% from 1433 mg/L up to 3600 mg/L which doubled up the concentration after 3 weeks. For Treatment MHL1 where EM Mud ball increased the Na+ concentration by 37% whereas Treatment MHL2 and Treatment MHL3 were increased slightly of 4.7% only. However, the Na+ concentration for a river system was ranged from 20 mg/L to 120 000 mg/L where the Na+ concentration for all Treatment MHL were still in the range.

For Ca^{2+} and K^{+} concentration, all the treatments were increased dramatically especially for Treatment MHLC where no vessel was added to treat the water quality compared to those with vessels in treatment. Both water parameters in all treatments were not in the safe range of a river system as they exceed the concentration limit where the concentration of Ca^{2+} and K^{+} should be in the range of 4 mg/L to 100 mg/L and 2 mg/L to 3 mg/L respectively. This might be due to the amount of metal products, disinfectants and fertilizers along Batu Pahat River flow into the water body that contributed to high concentration of Ca^{2+} and K^{+}. However, water hyacinth in Treatment MHL_2 was in lower concentration compared to Treatment MHL_1 and Treatment MHL_3 as water hyacinth has the capability in reducing metal ions. While EM Mud ball can promote excessive nutrients in the water which can enhance the phenomenon of algal bloom.

Conversion of NH_3 to NO_3^-

From table 6, NH_3 concentration before treatment was 5 mg/L which was light green color after reagents were added. The favorable range was between 0.02 mg/L to 0.4 mg/L. Hence, the water was considered not healthy and not safe for fish species as it was more than 0.03 mg/L. In Week 1 and Week 2, the NH_3 concentration for Treatment MHL1 and Treatment MHL2 were reduced to 0 mg/L whereas Treatment MHL_3 remained as 5 mg/L. Yet, the concentration of NH3 was reduced to 0 mg/L for all treatments in Week 3 while the concentration of NH_3 for Treatment MHLC increased to 10 mg/L which was toxic to the water and fish species.

The concentration of NH_3 reduced to 0 mg/L might because of the conversion of NH_3 to NO_3^- which was not harmful as NH_3 to aquatic organisms. NO_3^- acts as an essential nutrient for aquatic plants to enhance their growth and reproduction which in turn become food source to other organisms in higher food chain. From the results obtained, the NO3- concentration for all treatments was more than 80 mg/L which indicated the river of Batu Pahat was polluted. Excessive NO_3^- in the water can cause adverse effect to fish species as different fish have difference tolerance to NO_3^-. Concentration of 0 mg/L to 40 mg/L are still generally safe for fish species and when exceed 80 mg/L, it become toxic to fish species [5]. Besides, the concentration increased was contributed by runoffs from agricultural and construction site along the Batu Pahat River which wash off the nitrates in the fertilizers and wastes that flow into the river.

Neutralized Water pH

According to the results above, Treatment MHL_1 and Treatment MHL_3 using EM Mud ball and lotus pod respectively have the capacity to neutralize water pH of river water samples from selected site. Before treatment, the pH value of control treatment where no vessel was added was 6.375 which were higher than that of *in-situ* measurement. This was due to the events carried out in the temple where Chinese New Year festival was celebrated. As more locals and tourist visited and pray for blessing and fortune, the Chinese temple practices were organized more frequently than usual. More wastes and metal ions introduced into the water contributed to high level of minerals which increased the water pH. After running for 3 weeks, the pH value was lower to 5.441 which were acidic. Acidic water can speed up the leaching of heavy metals dissolved in the water and accumulate the metal ions in the body of aquatic organisms. Besides, acidic water was harmful to aquatic organisms especially to juvenile fish and water insects as well as hatching of eggs.

Fluctuation of water pH can bring adverse effect to aquatic organisms and water quality of ecosystem. The pH value of Treatment MHL1 was gradually increased to 8.747 which were slightly changed to basic water. Whereas Treatment MHL3 was increased the pH value and maintained in the range of 7.3 to 7.6 in these 3 weeks. In week 3, the pH value was 7.74 where it reached the optimum level of pH for river system. Hence, Treatment MHL3 was considered the best option among Treatment MHL_1 and Treatment MHL_2 to act as a buffer to control as well as maintain pH value of river water.

Reduction in COD concentration

From table 7, it can be seen that Treatment MHL_2 and Treatment MHL3 using water hyacinth and lotus pod respectively have the capability in reducing the COD concentration in the water sample. High COD concentration indicated high amount of oxidizable organic matter in the water sample and also required high amount of oxygen to oxidize them. COD concentration of Treatment MHL_2 and Treatment MHL_3 were reduced by 50% and 15% respectively which means that the amount of organic matter in the water body was reduced after 2 weeks. Meanwhile, the COD concentration for Treatment MHL1 was increased by 104% which doubled up the amount of organic matter and pollutants in the water sample and more oxygen needed to decompose them. This can decrease the amount of oxygen available for aquatic organisms as oxygen was competed by aerobic bacteria to degrade the pollutants. High COD concentration also affects the water quality due to high amount of pollutants and low level of DO available in the water column. EM Mud ball as a vessel used for wastewater treatment can induce more microbes into the

Table 7: COD Concentration in Treatment MHL for 2 weeks				
Weeks	**MHL_C**	**MHL_1**	**MHL_2**	**MHL_3**
1	358 mg/L	170 mg/L	38 mg/L	158 mg/L
2	351 mg/L	347 mg/L	19 mg/L	135 mg/L
Increased %	-2%	104%	-50%	-15%
Unit = mg/L				

water body and eventually reduce more DO level available in the water.

