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# Mobilization of Pseudominas Putida in Kaolin through Electrokinetic Bioremediation Technique in Borno State

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**ABSTRACT:** Contamination of soil are due to several factors which are caused as a result of presence of toxins, waste from industrial activities, heavy metal from landfill and organic and inorganic waste from fertilizer used by famers. This study aims to measure the mobilization of pseudomonas Putida cells through kaolin clay soil using electro kinetic bioremediation technique is a method were the electro kinetic energy is used to generate currents into the soil, thus migrating ions to opposite charge of electrodes. The Pseudomonas putida and distilled water was supplied into the anode and cathode reservoirs respectively. The electro kinetic bioremediation testing was conducted for five days duration using 50V of electric current. The bacterial count of pseudomonas SP. are enumerated high at 1.3 x  $10^7$ cfu/gww (near the anode), 5.0 x  $10^6$ cfu/gww (in the middle) and 8.0 x  $10^6$ cfu/gww (in the cathode). These result showed high serviceability of pseudomonas S.P. unit in day 5. It was demonstrated that the electro kinetic bioremediation technique can be used for pseudomonas Putida transportation in kaolin clay soil throughout the soil specimen and moving in the same direction with the electro-osmotic flow in the electro kinetic bioremediation system.

**KEYWORDS:** Bioremediation, electro kinetic, anolyte, catholyte

### I. INTRODUCTION

Bioremediation harness the action of natural organism in degrading unwanted chemical containment, natural and manmade, are susceptible to degradation by bacteria or fungi, or even higher organism such as plants (although often this apparent degradation will be due to microbial communities associated with plant), given the correct condition. The bioavailability chemical in soil describes how accessible it is to degrade organism and many factors play a role in determining this (N.M Sunar et al, 2014). Currently, electro kinetic bioremediation technique is been employed in the contaminated land industry in some developed countries, but it is not yet implemented in Nigeria. Electro kinetic bioremediation has the advantage of been applicable in Insitu, meaning that excavation is not necessarily required. While for bioremediation technique, it may be difficult to achieve a satisfactory result especially while dealing with high permeability of soil in addition, it is time consuming, and can take month or even a year to complete (Yeung T Albert 2010). However, together, as electro kinetic bioremediation, it will be an extremely manageable technique providing efficient ability to treat difficult areas. Moreover, it has been proposed that it may be even used to clean up soil underneath existing building without disturbing the foundations, due to its directional nature (S A 2012). When the electrolyte compartment was filled with bacteria pseudomonas putida solution and also allowed culture broth to flow due to external forces (under electrical gradient).



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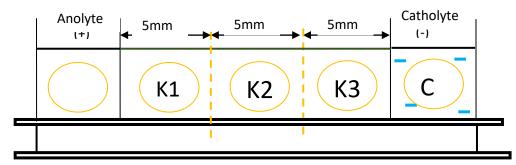


Fig 1: cross-section of the physical modeling of the electro kinetic cell

Table 1: Description of electro kinetic bioremediation with coding

Section (code)	description
A	Anolyte compartment
$\mathbf{K}_1$	Kaolin soil closeto theanode
$K_2$	Kaolin soil in the middle of the specimen
$K_3$	Kaolin soil close to the cathode
C	Catholyte compartment

**Soil Specimen Preparation:** A consistent method of soil specimen preparation was employed throughout the work. It was decided from the onsets to prepare soil spacemen prior to contamination, in order to ensure that a realistic soil structure was presented before zinc contaminant was added. The soil samples was dried for at least 3 days and was ground, and pass through a 2mm of aperture sieve size. The dried soil was mixed thoroughly with deionized water to achieve moisture content of 52% similar to liquid limit value. The mixture was then allow to homogenize overnight.

**Pseudomonas Putida Broth Culture:** Pure pseudomonas putida ATCC 49128 samples were purchased from an online vendor in dry culti-loop form. They were store in chiller at temperature 4°c prior of cultivation procedure. Approximately 16g of nutrient broth was transferred into 25000 mL Erlenmeyer flask containing 2000 mL of distilled water. The mixture were stirred gently and then dissolved by heating on hot plate temperature of 100°C for about two hours until dissolution. After that, the medium was sterilized in autoclave at temperature of 121°C for about 15mm. it was then allow to cool dawn for a few minute until its temperature drop dawn to around 37°C and 35°C. One loop shaft was removed from the handle straight into the medium following the manufacture instruction. The mixtures were then stirred until homogenous and kept in the chiller as 4°C for hours prior to further inoculation procedures.

**Pseudomonas Putida Cell by Electro kinetic:** The inert aluminum electrodes for anode and cathode were prepared by the exact dimension with holes at the grid section of the plate. The size of the electrode is 19.5 x 20.0 cm each. Filter paper (47 micrometer of pore size) was used and pasted it at the wall by using distilled water before the electrode plate are slot in. the containment soil sample was placed into the main compartment and distilled water was poured into the catholyte compartment.

**Preliminary Testing of Untreated Soil:** Several tests were conducted on the kaolin before running the electro kinetic bioremediation test such as specific gravity, Waterberg limits and soil pH. These test were conducted specifically to determine the characteristics of kaolin clay.



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Main Testing of Treated Soil: This experiment was conducted with two different suitable sample with bacteria (electro kinetic bioremediation) and without bacteria (electro kinetic remediation). Electro kinetic bioremediation (control test) was conducted using distilled water only for both anolyte and catholyte compartment. Both methods were to identify the effectiveness and the difference between electro kinetic and bioremediation methods are presented in the research so as to know the mobility of kaolin clay soil by using this technique except for preliminary testing. After the electro kinetic bioremediation test was conducted for pseudomonas putida –DW test using plate counts and soil microbial respiratory methods. Fig. 2 shows the electro kinetic bioremediation testing using pseudomonas putida and distilled water at the anolyte and catholyte compartments, respectively.

