Electricity Production By Different Microbes Isolated From Activated Sludge With Microbial Fuel Cell

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Abstract- *Microbial fuel cell (MFC) is the one of the alternative sources of bioelectricity generation from the sewage sludge. Microbial fuel cell technology used to produce the sustainable power production with the help of microbial consortium.*

Our study is aimed to study sustainable power production from activated sewage sludge with microbial fuel cell and we considered two chambered H shaped microbial fuel cell (MFC) with chamber capacity of 1 liter and having graphite electrodes in each chamber join together with salt bridge.The circuit was completed by using multimeter and electricity was measured in mV. From the highest electricity producer nine different bacterial isolate was obtained. An individual bacterial cell and bacterial consortium was run and optimization carried out.

In H shaped MFC good amount of electricity production was obtained in range of 220-300 mV in activated sludge. Among nine isolates, isolate 6 produced highest electricity and confirmed as best exo-electrogene.

Keywords- exo-electrogens, microbial fuel cell, sewage sludge, bioelectricity, mediator.

I. INTRODUCTION

Recently electricity is producing by using water but unfortunately it is limited energy source. Due to excess usage of this source, it will lead to water scarcity in near future. As the global requirement of water and other natural fuels are very high, combustion of that fuel will cause bad effect on global climate. Use of bioelectricity definitely reduces the use of other limited natural source and help to conserve the energy. Microbial fuel cell (MFC) is the technology and produce electricity by converting the chemical energy to electric energy. Bacteria work as biocatalyst and oxidize the biodegradable substrate and generate the electricity by the catalytic action(Jiang Hai-ming,2013). As bacteria can use the substrate and produce electricity can be used to treat waste water from various sources. (Schwartz,2007)

There are various types of MFC but two chambered MFC isbasic design. In two chambered MFC two chambers are

Separated and join together by salt bridge or proton exchange membrane (PEM). Salt bridge is a structure to have electrolyte and gel like agar (Supriya kumara et al, 2015). It will allow passing only protons. In H shaped MFC the two chambers have electrode and in cathode chamber provision of aeration. In H shaped MFC one chamber will anode and other will be cathode and circuit will complete by using multimeter. (Zhang et al, 2012)

Working principle is based on redox reaction. Bacteria can transfer electrons directly to the terminal electron accepter without help of mediator is known as exoelectrogens. In mediator less MFC, microorganisms consume the biodegradable substrate and generate the carbon dioxide, protons and electrons. During the catalysis of the substrate different energy pockets produced and pass through the electron transport chain (ETC)(Padma Sengodon et al, 2012) and pass through outer cell lipid membrane and electron accept by the terminal electron acceptor which is anode.The electron will pass through external wire circuit to cathode. Cathode chamber act as oxygen sink and oxidizing agent take up the electron at the cathode. By that potential difference will generate.(http://en.m.wikipedia.org>wiki>MFC)

- **Anodic Reaction**

$CH_3COOH + 2H_2O \rightarrow 2CO_2 + 7H^+ + 8e^-$
$C_6H_{12}O_6 + 6H_2O + 6CO_2 \rightarrow 24H^+ + 24e^-$

- **Cathodic Reaction**

$O_2 + 4H^+ + 4e^- \longrightarrow 2H_2O$
$24H^+ + 24e^- \longrightarrow 6O_2 + 12H_2O$
(Ishwar Chndra, 2012)

Some bacteria are non exoelectrogens and for the electron transfer mediator is require. Mediator works to transfer electron from the bacterial outer membrane to the anode. Mediator will tap into ETC and gain the electron and become reduced. Inorganic mediator pass through lipid

membrane and outer membrane and it will donate electron to electrode and become oxidized and that electrode will be anode. After release of electron mediator come back to original state and repeat the same process. Some mediators can be use like thionine, methyl viologen, neutral red, methyle blue, etc. (Nader Mokhtarian et al., 2012)

II. MATERIALS AND METHODS

2.1Sample collection:

Sewage sludge was collected from the sewage treatment plant of Bhatar (Sample no. S1) and Karanj,Surat (Sample no. S2), river sludge was collected from the river Tapi, Surat (Sample no. S3) and sewage slurry sample was collected from the sewage treatment plant of khajod, Surat (Sample no. S4).

