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Full Paper

Comparative evaluation of fibrous artificial carbons and bamboo charcoal in terms of recovery of current from sewage wastewater

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Summary

In this study, two fibrous carbon anodes (namely, pleated non-woven graphite (PNWG) and carbon brush (CB) made from artificial carbon) and bamboo charcoal (BC) were evaluated for current recovery from sewage wastewater. When these anodes were polarized at 0.2 V vs. Ag/AgCl in sewage wastewater, CB produced a maximum current of 2.9 A/m². This exceeded that produced by PNWG (1.5 A/m²) and BC (1.4 A/m²). The accumulative charge recovery achieved with CB was superior to those achieved with the other two (1.6- and 2.2-fold higher than that with PNWG and BC, respectively). During the cyclic voltammetry analysis, CB demonstrated the highest catalytic current with maximum potential in the range of -0.6 to 0.4 V vs. Ag/AgCl and the smallest anode resistance (0.20 Ω m²). Direct cell counting revealed that the fibrous anodes (CB and PNWG) attached most of the cells in the anodes (80%), whereas BC did not. In contrast, the proportion of *Geobacter* species, a representative electrogenic microorganism in the total bacteria, was observed to be similar among the three anodes (4.4–5.8%). The tubular microbial fuel cell (ϕ 5.0 cm) equipped with an air-chamber core wrapped with an anion exchange membrane (AEM) and the CB delivered a current of 1.8 A/m². This is higher than those reported in the existing literature for the same microbial fuel cell (MFC) configuration. This indicates that the alteration of the anode from planar to brush can contribute toward improving the current recovery through the air-cathode-AEM-MFC. The BC needs improvement to have more specific surface area, whereas it showed superiority in cost efficiency considering material and processing.

Keywords: microbial fuel cell (MFC), pleated non-woven graphite, carbon brush, bamboo charcoal, anion exchange membrane, *Geobacter*

1. Introduction

Greenhouse gas emissions from sewers account for approximately 0.5% of the total emissions in Japan (MILT, 2016). Meanwhile, the electricity consumption for sewage wastewater treatment accounts for 1%–3% of domestic electricity consumption in developed countries (Maktabifard et al., 2018). In contrast, sewage potentially has significantly more biomass energy (Shizas and Bagley, 2004). Thus, resource conservation and recovery during sewage treatment are essential for sustainable resource cycling. Although sewage sludge has been successfully transformed into solid fuel (Franz, 2008), biogas (Fountoulakis et al., 2010), and fertilizer (Franz, 2008), the practical application of sewage water as a biomass resource has not been advanced (McCarty et al., 2011). To achieve biogas production from sewage water, organic matter must first be concentrated from sewage water. This includes the concentrations of the membrane bioreactor (Martinez-Sosa et al., 2011), aquatic weeds (Kaur et al., 2018), and algae (Kaur et al., 2018). Microbial fuel cells (MFCs) constitute an alternative technology that directly recovers energy from sewage water (Logan et al., 2006) or bioelectrochemical systems (BESs) (Logan, 2010). Although MFCs have progressed rapidly (Munoz-Cupa et al., 2021), their configuration has not been optimized for practical applications (AlSayed et al., 2020). This is regardless of the presence of various configuration types (Du et al., 2007).

Four types of MFCs with a capacity of over 100 L (which can be used for treating municipal wastewater) have been reported: biocathode MFC (He et al., 2019), biocathode MFC with a cation exchange membrane (biocathode–CEM MFC; Liang et al., 2018), air-cathode MFC with a nonionic exchange separator (air–NIE MFC; Feng et al., 2014; Goto and Yoshida, 2019; Hiegemann et al., 2019; Rossi et al., 2019b,c), and air-cathode MFC with a CEM (air–CEM MFC) (Ge et al., 2015; Ge and He, 2016). In all the existing

MFC-based methods of municipal wastewater treatment, the electricity production and hydrocarbon degradation are determined by the anode-reaction rate (He et al., 2019) because of the low concentration of hydrocarbons. Thus, the MFC anode has a significant impact on the overall performance.

