

ELECTRICITY PRODUCTION BY BACTERIA

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ABSTRACT

Direct electron transfer from electrodes to cells has many rewards over indirect electrical stimulation of microbial metabolism through electron shuttles or hydrogen production. In these study MFCs screened from soil samples near college campus (Shri Shivaji Mahavidyalaya, Barshi). From which four isolates (B1, B2, B3 and B4) were select for electricity production. These isolates were identified by morphological and biochemical studies. The identified isolates were *Pseudomonas fluorescens*, *Bacillus pasteurii*, *Klebsiella ozaenae* and *Shigella spp.* These MFCs were grown in two different medium i.e. Succinate medium and nutrient medium while it showed more electricity generation in succinate medium. The physico-chemical parameters were optimized for more current formation. It was obtain that shaking condition was good for current production. The optimized condition showed that 5g/L succinic acid, 1g/L K_2HPO_4 , pH 7 and 37°C are good conditions for current generation. After 72 hrs incubation period MFCs produced more electricity i.e. 1.5V and LED bulb had produced light.

KEYWORDS: Succinate media, Nutrient medium and MFC.

INTRODUCTION

Bacteria able to transfer electrons to metals are key agents in biogeochemical metal cycling, subsurface bioremediation, and corrosion processes. More recently, these bacteria have gained attention as the transfer of electrons from the cell surface to conductive materials could be use in multiple applications.^[1] Research efforts are directed at utilizing the

biocatalytic electron transfer functions of enzymes to assemble biofuel cells that convert organic fuel substrates into electrical energy.^[2]

In the most general sense, microbial fuel cells function by oxidizing an electron donor with electron transfer to the anode under anoxic conditions. The electron donor can be a reduced product of microbial metabolism or more an added artificial mediator that facilitates electron transfer by accepting electrons from the microbes and donating them to the anode. In some instances the microorganisms can produce their own soluble electron transfer mediator. Alternatively, some microorganisms can directly transfer electrons to the anode surface. Electrons donated to the anode pass through a resistor or other type of electrical device to the cathode.

Although a strain of *Pseudomonas aeruginosa* capable of utilizing glucose and producing an electron shuttle was isolated from this system, the isolated strain was inefficient in converting glucose to electricity owing, at least in part, to the fact that it is only incompletely oxidized glucose to fatty acid.^[3] It is not clear what microorganisms were responsible for the high rates of power production in the mixed community. The production of electron shuttles is much less likely to be an important strategy for electron transfer to anodes in flow-through systems or other types of open systems in which there is a frequent change of the fluid around the anode. This is because, in an environment in which the shuttle might be rapidly lost to the external environment, the energetic cost of biosynthesizing an electron shuttle puts an electron-shuttle-producing microorganism at a severe disadvantage compared with microorganisms that directly transfer electrons to the anode.^[4] Manganese-oxidizing microorganisms served as the catalysts in a novel cathode system that increased the current by almost two orders of magnitude over plain electrodes in a microbial fuel cell. Mn(IV) precipitated on the cathode is reduced to Mn(II), which the manganese oxidizers recycle back to Mn(IV).^[5] For many applications microbial fuel cells rely on the selection of electricity-producing microorganisms from the natural community of microbes in the organic source material. However, pure culture studies are useful to evaluate the mechanisms of electricity production and strategies for optimizing this process.^[6] The possible extracellular electron transfer (EET) mechanisms for the microbial electron uptake from a cathode are reviewed, electron-accepting reactions. First a short classification of the underlying principles of microbial bioelectrochemical systems (BESs) are given to coupling electron-donating reactions. Initially, workers used the spontaneous electron movement between electronegative

bio anodes and electro positive abiotic cathodes in microbial fuel cells (MFCs) to generate electric power.^[7] Operated MFCs with bio cathodes at which bacteria catalyze the electron transfer from the cathode to electro positive terminal electron acceptors, such as oxygen or nitrate. This resulted in a complete biological MFC with both bacteria at the anode and cathode and therefore self-replenishing biological catalysts on just electrode materials, such as carbon or graphite the circumvention Besides of expensive metal catalysts, exciting recent work has shown that bio cathodes in photosynthetic MFCs also reduce carbon dioxide(CO₂).^[8]

