Biotechnology Research and Innovation (2019) 3, 144–158

Biotechnology
Research & Innovation

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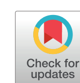
RESEARCH PAPER

Microbial fuel cells potential of marine actinobacteria *Actinoalloteichus* sp. MHA15 from the Havelock island of the Andamans, India



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Received 2 November 2017; accepted 22 January 2019
Available online 4 March 2019

KEYWORDS
Actinobacteria;
Actinoalloteichus sp.;
Bioelectricity;
Cellulase;
Havelock island;
Microbial fuel cells

Abstract In the recent past, use of fossil fuel is on the rise and has triggered global energy crisis. So, renewable bioenergy is viewed as one of the means to tackle this problem. In this context, interest in microbial fuel cells (MFCs) is increasing and gaining popularity due to their ability to convert the organic wastes into renewable energy. Present investigation is on the bioelectricity production by a marine actinobacterium isolated from the Havelock island of the Andamans. Actinobacterial colonies were isolated from the sediment samples, using Kuster's agar. 19 morphologically distinct strains were subjected to cellulase enzyme screening. Among them, higher cellulose degradation capacity was found in the strain MHA15. This potential strain was selected and identified as a species close to *Actinoalloteichus cyanogriseus*. The strain was subjected to bioelectricity generation using sugarcane bagasse as substrate and was evaluated in a dual chambered microbial fuel cell. In the MFC, initial voltage output started at 160 mV and it gradually increased, reaching a maximum of 257 mV at the 3rd hour in the actinobacterial incubated bagasse solution. From the MFC analyzed sludge bagasse, microbial diversity was searched using different media and only actinobacterial colonies were observed. Conventional taxonomic characters of the isolates were identical to the potential actinobacterial strain MHA15 which produced bioelectricity from bagasse. Marine actinobacteria, with their unique nature, differ very much in many aspects from their terrestrial counterparts and are known to produce diverse spectra of novel and useful substances and excellent bioactivity. Results of the present study have ascertained that the marine actinobacterial strain *Actinoalloteichus* sp. MHA15 is capable of generating bioelectricity and there is much scope for utilizing such marine actinobacteria for large scale production of bioelectricity, after further in-depth studies.

Introduction

Use of fossil fuels, especially oil and gas, for all human needs, has increased in recent years and this has triggered

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https://doi.org/10.1016/j.biori.2019.01.003
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the global energy crisis. In this context, renewable bioenergy is viewed as one of the means to decrease the current global warming effects. It is well known that fuels, such as ethanol, butanol, methane and hydrogen can be produced by microbes. But the microbial electricity production, known as microbial fuel cells (MFCs), is fairly a recent energy biology development (Lovley, 2006a, 2006b). MFCs have greatly attracted the attention of people in the recent past, as a sustainable technology for simultaneous bioelectricity generation and waste material treatment (Kim, Chae, Choi, & Verstraete, 2008). Numerous investigators have suggested that the microbial production of electricity may become an important form of bioenergy because MFCs offer the possibility of producing current from a wide range of complex organic wastes and renewable biomass (Lovley, 2008).

MFCs are devices which convert organic matter into energy (electricity), using microorganisms as catalysts. Generally, bacteria are used in MFCs to generate electricity while accomplishing the biodegradation of organic matters or wastes (Park & Zeikus, 2000; Venkata Mohan, Mohanakrishna, & Sarma, 2010). Primary mechanism of electrochemical active microorganisms, which are the key biocatalysts involved in the electricity generation in MFCs, transfer the electrons to the electrodes (Schroder, 2007). Use of the microorganisms in biological fuel cells eliminates the isolation of individual enzymes, thereby providing with cheaper substrates for biological fuel cells (Schroder, Niessen, & Scholz, 2003). MFCs offer a new source of electricity from wastes and other carbohydrate sources (Scott & Murano, 2007) and particularly, cellulose can serve as a fuel source (Mathis et al., 2008). MFC production process is eco-friendly (Muralidharan, Ajay Babu, Nirmalraman, & Ramya, 2011) and it clearly offers environmental benefits (Davis & Higson, 2007), with other opportunities like recovery of heavy metals from metal containing effluents (Mathuriya & Yakhmi, 2014), wastewater treatments (Li & Yu, 2014), hydrogen production (Call & Logan, 2008; Sharma & Li, 2010) and byproducts of energy-rich commercially valuable chemicals *i.e*. CH_4, H_2O_2, acetate and ethanol (Liu & Cheng, 2014), *etc*.

In addition, enormous amounts of agriculture, industrial and municipal cellulosic wastes are accumulating or used inefficiently due to the high cost of their utilization processes (Lee et al., 2008). Therefore, it has become a considerable economic interest to develop methods for effective treatment and utilization of cellulosic wastes as inexpensive carbon sources. One of the largest cellulosic agro-industrial by-products is sugarcane bagasse (or, 'bagasse' as it is generally called), a fibrous residue of cane stalks, left over after the crushing and extraction of the juice. It is a ligno-cellulosic residue (by-product) of the sugar industry and is almost completely used by the sugar factories themselves as fuel for the boilers. In recent years, there has been an increasing trend towards more efficient utilization of agro-industrial residues, including sugarcane bagasse for biofuel generation, pulp and paper production, and products based on fermentation (Pandey et al., 2000).

Since civilization, use of microorganisms to produce natural products and processes that benefit and improve our socio-economic lifestyles has been a part of our human history. They are the easiest manipulated sources of value added products like drugs, therapeutic proteins, vaccines, diagnostics and others. In this context, the term microbial bioprospecting refers to the search of microorganisms for biological products or the utilization of microbial cells as a whole for human benefits and environmental applications. In general, microbial bioprospecting starts from the collection of environmental samples to the identification and application of specific bioproducts (Mohanraj, Bharathi, Radhakrishnan, & Balagurunathan, 2011). Particularly, actinobacteria are the promising group of eubacteria in terms of biodiversity and bioactive metabolite production.

Actinobacteria have the capacity to withstand relatively low pH, high temperature, high sugar, salt and ethanol concentrations, as well as various other harsh conditions and they could be used to develop advanced biocatalysts and improve the commercial competitiveness of fuel production. In addition, actinobacteria produce a wide range of interesting enzymes which allow the degradation of complex glycans, such as cellulose and hemicelluloses (Hardter, Luzhetska, Ebeling, & Bechthold, 2012; Gobalakrishnan & Sivakumar, 2017). Because of the above potential characters of the actinobacteria, they are gaining great popularity and scientific importance, as energy sources (Lin & Tanaka, 2005). Hence, the present study was carried out at to generate bioelectricity from the waste cellulose substrate using a marine actinobacterium through microbial fuel cells (MFCs) assay and optimization of bioelectricity production, along with the confirmation of the identity of the potential strain.

Material and methods

Collection of samples and isolation of actinobacterial strains

Sediment samples were collected during the month of November 2011 at a depth of 25 cm from six locations of the Havelock island of the Andamans using a sterile spatula. The samples were placed in sterile polythene covers and brought to the field laboratory immediately and after arrival, necessary dilutions were made to carry out further microbiological analysis.

