



Impact of Salt on Bioelectricity Generation in a Dual-Chambered Microbial Fuel Cell Using Sago-Processing Wastewater

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Abstract: Effect of NaCl on electricity generation, COD removal, reduction in carbohydrate and starch content in dual chambered, salt bridge Microbial Fuel Cells (MFCs) employing raw sago-processing wastewater with an organic load of 14,400 mg COD/l as substrate was evaluated. Four dual chambered MFCs were constructed and the study aimed to find out the impact of addition of NaCl which is carried out for effective MFC performance. Isolation and identification of microbes from initial influent and final effluent was performed using serial dilution, spread plate and selective agar techniques. Interestingly, it was found that the MFC in which NaCl was added to its cathode chamber was best in performance compared to other three MFCs, with a maximum voltage of 603mV and current of 6.03mA. It also documented that the maximum COD removal efficiency of 83% with a total reduction of carbohydrate and starch content from the wastewater was obtained. Utilizing sago wastewater for the production of bioelectricity from MFC technique is considered as a feasible and sustainable process.

Key words: Sago wastewater • NaCl • Conductivity • Power density • Internal resistance • COD removal

INTRODUCTION

Increasing global energy demands and over-consumption of non-renewable sources of energy have led to search and use of renewable and cost-effective sources of energy. At present, global energy requirements are mostly dependent on fossil fuels. Combustion of fossil fuels also has serious negative effects on environment due to CO₂ emission like global climate change, environmental degradation and health problems. This has intensified the search for alternatives to replace fossil fuels [1, 2]. Many researchers predict that bioelectricity could play an important role as fuel in near future. In this perspective, wastewater which contains high levels of biodegradable material has gained importance as a source of electricity generation using a Microbial Fuel Cell (MFC) [3-5]. MFC technique offers the possibility of harvesting electricity from organic waste and renewable biomass. It has been developed as a novel technique to gain energy with simultaneous wastewater treatment.

Tapioca (*Manihot esculenta*) is a crop of great economic importance, both as human food, animal feed as well as raw material for industrial products. In India, tapioca is grown over an area of about 300,000 hectares, with a production of 5,800,000 to 6,800,000 tonnes of tubers [6]. There are about 1,000 sago and starch processing factories in Salem and Namakkal districts of the Tamil Nadu state in India and it ranks second in terms of cultivation and production of tapioca and first in processing of it into sago and starch, meeting about 80% of country's demand. The amount of water used for starch processing varies, depending on the processing scale and the level of technology sophistication. Processing of one tonne of sago releases 40,000 to 50,000 litres of wastewater and it takes about 10 days for the wastewater to be treated and discharged out of the factory [7]. The release of high content of organic load along with the wastewater when stored, results in obnoxious odour, irritating colour, low pH and high BOD and COD [8]. If the wastewater is released into the environment without

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Table 1: Methodology adopted for the experiment

Fuel cells	MFC-1		MFC-2		MFC-3		MFC-4	
Chambers	Anode	Cathode	Anode	Cathode	Anode	Cathode	Anode	Cathode
Contents	Wastewater	Phosphate buffer solution (PBS)	Wastewater + 50mM NaCl	PBS + 50mM NaCl	Wastewater + 50mM NaCl	PBS	Wastewater	PBS + 50mM NaCl
Initial pH	5.1	7.0	5.3	5.2	5.3	5.5	5.4	5.3
Initial Conductivity (mS/cm)	3.18	4.0	13.7	17.8	13.7	4.0	3.18	17.8

Table 2: Initial Physico-chemical parameters of the anolyte used for MFC operation

Parameters	Values
pH	5.0
Temperature	30 °C
Conductivity (mS/cm)	5.7
Total Suspended Solids (mg/L)	1464
Total Dissolved Solids (mg/L)	3272
Total Solids(mg/L)	4736
Chemical Oxygen Demand (mg/L)	14,400
Biological Oxygen Demand (mg/L)	8620
Starch (mg/L)	1.53
Carbohydrates (mg/L)	40
Sulphate (mg/L)	180

