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Authors' contributions

This work was carried out in collaboration between both authors. Author DNO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors DNO and OOO managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

Article Information

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ABSTRACT

Aim: This study investigates the physico-chemical and microbiological characteristics of a river mud (sediments) as substrates for biological generation of electricity.

Study Design: The study employed standard microbiological and physio-chemical experimental design and data interpretation.

Place and Duration of Study: Otamiri River, Etche Local Government Area of Rivers State, April to December, 2017.

Methodology: River sediments were collected from three different sampling stations at Otamiri River in Isu (E-510627.84; N-131566.60), Odagwa (E-520087.04; N-113278.48) and Imo River in



Umuebulu (E-521113.64; N-112436.74) in Etche Local Government Area of Rivers State, Nigeria. The samples from these stations were analysed using standard analytical methods.

Results: The results obtained show that the sediment had its particle size ranged from sand to silt and are porous. The pH of the sediments were acidic across the sampling points with the values ranging between 6.03 to 6.24, while conductivity values ranged from 53 to 75.4uS/cm. Nitrate content of the sediment ranged from 4.3 to 5.9mg/kg. The presence of organic content, phosphate, sulphate and total hydrocarbon were also recorded in good quantities. Microbial counts in sediment samples were carried out for both rainy and dry seasons. The results showed that the Total heterotrophic bacterial count from sediment samples from Otamiri at Isu River had more counts with 4.8x10⁶ colony forming units per gram of the sample while Odagwa River had 2.9x10⁶ cfu/g and Imo River had 2.2x10⁶ cfu/g during the rainy season. Fungal count was also higher at Isu River with 6.8x10⁵ cfu/g while Imo had the least count with 4.3x10⁵ cfu/g. Similar trend was observed in the dry season for all stations with Isu River recording the highest with 3.8x10⁵ cfu/g; Odagwa River had 3.2x10⁵ cfu/g while Imo River had 2.2x10⁵ cfu/g. Fungal count for dry season was similar with Isu River having the highest count while the Imo River at Umuebulu River recorded the least count in the sediment mud samples. The results of the microbial counts obtained shows that river mud (sediments) support microbial growth and can be used as a substrate to support microorganisms as biocatalysts for the conversion of organic and inorganic materials for alternative energy production. This is possible if the microorganisms in the sediments metabolise or convert the bio-wastes (organic and inorganic materials) to produce electrical energy. This trend can promote economic growth for domestic use and industrialisation to attain energy security and promote sustainable development.

Conclusion: It is concluded that the sediments can serve as substrates to support microorganisms as biocatalysts for the conversion of organic and inorganic materials to generate electricity if the microbial fuel cell technology is employed. This is because the results show that the physicochemical properties of the sediments and microbial populations found in the substrates can elicit adequate reactions to stimulate the production of alternative energy for electricity in this process.

1. INTRODUCTION

Rivers are the most important resource for man and socio- economic development because of its uses as sources of drinking water supply, irrigation of agricultural lands, industrial and municipal water supplies, industrial and municipal waste disposal, navigation, fishing, boating and body-contact recreation as well as aesthetic value. However, energy production and supply are challenging due to the depletion of fossil fuel. Presently, global energy requirements are mostly dependent on fossil fuel which eventually leads to the foreseeable depletion of limited fossil fuel resources [1,2]. Also, emission of global warming of gases such as CO₂ has been attributed to the combustion of fossil fuels. Concerns about climate changes and an increase in demand of energy resources are driving effort to search for alternative energy to fossil fuels [3]. Microbial energy technologies are an alternative to the fossil fuels that employ microbes for the conversion of chemical energy in the form of fuels (biogas, bioethanol,

biohydrogen) or directly to electricity by oxidation of organic substances [3]. Microorganisms are said to be ubiquitous and are known for essential functions such as the decomposition of organic materials, bioaccumulation of chemicals and biogeochemical cycling of elements. Their presence, abundance and growth in the environment are greatly influenced by factors such as substrate biodegradability, substrate concentration, pH, temperature, microorganisms used, area of the cell, anode and cathode material. For example, temperature changes could strongly affect microbial activity. Their dependence is linked to the kind of bacteria used and any species' response to temperature is characterised by upper and lower limits of temperature for growth [4]. When the temperature increases the rate of denaturation of particular cell components increases as well, with the consequent disruption of cellular function. On the contrary, when the temperature is too low a loss of efficiency of transport proteins embedded in the membrane, and thus a loss of affinity for substrates, occurs [2,5]. Temperatures below the

Keywords: Sediments; river mud; microorganisms; microbial fuel cells; otamiri river; biological generation of electricity; physico-chemical properties.

optimum typically have a more significant effect on growth rate than temperatures above the optimum [6].

The microbiota of freshwater and marine sediments serve similar roles in carbon degradation and nutrient regeneration. However, because of differences in the chemical environment between freshwater and marine systems, distinct physiological groups of bacteria dominate terminal carbon catabolism in each system. In general, the distribution and rates of microbial activities within a sediment are determined by availability of electron acceptors for respiration and metabolisable organic substrates. The oxidation of deposited organic matter, regeneration of inorganic nutrients and, in some cases, transformations of those inorganic materials, are generally attributed to the sediment microbiota [7]. The sources of organic matter to marine and freshwater sediments can be qualitatively different. Complex structural polysaccharides and phenolic polymers (i.e. ligno-cellulose) may comprise a greater fraction of the organic input to freshwater systems. Organic compounds that act as osmoregulatory solutes in marine plants and animals may be unique substrates for bacteria of marine sediments. It is likely that these differences also result in distinct assemblages of microorganisms responsible for the breakdown of organic carbon. The quantity of organic matter present in the sediments is also a major factor determining the magnitude and distribution of various microbial activities. Photoautotrophic processes of the water column supply the organic fuel to support the heterotrophic activities of the sediments. The sediments, in turn, may affect or regulate the production of new organic matter in overlying waters by the rate at which they oxidise organic material, thereby regenerating inorganic nutrients and returning them to the water column [8]

