ORIGINAL ARTICLE

Studies on agar salt bridge based dual chamber microbial fuel cells using sludge and dustbin waste

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Abstract

In the present study, the performance of dual chamber microbial fuel cells (MFC) was investigated employing sludges collected from drains, a textile effluent treatment plant, and the Buriganga river as the sources of microbes, while a mixed liquor prepared from dustbin waste and gruel was used as substrates for the growth of bacteria. Six experiments were carried out under aerobic conditions throughout the study and inspected for seven days. Agar salt bridge was used instead of the typical membrane for proton exchange. For analyzing the electrical parameters of the MFCs such as voltage, current density and power density were recorded for each experiment. Reduction of Biological Oxygen Demands (BOD_5) of the sludges was also observed in this study. Regarding electrical performance, the Buriganga sludge afforded the best results demonstrating maximum voltage, current density, and power density as 244.88 mV, 35.16 mA/m^2, and 8.61 mW/m^2, respectively. However, the BOD_5 reduction was found to be 50.15%, 43.64%, 47.32%, and 35.20% for sludges collected from a drain of a residential area, a textile effluent treatment plant, a tannery, and the Buriganga river, respectively. Counts of aerobic *Escherichia coli* and *Bacillus subtilis* in different sludges were also documented.

Keywords Agar salt bridge · Aerobic decomposition · Biological Oxygen Demands (BOD_5) · Current density · Power density · Proton exchangeable membrane

1 Introduction

The necessity to reduce carbon emissions globally has gained considerable attention in recent times, leading to the implementation of stricter policies and regulations [1]. As conventional fuel sources are of confined origins and a number of environmental issues are being brought on by carbon emissions, which pose a major threat to human existence and health, it is high time for the exploration for renewable energy sources [2, 3]. Biofuels can be the alternate sources to meet up the energy demand [4]. Microbial fuel cell (MFC) technology has found immense interest in recent years as a promising approach to mitigate energy scarcity globally [5].

MFC is a unique bio-electrochemical system, which can utilize different substances of organic origins as energy source [6], is able to generate electricity from wastes and reducing organic contaminants from wastewater, and is representing a sustainable technology for the future [7]. Exoelectrogens, a specific species of bacteria possessing the catalytic activity, are used in MFC for producing electricity from biomass. This type of bacteria is capable of transferring electrons also [8–10]. In a typical MFC, an anode chamber is fed with microbes and substrate; anode and cathode chambers are separated by proton exchangeable membrane (PEM) (Fig. 1). Available bacteria in the anode chamber usually function as catalysts [11–14]. Electrons generated due to the oxidation of organic substrates are transported from the anode to cathode through an external wire, while the liberated protons transfer through PEM [10]. Nowadays, ionogels are used in PEMs. Ionic liquids possess special characteristics composed of organic cations, and inorganic/organic anions are enclosed in a solid structure within hybrid materials called ionogels. Through the creation of nanochannels and the creation of extensive hydrogen bonding networks with matrix materials and other proton conductors, ionogels in PEMs

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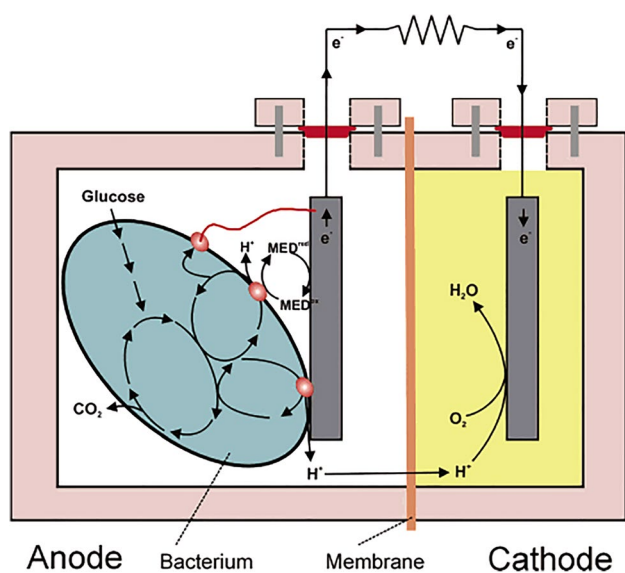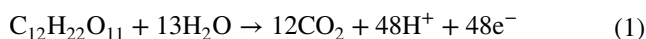
Fig. 1 A conventional model of MFC [10]