The general environmental pollutants effluents were at maximum of 250 mg/L. Thus, Treatment MHL_1 had a COD concentration of 347 mg/L which exceeded the tolerance limit of COD concentration into the surface water body as compared to Treatment MHL_2 and Treatment MHL_3 which had 19 mg/L and 135 mg/L respectively. The categories of Batu Pahat River based on WQI are Class V for Treatment MHLC, Treatment MHL1 and Treatment MHL3 whereas Class II for Treatment MHL_2 [32] figure 1.

Treatment ML

As water hyacinths have vigorous growth issues which can colonize the whole surface of water body, combination of EM Mud ball and lotus pod with different ratio were selected to be used in wastewater treatment (Treatment ML). Based on the results obtained from table 8, all parameters were rising gradually for all treatments. After a week, concentration of Na^+, Ca^{2+} and K^+ for Treatment MLC, Treatment ML_1, Treatment ML_2 and Treatment ML_3 were increased gradually.

Parameter	Class				
	I	II	III	IV	V
AN	<0.1	0.1 - 0.3	0.3 - 0.9	0.9 - 2.7	>2.7
BOD	<1	1 - 3	3 - 6	6 - 12	>12
COD	<10	10-25	25 - 50	50-100	>100
DO	>7	5 - 7	3 - 5	1 - 3	<1
pH	>7	6 - 7	5 - 6	<5	<5
TSS	<2.5	25 - 50	50 - 150	30 - 50	>300
WQI	>92.7	76.5 - 92.7	51.9 - 76.5	31 - 51.9	<31.0

Figure 1: Malaysia WQI categories. [Adapted from: http://file.scirp.org/Html/5-6701963_36314.html]

Table 8: Concentration of water parameters and microbe in Treatment ML						
Days **Na^+**		**Parameters**				
		NO_3-	**Ca^{2+}**	**K^+**	**Microbe**	
Before treatment						
No vessels		140 ± 0.00	128 ± 6.81	58 ± 2.08	45 ± 4.58	0.13 ± 0.06
After treatment						
D_1	ML_C	140 ± 0.00	121 ± 1.15	59 ± 2.65	46 ± 2.52	0.100 ± 0.01
	ML_1	140 ± 0.00	371 ± 7.02	37 ± 3.51	162 ± 3.21	0.16 ± 0.01
	ML_2	150 ± 0.00	406 ± 5.51	40 ± 3.51	214 ± 3.79	0.15 ± 0.02
	ML_3	160 ± 0.00	472 ± 2.52	41 ± 0.58	283 ± 3.79	0.22 ± 0.01
D_3	ML_C	180 ± 0.00	123 ± 1.53	70 ± 1.53	142 ± 2.08	0.18 ± 0.01
	ML_1	140 ± 0.00	353 ± 4.36	56 ± 1.53	141 ± 2.65	0.25 ± 0.02
	ML_2	150 ± 0.00	161 ± 1.15	68 ± 2.08	181 ± 7.02	0.48 ± 0.04
	ML_3	170 ± 0.00	438 ± 3.46	74 ± 3.61	262 ± 3.21	0.32 ± 0.01
D_5	ML_C	223 ± 5.77	126 ± 2.08	65 ± 1.53	148 ± 0.58	0.27 ± 0.05
	ML_1	183 ± 5.77	169 ± 1.15	94 ± 0.58	173 ± 4.16	0.21 ± 0.01

	ML_2	160 ± 0.00	64 ± 2.08	121 ± 1.15	239 ± 1.73	0.34 ± 0.03
	ML_3	180 ± 0.00	202 ± 3.21	121 ± 1.53	300 ± 2.52	0.28 ± 0.01
D_7	ML_C	240 ± 0.00	133 ± 4.58	71 ± 1.53	190 ± 3.51	0.16 ± 0.02
	ML_1	220 ± 0.00	204 ± 3.61	101 ± 1.53	163 ± 3.79	0.28 ± 0.01
	ML_2	200 ± 0.00	57 ± 0.58	120 ± 0.58	189 ± 1.73	0.26 ± 0.03
	ML_3	257 ± 5.77	242 ± 2.08	170 ± 0.58	301 ± 1.15	0.25 ± 0.01

The data in the table was mean ± SD. The unit for water parameters of Na^+, NO_3^-, Ca^{2+} and K^+ was milligram per liter (mg/L). Treatment of ML indicated combination of EM Mud ball (M), and lotus pod (L) with different ratio; MH_C = control treatment, ML_1 = EM Mud ball + lotus pod (1:4), MHL_2 = EM Mud ball + lotus pod (1:2), and MHL_3 = EM Mud ball + lotus pod (2:1).

