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Effect of pH and temperature on mediator-less Microbial Fuel Cells for waste water degradation and sustainable bioenergy production

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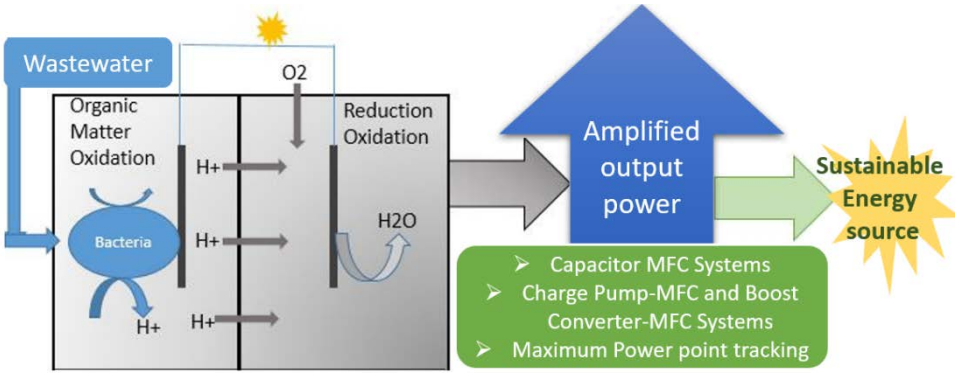
ABSTRACT



The unwanted and unmanaged waste disposal in the industrial sector thereby generating polluted waste water is one of the most critical issues in current scenario. This issue is being proactively addressed by several sectors to clean out the waste water without generation of any other secondary toxicants. However, application of microbes for such functions not only facilitates the sludge and waste water removal but also facilitates in sustainable generation of electricity in a carbon zero process. On this instance the holistic approach of microbial fuel cells has enormous efficiency. In the current study the pilot scale optimization of different types of microbes and their inoculum volume was done for highest efficiency in biodegradation of waste water and generation of maximum capacity of electricity. The study deals with waste water sample collected from sewage treatment plants in Jaipur city (Rajasthan) and its treatment procedure through microbial fuel cell technology in optimized manner. The results depicted that amongst all the four bacterial isolates, the bacterial strains, AKS2 strain was found to be the potent one at 15% of the bacterial inoculum volume. Similarly, amongst the three set temperature parameters (25°C, 35°C & 45°C), 35°C temperature and pH 7 was found to be the most effective for generation of higher Voltage (1.943±0.064V) and Current (7.793±0.007mA) and maximum rate of waste degradation (91.25± 0.03%). The current study can be utilised as an optimised pilot scale protocol for waste water bioremediation along with bioelectricity generation.

Keywords: Microbial fuel cell, bioelectricity, bioremediation, waste water, temperature, pH

INTRODUCTION

Lack of proper way to remove unmanaged industrial micro pollutant from the waste water is the major issue globally now-a-days.[1] Most of the industrial setups utilize the power of physicochemical techniques and sedimentation or absorption for removal of waste from wastewater. However, these methods add up to production of more sludge into the environment which becomes a source of secondary pollution.[2] Apart from this, the use of humongous chemicals questions its cost effectiveness.[3] Many chemical methods are even not effective enough to remove out all the waste compounds. This leads to the necessity of development of an effective method for wastewater treatment without any sludge formation.[4,5] The use of traditional methods for treatment of waste water by using physicochemical and some of the biological methods are unfavourable due to financial ineffectiveness.[6,7] and release of sludge to the water bodies.[8,2] Bacterial fuel cells are the major bio-green methodology that could be effectively applied for treatment and recovery of waste water by removal of toxic pollutants along with the utilization of organic and inorganic components for generation of electricity.[9] These cells have higher potency in systematic regulation and management of waste water without extra secondary sludge production for bioelectricity generation.[10,11] However, the mechanism and efficacy of microbial

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fuel cells not only depends up on the types of waste water used for treatment and types of bacterial isolates used; but also depends up on the concentration of bacterial isolates, environmental pH, temperature, time duration, cathode and anode material and chemical along with its concentration used for preparation of salt bridges.[5,12,13] The exoelectrogens facilitate electricity generation through the substrate oxidation.

