Journal of Science Research

Isolation, screening and anodic biofilm potentials of electrogenic microorganisms from urine and waste water samples

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Abstract

Microbial electrogenicity studies represent a new dimension in the development of renewable energy especially bioelectricity and other bioelectrochemical energy applications. Bioprospecting for electrogenic species that can subsequently be applied in this regard is ongoing in this budding field. In this study, bacteria were isolated from 5 human urine and 5 waste water samples collected within the campus of Modibbo Adama University of Technology in Yola. Pure cultures were screened for electrogenic activities by the 120 hour-Open Circuit Voltage (OCV) versus time determination using glucose minimal salt medium in a dual-chambered microbial electrochemical reactor connected to a digital mutimeter system. Anodic biofilm production by the electrogenic isolates was determined using the crystal violet binding assay method. A total of 37 bacteria were isolated from the samples out of which nine (9) were confirmed to be naturally electrogenic, while artificial electrochemical inducements using methylene blue as a redox mediator revealed that 16 of them were sub-electrogenic and 12 were non-electrogenic. The electrogenic isolates were identified as *Pseudomonas* species (3 isolates with OCV of 351.00±1.73mV; 288.67±2.52mV; 282.33±3.21mV) followed by *Enterobacter* species (221.00±2.65mV, 202.33±2.52mV), *Bacillus* species (182.00±2.65mV, 178.33±2.08). The least electrogenic isolates were identified as *Aeromonas* species with OCV values of 128.00±4.36mV and 106.33±0.58mV. Anodic biofilm production was highest with *Pseudomonas* species followed by *Enterobacter*, *Aeromonas* and *Bacillus* species respectively. The anodic biofilm production yield was an indication of the electrogenic mechanism adopted by the isolates. These strains can be further studied along the line of electrogenicity to enhance favourable application in bioelectricity generation.

Keywords: Electrogenic; anode; biofilm formation.

Introduction

Bacteria with the ability to transfer electrons outside their cellular structure onto an external material which is not an immediate electron acceptor are regarded as electrogenic in nature [1]. This ability has been tapped into in a wide variety of research works more frequently in recent times mostly in bioelectricity generation [2-4]. However, the prospects of this area of research are greatly dependent on our knowledge of electrogenic properties within microbial species around as well as their diversity and abundance.

Electrogenic species have earlier been isolated and identified and a basic insight into their mechanism of electron transfer has been researched upon [5, 6]. Kim [7], Park and Zeikus [8] and Ringeisen [9] identified *Shewanella* species as a major electrogenic bacteria and also determined their electrogenic capabilities. Electrogenic species like *Desulfuromonas*, *Desulfobulbus* and *Geobacter* have also been isolated and earlier characterized [10, 11]. *Geobacter* and *Shewanella* species have been the most researched-species, however, there are reports of the inadequacy of electrogenic characterization with regards to several environments as vast uncharacterized communities are still largely unknown, thus showing our inadequacy in knowledge of electrogenic species [4].

In line with the above, the search for species with electrogenic potentials within various environments and

climes is still an active one as well as the mechanisms governing electron transfer within species remains a subject of speculation as far as the species are diverse [12]. Community analyses of bacterial colonization on anodes of microbial electrochemical reactors, show the valuable contributions of biofilm development and structure on the electrogenic capabilities of the colonizing bacteria [1, 13], thus the claim is that biofilm structure and function by electrogenic organisms that dissipate electrons onto an anode within a bioelectrochemical reactor is a valid point of research [14].

This work seeks to search for bacteria with electrogenic capabilities from urine samples and waste water sites within a university campus in Nigeria as well as characterize their electrogenicity in line with their anodic biofilm formation potentials.

Materials and methods

Sample collection

Five (5) waste water samples were collected from sites within the campus of Modibbo Adama University of Technology in Yola in northern Nigeria. Five (5) urine samples were also collected from 3 female and 2 male adult students of the university. The samples were collected in 20 ml sterile plastic bottles before transferring to the laboratory for analysis.

