

**Improving Bioelectricity Generation of  
Microbial Fuel Cell (MFC) With Mediators  
Using Kitchen Waste as Substrate**

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**Abstract**

The enhancement of bioelectricity generation in the Microbial Fuel Cell (MFC) necessitated the introduction of exogenous compound (s) (i.e. mediators). The effect of 1ml of various synthetic exogenous mediators including dyes and metalloorganics such as Ethylene Diamine Tetra Acid [EDTA], potassium ferricyanide [ $K_3Fe(CN)_6$ ], methylene blue [MB], neutral red [NR] and potassium permanganate [ $KMnO_4$ ] was investigated in a 21day study during electricity generation in an MFC. The maximum Power Density (PD) obtained without the addition of any mediator was 84.58mW/m<sup>2</sup>, while those MFCs which utilized mediators recorded higher energy yield. The highest power density and percentage energy contribution of 924.79mW/m<sup>2</sup> (993.39%) was obtained using  $K_3Fe(CN)_6$ , while values obtained with EDTA [803.71mW/m<sup>2</sup> (850.24%)] MB [340.45mW/m<sup>2</sup> (302.52%)] and  $KMnO_4$  [192.14mW/m<sup>2</sup> (121.17%)] as mediators were appreciably higher. Further study on the use of these mediators showed inhibitory effects with the % reduction of microbial load in the following trend as MB (4.96%) < EDTA (6.13%) < NR (11.67%) < Ferricyanide (19.16%) <  $KMnO_4$  (21.89%) when compared to the control. Although the application of mediators improved energy production, minimum inhibitory concentration of the mediators should be ascertained to prevent the eradication of electrogens during electricity production.

**Introduction**

Microbial Fuel Cell (MFC), a novel Bioelectrochemical System (BES) with the potential of generating electrical energy from bulk biomass/organic waste through microbial metabolism, has recently attracted attentions as a potential method for clean energy production coupled with the added benefits of bioremediation [1-5]. The idea relies on the potential of bacteria to catalyze the breakdown of organic matter into carbon dioxide as end product via redox reaction, and utilize these reactions for electricity generation [6], thus developing an amazing device that consume our waste and light up our bulb [7].

Over the past years, efforts have been made to enhance the performance of microbial fuel cells (MFCs) for both clean bioenergy production and bioremediation. Reactor configuration, electrode construction, biofilm acclimation and feed nutrient adjustment, biomass/substrate, and addition of redox-active, electron donating mediators; among other factors that contribute to enhanced MFC performance [5], have recently been well focused on by researchers. Although, tremendous advances have been made, but further improvements are needed for MFCs to be economically practical.

Different organic compounds such as starch, cellulose, simple carbohydrates, organic acids, proteins/amino acids, chitin, toxic waste chemicals like as phenol; *p*-nitrophenol; nitrobenzene; polycyclic aromatic hydrocarbons; indole; ethanalamine; and sulphide have been reported as oxidizable substrates to power MFCs [8-11]. Sewage sludge [12]; municipal, paper mill and food industry wastewaters; as well as metal contaminated wastewater such as swine wastewater, brewery/distillery waste and marine sediments, have also been successfully used in laboratory MFC devices for bioelectricity generation [9,13,14]. Despite the number of exploitable substrates that have been reported, there are however, few reports on the use of kitchen waste in bioelectrochemical system.

In general, electrons are transferred in MFC either directly by electrogens without mediator or indirectly by electrogens with exogenous, redox-active mediator, which serves as transporter/shuttle for electrons [15,16]; and provide a platform for the microbes to generate reduced products that are electrochemically active [17]. The use of mediator in bioelectricity generation is essential because the outer layer of most microbial species consist of non-conductive lipid membrane, lipopolysaccharides and peptidoglycans which hinder the direct electron transfer to the anode [3]. Several mediators such as neutral red [18], methylene blue [1], HNQ and thionine [19], methyl orange, bromocresol green, methyl red, neutral red [20] and ferricyanide [12] have been reported. The toxicity of some of these mediators has also been reported [12]. The present study therefore aimed at evaluating the potentials of different exogenous compounds to enhance bioelectricity generation by MFC using kitchen waste as substrate.

