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Denitrification in Microbial Fuel Cells Using Granular Activated Carbon as an Effective Biocathode

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Abstract: Nitrate (NO_3^--N) and nitrites (NO_2^--N) are common pollutants in various water bodies causing serious threats not only to aquatic, but also to animals and human beings. In this study, we developed a strategy for efficiently reducing nitrates in microbial fuel cells (MFCs) powered by a granular activated carbon (GAC)-biocathode. GAC was developed by acclimatizing and enriching denitrifying bacteria under a redox potential (0.3 V) generated from MFCs. Thus, using the formed GAC-biocathode we continued to study their effect on denitrification with different cathode materials and circulation speeds in MFCs. The GAC-biocathode with its excellent capacitive property can actively reduce nitrate for over thirty days irrespective of the cathode material used. The stirring speed of GAC in the cathode showed a steady growth in potential generation from 0.25 V to 0.33 V. A rapid lag phase was observed when a new carbon cathode was used with enriched GAC. While a slow lag phase was seen when a stainless-steel cathode was replaced. These observations showed that effective storage and supply of electrons to the GAC plays a crucial role in the reduction process in MFCs. Electrochemical analysis of the GAC properties studied using electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV), and zeta potential showed distinct properties with different abiotic and biocathode conditions. We found that the enrichment of electrotrophic bacteria on GAC facilitates the direct electron transfer in the cathode chamber for reducing NO_3^--N in MFCs as observed by scanning electron microscopy.

Keywords: granular activated carbon; direct electron transfer; microbial fuel cells; biocathode; stored charge transfer; denitrification

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1. Introduction

Among the nitrogenous compounds, nitrate (NO_3^-) is the most widespread contaminant present in wastewater due to its high solubility in water and the thermodynamically difficulty to fix in the environment [1,2]. It is a highly mobile and stable anionic contaminant that is in the most oxidized state and exists both synthetically and naturally [3,4]. Due to its strong redox potential, it is considered a potent toxicant for cellular respiration in all living organisms [3,5]. The biogeochemical cycles taking place in sludge and similar anoxic environments demonstrate that NO_3^--N reduction is carried out by a group of denitrifying bacteria [6]. However, denitrification can be observed in an aerobic environment [7]. Electrotrophic denitrification, which differs from autotrophic and heterotrophic denitrification, is a peculiar phenomenon seen in bioelectrochemical systems (BES) [8].

Biological denitrification is a preferable route, wherein bacteria can reduce NO_3^- to N_2 with the combination of biological and electrochemical mechanisms in a BES [4,8,9]. Heterotrophic denitrifiers utilize organic substrates (electron donors) and reduce NO_3^- to N_2 under anoxic conditions, resulting in a high-denitrifying rate and treatment capacity in the treatment system [4,7] Nevertheless, nitrite (NO_2^-), the denitrification intermediate,

can accumulate in the process if the organic substrate does not fulfill the stoichiometric requirements [10]. Thus, autotrophic denitrification is an alternative process to heterotrophic denitrification, which is based on the activity of chemolithotrophic autotrophs which can accept electrons flowing through the cathode to reduce NO_3^- while fixing carbon dioxide (CO_2) [2,9,11]. In this process, autotrophic bacteria can derive their carbon source from inorganic compounds, such as bicarbonate, and can utilize hydrogen (H_2), reduced sulfur, iron, or manganese as their electron sources necessary for metabolism [11,12].

Nevertheless, low power generation is one of the main challenges for the application of microbial fuel cells (MFCs) in real-time. MFCs are a type of bioelectrochemical cells which uses biocatalysts, such as bacteria, for catalyzing the reaction. Ohmic losses, sluggish kinetics, and the development of a concentration gradient between the electrodes are the major reasons for the low performance of the MFCs [13,14]. Several attempts have been made to improve the performance of the MFCs by modifying the reactor configurations, using different electrode materials, applying transient operations, and adjusting the pH [4,12]. In recent years, the application of low-cost carbon-derived biomass, such as activated carbon powder (ACP), granular activated carbon (GAC), and activated carbon nanofibers (ACNs) has attracted a lot of attention due to their excellent electrochemical stability [9,15,16]. Among them, GAC is one of the most promising, readily available, and inexpensive materials, which can store electrons in the form of an electrical double layer (EDL), and a continuous current can be generated with intermittent contact between the GAC particles and the current collector [9,17].

It has been proven that GAC particles possess capacitive properties and thus, are used in MFCs to enhance power generation [9]. When GAC is used in an MFC, there is a possibility of charge storage by the formation of an EDL on the electrode–electrolyte interface [18,19]. However, there are very limited studies on the use of GAC in the cathode chamber for the denitrification process.