2.2Isolation of bacteria from sewage:

From the sample of Karanj sewage treatment plant, 1g of sludge sample was mixed into 10ml sterile distilled water. From that 1ml of sample was withdraw and serially diluted into the tube filled with 9ml sterile distilled water up to 10^{-6} dilutions. From each diluted sample 0.1ml sample was spread on sterile nutrient agar plate and incubated for 24 hour at 30°C. After incubation distinct 9 colonies were obtained and further purified on nutrient agar plat and then gram staining was done.(WMF Wan Ishak et al, 2011)

2.3MFC components and assembly:

- Electrode: Graphite electrode having dimension of 15 cm length and 1.5 cm diameter, were used as cathode and anode.
- Salt bridge: By dissolving 3 % Agar in 1 M NaCl solution boiled for 2 minute and cast in PVC pipe of 2 inch diameter and 3 inch length then keep it for proper settling. (Supriya Kumari, et al, 2015)
- Microbial fuel cell: Microbial fuel cell was made by using poly propylene container and capacity of 1200ml. Both chambers have electrodes and join together by using salt bridge. Anode chamber filled with freshly prepared sterile minimal broth [K_2HPO_4-7Gg/l, KH_2PO_4- 2g/l, Sodium citrate-0.50g/l, $MgSO_4$- 0.10g/l, $(NH_4)SO_4$-1.0g/l] 900ml and 7% glucose as a source of carbon and 200µmol/l neutral red as mediator(Nader et al, 2012). Isolated bacterial colonies were allowed to grow in sterile nutrient broth for 24-30 hour at 37°c. This previously enriched culture having 0.5 OD(DOO HYUN PARK et al, 2000). This 12 ml culture was inoculated to the anode chamber which having anaerobic condition. In cathode 900ml solution of 50mM K_2HPO_4 (Venkata et al,2009) was added and it was kept under continuous aeration.

From highest electricity producing bacterial isolate, effect of various concentration of neutral red(50µmol/l, 100µmol/l, 150µmol/l, 200µmol/l, 250µmol/l) (Nader et al, 2012), effect of various concentration of glucose (5g/l, 6g/l, 7g/l, 8g/l, 9g/l) (Supriya kumari et al, 2012), effect of various percentage of salt bridge having different agar percentage (3%w/v, 4%w/v, 5%w/v, 6%w/v, 7%w/v) and effect of KCl as a salt in salt bridge having concentration (1M, 2M, 3M, 4M) (Anand Prakash, 2015) was checked.

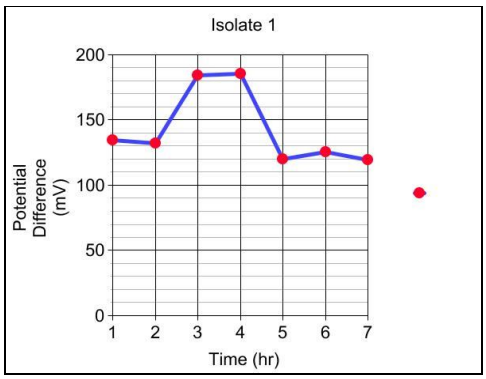
III. RESULTS AND DISCUSSION

3.1 Isolation and identification:

From all samples, sewage sample of Sample no. S2 gave the highest electricity of 320mV. This sample simultaneously processed to determine the presence of bacterial etiological agents by serial dilution method and resulted in to distinct 9 isolates (3gram negativeand 6 gram positive bacteria).

