

Removal Efficiency of Total Petroleum Hydrocarbons from Water by *Pseudomonas aeruginosa* A Case of Lake Albert, Uganda

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Abstract

Oil exploration and production has recently intensified in the Albertine Graben of Uganda with no emphasis placed on remediation methods to combat the spill that will happen. The current study was done to explore the extent to which *Pseudomonas aeruginosa* can remediate petroleum hydrocarbon (PH) contaminants of Lake Albert water in western Uganda. 2 liters of water of Lake Albert were collected and kept in sterile flask. The water was placed on Ice in a cooler box and transported to Makerere University Chemistry pesticide laboratory. The sample was then refrigerated for 10 hours. Ten percent (10% m/v) (100 g/L) contamination was intentionally made for this Lake Albert water sample using selected various amounts of PHs of known amounts. A sample of petroleum hydrocarbon contaminated water (10 ml), was quantitatively placed in a 250 ml flask containing a nutrient broth (100 mL). An aliquot of *Pseudomonas aeruginosa* starter culture (3.0×10^7 CFU/mL) was introduced. Amount of PHs left after the remediation was quantified using GC-MS unresolved complex mixtures approach. *Pseudomonas aeruginosa* significantly ($p < 0.05$) remediates the petroleum hydrocarbon from lake albert water with the highest removal efficiency registered at the end of the sixth day of the remediation process and this is 89.3% of *n*-hexane soluble PH. At the end of 6 days, the 100 g/L of PHs that was introduced on water was reduced to 10.7 g/L (89.3%) after inoculating with 3.0×10^7 CFU/mL at the beginning of the experiment. The control experiment also showed presence of PHs ranging from 9-32 g/L.

In conclusion, the Lake Albert water is already contaminated with PHs. However, *Pseudomonas aeruginosa* can remediate petroleum hydrocarbon (PH) contaminants significantly.

Keywords: Bioremediation; Lake Albert; Petroleum hydrocarbons; *Pseudomonas aeruginosa*; Removal efficiency

Introduction

Much as oil exploration and production has reached the final stages in the Albertine Graben of Uganda, no effort is put to remediation methods to fight the spill that may occur during extraction and production. One of such remediation approaches is by use of bioremediation. This is a restoration intervention method that uses microorganisms to degrade hazardous organic contaminants to environmentally less toxic compounds [1]. Such microorganisms include bacteria- *Pseudomonas aeruginosa*, and fungi [2]. *Pseudomonas aeruginosa* has long been considered as one of the predominant petroleum hydrocarbon degrading agents found in the environment, which are free living, meaning its use on water has no health effect since water is boiled before it is taken. The petroleum hydrocarbons are increasingly becoming pollutants of major concern within the environment [3] and some components of petroleum have the potential to bioaccumulate within susceptible aquatic organisms and can be passed by trophic transfer to other levels of the food chain [4]. Spills of petroleum Hydrocarbons that occur in aquatic environment are usually far further harmful than those that occur on land [5]. The emergency of oil exploration industry in Uganda makes it susceptible to petroleum related environmental challenges including spillage. Unfortunately, there are no well-documented environmentally friendly methods for remediation of petroleum hydrocarbons spillage in water bodies within the country. The study aimed at exploring the use of a pure culture of *Pseudomonas aeruginosa* in controlling such a case of petroleum hydrocarbons spillage into Lake Albert water.

Materials and Methods

Sampling of lake Albert water

Samples of Lake Albert water was collected in four quarter litre

sterile sample bottles from four points on Lake Albert near the oil exploration premise. The water collected was mixed into a 2 liter sterile sample bottle. This was made to a total of 2 liter sample of Lake Albert water. 2 liter samples was then kept in sterile flask and placed on Ice in a cooler container and brought to Makerere University Chemistry pesticide laboratory. In the laboratory, the sample was refrigerated for 10 hours. Every 10 mL of Lake Albert water was intentionally contaminated up to ten percent (10% m/v) (100 g/L) using selected various amounts of PHs of known amounts.

Culturing *Pseudomonas aeruginosa*

Nutrient broth (13 g) in 1liter of distilled water was made. This was a stock that was divided into 10 parts of 100ml volumes containing 1.3 g of nutrient broth each. The ten parts of nutrient broth were autoclaved at 121°C. The broth (1.3 g) (100 ml) solutions were allowed to cool. The resultant broth solutions were inoculated with a sterile micropipette tip full of *Pseudomonas aeruginosa* from the pure culture plate. The bacteria were allowed to grow at 37°C in the incubator shaker for 10 hours to a turbidity recording an absorbance of 0.04 at 600 nm measured from spectrophotometer serial number 122445 in the biochemistry laboratory containing about 3.0×10^7 colony-forming unit (CFU)/mL.

