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Research Article

Enhancing and optimization of electricity production by dye degrading bacteria in microbial fuel cell

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Abstract

In the recent years, various new technologies were used in the waste water treatment. The Microbial fuel cells (MFC) involved the bio electrochemical reaction which produces electricity by the microorganism. Microbial fuel cell is the alternative source of electricity generation from microorganisms for supplementing the high demand of energy. Electricity generates from the readily biodegradable organic substrate accompanied by decolourization of azo dye was investigated using a two-chamber microbial fuel cell (MFC). The azo dyes which constitute the largest chemical class of synthetic dyes extensively present in effluent discharged from dye manufacturing industries and dye consuming industries. The power output depends on the several factors to influencing the electricity generation. In this discussed about, Microbial fuel cell was constructed for bioelectricity production by using dye effluent collected from three different location of Coimbatore, Tiruppur and Chennai, Tamil Nadu, five dye degrading bacterial cultures to use for voltage production from dye effluent sample. The various parameters like Temperature, pH, Carbon source, Nitrogen source, different Surface area of electrodes, different concentration of Catholyte solution and different concentrations of salt bridge have been optimized for the maximum voltage production. The voltage was measured in Multimeter and compared among all the microorganisms.

Keywords: Optimization, Electricity production, Dye degrading Bacteria and Microbial fuel cells.

1. Introduction

The energy consumption in the present generations has been increased because of the usage of electrical devices in this modern world (Deepika *et al.,* 2015). The Energy generation is the serious environment issue of the world is to solve the problem of global warming and CO_2 emission. Using renewable energy from biomass is a promising method to overcome these problems (Sahar Bakhshian *et al.,* 2011). Microbial Fuel cell technology is a promising approach to waste water treatment as the process can convert the chemical energy of the contaminants to electricity while simultaneously completing wastewater treatment (Xuyun Wang *et al.,* 2014; Asodariya and Patel, 2011).

Electricity from microorganisms is alternative source for satisfying the high demand of energy. MFC is a bioelectrochemical device where electricity is produced from organic matter by biocatalytic reactions. In a MFC, the substrate (organic matter) is oxidized in a type of biological process in which microorganisms deliver electrons to the anode surface. This type of wastewater treatment has interesting advantages versus traditional (Logan, 2008; Merina Paul Das. 2013).

Currently, the discharge of dye wastewater is an important environmental hazard. These dyes are highly stable in light and during washing. They are also resistant to microbial degradation. Azo dyes, which are aromatic compounds with one or more –N=N– groups, are the most important commonly used synthetic dyes in commercial applications (Sriram *et al*., 2013; Saranraj *et al*., 2014). The waste water discharge is undesirable because of their color and their breakdown products are toxic and mutagenic. Their discharge in surface water leads to aesthetic problems and obstructs the light penetration and oxygen transfer into water, hence affecting aquatic life (Umbuzeiro *et al*., 2005; Saranraj and Stella, 2012;

Saranraj and Stella, 2014; Saranraj and Sujitha, 2014; Jayanthi *et al*., 2014).

In the past decade, researchers have made significant progress towards understanding fundamental issues of microbiology, electrochemistry and reactor architecture in MFCs (Arends and Verstraete, 2012). However, MFC development is still hindered by challenges such as system scaling up and further improvement of electric energy. However, implementation of MFCs for practical wastewater treatment is not straight forward yet because of the many remaining technical and economic obstacles (Sevda et al., 2013; Logan, 2010).

In a MFC, Optimizing operating conditions is another important approach to improve MFC performance. Besides factors like temperature and pH, different Surface area of anode, different concentration of Cathode solution, different concentration of salt bridge using mixing intensity to improve mass transfer could be an effective method to improve the performance in continuously operated MFCs. The objective of this study was the power generation by a MFC using the bacterial culture from the dye effluent samples and also optimize of the performance of the MFC.

2. Materials and Methods

2.1. Collection of Sample

The three different textile dye effluents were collected from the Tirupur, Coimbatore and Chennai in Tamil Nadu, India.

