Isolation and Screening of Exoelectrogenic Bacteria from Waste Water

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INTRODUCTION

Increasing human activities are consuming the natural energy sources leading to the depletion of fossil fuels. The present day energy scenario in India and around the globe is precarious. The need for alternate fuel has made to initiate extensive research in identifying a potential, cheap and renewable source for energy production (**Mahindra B**. *et al.*, **2013**). MFCs are an emerging technology that directly converts the chemical energy stored in organic matter to electricity (**Fatihah S**. *et al.*, **2013**). A Technology using MFC that convert the energy stored in chemical bonds in organic compounds to electrical energy achieved through the catalytic reactions by microorganisms.

Wastewater is an energy rich source for growth of several anaerobic and facultative bacterial species which have the capability to transfer electrons to an anode, as a terminal electron acceptor and thus are classified as electrogenic bacteria (Zakira N. et al., 2016). The activity of electrogenic microorganisms generally increases with the increase of the dissolved organic matter until the concentration reaches saturation. A typical MFC consists of two chambers, i.e. the anaerobic anode chamber and the aerobic cathode. The two chambers are separated by a membrane (e.g., proton exchange membrane or salt bridge), (Denny A. et al.,

ABSTRACT

Renewable energy is an increasing need in our society. Microbial fuel cell (MFC) technology is a bioreactor that converts organic waste material into electrical energy through catalytic reactions of microorganisms. This environmentally friendly process produces electricity without the combustion of fossil fuel. The objective of this study was to isolate Exoelectrogenic microorganisms with a potential to generate electricity and simultaneously treat waste water. The polluted water used in this study was obtained from Ulhas river Dist. Thane, Maharashtra and analysed for pH, salinity, hardness TDS, TSS, TS, COD and BOD. Exoelectrogenic bacteria were isolated from the polluted water sample and screened their ability to generate electricity by constructing cost effective MFC. The potential exoelectrogenic bacteria were then identified as *E.coli, Pseudomonas spp* and *S. aureus spp.* Thus, the selected exoelectrogenic bacteria can be used for waste water treatment alongwith generation of electricity thereby reducing the environmental pollution.

KEYWORDS: Microbial fuel cell, exoelectrogenic bacteria, biofilm

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2015) The protons generated pass from anode to cathode via the ion exchange membrane.

MFC is widely applied in following area such as Brewery Wastewater Treatment, Sewage Treatment, Hydrogen Production, Generation of Energy out of Bio-waste/Organic Matter, Remote Power Source, Omission of gas treatment, Sludge production, Microbial Fuel Cell generating electricity from wastewater, MFC for wastewater treatment: heavy metal removal, sewage sludge treatment, and its potential application in wastewater reuse in irrigation (Rohit M. et al., 2017). Thus, the present study is an attempt to isolate and select the potential exoelectrogenic bacteria from polluted water for generation of electricity and degradation of waste water as well.

MATERIALS AND METHODS

Collection and Physiochemical characterization of polluted waste water

The waste water was collected in clean plastic containers from Ulhas river near Ulhasnagar station, Dist. Thane. Maharashtra, India. The collected sample was studied for various physicochemical parameters like pH, total solid (TS), Total dissolved solids (TDS) and total suspended solids International Journal of Trend in Scientific Research and Development (IJTSRD) @ www.ijtsrd.com eISSN: 2456-6470

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(TSS), salinity, hardness, COD and BOD as per the standard procedure given in the standard methods for the examination of Water and Wastewater (APHA, 2012).

Isolation and screening of exoelectrogenic organisms

Ulhas River was used for isolation of exoelectrogenic organisms. The water sample was diluted and inoculated on Nutrient agar. The morphologically distinct bacterial colonies obtained on nutrient agar were studied for their cultural characteristics and further inoculated on sodium thioglycollate medium. Incubation was done under microaerophilic condition for 4-5 days at room temperature. All the isolates showing growth under on sodium thioglycollate medium were then screened for their ability to generate electricity by constructing MFC.

Study of exoelectrogenic property

Two plastic containers were used as a MFC chamber, one was anode chamber and another was cathode chamber as reported by **Prabu M.et al. (2012).** Anode chamber was filled with sodium thioglycolate with isolate, cathode chamber was filled with 0.2% NaCl solution. Aluminum mesh were used for both anode and cathode chamber. Biofilm was formed on anode chamber. Both connected to multi meter for measurement of output voltage. The suspension of the isolates was added to anode chamber along with growth medium and allowed to form bio film on anode surface separately and also in mixed form. After the formation of bio film, the growth medium was replaced by waste water and observed for exoelectrogenic property after 24 hours. The electricity generation was monitored using multi meter.

