

## Original Research Article

ELECTRICITY PRODUCTION POTENTIAL OF DECAYED *Tectona grandis* USING  
MICROBIAL FUEL CELL

## ABSTRACT

The potential of decayed *Tectona grandis* wood to generate current and voltage was determined in this study. The decayed wood was collected from the Federal University of Technology Akure forest plantation. Microorganisms were isolated from the decayed *Tectona grandis* wood and the organisms were identified using both cultural and molecular methods. The microbial fuel experimental set up was carried out for 14 days. Decayed *Tectona grandis* wood was placed in a microbial fuel cell and a multimeter was used to read the current and voltage that was generated. Results revealed that the microorganisms isolated include *Bacillus licheniformis*, *Micrococcus luteus*, *Bacillus sp*, *Acinetobacter iwoffii*, *Bacillus cereus*, *Pseudomonas putida*, *Bacillus thuringiensis*, *Penicillium notantum*, *Rhizopus stolonifer*, *Aspergillus penicilloides*, *Rhizopus oryzae* and *Aspergillus flavus*. It also showed that there was a continuous increase in the current generated which was within the range of  $0.032 \pm 0.00$  to  $0.441 \pm 0.02$  mA. The highest voltage was generated on day 12 with the value  $0.369 \pm 0.02$  mV. It was shown that there was a progressive increase in the voltage generated from day 1 to day 12 with the range of values  $0.023 \pm 0.01$  -  $0.369 \pm 0.02$  mV. Findings from this study affirmed that decayed *Tectona grandis* has the ability to generate current and voltage using microbial fuel cell.

Keywords: *Tectona grandis*, Microbial fuel cell, Electricity, Decayed, Potential.

## INTRODUCTION

Microbial fuel cell technology is a new type of renewable and sustainable method for the production of electric energy from the microbial breakdown of organic matter. It has also been considered a promising technology for power generation (Lee and Shih, 2010; Refaat, 2009). A Microbial Fuel Cell (MFC) is a device that converts chemical energy from bio-convertible organic substrate, directly into electrical energy through the metabolic activity of microorganisms. Fuel cells are able to generate electricity from many different chemicals by oxidation of the chemicals at the anode and reduction at the cathode. *Tectona grandis* Linn. commonly known as teak tree is known in the world for its dimensional stability, extreme durability and hardness in timber production (Sherifat *et al.*, 2013). Following the current global energy crises in relation to increasing demand for fossil fuels (particularly oil, coal and gas), as well as inadequate electricity supply various human services in a country like Nigeria, evaluating for newer sources of meeting required demand cannot be overemphasized, as future economic growth crucially depends on this, however this study attempts to isolate the organisms present in decayed wood and determine the feasibility of using *Tectona grandis* wood to produce current and voltage generation in a microbial fuel cell..

## 2.0 Methodology

### 2.1 Collection of samples

Decayed *Tectona grandis* wood was collected from the forest plantation located in the Federal University of Technology, Akure at Obanla. The decayed wood was collected into a sterile polythene bags and were transferred to the Microbiology Postgraduate Laboratory, Obanla, Federal University of Technology, Akure for microbiological analyses.

### 2.2 Isolation of microorganism

Five fold serial dilution was carried out on collected 1gram of decayed *Tectona grandis*. One millilitre of each diluent was pipeted into Petri dishes and pour plated with molten nutrient agar and potato dextrose agar media. Nutrient agar plates were incubated at 37 °C for 24 hours for bacteria and 28 °C for 3 to 5 days for fungi on potato dextrose agar plates respectively in duplicate before examination for microbial growth. The bacterial isolates were purified by streaking on fresh sterile nutrient agar before subculturing. Fungal isolates were also subcultured to obtain pure isolates. The pure isolates were stored temporarily on agar slants and kept at 4 °C for further use (Fawole and Oso, 2012). Colony count was carried out on plates (in duplicates) by using colony counter. Colony count was expressed as cfu x 10<sup>5</sup> and sfu x 10<sup>4</sup> per gramme of decayed wood for bacteria and fungi respectively.