In this study we investigated the NO_3^- reduction in an MFC complemented using GAC in the cathode compartment while monitoring its biochemical activity during the process. GAC for electron storage and transfer for NO_3^- reduction is a novel study conducted by this group. We also studied the role of GAC in NO_3^- reduction in a two-chambered MFC under different conditions, such as different cathode conditions, different electrodes, and the effect of rotations per minute (RPM). Deep analysis of the GAC properties was also studied using electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV), and zeta potential. In addition, the bacterial growth on the GAC was investigated. To the best of our knowledge colonization of electroactive bacteria on GAC for denitrification and MFC studies has not reported in the literature.

2. Materials and Methods

2.1. MFC Configuration

An H-type two-chamber MFC was designed for this study. The two chamber of the MFC were constructed by joining two Scott Duran glass bottles together with a working volume of 100 mL. Both the anode and cathode chambers contained two side ports for sampling. The anode and cathode chambers were separated by a cation exchange membrane (CMI 7000, Membrane International, Ringwood, NJ, USA) with a surface area of 8.04 cm^2. Before use in the MFC, the membrane was pre-treated by immersion in a 5% sodium chloride (NaCl) solution for 12 h to activate the membrane pores. A carbon cloth (Fuel cells store, Bryan, Texas, USA) of 2.5 cm × 5 cm was used as the anode and cathode electrodes, respectively, unless otherwise stated. A copper wire (2 mm) was used to connect the anode and cathode electrodes using a conductive epoxy, Eccobond 56 C (Emerson and Cuming, Randolph, MA, USA) followed by a waterproof non-conductive epoxy (Devcon, Solon, OH, USA).

2.2. Inoculum and Operating Conditions

The MFC was operated at 30 °C. The anode chamber was inoculated with 10 mL of anaerobic sludge from the Chuncheon wastewater treatment plant (Republic of Korea). Both the anode and cathode medium contained (per liter) 4.33 g Na_2HPO_4, 3.04 g NaH_2PO_4, 0.31 g NH_4Cl, and 0.13 g KCl. 10 mM of sodium acetate was added in the anodic chamber as the electron donor. The MFC was operated with an aerating cathode and continued operating for two to four weeks to enrich the electroactive bacteria in the anode. After reproducible cycles of electric potential generation, the cathode media was changed to an anoxic media with the following composition (per liter) of 4.4 g KH_2PO_4, 3.4 g K_2HPO_4, 0.5 g NaCl, 0.2 g $MgSO_4.7H_2O$, 0.014 g $CaCl_2$, and 2 g $NaHCO_3$. The cathode chamber was inoculated with 10 mL anaerobic sludge from the Chuncheon wastewater treatment plant (Republic of Korea) as an inoculum source for denitrifying bacteria. Potassium nitrate (KNO_3) was added at a concentration of 100 mg/L NO_3^--N which served as the sole electron acceptor for the cathode reduction. The anodic chamber was operated in semi-continuous mode by feeding 10 mM sodium acetate using a peristaltic pump (Longer Precision Pump Co., Tucson, AZ, USA) to maintain the stable condition of the anode.

2.3. Enrichment of Bacteria on GAC

Approximately 200 g of GAC was filled in the 250 mL media bottle. GAC particles, in the size range of 2–5 mm, were used in the cathode chamber to enhance electron storage and transfer efficiency. Before use, GAC flakes were washed by soaking in distilled water overnight followed by drying at room temperature to remove contaminants. Thereafter, 100 mL of cathode medium was filled inside the bottle. Finally, 20 mL of anaerobic sludge was added to the bottle as an inoculum for the electroactive bacteria. The cathode medium consisted of 100 mg/L NO_3^--N. The system was operated in anaerobic conditions and batch mode. To check the activity of the GAC, a three-electrode system was set up in the bottle: plain carbon cloth as the working electrode, Pt. wire as the counter electrode, and Ag/AgCl as the reference electrode. NO_3^- was fed into the system at 4-day intervals. Once the GAC was enriched with electroactive bacteria, it is used in the MFC.

2.4. MFC Operation with Enriched GAC

The cathode remained in batch operation during all experiments. The cathode chamber was supplied with a 100 mg/L nitrate-N whenever there was a decrease in potential, corresponding to a depletion of NO_3^--N in the media. 2 g of enriched GAC was added in the cathode chamber and operated at different RPM using a magnetic stirrer (2.5 cm). A fixed external resistance of 1000 Ω was plunged between the anode and cathode electrodes. The potential generated was measured every 10 min using a digital precision data acquisition system (Model 2700 Keithley Instruments, Inc., Beaverton, OR, USA) integrated into a personal computer.