A recently published life-cycle assessment showed that producing a chemical product, such as hydrogen (or in their case hydrogen peroxide), at the cathode would provide considerably larger environmental benefits compared to generating electric power with MFC₂. This can be performed with a 3-electrode BES for which the worker sets the working electrode potential (in this case for optimum anodic potentials) by using a potentiostat and a reference electrode, such as a Ag/AgCl reference system. Here, this BES is referred to as a microbial 3-electrode cell(M3C). Vice versa, the set potential at the working electrode can also maintain optimum cathodic potentials to, for example, support bioelectrochemical electron-accepting reaction. Thus, an M3C can be regarded as a special case of an MEC for which an power input helps to drive the reaction, while one electrode potential (working electrode) is controlled at favorable electrochemical conditions. Even more importantly, through optimization of the reaction rate, the M3C can boost the current density, which is also pertinent to ensure an economical scale.^[9]

During course of this investigation electricity production by bacteria from soil was used as a starting material for isolation and identification of electricity producing bacteria. A total 4 number of isolates were obtained from this screening. These isolates were subjected to colony characters and morphological studies. They were preserved on sterile nutrient agar slants. Then these electricity producing bacteria were added to the two different medium and measuring the current after 24 hrs. Then optimization of the different parameters as like succinic acid, K₂HPO₄, pH, Temperature were used to more electricity generation.

MATERIALS AND METHODS

Collection of soil samples

Samples of garden area collected in sterile polythene bags from Shri Shivij Mahavidyalaya, Barshi, India.

Isolation and identification of bacteria

Firstly soil samples were serially diluted and streaked on NB agar plate and these plates were kept incubated at 37⁰C for 24 hrs. After incubation colonies having different colony characters were selected for current formation and identified by the standard biochemical tests.

Maintenance of culture

Bacterial cultures were maintain on sterile nutrient agar slant and stored in a refrigerator 4⁰C for further use.

Detection of current formation

Different media like succinate broth and nutrient broth were prepared to check the efficiency current formation; each medium were inoculate separately and then incubated. After 24 hrs incubation time current formation was measured.

Effect of different physicochemical conditions on current production**Optimization of different incubation conditions**

Two sets were prepared and inoculated with the isolates in succinate broth and kept one set in static condition and other at shaking condition at 120 rpm for 48 hrs. After incubation time current formation was checked.

Optimization of succinic acid concentration

Succinate media having different concentrations of succinic acid (2, 3, 4, 5 & 6g/L) were used and separately inoculated with the bacterial isolates at 37⁰C for 48 hrs.

Optimization of K₂HPO₄

Succinate medium having different concentration of K₂HPO₄ (1, 2, 3, 4 & 5g/L) were used and separately inoculated with the bacterial isolates at 37⁰C for 48 hrs.

Optimization of pH

Succinate media having different concentration of pH (6, 6.5, 7, 7.5 & 8) were used and separately inoculated with the bacterial isolates at 37⁰C for 48 hrs.

Optimization of temperature

Succinate media flasks were separately inoculated with bacterial isolates for 48hrs. Flask were incubated at different temperature (20, 28, R.T, 37 & 40⁰C).

Formation of MFC in optimized media

For current formation (current & O.D) optimize media were prepared and sterilized. After sterilization the promising isolates were inoculated and kept for incubation. After each 24 hrs time interval current formation was checked as well as growth of bacteria till 4 days. After 4 days enriched broths were put into the ice tray & checked for current formation.

RESULTS AND DISCUSSIONS

Screening of current forming bacteria

After isolation of bacteria current forming bacteria were screened from isolates. During screening of current forming bacteria, it was noted that out of 10 isolates, only 4 isolates had current forming capacity.

