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Dye removal of AR27 with enhanced degradation and power generation in a microbial fuel cell using bioanode of treated clinoptilolite-modified graphite felt

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ABSTRACT

This work studied the performance of a laboratory-scale microbial fuel cell (MFC) using a bioanode that consisted of treated clinoptilolite fine powder coated onto graphite felt (TC-MGF). The results were compared with another similar MFC that used a bare graphite felt (BGF) bioanode. The anode surfaces provided active sites for the adhesion of the bacterial consortium (NAR-2) and the biodegradation of mono azo dye C.I. Acid Red 27. As a result, bioelectricity was generated in both MFCs. A 98% decolourisation rate was achieved using the TC-MGF bioanode under a fed-batch operation mode. Maximum power densities for BGF and TC-MGF bioanodes were 458.8 ± 5.0 and 940.3 ± 4.2 mWm⁻², respectively. GC-MS analyses showed that the dye was readily degraded in the presence of the TC-MGF bioanode. The MFC using the TC-MGF bioanode showed a stable biofilm with no biomass leaching out for more than 300 h operation. In general, MFC performance was substantially improved by the fabricated TC-MGF bioanode. It was also found that the TC-MGF bioanode with the stable biofilm presented the nature of exopolysaccharide (EPS) structure, which is suitable for the biodegradation of the azo dye. In fact, the EPS facilitated the shuttling of electrons to the bioanode for the generation of bioelectricity.

Keywords: Azo dye removal; Biodegradation; Bioelectricity; Microbial fuel cell; Treated clinoptilolite-modified graphite felt.

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Introduction

Microbial fuel cells (MFCs) are widely used as bio-electrochemical tools capable of converting chemical energy associated with chemical bonds of organic compounds into electrical energy through bacterial catalysis (Park and Zeikus, 2003). They are also regarded as innovative and environmentally friendly technologies, mainly because of energy recovered as a by-product in the course of degradation of organic pollutants in wastewater (Liu et al. 2004; Daniel et al. 2009; Hashemi and Samimi, 2012; Chen et al. 2011; Liu and Li, 2007; Miran et al. 2015). In the anaerobic compartment of an MFC, electrochemically active bacteria (EAB) can grow and form biofilms on the anode surface, and oxidise organic matter under non-aerated conditions, releasing electrons and protons (Song et al. 2015). The electrons are transferred to the anode surface and then moved towards the cathode by passing through the external electrical circuit. Meanwhile, the protons are moved to the cathode compartment through the electrolyte and the separator. Finally, the electrons and protons will then combine with oxygen on the cathode surface to form water molecules to complete the reaction scheme.

MFCs have been used for the removal of different types of wastewater pollutants, including dyes (Hou et al. 2011a; Li et al. 2010). Different types of dyes are utilised in many industries such as paper, textile, pulp, paints, carpets and printing, and a huge amount of effluent is generated (Liu et al. 2016). Effluents that contain dyes are intensely colored and are among the main causes of environmental pollution in such industries (Zouari-Mechichi et al. 2006). The most relevant of the dyes used are azo dyes. Azo dyes are characterized by the existence of one or more azo categories ($-N=N-$) and they are extensively used in the textile industry, where 10 to 15% of them are assumed to be lost in the effluent during the dyeing process (Liu et al. 2016). They are carcinogenic and mutagenic compounds, and different

dye-polluted wastewater treatment processes are currently under investigation (Saratale et al. 2011).

One option for the degradation of dye in wastewater is to use MFCs, which also offer the advantage of harvesting low amounts of energy. The double bonds ($-N=N-$) that exist in all azoic compounds are not easy to break because of their stable chemical structure. It has been previously reported that, under anaerobic or microaerophilic conditions, the azo bond ($-N=N-$) can be reduced as it can act as electron-acceptor (Miran et al. 2015; Chan et al. 2012a; Fang et al. 2015).