provide increased conductivity [15, 16]. Thus, in MFC, biodegradation of organic matters and production of electricity take place by the microbes [17, 18]. For example, fermentation of glucose in the presence of *Clostridium butyricum* produces CO_2, electron, and protons as shown in Eq. 1 [19].

$$C_{12}H_{22}O_{11} + 13H_2O \rightarrow 12CO_2 + 48H^+ + 48e^- \quad (1)$$

In the cathode compartment, electrons coming through an external load are recaptured by oxygen molecules, which combine with protons to form water [19]. The potential difference coupled to electron flow generates electricity in the fuel cell. Anaerobic MFC is also possible where electrons are generated by bacteria through the anaerobic oxidation of organic matter (by non-fermentative reactions) and passed to the respiratory enzymes in the inner cell membrane of the bacteria [17, 21].

The PEM is employed to separate the substrate molecules in the anode from electron capturing molecules (O_2) in the cathode while permitting transfer of H^+ from the anode to the cathode compartment. This allows the maintenance of charge balance and eliminates cross-contaminating reactions [20]. The PEM plays vital role in MFC construction. In recent years, many research groups are devoted in developing artificial PEM. Aromatic polymers such as *poly* (arylene ether ether ketone) (PEEK), *poly* (arylene ether sulfone), and their derivatives are generally used as high-performance PEMs [22]. Salt bridge has been used as an alternative to PEM by different research groups [23–28]. Salt bridges are generally made up of a mixture of agar ($C_{14}H_{24}O_9$), salt, and water which are less expensive than Nafion membranes and have a higher amount of self-resistance to proton transportation [28].

The effect of mediators likeanthraquinone-2,6-disulfonate (AQDS), neutral red, and humic acids to shift electrons to an external electrode from inside the cell was also studied [17, 21]. The laboratory-scale version of an MFC has been still lower than what is shown in an ideal case. The efficiency of an MFC is affected by several parameters [29]. Electrodes are important components of an MFC that help to improve its efficiency [30]. Carbon materials containing graphite fiber brush, carbon cloth, graphite rod, carbon paper, reticulated vitreous carbon (RVC), and carbon felt are the most commonly used materials in anodes because of their stability in microbial cultures, high electric conductivity, and large surface area [10, 31–35]. Furthermore, heterogeneous fabrication methods and modification methods incorporating nano materials have been tested for increasing the power density and expanding the electron accepting capability [36]. As cathode electrodes, carbon paper, carbon felt, carbon brush, carbon fiber, graphite of various types, Pt (Pt is commonly used as a cathode catalyst, while alternative polymer binders such as perfluoro sulfonic, Cu, Cu–Au, tungsten carbide, granular graphite, reticulated vitreous carbon (RVC) have also been tested [37–39]. A number of factors which control the release of electrons from substrate molecules in solution, their shifting through an external load, and their recapture by oxygen molecules. Fluctuations of temperature affect device kinetics, mass transfer (activation energy, mass transfer coefficient, and solution conductivity) [40], thermodynamics (free Gibbs energy and electrode potential), and microbial community existence and dispersion [40, 41]. Temperature has been shown to be a significant parameter for electricity generation [42, 43]. Temperature increases power generation of MFC exponentially owing to anodic biofilm growth, which impacts bio catalytic activity and therefore efficiency [44–46]. An MFC can operate at temperatures ranging from 5 to 40 °C, with an optimal working range of 18 to 35 °C [47–49].

As a process for both wastewater treatment and electricity production, MFCs are being studied in recent years [50]. Different types of wastewaters, which include wastewater from food production, swine wastewater, and sanitary waste, comprise numerous biodegradable natural materials that could be used to run MFCs [50]. MFCs have been first used to purify wastewater in 1991 [52].

