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ORIGINAL ARTICLE

Importance of Mixed culture in Generation of Electricity from Anaerobically Digested Distillery Wastewater through Microbial fuel cell

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ABSTRACT
Microbial fuel cell (MFC) is a device which converts chemical energy directly into electrical energy using microorganisms. MFC is becoming very important green biotechnological tool to generate clean energy simultaneously treating waste. Any organic biodegradable matter can be used as feed for microorganisms that has capacity to generate electrons and protons through their metabolism, thus help in generation of electricity. In this research, two chambered MFC has been used to treat anaerobically digested distillery wastewater (ADDW). ADDW generally goes to lagoons for further degradation and hence ADDW becomes ideal for extraction of further energy. Aerobes and anaerobes were isolated from ADDW and checked for the activity in MFC. Endogenous microbial consortium was found to be playing important role in generation of electricity as individual isolates failed to show the activity. Mixed consortia could generate 92.25±28.6 mW/m^3 power with reduction of 50% TOC within 48 hrs. Thus mixed culture proved to be useful in wastewater treatment simultaneously generating electricity.
Key words: *microbial fuel cell; distillery wastewater; anaerobic digestion; mixed consortium; power density*

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INTRODUCTION

The excessive utilization of energy to a greater extent around the world has contributed significantly to the energy crisis, especially from the environmental perspective. Population all over the world is dependent on conventional energy sources such as coal and oil which have led to the accumulation of harmful gases in the atmosphere leading to global warming. The high demand on fuels has led scientists and researchers to open a new area of research and development of renewable fuels from alternative sources. The application of microbial fuel cells (MFCs) represents a completely new approach to wastewater treatment processes while simultaneously producing sustainable clean energy [1]. MFC is a device which converts chemical energy directly into electrical energy using microorganisms as biocatalysts [2, 3]. In typical MFC configuration, bacteria at the anode metabolize such biodegradable matter, generating electrons and protons. Electrons are transferred to the cathode through external resistance and protons are transferred through proton exchanger to cathode. At cathode, electrons are accepted by an electron acceptor, thus completing the circuit and generating electricity [3, 4]. So, the biodegradable matter present in variety of sources such as agricultural wastewater, domestic wastewater and industrial wastewater would be preferable [4], as electricity can be generated simultaneously treating the wastewater, which is the need of the hour.

Various industries such as distillery, sugar industry, paper & pulp, chemical, pharmaceutical & tannery dispose off their improperly or partially treated effluent directly into the soil and water bodies, which has been causing major pollution problem. Manufacture of ethyl alcohol in distilleries based on cane sugar molasses constitutes a major industry in Asia and South America. The aqueous distillery effluent stream known as spent wash is a dark brown highly organic effluent and is approximately 12-15 times by volume of the product alcohol. It is one of the most complexes, worrying and strongest organic industrial effluents, having extremely high COD and BOD values [5].

Anaerobic digestion (AD) has been very successfully employed by large number of countries over recent years for purposes such as energy production and waste management. But even after anaerobic digestion, the COD of the wastewater remains high. Hence, MFC can be used as a commentary technique to anaerobic digestion for extraction of energy.

In the current study, comparative analysis of MFC based on isolated cultures from anaerobically digested wastewater and mixed consortium from the same wastewater has been performed.

MATERIAL AND METHODS

Material

For construction of MFC, customized Scott Duran glass bottles of 500 mL capacity used as reservoir in the preparation of MFC were acquired from Omega Glass Works, Mumbai, India. Graphite rods removed from exhausted Eveready AA batteries were used as electrodes. Electrical hardware like resistance and copper wires was bought from local vendor, Mumbai, India. Voltages for all experiments were recorded by Picolog 1216, bought from Picotech, UK. Anaerobically digested distillery wastewater used as feed throughout the experiments was procured from a distillery near Nasik, India. Sag Tex PHD antifoam was obtained from Momentive, India.

Bacteriological media like Nutrient broth, Nutrient agar, Alternative thioglycollate medium, Anaerobic agar as well as Anaerogas pack all were bought from HiMedia, India. All other chemicals used were of ExcelaR grade, used without further purification and obtained from Qualigens (Fisher Scientific), India.

