MASS TRANSPORT OF *Pseudomonas aeruginosa* THROUGH NATURAL ALLUVIAL AQUIFER MEDIA FROM COLUMN STUDY

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ABSTRACT

Riverbank filtration (RBF) is a widely practiced as a first-step pretreatment of surface water which employs bank and adjacent aquifer sediments to reduce concentrations of microbial pathogen and chemical contaminants in a cost-effective manner. To define protection zones around the abstraction well and self-setback distances for the RBF system in water treatment, quantitative information is needed about the removal of microorganisms during soil passage. Pseudomonas aeruginosa have been detected in the real location at the RBF study area using molecular method. The bacteria strain was cultivated in the river water sample and column experiments were conducted using natural soil aquifer collected from the RBF study site. The aim of the study was to investigate the mass transport of Pseudomonas aeruginosa strain and assessment of the water quality. The column experiments show that 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: Color; grain size distribution; pathogen; *Pseudomonas aeruginosa*; river bank filtration; turbidity.

INTRODUCTION

The sustainable development of humanity depends on our ability to bring mankind into a lasting equilibrium with nature. However, one third of the world's population live in countries with some level of water stress and water scarcity is expected to increase in the next few years due to increases in human population, per capita consumption and the resulting impacts of human activity on the environment [1]. Surface water has been used as a source of drinking water all over the world. However, microbial contamination of surface water is a wide-spread environmental problem, deteriorating drinking water quality and posing a great threat to human health. Usually, pollution of surface water consists of fertilizers, nutrients, pesticides and microorganisms. Microbial contamination is a major concern and the impact of water-borne pathogens in human health is expected to be significant [2].

Moreover, the risk of waterborne diseases is high when pathogens are spread through water supply systems. Even at low population levels (less than 1 organism per liter of water) such pathogens can be harmful to an at-risk segment of the population especially to a children and immune-compromised individuals [3]. The main symptom of the presence of pathogens in poor quality water is diarrhea. It causes 1.8 million deaths every year [4]. Microorganism pollution involves diverse groups of pathogens that can have different and complex survival mechanisms, which usually helps extend their presence and propagation in the environment.

As the demand for piped drinking water is increasing especially in developing countries, water utilities are facing the challenge of treating surface water that is often polluted. The aim of all water treatment technologies is to remove turbidity as well as chemical and microbial contaminants from water sources in the most affordable and expedient manner possible [5]. It is therefore critical to understand the relevance of natural and drinking water contribution to transmission of pathogenic microorganisms. In highlight of concern over pathogenic microorganism in drinking water, riverbank filtration (RBF) is the one of the best method to provide water from both river and groundwater. RBF, which is also referred to as bank filtration, is an old technology for treating surface water. Rather than obtaining water directly from a river or other surface water body and then treating it, surface water is drawn indirectly using wells located on land near the surface water body. It is typically used as a first step pre-treatment by drinking water companies preparing drinking water from surface water during passage through riverbed material to a production well [6-8].

RBF is a widespread water management operation where bank sediments are used as a pretreatment option for substantively reducing the quantity of many common microbial and chemical contaminants [9]. Most RBF systems use alluvial sand and gravel aquifers with hydraulic conductivities greater than $1x10^{-4}$ m/s (2.6 ft/day) [10]. Several studies have acknowledged the effectiveness of RBF in pathogen removal [9,11,12,13]. These studies were obtained from the field monitoring. Shamrukh and Abdel-Wahab (2008) compared the physical, chemical, and microbiological qualities of RBW with river water and background natural groundwater in a Nile valley region of Upper Egypt. They demonstrated that the RBF water qualities were superior to those of the other waters, especially in terms of turbidity, total coliform, and *Escherichia coli*.

Bacterial transport in soil and other consolidated or consolidated material depend strongly on material properties. Temperature, humidity, pH, the amounts of organic matter in soil and aquifer material, rainfall, sunlight, and competitive microorganisms will affect pathogenic survival in aquatic and soil environments as well as in other unconsolidated material, and even within aquifer matrices [15]. A number of field studies have been carried out that established either removal indigenous microorganisms or lab-cultured seeded microorganisms [16-19]. In fine sand, the migration of bacteria is limited and most bacteria are removed at the beginning infiltration-many even within the first 0.5m [15]. Pang et al. (1998) reported that in sandy gravel, the detection of *Bacillus subtilis* endospores after transport of 90m from an injection well. Sinton et al.

