Heavy Metal Uptake by Pseudomonas Sp. and Entrobacter Sp. and It Growth Rate Using Synthetic Water

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Abstract
Heavy metal pollution from development and human activities, badly impacts to human health. Chemical and physical treatment are the recently method used in heavy metal treatment. This research conducted the study on heavy metal removal by (Pseudomonas sp. and Entrobacter sp.) isolated from river. Heavy metal employed included sample of concentration of higher heavy metals such as Fe, Al, Mn and Cd obtained at initial. Synthetic water used to in this study to examine the effect of heavy metal removal and bacteria mass growth rate. Heterotrophic plate count with spread method used to isolate these two species using gram stain technique. Agar nutrient (LB) and EMB agar were used. Both bacteria placed in bioreactor which is aerobic for Pseudomonas sp. and anaerobic for Entrobacter sp. at different temperature which is 25°C and 38°C until 8 days. The biomass or pellet of bacteria was weight for 10 days to obtained the best condition of temperature and the trend of growth rate of these bacteria. Maximum uptake of Fe, Al, Mn and Cd were obtained for day 6 from initial concentration by pseudomonas sp. was 98.17% (25°C), 84.20% (38°C), 66.88% (38°C) and just 40% reduction from day 4 to 6 (25°C) respectively. For Entrobacter sp. the best removal was 98.26% (25°C), 94.81% (25°C), 75% (25°C) and no removal for Cadmium noted. Results showed that, referred to the biomass of bacteria taken and the graph trend using implementation of cubic polynomial and R^2 standard deviations, shows that 25°C temperature gave the best conditions of for these two species of bacteria growth rate.

Keywords: Biological Treatment, Heavy Metal, Growth rate

1.0 Introduction

Water is generally known as an important necessity for all activities such as living consumption, industries, agricultural and routinely human for human activities of drinking. However the sources of clean water drinking contaminated by chemical constituents in term of organics, inorganics and gases (Hassimi et al., 2011). In Malaysia we have two standard regulated water that show as limit which acceptable and safe drinking water quality of raw and treated water, National Standard Drinking Water Quality (NSDWQ).

Water treatment was developed from the sixteenth century onward (John et al., 2005). Water treatment can be defined as the manipulation of water from various sources to achieve a water quality that meets specified goals or standard set by community through its regulatory agencies. Natural water usually can possess common inorganic constituents such pH, chlorides, alkalinity, nitrogen, phosphorous, sulfur, toxic inorganic compounds and heavy metals (Joanne, 2001).

Mostly, inorganic chemical constituents commonly found in water in significant quantities (1.0 to 1000 mg/L) include calcium, magnesium, sodium, potassium, bicarbonate, chloride, sulfate, and nitrate and about (0.01 to 10mg/L) inorganic constituents that are generally present in lesser amounts include iron, lead, copper, arsenic, and manganese (John et al., 2005).

Metal contain in water that can harmful although in small amounts are classified as toxic and other metal are classified as nontoxic (Joanne, 2001). For safety water supply for drinking and human activities, WHO Standard has listed the maximum amount of heavy metal with that level of inorganic contamination can be interpreted (Chris Binnie, 2002). Nowadays many people concern about health and getting more sensitive to pollution. At the same time, biological treatment methods are now considerably as an economic drinking water treatment system and more natural.

From previous investigation, there are some microorganisms are able to accumulate metals, and this property exploited, using Enterobacter sp. and Pseudomonas sp. bacteria but the potential of two types of this bacteria to growth can be exploited by considering its life culture and temperature.

1.1 Problem Statement

Sungai Melaka is one of the important river that supply portable water to the community. This river remain polluted and have a problem with water quality. The aggravated problem of water quality in Sungai Melaka regarding on heavy metal, has inspired to determine the level of heavy metals Ferum (Fe), Aluminium (Al), Mangan (Mn), and Cadmium (Cd) itself. Heavy metals normally occurring in nature are not harmful to our environment because there are only present in very small amount (Sanayei et al., 2009).

However, if the levels of these metals higher than recommended limits, their roles change to a negative dimension. Drinking water is the one of important sources for heavy metals for human. Water contaminate such as bacteria, viruses, heavy metals, nitrates and salt have found their way into water supplier due to inadequate treatment and disposal of waste by human and livestock, industrial discharges and over-use of limited water resources (Singh and Mosley, 2003).

This site of research there are source of pollution that can contributed to heavy metal such as factory, chicken farm, and industrial. This problem will be more serious if there are no actions to be taken. In fact, water demand in most countries is steadily increasing as more and more people achieve higher standards of living and as lifestyles are changing steadily. Conventional physicochemical methods such as electrochemical treatment, ion exchange, precipitation, reverse osmosis, evaporation and sorption is commonly used for water treatment for heavy metal removal but this method or technique are not cost effective (Kadirvelu et al., 2001) Hence biological treatment approach as an alternative remediation for heavy metal contamination (Manisha et al., 2011).