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## Materials and Methods

### Source of kitchen waste

The kitchen waste was obtained from a popular restaurant in Ekiti State University, Ado Ekiti. The waste consists of leftovers of commonly consumed African dishes, which included pounded yam, rice, vegetable soup (prepared with vegetable and/or palm oil) and the waste washed in soap solution. The substrate (kitchen waste) was blended in a 12-speed warring blender (Excella) for 5mins. Five gram (5g) of homogenised waste sample was separated for physicochemical analysis to ascertain its nutritional composition/potential before the introduction of the mediators.

### Proximate and mineral analysis of kitchen waste

The physicochemical composition, including minerals and proximate properties of the kitchen waste was estimated following respective standard techniques described by AOAC (2012).

### Construction of MFC

Single chamber air cathode MFCs earlier described by [21] were constructed. The MFC consisted of an anode and cathode (with 11.6 cm<sup>2</sup> projected surface areas) placed in a plastic cylindrical chamber with an electrode spacing of 2cm. The anode and cathode electrodes were made of carbon (graphite) rod. The air-breathing cathodes consisted of carbon rod, a salt bridge serving as the Proton Exchange Membrane (PEM) separating the anode chamber from the cathode. A salt bridge was prepared into a polyvyline pipe. The electrode was placed in the biowaste to act as the anolyte. The biowaste suspension was placed in the cell and later sealed to stop the exchange of gas, hence forcing the microorganisms to undergo anaerobiosis.

### Preparation of Mediator

Five (5) different mediators were used in this study namely methylene blue (C<sub>16</sub>H<sub>18</sub>N<sub>3</sub>SCI), potassium ferricyanide [K<sub>3</sub>Fe(CN)<sub>6</sub>], Ethylene diamine tetra acid (EDTA), neutral red (C<sub>15</sub>H<sub>17</sub>ClN<sub>4</sub>): 3-amino-7-dimethylene-2-methylphanazine hydrochloride, potassium permanganate (KMnO<sub>4</sub>) with the molar mass of 319.85g/mol, 329.24g/mol, 292.24g/mol, 288.78g/mol and 158g/mol respectively [1,22,23]. One (1) ml of each mediator was added to the anode chamber for any probable increase in electrical output and the longevity of the MFC set up and mixed thoroughly before sealing.

### Monitoring Electrical Performances of Mediators

Electrical parameters such as the current and voltage were monitored using the multimeter (ALDA DT-830D) while the power density was determined by estimating the power generated per surface area of the electrode (i.e. power density= IV/Area) [24] for 21 days. A control experiment was set up as well to compare and ascertain the probable increase in the electrical output.

### Microbial composition of kitchen waste sample

Standard technique described by [25] was adopted to estimate microbial count in the kitchen waste sample using pour plate method. The plates were then incubated at 37°C for 24h, after which culture plates were examined for microbial growth and colonies were counted using the illuminated colony counter (Gallenkamp, England). The

counts for each plate were expressed as colony forming unit (CFU/ml) of the sample homogenate.

### Statistical analysis

The electrical and microbial load data obtained in this study were subjected to statistical analysis (t-test) using the Statistical Program for Social Sciences (SPSS) version 20.0 package.