3.2 Electricity produced by different isolates:

All isolated bacteria found to produce electricity individual. The graph was plotted of Time (hour) versus obtained potential difference at no load condition of each isolates are represented in figure below.By standard microbiological process identification (Bergis Manual 9th edition, Jean F Macfaddin, 3rd edition) and by 16s rRNA sequencing, highest electricity producer was identified as *Pseudomonas stutzeri*. (Isolate no. 6).



4Figure 1: Potential difference generated by isolate 1

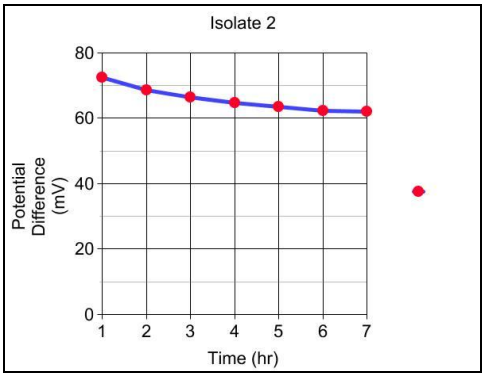


Figure 2: Potential difference generated by isolate 2

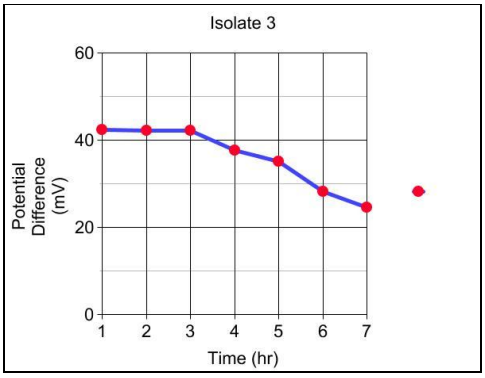


Figure 3: Potential difference generated by isolate 3

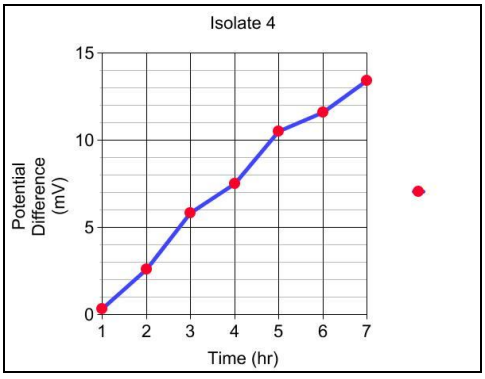


Figure 4: Potential difference generated by isolate 4

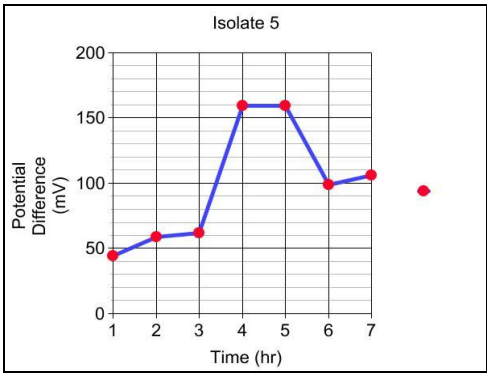


