

## Original Article

# Isolation and Characterization of Electrogenic Bacteria from Tannery Wastewater

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**Electrogenic bacteria are able to transfer electrons to extracellular electron acceptors as well as can be used in devices like bioelectrochemical systems (BES). This study was focused to produce electricity from wastewater using microbial fuel cell and find out potential electrogenic bacteria from liquid tannery wastes. After power generation study, six isolates were screened as potential electrogenic bacteria. Of them, two potential bacteria were identified based on their morphological and biochemical characteristics and confirmed by phylogenetic analysis based on 16S rRNA gene sequence. We also investigated the effect of anode surface area on electricity generation in the microbial fuel cells and found that the surface area had positive impact on electricity generation.**

**Keywords:** MFC, Anode, Cathode, Tannery wastewater, Electrogenic bacteria.

## Introduction

Currently, nonrenewable resources like fossil fuels such as coal, oil and natural gas are the main sources of our energy<sup>1</sup>. World is facing energy crisis due to its high global demand, depletion of fossil based fuels for energy generation and an explosion of world population which is rising with time<sup>2</sup>. Burning of fossil based fuels also has a serious effect on environment owing to emission of carbon dioxide and other greenhouse gases that cause global warming and atmospheric pollution<sup>3</sup>. Energy crisis as well as environmental pollution situations are very prominent in Bangladesh. Therefore, it is very necessary to find an effective alternative source for energy generation to meet energy demand and to mitigate environmental pollution as early as possible<sup>4</sup>. Indeed, renewable energy sources such as solar energy, energy produced from wind and water may be the pieces of cogent solution for this energy crisis<sup>5-6</sup>. Biomass, especially organic waste, is being considered as a valuable candidate. The use of biomass, in the case of waste organics, is environment friendly and regarded as a renewable energy source<sup>7</sup>. Energy from biomass can be harnessed in the form of ethanol, biodiesel, hydrogen cells as well as in the form of microbial fuel cells (MFCs) for power generation<sup>8</sup>.

Chemical energy of fuels such as hydrogen, natural gas, methanol, etc., existing in the chemical bonds is directly converted into electricity by fuel cells<sup>9</sup>. Biological fuel cells (BFCs) use biocatalysts (microbes or enzymes) instead of expensive metal catalysts in conventional fuel cells to produce bioelectricity<sup>10</sup>. Microbial fuel cells (MFCs) employ living cells for oxidation of

organic substrate, whereas enzymatic fuel cells use active enzymes for the same purpose<sup>11</sup>. Though the first fuel cell was built in 1839, Potter described the concept of microbial conversion in 1911 to create electrical current<sup>12</sup>. However, MFC gained much attention in 1999 once it was discovered that mediator was not a compulsory component within MFCs<sup>13</sup>. In addition, the discovery of electricity production from wastes and renewable biomass using bacteria paves the way to MFC research field<sup>14</sup>. Furthermore, MFC technology was highlighted more when Time Magazine declared *Geobacter sulfurreducens* KN400, a bacterial strain capable of high current production, as one of the top 50 most important inventions for 2009<sup>15</sup>. A great advantage of MFCs is that they typically have long lifetimes (up to five years)<sup>16</sup>. Additionally, they are able to oxidize simple carbohydrates to carbon dioxide via biochemical reactions as well as can be operated in mild conditions<sup>17</sup>. Therefore, MFC could play major role in green technology not only for the production of bio-energy but also for treating wastewater<sup>18</sup>. But a challenge is that MFCs produce low energy which is currently orders of magnitude lower compared to that of chemical fuel cells<sup>19</sup>. The aim of this study was to isolate and identify potential electrogenic bacteria from wastewater and to generate power using a basic MFC technology.

## Methods and Materials

### Collection of wastewater:

Tannery wastewater samples were collected aseptically from tannery industrial area located at Savar, Dhaka and hospital wastewater samples were collected from the Salimullah Medical College campus in Dhaka city. A total of 5 samples were collected

# Have equal contribution

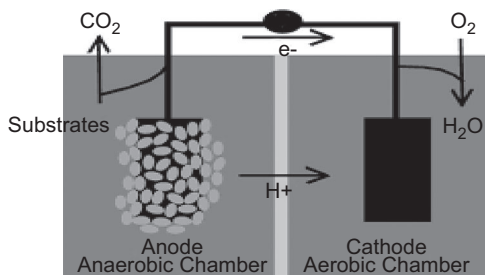
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from 4 locations. The color, odor and pH of the collected samples were recorded and samples were stored in normal refrigerator for further investigation.