Methods

Enrichment and isolation of bacteria from wastewater

Anaerobically digested distillery wastewater was used as a source of microbes for enrichment and isolation. Nutrient broth and Alternative thioglycollate medium were used for enrichment for aerobic and anaerobic microorganisms respectively. From enrichment media, aerobic organisms were isolated on Nutrient agar and anaerobic organisms were isolated on Anaerobic agar under anaerobic conditions created by Anaerogas Pack in anaerobic jar. Nutrient agar plates were incubated at 37°C for 24 hours, while anaerobic agar plates were incubated at 37°C for 72 hours. Single colonies were removed from these plates and sub-cultured on respective new plates. This procedure was repeated to obtain pure cultures. The outline of morphological and physiological characteristics such as spore stain, motility, pigment production were determined as per standard methods [6]. The gram nature of isolated colonies was determined by method described by Benson [7].

Wastewater sampling and analysis:

Wastewater was immediately stored at 4^0C. Characterization of the wastewater was carried out using APHA standard methods [8]. The wastewater characteristics are shown in Table 1.

Table 1 Characteristic of anaerobically digested distillery wastewater

Characteristics	Value
pH	7.96 ± 0.1[a]
Colour	Dark Brown
BOD (mg/L)	2330 ± 108[a]
COD (mg/L)	18560 ± 640[a]
TOC (mg/L)	6519 ± 89[a]
Total Solids (mg/L)	34658 ± 38.7[a]
Total Dissolved Solids (mg/L)	21914 ± 390.2[a]
Total Suspended Solids (mg/L)	12744 ± 135[a]

[a]values expressed as the mean ± the standard deviation (n=3).

Preparation of feed:

250 mL of wastewater was diluted to 1 L with distilled water. 2% Dextrose along with 1 mL each of 0.1 M phosphate buffer of pH 7.2, 0.1 M $MgSO_4.7H_2O$, 0.2 M $CaCl_2.2H_2O$ and 0.01 M $FeCl_3.6H_2O$ was added to 1 L

diluted anaerobically digested distillery wastewater to induce the growth of endogenous bacteria. To suppress the foaming, 1.5 mL of Sag Tex PHD antifoam was added to 1 L of wastewater. This standardized wastewater was used as feed throughout the experiments.

Microbial fuel cell set-up and operational conditions:

Two chambered MFC was constructed appropriate for the batch operation which is explained in details in previous study [9]. As shown in Fig. 1, the anodic and cathodic chambers of MFC of 500 mL each, which were joined by a salt bridge (15 mL) containing 10% KCl. Two identical cylindrical graphite rods (removed from exhausted Eveready AA batteries) were used as electrodes. Anode and cathode were connected to the copper wires, which in turn were connected to 1000 Ω external resistance. The anodic chamber was filled with 200 mL of standardized anaerobically digested distillery wastewater, while cathodic chamber was filled with 200 mL of 100 mM potassium ferricyanide prepared in 100 mM phosphate buffer of pH 7. Endogenous microflora from anaerobically digested distillery wastewater was used as a source of microorganisms. All the experiments were carried out at room temperature (25^0C to 30^0C).

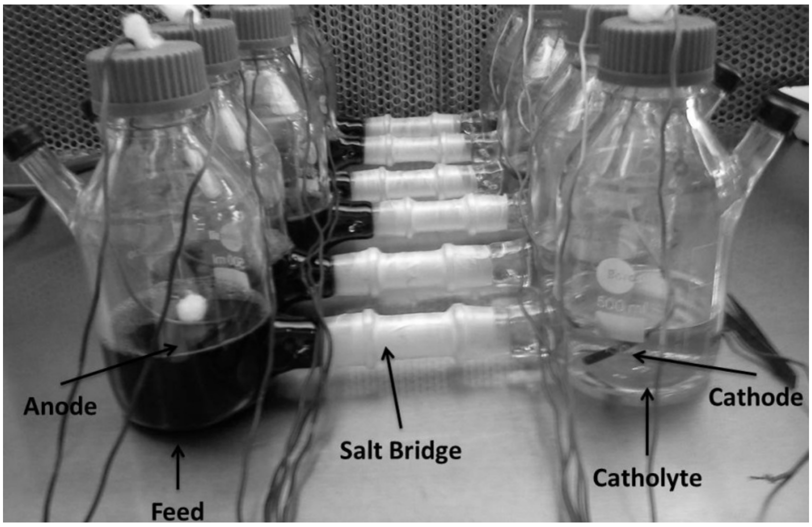

Fig. 1. A typical MFC set-up

Electrical measurements:

All experiments were performed using Picolog data logger (1216, Picotech, UK) to determine the electricity generation. The voltage was recorded every 5 minutes. The current was calculated using Ohm's law V=I x R, where V is the voltage across resistance, I is the current generated and R is the external resistance. The power generated by the system was calculated as P = V x I where, V is the voltage and I is the current generated by the system.