Microbial fuel cells (MFCs) are kind of bio-electrochemical device that facilitates the conversion of chemical energy into electrical form with in organic (wastewater) substrates by the applications of microbes.[14] MFCs are acknowledged to be a popular method to produce energy from wastewater, providing a new way to concurrently to deal with wastewater at the same time as acquiring a supply of source of clean and renewable energy.[15] Microbial fuel cell is one of the holistic approach, available recently, to extenuate the possible energy scarcity sustainably through utilisation of widely available bacterial agents by development of the waste-to-energy protocol that facilitate energy production along with subtending the available waste from waste water organically. However, efficiency of microbial fuel cells relies upon several factors. To ensure the effective, optimised microbial fuel cell formulations, so as to validate the power generation efficiencies and waste degradation from selected sewage treatment plants in Jaipur city (Rajasthan), the study is framed and designed. In this study we have use salt bridges instead of Nafion membrane as salt bridge facilitates flow of ions between the anode and cathode compartments, at the same time it separates the anode and cathode compartments, preventing direct mixing of the solutions maintaining the constant pH and preventing the microbial contamination.[14]

The current study basically facilitates the optimization and selection of a suitable bacterial isolate and the inoculum concentration for preparation of effective microbial fuel cells and get maximized waste degradation percentage and bioelectricity generation at optimised temperature and pH condition. The study is the first attempt to set a pilot scale microbial fuel cell model with optimized power generation through maximum waste degradation process.

MATERIALS AND METHODS

2.1 Chemicals/Reagents required

All the required chemicals, such as agarose, Graphite Carbon electrode, liquid broth, KCl, PVC pipes, Manganous sulphate, potassium iodide, sodium hydroxide, Sodium azide, Sodium thiosulphate and nutrient agar were from Sigma Aldrich and Merck, Bangalore, India.

2.2 Bacterial isolate Culture

All the four bacterial isolates (AKS2, AKS14, BKS2 and CKW5) were primarily collected from microbial samples were collected through the collection of Sewage samples from sewage treatment plants in Jaipur city (Rajasthan). These were then isolated, cultured and characterized before use in the process of microbial fuel cell development. The microbial samples were revived on Anaerobic BasalAgar Base by streaking and incubated at 37°C for 24-48 hrs. For the operation of microbial fuel cells, 2-3 isolated bacterial samples were inoculated in 20 ml of liquid broth and incubated at 37°C at 160 rpm.

2.3 MFC Design & Component

Graphite Carbon electrode (15cm×2cm) were used as cathode and anode and tightly fixed with the containers containing medium, culture and buffer. The salt bridge was formed by dissolving 3% agarose in 1M KCl (Figure 1). The mixture was boiled and casted in PVC pipes (10×3 cm) in aseptic condition and after proper sealing was kept in refrigerator. The 1 M of KCl was used for making the salt bridge along. Two holes were constructed in lower side for insertion of salt bridge. The lead was sealed with M-sealor epoxy adhesive.[16]

2.4 Circuit Assembly

Two chambers were internally linked by salt bridge and externally the circuit was connected with copper wires which were joined to the two electrodes at its two ends and to the multimeter (Model No- DT830D) by another two ends.

2.5 MFC operations

The bacterial isolates were transferred in 20 ml liquid broth and incubated at 37°C for 12 hours. The components were pre sterilized prior to operation by using 70% alcohol and 1% $HgCl_2$ followed by UV exposure for 20 minutes. The anodic chamber was filled with 350 ml of the substrate without any pre-treatment and aerobic cathodic chamber (where oxygen was used as the final electron acceptor) was filled with equal amount of phosphate buffer. The electrolytic solution is exposed to air for reduction reaction to occur.[17] The blackwire was connected to the electrode by making a hole in the bottle lid and another end is attachedwith a multimeter using alligator clips. The same procedure was for the red wire. It was placed in static condition. The potential difference was measured by digital multimeter. The MFC was operated at room temperature for 10-12 days and voltage and current was measured at each 1-hour difference.[18,5]

2.6 Optimization of Microbial Fuel Cell

All the selected four isolated bacteria were used for optimization of MFC for effect of temperature and effect of pH different Bacterial Concentration. Parameters have an important role in the bacterial growth and optimum production of bioelectricity.[15] For pH optimization pH 5, pH 7 and pH 9 were selected. Temperature effects were measured at three different levels including range of 25°C, 35°C, and 45°C. For each strain of bacterial isolates three concentration levels were selected such as volumes of 5%, 10%, and 15% (v/v).