### **2.3 Identification of microorganism**

The pure isolated bacteria and fungi were identified using cultural and morphological features. The bacteria isolate was subjected to various biochemical test while the fungi isolates were identified by viewing under a microscope (Samson and Varga, 2007). The isolates were further identified using molecular methods ascertain their identities ( Akinyemi and Oyelakin, 2014).

### **2.4 Microbial Fuel Construction**

The microbial fuel cell (MFC) was constructed according to (Yoganathan and Ganesh, 2015; Adegunloye and Olotu, 2017). The MFC was constructed using two screw capped plastic bottles which is made of two chambers; the anode (anaerobic) which contains the decayed wood and the cathode (aerobic) which contains water. In the case of the control, the decayed wood which was contained in the anode was sterilized which killed all microorganism present in it. Both anode

and cathode chambers were connected with 1.2 cm in diameter and 6 cm long tube which was filled up with salt bridge made of sodium chloride and agar in the ratio of 1:2. Agar salt bridge acted as a barrier between the anode and cathode chambers. The Purpose of an agar salt bridge is to provide an internal electrical connection between the chambers, while minimizing the transfer of ions from the electrical environment. The carbon rods of 1.5 cm diameter and 13.5 cm long served as anode and cathode. Before the MFC operation the electrodes were soaked in 1 mol/L HCL solution for a day to remove possible metal contamination and after the MFC operation the electrodes were washed with 1 mol/L NaOH solution to sterilize the attached cells. The electrodes were externally connected with copper wire and all exposed metal surface was sealed with non conductive epoxy. A multimeter was connected to the copper wires and it was used to read the current and voltage produced.

## RESULTS

### **Total microbial load of decayed wood samples**

Microbial load of the decayed wood indicated that there was significant difference ( $p \leq 0.05$ ) in total viable Bacterial and fungal counts of the decayed wood, the bacterial load of *Tectona grandis* was  $4.5 \times 10^5$  Cfug, while the fungal load was  $4.6 \times 10^4$  Sfu/g.

### **Morphological, biochemical characteristics and identification of bacterial isolates from decayed wood**

The biochemical tests carried out on the bacterial isolates (Table 1) are; Gram stain, Catalase, Coagulase, Motility, Citrate, Indole, Spore forming, Starch Hydrolysis and Urease. All the isolates showed different biochemical reactions and were morphologically characterized. The isolates were identified as *Bacillus thuringiensis*, *Acinetobacter iwoffii*, *Staphylococcus aureus*,

*Bacillus licheniformis* *Bacillus* sp *Pseudomonas putida*, *Bacillus cereus*, *Rhizopus stolonifer*,  
*Penicillium notatum*, *Aspergillus flavus*. *Aspergillus penicilloides*, *Rhizopus oryzae*

**Table 1: Morphological, Biochemical Characteristics and Identification of Isolates from decayed *Tectona grandis* wood**

Isolate No	Colony Morphology	Gram's Reaction	Catalase	Coagulase	Motility	Mannitol	Glucose	Fructose	Maltose	Lactose	Galactose	Citrate	Indole	Spore Forming	Methyl Red Test	Starch hydrolysis	Urease test	Probable Identity
1	Cream, circular, opaque, flat, rough	-	+	NA	+	A	A	A	A	-	NA	+	+	+	-	+	-	<i>Bacillus licheniformis</i>
2	Irregular, creamy-yellow, opaque, smooth, entire	+	+	+	+	+	A	A	A	-	NA	+	+	+	-	+	-	<i>Bacillus cereus</i>
3	Cream, circular, smooth, entire	+		-	+	-	A	A	A	A	A	-	+	+		-	-	<i>Bacillus thuringiensis</i>
4	Cream, circular, raised and smooth	+	+	NA	+	A	AG	AG	A	A	A	+	+	+	-	NA		<i>Bacillus subtilis</i>
5	Pale yellow, circular	+	+	+		A	AG			AG	A	+	-	NA	+	NA	+	<i>Staphylococcus aureus</i>
6	Cream , circular, smooth	+	+	-		AG	A		A	A		+	-	NA	-	NA		<i>Acinetobacter iwoffii</i>
7	Cream, circular, smooth, raised and lobate	-	+	-	-	A	-		-	-		+		-	-	NA		<i>Pseudomonas aeruginosa</i>

