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New fragmented electro-active biofilm (FAB) reactor to increase anode surface area and performance of microbial fuel cell

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Abstract

Background: Microbial fuel cell (MFC) technology is a promising sustainable future energy source with a renewable and abundant substrate. MFC critical drawbacks are anode surface area limitations and electrochemical loss. Recent studies recommend thick anode biofilm growth due to the synergetic effect between microbial communities. Engineering the anode surface area is the prospect of MFC. In this study, a microbial electrode jacket dish (MEJ-dish) was invented, first time to the authors' knowledge, to support MFC anode biofilm growth. The MFC reactor with MEJ-dish was hypothesized to develop a variable biofilm thickens. This reactor is called a fragmented electro-active biofilm-microbial fuel cell (FAB-MFC). It was optimized for pH and MEJ-dish types and tested at a bench-scale.

Results: Fragmented (thick and thin) anode biofilms were observed in FAB-MFC but not in MFC. During the first five days and pH 7.5, maximum voltage (0.87 V) was recorded in MFC than FAB-MFC; however, when the age of the reactor increases, all the FAB-MFC gains momentum. It depends on the MEJ-dish type that determines the junction nature between the anode and MEJ-dish. At alkaline pH 8.5, the FAB-MFC generates a lower voltage relative to MFC. On the contrary, the COD removal was improved regardless of pH variation (6.5–8.5) and MEJ-dish type. The bench-scale studies support the optimization findings. Overall, the FAB improves the Coulombic efficiency by 7.4–9.6 % relative to MFC. It might be recommendable to use both FAB and non-FAB in a single MFC reactor to address the contradictory effect of increasing COD removal associated with the lower voltage at higher pH.

Conclusions: This study showed the overall voltage generated was significantly higher in FAB-MFC than MFC within limited pH (6.5–7.5); relatively, COD removal was enhanced within a broader pH range (6.5–8.5). It supports the conclusion that FAB anode biofilms were vital for COD removal, and there might be a mutualism even though not participated in voltage generation. FAB could provide a new flexible technique to manage the anode surface area and biofilm thickness by adjusting the MEJ-dish size. Future studies may need to consider the number, size, and conductor MEJ-dish per electrode.

Keywords: Fragmented (fixed) electro-active biofilm or fragmented anode biofilm (FAB), Electro-active biofilms (EABs), Microbial fuel cell (MFC), Open-circuit voltage (OCV), Optimization setup, Bench-scale setup

Background

The electrochemical fuel cell was discovered early in 1839, but the bioelectricity generation through the microbial fuel cell (MFC) was known in 1910 after Michael Cresse Potter's observation (Flimban et al. 2019). However, MFC technology has been facing a critical challenge from commercialization (Do et al. 2018; Goto and Yoshida 2019; Guo et al. 2020). Hence, it calls for

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research on several aspects of MFC that require an innovative approach to increase anode surface area, electrode materials fabrication, terminal electron acceptor, proton exchange membrane, reactor design, and microbial dynamics, which needs small-scale investigation (Koroglu et al. 2019).

MFCs are a future promising renewable energy source, which recovers energy from wastewater or agricultural residue (Vicari et al. 2016). Biofilm coated electrodes for bioelectricity generation (MFC) attracted a wide area of research (Yu et al. 2017). It is applicable in biosensors, wastewater treatment, and seawater desalination (Guo et al. 2020). MFC differs from the conventional electrochemical cell (such as batteries and supercapacitors) and low-temperature fuel cell (proton exchange membrane or direct methanol fuel cell) (Borole et al. 2011; Erable et al. 2010). MFC electrochemical reactions are biotic (electroactive bacteria), which functions at a wider temperature (15 to 45 ℃), mostly neutral pH, a diverse range of biomass (liquid or solid) for anodic fuel, and an environmentally benign life cycle (Santoro et al. 2017).

MFC consists of anode and cathode separated by a membrane (double-chambered) or membrane-less (single-chambered), an external circuit, microorganisms, and substrate as a fuel. In MFC, microbes oxidize the substrate and release electrons to anode that flow as electrical current from the anode (negative terminal) to the cathode (positive terminal) through an external circuit, while proton travel via the proton exchange membrane or liquid (Logan 2008). The electrons, proton, and oxygen (e^- acceptor) react in the cathode to form water (Guo et al. 2020; Logan et al. 2006). There are two different cathode types: air and aqueous; the air cathode reduces the MFC cost because it does not require extra energy for aeration (Liu and Logan 2004). The critical research questions for MFC are (1) bioengineering exoelectrogen to become more efficient ones and (2) developing materials that are low cost, regenerate both energy production and wastewater treatment (Koroglu et al. 2019).

There are two major (direct and indirect) electron transfer mechanisms from the bacteria cell membrane to the electrode (Santoro et al. 2017). Direct electron transfer occurs without mediators at short-range (redox-active molecules) or long-range (pili or microbial nanowires); whereas, indirect electron transfer achieved using chemical mediators (neutral red or thionin) or bacteria owned mediators (pycocyanin) (Borole et al. 2011; Logan et al. 2006; Patil et al. 2012; Ucar et al. 2017). A recent publication on *Geobacter sulfurreducens* anode electro-active biofilm (EABs) reveals two additional electron transfer types. These were super-exchange that involves electron-transfer among a network of cytochromes (Strycharz-Glaven et al. 2011), and metallic-like conductivity by the biofilms that can conduct over a centimeters distance, and the biofilm conductivity increased as biofilm thickness increases (Malvankar et al. 2011). However, Strycharz and Tender (2012) argue that there was no evidence to support metallic conductivity. According to Ter Heijne et al. (2020), the electro-active biofilms (EABs) study is at an earlier stage. Meanwhile, most studies focus on how electron transfer, but there is an urgent research gap: (1) how to steer biofilm formation in MFC to increase energy generation and (2) lack of clear understanding on different EABs phenomenon. The MFC design and operation clear-cut understanding is ongoing research and debate, which requires a paradigm innovation from reactor design (anode surface area) to configuration.

The initial step in MFC is microbial acclimatization on the electrode surface (Kumar et al. 2018). It is followed by exoelectrogen colonization to form biofilm and transfer electrons (e^-) to the anode. During the biofilm formation, some proteins specifically pili, and outer membrane cytochromes (OMCs), such as *c*-type cytochromes (c-Cyts) (OmcZ and OmcS) facilitate the biofilm development (Nevin et al. 2008) and form a network of the nanowire (Strycharz-Glaven et al. 2011). Also, microbes in the biofilm produce extracellular polymeric substance composed of polysaccharides, proteins, lipids, and nucleic acids that contribute to the adhesion of biofilm to the electrode (Erable et al. 2010), and most biofilm polymers are semiconductor (10^{-9} to 10^{3} S cm^{-1}) (Borole et al. 2011). The MFC biofilm consists: electroactive microorganisms (EAMs) also called exoelectrogens, electrogens, electricegens, exoelectrogenic or anode respiring bacteria (e.g., *Geobacter* and *Shewanella* sp.) that oxidize organic matter and release e^- into the solid-state electrode (Logan 2009; Ramírez-Vargas et al. 2018). Non-EAMs are called endoelectrogens, or non-exoelectrogens (e.g., methanogens) either consume the e^- or release mediators that divert the e^- pathway (Chen et al. 2020; Umar et al. 2020). In the anode biofilm, non-electrogenic are essential to enhance MFC power production by creating the anaerobic condition for electro-active bacteria (Angelaalincy et al. 2018). The MFC ecology is dependent on the non-electrogenic microbes because they contribute to the development of thick biofilm and maintain an anaerobic environment by consuming oxygen that-contaminate anode (Guo et al. 2020). For instance, relative to pure exoelectrogens, the mixed culture provides advantages such as high power (Borole et al. 2011), higher robustness, degrade complex compounds, and electrical productivity (Ramírez-Vargas et al. 2018). Moreover, each species in the anode biofilm play a synergetic effect in pollutant removal and energy generation (Guo et al. 2020). However, according to Mancílio et al. (2020) the microbes in the anode biofilms may have antagonistic

effect that requires further study; however, increasing diverse anode biofilm communities can simultaneously improve energy generation and wastewater treatment (Abbassi et al. 2020; Santoro et al. 2017).

Another major drawback of MFC is the diffusion of oxygen from cathode to anode that hampers the energy yield, especially in membrane-less MFC (ML-MFC) (Jang et al. 2004). The optimum biofilm formation time on the anode electrode surface is seven days (Arbianti et al. 2018). The anode biofilm forms two layers; the inner layer generates electricity, whereas the outer layer consumes oxygen. Thick anode biofilms are essential because they minimize oxygen sensitivity (Yang et al. 2019). On the contrary, in pure *Geobacter sulfurreducens*, thin biofilm thickness (~20 μm) results in the highest power output, but the performance decline as thickness increases due to the dead biomass accumulates in the inner side of the electrode and develop resistance (Sun et al. 2016). Enhancing MFC bioelectricity generation under partially oxygenated conditions and thick anode biofilm are research hotspots in the future (Borole et al. 2011).