Microbial fuel cells (MFCs) are devices that use the natural metabolism of microbes to produce electrical power). Microbial fuel cell technology is very attractive because of wide range of potential applications, including energy recovery from wastewaters [6], Hydrogen production [7,8] and sulphide removal [9]. Within the MFC, microbes use sugars and other nutrients in the surrounding environment to release a portion of the energy contained within that food in the form of electricity. Bacteria inhabiting the sediment (river mud) oxidise organic compounds and supply electrons to the anode, while at the cathode, oxygen is reduced [5,6,7,8]. Microorganisms can be used to efficiently transform chemical energy into electrical energy [9]. Microorganisms in the anodic compartment utilise the biomass for growth-forming electrons and protons. These electrons can be transported out of cells using electron mediators or some microorganisms have the tendency to expel electrons for reducing the substrates which can be absorbed by electrodes. Several microorganisms have been reported to transfer electrons across the membrane by themselves to the anode [10]; they include Clostridium butyricum [11]. Shewanella putrefaciens [12], Geobacter sulferreducens [13], metallireducens Geobacter [14,15] and [13,16]. Rhodoferax ferrireducens These microorganisms are stable with high coulombic efficiency and can form film on the anode surface and transfer electrons directly to electrode across the membrane. These activities can be found in soil, river mud and decomposed organic materials. Within an oxygen-free environment, these bacteria are forced to use anaerobic (nooxygen) conditions to oxidise organic compounds such as glucose and acetate. The Sediment Microbial Fuel Cell (SMFC) therefore utilises the potential difference developed by the microbial oxidation of organic substances at the anode for the generation of electricity [11,17,18,19,20]. In this circumstance, mediators in the oxidised state are easily reduced by capturing electrons from within the membrane of microorganisms. These mediators then move across the membrane and release the electrons to the electrode and become oxidised again in the anodic chamber and thus are reutilised. Voltage gradient developed by the sediment microbes are utilised by the fuel cells to the connecting anode and cathode to an external load (resistance) capable of dissipating power at constant voltage. Microbes at the anode in anoxic conditions donates electrons (e-) to the electrode, whereas the protons (H+) are permeable through the sea sediment-water interface which acts as natural membrane instead of semipermeable membrane for power generation [21,22]. River Mud is rich in organic matter and have been considered as alternative carbon source. The aim of this study therefore is to determine the physico-chemical characteristics of a river sediment mud with their associated microbial flora, from a tributary of an Imo River in Etche Local Government Area of Rivers State, which hitherto could be used to generate electricity for sustainable development [23].

2. MATERIALS AND METHODS

2.1 Sediment Collection

Freshwater sediments and surface water samples were collected from three different locations in Etche Local Government Area of Rivers state. The samples were obtained in 20L sterilised air tight plastic containers. Sediments were collected anaerobically by immersing the plastic containers to the depth of 5cm from the surface of the sea bed. This is because electrode-reducing bacteria are usually present in the anaeorobic layer beneath 5cm from the surface of sediment [24]. One sample was collected from a tributary entering an Imo river popularly called Otamiri river at Isu Etche (E-510627.84; N-131566.60) while a second sediment sample was collected from another tributary of Imo river at Odagwa Etche (E-520087.04; N-113278.48) and the third sample was collected from another Imo river at Umuebulu (E-521113.64; N-112436.74) (Fig. 1). One litre surface water samples were also collected from each of the three sites. The collected samples were immediately transferred to the Institute of Pollution Studies Laboratory of the Rivers State University for simulation.



Fig. 1. Map of study area showing sampling stations Source: NDDC GIS laboratory.

2.2 Physico-chemical Analyses

2.2.1 Textural class of sediments

The sediment particle size was analysed by the Bouyoucos [25] hydrometer method modified by Day [26]. The sediment was dispersed with a solution of sodium hexametaphosphate (calgon 44g/L) and sodium carbonate (8 g/L). The pH of the solution was maintained at about 8.3. The textual class was determined using the triangular diagram.

2.2.2 pH and conductivity

The pH and conductivity of the sediment were determined by the method of Bates [27] Fifty grammes (50g) of sediment was diluted with 50 mL of distilled water in a 100 mL beaker to produce a ratio of 1:1 mixture. This was stirred with a stirring rod to homogenise the mixture and then left for 30 minutes to settle. pH and conductivity readings were taken by inserting their respective electrodes in the soil solution.

2.2.3 Nitrates, sulphates and phosphates

Nitrates, sulphates and phosphates in the sediment were analysed using APHA 4500-NO3B, APHA 4500-SO4B and APHA 4500 - P of APHA [28] Ten millilitres (10 mL) of sediment solution was transferred into sample cuvette. One reagent powder pillow was added to the contents, whether for the nitrates, sulphates and phosphates to complex the colours, if any of the nutrients were present in the sample. The readings (mg/L) were taken on the HACH DR 2400 spectrophotometer.

2.2.4 Total organic carbon content

Total organic carbon content of the sediment was determined using the Walkey - Black method of ASTM and APHA [28] This method is based on the theory that the colour of a soil sample determines the organic carbon content. Half the number (0.5 g) of sediment samples was sieved through a 2 mm mesh-size sieve and weighed into 250 mL conical flask. Ten millilitres (10ml) of potassium dichromate (K₂Cr₂O₇) and 20 mL H₂SO₄ were added and left to stand for 30 minutes on asbestos after intermittent swirls. One hundred mililitres (100 ml) of distilled water (spectator ion) was added. To this was added 3-4 drops of ferrous indicator and titrated with 0.5N FeSO₄. 7H₂O. If the soil sample is rich in organic carbon it will assume a greenish cast on adding

all reagents and indicators but if it is not rich in organic carbon it will assume an orange colour. Upon titration, an organic carbon rich soil goes from green to light green and finally to maroon red or brown; that is the end point. Total organic carbon was then calculated thus:

Organic Carbon (%) Titre value – Titre value × 0.195 (factor) $= \frac{\text{of blank of sample}}{\text{Weight of sediment sample (g)}}$

