



(RESEARCH ARTICLE)



Comparative study of bioelectricity generation by microbial degradation of organic wastes using microbial fuel cell

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Abstract

In the present project, we investigate to generate bioelectricity from organic wastes such as *Citrus sinensis* peel slurry and *Oryza sativa* waste water and characterize the electrogenic bacteria responsible for the generation of bioelectricity. The maximum voltage of about 0.8V was generated from *Citrus sinensis* peel slurry in 16 days, whereas 0.642V was generated in a period of 14 days from *Oryza sativa* waste water. In series connection of microbial fuel cells, voltage of 2.850V was measured. Four electrogenic bacterial isolates were obtained from the anode of microbial fuel cell and various biochemical characterization tests were performed. The effect of addition of different concentrations of glucose to the anode chamber of microbial fuel cell along with the organic wastes was analysed and 3g/l was found to be the optimum glucose concentration to increase the performance of microbial fuel cell. The output from the microbial fuel cells can be implemented to power low power consuming devices and biosensors.

Keywords: Bioelectricity; Microbial fuel cell; Electrogenic bacteria; *Citrus sinensis* peels; *Oryza sativa* waste water.

1. Introduction

Nowadays the world is observing an energy crisis due to huge energy demand and limited resources. Combustion of non-renewable energy emits a lot of greenhouse gas like carbon dioxide, which has shown alarming consequences to the environment [1]. An alternative strategy is direct conversion of sugars to electrical power [2]. Microbial fuel cells (MFCs) are emerging as promising technology for the treatment of wastewaters [3]. Microbial fuel cells (MFCs) directly convert biodegradable substrates to electricity and carry good potential for energy-positive waste water treatment [4]. Microbial fuel cells (MFCs) are devices that can use bacterial metabolism to produce an electrical current from a wide range organic substrate [5]. Recently great attentions have been paid to microbial fuel cells (MFCs) due to their mild operating conditions and using variety of biodegradable substrates as fuel [6]. The added advantage of using MFC technology for different effluent treatment is that several bio-based processes include removal of biochemical and chemical oxygen demand, nitrification, denitrification sulphate removal and removal of heavy metals can be carried out in the same bioreactor [7]. A typical MFC consists of anode and cathode compartments, which are separated by a cationic proton exchange membrane. Salt-bridge is the economic alternative to highly priced proton exchange membrane in the construction of a microbial fuel cell [8]. To improve the performance of microbial fuel cells (MFCs), the biocathode electrode material of double-chamber was optimized [9]. For practical applications of MFC technology, the design as well as the process of manufacturing and assembly, should be optimized for the specific target use [10]. A number of microorganisms having electricity generating efficiency such as *Geothrix* species and *Shewanella*, can produce their own electron shuttles. *Geobacter* species are advantageous due to the presence of the ability to directly transfer electrons to electrodes, when competing for space on the anode of sediment microbial fuel cells [11]. *Shewanella oneidensis* is able to conserve energy for growth by reducing a wide variety of terminal electron acceptors during anaerobic respiration, including several environmentally hazardous pollutants [12].

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The main objectives of this study were to generate bioelectricity from the two different organic wastes including *Citrus sinensis* peels and *Oryza sativa* waste water and to biochemically characterize the electrogenic bacterial isolates. Also, to optimize the effect of addition of glucose for enhancement of MFC performance.

2. Material and methods

2.1. Sample collection

The substrates for bioelectricity generation, *Citrus sinensis* peels and *Oryza sativa* waste water were collected from the domestic kitchen waste in a sterile plastic container and converted into slurry with a concentration of 50mg/ml. The soil sample was collected near juice shop of Prathyusha Engineering College, Chennai-602025, in aseptic manner for isolation of bacteria from soil.

2.2. Fabrication of microbial fuel cell setup

The H-type microbial fuel cell was constructed by connecting the anode and cathode compartments with a salt bridge composed of 5% sodium chloride and 5% agar. The anode was made up of zinc electrode whereas the cathode was made up of aluminum electrode with a dimension of 100mm*25mm.