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Bioremediation procedure

A sample of petroleum hydrocarbon contaminated water (10 ml), was quantitatively placed in a 250 ml flask containing a nutrient broth (100 ml). An aliquot of *Pseudomonas aeruginosa* starter culture (100 µl) whose turbidity absorbance was 0.04 at 600 nm was introduced. The resulting mixture was shaken at a speed of 180 r/min for 1, 3, 4, 5, 6 and 7 days at room temperature using a shaker model THZ-82. The bacterial activity was temporary stopped by reducing the temperature of the mixture to about 2°C to 8°C after every 24 hours.

Petroleum hydrocarbons extraction

Petroleum hydrocarbons were extracted from water with n-hexane following a method described by AUNEP/IOC/IAEA 1992 method for PHs. Gas Chromatography-Mass Spectroscopy following unresolved complex mixtures approach was used quantification of the removal efficiency.

Sample analysis

An Agilent 6890N gas chromatograph (GC), joined with Mass Selection detector (5975) on a fused silica capillary column coated with HP-5 MS 5% Phenyl methyl siloxane (30 m length and 0.25 mm ID 0.25 µm film thickness) was set. Injection of a 1.0 µL aliquot of the extract as the injector port is held at 150°C and operated in split mode and Helium carrier used to detect PHs at a split ratio of 1:20. Temperature-programme was as follows: Initial temperature at 95°C for 1 min, 95-190°C at 20°C/min, 190-250°C at 15°C/min, 250-300°C at 25°C/min for 3.0 min, giving a total run time of 18.5 min. The detector temperature was set at 150°C. Agilent Chemstation software was used to obtain the chromatogram and for data calculations. Integration of the entire peaks area was used to determine the total area counts for each sample chromatogram before and after bioremediation. The difference in total area counts was used to calculate the biodegrading efficiency [6]. The performance parameters used for the valuation of bioremediation were unresolved complex mixture (UCM) sum corrected area of chromatogram peaks and removal efficiency (RE). Removal efficiency (RE) was determined using the following equation, $removal\ eff = \frac{S_{ca1} - S_{ca2}}{S_{ca1}} \times 100\%$, [7] Where: S_{ca1} = sum corrected area of chromatogram peaks before bioremediation, S_{ca2} = sum corrected area of chromatogram peaks after bioremediation. $(S_{ca1} - S_{ca2})$ = the difference between sum corrected areas before and after bioremediation, $\frac{S_{ca1} - S_{ca2}}{S_{ca1}} \times 100g/L$ on Lake Albert water was computed for every 24 hours for 7 days.

Quality assurance and data analysis

Data quality was guaranteed by use of duplicate analyses and control experiment that ensured accuracy control. The results from this study were blank corrected. One procedural blank was made for every 4 samples. The limits of detection (LOD) were defined as 3 times the standard deviation divided by the slope of the regression line Figure 1. The limit of quantification (LOQ) was defined as 10 times the standard deviation divided by the slope of the line relating sum corrected area and mass of PHs. This method was described by [8] in the detection limit guidance. The limits of detection and quantification were reported as a function of mass of the PHs extracted. The statistical significance of the study results were defined by a $p < 0.05$. Minitab 17 [9], statistics package a reliable software statistical analysis program by NIST [10] was used in this study because it is a published data analysis software [11].

Results and Discussions

PHs removal from lake Albert water

The amount removed in this current study was considered to be a fraction of 100 g of PHs on a liter (or 100 g/L) of Lake Albert water contaminated with petroleum hydrocarbon. The study showed a significant ($p < 0.05$) reduction in PHs by *Pseudomonas aeruginosa*. The highest amount of PHs removed was 97 g/L (97%) and this was after 6 days as described in Table 1 but is 89.3 g/L in Figure 2 due the line of the best fit. This is in agreement with the findings of Xu, 2012. The author carried out a study in China to determine the remediation efficiency of *Pseudomonas aeruginosa* on the PHs of contaminated soils. Like in the current study, the maximum removal efficiency was on the day 6. However, his work did not cover the behaviour of PHs on water surfaces but the PHs on contaminated soils.

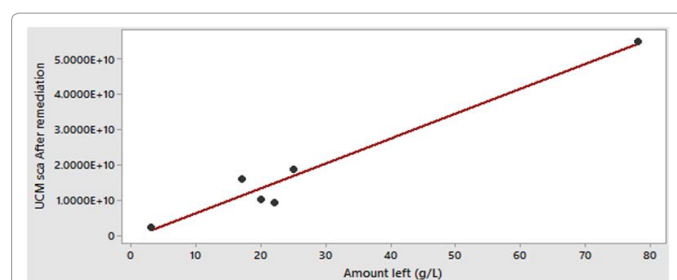


Figure 1: Unresolved Complex Mixture (UCM) Sum corrected area (sca) after bioremediation vs PHs left (g/L) ($p < 0.05$).