Biological treatment was choose for this study. The Enterobacter sp. and pseudomonas sp. bacteria with specific characteristics selected using isolation method. Enterobacter sp. and pseudomonas sp. which classified as heterotrophic from Sungai Melaka was isolated and tested for heavy metals removal such as Ferum (Fe), Aluminium (Al), Mangan (Mn), and Cadmium (Cd) (Manisha et al., 2011). For the result ordinary researcher used many types of graph and software in order to get the accurate analysis for heavy metal removal by bacteria. Hence there is an accurate and exactly graph to present growth rate (k) or mass of bacteria against time. In this research polynomial graph used as the best accurate result present. From the result of efficiency of two bacteria removed heavy metal hopely can be used as secondary treatment in treatment plant especially in Melaka.

1.2 Objective

The objectives of this study are as follows:

a. To carry out the ability of Enterobacter sp. and pseudomonas sp. in heavy metals removal such as Fe, Al, Mn, and Cd from synthetic water in temperature 25°C and 38°C.
b. To carry out the best of Enterobacter sp. and pseudomonas sp. bacteria growth rate, k using polynomial graph.

1.3 Scope

This research aim to remove Fe, Al, Mn, and Cd in synthetic water by biological treatment using Entrobacter sp. and Pseudomonas sp. The research only focused on the ability of Enterobacter sp. and Pseudomonas sp. isolated from Sungai Melaka water sample to remove the selected metals. The ability of Enterobacter sp. and Pseudomonas sp. will be monitored in reactor with temperature 25°C and 38°C. Entrobacter sp. will be culture in anaerobic batch and Pseudomonas sp. in aerobic batch. Result of heavy metal removal between Enterobacter sp. and Pseudomonas sp. is compared using linear graph. The growth rate, k of bacteria in 25°C and 38°C analyzed with polynomial, cubic graph. The relationship between k rate (mass) and absorption rate was analyze and concluded

2.0 Literature Review

Malaysia, as a developing country to another has goals to progress to a developed country-based industrial by the year 2020. In line with this goal the government has planned various development projects involving economic, social and infrastructure to improve the lives of people and thereby improving further economic growth. Therefore, since lately, whether in the city or rapid rural development projects implemented. In the human passion for high profits from this development process, intentionally or unintentionally have caused the environment affected.

This situation actually reflects that lack of awareness and concern for human beings to appreciate the environment. But instead they are only concerned with quality of life and forget the actual fact, the relationship between economic development and change are very complex environments. As a result of imbalance in the development of implementation appropriate environmental management then, a variety of environmental problems has exist. Among the pollution of water, air, noise, flash floods and so on. Incident this catastrophe is actually very closely related to the importance of planning joint development environment.

Over recent years, the issue of environmental pollution in Malaysia so debated by all communities. Among the issues of pollution the target of their discussion is water pollution. This issue is more getting public attention because water is an essential element to accommodate life of one of the three essential elements other than oxygen and sun in the basic needs of life in the ecosystem of the earth. In Malaysia, the river is the main source of water supply sources to all human activities. There are about 49 major river basins that the reason for these supplies.

Human use of water through two means of recruitment and flow of water, for example water intake for agriculture and domestic industry, while water flow is used to produce hydroelectric power, navigation, fishing and recreation. But what is worrying is that human lately, although water resources are easily accessible and a lot of lakes, rivers, sea and so on, but rather the quantity of water that is clean and safe to use is very limited. If scouting in general, water quality in the watershed regarding rivers are located on the East Coast of Peninsular Malaysia is more than 3 clean and safe to use compared to the rivers on the coast west. This is because on the east coast of the development process, urbanization and land use functions still manageable compared to the west coast, which became focus on various development projects, urbanization, industrialization and etc.

Therefore, the water quality will deteriorate if the course is near large cities, residential areas, industrial and etc. This is because the pattern of land use in the area was able to impact on the drainage system located in the surrounding area (Detwyler, 1975). A preliminary assessment of water quality was conducted by the Division Environment in 1976 showed that 42 river basins. Peninsular Malaysia can be categorized as highly polluted, 16 more are in moderate level and seven river basins found threatened.

Among regarding rivers include the Sungai Klang, Sungai Selangor, Sungai Stem, Sungai Langat, Sungai Melaka, Sungai Muda, Young, Sungai Muar, Sungai Segamat, Sungai Johor, Sungai Terengganu and Sungai Kelantan. River water quality monitoring was conducted in both 1978 and 1979, found that 55 percent of the 45 monitored river basins in Peninsular Malaysia can be considered clean. In addition, 29 percent of the water quality nothing changes include Sungai Orissa, Sungai Peri, Sungai Stem, Sungai Muar and Sungai Kemaman. While 14 percent have recovered and only Sungai Melaka available water quality got worse. (Tuan Pah Rokiah, 2001.)

3.0 Methodology

There were three methods which were used in this research of removal selected heavy metal using synthetic water. Stated method was isolation of bacteria, heavy metal testing and biomass bacteria monitoring. Removal of heavy metal by adsorption is a basically mass transfer process by metal ion transferred from solution to the surface of sorbent and bound by physical or chemical interaction. (Abdel-Raouf et al, 2017). These research focus on biological interaction using selected bacteria.