MFC with isolated microorganisms as well as mixed culture

All MFC experiments were performed under aseptic conditions. For all isolated cultures, above operating conditions were kept constant. 1 mL with approximate cell density of 1.5×10^8 cells (McFeland's Std 0.5) was used to inoculate in 200 mL of sterile wastewater. MFC experiments were repeated with standardized wastewater having mixed consortia.

Total Organic Carbon Analysis

TOC experiments were performed in Centre for Environmental Science and Engineering, IIT Mumbai using TOC analyser (TOC-VCSH, Shimadzu). Each sample was diluted 200 times before analysis.

Statistical analysis:

Results are expressed as Mean ± S.D. with experiments being conducted in triplicate. The statistical significance was determined by one-way analysis of variance (ANOVA) to determine if the data obtained is significantly different from each other. Statistical significance between different columns was determined by posthoc Tukey's Test using Graph Pad Prism software version 5.0 (GraphPad Software, San Diego, CA, USA).

RESULTS AND DISCUSSION

Isolation of bacteria from anaerobically digested distillery wastewater

The wastewater from distillery exhibits an extreme condition for growth of organisms. According to literature, either the consortium or some specific type of bacterium could be responsible for the production of electricity in MFC [10]. In case, some specific bacterium produces electricity, its activity can either be synergistically enhanced or instead reduced due to antagonism. The isolation of these organisms was carried out in order to check the production of electricity by individual bacteria and check for the type of association. In the extreme conditions of distillery waste water, either aerobes or anaerobes or both would grow depending upon the metabolism and level of oxygen supply to the particular layer of the stored water taken for study. The study involves the use of bottom layer of waste water tank.

Loopful of wastewater was inoculated to 20 mL of nutrient broth and alternative thioglycollate broth and incubated at 37^0C for 24 hours. Within 24 hours, good growth was observed in the broth which was then further used for isolation of microorganisms. The dominant bacteria were isolated from the cultures on nutrient agar and anaerobic agar plates. The morphological and physiological characteristics of the cultures are given in Table 2. As can be seen that out of four isolates, three of them were gram-negative. Gram positive organism was found to produce off-white to yellowish pigment on nutrient agar.

Table 2 The morphological and physiological characteristics of bacteria isolated from anaerobically digested distillery wastewater

Characteristic	Organism 1 (O1)	Organism 2 (O2)	Organism 3 (O3)	Organism 4 (O4)
Respiration	Aerobic	Aerobic	Anaerobic	Anaerobic
Shape	Irregular	Circular	Circular	Circular
Size	7 mm	2 mm	Pinpoint	Pinpoint
Color	Off white	Colorless	Colorless	Colorless
Margin	Serrate	Entire	Entire	Entire
Opacity	Opaque	Translucent	Translucent	Translucent
Elevation	Flat	Concave	Concave	Concave
Consistency	Dry	Sticky	Sticky	Sticky
Gram Nature	Gram positive	Gram negative	Gram negative	Gram negative

MFC with individual isolates

Fig. 2a showed the MFC pattern with isolated cultures. It was observed that no electricity was generated using individual isolates. Further experiment was carried out using consortium of all 4 isolated cultures in equal proportions, yet no electricity generation was observed (Fig. 2b).

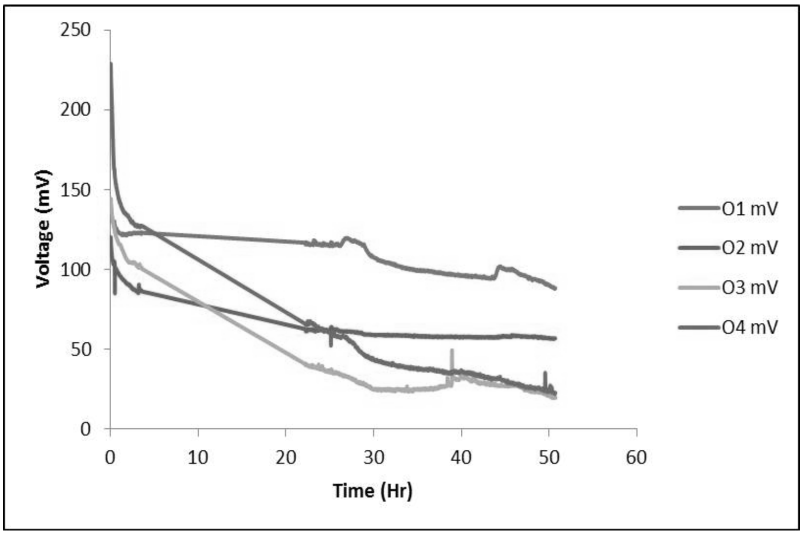

Fig. 2a MFC with Organism 1 (O1), Organism 2 (O2), Organism 3 (O3) and Organism 4 (O4)