Figure 5: Potential difference generated by isolate

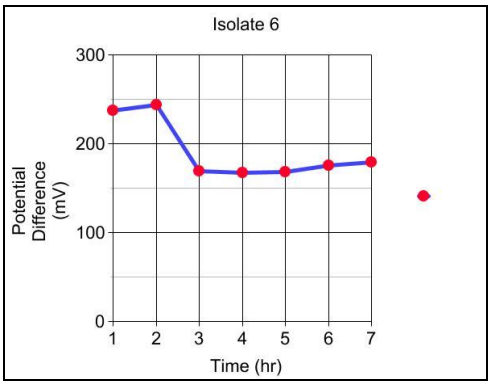


Figure 6: Potential difference generated by isolate 6

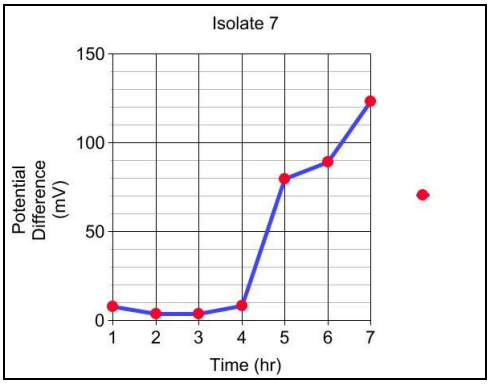


Figure 7: Potential difference generated by isolate 7

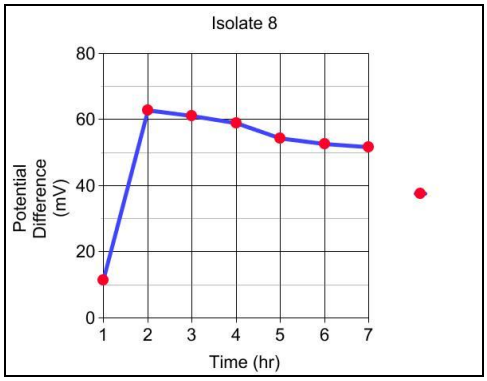


Figure 8: Potential difference generated by isolate 8

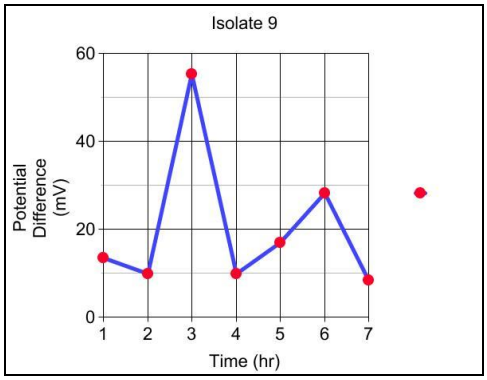


Figure 9: Potential difference generated by isolate 9

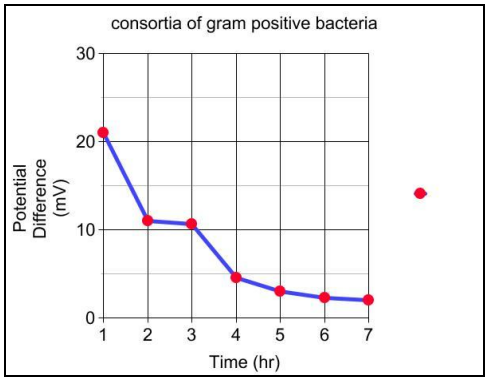


Figure 10: Potential difference generated by consortia of gram positive

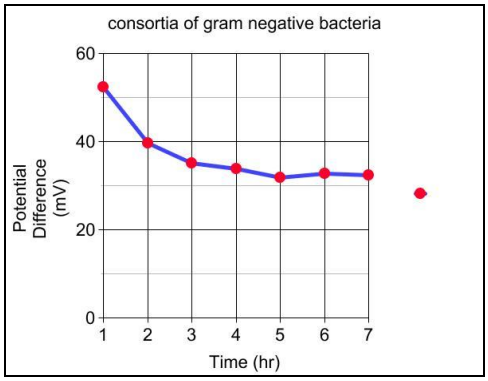


Figure 11: Potential difference generated by consortia of gram negative

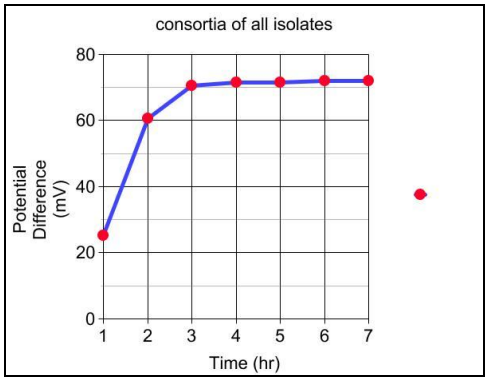


Figure 12: Potential difference generated by consortia of all isolates

Figure 13: Highest potential difference observed(isolate6)