2.5. Electrochemical Analysis and Calculation

The electrochemical properties of the cathode were performed by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) using a Ivium potentiostat (IviumStat, AJ Eindhoven, The Netherlands.) with a three- and four-electrode system. The cathode was used as the working electrode (WE), Ag/AgCl (+197 mV vs. SHE) served as the reference electrode (RE) and the anode chamber was used as the counter electrode (CE). The CV was performed in a fully operational MFC in the voltage range of −0.9 V to +0.3 V at the scan rate of 10 mV. sec^{-1}. Similarly, the EIS was performed in the frequency range of 10 kHz–0.1 Hz. Before polarization analysis, the MFC was left in open circuit mode for one to two hours to stabilize the voltage after which current was drawn from the system in a step-by-step manner from 0.01 mA to 1 mA and the corresponding voltage was measured at each step for 300 to 600 s. The current and power produced from the system were calculated using Ohm's law. For example, power (P) was calculated as $P = IV$ ($I = V/R$), where I (A) is the current, V (V) is the voltage, and R (Ω) is the external

load connected to complete the circuit. The power density was obtained by normalizing the power produced to the surface area of the electrode.

2.6. Scanning Electron Microscope

The morphology of the biofilms formed on the GAC was examined by a scanning electron microscope (SEM; S-4800, Hitachi, Japan). The samples for the SEM were processed for imaging according to the method described elsewhere [20]. In detail, the GAC from the cathode chamber was removed and immediately fixed with 2.5% glutaraldehyde overnight at 4 °C. After being dried, the samples were serially dehydrated in a series of ethanol solutions (i.e., 30, 50, 70, 80, 90, 95, and 100%), and then dried at a critical point for 12 h. All the above-mentioned steps were performed under sterile conditions to avoid contamination and the loss of desired outcomes.

2.7. Nitrate Analysis

NO_3^--N analysis was carried out in an Ion Chromatography Advanced Compact IC 813 with a conductivity detector (Metrohm, AJ Eindhoven, The Netherlands). The column used was a metrosep-Asup 5, eluent system composed of 1 mM $NaHCO_3$ and 3.2 mM Na_2CO_3, and 50 mM sulfuric acid as a suppressor. Samples for analysis were collected freshly every 12 and 24 h from the cathode, filtered, and performed with an optimized method with standards with a flowrate of 0.7 mL min^{-1} in an autosampler.

3. Results

3.1. Denitrifying Bacteria on GAC

In this work, we studied the electron transfer system in which GAC was used in the cathode chamber to store electrons. Enrichment of desirable bacteria on the GAC employed with two different strategies showed excellent results. The enrichment with electroactive bacteria on the GAC, as depicted in Figure 1a, showed similar results as those enriched in MFCs. The desired electrochemical potential and media composition resulted in the main role of acclimatizing and enriching the desired bacteria in the reactors which was noted by the potential measured from the system. To confirm this, when the thus-formed GAC-biocathode was inoculated and tested for NO_3^--N reduction, similar results were seen in the MFC.

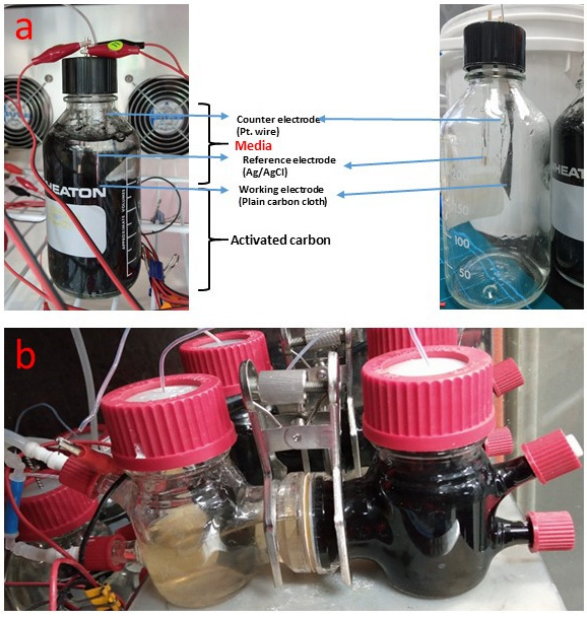

Figure 1. Enrichment of denitrifying bacteria on GAC by applying different strategies. (**a**) Bottle enrichment with a three-electrode system incubated at 30 °C and (**b**) the MFC with NO_3^--N (100 mg/L NO_3^--N)-composed media in a cathode operated at 30 °C.

3.2. NO_3^--N Removal in the MFC Using the GAC-Biocathode

The MFC was operated for at least three cycles with multiple replicates to reproduce the attainable voltage output with an air cathode. Once the MFC started to reproduce a voltage output of 0.30 V, the operating condition of the cathode chamber was then changed to NO_3^- media (per liter) containing 4.4 g KH_2PO_4, 3.4 g K_2HPO_4, 0.5 g NaCl, 0.2 g MgSO4.7H_2O, 0.014 g $CaCl_2$, and 2 g $NaHCO_3$ and inoculated the GAC-biocathode. Then, the cathode was operated in biocathode mode using a mixed culture (anaerobic sludge) as the inoculum. KNO_3 was added to a concentration of 100 mg/L NO_3^--N as the sole electron donor.

