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Original Research Paper

Generation of Electricity from A Low Cost Microbial Fuel Cell

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ABSTRACT

Using available resources, a dual chambered low cost microbial fuel cell was designed. Microbial fuel cell was tested for electricity production with microbial isolates like *Escherichia coli, Pseudomonas aeruginosa* and *Pseudomonas pseudoalcaligenes* using corn steep liquor as substrate. From the values recorded using a multimeter, it was observed that *Pseudomonas aeruginosa* was able to produce maximum electricity of I: 10.32 mA, V: 0.948 V, R: 91.88 Ω and P: 9.785 mW, at 45°C.

INTRODUCTION

Energy is an important input for development, which is required in household, agriculture, transport and industrial complexes. Energy resources are renewable as well as non-renewable. With the advancement in science and technology, mankind has invented a number of energy sources which include fossil fuel, hydro power, solar power, wind power, batteries, oil and natural gas, thermal power, fire wood (fuel wood), chemical fuel and nuclear power. A radical increase in the world population and globalization has led to improper power management, which in turn has created intense pressure on energy and power resources. Most of the commercially available energy sources involve high-tech methodologies which in turn have increased the expense for obtaining a refined final product. It also leads to increased market price and turns out to be unaffordable to the common public. Uncontrolled usage of fossil fuel has lead to the depletion of these energy sources which are non-renewable. All these discomforts have paved the way for advanced thinking for alternate sources, using acquired knowledge.

In recent years, scientists in various parts of the world are keen to develop more facile and cheaper power sources. One such advancement in research is fuel cell technology, and one major class of fuel cell techniques is biological fuel cell.

Biological fuel cell converts chemical energy directly into electrical energy. They operate under minimal or mild reaction conditions, namely ambient temperature, pressure, use of inexpensive catalyst i.e., microorganisms or enzyme. There are two types of biological fuel cells namely enzyme fuel cell (EFC) and microbial fuel cell (MFC). Problem with most of the enzyme fuel cells is that the majority of the redox enzymes do not take part directly in the transfer of electrons to the conducting supports. These, therefore, require a mediator to bond electrode surface with redox enzyme and the electrode with biocatalyst; which in turn elicits electrochemical transformation on the electrode surface.

An alternate method is making use of microorganisms in biological fuel cells, which minimize the use of isolated enzymes thereby providing affordable substrate for biological fuel cell. In others words it can be described as "microbial fuel cell" technology, as mentioned earlier.

MATERIALS AND METHODS

Sample Collection

Soil samples were randomly collected from ten sites at Manali, Chennai, which is an industrial hub, by scrapping the soil up to 50 mm depth using a sterile spatula. The pH and temperature of the soil were recorded at site during collection.

Sample Processing

1. 1g of soil sample was weighed and suspended in 10mL of sterile saline (stock solution).
2. 1mL of the stock solution was serially diluted up to 10^{-8} using sterile pipettes.
3. Spread plate technique was performed on to plate count agar for 10^{-5}, 10^{-6}, 10^{-7} dilutions.
4. Plates were incubated at 37°C for 18 to 24 hours.
5. Desired organisms were isolated from the processed sample by conventional method in reference with Bergey's Manual of Systematic Bacteriology.

Growth Curve

1. Bacterial cultures during their exponential growth phase were inoculated into individual 50mL sterile nutrient broth and incubated at 37°C.
2. 3mL of the suspension was pipetted out in a cuvette at the time of inoculation and the absorbance at 560nm (for the time 0) was recorded using appropriate blank.
3. The procedure was repeated for every half an hour and the absorbance value was recorded and tabulated accordingly for a period of 3 hours.
4. The generation time was calculated and the graph was plotted.

Calculation

$$n = \frac{\log N_t - \log N_0}{\log_2}$$

Generation time = t/n

Where, n: Number of generation in time "t"
N_t: Population number at time "t"
N_0: Initial number of population
t: Time

Preparation of Inoculums

1mL broth of the isolate was standardized using McFarland Standard (0.5). It contains 1×10^8 organisms per mL.