#### MFC design and operation:

Two separate chambers (1L capacity) were used to develop microbial fuel cell. The two compartments were connected by a U-shaped salt bridge which was filled with 5.0M NaCl and 10% agar for exchange of proton within anode and cathode<sup>20</sup>. In one chamber, Zinc plate was used as anode electrode and filled with the basal medium and in other chamber, Copper plate was used as cathode electrode and filled with distilled water and 0.1M phosphate buffer in 4:6 ratio. A single chamber microbial fuel cell with both anode and cathode electrode was designed to screen potential electrogenic bacteria (Figure 1). The single chamber MFC was filled with 900 ml basal medium and 100 ml of liquid pure culture of bacteria. The collected sample was directly added to anode chamber in the ratio of 6:4 with basal medium. The composition of basal medium (per liter) was 3.0g glucose, 0.25g NaHPO<sub>4</sub>, 0.25g Na<sub>2</sub>HPO<sub>4</sub>, 0.3g MgCl<sub>2</sub>, 0.005g CaCl<sub>2</sub>, 0.015g ZnCl<sub>2</sub>, 0.0105g CuCl<sub>2</sub>, 0.0105g MnCl<sub>2</sub>, and 0.5g NH<sub>4</sub>Cl. An electric multimeter was connected with both electrodes to record voltage from first day to last day with regular interval comparing with control.



**Figure 1.** Components of a Traditional Microbial Fuel cell.

#### Isolation of electrogenic bacteria from anode plate:

Basal medium with inoculated bacteria was incubated in microbial fuel cell for electricity generation. Zinc plate used as anode was the source of electrogenic bacteria (EB) which were capable of forming biofilm. After 15 days, the anode plate was collected from MFC aseptically. Sterile distilled water was rinsed over the anode surface containing biofilm by pipette and rubbed the surface with sterile toothpick for collection of the biofilm. Then, serial dilution and spread plate method were performed for isolation of individual bacterial cells.

#### Screening of potential electrogenic bacteria:

To screen the potential electrogenic bacteria, 100ml overnight culture of individual isolates was added to single chamber MFC. Then the chamber was maintained at room temperature for three days and electricity production was measured at 24-hour interval as voltage using an electric multimeter.

#### Identification of the selected isolates:

A number of six isolates were screened as potential electrogenic bacteria based on their three days electricity generation

performance and these were primarily identified on the basis of Morphological and a range of biochemical tests. Among the six isolates, two potential isolates (TWWS-14 and TWWS-15) were finally identified using 16S rRNA sequence analysis.

Primers set used to amplify 16S rRNA sequence were 27f (5'-AGA GTT TGA TCC TGG CTG AG-3') and 1492r (5'-GGC TAC CTT GTT ACG ACT T-3') as forward and reverse primer respectively in a PCR thermal cycler (Applied Biosystems, USA). The thermal cycling program was set with initial denaturation at 95°C for 5 minutes, followed by 30 cycles, denaturation at 95°C for 30 seconds, annealing at 57°C for 1 minute, extension at 72°C for 3 minutes and the final extension at 72°C for 10 minutes. The amplified DNA was visualized by gel electrophoresis. The 16S rDNA sequence was analyzed and compared with other deposited sequences in the GenBank via the online programme BLAST. Neighbor-joining phylogenetic tree was constructed based on the 16S rRNA gene sequences using MEGA6.

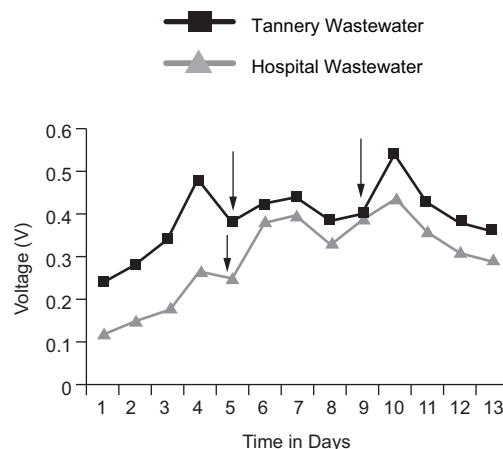
## Results

#### Characteristics of collected samples:

Among the collected samples, one wastewater sample collected from tannery disposal site had golden color, extremely bad odor and pH 7.77. Another wastewater sample collected from hospital area had tan color, bad odor and pH 8.09

#### Electricity generation in double chamber MFC:

The value of voltage in double chamber started to increase from 2<sup>nd</sup> day and continued up to 4<sup>th</sup> day as the growth of bacteria was in exponential phase and after that it dropped gradually due to depletion of nutrients and reached to minimum on 5<sup>th</sup> day. After addition of glucose (3gm/L) the voltage value started to regain and increased from the 5<sup>th</sup> day to the 8<sup>th</sup> in the anode chamber (Figure 2). At this stage, addition of glucose again (3gm/L) started to increase the voltage from 9<sup>th</sup> day and continued up to 10<sup>th</sup> day. Finally, the voltage started to decrease till 13<sup>th</sup> day as no



**Figure 2.** Voltage record for raw tannery (line imbedded square, red) and hospital wastewater (line imbedded triangle, green) with time during the treatment, followed by subsidiary addition of glucose, as shown by arrows.

additional glucose was supplied to anode chamber. The maximum voltage (0.54V) was observed for the tannery wastewater compared to hospital wastewater (0.44V). The voltage value did not vary for each repetition of tannery wastewater and hospital wastewater samples at each day (upto two decimal places).