2.2. Screening of Stable voltage Production by dye degrading bacteria

The five textile dye degrading bacterial isolates such as *Pseudomonas aeruginosa*, *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas fluorescens* and *Escherichia coli* were subjected to produce the maximum voltage production. The 25mL of 24h old bacterial culture in nutrient media were poured into the anodic chambers of MFCs containing respective 75mL sterilized Nutrient media as anolyte with 50mM Di Potassium hydrogen phosphate and Potassium Ferricyanide enriched in 100mM phosphate buffer as catholyte (Kassongo and Togo, 2010). The electricity generation was monitored from the MFC using multimeter.

2.3. MFC configuration and operation

Two MFC chambers were designed and fabricated using 250 ml conical flask in our laboratory. The fuel cell consisted of two equal volume (200 ml) chambers for anode and cathode connected by Salt Bridge. Plain graphite rods without any coating were used as electrodes for both anode and cathode. The electrodes were positioned at a distance of 6cm. Copper wires were used as contact with electrodes after carefully sealing the contact area with ‘epoxy’ material. Prior to use the electrodes were soaked in deionized water for a period of 24 hrs. The voltage production was monitored by Multimeter.

2.4. Effect of pH for voltage production

The MFC was constructed with carbon electrodes as it generates voltage, four different MFC each with different pH such as 5.0, 6.0, 7.0 and 8.0 was constructed and measurements were taken every 24 hrs for 15 days.

2.5. Effect of temperature for voltage production

For studying the role of temperature in electricity production, four different MFC were constructed each with different temperature such as 25°C, 30°C, 35°C and 40°C and measurements were taken every 24 hrs for 15 days.

2.6. Effect of Carbon sources for voltage production

The different carbon sources *viz*., 1% of Starch, Glucose, Fructose and Sucrose were used in MFC for electricity production. The voltage measurements were taken every 24 hrs for 15 days.

2.7. Effect of Nitrogen sources for voltage production

The different Nitrogen sources *viz*., 1% of Yeast extract, Urea, Ammonium chloride and Ammonium sulphate were used in MFC for electricity production. Various Nitrogen sources have been tried out for checking the voltage generation. The voltage measurements were taken every 24 hrs for 15 days.

2.8. Effect of different of Catholyte concentrations for voltage production

The 100mL of standard catholyte was also manipulated by different concentrations (25, 50, 75 and 100mM) of Di Potassium hydrogen phosphate and Potassium Ferricyanide enriched in 100mM phosphate buffer in the cathodic chamber. Voltage measurements were taken multimeter every 24 hrs for 15 days.

2.9. Effect of surface area of Electrodes for voltage production

The Carbon rod electrodes used in the MFCs were varied in their superficial surface area to observe their effect on the bioelectricity generation. The MFC setups were constructed keeping the different surface areas of electrodes in both chambers such as $8cm^2$, $16cm^2$, $32cm^2$ and $64cm^2$. Voltage measurements were taken in multimeter every 24 hrs for 15 days.

2.10. Effect of salt bridge concentration for voltage production

In this study, different concentrations of KCl and NaCl (1M, 2M, 3M, 4M,) and 3% agar concentration in salt bridge were tested for ability to transfer of ions through salt bridge for high electricity generation. The concentration which shows high conductivity was taken further for optimization. Voltage measurements were taken multimeter every 24 hrs for 15 days.

3. Result and Discussion

3.1. Screening of Stable voltage producing by dye degrading bacteria

The stable voltage generation from five different dye degrading bacteria was measured and the results were showed in Table - 1. In this study, the voltage production by the dye degrading bacteria was enhanced by optimizing different factors influencing voltage productions.