Despite the fact that there has been some improvement in the electrochemical performance of MFCs in recent years, their low power output is still deemed a major drawback, reducing their application in commercial wastewater treatment. Many research studies have been conducted in the past decade to explore the influence of various factors on the performance of the MFCs used in wastewater treatment (Song et al. 2015; Solanki et al. 2013).

One important factor under study is the anode material and configuration. Various types of carbon-based anode materials such as carbon paper (Hou et al. 2011a), graphite felt (Li et al. 2010) and carbon cloth (Zhang et al. 2010) have been used because of their good conductivity. It is often assumed that electron transfer from the EAB to the anode can be affected not only by the properties of the anode but also by its surface modification. Recent studies have reported that the use of acid and heat treatment (Feng et al. 2010), ammonia treatment (Cheng and Logan, 2007), carbon nanotube coating (Tsai et al. 2009), anionic surfactant addition (Song et al. 2015) and synthetic NaX zeolite addition can be successful methods for the surface modification of the anode (Wu et al. 2015), which have led to improvements in the performance of the MFC.

The power production enhancement through surface modification of the anode can be ascribed to factors such as the increase in the anode surface area available to active bacteria, better biocompatibility, and improved electron transfer rate from the bacteria to the surface of anode (Song et al. 2015; Cheng and Logan, 2007). The efficient transfer of electrons from the EAB to the anode and the enhanced power production can be directly related to bacterial adhesion and growth on the anode surface. EAB adhesion can be influenced by the hydrophilic, electrostatic and van der Waals interactions with the anode surface (Cheng and Logan, 2007). Thus, it is considered that investigation of the surface modification of the anode is important in understanding the efficient transfer of electrons, the adhesion and growth of bacteria on the anode surface, and the MFC performance.

Clinoptilolite is a member of the natural zeolite family and the chemical formula $(\text{Na,K})_6\text{Si}_{30}\text{Al}_6\text{O}_{72}\cdot n\text{H}_2\text{O}$, exhibits a three-dimensional crystal structure. The presence of negative charges on the surface of clinoptilolite enables it to repel bacteria that acquire negative charges due to the presence of lipoproteins in the bacterial cell wall along with carboxylates as their active functional groups (Gedikoglu et al. 2012). Moreover, it is well known that the cationic surfactants such as hexadecyl trimethyl ammonium bromide (HDTMA-Br) can be used to modify the negatively charged surface, and thus increase the adhesion of negatively charged bacteria due to hydrophobic and ionic interaction between the cells and the surface (Hrenovic et al. 2008).

A monolayer configuration can be formed, since the concentration of cationic surfactant HDTMA-Br is lower than the critical micelle concentration (CMC) (Nezamzadeh-Ejhi and Esmaeilian, 2012), and thus the surface of the clinoptilolite can be covered by a monolayer of surfactant molecules, provided they are present in low concentrations. The outer surface of the clinoptilolite undergoes a charge reversal because of this monolayer, resulting in the availability of hydrophobic sites for the retention of hydrophobic and organic

substances (Vaisman et al. 2006). In this way, the surface of the clinoptilolite becomes appropriate for the immobilization of bacteria and the attachment of organic and hydrophobic substances such as nutrients and azo dyes. Nevertheless, possible MFC anode improvement using a treated clinoptilolite with cationic surfactant HDTM-Br has not been studied until now.

In this context, the purpose of this research is to determine the feasibility of generating electrical energy from the treatment of wastewater containing synthetic azo dye by using a MFC with a treated clinoptilolite-modified graphite felt (TC-MGF) bioanode. An active biofilm of NAR-2 bacterial consortium was immobilized on the modified anode. A complete characterization of the properties of the modified anode as a biofilm carrier was carried out by different techniques, such as Fourier Transform Infrared Spectroscopy (FTIR), Field Emission Scanning Electron Microscopy (FESEM), and Cyclic Voltammetry (CV). The results of anode characterization and MFC performance were compared to those obtained using non-modified bare graphite felt (BGF) as the anode material.