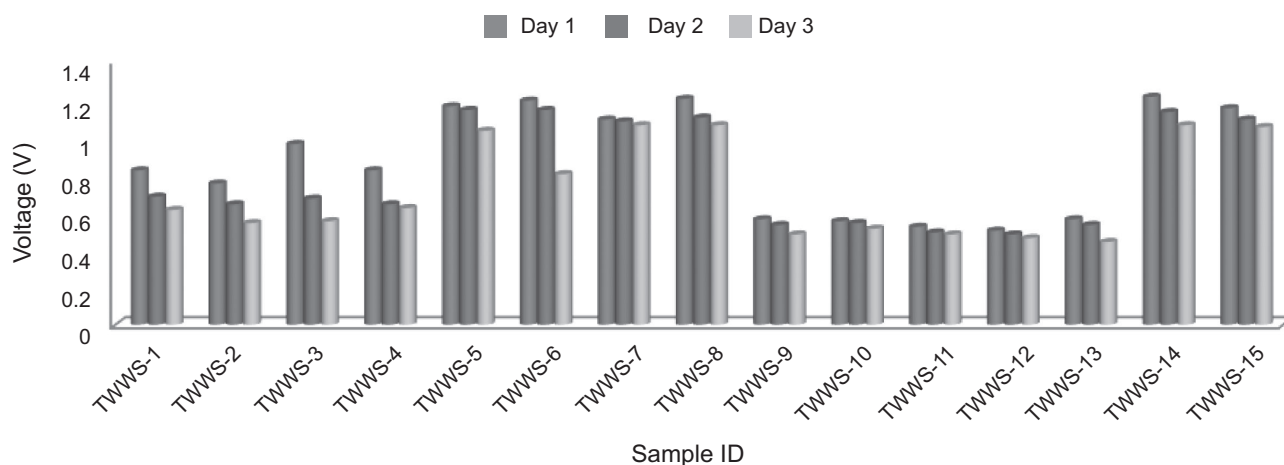
#### Isolation and screening of potential electrogenic bacteria:

To identify potential electrogenic bacteria, liquid pure cultures of 15 individual isolates from tannery wastewater were kept in different single chamber MFCs. From the study it was found that all the isolates generated maximum electricity after 24h (Figure 3). Among them, six isolates (TWWS-5, TWWS-6, TWWS-7, TWWS-8, TWWS-14 and TWWS-15) produced electricity above 1.0V were selected as potential electrogenic bacteria. It was observed that the highest voltage (1.21 V) was generated by

the isolate, TWWS-14. The voltage value did not vary for each repetition of same sample at each day (upto two decimal places).