To date, various types of anodes have been proposed (Yaqoob et al., 2020). In an anodic chamber filled with wastewater, microbes oxidize waste organics and negatively charge the anode through extracellular electron transfer (Lovley, 2012). Thus, the surface chemistry of the anode affects the microbial composition (Kaur et al., 2020), and the pore size, or 3D structure, regulates the specific area that supports microbial cells, substrate availability, and biofilm structure (Chong et al., 2019). Carbon-based anodes are becoming increasingly popular and are commercially available in various forms, such as felt, brush, cloth, and granules (Wei et al., 2011). Among these, fibrous carbons help achieve high specific surface areas and are representatively manufactured by the carbonization of polyacrylonitrile (a synthetic organic polymer resin). Notwithstanding its utility, there are problems of cost and carbon emissions of the manufacturing process (Chen et al., 2012b). The latter becomes particularly severe for MFC-based treatment of sewage wastewater, which requires large amounts of carbon anodes to treat large amounts of sewage wastewater.

Recently, biochar has received considerable attention owing to its carbon neutrality, resource cycling, and cost benefits (Chakraborty et al., 2020; Yaqoob et al., 2020). In addition, biochar has a natural 3D structure, the pores contribute to a high specific surface area, and its structure boosts electricity production in MFC (Chen et al., 2012a). Certain biochar contains C=O and C–N bonds on the surface, which enhances bacterial adhesion and electron transfer. However, the surface chemistry is attributed to the base material as well as carbonization conditions, i.e., heating temperature and atmosphere. However, the

applicability of biochar as an MFC anode has only been evaluated in a few cases.

In this study, three carbon anodes (namely, pleated non-woven graphite (PNWG), carbon brush (CB), and bamboo charcoal (BC)) were evaluated for current recovery from sewage wastewater. The former two represent anodes manufactured by carbonization of polyacrylonitrile that exhibit fibrous structures with high specific surface areas. The latter was selected because bamboo is an evergreen and fast-growing plant that is distributed in the Asia-Pacific region, and its tubular shape may enhance substrate accessibility. First, the three prepared carbon anodes were polarized at a constant voltage in sewage wastewater using a potentiostat to eliminate the effects of other constituents of the MFC, such as the separator and cathode. The three anodes were acclimated with sewage sludge and then analyzed using methods such as cyclic voltammetry (CV), direct counting of cell density, and 16SrRNA-gene pyrosequencing. Furthermore, the electricity production was evaluated in an MFC that was immersed in wastewater.

2. Materials and methods

Anode materials

In this study, three types of materials (PNWG, BC, and CB (Millrose, USA) (Fig. 1A)) were fixed on plastic panels (3.5×3 cm) and evaluated in sewage wastewater. The PNWG were pleated NWGs (TOYOBO) of 3.5×3 cm that were produced using a sewing machine. Four pieces were fixed to a panel. The CB was used in the form of two pieces ($\Phi 2.5 \times 3$ cm) constituting a set. For BC, 15 pieces of BC $\Phi 0.9\text{--}2.0 \times 1.0$ cm were fixed in a titanium cage. These were carbonized at 900°C by the company MET (Gamagori, Japan). The surface resistance of BC was 3–5 Ω/cm .

Electrochemical cultivation

As shown in Supplementary Fig. S1, the three anodes fixed on plastic panels were suspended as the working electrode in a 900 mL glass bottle filled with sewage wastewater. A platinum wire and an Ag/AgCl electrode (BAS Co., Ltd., Tokyo) were used as the counter and reference electrodes, respectively. To inoculate the microbes in the anode, the anodes were immersed in an activated sludge suspension and stirred for 30 min. The activated sludge was obtained from the aeration tank of a sewage treatment plant in Nagoya City. Subsequently, the anodes were polarized at +0.2 V vs. Ag/AgCl. The current was recorded by a data logger as described previously (Goto and Yoshida, 2017). The value of applied potential was conventionally used as the working potential and is convenient to compare with other anodes reported previously. This is notwithstanding that the anode potential of the MFC for treating sewage wastewater was below -0.1 V vs. Ag/AgCl. Thus, the anode potential (0.4 V) can permit the growth of electrogenic bacteria on the anode, although a lower potential could reflect the adaptation by more microbial communities to the anode in the MFC for treating sewage wastewater. Every 2-7 days, the wastewater was replaced with fresh wastewater kept at 4°C in a refrigerator. The culture was performed in three bottles for PWNG and BC, and two bottles for CB. The Ag/AgCl electrodes had a potential in the range of +0.199 to +0.209 V vs. SHE (Standard Hydrogen Electrode) in a 3 M NaCl solution.