Materials and Methods

Fine powder clinoptilolite and hexadecyl trimethyl ammonium bromide (HDTMA-Br) were purchased from Zeochem (Czechoslovakia) and Zeochem (Denmark), respectively. This work also used Amaranth (C.I. Acid Red 27), which was purchased from Sigma-Aldrich (USA).

Medium and cultures

Two types of media were used. The first was P5 medium, containing K_2HPO_4 (35.3 g L^{-1}), KH_2PO_4 (20.9 g L^{-1}), NH_4Cl (2.0 g L^{-1}), glucose (10.0 g L^{-1}), and nutrient broth (20.0 g L^{-1}),

with trace elements chosen as growth and feeding media in the growth phase. The second medium was modified P5, prepared as a mixture of a nutrient broth (5.0 gL^{-1}), glucose (2.5 g L^{-1}), NH_4Cl (2.0 g L^{-1}), KH_2PO_4 (20.9 g L^{-1}), K_2HPO_4 (35.3 g L^{-1}), trace elements, and AR27 (0.1 g L^{-1}). Three local strains, namely *Citrobacter freundii* A1, *Enterobacter cloacae* L17 and *Enterococcus casseliflavus* C1 were used in a combination formed and designed as the NAR-2 bacterial consortium. These organisms were isolated from the sewage oxidation pond of Universiti Teknologi Malaysia (Johor, Malaysia) (Kardi et al. 2016b).

In order to prepare the acclimated NAR-2 consortium to be used in the MFC, the following sub-culture procedure was performed. A 10 mL portion of the sterile P5 medium was placed in a conical flask. A wire loop was sterilised, and then a bacterial colony was picked from the nutrient agar plate and inoculated into the P5 medium. After the inoculation, the P5 medium was incubated overnight at 200 rpm and 37°C . To prepare the sub-culture, each overnight inoculum was formed by incubating independent cultures and transferring them into a conical flask containing 90 mL of the normal P5. The three subculture inocula were ready to be used after agitation until optical density reached 1 ± 2 at 600 nm using a UV-Vis spectrophotometer (Shimadzu UV-1280, Japan).

Preparation of TC-MGF anode

In order to fabricate the modified anode, clinoptilolite was first treated with the cationic surfactant HDTM-Br. For this purpose, 2 g of clinoptilolite was added to 100 mL 0.01 mM HDTMA-Br solution, which was then kept for 17 h at room temperature under agitation rate of 100 rpm (Kardi et al. 2016b). The solution was then filtered to obtain the surfactant-treated clinoptilolite (S-TC). After filtration, the surfactant molecules unattached to the clinoptilolite surface were removed by washing out with distilled water. The S-TC was left to dry

overnight at 70 °C. The next step was the dispersion of the dry S-TC in 100 mL of ethanol solution. Finally, the graphite felt added to the solution, followed by sonication for 30 min. The graphite felt was then left to dry out overnight at 100 °C (Wu et al. 2015). The above-mentioned procedure was developed for the synthesis of the TC-MGF anode. In order to discover the optimum amount of S-TC fine powder to be added to the modified anodes, the performance of such TC-MGF anodes with different amounts of S-TC (15, 25, 35, 45, 55, 75, and 95 wt. %) were subsequently evaluated by CV analysis.

