



RESEARCH ARTICLE

Investigation of electricity generation performance of grape marc in membrane-less microbial fuel cell

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ABSTRACT

Grapes are among the most widely grown fruits globally, with a third of the overall production used in winemaking. Both red and white winemaking processes generate significant amounts of solid organic waste such as grape marc that requires proper disposal. Grape marc, a natural plant product containing abundantly lignocellulosic compounds, is a promising raw material for production of renewable energy. In this study, the grape marc was used as an anode nutrient in the membrane-less microbial fuel cell (ML-MFC) system, and the electricity generation capacity of the grape marc as an environmentally friendly energy source was investigated in detail. The maximum power density produced in the ML-MFC reactor was determined as 274.9 mW m⁻², and the total internal resistance was 309.5 Ω. Cyclic voltammetry results showed the presence of electroactive microorganisms on the surface of the anode electrode provided a high biological activity. The presence of elliptical and round-shaped microorganisms on the anode electrode surface was observed. Quantitative polymerase chain reaction (qPCR) analyzes have shown that grape marc supports bacterial growth on the electrode surface.

Keywords: Grape marc, membrane-less microbial fuel cell (ML-MFC), electricity generation, microbial analysis

1. INTRODUCTION

Bioenergy is one of the most important alternatives for sustainable energy production. In addition to sustainable energy production, using organic pollutants as a bioenergy source is a powerful way to prevent environmental pollution [1, 2]. Energy generation using biomass is a sustainable process considering CO₂ emissions and the limitation in fossil fuels globally. Agricultural wastes are substantial natural resources because of their abundance and in the environment for energy production [1]. Various organic wastes such as rice husk, corn waste, palm waste, sugar cane, and grape marc can be used as renewable energy sources [3]. Grape marc, a typical form of waste biomass with organic residues produced from the process of wine production, has high lignocellulosic content resistant to biologic degradation in the environment [4].

In the world, millions of tons of grape marc are produced every year [5]. For this reason, it has a significant risk potential for the environment when

discharged without proper treatment. If the grape marc is discharged without stabilization, it causes many environmental problems such as surface and groundwater pollution, malodor, flies, and insect infestation [6, 7]. In addition to the grape marc, the wine production process produces winery wastewater containing high nutrient concentrations such as nitrates, phosphates, and different organic compounds [8, 9]. The contamination of these pollutants to the surface water sources causes eutrophication, which has an adverse effect on biotic components in the environment. Many previous studies reported that the grape marc causes many environmental problems such as soil pollution, surface water pollution, damage of plants, and odor [10]. For this reason, the wastes generated in the winery production process cannot be discharged without treatment [11]. This creates a considerable annihilation problem for wineries [4, 12, 13]. Various treatment strategies were used to remove pollutants in wastes produced by the winery production process to prevent these environmental problems. These treatment strategies include the extraction of

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commercial chemicals such as tannin, ethanol, and phenol from the grape marc and their use in technologies of energy conversion, including pyrolysis, combustion, and gasification [5, 14, 15]. The previous studies showed that the organic content and specifications of grape marc are suitable for thermochemical energy conversion [5, 16-18]. In previous studies, the energy generation potential of grape marc in the anaerobic digestion was investigated by Makadia, et al. [7] and Da Ros, et al. [19]. These studies demonstrated that the grape marc has sufficient bioenergy generation potential and would be promising biomass in the renewable energy generation process. However, the occurrence of highly endergonic reactions in energy recovery from grape marc by any of the thermal transformation (i.e., gasification) processes causes environmental pollution [4, 5]. To overcome the harmful effect of the classical energy production process, it is necessary to focus on a more environmentally friendly process that does not create any pollution during energy generation. Thus, bioenergy production can provide higher energy recovery efficiency and be a more environmentally friendly approach [7]. ML-MFC reactor is one of these processes and is among the promising electrochemical systems in energy storage and conversion.

ML-MFC is a promising technology among the electrochemical systems in terms of the direct electricity generation from organic wastes and environmentally friendly nature. ML-MFC does not need the proton exchange membranes (PEM) to physically separate anode and cathode compartments and transfer hydrogen ions between them [20, 21]. In MFC systems, bacteria produce electrons by oxidizing organic pollutants and transfer them to the anode electrode surface without producing any secondary pollutants [22-25]. Despite the lower bioelectricity generation of ML-MFC than MFC having PEM, they do not need expensive PEM during the operation. The current ML-MFC studies have focused on improving the electricity generation capacity by using novel electrode materials and substrate sources [26-28].

