Influence of Anodic Coculture on Dairy Wastewater Treatment and *Synecocystis* sp. Production in an Algal Assisted Microbial Fuel Cell

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Abbreviations

AAMFC, algal assisted microbial fuel cell; *MSW*, municipal solid waste; *AML*, Anolyte microbial load; OCV, open circuit voltage *; I, Current; FAME*, fatty acid methyl ester ; *COD*, Chemical oxygen demand; *BOD*, biological oxygen demand; *TDS*, total dissolved solids; *TSS*,total suspended solids; *TN*, total nitrogen; *TP*, total phosphorus; *DO*, dissolved oxygen; *CE*, Coulombic efficiency; *CN*, Cetane number ; *La*, Lauric acid ; *M* , Myristic acid; *P*, Palmitic acid; *Pt*, palmitoleic acid; *S*, Stearic acid; *O*, Oleic acid; *Li*, Linoleic acid; *Ln*, Linolenic acid ; *Ei*, Eicosanoic acid; *Er*, Erucic acid; BAPE, Bis-allyic position equivalent.

Abstract

The development of alternate energy resources is of great interest to meet the growing energy demand. Herein, we demonstrate the production of bioelectricity as well as *Synechocystis* sp. from dairy industry wastewater using an algal assisted microbial fuel cell (AAMFC) under different initial anodic microbial loads comprising *Enterobacter aerogenes* and *Rhodobacter sphaeroides*. *Synechocystis* sp. and municipal solid waste leachate present in the cathode chamber served as terminal electron acceptors. Synergistic interaction of dark and photo-fermentation at the anode region was better at a ratio of 1:9, which gave power density of 114±6 mW/m^2 and COD removal of 84%. This showed higher *Synechocystis* sp. and lipid productivity besides highest DO level of 9.2 mgL^{-1} in the cathode chamber. Better performance of AAMFC was observed at pH 7.5. *E. aerogenes* was found to grow much faster and dominant volatile fatty acid (VFA) produced was acetic acid. Carbon dioxide fixation by *Synechocystis* sp. exhibited biomass and lipid productivity of 156.3±1.5 and 28.8± 4.2 mg L^{-1} d^{-1}, respectively with 88.6% and 89.4% total nitrogen and phosphorous removal.

Keywords: Microalgae; Microbial fuel cell; *Synechocystis* sp,; Bioelectricity; Microbial load .

Nomenclature

X_a	Concentration of algae at time t, h,
K	Carrying capacity of algal biomass concentration, g/l
μ_a	Specific growth rate, d^{-1}
b	Constant
COD_R	COD removal efficiency, mgl^{-1}
C_i	Initial COD concentration, mgl^{-1}
C_f	Final COD concentration, mgl^{-1}
t_i & t_f	Initial and final time corresponding to the substrate concentration
bp_i	bis-allyic positions in a specific FAME
Ac_i	mass percent of each FAME

1. INTRODUCTION

The development of innovative renewable energy technologies are essential due to the depletion of fossil resources and its environmental pollution (Pant, Bogaert, Diels, & Vanbroekhoven, 2010). Bioelectrochemical systems (BES) are prominent and particularly, microbial fuel cell technology (MFC) is a sustainable bioelectricity production technique. MFCs are successfully used for wastewater treatment and CO_2 sequestration. Modified version of MFCs - microbial carbon capture cell (MCC) (Jadhav, Jain, & Ghangrekar, 2017), photosynthetic microbial fuel cells (Rong & Hu, 2017) and algal assisted microbial fuel cells (AAMFC) - offer higher efficiency than conventional cells (Saba, 2017; X. Wang et al., 2010b). AAMFC relies on photosynthetic process and consists of anode and cathode chamber separated by proton exchange membrane (PEM). The electrons generated in the anode chamber flow towards the cathode, where hydrogen ions are reduced to water (González et al., 2014; Nor et al., 2015; Zhang et al., 2013). In AAMFC, bioelectricity is produced due to the oxidation of organic substrate in the anode chamber as well as oxygen generated during algal growth in the cathode chamber (Rong & Hu, 2017). The anodic off gas (CO_2) produced is fed into the cathode region for fixation by microalgae. It is envisaged that production of biodiesel from microalgae is economically viable since CO_2 and light are sufficient for algal cultivation (Fornero, Rosenbaum, Cotta, & Angenent, 2010; Xu, Poon, Choi, & Wang, 2015). Furthermore, microalgae accumulate secondary metabolites such as proteins and carotenoids, which are of high commercial value in pharmaceutical and nutraceutical sector (Cui et al., 2014; Pandit, Nayak, & Das, 2012).

