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MICROBIAL FUEL CELLS- A GREEN INNOVATION FOR ELECTRICITY GENERATION

In this era, there is a huge demand for a technology that is economically feasible, scalable and stringent in energy consumption to avoid pollution and waste treatment. The increased use of fossil fuels will bring about the issues of global warming and global energy crisis in the nearby future. A solution for this crisis is the use of renewable bioenergy that does not result in any greenhouse gas emission. In this aspect, Microbial Fuel Cell (MFC) is going to be a promising technology for simultaneous electricity production and waste treatment. MFCs are bio-electrochemical reactors that convert chemical energy stored in organic and inorganic matter to electricity under anaerobic conditions with the help of microorganisms.

MFC technology is basically an anaerobic process in which anaerobic or facultative anaerobic bacteria form a biofilm on the anode. These bacteria have the ability to act as a biocatalyst to provide electrons to the anode surface and can even replace the expensive catalysts like platinum. The electrons produced can be transferred to the anode by electron mediators /shuttles by nano wires or by some other means. The systems in which bacteria does not have the ability to transfer electrons to the electrode can be added up with chemical mediators like neutral red or anthraquinone-2, 6-disulfonate.

TYPES OF MICROBIAL FUEL CELLS

Double chambered MFC

A double chambered MFC (Fig.1) comprises anodic and cathodic chamber separated by Proton Exchange Membrane (PEM) or salt bridge that mediates proton transfer from anode to cathode while blocking diffusion of oxygen into anode. In anode, the organic substrates are used by microbes which generate electrons and protons that are transported to the cathodic

chamber through circuit and membrane, respectively. Cathode is filled with a high potential electron acceptor like oxygen (ideal one) and ferricyanide. Microbes utilize organic substrates as their energy source and as a result, electrons are generated which are transferred to electron acceptors like molecular oxygen. In the absence of such electron acceptors in MFC, microorganisms transfer electrons onto anode surface that result in electricity generation. Scaling up two chambered MFCs to industrial size is very difficult. Periodic aeration of cathodic chambers also limits the MFC application. Two chambered MFCs are run in batch mode with a medium which is chemically defined with substrates to generate energy.

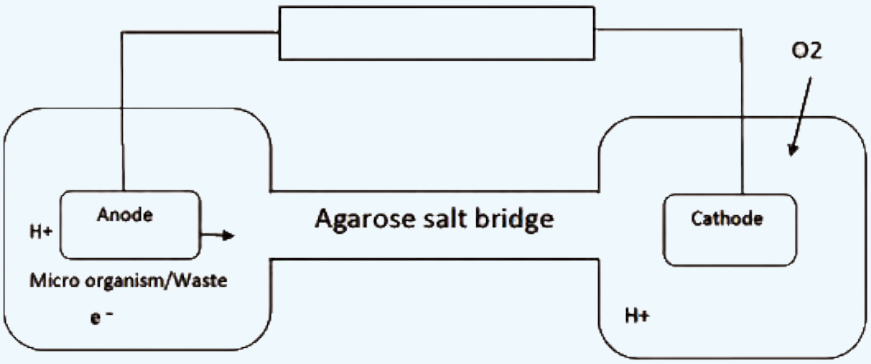

Fig.1 Schematic diagram of double chambered MFC

Single chambered MFC

In a single chambered MFC, anode and cathode are in the same chamber with cathode being exposed to air and separated by a gas diffusion layer or a Proton Exchange Membrane. Electrons are transferred to cathode to complete circuit. The main advantages of single chambered MFCs are low setup costs (due to absence of expensive membranes and cathodic chambers) and limited requirement of periodic recharging with an oxidative media and aeration. This makes them a flexible candidate for waste water treatment and power generation. Various types of single chambered MFC like upflow, flat and tubular MFCs have been developed.

