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Comparison of anodic metabolisms in bioelectricity production during treatment of dairy wastewater in Microbial Fuel Cell

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HIGHLIGHTS

- Energy generation from dairy wastewater was investigated using dual chambered MFC.
- Anaerobic metabolism showed higher current efficiency than aerobic metabolism.
- Aerobic metabolism showed higher power generation capacity.
- MFC operate at pH 7 and COD concentration of 1660 mg/L showed better performance.
- MFC operation using a buffer for pH maintenance given better power generation.

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ABSTRACT

Energy generation from dairy industry wastewater was investigated using a dual chambered Microbial Fuel Cell by aerobic and anaerobic anodic metabolism, operating with initial COD concentration of 1600 mg/L and anolyte pH of 7 produced highest power density of 192, 161 mW/m^2 and volumetric power of 3.2, 2.7 W/m^3 with COD removal efficiency of 91% and 90%, respectively. The columbic efficiency was 3.7-folds lower for aerobic metabolism compared to anaerobic metabolism with 17.17%. Effect of operating parameters such as anolyte pH and COD concentration on MFC performance was also evaluated. Anaerobic metabolism operated with COD concentration of 1600 mg/L and anolyte pH 7 showed best performances. Biofilm formation by inherent microbes of wastewater on anode was visualized by instrumental techniques. Milk processing operation runs almost through the year, hence MFC utilizing dairy industry wastewater would be a sustainable and reliable source of bio-energy generation.

1. Introduction

The rising concern over protection of environment and depleting energy resources has made it inevitable to taken over the waste management system from merely treating the waste to new horizon of recovery of energy from waste (Pant et al., 2009). Microbial Fuel Cell (MFC) a novel method of directly generating electricity from organic matter in wastewater, simultaneously treating waste water solves issues of energy crisis and environmental damage. MFC is a bioelectrochemical system exploiting bacterial oxidation of biodegradable organic matter, to generate electricity. The microorganism present in anode chamber of fuel cell acts as catalyst to convert chemical energy in wastewater to electrical energy. The microbial metabolism generates electrons (e^-) and protons (H^+) by oxidation of organic substrate, which leads to the development of bio-potential. The electrons are transferred to the anode by the bacteria through various mechanisms such as solid conductive matrix or by electron shuttles. Electrons are then transferred to cathode through external circuit (Rabaey and Verstraete, 2005). The protons in anode chamber pass through the proton exchange membrane into the cathode chamber, where they combine with the electrons and oxygen with the help of mediator to form water. The potential between the respiratory system of bacteria and electron acceptor generates the current and voltage needed to make electricity (Logan, 2007; Rabaey and Verstraete, 2005). Scaled up MFC system consisting of 40 individual cells, generating 4.1 W/m^3 of energy, capable of powering LED panel has been constructed and evaluated by Zhuang et al. (2012). Detailed research on MFC and its scale up would enable efficient conversion of waste to energy.

The pioneering research in MFC was performed using pure substrates. Though usage of pure substrate provided better understanding of the underlying process, the present day researchers are using more unconventional substrates with an aim of exploiting waste biomass or treating wastewater on one hand and improving MFC output on the other. Also MFC using complex substrates nurtures wide range of microbial communities enhancing

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the power generation (Pant et al., 2009). A range of organic wastewater including brewery industry wastewater (Feng et al., 2008), domestic (Min and Logan, 2004), rice mill (Behera et al., 2010), starch processing (Gil et al., 2003), landfill leachate (You et al., 2006), food processing (Oh and Logan, 2005), hospital wastewater (Aelterman et al., 2006), alcohol distillery (Huang et al., 2011), manure sludge waste (Scott and Murano, 2007), meat packing plant wastewater (Heilmann and Logan, 2006), paper recycling wastewater (Huang and Logan, 2008) have been used as substrate in MFC.

