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Bioelectricity Production from Oil Refinery Wastewater in Mediator-Less Microbial Fuel Cell Using Newly Isolated Native Gram Positive *Bacillus Sp.*

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Abstract

Microbial fuel cell technology is effective approach of generating electricity directly from the break-down of waste organic matter and renewable biomass using diverse metabolic activity of native electrigens. This communication reports electricity production of from cost effective mediator-less Microbial fuel cells (MFCs) fed with rice bran oil refinery wastewater using newly isolated native *Bacillus sp.* The performance of MFCs was improved by optimizing the operational parameters – the temperature and pH and represent easy-to operate fuel cell fabrications pretty much viable for generating electricity as well as wastewater treatment simultaneously.

Key Words: Microbial fuel cell, Wastewater treatment, *Bacillus sp.*, Bioelectricity

Introduction

Explosion in worldwide population and modernization has led to increase demand for energy. The traditional resources majorly the fossil fuels are being utilized for harnessing energy which extended the crises for not only energy but the clean and sustainable environment. The worldwide ongoing scenario of energy demand, consequences from overexploitation of resources to fulfill the demand, searching for sustainable resources and concerns towards these issues made researches think for every possible resources and technologies for its better utilization. One of such alternative for valuable energy resource to fulfilling the criteria is the high organic content of waste. The burning topics in The World Bank say that the waste generation rates are rising globally. In 2012, the amount of solid waste generated globally is 1.3 billion tons per year from cities only which accounts for footprint of 1.2 kilograms per person per day and expected to rise to 2.2 billion tons by 2025 only from municipal waste generation. An estimate says that around 22% of water is used for different industrial purposes worldwide. But, water withdrawal for agriculture and agriculture based industries is quite high. This account of wastewater just not only needs to be controlled but must be settled. According to World Water Development Report (2003), approximately 2 million tons of wastes per day are disposed of within receiving waters from human excreta; agricultural wastes in the form of fertilizers, pesticide residues and industrial wastes (solid as well as liquid) and chemicals etc. In the year 1995, the global industrial sectors were estimated to use about 725 km^3 of water annually. By 2025, a rise expected to about 1,170 km^3 water annually and with this raise, water usage by industrial sectors will represent 24% of all water abstractions. It is estimated that in developing countries 70% of industrial discharges is dumped untreated in water resources. In India, the wastewater generated from medium and large industries is 55,000 million m^3 per day, of which 68.5 million m^3 is dumped directly into local rivers and streams without any treatments (Pangare et al., 2006).

The situation heightened the efforts towards development and practices of the technologies and resources which should be renewable, eco-friendly and sustainable. Few of the developing techniques of renewable energy are wind energy, solar energy, bio-hydrogen (Thakur et al., 2015), bio-ethanol (Beliya et al., 2013), bio-electricity through microbial fuel cell (MFCs) (Kaushik and Jadhav, 2017). MFCs are of much important as it offers the degradation of wide range of organic compounds for producing energy as bioelectricity (Logan, 2009).

MFCs are the bio-electrochemical devices that convert the biodegradable waste organic matters directly into electrical energy without any combustion of substrate using diverse metabolic activity of the microorganism (Ahn and Logan, 2010). In other words, MFCs enable the diverse microbial catalytic activities to degrade the organic compounds from pure chemicals to waste organic matter, into electricity without any combustion via enabling direct oxidation and reduction of waste matter at electrode surface of fuel cells (Bond and Lovley, 2003). In these systems, the native microorganisms play crucial role in harnessing useful energy meanwhile treatment of the substrate in terms of removal of odors (Kaushik and Jadhav, 2017), dyes (Fernando et al., 2014), COD (Jiang et al., 2010), BOD, normalization of pH (Kaushik and Jadhav, 2017). Primarily there are two types of basic deigns; one is single chambered microbial fuel cell (SCMFC) and other is dual chambered microbial fuel cell (DCMFC). All the modifications are further derived from two of these basic configurations say for stacked (Choi & Ahn 2013), plate (Min and Logan, 2004), up flow, with membrane (Bond and Lovley, 2003), without membrane (Gajda et al., 2015) or with salt bridge (Min et al., 2005), open air cathode, single (Sund et al., 2007) or multiple electrodes (Liu et al., 2004) etc. The design and fabrication of the MFCs and the electrode materials being used in an MFC are the important aspect of the innovation and development. Early microbial fuel cells employed simple fermentable substrates like pure chemicals, for example- acetate (Pham et al., 2003), glucose (Potter, 1911; Park and Zeikus, 2000), xylose, sucrose and maltose (Chaudhuri and Lovley, 2003) etc as fuels and were powered by fermentative microorganisms (Lovley, 2006). Turning to more realistic, variety of wastewater has been used in MFC as fuel such as domestic sewage (Ahn and Logan, 2010; Liu at et., 2004), paper and pulp wastewater (Huang & Logan, 2008), rice mill wastewater (Daniel et al., 2009; Behera et al., 2010) brewery wastewater (Feng et al., 2008), swine wastewater (Kim et al., 2008), Oil refinery wastewater (Majumder et al., 2014), chocolate industry wastewater (Patil et al., 2009), and real dye wastewater (Kalathil et al., 2012) etc. One thing in common to this wastewater is high organic load and availability in bulk and that play as key to use it as fuel. They are rich in nutrients to support the bacterial growth and their biodegradability makes it possible to use it in MFCs. The MFCs can also be associated with the wastewater treatment units to purify the water to certain extend, especially when it took huge amount of energy to purify it.