a. Reduction in NO_3^- concentration

As for NO3- concentration, the percentage of ratio in Treatment MLC, Treatment ML_1 and Treatment ML_3 were increasing by 3.9%, 55% and 89% respectively while for Treatment ML_2, it decreased by 55%. Among these treatments, Treatment ML_2 with EM Mud ball and lotus pod at a ratio of 1:2, had better capacity in reducing and maintaining the water parameters at the acceptable range which were 57 mg/L as it was less than 80 mg/L that will not harmful to fish species. For Treatment ML_1 and Treatment ML_3, the ratio of EM Mud ball and lotus pod added were 1:4 and 2:1 respectively which might promote excessive nutrients into the water that can contribute to algal bloom due to more EM Mud ball were added as compared to Treatment ML_2.

b. Microbial Growth Enhancement

Microorganism in the water body can be naturally and synthetically induced that are non-native to the aquatic environment [1,3]. EM Mud ball contained lactic acid bacteria, photosynthetic bacteria and other beneficial microorganisms. These bacteria have the capacity to break down the organic pollutants and also eventually induced more microorganisms into the water body. Table 8 showed the concentration of microbe in all Treatment ML had increased in Day 7. This happened because the amount of minerals which are the food source of microorganisms had increased that enhanced the microorganism's growth. The bacteria species obtained from water samples of Treatment ML were shown in table 10.

Statistical Analysis

The significant different among Treatment MHL and Treatment ML were calculated using t-Test as shown in table 9. Based on the results, the Na^+, Ca^{2+} and K^+ concentration had significant difference between Treatment MHL and Treatment ML as the p-value were less than 0.05. These concentrations were much lower in Treatment ML compared to Treatment MHL as the data in Treatment ML were more consistency based on SD and the increasing ratio were much lower. The reason is that Treatment ML were using the combination of EM Mud ball and lotus pod rather than using only one vessel in each treatment as in Treatment MHL. On the other hand, concentration of NO_3^- showed no significant different between these treatments as the p-value was more than 0.05. Both Treatment MHL and Treatment ML had the capability to convert NH3 to non-toxic form of NO_3^-.

Soil Analysis

The 30 g of soil samples was collected at Si Hai Long Wang temple along the Batu Pahat River using soil tube. The analysis results from Bio Synergy lab were showed in table 10 and table 11 respectively.

Based on the results in table 10, the aerobic bacteria obtained from brown soil samples were 9.6 x 10^4 CFU/gm plate counts which was less than 10^6 CFU/g. There was also presence of mould and coli form with the plate counts of 4.2 x 10^2 CFU/gm and 3.8 x 10^2 CFU/gm respectively. No growth of yeast, *Escherichia coli* (*E. coli*) and Staphylococcus aureus in the brown soil sample as the plate counts for *E. coli* was less than 3 CFU/g. There was also no presence of *Salmonella* in the soil sample as *Salmonella* can result in waterborne or food borne illness. This soil sample was considered good microbiological quality as the microbes present in the soil was in the acceptable range.

Based on the results in table 11, the aerobic plate counts in black soil sample was 2.8 x 10^9 CFU/gm which was much lower than that in brown soil sample but it was more than 10^6 CFU/g. There was 1.2 x 10^2 CFU/gm of E. coli colony present in the soil sample whereas there was no growth of yeast, Staphylococcus aureus and absence of Salmonella. Presence of *E. coli* in the water indicated presence of sewage waste present in the water system. Water containing *E. coli* was not safe for drinking purpose as it indicated contaminated water and might introduced pathogenic bacteria. The microbiological quality of black soil sample was marginal [Microbiological quality of ready-to-eat foods 2009].

Microbe Identification

Microbe identification was analysed by using Gram staining, Oxidase test and Rap IDTM System test to identify the species of bacteria through ERIC. The gram staining results was observed under light microscope. After examined gram-type of bacteria, it proceeds to oxidase test to examine the bacteria oxidase positive or negative. As the results obtained were gram negative bacteria, Rap IDTM ONE System and Rap IDTM NF plus System were used to obtain the microcode of the bacteria and identified in ERIC.

Table 9: Comparison the significant difference between Treatment MHL and Treatment ML based on t-test

Parameters	Na^+	NO_3^-	Ca^{2+}	K^+
One-Tailed Test	0.00	0.41	0.00	0.04
p-value	0.05			

Table 10: Brown soil sample analysis at Bio Synergy Lab

Test parameter	Unit	Method used	Results
Aerobic plate counts	35°C, CFU/gm	AOAC Official Method 990.12 (3M Petri film)	9.6×10^4
Yeast	25°C, CFU/gm	AOAC Official Method 2014.05 (3M Petri film)	NG(<10)
Mould	25°C, CFU/gm	AOAC Official Method 2014.05 (3M Petri film)	4.2×10^2
Coli form	35°C, CFU/gm	AOAC Official Method 998.08 & 991.14 (3M Petri film)	3.8×10^2
E. coli	35°C, CFU/gm	AOAC Official Method 998.08 & 991.14 (3M Petri film)	NG(<10)
Staphylococcus aureus	35°C, CFU/gm	AOAC Official Method 2013.11, 2003.07, 2003.08 (3M Petri film)	NG(<10)
Salmonella	In 25 gm samples	AOAC Official Method 2014.01 (3M Petri film)	Absent