The need for continuous supply of electron donors and electron acceptors at the anode and cathode chamber, respectively, hinders the commercialization of MFC (Gajda et al., 2015; Lee, Chang, & Lai, 2015; D. Bin Wang, Song, Guo, Zeng, & Xie, 2014). Particularly, bio-

electrochemically active microorganisms called electricigens significantly affect the performance of MFC (Walter, Greenman, & Ieropoulos, 2013). Though studies have been reported on the performance evaluation of these electricigens, elaborate studies on synergistic effects of different microbial strains remain insufficient. MFC can utilize a variety of electron donors that ranges from wastewaters containing simple to complex substrates (Olivera et al., 2018; Khandelwal et al., 2018). Notably, wastewater generated from dairy industry has high organic load as well as COD level (Olguín, 2012; Wang et al., 2014). Bioelectricity can be produced by dark fermentation using electricigens such as *Bacillus* sp., *Citrobacter* sp., *Clostridium* sp., *Shewanella* sp., *Enterobacter* sp., *Geobacter* sp., and *Proteus* sp. (He, Kan, Mansfeld, Angenent, & Nealson, 2009; La Rotta Hernández et al., 2014). For photo fermentation, *Rhodobacter* sp., *Rhodopseudomonas* sp., and *Rhodospirillum* sp.,(W.-W. Li, Yu, & He, 2013; H. Wang & Ren, 2013; L. Xiao & He, 2014; N. Xiao, 2017) were employed. The usage of mixed culture/co-culture provides more stable power generation compared to pure culture. The synergistic interaction between microbial communities help long term operation of MFC (Bader, Mast-Gerlach, Popović, Bajpai, & Stahl, 2010). The co-culture/mixed culture contribution to power generation is dependent on the operational parameters of the AAMFC, which requires a detailed study. On the other hand, the usage of ferricyanide or other liquid based electron acceptors are not encouraged as they are not environmental friendly and have problems with total energy output limitations. In this study, bioelectricity was generated from dairy wastewater using co-culture comprising *Enterobacter aerogenes* and *Rhodobacter sphaeroides*. Importantly, we have attempted to study the influence of anodic microbial load (AML) on the performance of MFC. The COD removal, volatile fatty acids (VFA) accumulation, pH variation and shifts in bacterial population in the anodic chamber were monitored. For CO_2 sequestration, *Synechocystis* sp. – a potential strain for biodiesel production - were used in the cathode chamber and municipal solid

waste (MSW) leachate was used as the substrate. Algal growth, dissolved oxygen content and nutrient composition of algae were analyzed.

2 MATERIALS AND METHODS

2.1 Anodic and Cathodic Inoculum

E. aerogenes and *R. sphaeroides* were purchased from Microbial Type Culture Collection (MTCC), Chandigarh, India. *E.aerogenes* was cultivated in synthetic growth medium containing (per litre) glucose-5g, peptone -3g, yeast extract-1g, $KHPO_4$-2.8g, KH_2PO_4-3.9g, $MgSO_4.7H_2O$-0.2 g, NaCl-0.1 g, $CaCl_2.6H_2O$-0.01g, $FeSO_4.7H_2O$-0.05g, L-Cysteine-0.2g and micro nutrients solution ($CuCl_2$-$2H_2O$-0.02g, $NiCl_2.6H_2O$-0.02g, Na $MoO_4.2H_2O$-0.04g per litre)(Patel et al., 2014). *R. sphaeroides* was grown in Van Niel's medium containing (per litre) K_2HPO_4-1g, $MgSO_4.7H_2O$- 0.5g, yeast extract -10g, under continuous light irradiation (intensity: 250 Wm^{-2}) at 30°C (Basak & Das, 2007). These cultures were acclimatized systematically to dairy wastewater to achieve high power generation.