In rapidly developing country like India, the industrialization has triggered the recent trend of migration of rural people to urban areas for employment, raising the need of processing milk. The food processing industries have reported that 94.6 million tones of milk processed in the year of 2006–2007 and growing at the rate of 3–4% in India every year. Dairy industry wastewater consists of carbohydrates, proteins and fats, making it complex in nature. For every liter of milk processed, 2–2.5 L of wastewater is produced (Ramasamy et al., 2004). Hence the huge volume of dairy wastewater goes unutilized, pollutes the environment when released without treatment.

Current treatment methods include biological and physiochemical process (Kushwaha et al., 2011). Aerobic procedures are energy intensive increasing the cost of treatment, making it impossible to be implemented on large scales (Wheatley, 1990). Anaerobic treatment approach include production of biogas by Methanogenic bacteria, Inhibition of Methanogenic bacterial growth by long chain fatty acids produced by lipid hydrolysis present in wastewater results in lower biogas yield (Vidal et al., 2000). The presence of Methanogenic bacteria is the cause behind reduced electricity production in MFC, hence inhibition of methanogens growth by long chain fatty acid would prove to have positive influence over power generation in an MFC. The organic nature of wastewater makes it a suitable substrate for MFC operation (Pant et al., 2009).

Gil et al. (2003) has reported that increasing the fuel oxidation and electron transfer in anode compartment enhanced the power generation in MFC using starch processing wastewater. Rate of bio-oxidation can be improved by increasing microbial activity. Venkata Mohan et al. (2008) have studied MFC using synthetic wastewater, state that substrate degradation was higher using aerobic anodic metabolism than anoxic and anaerobic mechanism, which was due to higher substrate oxidation and good biofilm growth on anode surface. Most of the literatures on MFC include usage of inoculum from existing industrial wastewater treatment plants, (Venkata Mohan et al., 2010). These microbes require an initial lag phase to adapt itself to grow in dairy industry wastewater.

Studies have reported that dairy industry wastewater produces relatively lower power in comparison with other wastewater in MFC (Mathuriya and Sharma, 2010; Velasquez-Orta et al., 2011). Main components of dairy wastewater are carbohydrates and proteins. Apart from COD removal, influence of carbohydrates and proteins in power generation of MFC using dairy wastewater as substrate has been studied by Venkata Mohan et al. (2010) and has reported that reduction in carbohydrates and proteins did not show good relation to power generation. Researchers working with dairy industry wastewater have concentrated in improving the configuration of MFC to improve power generation. Mardanpour et al. (2012) tried to improve the power output in MFC using dairy wastewater by increased anode area using spiral anode where high open circuit voltage (OCV) of 810 mV was obtained. Ayyaru and Dharmalingam (2011) studies reveal that using SPEEK membrane in MEA–MFC assembly produced higher power in comparison to Nafion in MFCs treating dairy industry wastewater. The maximum voltage of 400 ± 15 mV was obtained in this system.

In this study, we concentrate on improving power generation by increasing fuel oxidation. The aim of investigation was to improve power production of dual chambered MFC employing plain graphite plate electrodes by varying anodic metabolism (aerobic and anaerobic) by increase rate of fuel oxidation, the native microbes present in the wastewater has been used as inoculum. The influence of operating parameters such as pH and concentration of Chemical Oxygen Demand (COD) in fuel oxidation and power production has been studied.

2. Methods

2.1. Dairy wastewater and inoculum

Dairy wastewater was collected from milk processing industry near Krishnagiri, Tamil Nadu. The wastewater was stored at 4 °C and brought to room temperature prior to use. The collected wastewater was foul smelling, with characteristically lower COD of 3200–3400 mg/L and pH value near neutral of 7.6.

For aerobic operations, single aerobic bacterial colony was isolated from dairy wastewater by serial dilution (Harley and Prescott, 2002). The isolated colony was maintained in slant cultures. The isolated colony was inoculated to sterilized dairy wastewater, to avoid the influence of other microbes on the MFC performance. Distilled water used in case of dilution was also sterilized. Continuous supply of oxygen was maintained for aerobic metabolism in the anode chamber. For operation of MFC, using anaerobic metabolism the wastewater was purged with nitrogen to remove dissolved oxygen and the anode chamber was completely sealed from external atmosphere. Unsterilized wastewater was used as inoculum for anaerobic metabolism. The anode plate was subjected to scanning electron microscopy (SEM JEOL JSM-6701F) to visualize the bacterial biofilm formed over the graphite plate. The electrode was air dried and thin layer of platinum was coated prior to imaging.

