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Assessment and Application of Plant-Bacterial Electrochemical Systems in Simultaneous Bioelectricity Generation and Biodegradation of Commonly Used Plant Pesticides

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Abstract: The study was conducted to explore potentials of electrochemical systems in simultaneous bioelectricity generation and biodegradation of commonly used plant pesticides. Sampling of various crop species and microbial isolation for subsequent electrogenic characterization of isolates and bioelectricity generation was carried out at the teaching and research laboratory of the department of animal and environmental biology, imo state university Owerri. Electrogenic screening of pure culture using Open Circuit Voltage (OCV) measurements in biolelectrochemical reactors and Cyclic Voltammetry were carried out. Bioelectricity generation measurements using multimeters and calculations of Voltage, Current, Power and Coulombic efficiency in Open and Closed-circuit systems were calculated. A total of 102 microorganisms were isolated and screened for electrogenicity. Samples from vegetables harboured the highest number of electrogenic isolates among all sites assessed. Four microorganisms elicited electrogenicity. Bioelectricity generation experiments showed that *P. aeruginosa* was the most electrogenic strain, eliciting the highest current of 556.03 ± 1.21 mA/m^2. The least current was observed for the *Bacillus strain* (554.11 ± 0.35 mA/m2). All isolates proved to be good electrogens and efficient candidates for optimising bioelectricity production.

Keywords: *biodegradation, plant pesticides; wastewater; electroactivity; bioenergy*

1. Introduction

Pesticide contamination in soil is a widespread environmental issue resulting from the intensive use of agricultural chemicals to control pests, diseases, and weeds. Pesticides such as herbicides, insecticides, fungicides, and nematicides often persist in soil for extended periods, leading to potential risks for human health, non-target organisms, and ecosystems. In recent decades, there has been an extensive use of synthetic pesticides and anthropogenic substances, which has generated serious environmental issues. These chemical substances cause severe effects on humans and other living organisms. Insecticides are harmful substances that are present in soil and aquatic systems and can migrate into the human body and cause various diseases. The chemical compounds are categorized based on their mechanism of action (Dai et al., 2016). Insecticides that enter target organisms disperse into the environment and may transfer from one area to another, increasing the likelihood of insecticides entering non-target organisms and causing harmful effects (Dai et al., 2016).

Pollution generated through industrial practices targeted at producing energy have also been implicated in the generally negative

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effects of ecological balance as noxious gases like carbon monoxide and sulphur dioxide are released as a result of this (Habibul et al., 2016). These gases translate into detrimental effects on the environment as they have been proven to cause respiratory and other health challenges for humans and animals as well as lead to phenomena such as global warming and climate change currently being experienced globally (Helder et al., 2012). This leads to the understanding that there is a need for a cleaner and more sustainable energy production system.

The twin challenges of clean energy need s as well as pollution control has led to the ingenious developments of latest technologies for meeting the two demands. One of such latest technologies involves the use of microbial electrochemical systems (MES) (Klindworth et al., 2013). These systems generally involve the use of electrogenic microorganisms. Electrogenic microbial communities have great importance in the natural environment, principally in metal oxidation and reduction and the associated effects on mineral dissolution, the carbon cycle, and the sorption and complexation of phosphorus and heavy metals. It is now been seen that they may have a larger role in fulfilling a need for bioenergy production through direct electricity generation (Kracke et al., 2015). Direct energy generation in the form of bioelectricity from electrogenic microorganisms has been a major focus in recent times as the quest for increased electricity generation through cleaner means has been intensified (Lee et al., 2003). To achieve this specialized reactor vessels - the MES have been applied. There have been different forms of MES based on the reactor architecture, and specie of fermenting microorganism, however, a novel kind of MES fuses together the functionality of plants-microbe rhizospheric interactions for the process of bioelectricity generation. This is known as plant-MES (Logan, 2008). The utilization of plant-MES brings to the fore the technique of improved energy harvesting as well as other added advantages that a plant system could bring.

Good examples of such chemosynthetic pollutants are agricultural pesticides used in the local farming and food production cycles (Phulpoto et al., 2016). Pesticides have a chemical composition that assures its efficacy, however their unique chemistry as well as their inordinate and uncontrolled use have been implicated as a major factor that results in their indication as environmental pollutants (Piemonte et al., 2016) Reports have illustrated the adverse impacts of pesticides that seep into water bodies through run-offs from rainfall as well as the persistence in soil layers (Ravikumar et al., 2012). There have also been indications of these compounds subsequently affecting aquatic and soil life forms (Sethy et al., 2011). These challenges have sparked interests in remediation systems that enable removal of pesticides as chemical pollutants from the environment. Bioremediation methods have been identified as cost effective, environmentally friendly, and mostly engaging microorganisms in waste degradation and removal (Soudi & Kolahchi, 2011).

