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Studies on bioelectricity production from microalgae *Spirulina platensis* cultivation; supplemented with tannery effluent in microbial fuel cell

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Abstract

In recent years the demand for the energy supply has become one of the major issues worldwide. Every day the energy requirement is an ever-increasing entity throughout the world due to the enormous increase in the vehicles and population. Continuous use of fossil fuels, especially petroleum oil and natural gas, has led to the environmental pollution. In this regard, renewable energy source has been viewed as a possible alternative to fossil fuels, which have potential to generate energy and also environment friendly energy source. Increasing human population and their activities are consuming the natural energy sources; it leads to depletion of fossil fuels. Active research for finding out alternate sources of energy generation especially from renewable sources is on the way. The microbial fuel cell (MFC) is a promising alternative technology that utilizes microbes as biocatalyst for power generation. Microalgae sp. offers an interesting step for wastewater treatments as well as it might be promising sources of biomass (waste water) for generating renewable energy sources.

Key words : Bioelectricity, *Spirulina*, Microbial fuel cell, tannery effluent, waste treatment.

As far as the data suggest that there are more than 2000 tanneries located all around India. Many of the tanneries (small scale and cottage sectors) are scattered in different states like West Bengal, Tamil Nadu, Maharashtra and Uttarpradesh. As per report of CLRI and State pollution control board, Chennai, 548 tanneries were situated in Vellore District (Vaniyambadi, Ambur, Melvisharam, Ranipet, and Perambat). Environmental pollution resulting from tanneries has become a huge problem in Vellore district. Tanning is the process that converts raw animal hides or skin into leather and their products. The effluents from tanning process may cause environmental pollution and ground water contamination.

Basically, during the processing of chromium tanning Cr (III) was converted into Cr (VI). Cr (VI) compounds are highly responsible for the majority of the health issues associated with all chromium compounds. The greatest environmental impact of hazardous chromium, Cr (VI), discharged from tanneries to the public, it has been the cause of various health hazards like allergic (skin rashes), nose irritation, breathing trouble, cancer, skin irritations, eye diseases, gastrointestinal problems, kidney failure, nose bleeds, weakened immune system, alteration of genetic materials, kidney and liver failure and often adversely affecting human life, livestock, and agriculture.

Increasing human population and their activities are consuming the natural energy sources; it leads to depletion of fossil fuels. Active research for finding out alternate sources of energy generation especially from renewable sources is on its way through Microbial Fuel Cell (MFC). Many microorganisms were used to produce food and fuel such as methanol, hydrogen and ethanol from the waste organic matters. It has been reported that the microorganisms can also produce electricity using MFC which degrades organic matter parallelly[14]. Microalgae species offer an interesting step for wastewater treatments as well as it might be promising sources of biomass for generating renewable energy sources. *Spirulina* can adapt to a wide range of environments like marshes, soil, sand, sea water, brackish water, and fresh water. In our earlier study we report the development of thermal tolerant *Spirulina* mutants for long lasting production of single cell protein under unfavourable climates of tropical regions[2].

MFC is a biochemically catalysed system, it can able to generate electricity by oxidizing organic matter with the presence of microalgae. The biocatalyst was taken in the anode chamber of MFC that can generate electron (e^-) and proton (H^+) through the anaerobic respiration of biocatalyst. The emitted electrons were transferred through the electrode anode (-) connected with the external circuit cathode (+) and the protons diffuse and transferred through PEM (Proton Exchange Membrane which bifurcates cathode and anode) and entered into cathode chamber and combined with electron acceptor. The potential difference between electron acceptor and anaerobic respiration of biocatalyst can generate current and voltage and produce electricity.

The aim of the present study is to investigate the methods of removing pollutant in tannery effluent with the recovery of oleaginous *Spirulina* microalgae used as biocatalyst cultivated in tannery effluent liquid medium and producing bioelectricity by using microbial fuel cell (MFC) to create pollution free environment. The present investigation was therefore undertaken with the aim of dual purpose of bioelectricity production and *spirulina* cultivation with focus on improvement of production efficiency, bio-electricity production and cost effectiveness in an eco-friendly manner. For the preparation of the manuscript relevant literature[1-14] was consulted.

