

## Microbial fuel cell powered by lipid extracted algae: A promising system for algal lipids and power generation

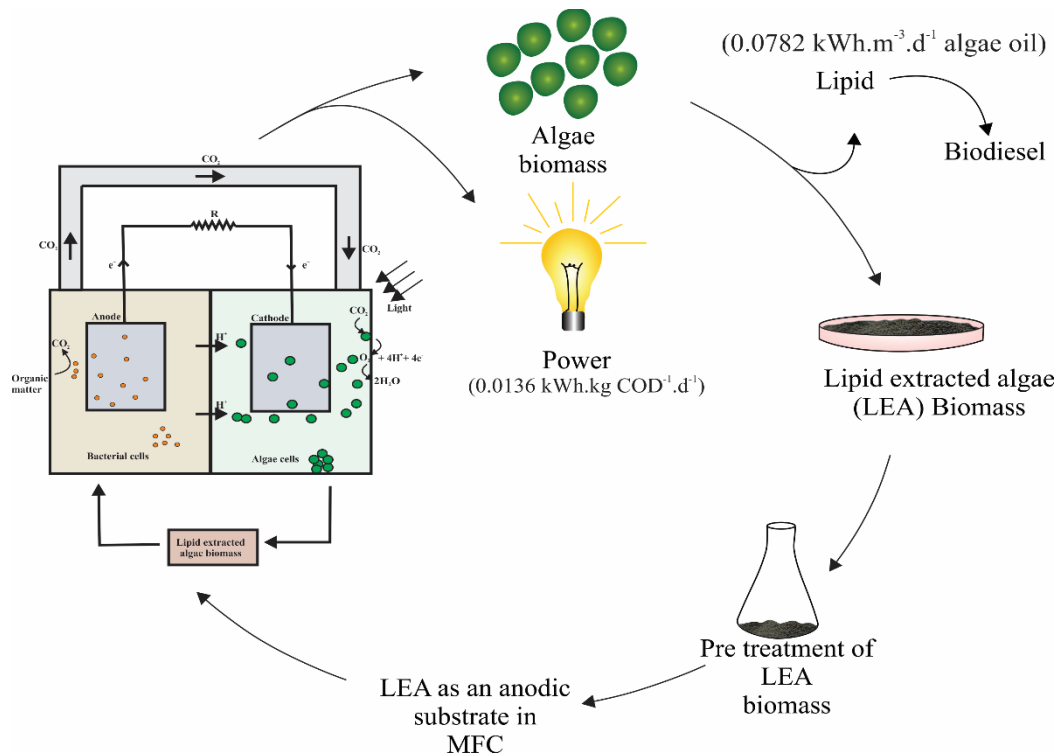
Microbial fuel cell (MFC) technology has established itself as a new energy alternative towards sustainable development. One of the modified versions of the MFC is the microbial carbon capture cell (MCC) or photosynthetic microbial fuel cell (PMFC) or algae assisted MFCs, which offers high efficiency for wastewater treatment, CO<sub>2</sub> sequestration, power generation and algae biomass production [Xiao et al., 2012].

Despite the potential MFC technology holds, it is far from being called a sustainable system because of several reasons. One of these is the need of the continuous supply of electron donors and the electron acceptor at anode and cathode respectively [Mateo et al., 2014]. There are various types of substrates used in MFC system in order to achieve high power output, high chemical oxygen demand (COD) removal rate, and high columbic efficiency. These range from simple or defined wastewater substrates like monosaccharides, sugar derivatives, polyalcohols, amino acids, organic acids, alcohols, nitrogenous heterocyclic compounds etc. to complex or undefined wastewater substrates like beverages industry wastewater, confectionary industry wastewater, dairy industry wastewater and much more [Pandey et al., 2016]. However, use of defined or undefined substrates is not economically viable. Hence, there is need to find renewable substrate which can be continuously produced and continuously fed to get consistent power output. Algal biomass either in the form of dry biomass or living cells can also act as an anodic substrate [Xiao et al., 2014; Garcia et al., 2016; Yuan et al., 2011]. Cui et al. (2014) and Velasquez-orta et al.(2009) reported 8.67 W/m<sup>3</sup> and 277 W/m<sup>3</sup>of power density respectively, using microalgae biomass as a substrate [Cui et al., 2014; Velasquez-orta et al., 2009]. *Chlorella vulgaris* biomass has high nutritional value comprising of proteins (42-55%) and carbohydrates (12-55%) which can be mineralized by electrogenic bacteria to generate electricity [Cui et al., 2014; Safi et al., 2014].

In this study, an attempt to increase the energy recovery from the system was made by extracting lipids from algae grown at the cathode and utilizing the lipid extracted algae (LEA) biomass as an anodic substrate. However, as of now an unsuccessful attempt to use lipid extracted algae (LEA) as an anodic substrate has been made wherein the authors reported an OCV (Open circuit voltage) of 0.021 V only [Rashid et al., 2013]. The reason behind the failure of LEA as an anodic substrate was attributed to the presence of possible inhibitory substances in LEA [Rashid et al., 2013]. The present study has demonstrated the use of LEA biomass as an anodic substrate, successfully first time in the literature. The algae is cultivated in the cathode chamber of MFC. This algae biomass is harvested and lipids are extracted. The extracted lipids can be further processed for biodiesel production. The residual biomass left after lipid extraction process i.e. LEA can be used as an anodic substrate which reduces the dependency on the external supply of electron donor substrate. LEA is also a waste byproduct of the algae based biodiesel industry. Hence, this strategy makes a system self-sustainable and economically viable. The proposed scheme is shown in figure 3.1. Keeping in view the above said points, the present study was conducted with following objectives:-

1. To evaluate the potential of LEA biomass as an anodic substrate.
2. To compare the performance of LEA biomass with another complex yet easily utilizable substrate like fruit waste/pulp.