Figure 2 shows the removal of NO_3^--N in the MFC under different cathode conditions. The GAC-biocathode showed a significant amount of NO_3^--N reduction at the same time compared to GAC and without the GAC cathode systems. Faster denitrification with the GAC-biocathode was due to the bacteria enriched on the GAC surface which resulted in a maximum reduction of NO_3^--N on the first day (close to 40%), thereafter the rate dropped slightly due to the availability of stored charge in the GAC. GAC without the enriched bacteria showed the next best results with an approximately 50% NO_3^--N reduction, observed over three days. However, the effect of GAC was noticeably higher irrespective of enrichment compared to the MFC without GAC. Throughout the four-day run experiment in the MFC, more than 90% denitrification was observed with the GAC-biocathode. The effect of denitrification was also observed with the potential generation in the MFC, where the electric potential ceased concerning NO_3^--N availability in the cathode chamber.

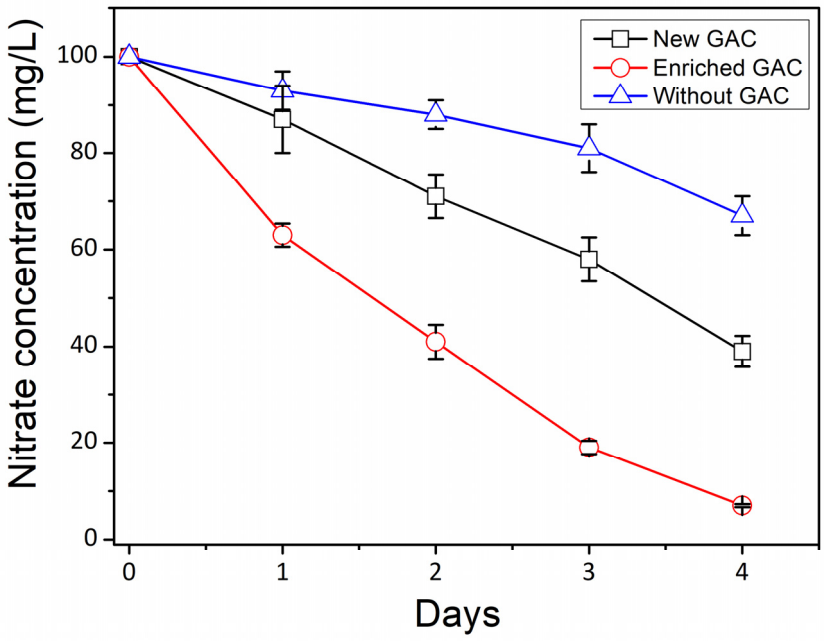

Figure 2. Denitrification observed in the MFC under different cathode conditions. Noticeably, the GAC-biocathode outperformed all the cathode systems tested in this study with the efficiency of reducing over 90% of NO_3^--N in four days.

3.3. Effect of GAC (Enriched and New GAC) in the Cathode Chamber

Figure 3 shows the effect of GAC on power generation. Once the voltage output was acclimatized in the biocathode mode, the effect of GAC on charge storage was examined in the cathode chamber at different operatic conditions. Using new media and the new cathode electrode, the MFC took approximately 14 h to regain its maximum voltage output. A similar result was observed when fresh media with the same composition was replenished in the subsequent cycle. The immediate potential generation in the new cathode electrode could be due to the charge stored in the GAC. It can be concluded that the enriched GAC with electroactive bacteria is efficient and reproducible in the biocathode.

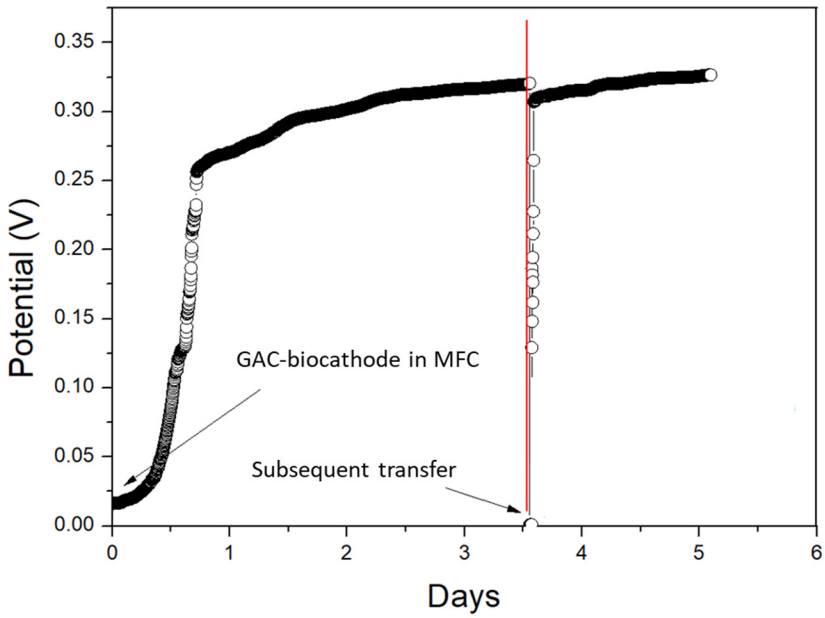

Figure 3. The potential generation from the MFC with the GAC-biocathode. The enriched GAC showed instant potential generation in the MFC with no lag phase.

