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Transport of *Pseudomonas aeruginosa* via column study trough aquifer at RBF study site, Lubuk Buntar, Kedah, Malaysia

Miskiah Fadzilah Ghazali[1], Husnul Azan Tajarudin[1,2*], Mohd Nordin Adlan[3], Mohd Remy Rozainy Mohd Arif Zainol[3]

[1]School of Industrial Technology, Universiti Sains Malaysia, 11800, Penang, Malaysia
[2]Solid Waste Management Cluster, Science and Engineering Research Centre, Engineering Campus, Universiti Sains Malaysia, 14300 Nibong Tebal, Seberang Perai Selatan, Penang, Malaysia.
[3] School of Civil Engineering Universiti Sains Malaysia 14300 Nibong Tebal, Seberang Perai Selatan,Penang, Malaysia
Email: azan@usm.my

ABSTRACT

Aims: This study is focused to explore *Pseudomonas aeruginosa* strain mass transport and assessment of the water quality.
Methodology and results: *Pseudomonas aeruginosa* have been detected in the real location at the RBF study area by using molecular method. The bacterium strain was cultivated in the river water sample and column study was performed using soil aquifer collected from the RBF study site.
Conclusions, significance and impact of the study: The column experiments show the removal of the bacteria was achieved almost 99%. Concentration of cultured bacteria was detected in range 0.26 g/L to 0.33 g/L and reduced after flow through the soil passage in range 0 to 0.148 g/L. This study also examines the water qualities that affected during the transport such as turbidity and color. The range of turbidity for initial concentration for both influents was 84-96 NTU was reduced to range 2.88-49.29 NTU. Meanwhile for color, the initial concentration was 51-58 PtCo was reduced to range 1-18 PtCo.

Keywords: Colour, grain size distribution, pathogen, *Pseudomonas aeruginosa*, river bank filtration, turbidity

INTRODUCTION

Surface water has been used as a of drinking water resource for entire world. However, microbial contamination of surface water is a major environmental problem. Drinking water quality was getting worse and posing a great threat to human health. Usually, pollution of surface water consists of fertilizers, nutrients, pesticides and microorganisms. Microbial contamination is a main concern and the effect of water-borne pathogens in human health is expected to be substantial (Suresh and Smith, 2004).

Furthermore, the threat of waterborne diseases is excessive when pathogens are spread out via water supply systems. Although at small population level (less than one organism per liter of water) such pathogens can be dangerous to an at-risk segment of the population exclusively to a children and immune-compromised persons (Ray and Melin, 2003). The major indicator of the existence of pathogens in poor quality water is diarrhea. Annually it causes 1.8 million deaths (WHO, 2006).

The target of all water treatment technologies is to eliminate turbidity as well as chemical and microbial contaminants from water resources in the maximum affordable and appropriate way possible (Ray, 2008). In highlight of concern upon pathogenic microorganism in drinking water, riverbank filtration (RBF) is the one of the effective technique to supply water from river and groundwater together. RBF is a longstanding technology for treating surface water. Instead of obtaining water directly from a river or other surface water body and then treating it, surface water is extracted indirectly using wells located on the bank near the surface water body. It is usually utilized as an initial step of pre-treatment by drinking water companies producing drinking water from surface water during travel throughout the riverbed material to the production well (Ray *et al*., 2002; Schijven *et al*., 2002; Vishal *et al*., 2009).

RBF is a well-known water management process where bank sediments are applied as a pre-treatment option for extensively decreasing the amount of many ordinary microbial and chemical contaminants (Tufenkji *et al*., 2002). Most of RBF technology use alluvial sand and gravel aquifers with hydraulic conductivities larger than $1x10^{-4}$ m/s (2.6 ft/day) (Grischeck *et al*., 2002). Numerous studies have recognized the effectiveness of RBF in pathogen removal (Berger, 2002; Tufenkji *et al*.,2002; Gollnitz *et al*., 2003). Shamrukh and Abdel-Wahab (2008) evaluated the qualities of riverbank water in term of physical, chemical, and microbiological characteristics

***Corresponding author**

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with background groundwater and river water of Nile valley region in Upper Egypt. This study proved that the qualities of RBF water were superior to those of the other waters particularly in terms of *Escherichia coli,* total coliform and turbidity.