Table – 1: Screening of stable voltage producing bacteria from dye effluents

S. No	Bacterial isolates	Stable voltage production (mV)
1	*Pseudomonas aeruginosa*	670
2	*Bacillus cereus*	610
3	*Bacillus subtilis*	560
4	*Pseudomonas fluorescens*	610
5	*Escherichia coli*	600

3.2. Effect of pH for voltage production

The effect of different pH *viz.* 5, 6, 7, and 8 for Voltage output by the five bacterial strains was investigated and the results are listed in Table- 2. The maximum voltage output was recorded at pH 7 by *Pseudomonas aeruginosa* (825 mV) followed by voltage output of *Bacillus cereus* (720 mV), *Bacillus subtilis* (695 mV), *Pseudomonas fluorescens* (690 mV) and *Escherichia coli* (670 mV). Similarly, pH plays a vital role in many biological experiments and, therefore, pH ranging from 5.0–9.0 has been tested in the anode chamber and the results showed that pH 7.0 recorded a maximum voltage of 590 mV and 420 mA. When the rumen fluid pH is changed to acidic, the voltage and current production is increased (Deepika *et al.*, 2015). Each of the microbial groups involved in the degradation had a specific pH optimum and could grow in specific pH range. The optimum range for all methanotrophic bacteria was between 6 and 8, whereas anaerobic bacteria were notably less sensitive to pH variations (Bailey and Ollis, 1986; Haandel and Lettanga, 1994). Similarly, Jadhav and Ghangerkar (2009) reported that highest current was generated at pH of 6.5 in the anodic chamber with CE of 4% and higher pH difference between both electrolytes favored higher current and voltage.

Table - 2: Effect of different pH for voltage production

S. No	Bacterial isolates	Different pH for voltage production (mV)			
		pH5	pH6	pH7	pH8
1	*Pseudomonas aeruginosa*	720	750	825	730
2	*Bacillus cereus*	640	660	720	590
3	*Bacillus subtilis*	580	635	695	560
4	*Pseudomonas fluorescens*	610	630	690	620
5	*Escherichia coli*	600	620	670	590

3.3. Effect of Temperature for Voltage production

The effect of different temperature *viz.* 25°C, 30°C, 35°C, and 40°C for Voltage output by the five bacterial strains was investigated and the results are listed in Table- 3. The maximum voltage output was recorded at 30°C by *Pseudomonas aeruginosa* (830 mV) followed by voltage output of *Bacillus cereus* (760 mV), *Bacillus subtilis* (670 mV), *Pseudomonas fluorescens* (660 mV) and *Escherichia coli* (640 mV). The results similar with Larrosa-Guerrero *et al.* (2010) reported the effect of temperature on the performance of MFCs; maximum power density was 174.0 mW m−3at 35 °C. In addition, microbial cellulase production was observed in the temperature optima of about 35ºC to 45ºC (Dutta *et al.*, 2008). Rathnan and Ambili (2011) reported that the optimal temperature for the cellulase enzyme production was 30ºC to 45ºC. Kumar *et al.* (2013) reported that the strain RK6 showed maximum cellulolytic activity and growth at 38ºC.

Table - 3: Effect of different Temperature for voltage production

S. No	Bacterial isolates	Different Temperature for voltage production (mV)			
		25 °C	30 °C	35 °C	40 °C
1	*Pseudomonas aeruginosa*	720	830	750	710
2	*Bacillus cereus*	670	760	660	590
3	*Bacillus subtilis*	610	670	580	550
4	*Pseudomonas fluorescens*	580	660	630	600
5	*Escherichia coli*	600	640	620	590