2.2. Fuel cell configuration and operation

Dual chambered Microbial Fuel Cell was fabricated using plexi glass material each chamber with total/working volume of 0.35/0.3 L operated in a batch mode. Ferricyanide was used as catholyte. Plain graphite plates (6.5 × 4 × 0.3 cm; surface area 50 cm^2) without any coating were used as electrodes. The electrodes were soaked in distilled water for 24 h and placed 1 cm away from the membrane in the MFC. Nafion 117 was used as proton exchange membrane separating the anode and cathode chamber. To increase porosity the Nafion membrane was pre-treated by boiling sequentially in 30% H_2O_2, distilled water pH 7, 0.5 M H_2SO_4 and distilled water (Venkata Mohan et al., 2007). Sampling ports were made to collect anolyte at particular intervals. These ports were sealed when anaerobic environment had to be maintained. Connections were made using copper wires.

MFC reactors were operated in fed batch mode and refilled each time when the OCV dropped below 200 mV. No extra inoculum of microbes from previous cycles was added. The MFC were operated at room temperature of 30 ± 2 °C. The effect of COD concentration was studied with dilution of wastewater made using sterilized distilled water.

2.3. Analyses and calculations

The cell potential (E in volts) of the system was measured using a digital multimeter (aplab vc97). The current (I in ampere) produced from a single MFC is small, so that the current is not measured, but instead it is calculated from the measured voltage

drop across the resistor as $I = E/R_{ex}$, where R_{ex} (Ω) is the external resistant. Power was calculated as P (Watts) = ER_{ex}. Polarization data was obtained by connecting resistance ranging 100 Ω to 30 kΩ. The voltage readings were taken after 15 min for voltage stabilization. Power density and current density were calculated by normalizing power and current with respect to anode surface area, respectively, as biological reactions occur at the anode. Polarization curve generated by plotting power density vs current density. Volumetric power and volumetric current is calculated on the basis of net liquid volume of anode compartment. The concentration of COD was measured by closed reflux titrimetric method using chromate as the oxidant. The treatment efficiency of MFC for the treatment of wastewater was calculated on the basis of percentage COD removal:

$$\text{Percentage COD removal } (\text{COD}_R) = \frac{\text{COD}_i - \text{COD}_t}{\text{COD}_i} \times 100 \quad (1)$$

COD_i is concentration of COD at $t = 0$ and COD_t is concentration of COD at $t = t$ in the wastewater.

The efficiency of conversion of chemical energy in the anolyte to electrical energy was measured as Columbic efficiency (CE) and was calculated by integrating the measured current possible based on the observed COD removal. The CE evaluated over a period of time t, is calculated as:

$$\text{CE} = \frac{M \int_0^t I dt}{FbV_{an}(\Delta\text{COD})} \quad (2)$$

where M is the molecular weight of final electron acceptor, F is Faraday's constant, b is number of electrons transferred/mole of O_2, V_{an} is the volume of anolyte and ΔCOD is the change in COD in the time t.