After reviewing the literature, Logan and Regan (2006) noted that MFC architecture is a critical challenge to improve energy output. The barriers to maximize MFC are: first, during the MFC operation, the H^+ accumulates at the anode and results in low pH; The second challenge was to enhance the growth and development of anode respiring bacteria (ARB) or exoelectrogen during the MFC process (He et al. 2017; Santoro et al. 2017). It is mainly due to the lack of a technique to increase the anode surface area for microbial attachment and biofilm development (Chaturvedi and Verma 2016; Choudhury et al. 2017). Increasing anode surface area increases microbial attachment and electricity generation (Di Lorenzo et al. 2010). The anode surface area is mainly modified through electrochemical oxidation, chemical, and heat treatment (Zhang et al. 2020; Zhou et al. 2012). The most adopted techniques to increase anode surface area were chemical treatment such as polymers (polydopamine and polyaniline) and carbon-based materials (graphene oxide) (Nosek et al. 2020). This method increases anode surface roughness and hydrophobicity and plays key role for microbial adhesion and biofilm formation that consequently increase energy generation.

Several authors attempted to increase anode surface area: packed beds of irregular graphite granules (Di Lorenzo et al. 2010), a dual-anode MFC (DAMFC) (Kim et al. 2011), spiral anode (Mardanpour et al. 2012), perfusion-electrode microbial fuel cells (MFCs) (Ledezma et al. 2012), thinner carbon materials (Nosek et al. 2020), and 3D anode (Michie et al. 2020). For instance, Mardanpour et al. (2012) observed that spiral anode increases surface area that resulted in high voltage (0.81 V) and 91% COD removal within 450 h. Michie et al. (2020) noted that 3D carbon materials influence biofilm development that directly affects electrogenic property and increases power generation. Ledezma et al. (2012), for the first time, reported the means to control the anodic biofilm through fluctuating substrate perfusion rate, but it requires knowing the microorganism and growth kinetics that challenge the practicality. The major weaknesses of so far invented mechanisms to increase anode surface area were inability to form dense and stable thick EABs, not low-cost option, not possible to manage the anode biofilm thickness through adjusting a controllable variable, and complicated to scale up. However, numerous efforts were conducted; this mystery was still unraveled since the problem was reported (Goto and Yoshida 2019; Guang et al. 2020; Li et al. 2013). Therefore, a novel approach that increases the anode surface area for microbial attachment and growth is required, which practically contributes to MFC technology advancement (Yu et al. 2017).

Therefore, this research objective was to increase anode biofilm growth and developing a simple novel approach that simultaneously enhances energy recovery and domestic wastewater treatment performance (with an eco-friendly single-phase process). In this study, the microbial electrode jacket dish (MEJ-dish) was invented, first time to the authors' knowledge, to increase anode surface area to enhance microbial attachment and biofilm growth, ultimately maximizing energy recovery and treatment performance. The MEJ-dish functions as MFC biofilm culture dish and grows a diverse microbial community, including exoelectrogens and non-exoelectrogens inside the reactor. It might shield the electro-active biofilms (EABs) through consuming oxygen (contaminating the anode) and contributes to a symbiotic relationship between microorganisms in the biofilm. Three different MEJ-dish types were considered, K3 filter media, 3D conical shape, and rubber. These MEJ-dishes have variable junction features (the point where the MEJ-dish was connected to the electrode), open (K3), partly opened (3D), and closed (rubber).

Materials and methods

Experimental setup

A lab-scale double-chambered fragmented electroactive biofilm-microbial fuel cell (FAB-MFC) reactors with two different setups were designed and constructed. The first was the optimization setup (Fig. 1), whereas the second setup was bench-scale (Fig. 2).

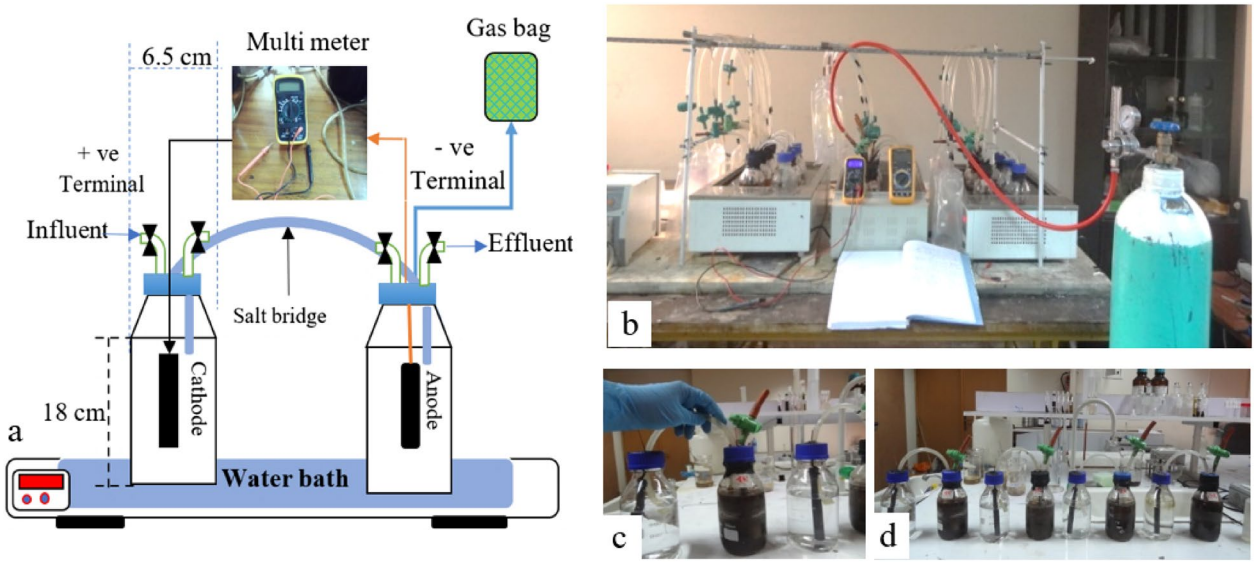

Fig. 1 Double-chambered FAB-MFC optimization setup **a** schematic diagram, **b** photo, **c**, **d** during the construction of the reactors. The cylinder is the N_2 gas sparged into the anode reactors and hardcover laboratory notebook to record the results

Optimization setup

The optimization setup, anode, and cathode were constructed using a Schott Duran bottle with 500 mL total working capacity. All gas and water valves' proper functioning were checked before installing into the reactors. A graphite electrode with 1 cm in diameter and 12.5 cm in length with a total surface area of 40.84 cm^2 was placed at the anode and cathode. In the FAB-MFC anode chamber, four similar MEJ-dishes were inserted per electrode, but MFCs were without MEJ-dish. The cathode chamber was not modified and the same in FAB-MFC and MFC (i.e., no MEJ-dish).

The anode and cathode were connected using a salt bridge as a proton exchange membrane. Salt bridge was prepared according to Sevda and Sreekrishnan (2012). Initially, distilled water warm on a hot plate with a magnetic stirrer; consequently, 5% (w/v) agar powder and 1 M KCL were added and stirred till a thick molten salt bridge formed. Then carefully, without creating air bubbles, poured into a plastic pipe (made from high-density polyethylene) closed in one end using cello tape and bent to a U-shape. It was placed in a 2 L beaker to maintain the shape and cool at room temperature. A salt bridge without any tiny air bubbles was used for the experiment. However, if any air bubbles were formed inside the tube, the salt bridge was refilled before being used for the experiment otherwise discarded.

Bench-scale setup

The bench-scale FAB setup was constructed from an overhead DC motor (24 V) stirrer, sample inlet, nitrogen gas sparging valve, and biogas (methane, CO_2, etc.) collection port. A pinched impeller was attached to the shaft to stir the reactor solution with a vertically agitating drive motor. The motor driver speed was controlled using a DC power supply unit (BK-1502DD, BAKU, China). The anode reactor was made from a polyacrylic tube. The reactor's total working volume was 800 mL (8.5 cm in diameter x 6.5 cm in depth x 18 cm in height) with multiple overhead ports. The cathode was made from a Schott Duran bottle with 1 L total working volume. The same gas pipe (thickness and length) was attached to all the reactors to avoid any biased contribution from the setup.

Two electrodes with and without MEJ-dish were inserted in the same reactor to observe the effect of pH near the anode electrode under similar operational parameters. Four MEJ-dishes were inserted per electrode. The distance between MEJ-dish was maintained equally. Both the anode and cathode electrodes were arranged vertically. As shown in Fig. 2, the anode and cathode were placed in a separate reactor and configured as double-chambered MFC. A salt bridge was used to connect the anode with a cathode and constructed as described under an optimization setup. Also, the configuration of the control setup was similar to the experimental design.