3.1 Site Description

Sungai Melaka is the main source of raw water treatment for plant. It supplies and produces treated water to the residence and industrial activities in Melaka. The river length 40.1 km initial from Muara Selat Melaka to Kg. Gadek. (JPS, 2012). Water samples were collected at Sungai Melaka location 2.34 latitude and 102.254 longitudes, by using Grab Sampling Method. Plastic bottle was used to take a sample, and it was cleaned using distilled water and rinsed at least three times with river water. The sample was used for bacteria isolation and the concentration of heavy metal in that river, was traced by using DR2010. The final amount of heavy metal left is important for comparison of reducing heavy metal after biological activities treatment done.

3.2 Sampling Bacteria Isolation

River water used in this study was collected from Sungai Melaka located at state of Malacca which main resources of water supply in Malacca residential area. The water samples were collected from this river by using Grab Sampling Method. Plastic bottle are used to take a sample, and prepared for laboratory testing.

3.3 Isolation of Gram Negative Bacteria.

Spread Plate method was chosen in this study for analysis Pseudomonas sp. and Entrobacter sp. bacteria growth in Sungai Melaka. The lab procedure are followed "Standard Method for Examination of Water and Wastewater". By using ISO standard the petri dish should incubated at 22°C ± 2°C for 24 hours (Paul Singleton, 1999). Gram stained conducted in this test to determine most bacteria as either Gram-positive or Gram-negative. Smear of bacteria cells use in this technique, and must be stained with a dye. Gram negative bacteria appear in pink color, while gram positive appear in dark purple (Ahmed and Carolyn, 2003).

3.4 Selective Media

Luria Bestanni (LB) and Eosin Methylene Blue Agar (EMB) were two media that important for growing the Pseudomonas sp. and

Entrobacter sp. colonies, and used for agar preparation to isolate these two bacteria in this study.

3.5 Inoculation Into Broth

This inoculation is carried out to culture or growing bacteria. The selected single colony are taken and put into the 1 liter broth in 1 liter breaker, than shake for 24 hours until the bacteria colonies appears.

3.5 Centrifuge

Centrifuge was used for broth preparation. 2L broth will be prepared and placed in each types of bioreactor. Centrifuge with 100ml in size used in this study, with centrifuge rate is 3000 rpm for 5 minutes. Centrifuge used for getting the mass or pellet as consider as bacteria mass for this research. The mass 0.0168 g/L was put in the bioreactor contained synthetic water for both condition aerobic and anaerobic which is 25°C and 38°C.

3.7 Bio-Reactors (Aerobic and Anaerobic)

In this study anaerobic and aerobic batch reactor used in order to study the effects of Pseudomonas sp. and Entrobacter sp. bacteria on heavy metal removal. This batch bio-reactor was placed with synthetic water, broth, and distilled water. The ions solution such as Fe $(N0_3)_3.9H_2O$, Al $(N0_3)_2.4H_2O$, Mn, and $Cd(N0_3)_2.4H_2O$ used and conducted 25°C at and 38°C, for 8 days retention time. The volume of reactor depend on the mass of broth that will be collected. This bio-reactor equipped with mixer and air diffuser to ensure the DO in this bioreactor always more than 2mg/L during the retention time.

3.8 Analytical Test

In this study, four parameters of heavy metals such as Fe, Al, Mn and Cd need to remove from Synthetic water. To prove that, AAS method was used to trace metal concentration from synthetic retention water. This method is very fast and accurate also can be made to be essentially free interferences. For analyze growth rate of bacteria during the study, about 10 ml water samples was taken every days, and by using a vacuum filter, water sample will be filtrate. This is very important to make sure water level still same until the end of test. Suspended solid that found on the filter paper will be balance for getting a mass of bacteria. Finally the bacterial growth rate against time will be plotted. For heavy metal reduction graph linear was used to see the pattern of graph and present the analysis. A part of determination of bacteria growth rate for two different temperatures, standard deviation R^2 results which is close to 1.00 was implemented using polynomial cubic graph.

4.0 Results And Analysis

The result of isolations of two selected species was presented in Table 1 The isolate obtained from the river water sample were Pseudomonas sp. and

Entrobacter sp. The effect of heavy metal concentration removed in percentages (%) by the species of bacteria was presented in Table 2.

Table 1: Result From EMB Colonies Identifications

EMB Identifications	Types of Bacteria	
	Pseudomonas sp.	**Entrobacter sp.**
Gram of Bacteria	Gram- Negative	Gram -Negative
Color of Colonies	Green/Drark Brown	Pink
Characteristic	Lactose Negative	Lactose Positive
	Not Produce asid	Produce acid
	Not generate energy from Lactose	Energy from Lactose

Table 2: Heavy Metal removal result by Pseudomonas sp. & Entrobacter sp.