3. Results and discussion

3.1. Performance of MFC under different anodic metabolism

Anodic microbial metabolism plays an important role in MFC performance. Each metabolism follows it own metabolic pathway for energy generation varying the power generation capacities. The MFC were operated with initial COD concentration of 1600 mg/L and anolyte pH 7. Phosphate buffer at working concentration of 10 mM was used to maintain pH of the anolyte. Voltage could be rapidly generated during the treatment of dairy wastewater of both aerobic and anaerobic anodic metabolism in the MFC. The highest OCV was recorded nearly 760 and 780 mV for anaerobic and aerobic metabolism, respectively. Fig. 1(A) shows the OCV variation with time, of aerobic and anaerobic anodic metabolism. It could be observed that the OCV of aerobic system reached the peak on 1st day, remained same for 2 days and then started to drop on 3rd day. While the system operating with anaerobic metabolism reached the peak on 2nd day and was stable for 4 days and started to drop 5th day onwards. The maximum OCV was observed from first cycle of operation while using both aerobic and anaerobic anodic mechanisms. Venkata Mohan et al. (2010); Mardanpour et al. (2012) studies shows the requirement of lag phase by microbes of 2–3 cycles after which maximum OCV was observed. Elimination of lag phase requirements may be a positive result of using inherent microbes of dairy wastewater which eliminates the adaptation phase in microbial growth. This behavior of MFC marks the opportunity to generate current from first cycle of operation. However there was marked difference in the power generation when different anodic metabolism was used. Fig. 1(B) shows the polarization behavior of MFCs using different metabolism. The polarization data suggest that both the MFC produced maximum power density at 470 Ω of external resistance; it was recorded to be 197

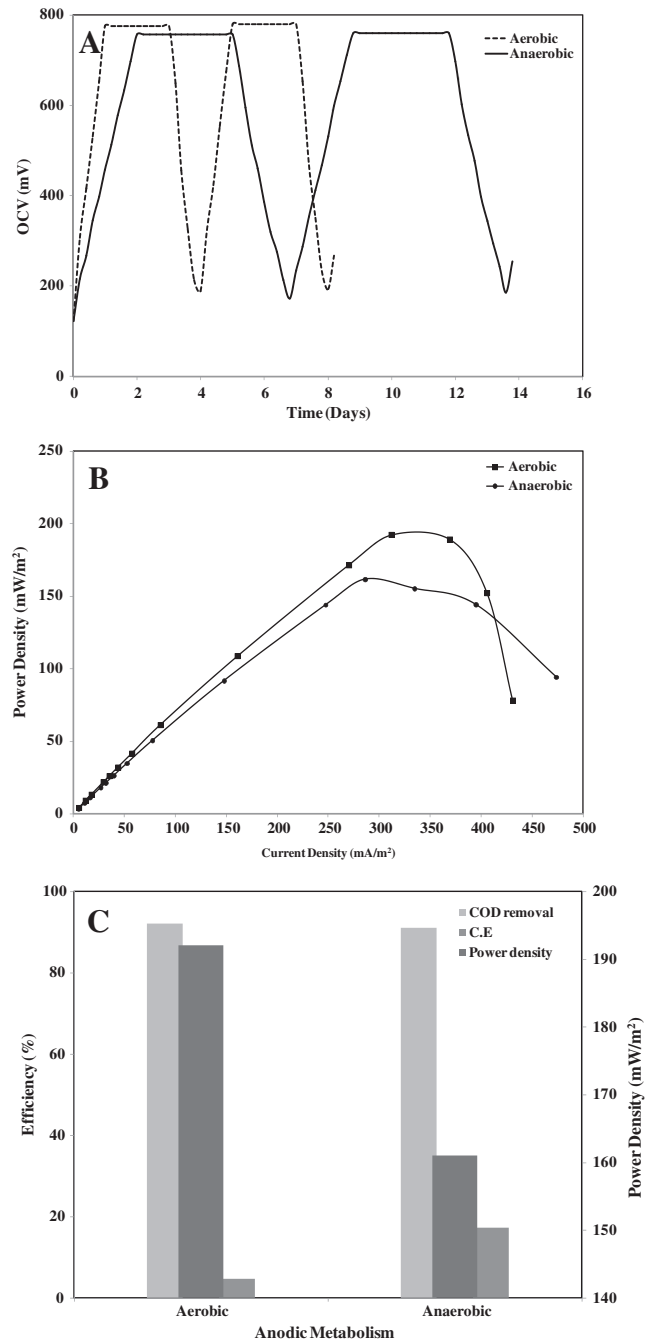

Fig. 1. (A) OCV variation along time using different anodic metabolism, (B) effect of aerobic and anaerobic metabolism on power production in dual chambered MFC with ferricyanide catholyte, and (C) comparison of percentage COD removal, columbic efficiency and maximum power density with different anodic metabolism.