Isolation of microorganism

Bacteria isolation from the samples was carried out using a 10-fold serial dilution and by pour plate technique onto nutrient agar. Pure cultures of isolated bacteria were stored in agar slants at 4°C.

Inoculum preparation

After the serial dilutions and isolation, the inoculum with the dilution factor 10^{-2} which had CFU values ranging from $4.2 \times 10^2 - 6.9 \times 10^2$ CFU/ml for all isolates were used for the electrogenic screening.

Electrogenic screening of isolates

This was carried out with modifications to the method of Biffinger [15] using a dual chambered microbial electrochemical reactor. The reactor specifications include a graphite anode/cathode, 150 ml anodic and cathodic chamber volume, 74.22 cm^2 electrode surface area, copper wire extensions from electrodes to a digital multimeter (model DM-87, HTC Instruments®), agar-agar membrane, 21.21 cm^3 membrane volume, and polymethyl metacrylate (PMMA) chamber material. The PMMA reactor was sterilized according to the protocol of Sharma [16] by a combination of treatment with 75% ethanol and ultraviolet radiation (254 nm UV-C; dose – 5000µW.s/cm^2) for 30 minutes at an exposure distance of 10 cm. For electrogenic screening, a glucose minimal salt media was prepared with the following composition: Glucose – 10g/l, $(NH_4)_2SO_4$ – 2g/l, KH_2PO_4 – 1g/l, K_2HPO_4 – 0.4g/l, $MgSO_4.7H_2O$ – 0.5g/l, $FeSO_4$ – 0.01g/l. the media was sterilized at 121°C for 15 minutes. The sterile glucose minimal salt media (pH 7.2) along with a prepared inoculum of each test isolate were aseptically introduced into the anode chamber, and a preparation of 0.5% potassium permanganate solution was used as the catholyte. The open circuit voltage readings were monitored via the digital multimeter at 12 hours interval for a total of 120-hr. Electrogenic isolates were regarded as isolates that produced a maximum OCV value of >100mV in a 120h run. To determine isolates with sub-electrogenic capabilities, isolates were subjected to a sub-electrogenic screening using methylene blue solution (30g methylene blue + 300ml 95% ethyl alcohol + 1sterile distilled water) as an artificial mediator to facilitate electrogenesis.

Identification of microorganism

Pure cultures of the electrogenic bacterial isolates were also subjected to standard microscopic and biochemical characterization. Characteristics of isolates were compared with that of known microbial identities [17].

Determination of anodic biofilm formation by isolates

The crystal violet binding assay as described by Stepanovic *et al* [18] was employed in the assessment of anodic biofilm formation by electrogenic isolates. At the end of the 120-hr electrogenic screening, the anodes were removed from the anodic chamber reactor set-up and washed thrice with sterile distilled water. The adhered cells on the anodes were then fixed with 2.5 ml of 95% ethanol. The anode was then stained with crystal violet for 12 minutes, and the excess stain was washed off with flowing water. The anodes were left to dry for about 3 minutes after which each anode with biofilm attached dye was re-solubilized with 3 ml of 30% glacial acetic acid, then the liquid from the re-solubilization was transferred into a cuvette for spectrophotometric determination of the optical density at 600nm.

Statistical analysis

Electrogenic screening was conducted in triplicates and

the standard deviation and mean values were calculated.

Results

A total of 37 bacteria were isolated from the samples and nine (9) isolates were confirmed to be naturally electrogenic, while 16 of them were sub-electrogenic and 12 were totally non-electrogenic. Table 1 shows the results of the electrogenic/sub-electrogenic screening with maximum OCV at 120-hr. Eight (8) of the electrogenic species were isolated from waste water samples while one (1) was isolated from urine sample. The electrogenic species were presumptively identified based on their morphology and biochemical properties as *Pseudomonas* species, *Bacillus* species, *Aeromonas* species and *Enterobacter* species (Table 2). The anodic biofilm production properties of these isolates was determined and the results showed that *Pseudomonas* species were the highest biofilm producers (0.9 OD at 600mn) followed by *Enterobacter* species (0.86 OD), *Aeromonas* species (0.51 OD), and *Bacillus* species (0.39 OD) respectively (Figure 1).