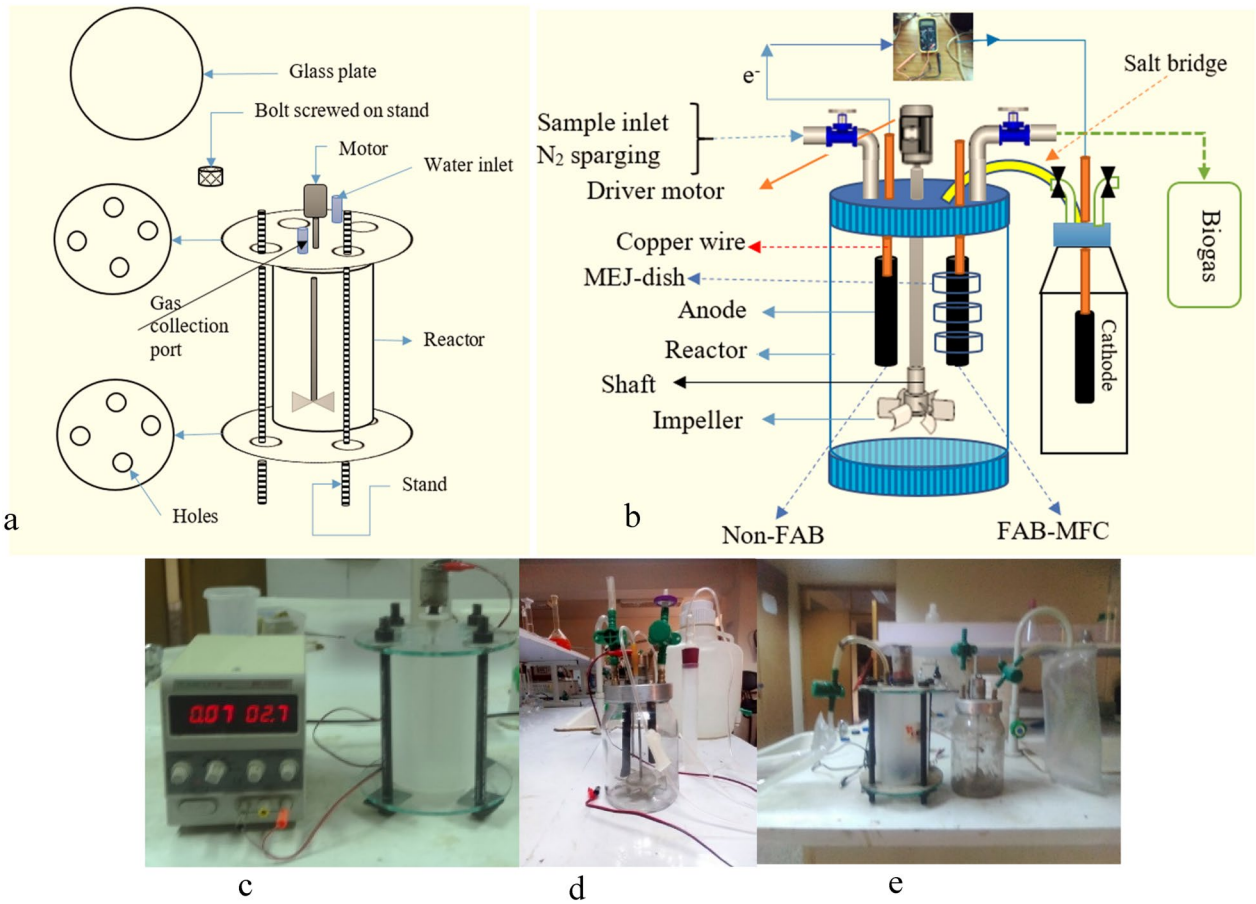

Fig. 2 Schematic diagram **a**, **b** and photo **c**, **d**, **e** bench-scale FAB-microbial fuel cell (FAB-MFC) of anode **a**, *c*, cathode **d**, and combined setup **b**, **e**

Microbial electrode jacket dish (MEJ-dish) fabrication for MFC

Bioelectrode modification was carried out to enhance the MFC performance. Microbial electrode jacket dish (MEJ-dish) or Jacketed microbial electrode (JME-dish) was designed and constructed to function as an electro-active biofilm growth support. As shown in Fig. 3, fragmented (fixed) thick and thin biofilms were hypothesized to develop over MEJ-dish and thin biofilms across the non-FAB or MFC electrodes without MEJ-dish.

The FAB concept was developed based on the graphical abstract shown in Fig. 4. A graphite electrode was inserted into the solid materials carrier substratum (e.g., funnel + rubber), which was used to imitate the MEJ-dish for microbial growth support media in the FAB reactor. First, this setup was inserted into raw domestic wastewater mixed with inoculum (cow manure: domestic wastewater in a 1:1 ratio). Second, the reactor contents were stirred using a magnetic stirrer (MS-H280-Pro, DIAB, USA) at a speed of 120 rpm (Fig. 4b). Third, the contents were settled for 10 min, then an auto-dosing peristaltic pump (DP-4, Jebao Inc., China) was used to draw the wastewater from the FAB reactor (Fig. 4c). Fourth, the MEJ-dish was dried in the open air for 24 h (Fig. 4d).

Consequently, a thick solid mass was developed across the electrode (Fig. 4e) that was assumed to be a retained biosolids and organic matter during practical application. This conceptual FAB demonstration was experimentally tested in an actual MFC reactor with an anode (cathode) and domestic wastewater. It was investigated in terms of electro-active biofilm growth, the voltage generated, and wastewater treatment performance under different shocking conditions such as pH.

Different MEJ-dish types were used for the FAB reactor study. First, smooth material that discourages the growth of biofilm on the electrode surface was selected. In this case, a rubber stopper was cut into a circular shape and plugged into the electrode. Second, a K3 filter media (Cz Garden, USA) with a dimension of 25 mm in diameter X 12 mm in depth was drilled at the center and inserted into the anode. Third, a 3D printer was used to fabricate a 3D conical MEJ-dish (2 cm long X 3 cm top diameter X

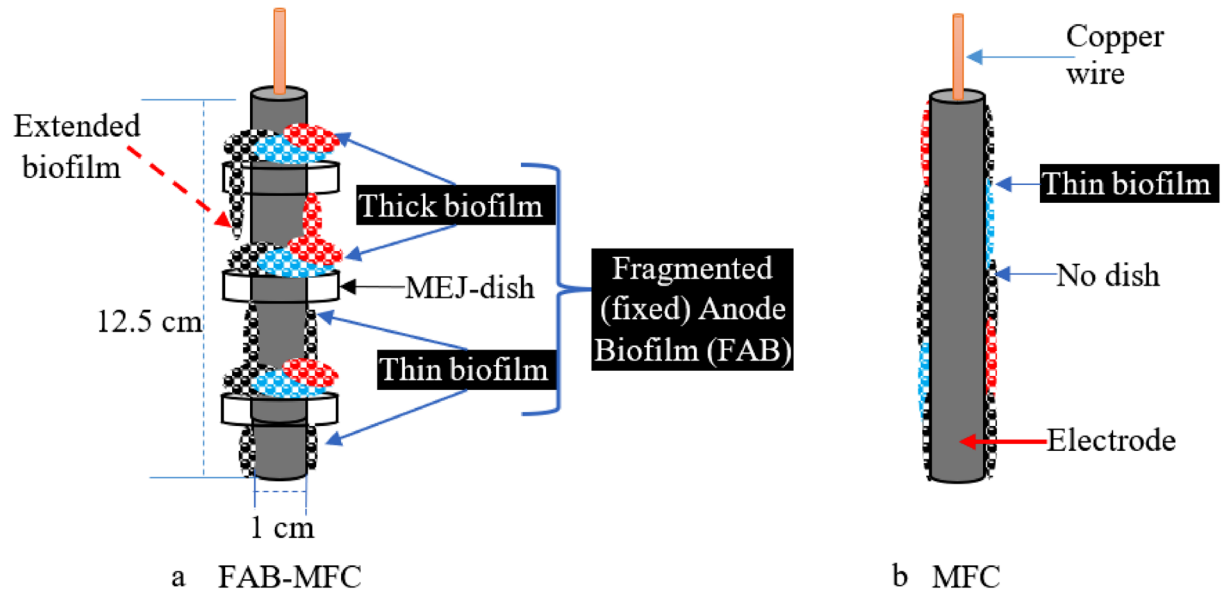

Fig. 3 Conceptual scheme of electro-active biofilm (EABs) formation on **a** microbial electrode jacket-dish (MEJ-dish) in fragmented electro-active biofilm-microbial fuel cell (FAB-MFC) anode, and **b** without MEJ-dish in MFC anode