The present work deals with Microbial Fuel Cells employ low-cost materials (zinc, carbon and copper electrodes), aerated cathode, simpler operational conditions (normal pH and ambient temperature) and native bacteria to generate electricity from waste materials.

1. Materials and Methods

2.1. MFC fabrication and operation

Double-chambered microbial fuel cells were architect using non-reactive, autoclavable, polyvinyl chloride (PVC) containers (volume of 750 mL), operated in the fed-batch mode under anaerobic microenvironment (Kaushik and Jadhav, 2017). Double-chambered microbial fuel cells was constructed comprising of an anode and an open air-Cathode chambers for minimizing the possibility of diffusion of oxygen to nearby anode and increase the efficiency of the system (Das and Mangwani, 2010) (Figure - 01). The major hurdles for the large scale implementation of MFCs in real world is the cost of MFCs due to expensive electrode materials assuming that the non-noble materials such as carbon rod, inexpensive metals were chosen and Zn-C combination were selected based on our previous studies (data not shown) (Liu et al., 2013). A UPVC pipe containing agar salt bridge was used to separate the chambers physically at distance of 3.2 cm. The aid of adhesive material (M-Seal) was used to fix both the chamber intact (Kumar et al., 2012; Kaushik and Jadhav, 2017). External copper wires were used to connect the electrodes to the digital multimeter by alligator clips. The open air-cathode chamber was filled with phosphate buffer and pH adjusted to 7.0 (Logan and Regan, 2006). In this set up oxygen was employ as the final electron acceptor (Liu et al., 2013). MFCs were surface sterilized as described by Kaushik and Jadhav, 2017. All tests were performed under room temperature, 30±2 ^{0}C, without pH control or any catalyst except where mentioned.

2.2 Isolation, Screening, biochemical characterization and Growth Condition of selected Electrogen

2.2.1. *Isolation and screening of electrogenic bacterial strain*

Sixteen bacterial isolates were isolated from anode of rice bran oil refinery wastewater fed MFC by serial dilution method. The dilutions were inoculated on plates containing nutrient agar medium (NAM). After 48 h of incubation, mixed bacterial colonies were observed which was further pure cultured through the streak plate method (Prescott and Harley, 2002). Screening process for confirmation of the electrogenic properties were carried out in sterile wastewater fed MFCs. The most potent bacterial isolate WRS-6 was subjected to further study and cross check the electrogenic property in synthetically designed wastewater (glucose 3.0 g/l, $FeCl_3$ 25.0 mg/l, NH_4Cl 0.5 g/l, $CaCl_2$ 5.0 mg/l, KH_2PO_4 0.25 g/l, K_2HPO_4 0.25 g/l, $CuCl_2$ 10.5 mg/l, $MgCl_2$ 0.3 g/l, $NiSO_4$ 16.0 mg/ l, $CoCl_2$ 25.0 mg/l, $ZnCl_2$ 11.5 mg/l and $MnCl_2$ 15.0 mg/l) under anaerobic microenvironment (Aldrovandi et al., 2009).

2.2.2. *Biochemical characterization of the most bacterial isolate*

Microscopic study was performed by Gram's staining, endospore staining and acid-fast staining. For biochemical characterization of selected bacteria, indole test, methyl red test, voges–proskauer test, citrate utilization test (IMViC test), fermentation of carbohydrate test and amylase production test were performed (Prescott and Harley, 2002).

2.2.3. ***Media and growth condition***

Nutrient agar and broth (HiMedia Laboratory Pvt. Ltd., Mumbai, India), were used for growth and maintenance of bacterial strain for further use in this study. The cell concentration was estimated by ultraviolet-visible spectrophotometric analysis and serial dilution colony-counting method. The bacterial isolate was grown at 36^0C temperature for 48h. The cells were harvested by refrigerated centrifugation at 6000 rpm for 10 min. The cell concentrations were set at 0.2 (OD_{600nm}), inoculum accounting for 16±2 x 10^4 CFU/mL throughout the experiments (Liu et al., 2010).

2.3 Wastewater characterization

The effluent was collected from the local rice based industry Shree Sita Refiners Pvt. Ltd, Arasnara, Durg, Chhattisgarh, India designated as SSR. Sample was collected in 5L sterile flask and stored at $4±1^0$C in a refrigerator for short term. Wastewater was used throughout the experiment without modification in organic load or pH adjustments except where mentioned. Typical physicochemical parameters of wastewater pH, color, odor, biological oxygen demand (BOD), chemical oxygen demand (COD) and electrical conductivity (EC), were analyzed before and after the experiment for the monitoring of wastewater treatment progress(APHA 1998). Then it was kept at low temperature ($4–5^0$C) for further study.