Table 1. Open circuit voltage readings of isolates screened for electrogenicity.

Isolate Code	Source	Electrogenic screening (mVolts)	Sub-electrogenic screening (mVolts)	Electrogenic status
MT02	Urine	22.33±1.53	200.33±0.58	**SE**
MT03	Urine	35.00±0.00	258.00±4.36	**SE**
MT04	**Waste water**	**128.00±4.36**	260.00±1.00	E
MT05	Waste water	20.33±2.31	119.00±1.00	**SE**
MT06	Waste water	26.67±3.51	130.67±2.08	**SE**
MT07	Waste water	4.67±1.53	62.33±2.08	NE
MT08	**Waste water**	**351.00±1.73**	417.67±3.79	E
MT09	Waste water	30.00±0.00	71.33±1.53	NE
MT10	Urine	12.67±0.58	148.33±2.08	**SE**
MT11	Urine	0.00±0.00	51.33±6.11	NE
MT12	Waste water	0.00±0.00	43.00±3.00	NE
MT13	**Waste water**	**182.00±2.65**	300.00±1.00	E
MT14	Urine	0.00±0.00	36.00±3.00	NE
MT15	Waste water	31.33±1.53	162.33±2.08	**SE**
MT16	Urine	26.33±1.53	130.00±0.00	**SE**
MT17	Urine	0.00±0.00	0.00±0.00	NE
MT18	Waste water	52.00±2.00	113.00±2.00	**SE**
MT19	**Urine**	**288.67±2.52**	380.67±1.15	E
MT20	**Waste water**	**202.33±2.52**	327.67±2.08	E
MT21	Waste water	0.00±0.00	14.67±1.53	NE
MT22	Waste water	27.33±1.53	300.00±5.00	**SE**
MT23	Waste water	66.00±1.00	350.00±1.00	**SE**
MT24	**Wate water**	**178.33±2.08**	302.33±3.21	E
MT25	Waste water	13.67±1.15	91.33±1.53	NE
MT26	Waste water	0.00±0.00	0.00±0.00	NE
MT27	Waste water	0.00±0.00	40.67±1.15	NE
MT28	**Waste water**	**106.33±0.58**	222.00±1.00	E
MT29	Urine	60.33±1.53	129.33±2.08	**SE**
MT30	Urine	79.00±2.65	202.00±3.46	**SE**
MT31	**Water water**	**282.33±3.21**	361.33±1.53	E
MT32	Wate water	30.00±0.00	118.33±2.08	**SE**
MT33	Waste water	0.00±0.00	0.00±0.00	NE
MT34	**Waste water**	**221.00±2.65**	350.67±7.02	E
MT35	Urine	24.67±4.16	202.67±4.62	**SE**
MT36	Urine	83.00±2.00	182.67±3.51	**SE**
MT37	Urine	93.33±3.51	104.33±4.16	**SE**

Triplicate values were obtained and the mean ± standard deviation was calculated and recorded.
Key: E – electrogenic, SE – sub-electrogenic, NE – non-electrogenic.

Table 2. Morphological and biochemical characteristics of the electrogenic isolates obtained from urine and waste water samples.