In this study, two types of MFC were constructed, one using PET bottles and the other of acrylic box, and in both cases, agar salt bridge was used instead of PEM, and sludges from drains, textile effluent treatment plant, and Buriganga river were used under aerobic condition. Mixed liquor prepared from dustbin waste and starch of boiled rice was used as source of carbohydrates (substrate) for the growth of bacteria. The Biological Oxygen Demand (BOD_5) of the sludges was also recorded before and after conducting each experiment. The counts of *Escherichia coli* and *Bacillus subtilis* (CFU/ml) in different sludges were also made.

Fig. 2 Electrode materials: **a** brass mesh and **b** copper mesh

The novelty of the current study is the use of cheap agar salt bridge instead of expensive nafion membrane, dustbin waste as substrate, and different types of sludges as the source of microorganisms. Moreover, MFCs were operated at ambient temperature and without agitation in the cathode chamber, so there was no external power consumption.

2 Materials and methods

Agar ($C_{14}H_{24}O_9$) and sodium chloride (NaCl) were used for constructing salt bridge, and Sorbitol MacConkey agar (SMAC) and NaCl-Glycine-Kim-Goepfect (NGKG)

were used for microbiological study. All analytical grade chemicals were purchased from Merck, Germany, and used as received. Copper mesh and brass mesh (shown in Fig. 2) were collected from local market (Dhaka, Bangladesh) and used as cathode and anode, respectively. An air compressor (Model no. SEBO Air Pump-348A) was used in experiment 6 to provide external air supply in the anode chamber.

Figure 3 illustrates the configuration of MFC that was used during this study. Two types of MFCs were constructed; one was H-shaped and the other was box type. Plastic (PET) bottles were utilized to construct anode and cathode chambers of H-shaped MFCs (experiments 1 to 4) shown in Fig. 4. For other types, acrylic boxes were used to construct anode and cathode compartments (experiments 5 and 6) as shown in Fig. 5. A cylindrical salt bridge having surface areas of 0.00025 m^2 was used for H-type MFC while in the box-type MFCs the salt bridge had surface area of 0.006 m^2 and 38.1-mm-thick membrane of agar placed between anode and cathode compartments. The dimensions of different components of MFCs are mentioned in Table 1.

2.1 Preparation of substrate

The non-degradable components (e.g., plastic products) were segregated manually from the waste collected from a dustbin and used without further processing. Gruel, a waste produces during cooking rice (1 l) was mixed with dustbin waste and blended for 10 min. The same proportions of starch and dustbin waste were used for the preparation of the substrate for all the studies.

2.2 Preparation of salt bridge

For H-shaped MFCs, agar salt bridges were prepared following a procedure reported previously elsewhere [23]. In brief, agar (4 g), sodium chloride (22 g), and water (180 ml) were mixed in a conical flask, and the mixture was heated

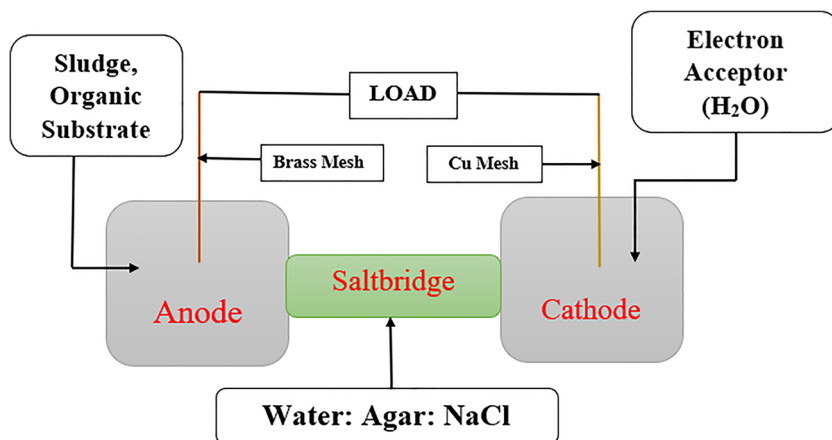**Fig. 3** Structure of microbial fuel cell (MFC) imitated in this study