In the present study 10 different isolates were obtained. Out of which 4 isolates generated electricity. Research conducted by Babu *et.al.*,^[10] showed a total of 12 bacteria isolates from 2 different sources namely S1 soil sample collected from agricultural land & S2 sample collected from dye industrial effluent soil near Madurai.

Identification of current forming bacteria

The organism showed current producing capacity those isolates were used for the identification purpose. With the help of morphological and biochemical characters 4 isolates were identified (Table 1).

Table 1: Identified Bacterium.

Sr. No	Isolate Code	Identified up to species level
1	B1	<i>Pseudomonas fluorescens</i>
2	B2	<i>Bacillus pasteurii</i>
3	B3	<i>Klebsiella ozaenae</i>
4	B4	<i>Shigella</i> spp

Detection and development of MFC

The isolated organisms had separately incubated on nutrient broth and succinate broth and kept for incubation at 37⁰C for 24 hrs. Table 2 showed the electricity production in nutrient and succinate medium after 24 hrs.

Table 2: Detection of Current on Different Media in Hrs.

Sr.no	Microorganism	Hrs	Current in nutrient medium	Current in succinate medium
1	<i>Pseudomonas</i>	0hrs	400 μ A	500 μ A
2	<i>Pseudomonas</i>	24hrs	600 μ A	800 μ A
3	<i>Pseudomonas</i>	48hrs	800 μ A	1000 μ A
4	<i>Pseudomonas</i>	72hrs	700 μ A	200mA
5	<i>Pseudomonas</i>	96hrs	900 μ A	600mA
6	<i>Bacillus</i>	0hrs	410 μ A	400 μ A
7	<i>Bacillus</i>	24hrs	800 μ A	810 μ A
8	<i>Bacillus</i>	48hrs	600 μ A	640 μ A
9	<i>Bacillus</i>	72hrs	520 μ A	500 μ A
10	<i>Bacillus</i>	96hrs	580 μ A	510 μ A
11	<i>Klebsiella</i>	0hrs	300 μ A	410 μ A
12	<i>Klebsiella</i>	24hrs	700 μ A	740 μ A
13	<i>Klebsiella</i>	48hrs	610 μ A	640 μ A
14	<i>Klebsiella</i>	72hrs	400 μ A	530 μ A
15	<i>Klebsiella</i>	96hrs	510 μ A	460 μ A
16	<i>Shigella</i>	0hrs	410 μ A	440 μ A
17	<i>Shigella</i>	24hrs	510 μ A	640 μ A
18	<i>Shigella</i>	48hrs	500 μ A	610 μ A
19	<i>Shigella</i>	72hrs	560 μ A	540 μ A
20	<i>Shigella</i>	96hrs	480 μ A	500 μ A

Optimization of different incubation conditions

Two sets were prepared. After 48 hrs incubation *Pseudomonas spp* had 1000 μ A current formation at shaking condition & 800 μ A at static condition (Figure. 1) from this result it was cleared that the shaking condition was good for current formation.