Isolation of actinobacteria was done in Kuster's agar medium. The autoclaved Kuster's agar medium containing petriplates were prepared aseptically. To minimize the fungal and bacterial contaminations, Kuster's agar medium was supplemented with cycloheximide (10 μg/ml) and nalidixic acid (10 μg/ml) respectively (Lee et al., 2014). One gram of pretreated sediment samples were serially diluted using sterile seawater and 0.1 ml of serially diluted samples were added to the petriplates containing Kuster's agar medium (Kuster & Williams, 1964) and spread using an 'L' shaped glass spreader. The plates were incubated at 37 °C for seven days in an inverted position. Leathery colonies of actinobacteria that appeared on the petriplates were counted from the 5th day onwards upto 28th day. After the incubation period, morphologically distinct colonies were picked up from the petriplates and restraecked in appropriate media and pure cultures were obtained and the slants were maintained at 4 °C for future studies.

Enzyme screening and preparation of crude enzyme, cellulase

Morphologically distinct actinobacterial strains MHA1to MHA19 were analyzed by the spot inoculation method using two different enzymatic agar media such as Cellulose Congo-Red agar medium and Carboxy Methyl Cellulose agar medium for the preliminary screening for cellulase enzyme activity (Gobalakrishnan et al., 2016). Ratio of the clear zone diameter to colony diameter was measured in order to select the highest cellulase activity producer.

A loopful of culture of the strain was inoculated in the carboxy methyl cellulose (CMC) medium (100 ml) in Erlenmeyer flasks (250 ml) in triplicate. Flasks were incubated at 37 °C for 5–7 days in a shaker incubator at 150 rpm. Part of samples (5 ml) was withdrawn from each flask after 7 days of fermentation and centrifuged at 2000 rpm for 10 min. The clear supernatant broth was collected aseptically to determine the enzyme yield (Gobalakrishnan & Sivakumar, 2017).

Taxonomical investigation of potential actinobacteria

Taxonomical investigation methods were adapted by Gobalakrishnan and Sivakumar (Gobalakrishnan & Sivakumar, 2017). Characterization and subsequent identification of the strains to genus level were made based on the criteria of Cummins and Harris (Cummins & Harris, 1958), Shirling and Gottlieb (Shirling & Gottlieb, 1966), Lechevalier and Lechevalier (Lechevalier & Lechevalier, 1970) and Nonomura (Nonomura, 1974) who have stated that chemical composition of the cell wall might furnish practical methods of differentiating various types of actinobacteria. Along with cultural characteristics, melanoid pigments, reverse side pigments, soluble pigments, spore chain morphology and assimilation of carbon source were studied using standard methodology, recommended by the International *Streptomyces* Project (Shirling & Gottlieb, 1966).

Species level identification of the potential strain was made based on the keys, using the Bergey's Manual of Determinative Bacteriology. Status of the strain was also determined based on its molecular characters (16S rDNA gene sequences), with the following methodology.

Genomic DNA isolation and amplification of 16S rDNA

Total genomic DNA of the strain MHA15 was extracted from the actinobacterial broth by phenol–chloroform isoamyl alcohol method, which removes the protein and the cellular components from the nucleic acid to obtain the pure DNA (Ahmed, Asghar, & Elhassan, 2014). DNA sample (10 μl) was mixed with 2 μl of loading (6X) dye and loaded in 1% agarose gel. The separated DNA was visualized by UV transilluminator.

Each 50 μl amplification reaction contained 1 μl template DNA (50–200 ng), 5 μl 10× PCR buffer, 1 μl both forward [27F (5′-AGAGTTTGATCMTGGCTCAG-3′)] and reverse [1492R (5′-GGYTACCTTGTTACGACTT-3′)] primers, 1 μl dNTP mix (10 mM), 6 μl $MgCl_2$ (25 mM), 2.5 U Taq DNA polymerase, 2.5 μl DMSO and 31.5 μl sterile water. The reaction conditions were initial denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 90 s. A final extension was performed at 72 °C for 10 min (Karuppiah, Aarthi, & Sivakumar, 2011). Reaction products were electrophoresed on a 1% agarose gel with 2 μl ethidium bromide and visualized under UV transilluminator and then purified.

16S rDNA sequencing and phylogenetic analysis

The purified fragment was directly sequenced using a Ampli Tag FS DNA sequencing Kit (Applied Biosystem). The data were analyzed using applied Biosystem DNA editing and assembly software and sequences comparisons were obtained using the Micro Seq Software.

Sequence similarity search was made for the 16S rDNA sequence of MHA15 by applying its sequence to BLAST search of the NCBI (National Centre for Biotechnological Information, USA). Final editing of the sequence alignment was done using BioEdit (Hall, 1999) and phylogenetic analysis was performed with version 5 of the MEGA (Molecular Evolutionary Genetics Analysis) software package (Tamura et al., 2011). A phylogenetic tree was constructed, using the Neighbour-joining tree-making algorithm (Saitou & Nei, 1987). Topology of the phylogenetic tree was evaluated, using the bootstrap resampling method of Felsenstein (Felsenstein, 1985) with 1000 replicates.

Collection of cellulosic substrate and pretreatment

Sugar cane bagasse was collected in sterile polythene bags from the local fruit juice shop, Chidambaram, Cuddalore District, Tamilnadu, India. Collected materials were kept in an ice box and transported to the laboratory. In order to minimize losses of cellulose and increase the extraction of hemicellulose and lignin, a kinetic study was performed to determine the best parameters for this step. Bagasse (15 g) and H_2SO_4 (10% v/v) were placed, under a consistency of 10% (w/v), in a polypropylene beaker with a capacity of 1 L. Reaction was carried out in a thermal bath with heating ramp for 40 min and when the temperature reached 100 °C, time counting was started as suggested by Candido et al. (Candido, Godoy, & Goncalves, 2012). At the end of the reaction time, the material was washed with distilled water until it reached neutral pH. The substrate was sun dried to reduce the moisture content and get the powder form. The powdered material was autoclaved (121 °C at 15 min). This pretreated substrate was used for the MFC process.

Preparation of actinobacterial inoculum and anolyte substrate

A loopful culture of the strain MHA15 was incubated into carboxyl methyl cellulose (CMC) medium (100 ml) in Erlenmeyer flasks (250 ml) in triplicate. Flasks were incubated at 37 °C for 5–7 days in a shaker incubator at 150 rpm; 100 ml of incubated CMC medium was dissolved in 1.9 L of seawater with pretreated bagasse (20 g) feed, as an anolyte substrate, which was incubated at 37 °C for 7 days in the shaker incubator at 150 rpm.