MFC Operation: Table 1 illustrates the experimental arrangements followed in this study. Air was purged into the cathode compartment with aquarium aerator (Shengze Electronic Co., Ltd, Shenzhen, China) in order to supply the oxygen required for the electrochemical reaction, at a rate of one liter per min, controlled with an air flow meter (Mahavir, SN 31, Coimbatore, India). Anode chamber was covered with aluminium foil and was placed in an anodic chamber to avoid air and light intrusion into the cell. The anolyte was continuously stirred with a magnetic stirrer (Superfit Equipments, Mumbai, India) to ensure uniform suspension of organic constituents. The performance of the fuel cell was evaluated under constant substrate loading of 14,400 mg/l and operated in batch mode at an initial pH of 5.5 up to a period of 34 days. Electricity generation, COD removal rate, reduction in carbohydrates and starch were monitored during the operation.

Electrical Parameters and Power Measurements: The voltage generated in the MFCs was measured after 3 hours incubation as lag phase, using a digital multimeter connected in parallel to an external resistance of 100 Ω in the external circuit. The readings were recorded after obtaining at stabilized voltage for at least 3 minutes and the average was calculated. Current (I) and Power (W) were calculated according to Patil *et al.* [17] and Behera *et al.* [18]. Power density (mW/m^2), current density

(mA/m^2), volumetric power density (mW/m^3) and volumetric current density (mA/m^3) were all calculated based on the procedures outlined by Venkatamohan *et al.* [19].

Quality Parameters Analyzed: The initial physico-chemical parameters for the sago wastewater were analyzed according to the standard methods [13] and are tabulated (Table 2). Starch and Carbohydrates were analysed according to Sadasivam and Manickam [20]. pH values were determined by (Susima pH Meter, India) and conductivity was measured by (ELICO-180 conductivity bridge, India). Performance of the fuel cells was evaluated by estimating the substrate (COD) removal efficiency once in three days and carbohydrates and starch removal efficiency once in five days during the experimental period.

Isolation and Identification of Bacteria: Isolation and identification of the bacteria present in the sample was carried out as per APHA [13]. Morphologically different bacterial colonies were isolated by spread plate technique and identified by biochemical methods and selective agar techniques. Selective media such as Eosin methylene blue (EMB) agar, Mannitol salt agar (MSA), *Salmonella Shigella* Agar, RS medium (Romler–Shotts Medium Base), KF– *Streptococcus* agar, MacConkey's agar were used. All media and agar used for microbiological estimations were purchased from Hi Media chemicals, Mumbai, India.

RESULTS AND DISCUSSION

Effect of NaCl on Electricity Generation: Electricity generation in MFCs is a function of various physico-chemical as well as biological parameters [21]. NaCl is generally used as the electrolyte to improve the mass transfer of charged particles and to increase the solution conductivity [22]. Electricity generation was estimated in all the four MFCs and the effect of NaCl addition was studied. The performance of the MFCs was monitored up to 34 days. The open and closed circuit voltage values generated in all the MFCs was measured and are

