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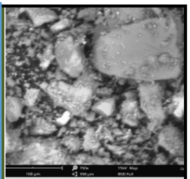

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Energy Production Using Microbial Fuel Cell from Sludge Produced from Potable Water Treatment

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ABSTRACT

A microbial fuel cell (MFC) is a type of device which is an electrochemical, after technology that is being to recover electricity from wastewater. This study's objective was to measure the potential difference from sludge collected from El-Sheikh Zayed water purification plant (Egypt in April 2021), isolate and identify bacteria present in sludge: Also, determine the effect of adding some metabolites on such physical and chemical properties. Potential difference measurement showed (192 mV) by using MFC. *Pantoea spp., Aeromonas salmonicida, Comamonas testosteroni* and *Staphylococcus lentus* were isolated and identified by biochemical reaction tests. *A. salmonicida* gave the highest potential difference (13mV). After adding some metabolites separately (albumen, dextrose, gelatin and casein), casein recorded 196Mv, a mixture was done between *A. salmonicida* and four metabolites measuring 50 mV. Physical and chemical analysis showed that biochemical oxygen demand (BOD) (55.0 P.P.M.), chemical oxygen demand (COD) (112.0 P.P.M.), and many minerals and heavy metals were also detected. Green synthesis of electricity from electrogenic bacteria using wastewater like sludge is a promising way to generate electricity by a cheap method.

INTRODUCTION

Sludge is one of the best materials for bioremediation because of its nutrient richness and year-round availability. It is made up of effluents obtained from urban, industrial, and other sources, which are regarded as the primary source of energy for the production of electricity [1]. Traditional wastewater treatment methods faced significant difficulties from high operational costs, high energy usage, and environmental pollution. According to estimates, the price of conventional technology for the treatment of waste amounts to about 3% of the world's energy consumption, with effluent disposal (or sludge disposal) accounting for 50% of the total cost of wastewater treatment [2]. MFC primarily both anodic and cathodic compartments that are divided by a salt bridge or a proton exchange membrane (PEM). In order to oxidise organic material at the anode while securing electrons and protons, microorganisms are used as biocatalysts. Protons enter the cathodic chamber through the PEM, and electrons are sent there via an external connection. As a result, in the cathodic chamber, oxygen, protons and electrons successfully mix. Complete the reduction process, which produces water [3]. Exo-electrogens are microorganisms that are used as biocatalysts because of their distinctive properties. Since they can facilitate lowering the electron acceptor (oxygen) at the cathode and bring electrons to the anode surface [3,4].

Pure or mixed cultures of microorganisms can be used in MFC to generate energy [5]. Microorganisms are obtained from widely used sources such as soil or marine sediment, the natural microbial population, and brewery wastewater and added to mixed cultures to increase biological constancy [6]. For microbial fuel cells, several sources of microorganisms have been considered, including drinking water, active sludge, material-reducing, municipal wastewater, and numerous other kinds of wastewater [7]. This study's objective is to introduce a new approach to using sludge that is collected during water treatment as a substrate to produce electricity. Chemical analysis, bacterial count, isolation and identification will be done throughout this study. Potential differences will be measured in the sludge containing all bacteria and compared to some isolated and purified bacteria in nutrient broth media. The effect of some metabolites will also be detected.

MATERIAL AND METHODS

Sludge collection

The sludge was collected from El-Sheikh Zayed water purification plant in 5-liter sterile containers in April 2021. The containers were transferred to the laboratory for measuring the potential difference by MFC [8].

Chemical and physical analysis of the sludge

Sludge samples were analyzed chemically Using ICP/MS (inductively coupled plasma/Mass spectrometry) (Model optima 7300 dv, PerkinElmer, USA). Lithium (Li), Barium (Ba), Mercury (Hg), Lead (Pb), Bismuth (Bi), Silver (Ag), Strontium (Sr), Cadmium (Cd), Copper (Cu), Chromium (Cr), Manganese (Mn), Nickel (Ni), Sodium (Na), Potassium (K), Magnesium (Mg), Calcium (Ca), Iron (Fe), Zinc (Zn), BOD and COD were measured. 2N HNO_3 was used to soak polypropylene tubes and caps for the entire night. The polyethylene rackets were triple-washed in metal-free water, allowed to dry overnight in a low oven temperature, and then preserved with 10 ml of sludge that had been well mixed with acid.