This study concentrated on using grape marc in the anode compartment of ML-MFC as a substrate source to produce electricity. For this purpose, the grape marc was added to the anode compartment of ML-MFC, and ML-MFC electricity generation performance was investigated in detail using electrochemical impedance spectroscopy (EIS), linear sweep voltammetry (LSV), and cyclic voltammetry (CV). The biofilm morphology and viability were examined using microscopic techniques. Additionally, the anode biofilm microbiome was analyzed by using the quantitative polymerase chain reaction (qPCR) method.

2. MATERIALS AND METHOD

2.1. ML-MFC setup and operation

In this study, a mixture of the anaerobic digester (Elazığ municipal wastewater treatment plant) sludge and sediment sludge obtained from the golden horn

(Istanbul, Turkey) was used as an inoculum source. The physical properties of grape marc containing C, H, N, O, and S; moisture 68.71%, volatile matter 75.83%, ash 6.52%. The grape marc was dried at room temperature (21 ± 2 °C), grounded, and then used as an organic substrate source for electroactive bacteria in the anode of ML-MFC (Fig 1a and Fig 1b). The inoculum was mixed with grape marc at the ratio of 1/5 and then added to the anode compartment of ML-MFC. A cylindrical plastic bottle with a height of 24 cm and a diameter of 8 cm was used as an ML-MFC reactor (Fig 2a). The cathode compartment was filled with tap water. A sand layer with a height of 2 cm was formed to physically separate anode and cathode compartments (the average diameter of sand particles is 1 mm). The height of the anode compartment was 11 cm. The anode electrode was set 7 cm below the anode surface. The ML-MFC was covered with aluminum foil to protect the growth of photosynthetic microorganisms and operated at room temperature. The cathode compartment of ML-MFC was continuously aerated by using an aquarium pump to provide oxygen for the cathodic reactions. The dissolved oxygen concentration of the cathode compartment was kept at between 5.5-6 mg L⁻¹.



Fig 1. Wet grape marc (a), dried-ground grape marc (b)

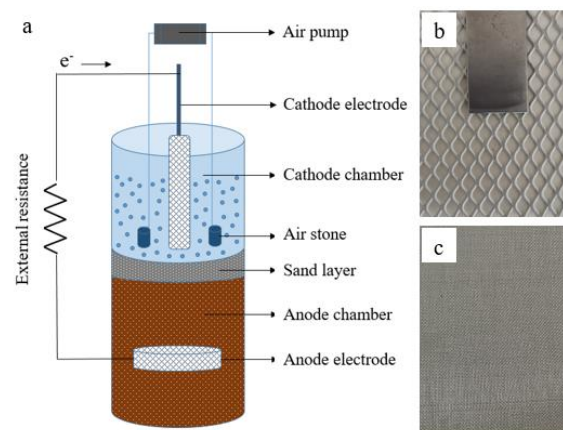


Fig 2. Schematic view of ML-MFC reactor (a), Platinum-coated titanium electrode (b), Stainless-steel electrode (c)

2.2. Anode and cathode electrodes

The stainless-steel and platinized titanium mesh were purchased from the Fuel cell store (www.fuelcellstore.com) and used as anode and cathode electrodes in ML-MFC. The electrodes were placed in ethanol for 15 min to remove micro and nano-scale particles and then washed with pure water. The 1000Ω resistance was used to fix anode

and cathode electrodes. The anode and cathode electrodes are shown in Fig 2b and Fig 2c.

2.3. Analysis

Electrochemical analysis

The voltages generated in the ML-MFC were measured by using an Agilent 34970a digital multimeter every 3 min (Data Acquisition/Switch Unit) and recorded to the computer using Agilent Benchlink Data Logger 3 V4.3 software. The electrochemical analysis was performed by using a Gamry interface 1000 potentiostat (Gamry, Warminster, PA). The EIS analysis was carried out in a frequency range of 100 kHz to 0.1 Hz with an amplitude value of 5 mV. The primary components of internal resistance of ML-MFC were determined using Gamry Echem Analyst V6.25 software according to the equivalent circuit model given by Abazarian, et al. [29]. The max power density of ML-MFC was measured using LSV analysis at the scanning rate of 2.5 mV s⁻¹. The CV analysis was carried out at the scanning rate of 5 mV s⁻¹ from 1.0 to -1.0 V.