PRINCIPLE OF ELECTRICITY GENERATION

MFC utilizes the metabolic potential of microbes for conversion of organic matter into electricity by transfer of electrons from cell to circuit. In the anode, the substrate is oxidized without oxygen by respiratory bacteria producing electron and proton that are transferred onto terminal electron acceptor [$O_{2,}$ nitrate or Fe (III)] through electron transport chain (ETC). But in the absence of electron acceptor in MFC, some microorganisms pass electron onto anode. Electrons transfer to anode can be achieved either directly or by mediators. Current generation through direct electron transfer to anode requires some physical contact of bacteria with electrode. In indirect electrons transfer, electrons are transported from bacteria to electrode through electron shuttle

compounds which are naturally produced by bacteria or present naturally. The electrons that are transported to the bacterial cell surface are collected by the shuttle compounds which are then transported to the electrode.

ELECTROCHEMICALLY ACTIVE BACTERIA IN MFCs

The metal reducing bacteria like *Shewanella putrefaciens*, *Rhodoferax ferrireducens*, *Geobacter metallireducens* and *Geobacter sulferreducens* show electron transport mechanism where in anode acts as final electron acceptor. Mediators also play an important role in electron transport especially in bacteria that cannot transfer electron directly to the anode. Common mediators involved are humic substances and dye molecule. Electron mediators like Neutral Red (NR) or Mn4+ incorporated into the anode enhance the performance of MFCs.

It is reported that *Pseudomonas aeruginosa* can produce compounds like phenazine pyocyanin and this compound functions as electron shuttles between the bacterium and an electron acceptor. MFCs inoculated with aquatic sediments were dominated with δ-*Proteobacteria* and a considerable amount of γ –*Proteobacteria* was reported in cystein-enriched MFC inoculated with sediments. ε-*Proteobacteria*, which were rare, were dominant in a unique sea water and marine plankton filled MFC.

There was a relative abundance of β-*Proteobacteria* and absence of γ-*Proteobacteria* observed in all MFCs except in propionate fed system. The propionate fed MFC was dominated by *Firmicutes*, followed by γ-*Proteobacteria*. Similarly, *Firmicutes* have been reported to be integral members of the MFC bacterial community; these can transfer the electrons retrieved from the metabolism of organic matter to the anode. The glucose fed MFC showed diverse community with a distribution of *Proteobacteria*, and *Firmicutes*.

SUBSTRATES USED IN MFCs

Acetate has been the choice of electricity generation in most of the MFCs studied so far. It is an easily utilizable carbon source and can be used to induce electrochemically active bacteria. Using a single chambered MFC, acetate (506 mW/m^2, 800 mg/L) produced 66% more power than butyrate (305 mW/m^2,1000 mg/L). Another commonly used substrate in MFC is glucose. It has been reported that maximum power density of 216 mW/m^3 was obtained from a glucose MFC with ferric cyanide as cathode oxidant.

Lignocellulosic materials can be a cost effective means for energy production as it is renewable and abundantly available. When cellulose is used as the substrate, the microorganisms should be capable of hydrolyzing cellulose anaerobically and should be electrochemically active. It is reported that power density of 55 W/m^2 was produced using cellulose in a double chambered MFC with the help of rumen microorganisms. Starch Processing Wastewater (SPW), a rich source of sugar, carbohydrates, starch and proteins, was used to enrich electricity generating microbes. In six weeks, a COD reduction of 97% and current of 0.044 mA/cm^2 was obtained. The maximum voltage obtained using SPW was 490.8 mW and power density of 239.4 mW/m^2.

Brewery waste water is a much preferred substrate for MFCs due to its low strength, absence of high amount of inhibitory substances and presence of easily utilisable organic matter. In an air cathode MFC, beer brewery waste achieved a maximum power density of 528 mW/m^2, but it was much lower than that produced by domestic waste water.

Landfill leachates, a highly polluted landfill effluent is also a good candidate for electricity generation. It was reported that the electricity was generated continuously from leachate using an upflow air-cathode MFC for 50 h and produced a maximum volumetric power and current density of 12.8 W/m^3 and 41 A/m^3, respectively.

APPLICATIONS

Electricity generation

One of the main and important applications of MFC is electricity generation. MFCs are capable of generating electricity from the biomass with the help of microorganisms at anaerobic conditions. MFC power generation is still very low and this issue can be solved by storing the generated electricity in rechargeable devices. Capacitors were used in robots called EcoBot I to accumulate the energy generated by the MFCs and operated in a pulsed manner. MFCs can be used to provide power to local uses, especially in those regions of the world that are not developed.

