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Electrogenicity assessment of *Bacillus subtilis* and *Bacillus megaterium* using Microbial Fuel Cell technology

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Abstract
Microbial fuel cell technology is a new type of renewable and sustainable method for production of electric energy from the microbial breakdown of organic matter. The present study focuses on the isolation of *Bacillus subtilis* and *Bacillus megaterium* from soil sample and also aimed to construct the dual chambered mediator less microbial fuel cell for appraise the electricity producing ability of isolated *Bacillus* sp. Minimal media containing 5% and 10% glucose as carbon source used as substrate for bioelectricity production. The maximum current of 691 mV was produced from 10% of glucose as carbon source by *Bacillus subtilis* followed by *Bacillus megaterium* with power production of 651 mV.

Keywords: Bio-electricity, MFC, electrogenicity and *Bacillus* sp.

1. Introduction
The use of fossil fuels, especially oil, coal and gas recently increased and this triggers a global energy crisis in recent years. Future economic growth crucially depends on the availability of fossil fuels for wide range of energy consumption. Utilization of fossil fuels resulted in increasing environmental problems like emission of green house gases which leads to global warming. New and alternative method of electricity production from renewable resources without environmental problems is much desired to save our environment.

A Microbial Fuel Cell (MFC) is a device that converts chemical energy from bio-convertible organic substrate, directly into electrical energy through the metabolic activity of microorganisms. A simple MFC setup contains two chambers respectively anode and cathode separated by Proton Exchange Membrane (PEM). The microorganisms are inoculated in anodic chamber they oxidize the substrate and generate protons and electrons. Through the external circuit the electrons are transferred from anode to cathode, the protons pass through the proton exchange membrane to cathode, where the proton meets the oxygen and electrons to form water [17].

Mechanism of electron transformation from bacterial cell to anode is known three ways, firstly, using exogenous mediators (those present outside the cell) such as thionine, methylene blue or neutral red and potassium ferricyanide. Secondly, using mediators produced by the bacteria and finally by direct transfer of electrons from the respiratory chain enzymes i.e. cytochromes, to the outer cell membrane, which in turn is reduced and then leaving it in a reduced state to shuttle the electrons to the electrode [4].

Clostridium butyricum [11], *Saccharomyces cerevisiae* [16] and *Proteus vulgaris* [2] are reported to generate electricity in mediated MFC while *Shewanella putrefaciens*, *Geobacter sulfurreducens*, *Geobacter metallireducens* and *Rhodoferax ferrireducens* have been shown to generate electricity in a mediator less MFC [3]. Bacteria present in mediator less MFCs have electrochemically active redox enzymes on their outer membranes that transfer the electrons to external materials and therefore, do not require exogenous chemicals to accomplish electron transfer to the electrode [12].

The present study electrogenic soil bacteria was isolated from garden soil sample to analyse

the bioelectricity producing potential in mediator less MFC using the substrate enriched with 5% and 10% glucose as soul of carbon source.

2. Materials and Methods

2.1. Isolation and Identification of microorganisms

The soil sample was collected from garden, Faculty of Agriculture, Annamalai University, Annamalainagar. The samples were brought to the lab in sterile air sealed packet to retain the moisture. Sample was serially diluted up to 10^{-8} dilution using 0.5 % of NaCl and 1 ml of sample from 10^{-8} was poured into nutrient agar plates. After incubation the colonies obtained were sub cultured subsequently to obtain pure culture. The pure cultures obtained were incubated in minimal broth (0.8% Glucose, 0.3% KH_2PO_4, 0.6% K_2HPO_4, 0.5% NaCl, 0.2% NH_4Cl and 0.01% $MgSO_4$) at 36 ˚C ± 2 for 48 hr. The isolated cultures were identified using various staining procedures and bio- chemical tests prescribed by Bergey's manual of systematic bacteriology *IV* Edition [21].