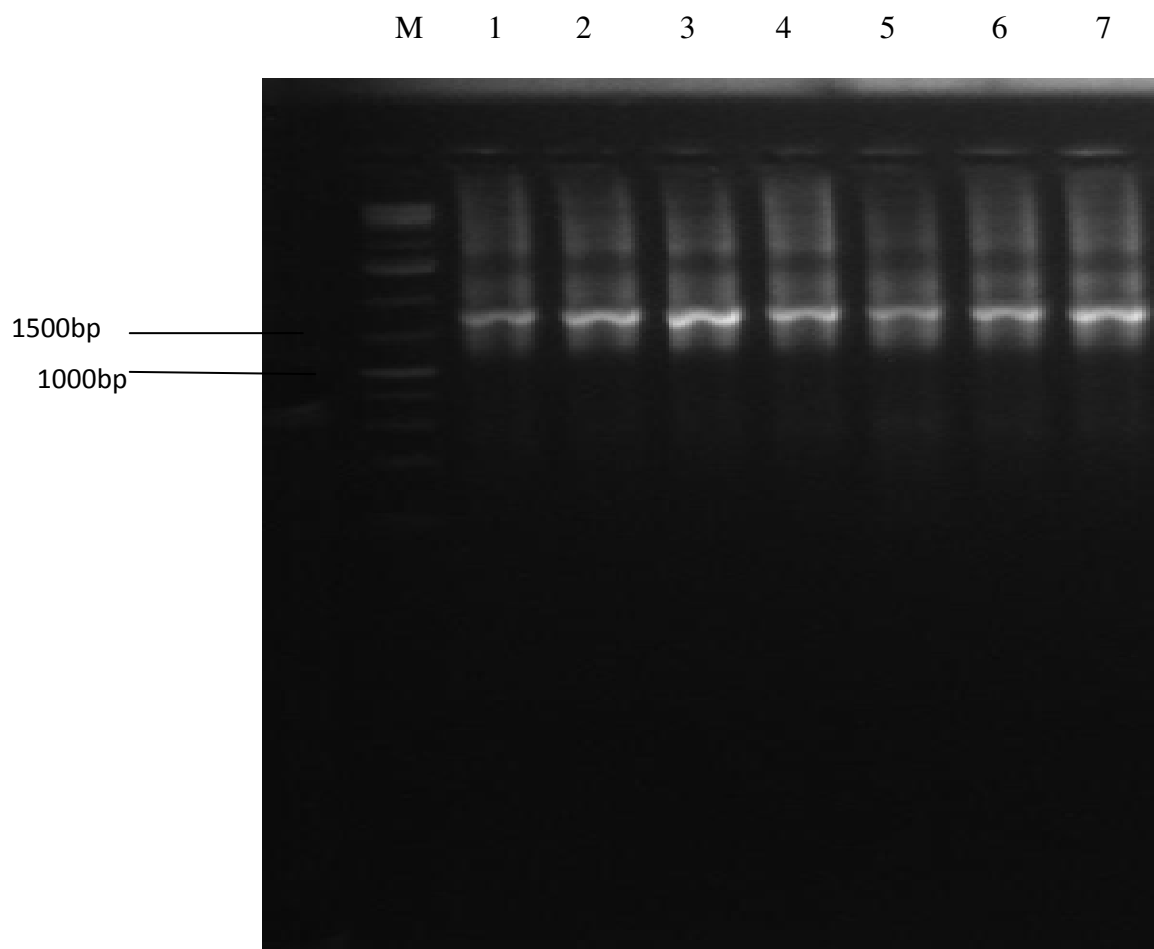
**Table 2: Fungal isolates obtained from the decayed wood**