MFCs are viewed by some researchers as a perfect energy supply candidate for Gastrobots that can use the biomass collected by themselves. MFCs can be used to power the EcoBot-II robot to perform behavior like motion, sensing, computing and communication.

Biohydrogen

MFCs can be easily modified to produce hydrogen. Under normal operating conditions, protons generated in the anodic chamber migrate to the cathode through salt bridge or proton exchange membrane and combine with oxygen to form water. For biohydrogen production, cathode becomes anaerobic so that the protons produced due to anodic reaction are combined at the cathode to form hydrogen. The external potential required for a MFC is theoretically 110 mV, much lower than the 1210 mV required for direct electrolysis of water at neutral pH. MFCs can produce about 8–9 mol H_2/mol glucose compared to the typical 4 mol H_2/mol glucose in conventional fermentation.

Wastewater treatment

The MFCs were used for treating waste water early in 1991. Municipal wastewater contains a high amount of organic compounds that can fuel MFCs. The power generated by MFCs using wastewater can complement the electricity needed for conventional waste water treatment process like aerating activated sludges. MFCs normally yield 50–90% less solids. Furthermore, organic molecules such as acetate, butyrate, propionate can be thoroughly broken down to CO_2 and H_2O.

Biosensor

Important application of the MFC technology is to use it as a sensor for pollutant analysis and *in situ* process for monitoring pollutant and control. The correlation between the Coulombic yield of MFCs and wastewater strength makes MFCs as possible Biological Oxygen Demand (BOD) sensors.

FUTURE OF MFCs

MFC technology has some potent advantages when compared with conventional anaerobic process as it produces electricity from different substrate even at low concentration directly without any intermediate steps. It produces less waste for disposal and handling. In spite of these advantages, the MFC technology yet faces problems in scaling up due to the costly catalyst used in cathode and due to the lack of economical proton exchange membrane. To overcome these limitations, research and development has to be done on various issues so that it can be made as an economical technology which can treat waste and produce energy simultaneously. Amongst the major concerns, the choice of appropriate electrode materials and the optimization of the reaction processes to achieve maximum power and reduce installation costs are to be taken care seriously. Even we can mutate microorganisms to produce superbugs which can efficiently transfer electron to the anode. Another option will be the use of a symbiotic microbial consortium in which a bacterium can synthesise electron mediators and another bacterium can use it to transport electron to anode effectively. Hence, an MFC functioning with a bio-cathode which is cost-effective and performing waste treatment would be a major interest.

MFC WORK INITIATED AT TANUVAS

Department of Animal Biotechnology, Faculty of Basic Sciences, TANUVAS developed a prototype model of Double Chamber MFC using slaughter house waste rumen fluid in anode and water as catholyte. The model was developed under funding by Government of Tamil Nadu, Tamil Nadu Innovation Initiatives (TANII). The prototype model uses agarose salt bridge for transfer of protons from anode to cathode. The outcome will be useful for mobile recharging and LED bulb glowing.

References on request

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RESEARCH HIGHLIGHTS (2019-20)

EFFECT OF DIETARY SUPPLEMENTATION OF POMEGRANATE (*Punica Granatum L.*) PEEL AND LACTIC ACID BACTERIA ON OXIDATION RESISTENCE AND MEAT QUALITY OF BROILER CHICKEN