2.2. MFC construction

The MFC was constructed using two screw capped plastic bottles with the total working volume of 1000 ml and it served as anode (anaerobic) and cathode (aerobic) chambers. Both anode and cathode chambers were connected with 1.2 cm in diameter and 6 cm long tube which is filled up with salt bridge made of sodium chloride and agar in the ratio of 1:2 [8]. Agar salt bridge acts as a barrier between the anode and cathode chambers. The Purpose of an agar salt bridge is to provide an internal electrical connection between the chambers, while minimizing the transfer of ions from the electrical environment [15]. The carbon rods of 1.5 cm diameter and 13.5 cm long served as anode and cathode. Before the MFC operation the electrodes were soaked in 1 mol/L HCL solution for a day to remove possible metal contamination and after the MFC operation the electrodes were washed with 1 mol/L NaOH solution to sterilize the attached cells [9]. The electrodes were externally connected with copper wire and all exposed metal surface was sealed with non conductive epoxy. Totally two MFC setups were constructed respectively for this present study.

2.3. MFC Operation

Initially the MFC setups were surface sterilized under the UV light for 20 min. The anode chambers of both MFC setups was filled with minimal media enriched by adding 5% and 10% glucose as carbon source and 100 ml of 24 hr old isolated bacterial cultures were inoculated into anode chambers respectively. To maintain anaerobic condition, both the anode chambers were continuously flushed with N_2:CO_2 (80:20) for 30 min and it was immediately sealed with non conductive epoxy. The cathode chambers (aerobic chamber where oxygen was used as electron acceptor) were filled with 1000 ml of 100 mM phosphate buffer solution and pH adjusted to 7.0 [1]. All the experiments were carried out under room temperature for 10 days incubation.

3. Results

3.1 Isolation and identification

Two electrogenic bacterial strains were isolated from the garden soil sample and designated as isolate 1 and isolate 2. After the biochemical character assessment the isolate 1 was identified as *Bacillus subtilis* and isolate 2 was identified as *Bacillus megaterium*. The biochemical characters of the both isolates were recorded in Table: 1

Table 1: Characteristics of Bacterial Electrogens

Morphological characteristics	***Bacillus subtilis***	***Bacillus megaterium***
Grams reaction	Positive	Positive
Morphology	Bacilli	Bacilli
Motility	Variable	Positive
Endospore	Positive	Positive
Biochemical characteristics	***Bacillus subtilis***	***Bacillus megaterium***
Indole production test	Negative	Negative
Methyl red test	Negative	Positive
Voges proskauer	Positive	Negative
Citrate utilization	Variable	Positive
Urease test	Negative	Negative
Coagulase test	Negative	Negative

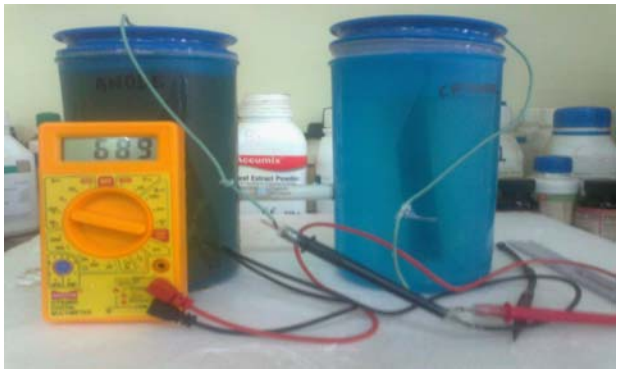
Table 2: Power generation of *Bacillus* sp with various concentration of glucose as substrate with minimal media