Types Of Bacteria		Pseudomonas sp.		Entrobacter sp.	
Types Of Bioreactor		Aerobic		Anaerobic	
Temperature		25°C	38°C	25°C	38°C
Heavy Metal (removal)	Ferum (Fe)	99.13%	68.22 %	98.57%	98.26 %
	Aluminium (Al)	90.28%	89.17 %	94.81%	92.59 %
	Mangan (Mn)	59.38%	66.88 %	7.50%	60.81 %
	Cadmium (Cd)	40%	1.69%	0%	0%
Growth rate, k	(Standard deviation) R^2	0.942	0.909	0.97	0.943
Other parameters	pH	8.53%	7.72%	8.04%	8.51 %
	Dissolved Oxygen (DO)	33.88%	42.12 %	47.04%	48.13 %
	Temperature (C)	25°C better than 38°C		38°C better than 25°C	
	Heavy Metal (reduction)				

4.1 Heavy Metal (Fe, Al, Mn, And Cd) Removal

All the information of iron removal can be seen at Table 2. For iron removal, Figure 1 shows that the trend of iron Ferum removal in 25°C and 38°C bioreactors. Enterobacter sp bacteria were more effective for iron removal in 25°C than 38°C with small differences percentage of iron removal. For Pseudomonas sp. in 25°C and 38°C result it proved that Pseudomonas sp. bacteria very effective in absorptions of ferum metal in room temperature rather than 38°C. At that time the membrane of this bacteria still accumulate the react with ferum. However, its effectiveness should be reviewed for a day more. As a result during 8 days the experiment done to this species the growth and the metabolism of bacteria were continued and still precede the reactions iron removed. From the result, Pseudomonas sp. at 25°C is the most effective removed ferum followed by Entrobacter sp at 25°C, Entrobacter sp. at 38°C and lastly Pseudomonas sp. at 38°C with 99.13% , 98.57%, 98.26% and 68.22% respectively. Lastly can be concluded Pseudomonas .sp at 25°C and Entrobacter sp at 25°C as the effective potential bacteria on removing Ferum in biological water treatment. Aluminium (Al) reading was shows in Figure 2, from the result Enterobacter sp. bacteria in two condition, decreased aluminum concentrations respectively until 6 days with high reduction. At 25°C which is room temperature about 94.81% aluminum was removed and for 38°C, 92.59% only. At 8th day, the amount of aluminum started increased 66.67% at 25°C and 33.33% at 38°C. The increased of aluminum concentration can be conclude as the effect of last stationary phase in exhibit bacteria growth, because of limited food supply, and the toxic waste start builds up (Ahmed and Carolyn, 2003). For aluminum, 25°C and 38°C temperature is suitable condition suggested for biological water treatment using Enterobacter sp. bacteria. For bioreactor Pseudomonas sp. 25°C and 38°C the amount of Aluminium continuously decreases respectively at 90.28% from the first day and 89.17% for 38C batch. It is shows that bacteria that culture in aerobic reactor Pseudomonas sp. more resistance to the metals ions and bioavailability than Entrobacter sp. at the conditions. Bioavailability of substance is defined as measure of its potential to interact with biological system and to cause a response. Lastly can be conclude Aluminium can be removed effectively by using at 25°C with 90.28% reduction compare to the 38°C bioreactor.

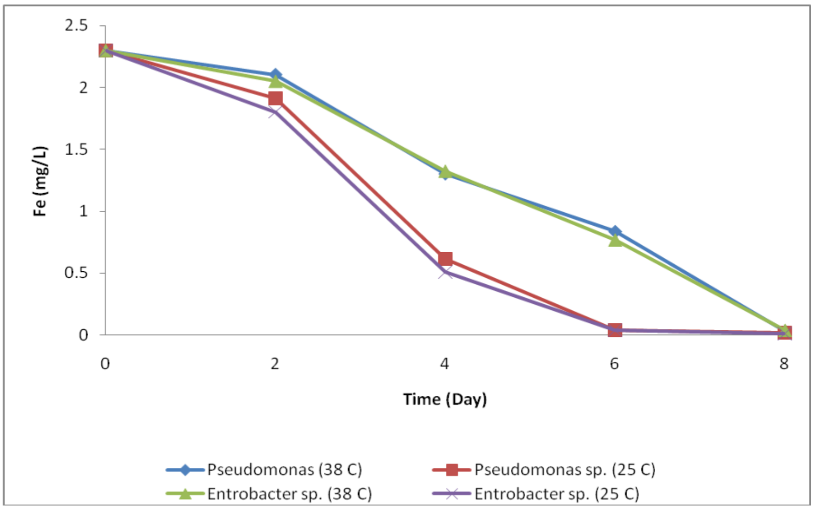

Figure 1: Fe (mg/l) versus Time (day)

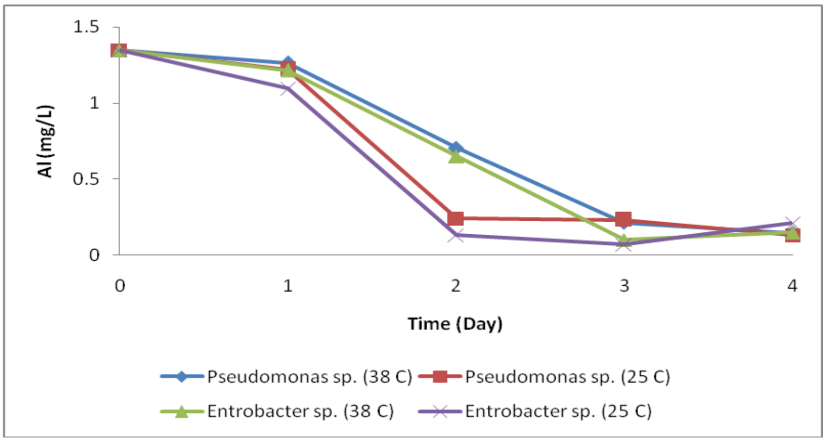

Figure 2: Al (mg/l) versus Time (day)