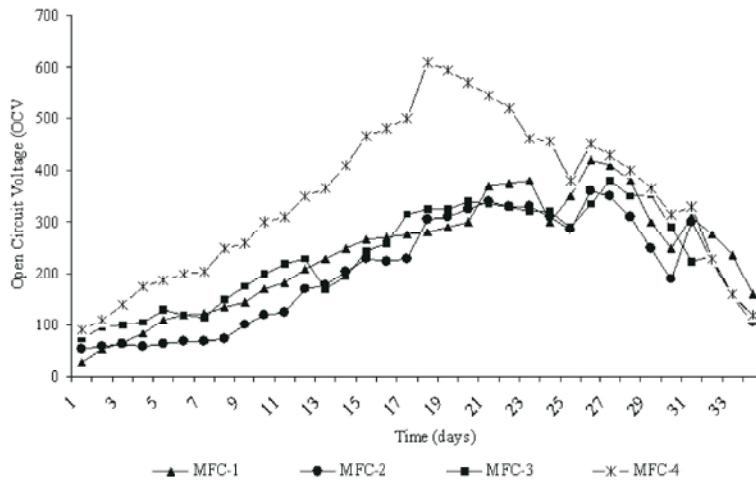


Fig. 2: Open circuit voltage generation in all the MFCs

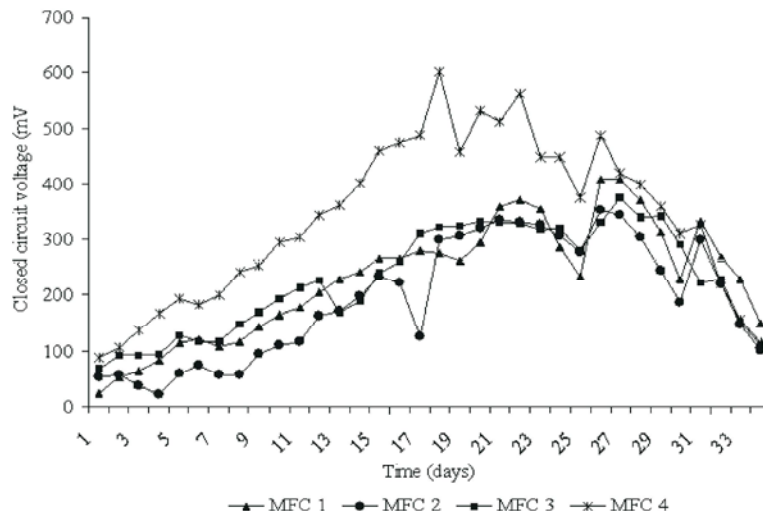


Fig. 3: Closed circuit voltage generation during the operation of fuel cells

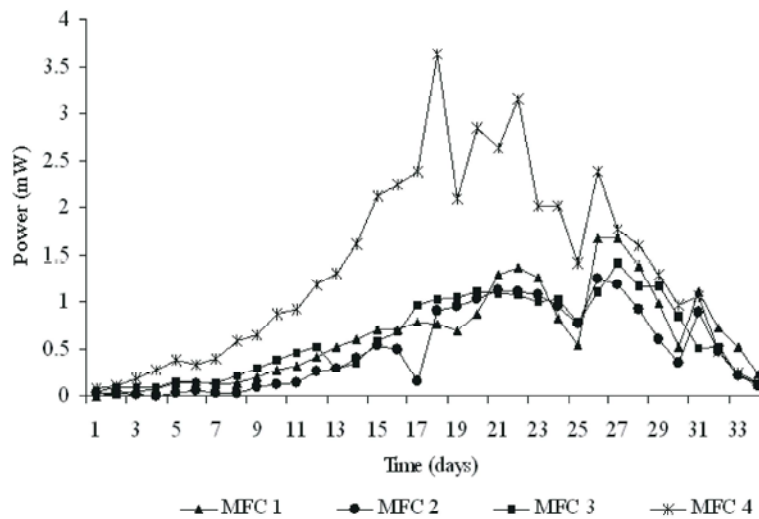


Fig. 4: Power generation during the operation of fuel cells with the function of time