All samples had HNO_3 conc. (0.5 mL) added to them. The hood's temperature was set to 105°C, and tubes were placed within. Cover each tube with a top to let acid fumes escape while preventing contamination. Until digestion is complete by observation of a clear solution, more concentrated nitric acid was added. Before the start of the chemical analysis, tubes were kept at 4°C. The wavelengths that pass through the optical spectrometer are counted by a computer-based data handling device, often employing a detector, and the results data are processed [9].

pH test

A pH meter was used to measure pH (Jenway model 3510, England) which was calibrated by using 3 solutions standard buffer pH levels are 4, 7, and 10.

Total Hardness test

20 mL of sludge sample were pipetted into a spotless conical beaker for the estimation of total hardness. The mixture was titrated against EDTA (Conc. 0.01 M) (Weight 3.723 g of EDTA, dissolved in D.W. and diluted to 1000 mL) from the burette after being added 5 mL of ammonia buffer and 2 droplets of Eriochrome Black T (EBT) indication. The end outcome is a change from wine red to steel blue. To obtain a concordant titer number, the titration is repeated [9].
Total hardness = Volume of EDTA solution ingested X 1000 / Volume of the hard water taken p.p.m.

Total dissolved solid and electric conductivity test

Connect the conductivity measuring cell to the measuring instrument. Immerse the conductivity measuring cell (electrode) in the test sample containing 10 mL sludge; check the measuring instrument by WTW-LF 330/SET LF, USA [10].

Measurement of bacterial count by colony-forming unit (CFU/ml) using spread plate method

Samples of sludge were cultivated in nutrient broth medium for 24 h at 37 °C. First, 100 µL of an overnight-grown culture were diluted in 900µl of sterile distilled water. On the solid growth medium, 100µl of bacterial cultures that had been grown overnight and serially diluted were uniformly dispersed with a glass spreader. The Petri dish was kept in the incubator for the following night's incubation, and we observed the colonies with our unaided eyes [11].

Bacterial isolation and identification

Samples of sludge were cultivated for 24 h at 37°C on nutrient agar, blood agar, and McConkey agar media. Bacterial isolates were identified by biochemical reaction tests [12]. These electrogenic bacteria were isolated first from the sludge and then tested separately after sterilizing the sludge.

Microbial Fuel Cell Structure

The microbial fuel cell (MFC) was built in the lab of the Faculty of Applied Health Sciences Technology on October 6. The MFC container's volume is set at 7 litres in order to accommodate the size of the electrodes being used and the amount of sample. Two graphite rods are used in the MFC to act as the two electrodes (cathode and anode). The two electrodes are intended to be placed 25 cm apart and doped in the sample. Anode (made of carbon paper) is connected with a positive heavy load clamp wire, and cathode (on the side that is exposed to the air, polytetrafluoroethylene (PTFE) diffusion layers are covered; 60% w/v, dispersion in water) is coupled with a negative heavy load clamp wire). As seen in **Fig. 1**, these two probes are attached to a digital multi-meter with the SUNDER Electronics, India, model number AVO DT-9205A. An external circuit's anode and cathode electrodes were positioned on opposing sides that connected them across various external resistances [13].

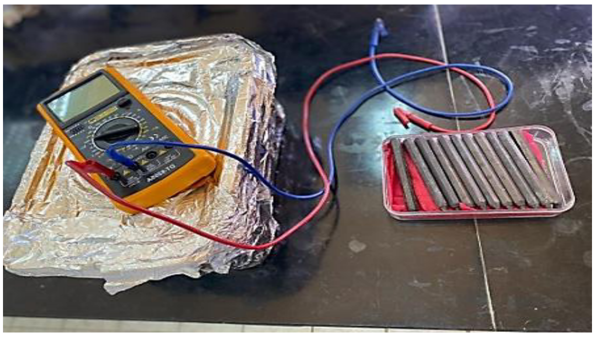

Fig. 1. Block diagram of microbial fuel cell, MFC instrument and AVO meter.