The amount of current and power generated in the reactor was calculated based on the equations below [20]:

$$V = I \times R \quad (1)$$

$$P = I \times V = I^2 \times R \quad (2)$$

Table 1. Primers used for qPCR amplification

Target microorganism	Primer names	Sequences
Bacteria	907R	5'-CCGTCAATTCMTTTGAGTTT-3'
Bacteria	BacV2f	5'-CCTACGGGAGGCAG CAG-3'
Eukaryote	Euk1A	5'-CTGGTTGATCCTGCCAG-3'
Eukaryote	Euk516r	5'-ACCAGACTTGCCCTCC-3'
Archaea	517F	5'-GCYTAAAGSRNCCGTAGC-3'
Archaea	909R	5'-TTTCAGYCTTGCGRCCGTAC-3'

The genomic DNA of biofilm samples was isolated by using PowerSoil DNA isolation kit (Mo Bio Laboratories, USA) according to the manufacturer protocol. The primer sets given in Table 1 were used to amplification of genomic DNA. Roche-Fast Start SYBR Green Master (Roche, Nutley, NJ, USA) was used in the Roche LightCycler® 96 real-time PCR system (Roche Applied Science) to perform the qPCR analysis in triplicates for each sample. The copy numbers of each gene were calculated using cycle threshold (CT) values based on the standard curve.

3. RESULTS AND DISCUSSION

3.1. Power generation in ML-MFC

The power generation capacity of MFCs depends on the organic content and degradation of substrate used

Where; V is the voltage in volts (V), I is the current in ampere (A), and R is the known value of the external load resistor in ohms (Ω).

Microscopic observations and microbial analysis

The biofilm samples on the anode electrode surface were obtained at the end of the operation period to perform microscopic analysis. The scanning electron microscopy (SEM) observations were performed using Zeiss electron microscopy (Carl Zeiss AG, Oberkochen, Germany) according to the protocol given by Taşkan, et al. [30]. According to the manufacturer protocol, the viability of anode biofilm was analyzed using the LIVE/DEAD® BacLight™ bacterial viability kit (Thermo Fisher Scientific, USA). Eclipse Ni-U microscope (Nikon, Japan) was used to perform viability analysis. The red color represents dead cells in the images, and the green color represents living cells [31].

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in the anode compartment. In this study, the electricity generation capacity of grape marc was determined by LSV analysis by plotting the power curve of ML-MFC (Fig 3). A maximum power density of 274.9 mW m⁻² and a maximum current density of 0.44 A m⁻² was achieved by ML-MFC fueled by grape marc. The obtained maximum power density of ML-MFC is higher compared with many similar previous studies. In a previous study, the treatment and electricity generation capacity of copper contained wastewater using a membrane-less double compartment MFC was investigated, and a maximum power density of 31.3 mW m⁻² was reported by Liu, et al. [32]. Morris and Jin [33] reported a maximum power density of 7 mW m⁻² with petroleum hydrocarbon at the concentration of 16 g k⁻¹ g by using an ML-MFC. In a study, the bioelectricity generation capacity of different electrode materials

(stainless-steel, aluminum, and titanium mesh) and sediment sludges having different sediment and salt ratio were investigated by Bartolome, et al. [34]. It was reported that the maximum power density of titanium mesh, aluminum, and stainless-steel electrodes were 400, 140, and 90 mW m⁻², respectively. In another study, the electricity generation capacity of ML-MFC reactor using sewage sludge as the substrate source was investigated, and it was reported that the maximum power density reached was 187 mW m⁻² [35]. Compared with previous studies, the maximum power density obtained by this study is higher than many previous studies. It seems possible that the high power density of ML-MFC is due to the high organic content of grape marc.