2.7 Determination of Percentage (%) waste degradation

The percentage change of physicochemical parameter in terms of BOD (Biological oxygen Demand) before and after operation of the MFC[20] was determined using the formula below:

$$Percentage\ (\%)\ \text{waste degradation} = \frac{initial - final}{initial} \times 100$$

The percentage change in physicochemical parameter gives an indication of increase or decrease of the pollutants measured in percentage after 10-12 days of operation of MFC.

2.8 Statistical analysis

Statistical validation was carried out utilising the Graph Pad PRISM software, 9.1.5. For validation of waste degradation ability of bacterial isolates and bioelectricity generation potency, two-way

repeated measure (RM) ANOVA was done. The analysis was carried out through utilisation of triplicate data set for waste degradation ability of individual bacterial isolate and in bioelectricity generation potency. Values are provided in mean ± SE format.

RESULTS AND DISCUSSION

Several studies are available on this aspect, however there is lagging in complete utilisation of bacterial fuel cells with optimized bioremediation protocol followed for the entire steps involved in this. Bioremediation of toxic pollutants is more frequently carried out by utilization of living forms like microbes (viz. bacteria).[21] The sustainable applications of microbes can facilitate elimination of waste without production of any associated sludge's through microbial fuel cells.[22] However, the efficient method is the use of bacterial fuel cells, having higher potency in systematic regulation and management of waste water without extra secondary sludge production for bioelectricity generation.[24] The microbial fuel cells have dual functions including bioelectricity generation and removal of pollutant from waste water along. Even many of the bacterial fuel cells also do not require external energy supply and aeration[23] and they require less secondary treatment system.[24] More over the waste water, as consisting of several organic compounds, can act as suitable substrate for microbial bioremediation to degrade all the toxic pollutants along with generation of electricity through application of microbial fuel cells. The diffusion rate of electrons can be elevated by the use of highly efficient cells with potent electrodes along with addition of certain mediators. Particularly the anodic compounds are selected in terms of suitable external environmental conditions.[11] For potential electron transfer, thereby resulting in electricity generation, the utilised microbe should be electrochemically active and contact the electrode surface. Along with this its standard potential should be nearer to substrate's redox potential.[25]

By utilising the microbial fuel cells wastewater can be treated which provides a solution for waste water treatment and energy shortages. The microbial fuel cell unit development design, type of substrate used is the vital factors that regulates the energy production rates of microbial fuel cells, while utilising different types of available microorganisms.[26]

These cells can easily function in very optimal conditions at a very minimal temperature range that facilitate the maximum amount of waste water treatment.[11] As the microbes used for such case is different, hence the physiology of the microbial cells also influences the environmental temperature and pH values within the microbial fuel cells. Besides these, the substrate oxidation rates, rate of oxygen supplementation, the microbial fuel cell circuit resistance, proton diffusion rate towards cathode compartment via proton exchange membrane, type of microbes utilised for application in bacterial fuel cells, microbial mediated electron diffusion to electrodes also plays a crucial role in the rate of energy generation through bacterial fuel cells.[27,22] The type of anodic and cathodic material, chemical used for preparation of salt bridge, distance between the two electrodes also affects the efficiency and potency of microbial fuel cells greatly. However, the cathode chamber material has a major importance as it is directly involved in energy production.