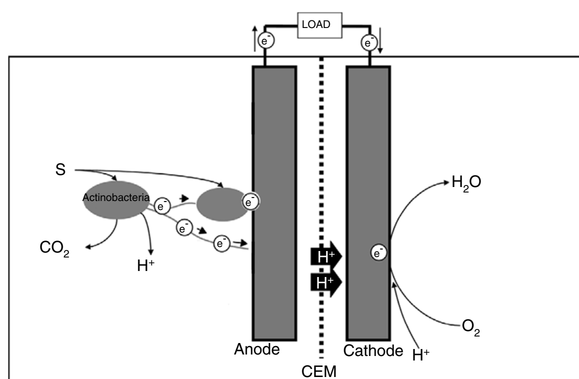

Figure 1 Conceptual diagram of a microbial fuel cell (Kim et al., 2008)

Table 1 Design criteria of duel chambered mediatorless MFCs.

Reactor configuration	Dual chamber
Anode chamber	Suspended growth
Anode inoculum	MHA15 actinobacterium strain
Mediator anode	Nil
Mediator cathode	Air
Volume of anode and cathode	5 cm
Anode and cathode material	Graphite rod
Surface of electrode (sq. cm)	17.5
Proton exchange membrane	Dialysis membrane
Operating temperature	37 °C
Operating pH	7

MFC configuration and operation

Essential physical components of MFCs are the anode, cathode and cation exchange membrane (CEM) and the conceptual diagram of the MFCs is shown in Fig. 1 and the design criteria of dual chambered mediatorless MFCs are shown in Table 1. After 7 days of incubation of the actinobacterial stain MHA15, bagasse solution was transferred to the duel-chambered MFC, designed in the laboratory using glass bottles (2.5 L, Himedia Pvt Lt.). Total volume of both the cathode and anode compartments was the same (5 cm) and each camber was provided with sample port, and wire point inputs (top). Pretreated proton exchange membrane (dialysis membrane-50, Himedia Pvt Lt.) was used to separate the two chambers. Contact between the electrodes and copper wires was sealed with soldering material. Prior to use, the electrodes were soaked in deionized water for a period of 24 h.

Cathode chamber of the MFC was filled with aerated deionized water (2 L) as catholyte. The anodic chamber prior to startup was filled with the anolyte substrate (2 L). No mediator was used in the anodic chamber of the MFC. Continuous monitoring of voltage output was done for five hours in both the control (without the actinobacterial strain) and treated bagasse (with actinobacterial strain).

Analytical methods

Continuous monitoring of current and electric potential (*V*) measurement were recorded using a digital multimeter (C€– IEC 1010-1), which was the indicator of bioelectricity production. A volt can be stated in SI base units as $1\,V = 1\,kg\,m^2\,s^{-3}\,A^{-1}$.

Optimization of growth characteristics of the potential strain for better bioelectricity production

In the bioelectricity generating potential strain MHA15, its growth characteristics were optimized for better bioelectricity production and the parameters tested were: different levels of temperature (29 °C–50 °C), pH (6–8), DO ($0\,ml\,l^{-1}$–$4.5\,ml\,l^{-1}$), substrate concentration (10 g–30 g) and nutrient concentration (0%–100%).

Isolation of the actinobacteria from MFC analyzed sludge bagasse

Isolation of the actinobacteria from the MFC analyzed sludge bagasse material was carried out using different media *viz*. Kuster's agar medium (KUA), starch casein agar medium (SCA), actinomycetes isolation agar medium (AIA) and Yeast Malt extract agar medium (ISP2). Zobell marine agar medium (ZMA) was used for the isolation of bacteria, if present. All the above autoclaved media containing petriplates were prepared aseptically; 1 g (approximately) of the MFC analyzed sludge bagasse sample (from MFC anode chamber) was serially diluted using sterile seawater (50%) and 0.1 ml of serially diluted samples were added to the petriplates containing the respective isolation medium and spread using a 'L' shaped glass spreader. The plates were incubated at 37 °C for 24–48 h for bacteria and 7 days for actinobacteria. Similar test was carried out for the control sample.

Results

Isolation and cellulase enzyme screening of the actinobacteria

Actinobacterial colonies were isolated from the sediment samples of six stations of the Havelock island, using Kuster's agar medium and a total of 19 morphologically distinct actinobacterial strains (MHA1 to MHA 19) were obtained. All these strains were subjected to enzyme screening using two different media. In both the Cellulose Congo-Red agar medium (CCR) and carboxy methyl cellulose agar medium (CMC), higher cellulose degradation zone (maximum cellulose degradation characteristic), was found in MHA3, MHA10, MHA13 and MHA15 (Fig. 2).

Cellulase enzyme assay

Based on the performance, MHA3, MHA10, MHA13 and MHA15 were selected as potential cellulase enzyme producers and these strains were subjected to cellulase enzyme screening, using the DNS method and glucose was used as the standard. By comparing the glucose standard curve, cellulase