2.3. Preparation of mixed culture

The mixed culture was prepared by adding 1g of soil to 250ml of sterile nutrient broth. It was incubated at 37^o C for 24 hours and used as inoculum for microbial fuel cell.

2.4. Loading of samples and measurement of voltage from MFC

Citrus sinensis peel waste and *Oryza sativa* waste water was loaded into the anode compartment of different MFCs and 0.1M phosphate buffer was added into the cathode compartment. The electrodes were inserted into the respective compartments. The mixed culture was inoculated into the anode compartment. The voltage generated from the microbial fuel cell was measured periodically using a digital multimeter FLUKE 17B.

2.5. Isolation of electrogenic bacteria from MFC

After a period of about 45 days, the enriched and pre acclimatized electrogenic bacteria capable of producing bioelectricity were isolated from the biofilm on the surface of anode of microbial fuel cells. The bacterial suspension was serially diluted up to 10⁻¹² dilutions and spread plates technique was carried out. Then, the number of bacterial colonies were counted. The quadrant streak technique was performed to obtain pure bacterial isolates, which were inoculated on surface of the slant and incubated at 37^oC for 24 hours.

2.6. Biochemical characterization

The various biochemical characterization tests such as Gram's staining, hanging drop technique, citrate utilization test, catalase test, urease test and sulphide indole motility test was performed for all the electrogenic bacterial isolates HST1, HST2, HST3 and HST4. The confirmatory test using King B agar medium was carried out to identify the electrogenic bacterial isolates.

2.7. Effect of different glucose concentrations in anode compartment

The glucose was added in three different concentrations including 1 mg/ml, 3 mg/ml and 5 mg/ml along with the organic waste into the anode chamber to optimize and enhance the performance of microbial fuel cell and the generated voltage was measured.

3. Results and discussion

The organic wastes including *Citrus sinensis* peels and *Oryza sativa* waste water was collected from the domestic kitchen waste in a sterile plastic container. The final concentration of both the organic samples was 50mg/ml. The mixed culture of bacteria from soil was inoculated into the anode chamber of microbial fuel cell. The voltage was generated from the microbial fuel cell by the degradation of *Oryza sativa* waste water and *Citrus sinensis* peel waste. The generated voltage was measured using a digital multimeter for 30 days (figure 1).



Figure 1 Voltage measurement for glucose optimization using digital multimeter

A stable voltage was recorded for 10 days from *Citrus sinensis* peel waste slurry. The maximum voltage of 0.8V was measured from *Citrus sinensis* peel waste inoculated with mixed culture. The generation of bioelectricity was low from *Citrus sinensis* peel wastewithout inoculation (figure 2).

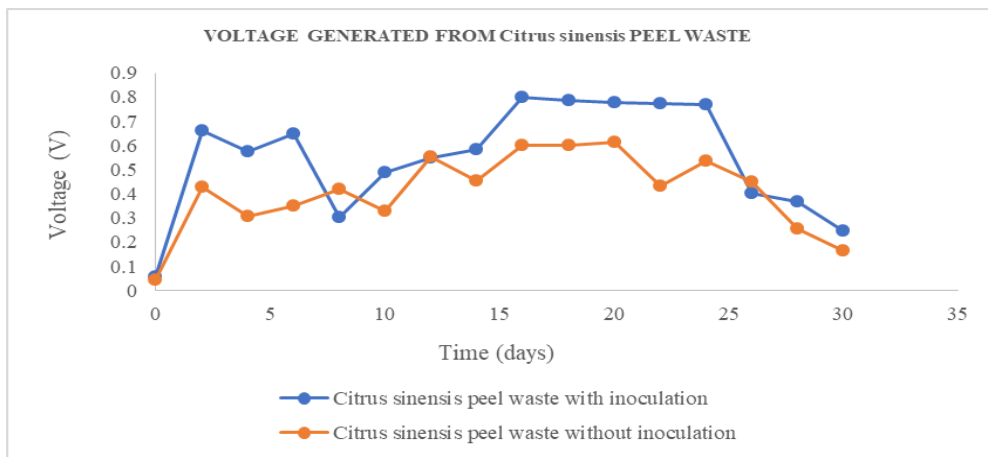


Figure 2 Effect of inoculation on voltage generation from Citrus sinensis peel waste

A stable voltage was recorded for 14 days from *Oryza sativa* waste water. The maximum voltage of 0.642V was measured from *Oryza sativa* waste waterinoculated with mixed culture.