The three anodes in the electrochemical cultures were subjected to CV using an electrochemical analyzer SP-150SH-Y (BioLogic SAS, France). The CV was addressed at a scan rate of 0.2 mV/s in the range of -0.6 to 0.4 V vs. Ag/AgCl.

Direct cell counting and microbial community structure analysis

The three anodes and liquids in the vessels were evaluated based on cell density by direct microscopic counting. This is as proposed in previous studies (Goto and Yoshida, 2017).

PNWG and BC samples acclimated for 115 days and CB samples acclimated for 45 days were analyzed. DNA was extracted from these three anodes of identical age as described by Ismaeil et al. (2018). Approximately 150 bps of the partial 16S rRNA gene was amplified using the 515F and 806R bacterial and archaeal consensus primers. These were subsequently sequenced using the Illumina Miseq platform as described previously (Ismaeil et al., 2017). An operational taxonomic unit (OTU) was defined as a phylogenetic group with $\geq 97\%$ sequence similarity. Its proportion was determined from the total reads. The analysis resulted in 34622, 34454, and 31244 reads for PNWG, BC, and CB, respectively.

Evaluation of current recovery in microbial fuel cell

The CB anodes acclimated in the electrochemical culture were placed in a tubular air-core MFC equipped with a carbon cathode and an anion exchange membrane (Sugioka et al., 2019) (Yamane et al., 2021). The MFC used in this study was smaller than the $\phi 5.0 \times 7$ cm set, with four CB pieces (Fig. 5A). The MFC had a styrene-foam on top and floated in a cylindrical reactor of polyvinyl chloride (Fig. 5B). The reactor was continuously supplied with sewage wastewater by a tubing pump at 0.3 hydraulic retention time (HRT) h. The MFC was polarized at 180 Ω , and the voltage was recorded. Linear sweep voltammetry of the MFC was performed using an electrochemical analyzer SP-150SH-Y (BioLogic SAS, France) at a scan rate of 1.0 mV/s. To record the cell current, the voltage was set from an open-circuit voltage of 0.46 to 0 V, and the anode and cathode currents were recorded in the range from -0.54 to 0 and -0.010 to -0.50 vs. Ag/AgCl, respectively.

3. Results

Electrochemical cultivation using three carbon anodes

The current densities produced during electrochemical cultivation with the three anodes are shown in Fig. 1B. To evaluate the association between the anode material and microbes,

the current was normalized by the projected area of anode for two reasons; the anode specific surface area (ASSA) was not measured in this study, and the projected area of anode equal to that of the ion exchange membrane (IEM), which accounted for a material cost of an air-cathode MFC equipped with IEM (Ge and He, 2016). The PNWG and BC were incubated for 100 days, whereas the CB was incubated for 30 days. This was owing to a delay in obtaining the material. In all the cultures, the current attained a peak at 6 h after the replacement and then decreased. This is notwithstanding immediate recovery by wastewater replacement. The wave-like trend of the current reflected the organic matter availability and was observed repeatedly in electrochemical cultures maintained in semi-batch mode (Goto et al., 2015; Yoshida et al., 2016a). The PNWG and BC anodes were set at room temperature (18°C) for the first month of cultivation. A maximum peak current density of 1.2 A/m^2 was observed for BC and PNWG. When the cultures were placed in an incubator maintained at 28°C, the peak current densities of BC and PNWG increased.

