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STUDIES ON ISOLATION, CONSORTIUM ESTABLISHMENT, INOCULUM DEVELOPMENT AND ELECTROPOTENTIAL ANALYSIS OF THE MICROORGANISMS USED IN MICROBIAL FUEL CELL

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ABSTRACT

Microbial fuel cells are new emerging technologies used as a sustainable source of electricity generation. Four sets of double chambered Microbial fuel cells were constructed and operated during current research. Synthetic waste water was initial substrate used during standardization. Primarily 20 municipal sewage isolates from Osmanabad city (Maharashtra, India) were used separately as inoculants. The electrogenic ability of 20 isolates was determined using Microbial fuel cells constructed during current research. Among these twenty, the four higher electrogenic bacteria (1, 3, 10, and 13) were selected to establish consortium by grouping them in eleven groups using permutation and combination statistical formula. The group of four isolates shows highest electrogenic ability (390 mV) constitutes the final consortium. The established consortium after 24 hr enrichment was used as inoculums. The primary substrate, in each set was separately replaced by waste water namely Agro industry waste water, Dairy industry waste water, Distillery industry waste water and Municipal waste water. Fifty-two microorganisms; 12 from Dairy waste water, 15 from Distillery waste water, 11 from Municipal waste water and 14 from Agro waste water were isolated and tested for electrogenic ability. Among them two isolates show electrogenic ability higher than 500 mV.

KEY WORDS

Microbial fuel cells, primary inoculants, consortium, electro potential.

INTRODUCTION

Microbial fuel cells (MFCs) are devices that utilize chemical energy of substrate to generate electrical energy by the catalytic activity of microorganisms (Allen and Bennetto, 1993, Gil *et. al.,* 2003 and Moon *et. al.,* 2006). The microbes can do this at the expense of their routine mechanism of the biodegradation of organic matters or wastes as substrate (Park and Zeikus, 2000; Oh, and Logan, 2009; Logan *et al.,* 2005 and Du *et. al.,* 2007). Microbial fuel cells are the means that involves the electricity generation by using the phenomenon of anaerobic respiration by microorganism which is common characteristic of anaerobic metal reducing bacteria (Matthew Charles Knighton, 2013). Microbial fuel cell is a new method for biological electricity generation with simultaneous waste treatment (Liu *et al.*, 2004; Logan and Regan, 2006). For successful utilization of MFCs, selection of electrogenic microorganism either pure culture or mixed culture is a critical point (S. K. Chaudhuri and D. R. lovely, 2003; Logan *et. al.,* 2006). Commonly mixed cultures are used as inoculum but MFCs can also run using single strain as inoculant (Rababe *et. al.,* 2005) but the results with consortium are satisfactory. Commonly used inoculum is anaerobic respiring bacteria, methanogenic micro flora, anaerobic digester sludge, domestic, municipal or industrial waste water, or even common laboratory isolated micro flora etc. It can be isolated from marine

sediment, fresh water sediment, waste water and activated sludge, (Niessen *et. al.,* 2006; Zhang *et. al.,* 2006).

MATERIAL AND METHODS

Isolation of Primary inoculants

The primary bacterial inoculants were isolated from Municipal sewage sample from Osmanabad city (Maharashtra, India). The sewage sample is streaked on nutrient agar plate and incubated at 37^0c for 24 hr. The twenty well isolated colonies from the plate were selected and slanted on nutrient agar media for further study.

Enrichment of Primary inoculants

Primary inoculants were enriched for 24hr in nutrient broth, 10% of enriched culture was used as inoculum to analyze their electro potential using MFCs.

Electro potential analysis of Primary inoculants

The four-double chambered MFCs sets constructed during current research were operated to observe the electro potential ability of the isolates. Each isolate from the twenty are analyze separately for the ability to produce electricity using synthetic waste water as substrate.

Establishment of consortia.

Four higher electrogenic isolates (1, 3, 10, and 13) indicated as 13-In1, 10-In2, 1- In3, 3-In4 among twenty were selected to establish consortia by grouping into different groups (Supriya kumari, 2012) using permutation and combination statistical formula that utilize different subsets of 'r' objects selected from 'n' objects ($r \leq n$) randomly.

$$^{n}C_{r} = \frac{n!}{(n-r)!\,r!}$$

Where; n - number of objects, r – subsets and
C – combination.

First lot (n=4, r=2), Total six groups; A= In1+In2, B= In1+In3, C= In1+In4, D=In2+ In3, E =In2+In4, F=In3+In4.

Second lot (n=4, r=3), Total four groups; G= In1+ In2+ In3, H= In1+ In2+ In4, I=In1+In3+In4, J= In2+ In3+ In4.