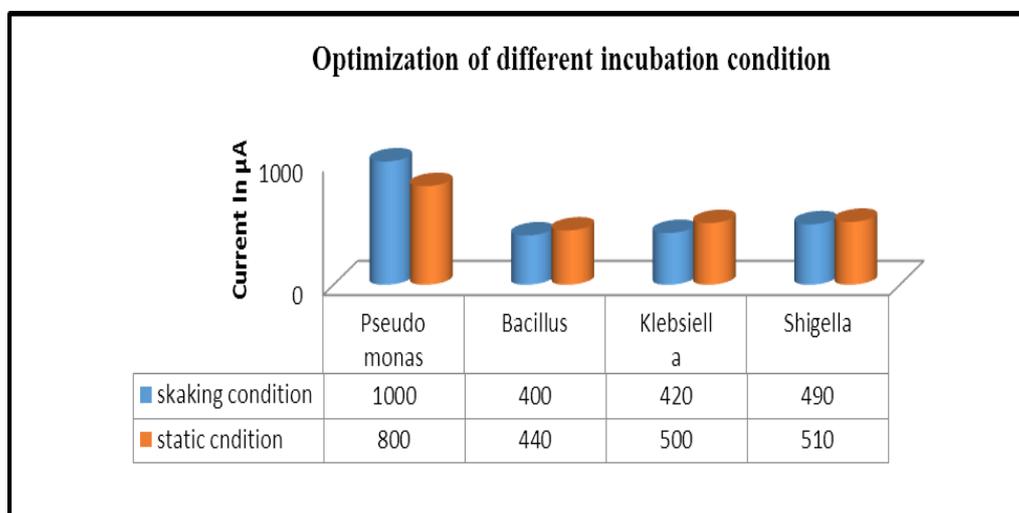


Figure 1: Optimization of Different Incubation Conditions.

Optimization of succinic acid for current formation

Optimization the succinic acid media by the MFCs were operated for different parameters (2, 3, 4, 5, 6g/liter) and after the 48 hrs incubation, 5g/liter showed the large amount of electricity production (figure 2). In research paper it was reported^[10] that different sugar sources like glucose, sucrose, lactose & mannitol, maximum potential was got in the glucose.

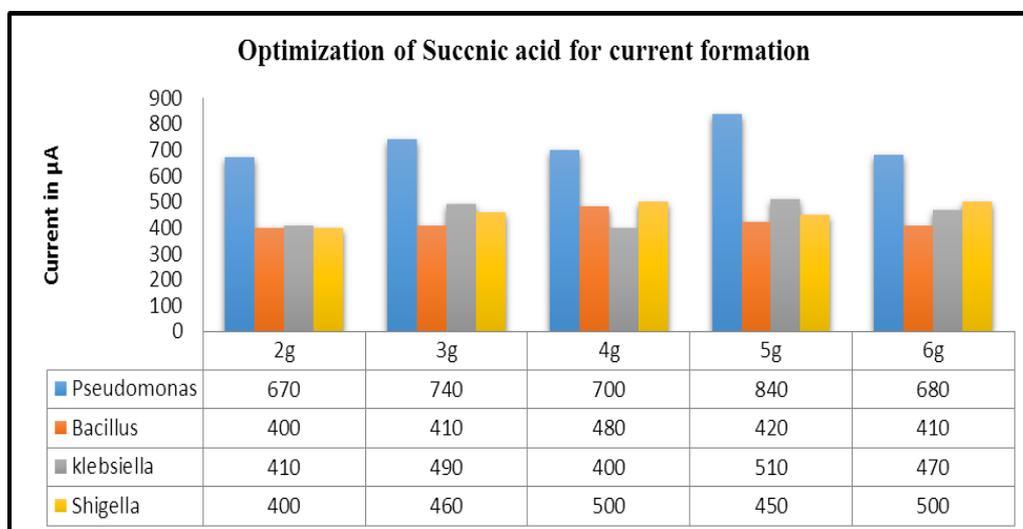


Figure 2: Optimization of Succinic acid for Current Formation.

Optimization of K_2HPO_4 for current formation

In succinic media different concentration of K_2HPO_4 having media were prepared after incubation period it was observed that 1g/liter concentration of K_2HPO_4 gave 800 μ A current formation by *Pseudomonas spp.*

In the optimization the K_2HPO_4 by different parameters (1, 2, 3, 4, 5g/liter) and after the 48 hrs results (figure 3) showed that the maximum electricity was produced in 1g/liter. In different papers safa sheikh took ammonium sulphate as nitrogen sources with different concentrations (0.5, 1, 1.5 and 2%) and he got maximum bioelectricity at 1%.