Manganese removal was shown in Figure 3. The Manganese in both reactors reduced to 0.0627 mg/L or 60.81% for 38C and only 7.5% or 0.148 mg/L for 25°C bioreactor respectively every 2 days in 6 days. At day 8, showed increment concentration of manganese, this happened may be because of limited available foods in the last stationary phase, the metabolism was lower and the energy cannot derive from oxidation organic compound (Ahmed and Carolyn, 2003). This also was may be these types of bacteria were utilizing the available substance containing manganese for stored inside, and was released manganese due to the usage of available substances stored in their body (Ho Siew Siong, 2005). If we looked at the Pseudomonas sp. tread from day 2 to day 6, also reduced about 59.38% for 25°C and 66.88% for Mn in 38C. If we look forward until day 8, the amount become increase drastically from 0.065 mg/L to 0.174 mg/L for 25°C bioreactor 0.053 to 0.192 mg/L. It about 167.69% at 25°C and 262.26% at 38°C. It is shows that Mangan only effective and can accumulate the Mn ion just for 6 day and believed that compare to fungi, fungi are more tolerant than bacteria to high concentrations of heavy metals and often leading to a prevalence of fungi in metal contaminated. Other than that, it can be proved that bacteria do not gain the energy from oxidation reaction. Compared with two different bacteria Entrobacter sp. and Pseudomonas sp. both are not effective in reducing Mn but for retention time below 6 days, Pseudomonas sp. 38C looked most effective with 66.88%, compare to Entrobacter sp. 60.81% (38°C), and Pseudomonas sp. 59.38% (25°C) and lastly 7.5% (25°C) for Entrobacter sp.

The exhibited of cadmium removal shows in Figure 4 clearly. Actually the initial cadmium concentration of synthetic water used was 0.000364 mg/L. During the experiment, cadmium in both reactors 25°C and 38°C was highly raised until 0.27 mg/L. This happened may be the Enterobacter sp. bacteria cannot bind the cadmium cations because it was so toxic complex-formers that they are too dangerous for any biological function (Saithong, 2002). At the same time, the reaction of cadmium in the water was very low, though in the acidic water only (Daniel, 1995). Due to the reason cadmium not so effective in this synthetic chemical water cause the synthetic water in neutral pH range 6.5 to 8.5. The complexation of the cadmium was not successful. It because complexation is only an efficient way of metal detoxification in slow-growing cells, and lives at low concentrations of heavy metals. In this experiment concentration of cadmium is very low than the others metal contain in synthetic water, so complexation happened not complete. In the real situation, in the effluent since reduction is not possible or may not be sensible, as a sole mechanism of detoxification, heavy metal ions have to be detoxified by efflux alone or in combination, in any organism growing fast in an environment contaminated with high concentrations of heavy metals. If we looked at Pseudomonas sp. this bacteria was effective at reducing Cadmium accumulation reduction of 1.69% at 38°C) and about 40% at 25°C which is room temperature. It proved that Pseudomonas sp. bacteria shows higher reduction at pH 7 (M.P.Krishna et al., 2012). During this acidic neutral pH the binding cell active binding sites on cell surface (Bai et al., 2002). From the result of Cadmium removing that can be concluded that the selected Pseudomonas sp. isolate are potential that Entrobacter sp. bacteria for bioaccumulation activity and appeared to be appropriate for bioremediation process (M.P.Krishna et al., 2012). In other situation, it can be summarized the effects of different external factors such as pH, contact time, temperature, concentration of biomass and metal ions, and the nature of aqueous environment in the degree of biosorption as conducted research. (Surajit ,2017).

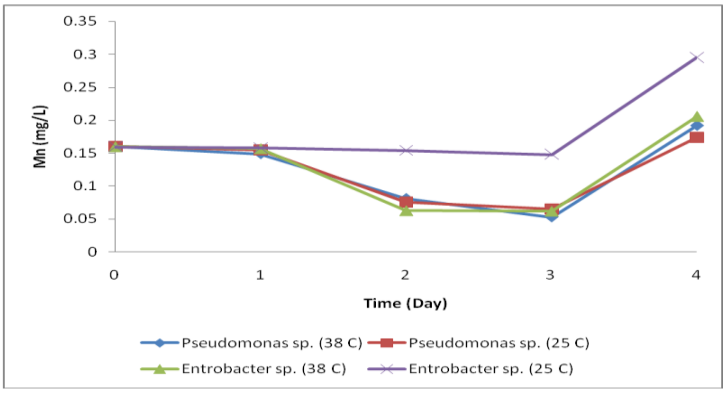

Figure 3: Mn (mg/l) versus Time (day)