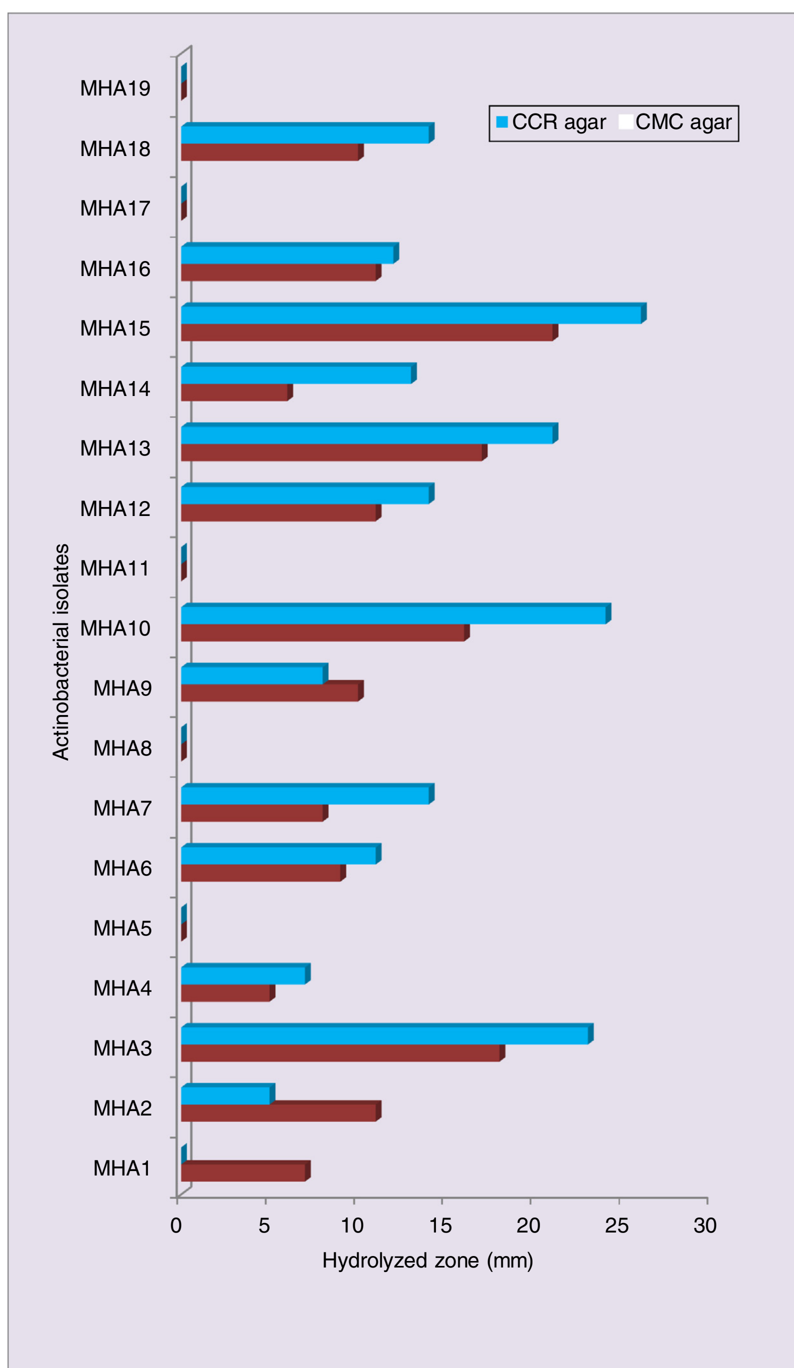

Figure 2 Screening for the cellulase enzyme activity in CCR agar and CMC agar media.

enzyme production of the selected actinobacterial strains was measured. Higher amount of cellulase enzyme production was found in MHA15 (14.379 U/ml), followed by MHA3 (14.213 U/ml), MHA13 (13.356 U/ml) and MHA10 (13.119 U/ml). Among them, MHA15 alone produced more quantity of cellulase enzyme. Therefore, this strain, MHA15 was chosen and subjected to conventional taxonomical investigation and bioelectricity generation study, for finding out its potential as microbial fuel cell (MFC).

Taxonomical investigation of the potential strain MHA15

Strain MHA15 is a mesophilic actinobacterium, which forms an extensively branched substrate mycelium and aerial hyphae that differentiate into long spore chains (Fig. 3a). Black with grey coloured aerial spores were formed in ISP2 agar (Fig. 3b). Reverse side and soluble pigments were produced on the Peptone yeast extract iron agar. Melanin

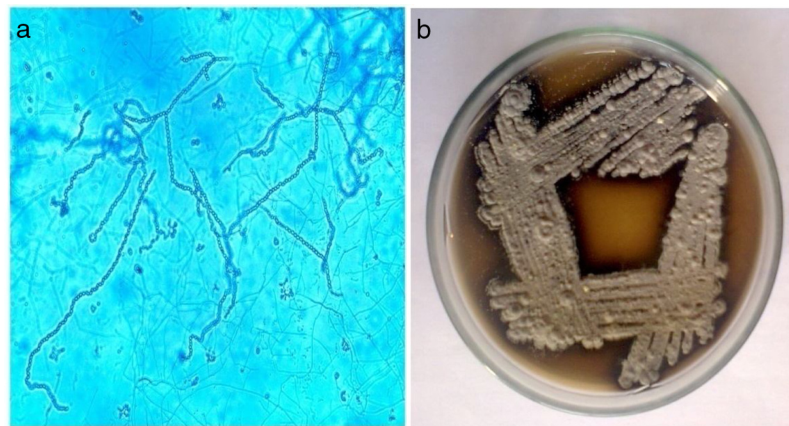

Figure 3 Morphological characters [long spore chain (a) & aerial mass colour (b)] of the strain MHA15.

Table 2 Comparative characteristics of the strain MHA15 with the *A. cyanogriseus*.

Characters studied	Strain MHA15	*A. cyanogriseus*
I. Cell wall amino acids		
LL-DAP	−	−
Meso- DAP	+	+
Glycine	−	−
II. Whole cell sugars		
Arabinose	−	−
Galactose	+	+
Rhamnose	+	+
Glucose	+	+
Mannose	+	+
III. Cell wall chemotype	III	III
IV. Characters studied (as perTamura et al., 2000)		
Colour of aerial mycelium	Blue-grey	Blue-grey
Melanoid pigment	Black	Black
Reverse side pigment	Black	Black
Soluble pigment	Black	Black
Spore chain	Long spore chain	Long spore chain
Carbon source assimilation		
Arabinose	+	±
Xylose	+	+
Inositol	+	−
Mannitol	+	+
Fructose	+	−
Rhamnose	+	+
Sucrose	+	±
Raffinose	−	−
V. Molecular characters		
Sequenced gene	16S rDNA	16S rDNA
NCBI Accession number	KF668663	NR_024650
No of bp.	873	1468
Similarity level with closest neighbour strain (%)	91.6	-

+, positive; −, negative; ±, weakly utilized.

pigment was also produced on ISP7 agar. The culture grew well when it was supplemented with the carbon sources *viz*. arabinose, xylose, inositol, mannitol, fructose, rhamnose and sucrose. No growth was observed in raffinose. In the strain MHA15, cell wall characteristics showed the presence of glucose, mannose, galactose and rhamnose as whole-cell sugars and meso-diaminopimilic acid as the amino acid in the cell wall and glycine was absent, indicating that this strain belongs to the cell wall chemo type III (Table 2).

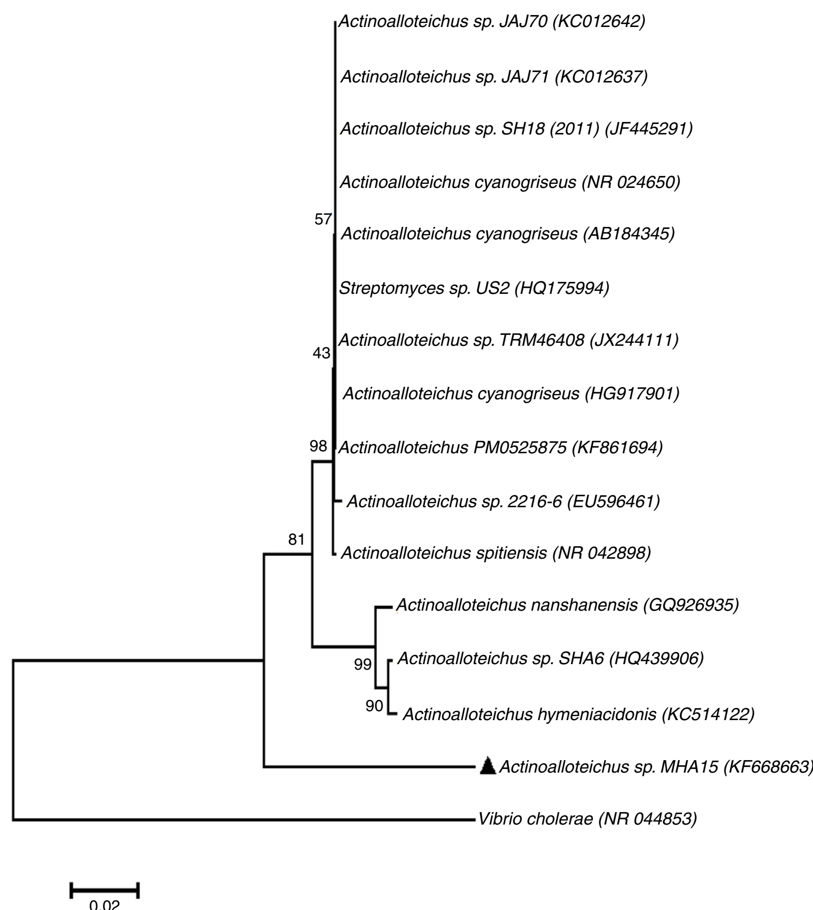

Figure 4 Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationship between the strain MHA15 and related and respective species of the genus *Actinoalloteichus*. Only bootstrap values (expressed as percentage of 1000 replications) are given at nodes.