Cultural and Microscopy description	Isolates
Hyphae broad, not or scarcely septate; rhizoids and stolons present; sporangiophores brown, solitary or in tufts on the stolons, diverging from the point at which the rhizoids form; sporangia rather round; apophysis absent or scarcely apparent; sporangiophores ovoid.	<i>Rhizopus stolonifer</i>
Yellowish green to dark green hyphae. Conidiophores arising from the mycelium singly or less often in synnemata, branched near the apex, penicillate, ending in a group of phialides	<i>Penicillium notatum</i>
Stipes are smooth, brown and pigmented, vesicles are globose, phialide biseriate, conidia are globose, conidial head are dark green and radiate.	<i>Aspergillus penicilloides</i>

### **Molecular Identification of bacteria isolate from decayed *Tectona grandis***

Molecular identification of the bacterial isolates are shown in Table 3. The lengths of amplified products were 1412, 1000, 1499, 1425, 1512, 1419, 1525 base pair for *Pseudomonas putida*, *Bacillus* sp, *Bacillus cereus*, *Bacillus thuringiensis* *Acinetobacter iwoffi*, *Bacillus licheniformis*, *Micrococcus luteus* respectively (Plate 1). The bacteria *Bacillus* sp, *Bacillus subtilis*, *Acinetobacter iwoffi*, *Bacillus cereus*, *Staphylococcus aureus*, *Bacillus thuringiensis*, *Pseudomonas aeruginosa*, were confirmed to be *Bacillus licheniformis* *Bacillus* sp *Acinetobacter iwoffi*, *Bacillus cereus*, *Micrococcus luteus* *Bacillus thuringiensis* and *Pseudomonas putida*.





**Plate 1:** PCR amplification of genomic DNA targeted to amplify the 16SrRNA gene of 7 bacterial isolate on 1.0% agarose gel electrophoresis.