Synechocystis sp. obtained from Algae Culture Collection Centre, University of Madras, Chennai, India was maintained in BG11 medium at 30°C under continuous illumination of fluorescence light. The stock cultures were maintained on liquid and agar slants of BG11 medium and regular sub-culturing was done at an interval of two weeks. The culture was added into MSW leachate and periodically agitated. This served as a stock inoculum for the rest of the studies. MSW leachate (COD- 1100 mg/l, BOD-273 mg/l, TDS-110.32 mg/l, suspended solids(SS)-12 mg/l, total nitrogen-74.63 mg/l, total phosphorus-12.35 mg/l, total alkalinity- 83.7 mg/l) present in the cathode chamber was inoculated with 5% by volume microalgae. No

additional nitrogen and phosphorus sources were added and the culture was maintained at 23±1°C at 2000 Klux light intensity in 16:8 h light dark cycles.

2.4 Microbial fuel cell

H-type dual chamber MFC constructed using quartz was used. The anode and cathode chamber - working volume of 250 ml - were separated by proton exchange membrane (Nafion 115, Dupont). Prior to use, the membrane was treated with distilled water at 80-90°C and then with 5% H_2O_2, which was followed by 0.5M H_2SO_4 for 1h at 70-80°C. Membrane was finally washed and stored in deionized water. A carbon plate with an effective surface area of 54 cm^2 was used as the anode and cathode. The electrodes were placed at a distance of 5 cm away from the membrane and each chamber was provided with inlet and outlet. The anode and cathode chamber was connected using silicon tube to allow CO_2 to pass into the cathode region from the anode chamber. The anode chamber filled with dairy wastewater was inoculated with a coculture comprising *E. aerogenes* and *R.sphaeroides.* Dairy wastewater was collected from milk processing unit located at the outskirts of Chennai, India and was stored at 4°C. This was used as the sole substrate in the anode chamber of AAMFC without any additional nutrients. The pH and temperature were maintained at 7.0 and 37℃, respectively. Prior to experiments, cultures were collected from their respective acclimatized medium by centrifugation at 10000 rpm for 30 min and suspended in 5 ml of dairy wastewater. The cell number in each solution was counted using haematocytometer. Five sets with different microbial concentrations were considered: 1) pure culture of *E.aerogenes*-AML-I (0.52×10^7 ml^{-1}); 2) pure culture of *R.sphaeroides*-AML-II (0.56×10^7 ml^{-1}); 3) co-culture consisting 0.72×10^7 ml^{-1} of *E.aerogenes* and 6.483×10^7 ml^{-1} of *R. Sphaeroides* (1:9)-AML-III; 4) co-culture containing 6.74×10^7 and 0.75×10^7 $ml^{-1}$of *E.aerogenes and R. Sphaeroides,* respectively (9:1)-AML-IV; and 5) co-culture containing 0.53

$\times 10^7$ $ml^{-1}$and 0.51×10^7 ml^{-1} of *E.aerogenes and R. Sphaeroides* (~ 1:1)-AML-V. After inoculation, the culture was kept under 100 W tungsten lamp with 150-200 W/m^2 illumination. The cathode chamber containing MSW leachate (pH:7.8) was inoculated with *Synechocystis* sp. (10% v/v). The cathode region was illuminated using 25 W fluorescent lamp throughout the experiment. The AAMFC was operated under batch mode at 30°C and 1 atm. pressure.