The importance of remediation measures in reducing the impact of pesticide pollution on the environment by direct biodegradative activity of plants and/or microorganisms on them cannot be over emphasized. This work thus seeks to bring to the fore the unique diversity of pesticide metabolizing microbial species found in plant roots and apply them in target bioremediation-based removal of unique pesticide types predominantly used in the Nigerian environment.

Recent research exploits have seen an increase in plant-MES applied research with targeted generation of energy and water/soil remediation. This is because of the ability to generate electricity from the biodegradation activities occurring within the root areas; such as the degradation of plant root exudates and deposits of plant roots by the activities of electrochemically active microorganisms (Tamura et al., 2004). The plant-MES can be

designed to have an anode area within the soil – having direct contact with the responsible electroactive microbial species and the soil roots. Electrons generated, generally flow through the anode into an external circuit and down into the cathode (Vidali, 2001).

Plant-MES provide a unique option that can enhance the process of bioelectricity generation as well as increase in bioremediation rates via synergistic interactions between plants and microorganisms. Enhancement of this technique will lead to and improved process of bioenergy generation as well as a defined path for bioremediation of agricultural pesticides in pesticide-laden soil environments. This research will lead to patentable technique that can be applied as a direct solution to the local challenge of pesticide pollution in Nigeria.

2. Materials and methods

The study was carried out at the teaching and research laboratory of the department of animal environmental biology, imo state university Owerri.

Isolation of bacteria from rhizosphere of selected plants

Sample collection

Target plants used in this experiment were vegetables and grasses. Identified plants were uprooted from their natural growth environments and the soils attached to the roots were carefully collected under aseptic conditions. Collected soil samples were serially diluted under aseptic procedures.

Processing of soil samples

Soil samples collected were processed based on the technique adopted by (Klindworth et al., 2013) with slight modifications. Precisely 1g of each sample collected from each site were dissolved in 50 mL of sterile distilled water and placed in flasks (250 mL capacity). The sample containing flasks were incubated at 37 ^{o}C for 2 hours. After incubation, approximately 5 mL of each sample were used as an inoculum for enrichment procedure.

Isolation of bacteria

Experimental set-ups with the highest absorbance values connoting higher microbial presence were be used for microbial isolation. Primary isolation of bacteria were carried out using nutrient agar by plating out 0.1 mL of samples on appropriately prepared culture.

Isolation of the Rhizobacteria from the Soil Sample Collected

Ten gm of collected soil was dissolved in 90 ml sterile deionized water and suspended soil particles by stirring for 10 min. One ml of the suspension was added to 9 ml of the deionized water to make a one-fold dilution; likewise, serial dilution was done for 10^{-2}, 10^{-3}, and 10^{-4}. An aliquot of 20µl of each dilution was spread on Nutrient Agar medium plates Containing agar ($20gL^{-1}$), peptone ($10gL^{-1}$), sodium chloride ($5gL^{-1}$) and yeast extract ($10gL^{-1}$). Based on the morphological characteristics, bacterial isolates were selected and sub-cultured until the pure culture was obtained. The pure isolates were preserved for future use in 80% glycerol stock at -60°C. Determination of the biodegradation rate of exogenously introduced pesticides on the plant-MES soils using efficient electrogenic bacteria isolates and plant combinations.

The plant-MES with most electrogenic bacteria and plant combinations were selected based on the bioelectricity yield and utilized in the biodegradation experiments. Selected pesticides (Chlorpyrifos and Dichlorvos) at different doses (5 – 25%) were applied unto the soil surface of the plant-MES and assessed for rate of biodegradation over a 30 day period.

Bioelectricity Measurements

Bioelectricity measurements of MER systems of each isolate were made by taking open and closed circuit voltage readings using a digital multimeter, and a 100Ω resistor. To determine the current, Ohm's law (Voltage = Current x Resistance) was used. Voltage plots against reaction time (hours) were measured and used as the volume of bioelectricity generated

(Yonetani, 2004). Power was determined by multiplying the value of the Voltage (Closed Circuit Voltage, CCV) and current measured (Helder et al., 2012).

Current density (mA/m2) and Power density (mW/m2) values were then deduced (Helder et al., 2012) as stated in the formulae below of which the maximum values (Current density max and Power density max) was recorded for each isolate.