Key: **M** = Molecular marker

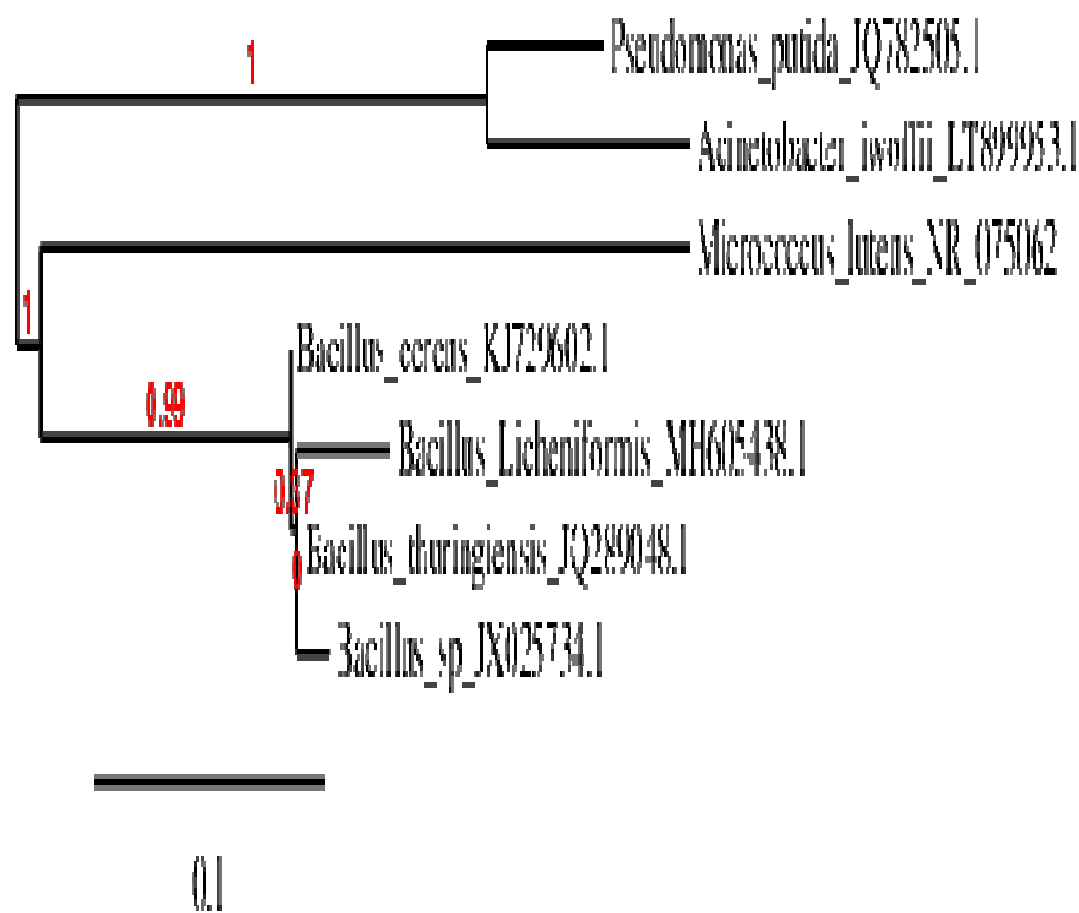


Plate 2: Phylogenetic tree of bacteria isolate from *Tectona grandis* wood

**Table 3: Molecular identification of isolated bacteria from decayed *Tectona grandis***

<b>Cultural and Biochemical identification</b>	<b>Gene sequence identification</b>	<b>Max identity</b>	<b>Accession number</b>
<i>Bacillus sp</i>	<i>Bacillus licheniformis</i>	100	MH605438.1
<i>Bacillus subtilis</i>	<i>Bacillus spp</i>	100	JX025734.1
<i>Acinetobacter Iwoffii</i>	<i>Acinetobacter iwoffii</i>	98	KT997475.1
<i>Bacillus cereus</i>	<i>Bacillus cereus</i>	100	KJ729602.1
<i>Staphylococcus aureus</i>	<i>Micrococcus luteus</i>	98	NR_075062.2
<i>Bacillus thuringiensis</i>	<i>Bacillus thuringiensis</i>	100	JQ289048.1
<i>Pseudomonas aueriginosa</i>	<i>Pseudomonas putida</i>	100	JQ782505.1

#### **Voltage and current generated from the decayed *Tectona grandis* wood**

The voltage generated from the decayed wood during the period of 14 days is represented in figure 1. The voltage produced were within the range of  $0.023 \pm 0.01$  -  $0.369 \pm 0.02$  mV. Figure 2 shows the current generated from the decayed wood. The current produced were within the range of  $0.032 \pm 0.00$  to  $0.441 \pm 0.02$  mA.