Direct production of potential difference from sludge in MFC

Five litres of sludge sample were put in MFC. First, secure the Avometer's two wires to the cathode and anode poles in the cell in the laminar. The Avometer readings were observed at different times [14].

Production of potential difference after adding some metabolites

The effects of some metabolites such as albumen, dextrose, gelatin and casein on potential difference production from sludge were studied. The previous metabolites were added separately.

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Production of potential difference from bacteria

Bacteria samples were separated from sludge. Samples were cultured on nutrient agar media and incubated at 37°C for 24 h, the separated colonies were collected and suspended in 5ml of brain heart broth for 24 h at 37°C. 100 ml of the bacterial solution (95 mL of broth plus 5 mL of the bacterial broth) was cultured for 24 h at 37°C without any further ingredients. The effect of a mixture combination between 4 metabolites and the tested bacteria was observed by reading the Avometer at different times. Only the bacteria that had high values from potential difference production were tested.

Statistical analysis

Using the SPSS package program, data were subjected to the analysis of variance (ANOVA) Version 26; Software, USA. The differences among means of treatments were calculated using the least significant difference.

RESULTS AND DISCUSSION

Sludge production volumes per year have increased steadily and will do so in the future [15]. Major issues arise when large amounts of sludge are dumped into the environment, including worsened odours, an increased risk of pathogenic microorganisms, and heavy metal pollution [16]. In the current study, the sludge was collected from El-Sheikh Zayed water purification plant, in Egypt. Heavy metals in parts per million (p.p.m.), BOD and COD were all determined using ICP/MS and also some chemical reactions and physical tests such as pH, total hardness, total dissolved solid (TDS) and electric conductivity were measured by calibration method (**Table 1**).

Table 1. Results of chemical and physical analysis of sludge sample.

Analysis type	unit	Result
Temperature	°C	15.5
pH	-	7.37
Total Dissolved Solid	mg/l	338
Electric conductivity	Us/cm	512
Total hardness	mg/l	540
Ca	p.p.m.	120.0
Mg	p.p.m.	57.6
Fe	p.p.m.	8.03
Li	p.p.m.	0.20
Na	p.p.m.	122.0
K	p.p.m.	5.0
Cr	p.p.m.	1.2
Mn	p.p.m.	1.3
Ni	p.p.m.	1.3
Cu	p.p.m.	5.2
Zn	p.p.m.	4.4
Sr	p.p.m.	0.6
Ag	p.p.m.	1.2
Cd	p.p.m.	1.3
I	p.p.m.	2.0
Ba	p.p.m.	1.0
Hg	p.p.m.	0.11
Pb	p.p.m.	0.02
Bi	p.p.m.	0.3
BOD	p.p.m.	55.0
COD	p.p.m.	112.0

The current study indicated that chemical and physical analyses of sludge were measured in conditions of temperature and pH (7.37 at 15.5°C). Results showed that sludge samples had the largest no. of CFU/mL, chemical and mineral measures. Irshaid conducted an investigation [17] and discovered that at the time of collection, sludge had an average of 80% total solid, 20% moisture and a slightly alkaline pH of 7.6: The total solid presence was 5.1%, and 94.9% was noticed as total volatile solid content. These numbers are different from what was reported in earlier studies [18-20].