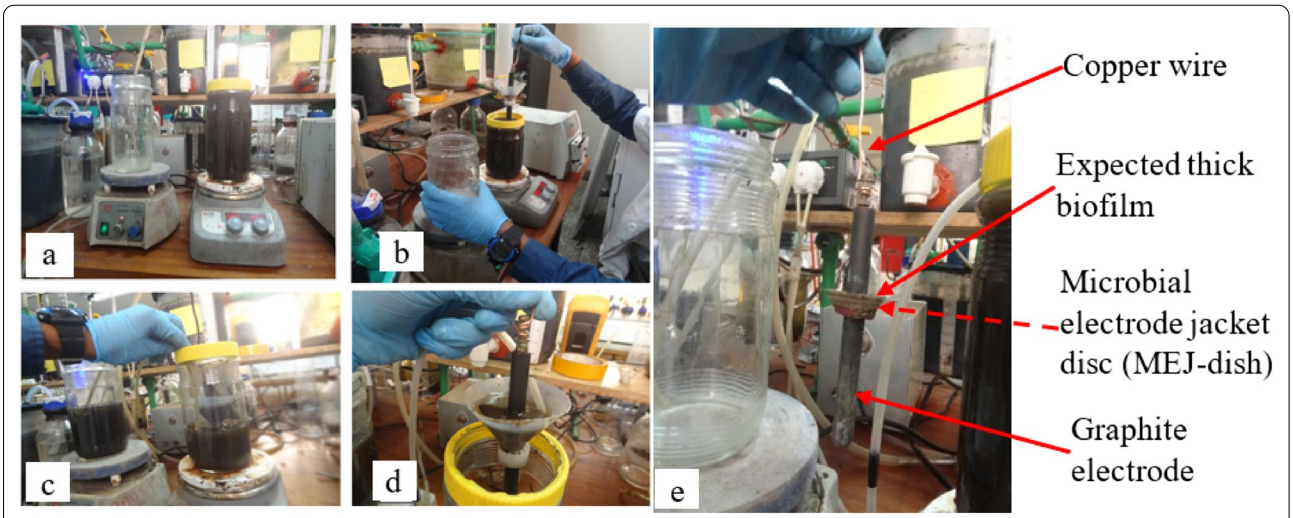

Fig. 4 Fragmented electro-active biofilm (FAB) reactor conceptual scheme **a** raw domestic wastewater and inoculum fed into the reactor, **b** microbial electrode jacket dish (MEJ-dish) inserted to the graphite electrode, **c** pumping wastewater from the reactor, **d** removing the electrode, and **e** solid mass collected on the MEJ-dish

1.2 cm bottom diameter). It consists of a perforated wall for easy movement of nutrients in the reactor and a base that supports biofilm growth (Fig. 5).

Startup of FAB-MFC

During startup, inoculum and raw domestic wastewater were mixed in a 1:3 ratio (v/v). Raw domestic wastewater was collected from the Mickey Leland condominium wastewater treatment plant at the primary clarifier (Asko, AA, Ethiopia). The cow manure was used as inoculum in both the optimization and bench-scale setup. In both setups, the anode surface was abraded with sandpaper and rinsed with tap water to aid bacteria attachment. An equal length of copper wire (10 cm) was attached to all the electrodes while constructing the reactors. A tiny hole was drilled at one

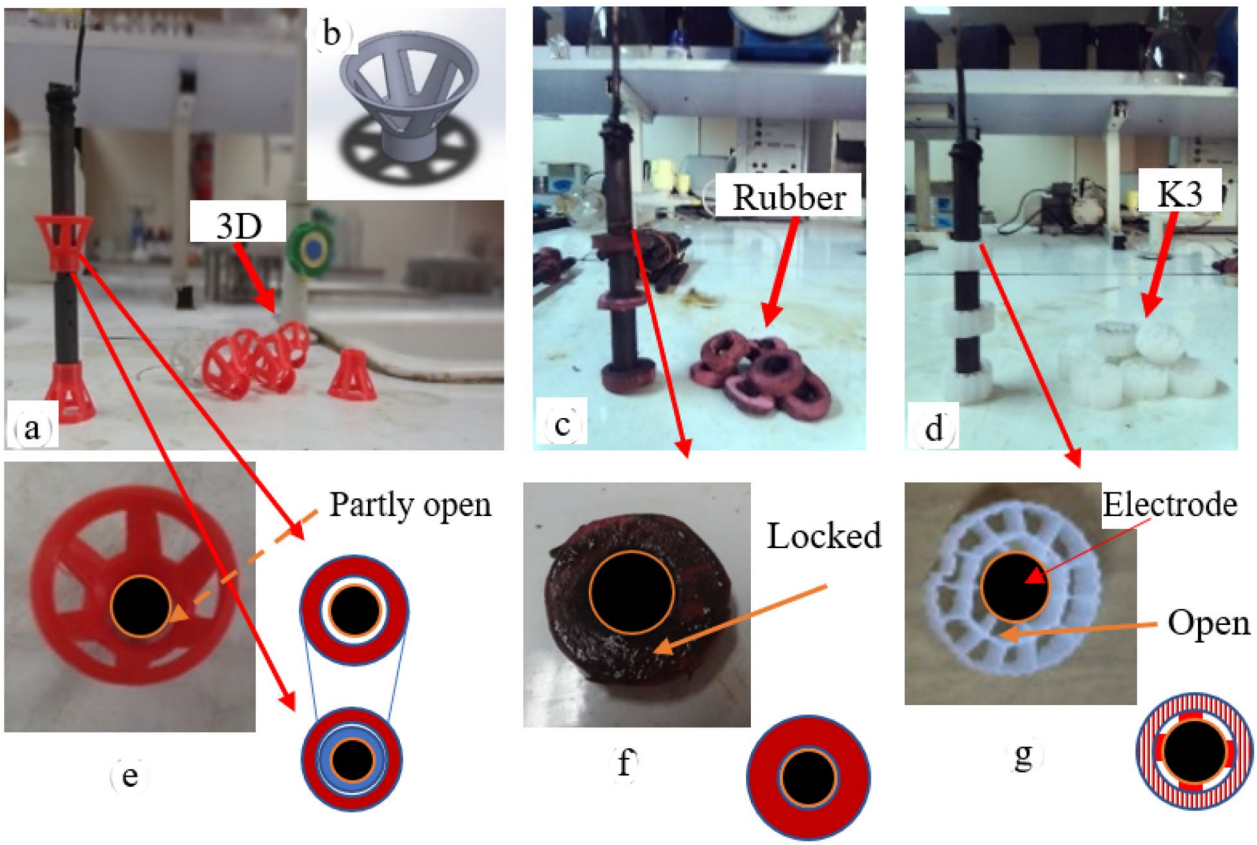

Fig. 5 Different microbial electrode jacket-dish (MEJ-dish) used during this study **a** 3D, **b** 3D design, **c** Rubber, **d** K3 MEJ-dish, and **e**–**g** junction between the electrode and MEJ-dish: partly opened (closed), open, and locked (closed). Locked (closed) indicates no space between the electrode and MEJ-dish, partly open shows the upper part is open, but the lower part is partially open or closed, and open means the connection part is exposed for microbial attachment

end of the electrode (anode or cathode), and a peeled part of insulated copper wire was inserted into the hole and firmly coiled. The copper wire section that remains inside the reactor was sealed with epoxy (Dexter Corp., NJ, USA) to protect corrosion or contact the wastewater. Then, it was open-air dried for about 48 h before inserted into the reactor. The copper wire part connected to the electrode remains inside the reactor, and the stranded part was exposed outside the reactor and combined as required to measure voltage.

Operation and maintenance

Optimization setup

Before the operation startup, the anode compartment was sparged with nitrogen gas. The aqueous-cathode (hybrid) was fed with tap water, not aerated, and the lid was loosely closed to allow airflow (9 cm submerged and 3.5 cm exposed to air, Fig. 1c and d). There were two reactors with microbial electrode jacket (MEJ-dish) as FAB-MFC, two reactors without the MEJ-dish as MFC, and two FAB-MFC autoclaved at 120 ℃ for 30 min as control.

Samples were drawn periodically to analyze the COD concentration. Gasbags were emptied (squeezed) and connected to the anode gas outlet port. During and after water sampling, all anode reactors were sparged with nitrogen gas before starting the operation. Like the anaerobic reactor, the anode chamber coverlid, tube junctions, including all other openings, were closed and sealed with a gasket maker (ABRO Inc., USA). Daily, all gas and water valves were checked for proper functioning during the reactor operation and maintained if necessary.

The FAB-MFC was optimized based on anodic pH at a specific temperature and organic loading rate (OLR). All the setups were placed in a thermostatic water bath at 27 ℃ (DK-98-II/DK-98-II-A, Faithful Instruments Co., China) and mixed once a day using initially inserted magnetic stirrer bead to ensure complete mixing and mass

transfer. The influent anodic pH was adjusted to 6.5, 7.5, and 8.5 using 0.1 M NaOH and HCl. All MFC tests were conducted in duplicate and operated in a batch mode. All the cathode and anode contents were removed, and a fresh salt bridge was used between the various experimental parameters, such as changing the MEJ-dish. The treatment retention time undertook a total of 25 days.

