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Confectionery wastewater treatment through upflow microbial fuel cell

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ABSTRACT

In the present study, upflow microbial fuel cell was setup for the treatment of confectionery wastewater. Upflow anaerobic sludge blanket reactor was used as anaerobic unit which was connected with aerobic units with salt bridge. For optimization of reactor startup synthetic wastewater was used for 30 d then different concentrations of confectionery wastewater were treated. Samples were analyzed for physicochemical parameters and chemical oxygen demand (COD) and Pearson's correlation matrix was applied. Biogas was collected and electricity was measured through multimeter. Total anaerobic microbial count was performed on thioglycollate media (CFU mL^{-1}) and biofilm formation was observed through scanning electron microscopy (SEM). After startup COD removal efficiency with 50% diluted wastewater was 100%. Highest voltage (982 mV), power density (0.042 W cm^{-2}) and current (797 μA) were observed when 1 mL nutrients added wastewater was used. Maximum biogas 472 mL d^{-1} production was achieved in undiluted wastewater sample. Highest anaerobic count 1.24×10^4 CFU mL^{-1} was observed when 1.5 mL nutrient added wastewater was used. SEM analysis showed biofilm formation on C anode with filamentous microbes.

Keywords: Bioelectricity; Confectionery; Microbial fuel cell; Pearson correlation; Scanning electron microscopy; Salt bridge

1. Introduction

Anaerobic treatment is a popular and well-known process in wastewater treatment since 1980s, because of its high efficiency for low and high strength wastewater treatment and due to its cost-effectiveness [1]. For the control of pollution and infinite dependence on fossil fuels, it is well accepted that alternative sources of energy are immediately required. Bacteria can be used to generate electricity from waste and renewable biomass. High content of organic matter in the wastewater is an ideal substrate to produce bioelectricity and biogases [2]. Confectionery industry is categorized into three main sections, sugar, chocolate and flour confectioneries. During confectionery manufacturing process, liquid raw material and wastewater are produced with high biochemical oxygen demand and chemical oxygen demand (COD) [3], which should be treated before discharged into natural watercourses [4].

In upflow anaerobic sludge blanket (UASB) reactor, wastewater flows in sludge bed, in which different

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biochemical and physical mechanisms take place in order to acidify and biodegrade organic contents, and convert them into biogas and bioelectricity [5].

Microbial fuel cell (MFC) is a bioreactor which changes chemical energy into electrical energy through catalytic reactions of microorganisms under anaerobic conditions [6]. In upflow microbial fuel cell, at anode chamber microorganisms oxidized the substrate which are added to the system, for example, in the present study confectionery wastewater was used, which formed electrons and protons. Through external circuit, free electrons from anode are transported to cathode. Protons passes through the salt bridge or proton exchange membrane and arrived at the cathode chamber, in the presence of electrons and oxygen water molecules were formed [7,8]. In this research, UASB reactor was used as an anode chamber which provides anaerobic condition with acclimatized microbial community. These microbes served as a biocatalyst for bioelectricity production [9]. Anode electrode more suitably receives electron during organic waste degradation. It is hypothesized that UASB might be proved as a suitable alternative for anode and confectionery wastewater would be useful substrate for biogas and electricity production.

2. Materials and methods

2.1. Wastewater sample and sludge collection

The research was conducted for the treatment of confectionery industry wastewater, located at Hattar Industrial Estate, Pakistan. Sample of wastewater was collected from effluent's drain in the prerinsed plastic bottle and all physiochemical parameters were analyzed in the laboratory within 24 h. For anaerobic bioreactor the sludge was collected in a cane of 4 L capacity from Municipal Committee, Abbottabad.

2.2. Construction of upflow microbial fuel cell

In lab scale experimental setup, UASB reactor was used as anaerobic unit (anode chamber) of MFC. This airtight unit was made with acrylic material, 18 cm height and 2.5 cm diameter, with 2 L capacity. Appropriate amount of sludge was transferred in this unit and anaerobic conditions were maintained. Aerobic unit was made by plastic bucket with 7.5 cm length, 3.8 cm width and 4.5 L capacity. Aeration pump was used in aerobic unit for oxygen supply which reacted with protons from anaerobic unit. Both anaerobic and aerobic units were connected through salt bridge and external electrical circuit (Fig. 1).