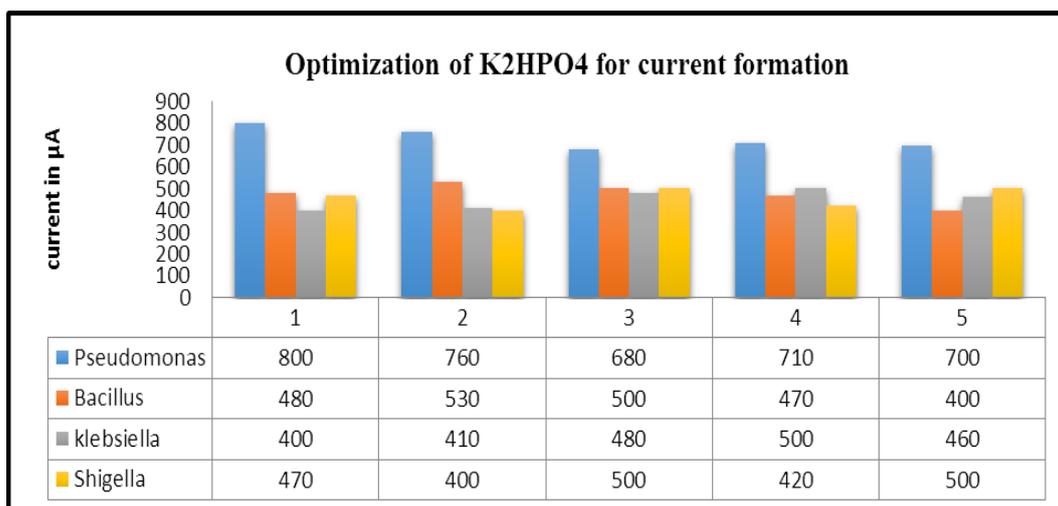


Figure 3: Optimization of K₂HPO₄ for current formation.

Optimization of pH for current formation

In the optimization we took different pH (6, 6.5, 7, 7.5, 8) and after 48 hrs we got maximum electricity at pH 7 (figure 4). In research papers safa sheikh^[10] conducted (5, 6, 7, 8) after 24 hrs pH 7 showed highest electricity.

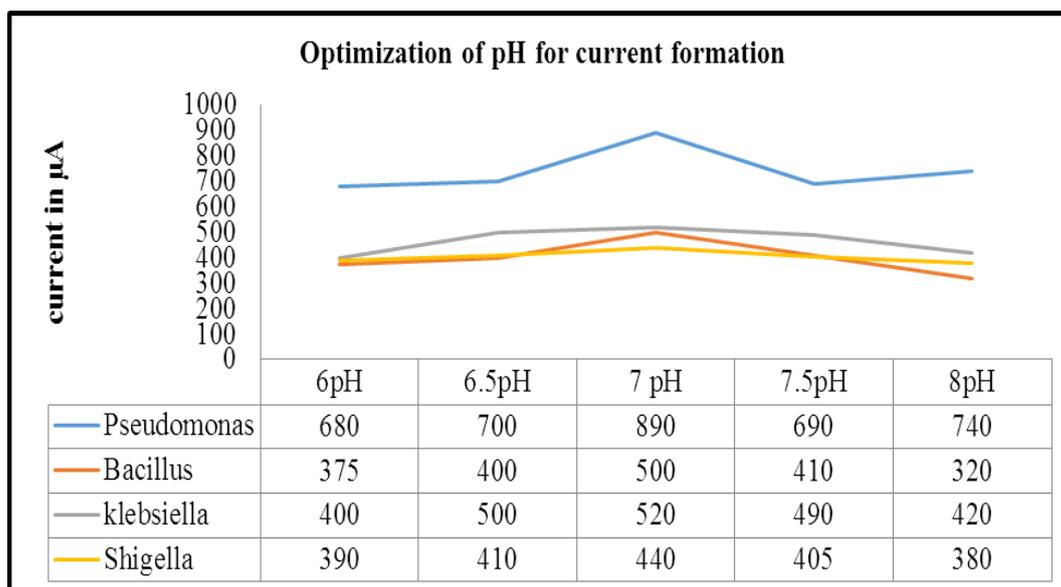


Figure 4: Optimization of pH for current formation.