- To perform the energy analysis of the system to determine whether the system is a net energy producer.



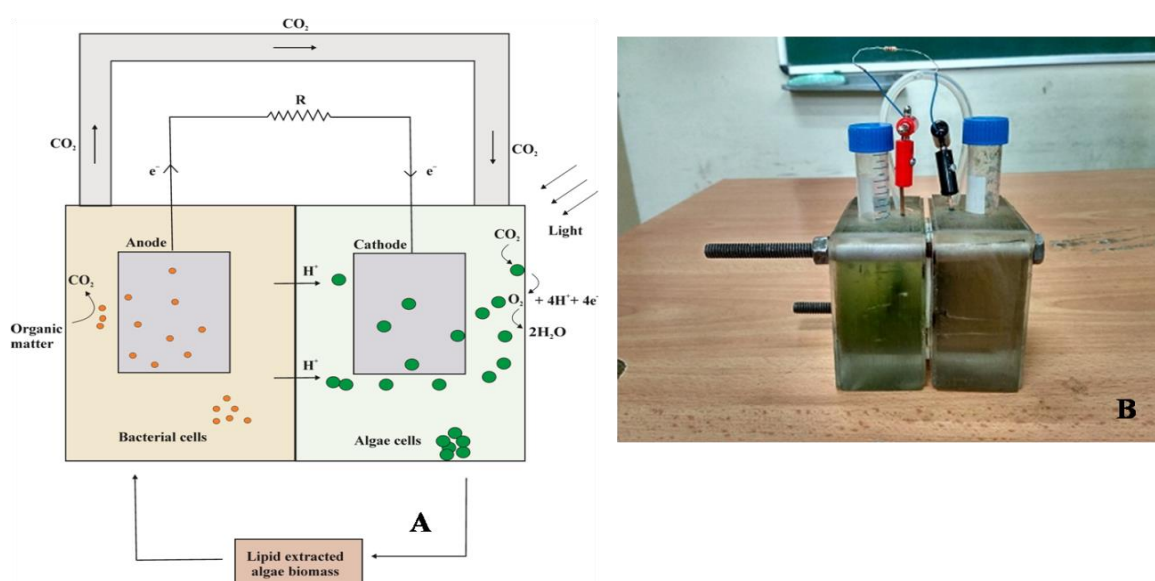
**Figure 3.1:** Schematic presentation of the strategy employed in the current study.

### 3.1 MFC CONSTRUCTION & OPERATION

Dual chamber MFC reactors were fabricated using acrylic material. The working volume of both the chambers was 100 ml. Graphite felt (4 cm×4 cm×0.56 cm) was used as the anode and the cathode. Both the anode and the cathode were connected by a Cu wire (gauge 2mm). A schematic diagram of a respective chemical process and the photograph of actual MFC reactor, used for the proposed system are shown in Figure 3.2 A and 3.2 B. Nafion-117(DU Point, USA) was used as a separator between anode and cathode chamber. Pre-treatment of nafion was done as described previously [Ghasemi et al., 2013]. Pre-treated cow manure was used as the source of exo-electrogens. Pre-treatment of cow manure was carried out as described previously [Vijay et al., 2016]. The anode chamber was fed with pre-treated cow-manure, anolyte, and a substrate. Anolyte was composed of  $\text{KH}_2\text{PO}_4$  4.4 g/l,  $\text{K}_2\text{HPO}_4$  3.4 g/l, NaCl 0.5 g/l,  $\text{MgSO}_4$  0.2 g/l,  $\text{CaCl}_2$  0.014 g/l and  $\text{KNO}_3$  1 g/l [Clauwaert et al., 2007].

The anodic bacterial community was acclimatized with LEA by mixing the pre-treated cow manure with LEA [3.5 g/l], followed by anaerobic incubation at 30 °C for a week. Three types of MFCs were constructed with the following experimental conditions. **LEA-fed MFCs** anode consisted of pre-acclimatized microbial consortia, obtained from cow manure, (50 ml) as described in section 2.7+ LEA biomass (2 g/l) + anolyte to make up the volume. **FP-MFC** consisted of pretreated cow manure slurry (50 ml) + orange fruit pulp (FP) (32 g/l) + anolyte to make up the volume. Control-MFC contained 50 ml of cow manure and 50 ml anolyte. The concentrations of LEA and FP in different reactors, except control, were adjusted in such a way that the initial COD (Chemical oxygen demand) is nearly equal to make a performance comparison. Cathode was same for all the MFC reactors with catholyte containing BG 11 media inoculated with *C. vulgaris* at an initial concentration of  $21.12 \times 10^4$  cells/ml. Prior to incubation,

all MFCs anodes were flushed thoroughly with oxygen free nitrogen gas. A fixed 1000 ohm resistor was used as an external load in all MFC reactors. All the experiments were done in a fed-batch mode, wherein a single batch of LEA-fed MFCs sustained 12-120 hours of continuous power generation. After a drop in voltage in each batch cycle, MFC reactors were supplemented with LEA/FP to regain voltage and current.