Transport of bacteria in soil and other consolidated or unconsolidated material is greatly controls by the materials properties. Survival of pathogenic bacteria in soil environment and marine as well as in other unconsolidated material, and even inside aquifer matrices is influenced by temperature, humidity, pH, the amounts of organic matter in soil and aquifer material, rainfall, sunlight, and competitive microorganisms (Dash *et al*., 2010).

Several field studies were conducted and verified either elimination of aboriginal microorganisms or lab-cultured seeded microorganisms (Medema *et al*., 2000; Schijven *et al*., 2002; Schijven *et al*., 2003; Hijnen and Brouwer-Hanzens, 2006). The movements of bacteria are restricted and mostly are removed when passing through in fine sand. The process happened at the beginning infiltration-many even within the first 0.5 m (Dash *et al*., 2010). Pang *et al*. (1998) reported that in sandy gravel, the discovery of *Bacillus subtilis* endospores after passage of 90m from an injection well. Sinton *et al*. (1997) stated that *E. coli J6-2* was retrieved in a gravel aquifer at 401 m from an injection well. These data propose that some coarse-grained aquifers could afford high amount of water yields, however possibly has low effectiveness in removing bacteria by RBF when fine-grained river bed sediments are not present.

These findings presented migration of bacteria through soil poses a very valuable barrier to microorganisms, but acute circumstances could happen such as intrusion of contaminants to unconfined aquifers above groundwater wells, water abstraction during RBF from a gravel aquifer, with increased risk during high flow events, or short circuiting during recollection. Field studies were helpful but hindered by some weaknesses. Normally, concentrations of pathogenic bacteria were very low in the field to evaluate removal and non-hazardous model microorganisms only such as *E. coli,* bacteriophages and *clostridia* spores could be applied in spiking research. Consequently, the impact of specifics situations (e.g. soil characteristics, water velocity, water quality variations) is hard to measure under field conditions. Column-based studies with injected microorganism are helpful to overcome all those drawbacks.

To classify protection zones nearby the abstraction well and self-setback distances for the RBF system in water treatment, quantitative data of microorganism removal during the soil passage is required. Accordingly, quantitative evaluation for microbial transport in various soils could assist to assess the risk of riverbank filtered water contamination. Thus, the objectives of this study were to investigate the mass transport of bacteria and retention in two different soils at the zone around of abstraction well as well as water quality of the filtered water sample through column experiments. The soil samples were collected at the screen layer of the test well that was constructed at the RBF study site.

MATERIALS AND METHODS

Study area

The site was located in Lubuk Buntar area, District of Bandar Baharu, Kedah Darul Aman with longitude and latitude of 5° 7'37.60"N, 100°35'42.97"E as shown in Figure 1. The area was near to raw water intake of SADA (Syarikat Air Darul Aman) water treatment plant at Kerian River. Kerian River is the main river at the study area which is the border between Kedah and Perak. The upper stream of Kerian River is Selama and the downstream is Nibong Tebal. Nur Aziemah *et al*. (2015) stated that, the upper stream of Kerian River will face an increase of annual rainfall, while the downstream of the river will face a decrease of annual rainfall from 2011 until 2099. Due to that, the site has experienced flooding for several times. Kerian River has been classified as river class II and III, which indicated that extensive treatment is needed in order to make the water supply using water from this river. Alternatively, RBF is a one of the potential initiatives to be initiated as an additional source to meet the high supply demand in the region.