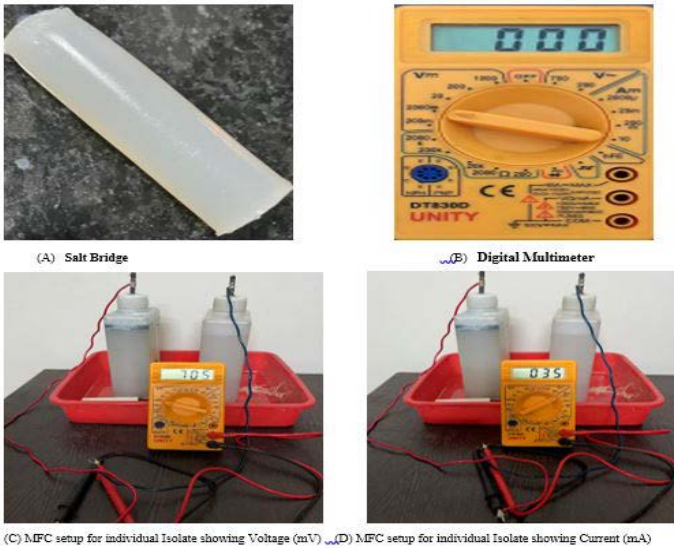

Figure 1. Microbial Fuel Cell (A) Salt Bridge (B) Digital multimeter (C) MFC set up for individual isolates showing Voltage (mV) (D) MFC set up for individual isolates showing Current (mA)

Voltage and Current generation potential of different organisms used as pure culture

The voltage generation was recorded every hour for nearly up to 18-21 days for all the bacterial strains individually. There was a uniform increase in voltage with the increase in time and bacterial inoculum as we can see from Table 1, 2 & 3. Maximum voltage and current generation in the microbial fuel cells can be observed at pH5, temperature 35°C and 15% of the bacterial inoculum volume. However out of the four selected bacterial strains, AKS2 strain was found to be the potent one with generation of 1.954±0.004 of Current (mA) and 0.923±0.003 of Voltage (V) (Table 1). When the four selected bacterial isolates were considered for generation of higher voltage (v) and current (mA), AKS2 showed the best potency followed by AKS14, CKW5 and then by BKS2.

Table 1: Voltage and Current generated by all strains at pH 5 and temp. 25, 35 & 45^{0}C at different volume of Bacterial inoculum (5, 10 & 15%)

	Bacerial inoculum	Voltage & Current	AKS2	AKS14	BKS2	CKW5
pH 5, Temp 25°C	5%	Voltage (V) Mean ±S.E.	0.201±0 .003	0.187±0 .006	0.174±0 .008	0.193±0 .007
		Current (mA) Mean ±S.E.	0.267±0 .078	0.253±0 .091	0.247±0 .031	0.283±0 .166
	10%	Voltage (V) Mean ±S.E.	0.339±0 .074	0.324±0 .053	0.296±0 .034	0.351±0 .015
		Current(mA) Mean ±S.E.	0.662±0 .002	0.637±0 .003	0.566±0 .008	0.537±0 .056
	15%	Voltage (V) Mean ±S.E.	0.603±0 .004	0.572±0 .003	0.415±0 .008	0.385±0 .02
		Current(mA) Mean ±S.E.	0.992±0 .009	0.943±0 .025	0.84± 0.046	0.798±0 .013

pH 5, Temp 35°C	5%	Voltage (V) Mean ±S.E.	0.286±0.016	0.248±0.003	0.22±0.012	0.275±0.006
		Current (mA) Mean ±S.E.	0.653±0.004	0.434±0.022	0.409±0.009	0.388±0.01
	10%	Voltage (V) Mean ±S.E.	0.492±0.01	0.41±0.011	0.419±0.008	0.449±0.096
		Current (mA) Mean ±S.E.	1.302±0.003	1.03±0.187	0.697±0.089	0.714±0.049
	15%	Voltage (V) Mean ±S.E.	0.923±0.003	0.746±0.002	0.622±0.002	0.597±0.002
		Current (mA) Mean ±S.E.	1.954±0.004	1.175±0.048	0.98±0.019	1.036±0.038
pH 5, Temp 45°C	5%	Voltage (V) Mean ±S.E.	0.218±0.015	0.208±0.012	0.21±0.014	0.232±0.007
		Current (mA) Mean ±S.E.	0.327±0.027	0.356±0.021	0.276±0.036	0.361±0.028
	10%	Voltage (V) Mean ±S.E.	0.42±0.027	0.394±0.009	0.383±0.011	0.418±0.018
		Current (mA) Mean ±S.E.	0.671±0.015	0.704±0.04	0.577±0.009	0.635±0.093
	15%	Voltage (V) Mean ±S.E.	0.609±0.009	0.578±0.023	0.484±0.006	0.529±0.024
		Current (mA) Mean ±S.E.	0.872±0.011	0.993±0.008	0.699±0.003	0.809±0.004