Molecular identification of the strain MHA15

Further, for molecular identification, genomic DNA of the strain MHA15 was extracted and purified and genomic DNA's 16S rDNA gene was amplified using Universal primers and the amplified products were sequenced. A 873 bp of 16S rDNA sequences was determined for the strain MHA15, which was submitted to the GenBank (National Center for Biotechnology Information, USA) and the accession number (KF668663) was obtained. Based on the molecular data (16S rDNA sequences), phylogenetic tree was constructed on the comparison of 16S rRNA gene (873 bp) of the strain, MHA15 with the previously reported sequence of *Actinoalloteichus* species (accession number: NR_024650) deposited in the GenBank and the molecular data of the closest species were also obtained from the GenBank. *Vibrio cholerae* rooted phylogenetic tree (Fig. 4) indicated that the strain MHA15 is related to the members of the genus, *Actinoalloteichus* and the strain MHA15 forms a branch with *A. cyanogriseus* with 91.6% similarity.

Results of the cultural, cell wall chemotypical, morphological, physiological and molecular characters were compared between the strain MHA15 and its closest phylogenic member. The strain MHA15 showed no variation in any of the characters when compared to those of the reference species *A. cyanogriseus*. All the conventional characters were similar to those of *A. cyanogriseus*. But, the molecular characters showed distinct variation in the phylogenetic relationship (8.4%). If detailed polyphasic taxonomic analysis of the strain is made, it could be a new species.

Operation of MFCs assay

After a lag period of 7 days, first inoculation of actinobacterial MHA15 treated 2 L agitated bagasse solution was transferred to the duel-chambered MFC, designed using glass bottles. Performance of MFC with respect to voltage output during operations is depicted in Fig. 5. At the initial stage, voltage output was 160 mV and it gradually increased in the MFC assay, reaching a maximum of 257 mV at the 3rd hour in the actinobacterial incubated bagasse solution. In the untreated actinobacterial bagasse solution (control), the voltage was only 90 mV at the first hour and it gradually decreased to 63 mV at the 5th hour (Fig. 6).

Figure 5 Dual-chambered microbial fuel cell.

Optimization of growth characteristics of potential strain for better bioelectricity production

Temperature

During the performance of MFC, bioelectricity production was studied with different temperature *viz.* 29 °C, 33 °C, 37 °C, 41 °C and 45 °C. Voltage output during the operations is depicted in Fig. 7a. Higher level of voltage output (238 mV) was recorded at 37 °C at the 3rd hour, in the actinobacterial (strain MHA15) incubated bagasse substrate.

pH

During the performance of MFC, bioelectricity production was studied with different pH *viz.* 6, 6.5, 7, 7.5, and 8. Voltage output during the operations is shown in Fig. 7b. Higher level of voltage output (240 mV) was recorded at pH 6.5 at the 3rd hour, in the actinobacterial (strain MHA15) incubated bagasse substrate.

Dissolved oxygen

During the performance of MFC, bioelectricity production was studied with different DO levels *viz.* 0 ml/l, 1.5 ml/l, 2.5 ml/l, 3.5 ml/l and 4.5 ml/l. Voltage output during the operations is depicted in Fig. 7c. Higher level of voltage output (236 mV) was recorded at 4.5 ml/l at the 3rd hour, in the actinobacterial (strain MHA15) incubated bagasse substrate.

Substrate concentration

During the performance of MFC, bioelectricity production was studied with different substrate concentrations *viz.* 10 g, 15 g, 20 g, 25 g and 30 g. Voltage output during the operations is shown in Fig. 7d. Higher level of voltage output (244 mV) was recorded at the 25 g substrate concentration at the 3rd hour in the actinobacterial (strain MHA15) incubated bagasse substrate.

Nutrient concentrations

During the performance of MFC, bioelectricity production was studied with different nutrient concentrations *viz.* 0%, 25%, 50%, 75% and 100%. Voltage output during the operations is depicted in Fig. 7e. Higher level of voltage output (242 mV) was recorded at 100% nutrient concentration at the 3rd hour in the actinobacterial (strain MHA15) incubated bagasse substrate.

Testing the MFC analyzed sludge bagasse for confirming the presence of the strain MHA15 and its bioelectricity potential

In the present study, different media *viz.* Kuster's agar medium, Starch casein agar medium, Actinomycetes isolation agar medium and Yeast Malt extract agar medium were used for the isolation of actinobacteria and Zobell Marine Agar (ZMA) was used for the isolation of bacteria. After 24–48 h of incubation, no bacterial colonies were found in the ZMA medium (Fig. 8). After 7 days, identical actinobacterial colonies were observed in KUA, SCA, AIA and ISP2 and the population density was 14.5×10^3 CFU ml^{-1}, 10.7×10^3 CFU ml^{-1}, 9.9×10^3 CFU ml^{-1} and 4.9×10^3 CFU ml^{-1}, respectively; while, no microbial growth was found in the control and ZMA medium (Fig. 8).

Cell wall characteristics showed the presence of glucose, mannose, galactose and rhamnose as whole-cell sugars; and meso-diaminopimilic acid as the amino acid in the cell wall

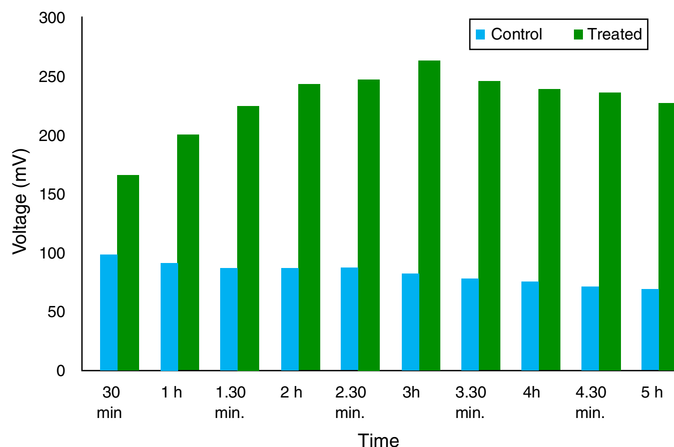

Figure 6 Variation in voltage and current generation during the operation of MFC.