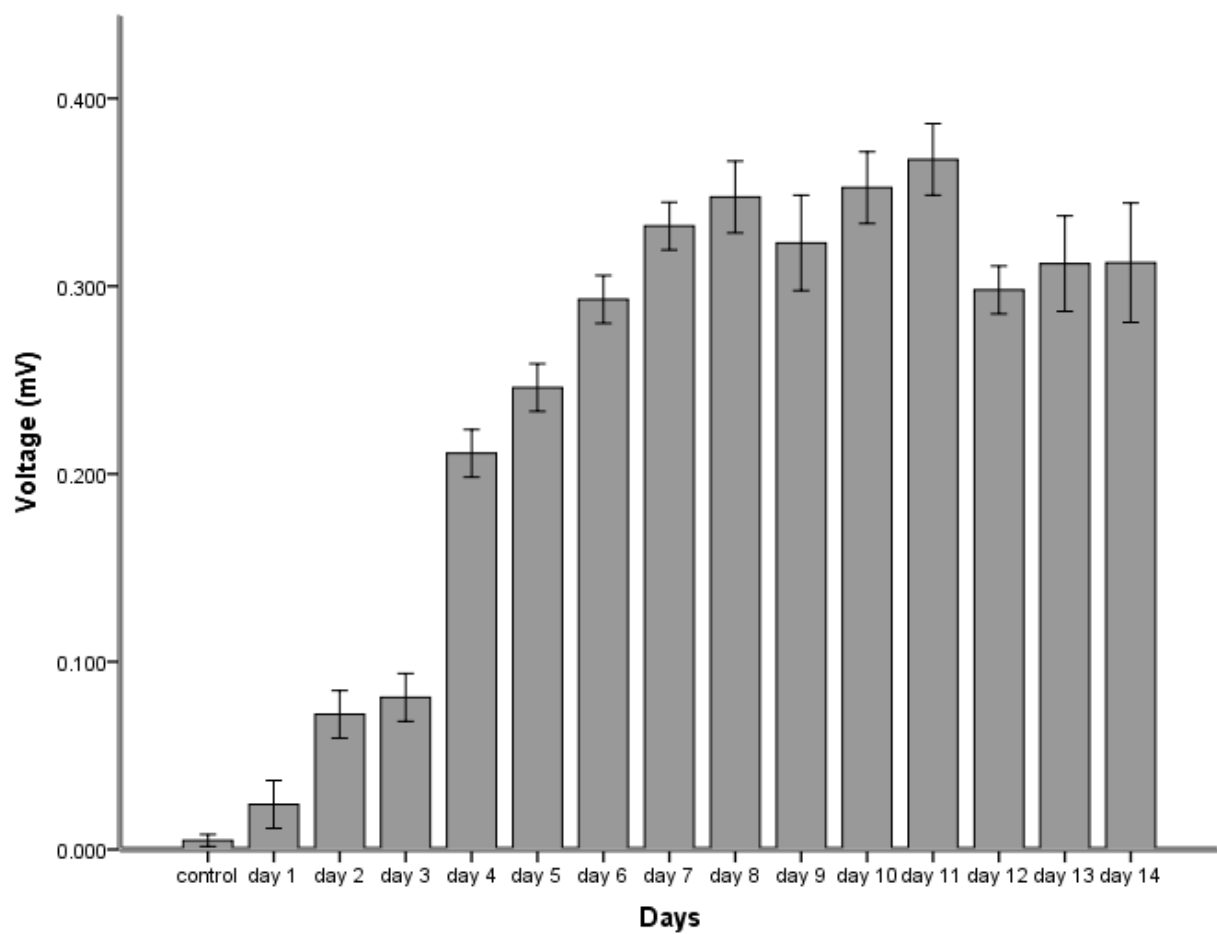


Figure 1: Voltage produced from decayed *Tectona grandis* wood within 14 days

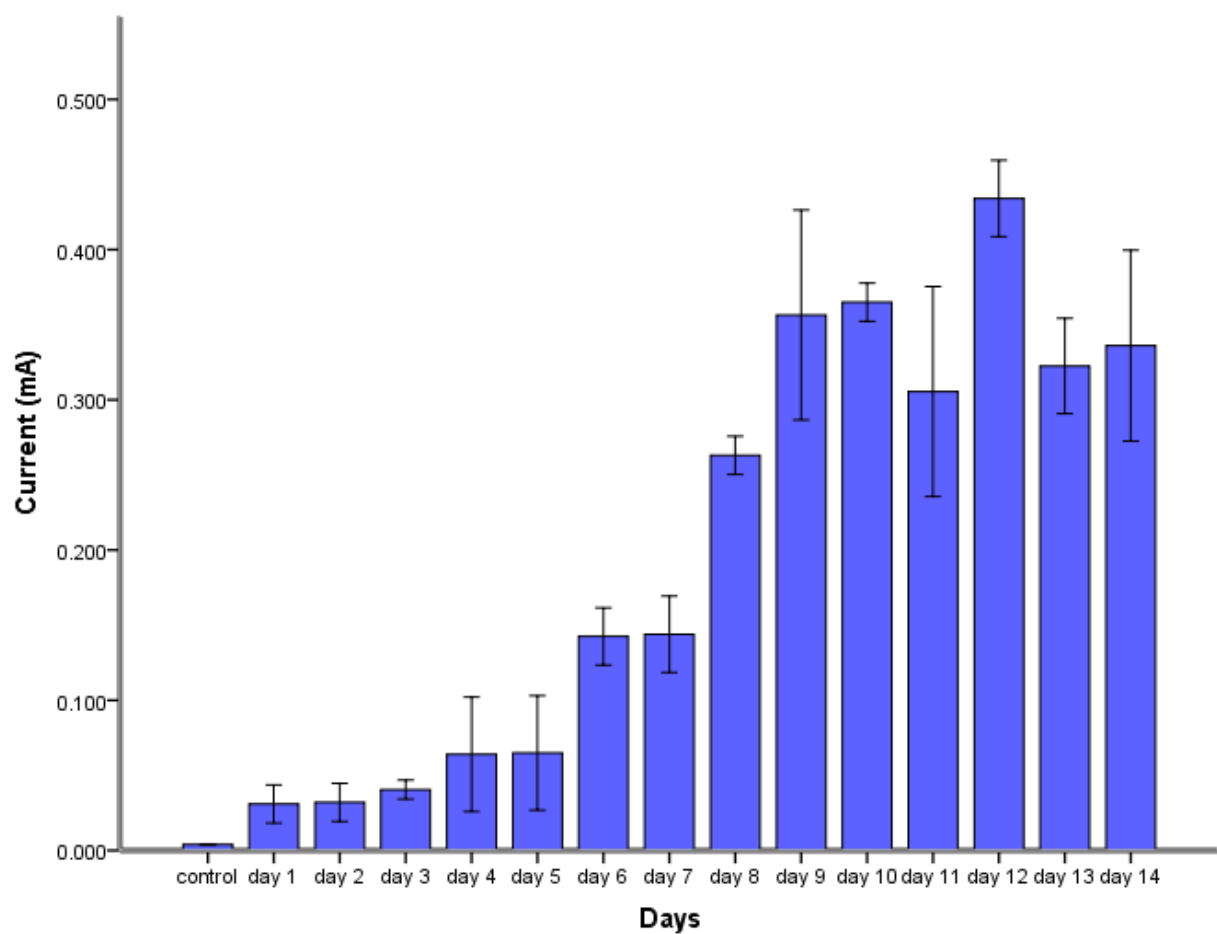


Figure 2: Current produced from decayed *Tectona grandis* wood within 14 days

## DISCUSSION

In this study, the potentials of decayed *Tectona grandis* Linn. to produce current and voltage was evaluated. The microbial load obtained in this study has shown that decayed wood harbour bacteria and fungi. However, there were differences in total viable bacterial and fungal counts of *Tectona Grandis*. It was observed that bacteria counts were higher than fungal counts. High microbial counts in the decayed woods could be attributed to high moisture content and nutrients present in the soil where the woods are fallen. These findings are in agreement with the reports of Janet and Kelechi, (2015)

Morphological, biochemical and cultural characteristics of bacterial and fungal isolate revealed the microorganisms that was isolated to include; *Bacillus thuringiensis*, *Acinetobacter iwoffii*, *Micrococcus luteus*, *Bacillus* sp *Pseudomonas putida*, *Bacillus cereus*, *Bacillus licheniformis* while the fungi isolates includes; *Rhizopus stolonifer*, *Penicillium notantum* and *Aspergillus penicilloides*. These microorganisms were probably found on these decayed wood due to high moisture content, the nutrients they derive and their attachment with the soil since soil harbors many organism. However, the presence of *Pseudomonas putida* and *Acinetobacter iwoffii* is highly uncommon and could have been as a result of contamination or environmental factors as reported by Chenhui *et al.