The CB maintained at 28°C demonstrated a peak current of $2.6 \pm 0.30 \text{ A/m}^2$. The values were 3.3- and 3.0-fold higher than those of PNWG and BC, respectively (Fig. 2A). The PNWG and BC showed similar peak current densities, 0.88 ± 0.34 and $0.79 \pm 0.31 \text{ A/m}^2$, respectively, and those values were equivalent to that observed with the graphite felt (GF) presented in a previous study (0.8 A/m^2) (Fujii et al., 2021). Addition to the temporal peak current densities, the cumulative charge for 40 h in each semi-batch is compared in Fig. 2B. The cumulative charge densities of PNWG, BC, and CB were 0.53 ± 0.20 , 0.64 ± 0.34 , and $1.8 \pm 0.15 \times 10^5 \text{ C/m}^2$, respectively. It is evident that only the value for CB was higher than that for the GF ($0.74 \pm 0.0025 \times 10^5 \text{ C/m}^2$) in the previous study.

CV measurements

The cyclic voltammograms acquired in the cultures using the three anodes are shown in

Fig. 3. In the CB culture, the current was produced from -0.48 V vs. Ag/AgCl. It increased linearly with increasing potential and attained the maximum value of 4.5 A/m² at 0.4 V vs. Ag/AgCl. The currents for BC and PNWG were less than that for CB in the range of -0.6 to 0.4 vs Ag/AgCl. These showed maximum currents of 2.3 A/m² and 1.4 A/m², respectively. The CB resistance with the linear increase in current was calculated to be 0.20 Ωm². This was less than those of BC (0.40 Ωm²) and PNWG (0.58 Ωm²).

Biomass distributions in electrochemical cultures

Direct counting of microbes in the anode and liquid phase in these three cultures revealed differences in biomass distribution among these (Fig. 4A). The total biomass in the vessels and the planktonic cells in the liquid were of equal magnitude (Fig. 4B). In contrast, the anode biomass displayed a large difference among the anodes. The CB and PNWG cultures had 79% and 82%, respectively, of the total biomass on the anodes, whereas the BC anode had 1.7%. These results indicate that the fibrous structure of CB and PNWB captured more biomass than BC (which has a smooth surface).

Comparison of microbial community structure on anode biofilm

The composition of the 16S rRNA gene amplicon targeting prokaryotes on the anode and OTUs with proportion > 1% is presented in Supplementary Fig. S2. The three anode-biofilms displayed highly similar microbial compositions. This indicates that genomic drift, wastewater, and anode potential (rather than the anode material) determined the microbial composition. In the taxonomic hierarchy of phylum, *Proteobacteria* was the most abundant in all the three cultures (41%–44%). This is followed by *Bacteroidetes* (21%–23%) and *Firmicutes* (4.0%). Focusing on the known electrogenic genera, OTUs affiliated with the genus *Geobacter* were present in the

three anodes, in the range of 4.4–5.8%. Meanwhile, other phylotypes from known electrogenic bacteria were not detected. Among the *Geobacter* species, most OTUs were closely related to *G. psychrophilus* and accounted for 1.9–2.6% of the three anodes. *G. psychrophilus* was isolated as an iron-reducing bacterium from underground soil (Nevin et al., 2005). It is frequently detected in MFCs. These results indicate that bacteria corresponding to the phylotype were involved in the current production on the anodes. The OTU had a similarity of 97.6% with *G. psychrophilus*. This indicates that the corresponding bacteria were possibly a novel species of the genus *Geobacter*.

MFC reactor

The CB with the highest current was evaluated further in terms of current and power production in an air-cathode AEM-MFC (Sugioka et al., 2019). The CB in the MFC increased the current from 0.2 to 1.0 A/m² within 20 days of running with a continuous supply of wastewater (Fig.5C). The current decreased owing to the depletion of influent and then recovered by supplying wastewater to attain 1.8 A/m². After another cycle of depletion and supplementation of wastewater, the current became stable at 1.0 A/m². The differences in peak current were affected by the anode as well as by the availability of organic matter and the ionic strength in wastewater, which varies with rainwater dilution and time throughout the day (Sugioka et al., 2019). Although the differences in current depended on wastewater, these values were apparently higher than those of the MFCs with an identical configuration. This is except for the one equipped with the NWG-anode, which showed a maximum current of 0.6 A/m² throughout the 230 days of operation (Sugioka et al., 2019) with a continuous supply of the same influent. The external resistances used in the two MFCs were different: 180 Ω for this study and 3 Ω for a previous study (Sugioka et al. 2019). This hinders comparison. However, a lower resistance generally increases the current in an MFC, and the MFC equipped with CB polarized via 180 Ω showed a

higher current density than the MFC using NWG in the previous study. This indicates that the replacement of the NWG with CB (as the anode) contributed toward improving the current recovery in the air-cathode AEM-MFC.