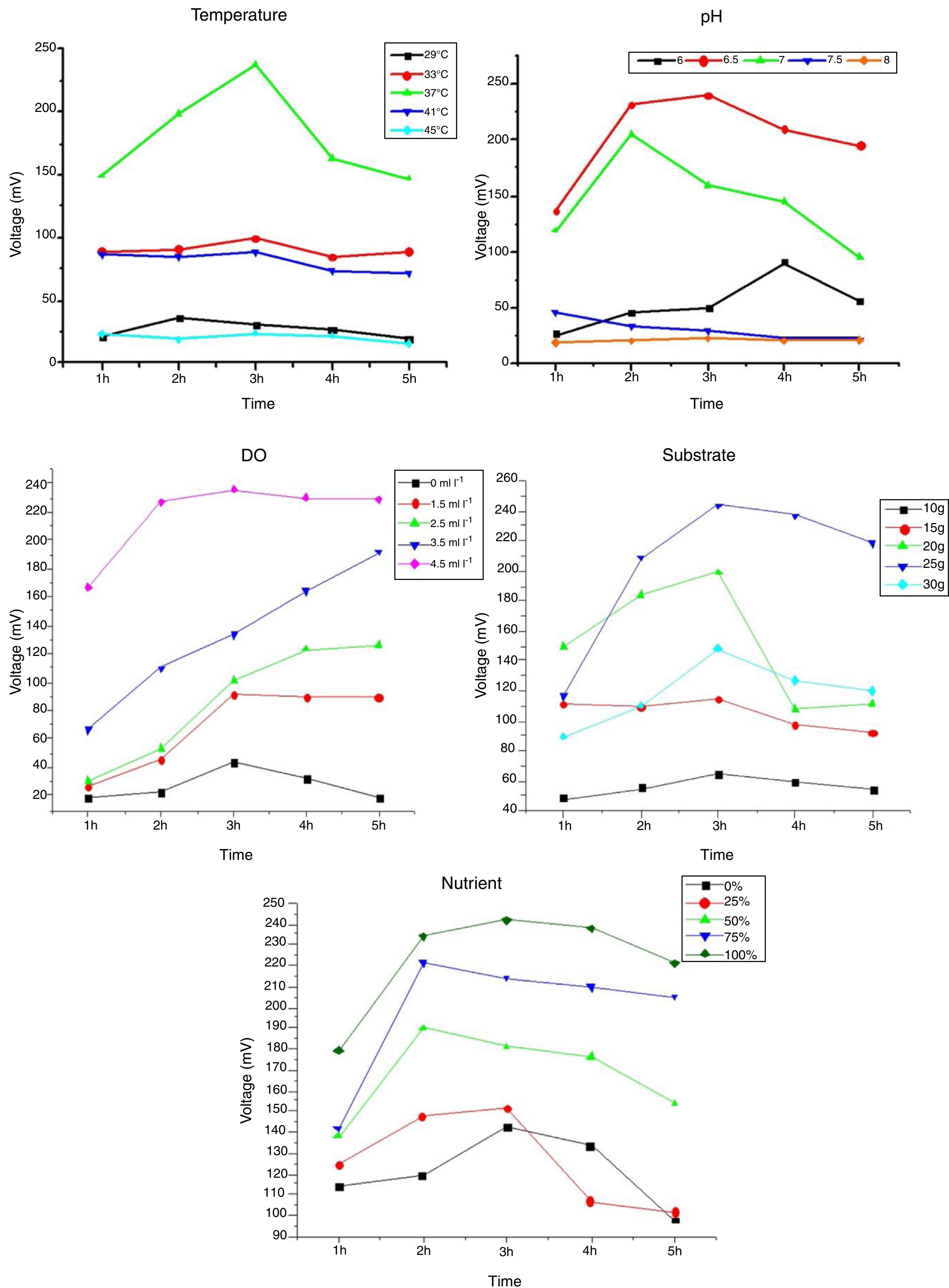

Figure 7 Effect of temperature, pH, DO, substrate and nutrient on the electricity generation during the operation of MFC.

and glycine was absent, indicating that this strain belongs to the cell wall chemo type III.

The strain obtained from the identical colony formed an extensively branched substrate mycelium, producing black with grey coloured aerial spores and aerial hyphae that differentiate into long spore chains, in ISP2 agar (Fig. 9). Reverse side and soluble pigments were produced on Peptone yeast extract iron agar. Melanin pigment was also