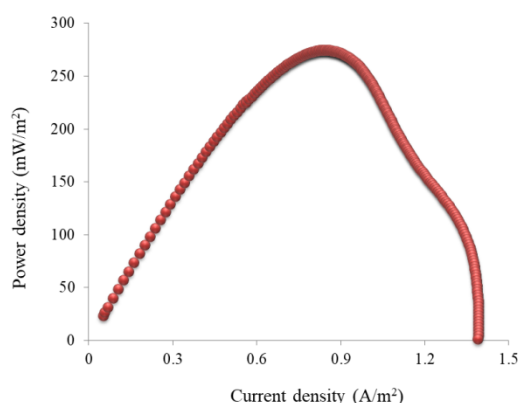


Fig 3. The power density produced in the ML-MFC reactor

3.2. Electrochemical performance of ML-MFC

In the current study, CV and EIS analysis were performed to evaluate the electrochemical performance of ML-MFC. EIS is one of the most powerful analyses that inform the ohmic resistance (R_{Ω}), mass transfer resistance (R_{mt}), and charge transfer resistance (R_{ct}) of ML-MFC. The internal resistance of MFC significantly affects the electricity generation performance. The equivalent circuit model given by Abazarian, et al. [29] was used to analyze the internal resistance of ML-MFC. The Nyquist plot and equivalent circuit model are shown in Fig 4. The equivalent circuit model includes solution resistance (R_s), mass transfer resistance (R_{mt}), cathode charge transfer resistance (R_{ct}), and other resistances (R_o) present at the cathode. Additionally, constant phase elements (CPEs) representing the capacitance of electrical double layer are also available in the model to consider for the structure and porosity of the electrode [41]. The CPE_{ct} , CPE_{mt} , and CPE_o are related to mass transfer, charge transfer, and other processes contained in the cathode. The R_s , R_{ct} , R_{mt} , and R_o are 24.1, 206.5, 70.3 and 8.6 Ω , respectively. The total internal resistance of the ML-MFC reactor was calculated as 309.5 Ω from the formula $R_t = R_s + R_{ct} + R_{mt} + R_o$. Hou, et al. [36] investigated the effect of two different anode electrode materials (carbon cloth and stainless-steel fiber felt) modified and unmodified with graphene on MFC performance. It was reported that the total internal resistance of MFCs with unmodified carbon cloth and stainless-steel fiber felt

were 235.4 and 974.5 Ω , respectively. In another study, a total internal resistance of 437.7 Ω was reported by Taşkan, et al. [20] with a graphene-coated nickel-titanium (NiTi) alloy electrode. Another study reported that the total internal resistance of a double chamber MFC with graphite plate and tin-coated copper mesh electrodes were 408 and 477 Ω , respectively [37]. In another study, a tubular separating electrode assembly with graphite granule bed anode and simple carbon-fabric cathode was investigated as nylon fabric, J-fabric, and glass fiber separators in MFCs. It has been reported that the internal resistance of these three different MFCs is in the range of 614.71–1711.59 Ω . [38].

CV analysis is used to measure the catalytic processes, and redox reaction that occur in the anode and cathode electrodes of MFC [39]. Additionally, the CV helps to profoundly understand the biological activity of the anode biofilm, which converts organic matter in the grape marc into bioelectricity. Fig 5 shows the CV curves at the beginning (red) and at the end of the operating period (blue). Due to the insufficient of electroactive microorganism colonization on the surface of anode, no clear oxidation/reduction peak was detected in the CV curve obtained in the initial phase of the reactor. The CV results at the end of the operation showed that an oxidation peak of 0.55 mA was detected at -0.070 V, while two cathodic reduction peaks of -0.61 mA and -0.42 mA at -0.50 V and 0.01 V, respectively. The anodic peak confirms the presence of electroactive bacteria and electrogenic activity on the anode electrode. The cathodic peaks show that effective hydrogen (H^+) transfer from anode to cathode and the reduction of oxygen cathode electrode surface. Nimje, et al. [39] investigated simultaneous nitrate reduction and electricity generation by *Bacillus subtilis* under anaerobic conditions, using glucose and nitrate as the carbon source in a single chamber MFC. They reported two cathodic reduction peaks at about 0.1 V and -0.4 V and two anodic oxidation peaks at 0.05 V and 0.25 V. In another study, an anodic peak of 0.6 mA at -0.05 V was detected in MFC with modified polyaniline-coated carbon fabric anode, while a reduction peak of -1.28 mA at -0.5 V was observed by Hou, et al. [40].

3.3. Microscopic and molecular analysis

The SEM and viability images of anode biofilm are given in Fig 6. The biofilm structure and bacterial morphology are shown in the SEM image of anode biofilm in Fig 6a. It is observed from the SEM image that the presence of abundant round and elliptical bacteria on the anode surface, while rod-shaped bacteria were rarely found. The SEM image demonstrated that the anode surface provides a suitable environment for attachment and colonization of anode microorganisms. The bacterial viability image showed live (green) and dead (red) bacteria in anode biofilm. It can be concluded that the stainless-steel mesh anode has good biocompatibility with electrogenic bacteria, and provide relatively high viability of biofilm (Fig 6b).