Discussion

This study evaluated the current recovery of three anodes: PNWG, CB, and BC. CB showed the best current recovery among the three carbon anodes. This agrees with previous reports that demonstrated the superiority of CB over carbon paper (Logan et al., 2007), carbon cloth (Nam et al., 2017), and carbon mesh (Wang et al., 2009). BC yielded a current that was approximately half of that by CB and was comparable to that for PNWG in this study. BC increased current recovery by 1.5 compared with that for graphite rods (Zhang et al., 2014), whereas it was comparable to that for fibrous carbon (Moqsud et al., 2014). Overall, CB has two important physical characteristics: it is fibrous and macroporous. Thereby, it displayed the highest current recovery. BC can be used as an anode in MFC. However, it requires a higher ASSA while having micropores identical to those of CB. Recently, a 3D-weaved carbon mesh with 1.0 cm intervals yielded more current than CB (Luo et al., 2020). This was owing to the presence of fibrous carbon with centimeter-scale pores. Other papers contended that the optimum pore width is 0.3–0.5 mm (Bian et al., 2018; Chong et al., 2019). In another study, the optimum size increased from 1.0 mm at an early stage to 3.0 mm after one month of operation (Li et al., 2014). The pore size is a critical factor that directly affects the specific surface area of metabolically active microbes rather than the electrode (Luo et al., 2020). It should be optimized to prevent cell clogging, which hinders substrate accessibility during long-term operation.

Supplementary Table S1 compares the manufacturing costs of CB and BC, including the material and processing costs. In general, only the material cost is considered because of the variability of processing cost depending on the production scale and process (He et al., 2019).

However, the table shows the material cost of CB as that of carbon fiber and brush, which includes the fiber-to-brush processing cost. Presently, the processing cost of CB (i.e., twisting of wires together with cut carbon-fiber) is significant and becomes more expensive with reduction in diameter because of the need to fill a larger number of CBs in a volume. In large MFCs for treating municipal wastewater (> 85 L), CB has an average diameter of 50 mm and is filled with 32%–56% (v/v) (see Supplementary Table S2). According to our calculation, the cost efficiency of the cost-normalized volume of the anode in the BC was 64% of that of CB of $\phi 50$ mm, although the cost is variable and is a tentative value based on the company's estimation at the current instant. According to Supplementary Table S2, MFCs having 4 or 15 m²/m³ and 30% of anode volume ratio were considered as model MFCs and evaluated in the cost balance between the ion exchange membrane, CB of $\phi 50$ mm, and BC. The material cost was accounted for by the ion exchange membrane (IEM), whereas the total cost of material and processing was accounted for by carbon anodes in all the cases (Supplementary Fig. S3). The MFCs with 14 and 4 m²/m³ ASSA using BC incurred 30% and 58%, respectively, of the cost of those using CB in terms of the processing cost. These results indicate the potential advantage of using BC.

The results of the three anodes used in this study in terms of current recovery from sewage wastewater were compared with those of the other three anodes: namely, GF, electrochemically oxidized GF (EOGF), and reduced graphene oxide (rGO) (Table 1). These anodes were evaluated in terms of current production using the same electrochemical cultivation vessels filled with wastewater obtained from the same wastewater treatment plant. The anode resistance tends to be negatively correlated with current and the value could be an efficient index marker for the goodness of the anode-microbes complex. CV analysis was applied to determine the anode resistance in this study, whereas linear sweep voltammetry (Sugioka et al., 2019) and electrode potential slope analysis (Rossi et al., 2019a) could determine the anode resistance. The