2.2.5 Total hydrocarbon content

Total hydrocarbon content of the sediment was determined using American Standard tests and method (ASTM D 3921) 1995.5 g of sediment sample after air -drying was sieved through a 2 mm mesh-size sieve. The essence of sieving was to obtain particle sizes of almost uniform diameter, increase surface area so as to enhance the contact/spreading of organic solvent within the soil matrix. Twenty five milliliter (25 ml) of chloroform was added to soil sample in a beaker, stirred carefully to allow for proper extraction of organic materials or extract. The organic extract was used to dehydrate the sample of any excessive moisture so as to avoid interference of moisture within the organic extract. Chloroform (CHCL₃) was used as blank and the organic extract as sample. The blank was inserted into the cell holder of the spectrophotometer. The blank was zeroed and removed from the cell holder. In turn, the sample was inserted: the concentration was obtained on the digital read-out of the spectrophotometer when it was stable.

Total hydrocarbon content was calculated as:

	Dilution x Spectrophotometer reading
$ruc\left(\frac{g}{g}\right)$	x volume of solvent
$\left(\frac{1}{\text{kg}}\right)^{-1}$	Weight of soil (g)

2.2.6 Bulk density and particle density

Bulk density and Particle density were determined by core method. Total porosity was calculated by substituting the bulk and particle densities into the formular below:

Total porosity =
$$1 - \frac{Bd}{Pd} \times \frac{100}{1}$$

Where
Bd = Bulk density g/cr

Bd	=	Bulk density g/cm ³
Pd	=	Particle density g/cm ³

2.3 Microbiological Analyses

The presence of various microorganisms in the sediments from the three sample stations were analysed using standard procedures. Ten-fold serial dilutions of the samples were made according to the methods described [29,30]. One millilitre each of the waste water samples was separately added to 9 ml of 0.1% peptone water diluents to give a 10⁻³ dilution. Aliquots (0.1 ml) of various dilutions were transferred to plates of surface dried nutrient agar in duplicates and inoculated by spreading with flamed glass spreaders and incubated at 37°C for 24 hours. Bacterial isolates were subjected to further identification according to determinative schemes of Cowan and Steel [31].

2.3.1 Total heterotrophic bacterial counts

This was determined with the nutrient agar using the spread plate technique as described by [32]. Here 0.1ml of the serially diluted samples was inoculated onto different sterile nutrient agar plates in triplicates. The plates were incubated for 24 hours at 37°C. After incubation, colonies that appeared on the plates were counted and the mean expressed as colony-forming units per gram (cfu/g) for the sediment samples.

2.3.2 Total coliform counts

The method of Cowan and Steel [32] was adopted where 0.1 milliliter of the serially diluted samples were inoculated onto different sterile MacConkey agar plates in triplicates, the inocula were then spread evenly on the surface of the media using a sterile spreader. This was followed by incubation at 37°C for 24 hours, after which the colonies were counted and the mean total coliform count expressed as cfu/g.

2.3.3 Total Salmonella-Shigella counts

This was determined with the Salmonella-Shigella agar using the spread plate method as described by Cowan and Steel [32]. One milliliter of the serially diluted samples was inoculated onto sterile pre-dried Salmonella-Shigella agar plates in duplicates. The inocula were then spread evenly on the surface of the media using a sterile spreader. The plates were then incubated at 37°C for 24 hours, after which the colonies that developed were counted and the mean total Salmonella-Shigella counts were recorded from sample from each station.

2.3.4 Total Vibrio count

Total *Vibrio* count was determined with the thiosulphate citrate bile salt (TCBS) agar using the spread plate technique as described [32]. One milliliter of the serially diluted samples were inoculated onto sterile pre-dried TCBS agar plates in triplicates and then spread evenly with a sterile bent glass rod. The plates were incubated at 37°C for 24 hours, after which the colonies that developed were counted and the mean recorded accordingly for sediment from each sampling station.

2.3.5 Total fungal counts

This was determined using the potato dextrose agar (PDA) onto which sterile streptomycin (50 mg/ml) had been added to suppress bacterial growth [33]. The spread plate technique as described [32] was adopted. An aliquot (0.1ml) of the serially diluted samples were inoculated in triplicates onto sterile pre-dried PDA plates and then spread evenly with a sterile glass spreader. The plates were incubated at room temperature for about 3-5 days after which the colonies were counted and the mean of the count recorded accordingly.

2.3.6 Total hydrocarbon utilising bacterial counts

The population of hydrocarbon utilising bacteria was determined by inoculating 0.1ml aliquot of the serially diluted samples onto mineral salt agar media using the spread plate technique as described by <u>Okerentugba and Ezeronye</u> [34]. A vapour phase transfer method of Mills and Colwell [35] was adopted. It employed the use of sterile filter paper discs soaked in filter-sterilised crude oil which served as the only carbon source in the mineral salt agar. The sterile crude oil-soaked filter papers were then aseptically transferred to the inside covers of the inoculated petri dishes and incubated for 5 days at room temperature. After the incubation period, mean of the colonies.

2.3.7 Total hydrocarbon utilising fungal counts

Total hydrocarbon utilising fungi was determined by inoculating 0.1ml of the serially diluted samples onto mineral salt agar using the method of Okerentugba and Ezeronye [34]. Eight hundred milliliter of the mineral salt medium was supplemented with 70mg of Aureomycin hydrochloride in 200ml of sterile distilled water as mentioned earlier [34]. A vapour phase transfer method of Mills and Colwell [35] was adopted. It employed the use of sterile filter paper discs soaked in filter-sterilised crude oil which served as the only carbon source in the mineral salt agar. The sterile crude oil-soaked filter papers were then aseptically transferred to the inside covers of the inoculated Petri dishes and incubated for 5-10 days at room temperature. After the incubation, mean count of the colonies from the triplicate plates were calculated and recorded accordingly

3. RESULTS AND DISCUSSION

3.1 Physico-chemical Characteristics

3.1.1 Particle Sizes of Sediment Samples

The results of the particle size of the sediment samples from the various Imo Rivers are presented in Table 1. The texture of the particle size of sediments in sample points ranged from sand to silt. The result indicated that the sediment is sandy in Otamiri- Odagwa River and Imo River and loamy sand in Otamiri River at Isu. Bulk densities of (2.43), (3.01) and (3.22) g/cm³ and particle densities of (5.00), (2.70) and (2.59) g/cm³ were recorded for Otamiri- Isu, Otamiri-Odagwa and Imo river respectively. This finding compared favourably with the observation of Mills and Colwell [36] who reported similar sediment particle size for Okpoka creek in the Niger Delta which also consists of sand, clay and silt.