Isolation and identification of Spirulina platensis micro algae from water bodies:

The microalgal samples were collected from aquatic habitats like ponds, tanks and lakes in different locations. Morphological characters (size, shape, color) were observed under low power and high-power objectives of the compound microscope and measured by micrometry method and confirmed as *Spirulina Platensis.* The identified *Spirulina Platensis* microalgae was grown in Zarrouk's medium under laboratory condition at 30ºC in the light chamber for 30 days and estimated the growth parameters like cell population, specific growth rate and doubling time at 10 days interval on 10th, 20th and 30th days. Biomass concentration was calculated by measuring dry weight of *Spirulina platensis*[13], Protein content was estimated by Lowry *et al.,*[9] and Lipid content was estimated by Chlorophyll content method given by Mc Kinney[10].

Collection and characterization of physio-chemical nature of tannery effluent :

Collection of tannery effluents from Vellore District. Various physio-chemical like color, odor, water temperature, electrical conductivity, pH, total solids, suspended solids, dissolved solids, BOD, COD, DO, Chromium, sulphide, Nitrate, minerals and heavy metals were estimated. The tannery effluent sample was kept in a refrigerator at 4°C before use. The collected tannery effluent sample was used with full strength to analyse all the physio-chemical nature for the MFC setup without any modifications such as addition of nutrients, pH adjustments, filtration and removal of impurities and trace metals. The effluent was kept in a refrigerator at 4°C for further studies.

Enumeration of microbial population in tannery effluent :

The biological nature of tannery effluent was (Bacteria, actinomycetes and fungi) determined by standard methods like spread plate on Nutrient agar medium, Rose Bengal Agar medium and Kenknight's agar medium respectively. The bacterial, fungal and actinomycetes colonies on the respective plates were counted and CFU calculated.

CFU/mL = Number of colonies counted × Amount of sample taken × Dilution factor

Formulation of novel tannery effluent medium :

The suspended particles and impurities were removed from Tannery effluent by filtration method and supplemented with various minerals like $NaNO_3$ 2.5 g L^{-1}, K_2HPO_4 0.5g L^{-1} and $CaCl_2$ 0.04g L^{-1} and formulate new tannery effluent base medium for cultivation of ***Spirulina platensis.***

MFC Construction :

The design of the microbial fuel cell was constructed by single chamber setup[11]. Two glasses that consist of tannery effluent and microalgae (glass A) and phosphate buffer (glass B) respectively are separated by a glass bridge (of hole size 6x6 cm) covered with proton exchange membrane. An ionic salt of KCl or NaOH is melted with agar, transferred into a cylindrical cast and left undisturbed for solidification. Following the solidification of components, it is used as a bridge that is placed between the two chambers of the MFC which, in turn, acts as a PEM aiding the transfer of protons. Glass A consists of a plain carbon rod as an anode while, glass B contains graphite that acts as a cathode and are attached to each other with the help of a copper wire wrapped in a nonconductive epoxy. The chamber with the anode is constantly flushed with N_2/CO_2 to uphold anaerobic condition. The cathode chamber, on the other hand, comprises phosphate buffer with pH 7 to ensure aerobic condition. MFCs are devices that can convert chemical energy into electrical energy that uses microalgae to oxidise the organic matter present in the tannery effluent.

MFC operation, monitoring and production of bioelectricity :

The microalgae *Spirulina platensis* slurry (5g) was diluted with the tannery effluent medium (700 ml) taken in one litre capacity anode chamber of Microbial fuel cell and filled phosphate buffer in cathode chamber. Anode and cathode chamber was connected by glass tube with membrane as the media of ion proton transfer. The chambers were connected with electrodes, the transferred electron was measured with multi meter for 24-hour illuminated for 8 days. After all material and chambers are set, measure bioelectricity and test the sample every 24 hour with 6 h interval.

Monitoring and production of bioelectricity:

Current (*I*) was measured using a Digital Multimeter (Model - 108, Kusam electrical industries, India) by external circuit connection with 10Ω. According to Greenberg *et al.*, (1992), COD was measured using standard procedures. Filtrate collected from all the samples using 0.22 µm (pore size) membrane filter were used for COD estimation.

COD removal was calculated as follows:

$$ECOD= [COD_{in} - COD_{out} / COD_{in}] \times 100\%,$$

Where, COD_{in} is the COD of influent and COD_{out} is the COD of effluent.