Unit = CFU/gm
E. coli = Escherichia coli

Table 11: Black soil sample analysis at Bio Synergy Lab

Test parameter	Unit	Method used	Results
Aerobic plate counts	35°C, CFU/gm	AOAC Official Method 990.12 (3M Petri film)	2.8×10^9
Yeast	25°C, CFU/gm	AOAC Official Method 2014.05 (3M Petri film)	NG(<10)
Mould	25°C, CFU/gm	AOAC Official Method 2014.05 (3M Petri film)	3.9×10^2
Coli form	35°C, CFU/gm	AOAC Official Method 998.08 & 991.14 (3M Petri film)	6.8×10^2
E. coli	35°C, CFU/gm	AOAC Official Method 998.08 & 991.14 (3M Petri film)	1.2×10^2
Staphylococcus aureus	35°C, CFU/gm	AOAC Official Method 2013.11, 2003.07, 2003.08 (3M Petri film)	NG(<10)
Salmonella	In 25 gm samples	AOAC Official Method 2014.01 (3M Petri film)	Absent

Unit = CFU/gm
E. coli = Escherichia coli

Gram-negative Bacteria

After observation of glass slides containing bacteria stain from bacteria culture of Treatment ML under light microscope, the examination of gram type of the bacteria was tabulated in table 10. The gram type of bacteria was gram negative for each treatment that cultured on nutrient agar and MacConkey agar. The gram negative bacteria was pink or red in color after gram staining as they did not retain the stain of crystal violet that cause blue. Bacteria cultured on nutrient agar from water sample 1, water sample 2 and water sample 3 from Si Hai Long Wang temple were named N1, N2 and N3 respectively. Whereas bacteria cultured on MacConkey agar for water sample 1, water sample 2 and water sample 3 from Si Hai Long Wang temple were named M1, M2 and M3 respectively.

Gram-negative bacteria can cause wide spread of waterborne and food borne diseases to human through direct contact of water or bacteria. It can cause infection to human by disruption of outer membrane of the bacteria cell which antibiotic or immune cells of human failed to destroy them and released toxic substances that are harmful to human [8].

Oxidase-positive and Oxidase-negative Bacteria

Oxidase test was carried out to determine the capability of bacteria to produce enzyme cytochrome c oxidase. This enzyme determines whether the bacteria are oxidase positive or negative. Cytochrome c oxidase is a Tran's membrane protein complex that present in the inner membrane of mitochondria. Bacteria containing this enzyme turns oxidase reagent to blue on the filter paper whereas if the bacteria lack of this enzyme, it remain colourless. It catalyses the electron transport chain to oxygen and produce by-product of water and hydrogen ions. Only bacteria cultured of N1 and N2 were oxidase-negative and the rest of the bacteria cultured were oxidase-positive.

a. Bacteria Identification

The bacteria species identified using ERIC based on the microcode were tabulated in table 12. Pure bacteria colony obtained from nutrient agar N1 and N2 were using Rap IDTM NF Plus System as the bacteria was oxidase positive where the bacteria possess of cytochrome c oxidase that turned the oxidase reagent to blue. Meanwhile, bacteria colony from N3 (nutrient

agar), M1, M2 and M3 (MacConkey agar) were using Rap IDTM ONE System as they lack of cytochrome c oxidase for electron transport chain. Each reaction cavities have specific value to determine the results obtaining microcode. Based on the results obtained, most of the bacteria presence in Batu Pahat River was opportunistic bacteria.

From the report form for N1, only cavity 3, 4, 5 and 10 were positive results in the first reaction before adding Rap ID reagents. After adding reagents from cavity 4 to 10 for second reaction, only cavity 10 showed positive results. The negative results will considered as zero value. Hence, the microcode for N1 was 430102 and identified as *Burkholderia cepacia* (99.9%). *B. cepacia* is an aerobic gram negative bacillus which commonly found in aquatic environments including water and soil [15]. This bacterium can infect and pose risk to healthy individual from contact with contaminated water. It is an opportunistic pathogen which can cause high mortality and morbidity as this bacterium is resistant to antibiotic treatment [4]. It normally causes respiratory issues when these bacteria invade the individual especially to cystic fibrosis patients. It can weaken immune systems where the immune response fails to detect the invaded bacteria.

The identified bacteria in N2 were *Acinetobacter* spp. (99.9%) with microcode of 110100. In the first reaction after incubation, cavity of 1, 4 and 10 showed positive results. In the second reaction, cavity 4 to 10 showed negative results where these results were valued as zero. *Acinetobacter* spp. was gram-negative coccobacillus which found in water, soil and sewage waste [29]. It also can grow on human skin areas and colonize gastrointestinal tract when ingest food or water containing the bacteria [13]. This bacterium is non-pathogenic bacteria but become pathogenic when unfavourable condition activated them to invade human when direct contact to the environment or drink contaminated water. It has the ability to escape from phagocytosis by immune cells of the host as it has thick polysaccharide capsule. *Acinetobacter* can infect human causing pneumonia, urinary tract infection and bloodstream infection that showed symptoms of fever, vomit, pain and burning during urination which can cause human death in chronic case [34]. Water with high nitrogen sources become food source which promote the population of *Acinetobacter* [30].