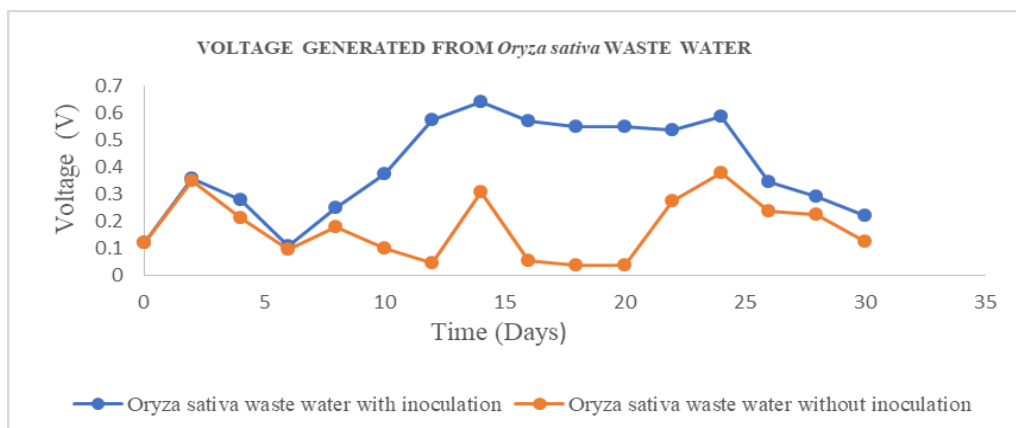


Figure 3 Effect of inoculation on voltage generation from Oryza sativa waste water

It was observed that there was fluctuation in the voltage generation in the initial period and then there was increase in voltage generation. In series connection, a stable voltage obtained was $2.5 \pm 0.3V$. A maximum of 2.8V was produced from four microbial fuel cells in series connection (figure 4).

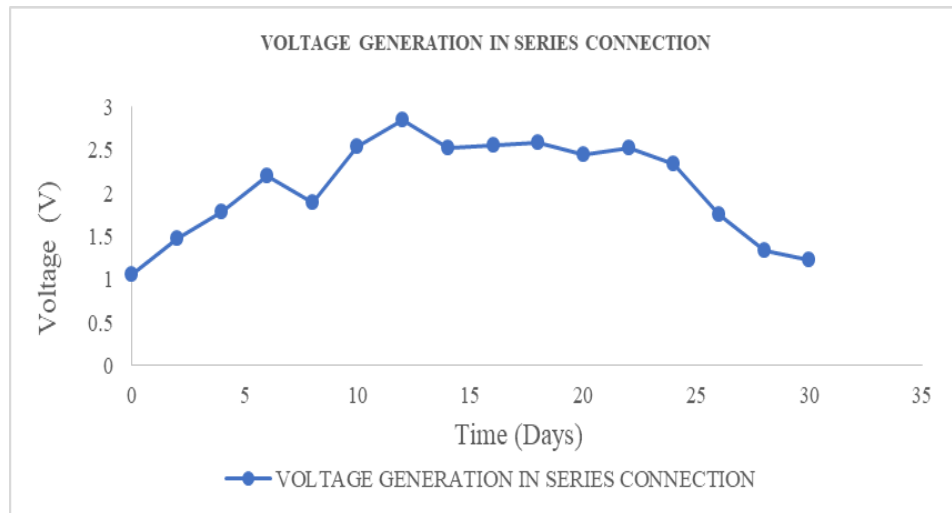


Figure 4 Generation of voltage from four microbial fuel cells connected in series.