Drilling, construction of wells and soil sampling

The drilling of the exploration test well (PW) was carried out using Truck Mounted Rig with mud rotary drilling technique and drilling with 20 inches diameter craw bit. The construction of the exploration alluvium tube well consists of installation of 10-inch stainless steel screen from 24 to 30 m, 1 m sand trap from 30 to 31 m and 10 inch diameter PVC blank from ground level to 24 m followed by 1 m top of well with steel wellhead protector. The develop 10 inch tube well also consist of 1 installation of 2 inch diameter PVC monitoring well from ground level up to 30m with screen from 24 to 30 m. During the drilling, samples were collected and described for every meter depth of the borehole and placed in the plastic bag and were then transported to labs. These soil samples were then dried for analysis. This study only focused on the soil samples from PW at the screen layer. There were two layers of soils which at depth 23-29 m and 29-33 m. Figure 2 illustrated the drilling log and the design of the test well.

Sieve analysis of soil samples

Sieve analyses carried out on the soil samples are purposely to justify on the geological bore log results and also to indicate on the proportion of the soil sizes

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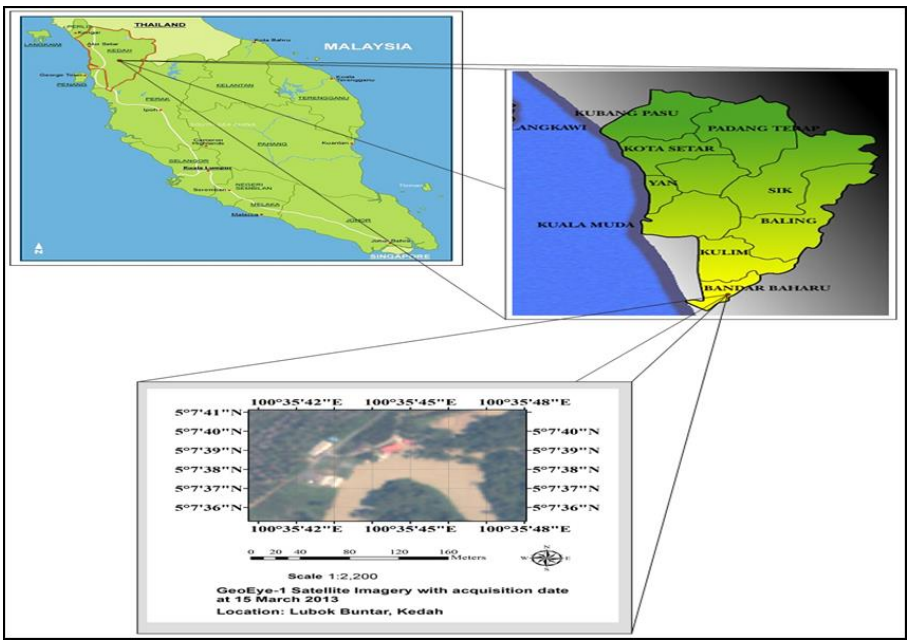

Figure 1: Location of the RBF study area.