Table 2: Voltage and Current generated by all strains at pH 7 and temp. 25, 35 & 45^{0}C at different volume of Bacterial inoculum (5, 10 & 15%)

	Bacterial inoculum	Voltage & Current	AKS2	AKS14	BKS2	CKW5
pH 7, Temp 25°C	5%	Voltage (V) Mean ±S.E.	0.488±0.021	0.352±0.036	0.338±0.017	0.379±0.011
		Current (mA) Mean ±S.E.	1.133±0.297	1.029±0.201	1.22±0.004	1.144±0.015
	10%	Voltage (V) Mean ±S.E.	1.006±0.009	0.723±0.019	0.654±0.027	0.634±0.108
		Current (mA) Mean ±S.E.	1.738±0.013	1.855±0.046	2.442±0.1	1.911±0.275
	15%	Voltage (V) Mean ±S.E.	1.191±0.069	1.076±0.062	0.916±0.01	0.899±0.023
		Current (mA) Mean ±S.E.	2.173±0.042	2.406±0.133	2.554±0.03	2.339±0.018
pH 7, Temp 35°C	5%	Voltage (V) Mean ±S.E.	0.647±0.02	0.624±0.008	0.615±0.005	0.706±0.013
		Current (mA) Mean ±S.E.	1.715±0.223	2.382±0.023	2.195±0.073	2.395±0.006
	10%	Voltage (V) Mean ±S.E.	1.319±0.004	1.252±0.002	1.623±0.162	1.519±0.019
		Current (mA) Mean ±S.E.	5.192±0.01	4.793±0.007	4.24±0.197	4.781±0.006
	15%	Voltage (V) Mean ±S.E.	1.943±0.064	1.871±0.006	1.843±0.129	1.863±0.083
		Current (mA) Mean ±S.E.	7.793±0.007	7.192±0.009	7.131±0.114	7.173±0.008
pH 7, Temp 45°C	5%	Voltage (V) Mean ±S.E.	0.551±0.006	0.548±0.014	0.57±0.028	0.644±0.011
		Current (mA) Mean ±S.E.	1.652±0.099	1.845±0.005	1.538±0.016	1.636±0.077
	10%	Voltage (V) Mean ±S.E.	1.11±0.003	0.959±0.122	1.314±0.155	1.339±0.061
		Current (mA) Mean ±S.E.	3.671±0.491	3.693±0.011	2.759±0.101	2.576±0.089
	15%	Voltage (V) Mean ±S.E.	1.66±0.007	1.632±0.015	1.702±0.068	1.915±0.013
		Current (mA) Mean ±S.E.	5.903±0.06	5.49±0.066	5.613±0.053	5.373±0.142

Similarly, maximum voltage and current generation in the microbial fuel cells can be observed at pH7, temperature 35°C and 15% of the bacterial inoculum volume. However out of the four selected bacterial strains, AKS2 strain was found to be the potent one with generation of 7.793±0.007 of Current (mA) and 1.943±0.064 of Voltage (V) (Table 2). When the four selected bacterial isolates were considered for generation of higher voltage (v) and current (mA), AKS2 showed the best potency followed by AKS14, CKW5 and then by BKS2.

However similar pattern was also observed for pH 9. The maximum voltage and current generation in the microbial fuel cells can be observed at pH7, temperature 35°C and 15% of the bacterial inoculum volume. However out of the four selected bacterial strains, AKS2 strain was found to be the potent one with generation of 0.865±0.015 of Current (mA) and 0.574±0.015 of Voltage (V) (Table 3). However, when the optimized conditions for the bacterial isolate inoculum was to be selected for considering all other parameters fixed, amongst all the four bacterial isolates, the bacterial strains, AKS2 strain was found to be the potent one at 15% of the bacterial inoculum volume. Similarly, amongst the three set temperature parameters (25°C, 35°C & 45°C), 35°C temperature was found to be the most effective one for generation of higher Voltage (V) and Current (mA). Similarly, in case of pH optimization, pH 7 was found to be the most effective pH for higher bioelectricity generation. When the four selected bacterial isolates were considered for generation of higher voltage (v) and current (mA), AKS2 showed the best potency followed by AKS14, CKW5 and then by BKS2.