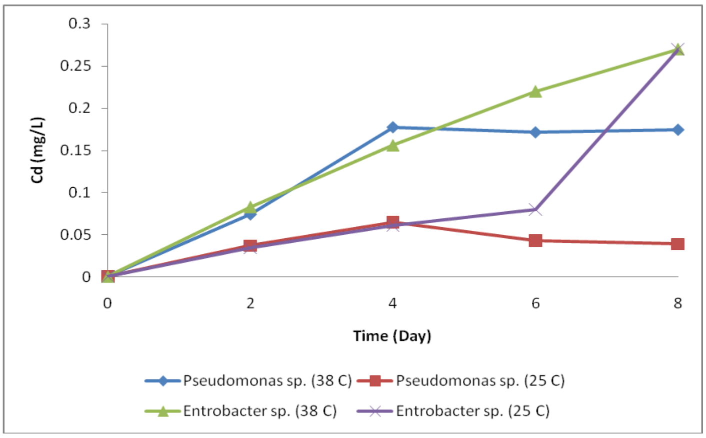

Figure 4: Cd (mg/l) versus Time (day)

4.2 Dissolved Oxygen (DO)

Level of Dissolved Oxygen concentration is measured every day from day one to day eight for both reactors shows in Figure 5. From this experiment, DO was decreased slowly due to the substrate adaptation and consumption by time. It is also because the activities of bacteria that used oxygen for their metabolism processes reduced heavy metals. Range of reduction can be conclude as 42.13% for Pseudomonas sp. (38°C), 48.13% (Entrobacter sp. 38%), 3.79% increase from day one to four and decrease 49.05% day four to eight (Entrobacter sp. 25°C), it shows the Entrobacter sp at 25°C is actively accumulate start from day four to eight. Lastly, 33.88% decreasing of Pseudomonas sp. (25°C) from day one two eight recorded. Dissolved oxygen is the best indicators show the health of water ecosystem through the action of the bacteria. Decreases in the dissolved oxygen levels can cause by changes in the types and numbers of aquatic microorganism which live in this bioreactor.

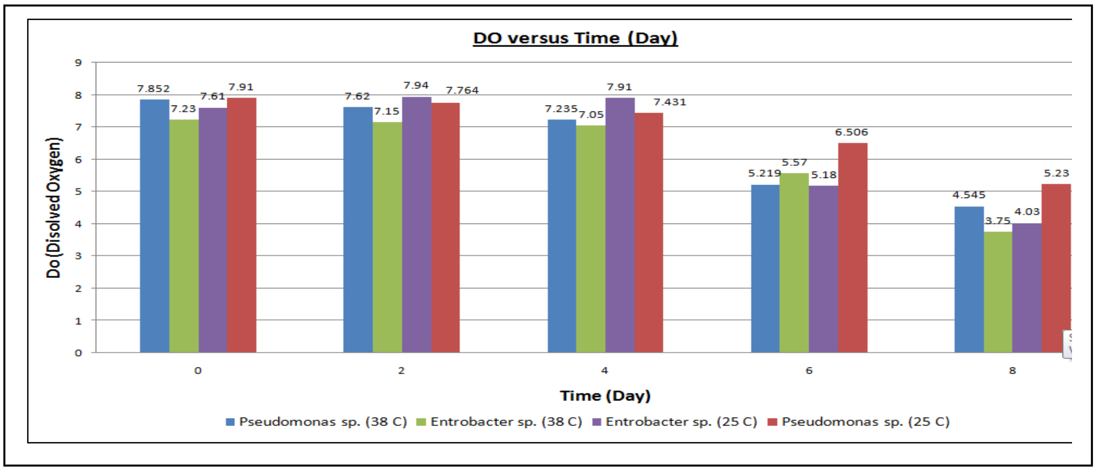

Figure 5: Dissolved Oxygen (DO) for bioreactors

4.3 pH Reading

Figure 6 exhibited the pH in each reactor. pH needed to be kept constant during chemical and biological treatment processes to provide an optimal environment for microorganisms during bioremediation of inorganic pollutants. In this experiment pH range was fixed 6.5 to 8.5 which is well-defined pH optimum. The initial pH for Entrobacter sp. was 7.38 for 38°C bioreactor, and 7.45 for 25°C bioreactor. For Pseudomonas sp. was 7.46 for 38C and 7.35 for 25C. The pH constantly decreased from basic solution 7 something to having a pH near 7 which is neutral solution. From the result the medium pH affects solubility and ionization of metal and interaction between bacteria and metal accumulation. For Pseudomonas bacteria pH 7 is the best condition higher removal of metal (M.P.Krishna et al., 2012). Another factor that influenced the change of pH is because the Entrobacter sp. will released acid during digestion of metal from the body due to chemical reaction in the bioreactor (John Wiley and Son, 1999).