(1997) reported that *E. Coli J6-2* was recovered in a gravel aquifer at 401m from an injection well. These data suggest that some coarse-grained aquifers may provide high water yields, but may have low efficiency in removing bacteria by RBF when fine-grained river bed sediments are absent. Under steady-state conditions, RBF with an average residence time of 2 weeks showed 5 log removals for pathogenic microorganism surrogates, bacteria and bacteriophages.

These studies showed that soil passage poses a very effective barrier to microorganisms, but critical situations may arise 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 are valuable but hampered by some drawbacks. The concentration of pathogens in the field is generally too low to assess removal, and only nonhazardous model microorganisms such as *E.coli*, bacteriophages and *clostridia* spores can be used in spiking studies. Hence, the effect of specifics conditions (e.g. soil characteristics, water velocity, water quality variations) is difficult to assess under field conditions. Column-based studies with spiked microorganism can be overcome all those disadvantage.

To define protection zones around the abstraction well and self-setback distances for the RBF system in water treatment, quantitative information is needed about the removal of microorganisms during soil passage. Therefore, quantitative assessment of microbial transport in different soil types would help to evaluate 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 study site is located in Lubuk Buntar, 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. Ismail et al. (2014) 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. This study site was chosen due to the high water demand in the area and the river has high pollution level. 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 and RBF, is seen as an alternative source with very high potential to be developed as a supplementary source to meet the high public water supply demand.

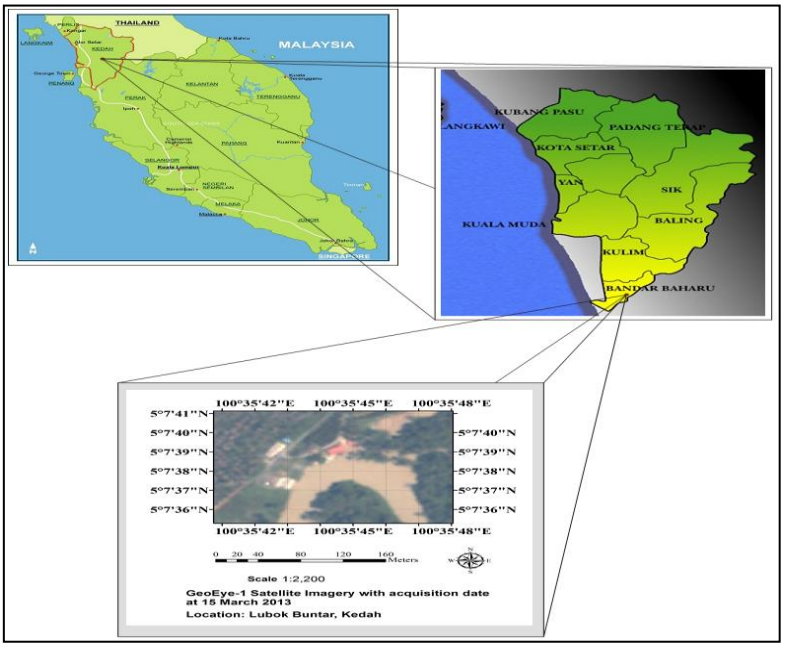

Figure 1. Location of the RBF study area

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 consist 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 meter 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 30 m 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 23-29m and 29-33m. Figure 2 shows the drilling log and design of the test well.