	Time [days]	Amount [g/L]		
		Experimental amount removed	Experimental amount Left	Experimental control results
Sample 1	1	22	78	31
Sample 2	3	80	20	12
Sample 3	4	75	25	22
Sample 4	5	78	22	9
Sample 5	6	97	3	21
Sample 6	7	83	17	32

Table 1: Time in day of bioremediation and amount removed, amount left including the control experiment.

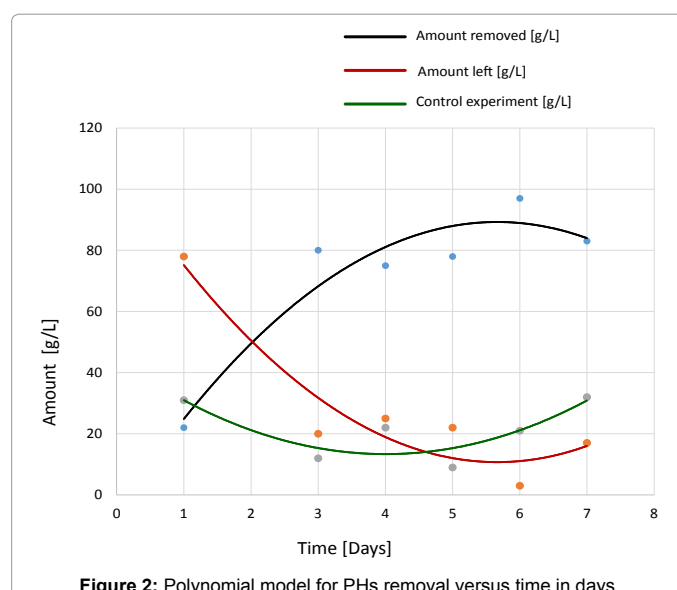


Figure 2: Polynomial model for PHs removal versus time in days.

Using polynomial model to establish a relationship between bioremediation and time, the model revealed that the maximum amount of PHs removed from water by *Pseudomonas aeruginosa* was 89.3 g/L between days 5 and 6 as shown in Figure 2. This showed that *Pseudomonas aeruginosa* removed almost 89.3% of contaminants from lake water. Similar results were obtained by Ref. [12]. Between days 1 and 3, the amount removed varied almost linearly with time, suggesting that bioremediation using *Pseudomonas aeruginosa* on Lake Albert water is more active in this period. It was noted that, beyond the sixth day the amount removed reduced from 89.3 to 84.7 g/L. The current result (Figure 2) agrees with the results reported by Ref. [13]. However, his study was done using microbial in nutrient broth. The significant death phase in these bioremediation studies could be due to the exhaustion of the PHs to support *Pseudomonas aeruginosa* as the carbon source. This suggests that bioremediation process is less active in this period.

In the control experiment the amount of PHs added and the final amount of PHs was expected to be zero since the remediation agent (*Pseudomonas aeruginosa*) was not added. However, in this current study, the difference was more than zero. It was found to range from 9 to 32 g/L of PHs. This suggests that some PHs could have been present already in the Lake Albert water as contaminants in addition to intentional PHs that was added. This is supported by the historical record [14]. Who reported the seepage in Lake Albert as the indication of oil in and around Lake Albert? In addition, verbal communication from three people in Albertine graben did mention that seepages are commonly seen on the shores of the lake.

PHs left in lake Albert water after bioremediation

In this study, the initial amount of PHs was recorded on day one on the amount left-time curve as 78.0 g/L on Figure 2. This reduced to 10.74 g/L between 5th and 6th day. This further indicates that bioremediation process is low during this period but relatively high in the first three day of the process. It was also observed that after the 6th day, the amount left started to increase. Here, the study suggest that, after 6 days the residues produced during bioremediation start to chock *Pseudomonas aeruginosa* population making them inactivated and hence biodegrading fewer petroleum hydrocarbons from water [13]. When they become inactivated their usefulness on the removal of PHs starts to reduce, a reason why this graph flattens on the between 5th and 6th day. It is also seen that, in this period, *Pseudomonas aeruginosa*'s capacity to degrade PHs reaches a saturation point beyond which they start to die off. This maximum saturation is the value of bioremediation amount left that explains the lowest level of PHs concentration reached between days 6 and 5 of this study.

Conclusions

The Lake Albert water is already contaminated with PHs. The

Pseudomonas aeruginosa can remediate petroleum hydrocarbon (PH) contaminants from Lake Albert water in western Uganda with the highest removal efficiency of 89.3 g/L (89.3%) for *n*-hexane soluble PHs. The maximum removal efficiency of petroleum hydrocarbons (PHs) from water by *Pseudomonas aeruginosa* is achieved between 5th and 6th day.

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