RESULTS AND DISCUSSION

The collected waste water was analysed for pH, salinity, hardness, TDS, TSS, TS, COD and BOD (Table 1). The pH of the collected water sample was found to be to be near neutral range. The value of TDS, TSS and TS of the collected water sample was found to be very high indicating poor quality of water due to the various undesirable suspended and dissolved components in the sample. COD and BOD are a measure of pollution in aquatic ecosystem and estimate carbonaceous factor of organic matter. The high values of BOD and COD reflects the high pollutant content of the water (Chaudhari and Deshmukh, 2015).

Isolation and screening of exoelectrogenic microorganisms from waste water

The collected sample was diluted and used for isolation of organisms, using nutrient agar which were further screened for their exoelectrogenic property. 12 isolates with different cultural features were obtained from the collected waste water. The colony size of the isolates ranged between pinpoint to 3mm. Isolate no. 3 showed typical bluish-green pigment on NA plate, while isolate 12 showed golden yellow pigment **(Table 2)**. Screening study of the isolate for exoelectrogenic property was done using sodium thioglycollate medium. Mainly gram-negative, facultative anaerobic bacilli, gram- positive cocci, strict anaerobic bacteria can grow on this media. Because of this property it is very useful for microbial isolation of exoelectrogenic bacteria. Mainly, exoelectrogenic bacteria are anaerobic and facultative anaerobic in nature.

Table 1: Physicochemical analysis of waste water

Parameter	Value
pН	6.8±0.2
TDS	194.6±2.50mg/L
TSS	132.600±1.50mg/L
TS	326.6±3.50mg/L
Salinity	2.58±0.5%
Hardness	264±10mg/L
BOD	330±10mg/L
COD	842±mg/L

Table 2: Colony characteristics of isolates obtainedfrom waste water

ii olii waste water		
	Isolate no.	Colony characteristic on nutrient agar
	1	2mm, circular,off white, convex, transparent, smooth, gram negative rods
	2	1mm, circular, entire, light yellow, dry, flat, opaque, gram negative rods
	3	2mm, circular, bluish-green, opaque, flat, entire, smooth, gram negative rods
	4	1mm, circular, entire, off white, dry, flat, transparent, gram positive cocci
S	150	1mm, circular, off-white, entire, flat, opaque, gram negative rods
	6 A	Pin point, circular, seriate, white, low convex, smooth, gram negative rods
	7	3mm, circular, entire, white, dry, flat, transparent, gram negative rods
al	Joårna	1mm, circular, entire, colorless, smooth, flat, opaque, gram negative rods
S	cieștific and	1mm, circular, entire, white, smooth, flat, transparent, gram positive cocci
	ne ¹⁰	2mm, circular, golden yellow, entire, covex, gram positive cocci
6	-6410	1mm, circular, entire, white, smooth, low convex, opaque, gram negative rods
	12	1mm, circular, irregular, off white, rough, concave, transparent, gram positive rods
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Identification and characterization of microorganisms

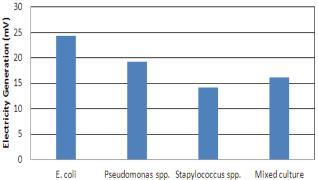
Identification of the isolates was done on the basis of morphological, cultural and biochemical characteristic. Cultural features of the isolates were studied by streaking them on selective media like MacCkonkey's agar, Baired Parker medium and cetrimide agar. Isolates with exoelectrogenic property were identified as *E.coli*, *Pseudomonas* and *S. aureus spp.*

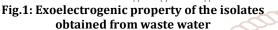
Exoelectrogenic potential of the isolate

In the present study, biofilm was formed on anode surface using all the three isolate in pure culture form separately and also biofilm was using all the three isolates in mixed culture form and then checked for electricity generation using multi meter. The generated electricity was measured in mV after 24hrs (**Fig. 1**). The maximum electricity generation was seen with *E. coli* compare to *Pseudomonas spp, Staphylococcus spp* in pure culture form and in mixed form. Mixed culture generally produced more electricity than pure culture, due to complex syntrophic relationship between the microorganism species in the MFC but in the presence study *E.coli* in pure culture form showed more electricity generation. Individual bacterial species in mixed culture was assumed to have specific mechanism in transferring electrons to produce high electricity generation,

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but the complex relationship has yet to be explained scientifically, but using mixed culture increase the start up time and longer time is required to reach stability of the power generated by the fuel cell. Furthermore, handling mixed culture is more challenging than pure culture. Surprisingly the electricity generation with mixed culture biofilm which was less but it was more than *Staphylococcus spp.* **Denny A.** *et al.*, **(2015)** have reported the generation of electricity from *Shewanella putrefaciens, Pseudomonas aeruginosa and Geobacter sp*





CONCLUSION

The findings indicate the potential of the exoelectrogenic *E. coli* isolated from waste water for development of MFC technology for practical applications. MFC offers an eco-friendly way for the generation of electrical energy along with effective treatment of waste water.

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