The tannery effluent was collected from Vellore District, Tamil Nadu, India. The base Tannery Effluent Medium (TEM) was brownish in colour, the odour was unpleasant with pH 8.2 at 25ºC. All the physio-chemical contents were much higher than the recommended standards set by IS 17374: 2020 (Table-2). The high BOD level was decreased by Millipore (0.45µm pore diameter) filtration technique. From the table-1, it is observed that total viable bacterial count was two folds higher than fungal count which in turn would two-fold higher than actinobacteria. Moreover, the tannery effluent was rich in various heavy metals like Chromium (103.6) followed by iron (39.8), zinc (7.50), arsenic (2.80) copper (1.42), nickel (1.32), lead (1.25), magnesium

Table-1. Enumeration of microbial population in Tannery effluent

Organisms	Medium	Population per ml (Log_{10} CFU g^{-1})
Bacteria	NA	10.34
Fungi	RBA	5.69
Actinomycetes	KKA	2.34

Note: NA - Nutrient agar; RBA – Rose Bengal Agar; KKA – Ken Knight Agar

Table-2. Physico-chemical nature of Tannery Effluent Medium

S.No	Test parameters	Unit	Test results
1.	Colour	Hazen	Brownish
2.	Turbidity	NTU	60
3.	Electrical conductivity	Mu	1.75
4.	pH@25°C	No unit	7.82
5.	Specific conductance@25°C	mS/cm	10060
6.	Total dissolved solids (TDS)	mg/lit	6280
7.	Residual free chlorine as Cl_2	mg/lit	13.3
8.	Total suspended solids (TSS)	mg/lit	8.34
9.	Total alkality as $CaCO_3$	mg/lit	3230
10.	Total hardness as $CaCO_3$	mg/lit	3050
11.	Biological oxygen demand (BOD-27°C for 3 days)	mg/lit	148
12.	Chemical Oxygen demand (COD)	mg/lit	824
13.	Dissolved Oxygen (DO)	mg/lit	4.45

(1.18), cadmium (1.15), mercury (1.12) and selenium (1.10) (Table-3). Unsafe release of the heavy metal into the soil or water bodies may result in bioaccumulation within plant or animal systems thereby contamination of food chain occurs causing severe threat to humans[12].

The microbial populations of tannery effluent medium as shown in table-1. Among the microbial populations bacteria were dominant (10.34 Log_{10} CFU g^{-1}) followed by fungi (5.69 Log_{10} CFU g^{-1}) and actinomycetes (2.34 Log_{10} CFUg^{-1}). The result of bioelectricity produced from dual chamber (1:1 and 2:2 electrodes) on the microalgae-microbial fuel cell (MMFC) technology is given in the table 4 and table-5.

The results shown in the graph above is the voltage (mV) obtained from the different electrodes (1:1 and 2:2), where the highest voltage was obtained in 2:2 electrodes on the eighth day of 122 mV with the absorbance value of *Spirulina* cell growth of 1.330 followed by 62 mV observed on 1:1 electrode on the same day with the absorbance value of 0.910 indicating the biomass increase of *Spirulina*. The *Scenedesmus* microalgae

Table-3. Estimation of minerals and Heavy metals in TEM

S. No.	Test parameters	Unit	Test results
1.	Calcium (Ca)	mg/lit	926
2.	Magnesium (Mg)	mg/lit	423
3.	Total sulphide (SO_2)	mg/lit	20.8
4.	Chloride (Cl^-)	mg/lit	2540
5.	Sulphate (So_4^-)	mg/lit	712
6.	Total phosphorus (Po_4^-)	mg/lit	3.50
7.	Nitrate (NO_3)	mg/lit	73.2
8.	Nitrite (NO_2)	mg/lit	2.5
9.	Fluoride (F)	mg/lit	6.0
10.	Sodium (Na)	mg/lit	22.0
11.	Potassium (K)	mg/lit	10.2
12.	Arsenic (As)	mg/lit	2.80
13.	Cadmium (Cd)	mg/lit	1.15
14.	Copper (Cu)	mg/lit	1.42
15.	Lead (Pb)	mg/lit	1.25
16.	Mercury (Hg)	mg/lit	1.12
17.	Zinc (Zn)	mg/lit	7.50
18.	Total chromium (Cr)	mg/lit	103.6
19.	Iron (Fe)	mg/lit	39.8
20.	Nickel (Ni)	mg/lit	1.32
21.	Selenium (Se)	mg/lit	1.10
22.	Manganese (Mn)	mg/lit	1.18

Table-4. Bioelectricity production and cell growth in days on 1:1 electrode

S.No.	Days	Electricity production (mV)	*Spirulina* cell growth (OD)
1.	0	0	0.000
2.	2	32	0.057
3.	4	45	0.102
4.	6	59	0.544
5.	8	62	0.910
6.	10	72	0.855

Table-5. Bioelectricity production and cell growth in days on 2:2 electrode

S.No.	Days	Electricity production (mV)	*Spirulina* cell growth (OD)
1.	0	0	0.000
2.	2	75	0.452
3.	4	92	0.750
4.	6	109	1.019
5.	8	122	1.330
6.	10	92	1.011