2.3. Preparation of salt bridge

Salt bridge was prepared by dissolving 15 g agarose and 10 g NaCl in 300 mL distilled water and boiled together in an oven for 3 min [10,11], after boiling the mixture was poured in 6 inches long polyvinyl chloride pipe and tightly sealed. Pipe was then placed in the refrigerator for proper solidification and then cut into two pieces of 3 inches length. These two pipes were connected between two units; one was attached with wastewater portion and another with sludge at the bottom for upflow.

2.4. Electrical circuit setup procurement

Anaerobic and aerobic units were externally connected by an electric circuit, made by 182 cm long copper wire. Anode was prepared from C rods by using consumed batteries, and each had 50.8 mm length and 8.02 mm diameter. On the other end of wire seven aluminum rods of 50 mm length and 9 mm diameter were connected as cathode electrode, and were placed in the aerobic unit. Both anode and cathode points were outside the reactor to measure the current.

2.5. Upflow microbial fuel cell operation

The duration of startup phase was 1 month, synthetic wastewater containing nutrients and trace elements (Tables 1 and 2) were fed in anaerobic unit through peristaltic pump. Nutrients solution was prepared by adding 10 mL

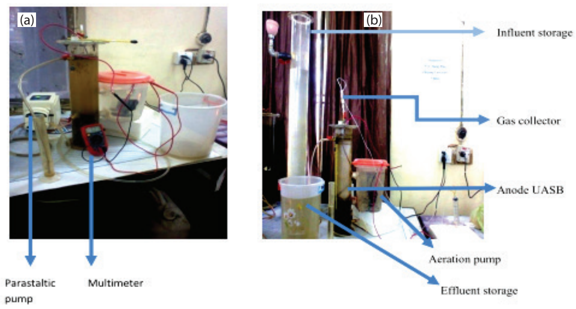

Fig. 1. Upflow microbial fuel cell (a) before treatment and (b) after treatment, sludge settlement can be seen in UASB.

Table 1
Composition of trace elements in synthetic wastewater

Components	Amount (g L^{-1})
Na_2-ETDA	5
NaOH	11
$CaCl_2.2H_2O$	7.34
$FeCl_2.4H_2O$	3.58
$MnCl_2.2H_2O$	2.5
$ZnCl_2$	1.06
$CoCl_2.6H_2O$	0.5
$(NH_4)_6MnO_{24}.4H_2O$	0.5
$CuCl_2.2H_2O$	0.24

Table 2
Nutrient composition in synthetic wastewater

Nutrients	Concentration (g L^{-1})
$NaHCO_3$	1.0
Trace element solution	1.0
$MgCl_2$	1.0
KH_2PO_4	1.0
$(NH_4)_2SO_4$	0.24
Glucose	2.5

trace elements, 10 mL synthetic solution and 2.5 g glucose in 10 L tap water [12].

After 1 month reactor was directly fed with confectionery wastewater in different concentration and treated wastewater was collected from an outlet valve in the UASB reactor. The treatment efficiency was checked for 3 months and biogas produced during anaerobic digestion was collected and measured in plastic syringe of 60 mL volume. Protons produced in the anaerobic unit were transferred to aerobic unit through salt bridge, whereas electrons produced in the anaerobic unit were captured by carbon rods and transferred to external circuit connected to cathode.

Initially flow rate at 0.5 rpm was adjusted as 21 mL h^{-1} and the hydraulic retention time was 95 h (HRT = volume/flow rate), later on flow rate increased to 0.6 and 1 rpm and the HRT reduced to 55.5 and 21 h.