Preparation of the Substrate

Blank solution: (For multimeter calibration) 100mL of distilled water was autoclaved at 121°C at 15 lbs for 15 min.

Substrate: 100 mL of corn steep liquor was autoclaved at 121°C at 15 lbs for 15 min. Initial pH of the medium was 3.8. Since this pH was not suitable for growth of the organisms, it was adjusted to 7.0 using 1 N sodium hydroxide.

Mediator: Concentration of 0.1 M of methylene blue for 100mL of the substrate was added after the substrate was autoclaved and cooled to room temperature. Mediator is required for MFC to be operated using *Pseudomonas* species (Scott et al. 2006).

Microbial Fuel Cell setup: as per Guzzetta et al. (2007) and Yang et al. (2012).

Materials Used

Anode: Carbon rod (135mm × 11mm)
Anolyte: 100 mL of the substrate
Cathode: Zinc sheet (120mm × 80mm)
Catholyte: 100 mL of saturated zinc sulfate solution
Salt bridge: Glass tube with sieve filter
Salt solution: 25mL of saturated potassium chloride
Hook up wire: 2 × 75 mm

Assembly (Fig. 1)

1. Anode was connected to one end of the wire externally and placed inside the conical flask (250mL capacity) containing 100mL of the substrate (control without the inoculum and test with the inoculum).
2. Cathode was connected to the wire externally and placed in 100mL of the saturated zinc sulfate solution taken in a beaker and was closed air tight.
3. Salt bridge containing 25mL of saturated potassium chloride solution was placed in both the compartments with the sieve filter immersed in the liquid.
4. Anode compartment was cotton plugged air tight using non-absorbent cotton.
5. The other ends of the wires from cathode and anode were connected externally.
6. The potential difference and current across the electrode was measured using a multimeter.
7. Wires from the multimeter were placed across the electrode i.e., black wire on anode and red wire on cathode respectively. While taking readings, the point at cathode was kept constant. This point was attained during calibration of the multimeter.
8. Readings were noted at the time of inoculation and at the end of 6 hours of incubation.
9. Absorbance values of the substrate were also recorded correspondingly at 560 nm.
10. From the above readings obtained; resistance, electro motive force (emf) of the cell and power was calculated by applying the formula recommended by Premkumar (2010).
11. The experiments were repeated by altering the incubation temperature i.e., 25°C, 35°C and 45°C respectively.

The calculation was done as per Premkumar (2010).

$$\text{emf of cell} = E_R - E_L \qquad \text{(Equation 1)}$$

Where, emf: electromotive force
E_R: emf of the anode
E_L: emf of the cathode

$$V = IR \qquad \text{(Equation 2)}$$

$I = V/R$ and $R = V/I$

Where, V: Voltage, unit - Volt (V)
R: Resistance, unit - Ohm (Ω)
I: Current, unit - Ampere (I)

$$P = VI \qquad \text{(Equation 3)}$$

Where, P: Power, unit - Watt (W)

Calibration of Multimeter

MFC assembly was made with 100mL of distilled water (blank solution) as anolyte solution. Wires from the multimeter

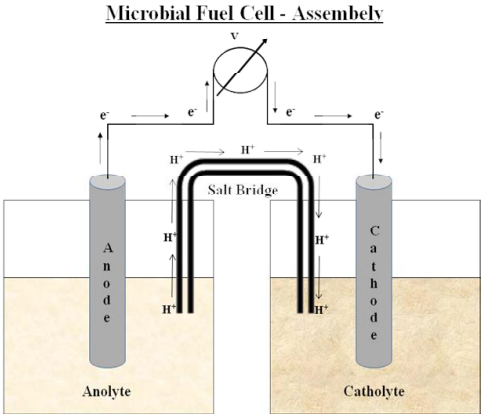

Fig. 1: Microbial fuel cell assembly.