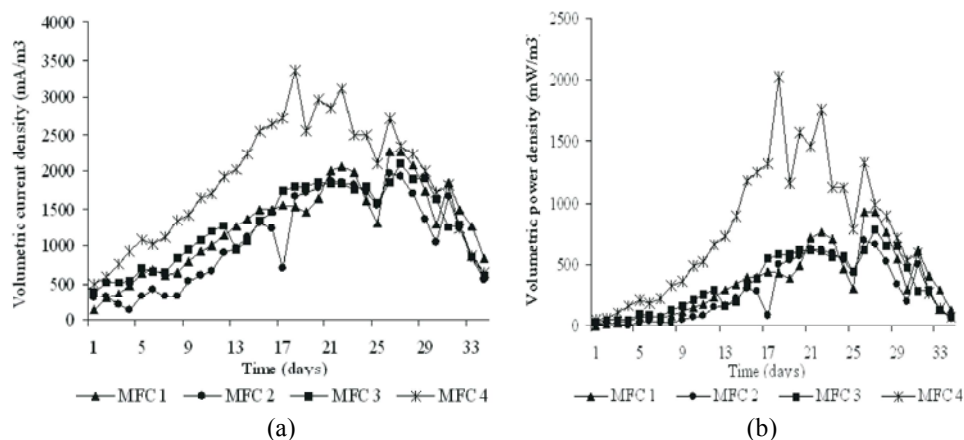


Fig. 5: Power details of the fuel cells (a) Volumetric current density (b) Volumetric power density

illustrated (Figs. 2 and 3). MFC- 4 documented the maximum open and closed circuit voltages of 610 mV and 603 mV on the 18th day of the experimental period. The details of power (mW) produced in the fourth MFCs are depicted (Fig. 4). Consistent increase in voltage and current output was observed in the fuel cells with exhaustion of time, accounting for a maximum power production of 3.63 mW in MFC- 4 after 17 days of start up. Power output of 1.67 mW was observed in MFC- 1 on 26th and 27th day, whereas MFC- 2 and MFC- 3 produced 1.25 mW on 26th day and 1.4 mW on 27th day, respectively. The experimental data revealed that the fuel cell with NaCl addition in its cathode chamber showed better power production than the other MFCs.

The experimental data documented the details of power generation with respect to volumetric current density (a) and volumetric power density (b) (Fig. 5). MFC-1 produced a current density of 151.4 mA/ m² on 26th and 27th day of experimental period where as MFC- 2 and MFC- 3 produced a current density of 130.7 and 139.2 mA/ m² on 26th and 27th day, respectively. In the case of MFC-4, maximum current density of 223.3 mA/ m² and a corresponding maximum power density of 134.6 mW/m² were detected on 18th day of fuel cell operation. A power density of 61.9 mW/m² was observed in MFC- 1 on 26th and 27th day of the experimental period, whereas MFC- 2 and MFC- 3 produced a power density of 46.1 and 52.3 mW/m² on 26th and 27th day, respectively. MFC-1, MFC-2 and MFC-3 generated volumetric current densities of 2272.2, 1961 and 2088.9 mA/m³, respectively with MFC-4 documenting a maximum of 3350.0 mA/m³. A maximum volumetric power density of 2020 mW/m³ was noticed in MFC- 4, whereas MFC- 1, MFC- 2 and MFC- 3 produced

volumetric power densities of 929.4, 692.3 and 785.4 mW/m³, respectively. Power generation was higher in MFC- 4 compared to the other three MFCs.

The reason for increased power production in MFC- 4 may be due to the increased ionic strength of electrolyte by the addition of NaCl in cathodic chamber. Power generation was higher in MFC- 4 compared to the other three MFCs and that may be due to the increased ionic strength of electrolyte by the addition of NaCl which increases the cathodic proton transfer rate in cathodic chamber. NaCl dissociates into cations (Na⁺) and anions (Cl⁻) in a solution, thus increasing the number of anions and cations. This will increase the conductivity of the solution which is nothing but the ability of the ions (anions and cations) to conduct electrons (e⁻) and protons (H⁺). Na⁺ will conduct e⁻, as sodium ion is a known electron acceptor and Cl⁻ will conduct H⁺ to the cathode electrode thus making protons and electrons available for oxygen reduction. Thus increased proton availability results in an increase in the rate of oxygen reduction, which indirectly increases the microbial and anodic activity. Similar results were reported by Gil *et al.* [22], where the cathode chamber was operated with various electrolytes like distilled water, phosphate buffer, NaCl solution and phosphate buffer. Maximum current was produced by the MFC which used phosphate buffer with NaCl solution as the catholyte. The reason is because of the combined effect of buffer and NaCl in increasing the electrical conductivity of the solution. Jang *et al.* [23] has reported that NaCl was generally used as an electrolyte to improve the mass transfer of charged particles. They observed an increase in current production from 3.5 to 4.7 mA when NaCl was added to the cathode and a maximum current of 7.7 mA was generated with continuous salt feeding.