2.4 Optimization of operating conditions

Some important parameters required for maximum production of bioelectricity were optimized including Inoculum size (refers to the amount of bacterial culture) and incubation of inoculum for different time period. 48-h-old bacterial culture of isolate WRS-6 (maximum bioelectricity producing bacterial strain) was independently studied at various inoculum sizes of 0.1, 0.5, 1.0, 1.5 and 2.0% (v/v). Previous reports showed that pH has vital role in growth of microorganisms which is directly linked to the rate of bioelectricity production. In this regards MFCs were operated at different pH ranging from 4.0 to 9.0. The pH was adjusted using freshly prepared acid and alkali stock solutions. The operating temperature influences the growth, rate of metabolic activity and survival of bacteria which is directly related to bioelectricity production. So, a series of experiments executed under identical conditions after maintaining the pH of MFCs at different temperature range of 20, 25, 30, 35 and 40^0C.

2.5 Statistical Analysis

The performance of MFCs were continuously operated and monitored. Electrode output was evaluated in terms of voltage (V) and current (mA) in the open circuit using auto-range digital multimeter (KUSAM-MECO 603) after one hour time intervals. The COD and BOD were determined by standard methods as per APHA. EC and TDS were analyzed using EC-TDS analyzer (ELICO CM-183 VER. 2.3), DO was measured using a DO analyzer (ELICO PE-135) and the pH was measured using a pH meter (ELICO LI-120). All experiments were performed in triplicate and repeated at least thrice to measure the reproducibility. Descriptive analysis of data was performed by one-way analysis of variance (ANOVA), least significant difference (LSD) for test of significant difference among means using Duncan's multiple range test (DMRT). > 0.05 (P values) were considered as significant. All the analysis was performed at the 0.05 probability level by SPSS version 16.0 and typical values are presented.

3. Results and Discussion

3.1. MFC Operation and Performance

The non-noble materials zinc and carbon owing strong appeal to their low cost and ubiquity were selected to operate MFCs in present study. These materials are implicated with 2- electron pathway for oxygen reduction reactions (Liu et al., 2013). The anodic chamber was filled with 750 ml of sterile wastewater samples and cathode chamber was filled with phosphate buffer. Anode and cathode was connected to external wiring to complete the circuit and voltage and current were measured via auto-range digital multimeter. Anaerobic micro-environment was maintained in the anode compartment throughout the experiment. Studies showed that, lack of oxygen as terminal electron acceptor in anode chamber forces bacteria to transfer their electrons outside the cell hence maintaining anaerobic condition is important (Lovley et al., 1993). MFCs were operated in batch mode at ambient room temperature (30 ± 2^0C) and pressure. The electrolytic solution are exposed to air for reduction reaction to occur where oxygen work as final electron acceptor, because the ambient oxygen has ubiquity, low cost and high standard equilibrium potential (Liu et al., 2013; Feng et al., 2008). In the mediator-less anode compartment, microbial consortium oxidizes fuel resulting in production of electrons and protons (Jung and Regan, 2007). The electrons travel through the external circuit and the protons are transferred to the cathode compartment through the salt bridge (Gil et al., 2003, Gregory et al., 2004). Electron coming from anode are responsible for electrolytic reduction of oxygen on cathode side, thus the overall performance is strongly affected by the oxygen reduction reaction efficiency especially in present case where MFCs are being operated at neutral pH and room temperature (Liu et al., 2013; Merino-Jimenez et al., 2017).

3.2. Characteristics of bacteria

The colony characterization of selected bacterial species (WRS-6) was done and it was found to be irregular in form with lobate margin, convex elevation and opaque in optical character on Nutrient Agar plates at 37°C. The bacterial colony was cream-white and rough. After this, microscopic study was performed with different staining techniques such as Gram's staining, Endospore staining and Acid-fast staining. It was found to be large rod-shaped, gram positive, acid-fast negative, and Endospore forming. Different biochemical tests were performed (Table-01).

3.3. Bioelectricity production from WRS-6 in sterile wastewater and synthetic wastewater

The isolate WRS-6 was used as inoculum in sterile wastewater fed mediatorless MFC. The electricity generation is directly from microbes and the transfer of electrons from anode to cathode is mediated by microbes itself as no external mediators were used. In present study, the sterile wastewater from SSR produced maximum open circuit voltage of 1.21033 ± .001 V with corresponding current of 2.5024 ±0.307 mA in mediator-less MFCs (Figure-02). When electrogenic property was checked in synthetic wastewater the maximum electricity in terms of both the current as well as OCV was 1.316 ± .027 mA and 1.161 ±0.034V (Figure-03). Hence, the isolate WRS-6 was efficient in producing electricity.

Previous studies also report that the wastewater from different industrial sectors is the potential candidate for fuel in the MFCs. Liu et al. (2004) utilized domestic wastewater to produce 26 mW/m^2 power output. Oh and Logan (2005) reported food processing wastewater as fuel option. Aelterman et al. (2006) studied industrial organic influent and effluent streams from a potato processing factory and found highest power output of 58±2.1 W/m^3 from the influent of the anaerobic digester. Feng et al. (2008) examined effect of temperature on brewery wastewater and recorded that, decrease in temperature from 30 °C to 20 °C reduced the maximum power density from 205 mW/m^2 to 170 mW/m^2. This section of the study elucidates that high organic strength of wastewater from rice bran oil refinery make them suitable for the MFCs experiments. Also, the optimization of the operating conditions is the important part in these experiments which may enable the utilization of the maximum ability of the electrogenic bacteria for optimum performance of the fuel cell. Therefore, the further section deals with optimization of operating conditions.