Bench-scale setup

The bench-scale setup operation was similar to the optimization, except particular modifications were made on reactor size and temperature. Two control systems were used to test the bench-scale FAB-MFC operation. Voltage output control was measured through autoclaving the FAB-MFC at 120 ℃ for 30 min. The control setup COD removal was investigated using a similar FAB-MFC influent but without electrode and sterilization. The cathode compartment was not aerated. Bench-scale FAB-MFC operation was conducted at room temperature (25 ± 1 ℃) without pH modification of the influent. Mixed microbial consortia were used to run all the MFC systems. The treatment period was maintained for 15 days.

Bioelectrochemical analysis

The anode and cathode were disconnected to measure open-circuit voltage (OCV) using a digital multimeter (XL830L, China), then connected with the external resistor (100 Ω) to measure voltage and calculate the current (Eq. 3). The measurements were conducted at least three times per day, and the daily average results were presented. Chemical oxygen demand (COD: closed reflux method) and pH (HQ440D, HACH, USA) were monitored using the standard method (APHA 2005), and a UV-Vis spectrophotometer (DR 6000, HACH, USA)) was used to determine the chemical compositions.

Calculations

Process performance calculation

The process performance was measured in terms of Coulombic efficiency, voltage generation efficiency, and COD removal efficiency. The removal of COD is calculated based on Eq. 1.

$$R\ (\%) = \left(1 - \frac{C_{out}}{C_{in}}\right) x\ 100\% \tag{1}$$

where R (%) is removal efficiency, C_{in} is an influent concentration (mg/L), and C_{out} is an effluent concentration (mg/L).

The bioelectrochemical performance was determined based on MFC Coulombic efficiency. It is the fraction (percent) of electron recovered as current from the total electron present in the substrate (wastewater) through complete oxidation (Logan et al. 2006). Coulombic efficiency (C_E) was calculated using Eq. 2, considering the wastewater COD concentration as substrate and batch operation.

$$C_E = \frac{8 \int_0^{t_b} I\ dt}{F \cdot V_{an} \cdot \Delta COD} \tag{2}$$

where F is Faraday's constant (96,485 C mol^{-1}), ΔCOD is the difference in the influent and effluent COD (mg L^{-1}) over time = t_b, I is the current (A), and V_{an} is the volume of liquid in the anode (L).

The current (I) generated by the MFCs was calculated according to Ohm's law using Eq. 3.

$$I = \frac{V}{R_{ext}} \tag{3}$$

where I is current (A), V is voltage (V), and R_{ext} is the external resistor (Ω).

Data analysis

All samples were analyzed in triplicate and presented as mean ± standard deviation (SD). A significant difference ($p < 0.05$) between the study parameters were compared by a one-way ANOVA followed by the Games-Howell post-hoc test. The analyses were carried out using IBM SPSS Statistics, version 20 (IBM Corp., NY, USA).

Results and discussion

Wastewater and inoculum characterization

Table 1 shows the physicochemical composition of the raw wastewater and inoculum. The pH of 6.5 to 7.5 is favorable for anaerobic microbial growth (Liu et al. 2008). According to this table, both the raw wastewater and inoculum were applicable for biological treatment without further pH adjustment. Additionally, the raw domestic wastewater lower alkalinity content (35 mg/L as $CaCO_3$) might reduce the buffering capacity.

The solid content of wastewater is an important parameter that affects treatment efficiency. The total solids (TS) found in wastewater are either in volatile or fixed form, further classified into suspended and dissolved solids (APHA 2005). However, the most determinant in wastewater treatment is volatile solids (VS), showing the microbial biomass (Takashi et al. 2007). Based on Table 1, the TS and VS concentration of the inoculum wastewater was four times higher than the raw domestic wastewater. The mean total chemical oxygen demand (TCOD) observed in raw domestic wastewater and inoculum was 795 and 1678 mg/L, respectively. This value was within the typical wastewater COD content (Edwin et al. 2014).

Table 1 Characteristics of raw wastewater and inoculum used in this study

Parameters	Raw wastewater	Inoculum	Influent
pH	7.51 ± 0.07	6.43 ± 0.06	7.42 ± 0.26
Total alkalinity (mg/L as $CaCO_3$)	35.01 ± 2.87	144 ± 4.59	84.29 ± 0.37
Total solid (mg/L)	157.93 ± 28.29	3,277.35 ± 416.2	2396.52 ± 0.19
Volatile solid (mg/L)	366.14 ± 58.99	1,559.62 ± 210.89	793.86 ± 0.48
Total COD (mg O_2/L)	795.34 ± 160.01	9,760.4 ± 550.97	1678.29 ± 0.72
Ammonia N (mg NH_3-N/L)	19.75 ± 1.77	272.35 ± 11.97	152.62 ± 0.60
Total phosphorus (mg P/L)	8.12 ± 1.59	21.64 ± 0.9	11.27 ± 0.15

The value indicates means ± SD (n = 3)

Biofilm growth and development in FAB-MFC

After finalizing the FAB-MFC and MFC operation, the reactors were dismantled to observe biofilm growth and development on the electrode surface. As shown in Fig. 6, FAB-MFC with MEJ-dish (MEJ+) develops naked eye observable fragmented anode biofilm compared to MFC without MEJ-dish (MEJ-).

Based on Fig. 7, two different types of extended biofilm growth were observed in the FAB-MFC bench-scale setup. These were intra-electrode and inter-electrode extended biofilm. This nature of biofilm formation might critically influences and mislead the conclusion drawn from dual FAB and non-FAB electrode inserted in the same reactor.

Oxygen diffusion decreases as biofilm thickness increases (Ntwampe et al. 2008; Piculell et al. 2016). The FAB could potentially minimize oxygen diffusion into the inner layer of the thick anode biofilm. However, the energy production efficiency might be affected by the dominant microbial community structure across the biofilm. If exoelectrogen dominates the thick biofilm inner part, may reduce the oxygen penetration and contributes to the MFC performance. Otherwise, it might be an electron sink and expose the electrogenic biofilm to oxygenated conditions. However, the FAB-MFC could subdues this negative effect by creating a variable biofilm thickness across the anode.

Performance of the FAB-MFC optimization setup

COD removal efficiency

The COD removal in the FAB-MFC and MFC anode reactor was measured at 27 ℃ to compare the performance. Regardless of pH and MEJ-dish type variation, the COD removal in the FAB-MFC was significantly

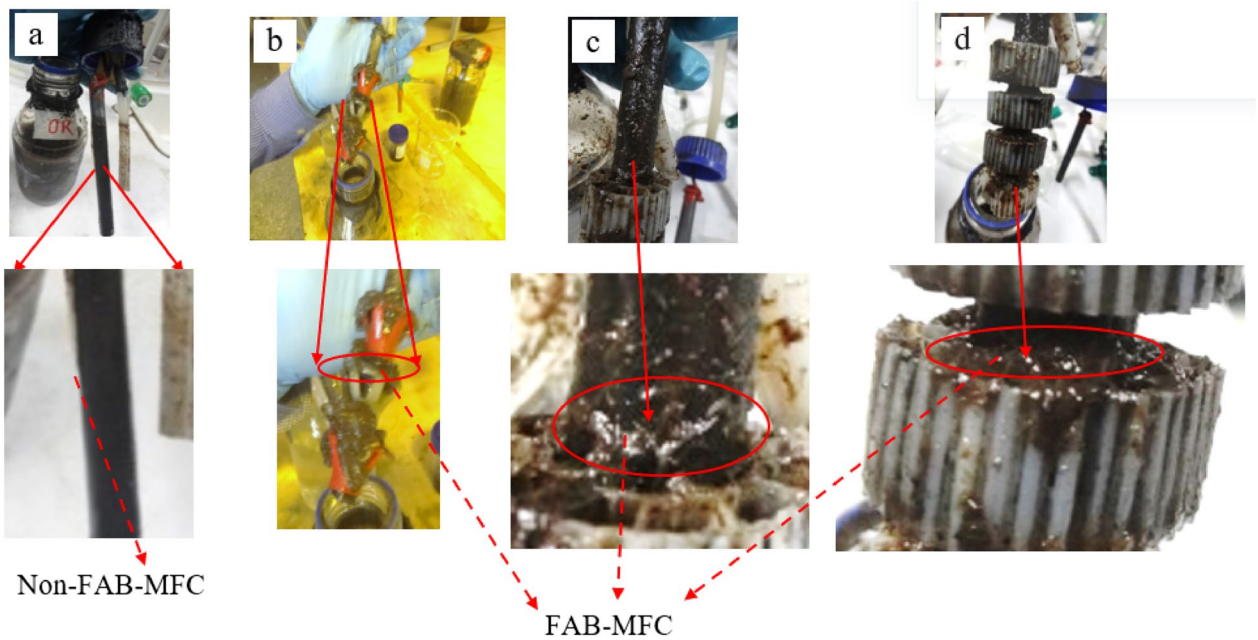

Fig. 6 Biofilm growth and development on **a** non-FAB-MFC, **b** 3D, **c**, **d** K3 MEJ-dish