## Result and Discussion

In order to ascertain and document the nutritional composition and potential toxicity of the substrate for bioelectricity generation before introducing mediators, the mineral and proximate components of the kitchen waste were analysed. The waste sample had its proximate component as presented in Table 1, carbohydrate [40.49mg/g]; crude protein [10.69mg/g]; crude fat [5.84mg/g]; ash content [10.58mg/g]; crude fibre [8.94mg/g]; moisture content [23.36%]; TTA [0.187%] and pH [6.29]; and the mineral components as sodium (1.878mg/g), potassium (83.016mg/g), calcium (7.593mg/g), magnesium (0.015mg/g), iron (0.001mg/g), zinc (0.25ppm), lead (0.04ppm) and cobalt (0.03ppm). With the physicochemical component, the kitchen waste like other municipal wastes, showed wide spectrum of feedstock which produces vast array of pollutants that are biodegradable in nature and less toxic, making it a suitable candidate for electricity generation in MFC. This report is similar to that of [7] who described kitchen waste as the major component (15-20%) of municipal solid waste that is composed of rich organic fractions with high moisture content. The choice of the kitchen waste as substrate in this study for MFC was based on the report of [26] on the feasibility of bioelectricity generation from solid state fermentation of canteen-based food waste.

The high moisture content observed for the kitchen waste substrate in this work apparently facilitates the formation of more electron-mobile solutions and promotes the transfer of electrons to the cathode in the MFC. Similarly, [9] reported that a 10% greater content of water in the MFC resulted in 3-fold greater voltage output. Furthermore, it has been established that the nature of the carbon source in a substrate for MFC plays vital role in selecting microbial population, thus the development of optimal electrogenic biofilms in the MFCs [27,28]. The suitability of kitchen waste in the study as substrate for bioelectricity generation using MFC is hence justifiable with the high content of carbohydrate (40.49mg/g).

**Table 1:** Physicochemical properties of Kitchen waste as substrate for MFC.

| Proximate component |               | Mineral components |               |
|---------------------|---------------|--------------------|---------------|
| Element             | Concentration | Element            | Concentration |
| Carbohydrate        | (40.49mg/g)   | Sodium (Na)        | (1.878mg/g)   |
| Crude Protein       | (10.79mg/g)   | Potassium (K)      | (83.016mg/g)  |
| Crude Fat           | (5.84mg/g)    | Calcium (Ca)       | (7.593mg/g)   |
| Ash                 | (10.58mg/g)   | Magnesium (Mg)     | (0.015mg/g)   |
| Crude Fibre         | (8.94mg/g)    | Iron (Fe)          | (0.001mg/g)   |
| Moisture            | -23.36%       | Zinc (Zn)          | (0.25ppm)     |
| pH                  | -6.29         | Lead (Pb)          | (0.04ppm)     |
| TTA                 | -0.187        | Cobalt (Co)        | (0.03ppm)     |

**Key:** TTA- Total titratable acidity.

Enhanced electricity generation using the MFC has long been attempted by introducing various synthetic exogenous mediators such as neutral red [18], methylene blue [1,12] HNQ and thionine [19], methyl orange, bromocresol green, methyl red, neutral red [20] and potassium ferricyanide [12]. The effect of exogenous compounds (mediator), EDTA,  $K_3Fe(CN)_6$ , methylene blue (MB), neutral red (NR) and  $KMnO_4$ , on power generation in the MFC, was evaluated using the kitchen waste as the fuel source compared to the control (Kitchen waste without mediators). Figures 1 and 2 present the electrical output in a single chambered air-cathode MFC motivated by the presence of mediators for 3 weeks. The power density recorded for the mediated MFC ranged from 1.11mW/m<sup>2</sup> in  $KMnO_4$  to 924.79mW/m<sup>2</sup> in  $K_3Fe(CN)_6$ . The amount of current and voltage generated in an MFC at zero hour when motivated with methylene blue (MB) was 0.596mA and 0.51V; potassium ferricyanide ( $K_3Fe(CN)_6$ ), 0.895mA and 0.47V; Ethylene diamine tetra acid (EDTA), 1.64mA and 0.74V; Neutral Red ( $C_{15}H_{17}ClN_4$ ), 0.280mA and 0.32V; potassium permanganate ( $KMnO_4$ ), 0.393mA and 0.50V and control 0.26mA and 0.19V. Following the report of [20] on the efficiency of mediators in enhanced bioelectricity generation, the relatively high current and voltage recorded for MFC with exogenous mediators may be due to combined effect of the mediators, high carbohydrate and high moisture contents of the kitchen waste.