The denitrification MFC was run with 2 g of enriched GAC and enriched cathode electrode in the new cathode media to investigate the electron transfer phenomenon of GAC in the cathode chamber. Even though enriched GAC and enriched cathode electrodes were employed, a modest increase in voltage was seen. To reach its maximum voltage output again, it took roughly 55 h. The adaption of or the use of novel cathodic media may be the primary cause of this constant potential. The enriched cathode was changed for a new cathode electrode after the MFC had reached its maximum voltage output. In this case, the voltage output quickly increased. The voltage output started at 0.16 V and increased to its maximum voltage output in 2 h. When the stainless-steel cathode was employed, and the cathode chamber was refilled with 100 mg/L NO_3^--N in subsequent transfer, the voltage output also increased quickly even with the stainless steel cathode.

When the voltage output reached approximately 0.26 V, the effect of different RPMs (0, 50, 100, 150, and 200) was tested in the cathode chamber. The cathode electrode was replaced with an enriched GAC and a stainless steel electrode. With the stainless steel, the power did not increase rapidly and took approximately 2 days to generate 0.25 V. Figure 4 shows the potential generation in the denitrification MFC operated under different cathode conditions.

Figure 5 shows the potential generation in the denitrification MFC without enriched GAC. To further investigate the electrochemical properties of GAC in the MFC, enriched carbon cloth was used as a replacement for the GAC-biocathode and operated under similar conditions. With the enriched biocathode, a similar efficiency was observed as the GAC-biocathode. Upon the addition of GAC particles in the media, the potential was found to decrease steadily even in the presence of NO_3^--N. This could be due to the phenomenon that electrons were utilized for charging GAC rather than reducing the NO_3^--N and potential generation. When operated at different RPMs (0, 50, 100, 150 and 200), there was no noticeable effect on potential or denitrification as observed with the enriched GAC-biocathode. The power gradually decreased after the introduction of a new cathode electrode in the cathodic chamber. The voltage output decreased from 0.32 V to 0.22 V within 10 h and continued to drop. The potential effect was further decreased when the cathode was changed to stainless steel, the maximum voltage output was only 0.07 V. E_{cell} is the total cell potential, which is cumulative of the anode and cathode. The similar E_{cat} of 0.12 V suggests that the reduction reaction in the biocathode (denitrification)

was taking place at a similar rate, while the oxidation of organics in the anode resulted in E_{an}. The same E_{cat} dropped down when switched to a new cathode and stainless steel, indicating that the enriched electro-trophic denitrifying bacteria were distracted during the transfer process. There is a strict need for this biocathode for acclimatizing, enriching and culturing. This observation strongly recommends that the GAC-biocathode enriched with electrotrophic, denitrifying bacteria can serve as an excellent material for reduction reactions in MFCs. The capability of GAC to store and release charge depending on the potential makes it an excellent material for the source and sink of electrons for electrochemical and bio-electrochemical studies.