A total of four enriched electrogenic bacteria were isolated by nutrient agar from the biofilm on the surface of anode of *Oryza sativa* waste water and *Citrus sinensis* peel slurry microbial fuel cells (figure 5). These isolated bacterial strains were named as HST 1, HST 2, HST 3 and HST 4. The morphology of the bacterial colonies such as colour, shape, size, opacity, elevation and texture were observed and noted (table 1). The quadrant streak plate technique was performed (figure 6).

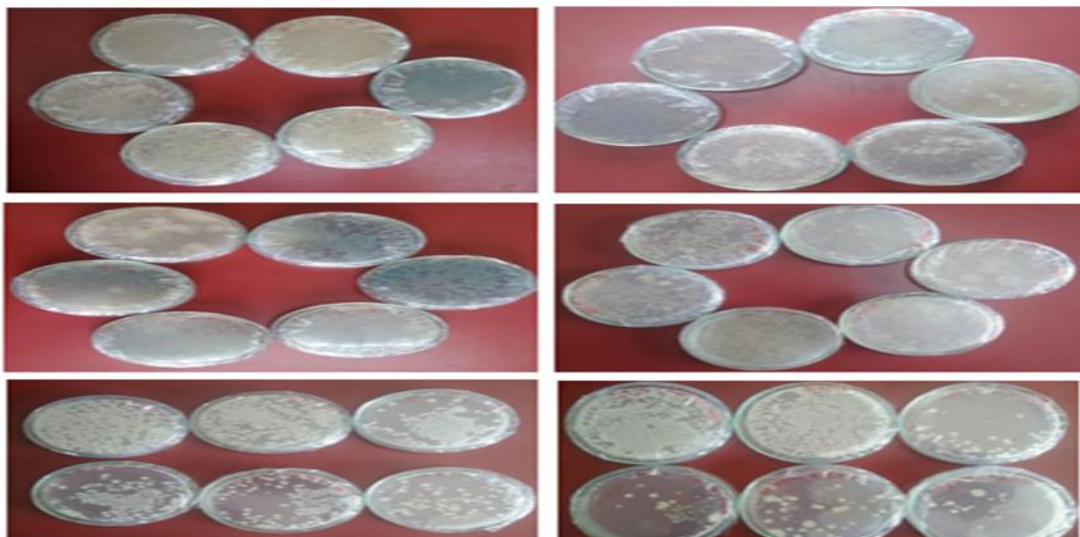
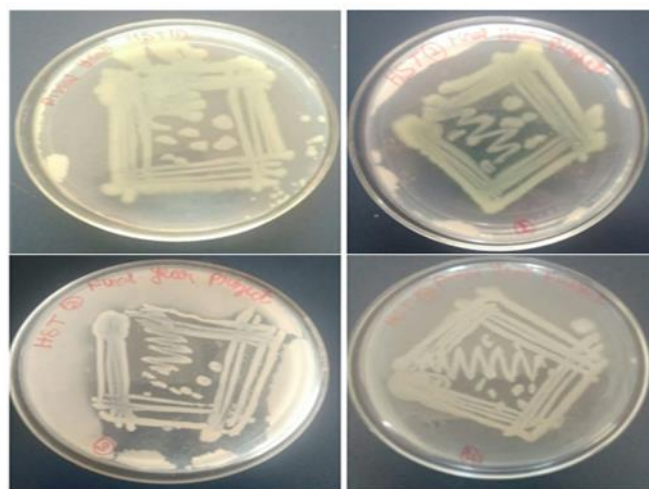


Figure 5 Enumeration of electrogenic bacterial isolates from different samples

Table 1 Colony morphology of the electrogenic bacterial isolates

CHARACTERISTICS	HST 1	HST 2	HST 3	HST 4
Colour	Orange	yellow	white	white
Shape	circular	circular	circular	filamentous
Size (mm in diameter)	3	4	3	5
Optical property	transparent	opaque	opaque	opaque
Elevation	flat	raised	raised	flat
Texture	sticky	sticky	sticky	sticky

**Figure 6** Quadrant streak plates of electrogenic bacterial isolates

All the four electrogenic bacterial isolates HST 1, HST 2, HST 3 and HST 4 were biochemically characterized. Gram's staining, hanging drop technique, citrate utilization test, catalase test, urease test and sulphide indole motility test was performed (table 2).