Isolate Code	Gram reaction and Morpho-logy	O_2 Preference	H_2S prod.	Spore	Motility	Growth on MAC	Pigment on Nut. agar	Growth in MSA	Growth on Indole	Catalase	Citrate utilisation	Oxidase	M R	Coagulase	VP	Urease	Nitrate reduction	Glucose	Lactose	Mannitol	xylose	Maltose	Probable organism
MT 13	Gram positive large bacilli in chains	Aerobic	ND	+	+	-	-	-	-	-	+	+	-	-	-	+	-	+	+	-	-	-	-	*Bacillus* sp
MT 24	Gram positive baciili	Aerobic	ND	+	+	-	-	-	-	-	+	+	-	-	-	+	-	+	+	-	-	-	+	*Bacillus* sp
MT 34	Gram negative bacilli	Faculta-tive anaerob ic	-	-	+	+	-	-	-	-	+	+	-	-	-	-	-	ND	+	+	+	+	+	*Entero-bacter* sp
MT 20	Gram negative bacilli	Faculta-tive anaerob ic	-	-	+	+	-	-	-	-	+	+	-	-	-	-	-	ND	+	+	+	+	+	*Entero-bacter* sp
MT 08	Gram negative bacilli	Faculta-tive anae-robic	-	-	+	+	+	+	+	-	+	-	+	-	-	-	-	+	+	-	+	+	-	*Pseudo-monas* sp
MT 28	Gram negative bacilli	Faculta-tive anae-robic	+	-	+	-	-	-	-	+	+	-	+	-	-	+		+	+	-	-	-	-	*Aeromo-nas* sp
MT 19	Gram negative bacilli	Faculta-tive anaerob ic	-	-	+	+	+	+	+	-	+	-	+	-	-	-	-	+	+	-	+	+	-	*Pseudo-monas* sp
MT 31	Gram negative bacilli	Faculta-tive anaerob ic	-	-	+	+	+	+	+	-	+	-	+	-	-	-	-	+	+	-	+	+	-	*Pseudo-monas* sp
MT 04	Gram negative bacilli	Facultat ive anaerob ic	+	-	+	-	-	-	-	+	+	-	+	-	-	+		+	+	-	-	-	-	*Aeromona s sp*

Key: MAC – MacConkey agar; MSA – Mannitol salt agar; ND – Not determined; VP – Voges Proskaeur.

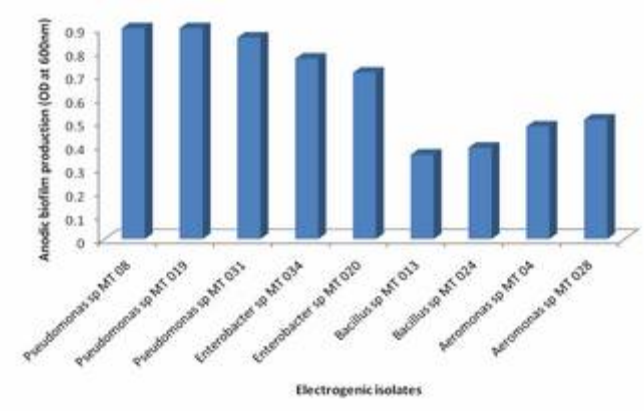

Figure 1. Anodic biofilm production by electrogenic isolates.

Discussion

The diversity of electrogenic species in nature are still being studied. The nine (9) bacteria isolated namely *Pseudomonas* species, *Enterobacter* species, *Bacillus* species and *Aeromonas* species, were isolated from urine and waste water samples. The occurrence of electrogenic bacteria within many environmental sites and samples show that a great deal of metabolism and activity are responsible for electrogenesis within these organisms. Results of bacterial isolation in this work were in agreement with previous research. Phung *et al* [19] identified electrogenic microorganisms from river sediments with about 65% as alphaproteobacteria, 21% betaproteobacteria, 3% gamaproteobacteria, about 8% bacteriodetes and 3% for other bacteria classes. From waste water samples Lee *et al* [20], Methe *et al* [21] and Kim *et al* [22] isolated bacteria and classified them as Alphaproteobacteria, Cytophaga, *Flexibacter*, *Bacterioides*, deltaproteobacteria, gammaproteobacteria, and betaproteobacteria. Recently, more diverse microbial species like *Advenella kashmirensis* and *Desulfovibrio aminophilus* have also been implicated as electrogenic species from waste water sources [23]. This thus shows that there is a greater diversity of electrogenic bacteria than earlier understood, thus leaving a wider scene for biodiversity research on electrogens, especially with respect to pure/axenic culture.