Fig. 4 H-type MFC for experiments 1–4 (left) and for box-type MFC for experiments 5–6 (right)
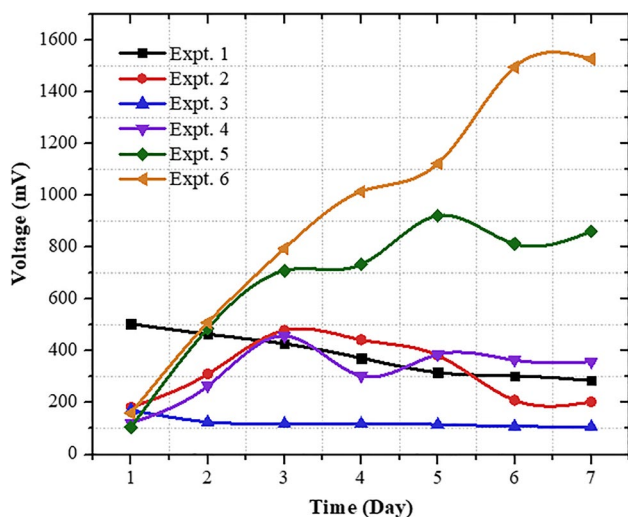

Fig. 5 Change in open-circuit voltage (OCV) with time for different experiments

with stirring at 95 °C for 10–12 min, and the hot solution was poured into a PVC pipe (4 inches, 0.1016 m long and 0.5 inches, and 0.0127 m diameter). Then it was cooled to room temperature. For box-type MFCs, the same procedure was followed to prepare the solution using 15 g agar, 90 g sodium chloride, and 450 ml water. The hot solution was poured into the empty area created in between the boundaries of the anode and the cathode chamber.

2.3 Amounts of ingredients

Four types of sludges and same organic substrate were fed in an anode chamber, and water was taken as an electron acceptor in the cathode chamber. Different amounts of sludge, substrate and electron acceptor in different experiments of this study is mentioned in Table 2.

2.4 Measurement of electrical parameters

A multimeter (Model no. DT-9205A) was used the measurement of electrical parameters. All the cells were kept in aerobic condition, and readings were taken for seven days across 101 Ω, 261 Ω, 472 Ω, 991 Ω, 1950 Ω, 4910 Ω, and 9850 Ω external loads [23]. The current density was obtained from the current that was calculated using the Ohm's law (Eq. 2).

$$I = \frac{V}{R} \tag{2}$$

where I = current, V = measured voltage, and R = resistance.

2.5 BOD measurement

Biological Oxygen Demand (BOD_5) values of sludges before and after the experiment were recorded using Lovibond BD 600 measuring system.

Table 1 Physical parameters of constructed MFCs

Expt. No	Chamber (anode & cathode) area (m^2)	Electrode (anode & cathode)		Salt bridge	
		Length (m)	Width (m)	Surface area (m^2)	Length (m)
1	0.069	0.1524	0.0762	0.00025	0.1016
2	0.069	0.1524	0.0762	0.00025	0.1016
3	0.069	0.1524	0.0762	0.00025	0.1016
4	0.069	0.1524	0.0762	0.00025	0.1016
5	0.248	0.1524	0.1524	0.006	0.0305
[a]6	0.248	0.1524	0.1524	0.006	0.0305

[a]External air supply in the anode chamber was made

Table 2 Amount of ingredients used in different experiments

Expt. No	Sludge type	Amount of sludge (ml)	Amount of substrate (ml)	Amount of electron acceptor (ml)
1	Drain	1000	200	1000
2	Textile ETP (effluent treatment plant)	1000	200	1000
3	Tannery	1000	200	1000
4	Buriganga river	1000	200	1000
5	Drain	8000	800	8000
6	Drain	8000	800	8000

2.6 Microbiological study

The amount of aerobic *Escherichia coli* and *Bacillus subtilis* was also inspected in this study. Sorbitol MacConkey Agar (SMAC) and NaCl-Glycine-Kim-Goepfect (NGKG) medium were applied for determination of the presence of aerobic *Escherichia coli* and *Bacillus subtilis* in the sludges.