Bacteria identification for N3 was *Enterobacter cloacae* with 99.9%. According to [11], E. cloacae were reported as opportunistic and multi resistant bacteria pathogens to human. It is a gram-negative rod-shaped bacterium from family Enterobacteriaceae. This bacteria is facultative anaerobic as it can be aerobic when oxygen present and anaerobic when oxygen absent. *Enterobacter cloacae* are the most common and widespread in aquatic environment. When direct contact with the environment containing this bacteria, it can colonize the gastrointestinal tract of fish and human [9]. The bacteria can affect the health of organisms by secondary wound infection, respiratory tract issues, skin and soft tissues infection and bacteraemia [21]. The common disease infected by these bacteria is food poisoning [2,25]. Diseases caused by these bacteria are difficult to manage due to its multi resistant properties to drug.

Cronobacter sakazakii was identified from M1 with 99.9%. The first reaction showed positive results in cavity [3,7,8,10,11,12,13,14,16]. After addition of Rap ID Spot In dole Reagent in cavity 18, the reaction cavity turned from dark red-orange to dark purple which showed positive result. Thus, the microcode obtained from the result was 4037311. It is gram-negative rod-shaped bacilli that are pathogenic for both fish and human. However, these bacteria can cause life-threatening disease but in rare case such as meningitis, bacteraemia and necrotizing enter colitis [12]. Water outlets and aquatic environments are the possible sources of contamination and infection of the bacteria when direct contact. The general diseases caused by *C. sakazakii* were conjunctivitis, pneumonia, diarrhoea, wound infections and urinary tract infection [20].

Bacteria cultured from M2 were identified as Klebsiella pneumoniae (99.9%). The microcode was 1137250 where cavity [1,4,7,8,10,11,12,14,16,18] showed positive results in the first reaction. Cavity 18 was added with Rap ID Spot In dole for second reaction and obtained negative result which turned the color from light orange to red. *K. pneumoniae* is an encapsulated gram-negative bacillus with rod-shaped. It is common infectious agents that cause variety of infections such as pneumonia, urinary tract infection, bacteraemia, and liver ulceration [7,26]. These bacteria also cause infection to fish species when the condition is favourable to thrive the population of the bacteria such as high level of NH_3 in the water body. Such condition can cause a disease outbreak among fish population when aspiration with the symptoms of skin and fin haemorrhage, ulceration, swelling of visceral organs and respiratory difficulties [10,17]. In addition, it cause seafood poisoning to individuals when consuming the fish from the contaminated water or direct consume the water.

Lastly, the bacteria identified from M3 were *Enterobacter cloacae* (98.51%) which were similar to N3 and *Enterobacter asburiae* (1.48%). In the first reaction, the positive results obtained from cavity [3,7,8,10,11,12,13,14,16]. Cavity 18 remained negative result after addition of RapID Spot In dole in both first and second reaction with the microcode of 4037310. *E. asburiae* is similar to *E. cloacae* which come from same family and genus. This bacterium is often found in fish respiratory tract, faeces, blood specimen and urinary tract when aspirated water containing the bacteria [28]. It can become an opportunistic pathogen when favourable condition such as excessive nutrients or waste flows into the river water. This condition can increase the susceptibility of fish and human to infect by *E. asburiae* when there are open wounds on the surface that cause secondary infection. Direct contact of the water environment can result in illness such as fin haemorrhage and ulceration for fish and bloodstream infection, infection of skin and soft tissues, respiratory tract, bone and joint as well as gastrointestinal tract for human infection [22,27].

All these bacteria identified from water sample of Batu Pahat River were opportunistic bacterial pathogens where it can cause chronic disease to both aquatic organisms and human when aspirated or direct contact with the environment. As more wastes threw from the selected site into the river water, it can thrive and activate the bacteria population to become pathogenic. Since the water from Batu Pahat River was polluted, it is not considerable as clean and safe for drinking as many activities were carried out in the temple such as there was a well that available for washing and cleansing without drinking. This action might infected healthy individuals as they direct contact with the water contaminated with pathogenic bacteria. The reason is that the pollutants and the pathogenic bacteria may seep into the groundwater. Water from the wells is get from aquifers in the layer of groundwater.

Efficacy of Lotus Pod

Lotus pod as bio filter in the water filtration system had the capability in reducing the concentration of water minerals such as Na^+, Ca^{2+} and K^+ in the artificial green water that is nutrient-rich.