Current density (mA/m2)

Current generated (mA)

Anode surface area (m^2)

Power density (mW/m^2)

Power generated (mW)

Anode surface area (m2)

Statistical Analyses

Experiments were carried out in triplicates and values were expressed ad as mean±standard deviation. Results were presented in tabular and graphical formats. Data obtained were statistically analyzed using different Analysis of variance (ANOVA) adopting probability levels below 5%.

3. Results and discussion

Physicochemical analysis of the soil sample showed that the pH of the soil was 4.94 which indicate an acidic nature of the soil. Electrical conductivity of tested rhizospheric soil was 0.602dS/m which shows highly saline environment of the soil with high soluble salt content. Available nitrogen was found to be 22.41 kg/ha, whereas available phosphorus and organic carbon were measured to be 2.71 kg/ha and 0.66 kg/ha, respectively after analysis of soil sample (Table 1).

Table 1: Physicochemical properties of the soil samples at the rhizosphere

Samples	pH	Tempt(^{0}C)	Nitrogen	P	OC	MC	EC
Sam A	5.02	25.28	18.20	3.44	0.66	44.6	0.602
Sam B	4.91	26.69	22.41	2.71	0.91	51.6	0.488

Cultivable bacteria isolated from vegetable and grass rhizosphere

A total of 102 bacterial strains associated with vegetable and grass *rhizosphere* were isolated from two sampling sites in different geographic locations. *Bacillus* and *Pseudomonas* were the most frequently observed genus in the collection at 25 % and 22.5 % respectively. The phylogenetic result also showed a slight predominance of Gram-positive bacteria (51.5 %). For Gram-negative bacteria (47.5 %), *Bacillus* (35 %) *Chryseobacterium* (10 %) and *Bacillus flexus* (2.5 %) were present

Figure 1: Diversity of rhizospheric bacteria of Ficus carica.

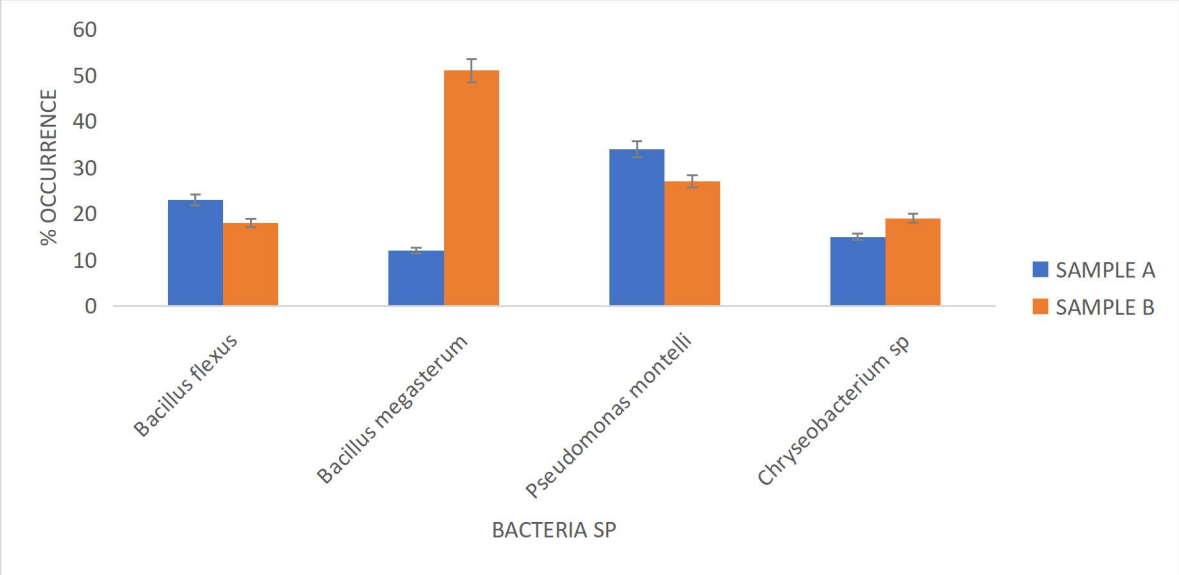

Table 2: Overview of number of electrogenic microorganisms isolated

Isolates source	Total number of isolates from source	No of electrogenic Bacteria isolated
Sample A	11	2
Sample B	7	1

Table 3: Data on screened electrogenic isolates based on amount of electrogenic potentials

Source	open circuit voltage (mV)	status remarks
Sample A	556.03 ± 1.21	Electrogenic bacteria
Sample B	554.11 ± 0.35	Electrogenic bacteria