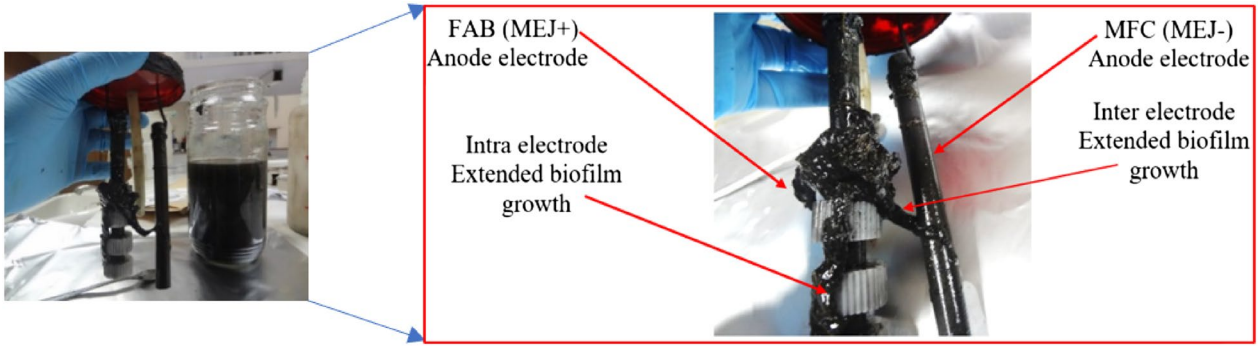

Fig. 7 Biofilm growth and development in bench-scale FAB-MFC setup. The biofilm extended in between the FAB (MEJ+) and MFC (MEJ-) anode electrode inserted in the same reactor

increased than MFC during the first five days (Fig. 8). The overall COD removal was more than 80% during the treatment period (25 days). A closer COD removal (79%) was reported in another study (Tamilarasan et al. 2017). However, a comparison of COD removal between different MFCs studies might be difficult because of variation in wastewater source and initial COD concentration, MFC configuration and operation, and electrode properties. The effluent COD concentration was in the range of 120–249 and 195–327 mg/L in FAB-MFC and MFC, respectively. It shows the fragmented anode biofilm in FAB-MFC might contribute to the lower effluent COD load. In support of this, previous study reports the synergetic effect between electrogenic and non-electrogenic improves COD removal in dual chambered MFC (Logan 2008).

Furthermore, these findings implicate that extended biofilm in FAB-MFC could contribute to additional COD removal. However, the extended biofilm (Fig. 7) might not contribute to voltage generation, particularly in the intra-electrode extended biofilm formed within the MEJ + electrode. It might be difficult for the microbes to transport electrons over such a long-range distance (ca. > 1 cm) to the electrode. But, it might contribute indirectly through degrading organic contents in the solution and releasing metabolites to the electro-active microbes that can be later used at a distance. Additionally, it minimizes the effect of oxygen intrusion into the system as this extended biofilm might consume the anode contaminating oxygen. Several biofilm substances such as EPS and bacterial nanowire are reported as electron conductors (Angelaalincy et al. 2018; Ucar et al. 2017) and contribute to electrode electron transfer reactions (Kumar et al. 2017). On the other hand, besides the endoelectrogens (Borole et al. 2011; Patil et al. 2012) and electron storage compounds (Ter Heijne et al. 2020), there might be non-conductor materials in the biofilm that block the long-range electron transfer to the electrode. Depending on the surrounding materials, the biofilms might contain mineral crystals, corrosion particles, clay, or silt particles (Donlan 2002); this might influence the biofilm matrix's electron conductivity nature, which may affects the FAB performance.

There were contradictory effects of enhancing biofilm development, voltage generation, COD removal, and substrate pH. The voltage generation was significant at acidic and near-neutral pH. Still, at all studied pH values (6.5–8.5), the COD removal was significantly higher within 3–10 days in the supported growth reactor (FAB-MFC) relative to the one that deprives MEJ-dish (MFC). However, as the reactor age increases, there was no observable COD difference between FAB-MFC and MFC but voltage generation maximized in FAB-MFC, which entails suspended biofilms (not on the anode) might slowly developing in the non-FAB reactor to remove the COD. In MFC, the optimal period for biofilm growth was seven days (Arbianti et al. 2018), whereas FAB (MEJ-dish) might facilitate the attachment and biofilm formation that shorten this period. Therefore, to enhance the COD removal, the FAB could be advisable; but the voltage generation was another critical concern. Hence, a hybrid reactor that contains both FAB and non-FAB in a single MFC reactor might maximize the two contradictory points at higher pH.

Bioelectricity generation

Figure 9 shows the performance of each MFC reactor in terms of daily voltage generation. During the first five days and pH 7.5, except K3 MEJ-dish FAB, the generated voltage was significantly ($p < 0.05$) higher in MFC than FAB-MFC. However, as the age of the reactor increases, the super advantage of high voltage generation and thick biofilm formation was recorded in all types of MEJ-dish

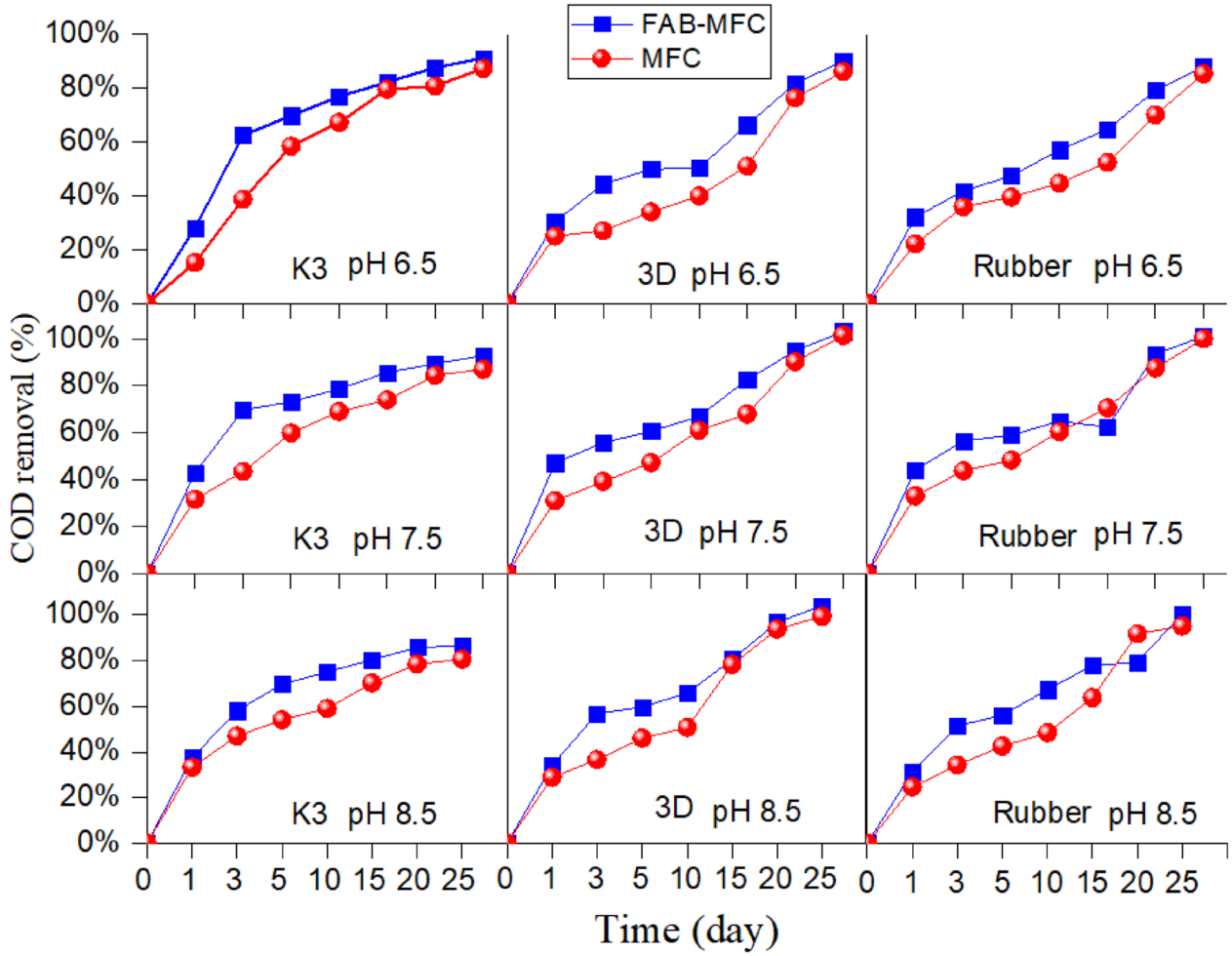

Fig. 8 COD removal efficiency of FAB-MFC and MFC using different MEJ-dish (K3, 3D, and rubber) at 27 ℃ and anodic pH 6.5, 7.5, and 8.5

FAB reactor than MFC. At startup, EABs might colonize rapidly in both FAB-MFC and MFC electrodes, but the MEJ-dish junction type (open/closed) and thickness determine the inactive anode surface area that consequently lowers voltage generation in FAB-MFC. The difference was observed among different MEJ-dish types, where K3 FAB narrow the gap in MFC (within 3 days), and the effect was more revealed on junction locked rubber MEJ-dish (8 days).