Experimental set-up

Immobilization of NAR-2 consortium on bioanodes

The immobilization of the NAR-2 bacterial consortium on the anode surface was carried out in two steps: first, initial attachment of bacteria, and second, the development of the biofilm (Ginsburg and Karamanev, 2007; Kang et al. 2012). Three bacterial strains were grown in the P5 medium overnight. The culture was then centrifuged at 4500 rpm for 15 min. The pellets obtained by centrifugation were added in equal proportions to 0.1 M phosphate buffer solution (PBS) (pH 7.0) with no carbon source. The initial attachment of the bacterial cells was performed by dipping the anode (unmodified or modified) into a glassy up-flow column bioreactor and incubated at 37 °C for 24 h (Fig. 1a), using the inoculated buffer solution and thus allowing the NAR-2 bacterial consortium cells to be attached to the surface of the anode. Then, in order to achieve an active developed biofilm, the initial attachment process was followed by running the bioreactor in a co-current up-flow mode. During this process, which lasted for six days, a P5 medium of enriched nutrients was pumped (peristaltic pump, Watson Marlow, USA) into the column in a closed loop mode at 37 °C. However, the P5 medium was replaced at intervals of 48 h (Kardi et al. 2016b).

Microbial cell configuration and operation

A comparative analysis was conducted for two identical MFCs but using different anode material, namely BGF-MFC and TC-MGF-MFC. The anode dimensions of these MFCs were 10 × 15 × 1 cm, and they were made up of bare graphite felt (BGF) and treated clinoptilolite-modified graphite felt (TC-MGF) respectively. A pair of 1400 mL anode and cathode chambers (with a working volume of 1200 mL) were connected by a short tube and separated by a salt bridge, which in this case was made up of 10% agarose mixed with 1 M NaCl solution. The cathode and catholyte used were as previously described (Kardi et al. 2016a). A copper wire passing through an external resistor (300,000 Ω) linked the two electrodes of the MFC with 3 cm electrode space. A magnetic stirrer was used to homogenise the medium in the anode chamber. In addition, an air stone linked to a blower supplied oxygen to the cathode chamber (Fig. 1b).

Once the bacterial cultures were immobilized on the anode materials, they were then placed in the TC-MGF-MFC and GF-MFC anode chambers. First, the MFCs were operated under semi-continuous mode in four successive cycles, and the cycle retention time was 100 h. The modified P5 medium containing AR27 at a concentration of 0.1 gL⁻¹ was serially fed to the MFC at 30 °C and under closed circuit voltage (CCV) 10 Ω. Then, for the execution of power generation, after running the four 100 h cycles, the MFC worked under stable open circuit voltage (OCV) and then, subsequently connected to the resistance box from the highest to the lowest resistance (300,000–10 Ω) at time intervals of 15 min in order to determine the polarisation curves.

Power calculations

A precision multimeter (Keithley Instruments 2700, USA) was used to measure the OCV and voltage output of the MFC every 30 min during the experiments (Fig. 1b (i)). In order to

perform the polarisation curve tests, the MFC electrodes were linked to a variable resistance box (300,000–10Ω). This was followed by the determination of stable voltage values at different resistances under CCV (Fig. 1b (ii)). The power curves, which indicate the power density as a parameter determined by the current density, were obtained by means of the polarisation curve. The power and current densities were determined based on the anode surface area. Moreover, Ohm's law ($V = IR$) was used to compute the current (I). In this equation, V and R refer to the voltage and external resistance, respectively. The equation $P = IV$ was used to compute the power (P). Finally, the polarisation curve slope method was used to determine the internal resistance (Logan et al. 2006).

Fig. 1

Analytical measurements in the anode chamber

Color removal of AR27 by the NAR-2 bacterial consortium was measured as follows. Liquid samples were taken from the anode chamber in quartz cuvettes (1 mL) at time intervals of 30 min, and the absorption spectra of the samples were determined with a UV-Vis spectrophotometer (Shimadzu UV-1280, Japan). The blank used in this experiment was modified P5 medium, and the wavelengths used ranged from 200 to 800 nm. The maximum absorbance of AR27 and the absorbance values of the samples were determined at 521 nm. The decolorization rate was calculated based on the following equation (1):

$$\text{Decolorization (\%)} = \frac{A_i - A_f}{A_i} \times 100\% \quad (1)$$

where A_i and A_f stand for initial and final absorptions, respectively.