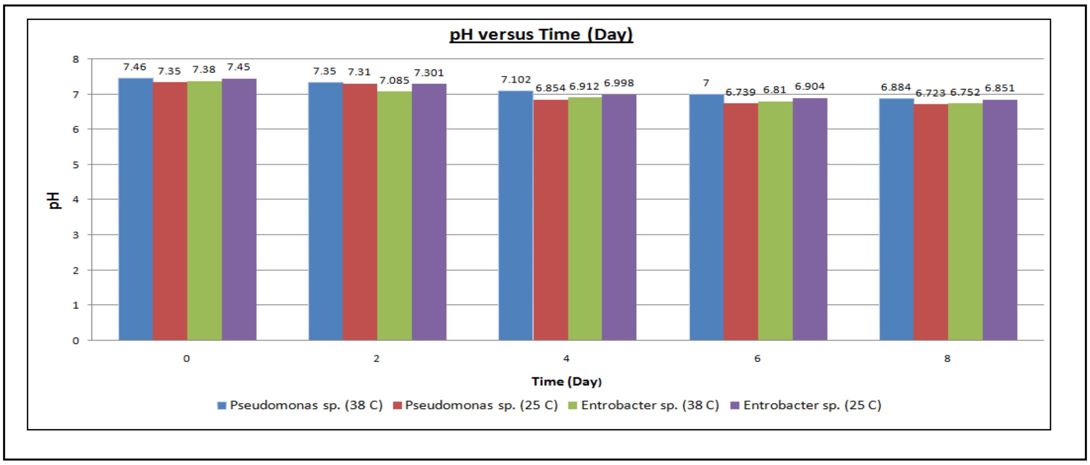

Figure 6: pH reading for bioreactors

4.4 Bacteria Growth Rate

Cubic graph was carried out in Figures 7 and 8, to find the *k* rate which is close to 1.00. Regarding to the results from liner and polynomial graph, the cubic graph played the most accurate results for Entrobacter sp. with 0.97 and 0.943 standard deviation *k* rate bacteria, for 25°C and 38°C bioreactors. It indicated a potentially truly maximum cell growth obtained in two difference water temperature. In this graph also it can viewed the bacteria life or phase, which is shows the bacteria increased the populations until day 5 and was a small decrease on mass for the day after. k rate of bacteria was study to carried out the best condition for bacteria life in two different temperature. So, it was established that biological water treatment by using Enterobacter sp. bacteria was more suitable launch in 25°C which is in room temperature. These two graphs at Figures 9 and 10 shows the implementing of polynomial (cubic graph) x^3 that carried out two get the closure analysis and standard deviation for graph plotted for Pseudomonas sp. 25°C and 38°C bioreactor. Compare to linear and quadratic polynomial graph, cubic graph represented the nearest value to 1.00 which is 0.942 and 0.909 respectively for 25°C and 38°C. This usage of this graph polynomial cubic function is accurate because the plotting means the graph has no or less breaks. The sketching of the graph smooth and it can produce the best standard deviation that shows the precise value. From the results it was established that biological water treatment by using Pseudomonas sp. bacteria was more suitable and effective in 25°C which is in room temperature compare to 38°C. For water treatment application using Pseudomonas sp., 25°C is recommended condition to be implemented.