2.5 Electrochemical analysis

The cell voltage was measured using an auto range digital multimeter. Under steady state conditions, the circuit is kept open to obtain the open circuit voltage (OCV). The polarization behavior was studied by measuring the change in voltage with external resistance (15000 Ω to 100 Ω). For electrochemical analysis, the cell was connected to potentiostat-galvanostat (VMP3, Biological instruments, France) in three electrode setup with an Ag/AgCl electrode (3M KCl, + 205 mV Vs. standard hydrogen electrode (SHE)). To calculate the current density (mA/m^2) and power density (mW/m^2), current and power obtained were normalized based on the anode surface area.

2.6 Chemical analysis

Dairy wastewater and MSW leachate quality parameters were analyzed by following methods prescribed by APHA (1998)(Arnold E. Greenberg, 1992). Gas samples from headspace of the anode and cathode chamber were analyzed using gas chromatograph (Agilent 7890b GC) with thermal conductivity detector (TCD) and a 2 m stainless porapak column using N_2 as the carrier gas. Volatile fatty acids (VFA) were determined using GC (Agilent 7890b GC) equipped with flame ionization detector (FID) and capillary column (30 m) with helium as the carrier gas.

2.7 Biochemical, Lipid and Bacterial analysis

Algal cells were harvested by centrifugation at 10000 rpm for 20 min at 4°C. Total nitrogen and phosphorus present in supernatant was determined calorimetrically by following standard methods (Arnold E. Greenberg, 1992). The biomass dry weight was determined gravimetrically. The dissolved oxygen concentration and light intensity were measured using DO probe (Lutron DO-5510 Oxygen Meter) and Lux meter (Luxeron LX-1108), respectively. Modified version of Bligh and Dyers method was followed to estimate lipid concentration (Bligh and Dyer, 1959). Fatty acid methyl esters were analyzed using high performance liquid chromatograph (Agilent 1260) with RI detector and Hi-Plex H column (300 × 7.7 mm) operated with 5 mM H_2SO_4 as mobile-phase at a flow rate of 0.6 mL/min. The anodic bacterial cell density was determined based on protein extraction methods (Kakarla & Min, 2014). The whole protein was measured by bicinchoninic acid method against bovine serum albumin as standard and estimated using HACH spectrophotometer (Smith et al., 1985). Similarly, bacterial biomass in the anolyte culture broth was measured by following the same procedure after the cells were harvested by centrifugation at 15000 rpm for 20 min. Further, the cells were stained with 2 mg/l of 4,6-diamidino-2-phenylindole (DAPI) for 5 min. The DAPI stained cells were collected on an isopore membrane and were analyzed using fluorescence microscopy (Nikon Epifluorescence microscopy).

2.10 SEM analysis

Microbial adhesions on the cathode and anode were analyzed using scanning electron microscope. A small portion of electrode containing cells were removed and the cells were fixed using 1.25% glutaraldehyde in a sterilized phosphate buffer solution (PBS) for 1 h at room temperature and subsequently washed thrice using PBS solution. The samples were stored at 4℃ overnight and dehydrated using graded ethanol solution. The electrodes were attached to the

copper stud using carbon adhesive tapes and visualized using scanning electron microscope (FESEM-SUPRA 55).

2.11 Growth kinetics and COD Removal

Logistic model was used to describe the growth of algae and is given in Eqn. (1).

$$X_a = \frac{K}{1+e^{b-\mu_a t}} \qquad (1)$$

where X_a is the concentration of microalgae at time t (h), K is the carrying capacity(g/l), b is the constant and μ_a is the specific growth rate (d^{-1}) (Eze et al., 2018). The COD was determined by potassium dichromate (open reflux) titration method (Arnold E. Greenberg, 1992). The COD removal efficiency (COD_R) was calculated using Eqn. (2).

$$COD_R = \frac{C_i - C_f}{C_i} \times 100 \qquad (2)$$

where C_i is the initial COD concentration (mg/l) and C_f is the final COD concentration (mg/l) of the wastewater in the anode chamber.