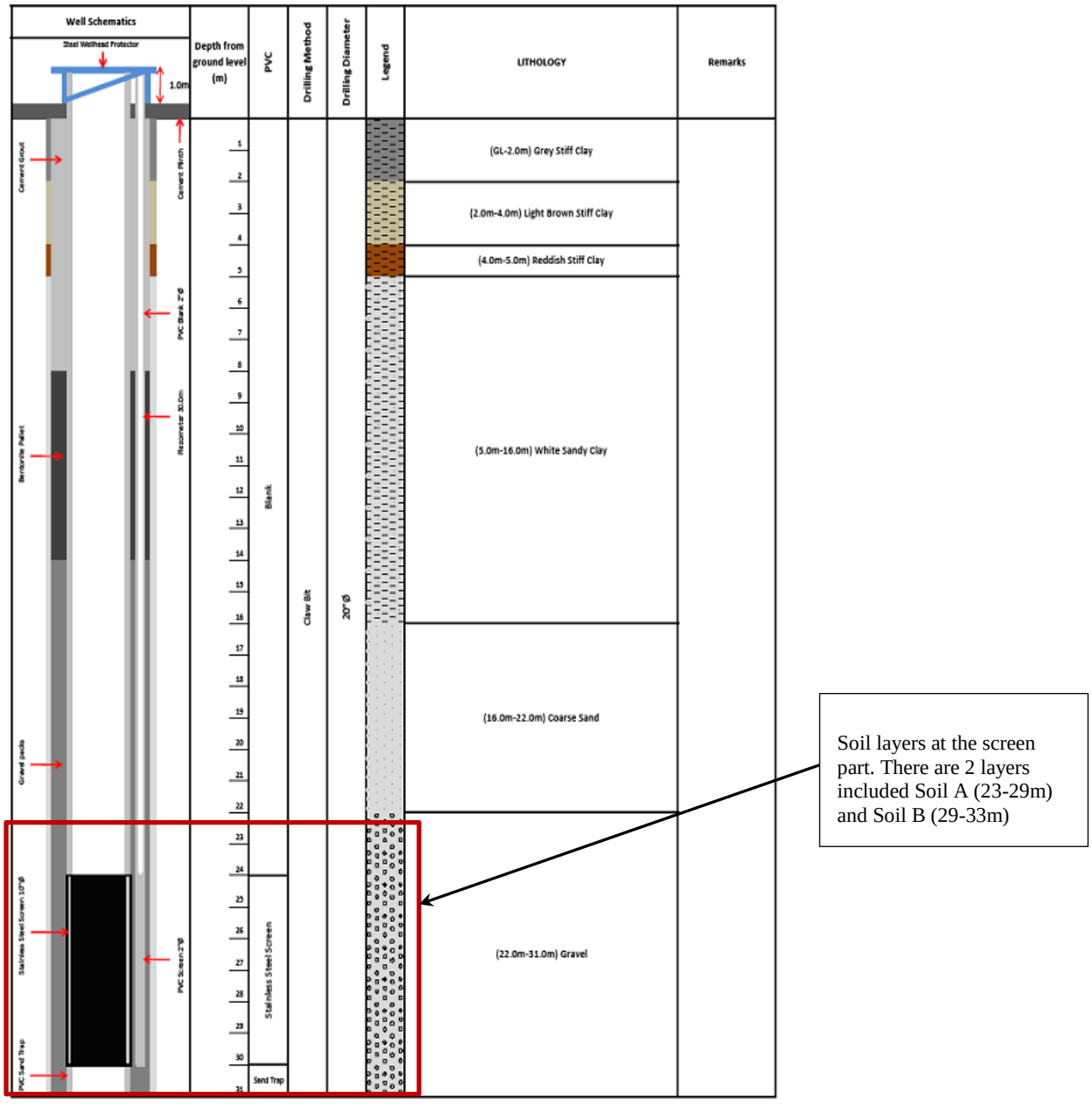

Figure 2. Drilling log and well design of the test well (PW)

Sieve Analysis of Soil Samples

Sieve analyses of the soil material were performed to support the results of geological bore logs and also to determine the percentage of different grain sizes contained from the wells. The mechanical or sieve analysis is performed to determine the distribution of the coarser, larger-sized particles, while the hydrometer method is used to determine the distribution of the finer particles. This analysis 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 hours. After that, the soil was washed using 63 µm sieve size until the water was clear. The remaining soil on that sieve was dried in the oven for 24 hours. 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. The weight of the soil that was retained on each sieve was measured after completing the shaking process in 10 minutes. The soil that passed through the finest sieve (63µm) was collected and undergone for hydrometer test. The hydrometer test was conducted using the measuring cylinder (1000ml) 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 hours at 37ºC. 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 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 (5' AGAGTTTGATCMTGGCTCAG 3') and 16S-1429R (5' TACGGYTACCTTGTTACGACTT3'). PCR was carried out under the following conditions: 94°C (3 min), 30 cycles of 94°C (30 s), 55°C (30 s) and 72°C (1.4 min) and a cycle of final extension at 72°C (5 min). All the PCR products were subjected to electrophoresis and the gel band containing desired DNA fragment was then excised and purified for cloning and sequencing purpose. The obtained sequences were analyzed using BLAST analysis 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 stationary growth phase was used in the column experiment. The bacterial strain was cultivated at 37°C in a LB broth that was agitated at 150 rpm using an incubator shaker. 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 20L in a vessel. Two sets of column experiments were performed using stainless steel column with length 60 cm and inner diameter 75 cm. At the column inlet and outlet was replaced with a screen (103 µm mesh spacing) to uniformly distribute the influent solution at the soil surface. The stainless steel column were equipped with tubing to and from the columns, fitting, column O-rings, and flow adapter were composed of chemically and heat resistant 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 minutes. From the respective culture vessel the columns were connected with a peristaltic pump and the cultured bacteria strain was pump upward through the vertically oriented columns with a constant flow rate. The suspension was pumped upward to minimize any sedimentation that may occur as a result of the difference in density between the bacteria strain and eluent. The flow rate of the columns was 95ml/sec which is according to the discharge rate of the test well (PW) at the RBF study site. Figure 3 shows the schematic diagram of the column experiment set up. The concentration of the cultured bacteria cell was estimated using a calibration between optical density at 600nm and measured concentration cell prior the experiment. The effluents from the column experiment were collected every 15 minutes at first hour and every 30 minutes 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.