S. No	**Incubation time (hours)**	**power generation in millivolts by *bacillus subtilis* with 5% glucose**	**power generation in millivolts by *bacillus megaterium* with 5% glucose**	**power generation in millivolts by *bacillus subtilis* with 10% glucose**	**power generation in millivolts by *bacillus megaterium* with 10% glucose**
1	12	92	104	151	159
2	24	234	224	362	224
3	36	254	305	420	392
4	48	256	364	503	477
5	60	276	369	541	522
6	72	298	375	578	559
7	84	326	374	589	579
8	96	365	379	601	590
9	108	364	389	620	618
10	120	369	398	638	621
11	132	387	410	635	651
12	144	433	422	650	651
13	156	498	436	659	641
14	168	502	450	668	637
15	180	504	456	689	597
16	192	508	459	691	574
17	204	519	467	672	580
18	216	520	466	671	571
19	228	501	458	671	568
20	240	486	440	669	552

3.2 Power generation

MFC setups having 5% and 10% glucose as carbon source loaded in anode chambers respectively with the isolated bacterial electrogens for operated for 10 days. The bioelectricity production was recorded in millivolts for every 12 hours interval. At 5 % concentration of glucose supplemented with minimal media, *Bacillus subtilis* produced maximum voltage of 520 mV at 216th hour (9th day) followed by *Bacillus megaterium* of 467 mV at 204th hrs (8-9 days) of 240 hours (10 days) total incubation. The electricity production by *Bacillus subtilis* was maximum of 691 mV at 192th hour in 10 % of glucose substrate, followed by *Bacillus megaterium* of 651 mV at 144th hour of 240 hrs (10 days) total incubation and the results were recorded in table 2.

3.3 MFC set up used in this study



4. Discussion

The current study was carried out for the electrogenic efficiency of bacterial isolates from garden soil sample. The potential isolates were identified as *Bacillus subtilis* and *Bacillus megaterium* on the basis of morphological, biochemical characteristics and staining techniques. Debajit *et al.* (2013) [6] reported that *Bacillus megaterium* is one of the best strains for bioelectricity production up to 698 mV. Similarly, in our study maximum current of 691 mV was generated by *Bacillus subtilis* after 8 days of MFC operation.

Most of the previous results showed that the microorganisms *viz*., *Clostridium butyricum*[11], *Saccharomyces cerevisiae* [16], *Proteus vulgaris* [2] *Clostridium acetobutylicum*, *Clostridium thermohydrosulfuricum*[10,7] *Shewanella putrefaciens*, *Geobacter sulfurreducens*, *Geobacter metallireducens* and *Rhodoferax ferrireducens* [3] as well known potential strains for the production of bioelectricity, similarly our current study showed that *Bacillus subtilis* and *Bacillus megaterium* as one of the best electrogenic bacteria.

Findings of the present study followed the same trends reported by earlier researchers, where individual microbes with a known substrate, such as glucose, fructose, lactose and sucrose, were used in indigenous mediated MFC for bioelectricity production. Saravanakumari and Angel, (2014) [18] reported that the *Bacillus* sp produced 336 mV current at 4 hours of incubation with glucose containing medium. Thurston *et al*. (1985) [19] reported that the production of a power density average voltage of 300 mV with the glucose as substrate and mediator thionine, employing *Proteus vulgaris* in nafiane membrane MFC. Similarly, in another study *E. coli* produced high voltage up to 680 mV with the substrate glucose and neutral red as mediator in proton exchange membrane MFC [14].

Similar results were obtained by several other workers, who have reported the electricity generation from a variety of microorganisms like *Shewanella putrefaciens*, *Geobacter sulfurreducens*, *Lactobacillus plantarum*, *Streptococcus lactis* and *Erwinia dissolvens*, employing several different nutritional sources [20, 3, 13, 5].

5. Conclusion

The present study emphasizes that the *Bacillus subtilis* and *Bacillus megaterium* both are good strains for bioelectricity production via Microbial Fuel Cell technology. Using the glucose as carbon source *Bacillus subtilis* has high bioelectricity producing ability than *bacillus megaterium.* Increasing of glucose concentration resulted in enhancement of power production and it is economically considerable. From the present study we conclude that *Bacillus* electrogens are economically important bacterial strains for electricity production because they have ability to produce electric power in mediator less Microbial Fuel Cell substrate as glucose and glucose containing wastewater.

6. Reference

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