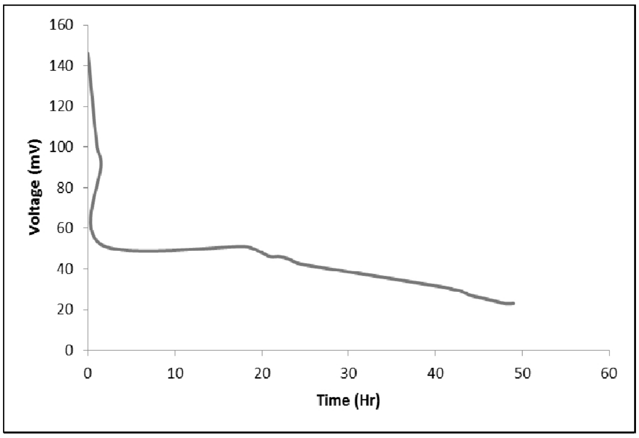

Fig. 2b MFC inoculated with all four isolated cultures simultaneously

MFC with mixed consortium

The experiment was carried out using endogenous microflora used as inoculum and standardized wastewater as feed. Fig. 3a showed generation of electricity at 36.5±3.54 hr with highest voltage generated 0.135±0.02 V. The power generated by the system was found to be 18.45±5.73 µW. Volumetric power density was calculated power generated per meter cube. Since 18.45±5.73 µW was generated from 200 mL of wastewater, power density was found to be 92.25±28.6 mW/m^3. Mixed consortium was found to be successful in generation of electricity as compared to individual isolates; also various controls were maintained in order to prove that the electricity generation was by bacterial activity. Fig. 3b showed reduction in time required to attain highest voltage when 10 fold concentrated bacterial culture was inoculated in the anodic chamber. For this experiment, normal MFC with mixed consortia was carried out; following the electricity generation, 200 mL feed was centrifuged and 20 mL of the residual pellet was used as inoculum in the subsequent experiment. Because of high bacterial load (Fig. 3b), the rate of degradation of organic matter was high and hence was responsible highest peak much earlier than that of MFC with mixed consortium (Fig. 3a). The highest peak for MFC with mixed consortium (Fig. 3i) and MFC with high microbial load (Fig. 3b) were found to be non-significantly different possibly because, the available surface area for electron transfer was limited thus high current was not obtained. From the above results it can be said that higher density of bacterial inoculum was useful in faster electricity generation rather than obtaining higher voltage. This result can be useful in the future research especially when MFC will be used at industrial scale. When bacteria culture was metabolically poisoned by addition of disinfectant in anodic chamber, no electricity generation was observed (Fig. 3c). Fig. 3d showed importance of anaerobic cultural in generation of electricity. When anodic chamber was continuously aerated with the help of an aerator, no power generation was observed. This proved the known theories regarding the importance of nature of bacterial respiration and thus authors prefer maintaining anaerobic condition at anodic [11, 12, 13]. Though Rabaey & Verstraete (2005) in a review reports the use of aerobes, facultative anaerobes to strict anaerobes in MFC [4]. Fig. 3e showed no culture control, as the feed in anodic chamber was autoclaved before use. Due to lack of bacterial culture, no electricity generation was observed. This result confirms the role of bacteria in electricity generation.

Organic removal efficiency

The TOC of initial standardized wastewater was found to be 14660±105 mg/L. The TOC after 48 hours was found to be 7297.67± mg/L. Thus % TOC reduction calculated was found to be 49.78.

Since the wastewater was collected after biogas formation, primary bacteria growing on easily biodegradable matter must be generating by-products (Lag phase in Fig. 2a) which were in turn utilized by secondary bacteria generating electricity (Log phase in Fig. 2b). The isolated colonies must be of primary organisms as they fail to generate electricity. The current study also shows that new technology of microbial fuel cell can be used as a complementary technique to old technology like anaerobic digestion. In the future, it will be interesting to investigate the feasibility of simultaneous generation of methane along with electricity at industrial level. It has been already shown that mixed communities typically produce more power in microbial fuel cells than pure cultures [14].