The organic compounds of the anolyte chambers were analysed by gas chromatography-mass spectrometry (GC-MS) (PerkinElmer Clarus 680-SQ8S, USA), which was carried out at the end of the fed-batch cycles, and the predicted metabolites were drawn using ACD ChemSketch (Version 1.0) (Karuppiyah and Raju, 2009).

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2 Finally, to determine the profile of bacterial species of the NAR-2 bacterial
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4 consortium leached out from the anode surface, sampling for viable plate count (CFU mL⁻¹)
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6 was performed.
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10 11 *Anode and bioanode characterization* 12 13

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15 A digital multimeter (Sanwa, Model 76500, Japan) was used to determine the resistance of
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17 the BGF and TC-MGF anodes. An N₂ adsorption-desorption experiment was performed to
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19 compute the specific surface area (SSA) of both anodes. This experiment involved the
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21 utilisation of the Brunauer Emmett Teller (BET) method for the surface area analyser
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23 (Hitachi-450, Japan). Field emission scanning electron microscopy (FESEM) (Hitachi,
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25 SU2080, Japan) was used to study the morphological features of the anodes. The Cam Scan
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27 FESEM (Model S2-80DV) was used to perform the energy dispersive X-ray analysis (EDX).
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29 Similarly, Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR)
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31 (Perkin Elmer, USA) was used to verify the functional groups of S-TC attached to the
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33 modified anode. In order to optimize the amount of S-TC fine powder in the modified anode,
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35 the performance of TC-MGF anodes with different amounts of S-TC fine powder (15, 25, 45,
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37 55, 75, and 95 wt. %) were evaluated by cyclic voltammetry (CV) analysis. NaCl solution
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39 (0.1 M) was used in an electrochemical cell equipped with three electrodes, including anodes
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41 (i.e. TC-MGF and BGF) functioning as a working electrode, a saturated calomel electrode
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43 (SCE) functioning as reference electrode, and platinum wire functioning as a counter
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45 electrode. A potentiostat (Metrohm Autolab, Netherlands) device was employed for
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47 controlling the system range versus scan rate (Fig. 1c). The scan was conducted between -1.5
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49 V and +1.0 V with a scan rate of 0.02 mV/s.
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Regarding the bioanode (that is, the anode including the developed biofilm) characterization, FESEM (Hitachi, SU2080, Japan) was used to study the morphological features of the bioanodes and to validate the exopolysaccharide (EPS) structure. After 16 days of operation the modified and non-modified bioanodes were removed from the fuel cells. The bioanodes were then washed with nutrient-free phosphate buffer solution. In addition, CV was employed to evaluate the electrochemical activity of the biofilms developed on the surfaces of the modified and non-modified anodes. Prior to any characterization test, the anodic medium was replaced with fresh P5 medium to evaluate the EPS structure of the biofilm. The scan was conducted between -0.6 V and $+0.6$ V with the scan rate of 0.02 mV/s. Moreover, ATR-FTIR was performed to determine the loading EPS of biofilm on the surface of modified and non-modified bioanodes.

Results

Optimization of the amount of S-TC fine powders on modified anodes

Prior to application of the non-modified and modified anodes in the MFCs, the effect of the amount of S-TC fine powder on the modified anode was studied through CV analysis, in order to define the optimum amount. Fig. 2a shows that using 25% S-TC fine powder on the modified anode produced the largest redox current in the potential range of 1.0 to -1.5 V; this result suggests that this amount of S-TC fine powder would lead to excellent electrochemical activity. Thus, 25% S-TC fine powder was considered the optimum ratio for further study of characterization and immobilization of biofilm of bacterial consortium on the fabricated modified bioanode.