In previous studies, the sludge contains all of the essential elements which are Pb (0.340 p.p.m.), Zn (2.57 p.p.m.), Cu (0.49 p.p.m.), Ga (0.06 p.p.m.), Ba (0.86 p.p.m.), Mn (1.38 p.p.m..), Ti (13.31 p.p.m.), K (28.59 p.p.m.) and SO_4^2 (6.85 mg/L). Major elements are present in the sludge because rain causes soil erosion. The wastewater treatment plant receives these components after being carried there by sewage from the combined sanitation network [8]. In the present study, sludge was inoculated on nutrient broth and serial dilutions were done to measure the bacterial contents (CFU/mL). The results showed that the count was 14100 CFU/mL. The sludge sample was then cultured on different media to isolate and identify bacterial load. Biochemical reaction tests were done for bacterial identification and confirmation. Four different bacteria (*Pantoea* sp., *Aeromonas salmonicida, Comamonas testosteroni* and *Staphylococcus lentus*) were detected (**Table 2**).

Table 2. Bacterial identification by biochemical reaction tests.

Test	*Pantoea* sp.	*S. lentus*	*A. salmonicida*	*C. testosteroni*
L-Pyrrolidonyl Arylamidase	- ve	- ve	-ve	+ve
Fermentation/Glucose	- ve	- ve	-ve	-ve
Fermentation D-Maltose	+ ve	- ve	-ve	-ve
Fermentation D-Mannitol	+ ve	+ ve	-ve	-ve
Fermentation D-Mannose	+ ve	+ ve	-ve	-ve
Beta-Alanine arylamidase pDNA	- ve	- ve	-ve	-ve
L-Proline Arylamidase	- ve	- ve	+ve	-ve
Lipase	- ve	- ve	+ve	-ve
Urease	- ve	- ve	-ve	-ve
D-Sorbitol	- ve	- ve	-ve	-ve
Sucrose	- ve	- ve	-ve	-ve
Citrate	- ve	- ve	-ve	-ve
Phosphatase	- ve	+ ve	-ve	-ve
Glycine Arylamidase	- ve	- ve	-ve	-ve
Ornithine Decarboxylase	- ve	- ve	-ve	-ve
Lysine Decarboxylase	- ve	- ve	-ve	-ve
Beta-Glucuronidase	- ve	- ve	-ve	-ve
Ellman	- ve	- ve	-ve	-ve

Measurements of potential difference from the sludge sample were done using an AVO meter in the MFC. **Fig. 2** shows the potential difference was high in the first 30 minutes (192Mv) then it began to decrease gradually till it became (5 Mv) after 21 h. The effect of some metabolites (albumen, dextrose, gelatin and casein separately) on potential difference production from sludge sample were measured. Potential difference recorded 185, 190, 190 and 196 mV respectively after 30 minutes, then decreased gradually until 4 days recorded 35 mV, 6 days recorded 30 mV, 3 days recorded 17 mV and 1 day recorded 3.1 mV respectively, as shown in figure 3a and 3b.

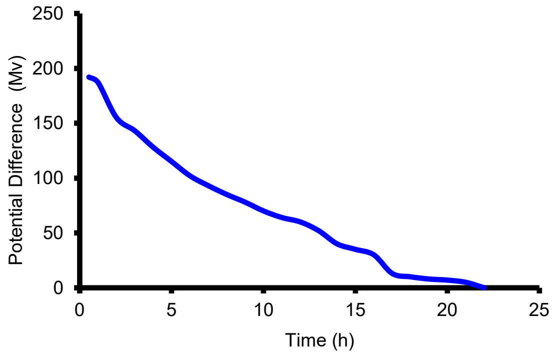

Fig. 2. Chart of production of potential difference (Mv) from sludge sample. The value represents the mean ± SE. Data shown are mean± stander deviation number of observations within each treatment.

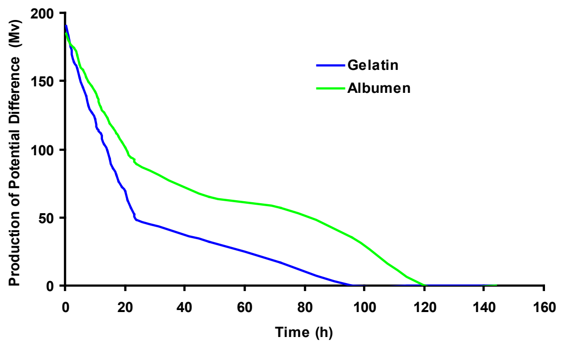

Fig. 3a. Chart of the effect of some metabolites (gelatin & albumin) on the potential difference (Mv) production from sludge. The value represents the mean ± SE. Data shown are mean± stander deviation number of observations within each treatment.