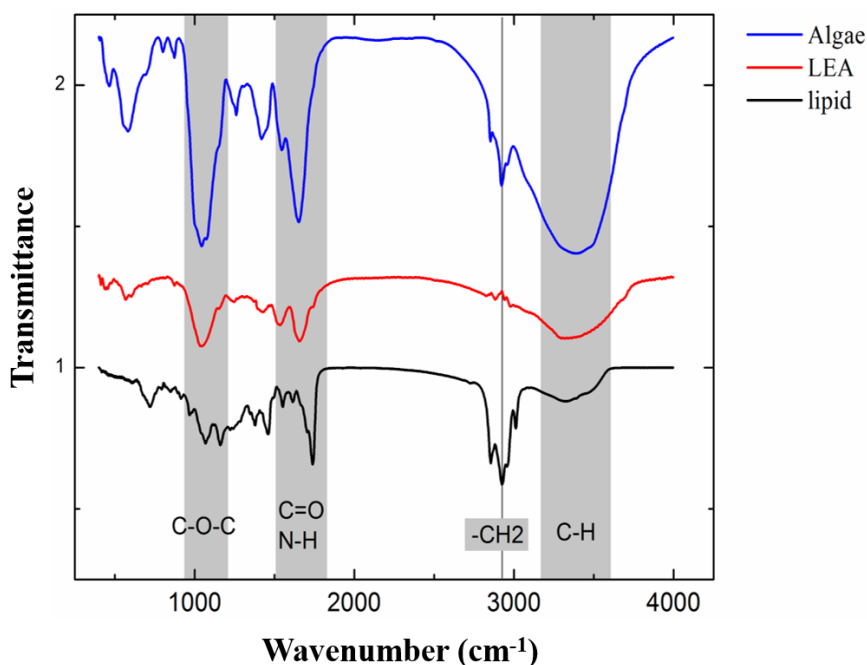


**Figure 3.2:** A) Schematic representation of microbial fuel cell (MFC), and B) MFC reactor used in the study.

## 3.2 RESULTS AND DISCUSSION

### 3.2.1 FTIR analysis of algae biomass, LEA biomass and algae-derived lipid.

Total lipid content was calculated gravimetrically. Without imposing any nitrogen limitation,  $36 \pm 6\%$  of lipid content was estimated. FTIR analysis (Figure 3.3) confirmed that both algal lipid sample and intact algal biomass sample has  $-\text{CH}_2$  symmetric and asymmetric stretching vibrations at  $2922\text{--}2927\text{ cm}^{-1}$  and  $2850\text{ cm}^{-1}$  respectively, which substantiates the presence of lipid. The lipid sample has characteristic lipid peaks at  $2927\text{ cm}^{-1}$  and  $2850\text{ cm}^{-1}$  respectively. Apart from this, lipid sample also exhibit absorption shoulder at  $1737\text{ cm}^{-1}$  which is due to the presence of  $\text{C}=\text{O}$  group of esters and fatty acids. On the other hand, LEA biomass or de-oiled cake sample did not exhibit such lipid characteristic absorption bands, indicating the removal of lipid content from the algae. Also, LEA biomass showed absorption at  $1652\text{ cm}^{-1}$  and  $1525\text{ cm}^{-1}$  which were due to  $\text{C}=\text{O}$  stretching vibration and  $\text{N-H}$  bending vibrations of peptide bond respectively [Stehfest et al, 2005]. Furthermore, LEA biomass is exhibiting absorption spectra at  $1058\text{ cm}^{-1}$  to  $570\text{ cm}^{-1}$ . This spectrum scale covers the  $\text{C-C}$ ,  $\text{C-O}$ ,  $\text{C-O-C}$  and  $\text{C-O-P}$  stretching vibrations of polysaccharides [Phukan et al., 2011]. These observations suggest that LEA biomass is essentially composed of carbohydrates and proteins and is nearly lipid free.



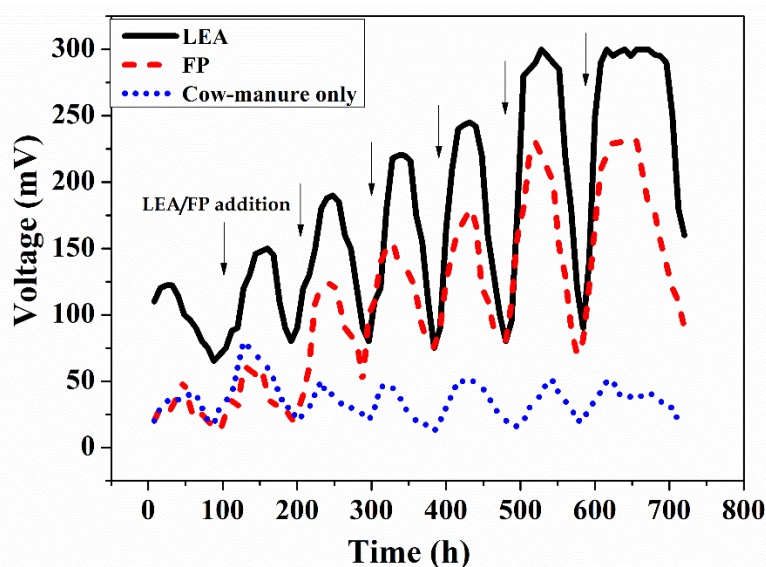
**Figure 3.3:** FTIR spectrum obtained for intact algae biomass, lipid extracted algae (LEA) biomass and isolated lipids.