Table 3: Voltage and Current generated by all strains at pH 9 and temp. 25, 35 & 45^{0}C at different volume of Bacterial inoculum (5, 10 & 15%)

	Bacterial inoculum	Voltage & Current	AKS2	AKS14	BKS2	CKW5
pH 9, Temp 25°C	5%	Voltage (V) Mean ±S.E.	0.167±0.007	0.171±0.008	0.188±0.008	0.163±0.007
		Current (mA) Mean ±S.E.	0.209±0.005	0.204±0.005	0.152±0.011	0.125±0.007
	10%	Voltage (V) Mean ±S.E.	0.342±0.006	0.336±0.01	0.283±0.067	0.259±0.033
		Current (mA) Mean ±S.E.	0.441±0.03	0.415±0.006	0.31±0.011	0.271±0.017
	15%	Voltage (V) Mean ±S.E.	0.518±0.006	0.511±0.01	0.587±0.022	0.507±0.006
		Current (mA) Mean ±S.E.	0.674±0.033	0.652±0.043	0.488±0.012	0.409±0.01
pH 9, Temp 35°C	5%	Voltage (V) Mean ±S.E.	0.255±0.013	0.221±0.022	0.217±0.006	0.257±0.016
		Current (mA) Mean ±S.E.	0.29±0.009	0.279±0.004	0.278±0.008	0.269±0.011
	10%	Voltage (V) Mean ±S.E.	0.413±0.067	0.377±0.055	0.352±0.059	0.379±0.02
		Current (mA) Mean ±S.E.	0.557±0.031	0.555±0.015	0.547±0.009	0.525±0.007
	15%	Voltage (V) Mean ±S.E.	0.574±0.015	0.533±0.007	0.519±0.017	0.52±0.011
		Current (mA) Mean ±S.E.	0.865±0.015	0.836±0.013	0.802±0.025	0.786±0.013
pH 9, Temp 45°C	5%	Voltage (V) Mean ±S.E.	0.181±0.012	0.17±0.01	0.19±0.008	0.168±0.009
		Current (mA) Mean ±S.E.	0.227±0.011	0.207±0.011	0.209±0.008	0.19±0.016
	10%	Voltage (V) Mean ±S.E.	0.372±0.011	0.347±0.011	0.333±0.041	0.336±0.021
		Current (mA) Mean ±S.E.	0.463±0.009	0.428±0.01	0.419±0.017	0.401±0.013
	15%	Voltage (V) Mean ±S.E.	0.541±0.029	0.527±0.006	0.513±0.014	0.508±0.017
		Current (mA) Mean ±S.E.	0.698±0.012	0.654±0.004	0.64±0.008	0.61±0.01

From the above results, AKS2 showed higher potential to generate energy by 1.98±0.005 V and 7.793±0.007 mA followed by AKS14 which generated potential difference by 1.871±0.006 V and 7.192±0.009 mA. Whereas BKS2 have the capacity to generate potential difference of 1.695±0.009 V and 6.194±0.004 mA which was lesser than CKW5 which generate nearly about 1.801±0.005 V and 7.173±0.008 mA. In one of the studies the benthic microbial fuel cell (BMFC) was utilised to produce renewable energy along with bio-remediate wastewater containing aromatic hydrocarbons.[28] In another study, integrated drip hydroponics-microbial fuel cell system was used for wastewater treatment.[29] In another study, sea-water fuel cell with stainless steel cathode and platinum anode, the power supply lowers from up to 2.8 mW/m^2 after biofilm removal.[30] From the study it was evident that the development of biofilm accelerates oxygen reduction and eliminates the bad smell. In the study H. Song et.al.[31], the Constructed wetland-coupled microbial fuel cell system was being optimized to get higher bioelectricity generation. In their study, 20 cm distance within anode and cathode facilitated efficient chemical oxygen demand removal (94.90%), 0.31% columbic efficiency and 0.15 W/m^3 power generation. Furthermore, addition of 50 mM phosphate buffer solution to synthetic wastewater enhances the microbial fuel cell capacity. In another study it was being validated that bioenergy production of 1.93Wm^{-2} with 6.3% columbic potency and 2500 mg COD L^{-1} from 0.5 g L^{-1} microalgae biomass.[32]