*, 2017 who confirmed that the microbial community compositions of fallen logs are affected by both physicochemical wood properties and environmental factors. In addition most of the isolated microorganisms from the decayed wood owned their origin from air and soils this is in agreement with the findings of Singh *et al.*, 2014.

Polymerase Chain Reaction revealed that the molecular weight of the genomic DNA of sequenced bacteria in this study is 1500bp. According to the 16S rDNA analyses, selected bacteria showed more than 80% similarity in the NCBI Gene Bank by BLASTn. Based on the

BLASTn results, the isolates confirmed were *Bacillus licheniformis*, *Bacillus sp*, *Acinetobacter iwoffii*, *Bacillus cereus*, *Pseudomonas putida*, *Micrococcus luteus* and *Bacillus thuringiensis*. The result also revealed a difference in cultural identification of *Micrococcus luteus*, *Bacillus subtilis* and *Bacillus cereus*. This was also reported by Akinyemi and Oyelakin, 2014, who reported differences in conventional method and molecular method of bacteria identification. However, the results of this study demonstrate clearly the interest and feasibility to introduce the 16S rDNA gene sequencing method in identification of bacteria, combination of conventional techniques and molecular approach will improve bacteriological investigation and authentication, allowing specific and efficient identification of microorganisms as against cultural method that is probable.