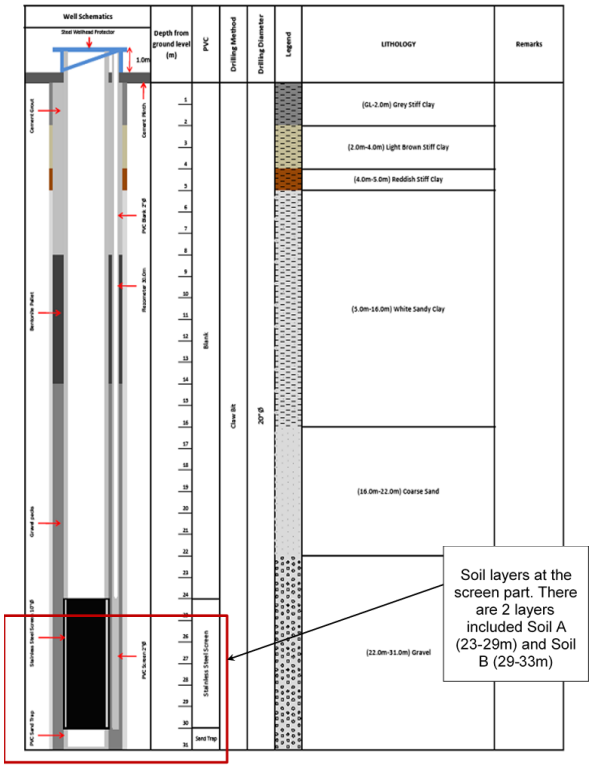

Figure 2: Drilling log and well design of the test well (PW).

particular formed from the constructed wells. The sieving process is applied to specify the percentage of coarse-size particles contained in the soil samples, while the hydrometer method is purposely to identify the percentage of lower size or finer particles. This process was performed based on British Standard 1377: Part 2:1990. The initial mass of the soil in the container was measured and then it was soaked in distilled water for 24 h. After that, the soil was washed using 63 µm sieve size until the water was clear. The remaining soil left on the sieve was put into the oven for 24 h to dry the sample. The dried soil was weighed again and started with sieve analysis. The sieves were fixed in the order of 14 mm, 10 mm, 6.3 mm, 5.0 mm, 3.35 mm, 2.0 mm, 1.18 mm, 600 µm, 425 µm, 300 µm, 212 µm, 150 µm and 63 µm. Retained sample from each sieve were weighted upon completing the shaking process in 10 min. The final undersize soil (63µm) was collected and was proceeded for hydrometer test. The hydrometer test was conducted using the measuring cylinder (1000 ml) and sodium silicate (Na_2SiO_3) as a dispersing agent. The test was performed using British Standard 377: Part 2:1990:9.6.

Isolation of pathogenic bacteria

River water sample was collected at Sungai Kerian (Kerian River) which is the location of RBF study area. Pathogenic bacterium was isolated from river a water sample that has been diluted serially using the streaking technique. The media used to culture the bacteria was chocolate agar and cultured for 24 to 48 h at 37 °C. All steps were done aseptically to avoid any contamination. Only single colony of bacterium was selected and preceded for the next step. The selected colony was streaked on a fresh nutrient agar (Sigma-Aldrich). Sub-culturing was done many times to achieve pure culture. Gram staining method was done to classified bacteria into Gram-positive and Gram-negative bacteria and was

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observed its cell morphology under microscope observation. For identification of bacteria isolated, GF-1 Bacteria DNA Extraction Kit was used to obtain pure DNA samples and purity of the DNA was determined using the Nanodrop 2000 Spectrophotometer.

Molecular identification

Molecular analysis was carried out by Centre for Chemical Biology, Penang, Malaysia (CCB. The 16s rRNA gene was amplified by PCR using universal primers 16S-27F and 16S-1492R. PCR was conducted under 94 °C (3 min), 30 cycles of 94 °C (30 sec), 55 °C (30 s) and 72 °C (1.4 min) and a cycle of final extension at 72 °C (5 min) conditions. The PCR product was subjected to electrophoresis. The required DNA fragment that contained in the gel band was then extract out and purified for cloning and sequencing process. BLAST analysis was applied to analyze the obtained sequences. BLAST analysis was provided by National Centre for Biotechnology Information (NCBI), (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Column experiment

The identified bacterial strain from the molecular identification in the late exponential growth phase was used in the column experiment. The strain was cultivated at 37 °C in LB broth that was shaken in an incubator shaker at 150 rpm. The inoculum was harvested from the LB broth and cultured in the sterilized river water sample at the study site. 10% of the inoculum was scale up until 20 L in a vessel. Two sets of column experiments were performed using stainless steel column with length 60 cm and inner diameter 75 cm. Column inlet and outlet were replaced by set of screens (103 µm mesh spacing) to distribute the influent solution at the soil surface homogeneously. The column was fitted with tubing and other ancillary components such as column O-rings, and flow adapter. These components were made from the chemical and heat resistance materials such as Teflon and stainless steel.