The analysis of particle size for the three stations are presented in Table 1. The percentage values of sand recorded (80.24%), (83.82%), (90.64%); silt (10.17%), (7.48%), (2.42%); clay (15.72%), (6.96%), (7.00%) and porosity (63.24%), (79.34%), (75.22%). These values obtained in this study compared favourably with the observation of Mills and Colwell [36] who recorded mean values of 73, 97, 22 and 27% for sand, porosity, clay and silt respectively. However, it was observed that the percentage sand content of Imo River was higher than that of Otamiri River at Odagwa and Isu while the clay and silt contents of Otamiri- Isu River were higher than those of Otamiri Odagwa and Imo River. The percentage porosity of Otamiri-Odagwa River was higher, followed by Imo River and Otamiri Isu River. Also the bulk density of Imo River was higher than those of Otamiri-Odagwa and Isu River while particle density was highest at Otamiri- Isu River followed by Otamiri-Odagwa River and Imo River. These variations may be attributed to the fact that Otamiri- Isu and Odagwa are tributaries of the Imo River and are at some distance apart and their sediment loads may vary because they may have different parent materials as well as other deposits. The values obtained for sand, silt and clay samples could be attributed to high organic discharge due to regular sand mining activities in the Imo Rivers. Similar reports have been observed for Okpoka creek in the Niger Delta by Mills and Colwell [36].

The pH of the sediments ranged from 6.03 to 6.24 (Table 2). This indicates that pH of the river sediment is acidic across the stations. Imo River had 6.03, this was the most acidic, followed by Otamiri- Odagwa with 6.12 while Otamiri- Isu had 6.24. the least. Comparatively, the pH of the sediment obtained in this study differs with that reported by George et al. [37] for Minichida stream in the Niger Delta. This difference may be attributed to the fact that the Imo River is characterised by land drainage pollution arising from the presence of automobile and photographic workshops and other commercial activities around the area. This may further be attributed to the dredging activities and discharge of oils used by the dredging engine and indiscriminate discharge of wastes into the water body as Imo River at Umuebulu Etche is located within the urban centre of Rivers state, Nigeria [38,39]. Poor management of contaminated sediments due to industrial activities may

	Table 1. A	Analysis of	f sediment	particle size
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Parameters	Sampling stations									
	lsu	Odagwa	Imo river							
Sand (%)	90.44	85.82	82.24							
Silt (%)	2.22	8.48	11.17							
Clay (%)	7.50	6.96	14.72							
Porosity (%)	75.22	69.34	64.24							
Bulk Density(g/cm ³)	4.22	4.01	3.43							
Particle Density (g/cm ³)	3.59	3.70	5.50							
Textural Class	Sand	sand	Loamy sand							

contribute to the problem [40]. However, these levels of conductivities are fair for bioelectricity production. This agrees with the observations reported by other workers [57]. They maintained that low ionic conductivity limits the transfer of cations from anode to cathode and would retard the balance of electro-neutrality of the system. This means that the value obtained in this study is good enough for bioelectricity generation. Imo River had the highest value of 75.4µS/cm, followed by Otamiri- Odagwa River that had 60µS/cm while the least value of 53 µS/cm was obtained from Otamiri- Isu. All are freshwater sediments. The close variation between the conductivity of the sediment at the three different sampling stations may be attributed to the fact that water from the sampling stations is a freshwater. Similar reports had been made for Minichida stream in the Niger Delta [37].

The organic carbon content of Otamiri- Isu River had 6.463, Otamiri- Odagwa River 4.046 and Imo River 3.943%. The level of organic matter decomposition may be attributed to the variation in organic carbon content. Sediment however, is a major site for organic matter decomposition which is largely carried out by bacteria [41]. The sediment dwelling colony of bacteria are capable of degrading toxic organic pollutants arising from subsurface contamination of soil resulting from hydrocarbon pollution and its derivatives which is prevalent in the Niger Delta region occasioned by petroleum-related activity. Bacteria in a family of Geobacteraceae breaks down organic matter to obtain energy, and the ensuing process is now known to release a stream of electrons to minerals rich in iron [42]. Human activities in Isu and Odagwa Otamiri Rivers and Imo River may vary in terms of agricultural practice. Agricultural land drainage includes channelisation of water courses and such drainage schemes can have a considerable impact on hydrology, sediment load, water temperature, chemistry and aquatic biology of the river. Otamiri Isu River had the highest organic carbon content of 6.463% than Otamiri- Odagwa and Imo river stations with 4.046 and 3.943%, respectively. This difference may be attributed to the deposition of organic matter at the various stations. High density of microbenthic invertebrates in the drv season months had been reported significantly and this was attributed to the unstable nature of the substrate during the rainy season months arising from inputs of storm water thus accounting for the low density of organisms [43]. Similar observations have been reported in similar streams elsewhere [44,45]

The nitrate content of sediments is 5.9 mg/kg for Imo River, 5.4 mg/kg for Otamiri- Isu and 4.3mg/kg for Otamiri- Odagwa. These values are generally low and are attributed to the low nutrient level of the rivers. The Niger Delta is not essentially rich in nitrate [46]. Nitrate concentration is uniformly distributed in the three sampling points. This is attributed to similarity in sediment nutrient input from the drainage systems of the various sampling points. Land disturbing activities may cause introduction of large amounts of sediment into nearby streams and rivers.