2.6. Analysis of physicochemical parameters

Untreated and treated wastewater samples were analyzed daily for pH, total dissolved solids (TDS), electrical conductivity (EC), temperature, turbidity, COD according to standards methods of analysis [13]. pH of the influent and effluent samples was determined by Digital desktop pH meter (Jenway model 520). Temperature, EC and TDS were measured by potable meter (Jenway model 470). Turbidity of influent and effluent samples was determined by Turbidometer (Eutech, TN-100). COD was determined by closed reflux chlorimeteric method using digester (HACH-LTG 082.99.40001) and Lovibond COD meter [12]. Electric current and voltage were measured by multimeter. Electric current was measured in microamperes (μA) and voltage was measured in millivolts (mV).

2.7. Anaerobic total plate count

Sludge sample was taken from the outlet valve of anode chamber of upflow MFC for microbial analysis. Total anaerobic plate count (CFU mL^{-1}) was enumerated on thioglycollate medium, briefly 8.92 g thioglycollate fluid media and 3.5 g agar were dissolved in 300 mL distilled water (already passed through nitrogen gas in order to remove oxygen). After mixing it was boiled on hot plate [14], sterilized and was poured in Petri plate and allowed to solidify. After solidification, samples were taken in syringe for anaerobic condition, 10 mL syringe was filled with 9.5 mL of nitrogen treated distilled water and 0.5 mL sludge sample, mixed and diluted, then spread on media plates. These plates were then wrapped with paraffin film to avoid any air contamination, to maintain anaerobic condition, these plates were placed in desiccators, which were wrapped with paraffin film and incubated at 37°C for 4–6 d.

2.8. Scanning electron microscopy of anode

The surface of the anode electrode was visualized by scanning electron microscopy (SEM) to determine microbial attachment and formation of a biofilm on the electrode surface. Anode samples with a geometric surface area of 1 cm^2 were rinsed with fresh MFC medium to remove suspended bacteria and fixed for ~15 min with 2 mL of 1% glutaraldehyde. The samples were then washed three times with Milli-Q water (2 mL) and dehydrated by immersion in a series of 2 mL ethanol solutions of increasing concentration (25%, 50%, 70%, 85%, 95% and 100%, v/v) for 5 min each and incubated in hexamethyldisilazane for 5 min and air-dried prior to coating with gold [15]. SEM was performed by using scanning electron microscope JEOL, model JSM 5910, Japan.

2.9. Statistical analysis

In order to check the significance difference (at $p < 0.05$) of various parameters with time, XLSTAT, 2017 version was used to perform multivariate analysis of variance. Pearson correlation model was constructed to check the correlation coefficient among different parameters and $r \geq 0.5$ considered as significant correlation.

3. Results and discussion

3.1. Pearson correlation model of different parameters in the startup of upflow microbial fuel cell

Initial physicochemical parameters of confectionary wastewater show acidic pH of 4.6 with higher values of COD and total suspended solids (Table 3). In the early days of lab experiment sludge was in semisolid form, as the time passed the sludge became thick and small granules formed. In the anodic compartment microbes consumed different components of organic substrates (Fig. 2).

In the startup period sludge was not properly settled and pH variation occurred because microbes undergone stabilization. Due to glucose fermentation acid formation resulted in lower pH, later on volatile fatty acid converted to CO_2 and CH_4 which contributed in raised pH [16]. Influent temperature was uncontrolled and ranges from 9.9°C to 15.9°C and effluent temperature ranges from 8.3°C to 16°C (Table 4).

In early days, effluent TDS increased but as the time passed it was decreased, and ranges from 202 to 599 mg L^{-1}. At day 14, influent conductivity increased to 696 μS cm^{-2} and on 8th day, effluent conductivity was maximum 1,547 μS cm^{-2}. Turbidity varied greatly during startup, the maximum effluent turbidity was 742 NTU at day 2 and it decreased to 20 NTU at day 30. Effluent COD range decreased from 1,262 to 0.0 mg L^{-1} from day 2 to 31 (Table 3). EC, TDS, turbidity

Table 3
Physicochemical parameters of raw confectionary industrial wastewater

Parameters	Values
pH	4.60
TDS (mg L^{-1})	880.00
COD (mg L^{-1})	2,500.00
TSS (mg L^{-1})	443.00
VSS (mg L^{-1})	171.00
VSS/TSS	0.1603
Turbidity (NTU)	200.00
DO (mg L^{-1})	0.00

TSS, total suspended solids; VSS, volatile suspended solids; DO, dissolved oxygen.