and 161 mW/m^2 for aerobic and anaerobic metabolism, respectively. Fig. 1(C) shows the comparison between aerobic and anaerobic metabolism influence on the MFC performance on the basis of power density, COD removal efficiency and CE. The COD removal efficiency of 91% for anaerobic and 92% for aerobic metabolism was observed in 8 and 4 days respectively. The major drawback of aerobic system was that the efficiency of conversion of chemical energy to electrical energy was 3.7-folds lower than anaerobic metabolism whose efficiency was 17.16%. In aerobic mode the microbes used oxygen as terminal electron acceptor, hence loss of electrons occurred reducing the CE. Though the CE for aerobic

metabolism was lower than anaerobic metabolism it could produce higher power density, this could be the result of rapid growth and hyper metabolic activity of aerobic bacteria, which would result in higher rate of protons and electrons generation. The faster COD removal by aerobic metabolism is a result of quick substrate utilization (Venkata Mohan et al., 2008).

3.2. Effect of operating pH of anolyte

3.2.1. MFC operation with pH buffer

The effect of pH remains to be one of the most important parameters determining microbial activity. The systems were operated using anaerobic metabolism due to very low CE of aerobic metabolism. The influences of Anolyte pH on MFC performance was investigated by varying pH 5, 7 and 9 with initial COD concentration of 1600 mg/L. Citrate and phosphate buffers were used to maintain pHs in the anolyte and the working concentration of buffer in the anolyte was 10 mM. Fig. 2(A) shows the variation in OCV on increasing time in MFCs operating at various anolyte pH. The maximum OCV was observed of MFCs operating at pH 5, 7 and 9 were 754, 774 and 675 mV, respectively. The MFC at pH 7 was recorded with maximum OCV in 2 days which was stable for 4 days; drop in voltage was observed on 5th day and reached 202 mV on 7th day. However MFC operating with pH 5 and 9 attained peak OCV only on 4th day and was stable for 3 days and dropped on 7th day and reached 154 and 143 mV on 8th day, respectively. The lag time of 3 days for MFC operating at pH 5 and 9 compared to pH 7 is due to the requirement of microbial adaptation to anolyte pH. The wastewater pH is near neutral 7.6, hence the inherent microbes would have required adapting to pH 5, 9 leading requirement of lag time of 2 days to reach maximum OCV.

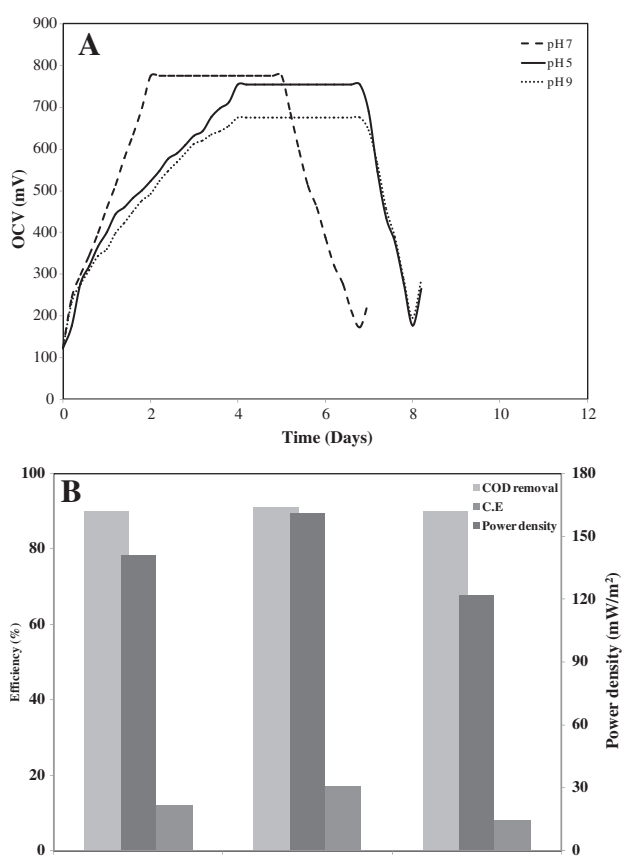

Fig. 2. (A) Effect of anolyte pH on open circuit voltage, and (B) effect of pH of anolyte on columbic efficiency, percentage COD removal and maximum power density.