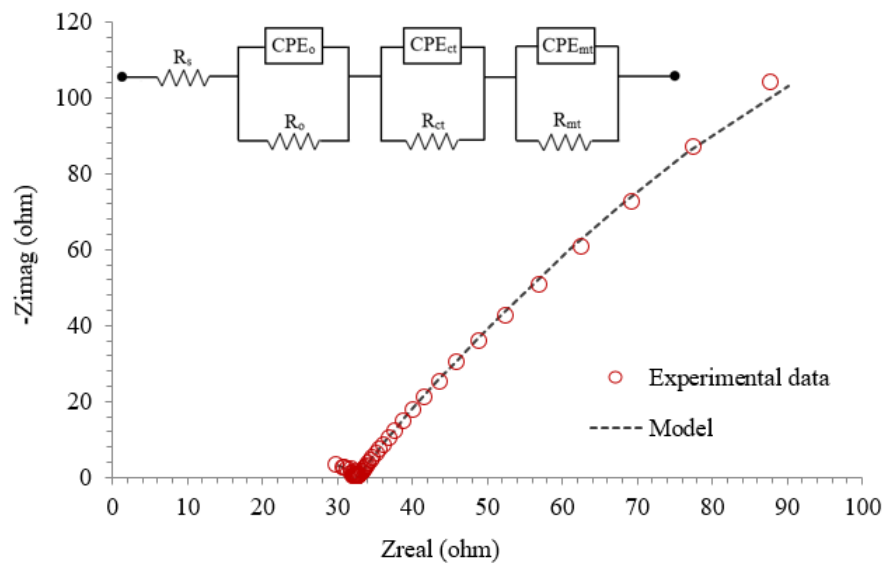


Fig 4. EIS diagram of anode electrode in ML-MFC reactor

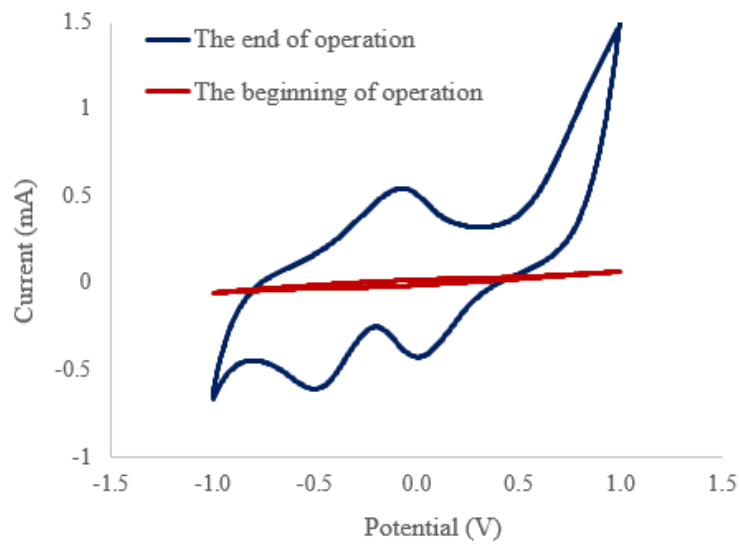


Fig 5. CV curves obtained at the beginning and end of the operation in the ML-MFC reactor

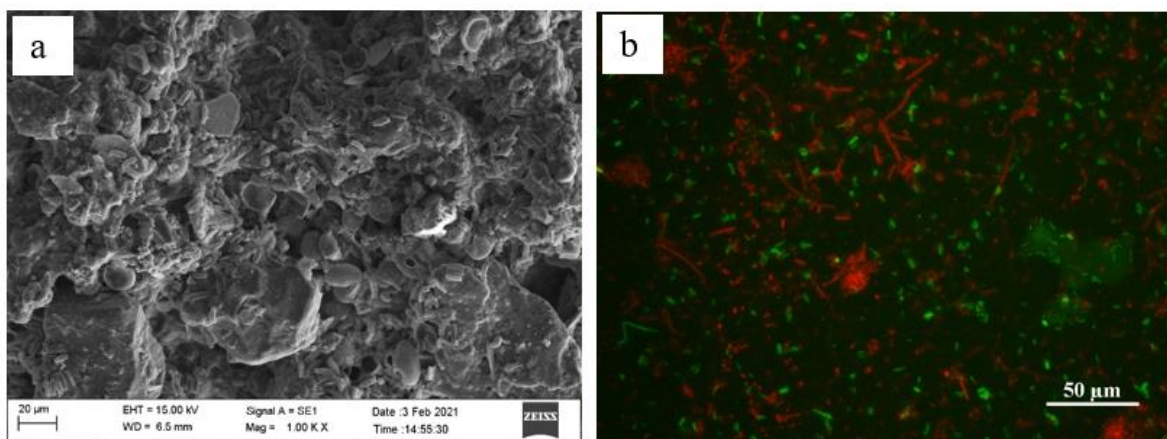


Fig 6. SEM image (a) and fluorescence microscopy image (b) of the biofilm on the anode electrode surface at the end of the operation