total cell density on the anode correlated effectively with the current density. This agrees well with the previous observation that planktonic cells are less involved in the production of electricity than cells in biofilms (Fan et al., 2008; Rabaey et al., 2005). The higher total biomass growth on PNWG and CB than on BC was owing to the fabric structure, and the high ASSA enabled cell adhesion via π - π stacking of the aromatic rings of the carbon anode and microbial surface (Erbay et al., 2015). BC had an apparently smooth surface and difficult-to-strong cell adhesion under the stirring conditions in this study. Although the genus *Geobacter* in the total prokaryotes has been acknowledged as an indicator of a good anode biofilm, the proportion or cell density of electrogenic bacteria is not correlated with the production of current. The predominance of *G. psychrophilus* was not observed in our previous analysis of GF, EOGF, and rGO, where *G. bremensis* and *Geothrix* species were detected. The presence of *G. psychrophilus* in all the three anodes in this study indicated that the inoculum and wastewater obtained in different years and seasons, or the stirring conditions in three electrochemical cultivations affect the microbial community. Considering all of the above, CB can be concluded to be the best among these anodes. This is because of the large ASSA that harbors many microbes, which contributed to a decrease in the anode resistance.

In conclusion, the fibrous structure of CB with macropores yielded the best current recovery from sewage wastewater among the three anodes. It was expected to eliminate the anode-limiting rate of MFC for treating wastewater at a low COD. BC needs to be improved to have an ASSA that is necessary for further current recovery. However, it displayed superiority in terms of cost efficiency considering material and processing.

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Figure legends

Figure 1 The three anodes used in this study and the current recovery. Panel A indicates the apparatus of the three anodes. Panels B illustrates the current recovery from sewage wastewater using PNWG, BC, and CB. Arrows and asterisks indicate the timing of the wastewater replacement and shifting of the culture into the incubator maintained at 28°C.

Figure 2 Comparison of peak current and accumulative charge. Panel A indicates the peak current density and B shows the accumulative charge density by using three anodes. * indicates a significant difference ($p < 0.05$) between CB and the other two anodes, in an unpaired Student's t-test. Whiskers indicate the 95% range, boxes indicate the medians and quartiles, and the cross marks are the average values.

Figure 3 Cyclic voltammograms obtained from the three anodes in wastewater.

Figure 4 Comparison of biomass on the three anodes. Panel A and B show apparatus of the biofilm and cell distribution in the three cultures, respectively.

Figure 5 An air-cathode-AEM MFC unit used in this study and the current production. Panels A and B indicate apparatus of air-cathode-AEM MFC unit equipped with four pieces of CBs and the reactor where the MFC unit was installed, respectively. Panel C shows the current production by the MFC.

333 **Table 1 Comparison of carbon anodes in current recovery from sewage wastewater.** The referred areas and volumes are the projected area and
334 volume of the anode, respectively.
335

	GF	PNWG	BC	CB	EOGF	rGO
Anode specific surface area / volume [m ² /m ³]	340	1700	590	18200 ¹	340	340
Peak current density [A/m ²]	0.95	1.5	1.4	2.9	1.5	2.5
Anode resistance [Ωm ²]	0.50	0.58	0.40	0.20	0.20	0.10
Cell density [10 ⁸ cells/cm ²]	6.5	30	0.35	40	N.D.	22
Electrogenic bacterial genera (%)	<i>Geobacter</i> (28%)	<i>Geobacter</i> (5.1%)	<i>Geobacter</i> (5.6%)	<i>Geobacter</i> (4.4%)	<i>Geobacter</i> (23%)	<i>Geobacter</i> (25%) <i>Geothrix</i> (7.6%)
Electrogenic bacterial population [10 ⁸ cells/cm ²]	1.3	1.5	0.020	1.8	N.D.	7.2
Culture condition	Static	Stirring	Stirring	Stirring	Static	Static
Reference	(Yoshida et al., 2016b, 2019)	This study	This study	This study	(Yoshida et al., 2019)	(Yoshida et al., 2016b)

¹Logan et al., 2007b

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485

Fig1

A

B

Fig2

Fig3

Fig4

A

PNWG

BC

CB

B

Fig5

A

B

C