The columns were packed with the Soil A and Soil B and prior to experiment all porous media were sterilized in autoclaved at 121 °C for 30 min. From the respective culture vessel, the columns were fixed with a peristaltic pump then the cultured bacteria strain was pumped upward throughout the columns with a constant flow rate vertically. The suspension was injected upward to diminish any sedimentation that can happen as a result of the dissimilar in density among the bacteria strain and eluent. The flow rate of the columns was 95 mL/sec which is according to the discharge rate of the test well (PW) at the RBF study site. Figure 3 illustrates the schematic diagram of the column experiment set up. The concentration of the cultured bacteria cell was estimated by a calibration between optical density (OD) at 600nm and evaluated concentration cell prior the experiment. The effluents from the column experiment were collected every 15 min at first hour and every 30 min for the next hours. Samples for optical density measurement were collected in glass test tube (cuvette) and were measure using DR2800 spectrophotometer over the course of each column experiment. For turbidity and color were tested in accordance to APHA 2005.

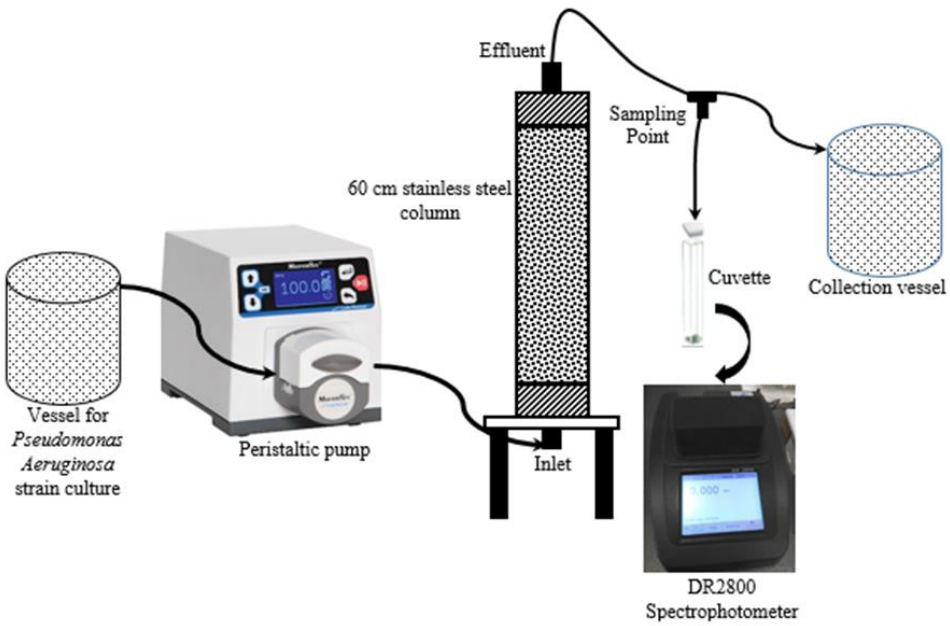

Figure 3: Schematic diagram of column experiment.

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RESULTS AND DISCUSSION

Grain size distribution of soil samples

From mechanical sieve analysis and the hydrometer test could be stated in terms of the soil particles weight percentage to the overall weight of the soil sample. Table 1 lists the the classification of soil for the test well (PW) at the depths of 23-29 m and 29-33 m which were at the screen part. Table 1 show that Soil A (23-29 m) has a proportion of fine gravel, coarse sand, medium sand and fine sand as 19%, 59%, 11.5% and 3.6% respectively. For proportion of coarse silt, medium silt, fine silt and clay for Soil A as 2.9%, 1.7%, 2.5% and 0% respectively. While Soil B (29-33 m) has a proportion of fine gravel, coarse sand, medium sand and fine sand as 25.6%, 46.1%, 20.2% and 2.0% respectively. For proportion of coarse silt, medium silt, fine silt and clay for Soil B as 1.1%, 0.8%, 4.1% and 0% respectively. The results show that Soil A has lower percentage of gravel and sand than Soil B but higher content of silt. Sieves analysis results are shown in Table 2. A type of soil for Soil A is poorly sorted gravelly sand low in fines and for Soil B is moderately well sorted gravelly sand low in fines.