Table 12: Identification of bacteria species from water samples collected from Si Hai Long temple using ERIC

Bacteria sample	Gram-type	Oxidase test	Rapid system	Microcode	Bacteria species
Nutrient agar					
N1	Negative	Positive	NF Plus	430102	Burkholderia cepacia
N2	Negative	Positive	NF Plus	110100	Acinetobacter
N3	Negative	Negative	ONE	4034310	Enterobacter cloacae
MacConkey agar					
M1	Negative	Negative	ONE	4037311	Cronobacter sakazakii
M2	Negative	Negative	ONE	1137250	Klebsiella pneumoniae
M3	Negative	Negative	ONE	4037310	Enterobacter cloacae, Enterobacter asburiae

Reduction in Na^+, Ca^{2+} and K^+ Concentration

Comparison between Treatment LC and Treatment L_1 from table 13 and graph of (a), (b) and (d) from figure 2, the concentration of Na^+, Ca^{2+} and K^+ had reduced after 6 days. Na^+, Ca^{2+} and K+ concentration for Treatment LC at D6 were 1680 mg/L, 160 mg/L and 364 mg/L respectively which were much higher than that of Treatment L_1 with the concentration of 1133 mg/L, 107 mg/L and 277 mg/L respectively. There was a significant difference between Treatment LC and Treatment L_1 as the p-value for these water minerals were less than 0.05 table 14. Reduction of these water minerals proved that lotus pod had the capacity to reduce sediments and nutrients in the water body.

Table 13: Concentration of water parameters and microbe in Treatment L

Treatment L_C						
Day	**D_1**	**D_2**	**D_3**	**D_4**	**D_5**	**D_6**
Na^+	1700 ± 0.00	1700 ± 0.00	1728 ± 46.77	1677 ± 141.55	1701 ± 0.00	1680 ± 36.37
Ca^{2+}	165 ± 4.51	160 ± 10.00	165 ± 4.62	158 ± 7.39	160 ± 0.00	160 ± 0.00
NO_3-	190 ± 2.00	193 ± 26.68	189 ± 56.00	192 ± 0.00	189 ± 16.65	193 ± 3.98
K^+	369 ± 2.65	370 ± 0.00	385 ± 19.63	381 ± 12.70	370 ± 0.00	364 ± 24.98
NH_3	0.50 ± 0.00	0.28 ± 0.00	0.14 ± 0.00	0.13 ± 0.00	0.15 ± 0.00	0.13 ± 0.00
pH	8.59 ± 0.00	8.27 ± 0.02	8.17 ± 0.20	8.03 ± 0.06	8.08 ± 0.10	8.34 ± 0.08
DO	4.06 ± 0.05	4.08 ± 0.11	4.24 ± 0.18	5.31 ± 0.33	4.87 ± 0.18	5.02 ± 0.33
Microbe (M)	1.09 ± 0.03	0.25 ± 0.05	0.20 ± 0.01	0.16 ± 0.01	0.17 ± 0.00	0.18 ± 0.04
Treatment L_1						
Day	**D_1**	**D_2**	**D_3**	**D_4**	**D_5**	**D_6**
Na^+	1700 ± 0.00	1167 ± 57.74	1200 ± 0.00	1333 ± 57.74	950 ± 0.00	1133 ± 57.74
Ca^{2+}	161 ± 1.73	101 ± 7.81	106 ± 6.93	123 ± 5.77	94 ± 6.00	107 ± 11.55
NO_3-	189 ± 1.15	267 ± 55.08	163 ± 15.28	193 ± 5.77	220 ± 0.00	170 ± 0.00
K^+	371 ± 2.08	257 ± 5.77	267 ± 20.82	293 ± 25.17	237 ± 5.77	277 ± 5.77
NH_3	0.50 ± 0.00	0.47 ± 0.00	0.37 ± 0.00	0.39 ± 0.00	0.36 ± 0.00	0.23 ± 0.00
pH	8.58 ± 0.02	8.07 ± 0.04	8.21 ± 0.02	8.10 ± 0.07	8.03 ± 0.03	7.97 ± 0.07
DO	4.13 ± 0.13	7.37 ± 0.35	7.03 ± 0.09	7.45 ± 0.34	7.59 ± 0.04	7.26 ± 0.24
Microbe (M)	1.09 ± 0.04	0.82 ± 0.00	0.75 ± 0.03	0.69 ± 0.06	0.52 ± 0.03	0.65 ± 0.06

The data for the table was mean ± SD. The unit for water parameters of Na^+, NO_3^-, Ca^{2+}, NO_3^-, K^+, NH_3 and DO was milligram per liter (mg/L). This treatment was run for 6 days where D_1 = Day 1, D_2 = Day 2, D_3 = Day 3, D_4 = Day 4, D_5 = Day 5 and D_6 = Day 6. Treatment of ML indicated combination of EM Mud ball (M), and lotus pod (L) with different ratio; MH_C = control treatment, ML_1 = EM Mud ball + lotus pod (1:4), MHL_2 = EM Mud ball + lotus pod (1:2), and MHL_3 = EM Mud ball + lotus pod (2:1).