Characterization of BGF and TC-MGF anodes

In order to evaluate the morphological features of the fabricated anodes, the S-TC fine powder and the two types of anodes were subjected to FESEM analysis. The FESEM images

of the S-TC fine powder and the BGF and TC-MGF anodes are shown in Fig. 2b–d. The microscopic examination revealed that the BGF comprised a complex network of carbon microfilaments (Fig. 2c). In the case of the TC-MGF anode, it was observed that certain crystalline fine powders (Fig. 2b) were randomly distributed on the graphite felt surface (Fig. 2d). This change in the microstructure of the altered graphite felt surfaces could have caused a substantial increase in the specific surface areas, as well as in the number of active sites that were conducive for the catalytic process. The EDX image shows (Fig. 3a) that Si and Al peaks were observed in the S-TC fine powders, and the Si and Al peaks were present because of the clinoptilolite elements. In addition, Fig. 3b shows the EDX spectrum, which revealed that the BGF anode was comprised of oxygen and carbon only. It was also confirmed through EDX that the surface of the TC-MGF anode was efficiently coated with S-TC fine powder (Fig. 3c).

Fig. 2

ATR-FTIR was performed to determine the loading of the S-TC fine powder on the surface of the graphite felt. The ATR-FTIR spectra of the S-TC fine powder, the BGF anode, and the TC-MGF anode are shown in Fig. 3d. The TC-MGF anode gave rise to a sharp band in the range of 1054.25–1067.93 cm^{-1} , which corresponds to the asymmetric vibration patterns of the T–O bonds found within the tetrahedral TO_4 structure (T = Si and Al). On the other hand, the sharp and intense band formed at 797.79–795.87 cm^{-1} was related to the stretching vibration modes of the O–T–O groups. Moreover, the bands formed at 2864.22 and 2924.98 cm^{-1} would be related to the CH_2 symmetric and asymmetric stretching modes because of the adsorption of the cationic surfactant HDTMA-Br on the surface of the clinoptilolite. Some peaks were found exclusively in the analysis of the TC-MGF anode

while there were no such peaks in the BGF anode analysis, and thus validating that the S-TC fine powders were successfully loaded onto the graphite felt surface.

Fig. 3

The BET method was used to evaluate the SSA of both anodes. The results of the analysis are shown in Table 1 and they indicate that the SSA value of the BGF anode was $1.2 \text{ m}^2\text{g}^{-1}$, while the SSA value of the TC-MGF anode was found to be quite high ($9.8 \text{ m}^2\text{g}^{-1}$). The larger area of the modified anode would offer greater porosity compared to the non-modified anode. The modified anode exhibited an extensively greater SSA value (more than 8-fold) that was related to the high porosity of clinoptilolite. The resistances of both of the anodes were also determined. The resistance of BGF was found to be 0.7Ω , whereas the resistance of TC-MGF was defined a bit higher at 1.0Ω .

Table 1

Fig. 3e presents the results obtained in the CV tests of the two different types of anode. It is evident from the CV that the redox peaks of TC-MGF anode were bigger than those of the BGF anode. This result implied that the TC-MGF anode should exhibit a greater electrochemical activity compared to the BGF anode, and thus the modified anode would be an appropriate material to be used as a biocatalyst support and anodic electrode.

FESEM characterization of bioanodes in immobilization step

The morphological features of the bacterial cells in the immobilization stage of NAR-2 over the surface of the BGF and TC-MGF bioanodes were scanned by FESEM and the images are shown in Figs. 4a–d. These images were taken after feeding the bioreactor with a fresh P5 medium for six days. It can be stated that the morphological characteristics were different,

1 according to the significant differences in the images of the biofilms formed over the BGF
2 and the TC-MGF anodes. The biofilm over the TC-MGF anode (Fig. 4b) was visibly higher,
3 with an expected greater population of bacteria compared to the biofilm over the BGF anode
4 (Fig. 4a). In fact, no dense biofilm was formed on the BGF surface. In principle, the surface
5 of the negatively charged compounds decreased the adhesion of negatively charged bacteria
6 due to repulsive force. Yet, the negative charges existing on the BGF anode might have
7 repelled the bacterial biofilm, resulting in the attachment of a small population of bacteria to
8 the surface of the BGF anode (Song et al. 2015; Wang et al. 2009).
9