The performance of potassium ferricyanide [ $K_3Fe(CN)_6$ ] was the best on the average producing an initial current and voltage of 0.895mA and 0.47V respectively which continuously increased to the highest yield of 1.724mA and 0.81V after the 10th day. The performance of the mediator was probably due to the fact that ferri/ferrocyanide [ $Fe(CN)_6^{3-}$ ] is highly diffusible and can be easily reduced to its ferrous counterpart by the well-defined reversible reaction simultaneous to an increase in the redox potential of the solution [12]. However, the reduction noticed after the 10th day till the 21<sup>st</sup> day may be an indication that the ferricyanide requires continuous replacement after microbial utilization [29]. EDTA showed a promising performance recording the highest current (1.640mA) and voltage (0.74V) values within the first five days. Its degeneration to 0.037mA and 0.15V on the last day indicated that EDTA has been used up. Similarly, Methylene blue had an enhanced performance from 0.51V and 0.596mA on the 1st day to 0.64V and 0.816mA after

the 10th day (125 and 137% increment), thereafter degenerating steadily to 0.31V and 0.404mA.

Toxicity and relative expensive nature of many of the exogenous mediators that have been exploited are among the bottlenecks in their application [3,30,31]. Potassium permanganate ( $KMnO_4$ ) which continuously degenerated after reaching its peak value for current and voltage (0.509mA and 0.57V) on the 3rd day to 0.012mA and 0.14V on the 21st day may have been influenced by the toxicity of the mediator ( $KMnO_4$ ) as seen in the drastic reduction in the microbial load (Figure 2). Neutral red which produced the least current and voltage values on the average, ranging from 0.255mA and 0.29V to 0.356mA and 0.35V, maintained a near-stable generation of energy throughout the study. The low values resulting from the presence of methylene blue and neutral red in the MFCs may be due to the irreversible behaviour of these mediators based on the cyclic voltametric results [32]. Another reason may be due to the presence of some Gram-negative (the ethanol degrading) bacteria that react with MB, forming a distinctive metallic green sheen (seen as sediment at the base of the MFC reactor) due to the metachromatic properties of the dye [32]. The performance of the control MFC (kitchen waste without mediators) was the least [current, 0.26mA and voltage, 0.19V] compared to the performance of all the mediators. These results revealed that the various mediators encouraged energy generation at varying capacity with the trend of activity of the mediators being potassium ferricyanide > EDTA > methylene blue >  $KMnO_4$  > neutral red > control. Better performance of methylene blue to neutral red recorded in this study is in line with the result of [20] which documented the order of mediator potentials as bromocresol blue < neutral red < methyl red < methyl orange < methylene blue in terms of power generation in MFC.

Furthermore, the application of mediators in the generation of energy in this study also showed that the exogenous compounds exhibited antimicrobial effect on the electrogens in the MFC (Figure 2). The drastic reduction in the microbial load of the samples where mediators were added as against the microbial load of the control sample after 21 days can be attributed to inhibitory effects of the mediators. In this study, the trend of toxicity of the mediators presented as methylene blue < EDTA < neutral red < Ferricyanide <  $KMnO_4$  was considered based on the reduction in microbial population from 11.15 log<sub>10</sub> CFU/ml (fresh domestic waste) to 9.77 log<sub>10</sub> CFU/ml (with methylene blue), 9.65 log<sub>10</sub> CFU/ml (EDTA), 9.08 log<sub>10</sub> CFU/ml (neutral red), 8.31 log<sub>10</sub> CFU/ml (ferricyanide) and 8.03