### 3.2.2 Profile of voltage v/s time as obtained for different experimental MFCs.

The variation of voltage with time is shown in figure 3.4 for all MFCs. The acclimatized LEA-fed MFC didn't take any start-up time and exhibited a voltage of  $120 \pm 11.5$  mV after 1 day of operation and it reached up to 300 mV at 1000 ohm of external resistance. The possible reason for lower lag period can be that the pre-incubation of LEA biomass with cow manure in LEA-fed MFC helped the bacteria, present in the cow manure to degrade LEA biomass into small chain fatty acids. These fatty acids can be easily mineralized in the MFCs anode thereby exhibiting a lower start up time and better power output. Similar observations were made by Walter et al.(2015) wherein introducing a pre-digester for intact algae biomass mineralization helped in obtaining higher voltage and higher power output in cascade MFCs [Walter et al, 2015]. During the last batch cycle, voltage value reached to 250 mV and continued for 104 hours in LEA-fed MFC. The steady voltage generation during this period can be attributed to continuous supply of electrons from the anode and continuous acceptance of electrons at the cathode. The decline in anodic COD (measured during the course of analysis) or LEA depletion, after 104 h was held responsible for the performance decay. As the performance decayed, anodes were supplemented with 2 g/l of LEA, which helped in regaining the power output. Another very important factor which affects the power output is the electron acceptor at the cathode. Since algae based oxygen was the intended electron acceptor, the voltages are also expected to drop during dark periods as observed by previous researchers [Walter et al., 2013; González del Campo et al., 2015]. In our study, however, during dark periods (considering electron donor at the anode is not limiting), no substantial decline in the voltage and current outputs were observed. The fluctuations in voltage outputs were present but these fluctuations were not coincident with the dissolved oxygen (DO) concentrations. The concentration of DO in our study varied from 3.5 mg/l in the dark period to 6 mg/l during the day period. Absence of organic matter and not very high algae concentrations at the cathode probably resulted in good enough oxygen concentration at the cathode. These observations further point to the fact that the electron acceptor at cathode was not the limiting factor for power output. Similar observations have been made earlier [Wang et al., 2010; Colombo et al., 2017]. Previous researchers had also observed considerable power outputs during dark periods even when the DO concentration approaches zero and it was believed that ions such nitrate (present in the

algae growth medium) can support power output [Rodrigo et al., 2010; González del Campo et al., 2013; González del Campo et al., 2015]. Also, the reduction potential of nitrate ion is close to that of oxygen, even though activation over potentials differs, the expected voltage outputs are not drastically different with such alternative electron acceptors. Nevertheless, in our study oxygen concentration never came below 3.5 mg/l.

FP-MFC, on the other hand, exhibited the voltage output of  $30 \pm 7$  mV after 1 day of operation and took 232 hours to reach the magnitude of  $120 \pm 16.5$  mV. Further, the voltage in FP-MFC increased up to 230 mV and then decreased rapidly. Unlike LEA-fed MFC, the voltage was not sustained for the longer period in FP-MFC. This suggests that LEA is more energy dense substrate as compared to fruit pulp. *C. vulgaris* has 42-58% of total protein content, which is variable according to growth and environmental conditions. Similarly, carbohydrate profile of *C. vulgaris* includes polysaccharides like starch, cellulose,  $\beta(1\rightarrow3)$  glucan etc [Safi et al., 2014]. The sugar composition of cell wall polysaccharides includes rhamnose, arabinose, xylose, mannose, galactose and glucose [Takeda, 1991]. Cow manure was chosen as the source of bacteria because it is expected to contain microbial consortia which can mineralize the heterogenic mixture of organic compounds present in algae biomass. It was observed that LEA-fed MFC exhibited three stages in every batch cycle:-an initial, continuous, and steady build up in voltage-exponential phase, a plateau of maximum voltage which sustains for many days-stationary phase, and a gradual decline in voltage-decay phase, as showed in figure 3.4. Similar voltage v/s time profile has been reported earlier [Cheng et al.,2011].



**Figure 3.4:** The profile of voltage (across external resistor 1000 ohm) with time, obtained for different experimental MFCs. Arrow indicate addition of LEA/FP.

### 3.2.3 COD degradation rate obtained for different experimental set ups.

COD degradation rate reflects the adaptability of microbial consortia for a particular substrate and also determine the overall efficiency of the system. This also decides the hydraulic retention time (HRT) for continuous flow systems. Higher COD degradation rates impart lower HRTs and lower reactor cost to the process under consideration. The COD removal by electrogens in present system is also responsible for the  $\text{CO}_2$  generation in anode chamber which in turn controls algal growth rate in the cathode chamber. The COD removal rate ( $\text{kg}/\text{m}^3/\text{d}$ ) obtained for LEA, FP, and control MFCs are  $0.29 \pm 0.11$ ,  $0.28 \pm 0.04$  and  $0.05 \pm 0.03$   $\text{kg}/\text{m}^3/\text{d}$  respectively (Table 3.1).