10 Moreover, the bacterial cells covering the anode electrode surface also demonstrated
11 the production of filaments in both anodes. These filaments or nano-pili facilitated the
12 adherence of additional cells to the electrode. Previous research has reported that the bacterial
13 filaments of *Geobacter* and *Shewanella* genus are able to conduct electricity, hence, they may
14 contribute to the electron transfer (Eaktasang et al. 2013). The highlighted portion in the
15 images indicates interaction between the anode and nano-pili of the immobilized bacterial
16 cells (dotted ovals) (Fig. 4c). Thick biofilms can be observed in Fig. 4d (modified anode).
17

18 **Fig. 4**

19 ***MFCs performance on color removal and bioelectricity generation***

20 Once the MFCs with BGF or TC-MGF bioanodes started, their operation involved the
21 periodic feeding of synthetic wastewater comprising 2.5 gL⁻¹ of glucose and 0.1 gL⁻¹ of AR27
22 in a semicontinuous operation mode (4 cycles, 100 h per cycle). The results of the
23 performance of the MFCs are shown in Figs. 5a and 5b. It is clear that the voltage generation
24 and the decolorization rate of the TC-MGF-MFC was much higher compared to those of the
25 BGF-MFC.
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27 The maximum voltage recorded during the first feeding cycle of the MFC operated
28 with a TC-MGF bioanode was found to be 357.7 mV and that of the MFC having BGF anode
29

was 137.3 mV (Fig. 5a). In addition, more than 78% decolorization of AR27 was obtained in the TC-MGF-MFC within 2 h of the injection of the first feeding cycle, giving it much faster decolorization rate than that of BGF-MFC (Fig. 5b). This would be very likely due to the non-stable adhesion of the NAR-2 bacterial consortium on the BGF anode and bacteria cell leached out increased as illustrated in Fig. 5b. The rate of color removal in TC-MGF bioanode showed a 1.5-fold increase compared to the unmodified anode. The reduction in decolorization rate may be directly linked to a lower anode surface area which reduces the contact time between the dyes and the bacteria. This could be the reason for the relatively lower decolorization efficiency shown by the BFG bioanode. In the case of the second feeding cycle, there was a considerable increase in the maximum voltage of the TC-MGF-MFC at a fixed lag time (mean 0.5 h). However, a very low increase in the maximum voltage was recorded in the case of the MFC containing a BGF anode. Moreover, the maximum voltage recorded for the MFC containing a TC-MGF bioanode was 0.558 V. This voltage was 101% greater than that of the MFC equipped with a BGF anode (0.278 V), and had 1.4 times faster color removal than the MFC containing the BGF anode. In addition to these findings, a significant improvement was noted in the maintenance of a relatively high and constant voltage near the maximum values in the case of the MFC equipped with TC-MGF bioanode compared to the MFC having a BGF bioanode as shown in cycles 3 and 4. Regarding the biomass leaching out, Fig. 5b shows that leaching out of NAR-2 with BGF bioanode (1.3 and 1.1 log(CFU mL⁻¹)), was approximately three times higher than with TC-MGF bioanode (0.44 and 0.4 log(CFU mL⁻¹)) in cycles 3 and 4, respectively. Lower values of biomass leaching with TC-MGF bioanode might be caused by better bacterial adhesion to the anode material.

Polarisation curves were obtained from studies of the two different bioanodes in MFCs under closed circuit situation (300,000–10 Ω) after 368 h and they are shown in Fig.