3.4. Effect of Carbon for Voltage production

In the present study, the effect of different carbon source *viz.* Starch, Glucose, Fructose and Sucrose. Voltage output by the five bacterial strains was investigated and the result shown in Table- 4. The maximum level of voltage was recorded in glucose as a carbon source by *Pseudomonas aeruginosa* (870 mV) followed by *Bacillus cereus* (750 mV), *Bacillus subtilis* (710 mV), *Pseudomonas fluorescens* (670 mV) and *Escherichia coli* (660 mV). Effect of carbon source in bioelectericity production many reports are available on the bioelectricity, generated by many bacterial organisms with different waste materials as substrates. Substrate is important for any biological process as it serves as a carbon and energy source. The efficiency and economic viability of converting organic wastes to bioenergy would depend on the characteristics and components of the waste materials (Deepak Pant *et al.*, 2010). A great variety of substrates can be used in MFCs for electricity production ranging from pure compounds to complex mixtures. MFC's can use bacteria from the natural environment to generate electricity from various substrates such as glucose, acetate, butyrate, lactate, ethanol, cysteine and bovine serum albumin as well as those from waste streams such as domestic waste waters and various food-industry waste waters (Rabaey *et al.*, 2005; Rezaei *et al.*, 2007; Liu and Logan 2004.

Table - 4: Effect of different Carbon Source for voltage production

S.No	Bacterial isolates	Different Carbon Source for voltage production (mV)			
		Starch	Glucose	Fructose	Sucrose
1	*Pseudomonas aeruginosa*	840	870	820	780
2	*Bacillus cereus*	680	750	710	680
3	*Bacillus subtilis*	650	710	660	650
4	*Pseudomonas fluorescens*	640	670	650	630
5	*Escherichia coli*	620	660	620	620

3.5. Effect of Nitrogen for Voltage production

In the present study, the effect of different Nitrogen source *viz.* Yeast extract, Urea, Ammonium chloride and Ammonium sulphate. Voltage output by the five bacterial strains was investigated and the result shown in Table- 5. The maximum voltage output was recorded in urea as a nitrogen source by *Pseudomonas aeruginosa* (870 mV) followed by *Bacillus cereus* (710 mV), *Bacillus subtilis* (680 mV), *Pseudomonas fluorescens* (660 mV) and *Escherichia coli* (650 mV). Mannarreddy Prabu *el al.*, 2012 also reported beef extract as a nitrogen source to produce maximum electricity production followed by malt extract and yeast extract. Peptone and ammonium molybdate showed moderate electricity production.Urea as a nitrogen source to yielded maximum bioelectricity of 0.30V at 1% concentration (Safa Sheikh *et al.*, 2015). The result also reported by (Shiv Kumar *et al.*, 2014) for urea as nitrogen source gave maximum yield of 1.575V.

Table - 5: Effect of different Nitrogen Source for voltage production

S.No	Bacterial isolates	Different Nitrogen Source for voltage production (mV)			
		Yeast extract	Urea	Ammonium chloride	Ammonium sulphate
1	*Pseudomonas aeruginosa*	820	870	820	750
2	*Bacillus cereus*	650	710	680	670
3	*Bacillus subtilis*	620	680	650	630
4	*Pseudomonas fluorescens*	630	660	630	650
5	*Escherichia coli*	680	650	620	610

3.6. Effect of different Catholyte concentration for Voltage production

The bioelectricity production was studied with different concentrations of Di-potassium hydrogen phosphate and Potassium Ferricyanide for cathyote viz. 25mM, 50mM, 75mM and 100mM. Voltage output by five bacterial strains was investigated and the result shown in Table - 6. The maximum voltage output was recorded in 50mM catholyte concentration by *Pseudomonas aeruginosa* (810 mV) followed by *Bacillus cereus* (680 mV), *Bacillus subtilis* (670 mV), *Pseudomonas fluorescens* (660 mV) and *Escherichia coli* (640 mV). Optimization cathyote also recorded in mediator less microbial fuel cell losses occur in the cathode compartment due to activation over potentials which can bedecreased by adding K3Fe (CN) 6 to the liquid catholyte (Park *et al*., 2000; Araceli González del Campo *etal.*, 2014). The maximum current and the voltage in the MFC with the potassium permanganate as the electrolyte solution showed an increase of 19% and 12% respectively (Rita Arbiant*i et al.*, 2013).