In line with isolation of pure culture of electrogenic species, the presence of electrogenic *Pseudomonas* species within the samples tested in this work was also in agreement with the research of [24] which characterized *Pseudomonas aeruginosa* strain as a potent electrogenic strain. Rabaey and co-researchers also had a deeper experimentation into the potentials of *Pseudomonas* species for electrogenicity [25, 26]. The mechanisms of electrogenicity of *Pseudomonas* species were also understudied by Hernandez *et al* [27] and Voggu *et al* [28], though this area of research has been subjected to a lot of investigation recently [12]. The isolation of electrogenic *Enterobacter* species in this work is corroborated by the research of Feng *et al* [29] which characterized the ability of *Enterobacter* species to transfer electrons to an electrode. A variety of nutrient sources/substrates were investigated with *Enterobacter* species to properly characterize the electrogenicity of the species, and it showed in their work that the species were truly electrogenic under all conditions of test.

Pham *et al* [30] worked on isolating and characterizing an *Aeromonas hydrophila* as an electrogenic specie under Iron reducing conditions. They explained that the specie which they first characterized at that time as electrogenic was proven to be functional in electron excretion onto the electrode. Their work is in correlation with the results of isolation of electrogenic *Aeromonas* species as obtained during the course of this research as the species were proven to be electrogenic from the results of the electrogenic screening carried out. *Bacillus* species isolated in this work were also in agreement with the research of Saravanakumar and Angel [31] that identified *Bacillus* species and Yoganathan and Ganesh [32] who identified *B. subtilis* and *B. megaterium* as electrogenic species and applied them in bioelectricity generation. The isolates were grown on glucose-augmented minimal media which was similar to that used in this research. The *Bacillus* isolates proved to be promising isolates for application in bioelectricity generation using microbial electrochemical technologies.

The results of this work corroborate the direct relationship between the amounts of electrogenic discharge with the amount of biofilm produced on the anode by *Pseudomonas* and *Enterobacter* species. There have been reports of relationship between anodic biofilm formation and bioelectrogenesis [1, 4, 14, 33]. It was observed that a mechanism of electrogenicity by microorganisms is by direct attachment to the electrode surfaces as there is a plethora of cell surface bound cytochromes, surface blebs, and extra-polymeric substances that help in bridging the distance between the cell walls and the electrode surface [34]. *Pseudomonas* species were the highest biofilm producers and at the same time the most electrogenic indicationg the possible activity of anodic biofilms as a mechanism of bioelectrogenicity. However, *Bacillus*

species which were the least anodic biofilm producers were not the least electrogenic isolates as they produced more electrons than *Aeromonas* species even if their anodic biofilm production was less than *Aeromonas* species. This thus highlights the fact that there is another possible mechanism of electrogenicity from *Bacillus* species aside the anodic biofilm. This thus calls for deeper research into the mechanisms of electrogenicity within identified electrogenic species.

The abilities of electrogenic bacteria can be applied in bioelectricity generation and used as another form of renewable energy resource [35]. There is also a need for deeper insights into means of full elucidation of mechanisms of electrogenicity as well as means of optimizing such mechanisms.

References

[1] Logan, B.E. 2008. *Microbial Fuel Cells*. John Wiley and Sons. Inc. New Jersey.

[2] Rabaey, K. and Verstraete, W. 2005. Microbial fuel cells: Novel biotechnology for energy generation. *Trends in Biotechnology, 23(6),* 291-298.

[3] Rezaei, F., Richard, T. L., Brennan, R. and Logan, B. E. 2007. Substrate-enhanced microbial fuel cells for improved remote power generation from sediment-based systems. *Environmental Science Technology, 41(1 l),* 4053-4058.

[4] Logan, B.E. and Regan, J.M. 2006. Electricity-producing bacterial communities in microbial fuel cells. *Trends in Microbiology, 14(12),* 512-518.

[5] Gorby, Y. A. and Beveridge, T. J. 2005. Composition, reactivity, and regulation of extracellular metal-reducing structures (nanowires) produced by dissimilatory metal reducing bacteria, Warrenton, VA.

[6] Reguera, G., McCarthy, K.D., Mehta, T., Nicoll, J. S., Tuominen, M. T. and Lovley, D. R. 2005. Extracellular electron transfer via microbial nanowires. *Nature, 435,* 1098-1101.