3.4. Electrochemical Activity of native *Bacillus sp.* (WRS-6)

In present study rice bran oil refinery wastewater is being used as substrate which is result of washing of extraction units and may contain some complex components including poly-unsaturated Fats, mono-saturated fats, saturated fats, rich amount of essential fatty acids, Linoleic fatty acid, Linolenic acids and trace amount of other nutrients that are recommended for edible oils (Ghosh, 2007). Therefore, the wastewater from the industry might be rich in nutritional values as well as high organic load (Akhter et al., 2014). The members of *Bacillus sp.* are one of the widely distributed bacterial candidates. The members of *Bacillus sp.* owe strong survivability even in hostile conditions probably due to the metabolic diversity resulting from ecological adaptation in different environments (Ivanova et al., 2003) Member of *Bacillus* sp. are known to have good capability of degrading organic waste compounds and use them as carbon source at moderate temperatures. Member of *Bacillus* sp. pose specific inducer and a conserved transcriptional repressor as malonyl CoA (coenzyme A) which is involve in regulation of the expression of several genes that are engaged in bacterial fatty acid synthesis (Islam et al., 2017). Also, some of the members such as *B. subtilis* and others have characteristic feature of the multiplicity of carbohydrate catabolic pathways due to variety of carbohydrates present in the soil majorly from plant-derived materials that may have helped in utilization of peptides, amino- acids and vitamins as nutrient source (Ivanova et al., 2003).

3.5. Effect of different parameters on bioelectricity production

3.5.1. *Effect of different inoculum size:*

Effect of inoculum size on bioelectricity production was studied with a concentration range of 0.5, 1.0, 1.5, 2.0 and 2.5% (v/v). Isolate WRS-6 was inoculated on nutrient broth (NB) for 48h and was used for making different inoculum size in NB. Inoculum was added to the MFCs containing sterile wastewater with respective inoculum size and OCV and current were recorded using auto-range multimeter. All the experiments were performed in triplicate and data was further analyzed. It was found that increasing the concentration of bacterial culture led to increased production to maximum 1.239±0.012V with corresponding current 9.535±0.148mA at 1.0 % v/v concentration (Table -02). Further increase in concentration of bacteria resulted in decreased OCV which may be the effect of toxins produced by the over growth of bacterial cultures. It implies that 1.0 % concentration of bacterial culture would be optimum to produce maximum OCV in rice bran oil refinery wastewater fed MFC assisted with newly isolated electrogen WRS-6. Significant impact on characteristics of wastewater was observed. 17.96 to 37.34 % removal of COD and 69.20 to 84.60 % removal of BOD were noted. Lowering of pH was noted for all the set up might be due to production of fermentative acids (Table-03). Alteration in catholyte characteristics also noted in terms of EC, TDS and pH (Table-04).

3.5.2. Effect of operating temperature:

In MFC, maintenance of optimal temperature condition inside the vessel are essentially required to generate maximum amount of electricity as it directly affects the metabolic activity of the bacterial counter part of MFC. Therefore, study was carried out to find out the optimum temperature for maximum production of bioelectricity at different temperature ranges from 20 to 40^0C. In MFC assisted with WRS-6, bioelectricity production increased considerably with increase in temperature from 20 to 35^0C with a maximum production in OCV was 1.157±0.009 V and Current 4.417±0.269 mA was achieved at 35 ^{0}C followed by 30^0C (1.103±0.012 V, 3.767±0.189 mA), 25 ^{0}C (1.068±0.010 V, 3.206±0.148 mA), 20 ^{0}C (1.041±0.014 V, 2.977±0.244 mA), and 40 ^{0}C (0.846±0.010 V, 2.832±0.174 mA) (Table-05). Temperature variation led to near about two fold increase in electrical conductivity of catholyte solution as well as anolyte after the completion of experimental run (Table -06 and 07). At lower and higher operating temperature (20 ^{0}C and 40 ^{0}C) COD removal efficiency decreased from 60.27% to 35.95% and 45.95% respectively.

Earlier report also suggests that MFC operated at 35^0C temperatures can covert substrate efficiently in mediator-less MFC. Kumar et al. (2012) and Kaushik and Jadhav (2017) reported maximum electricity production at 35 ^{0}C while testing different temperature range. Wei et al. (2013) revealed that stable power output in MFC operated at 35 ^{0}C, although good power at high temperature of 35 and 40 ^{0}C was also noted. Our findings are contrary to report from Jadhav and Ghangrekar (2009) that lower operating temperature (8–22 ^{0}C) favors the higher current output (maximum current of 1.4 mA) as compared to 0.7 mA current at higher temperature (20–35 ^{0}C). Earlier report also suggests that MFC operated at as low as 20 ^{0}C temperatures can covert substrate efficiently in MFC and are efficient under higher temperatures also. This shows the flexibility of these systems for operating it under diverse environmental temperature (Min et al., 2008). This section of the study implies that various electrochemically active bacteria may work at varying operating temperatures but for maximum bioelectricity production optimum temperature is rather important.