Fig. 2(B) represents the comparison of MFC performance in energy generation and treatment of wastewater when operating at varying anolyte pH. Maximum power density produced was 161, 144 and 122 mW/m^2. The COD removal efficiency was found to be 90%, 89% and 86% for pH 5, 7 and 9, respectively. CE was highest for pH 7 of 17% followed by pH 5 of 12% and lowest for pH 9 of 8%. The batch time for MFC at pH 5, 7 and 9 were 8, 7 and 8 days, respectively. The final pH of anolyte was found to be reducing for initial pH of 7 and 9 to 6.9 and 8.5, respectively. While the pH of system operating with initial pH 5 increased to 6.8. Lower power production by MFCs operating with initial pH 5 and 9 would be the consequence of slower microbial activity at suboptimal pH than optimal pH. Poor proton transfer at reduced proton gradient across anode and cathode at higher pH of 9 might be one of the reasons for low performance of the system. The slower activity of microbes contributes to the increased batch time of 9 days for pH 5 and 9, for treatment of wastewater. The variation of pH in anolyte can be as a result of both electrochemical and microbial reactions. (Gil et al., 2003; He et al., 2008)

3.2.2. MFC operation without pH buffer

Anolyte system using phosphate buffer (pH 6.9) of 10 mM concentration bringing the initial concentration of anolyte to 7.2 showed gradual decrease of pH to 6.9 in 8 days. When the system was operated in absence of buffer the pH was adjusted to 7 by using orthophosphoric acid. Fig. 3(A) depicts the pH variation in the MFC when buffer is not used in comparison to MFC operating with buffer. The pH fluctuations were found to be significant in absence of buffer. The pH of MFC in absence of buffer system increased gradually to 7.52 on the 3rd day and then decreased to 7.02 on the 6th day. Though the OCV and treatment efficiency were same, marked difference were observed in polarization of the system. Fig. 3(B) shows the polarization behavior of the MFC with and without pH buffer system. The maximum power density of the system without buffer was 85.98 mW/m^2 which was nearly half the value of system using buffer system for pH maintenance being 161 mW/m^2. The requirement of batch time of 8 days for both the reactors for COD removal of 90% suggests that microbial activity was not affected by elimination of the pH buffer.

The phosphates and citrate remain as carriers of protons in MFC. Though the diffusion coefficient is lower for these carriers, the concentration gradient across the membrane is higher. Higher concentration gradient is due to absence of phosphates and citrates in cathode chamber. Due to increased proton transfer there was reduction in internal resistance usually caused by proton concentration polarization, thus improving power production in system using pH buffer (Fan et al., 2007). Phosphate buffer system have a magnificent effect in increasing power generation by affecting the electrochemical reactions though it did not affect the microbial growth and COD removal efficiency of MFC. Higher power density could be achieved at pH 7 of anolyte. Addition of phosphate buffer for pH modification is not required as pH of wastewater without modification is near neutral, but addition of phosphates is essential to increase the proton transfer in MFC.

3.3. Influence of substrate concentration

Concentration of substrate in the anode compartment has a great influence on microbial growth. The MFCs were operated with anaerobic metabolism with initial anolyte pH of 7 using buffer system. The concentration of substrate of the system varied as a function of initial COD concentration of 800, 1600 and 2800 mg/L. Marked difference could be observed in the maximum OCV attained by the MFCs. The maximum OCV of 760 mV was recorded