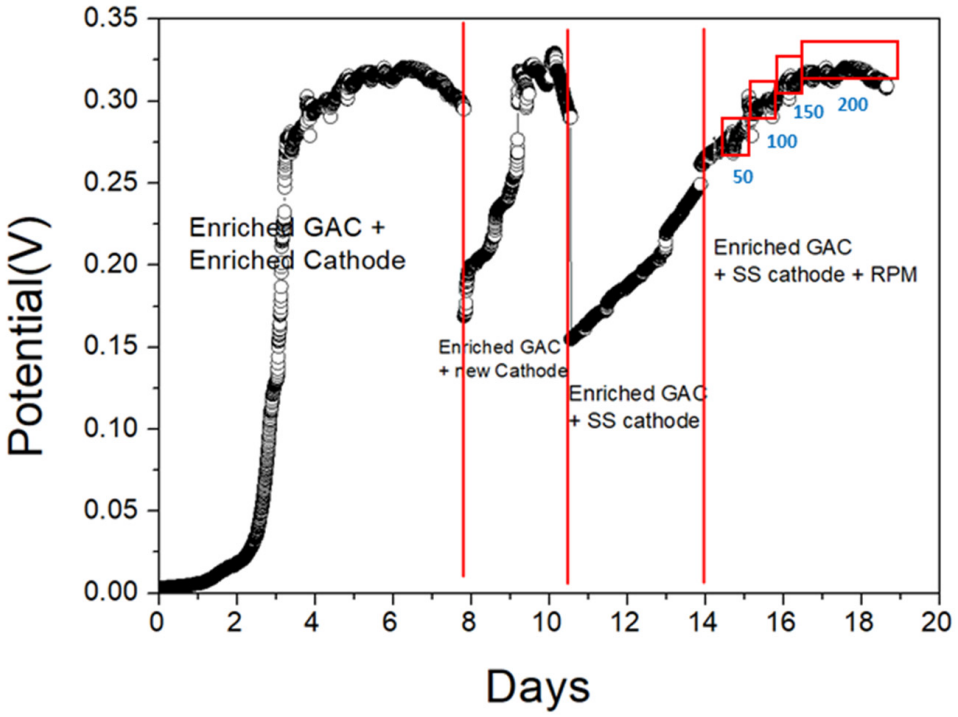

Figure 4. Denitrification MFC operated under different cathode conditions with an enriched GAC-biocathode.

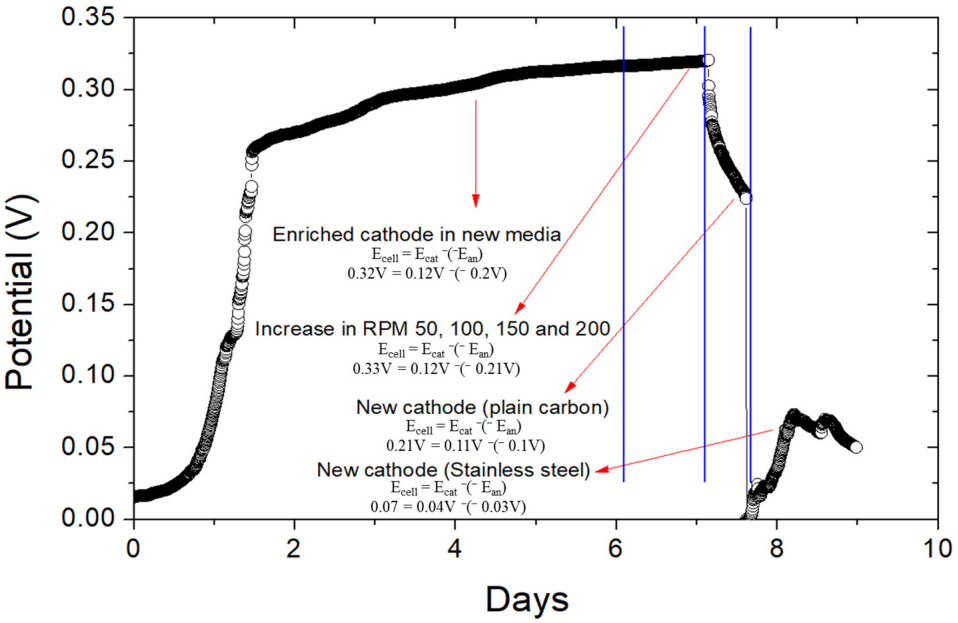

Figure 5. Potential observed in the MFC with fresh GAC particles and different cathode systems.

3.4. Electrochemical Properties of the Biocathode

The electrochemical properties of the cathode were investigated by CV and EIS. The EIS test performed in the presence and absence of GAC-biocathode enrichment showed that the internal resistance and charge transfer of the MFC decreased with the GAC-biocathode compared to one without (Figure 6). These results strongly suggest that the electrochemical performance of the MFC in power generation can be improved with the application of a biocathode and can minimize the losses associated with the system. The use of GAC helps to decrease the internal resistance of the MFC thereby improving charge/electrons recovery.

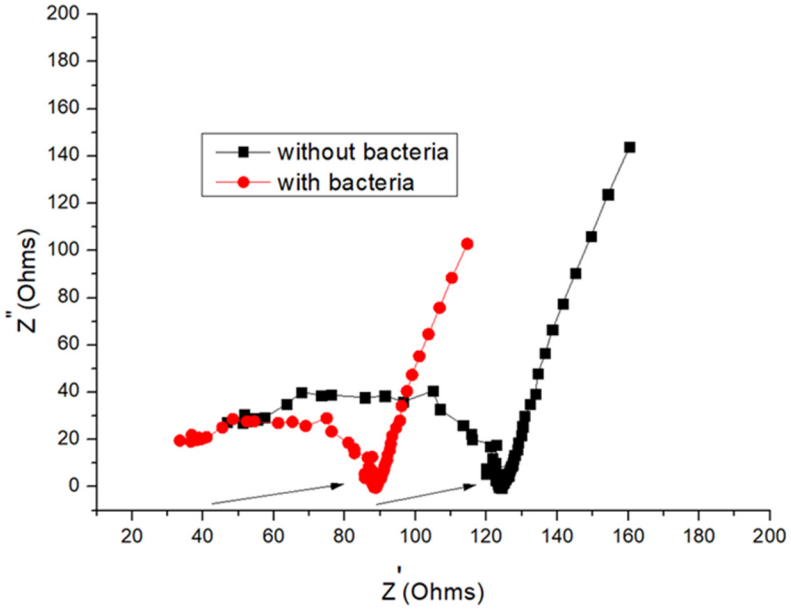

Figure 6. Shows the internal resistance of the MFC with and without bacteria.