2.13 Coulombic efficiency

The Coulombic efficiency was calculated using Eqn. (3).

$$CE = \frac{\int Idt}{[(\Delta COD/32\times 1000)\times b\times V\times 96{,}480]} \qquad (3)$$

where I is the current, ΔCOD is the COD difference between influent and effluent, V is the volume of wastewater, b represents the number of electrons exchanged per mol of O_2(4), 32 is the molecular weight of O_2. (Li et al ., 2013)

2.14 Cetane number (CN)

Piloto-Rodrigueza (2016) proposed a model based on fatty acid profile for calculating the cetane number of biodiesel (Rodríguez et al., 2016)

$$CN = 56.16 + 0.07\,La + 0.1M + 0.15P - 0.05Pt + 0.23S - 0.03O - 0.19Li - 0.31Ln + 0.08Ei + 0.18\,Er - 0.1Ot$$

where, La is the percentage of lauric, M is myristic, P is palmitic, Pt is palmitoleic, S is stearic, O is oleic, Li is Linoleic, Ln is Linolenic, Ei is eicosanoic, Er is erucic, and Ot is the sum of the other FAMEs detected. The bis-allyic position equivalent (BAPE) values were calculated using the following equation proposed by Buscy and Marchese (2012)

$$BAPE = \sum_{i=1}^{n} bp_i \,.\, Ac_i \qquad (4)$$

where bp_i denotes the number of bis-allyic positions in a specific FAME, Ac_i is the mass percent of each FAME, and n is the total number of FAMEs(Bucy & J. Marchese, 2012)

3. Results and Discussion

3.1 AAMFC performance

Anodic compartment was filled with sterilized dairy wastewater (COD = 3500 mg/l, pH-6.5) and different AML. AAMFC was operated for a period of 16 days (per cycle) and the initial shoot up time for the AAMFC operation was around 10 h. During the cycle, voltage generation increased due to anodic microbial activity and reached peak voltage under stable conditions. Under open circuit conditions, cell voltage was measured. As shown in **Figure 1a**, highest OCV value 850 ± 2 mV was observed for AML-III. To verify the synergistic effect, the electrochemical performance was investigated under closed circuit conditions. At stable conditions, anodic bacterial cultures gave significant power generation. The effect of different inoculum was compared based on polarization behavior and power density (**Figure 1b & c**). As shown in **Figure 1b,** voltage output order is: AML -III >AML–II>AML-IV>AML-I>AML-V. AML-III was more susceptible to polarization than pure cultures (**Figure 1b**). This result suggests that AML-III is well acclimatized with dairy wastewater. From polarization curves

(**Figure 1c**), power densities of 49±5, 92±2,114± 6,62 ± 4 and 32 ±5 mW/m^2 with corresponding current densities of 125±4, 124±8, 126±6, 125±7 and 72± 8 mA/m^2 were observed at 450 Ω for AML-I, AML-II, AML-III, AML-IV and AML-V, respectively. The current density and power density observed for AML-III were higher. Under open circuit conditions, power density significantly varied with pure and co-culture due to varied substrate degradation pathways. Nimje et al (2011) reported variations in current generation with different inocula such as endogenous microbe (MFC1), *Shewanella oneidensis* MR-1(MFC2), and endogenous microbes with MR-1(MFC3) in an anaerobic environment utilizing agriculture and domestic wastewater as a substrate (Nimje et al., 2011). Samsudeen et al also reported different current generation capacities for *Bacillus* species (SN-1, SN-2, SN-3). The maximum open circuit voltage (OCV) of 646 mV and power density of 104 mW/m^2 were reported for distillery wastewater (Samsudeen, Radhakrishnan, & Matheswaran, 2016). Bio-electrochemical activity of the coculture increased the charge transfer and reduced the diffusion resistance, which in turn accelerated the power generation (Rabaey & Rozendal, 2010). However, AML-V showed lower power density due to equal distribution of electro -active microbes as well as metabolizing the substrate for survival rather than generating the current. The synergistic effect of microbes in AML-III improved the electron transfer performance and reduced the transmission resistance of electrons to the cathode. The power density of 114 mW/m^2 was observed with dairy wastewater in the anode region and *Synechocystis* sp. in the cathode region utilizing MSW leachate as the substrate. This value is comparatively higher than the values reported in the literature (**Table 1**).