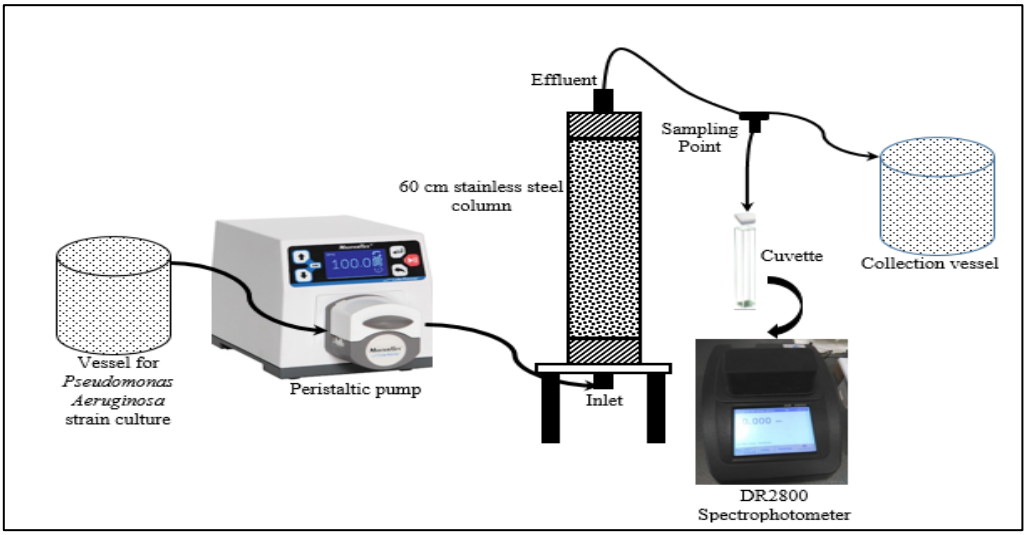

Figure 3. Schematic diagram of column experiment

RESULTS AND DISCUSSION

Grain Size Analysis of Soil Samples

The results of the mechanical sieve analysis and the hydrometer test can be expressed in terms of the weight percentage of the soil particles to the total weight of the soil sample. Table 1 show the classification of soil for the test well (PW) at the depths of 23-29m and 29-33m which were at the screen part. Table 1 show that Soil A (23-29m) 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 (29-33m) 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.

Table 2 shows the sieve analyses results for both soil samples. 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.

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

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, meaning that it exploits break in host defense to initiate an infection. It has become increasingly recognized as an immerging opportunistic pathogen of clinical relevance. It causes urinary tract infection, respiratory system infection, dermatitis, soft tissue infection, bacteremia, bone and joint infection gastrointestinal infection and a variety of systemic infection particularly in a patient with a severe burn, in cancer and AIDS patient who are immunocompromised [22]. These bacteria might cause minor problems associated with color, taste, odor, and turbidity of the water. The main concern was related to the biological slime they form. This slime, also had the ability to harbor other disease-causing bacteria such as coliforms [23]. 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 0.26mg/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 minutes time. Meanwhile for Soil B the biomass of bacteria cell was decreased to 0 mg/l and started to increase at 90 minutes 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. Bacteria retention in saturated soil occurs by attachment and straining mechanisms. In many experiments it has been observed that attachment is important mechanism influencing bacterial retention in porous media [24]. The bacteria attachment to a solid matrix is influenced by solution chemistry (ionic strength and pH), properties of porous media (surface charge and grain size) and characteristics of bacteria [25].