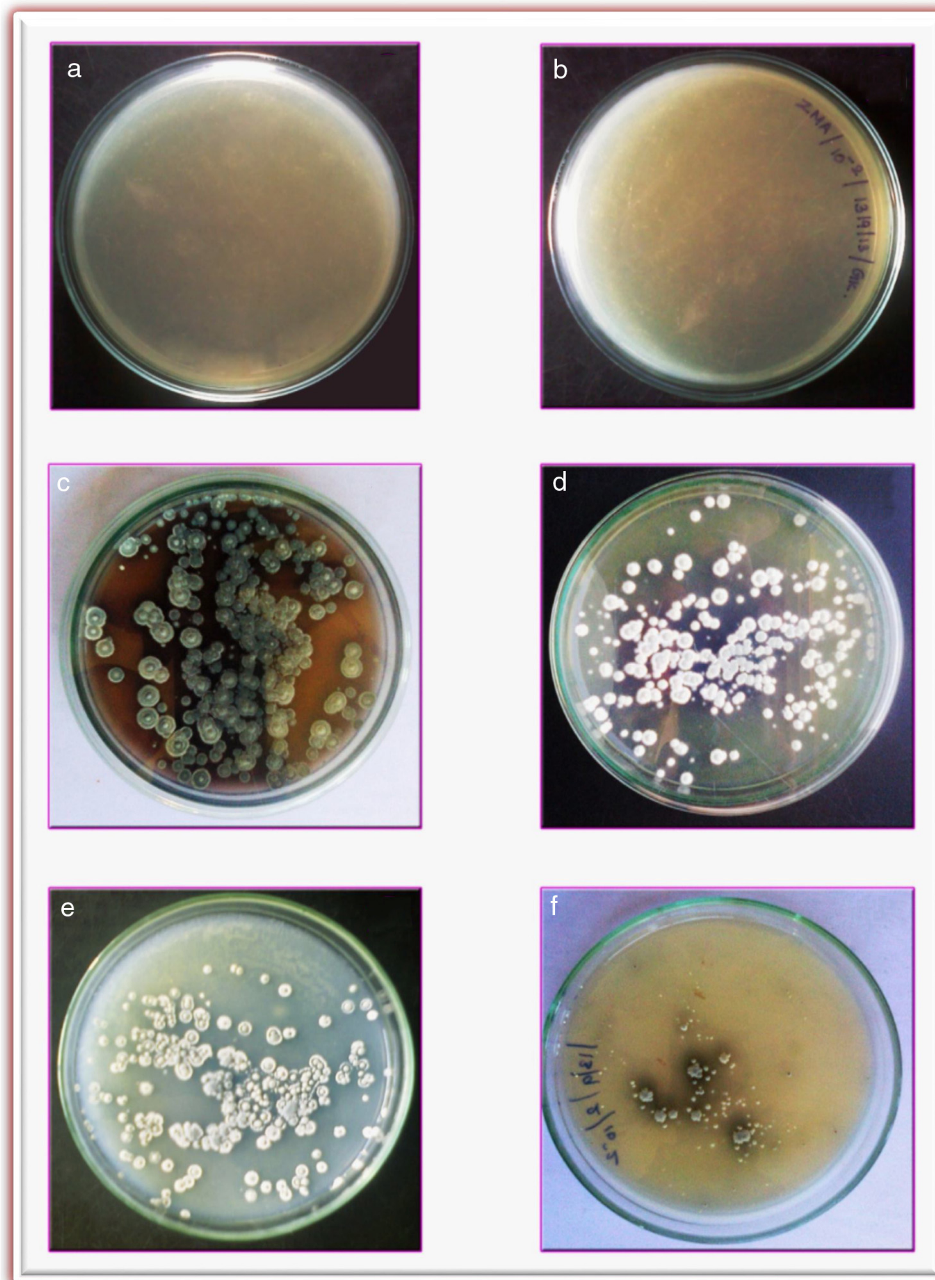

Figure 8 Identical colonies of the actinobacterial strain, MHA15 in different media (c, KUA; d, SCA; e, AIA; f, ISP2) except, (a) control and (b) ZMA.

produced on ISP7 agar. The culture grew well when it was supplemented with the carbon sources *viz*. arabinose, xylose, inositol, mannitol, fructose, rhamnose and sucrose. No growth was observed in raffinose.

Results of the cultural, cell wall chemotypical, morphological and physiological characters were the same as that of the actinobacterial strain, MHA15, without any variation in any of the characters (Table 3). Considering these, the isolated identical strain was confirmed as the strain MHA15, which alone has contributed to bioelectricity production, in the present study, and not any other microbe.

Discussion

Microbial fuel cells (MFCs) convert biodegradable materials into electricity, potentially contributing to an array of renewable energy production strategies, tailored for specific applications. Cellulose is the main renewable energy source and so, formation and microbial degradation of cellulose represent the major energy flux in our biosphere (Niessen, Schroder, Harnisch, & Scholz, 2005). Therefore, present study was carried out, to generate electricity from cellulose (bagasse), employing a marine actinobacterium. Since there are no known microorganisms that can both

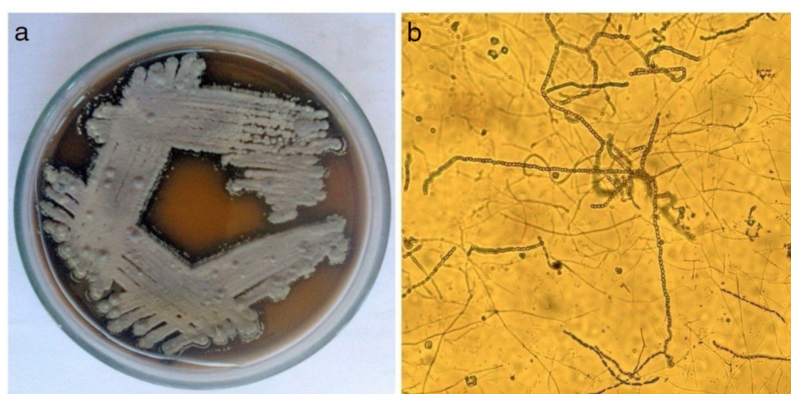

Figure 9 Morphological characters [aerial mass colour (a) and long spore chain (b)] of the isolated strain in the MFC analyzed sludge.

Table 3 Conventionally identified characteristics between the strain MHA15 and isolated strain from MFCs analyzed sludge bagasse.

Characters studied	Isolated strain	Strain MHA15
I. Cell wall amino acids		
LL-DAP	−	−
Meso-DAP	+	+
Glycine	−	−
II. Whole cell sugars		
Arabinose	−	−
Galactose	+	+
Rhamnose	+	+
Glucose	+	+
Mannose	+	+
III. Cell wall chemotype	III	III
IV. Characters studied		
Colour of aerial mycelium	Blue-grey	Blue-grey
Melanoid pigment	Black	Black
Reverse side pigment	Black	Black
Soluble pigment	Black	Black
Spore chain	Long spore chain	Long spore chain
Carbon source assimilation		
Arabinose	+	+
Xylose	+	+
Inositol	+	+
Mannitol	+	+
Fructose	+	+
Rhamnose	+	+
Sucrose	+	+
Raffinose	−	−

+, positive; −, negative.

metabolize cellulose and transfer electrons to solid extracellular substrates, conversion of cellulosic biomass to electricity requires a syntrophic microbial community that uses an insoluble electron donor (cellulose) and electron acceptor (anode) (Ren, Ward, & Regan, 2007). Present study has shown that electric current can be generated in MFCs from microbial cellulose degradation by the utilization of insoluble macromolecular biomass (bagasse) components. Previously, conversion of cellulose to electricity in MFCs required mixed cultures or separate microorganisms to hydrolyze cellulose and generate electricity (Rezaei et al., 2009) but, the present study has proved that the strain MHA15 could be used as the sole microorganism to accomplish both cellulose degradation and electricity generation.

Actinobacteria play an important role in the biodegradation processes during the biological treatment. They have

the advantage over the other microbes to adhere to water immiscible interfaces due to the hydrophobicity of their cell wall and are able to degrade the complex organic materials (Lemmer & Baumann, 1988; Soddell & Seviour, 1998). In addition, microbes capable of complete oxidation of organic compounds can be the most significant direct contributors to power production (Lovley, 2008). In the present study, the actinobacterial strain MHA15 has degraded the bagasse material through the cellulase enzyme and generated electricity. This is possible because actinobacteria can produce cellulolytic enzymes, regulated by CleR (Anderson & Wellington, 2001). Very recently, Gobalakrishnan and Sivakumar (Gobalakrishnan & Sivakumar, 2017) reported that the strain MHA15 was identified and the molecular characters showed in 8.4% of distinct phylogenetic relationship with the species *A. cyanogriseus* using systematic characterization approach for their potential of cellulolytic nature.

Many reports are available on the bioelectricity, generated by many bacterial organisms with different waste materials as substrates. Substrate is important for any biological process as it serves as a carbon (nutrient) and energy source. The efficiency and economic viability of converting organic wastes to bioenergy would depend on the characteristics and components of the waste materials (Deepak, Bogaert, Diels, & Vanbroekhoven, 2010). A great variety of substrates can be used in MFCs for electricity production ranging from pure compounds to complex mixtures. In the present study, sugarcane bagasse was used as the substrate.