Table 4: Bioremediation potential by all strains at different pH and Temp (pH 5,7 & 9 and temp 25, 35 & 45C) before and After Treatment

pH & Temp	Treatment	AKS2	AKS14	BKS2	CKW5
pH 5, Temp 25°C	Before Treatment	340.21	325	325	348.64
	After Treatment	62.26	68.54	46.57	50.24
	% Degradation	81.7 ± 0.01	78.91 ± 0.01	85.67 ± 0.02	85.59 ± 0.02
pH 5, Temp 35°C	Before Treatment	320.69	328.91	328.91	342.69
	After Treatment	36.63	63.74	45.09	49.1
	% Degradation	88.58±0.02	80.62± 0.02	86.29± 0.01	85.67± 0.01
pH 5, Temp 45°C	Before Treatment	325.44	319.84	319.84	372.58
	After Treatment	63.4	67.1	48.3	38.82
	% Degradation	80.52±0.01	79.02± 0.01	84.9± 0.01	89.58± 0.02
pH 7, Temp 25°C	Before Treatment	380.25	415.78	415.78	300.92
	After Treatment	33.27	49.89	58.29	40.41
	% Degradation	91.25±0.03	88± 0.03	85.98± 0.01	86.57± 0.02
pH 7, Temp 35°C	Before Treatment	320.92	360	360	306.68
	After Treatment	32.47	52.27	43.2	33.73
	% Degradation	89.88±0.01	85.48± 0.01	88± 0.01	89± 0.01
pH 7, Temp 45°C	Before Treatment	416.32	495.85	495.85	370.66
	After Treatment	54.2	76.46	94.21	41.4
	% Degradation	86.98±0.02	84.58± 0.02	81± 0.03	88.83± 0.01
pH 9, Temp 25°C	Before Treatment	312.25	342.95	342.95	369.18
	After Treatment	58.77	60.98	68.11	73.95
	% Degradation	81.18±0.02	82.22± 0.01	80.14± 0.03	79.97± 0.02
pH 9, Temp 35°C	Before Treatment	300.75	352.28	352.28	357.62
	After Treatment	57.5	78.59	54.71	59.4
	% Degradation	80.88±0.01	77.69± 0.02	84.47± 0.01	83.39± 0.03
pH 9, Temp 45°C	Before Treatment	480.25	350.81	350.81	372.41
	After Treatment	83.61	68.34	58.3	74.07
	% Degradation	82.59±0.03	80.52± 0.02	83.38± 0.01	80.11± 0.01

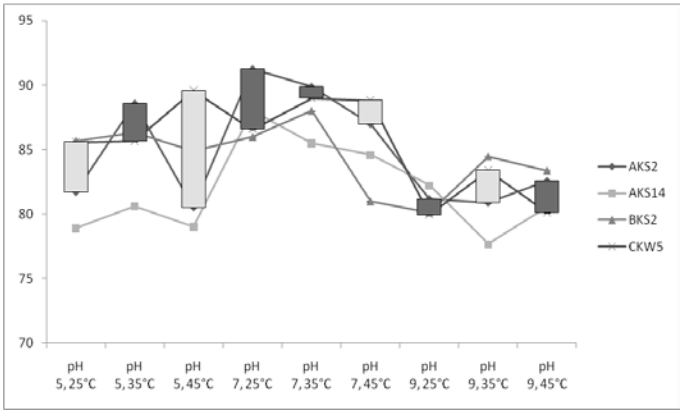

Figure 2: % of Bioremediation by all strains at different pH and Temperature (pH 5, 7 & 9 and temperature 25, 35 & 45^{0}C).