The phosphate concentration was 16.0 mg/kg for Imo River, 16.20 mg/kg for Otamiri- Isu River, and 8.43mg/kg for Otamiri- Odagwa River. This compares favourably with the range of values of 0.29-244 mg/kg that was recorded earlier [36]. The phosphate concentration distribution follows the same pattern as nitrates. The factors which affected nitrate concentration also affect phosphate distribution in the stations. Similar observations have been reported that large tonnage of phosphate enters rivers and lakes through super-phosphate fertiliser from soil and from chemicals used to improve the performance of detergents [41]. Farm activities along this river need to be regulated to avoid algal bloom in this river. The sulphate concentration had 34.4mg/kg at Otamiri-Isu, 26.4mg/kg at Imo River and 27.7 mg/kg at Otamiri- Odagwa. This is in line with the mean sulphate concentration of 25.8 mg/kg in the sediments of Sombreiro River [47]. The values also compares favourably with the range of 36.7-92.9 mg/kg that was obtained [36]. The assertion that the Niger Delta is rich in sulphate is observed in the uniform distribution of sulphates in the three sampling points and across the stations of Sombreiro River as reported [45,47].

The Total Hydrocarbon content (THC) recorded in this study was 52.4 mg/kg for Imo River, 32.4 mg/kg for Otamiri- Odagwa River and 26.3 mg/kg for Otamiri- Isu River. This also compares favourably with the total mean values of hydrocarbon content obtained from sediments at Sombreiro River [47]. The presence of commercial activities especially transportation and frequent oil spillages in the Niger Delta waterways may be attributed to the occurrence of hydrocarbon content in the river mud (sediment) of the Imo Rivers. Hydrocarbon content in the sediment of the Imo River may produce adverse effect on the benthic communities. The presence of hydrocarbon in water may have adverse

Parameters	Sampling points								
	lsu	Odagwa	Imo River						
BH	6.24	6.12	6.03						
Conductivity (µS/cm)	53	60	75.4						
Organic Carbon (%)	6.463	4.046	3.943						
Nitrate (mg/kg)	5.4	4.3	5.9						
Phosphate(mg/kg)	16.2	8.43	16.0						
Sulphate (mg/kg)	34.4	26.4	27.7						
THC (mg/kg)	26.3	32.4	52.4						

Table 2. Physico-chemical properties of the sediments

effects on the phytoplankton community structure and abundance [46]. Higher concentrations of total hydrocarbon was observed in Imo River, followed by Otamiri Odagwa while the least value was observed in Otamiri- Isu River.

3.2 Microbiological Characteristics

The results of the population of various microorganisms in the sediments from the three sample stations at both rainy and dry seasons are shown in Tables 3 and 4 respectively.

The results of microbial counts in Table 3 revealed that sediment from the Otamiri River at Isu had more microorganisms followed by Otamiri-Odagwa River and Imo River except total *Salmonella-Shigella* counts that had higher counts in Odagwa River. The presence and population of these microorganisms were more in the rainy season than in the dry season. This is as a result of increased microbial load incorporated into the river from the surface soil through run off by the rain and the fact that more nutrients are washed into the river through leaching of the soil which eventually settles at the bottom of the river, leading to increased nutrient

levels which encouraged rapid multiplication of bacteria and fungi. This increase in microbial population may be due to nutrient enrichment and other environmental perturbations [48].

During the dry season, the sediment had higher total heterotrophic bacterial count of 4.2x10⁶cfu/g than the other samples as shown in Table 3. This could be as a result of destabilisation of the sediment ecological balance arising from contamination [49]. Another possible reason for this high bacterial count in the sediment during the period of the rainy season could be as a result of accumulation of wastes which are sources of microbial nutrients. The presence of this physiologic group in these samples could also be an indication of faecal contamination of the samples [31] arising from flooding and storm water percolation directly into the Imo River (water body). The presence of faecal materials may be possible sources since there is rearing of cows within Etche by Fulani herdsmen where cow dung is indiscriminately deposited within and around the place. Through surface run-off, some of the faecal materials are carried to the nearby water body, leading to the presence of coliforms in such water body.

Table 3. Microbial counts of sediment samples at rainy season (cfu/g)

Stations	THBC	TFC	THUB	THUF	тсс	TSSC	TVC
lsu	4.8 x 10 ⁶	6.8 x 10⁵	4.3 x 10⁵	3.3 x 10⁵	2.6 x 10 ⁶	7.8 x 10⁵	4.2 x 10⁵
Odagwa	2.9 x 10 ⁶	6.2 x 10⁵	3.8 x 10⁵	2.4 x 10⁵	2.1 x 10 ⁶	7.9 x 10⁵	3.3 x 10⁵
Imo River	2.2 x 10 ⁶	4.3 x 10⁵	4.2 x 10⁵	1.8 x 10⁵	1.6 x 10 ⁶	4.4 x 10⁵	2.1 x 10⁵

Key: THBC: Total heterotrophic Bacterial count, TFC: Total Fungal count. THUB: Total hydrocarbon utilising bacteria, THUF: Total hydrocarbon utilising fungal count, TCC: Total coliform count, TSSC: Total Salmonella- Shigella count, TVC: Total Vibrio count

Table 4. Microbial counts of sediment samples at dry season (cfu/g)

Stations	THBC	TFC	THUB	THUF	тсс	TSSC	TVC
lsu	4.2 x 10 ⁶	3.8 x 10⁵	2.2 x 10⁵	1.5x 10⁵	1.1 x 10 ⁶	2.5 x 10⁵	3.5 x 10⁵
Odagwa	2.6 x 10 ⁶	3.2 x 10⁵	1.4 x 10⁵	1.3 x 10⁵	8 x 10 ⁶	1.5 x 10⁵	1.4 x 10⁵
Imo River	2.2 x 10 ⁶	2.2 x 10 ⁵	1.2 x 10⁵	1.2 x 10⁵	0	1.1 x 10⁵	1.1 x 10⁵