Values of voltage (mV) are represented as Mean±SD (standard deviation)

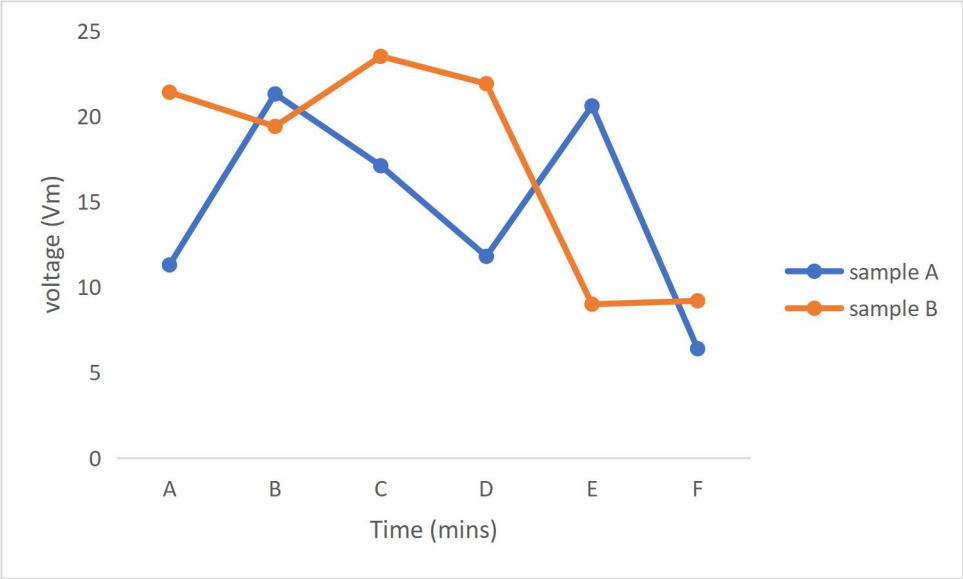

Figure 2: Electricity yield with time

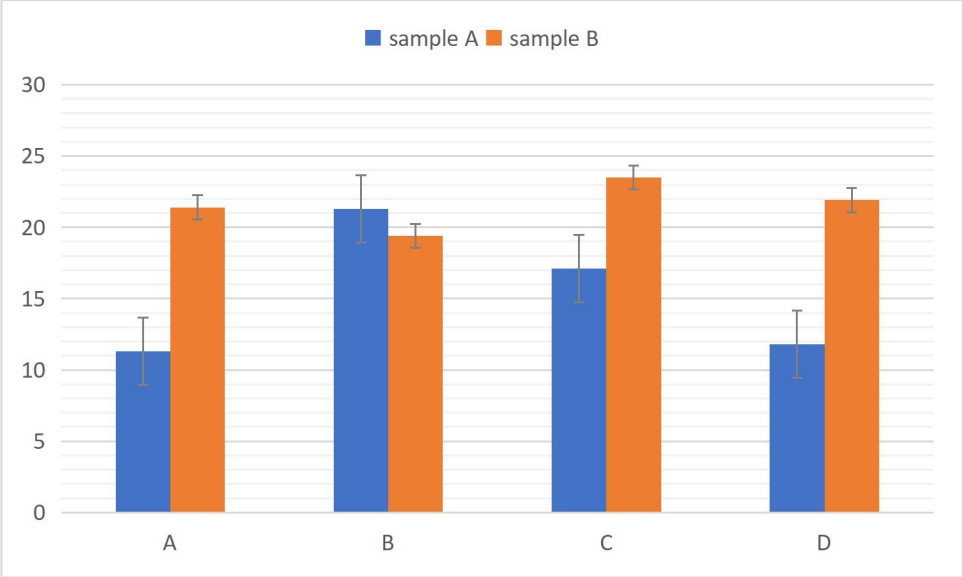

Figure 3: Rate of biodegradation

4. Conclusion

The experiments conducted in this work have shown that there is a prevalence of electrogenic species in environmental sites around known human habitats. The geologic/environmental uniqueness of each site was not considered; however, it is clear that the microbial diversity of electrogens can be strongly linked with electrogenicity based on specie/strain specificity. All genera identified have been earlier implicated as electrogens by previous research works. This thus implies that to obtain novel genera and species, it is important to screen more environmentally diverse sites, as well as enhance the selectivity process of extracting high electrogenic strains from those environments. The strains isolated proved to have good bioelectricity generation potentials in single culture experiments. This buttresses the point that single culture bioelectricity generation in MER can be achieved with the aid of defined conditions.

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