3.1.1. Effect of Initial pH of the anolyte medium:

Initial pH of the anolyte medium (wastewater) was optimized. For this the pH of the wastewater was set above and below the neutral pH (5.0 to 9.0) using standard acid and alkaline solution (Gil et al., 2003; Kaushik and Jadhav, 2017). The effect of initial anodic pH on the performance of MFC was studied in terms of electricity production and COD removal efficiency. pH 6.0 was found to be more effective (OCV 1.190±0.005 V and current 4.957±0.192 mA) and at neutral pH production was very close to OCV (1.161±0.007 V) produced at pH 6.0. The trend for bioelectricity production in ascending order was alkaline MFCs < acidic MFCs < neutral MFCs < slightly acidic (Table-08). Each value of pH significantly differed in the production of bioelectricity, but they are less likely to help in increasing the production (Figure-04). Also, variation in pH may lead to change the internal resistance of MFC due to change in the anodic and cathodic solution chemistry. From the results, it was clear that alkaline anolyte hinders the production of bioelectricity form isolate WRS- 6. Earlier reports suggest that neutral pH of the anolyte supports faster growth of microorganisms inside the anode chamber enabling effective performance of MFCs for wide range of substrate (Gil et al. 2003; Kaushik and Jadhav, 2017).

Our findings are similar to report from Jadhav and Ghangrekar (2009) that slightly acidic (pH- 6.5) conditions resulted in highest current as well as coulombic efficiency. Raghavulu et al. (2009) could achieve current output of 5.18 mA in acidic microenvironment. Similarly, Nimje et al. (2013) found current of 0.46 mA, 0.40 mA and 0.16–0.19 mA using distillery spent wash (DSW) wastewater of varying pH (pH 6, 7 and 8-9) respectively. Findings of our study are contrast to the some earlier reports. Gil et al. (2003) and Kumar et al. (2012) found pH 7 as optimum condition. Krishna et al. (2014) also reported that neutral pH microenvironment in paper and pulp wastewater MFCs showed higher efficiency.

Significant improvement on wastewater characteristics was noted after experimental run ended. Up to 3 fold increase in elecyrical conductivity and TDS has been recorded. 64.92% to 73.13% removal of BOD and 50.00% to 67.11% removal of COD were observed (Table-09). pH of catholyte increased up to 7.277±0.018 from initial pH of 7.06 ± 0.02 (Table-10).This indicates that, the higher amount of electrons were transferred to the cathode chamber, but the process was limited by the availability of the protons from anode chamber to final reduction reaction of the oxygen (Jadhav and Ghangrekar, 2009). The initial pH of the fresh feed catholyte was 7.0, during the operating conditions pH of cathode solution decreased from neutral to slightly acidic in case of isolate WRS-6 (Table – 08). This indicates that the proton transport through the bridge is quite good between two of the chambers which resulted in efficient performance of MFCs and the performance limiting factor could be the cathodic reactions or the ability of bacterial isolate WRS-6 to transfer the electron to the electrode. This could be further remediate by equipping the cathode with stirring conditions or the air sparger for the enhanced cathodic reactions.

Conclusions

Here, we report that newly isolated *Bacillus sp.* (designated as WRS-6) from rice bran oil refinery wastewater fed MFC can be utilized in mediator-less MFC for efficient current production as well as its treatment using inexpensive metal electrodes as electron acceptors. The experimental data showed that optimization of size of inoculum, pH of anolyte and operating temperature are important factor to achieve the higher electricity from the microbial fuel cell which have direct influence on bacterial metabolic capacity to utilize the substrate.

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Declaration of interest

Authors have no conflicts of interest to disclose regarding any financial and personal relationships with other people or organizations that could inappropriately influence (bias) our work which includes employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/ registrations, and grants or other funding.

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Figure – 01. Schematic diagram of dual chambered MFC

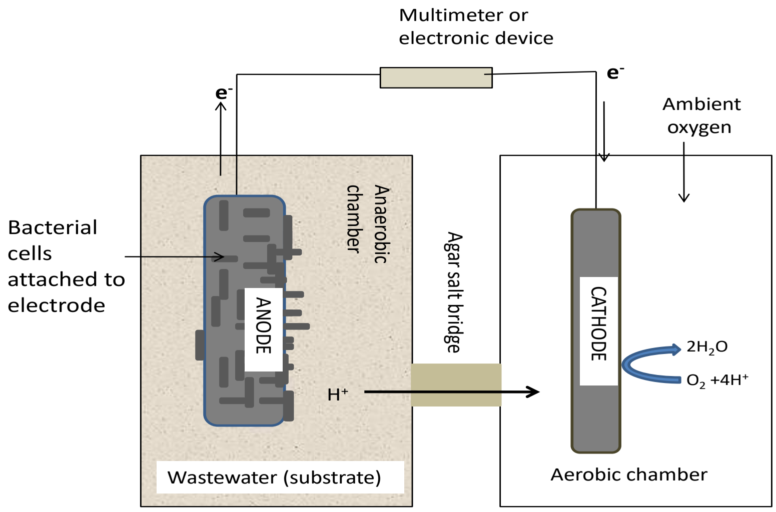

Figure-02. Showing bioelectricity production from *Bacillus sp.* (designated as WRS-6) using sterile wastewater