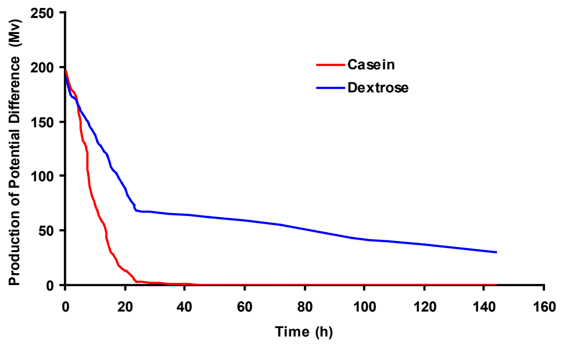

Fig. 3b. Chart of the effect of some metabolites (casein and dextrose) on potential difference (Mv) production from sludge. The value represents the mean ± SE. Data shown are mean± stander deviation number of observations within each treatment.

The main focus is on isolating and enlarging consortia of microbes producing electricity from samples of organic waste. Because the bacteria grow in a chamber on an electrode without oxygen, an MFC is essentially an anaerobic treatment method. In order to generate energy, the bacteria in the wastewater oxidise organic materials and transmit the electrons to an anode. Electricity doesn't start to flow until electrons are moved from the electrode to the electrode. To allow protons to mix with electrons and oxygen to make water, they must be able to get to the counter electrode via the salt bridge. To keep the equilibrium of charges, protons are also created. The potential difference between the cathode and anode chambers and current production are the foundations upon which the MFC is constructed. The potential is 0.9 V at its peak. This low voltage was increased to a greater voltage that could power a lid light by putting the reactors in series (21).

In the current study, the four different identified bacteria (*Pantoea spp., Aeromonas salmonicida, Comamonas testosteroni* and *Staphylococcus lentus*) were grown separately on nutrient broth to measure the potential difference by MFC and it was found that *A. salmonicida* showed the highest potential difference measurement at 30 min. (13 mV), then decreased gradually until 20 h recording 1.7 mV. *C. testosteroni* was produced of potential difference at 30 min. (9 mV), then decreased gradually until 15 h recording 2.7 mV. *S. lentus* was produced of potential difference at 30 min. (4 mV), then decreased gradually until 7 h recording 0.1 mV while *Pantoea spp.* showed non potential difference measurement (0.0 mV) (**Fig. 4**).

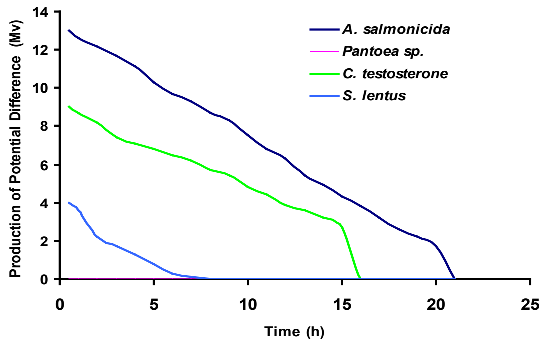

Fig. 4. Chart of production of potential difference (Mv) from different bacteria by MFC. The value represents the mean ± SE. Data shown are mean± stander deviation number of observations within each treatment.