Key: THBC: Total heterotrophic Bacterial count, TFC: Total Fungal count. THUB: Total hydrocarbon utilising bacteria, THUF: Total hydrocarbon utilising fungal count, TCC: Total coliform count, TSSC: Total Salmonella- Shigella count, TVC: Total Vibrio count

lso.	Tex.	Colour	Elev.	Transl	Gt	Shape	Ind	Cat.	Mot	Coa	Cit	MR	G	L	М	S	F	Identification
Α	Mucoid	Creamy	Raised	Opaque	+ve	Rod	-	+	+	-	+	+	+	-	-	-	-	Bacillus Sp
В	Moist	Yellow	Smooth	Translucent	+ve	Cocci	-	+	-	+	+	+	+	+	+	+	+	Staphylococcus sp
С	Moist	Milky	Smooth	Translucent	+ve	Cocci	-	+	-	-	+	+	+	+	+	-	-	Streptococci spp
D	moist	Light pink	Raised	Opaque	+ve	Cocci	+	+	-	-	+	+	+	-	+	-	-	Proteus sp.
Е	Moist	Creamy	Raised	Opaque	-ve	Rod	-	+	+	-	-	+	+	-	+	-	-	Salmonella sp
F	Smooth	Clear	Raised	Translucent	-ve	Rod	-	+	-	-	-	+	+	-	-	+	-	Shigella sp
G	Dried	Green	Flat	Opaque	-ve	Rod	+	+	+	-	+	+	+	-	+	+	-	Pseudomonas sp.
Н	Moist	pale	Raised	Opaque	-ve	Rod	+	+	+	-	-	+	+	-	+	+	+	Enterobacter sp.
Morp	Morphological and biochemical Characteristic of Isolates on SSA																	
Ι	Moist	Black center	Raised	Opaque	-ve	Rod	-	+	+	-	-	+	+	-	+	-	-	Salmonella sp
J	Smooth	Pinkish	Raised	Translucent	-ve	Rod	-	+	-	-	-	+	+	-	-	+	-	Shigella sp
Morp	hological	and biochemical Chara	cteristic of	f isolates on	TCBS	5												
Н	Moist	Yellow	Raised	Opaque	-ve	Rod	-	+	-	-	+	-	+	+	-	-	+	Vibrio sp.
K	Moist	Green	Raised	Translucent	-ve	Rod	-	+	-	-	+	-	+	+	-	+	+	Vibrio sp.
L	Moist	Deep green	Flat	Opaque	-ve	Rod	-	+	-	-	+	-	+	+	+	-	+	Vibrio sp.
Morp	hological a	ind biochemical Charac	cteristic of I	solates on EME	3													
М	Moist	Purple Metallic sheen	Raised	Opaque	-ve	Rod												E. Coli
Ν	Moist	Pinkish	Raised	Translucent	-ve	Rod	-	+	+	-	+	-	+	+	-	+	+	Klesiella sp.
0	Moist	pale	Raised	Opaque	-ve	Rod	+	+	+	-	-	+	+	-	+	+	+	Enterobacter sp.
Р	moist	Light pink	Raised	Opaque	-ve	Rod	+	+	-	-	+	+	+	-	+	-	-	Proteus sp.
Q	smooth	pinkish	Raised	Translucent	-ve	Rod	-	+	-	-	-	+	+	-	-	+	-	Shigella sp.
Morphological and biochemical Characteristic of Isolates on minimal salt																		
agar																		
R	smooth	Golden yellow	Raised	Opaque	+ve	cocci	-	+	-	+	+	+	+	+	+	+	+	Staphylococcus sp

Table 5. Biochemical characteristics of the isolates

Salmonella and Shigella were present in the samples collected from the three sampling stations. Their presence was not astonishing since they cohabit with coliforms in the intestinal tract of warm blooded animals. However, cow dung could be a good source of coliforms around the abattoirs. This could be due to the random and uncontrolled deposition of wastes including cow dung within and around the abattoir environment. This observation is in agreement with the result of [38] in his study of distribution of microorganism in water, soil and sediment from abattoir wastes from Southern Nigeria. Salmonella-Shigella counts from samples during the rainy season especially the Otamiri River at Odagwa was higher with 7.8x10⁵ cfu/g than other stations. This may be as a result of increased surface run-off from the abattoir into the river in the rainy season and it could probably be due to domestic activities that take place at different points around these abattoirs which can equally be a means of contaminating the environments outside the abattoirs with pathogenic organisms like Salmonella and Shigella.

Vibrio was also isolated from the sediment samples from the Imo River at Umuebulu during the dry season. This indicates that the sampling points across the area was impacted by human activities. However, Vibrio was isolated from sediment from Imo River during the dried which recorded low count of season. 1.1x10⁵cfu/q. Vibrio was found in the other sampling points both in rainy and dry seasons, but were more during the rainy season. It is possible that the samples were contaminated with organic wastes that were devoid of Vibrio species. This result was similar to the observations [38]. The relatively high incidence Klebsiella. Enterobacter. of Escherichia. Salmonella, Shigella, Citrobacter, Serratia and Proteus around the sampling points may be connected with a high rate of cattle defecation around the rivers each time they graze near the rivers to drink water.

The presence of *Geobacter sulfurrenducens*, *Geobacter metallireducen*, and *Rhodoferax ferrireducens* indicates the presence of fossil fuel decomposing resources and microbial oxidation of organic substances [2,13,17,18]. The introduction of wastes from the abattoir and the surface run-off into the sites and nearby rivers during the rains are also contributory factors [49]. Their presence in this study gives credence to these findings. The isolation of *E. coli* and other coliforms is an indication of recent human contamination of the sampling points, and is of great public health concern [49; 50]. The organism is mostly a soil inhabitant and its presence could be as a result of contamination from overland runoff and organic matters in the rivers.