### 3.2.4 Polarization studies and power output as obtained for different experimental set ups

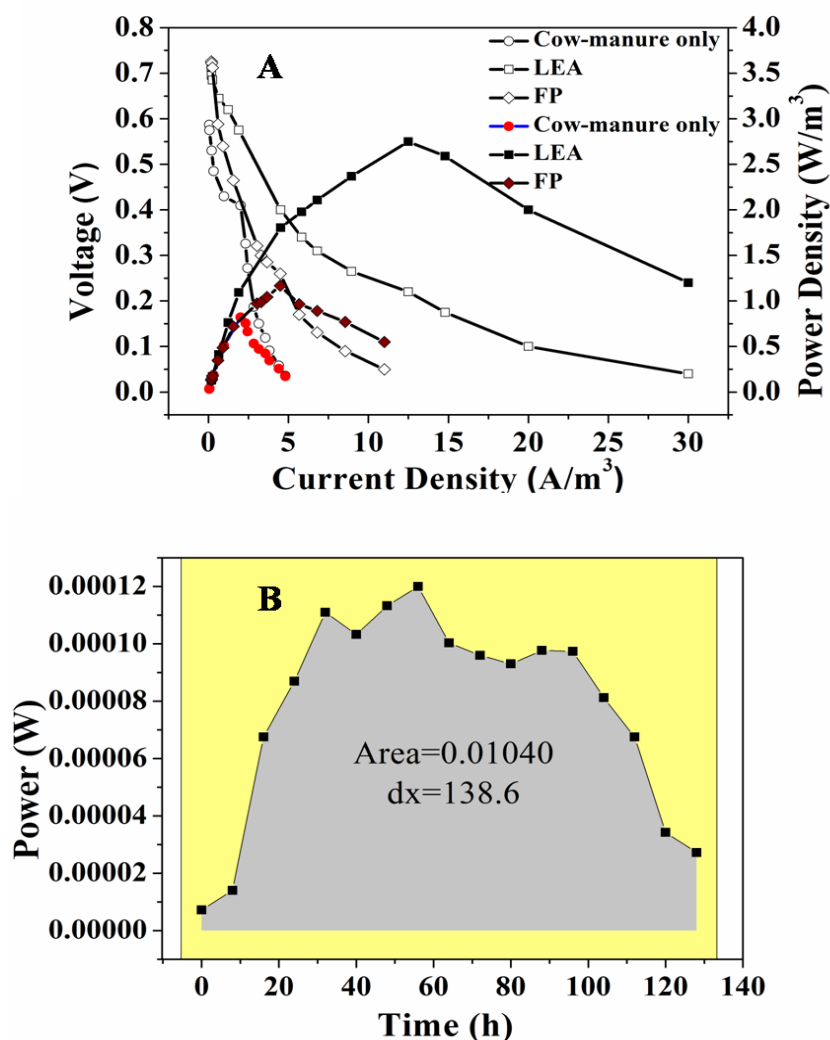
Polarization curves were obtained by varying resistance from 39 k ohm to 51 ohm. Figure 3.5 A shows the polarization curves depicting the highest power outputs for different MFCs. Highest power output generated by LEA, FP, and control MFCs was 2.7 W/m<sup>3</sup> (67.07 mW/m<sup>2</sup>), 1.1 W/m<sup>3</sup> (28.47 mW/m<sup>2</sup>), and 0.75 W/m<sup>3</sup> (17.15 mW/m<sup>2</sup>), respectively. Volumetric power density exhibited by LEA-fed MFCs was 145% and 260% higher than that of FP and control MFCs respectively. Also, LEA-fed MFCs exhibited 0.742 ± 0.043 W/m<sup>3</sup> of operating power density during last batch cycle. A comparison of power density, current density, maximum voltage, columbic efficiency, total COD removal (%) specific algal growth rate, and biogas analysis (CH<sub>4</sub>% and CO<sub>2</sub>%) has been summarized in Table 3.1. The LEA-fed MFCs showed the highest current density i.e. 30 A/m<sup>3</sup> from polarization studies at the external resistance of 51 ohm. Power generated by LEA-fed MFC is higher than that of FP-MFC, indicating the utility of LEA biomass as a useful anodic substrate. The OCV generated by LEA-fed MFCs is 885 ± 15 mV which is much higher than that of previously reported value ~21 mV [Rashid et al., 2013]. This signifies the importance of pre-treatment of LEA biomass, as done in this study before using it as an anodic substrate. After lipid extraction process, the inhibitory traces of organic solvents may exist, such as chloroform and methanol, which are detrimental to the MFC performance. Therefore, incubating it at around 82°C temperature, overnight helped getting rid of these inhibitory traces rendering it suitable as the anodic substrate. By passing this step led to the complete failure of MFC system in terms of voltage, power output etc. The sunlight exposure to LEA biomass instead of heat treatment at 82°C was also observed as an effective strategy for pretreatment.

**Table 3.1:** Summary of results obtained in different MFC setups.

Parameters	LEA	FP	Control
OCV (mV)	885 ± 15	692 ± 33.5	530.5 ± 52.5
Voltage across 1000 ohm (mV)	300 ± 14.5	230 ± 37.5	80 ± 29.5
Power Density (W/m <sup>3</sup> )	2.7	1.1	0.75
Power Density (mW/m <sup>2</sup> )	67.07	28.47	17.15
Current Density (A/m <sup>3</sup> )	30	11	4.8
COD Removal (%)	70.8 ± 4	67.27 ± 0.5	51.8 ± 0.5
COD degradation rate (Kg /m <sup>3</sup> /d)	0.29±0.11	0.28± 0.04	0.05 ± 0.03
Columbic efficiency (%)	15.3 ± 0.04	12.7 ± 0.06	5.78 ± 0.05
Specific Algal growth rate (d <sup>-1</sup> )	0.275 ± 0.02	0.208 ± 0.015	0.194 ± 0.014
CH <sub>4</sub> (%)	1.2	0	0
CO <sub>2</sub> (%)	13.5	22.8	7.1

Previous study has reported power density of 2.4 W/m<sup>3</sup> and 153 mW/m<sup>2</sup> using glucose and acetate as substrate, respectively [Zhou et al., 2012; Kakarla et al., 2014]. Substrates like glucose and acetate add cost to the system and thus are not sustainable. Cui et al. (2014) obtained power density of 8.67 W/m<sup>3</sup> using intact microalgae biomass as an anodic substrate [Cui et al., 2014]. The difference in the power output, when compared with other studies, could be due to the difference in MFC reactor configuration, electrode types and reactor volume [Wang et al., 2012]. The power output can be enhanced using stacked MFCs (series or parallel). Asensio et al. (2017) used intact algae biomass in twenty stacked MFCs which in turn produced