Figure 7 shows the CV graphs plotted at different concentrations of NO_3^--N and NO_2^--N. The CV analysis was performed to assess the redox activity of the biocathode in the MFC for reduction reactions. NO_3^--N and NO_2^--N were used as reducing species for this purpose due to their similar properties to become reduced by a similar bacteria during denitrification reactions when occurring in wastewater, anaerobic sludge, and other environmental conditions. A small hump during the forward scan was observed with NO_3^--N indicating there was an oxidation reaction at high concentrations (100 mg/L NO_3^--N concentration) which was inconsistent with low concentrations under the same conditions. However, the reduction peaks showed consistency in all reverse scans in CV indicating the effectiveness of the GAC-biocathode in reduction reactions. Similar observations were also noted when operated with NO_2^--N. The reduction peaks observed with NO_2^--N were more consistent with all tested concentrations. There was no observance of the peak current at 0 mg/L of NO_2^--N and NO_3^--N in the MFC.

3.5. SEM Images of Bacteria Grown on Enriched GAC on the Biocathode

The micrographs from scanning electron microscopy are shown in Figure 8. A group of bacteria appearing to be similar in morphology (slender rods with 0.5 µm) were found adhered to the surface of the GAC. These bacteria were seen to have adhered to the surface of the GAC and had direct communication with it rather than forming a biofilm, which is a commonly observed phenomenon in electroactive bacteria. In general, GAC has a large porosity and adsorptive capacity that can spread clumped microbes adsorbed onto the electrode surface and increase the surface area of the electrode [21]. Consequently, the higher specific area enabled the more effective collection of electrochemically active microorganisms and the performance of GAC increased accordingly. The GAC acted as a porous material with a variety of internal large holes and mesoporous structures. The

rough surface of the GAC facilitated the attachment and growth of these bacteria and possibly directed electron sharing. The attachment and enrichment of bacteria as well as the colonization of the surface had a direct impact on the voltage output of the MFC.

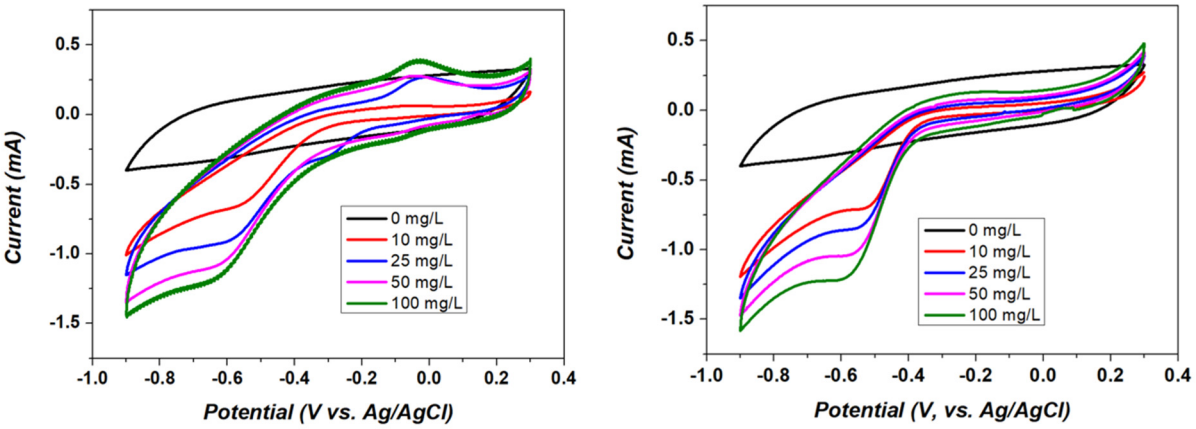

Figure 7. CV tests performed for redox reactions at different concentrations of NO_2^--N and NO_3^--N.