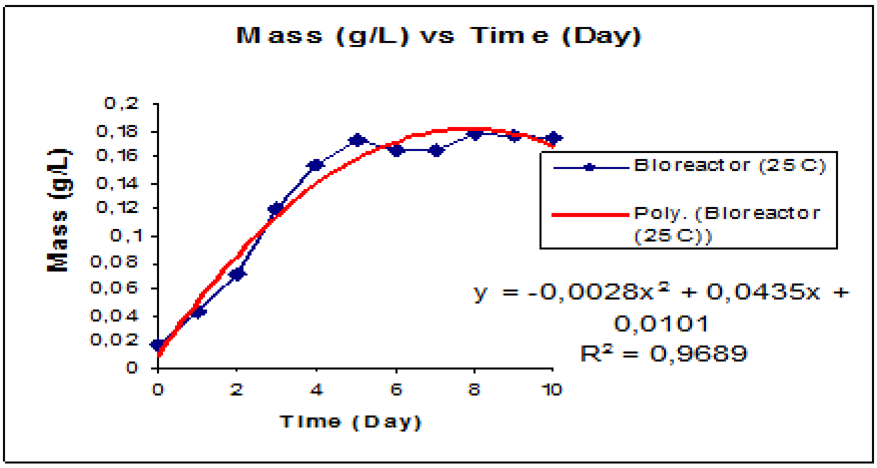

Figure 7: Entrobacter sp. (25°C) cubic graph

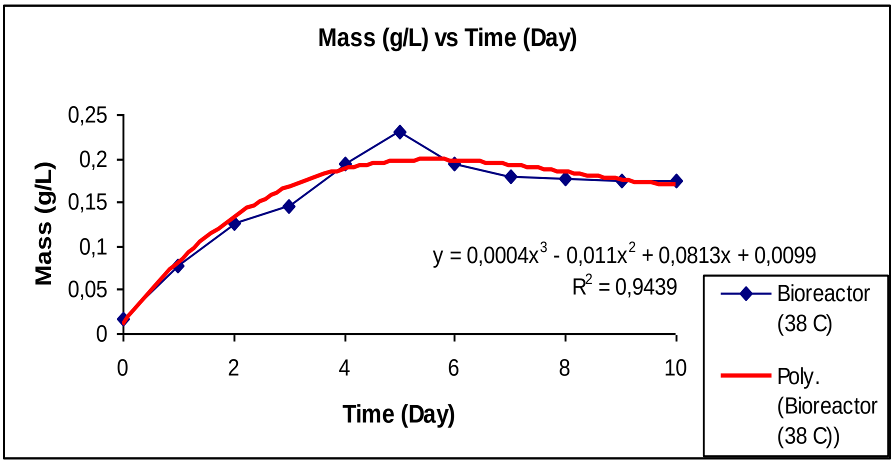

Figure 8: Entrobacter sp. (38°C) cubic graph

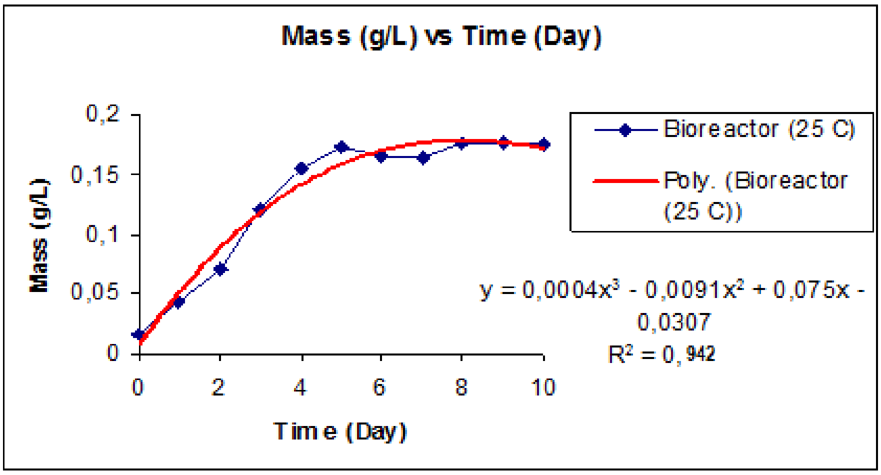

Figure 9: Pseudomonas sp. (25°C) cubic graph

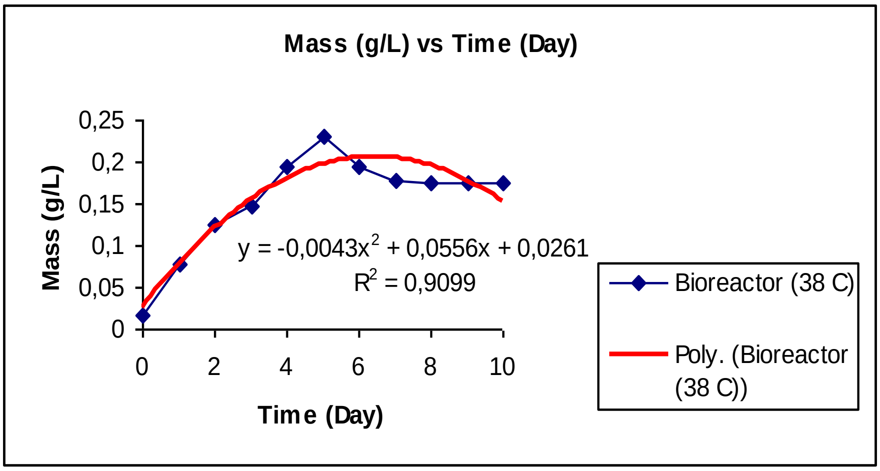

Figure 10: Pseudomonas sp. (38°C) cubic graph

5.0 Conclusion

The two test organisms Pseudomonas sp. and Entrobacter sp. had the capability to accumulate all the tested metal. Regarding on the results shows, Pseudomonas at 25°C temperature more effective for Fe removal, than 38°C. For Entrobacter sp. temperature 25°C more effective than 38°C. Temperature 25C for both bacteria was suggested as a suitable condition for Fe removal with 99.13% Pseudomonas p. and 98.57% for Entrobacter sp. While for Al, Entrobacter sp. was recommended at 25°C and 38°C for best of Aluminium removal. For day fist day to day 4. But the best removal was Pseudomonas sp. at 38°C with 89.17% day 1 to day 8. For Mn, the trend show Pseudomonas sp. and Entrobacter sp. at 38°C is recommended for Mangan (Mn) elimination in water treatment with about 66.88% and 60.81% reduction from day 2 to day 6. Lastly from observation Pseudomonas sp. in 25°C is recommended for Cadmium removal with 40% reduction. However the implementation in water treatment from the research showed it effective just after day 4 to day 8 in bioreactor.

For relationship between that three related parameters temperature (°C), pH and dissolved Oxygen (DO) was analyzed from the reading of parameters. DO and pH were strongly positively correlated revealing that organisms on this upwelling shelf not only exposed to low pH but also low DO (Frieder, 2012). That can be conclude that, Lower pH contributed to low dissolved oxygen (DO). Lack of dissolved oxygen was caused the stagnant or reduction of certain heavy metal removal. However in this research DO range 8.00 to 5.00 mg/L is seem to be practice for removal Ferum (Fe) from day 2 to day 8, Aluminium (Al) for day 2 to 6, Mangan (Mn) start day 2 to 6 and Cadmium (Cd) removal using Pseudomonas sp. only from day 4 to 8. By referring to the polynomial cubic graph and the standard deviation which is close to 1.00 showed that retention time for bioreactors at 25°C anaerobic and aerobic condition give the best growth curve for these two bacteria than in 38°C. The relations were proved by result stated temperature 25°C give the best removal of heavy metals compare to 38°C. It happened may cause of using synthetic water as a medium for metal removal. Thus, in general, it proved bacteria in different temperature can be used to accumulate heavy metals in water and can be determined as well as the bacteria growth, *k*. Hopefully, this study could be used as a guidance and alternative for biological water treatment to treat heavy metal contaminate.

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