Identification of bacteria

From the DNA sequences, it was identified that bacteria strain isolated from raw water sample was *Pseudomonas Aeruginosa* strain DSM 50071. Based on the morphology, the organism was a Gram-negative bacterium, one or more polar flagella providing motility, aerobic, non-spore-pore forming, catalase positive and oxidase positive. *P. aeruginosa* is an opportunistic pathogen. Where, this bacterium would manipulate to breaks into host defence to start the infection. It has become known as an immerging opportunistic pathogen of clinical relevance. It causes the urinary tract infection, respiratory system infection, dermatitis, soft tissue infection, bacteraemia, bone and joint infection gastrointestinal infection and a various of systemic infection especially for the patient with a high degree of burn, in cancer and AIDS patient who are immunocompromised (Ashish *et al*., 2011). Besides, it might become a minor source of problem which related to the physical characteristics such as colour, taste, odour, and turbidity of the water. The major anxiety was about the biological slime they develop. This is because it also had the ability to be a shelter for other disease-causing bacteria such as coliforms (Hardalo and Edberg, 1997). Because this type of bacteria exists in the RBF study area, hence, the transport of bacteria travel through soil passage need to be studied.

Bacteria mass transport

Prior to the column experiment running, the concentration of the bacteria cells in the feed water were 0.33mg/L and 0.26 mg/L in vessel for Soil A and vessel for Soil B respectively. Figure 4 shows the result of biomass removal during the transport experiments. Biomass of bacteria cell was decreased at 0 mg/L for Soil A and started to increase after 60 min time. Meanwhile for Soil B the biomass of bacteria cell was decreased to 0 mg/L and started to increase at 90 min time. It shows that the foul point of Soil B occurred later than Soil A. the results show the removal of bacteria cell almost 99% in both soil column. Attachment and retaining mechanisms cause the retaining of bacteria in saturated soil. In numerous researches stated that attachment is the major mechanism cause of bacteria retaining in porous media (Ahmad Farrokhian *et al*., 2015). Attachment of bacteria to soil matrix is caused by the characteristics of the solution, porous media and bacteria involved in the transport process. They are including pH and ionic strength of the solution, surface charge and the grain size of the porous media, characteristics of bacteria itself (Song-Bae *et al*., 2008).

Table 1: Soil classification at the Test Well (PW).

Soil	Depth (m)	Soil Types (%sample)									
		Gravel			Sand			Silt			Clay
		Coarse	Med	Fine	Coarse	Med	Fine	Coarse	Med	Fine	
Soil A	23-29m	0	0	19.0	59.0	11.5	3.6	2.9	1.7	2.5	0.0
Soil B	29-33m	0	0	25.6	46.1	20.2	2.0	1.1	0.8	4.1	0.0

Table 2: Sieve analyses results for soil samples within PW.

Depth (m)	[a]D_{10} (mm)	[b]D_{30} (mm)	[c]D_{60} (mm)	C_u	C_c	Types of samples
23-29m	0.267	1.104	1.357	5.09	2.9	Poorly sorted gravelly sand low in fines
29-33m	0.371	1.211	1.534	4.14	2.12	Moderately well sorted gravelly sand low in fines

[a,b,c] Characteristics grain (D^{10},D^{30},D^{60}) were determined from the grain size distribution
C_u Coefficient of uniformity
Cc Coefficient of gradation

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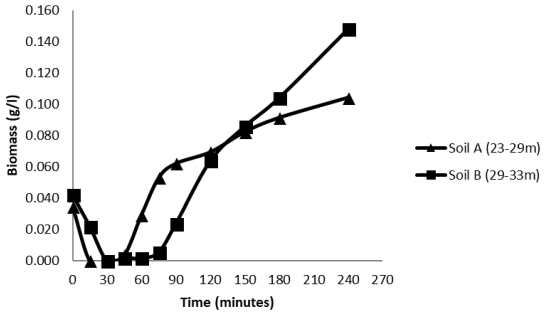

Figure 4: Biomass of *Pseudomonas aeruginosa* through column experiment.