However, the optimised conditions of bacterial isolates, inoculum size, temperature and pH were also validated for the efficacy in terms of waste water treatment by checking the percentage of waste degradation (Table 4; Figure 2). More than one type of organic sources, when utilised in bacterial fuel cells, the bioremediation rate gets improved greatly.[33] When it has used a mixed waste water pattern including both domestic and brewery wastewater in a double-chambered microbial fuel cell, it got better result in the mixture of waste water system. The experiment was set by taking all the three temperature range (25°C, 35°C & 45°C) and all the three pH range (pH 5, pH 7 & pH 9).

From the results, in terms of % Bioremediation potential as well AKS2 showed higher % Bioremediation potential by 89.88% followed by CKW5 (89%), BKS2 (88%), AKS14 (85%). All the bio remedial percentages were validated statistically through Graph Pad PRISM software, 9.1.5 taking two-way repeated measure (RM) ANOVA. The results were found to be statistically significant at $p < 0.01$ with R squared value at 0.1017. The less % Bioremediation potential and energy generation was observed by the bacteria in pH 9 and 25^{0}C, 35^{0}C, 45^{0}C temperature. In general, the desired microbes behave as a prime biological catalyst that accelerates the biological degradation of given substrate i.e. waste water containing organic materials for electron generations diffusing to cathodic compartment [34]. In our study, AKS2 showed the best potency followed by AKS14, CKW5 and then by BKS2. All the selected four bacterial isolates were deposited for validation of their accession report depicted in Table 5.

Table 5: Accession report of selected bacterial isolates

Sl. No	Bacterial isolate code	Accession No.	Submission No.	Isolate scientific names according to 16s rRNA Sequencing
1	AKS2	OR144374	SUB13559118	*Escherichia coli*
2	AKS14	OR146961	SUB13560869	*Salmonella enterica* subsp. enterica serovar *Typhimurium*
3	BKS2	OR16961	SUB13559171	*Bacillus cereus*
4	CKW5	OR146746	SUB13560835	*Klebsiella pneumoniae*

From the accession report, the most potent bacterial isolate AKS2 was being validated with accession number as OR144374 and found as *Escherichia coli*. Similarly, the accession number for AKS14 was found to be OR146961. In case of BKS2 the accession number was found to be OR144416 and for CKW5 the accession number was found to be OR146746. Our findings were being validated by several other studies.[35-42] In the study of T. Aswin et.al.[37], dairy, leather and sewage wastewater was taken in microbial fuel cells with COD and BOD removal values as 80% and 64% for leather effluent, 85.4% and 79% for dairy effluent and 65% and 47% for domestic wastewater with 1.98 mW, 1.95 mW and 1.28 mW of power generations respectively. In another study carried out by K. Tota-Maharaj et.al.[36], results depicted that microbial wastewaters degradation facilitated bioelectricity production (84 and 96 mW/m^2) along with reducing the organic matter in form of BOD and COD up to 75 %. In the study of [40], the efficacy of microbial fuel cells was monitored by several internal and external parameters such as substrate feeding interval, electrode material and their spacing etc. The results depicted maximum energy generation in the range of 782 ± 12.2 mV.

CONCLUSION

Microbial fuel cell is one of the holistic approach, available recently, to extenuate the possible energy scarcity sustainably through utilisation of widely available bacterial agents by development of the waste-to-energy protocol that facilitate energy production along with subtending the available waste from waste water organically. However, efficiency of microbial fuel cells relies upon several factors such as type of fuel cell, type of electrodes, distance between them, type of microbes and their concentration, temperature, pH and many more. In the current study, the pilot scale optimization of different types of microbes was done for achieving the highest efficiency in biodegradation of waste water and generation of maximum capacity of electricity. The results depicted AKS2 strain as the potent one at 15% of the bacterial inoculum volume. Similarly, amongst the three set temperature parameters (25°C, 35°C & 45°C), 35°C temperature and pH 7 was found to be the most effective one for generation of higher Voltage (1.943±0.064V) and Current (7.793±0.007mA) and maximum rate of waste degradation (91.25± 0.03%). The current study can be utilised as an optimised pilot scale protocol for waste water bioremediation along with bioelectricity generation.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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