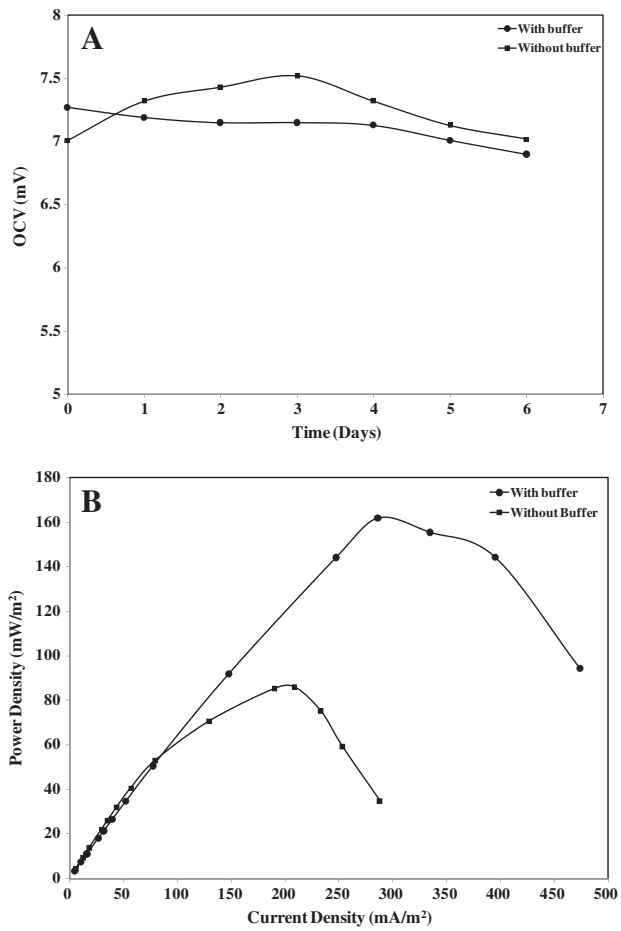

Fig. 3. (A) Comparison of pH variations with and without addition of buffer in anode compartment, and (B) polarization behavior of MFC operated with addition of buffer.

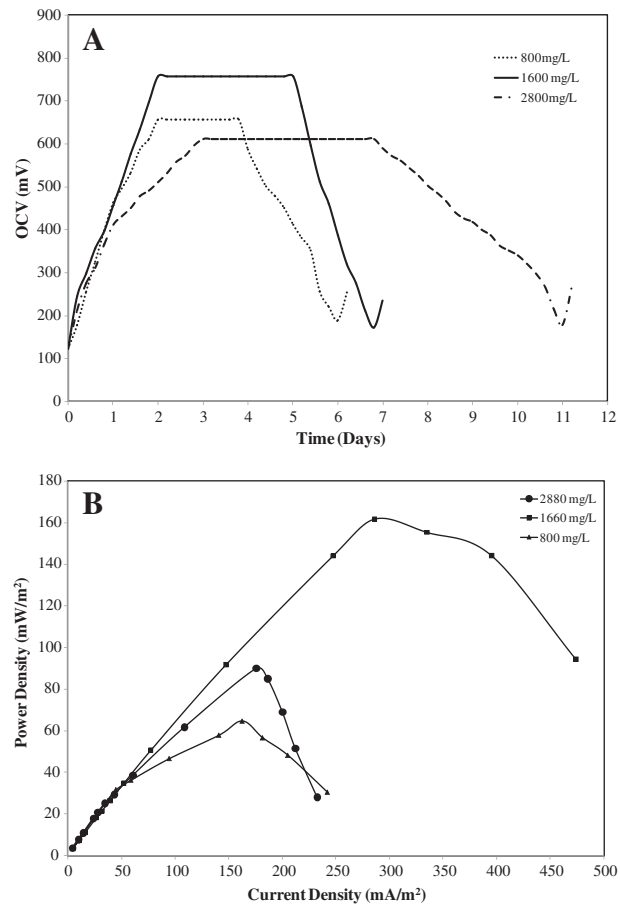

Fig. 4. (A) Effect of COD concentration on OCV in dual chambered MFC, and (B) polarization behavior of MFC operating at different COD concentration in anolyte.