There was a progressive increase in the current generated within the period of 14 days. It was observed that as the current generated increased, there was decrease at some point in the current generated. This could be as a result of low proton transfer between the anode and cathode when the decayed woods immersed in water was kept in the same position throughout the experiment which limited power generation. This is in agreement with the findings of Liu *et al.*, 2005 who reported the same. The highest current generated from the decayed *Tectona grandis* was recorded on day twelve after which it started decreasing gradually. This is similar with the finding of Sonal and Prakash, 2013 who used waste water to generate current and had the highest current generated on day 8 after which there was a decrease in current generated.

Voltage generated from the decayed wood was recorded daily for the entire time period of 14 days. The results showed a general increase across the number of days. The maximum voltage generated within 14 days was on day eleven, after which there was a definitive decrease,

was noticed. The result obtained is comparable with that of Parkash, (2016) who reported a similar result that initially the voltage rises rapidly but voltage starts falling down.

## CONCLUSION

In this study, it can be concluded that decayed *Tectona grandis* wood have the ability to generate current and voltage and hence can be employed as an alternative to electricity generation and inturn reduce energy crisis.

## REFERENCES

- Adegunloye, D. V and Olotu, T. M. (2017). Generating Electricity Using Microbial Fuel Cell Powered by Benthic Mud Collected From Two Locations in Akure, Nigeria. *European Scientific Journal*. Vol.13 No.18 ISSN: 1857 – 7881
- Akinyemi, A. A., and Oyelakin, O.O (2014). Molecular characterization of bacteria isolates from farm-raised cat fish *Clarias garipinus*. *British Microbiology Research journal*. **4**(12): 1345-1352
- Chenhui, C., Fuzhong, W., Wanqin, Y, Zhenfeng, X., Rui, C., Wei, H., Bo, T. and Meta F. (2017). The microbial community in decaying fallen logs varies with critical period in an alpine forest. *Environ Sci Technol* **6**:3
- Chonde, S., Mishra, A. and Raut P.(2013) Bioelectricity production from wastewater using Microbial Fuel Cell (MFC).
- Fawole M and Oso, B. (2012). Laboratory Manual of Microbiology, Spectrum Books Limited 2:112-121
- Janet, O. W. and Kelechi, H. (2015). Microorganisms associated with dumpsites in Port Harcourt Metropolis, Nigeria. *Journal of Ecology and the Natural Environment*.**8**(2): 9-12.
- Lee, S. C. and Shih, L. H., (2010). Renewable energy policy evaluation using real option model- The case of Taiwan. *Energy Econ.*, **32**; 567-578



Liu H, Cheng S. and Logan, B. (2005). Power generation in fed-batch microbial fuel cells as a function of ionic strength, temperature and reactor configuration. *Environ Sci Technol* **39**: 5488-5493.

Singh P, Kumar V and Agrawal S (2014) Evaluation of phytaseproducing bacteria for their plant growth promoting activities. *Int J Microbiol* 1;7

Parkash A. (2016). Microbial Fuel Cells: A Source of Bioenergy. *J Microb Biochem Technol* 8: 247-255.

Refaat, A. (2009). Correlation between the chemical structure of biodiesel and its physical properties. *Int. J. Environ. Sci. Tech.*, **6** (4), 677-694

Samson, R. and Varga, J. (2007). *Aspergillus* systematic in the genomic era. CBS fungal Biodiversity centre Utrecht p. 206

Sherifat, A., Akinsola, A., and Guido, F. (2013). Chemical constituents, toxicity and antimicrobial activities of the essential oil from the leaves of *Tectona grandis*. *Elixir Bio Technology*, **61**; 6795-16798

Yoganathan, K., and Ganesh, P. (2015). Electrogenericity assessment of *Bacillus subtilis* and *Bacillus megaterium* using Microbial Fuel Cell technology. *International Journal of Applied Research* **1**(13): 435-438