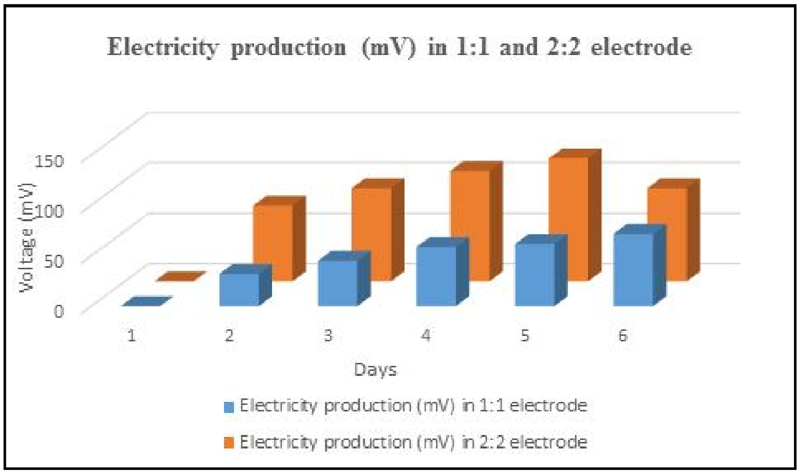

Figure 1. Bioelectricity production (mV) under the growth of *Spirulina platensis* in Tannery effluent medium filled Microbial Fuel Cell (MFC)

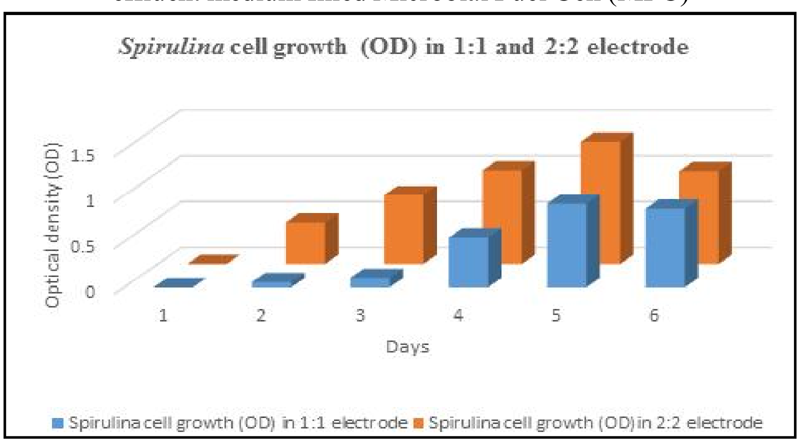

Figure 2. Effect of Bioelectricity producing Tannery effluent medium filled Microbial Fuel Cell (MFC) on the growth (OD value) of *Spirulina platensis* biomass

carried out the phycoremediation of tannery waste water and reduce the pollution load of heavy metals (Cr-8.2-2.96%, Cu- 73.2- 98%, Zn- 65-98% and Pb- 75-98%). Tannery effluent making as a growth medium for *Scenedesmus* algae for phycoremediation of toxicants[1].

During MFC operation, all the MFC systems were constantly monitored for the removal of COD that enumerate the potential of fuel cell to act as wastewater treatment system.

After production of bioelectricity, the total COD range 80% was removed. The higher range of COD removal that indicate the growth of *Spirulina* cells will be higher, which showed higher production of bioelectricity. The tannery biocatalyst exhibited potentials for COD removal indicating the massive growth of microalgae that helps to catabolize the components of tannery effluent utilizing the carbon source as electron donors. It shows that current generation and COD removal in a relative compatibility and COD was observed to be continuously decreased in the MFC system.

In the present context of energy need, it is essential to develop a cost-effective way of energy production by exploiting the bioenergetics of microbial cell factory. In parallel, everyday accumulation of untreated effluents resulting from tanneries alarm the terrestrial and aquatic landscape due to undesirable toxicity. In this study, we make an *Art from waste* model to generate electricity using biological system. *Spirulina platensis* was cultivated in tannery effluent supplemented medium to reduce the overall cost for cultivation in a specially designed MFC which produced considerable electricity for future possible applications.

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: All the authors have read the manuscript and approved for its submission

Availability of data and materials: All the datasets are included in the manuscript

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Authors' contributions:

Dr. KA devised, conceived the study, performed the experiments, analysed the data, and wrote the first draft of the manuscript. Mr. KV and Mrs.MK assisted data analysis, revised and prompted the final draft of the manuscript. Ms.AA and Mr.KBSB assisted experimentation and data representation.

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