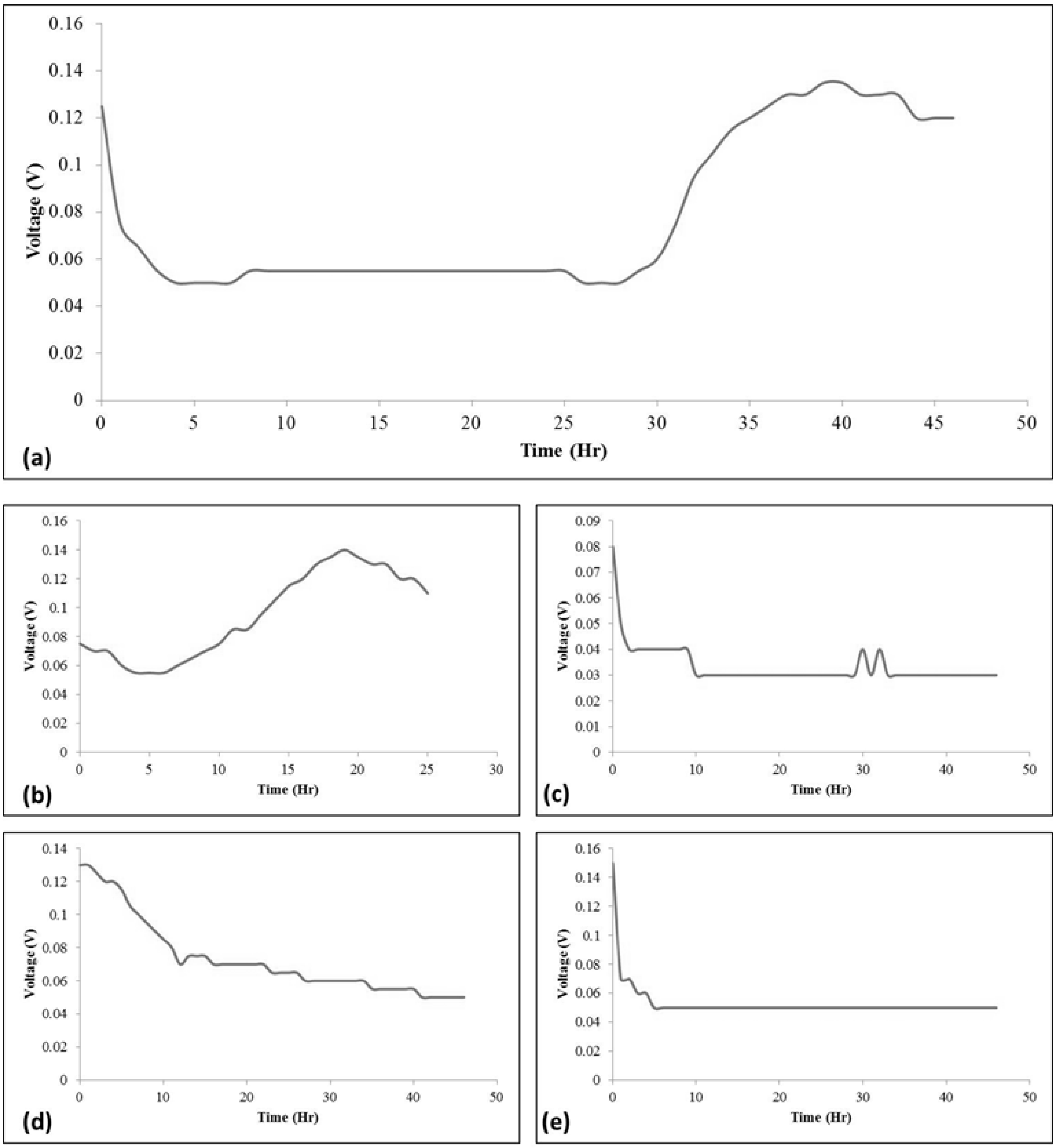

Fig.3 Electricity generation with endogenous mixed consortia along with few controls

a) MFC with mixed consortia
b) MFC with addition of 10x culture from previous batch in anodic chamber
c) MFC with addition of disinfectant in anodic chamber
d) MFC with aeration at anode
e) MFC with autoclaved wastewater in anodic chamber

CONCLUSION

Mixed consortium from anaerobically digested distillery wastewater was found to produce electricity as oppose to individual isolates. The advantages of using mixed cultures in an MFC include no requirement for sterilization and the possibility of using MFC in a continuous process. The current study opens the view of an integration of two technologies that is anaerobic digestion and microbial fuel cell for enhancement of energy extraction from distillery wastewater. Though MFC offers a promising prospect in electricity generation along with biogas formation from distillery wastewater, the improvements and optimization are needed to achieve better results for electricity generation and pollutant removal.

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