Table - 6: Effect of different Catholytes concentration for voltage production

S. No	Bacterial isolates	Different concentration of Di Potassium hydrogen phosphate and Potassium Ferricyanide for voltage production (mV)			
		25mM	50mM	75mM	100Mm
1	*Pseudomonas aeruginosa*	725	810	780	710
2	*Bacillus cereus*	620	680	640	615
3	*Bacillus subtilis*	570	670	560	600
4	*Pseudomonas fluorescens*	630	660	650	620
5	*Escherichia coli*	610	640	630	605

3.7. Effect of different surface area for Voltage production

The bioelectricity production was studied with different surface area of electrodes for both anode and cathode chamber *viz.* 8cm^2, 16cm^2, 32cm^2 and 64cm^2. Voltage output by five bacterial strains was investigated and the result shown in Table - 7. The maximum voltage output was recorded in 32cm^2 surface area of electrodes by *Pseudomonas aeruginosa* (820 mV) followed by *Bacillus cereus* (710 mV), *Bacillus subtilis* (680 mV), *Pseudomonas fluorescens* (670 mV) and *Escherichia coli* (660 mV) Similarly, the maximum output voltage (595 mV) and power density (393.4 mWm-2) were produced when the electrode spacing was 2 cm (Da-yu Yu *et al.*, 2012). Evans M.N. Chirwa *et al.*, 2010 reported the anode and cathode's surface area and adding more oxygen to the cathode. The increase in surface area of the anode and cathode increased the power output from 0.026 mW to 0.054 mW. The increase in power output was due to an increase in area at which the respective anode and cathode mechanisms could take place.

Table - 7: Effect of different anode surface area for voltage production

S. No	Bacterial isolates	Different anode surface area for voltage production (mV)			
		8 cm^2	16 cm^2	32 cm^2	64 cm^2
1	*Pseudomonas aeruginosa*	720	750	820	780
2	*Bacillus cereus*	610	630	710	680
3	*Bacillus subtilis*	580	610	680	650
4	*Pseudomonas fluorescens*	610	630	670	650
5	*Escherichia coli*	600	620	660	630

3.8. Effect of salt bridge concentration for Voltage production

In this study, the effect of salt bridge concentration *viz.* 1M, 2M, 3M and 4M. Voltage output by five bacterial strains was investigated and the result shown in Table - 8. The maximum voltage output was recorded in 1M concentration NaCl and KCL by *Pseudomonas aeruginosa* (780 mV) followed by *Bacillus cereus* (680 mV), *Bacillus subtilis* (670 mV), *Pseudomonas fluorescens* (650 mV) and *Escherichia coli* (630 mV). Similarly, Safa Sheikh *et al.*, 2015 Reported different molar concentration of NaCl and KCl. The 1M Nacl and KCl gave highest yield of 0.17V while 2M, 3M and 4M KCl and NaCl showed 0.11, 0.14 and 0.13V respectively. Deepika *et al.*, 2015 performed the salt bridge made of sodium chloride and agar in the ratio of 1:2.

Table - 8: Effect of salt bridge concentration for voltage production

S. No	Bacterial isolates	Different Salt bridge concentration for voltage production (mV)			
		1M	2M	3M	4M
1	*Pseudomonas aeruginosa*	790	760	780	680
2	*Bacillus cereus*	740	555	640	585
3	*Bacillus subtilis*	710	530	650	550
4	*Pseudomonas fluorescens*	680	635	630	650
5	*Escherichia coli*	660	600	620	625

4. Conclusion

The present study revealed that the physical parameters involved a importance role in the bioelectricity production in MFC developed with dye effluent collected from three different regions viz., Tiruppur, Coimbatore and Chennai in Tamil Nadu, India. The Carbon electrodes produced a maximum bioelectericity at pH 7.0 and temperature at 30°C, Glucose as a carbon source, Urea as a nitrogen source, 32 cm of cathode surface area, 1M salt concentration of NaCl and KCL and catholytes of each 50 mM concentration of Di Potassium hydrogen phosphate and Potassium Ferricyanide. This study concluded that the above physical and chemical parameters were observed as the optimum condition for the enhanced bioelectericity production.

5. References

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