Additionally, in another lab-scale experiment, data not presented, FAB forms thick biofilms on the anode surface and improved the voltage generation when the number of MEJ-dish reduced from 4 to 3 per electrode. The contribution was found magnificent, especially when substrate feeding was interrupted (no feeding), and the age of the MFC increases. It implies the system might play a vital role in the survival of EABs during starvation. Hence, one of the significant factors that affect the result could be the type and number of MEJ-dish per electrode. It could be advisable to study the effect of size, number, and conductor MEJ-dishes on bioelectricity generation and treatment performance.

The biofilm may also contain an extracellular polymer substance (EPS) (Donlan 2002), which might be an electro conductor and contribute to the MFC electron transport chain (Angelaalincy et al. 2018). However, a clear understanding of electron transfer (ET) mechanisms in EABs requires further research (Li et al. 2013). Recent publications revealed a network of the nanowire, cytochromes, and some conductive proteins involved in the long-range ET through cell-to-cell or cell to the electrode (Patil et al. 2012). Based on the observed thick biofilm over the FAB electrode surface, the FAB reactor's voltage could be due to the cell-to-cell metallic-like conductivity at a distance than direct electron tunneling through *c*-type cytochrome to the electrode.

According to Abbassi et al. (2020), metallic electrodes were less used than carbon-based electrodes, although

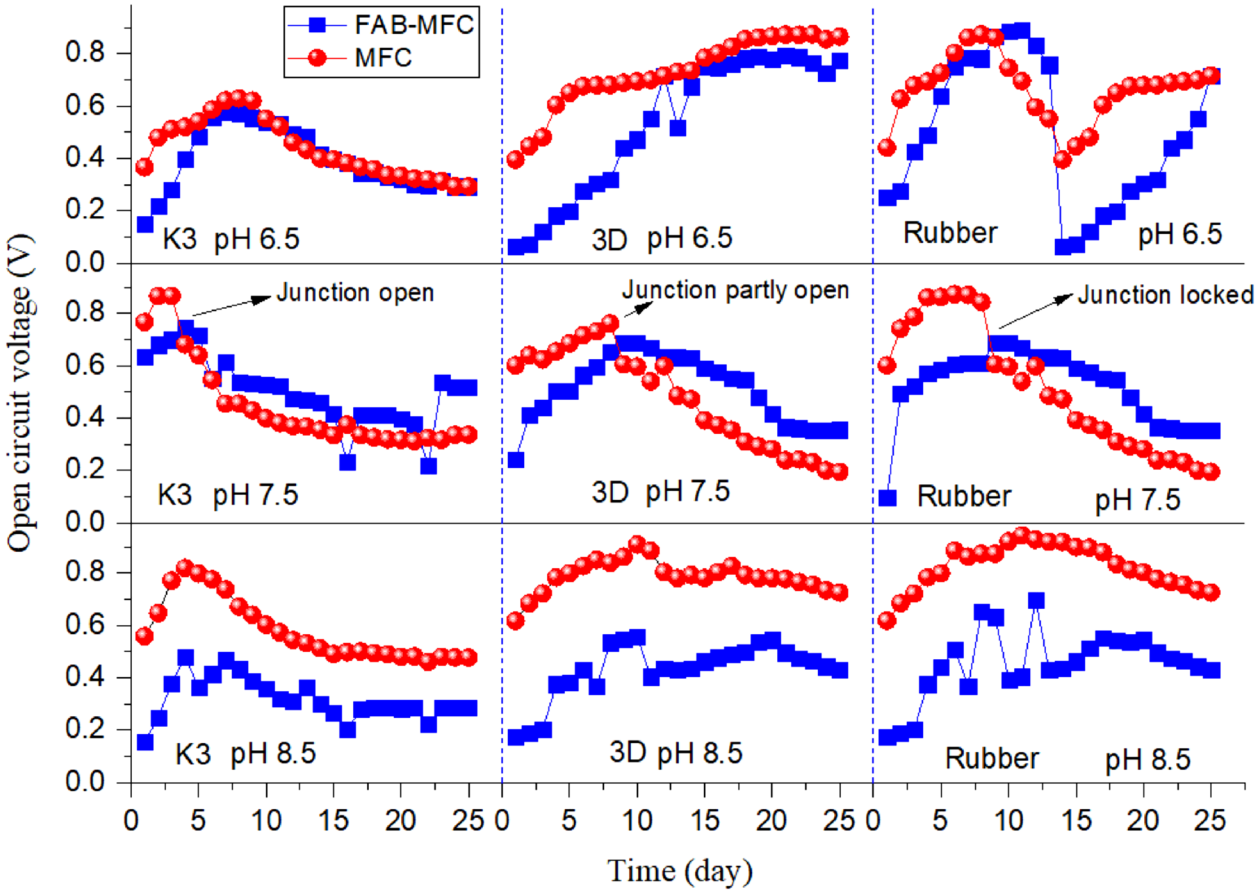

Fig. 9 Open circuit voltage (OCV) generated at different anodic pH in MFC and FAB-MFC reactor using rubber, 3D, and K3 MEJ-dish at 27 ℃. Junction refers to the space between the electrode and MEJ-dish (where MEJ-dish was connected to the electrode); K3 (open), 3D (partly opens), and rubber lock (close) the junction

they have higher conductivity and mechanical strength. One of the difficulties of using metallic electrodes (e.g., stainless steel) for MFC is the lower surface area that discourages biofilm attachment and development. Future studies might apply the FAB concept on the metallic electrode to investigate the effect on biofilm formation and associated contribution for MFC development. The findings show that fragmented anode biofilms were crucial for electricity generation and mediating favorable conditions for electro-active microorganisms. It could be creating an anaerobic environment, minimizing the effect of pH (6.5 to 7.5) for exoelectrogens, and probably providing suitable intermediate metabolites. Overall, there might be a mutualistic effect within the anode biofilm. Similar results were reported somewhere else (Borole et al. 2011).

On the contrary, at alkaline pH 8.5, the MFC voltage was significantly higher than FAB-MFC. There could be multifarious interaction that results in voltage output change with pH perturbation during FAB-MFC operation. Previous studies noted that the optimum pH for most exoelectrogens is between 6 and 7 (Guang et al. 2020), and the maximum power production occurs at pH 7 (Michie et al. 2020). The extracellular e^- transfer might be effective in acidophilic (and neutral) than alkaline pH (Raghavulu et al. 2009). At alkaline pH, endoelectrogens might first colonize the MEJ-dish and hinder the EABs development as the reactor age increases. The e^- generated might be more consumed in alkaline pH compared with acidic conditions. As the biofilm thickness increases in the FAB-MFC, the long-distance e^- transfer might decrease due to the endoelectrogens dominance and biofilm electrochemistry that consequently hampers voltage output. Additionally, the availability of e^-, H^+, and metabolites are dependent on pH (Venkatamohan et al. 2009). H^+ reduction was noted in the neutral and basic environment, which was associated with dehydrogenase activity. Venkatamohan indicate, in basic pH,

the dehydrogenase is more active and reduces H^+ into a product that minimizes e^- releases to the environment.

Furthermore, electrons generated during organic matter oxidation in MFC could have different fate such as gas production, metabolites generation, consumed by endoelectrogens, or electron acceptors (oxygen) penetrating the anode chamber (Kim et al. 2011). Hence, suppressing these factors could improve the Coulombic efficiency of the system. For instance, Wagner et al. (2009) noted that suppressing CH_4 production could improve the bioelectrochemical electron recovery efficiency.

Overall, the FAB-MFC increased the MFC Coulombic efficiency (CE) by 7.4–9.6%. According to Stoll et al. (2018), 10% CE or 10% higher voltage improvement in MFC is a notable achievement. It indicates EABs degrade more organic matter and release electrons in FAB-MFC than MFC due to reduced oxygen contamination. Within 3–10 days of the wastewater treatment, the FAB reactor enhances 10–23% COD removal. In turn, the overall voltage generated decreases as the treatment period increases. It might be attributed to the fed substrate reduction associated with COD removal increase when the residence period increases. It was reported that the substrate (COD) consumed by microorganisms used for electricity generation (Tamilarasan et al. 2017), but a substantial portion was not used for power generation (Liu et al. 2004). The CE and voltage generated in FAB-MFC might be related to the COD concentration and conversion efficiency, dependent on the electrode conductivity, EABs efficiency, microbial diversity in the anode biofilm, and nature of the wastewater. Relatively, FAB-MFC may provides a more convenient niche for EABs.