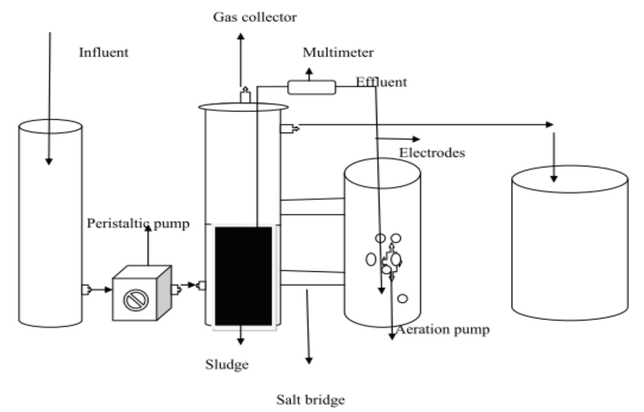

Fig. 2. Upflow microbial fuel cell setup, sludge in UASB reactor connected with fuel cell through salt bridge.

Table 4
All parameters range during startup of reactor from day 1 to 30

Parameters	Effluent (±SD)
pH	7.3–8.7 ± 0.02
Temperature (°C)	8.3–16 ± 0.057
TDS (mg L^{-1})	202–599 ± 0.7
EC (μS cm^{-2})	381–1,547 ± 0.57
Turbidity (NTU)	10.5–742 ± 0.1
COD (mg L^{-1})	0–1,260 ± 10
Volts (mV)	224–694 ± 50
Current (μA)	26–71 ± 8
Gas production (mL d^{-1})	200–270 ± 71
Power density (W cm^{-2})	0.000–0.003 ± 0.001

and COD decreased with the passage of time as significant negative correlation was found between these parameters with days. TDS, turbidity and COD showed significant positive correlation of r = 0.9, 0.7 and 0.79 (n = 30), respectively, with EC (Table 5).

3.2. Pearson correlation model of different parameters during confectionery wastewater treatment

After 1 month of upflow microbial fuel cell optimization with synthetic wastewater, confectionery wastewater sample was treated. During treatment process different parameters were observed and Pearson correlation model was drawn which showed significant negative correlation (n = 32) between sample TDS, EC and turbidity with time and temperature (Table 6).

COD shows strongly negative correlation (r = –0.62) with pH, which means that COD decreased with alkalinity and increased with acidity. EC showed strongly positive correlation (0.92 and 0.6, n = 32) with TDS and turbidity, respectively. Decreased in turbidity was due to upward flow of wastewater, all the minute impurities and large particles trapped in the sludge blanket and consumed by the anaerobic microbes of the reactor [17].

3.3. Effect of confectionery influent concentration and nutrients addition on treatment efficiency

From 32nd to 34th day, 50% diluted wastewater was fed in the reactor, from 35th to 54th day undiluted wastewater was used, from 55th to 59th day, 1 mL synthetic nutrients was added, from 60th to 63rd day wastewater plus synthetic nutrients in 1.5 mL concentration were used as influent.

Table 5
Pearson's correlation matrix of various parameters on different days of startup

Variables	Days	Temperature	pH	EC	TDS	Turbidity	COD
Days	1	–0.265	0.570	–0.779	–0.859	–0.822	–0.841
Temperature	–0.265	1	–0.076	0.236	0.401	0.627	0.477
pH	0.570	–0.076	1	–0.264	–0.263	–0.396	–0.393
EC	–0.779	0.236	–0.264	1	0.942	0.717	0.794
TDS	–0.859	0.401	–0.263	0.942	1	0.867	0.892
Turbidity	–0.822	0.627	–0.396	0.717	0.867	1	0.907
COD	–0.841	0.477	–0.393	0.794	0.892	0.907	1

Table 6
Pearson's correlation matrix of various parameters on different days of confectionery wastewater treatment