Present study achieved a maximum electric potential (237 mV) from the bagasse substrate at 10 g/l. Rezaei, Richard, and Logan (2008) reported that the maximum power density ($100 \pm 7\,mW/m^2$) was measured when cellulose was added to MFC with cellulase. Similarly, Nieessen et al. (Niessen et al., 2005) achieved current densities of $130\,mA\,l^{-1}$ from 3 g of cellulose substrate. Ren et al. (2007) reported that the co-culture of microorganisms (*Clostridium cellulolyticum* and *Geobacter sulfurreducens*) generated a maximum power density of $143\,mW/m^2$. Rismani-Yazdi et al. (2007) have reported power density up to $55\,mW/m^2$ using cellulose as a substrate and cattle rumen microorganisms as the catalyst. Sethubathi (2011) has stated that the bioelectricity can be harvested from the industrial effluents using the marine actinobacterium (*Streptomyces albidoflavus*). He observed that during the dual-chamber MFC performance, voltage and current output reached a maximum of 524 mV at the 4th hour. Very recently, Sarranyadhevi, Shanmugasundaram, Thirumalairaj, and Balagurunathan (2014) have reported that the actinobacterium, *Streptomyces silaceus* (KBA8) has generated nearly 1.5 mV of current at the end of 5th day reaction from the waste water and waste product rich in ions. But, Nevin et al. (2008) reported that *G. sulfurreducens* grown on acetate produced $2.15\,kW/m^3$ anode volume, which is the highest MFC power density reported to date. It could be due to the fact that acetate is a simple substrate and this is the end product of several metabolic pathways for higher order carbon sources (Biffinger, Byrd, Dudley, & Ringeisen, 2008). So, it is extensively used as the carbon source to induce electroactive microbes.

Performance of a MFC is affected by a variety of factors, such as reaction configuration, substrate and various operational parameters, such as pH and temperature (Kim et al., 2008) and other nutrient sources. Hence, the present study was carried out with the bioelectricity generating strain MHA15 and its growth characteristics were optimized for better bioelectricity production and the parameters tested were different levels of temperature, pH, DO, substrate concentration and nutrient concentration.

Temprature plays an important role in the cell growth as well as electricity production. In the present study, higher level of electricity production was recorded at 37 °C. Larrosa-Guerrero et al. (2010) reported the effect of temperature on the performance of MFCs; maximum power density was $174.0\,mW\,m^{-3}$at 35 °C. In addition, microbial cellulase production was observed in the temperature optima of about 35 °C–45 °C (Dutta et al., 2008). Rathnan and Ambili (2011) reported that the optimal temperature for the cellulase enzyme production was 30 °C–45 °C. Kumar et al. (2013) reported that the strain RK6 showed maximum cellulolytic activity and growth at 38 °C and Alam, Manchur, and Anwar (2004) have observed maximum cellulase activity at 37 °C in *Streptomyces omiyaensis*. This higher cellulolytic enzyme production could be the reason for a higher degree of organic degradation, which may lead to more electricity generation. In addition, MFCs cannot operate at extremely low temperatures due to the fact that microbial reactions are slow at low temperature (Rahimnejad, Adhami, Darvari, Zirepour, & Oh, 2015).

Microbial groups involved in the degradation of organic matter have a specific pH optimum and could grow in specific pH range. Optimum range for all the methanotrophic bacteria is between 6 and 8, whereas anaerobic bacteria are notably less sensitive to pH variations (Bailey & Ollis, 1986; Haandel & Lettanga, 1994). In the present study, pH 6.5 showed higher electricity production on both electrodes. Similarly, Jadhav and Ghangrekar (2009) reported that highest current was generated at pH of 6.5 in the anodic chamber with CE of 4% and higher pH difference between the electrolytes favoured higher current and voltage production. Raghavulu, Venkata Mohan, Venkateswar, Mohanakrishna, and Sarma (2009) demonstrated the effectiveness of acidophilic operation of MFC anodic chamber on bioelectricity generation compared to neutral and alkaline operations. A decrease in pH in the anodic chamber due to the proton accumulation during the substrate degradation and a corresponding increase in pH were observed in the cathode chamber of the prolonged-run MFCs (Chae, Choi, Ajayi, & Park, 2008; Rozendal, Hamelers, & Buisman, 2006).

DO level would affect the performance of MFCs and it is the major limiting factor when oxygen is unsaturated. Oxygen is the most favourable final electron acceptor in MFCs for the cathodic reaction and power output strongly depends on the concentration level of electron acceptors (Kim et al., 2008). In the present study, the microbial cell was operated with aeration in the cathode compartment; when DO was increased, voltage output also increased sharply and when DO was low, the voltage output was also reduced. These results show that DO is an important limiting factor for the operation of microbial fuel cell, with graphite electrode.

In MFCs, substrate is regarded as one of the most important biological factors, affecting electricity generation (Liu, Liu, Zhang, & Su, 2009). One way to improve the power generation is to use enzymes to increase the hydrolysis rate of cellulose (Rezaei et al., 2008). In the present study,

maximum electric potential (244 mV) was observed in 25 g of bagasse in 2 L.

The optimal levels of the growth parameters observed in the present study for the production of bioelectricity, with respect to the potential actinobacterial strain MHA15 are: Temperature 37 °C, pH 6.5, DO 4.5 ml/l, Substrate 25 g and Nutrient solution 100%.

It is worth mentioning that in the past years, various ideas and approaches have been reported about the application of MFCs but information is scattered (Li & Yu, 2014) and various review articles have been published on the MFCs configuration, substrates, electrode materials, separators and microbiology (Kim et al., 2008; Pant et al., 2010; Reddy, Pradeep Kumar, & Wee, 2010; Zhou, Chi, Luo, He, & Jin, 2011). Very recently, Rahimnejad et al. (2015) have presented a detailed review on the MFC as new technology for bioelectricity generation and Logan et al. (Logan et al., 2015) have also given a review on the assessment of MFC configuration and power density. Similarly, Sivakumar et al. (Sivakumar et al., 2015) have given a detailed account of bioelectricity production by marine bacteria, actinobacteria and fungi. Mustakeem (Mustakeem, 2015) and Ramachandran et al. (Ramachandran, Chen, & Gnanakumar, 2015) have reviewed the electrode materials for microbial fuel cells in nanomaterial and fabricated materials. In this context, present study is significant as it would pave way to the willing researchers to pursue bioelectricity production from waste materials, using marine actinobacteria.

Conclusion

Currently, MFCs have become an interesting and promising area of research and applications of MFCs will help reduce the use of fossil fuels and allow for energy gain from wastes. In this perspective, results obtained in the present investigation have demonstrated that the marine actinobacterium, strain MHA15, is capable of generating bioelectricity (257 mV) under optimal culture conditions (growth parameters) and this can be scaled-up. There is much scope for utilizing such of those marine actinobacteria for large scale production of bioelectricity, after further in-depth studies.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

Authors thank the Dean, Faculty of Marine Sciences and the authorities of Annamalai University and one of the authors (R.G.) thank the Principle, District Institute of Education and Training, Pudukkottai, for providing with necessary facilities. They also thank Dr. K. Sivakumar, Assistant Professor, CAS in Marine Biology, Annamalai University and Prof. L. Kannan, Former Vice Chancellor, Thiruvalluvar University, Vellore, for their guidance and editorial support. The contents and views reported in this manuscript are of individual authors and not reflect the views and/or positions of the institutions they belong.

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