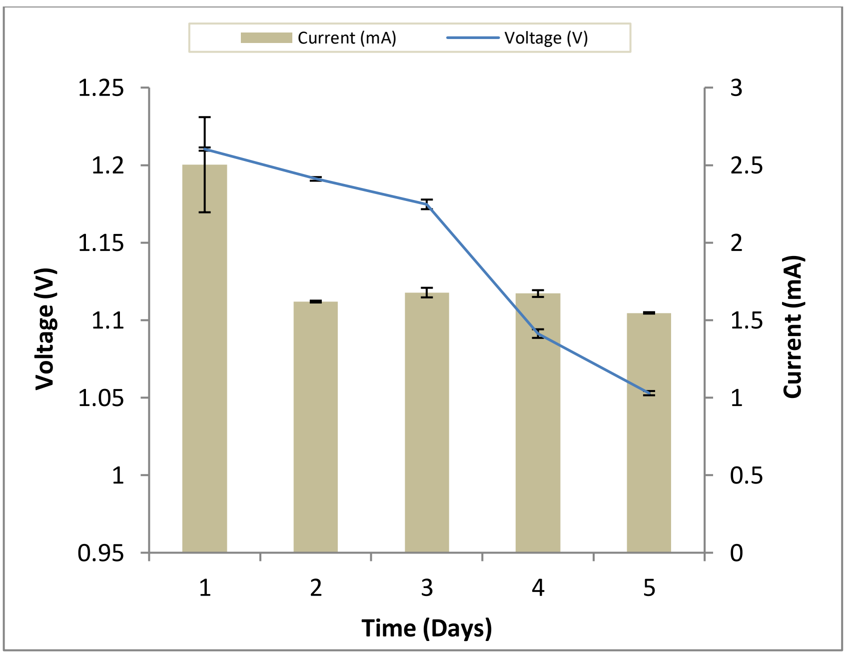

Figure-03. Showing bioelectricity production from *Bacillus sp.* (designated as WRS-6) using synthetic wastewater

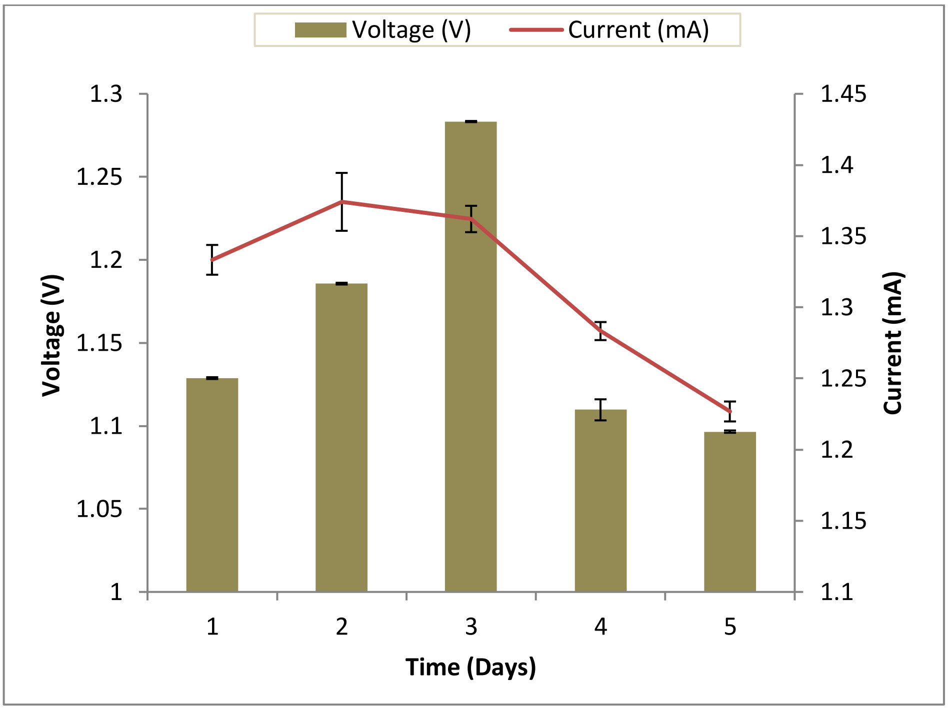

Figure-04. Effect of pH on bioelectricity production in mediatorless MFC using WRS- 6