Pomegranate peel (PP) is a good source of total phenols (43.84 mg gallic acid Equivalents/g) and flavonoids (10.04 mg quercetin Equivalents/g) and has radical scavenging activity of 59.44 %. Out of twelve species of lactic acid bacteria (LAB) isolated from chicken gut, three species viz., *Enterococcus hirae, Enterococcus faecalis and Enterococcus thailandicus* were found to possess pronounced antioxidant potential, in addition to acid and bile tolerance, antimicrobial activity against *E.coli O157*: *H7*, bile salt hydralase activity and adherence to Caco2 cell lines. Supplementation of pomegranate peel and lactic acid bacteria improves overall weight gain and FCR and reduces the serum LDL and triglycerides concentration. Pomegranate peel supplementation (15 g/kg) and LAB increases PUFA concentration by 10.4% and omega-3 fatty acids by 20.9% compared to other treatments and there was a significant reduction in SFA content of breast muscle of birds fed with 15g/kg PP and 109 cfu/kg LAB (36.25%) from control (39.91%). Inclusion of pomegranate peel and LAB increases the serum superoxide dismutase (23.53%), glutathione reductase activity (2.18%) and reduces the serum malondialdehyde concentration (37.33%). Muscle malondialdehyde concentration has been decreased by 22.48% when compared to control. Additionally, the pH, water holding capacity (WHC) and shear force value of meat have been retained for 7 days in refrigerated storage. Supplementation of PP and LAB increased the villus height and crypt depth ratio and decreased the villus height to crypt depth ratio. Differentially expressed proteins were present in the pectoralis major muscle of broiler chickens fed with control diets and control diets supplemented with 15 g/kg PP and 109 cfu/kg LAB. Integrative data indicates that PP (15g/kg) and LAB (109/kg) supplement resulted in significant changes in the development of gut microvilli, gut microbiota, serum lipid profile, antioxidant status, meat quality parameters and proteome changes in broiler chicken. These changes might be attributed to the well-balanced microbial consortium with the presence of pomegranate peel.

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ELECTROCHEMOTHERAPY FOR MAMMARY TUMOUR IN DOGS

The study was carried out on clinical cases of canine mammary tumour presented to the Small Animal Surgical Out-Patient unit of Madras Veterinary College Teaching Hospital during the period from September, 2018 to May, 2019. Routine clinical, haematological, biochemical and radiological evaluations were done. Twelve dogs with mammary tumours, in which a single solitary lump was present, were selected for this study. Six of the twelve dogs were subjected to electrochemotherapy with Cisplatin (Group I) and six of them were subjected to electrochemotherapy with Bleomycin (Group II). Haematology and biochemistry tests were repeated on days 3, 7, 14, 21 and 30 post-therapy to evaluate any chemotherapy induced haemato-biochemical changes. The size of the tumour was measured at the end of 30 days and the response was calculated based on Response Evaluation Criteria in Solid Tumours (RECIST). At each visit, the tumour was measured using a digital vernier calliper and documented with photographs. At the end of 4 weeks, the response based on RECIST criteria was evaluated and classified. On response evaluation, cases 1, 2 and 3 of Group

I and case 6 of Group II showed partial responses (PR). Cases 4 and 6 of Group I and cases 1,2,3,4 and 5 of Group II showed complete responses (CR). Case 5 of Group I showed stable disease (SD). The mean response (%) of Group I (Cisplatin) was 64.36 ± 4.53 and that of Group II (Bleomycin) was 99.24 ± 0.75.

The adverse effects were categorized as groups based on Veterinary Co-operative Oncology Group – Common Terminology Criteria for Adverse Events (VCOG-CTCAE, 2016) and were graded. Mild anorexia and local necrosis were the most common adverse effects noticed. Cases 1 and 2 of Group I were graded as VCOG- CTCAE Grade 1 while all other cases were graded as Grade 2. The cases that showed partial response were subjected to simple mastectomy. A follow-up cytology was performed after four weeks following therapy. In Group I, cases 1, 2, 3 and 5 and case 6 from Group II showed fewer clusters of neoplastic cells when compared to the pre-chemotherapy cytological slide. All the other cases (cases 4 and 6 of Group I and cases 1, 2, 3, 4 and 5 of Group II) did not show any signs of malignancy. Hence, it was concluded from the study that electrochemotherapy using Cisplatin and Bleomycin was effective in treating canine mammary tumours with Bleomycin showing higher efficacy when compared to Cisplatin.

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EXTRACTION OF BIOACTIVE PEPTIDES FROM POULTRY BY-PRODUCTS

The bioactive peptides were extracted from the chicken intestine by enzymatic hydrolysis by using fungal protease P (phycomycetes), followed by ultrafiltration. The extracted bioactive peptides were characterised by physico – chemical properties, bioactive assays and SDS PAGE. The yield of the bioactive peptide was 4.28 ± 0.06 per cent. The molecular weight of the extracted bioactive peptides of <10 KDa was 9.6 KDa. The IC_{50} value for DPPH Radical scavenging assay for antioxidant activity of the bioactive peptides of <10 KDa and standard butylated hydroxyl toluene were 0.15 ± 00 mg/ml and 0.23 ± 0.3 mg/mL, respectively. It was found that lower the IC_{50} value higher was the potency of the peptides to exhibit better antioxidant activity. Thus, the extracted bioactive peptides of <10 KDa were found to have better antioxidant activity than standard butylated hydroxyl toluene, which is a commercially used antioxidant in the food industry.