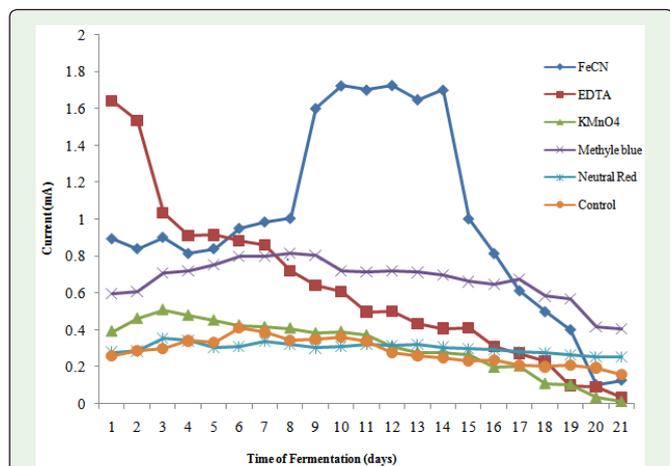


Figure 1: Current generated with exogenous mediators in MFC using kitchen waste as substrate.

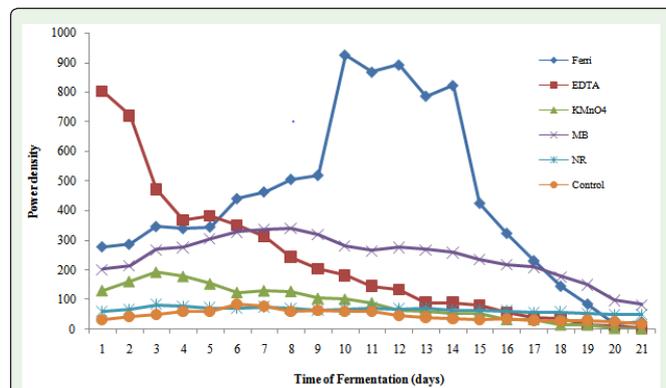
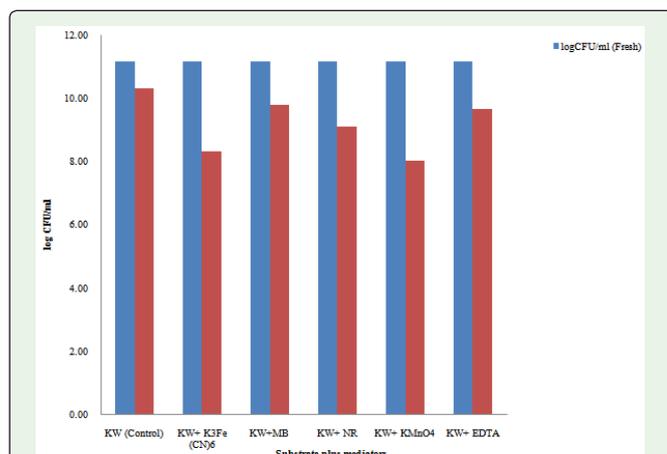


Figure 2: Power density generated with exogenous mediators in MFC using kitchen waste as substrate.



**Figure 3:** Antimicrobial effect of mediators on microorganisms in kitchen waste during fermentation.

**Key:** KW: kitchen waste; K<sub>3</sub>Fe (CN)<sub>6</sub>: potassium ferricyanide; MB: methylene blue; NR: neutral red; KMnO<sub>4</sub>: potassium permanganate; EDTA: Ethylene diamine tetra acid.

log<sub>10</sub> CFU/ml (KMnO<sub>4</sub>) respectively, in relation to that of control (kitchen waste without mediators) (10.28 log<sub>10</sub> CFU/ml) (Figure 3).

## Conclusion

The effect of different mediators on electricity generation by MFC was investigated. The generation of energy was enhanced at varying capacities with the trend being Potassium ferricyanide > EDTA > methylene blue > KMnO<sub>4</sub> > Neutral red > control using MFC. The various mediators displayed some toxicity or inhibition on the microorganisms. In this study, the trend of toxicity of mediators is presented as methylene blue < EDTA < Neutral Red < Ferricyanide < KMnO<sub>4</sub>. The investigation carried out on the effect of addition of mediators showed the importance of mediators and the detrimental effect on the survival of the electrogens and the longevity of the MFC.

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