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# The Consortia of Fungi GPPV1 and GPPV2 as a Biocatalyst in Microbial Fuel Cell for the Production of Electric Current

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**Abstract** In the present studies, the potential for energy production in the form of electric current has been exploited using a consortia of fungi GPPV1 and GPPV2 as a biocatalyst in Microbial Fuel Cell. The MFC system were assembled and the electricity generation was measured after different intervals using various parameters and substrates, 68 mV electricity was generated after 1 h. Among 10 fungal isolates, GPPV1 and GPPV2 were selected for the further investigation of MFC in consortia. The MFC was constructed in presence of GPPV1 and GPPV2, electricity generation was measured at various intervals. The GPPV1 and GPPV2 were produced electricity 252 and 91 mV after 74 h, respectively. After the addition of various sugars, the electricity generation was found to be increased, upto 127 mV at 74 h by glucose and after supplement with maltose 195 m V at 75 h. Now time came to not only explore fungal diversity but also utilization of them require for overcome on needs and challenges in sustainable energy development and biotechnological potential.

**Keywords** Fungi GPPV1 and GPPV2, Microbial fued cell (MFC), Biotechnological potentials, Biocatalyst, Production of electric current.

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## Introduction

From last two decades the microbial fuel cells (MFCs) has been extensively studied for energy production in the form of electric current (Logan 2008, Franks and Nevin 2010). It is specific designs that cause microbial metabolic or enzyme catalytic activity into electricity by using electrochemical technology (Dhundale et al. 2017). The MFC has been best option for the production of electric current from the wide range of substrate by using different types of microorganisms such as bacteria, fungi, or algae (Toczylowska-Mami et al. 2018). The microorganism is established in anode chamber of MFC, where they oxidise the organic substrate for the electron released. The sudden transfer of electron from anode to cathode result in the production of electric current in the MFC assembly *Shewanella* sp. or *Geobacter* sp. were exploited for the first time as electrogenic strains for production if electric current. Improvement and stable high bioelectricity generation using Alkaliphilic *Oceanobacillus iheyensis* in microbial fuel cells and effect of different anodic operating conditions was reported by Dhundale et al. (2018) MFCs which were Gram-negative and most-exoelectrogens are cultivated in MFCs under 7 pH such as *Aeromonas hydrophila, Rhodoferax ferrireducens, Geobacter sulfurreducens, Klebsiella pneumoniae, Desulfobulbus propionicus* (Dhundale et al. 2018,Kim et al. 2002, Pham et al. 2003, Chaudhuri and Lovley 2003, Bond and Lovely 2003, Holmes et al. 2004, Zhang et al. 2008). But very less studies have been reported using Gram-positive bacteria as biocatalyst in MFCs like *Clostridium butyricum* EG3, *Bacillus subtilis, Corynebacterium* sp. strain MFC0), *Thermincola ferriacetica* Z-0001 (Park et at. 2001, Nimje et al. 2009, Liu et al. 2010, Marshall and May 2009) and were performed to be enable producing bioelectricity. Electrochemical mechanisms have been used in different fields of biotechnology including biosensors, bioelectrochemical synthesis and biofuel cells (Dhundale et al. 2017). The effect of salt on MFC, NaCl was enhance electricity generation indicating some bacteria required NaCl for the MFC and salt, sugar had an even greater effect on execute the electricity generation by MFCs (Dhundale et al. 2018). Haloalkaliphiles, representing most diverse group of holophiles could grow optimally at high salt concentration along with grow optimally at pH values at or above 10. This regards, the outstanding stability of haloalkaliphilic organism toward the bioelectricity generation, only few of them have been explored from the soda lake (Dhundale et al. 2018).