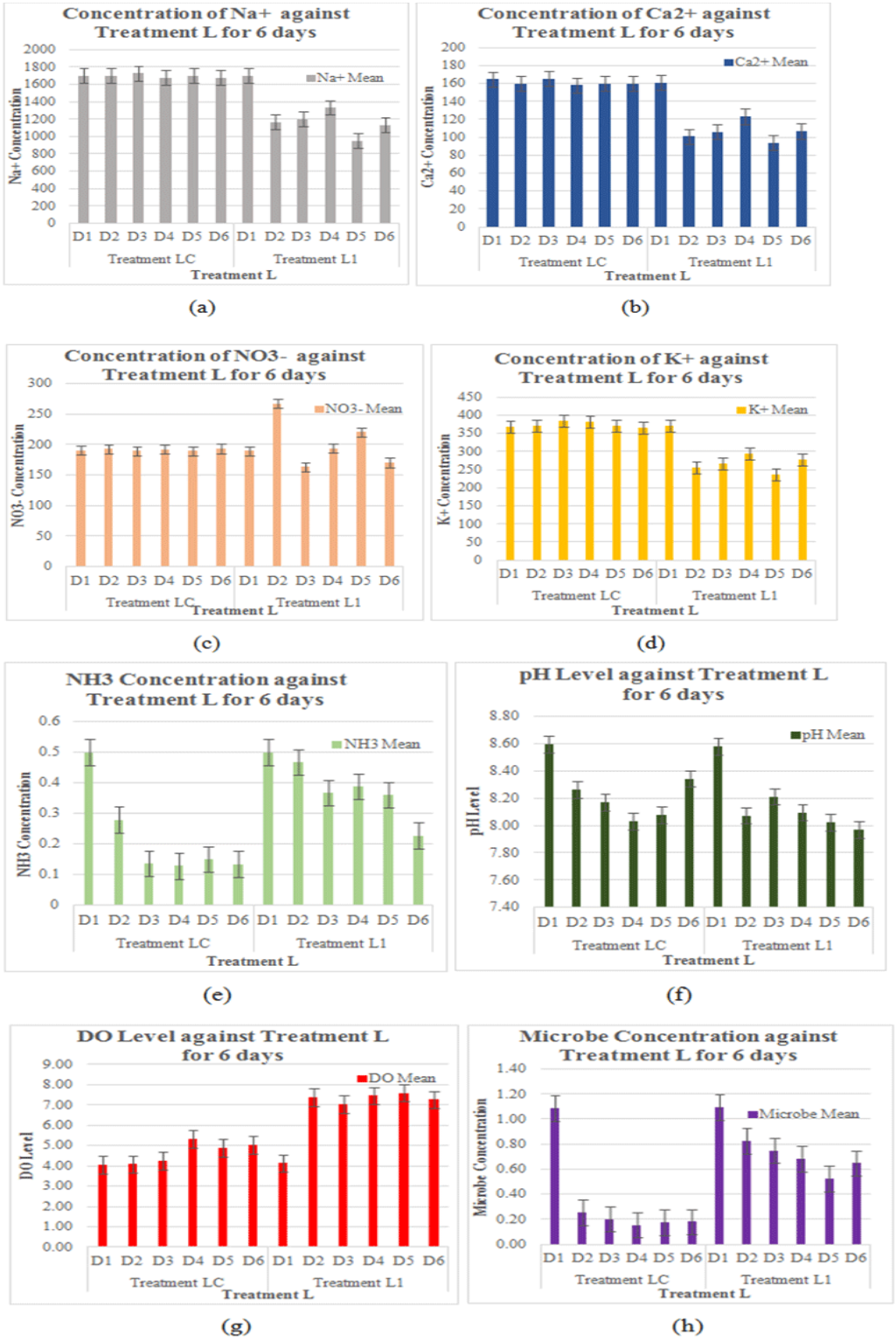

Figure 2: Graph of water parameters against Treatment L for 6 days. (a) Na+ concentration, (b) Ca2+ concentration, (c) NO3- concentration, (d) K+ concentration, (e) NH3 concentration, (f) pH level, (g) DO level and (h) Microbe concentration.

Table 14: Comparison the significant difference between Treatment L_C and Treatment L_1 based on t-test

Parameters	Na^+	Ca^{2+}	NO_3-	K^+	NH_3	pH	DO	M
One-Tailed Test	0.004	0.003	0.288	0.002	0.025	0.248	0.004	0.162
p-value	0.050							

Increasement of DO Level

Concentration of DO in Treatment LC in these 6 days were approximately around 4.6 mg/L and reached 5.02 mg/L at D6 which just right reaching the lower limit of desirable DO level. At the same time in Treatment L_1 which using lotus pod as bio filter in the water filtration system increased and maintained the DO level at above 7 mg/L which was in the range of 5 mg/L to 9 mg/L that support variety fish populations as well as promote spawning and reproduction.

Treatment L_1 had better water quality compared to Treatment LC as they DO level was higher. This indicated the free and available oxygen dissolved in the water are adequate and able to support aquatic organisms for their physical activities and metabolism as well as decomposition by aerobic microbes. A high DO level in the water system provides good water supply as it was good palatability. Based on table 4.8, the p-value obtained from t-test was 0.00 which was less than 0.05 showed a significant difference between Treatment LC and Treatment L_1 as lotus pod was more effective in maintaining and rising DO level to the optimum range.

Lotus Pod as Buffer Agent

Based on the previous treatment in Treatment ML, the efficiency of lotus pod able to neutralize the pH to optimum level (pH 7.4). In this water filtration system, the water pH was maintained at approximately pH of 8 which was in the acceptable range of 6.5 to 8.5 [33]. Water pH for Treatment L1 in D6 reached pH of 7.97 which was slowly reduced from pH of 8.58 to be reached at the optimum pH level of a river system. Lotus pod act as a buffer which prevent fluctuation of water pH that can affect the water quality and the health status of aquatic organisms. Contaminated or polluted water often become acidic than a good water quality of aquatic environment. Introduction of lotus pod can eventually increase the pH to the desirable range to be less acidic.