[7] Kim, B.-H., Ikeda, T., Park, H.-S., Kim, H.-J., Hyun, M.-S., Kano, K., Takagi, K. and Tatsumi, H. 1999. Electrochemical activity of an Fe(II1)-reducing bacterium, *Shewanella putrefaciens* IR-I , in the presence of alternative electron acceptors. *Biotechnology Techniques, 13(7),* 475-478.

[8] Park, D. H. and Zeikus, J. G. 2002. Impact of electrode composition on electricity generation in a single-copartment fuel cell using *Shewanella putrefacians*. *Applied Microbiology and Biotechnology, 59*, 58-61.

[9] Ringeisen, B. R., Henderson, E., Wu, P. K., Pietron, J., Ray, R., Little, B., Biffinger, J. C. and Jones-Meehan, J. M. 2006. High power density from a miniature microbial fuel cell using *Shewanella oneidensis* DSPIO. *Environmental Science Technology, 40(8),* 2629-2634.

[10] Bond, D. R., Holmes, D. E., Tender, L.M. and Lovley, D. R. 2002. Electrode-reducing microorganisms that harvest energy from marine sediments. *Science, 295(5554),* 483-485.

[11] Reimers, C. E., Tender, L. M., Fertig, S. and Wang, W. 2001. Harvesting energy from the marine sediment-water interface. *Environmental Science and Technology, 35:* 192-195.

[12] Kracke, F., Vassilev, I. and Krömer, O. J. 2015. Microbial electron transport and energy conservation – the foundation for optimizing bioelectrochemical systems. *Fronteirs in Microbiology, 6:* 575.

[13] Logan, B. E., Murano, C., Scott, K., Gray, N. D. and Head, I. M. 2005. Electricity generation from cysteine in a microbial fuel cell. *Water Research, 39:* 942-952.

[14] Deval, A. S., Parikh, H. A., Kadier, A., Candrasekhar, K., Bhagwat, A. M. and Dikshit, A. K. 2017. Sequential microbial activities mediated production from distillery wastewater using bio-electrochemical system with simultaneous waste remediation. *International Journal of Hydrogen Energy, 42:* 1130-1141.

[15] Biffinger, J., Ribbens, M., Ringeisen, B., Pietron, J., Finkel, S. and Nealson, K. 2009. Characterisation of electrochemically active bacteria utilizing a high-throughput voltage based screening assay. *Biotechnology and Bioengineering, 102 (2):* 436-444.

[16] Sharma, A. 2012. An ultraviolet-sterilisation protocol for microtitre Plates. *International Journal of Experimental Microbiology and Immunology, 16:* 144-147.

[17] Barrow, G. I. and Feltham, R. K. A. 1993. *Cowan and Steels's Manual for the Identification of Medical Bacteria. 3rd Edition.* Cambridge University Press, Cambridge, UK.

[18] Stepanovic, C. S., Cirkoric, M. L., Ranin, L. and Svabicviahocic, A. L. 2004. Biofilm formation by *Salmonella* spp and *Listeria monocytogenes* on plastic surface. *Applied Microbiology, 28:* 326-432.

[19] Phung, N. T., Lee, J., Kang, K. H., Chang, I. S.,Gadd, G. M. and Kim, B. H. 2004. Analysis of microbial diversity in oligotrophic microbial fuel cells using 16s rDNA sequences. *FEMS Microbiology Letters, 233(I):* 77-82.

[20] Lee, J., Phung, N. T., Chang, I. S., Kim, B. H. and Sung, H. C. 2003. Use of acetate for enrichment of electro-chemically active microorganisms and their 16s rDNA analyses. *FEMS Microbiology Letters, 223(2):* 185-191.

[21] Methe, B. A., Nelson, K. E., Eisen, J. A., Paulsen, I. T., Nelson, W., Heidelberg, J. F., Wu, D., Wu, M., Ward, N., Beanan, M. J., Dodson, R. J., Madupu, R., Brinkac, L. M., Daugherty, S. C., DeBoy, R. T., Durkin, A. S., Gwinn, M., Kolonay, J. F., Sullivan, S. A., Haft, D.H., Selengut, J., Davidsen, T.M., Zafar, N., White, O., Tran, B., Romero, C., Forberger, H. A., Weidman, J., Khouri, H., Feldblyum, T. V., Utterback, T. R., Van Aken, S. E.,

Lovley, D. R. and Fraser, C. M. 2003. Genome of *Geobacter sulfurreducens:* Metal reduction in subsurface environments. *Science, 302(5652):* 1967-1969.