Soda lakes are highly alkaline extreme environments that form in closed drainage basins exposed to high evaporation rates with pH values generally higher than 10 and occasionally reaching 12 (Joshi et al. 2008, Hemke and Dhundale 2017, Dhundale and Hemke 2017, Hemke and Dhundale 2017). Hyper alkaline saline lakes, with salinity and alkalinity ranges at or near saturation are extreme environments; yet, they often harbor remarkably high microbial cell densities and are biologically very productive ecosystems (Dhundale et al. 2017). The Indian soda lake, Lonar crater is unique soda lake in the world due to alkaline environment and unique basaltic rock meteorite impact crater, ranking third in the world. Lonar crater is filled with saline water. The lake water is alkaline having an average pH of 9.5-10. Due to the uniqueness, the lake has evoked much scientific value among researchers (Dhundale and Hemke 2015). The ecology and diversity of an Lonar soda lake was studied and extensively reviewed for their biotechnological potential (Hemke et al. 2015). The microbial diversity of saline lakes has been studied primarily by focusing on the isolation and characterization of individual organisms with potential industrial application. Lipase, amylase and protease producing microorganisms were isolated from alkaline Lonar soda lake (Tambekar and Dhundale 2012, 2013). Deshmukh and Verekar (2006) presented the results of a study of the occurrence of keratinophilic fungi from the vicinity of meteorite crater soils. The frequency of alkaliphilic fungi is low, while alkalitolerants seem to be far more widespread, as alkalitolerants may often be encountered in many neutral types of soil (Alexey et al. 2006). Recent studies showed that, few new taxonomically diverse alkaliphilic and alkalitolerant filamentous fungi survive in soda soils (Grum-Grzhimaylo et al. 2013a, 2013b). Being polyphyletic, halophilic filamentous fungi have been shown to utilize different strategies of dealing with extreme halophilic environment (Kis-Papo et al. 2014). Fungi have established in different habitats like tropical area. Fungi are among the microorganisms enable to produce the electricity due to their metabolic process. From the last several years, a lots of bacterial MFC has been extensively studied for the production of electricity, fungi still lack sufficient evaluation in this regard (Sekrecka-Belniak and Toczylowska- Mami 2018). This study aims to investigate the feasibility of using *in situ* fungus to improve the electricity generation performance of MFCs. The potential of fungal biomass production to power generation within a fully biotic Microbial Fuel Cell (MFC). In this study, the fungus GPPV1 and GPPV2 were planted on the anode chamber MFC to generation of electricity.

## Materials and Methods

Enrichment and isolation of microorganisms

Lonar lake soil sample were collected in polythene bags from defined sampling site. Enrichment of soil were carried out in sabouraud broth and potato dextrose broth. All flasks were incubated at 37°C on a rotary shaker (100 rpm ) for 48 h. After enrichment, the fungi were isolated on respective media agar plates and incubated at RT for one week. Well isolated and morphologically distinct colonies from these plates were transferred on the respective medium slants and maintined as stocks. Fungal cultures were examined for their cultural, morphological character.

Cultural condition

Culture was retrieved by streaking on sabouraud agar and potato dextrose agar plates and incubating at RT. For MFC operation, 2-3 isolated colonies were inoculated in 100 ml of sabouraud broth and potato dextrose broth and incubated for 48 h at RT (160 rpm) in shaking conditions.

*Enrichment of culture* : Culture was inoculated in 100 mL sabouraud broth and potato dextrose broth and incubated for 48 h at RT. Incubation was done at room temperature on a rotary shaker (120 rpm) for 48 h. After enrichment, these cultures now serve as a MFC for the electricity generation.

MFC operations

In MFC, all components are connected internally with the help of salt bridge and externally with wires to the multimeter. The pure colony was aseptically transferred in 100 ml specific broth and incubated at 37° C at 160 rpm for 48 h . Then under aseptic conditions salt bridge was sealed inside the holes of bottles. First sterile soil was placed in and then culture broth was poured to prepare liquid suspension in anodic chamber. Sterile distilled water was poured in cathodic chamber. MFC set up was kept at static conditions and its operation was carried out at room-temperature. To check the isolates ability to generate potential difference, these isolates were tested by varying carbon source, pH, organic acids and salts concentration. The MFCs was run up to 48 h and in every 2 h interval voltage was recorded in all cases.

*Measurement of potential difference and current* : The potential difference generated by the Fuel Cell was measured by using multimeter from HAOYUE-DT830D.

## Results and Discussion

Total ten fungal isolates obtained in the isolation exercise, cultural, morphological characteristics were performed for of all the isolates. In the present study, the 72 h alkaline pH (Holmes et al. 2004) culture was prepared and aseptically pours into anodic chamber which contain sterile soil and to analyze the production rate. All ten cultures were selected for the electricity generation, GPP9 produced highest electricity 460 m V at 72 h but after 73 h its production rate was dramatically decrease. But GPPV1 and GPPV2 were produced electricity 252 and 91 mV after 74 h respectively. All the fungal interactions enhance power generation in microbial fuel cells with different combination. Out of ten, two fungal isolate GPPV1 and GPPV2 were selected for the further MFC studies due to constant production of electricity in consortia. Electricity can be generated directly from sewage sludge with a microbial fuel cells (MFCs), combining degradation of organic matter and MFC for the generation of bioelectricity and the degradation of sewage sludge organic matter under the alkaline condition.