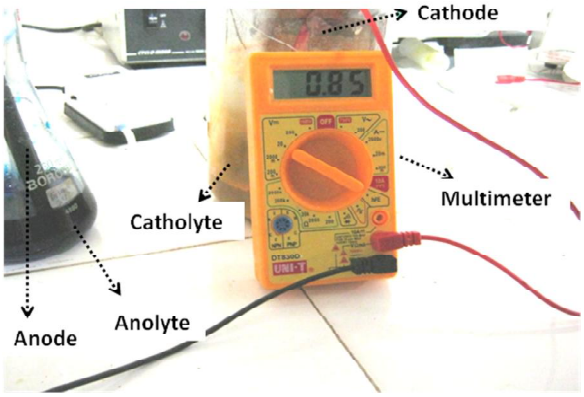

Fig. 2: Multimeter used for recording electricity.

were respectively placed across the electrodes. Readings were noted using digital display (Fig. 2). This procedure was performed every day for the reliability of the multimeter.

RESULTS

Five soil samples were obtained for this study. The soil temperature was 35°C and pH was 7.5. The colonies obtained from the processed samples were tested systematically by conventional methods and the desired isolates were identified and characterized as given in Tables 1 and 2.

Isolate 1: Isolate 1 was found to be Gram negative, motile and produced pink colored colonies on MacConkey's agar. Isolate also reported positive results for Indole, methyl red and hydrogen sulfide production tests and negative for Voges Proskauer, Citrate and Urease tests. It produced acid slant and acid butt on TSI agar. The above results suggest that the organism may be *Escherichia coli*. The results obtained from the above isolate were compared with standard *E. coli* Microbial Type Culture Collection (MTCC) 443 for confirmation.

Isolate 2: Isolate 2 was found to be Gram negative, motile and produced diffused bluish green color pigment on nutrient agar and colorless colonies on MacConkey's agar. Isolate also reported negative results for Indole, methyl red, Voges Proskauer and urease tests; negative and positive for citrate utilization test. It produced alkaline slant and alkaline butt on TSI agar. The above results suggest that the organism may be *P. aeuroginosa.* The results obtained from the above isolate were compared with standard *P. aeuroginosa* MTCC 5113 for confirmation.

Isolate 3: Isolate 3 was found to be Gram negative, motile and produced no diffusible pigment on nutrient agar with colorless colonies on MacConkey's agar. Isolate also reported negative results for Indole, methyl red, Voges Proskauer and urease tests; negative and positive for citrate utilization test. It produced alkaline slant and alkaline butt on TSI agar. The above results suggest that the organism may be *P. pseudoalcaligenes.* The results obtained from the above isolate were compared with standard *P. pseudoalcaligenes* MTCC 2651 for confirmation.

Generation Time Calculation

The genaration time calculated for various isolates is given in Table 3, showing maximum value for *P. aeruginosa* (24.24 min) followed by *P. pseudoalcaligenes* (22.06 min) and *E. coli* (20.02 min).

Calibration of the Multimeter

Since, surface area of the cathode was large, the red wire from the multimeter was moved over the surface of the cathode till digital meter showed 0.00 by keeping the black wire constant over the anode. This point of null deflection obtained by using distilled water as the substrate was marked and used as a standard cathode point to take readings for further proceedings of the experiment.

Electricity Production by by Microbial Fuel Cell

The electricity generation by microbial fuel cell with corn step liquor at various temperatures is given in Tables 4-7. The efficiency of the organisms was found to be *P. aeruginosa* > *E. coli* > *P. pseudoalcaligenes.* The absorbance for different isolates at different temperatures at 560 nm is shown in Figs. 3-6.

DISCUSSION

Momoh et al. (2010) in his experiment generated electricity from high strength abattoir wastewater and recorded it to be feasible at room temperature using a novel electron acceptor as catholyte in a dual chambered microbial fuel cell system with agar-salt bridge interconnection. The utilization of this electron acceptor in the single unit dual-chambered and double unit dual-chambered in parallel and series was observed

Table 1: Morphological characteristics of the isolates.