A previous study illustrated that the Enterobacteriaceae family (10 bacterial species), were represented in the biochemical profile of suspected bacteria in sludge (54.54%). *Pseudomonas* and *Moraxella* (18.18%) were the two most common bacterial genera. *Burkholderia* and *Vibrios* were the second-most prevalent bacterial genus (9.09%). *E. coli* (4.54%), *Enterobacter, Photobacterium* and *Raoultella* were also present, and the presence of these bacteria and others supported the involvement of various microorganisms in the production of electricity [22]. In a different study, Páez *et al.* [23] discovered that the combinations that produced the most electricity, as measured in voltage, were those that lasted 4 and 10 h as observed. *Pseudomonas aeruginosa* measured at 414 mV and *Escherichia coli* at 464 mV. This demonstrates that *E. coli* were the bacterium that produced the most electricity under the tested conditions. Additionally, each strain's mediator's beneficial effect was seen.

E. coli test results reveal that methylene blue was used to produce the most electricity. For *P. aeruginosa*, a similar pattern was seen, but the outcomes are dependent on the initial pH value and the type of electrode used. A second test was conducted for each combination to emphasize how both bacteria behaved under the chosen combinations. *E. coli* values of 386 mV and *P. aeruginosa* values of 334 mV were as a result obtained. The results confirm a higher voltage for the *E. coli* strains and reveal a slight difference from the initial test. Additionally, the voltage values are in line with what other authors have reported [24,25]. In a prior study, Jamlus *et al.* [26] discovered that *K. pneumonia, K. variicola, B. licheniformis,* and *B. velezensis* were the strains of electroactive bacteria that generated electricity, whereas abiotic experiments were performed without the substrate containing any electroactive bacteria. Both electroactive bacteria and abiotic sources of energy produced positive overall values.

According to 5 electroactive bacteria, *K. pneumonia* generates the most electricity, at 222.08 mV, while abiotic bacteria generate the most electricity, at 51.54 mV. Among five different strains of electroactive bacteria, *B. velezensis* produced the least amount of electricity (44.82 mV). The lowest value, 44.19 mV, was for the abiotic value. Overall, electroactive bacteria demonstrate the growing importance of electricity production. As the 24 hour draws near, these bacteria gradually increase their electricity production. From 1 to 20 h, the trend for electricity production by *K. pneumonia* steadily increases. The growth of *K. variicola* was in the range of 4 mV/h and the graph shows a pattern that slightly increases from 1 to 19 hr. The value for *K. variicola* is highest at 19 h. The value for producing electricity gradually decreased after that. In the current study, the effect of a combination of mixture between the four metabolites

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(Albumen, Dextrose, Gelatin and Casein) and *A. salmonicida* showed higher values from potential difference production after 30 min. recording 50 mV, then decreased gradually (**Fig. 5**)

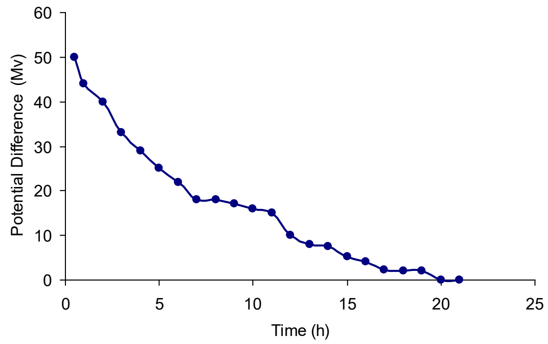

Fig. 5. Chart of production of potential difference of combination between mixture 4 metabolites (Albumen, Dextrose, Gelatin and Casein) and *A. salmonicida* (high values from potential difference). Value represents the mean ± SE. Data shown are mean± stander deviation number of observation within each treatment.

CONCLUSION

The sludge sample contained a high concentration of chemicals and minerals such as Li, I, Ba, Hg, Pb, Bi, Ag, Sr, Cd, Cu, Mn, K, Zn, BOD, COD and electric conductivity. A high production of potential difference from sludge after 30 min. (192 mV), then decreased gradually. Casein mixed with sludge to produce a high potential difference after 30 min. (196 mV), then decreased gradually. *A. salmonicida* was produced a higher potential difference after 30 min. (13 mV) than other different bacteria in this study and the highest value of potential difference recording by combination of a mixture of four metabolites (Albumen, Dextrose, Gelatin and Casein) and *A. salmonicida* (50 mV) after 30 min.

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