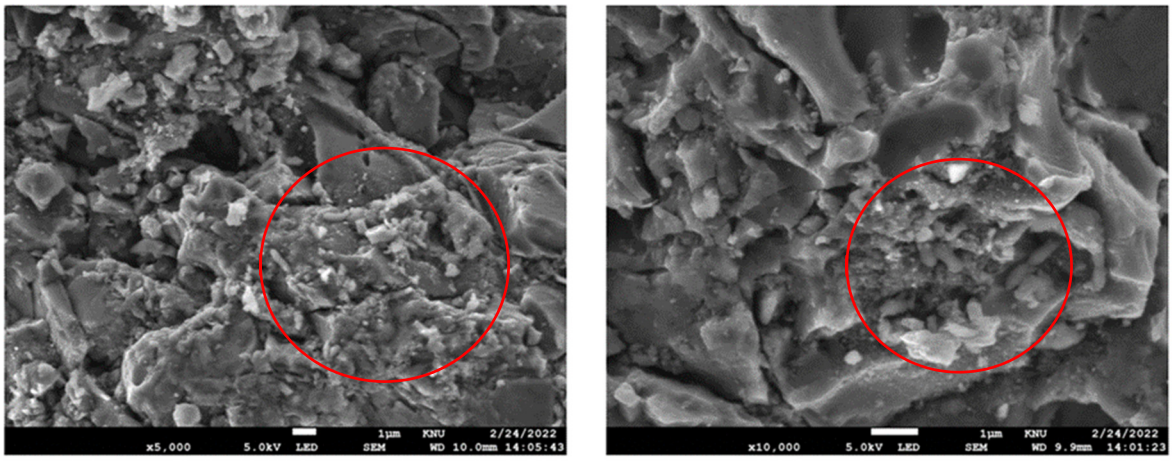

Figure 8. SEM images of bacteria grown on enriched GAC.

4. Discussion

The potential generation test, EIS, and CV characterizations demonstrate that enriched GAC with electroactive bacteria is efficient for charge storage and transfers to the electrodes. The SEM images also revealed that GAC entrapped bacteria on its surface. The presence of enriched GAC in the biocathode helped to transfer electrons to the electrode thereby assisting in generating the reproducible potential even when a new cathode electrode was used. However, this scenario was different when the biocathode was operated without enriched GAC.

In recent years, materials with electrochemical capacitive properties are used in MFCs to improve the overall performance of the system [15,22]. GAC is increasingly used as a capacitor in MFCs because it has a high specific surface which is the surface area created by the porous structure of carbon granules, and an EDL can form on this porous surface when the electrolyte is present [17,23,24]. When GAC is used in MFCs, two phenomena can occur: (i) the electroactive biofilm releases electrons during the oxidation of organics, and (ii) these electrons are stored at the pore surface of the carbon, while cations are required to maintain the charge balance in the EDL [17,24]. Using this concept, GAC has previously been used in the anode of MFCs to form a capacitive bioanode enhancing the performance of MFCs [15,22,24,25]. Liu et al. 2020 reported that integration of GAC in the anode chamber provides a larger surface area for the growth of exoelectrogenic bacteria

at the anode, improving the power output as well as decreasing the internal resistance of the system.

GAC has previously been integrated into the anode to form a capacitive bioanode to increase the power density. Thus far, GAC has not been integrated with the biocathode to form a capacitive biocathode. We conducted this novel study by integrating GAC into the biocathode to form a capacitive biocathode and evaluated the overall performance of the denitrification MFC. The study revealed that the integration of GAC into the biocathode enhanced the performance of the system. The enhanced performance of the MFC could be the result of the highly conductive properties and high specific surface area of GAC. When the amount of GAC increased in the cathode chamber, the denitrification rate also increased. The high-denitrification efficiency is the result of the high specific surface area that provides sufficient surface area for the attachment of cells.

5. Conclusions

The study extensively investigated the power generation and NO_3^--N removal in the MFC with and without GAC. The electrochemical and biochemical characteristics of GAC in the cathode chamber were comprehensively explored. Moreover, the performance of enriched and non-enriched GAC with electroactive bacteria in the cathode chamber were compared. Four major conclusions were drawn from this study. First, the enrichment of GAC with electroactive bacteria is essential for enhancing the overall performance of the MFC. Second, enriched GAC is efficiently aids in generating a reproducible potential. Third, the denitrification rate increased when the amount of GAC in the biocathode increased. Finally, the MFC with enriched GAC resulted in a lower internal resistance of the overall system. As evidenced by this study, the use of GAC may result in lower cathode overpotentials compared to non-conductive granules. Recently, many studies have investigated the effect of GAC on methanogenesis. In future, we will investigate the effect of GAC on hydrogen generation in microbial electrolysis cells (MEC). As GAC allows for direct interspecies electron transfer in anaerobic granules it may consequently improve the hydrogen production rate and overall performance of the anaerobic system. In addition, we will study the absorption of heavy metals using GAC in the BES.

Author Contributions: Conceptualization, B.S.T., S.-Y.K. and S.-E.O.; methodology, B.S.T. and A.G.; software, B.S.T. and S.-Y.K.; formal analysis, B.S.T., S.-Y.K. and E.A.; investigation, B.S.T., A.G., S.-Y.K. and S.-E.O.; resources, S.-E.O.; data curation, B.S.T., S.-Y.K. and A.G.; writing—original draft preparation, A.G. and B.S.T.; writing—review and editing, B.S.T., A.G., S.-E.O. and U.A.T.; visualization, B.S.T.; supervision, B.S.T. and S.-E.O.; project administration, B.S.T. and S.-E.O.; funding acquisition, S.-E.O. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data will be provided on request to Sang-Eun Oh, Kangwon National University.

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Conflicts of Interest: The authors declare no conflict of interest.

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