Water pH that exceeds 8.5 was usually caused by industrial wastes including steel production. These wastes might flow into river water when runoffs from the disposal and abandoned landfills as well as construction sites. High minerals concentration in the water also contributed to high water pH. This eventually increased the COD concentration and declined the DO level as more metals ions organic pollutants were dissolved in the water to be degraded. Mineral precipitation including Na+ and Ca^{2+} increased the water pH and affect the environmental issues such as reducing the light penetration into the water column as well as overwhelm the population of fish, macrophytes and macro invertebrates [16].

Improvement of Microbe Performance

The properties of lotus pod were large surface areas that providing sufficient space and adequate nutrients for microorganisms to grow. It also has the ability to prevent introduction of new microorganisms that are not native to the water system. In D1 for both Treatment L_C and L_1, the concentrations of microbes were 1.09 which were considered high and might cause negative effect to the water system and organisms in the water. This can cause bacterial contamination and disease outbreak can occur when high level of nutrients was available in the water to thrive the bacterial to become opportunistic pathogen. In Day 6 for Treatment L1, the microbe concentration was reduced to approximately 0.65 which was sufficient for bacterial activity in degradation of organic pollutants at faster rate.

Whereas the concentration of microbes in Treatment LC was approximately 0.18 which was low for decomposition of organic pollutants. Decomposition of organic pollutants would be much slower when the water system has low microbe concentration. This might be due to low concentration of DO and accumulation of organic waste that contributed to low microbe concentration [24]. Lotus pod provided more space and nutrients which enhanced the performance of microbes in decomposition.

Conclusion

The overall aim of this study was to evaluate the water quality of Batu Pahat River and the efficiency of lotus pod as a vessel for wastewater treatment. In this study, EM Mud ball, water hyacinth and lotus pod were used as treatment for water samples from Si Hai Long Wang temple. This selected site was the center of Batu Pahat River and most of the activities were carried out in this area. Among these vessels, lotus pod act as the best option in reducing sediments and water nutrients in the water. Although EM Mud balls and water hyacinth also had the capacity in reduction of water minerals and organic waste, however, they introduced much more nutrients which favorable to cyanobacteria growth and acidified the water as lactic acid bacteria were introduced into the water system. Fast propagation of water hyacinth can colonize the whole surface area of the water ecosystem which happens to affect the water flow that have directly affected on water temperature, DO level and community of aquatic organisms including zooplankton, macro invertebrates and fish.

Batu Pahat River was in the polluted range where the concentrations of water parameters were exceeded the acceptable range. Besides, the water pH was slightly acidic which was not suitable for most freshwater aquatic organisms to support growth and reproduction. High organic matter present in the water body contributed to low DO level, high ammonia, low pH and high

concentration of microbes. To evaluate the efficiency of vessels in water bioremediation, three vessels were tested in Treatment MHL and the results showed EM Mud ball increased the water mineral concentration and COD concentration in the water. Water hyacinth was able to reduce 50% of COD concentration but it can acidify the water when the plant decay as the water pH using water hyacinth was only reaching the border line of 6.5. Whereas lotus pod had the capability to reduce COD concentration, neutralize water pH to optimum level of 7.4.

Vigorous growth of water hyacinth that might contribute to poor water quality was not considered in the next treatment. Combination of EM Mud ball and lotus pod treatment with the ratio of 1:2 had the highest capability in reducing the concentration of NO_3- and enhancing microbial growth as they provided sufficient nutrients and space for beneficial microbes to grow which performed the degradation of organic waste. This combination in Treatment ML showed significant difference as compared to Treatment MHL.

Most of the microbes identified in the water samples from Si Hai Long Wang temple were opportunistic pathogenic bacteria if the concentration of the microbe was high which more than 1 was. The bacteria species present in the water sample were gram-negative bacteria including *Burkholderia cepacia, Acinetobacter, Enterobacter cloacae, Cronobacter sakazakii, Klebsiella pneumoniae* and *Enterobacter asburiae*. These bacteria might pose serious health effect to both aquatic organisms and human when direct contact with the contaminated water. Hence, the water from Batu Pahat River was not suitable for drinking purpose.

Vessel such as lotus pod in the water filtration system can decrease the water minerals concentration including Na+, Ca2+ and K+, increase the DO level which is in the range of 5 mg/L to 9 mg/L that able to support wide population of fish as well as spawning and hatchability rate. Moreover, lotus pod can act as buffer agent where it can maintain the pH at approximately 8. Introduction of lotus pod can prevent fluctuation of pH so that the aquatic organism and water quality can be preserved. It also can improve microbe performance as lotus pod has large surface area which providing adequate space and nutrients for microbe to grow and able to perform degradation of organic waste in the water system.

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Declarations

Author declares that this project is based on original work except for quotations and citations, which have been duly acknowledged. This manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

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