3.2 COD removal, off gas analysis, volatile fatty acids and Coulombic efficiency

The effect of AML on anodic off gas production and COD removal is shown in **Figure 2a**. The pure culture of *E.aerogenes* (AML-I) and *R.sphaeroides* (AML-II) produced 0.8 and 2.4

mM of CO_2, respectively. The CO_2 concentration in the anodic region increased rapidly for co-culture and highest value of 3.2 mM was observed for AML-III. Synergistic mechanism of anodic inoculum through anoxygenic - photoherterotrophic mode generates H^+ and e^-. AML-III produced high VFA and CO_2 due to its facultative nature. About 1.6 and 0.5 mM CO_2 production was observed for AML - IV and AML –V, respectively. Also, traces of hydrogen were detected and no methane production was observed (**Figure 2a**.). However, no CO_2 was observed in the cathodic headspace. This confirms that CO_2 generated was completely absorbed by microalgae present in the cathode region (Wang et al., 2010a). Hydrogen production observed with AML-V was 1.2 mM and this is due to excessive reduction of H^+ and electrons generated during substrate oxidation, which in turn affected the power generation. The performance of AAMFC on wastewater treatment was analyzed and is shown in **Figure 2a**. The COD removal of 84 and 78% was observed with AML-III and AML-II, respectively. In this study, electrons generated by microbial metabolic activity were transferred to anode surface in a timely manner and degradation of dairy wastewater was facilitated. Lower COD removal (60%) was observed with AML-V and this may be due to the inhibition of microbial electron transfer performance by antagonistic reaction. The electron transfer process was blocked and accumulated electrons around the anode region restrict the degradation of dairy wastewater, which in turn affect the COD removal. It is apparent that wastewater treatment efficiency was influenced by microbial synergism. **Figure 2b** illustrates the changes in VFA production for different microbial populations in the anode chamber. Irrespective of AML, neither valeric acid nor hexanoic acid were detected. Major fatty acids present were acetic acid, butyric acid and proponic acid. However, dominant VFA produced was acetic acid and its concentration observed was 8.46 g/L. The total VFA content observed with pure culture was higher compared to coculture. This is due to the fact that the VFAs produced were utilized by the phototrophic bacteria to generate

electricity. The CE obtained in this study was in the range of 1-7% (**Figure 2c**) and higher CE was observed for AML-III. The slow and gradual decrease in anolyte pH was observed for different microbial loads (**Figure 2c**). This acidification was due to production of VFAs or other organic compounds such as amino acids and long chain fatty acids by the metabolic activity of electrochemically active bacteria (Rabaey & Verstraete, 2005).

3.3 Anodic biomass

Figure 3a shows that facultative bacteria entered into the exponential growth phase after 12 h. *E.aerogenes* count increased after a minimum lag phase and reached the maximum at the end of 96 h. The increase in *R.sphaeroides* count started at 40 h (**Figure 3b)**. **Figure 3c** illustrates that *R.sphaeroides* count in the co-culture (1:9) increased rapidly after a short lag phase of 20 h and maximum was observed at 160 h. *E.aerogenes* count in the co-culture increased without any lag and attained highest production at 72 h. **Figure 4** shows the fluorescence image of co-culture, which illustrate the synergism between *E.aerogenes* and *R.sphaeroides*. Based on total cell counts and FISH images of the samples, population changes in *E.aerogenes* and *R.sphaeroides* were examined. Facultative bacteria, *E.aerogenes,* was found to grow much faster than the phototrophic bacteria in the dairy wastewater The results observed are in accordance with the results observed by Liu et al (2015) (Liu, Rao, Yuan, & Zhuang, 2015). We observed an imbalance in cell number for the pure culture of *E. aerogenes* and *R. sphaeroides*. The dark-to-phototrophic anodic bacterial inoculation ratios of 9:1; 1:9 and 1:1 were studied and increase in cell number was observed for 1:9. This indicated that the dark and phototrophic organism complements each other. The facultative bacteria were able to grow utilizing sugar compounds present in the wastewater, whereas the photo fermentative bacteria utilized VFAs produced by the facultative bacteria. The addition of the phototrophic bacteria to