Third lot (n=4, r=4), single group K= In1+ In2+ In3+ In4

Inoculum development Highest electrogenic group of consortia (K) after 24 hr of enrichment were used as final inoculum in each set of MFCs.

Isolation of electrogenic microorganism from different waste water.

Samples from working anode chambers of each set of MFCs were streaked on nutrient agar to get isolated bacteria. Well isolated colonies were slanted on nutrient agar for further study.

Electro-potential analysis of isolates from different waste water.

Each MFCs anode was fed with synthetic waste water and inoculated with single separate isolates from the waste water samples. The electro-potential was measured in millivolt (mV).

Results and discussion

Isolation and electro-potential analysis of Primary inoculants

(Fig. 1) indicates electrogenic ability 20 municipal sewage isolates using them separately as an inoculant to run MFCs. Rabaey *et. al.,* (2004a) was isolated electrogenic bacteria from methanogenic sludge which are facultative anaerobic bacteria, Bond and Lovley 2003, isolated mixed cultures from sediment and waste water treatment plant and Hampannavar *et. al.,* 2011, isolated from distillery waste water. Our findings are analogous to all of them.

Establishment of consortia for development of inoculum

The consortia of four primary inoculants (isolate13-In1, isolate10-In2, isolate1- In3, isolate 3-In4) were established by grouping them in 11 groups**.** The highest electrogenic (390 mV) group of four isolates selected as consortium (Fig.2) which after 24 hr enrichment was used as final inoculums in all MFCs sets. (Plate 1) shows MFCs sets used during work.

Supriya kumari, 2012 perform research regarding marine MFCs using combination of four microorganisms in eight groups among which seven were of two organisms and a single was of four microorganisms, better results were obtained with combination of two micro-organisms viz., Pseudomonas and Penibacillus and not with the grouping of four micro-organisms. While the current research involves 11 combinations of four microorganisms among which six of two isolates, four were of three and one was of four. The group of four organisms show highest electrogenic ability. Pethkar *et. al.,* 2012 utilizes anaerobic sludge and cow dung samples and studied electrogenic pattern of both pure as well as mixed culture enriched with thioglycolate media and synthetic waste water

respectively, same media was used for isolation similarly we used nutrient media for both purpose. Result he got with consortia (3.3 mV) and of pure culture (7.2 mV) our results were quite opposite the pure culture, isolate 13 (3.1 mV) while with consortia (3.9 mV).

Isolation of electrogens from different waste waters

Fifty-two microorganisms ;12 from Dairy waste water, 15 from Distillery waste water, 11 from Municipal waste water and14 from Agro waste water were isolated from four MFCs sets. All isolates were tested for electrogenic ability, twelve being less electrogenenic were not used further.

Electopotential analysis of isolates from different waste water samples.

The (fig.3) show the graphical representation of electrogenic ability of forty isolates from four waste samples. Two isolates (5, 37) show electropotential higher than 500 mV, maximum with isolate 37 (570 mV). The comparision of electropotentials of primary inoculants from municipal sewage (fig.1) and the electrogens from local industrial waste (fig. 3) shows that the local industrial waste isolates were more prominent electrogens than primary isolates from sewage.

SUMMARY

There is isolation of twenty primary Municipal waste water isolates among these four higher electrogenic isolates were selected for consortium development forming 11 different groups. The group of four microorganisms showing highest electrogenic ability (390 mV) was selected as final inoculum to run four double chambered MFCs constructed during current research. Forty electrogenic microorganisms were isolated from different waste waters. The isolate 37 is highest electrogenic (570 mV).

CONCLUSION

It is concluded that there is isolation of primary inoculants from municipal sewage to establish consortium for development of final inoculum to run Microbial Fuel Cells. Also, there is isolation of forty electrogenic microorganisms from four different waste water samples and their electrogenic ability were studied.

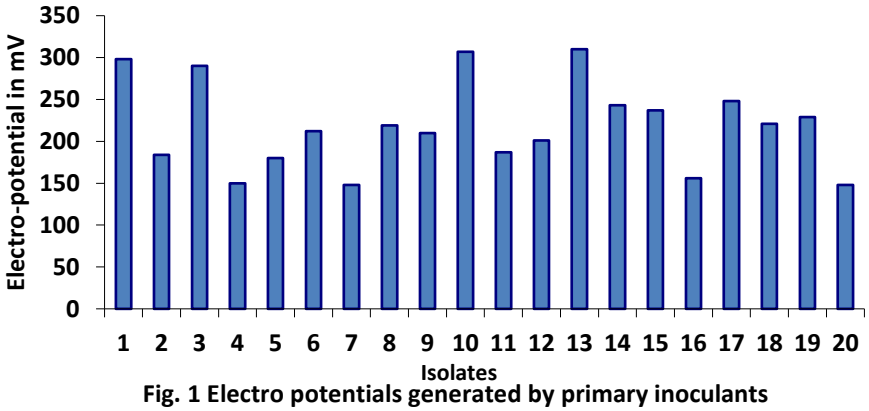

Fig. 1 Electro potentials generated by primary inoculants

(Note: The four isolates (1, 3, 10, 13) having higher electrogenic ability were selected for establishment of consortia.)