Huang *et al.* [24] also documented a rapid increase in power density from 3.6 mW/m² to 9.6 mW/m², when the ionic strength of the catholyte was increased from 291 to 1,146 mM. The use of NaCl in the cathode might have enhanced the cathode reaction by increasing its conductivity and proton availability, thereby resulted in high power output.

Addition of NaCl to the anode chamber had a negative impact on power generation in MFC- 2 and MFC- 3, where less power output was documented compared to MFC- 1 and MFC- 4. The reason for decreased power production might be due to the fact that microorganisms in the sago wastewater are less tolerant to salt addition and their growth and multiplication was limited or attenuated by the presence of NaCl in anode chamber. All organisms with a semi permeable plasma membrane are subject to osmotic pressure, which is the effect of water moving in and out of the cell. Bacteria have a cell membrane and a cell wall and most of them thrive in a hypotonic environment, where the concentration of water outside the cell is greater than the concentration of water inside the cell. So when microbes are exposed to NaCl, the medium around it is hypertonic and it causes the microbe to dehydrate and perish. Mohan *et al.* [25] observed a similar decrease in voltage from 0.3 to 0.2 V when 15 mM NaCl was added to the anode chamber and complete inhibition of bacterial growth when the concentration was further increased to 100mM. The performance of the MFC is microbial growth dependent and addition of salt in the anode chamber is limited by the tolerance of the microorganisms to the ionic strength of the solution. Thus, decrease in the performance of MFC when NaCl was used in the anode chamber may be attributed to the effect of added salt on the growth of the organism [26]. These facts substantiate that employing NaCl exclusively in the catholyte is better than using it in the anode chamber or rendering the cathode devoid of NaCl.

Effect of NaCl on Internal Resistance: Internal resistance is the resistance inside a solution and electrons need to overcome that hindrance before passing through a circuit. Liu *et al.* [27] have reported that increasing the ionic strength of the catholyte in a MFC can remarkably increase power output due to the reduction of internal resistance. The internal resistance (R_{int}) of all the MFC's was calculated using the equation (1) according to Ieropoulos *et al.* [28, 29]

$$R_{int} = OCV / R_r - R_i \quad (1)$$

where,

OCV = Open Circuit Voltage (V)

R_i = Current under a load (A)

R_r = Value of the load (Ω)

The internal resistance of all the MFCs were 3k Ω before NaCl addition and it was reduced to 2K Ω (MFC- 1), 1K Ω (MFC- 2), 1K Ω (MFC- 3) and 500 Ω (MFC- 4). This data revealed that NaCl addition has considerably reduced the internal resistances of the fuel cells and MFC- 4 documented maximum reduction. Low concentration of protons in the cathode raises the internal resistance of the MFC as there is a hindrance in movement of electrons from anode to the cathode chamber due to decrease in oxygen reduction at the cathode electrode and reduced anodic activity. This will eventually result in less power generation. So, addition of NaCl into the cathode will increase the proton availability, thereby decreasing the internal resistance and increasing the power generation. Therefore, internal resistance and electricity generation are inter-related and both are affected by NaCl addition. This result was supported by Liu *et al.* [27], where, the internal resistance of the cell was 161 Ω and it eventually decreased to 91 Ω with the addition of 100 mM NaCl. Huang *et al.* [24], have supported the results of this study by demonstrating that the internal resistance of their MFC decreased from 2 K Ω to 900 Ω , when the concentration of NaCl was gradually increased from 300 to 1200 mM. These reports have substantiated that the reduction of R_{int} was the reason for high power output in MFC- 4, where an increase in ionic strength of the catholyte improved the cathodic proton transfer rate resulting in better MFC performance.