From all the nine isolates, isolate number 6 gave highest electricity production as stated earlier, about 244 mV within 24 hour but it is in contrast to Zakira Naureen et al, 2016 who reported gram negative organisms produced highest electricity (143 mV) after 7 days.Similar to our study, Borole et al observed Gram negative bacteria more electrochemically active than gram positive bacteria (Borole et al, 2011).

In present study, mix consortia of the all isolates were also runfor electricity generation, in combination of all gram positive, gram positive and gram negative, gram positive and gram negative and actinomyces. Highest electricity generation was observed when MFC was run by prepared consortia of all isolate together compared to individual isolates. However, the consortia of gram negative bacterial isolates produced higher electricity than consortia of gram positive isolates.

3.3 Effect of Neutral Red on Potential Difference :

In all the MFC, concentrations of neutral red were 50µmol/l, 100µmol/l, 150µmol/l, 200 µmol/l, 250µmol/l. The potential differences were observed for seven hour time period.

Table 1: Effect of Neutral Red on Potential Difference

Concentration of Neutral Red (µmol/l)	Highest Potential difference observed (mV)
50	123.3
100	128.4
150	23.5
200	244
250	56.6

For*Pseudomonas stutzeri* 200 µmol/l concentration of neutral red observed as optimum concentration. In accordance of our study, *Sacchromyces cerevisiae*also 200 µmol/l concentration of neutral red was reported as optimum (Nader Mokhtarian et al, 2012).

3.4 Effect of Glucose on Potential Difference :

As well as the different concentrations of glucose (5g/l, 6g/l, 7g/l, 8 g/l, 9g/l) were analyzed.

Table 2: Effect of glucose concentration on potential difference

Concentration of glucose (g/l)	Highest potential difference observed (mV)
5	64.4
6	41.7
7	244
8	144
9	231

For *Pseudomonas stutzeri*, 7 g/l concentration of glucose found to be as optimum concentration. In their study, Supriya kumari et al, 2015 also accounted 7g/l as optimum concentration.

3.5 Impact of salt bridge on potential difference :

3.5.1 Concentration of Agar :

Various concentrations of agar in salt bridge used for optimization were 3%w/V, 4%w/v, 5%w/v, 6%w/v and 7%w/v (Table:3)

Table 3: Effect of agar percentage in salt bridge on Potential Difference

Concentration of Agar (%w/v)	Highest Potential difference observed (mV)
3	244
4	386
5	323
6	106
7	499

For *Pseudomonas stutzeri*, 7 %w/v concentration of agar in salt bridge can be considered as optimum concentration but in contrast to present study, Ramya Nair, 2013 detailed 10% of agarose concentration for highest electricity production.

3.5.2 Concentration of KCl :

KCl with various concentrations (1M, 2M, 3M, 4M) were studied for better production.

Table 4: Effect of KCl in salt bridge on Potential Difference

Concentration of KCl (M)	Highest Potential difference observed (mV)
1	180
2	653
3	164
4	367

For our highest producer, 2M concentration of KCL in salt bridge found as optimum concentration. Our finding is in contrast to study of Anand Prakashwho reported highest electricity produced at 1M concentration of KCl (Anand Prakash, 2015).

IV. CONCLUSION

According to the results of all experiment were carried out in dual chambered MFC having salt bridge, isolate no. 6 was identified as*Pseudomonas stutzeri* gram negative rods, is giving highest electricity production and optimized conditions for that organisms are 200 µmol/l neutral red, 7 g/l glucose in minimal media and 7 %w/v agar, 2 M KCl salt in salt bridge optimized.

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