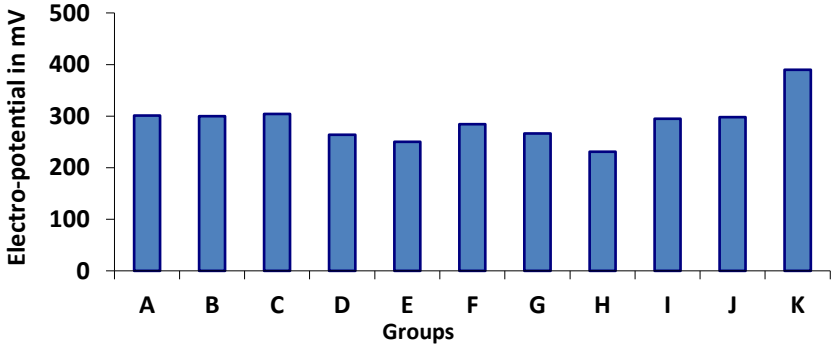

Fig. 2 Establishment of consortia

(A= In1+In2, B= In1+In3, C= In1+In4, D=In2+ In3, E =In2+In4, F=In3+In4, G= In1+ In2+ In3, H= In1+ In2+ In4, I=In1+In3+In4, J= In2+ In3+ In4, K= In1+ In2+ In3+ In4.)

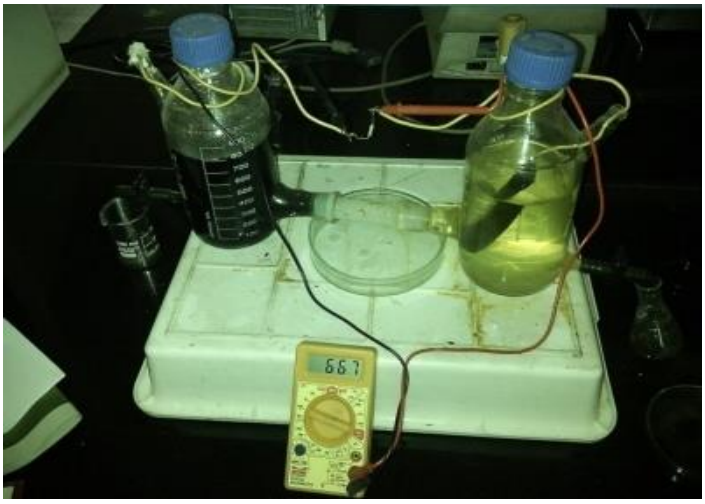

Plate 1: A model MFCs set used during current research

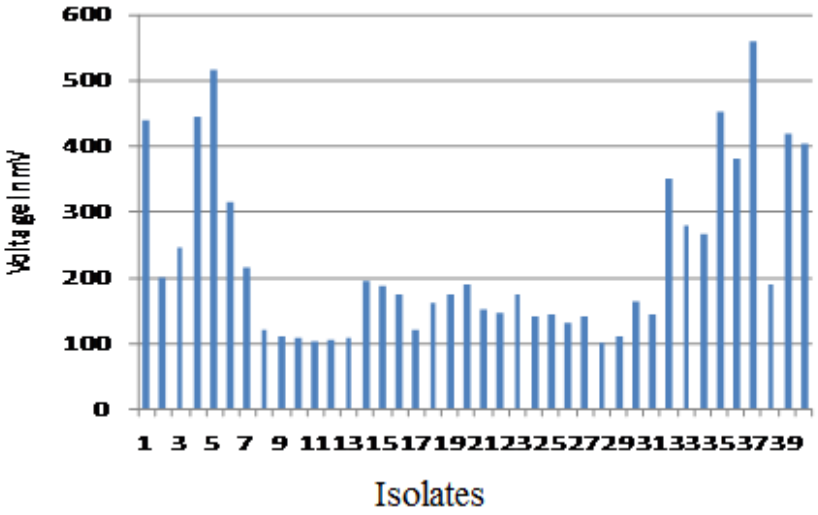

Fig. 3: Electro potential analysis of isolates from four different waste water

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