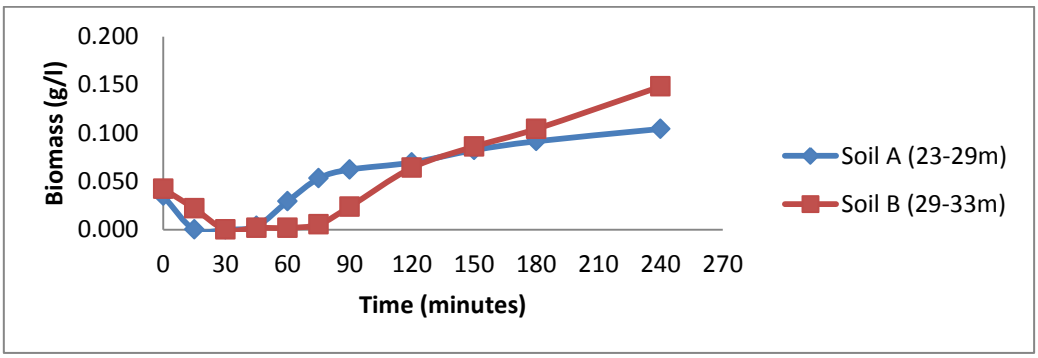

Figure 4. Biomass of *Pseudomonas aeruginosa* through column experiment

Turbidity and Color

Particles measured as turbidity is a typical general water quality parameter for most surface waters and is a useful measurement tool for water quality analyses. Although turbidity is not an inherent property of water, as is temperature or pH, the recognition of turbidity is an indicator of the environmental health of water bodies [26]. Turbidity is an important water quality indicator because Bacteria, viruses and parasites can attach themselves to the suspended particles in turbid water. Color is organic material that has dissolved into solution. Turbidity and color are two water quality parameters that detract from the appearance of water making it unpleasing to drink for aesthetic reasons. *Pseudomonas aeruginosa* can cause

problems with color, taste, odor and turbidity if found in high numbers. Hence, the effluents from the column experiment were tested for turbidity and color. 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 color, the initial concentration was 51-58 PtCo was reduced to range 1-18 PtCo. Figure 5 and Figure 6 show the removal of turbidity and color respectively. The results indicate that turbidity and color decrease when the biomass of bacteria is reduced. A high turbidity can interfere with the disinfection process and can provide a medium for microbial growth. It may also indicate the presence of microbes. While, the color of water, whether as a result of dissolved compounds or suspended particles, could affect the turbidity measurement [27].

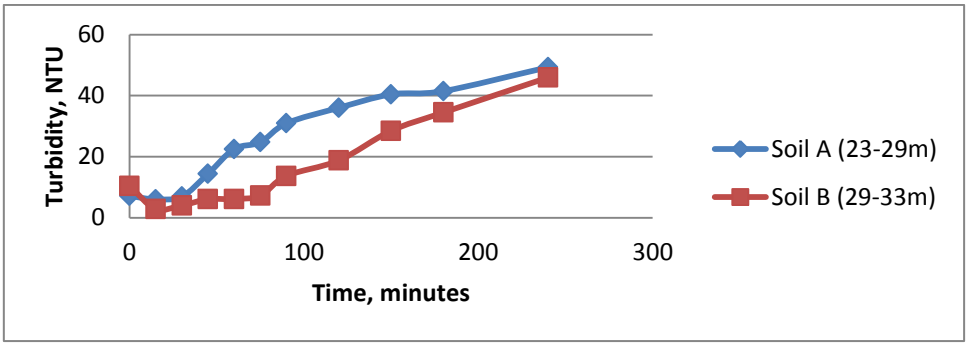

Figure 5. Removal of turbidity from column experiment

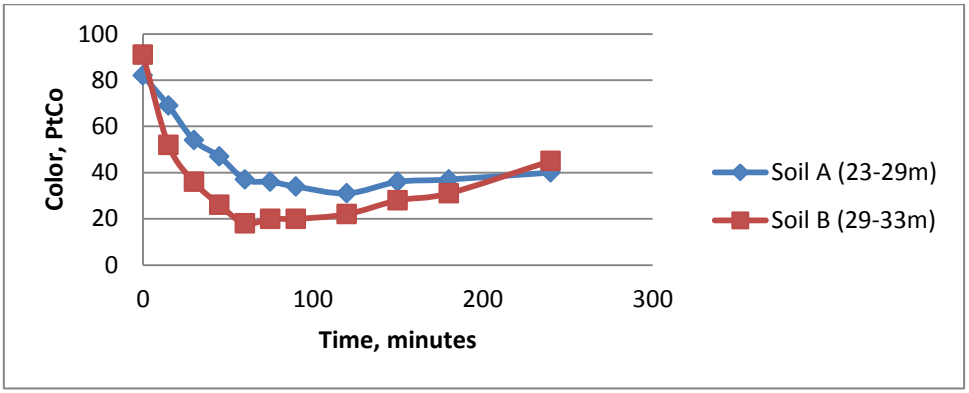

Figure 6. Removal of color from column experiment

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. Additional research is warranted to better quantify and investigate the influence factor of straining deposition and mobilization of the bacteria transport. This information is believed to be essential for predicting the fate and transport of pathogenic bacteria trough soil passage.

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