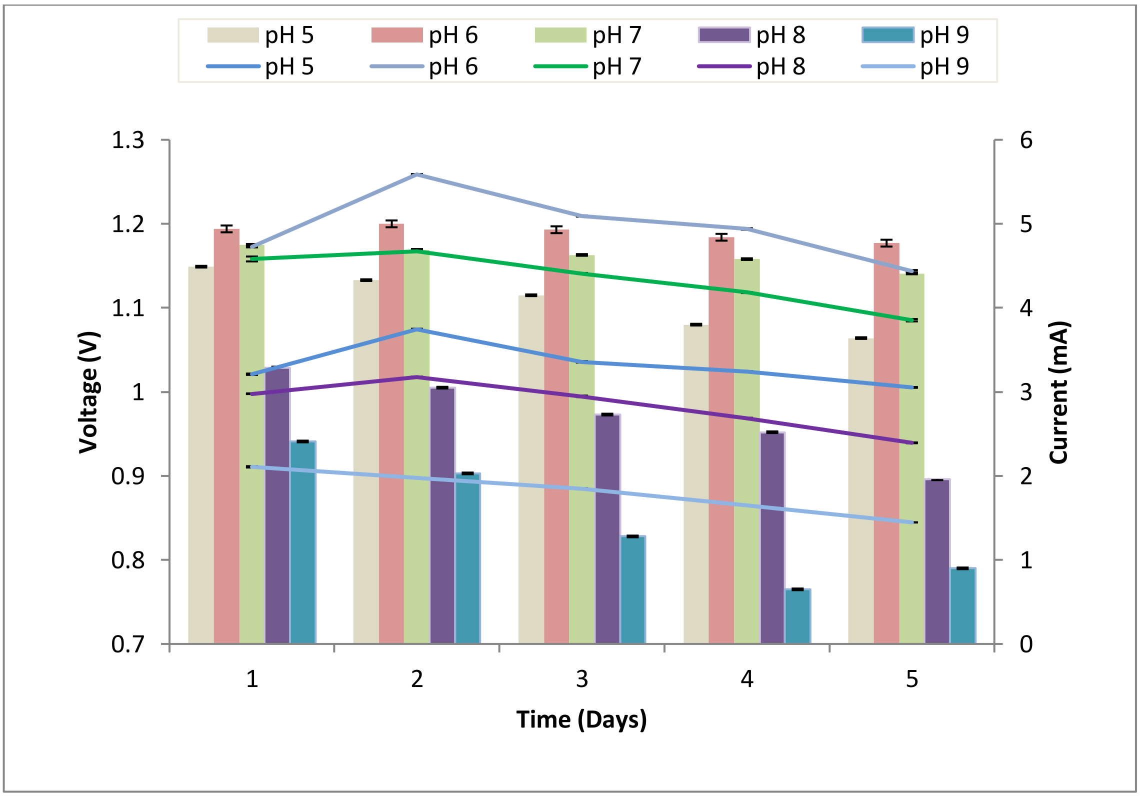

Table-01. Showing the biochemical Characteristics of bacteria isolate WRS-6

S. No.	Biochemical Test	Results
1.	Fermentative Test (a) glucose	Positive
	(b) lactose	Negative
	(c) sucrose	Positive
2.	Starch hydrolysis	Positive
3.	Urease test	Positive
4.	Citrate	Positive
5.	MR	Negative
6.	VP	Negative
7.	Indol	Negative
8.	Citrate	Negative

Table-02. - Effect of size of inoculum on bioelectricity production using WRS - 6

size of inoculum (in % v/v)	Voltage (V)	Current (mA)
0.5	1.137 ± 0.010^{c}	3.998 ± 0.313^{b}
1.0	1.239 ± 0.012^{a}	9.535 ± 0.148^{a}
1.5	1.170 ± 0.020^{b}	9.229 ± 0.496^{a}
2.0	1.028 ± 0.011^{d}	3.454 ± 0.100^{bc}
2.5	1.027 ± 0.023^{d}	2.983 ± 0.239^{c}

*ANOVA for Voltage Sum of Squares Between Groups 0.1636, Mean Square 0.04091, F 121.9882,sig >0.01.

* ANOVA for Current Sum of Squares Between Groups 211.964, Mean Square 52.991, F 73.50201, sig >0.01

Table -03. Wastewater characteristics before and after experimental run

Parameter	Before Experimental Run	After Experimental Run				
		0.5% v/v	**0.1% v/v**	**1.5% v/v**	**2.0 %v/v**	**2.5% v/v**
Colour	Pale Yellow	Translucent	Translucent	Translucent	Translucent	Translucent
Odour	Foul smelling	Less-Odorous	Less-Odorous	Less-Odorous	Less-Odorous	Less-Odorous
pH	6.62	5.74 ± 0.04	5.85 ± 0.032	6.08 ± .108	6.28 ± .238	6.11 ± .116
TDS (ppt)	2.656	3.51 ± 0.081	4.640 ± 0.22	4.719 ± 0.16	4.719 ± 0.16	4.719 ± 0.16
EC (mS/cm)	4.473	5.712 ± 0.326	7.475 ± 0.34	8.158 ± 0.08	7.235 ±0.063	7.424 ±0.237
DO (mg/L)	2.5	1.2	0.9	0.7	0.7	0.7
BOD_3 (mg/L)	26.00	8.00 (69.2% Removal)	4.00 (84.6% Removal)	6.00 (73.08% Removal)	6.00 (73.08% Removal)	6.00 (73.08% Removal)
COD (mg/L)	2560	1920 (25% Removal)	1600 (37.5% Removal)	1732 (32.34% Removal)	1960 (23.43% Removal)	2100 (17.96% Removal)

Table -04. Characteristics of catholyte before and after experimental run

Parameter	Before Experimental Run	After Experimental Run				
		0.5% v/v	0.1% v/v	1.5% v/v	2.0% v/v	2.5% v/v
pH	7.06	6.967±0.019	6.72±0.012	6.780±0.140	6.700±0.012	6.82±0.230
TDS (ppt)	2.656	4.775±0.104	6.485±0.193	6.719±0.150	7.43±0.302	7.119±0.160
EC (mS/cm)	4.473	7.760 ±0.210	8.950±0.244	12.158±0.08	16.55±0.658	15.158±0.08

Table-05. Effect of operating temperature on electricity production for five consequent days by isolate WRS - 6