Effect of NaCl on COD Removal: The anodic chamber of the fuel cells was monitored for substrate (COD) removal efficiency once in 72 h during the experimental period. The experimental data documented that the COD removal efficiencies were 14, 7, 18 and 27% in MFC- 1, 2, 3 and 4, respectively on 4th day of operation and a gradual increase in the efficiencies was observed during the experimental period (Fig. 6). The removal efficiencies were 75, 74, 75 and 83% in MFC- 1, 2, 3 and 4, respectively on the 34th day. MFC- 4 was observed to be the best in substrate removal compared to the other MFCs and it may be due to the increased oxidation of organics in the anode chamber. The increased proton availability in the cathode chamber after NaCl addition resulted in better oxygen reduction at the cathode which might have indirectly triggered anodic activity. The observations by Feng *et al.* [30]

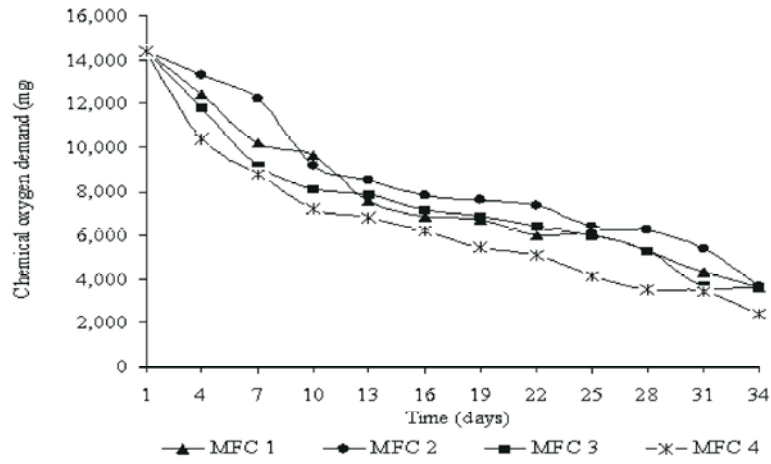


Fig. 6: COD removal efficiency over time in all the four fuel cells

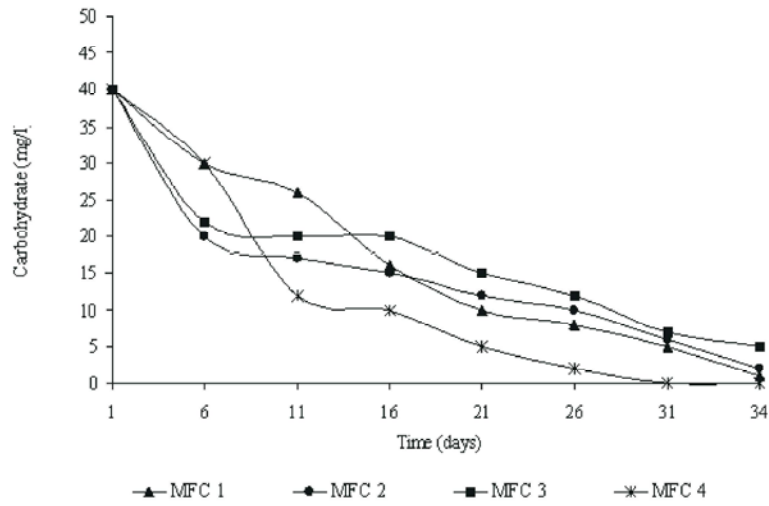


Fig. 7: Effect of salt addition on Carbohydrate reduction in MFCs

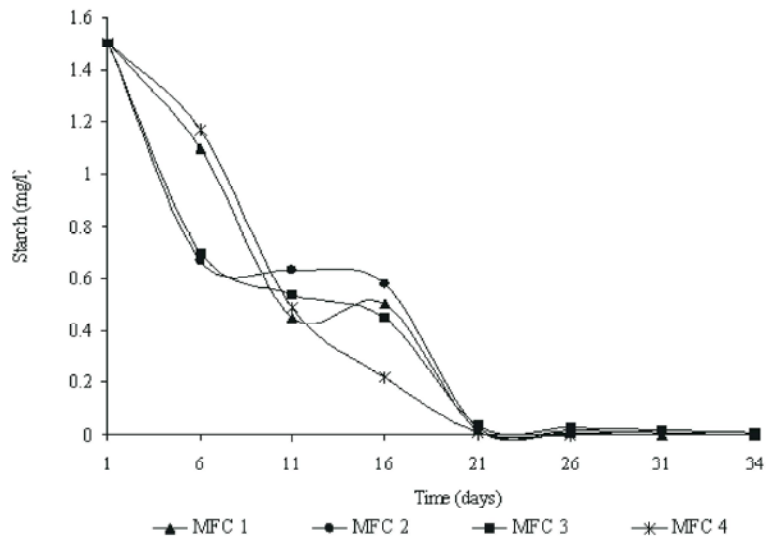


Fig. 8: Effect of salt addition on the reduction of starch in MFCs

Table 3: Physico-chemical parameters of the treated effluent from the anode of all the four MFCs

Parameters	MFC-1 (mg/L)	MFC-2 (mg/L)	MFC-3 (mg/L)	MFC-4 (mg/L)
Chemical Oxygen Demand	3,610	3,680	3,610	2,400
Carbohydrate	1	2	5	Nil
Starch	Nil	Nil	0.01	Nil

Table 4: Identification and confirmation of microbes from sago wastewater before and after MFC operation

Organisms in the initial effluent	Gram's staining	Catalase test	Oxidase test	Cultural characteristics	Carbohydrate fermentation test					IMVIC tests				