Variables	Days	Temperature	pH	EC	TDS	Turbidity	COD
Days	1	0.848	–0.168	–0.573	–0.648	–0.759	0.087
Temperature	0.848	1	–0.240	–0.509	–0.585	–0.641	0.014
pH	–0.168	–0.240	1	–0.310	–0.202	–0.037	–0.622
EC	–0.573	–0.509	–0.310	1	0.920	0.525	0.404
TDS	–0.648	–0.585	–0.202	0.920	1	0.602	0.346
Turbidity	–0.759	–0.641	–0.037	0.525	0.602	1	0.083
COD	0.087	0.014	–0.622	0.404	0.346	0.083	1

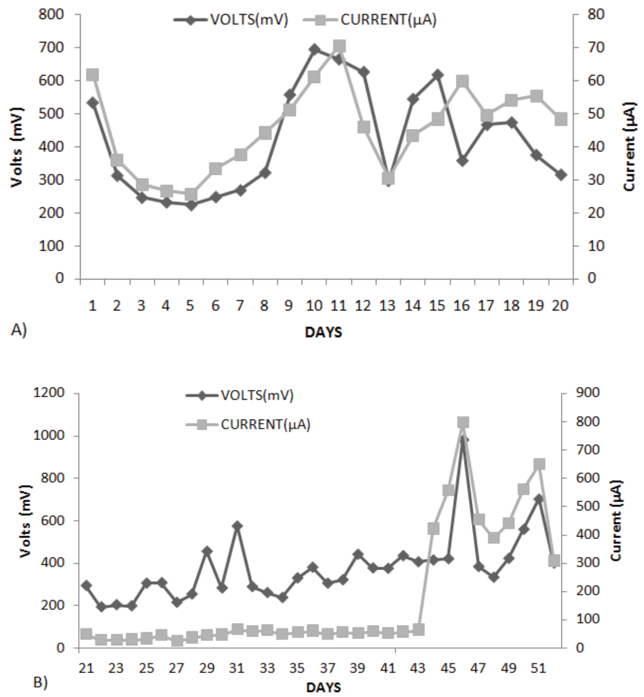

Fig. 3. Values of potential difference and current during (A) startup and (B) confectionery wastewater treatment process on daily basis.

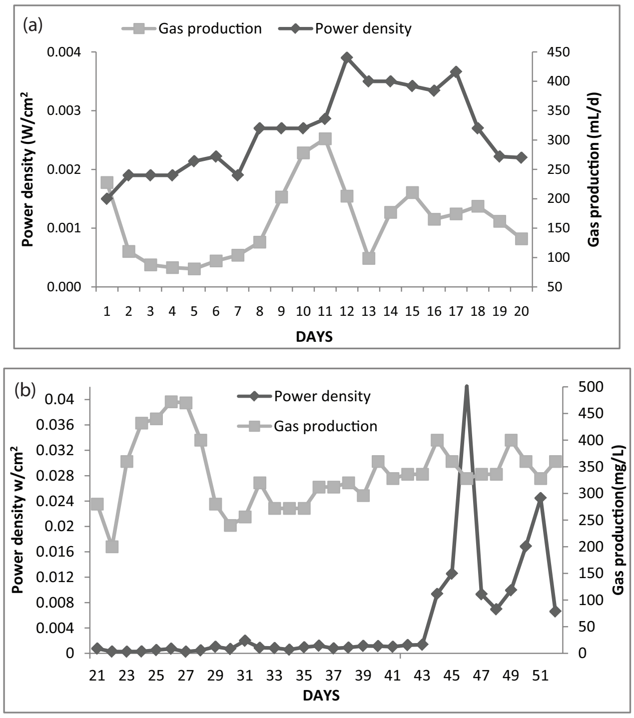

Fig. 4. Values of power density and gas production during (A) startup and (B) confectionery wastewater treatment process on daily basis.