2.5 mW of power [Asensio et al., 2017]. This stacked MFC system was reported as sustainable and carbon neutral. In the present study also, the system is sustainable as it is a net energy producer and carbon neutral. Lakaniemi et al. (2012), obtained  $722 \pm 62$  mW/m<sup>3</sup> of power density, using ferricyanide as an electron acceptor in the cathode chamber [Lakaniemi et al., 2012]. *C. vulgaris*, on the other hand, is a better candidate in the cathode chamber, since it generates oxygen by photosynthesis, rendering the system cost-effective.

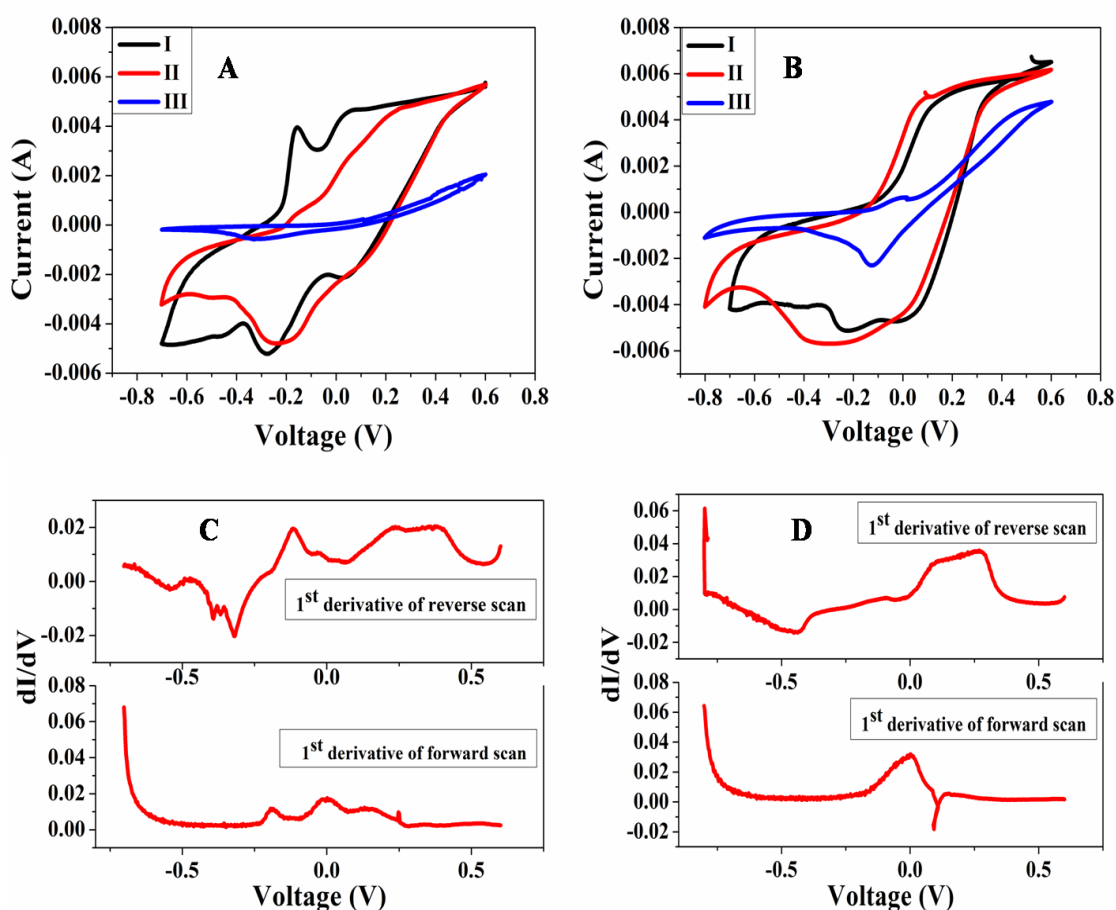


**Figure 3.5:** A) Polarization curve obtained for different experimental MFCs. B) Graph showing area under the curve as energy produced by LEA-fed MFC during last batch cycle.

### 3.2.5 Cyclic voltammetry (CV) analysis

In order to investigate the role of substrate in the current generation and the presence of electron shuttles produced by bacterial biofilm, CV experiments were performed for three different samples- 1) MFCs operating during the 7th batch cycle and showing steady voltage and current generation. 2) Anodes (with biofilm and fresh anolyte), taken at the end of a batch cycle. 3) CV of a fresh anode (without biofilm) immersed in the same medium used in above experiments [Liu et al., 2005]. CV analysis of LEA fed MFC (1) revealed that there is a clear distinguishable oxidation peak in the forward scan at -154.41 mV (vs Ag/AgCl), which is most likely due to oxidation of LEA biomass through bio-electrocatalytic processes. In reverse scan, a reduction peak was obtained at -276.48 mV (Figure 3.6 A). Also, the first derivative of CV of the anode with biofilm (2) showed an oxidation peak current at -191 mV (0.1 mA) in the forward scan. In