The IC_{50} values of alpha amylase inhibitory activity assay of the extracted peptides and standard acarbose (a drug for type 2 diabetes mellitus) were 959.0 ± 8.1 µg/mL and 158.42 ± 10.1 µg/mL, respectively. The results indicated that higher the IC_{50} value, lower was the potency of the peptides to exhibit better antidiabetic activity. Thus, on comparision with the standard acarbose, the potency of the extracted peptides were low in antidiabetic activity. The extracted bioactive peptides of <10 KDa were then incorporated in canine feed mash at three different inclusion levels of 5 per cent, 6 per cent, and 7 per cent. The activity of the bioactive peptides in the above incorporation levels in canine pet food were assessed by *in vitro* assays. The results of DPPH radical scavenging activity among different levels were compared with canine pet food which was taken as control. Among the different levels of incorporation, 7% level of inclusion of extracted bioactive peptides was found to have more antioxidant activity with an IC_{50} value of 0.20 ± 0.70 when compared with other inclusion levels of 5%, 6% and the control with an IC_{50} value of 1.80 ± 0.5 mg/mL, 1.41 ± 0.6 mg/mL and 1.84 ± 0.4 mg/ml respectively.

The results of alpha amylase inhibition between different levels of inclusion of bioactive peptide in canine pet food revealed a highly significant difference. The 5% inclusion level of bioactive peptide was found to have lower IC_{50} value of 59.26 ± 4.4 µg/mL and thus better alpha amylase inhibition activity when compared with the IC_{50} values of control

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canine pet food, while 6% and 7% inclusion levels were 1313.85 ± 52.8 µg/mL, 364.28 ± 13 µg/mL and 163.57 ± 5.04 µg/mL, respectively. Thus, it is concluded that, low value poultry by-products like intestines can be effectively utilized for the extraction of bioactive peptides. The bioactive peptides could be used as an additive in pet food in the above mentioned levels. The extracted bioactive peptides can thus be used as nutraceuticals and therapeutic diet because of their high antioxidant activity.

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TOXINOTYPING AND ANTIMICROBIAL PROFILE OF *Clostridium Perfringens* OF LIVESTOCK AND POULTRY

Clostridium perfringens is a saprozoonotic Gram positive, anaerobic bacteria which causes enterotoxaemia in lambs and calves; enteritis in pigs, cattle, dogs, horses and necrotic enteritis in poultry. A total of 434 (414 fecal and 20 intestinal) samples were collected from cattle, sheep, goat, pigs and poultry, from which 48 isolates of *C. perfringens* were isolated and identified by cultural characteristics, biochemical characters and species specific 16S rRNA PCR, followed by sequencing and BLAST analysis. All the isolates were subjected to toxinotyping by multiplex PCR which revealed 97.91% belonging to type A and 2.08% belonging to type C. Minor toxin cpb2 was also identified among 27.08% of the isolates. Antimicrobial resistance pattern among the isolates showed, 97.91% isolates were resistant to penicillin, followed by 85.41% isolates to cefoxitin, 81.25% isolates to metronidazole, 77.08% isolates to amoxicillin, 64.58% isolates to ampicillin and 62.5% isolates to tetracycline. All the isolates were found to be multidrug resistant (MDR). Genotyping to detect resistance genes for enrofloxacin (gyrA), lincomycin (inuA and inuB) and tetracycline (tetM) were done and found that 93.75% of that isolates possessed tetM gene and 58.33% and 39.58% possessed inuA and inuB, respectively. The correlation between phenotypic and genotypic resistance for tetracycline and lincomycin showed highly significant difference which could be due to point mutation or defective expression of the resistance genes. Increased drug resistance to most of the commonly used drugs in veterinary practice, along with increased resistance to metronidazole and presence of MDR is serious concern which needs to be addressed effectively with suitable control strategies.

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