Operating temperature (^{0}C)	Voltage (V)	Current (mA)
20	1.041±0.014^c	2.977±0.244^c
25	1.068±0.010^c	3.206±0.148b^c
30	1.103±0.012^b	3.767± 0.189^b
35	**1.157±0.009^a**	**4.417±0.269^a**
40	0.846±0.010^d	2.832±0.174^c

ANOVA for Voltage Sum of Squares Between Groups 0.28, Mean Square 0.07, F 113.931,sig >0.01.
* ANOVA for Current Sum of Squares Between Groups 8.503, Mean Square 2.126, F 9.685, sig >0.01

Table -06. Effect of operating temperature (in ^{0}C) on typical wastewater characteristics before and after experimental run simultaneously with electricity production for five consequent days by isolates WRS - 6

Parameter	Before Experimental Run	After Experimental Run				
		20^{0}C	25^{0}C	30^{0}C	35^{0}C	40^{0}C
Colour	Pale Yellow	Translucent	Translucent	Translucent	Translucent	Translucent
Odour	Foul smelling (++++++)	Less-Odorous (+++)	Less-Odorous (+++)	Less-Odorous (+++)	Less-Odorous (+++)	Less-Odorous (+++)
pH	5.4±0.057	5.683±0.059	5.707±0.099	5.750±0.183	6.315±0.168	6.192±0.039
TDS (ppt)	2.936±0.031	3.594±0.191	4.504±0.095	4.931±0.116	5.554±0.141	6.174±0.034
EC (mS/cm)	3.612±0.113	4.388±0.259	6.445±0.082	6.838±0.076	7.325±0.021	8.078±0.124
BOD_3 (mg/L)	23.4	11.4 (51.28% Removal)	10.66 (54.44% Removal)	8.66 (62.99% Removal)	7.46 (68.12% Removal)	9.46 (59.57% Removal)
COD (mg/L)	5920 ±160	3792±528 (35.95% Removal)	3392±128 (42.70% Removal)	2656±64 (55.14% Removal)	2352±48 (60.27% Removal)	3200±320 (45.95% Removal)

Table -07. Effect of operating temperature (in ^{0}C) on characteristics of catholyte solution before and after experimental run simultaneously with electricity production for five consequent days by isolates WRS - 6

Parameter	Before experimental run	After experimental run				
		20^{O}C	25^{0}C	30^{0}C	35^{0}C	40^{0}C
pH	7.103±0.032	6.907±0.092	6.72±0.140	6.84±0.055	6.69±0.06	6.753±0.132
TDS (ppt)	4.323±0.048	4.892±0.174	5.285±0.244	6.802±0.486	7.696±0.553	8.857±0.396
EC (mS/cm)	5.347±0.132	6.142±0.039	7.067±0.181	7.348±0.081	8.828±0.353	10.64±0.343

Table-08. Effect of pH on bioelectricity production for WRS – 6

pH range	Voltage(V)	Current (mA)
pH 5	1.108±0.008^{b}	3.321±0.160^{c}
pH 6	1.190±0.005^{a}	4.957±0.192^{a}
pH 7	1.161±0.007^{ab}	4.340±0.148^{b}
pH 8	0.971±0.023^{c}	2.836±0.135^{d}
pH 9	0.845±0.033^{d}	1.807±0.117^{e}

*ANOVA for Voltage Sum of Squares Between Groups 0.416, Mean Square 0.104, F 53.585, sig >0.01.
* ANOVA for Current Sum of Squares Between Groups 30.782, Mean Square 7.696, F 73.805, sig >0.01

Table-09. Wastewater characteristics before and after experimental run

Parameter	Before Experimental Run	After Experimental Run				
		pH 5	**pH 6**	**pH 7**	**pH 8**	**pH 9**
Colour	Pale Yellow	Translucent	Translucent	Translucent	Translucent	Translucent
Odour	Pungent	Less-Odorous	Less- Odorous	Less-Odorous	Less- Odorous	Less- Odorous
pH	3.497±0.012	5.11±0.017	5.707±0.099	6.347±0.252	7.277±0.182	8.037±0.332
TDS (ppt)	2.171±0.024	3.044±0.273	4.418±0.022	4.498±0.259	5.349±0.104	5.953±0.252
EC (mS/Cm)	3.129±0.020	3.994±0.045	6.491±0.197	6.655±0.254	7.335±0.163	9.342±0.245
DO (mg/L)	1.7	0.73	0.53	0.63	0.63	0.63
BOD_3 (mg/L)	26.8	9.4 64.92%	7.2 73.13%	8 70.15%	8.6 67.91%	9.2 65.67%
COD (mg/L)	6080 ±320	2560±640 57.89%	2000±80 67.11%	2280±360 62.50%	2824±616 53.55%	3040±160 50.00%

Table-10. Wastewater characteristics before and after experimental run

Parameter	Before Experimental Run	After Experimental Run				
		pH 5	**pH 6**	**pH 7**	**pH 8**	**pH 9**
pH	7.103±0.032	6.833±0.147	6.720±0.140	6.84±0.056	6.690±0.057	6.753±.132
TDS (ppt)	0.011±0.001	4.042±0.247	5.751±0.052	8.068±0.532	9.363±0.352	9.617±0.347
EC (mS/Cm)	6.114±0.013	6.142±0.039	8.734±0.335	10.983±0.352	12.547±0.343	14.041±0.409