Effect of lactate and acetate on bioelectricity generation

Biochemical routes that lead to acetate produce more hydrogen than those that lead to butyrate production (Fig. 1). However, there are previous studies on electricity production from lactate, while bioelectricity generation from acetate in two-chambered MFCs is well known, systems. Here demonstrates that electricity can be generated from lactate and acetate in a double-chambered MFC, and compare power densities obtained from acetate and lactate with those previously obtained in the same system using sugar. Here in the present investigation the 310 mV and

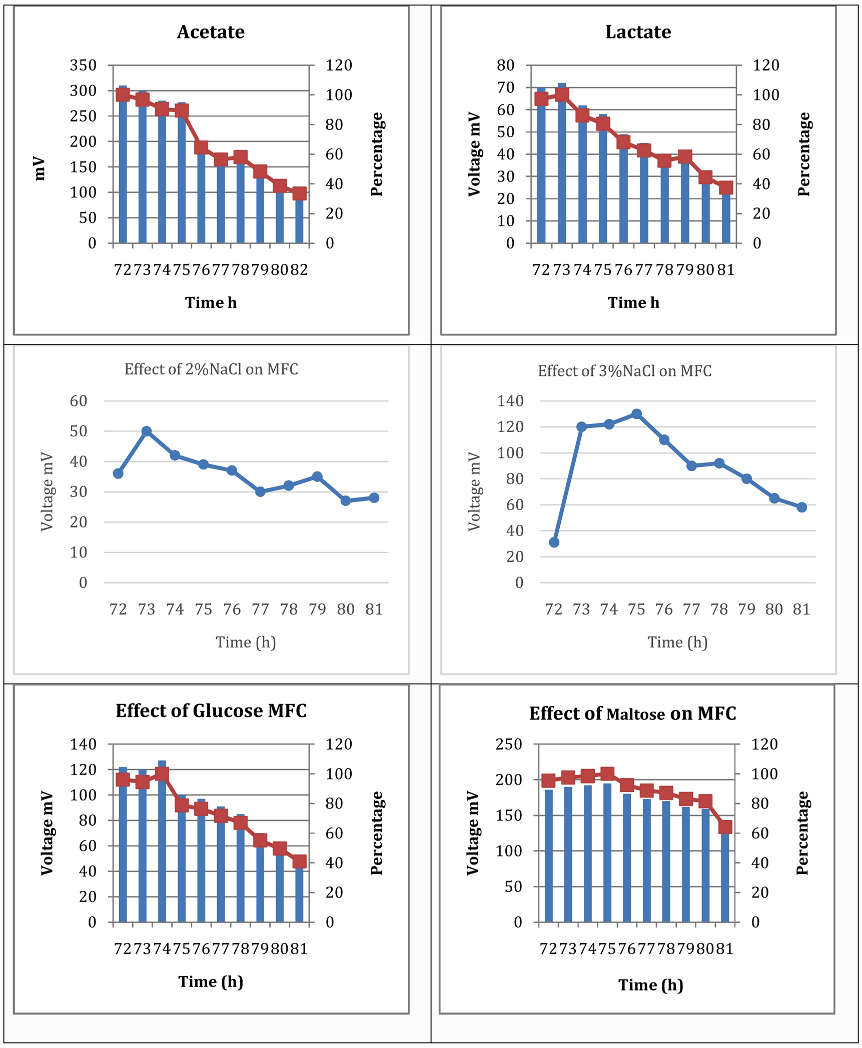


**Fig. 1.** (A) Effect of acetate and lactate on bioelectricity generation. (B) Effect of NaCl on MFC. (C) Effect of glucose MFC and maltose on MFC.

72 mV bioelectricity were generated in presence of acetate and lactate respectively.

Effect of NaCl on bioelectricity generation

Salinity effect on the microbial fuel cell performance was investigated. The 2% and 3% of NaCl was added into the MFC, after the addition of NaCl, the eletricity generation was found to be 50 mV at 2% NaCl after 73 h of inoculation while 130 mV electricity produced at 3% NaCl after 75 h. Muralidharan et al. (2011) studied the limpact of various salt and concentration on the electricity generation based on dual chambered MFC. Parkash et al. (2011) studied on the impact of salt concentration on electricity production in microbial fuel cells. They revealed that the KCl salt bridge was efficient in electricity generating that the NaCl. Dhundale et al. (2017, 2018). studied , the effect of salt on MFC, NaCl was the prominent electricity generation than the KCl, indicating a bacterium ARS4 was required NaCl for the MFC.