The presence of Pseudomonas, Acinetobacter, and Lactobacillus around the rivers is possible since they have been reported to be agents of meat spoilage [51]. Occurrence of Pseudomonas sp as a heterotrophic and hydrocarbon utilising bacteria has been reported [52]. The presence of Pseudomonas sp within the environment is probably due to the presence of hydrocarbons (PAHs) from oil spillages within the study area. This observation supports the report that Pseudomonas sp is widespread in the environment and concluded that they could contribute to the oxidation of hydrocarbons in the environment [53]. The same reason is applicable to Achromobacter and Acinetobacter, which are among the hydrocarbon degraders. The incidence of Staphylococcus species in this study is also in agreement with the report that Staphylococcus species is naturally found in the hides of cattle and *Flavobacterium* which is said to be authochthonous to the environment [54].

4. CONCLUSION

This study illustrates that that river mud (sediments) could be used as a MFC feedstock for electrical energy recovery because the microorganisms in the sediments are capable of converting organic matters in waste streams directly into electricity. The high organic matter content makes sediments a desirable MFC feedstock for this purpose. River mud (sediments) can act as self-supporting electrolyte and could be used for energy recovery given that the ionic conductivity of a river mud can lead to an increased MFC performance. River mud can act as self-supporting electrolyte and could be used for energy recovery given that the ionic conductivity from 43 to 73.4 µS/cm obtained in this study is fair enough for bioelectricity production. The microbes could degrade or complex such transform materials as polysaccharides, lipids and peptidoglycans to simpler molecules, which in turn serve as the substrates for the electrogenic bacteria that can either accept or donate electrons to the electrode. This study realises that if these compounds in sediments are employed in the microbial fuel cell technology, electrons from these sediments can be transferred to bacteria to

the anode and to the cathode as they diffuse into the water column to generate electricity.

DISCLAIMER

This paper is based on the preliminary dataset. Readers are requested to consider this paper as preliminary research article. Authors are aware that detailed statistical analysis is required to get a scientifically established conclusion. Readers are requested to use the conclusion of this paper judiciously as statistical analysis is absent. Authors also recommend detailed statistical analysis for similar future studies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- 1. Amann CA. Alternative fuels and power systems in the long term. Int. J. Vehicle Des. 1996;17:510-517.
- Das D, Veziroglu TN. Hydrogen production by biological process: a survey on literature. Int. J. Hydrogen Energy. 2001;26:13-58.
- Logan BE. Biologically extracting energy from wastewater: Biohydrogen production and microbial fuel cells. Environ. Sci. Technol. 2004;38:160-167.
- Ferrentino R, Langone M, Andreottola G Temperature effects on the activity of denitrifying phosphate accumulating microorganisms and sulphate reducing bacteria in anaerobic side-stream reactor. Journal of Environment and Bio Research; 2017.
- Nedwell DD. Effect of low temperature on microbial growth: lowered affinity for substrates limits growth at low temperature. FEMS Microbiol Ecol. 1999;30:101-111.
- Mulkerrins D, Dobson ADW, Colleran E. Parameters affecting biological phosphate removal from wastewaters. Environ Int. 2004;30:249-259.
- Jarregensen L, Dicks A. Fuel cell systems explained, 2nd Ed, West Sussex, England: Wiley. 2006;331-336.
- Rabaey K, Verstraete W. Microbial fuel cells: Novel biotechnology for energy generation. Trends Biotechnol. 2005;23: 291-298.

- Kim BH, Kim HJ, Hyun MS, Park DH. Direct electrode reaction of Fe (III)reducing bacterium, Shewanella putrifaciens. J. Microbiol. Biotechnol. 1999;9:127–131.
- Park HS, Kim BH, Kim HS, Kim HJ, Kim GT, Kim M, Chang IS, Park YK, Chang HI. A novel electrochemically active and Fe (III)-reducing bacterium phylogenetically related to Clostridium butyricum isolated from a microbial fuel cell. Anaerobe. 2001;7:297–306.
- 11. Kim HJ, Park HS, Hyun MS, Chang IS, Kim M, Kim B. H mediatorless microbial fuel cell using a metal reducing bacterium, Shewanella, putrefaciens. Enzyme. Microb. Tech. 2002;30:145–152.
- Bond DR, Lovley DR. Electricity production by Geobacter sulfurreducens attached to electrodes. Appl. Environ. Microbiol. 2003;69:1548-1555.
- 13. Bond DR, Holmes DE, Tender LM, Lovley DR. Electrode-reducing microorganisms that harvest energy from marine sediments. Science. 2002;295:483-485.
- 14. Min B, Cheng S, Logan BE. Electricity generation using membrane and salt bridge microbial fuel cells. Water Res. 2005;39:1675–1686.
- 15. Chaudhuri SK, Lovley DR. Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells. Nat. Biotechnol. 2003;21:1229-1232.
- 16. Park DH, Zeikus JG. Improved fuel cell and electrode designs for producing electricity from microbial degradation. Biotechnol. Bioeng. 2003;81:348–355.
- Rabaey K, Lissens G, Siciliano SD, Verstraete W. A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency. Biotechnol Letters. 2003;25:1531–1535
- Reimers CE, Tender LM, Ferig S, Wang W. Harvesting energy from the marine sediment–water interface. Environ. Sci. Technol. 2001;35:192–195.
- Tender LM, Reimers CE, Stecher III, HA, Holmes DE, Bond DR, Lowy DA, Pilobello K, Fertig SJ, Lovley DR. Harnessing microbially generated power on the seafloor. Nat. Biotechnology. 2002;20(8): 821-825.
- 20. Logan BE, Murano C, Scott K, Gray ND, Head IM Electricity generation from cysteine in a microbial fuel cell. Water Resources. 2005;39:942–952.