Table 7
All parameters range of confectionery wastewater at different influent concentration

Parameters	50% dilution	Undiluted	1 mL L^{-1} nutrient solution + w.w[a]	1.5 mL L^{-1} nutrient solution + w.w[a]
pH	7.8–8.8	7.0–8.0	5.42–7.26	7.2–7.7
Temperature	8–10	9.5–15	15–17	16–20
TDS (mg L^{-1})	217–222	215–244	179–238	150–197
EC (µS cm^{-1})	419–428	415–431	346–483	208–381
Turbidity (NTU)	16–32	30–47	15–28	12–13
COD (mg L^{-1})	0–80 (100%)[b]	108–392 (0%)[b]	277–670 (58%)[b]	5–131 (96%)[b]
Volts (mV)	203–294	197–575	333–982	400–702
Current (µA)	25.3–47.8	23.5–59.9	62.5–797	307–648
Power density (W cm^{-2})	0.00028–0.00076	0.00027–0.00936	0.00694–0.042	0.0066–0.0244
Biogas (mL d^{-1})	200–360	240–472	328–400	328–400

[a] Confectionary wastewater.
[b] COD removal percentage.

Table 8
Pearson's correlation matrix of bioelectricity parameters on different days

Variables	Days	Volts	Current	Power density	Temperature
Days	1	0.594	0.743	0.723	0.805
Volts	0.594	1	0.734	0.681	0.521
Current	0.743	0.734	1	0.929	0.732
Power density	0.723	0.681	0.929	1	0.748
Temperature	0.805	0.521	0.732	0.748	1

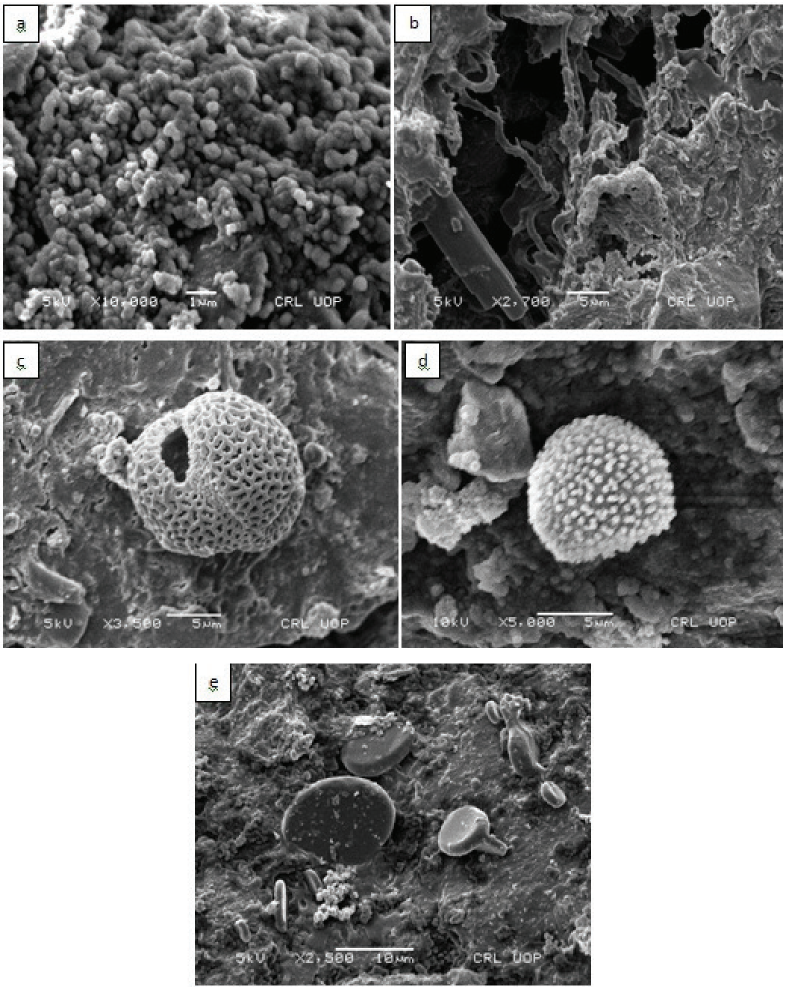

Fig. 5. SEM images of carbon anode at (a) 10,000×, (b) 2,700×, (c) 3,500×, (d) 5,000× and (e) 2,500× resolution, showing biofilm formation with filamentous microbes.