					Glucose	Sucrose	Lactose	Maltose	Mannitol	Indole	MR reaction	VP reaction	Citrate test	Urease test
<i>Staphylococcus sps</i>	G+ Cocci	+	-	Opaque	+	A	A	+	+	-	+	+	-	-
<i>Streptococcus sps</i>	Cocci+	-	-	Thin, even growth	+	A	A	A	A	-	+	-	-	-
<i>E. coli</i>	Rod, -	+	-	White moist, glistening	+	A+	AG	A	A	+	+	-	-	-
<i>Proteus vulgaris</i>	Rod -	+	-	Thin blue grey spreading growth	AG	AG+/-	-	A	A	+	+	-	-	+
<i>Klebsiella sps</i>	Rod -	+	-	Slimy, white, translucent growth	AG	AG	AG	A	A	-	-	+	+	+
<i>Salmonella sps</i>	Rod -	+	-	Soft, black colonies	AG	AG	AG	A	A	+	+	-	-	-
<i>Micrococcus sps</i>	Cocci +	-	-	Soft, smooth, yellow growth	-	-	-	-	-	-	-	-	+	+
<i>Pseudomonas aeruginosa</i>	Rod -	+	+	Abundant, thin white medium to yellow	-	-	-	-	-	-	-	-	+	-
<i>Aeromonas sps</i>	Rod -	+	+		A	A	-	A	A	+	-	+	+	-
Organisms in the final effluent														
<i>E. coli</i>	Rod, -	+	-	White moist, glistening	+	A+	AG	A	A	+	+	-	-	-
<i>Aeromonas sps</i>	Rod -	+	+		A	A	-	A	A	+	-	+	+	-
<i>Streptococcus sps</i>	Cocci+	-	-	Thin, even growth	+	A	A	A	A	-	+	-	-	-
<i>Pseudomonas aeruginosa</i>	Rod -	+	+	Abundant, thin white medium to yellow	-	-	-	-	-	-	-	-	+	-
<i>Salmonella sps</i>	Rod -	+	-	Soft, black colonies	AG	AG	AG	A	A	+	+	-	-	-

A, Acid production; G, Gas production

have supported the results of this study and they reported that the COD removal efficiency was increased from 87 to 90% when 50mM of NaCl was added into the cathode chamber. These supporting results have clearly demonstrated that highest reduction in COD was due to the effective functioning of the anode compartment of MFC 4 in response to NaCl addition.