- Lowy DA, Tender LM, Zeikus JG, Park DH, Lovley DR. Harvesting energy from the marine sediment-water interface II – Kinetic activity of anode materials. Biosens. Bioelectron. 2006;21: 2058–2063.
- Tender LM, Gray SA, Groveman E, Lowy DA, Kauffman P, Melhado J, Tyce RC, Flynn D, Petrecca R, Dobarro J. The first demonstration of a microbial fuel cell as a viable power supply: Powering a meteorological buoy. J. Power Sources. 2008;179:571–575.
- 23. Saravanan P, Pakshirajan K, Saha P. Hydrodynamics and batch biodegradation of phenol in an internal loop airlift bioreactor. Int. J. Environ. Eng. 2010;2: 303-315.
- 24. Day JA. A monograph on the polychaeta of Southern Africa. Part 1 Errantia. British Museum of Natural History, London. 1967;458.
- 25. Bouyoucos GH. A recalibration of the hydrometer for making mechanical analysis of soils. Agronomy Journal. 1961;43:434-438.
- Bates RG. Electronic pH determinations. John Wiley and Sons Inc., New York; 1954.
- 27. APHA. Standard Methods for the Examination of Waste Water. 14th Edn., APHA, AWIWA -WPCHCF, Washington; 1995.
- Ogbonna DN, Ideriah TJK. Effect of Abattoir wastewater on the physicochemical characteristics of soil and ediment in Southern Nigeria. Journal of Scientific Research and Reports. 2014;3(12):1612-1632
- 29. Oliveira HMB, Santos C, Russell R, Paterson M, Gusmão NB. Fungi from a groundwater-fed drinking water supply system in Brazil. International Journal of Environmental Research and Public Health. 2016;13:304-315.
- 32. Cowan ST, Steel KJ. Manual of the identification of medical bacteria. 2nd edition Cambridge University Press, Cambridge; 1994.
- Prescott LM, Harley JP, Klein DA. Microbiology. 6th ed. McGraw Hill, London. 2005;135-140.
- 34. Okerentugba PO, Ezeronye OU. Petroleum-degrading potentials of single and mixed microbial cultures isolated from rivers and refinery effluents in Nigeria.

African Journal of Biotechnology. 2003;2(9):288-292..

- Odokuma LO. The techniques in aquatic microbiology. In: Onyeike, E.N. and Osuji, J.O. (eds), Research Techniques in Biological and Chemical Sciences. 1st ed. Springfield Publishers Ltd, Owerri. 2003;156-173.
- Mills AL, Colwell RR. Enumeration of petroleum-degrading marine and estuarine microorganisms by the most probable number method. Canadian Journal of Microbiology. 1978;24:552-557.
- George ADI, Abowei JFN, Allison ME. The sediment characteristics of Okpoka Creek, Niger Delta, Nigeria. Asian Journal Agricultural Science. 2010;2(1):9-14.
- Braide SA, Izonfuo WAL, Adakwu PU, Chinda AC, Obunwo CC. Water quality of miniweja stream, a swamp forest stream receiving non-point source waste discharge in Eastern Niger Delta, Nigeria. Sci. Afric. 2004;3(1):1-8.
- Ogbonnaya C. Analysis of groundwater pollution from Abattoir waste in Minna, Nigeria. Research Journal of Dairy Sciences. 2008;2(4):74-77.
- Ogbonna DN, Ajubo TA Assessment of the Impact of Municipal Sewage Disposal on the Water Quality in Obio/Akpor LGA, Rivers State. IIARD International Journal of Geography and Environmental Management. 2017;3(1):13-22.
- 41. Obande OO. Construction of Sediment battery from River mud for Biological Generation of Electricity. MSc Thesis. Rivers State University, Nkpolu-Oroworukwo, Port Harcourt. 2017;62
- Prygiel J, Rosso-Darmet A, Lafont M, Lesmak C, Durbec A, Ouddane B. Use of oligochaete communities for assessment of ecotoxicological risk in fine sediment of Rivers and Canals of the Artois-Picardie Water Basin (France). Hydrobiologia. 2000;410:25-37.
- 43. Abowei JFN, Sikoki FD. Water pollution management and control. Double Trust Publications Company, Port Harcourt. 2005;236.
- Ikomi RB, Arimoro FO, Odihirin OK. Composition, distribution and abundance of macroinvertebrates of the upper reaches of River Ethiope, Delta State, Nigeria. The Zoologist. 2005;3:68-81.
- 45. Edokpayi CA, Okenyi JC, Ogbeibu AE, Osimen EC. The effect of human activities on the macroinvertebrates of Ibekuma

Stream, Ekpoma, Nigeria. Biosci. Res. Commun. 2000;12(1):79-86.

- 46. Tumwesigye C, Yusuf SK, Makanga B. Structure and composition of benthic marcoinvertebrate of a tropical Forest Stream. River Nyanweru, Western Uganda. Afr. J. Ecol. 2000;38(1):72-77.
- Chinda AC, Braide SA. Epipelic algae of tropical estuary: Case of stable and invariable seasonally community. Pol. J. Ecol. 2003;51(1):91-99.
- 48. Ezekiel EN. Comparative studies of the flood plains and Major Rivers in Odiokwu -Ekpeye, Niger Delta. M.Sc. Thesis, University of Port Harcourt, Choba. 2001;77.
- Adesemoye AO, Opere BO, Makinde SCO. Microbial content of abattoir waste water and its contaminated soil in Lagos, Nigeria. African Journal of Biotechnology. 2006;5(20):1963-1968.
- 50. Ezeronye OU, Ubalua AO. Studies on the effect of abattoir and industrial effluents on

the heavy metals and microbial quality of Aba River Nigeria. African Journal of Biotechnology. 2005;4(3):266-272.

- Ezeama CF, Nwamkpa F. Studies on the longitudinal profile of the bacteriological quality of Aba River, Nigeria. Global Journal Pure & Applied Science. 2002;8(4):469-473.
- 52. Frazier WC, Westhoff DC. Food microbiology, 4th ed. Tata McGraw-Hill Publishing Company Ltd. New Delhi. 2003;218 242.
- Loureiro STA, Calvalcanti MAD, Neves RP, Passavante JZD. Yeasts isolated from sand and sea water in beaches of Olinda, Pernambuco state, Brazil. Brazillian Journal of Microbiology. 2005;36:333-337.
- 54. Faria D, Bharathi, L. Marine and estuarine methylotrophs: Their abundance, activity and identity. Current Science. 2006;90(7): 984-989.

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