in MFC operating with COD concentration of 1600 mg/L. System operating with COD concentration of 800 and 2800 mg/L attained peak value OCV of 656 and 612 mV, respectively. Fig. 4(A) shows the variation in OCV by increasing time for MFC operating at varying COD concentration. The batch time requirement of MFC operating with COD concentration of 800, 1600 and 2800 mg/L were 6, 7 and 11 days. Maximum power density of 161 mW/m^2 was recorded for 1600 mg/L of COD concentration and is 2.5- and 1.8-folds lower for MFC operating with COD concentration of 800 and 2800 mg/L, respectively. Fig. 4(B) shows the polarization behavior of these MFCs with different initial concentration of COD. The columbic efficiency was 2.6- and 1.7-folds lower for MFC with 800 and 2800 mg/L, respectively compared MFC at 1600 mg/L COD concentration having 17.17%.

On using wastewater at higher COD results in reduction in power generation, which may be due to substrate mediated inhibition of microbial growth (Mathuriya and Sharma, 2010). Organic fouling of the membrane was found to be very high on the anode side in MFC operating with initial COD concentration of 2800 mg/L which could be the reason for poor performance in power production. When 800 mg/L of initial COD concentration was used there was drastic decrease of power production. The power production reduced with decrease in initial substrate load (Venkata Mohan et al., 2010; Feng et al., 2008). The variation of initial COD did not affect the treatment efficiency of the MFC but the batch time for treatment increased with increase in substrate concentration (Feng et al., 2008). Venkata Mohan et al. (2007) also reported that there was no correlation between the treatment efficiency and power generation.

The earlier literature data on dairy wastewater and their performance has been compared and summarized in Table 1. The maximum OCV of 308 mV and volumetric power of 1.1 W/m^3 was reported when single chambered MFC using graphite plate anode. The results observed in this study are higher OCV of 760 mV and power density of 2.7 W/m^3, this tremendous increase in power may be due to use of pH buffer system to maintain pH, optimal operating parameters of pH 7 and COD feed rate of 1600 mg/L and use of inherent microbes of wastewater in power generation.

3.4. Difference in biomass community structure of different anodic metabolism

Attachment of microbes to plain graphite plates could be visualized in SEM images. Development of mixed microbial bio-film over the plain graphite plates was visible. The microbes present in the wastewater are sufficient to form bio-film over the anode. Plain graphite plates provide good surface for bacterial attachment. The SEM images of aerobic and anaerobic mode show difference in the biomass community structure. In aerobic mode community structure consists of both rod and clusters but in anaerobic mode clusters are predominant. The microbial clumps were larger in aerobic than anaerobic mode which may be due to rapid microbial growth. Analyses of microbial community by 16SrRNA pyrosequencing would reveal the details of difference in community structure of aerobic and anaerobic modes (Lu et al., 2012).

Table 1
Comparison of performance of MFCs using dairy industry wastewater as substrate.

S. No.	MFC type	Inoculum	COD (mg/L)	Power (W/m^3)	COD_R (%)	CE (%)	References
1	Dual chambered	Dairy wastewater	1600	2.7	91	17	This study
2	Single chambered	Dairy wastewater	1487	1.157	81	–	Mathuriya and Sharma (2010)
3	Single chambered	Activated sludge from DW treatment plant	1000	20.2	91	26	Mardanpour et al. (2012)
4	Single chambered	Anaerobic inoculum from industrial wastewater treatment plant	4440	1.1	95.5	14.2	Venkata Mohan et al. (2010)
5	Single chambered	Anaerobic digested sludge from municipal wastewater treatment plant	2800	0.442	82	3	Velasquez-Orta et al. (2011)

4. Conclusion

This study demonstrates that anaerobic metabolism has better columbic efficiency than aerobic metabolism, though higher power generation was observed while using aerobic metabolism. Aerobic metabolism exploitation in MFC would involve continuous supply of oxygen for growth increasing the energy requirements and cost of installation. Pre-existing aerobic treatment plants can be modified to suit MFC operation which could supply energy requirements of aeration, when considering new installation it would be wise to use anaerobic systems which are more efficient in recovering energy from the waste biomass. Dairy industries operate all through the year making MFC operation reliable and sustainable.

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