the dark fermentation stabilized the pH and decreased the oxidation-reduction potential of the co-culture system besides extending power generation (R. Y. Li & Fang, 2009). The average anodic biomass obtained with AML III showed 0.756 ± 0.06 mg protein/cm^2, which was followed by other co culture ratios AML-IV (0.312 ±0.02 mg protein /cm^2) and AML-V (0.228 ±0.013 mg protein /cm^2). AML III, which is three times higher than AML–I and AML-II value of 0.258 ± 0.08 mg protein/cm^2 and 0.523 ± 0.002 mg protein /cm^2, respectively. The total biomass growth obtained with substrate removal indicated that the growth with AML-III favored higher power generation**.** **Figure 5** shows that current generation is linearly correlated to the anodic biomass density for AML-III. This suggests that increase in exoelectrogenic bacteria in the anode at optimum operational conditions can produce higher current density.

3. 4 Power Generation

3.4.1 Effect of Anodic COD

The polarization behavior of AAMFC- voltage and power density as function of current density - at different COD loadings (750 mg/l-3100 mg/l) is shown in **Figure 6 (a&b)**. Under closed circuit conditions, highest voltage recorded for 750, 1600 and 2550 mg/l COD was 550 ±2.4, 654±4 and 730 ±1.2 mV, respectively. The voltage generation increased up to 2550 mg/l and further increase in organic load dropped the voltage to 700 mV (3100 mg/l). The highest power density of 195±2.1 mW/m^2 was observed for 2550 mg/l. whereas lowest power density of 38 ± 4 mW/m^2 was observed at a COD of 750 mg/l.

3.4.2 Effect of pH

The effect of anodic pH on power generation was investigated by adjusting the pH to 5.5, 6.5, 7.5 and 8.5. AAMFC was filled with dairy wastewater (COD: 2550 mg/l) and 5% of AML

IV. During the treatment, the pH value changes due to electrochemical and metabolic activity of microbes. The polarization and power density behavior (350 Ω) of AAMFC with AML –IV under different pH is shown in **Figure 6c.** The maximum performance of AAMFC was observed at pH 7.5 whereas the power density declined when the pH was increased above 8.5. This might be attributed to slow electron discharge activity of electrochemical bacteria at alkaline pH. The coculture of electrochemically active bacteria could survive in the dairy wastewater and enhance the power generation around neutral pH. The results observed in this study were similar to that of the results observed by Behera et al (Behera, Jana, & Ghangrekar, 2009)

3.5 Algal growth, dissolved oxygen content and nutrient removal

Figure 7a shows the growth *Synechocystis* sp. as a function of time at different AMLs. The modified logistic equation kinetic parameters of *Synechocystis* sp. are presented in **Table 2**. Higher biomass concentration of 1.54 g/l was observed for AML-III. This indicates that the growth of microalgae depends on the supply of CO_2 produced in the anode region. It has been reported that gaseous CO_2 generated in the anode chamber can be converted into algal biomass in cathode chamber (Subhash, Chandra, & Mohan, 2013). The maximum carrying capacity of 0.0392 ± 0.40 g/l and specific growth rate of 0.422 ± 0.12 $d^{-1}$was obtained for AML-III. Logistic equation fitted the experimental data well and the R^2 value obtained were above 0.99. DO content in the cathode chamber influenced the bioelectricity generation and highest DO observed in the cathode chamber was 9.2 mg/L (**Figure 7b**) for AMLIII, which generated the maximum power density of 114 mW/m^2. The electron discharge from the anodic region is highly influenced by the dissolved oxygen reduction mechanism at the cathode (Kakarla & Min, 2014). Microalgal photosynthesis mechanism in the cathode chamber liberates oxygen and this acts as a terminal electron acceptor. Catholyte DO content varied between 6.5-11.2 mg/l during operation