2.6.1 *Escherichia coli* count

Sorbitol MacConkey Agar (SMAC) (51.5 g) was mixed with distilled water (1 l), and the solution was sterilized by autoclaving at 121 °C for 15 min. After that, the medium was cooled down to 50 °C and poured on Petri dishes in a laminar flow. After drying, the samples of the sludge were dispersed on the surface of the plate, and the plates were incubated at 30 °C for 24 h. After 24 h, the colonies of *Escherichia coli* were visually inspected and counted manually.

2.6.2 *Bacillus subtilis* count

NaCl-Glycine-Kim-Goepfect (NGKG) (13.3 g) was mixed with distilled water (450 ml), and the solution was sterilized by autoclaving at 121 °C for 15 min. After that, the medium was cooled down to 50 °C followed by the addition of 100 ml 20% egg yolk with the solution. 20% egg yolk was prepared adding egg yolk (20 ml) with sterilized saline solution (80 ml) aseptically. Next it was poured on Petri dishes in a laminar flow. After drying, the samples of the sludge were dispersed on the surface of the plate, and the plates were incubated at 30 °C for 24 h. After 24 h, the colonies of *Bacillus subtilis* were visually inspected and counted manually.

3 Results and discussion

3.1 Performance of MFC

Total six experiments were carried out in this study; experiments 1–4 were conducted in H-shaped MFCs) whereas experiments 5–6 in box-type MFCs (Fig. 4). In experiment 1, experiment 2, experiment 3, and experiment 4, sludges collected from drain, textile effluent treatment plant, drain adjacent to tannery, and the Buriganga river were used as the source of microorganisms respectively. In experiments 5 and 6, drain sludge was used and external air supply was provided with experiment 6.

For each experiment, the open-circuit voltage (OCV) and voltages across different loads (e.g., 101 Ω, 261 Ω, 472 Ω, 991 Ω, 1950 Ω, 4910 Ω, and 9850 Ω) were measured for seven days. First reading of open-circuit voltage (OCV) was taken after 4 h of starting the MFC and data were recorded at a fixed interval of 24 h (Fig. 5). At the beginning OCV for all the experiments were close to 100–200 mV. For experiment 1 (drain sludge), OCV decreased steadily from days 1 to 7, while for experiments 2 (textile effluent treatment plant sludge) and 4 (Buriganga sludge) OCV slowly increased to a maximum of 500 mV on day 3 and then reduced steadily to 204 mV. For experiment 3 (tannery drain sludge), OCV was almost constant at 119 mV from day 1 to day 7. For box-type MFCs, OCV reached a maximum of 922 mV on day 5 then started to decay (experiment 5). For experiment 6, OCV increased until day 6 and reached at 1496 mV and then reduced. This phenomenon could be explained with the fact that under additional air supply and nutrients contents microbial growth was enhanced and after 6 days nutrients were not sufficient for the growth although air supply was continued. Another interesting finding was that tannery drain sludge (experiment 3) demonstrated the lowest output which may be due to the presence of different types of fungicides used in tanning hindered the growth of microorganisms.