Optimization of temperature for current formation

At 37°C *Pseudomonas spp* were formed 900µA current. As temperature increased or decreased current formation were also fluctuated. In the optimization of temperature (room temp, 37, 28) but maximum electricity was got at 37°C (figure 5). Safa sheikh^[10] got high electricity produced at room temp. This result is similar with Larrosa-Guerrero *et al.*^[11] who

reported the effect of temperature on the performance of MFCs, maximum power density was 174.0mWm⁻³ at 35°C

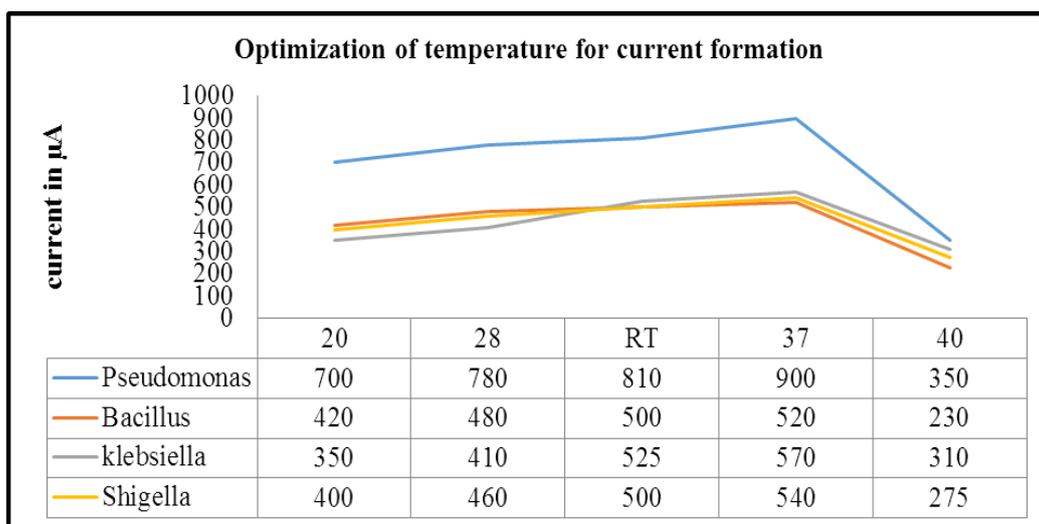


Figure 5: Optimization of Temperature for Current Formation.

Formation of MFC in optimized media

In optimized media having (temperature, succinic acid, K₂HPO₄, pH) was inoculated with promising isolates. After every 24 hrs incubation period increasing of current as well as growth of bacteria was observed (Table 3). After 72 hrs maximum current formation was observed. At that point LED bulb was produced light.

In construction of MFC, the MFC was used as the cathode & anode. The wire and different meters were used to the measure current. Current flow was observed by the µA, mA, volts by these different parameters. Ice tray was used to measuring the electricity and LED were produced the light. In different paper conducted by the Safa Sheikh^[10] as they used a salt bridge 15cm×13cm×13cm & volume 2.5L. Anode & cathode electrodes with to recorded the readings in volts.

Table 3: Formation of MFC in optimized media.

Time in hrs	Current	O.D
0	400µA	0.10
24	800mA	0.27
48	1000mA	0.33
72	1.5V	0.46
96	800mA	0.38

CONCLUSIONS

The four isolates (B1, B2, B3 and B4) were produced electricity. The identified isolates were *Pseudomonas fluorescens*, *Bacillus pasteurii*, *Klebsiella ozaenae* and *Shigella spp* respectively. *Pseudomonas fluorescens* B1 isolate generates more electricity than the other isolates. These MFCs were produced more electricity in succinate medium as compared to nutrient medium. The 5g/L succinic acid, 1g/L K₂HPO₄, pH 7 and 37°C are the good conditions for current formation.

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