Effect of NaCl on Carbohydrate and Starch Utilization:

Carbohydrate and starch reduction were monitored once in five days during the experimental period of the MFCs. The sago wastewater had an initial carbohydrate content of 40 mg/l and an initial starch content of 1.53 mg/l. Carbohydrate reduction observed in all the MFCs was calculated and plotted (Fig. 7). MFC- 4 showed a steep reduction in carbohydrate level by reaching complete removal on the 31st day, whereas the other MFCs had residual levels of carbohydrates in their anolyte. Starch reduction during the experiment was depicted (Fig. 8). MFC- 1 and MFC- 4 portrayed a rapid reduction in starch and complete removal was observed on the 31st day. Though starch removal rate in MFC- 4 was initially less compared to other fuel cells it reached a maximum of 98% on 26th day and documented complete removal on the 31st day. It is evident from the above results that NaCl addition in the cathode chamber had a positive influence on MFC performance.

The physico-chemical parameters of final effluent from the anode chamber of all the MFCs were analyzed and are summarized in Table 3.

Microbiological Analysis: The sago wastewater was subjected to microbial analysis before and after employing it in the fuel cells and the details is shown in Table 4. A bacterial count of 176×10^{-6} CFU/ml was present in the initial influent and 140×10^{-6} CFU/ml was present in the final effluent. Microbes such as *Escherichia coli*, *Staphylococcus sps*, *Proteus vulgaris*, *Micrococci sps*, *Salmonella sps*, *Aeromonas sps*, *Streptococci sps*, *Klebsiella sps* and *Pseudomonas aeruginosa* were identified in the initial sample, where as microbes such as *E.coli*, *Aeromonas sps*, *Streptococci sps*, *Pseudomonas aeruginosa* and *Salmonella sps* were found to be dominant in the final effluent. Microbes such as *Alcaligenes*, *Bacillus*, *Corynebacterium*, *Micrococcus* and *Pseudomonas sps* have already been identified from sago wastewater and are reported to be starch degrading bacteria [31, 32, 33]. *Pseudomonas aeruginosa* have already been used in a dual chambered MFC, employing potato-processing wastewater sludge diluted with nutrient broth. Power production of 23.3W/m² was documented in the MFC [34]. Gallagher *et al.* [35] and Hernandez and Newman [36] reported that *Pseudomonas aeruginosa* produces a phenazine blue pigment called

pyocyanin which functions as an extra cellular electron shuttle and is responsible for the electrochemical activity of the microbe. *Aeromonas* sps have been isolated by Pham *et al.* [37], from mediator less microbial fuel cell and their electrochemical activities have been proven by cyclic voltammetry.

CONCLUSIONS

MFC is a novel wastewater treatment process in which energy can be recovered from waste. Experiments were conducted to demonstrate the feasibility of electricity production from sago wastewater in a dual chamber, salt bridge MFC. The influence of NaCl addition on MFC performance was evaluated in terms of power generation, COD, carbohydrate and starch reduction. Better performance and stabilization tendency was found in the MFC in which NaCl was added only to its cathode chamber. A maximum voltage of 603mV, current of 6.0 mA, COD removal of 83% and a total reduction of carbohydrate and starch content was recorded in it. While our results show that power production and substrate removal is feasible for sago industry wastewater, pilot-scale and additional studies are further required for better evaluation.

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Persian Abstract

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