Polarization curve is also widely used for analyzing the performance of MFC, which is the representation of the cell voltage and power density in terms of current (or current density) [9]. In the present study, after 48 h (2 days) of operation MFCs, the output voltage was almost constant for the following 72 h (day 3, day 4, and day 5). Figures 6, 7, and 8 represent the polarization curve on day 3, day 4, and day 5, respectively. Polarization curve was propagated by varying loads as 101 Ω, 261 Ω, 472 Ω, 991 Ω, 1950 Ω, 4910 Ω, and 9850 Ω after obtaining stable voltage. Among the H-shaped MFCs (expt. 1–4), the highest output was provided by the Buriganga sludge on day 3 which produced maximum current density and power density of 35.16 mA/m^2 and 8.61 mW/m^2, respectively (Fig. 6). This may be attributed to the presence of some bacterial strains in Buriganga sludge, which are more effective in bioenergy
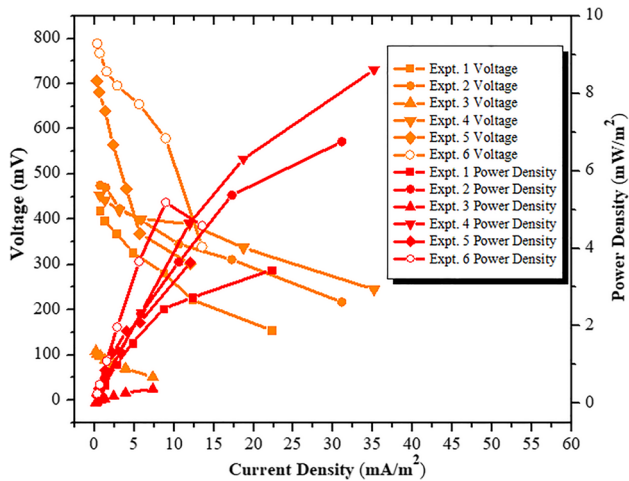
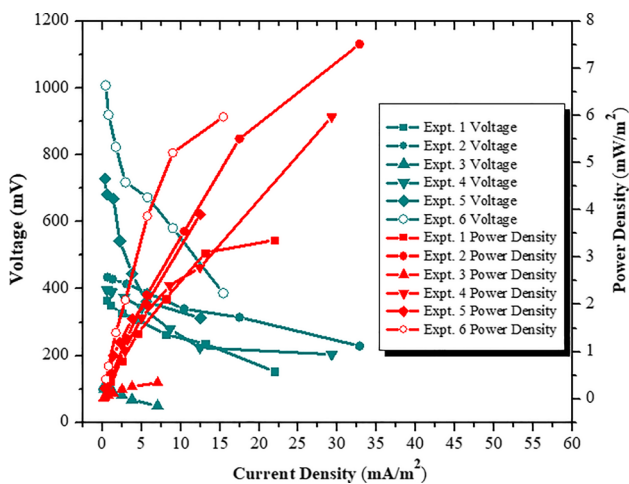**Fig. 6** Polarization curve on day 3
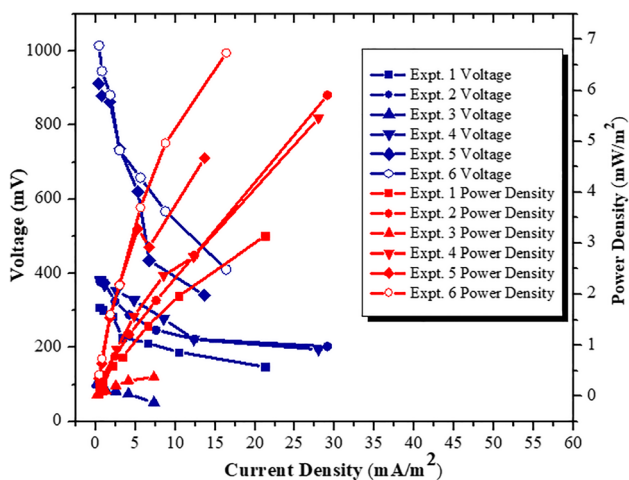

Fig. 7 Polarization curve on day 4

Fig. 8 Polarization curve on day 5

production. In case of box-type MFCs, experiment 6 with external air supply gave better results compared to experiment 5 providing 16.4 mA/m² and 6.74 mW/m² as peak current density and power density, respectively, at day 5 (Fig. 8). The value of electrical parameters (voltage, current density, and power density) started to decrease after a certain period of time for each experiment where there was no air supply, while experiment 6 with air supply demonstrated upward trend (Table 3). The reason behind is that the amount of oxygen and inside the chamber started to reduce from day one and consequently hindered the growth of aerobic bacteria in the sludges.

Table 3 represents the comparison for electrical parameters obtained from different MFCs constructed in this study with previous research works.