Isolates	Gram staining	Nutrient agar	MacConkey agar
Isolate 1	Negative	Greyish white, moist, smooth, opaque colonies.	Lactose fermenting colonies
Isolate 2	Negative	Large, opaque, irregular colonies with diffusible bluish green color pigment.	Non-lactose fermenting colonies.
Isolate 3	Negative	Large sized irregular colonies with no diffusible pigment	Non-lactose fermenting colonies.

The colonies were identified based on the morphology; and the color of the colonies produced on the selective and differential medium.

Table 2: Biochemical characteristics of the isolates.

Isolates	Motility	Catalase and Oxidase	I	MR	VP	C	U	TSI	G	L	S	M
Isolate 1	+	+ and -	+	+	-	-	-	A/ AG	AG	AG	-	AG
Isolate 2	+	+ and +	+	-	-	+	+	AK/AK	A	-	-	-
Isolate 3	+	+ and +	+	-	-	+	+	AK/AK	A	-	-	-

Note: I: Indole, MR: Methyl red test; VP: Voges Proskauer test; C: Citrate utilization test; U: Urease Test; TSI: Triple sugar iron agar test; Sugar fermentation test:- G: Glucose; L: Lactose; S: Sucrose; M: Maltose
+ : Positive, - : Negative, A: Acid, AK: Alkaline, AG: Acid and Gas Production

Table 3: Generation time calculation.

S.No.	Organism	Time (in minutes)					Generation time (in minutes)
		t_0	t_{30}	t_{60}	t_{90}	t_{120}	
1	*E. coli*	0.010	0.109	0.187	0.375	0.638	20.02
2	*P. aeruginosa*	0.010	0.037	0.165	0.227	0.311	24.24
3	*P. pseudoalcaligenes*	0.010	0.032	0.129	0.256	0.436	22.06

Table 4: Electricity production using microbial fuel cell with corn steep liquor at 37°C.

Organism		Control					Test				
		I(mA)	V(V) E_R	V(V) E_L	R(Ω)	P(mW)	I(mA)	V(V) E_R	V(V) E_L	R (Ω)	P(mW)
E. coli	Initial	1.54	-0.612	-0.762	96.90	0.231	1.124	-0.652	-0.762	97.80	0.123
	Final	2.65	-0.502	-0.762	97.83	0.689	7.460	-0.006	-0.762	101.3	5.640
P. aeruginosa	Initial	1.93	-0.573	-0.762	97.70	0.360	1.090	-0.653	-0.762	99.37	0.119
	Final	2.89	-0.473	-0.762	99.78	0.835	6.650	0.015	-0.762	116.7	5.160
P. pseudoalcaligenes	Initial	1.97	-0.573	-0.762	97.70	0.360	1.117	-0.651	-0.762	99.34	0.124
	Final	2.89	-0.473	-0.762	99.78	0.865	6.439	-0.024	-0.762	114.6	4.752

Table 5: Electricity production using microbial fuel cell with corn steep liquor at 25°C.

Organism		Control					Test				
		I(mA)	V(V) E_R	V(V) E_L	R(Ω)	P(mW)	I(mA)	V(V) E_R	V(V) E_L	R (Ω)	P(mW)
E. coli	Initial	0.572	-0.691	-0.762	124.05	0.040	0.725	-0.671	-0.762	125.36	0.066
	Final	1.580	-0.563	-0.762	125.59	0.315	3.020	-0.337	-0.762	140.59	1.284
P. aeruginosa	Initial	0.384	-0.715	-0.762	122.40	0.018	0.367	-0.717	-0.762	122.53	0.016
	Final	1.857	-0.534	-0.762	122.77	0.423	3.240	-0.314	-0.762	137.91	1.455
P. pseudoalcaligenes	Initial	0.384	-0.715	-0.762	122.40	0.018	0.334	-0.721	-0.762	122.58	0.032
	Final	1.857	-0.534	-0.762	122.77	0.423	3.240	-0.275	-0.762	144.93	1.582

Table 6: Electricity production using microbial fuel cell with corn steep liquor at 35°C.