Table 2 Various biochemical characterization tests for electrogenic bacteria

S.NO	BIOCHEMICAL TEST	HST1	HST2	HST3	HST4
1	gram staining	gram negative(rod)	gram negative(rod)	gram negative(rod)	gram positive (rod)
2	catalase test	positive	positive	negative	positive
3	urease test	negative	positive	negative	negative
4	motility test	positive	positive	positive	negative
5	indole test	positive	positive	positive	negative
6	H2S production	positive	positive	negative	negative
7	citrate test	positive	positive	negative	positive

It confirmed that HST1 electrogenic bacterial isolate was *Pseudomonas sp* by the appearance of fluorescent greenish yellow colour in the King B agar plate containing HST1 bacterial isolate (figure 7).The yellow colour was not produced by the remaining bacterial isolates.

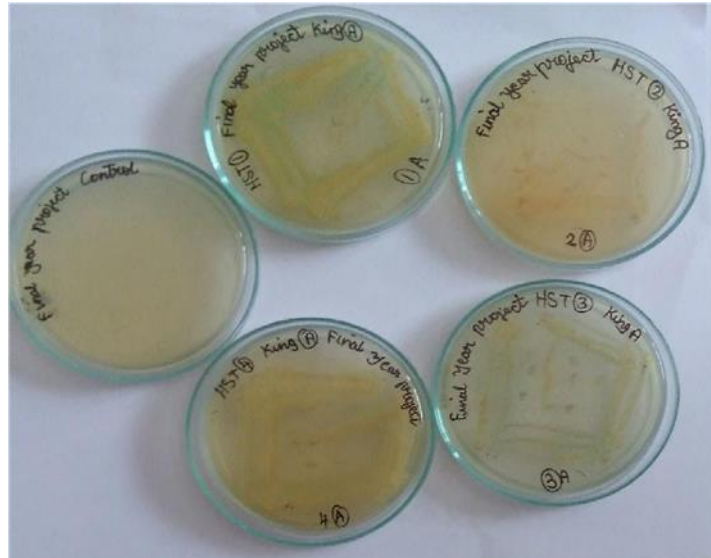


Figure 7 Electrogenic bacterial isolates on King B agar plates

The performance of microbial fuel cell was analyzed by the addition of glucose into the anode chamber of microbial fuel cell along with the substrates *Citrus sinensis* peel waste and *Oryza sativa* waste water. It was observed that 3mg/ml of glucose concentration had maximum effect on voltage generation due to the enhanced activity of electrogenic bacteria. The stable voltage was generated for 5 days from *Oryza sativa* waste water (figure 8) and 3 days from *Citrus sinensis* peel waste (figure 9).