3.2 Reduction of BOD_5 of the sludges

Figure 9 illustrates the change in BOD_5 of the sludges before and after conducting the experiments. In H-type MFCs, the BOD_5 values for drain sludge, textile ETP sludge, tannery drain sludge, and the Buriganga sludge had been reduced by 48.47%, 43.64%, 47.32%, and 35.20%, respectively (experiments 1, 2, 3, and 4), while for box-type MFCs, the reduction of BOD_5 value for drain sludge was found to be 46.88% and 50.15% (experiments 5 and 6) respectively. The maximum BOD_5 reduction in this study was found for experiment 6 where additional air was pumped into the anode chamber. The organic substances were decomposed during the bacterial growth and thus BOD_5 of each sludge reduced significantly. When there was an additional air supply in experiment 6, the BOD_5 removal was highest (50.15%) which further supports the aerobic decomposition of the substrates.

3.3 Microbiological studies

The colonies of bacteria were visually inspected. The measuring unit was colony-forming unit per milliliter (CFU/ml). Figure 10 shows the microscopic view (using 40×lens) of *Escherichia coli* and *Bacillus subtilis* in the drain sludge. Figure 11 represents count of *Escherichia coli* [log(CFU/ml)] and *Bacillus subtilis* [log(CFU/ml)] in different types of sludges, respectively.

Significant counts of *Escherichia coli* and *Bacillus subtilis* were found in all sludges except the one collected from a drain close to a tannery. Although the tannery drain sludge contained a good number of *Bacillus subtilis*, it contained a negligible number of *Escherichia coli*. The maximum electrical output from the Buriganga sludge (voltage 244.88 mV) and the minimum output from the tannery drain sludge (84.44 mV) indicated that both *Escherichia coli* and *Bacillus subtilis* played a significant role in energy production of the MFCs studied in the present investigation, and the results were in good agreement with previous study [57]. However, the best result for the Buriganga sludge may be attributed to

Table 3 Comparison of electrical parameters of different MFCs of this study with previous works

No	System configuration	Max. voltage (mV)	Max. current density (mA/m^2)	Max. power density (mW/m^2)	References
1[a]	Two-chamber	679	1.67×10^{-3}	315	53
2[a]	Two-chamber	575	6×10^{-3}	77	54
3[b]	Two-chamber	742.3	3145	539	54
4[c]	Two-chamber	408	0.65	770	55
5[c]	Two-chamber	560	0.552×10^3	214	56
6[d]	Two-chamber	192.01	27.55	5.29	Expt. 1 (this study)
7[d]	Two-chamber	229.10	32.83	7.52	Expt. 2 (this study)
8[d]	Two-chamber	84.44	12.20	1.03	Expt. 3 (this study)
9[d]	Two-chamber	244.88	35.16	8.61	Expt. 4 (this study)
10[d]	Two-chamber	341.64	13.64	4.66	Expt. 5 (this study)
11[d]	Two-chamber	431	17.19	7.41	Expt. 6 (this study)

[a]Nafion membrane was used

[b]Bipolar membrane was used

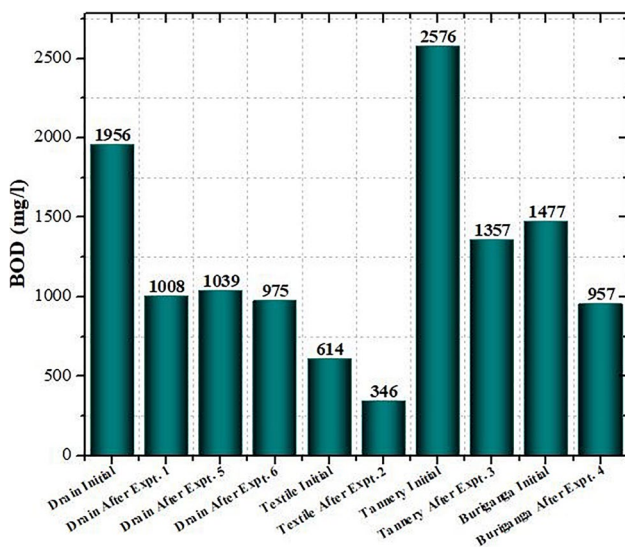[c]Micro filtration membrane was used

[d]Agar salt bridge was used

Fig. 9 Change in BOD$_5$ (mg/l) for different sludges

the presence of some more effective microorganism which were not identified in the current investigation.