Organism		Control					Test				
		I(mA)	V(V) E_R	E_L	R(Ω)	P(mW)	I(mA)	V(V) E_R	E_L	R (Ω)	P(mW)
E. coli	Initial	0.98	-0.671	-0.762	93.08	0.0889	1.260	-0.643	-0.762	93.87	0.105
	Final	2.14	-0.559	-0.762	94.57	0.435	6.070	-0.125	-0.762	104.93	3.867
P. aeruginosa	Initial	1.48	-0.609	-0.762	103.03	0.2272	1.150	-0.6419	-0.762	103.93	0.139
	Final	2.57	-0.494	-0.762	103.91	0.6912	5.930	-0.072	-0.762	117.7	4.151
P. pseudoalcaligenes	Initial	1.48	-0.609	-0.762	103.03	0.2272	0.992	-0.659	-0.762	103.84	0.102
	Final	2.57	-0.494	-0.762	103.91	0.6912	3.536	-0.373	-0.762	109.99	1.375

Table 7: Electricity production using microbial fuel cell with corn steep liquor at 45°C.

Organism		Control					Test				
		I(mA)	V(V) E_R	E_L	R(Ω)	P(mW)	I(mA)	V(V) E_R	E_L	R (Ω)	P(mW)
E. coli	Initial	1.915	-0.609	-0.762	79.89	0.2930	1.689	-0.611	-0.762	89.39	0.255
	Final	4.118	-0.424	-0.762	80.84	1.4132	6.580	-0.14	-0.762	94.6	4.095
P. aeruginosa	Initial	3.51	-0.481	-0.762	80.85	0.9863	3.200	-0.503	-0.762	80.77	0.831
	Final	5.78	-0.294	-0.762	80.99	2.706	10.320	0.1867	-0.762	91.88	9.785
P. pseudoalcaligenes	Initial	3.51	-0.481	-0.762	80.85	0.9863	3.122	-0.51	-0.762	80.71	0.787
	Final	5.78	-0.294	-0.762	80.99	2.706	9.770	-0.094	-0.762	99.79	9.525

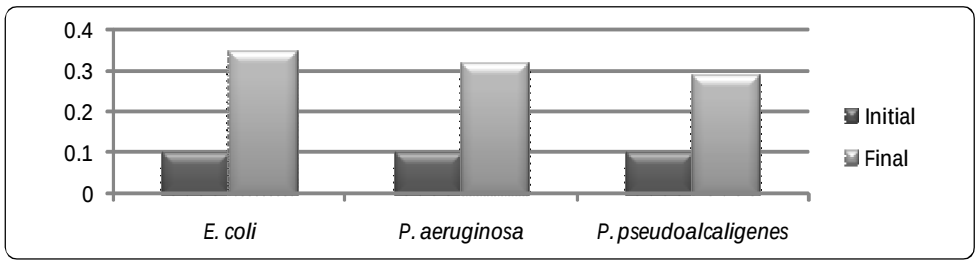

Fig. 3: The initial and final absorbance at 560nm for different isolates at 37°C.

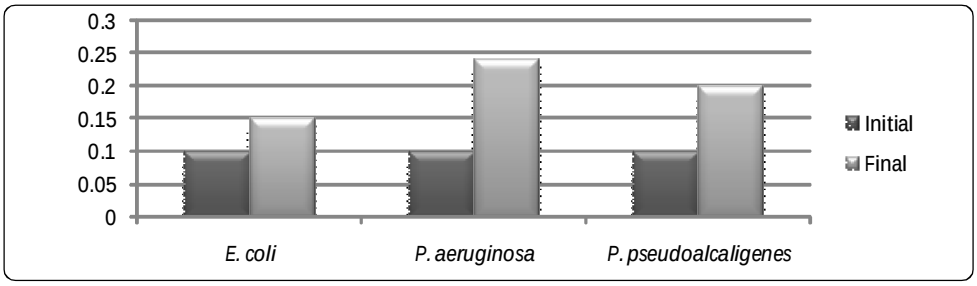

Fig. 4: The initial and final absorbance at 560nm for different isolates at 25°C.

by them to produce an open circuit voltage of 1560 mV, 1400mV and 2860 mV respectively.