At 50% dilution, and undiluted influent, pH was alkaline but when 1 mL L^{-1} nutrient solution was added to wastewater pH became acidic (Table 7). Nutrient solution concentration when increased to 1.5 mL L^{-1} then pH again became alkaline and highest temperature was observed. Optimum temperature between 35°C and 38°C best for anaerobic degradation rate, which decreases by 11% for each degree Celsius decrease in temperature below this range [18]. In the present study, biogas production might be increased if the reactor and influent's temperature was set to optimum.

At 50% influent's dilution COD removal efficiency was 100%, when wastewater without dilution was used, COD removal was not observed. When wastewater in addition to 1.5 mL nutrient solution were used then 96% COD removal was observed. COD removal efficiencies mainly based on influents of the systems [19], not on the nutrient bioavailability. Current and voltage showed day wise similar trend during startup and maximum values were observed on day 10 and 46 during startup and wastewater treatment, respectively (Fig. 3). Bioelectricity and biogas production were significantly increased with addition of nutrient solution, highest voltage, current and power density were observed when 1 mL nutrients added wastewater was used as 982 mV, 797 μA and 0.042 W cm^{-2}, respectively, on day 46 (Figs. 3(B) and 4(B)).

Table 9
Anaerobic total plate count, CFU mL^{-1} was analyzed at different stages

Days	CFU mL^{-1} (±SD)
Startup (day 25)	$6.06 \times 10^3 \pm 252$
Undiluted (day 36)	$2.33 \times 10^3 \pm 153$
1.5 mL SN + w.w (day 63)	$1.24 \times 10^4 \pm 200$

SN, synthetic nutrient solution.

Comparing with other studies, confectionary wastewater showed higher production of voltage than 779 mV from river water [9], 450 mV from sewage sludge in the presence of Mn–graphite anode in MFC [20]. In the present study, maximum power density was also higher than 0.028 W cm^{-2} [20] and 22 mW cm^{-2} when oxidation of cathode chamber with H_2O_2 was performed [21]. Nutritional conditions are very important for chemical environment of microbial cell. For development of microorganism's proper supply of energy, carbon, nitrogen, minerals and micronutrients are needed [22]. In MFC biological oxidation of pure carbon sources, for example, lactate, acetate and glucose take place and carbon source transform into biogas [23,24].

Highest biogas production was achieved as 472 mL d^{-1} in undiluted wastewater sample (Table 6). Voltage has a significant effect on the power density, as p value was lower than the significance level α = 0.05. Voltage, current, power density and temperature showed significantly positive correlation with each other and day wise (Table 8).

3.4. Anaerobic plate count and biofilm formation on anode

Anaerobic microbial count was observed in startup and during the confectionery wastewater treatment. It was high in the beginning but when undiluted wastewater was ejected anaerobic count was declined, highest count 1.24×10^4 CFU mL^{-1} was observed on the addition of 1.5 mL nutrient solution (Table 9). SEM analysis showed biofilm formation on C anode with filamentous microbes, these microcolonies were seen throughout the electrode (Fig. 5). Logan and Regan [25] studied about microbial community in MFC and used the term 'exoelectrogens' for these bacteria capable of exocellular electron transfer [25]. Firmicutes distinguished character of porous pseudo-outer membrane was observed under SEM. In another study, it was found that extracellular electron transferring bacteria move fastly toward electrodes due to bacterial cytochrome and electrode potential which helped in formation of biofilm. Similarly they also found *Clostridium* (which is the member of firmicutes group) and *Arcobacter* first time in the microbial fuel cells [26].

4. Conclusions

It was concluded that confectionery wastewater contains higher amount of glucose, when this water was used in addition with synthetic nutrients, maximum voltage and current were observed while biogas production and COD removal efficiencies were not affected with nutrient addition in wastewater. Reactor temperature is important factor which was not controlled in the present research, it should be maintained above 30°C for maximum biogas production. Scanning electron micrograph showed biofilm formation on anode due to filamentous bacteria and firmicutes, further study on microbial community structure of anode are recommended which could help in better understanding of microbial role in electricity production.

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