#### Identification of Selected Strains:

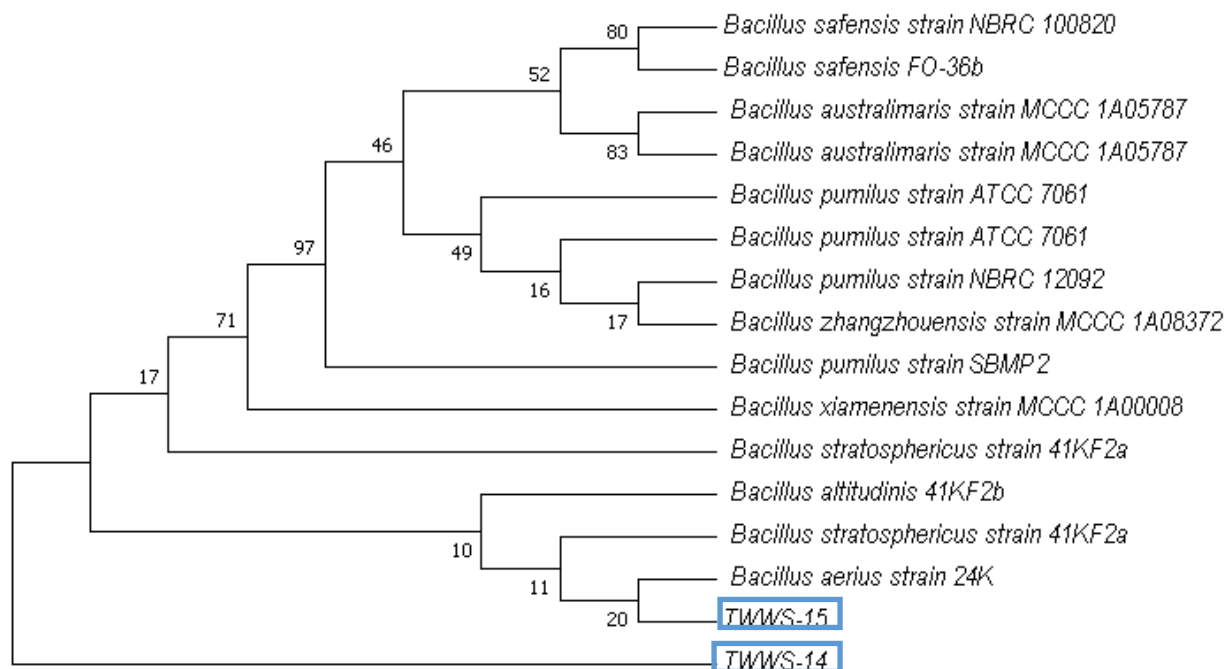
The selected six isolates were identified using morphological and biochemical characterizations (Table 1). To confirm the identification of two most potential bacterial isolates (TWWS-14 and TWWS-15), 16S rRNA gene sequence analysis was performed. The obtained sequences were compared with those of 16S rRNA gene sequences deposited at GeneBank by using BLAST program ([blast.ncbi.nlm.nih.gov/Blast.cgi](http://blast.ncbi.nlm.nih.gov/Blast.cgi)). Phylogenetic and molecular evolutionary analyses were constructed using MEGA version 6. From the data it was found that the TWWS-14 and TWWS-15 isolates were closely related with *Bacillus altitudinis* and *Bacillus aerius* respectively (Figure 4).



**Figure 3.** Electricity generation by individual isolates in single chamber

**Table 1.** Biochemical Characteristics of the isolates

Isolates Id	Gram staining	Indole test	Motility test	Citrate test	Urease test	Gelatin hydrolysis test	Starch hydrolysis test	H <sub>2</sub> S	Catalase test	MR test	VP test	Oxidase test
TWWS-5	-	-	-	-	-	-	C/+	-	-	+	+	+
TWWS-6	-	-	+	+	-	-	C/+	-	-	+	+	-
TWWS-7	+	+	+	-	-	+	C/+	-	+	+	-	+
TWWS-8	+	-	-	+	-	+	C/+	-	+	-	-	+
TWWS-14	+	+	-	-	-	+	D/-	-	+	+	-	+
TWWS-15	+	-	-	+	-	+	C/+	-	+	+	+	+



**Figure 4:** Phylogenetic Tree Analysis for TWWS-14 and TWWS-15

## Discussion

Microbial fuel cells are the promising source of power supplies with high longevity and the capability of using complex biofuels formed from biological wastes. The development field of microbial fuel cell for practical applications is still in its infancy, though there are huge opportunities for further improvement. The focus of this study was to identify the potential electrogenic bacteria isolated from wastewater using both single and double chambered microbial fuel cells. To study the power generation and current output using tannery wastewater, a basic double chambered MFC was designed and operated. The values of voltage output were higher compared to previous study of mixed culture MFC<sup>21</sup>.

Power generation during the operation of MFC with tannery and hospital wastewater was successfully demonstrated in this study. This experiment showed that MFC with tannery wastewater generated higher voltage than MFC with hospital wastewater. This indicated that microbes present in the anode chamber of MFC with tannery wastewater effectively degraded the organic matter present in tannery wastewater and produced more electrons compared to hospital wastewater<sup>18</sup>. The aim of this study was to isolate and identify most potential electrogenic bacteria from wastewater. Studies showed that the organisms that have been observed in details, among them *Bacillus licheniformis* and *Geobacter sulfurreducens* produce electricity above 1V per day<sup>22</sup>. In this study, we have identified two potential isolates as *Bacillus stratosphericus* and *Bacillus aerius* using both biochemical and

morphological tests which were further confirmed by 16S rRNA sequence analysis. The two bacteria produced 1.21V and 1.15V respectively which are relevant with another study<sup>23</sup>. So, the present study also support that application of MFC to generate electricity from organic liquid wastes. Indeed, an important limitation of this technology is low power output and therefore, a lot of further work is needed to make this technology feasible and widely acceptable.

## Conclusions

Microbial fuel cell technology offers a new technique which would be used in power generation as well as wastewater treatment in future. In the present study, the generation of voltage value is very significant with the identified isolates which can be used as a sustainable energy resource in MFC technology in the near future.

Accession number(s): The 16S rRNA sequences of the isolates TWWS-14 and TWWS-15 have been deposited in GenBank under the accession number MN148700 and MK757700 respectively.

## Acknowledgement

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## Conflict of interest

Authors have declared that they have no competing interest.

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