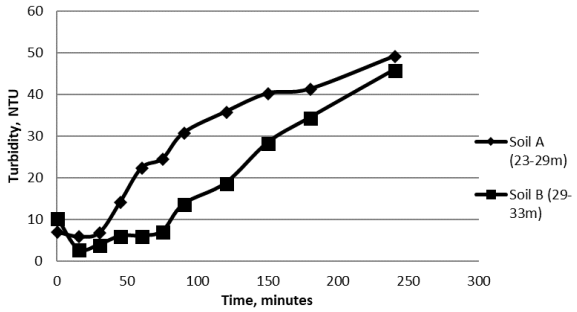

Figure 5: Removal of turbidity from column experiment.

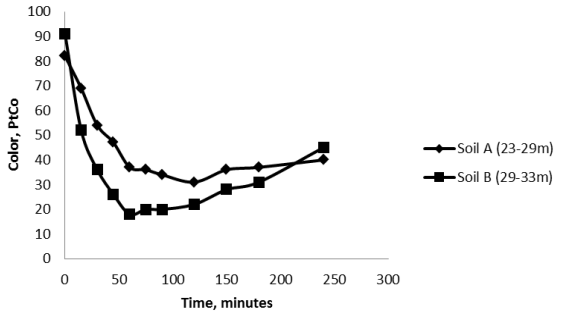

Figure 6: Removal of color from column experiment.

Turbidity and colour

Particle measured which known as turbidity is a standard universal water quality parameter for most of surface waters to measure the water quality. Turbidity is a guide of the environmental health of water bodies even though it is not an inherent property of water such as temperature and pH (Davies-Colley and Smith, 2001). This parameter is essential parameter of water quality indicator where bacteria, viruses and parasites could attach their body to the suspended particles in turbid water. While, colour is a water quality parameter which is solution that form from dissolved organic material.

Both turbidity and colour are water quality parameters that that detract from the appearance of water making it not suitable to drink because of the aesthetic reasons. *Pseudomonas aeruginosa* can cause problems with colour, taste, odour and turbidity if found in high numbers. Hence, the effluents from the column experiment were tested for turbidity and colour. The results show that the range of turbidity for initial concentration for both influents was 84-96 NTU was reduced to range 2.88-49.29 NTU. Meanwhile for colour, the initial concentration was 51-58 PtCo was reduced to range 1-18 PtCo. Figures 5 and 6 show the removal of turbidity and colour respectively. The results indicate that turbidity and colour decrease when the biomass of bacteria is reduced. Turbidity could be as indicator for the presence of microbes. If the turbidity has the excessive value, it could disturb the disinfection process and it is affordable for the growth of microbial. While, the colour of water, whether as a result of dissolved compounds or suspended particles, might influence the turbidity measurement (Shamsuddin *et al*., 2014).

CONCLUSION

The study investigates the mass transport of Pseudomonas aeruginosa bacteria cell that was isolated from river water at RBF designated study area. Column experiments were conducted for two types of soil at the screen part of the test well (PW). Soil A has lower percentage of gravel and sand than Soil B but higher content of silt. The column experiment shows that the transport of bacteria cell for Soil A foul point was occurred faster than Soil B. A further study is warranted to better measure and investigates the affect factor of straining deposition and mobilization of the bacteria transport. This knowledge is assumed to be necessary for predicting the fate and transport of pathogenic bacteria trough soil passage.

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