In addition to the microscopic observations, the gene copy numbers of bacteria, eukaryotes, and archaea were determined by qPCR analysis. The qPCR analysis was effectively used to determine the cell concentration by previous researchers [41, 42]. The gene copy numbers of bacteria (16S rRNA) in anode medium at the beginning of the operation period were 1.26×10^{10} copies μL^{-1} while it was 3.19×10^{10} copies μL^{-1} anode biofilm at the end of the operation period. The number of bacteria in the anode electrode surface increased approximately 2.5 times from the beginning to the end of the operation period (Fig 7). This finding suggests that grape marc contributes to the growth of bacteria and accordingly supports electricity generation. The copy numbers of eukaryotic genes (18S rRNA) were 3.84×10^6 copies μL^{-1} at the beginning of the operation, while it was 1.72×10^5 copies μL^{-1} at the end of the operation period of ML-MFC on the anode electrode surface. The number of eukaryotic

microorganisms in the anode compartment of ML-MFC was significantly decreased during the operation period of ML-MFC. The decrease in the number of eukaryotic microorganisms might be insufficient light and inorganic carbon sources for photosynthetic eukaryotes. In addition to the bacteria and eukaryotes, the number of archaea was investigated in anode biofilm. While the gene copy number of archaea in ML-MFC anode was 4.66×10^7 copies μL^{-1} at the beginning of the operation period, it increased to 6.19×10^7 copies μL^{-1} (1.3 times more) in the anode biofilm at the end of the operation period. This result confirms that the anode compartment of the ML-MFC was suitable for the growth of archaea. However, the environmental operation conditions relatively limited the growth of archaea such as low operation temperature (21 ± 2 °C) of ML-MFC for archaeal microorganisms.

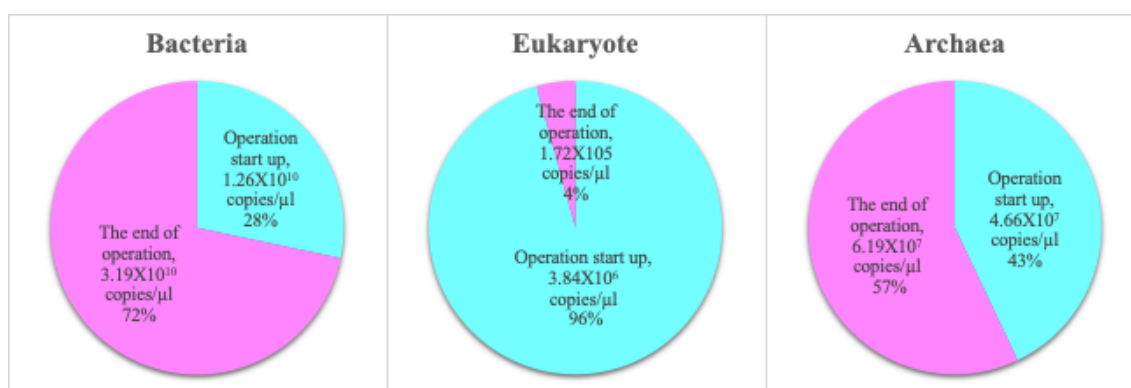


Fig 7. Quantification of bacteria, eukaryote, and archaea cells in the anode compartment at the beginning and end of the operation period by qPCR analysis

4. CONCLUSIONS

In this study, the grape marc was used as a substrate source for anode microorganism in ML-MFC for the first time, and the electricity generation capacity was investigated in detail. The maximum power density of ML-MFC was 274.9 mW m^{-2} , which is comparable to the literature studies. The CV results showed that the microbial colonization on anode biofilm did not occur at the beginning of the operation period, while the electroactive biofilm occurred during the operation period. The qPCR results proved that the number of bacteria on the anode surface increased approximately 2.5 times from the beginning to the end of the ML-MFC operation period. The microscopic observations of anode biofilm confirmed the presence of viable bacterial colonization on the anode electrode surface. As a result, the power generation performance of ML-MFC fueled with grape marc is relatively high compared to some literature studies, and this study has the potential to shed light on future MFC studies for the use of grape marc as a substrate source.

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