reverse scan a reduction peak was observed at -321.35 mV (4 mA) (Figure 3.6 C). These peaks exhibited by anodic biofilm might be due to the presence of mediators generated by bacterial culture [Liu et al., 2005]. However, the current produced by these redox couples is in the range 0.1-4 mA. This implies that some of these mediators were present in high concentration in the biofilm which gave rise to the high current. CV analysis of a fresh anode (3) exhibited no significant redox couples. This suggests that the mediators, if present, were held in biofilm itself that is why no peaks were obtained during CV scan of fresh anode [Liu et al., 2005]. This suggests that biofilm holding the active mediators is responsible for catalysis and the main mechanism of the current generation was the direct electron transfer. On the other hand, FP-MFC (1) showed a reduction peak in reverse scan at -212.4 mV (-5mA) (Figure 3.6 B). No oxidation peak was observed here, indicating an irreversible reaction. Colonized anode of FP-MFCs (2) showed one redox couple center in the first derivative of CV scan i.e. at 2 mV (3 mA) in the forward scan and -434.88 mV (5 mA) in reverse scan (Figure 3.6 D).



**Figure 3.6:** Cyclic voltammograms of A) LEA-fed MFCs and B) FP-MFCs respectively, (I) CV of MFCs during stable power generation, (II) CV of anodes having biofilm after the end of batch cycle (III) CV of a fresh anode. C) and D) first derivative of colonized anodes of LEA-fed and FP MFC respectively.

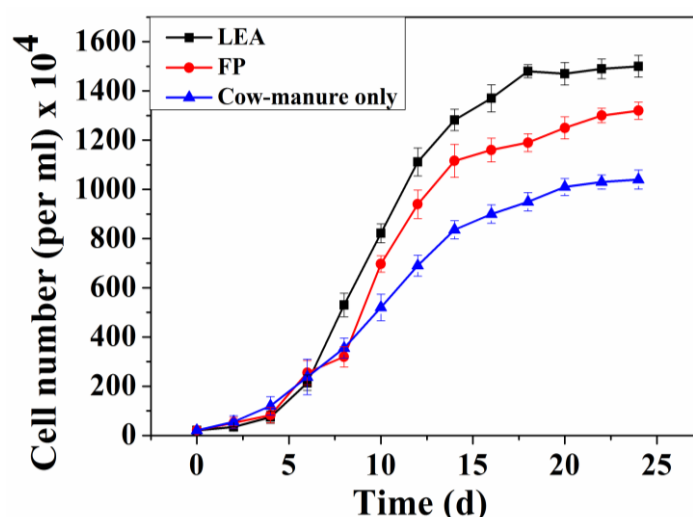
Higher magnitude of current (3-5 mA) obtained in CV scan is attributed to the high concentration of mediators held in the biofilm. CV scan of the fresh anode in FP MFC (3) showed a reduction peak at the same position where anodes during stable power generation were showing. So, in this case the mediators are expected to be released in the anolyte instead of being held in a biofilm [Rabaey et al., 2004]. Therefore, it can be stated that the biofilm holding



mediators as well as the mediators released in the anolyte played a pivotal role in the oxidation of fruit pulp [Rabaey et al., 2004; Nimje et al., 2012].

### 3.2.6 Algal growth at cathode chamber

Algae growth at cathode chamber is one of the important parameters in assessing the performance of a photosynthetic MFC. At cathode chamber, algal growth is controlled by CO<sub>2</sub> generation rate at anode chamber which in turn is influenced by COD removal rate. As shown in Figure 3.7, the algae count reached up to  $1370 \pm 54.8 \times 10^4$  and  $1160 \pm 48.4 \times 10^4$  cells/ml in LEA-fed and FP MFCs respectively. Algae productivity (kg/m<sup>3</sup> /d) was observed as 0.0288, 0.017 and 0.008 in LEA-fed MFC, FP MFC and control MFC respectively. Algae growth also provides oxygen for oxygen reduction reaction (ORR) at the cathode [Lobato et al., 2013; González del Campo et al., 2015]. So, higher algae growth rate is associated with high oxygen concentration or higher power output in LEA-fed and FP MFCs. So adding microalgae in the cathode is beneficial in multiple aspects and it also significantly reduces the cost of the MFC system which will otherwise depend on oxygenated water. Also, algae growth rates as achieved in photo bioreactors are achievable in such systems. Photo bioreactors are generally equipped with de gasifiers as high oxygen concentration inhibits the algae growth, but continuous quenching of oxygen via ORR in MFC eliminates the need of a de gasifier. Thus, the high power output complements high algae growth rate in such systems.



**Figure 3.7:** Algae growth as observed in cathode chamber with different experimental MFCs.

The algae cultivation is an environment friendly process and is an attractive way of greenhouse effect mitigation. However, the algae exhibit low production rates at the ambient CO<sub>2</sub> concentration (0.035 %). A carbon dioxide concentration of 2-6% supports the enhanced photosynthetic activity and biomass growth simultaneously [Chinnasamy et al., 2009]. For culturing algae on the large scale, the high cost of CO<sub>2</sub> as a feed stock is a matter of utmost concern [Gajda et al., 2015]. It was revealed by anodic off gases analysis that the major gas produced in the anodic chamber is CO<sub>2</sub> (7-23%), which can easily support the growth of algae at the cathode. González del Campo et al., (2014) systematically studied the effect of CO<sub>2</sub> supplied either in form of gas bubbled in the catholyte or added as bicarbonate ion. It was found that in case of bubbling, the CO<sub>2</sub> concentration goes up and so is the rate of photosynthesis. However, voltage drop ensues immediately after the bubbling step as the concentration of dissolved oxygen goes down [González del Campo et al., 2014]. In our study, a steady supply of CO<sub>2</sub> from anode supports algae growth and the sharp decline in the voltages are avoided.