4 Conclusion

A total number of six experiments were investigated throughout the project, and all of them were maintained under aerobic conditions and observed for seven days. Among six experiments, only experiment 6 was provided with external air supply, which facilitated the bacterial growth and consequently stable outputs were attained. Besides this, two types of bacteria were detected and counted in the sludges which were *Escherichia coli* and *Bacillus subtilis*. The reduction of BOD$_5$ was also measured, and the maximum reduction was 50.15% for drain sludge (expt. 6) where additional air played a vital role to reduce BOD$_5$ due to ease of oxidation of the substrates. The result in the current investigation revealed that organic waste

Fig. 10 Microscopic view of **a** *Escherichia coli* and **b** *Bacillus subtilis* using 40× lens in drain sludge
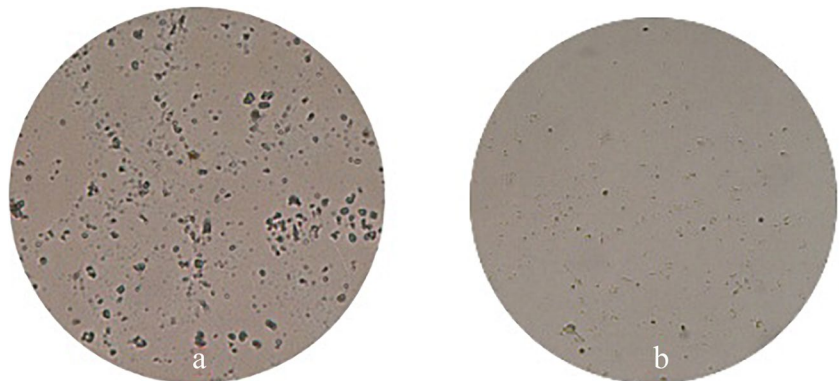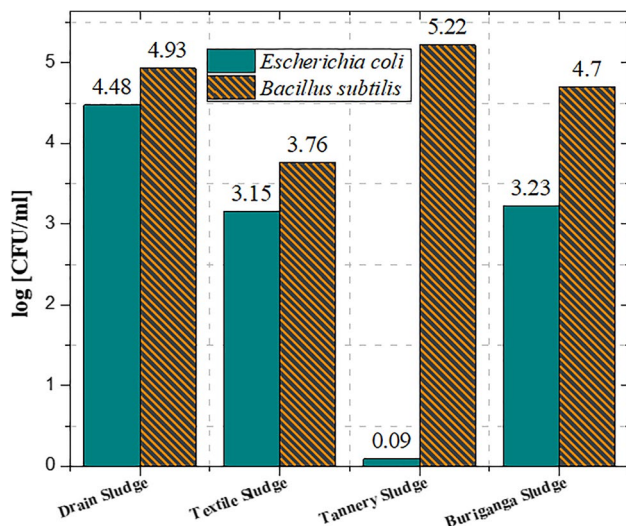
Fig. 11 Amount of *Escherichia coli* and *Bacillus subtilis* [log(CFU/ml)] present in different sludges

can be used as a substrate and different sludges can be used as the sources of microorganisms in MFC. The reduction of Biological Oxygen Demand (BOD_5) of the sludge implied that in MFC both power generation and effluent treatment can be done simultaneously.

However, due to lack of laboratory facilities and financial support, it was not possible to identify the proportion of particular carbohydrate (e.g., starch, glucose, and sucrose) in organic substrate, and biochemical test of the sludge was not done to identify the composition of the sludge.

Author contribution Sheikh Shehab Uddin: conducting experimental studies, formal analysis, and writing draft manuscript. Md. Mahidul Haque Prodhan: manuscript proofreading and editing draft manuscript. Mohammad Nurnabi: conceptualization, formal analysis, editing draft manuscript, and supervision.

Declarations

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