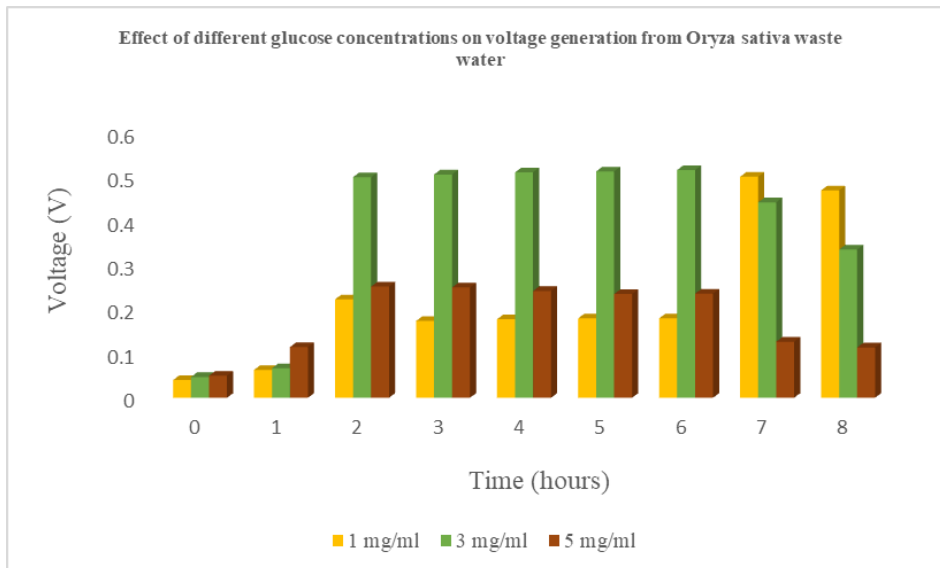


Figure 8 Effect of different glucose concentrations on voltage generation from Oryza sativa waste water

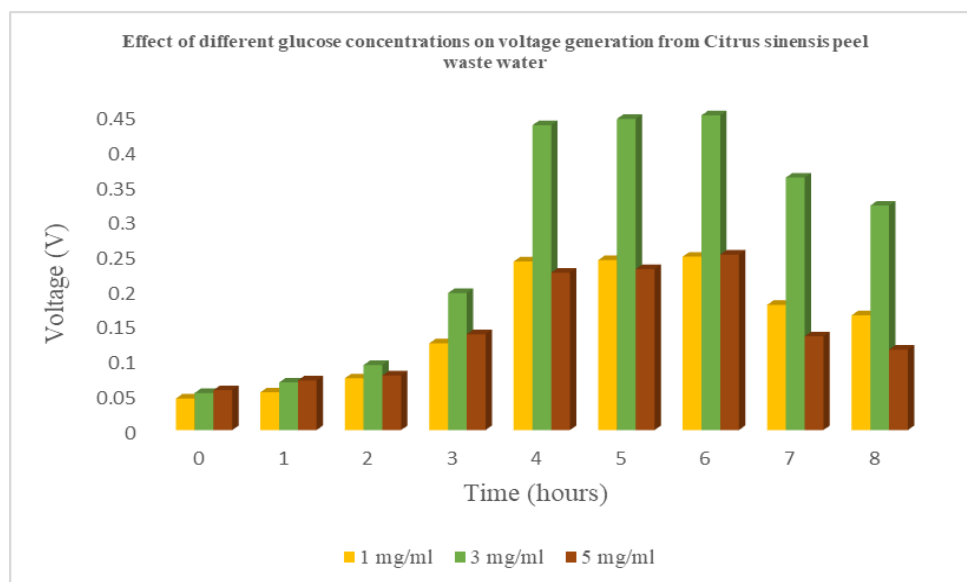


Figure 9 Effect of different glucose concentrations on voltage generation from Citrus sinensis peel waste water

According to the previous reports, Washington Logrono *et al.*, [13] demonstrated the bioelectricity generation from vegetable and fruit wastes with high Andean soil as microbiological resources and reported the highest value of output voltage of 330 mV over a testing period of 60 days by the activity of electrogenic microorganisms. Elviliana *et al.*, [14] analyzed the generation of bioelectricity from banana and orange peel waste using microbial fuel cells. The cathode was perforated copper plates coated with activated carbon powder and anode consisted of zinc plates coated with graphite powder. The voltage and current were measured for 10 days and the highest reported was 0.492 V and 0.101 mA for banana peel waste and 0.563 V and 0.017 mA for orange peel waste respectively. The study concluded that the organic content of substrates and the microbial activity influenced the electricity production from the MFCs.

4. Conclusion

The maximum voltage was generated from *Citrus sinensis* peel slurry compared to *Oryza sativa* waste water sample using double chamber microbial fuel cell. The electrogenic bacteria were isolated from MFC and biochemically characterized. The performance of MFC was enhanced by optimizing the addition of glucose along with substrates into MFC.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors do not disclose any conflict of interest.

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