[22] Kim, B. H., Park, H. S., Kim, K. J., Kim, G. T., Chang, I. S., Lee, J. and Phung, N. T. 2004. Enrichment of microbial community generating electricity using a fuel-cell-type electrochemical cell. *Applied Microbiology and Biotechnology, 63(6):* 672-681.

[23] Kumar, S. S., Basu, S., Bishnoi, N. R. 2017. Effect of cathode environment on bioelectricity generation using a novel consortium in anode side of a microbial fuel cell. *Bioechemical Engineering Journal, 121:* 17-24.

[24] Rabaey, K., Lissens, G., Siciliano, S. D. and Verstraete, W. 2003. A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency. *Biotechnology Letters, 25(18):* 1531-1535.

[25] Rabaey, K., Boon, N., Siciliano, S. D., Verhaege, M. and Verstraete, W. 2004. Biofuel cells select for microbial consortia that self-mediate electron transfer. *Applied Environmental Microbiology, 70(9):* 5373-5382.

[26] Rabaey, K., Boon, N., Hofte, M. and Verstraete, W. 2005. Microbial phenazine production enhances electron transfer in biofuel cells. *Environmental Science and Technology, 39(9):* 3401-3408.

[27] Hernandez, M. E., Kappier, A. and Newman, D. K. 2004. Phenazines aqnd other redox-active antibiotics promote microbial mneral reduction. *Applied Environmental Microbiology, 79(2):* 921-928.

[28] Voggu, L., Schlag, S., Biswas, R., Rosenstein, R., Rausch, C. and Gotz, F. 2006. Microevolution of cytochrome *bd* oxidase in *Staphylococci* and its implication in resistance to respiratory toxins released by *Pseudomonas*. *Journal of Bacteriology, 188(23):* 8079-8086.

[29] Feng, C., Li, J., Qin, D., Chen, L., Zhao, F., Chen, S., Hu, H., Yu, C. 2014.Characterization of Exoelectrogenic Bacteria *Enterobacter* Strains Isolated from a Microbial Fuel Cell Exposed to Copper Shock Load. *PLoS ONE 9(11):* e113379.

[30] Pham, C. A., Jung, S. J., Phung, N. T., Lee, J., Chang, I. S., Kim, B. H.., Yi, H. and Chun, J. 2003. A novel electrochemically active and Fe (III)-reducing bacterium phylogenetically related to *Aeromonas hydrophila*, isolated from a microbial fuel cell. *FEMS Microbiology Letters, 223:* 129-34.

[31] Saravanakumari, P. and Angel, D. 2014. Two chamber microbial fuel cells for electricity generation using different carbon sources. *British Microbiology Research Journal, 5(1).*

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

[33] Ren, Z., Steinberg, L. M. and Regan, J. M. 2008. Electricity production and microbial biofilm characterisation in cellulose-fed microbial fuel cells. *Water Science and Technology, 58(3):* 617-622.

[34] Lower, S. K., Hochella, M. F. and Beveridge, T. J. 2001. Bacterial recognition of mineral surfaces: Nanoscale interactions between *Shewanella* and a-FeOOH. *Science, 292(5520):* 1360-1363.

[35] Deval, A, Bhagwat A, Dikshit A. K. 2014. Significance of surface area of anode in generation of electricity through microbial fuel cell fed with anaerobically digested distillery wastewater. *International Journal of Pharma and Bio Sciences, 5(B):* 35e43.

Journal of Science Research
Volume 15, 2016, ISSN 1119 7333
Citation: Onilude A. A., Odeniyi, O. A. and Ajunwa O. M. Isolation, screening and anodic biofilm potentials of electrogenic microorganisms from urine and waste water samples.

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