depending on the CO_2 supply and microalgal growth. Maximum DO level was maintained by AML- III whereas AML-I gave low DO level (6.5 mg/l) due to the reduction in algal growth. *Synechocystis* sp. followed a power output that was closely related to the DO concentration (**Figure 7b**). Microalgae serve as an important agent for bioremediation due to their ability to assimilate the organics and nutrients present in wastewater for their growth. The nutrient removal aspects of *Synechocystis* sp., are shown in **Table 2**. TN and TP removal efficiency of 88.6 and 89.4 % were, respectively, observed with a CO_2 supply of 3.2mM.

3.6 Microalgae and lipid production

Table 2 shows the cathodic algal biomass kinetic parameters along with *Synechocyctis* sp. and lipid productivities**.** Highest biomass and lipid productivity observed with 3.2 mM CO_2 supply were 156.3 ± 1.5 and 28.8 ± 4.2 mg L^{-1} d^{-1}, respectively. The overall lipid content of *Synechocyctis* sp. was in the range of 10.25- 15.8% and it did not change significantly. AML-III showed higher *Synechocystis* sp. and lipid productivity. **Figure 7c** shows the fatty acid profile of *Synechocystis* sp. when the AAMFC was operated with AML III and the results showed higher content of palmitic acid (62.5%) and linoleic acid (14.5%). The supplementation of 3.2 mM CO_2 increased and enhanced the biodiesel production potential of the algae. Cetane number (CN) determines the biodiesel quality and high cetane number indicates easier start up and less knocking. The calculated CN was 53 ± 0.8 respectively. *Synechocystis* sp. lipids contained higher level of saturated fatty acids (70.8%) but low level of unsaturated fatty acids (28.5%). Lipids having less bis-allylic position equivalent (BAPE) value < 50 have been approved (for oxidative stability) by ASTM. In this study, BAPE value observed was 22.3 ±1.2. This suggests that S*ynechocystis* sp. lipids ($p<0.05$) have high oxidative stability.

3.7 Microbial morphology

Figure 8a shows the SEM image of anodic biofilm composed of mixed cells of heterogeneous morphology. Co-culture of *E.aerogenes* and *R.sphaeroides* shows uniform stratified biofilm layer on the anode electrode. This helps release of more electrons and hence higher power density obtained was obtained from consortium. **Figure 8b** shows the SEM image of *Synechocyctis* sp. The presence of numerous elongated cells was clearly observed on the cathode electrode surface. CO_2 uptake by algal cells expands their shape and increases the accumulation of lipids in the thylakoid membrane region

4 CONCLUSION

This study demonstrated the influence of anodic co-culture on the performance of algal assisted microbial fuel cells. The system utilized the synergistic effect of dark and photo fermentation by employing *E. aerogenes* and *R. sphaeroides.* AML with a coculture ratio of 1:9 (*E.aerogenes* to *R.sphaeroides*) (AML-III) gave highest power generation (114 mW/m^2) and better efficiency. Highest anodic biomass growth observed was 0.756 ± 0.06 mg protein/cm^2 and the current generation by AAMFC was linearly correlated to the anodic biomass. The anodic CO_2 off gas produced - in the range of 0.5-3.2 mM, - was effectively utilized for algae cultivation. The COD and pH played a significant role in power generation. In the cathode region, *Synechocystis* sp. production and its lipid content at a CO_2 concentration of 3.2 mM were 156.3±1.5 and 28.8±4.2 mg L^{-1} d^{-1} respectively. The catholyte DO content increased from 6.5-11.2 mg/l during operation depending on the CO_2 supply and algal growth. The cetane number of *Synechocystis* sp. lipids was significantly higher, which indicates that this can be utilized for biofuel production. The utilization of AAMFC for concurrent wastewater treatment and electricity production is promising and cost-effective besides offering a sustainable solution.

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Declaration of Interest:

The authors declare that they have no conflict of interest

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