Effect of sugar on bioelectricity generation

The most extensive studies has been reported on the bioelectricity generation by using carbohydrate-rich

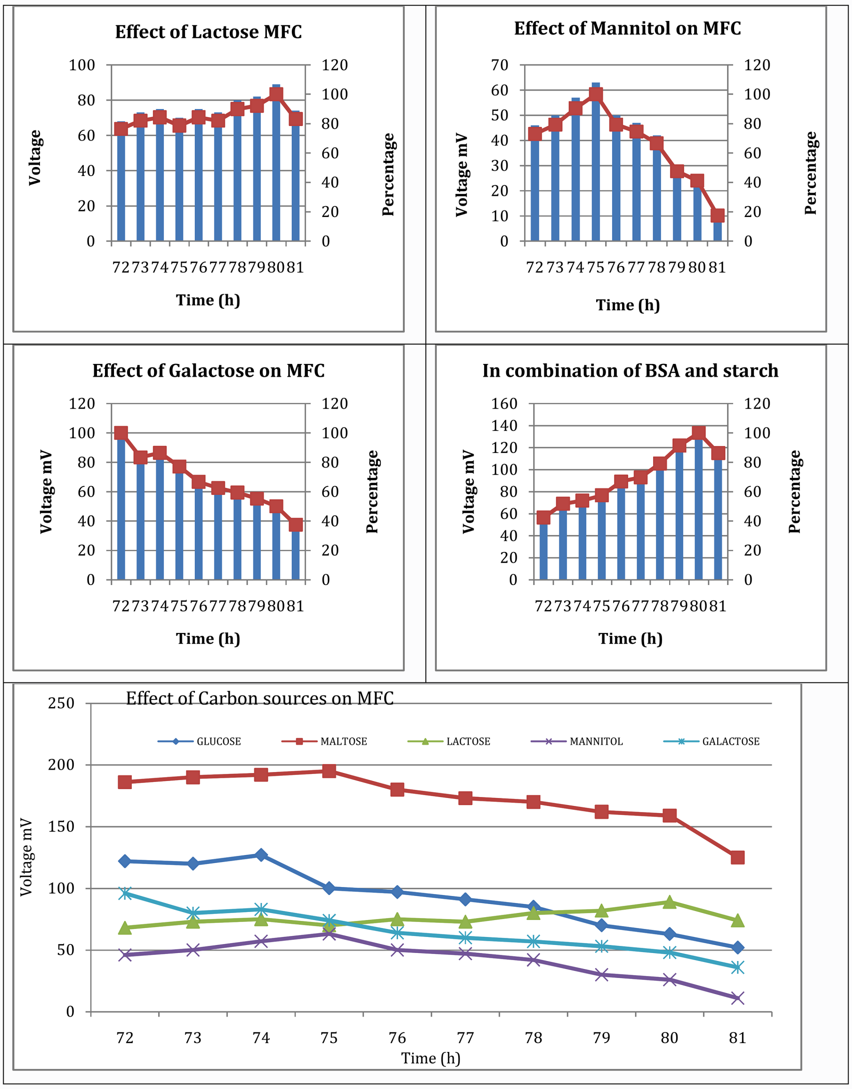


**Fig. 2 .** (A) Effect of lactose MFC and mannital on MFC. (B) Effect of galactose on MFC and in combination of BSA and starch (C) Effect of carbon source on MFC.

wastes such as food processing wastewater, starch processing wastewater and chocolate based wastewater (Chaturvedi and Verma 2016). Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells was reported by Chaudhuri and Lovley (2003). In the present studies, anodic chamber was supplement with various sugar for the bioelectricity generation. Tarte et al. (2015) effect of NaCl and Glucose on generation of electricity was also studied. MFC was prepared from waste water and different sugars were mixed with it to see effect of sugar on electricity generation. The fungal isolate GPPV1 and GPPV2 was supplemented with the various sugars like in glucose, sucrose, lactose, maltose, mannitol and starch. After the addition of various sugars, the electricity generation was found to be increased, upto 127 mV at 74h by glucose and after supplement with maltose (195 mV at 75 h), manitol (63 m V at 75 h), galactose (96 mV), but the optimum electricity generation was found after addition lactose 75 mV.

Microbial Fuel Cells (MFCs) are most fascinating bioelectrochemical devices that use living catalysts to produce electric energy from organic matter present naturally in the enviroment or in waste. Kumar et al. (2016) investigate bioelectricity generation and treatment of sugar mill effluent using a microbial fuel cell. MFCs can also aid in waste water treatment (Fig.2).

## Conclusion

Among ten fungal isolates, GPPV1 and GPPV2 were selected for the further investigation of MFC in consortia. The MFC was constructed in presence of GPPV1 and GPPV2, electricity generation was measured at various intervals. The GPPV1 and GPPV2 were produced electricity 252 and 91 mV after 74 h respectively. After the addition of various sugars, the electricity generation was found to be increased , upto 127 m V at 74 h by glucose and after supplement with maltose 195 m V at 75 h. Now time came to not only explore fungal diversity but also utilization of them require for overcome on needs and challenges in sustainable energy development and biotechnological potential.

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