### 3.2.7 Energy output

In order to evaluate system efficiency, energy output was calculated in all the MFC reactors. LEA-fed MFC generated 0.1 kWh/m<sup>3</sup> as energy output during the last batch cycle, while FP MFC and control MFC could generate only 0.03 kWh/m<sup>3</sup> and 0.0014 kWh/m<sup>3</sup> respectively. Electrical energy density generated by LEA-fed MFC is 10 times and 100 times more than that of FP MFC and control MFC respectively. Volumetric energy productivity of lipids (kWh/m<sup>3</sup>/d) was 0.0782, 0.047 and 0.023 in LEA-fed MFC, FP MFC and control MFC respectively. As mentioned earlier, the voltage and current outputs did not drop drastically during the dark period. The fluctuations in power output were observed and the area under the curve (Figure 3.5 B) which depicts energy output accounts for these variations. However, the power fluctuations were not coincident with the DO concentrations or light/dark cycle.

### 3.2.8 Energy analysis

The process (LEA-fed MFC) studied above, generated electrical energy concomitant with algae growth at the cathode. The electrical energy yield from the system is 0.068 kWh/kg COD or 0.1 kWh/m<sup>3</sup> (Figure 3.5 B). In general, power produced by MFC systems is low and does not impart any significant process sustainability. In order to render MFC technology sustainable, it has to be integrated with some other value added product generation and algae biomass cultivation at the cathode chamber, certainly offer a significant value addition. In the present process, algae productivity of 0.028 kg/m<sup>3</sup>/d was achieved. This productivity was achieved under unstirred, unaerated, and autotrophic mode of cultivation. When compared with previous reports, discussing cultivation of *Chlorella* under similar conditions (Table 3.2), productivity attained is higher possibly due to anodic off gas (CO<sub>2</sub>) that is being continuously fed to the cathode [Liang et al., 2009]. Also, the algae accumulated lipids and exhibited high lipid yield (36%) which led to the lipid productivity of 0.010 kg lipids/m<sup>3</sup>/d. Considering that 1 kg of algal lipids contains 10.52 kWh of the energy, of which 2.7 kWh is needed for lipid extraction, a net energy yield of 7.82 kWh/kg algae lipids can be obtained [Chisti et al., 2008; Ou et al., 2013]. This accounted for additional and significant energy output from a system that does not rely on any external electron donor or CO<sub>2</sub> source.

The specific electrical energy productivity was 0.0136 kWh/kg COD/d which was higher than the electrical energy produced from the anaerobic digestion of LEA [Sfroza et al., 2017]. Sforza et al., (2017) reported a biogas yield of 150 ml CH<sub>4</sub> /g VS [Sfroza et al., 2017]. Considering that 1 m<sup>3</sup> methane corresponds to 10 kWh of the energy, of which about 35 % can be recovered as electrical energy [Nguyen et al., 2016; McCarty et al., 2011]. So the specific electrical energy productivity obtained from the anaerobic digestion of LEA is in the order of 0.009 kWh/kg COD/d. Specific electrical energy productivity from this closed loop system is thus 43 % higher than what is attained from a conventional anaerobic digestion unit. Also, electricity generation from biogas plant needs several unit operations like anaerobic digestion followed by biogas clean-up, CH<sub>4</sub> concentration, and electricity generator. The present closed loop process is a unit operation associated with the simultaneous generation of electrical energy and algae.

The energy output and algae productivity from the system can be further enhanced by process modification such as nutrient recycling from the anode, mixotrophic algae growth, and effective anode/cathode catalyst and separator membranes. The study proves the potential of the process which certainly needs substantial enhancement for commercial applicability.

**Table 3.2:** Comparison of energy output from the closed loop process presented in this study with conventional algae cultivation and algal biomass utilization.

Parameter	Present closed loop Process	Conventional (algae cultivation on lab scale followed by anaerobic digestion of the residual algae biomass)
Algae productivity	0.0288 kg/m <sup>3</sup> /d	0.010 kg/m <sup>3</sup> /d (Liang at al., 2009)
Lipid productivity	0.010 kg/m <sup>3</sup> /d	0.004 kg/m <sup>3</sup> /d (Liang at al., 2009)
Energy savings from mixing and CO <sub>2</sub> transport	1.94 kWh/kg algae oil (Ou et al., 2013)	NA
Energy yield from lipids	7.82 kWh/Kg algae oil (Ou et al., 2013)	<7 kWh/Kg algae oil
Volumetric energy productivity of lipids	0.0782 kWh/m <sup>3</sup> /d	~0.027 kWh/m <sup>3</sup> /d
Electrical energy recovery	0.0136 kWh/kg COD/d	0.0090 kWh/kg COD/d (Sfroza et al., 2017)*
Process complexity	Unit operation, simultaneous generation of algae and electrical energy	Multiple operations (Algae cultivation, Anaerobic digestion, Biogas purification)
Infrastructural requirements	Low	High

\*calculated from the biogas yield and batch cycle length given by Sforza et al., 2017.

### 3.3 CONCLUSIONS

The study demonstrates the successful use of LEA biomass as an anodic substrate for the first time in literature. A process which does not rely heavily on the external supply of carbon sources is presented. The process offers a zero waste and zero carbon system turning out to be a net energy producer. The power density and electrical energy generated are 2.7 W/m<sup>3</sup> and 0.1 kWh/m<sup>3</sup> respectively. Further research is needed on optimization of the process for obtaining higher power output, continuous operation, and higher algae growth rates.

...