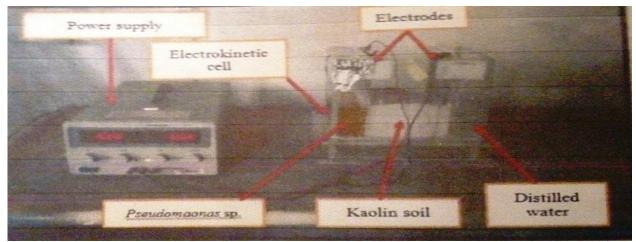


Fig 2: Electro kinetic Bioremediation Testing

### II. RESULT AND DISCUSSIONS

The initial characteristics of kaolin soil were taken before the electro kinetic bioremediation and electro kinetic remediation technique were conducted. The plastic and liquid limit recorded percentages of 36.25%, while the specific gravity value is 2.58. The pH values for both experiments using bacteria and without bacteria shows lightly similar result as shown in table 2. The pH at the initial testing for sample treatment is 4.6 while the pH of the soil is acidic. After the 5 days treatment, the result for both samples increase to pH 5.4 for electro kinetic bioremediation and pH 5.8 for the electro kinetic remediation.

Table 2: pH values of the kaolin soil before and after treatment

pH value	electro kinetic bioremediation electro kinetic remediation		
	(Bacteria and distilled water)(Both using distilled water)		
Before experiment	4.6	4.5	
After experiment	5.4	5.8	

Bacterial count method was conducted on the kaolin clay the soil sample and nutrient broth after the experiment was complete to ensure that the bacteria are in good condition. In fact, Li .s.et al (2010) suggested using electro kinetic bioremediation technique can be used to treat petroleum in soil. They explained that electro kinetic accelerated the biodegradation in soils stronger field intensity stimulated the bacteria activities, and accelerated the petroleum removal



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efficiency. Even though natural soil bacteria such as pseudomonas can be present in dormant or slow growing state, but when stimulated by optimum environmental conditions, they multiply rapidly and then adapt to a new environment N.M et al and N M et al(,2014). The microbial growth of the biodegradability soil pollutants N M et al (2014) Therefore, this aims to evaluate the mobility of pseudomonas putida through kaolin clay soil electro kinetic bioremediation technique.

### III. MATERIALS AND METHODS

**Development of experimental apparatus:** Schematic diagram of physical modeling is present in Fig. 1 where two small electrolyte compartments were designed to be separated from main compartment. To perform the test, kaolin soil was placed on a solid base plate in the main compartment then the electrolyte with culture broth of bacteria pseudomonas putida was introduced into the analyte compartment. Both facing of internal wall between the small electrolyte compartment and main compartment are perforated with small holes (approximately 4mm diameter) and covered by a perforated electrode plate made of aluminum to ensure the electros made good contact with soil. The hole in both walls allowed electrolyte fluid to flow into the soil specimen and also enabling pseudomonas putida to flow through the system. Filter papers were attached between perforated Perspex walls and an electrode plate which is sufficient to permit the passage of culture broth under working hydraulic gradient (i.e. result shown in Fig. 3) represent the mobilization and to ensure that the bacteria count analysis. At the anolyte compartment {A} recorded a value of 1.0x108cfu/gww, whereas K1 K2 and K3 recorded bacterial counts of 1.3x10<sup>7</sup>cfu/gww, 5.0x10<sup>6</sup>cfu/gww, respectively. According to Gill et al Electro kinetic enhance bioremediation by making bio accessible contaminants, nutrients, electron acceptors and electron donors more bioavailable to catabolically active microorganisms. Microbe distribution is influenced by cell surface charge and tendency for attachment to sediments, where increase contaminant transport relates to the water partition Coefficient of the specific compound; more soluble compound have greater mobility under an electric field. When an electric field is applied, pseudomonas putida migrates from the anode to the cathode electrode. No pseudomonas sp. was found in the kaolin soil sample. Microbial transport research has been carried on primarily in saturated soil system, simulating either horizontal movement through an aquifer or vertical water Movement. Previous studies indicated that water flow is the main is the contributor to bacteria movement in soil and is smaller than cell, passive diffusion of the microorganism is not possible.

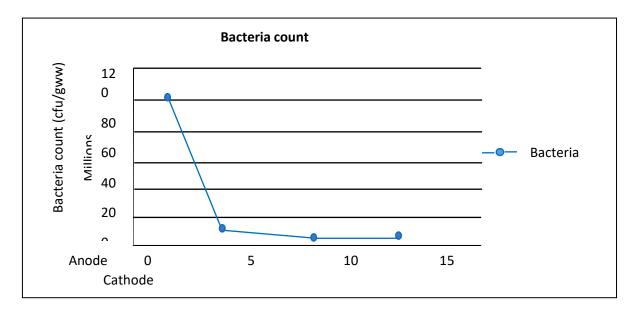


Fig 3: shows the bacterial counts of pseudomonas putida mobilization through the electro kinetic technique.



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### IV. CONCLUSION

Electro kinetic bioremediation is an effective coupled technology for contaminants removal, working through the combined effects of bioremediation and electro kinetic. This is mainly caused by changes in pH and a series of electric field effects. Furthermore, this technology can lengthen the growth time of the pseudomonas putida and at the same time support the amount of bacteria. As a conclusion, the study shows promising results and good indication technology by electro kinetic bioremediation technique.

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