Performance of the FAB-MFC bench-scale setup

COD removal efficiency

In agreement with the optimization results, the COD removal in bench-scale FAB-MFC setup was significantly higher than MFC during the first week of the treatment period, as shown in Fig. 10. It signals the FAB reactor facilitates the microbial attachment on the anode surface and shortens the biofilm acclimatization period in a scale-up reactor. The effluent COD concentration in FAB-MFC, MFC, and control were 134 mg/L, 168 mg/L and 218 mg/L, respectively. It implicates the COD removal was achieved by interdependent functions between anode biofilm attached to the electrode and suspended in the liquid. All the biofilm communities might be involved in the COD removal; especially the enhanced biofilm growth contributes to the additional removal in FAB-MFC than MFC. However, FAB might result in excessive biofilm growth or promotes endoelectrogens. It affects the EABs formation for enhanced voltage generation at the early stage of treatment. On the other hand, MEJ-dish covers some part of the electrode surface area. It depicts the need for novel MEJ-dishes that nil the junction effect and intensify the biofilm growth and voltage generation.

Against the improved COD removal within the first week, the voltage generation was suppressed in FAB-MFC relative to MFC (Fig. 10a, b); whereas, at later age of the reactor (>9 days) when COD removal was not significantly different between the FAB-MFC and MFC higher voltage output was recorded in FAB-MFC than MFC. This proof the improved COD removal was not always accompanied by high voltage output. It could be

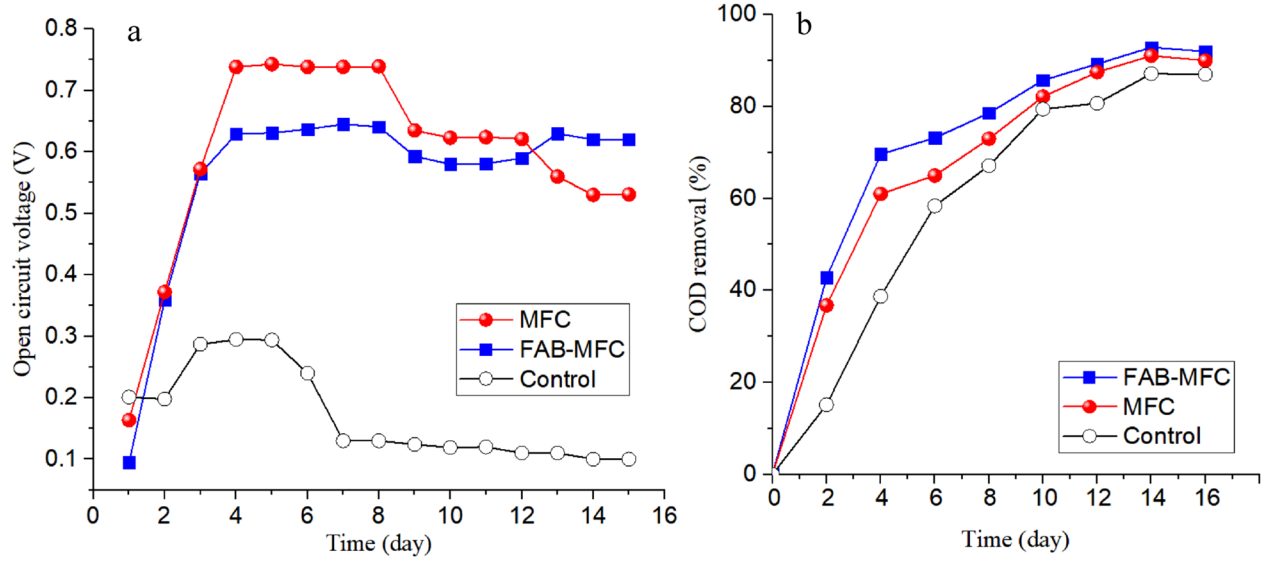

Fig. 10 Bench-scale **a** voltage output and **b** COD removal during the study period

either the enhanced biofilm growth dominated by non-EAM, or the extended EABs could not contribute voltage generation at a centimeter distant to the anode but remove the COD, or the type of MEJ-dish were inhibiting the electron generation, particularly at the beginning of the reactor operation. Therefore, it might be essential to determine the number of MEJ-dish per electrode and wastewater characteristics in future studies.

Bioelectricity generation

Based on the optimization result, a 3D MEJ-dish with intermediate voltage efficiency during startup (1–5 days) and junction partly open (Fig. 9) were selected to study the bench-scale FAB-MF. The bench-scale results were related to the optimization experimental findings. The maximum 0.74 V was generated in MFC compared with the FAB-MFC during 4–10 days (Fig. 10). FAB-MFC generates a higher voltage than MFC as the age of the system increases. The FAB might boost anode respiring EABs through mutualistic association, but different mechanisms previously mentioned might still undermine the startup voltage in the bench-scale FAB reactor. The long period operation of MFC might develop dead biomass on the electrode and prevent new active EABs growth (Sun et al. 2016). These could block electron flow from EABs to the electrode and decrease the energy generation performance. To the best of the authors' knowledge, the in situ electrical conductivity of inactive EABs mass due to pili or the nanowire over the electrode surface was not reported (Borole et al. 2011). Hence, additional studies or MEJ-dish modifications might be required to conclude FAB-MFC sustain the bioelectrochemical merit during extended period operation.

This study initiated MEJ-dish containing electrode fabrication, which forms a T-shape electrode (T-electrode). Based on this study, several MEJ-dishes were proposed for future studies. These electrodes might contribute to minimizing oxygen penetration to the anode chamber by creating small pockets of a strict anaerobic zone for exoelectrogens. However, there could be competition among the anaerobes to colonize this favorable niche. It requires specific modalities to undermine the dominance of prokaryotic bacteria. Although it was not covered in this work and further study required, the biofilm formation on the anode might be controlled by adjusting the MEJ-dish size. In the long-run operation, it might be necessary to periodically expose the FAB electrode to an aerobic environment to manage electrode biofouling and degrade dead biomass. Stirring the contents and substrate shock (variable COD or DO load) could play an additional role in controlling biomass thickness in FAB-MFC. These FAB techniques might substantially contribute to oxygen exposed anode MFC setup such as ML-MFC with air-cathode configuration.

Conclusions

In this study, the fragmented electro-active microbial fuel cell (FAB-MFC) reactor was invented to increase bioelectricity generation and treatment performance simultaneously. The microbial electrode jacket-dish (MEJ-dish) was used as a fixed anode biofilm support media. MEJ-dish improves the anode biofilm thickness. During the startup (first five days) at pH 7.5, the voltage generated was higher in MFC than FAB-MFC, but as the age of the reactor increases, all the FAB-MFC (with different MEJ-dish) gains momentum. During the startup, the FAB voltage output depends on the pH and MEJ-dish type, which determines the junction nature between electrode and MEJ-dish (open or close) that affects the microbial attachment and additional electron collection. In contrast, the COD removal was improved regardless of pH difference (6.5–8.5) and MEJ-dish type. It indicates all the COD removal was not directly associated with voltage generation and electro-active biofilm formation. The bench-scale experiments also support the optimization findings, where COD removal was higher in the FAB-MFC, but the voltage generation was lower at the early stage of the treatment period and became higher as the age of the reactor increases. Overall, FAB improves the voltage generation within a limited pH range (6.5–7.5), COD removal over a broader pH spectrum (6.5–8.5), and Coulombic efficiency by 7.4–9.6 %. This study shows the development of fragmented anode biofilms (FABs) in the MEJ-dish FAB-MFC reactor than MFC. It could be possible to control the anode biofilm thickness by adjusting the size of MEJ-dish. The observed results indicate FAB provides a new flexible technique to increase anode surface area, manage the anode biofilm, and could contribute to the (in situ) scientific understanding of the MFC biofilm thickness and performance. Future studies may need to consider the number, size, and conductor MEJ-dish per electrode under various operations or membrane-less microbial fuel cell (ML-MFC).

Abbreviations

FAB: Fragmented electro-active biofilm or fragmented anode biofilm; MFC: Microbial fuel cell; FAB-MFC: Fragmented electro-active microbial fuel cell; MEJ-dish: Microbial electrode jacket dish.

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Authors' contributions
TA design the experimental setup and run the analysis. SL supervised and evaluated the work and participated during the design and development of the study. All authors read and approved the final manuscript.

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The data sets used in this study are available from the corresponding author on reasonable request.

Declarations

Ethical approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that no conflict of interest.

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