In the present study, with corn steep liquor as a growth medium using a single unit dual chambered microbial fuel cell with salt bridge interconnection, all the three isolates were tested for electricity production but the only promising isolate which showed optimal activity in the current medium was *P. aeruginosa* as it gave satisfactory results under 37°C as I: 6.650mA, V: 0.776 V, R: 116.7Ω, P: 5.16 mW.

Farida & Markx (2008) used batch system for microbial fuel cell operation. The pure strain of *P. aeruginosa* was fed to the anode using artificial wastewater as substrate or fuel

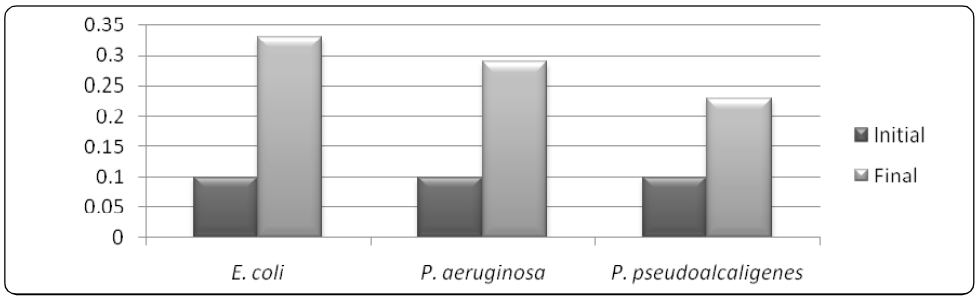

Fig. 5: The initial and final absorbance at 560nm for different isolates at 35°C.

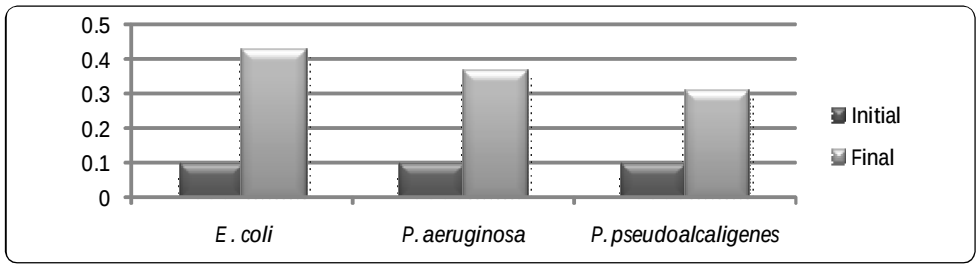

Fig. 6: The initial and final absorbance at 560nm for different isolates at 45°C.

which consisted of glucose-glutamic acid in range of 100-400 mg/mL BOD. As a result, the optimum condition for *P. aeruginosa* at concentration of 8mg/mL the value of the power density and volumetric power in this condition was 1198.61 mW m^{-2}. For microbial aggregation, voltage and current obtained were 0.2 V and 0.27 to 0.3 mA respectively.

In the current study, by experimenting using *P. aeruginosa* in a single unit dual chambered microbial fuel cell with salt bridge inter connection, the substrate with corn steep liquor at 45°C obtained a maximum result of I: 10.32 mA, V: 0.948, R: 91.88 Ω and P: 9.785 mW. Wherein *E. coli* (mediator-less MFC) showed moderate results in all the experiments with the maximum result of I: 10.003 mA, V: 0.987 V, R: 98.7Ω, P: 9.603 mW in corn steep liquor as the substrate.

CONCLUSION

The low cost microbial fuel cell was found to be efficient and consistent in all operating conditions. It is also proved that increase in temperature will result in decrease in resistance and increased output as per the experimental value. *P. aeruginosa* was recorded to give satisfactory results in corn steep liquor. Since both the species of *Pseudomonas* were operated in mediator based microbial fuel cell, the efficiency of rate of transfer of electrons to the anode surface was high and reliable when compared to that of *E. coli* which was operated in mediator-less microbial fuel cell obtained only moderate output under all operating conditions.

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