5c. It can be observed from the curves that the highest value obtained for the power density in the MFC having the TC-MGF bioanode was $940.3 \pm 4.2 \text{ mWm}^{-2}$, and this value was about two times greater than the power density of the MFC with BGF anode (i.e. $458.8 \pm 5.0 \text{ mWm}^{-2}$). It is clear that the lowest internal resistance (i.e., $601.9 \pm 13.0 \Omega$) was recorded in the case of the MFC with the TC-MGF bioanode, and this value was 48% lower than the internal resistance of the BGF anode (i.e. $925.1 \pm 10.0 \Omega$). These results showed that the efficiency of the MFC increased substantially when a modified anode was used; that was a great indication demonstrated by output voltage and improved maximum power density.

Fig. 5

Compared to the MFC with a modified bioanode, the MFC with the BGF bioanode demonstrated a very poor performance in terms of output voltage and maximum power density. The generation of power in an MFC, a process that utilises the biofilm, is influenced by the interaction between the surface of the anode and the bacteria. A greater current density and power density were generated in the TC-MGF anode upon alteration of the external resistances from 1000 to 500 Ω . The internal resistances of the BGF and TC-MGF were 925 and 601 Ω , respectively. The distribution of the internal resistance was analysed in detail through the slope analysis method, and the results revealed that the TC-MGF bioanode had greater electron transfer properties than the BGF bioanode. This might have been due to greater surface area and lower internal resistances available in the TC-MGF bioanode compared to the BGF bioanode. In addition, the increased surface area resulted in the formation of more biofilms and active sites which facilitated a reduced charge transfer resistance in the anode (Hou et al. 2011b; Fan et al. 2008). Therefore, it can be concluded that the biofilm on the TC-MGF bioanode was more efficient; therefore this type of MFC generated additional bioelectricity.

EPS Characterization of bioanodes

Once the fed-batch operation was completed, the anodes were taken out of the anodic chamber, and FESEM analysis were carried out to validate the EPS structure and to analyse the biofilm developed on the surface of the electrodes (Fig.6).

The FESEM images of the NAR-2 biofilms are shown in Fig. 6. The morphological features of the biofilm on the TC-MGF bioanode (Fig. 6a) and those of the BGF bioanode (Fig. 6b) are definitely different. Rod-shaped bacteria were identified on the BGF anode as observed in FESEM image at high magnification (Fig. 6e). However, a considerably dense and thick biofilm had developed on the TC-MGF bioanode and the attachment of cells is shown (Fig. 6c). A gradual increase in the generated current occurred because of the development of biofilms (i.e. primary, and then secondary biofilms, upon further development). In the final cycle, a quick current generation and high magnitude of maximum current generation were achieved.

In the case of the TC-MGF bioanode, it was found that the EPS remained intact for 16 days while the MFC was operating at 30 °C in at microaerophilic condition. The squared area is shown in Fig. 7a and the image with a high magnification is shown in Fig. 7d. The immobilized cells on the BGF bioanode demonstrated an altered morphology (i.e. rod shape) unlike the bacteria that were attached to the TC-MGF bioanode (i.e. A1, L17 and C1) (Fig. 6a). Chan et al. (2012b) published the draft genome sequence of C1. The C1 produced alginate, possibly by the glycosyl-transferase group 1 family protein OS, while at the same time, C1 possessed the enzyme alginate lyase. Alginate lyase can dissolve alginate under limited nutrient conditions as the breakdown product can serve as a nutrient for the bacteria. This may explain the simple leaching out of C1 from the biofilm relative to A1 and L17 on the BGF anode, as indicated by the arrows in Fig. 6e.

Fig. 6

In addition, researchers have used cyclic voltammetry extensively to evaluate the redox characteristics of bioanode components and to validate the potential of anodic biofilms to act as bioelectrocatalysts for the oxidation of P5 medium. The CV curves of the two different bioanodes covered with biofilm are shown in Fig. 7a. The TC-MGF bioanode displayed quite superior electron transfer characteristics compared to the BGF bioanode, as indicated by the greater redox currents obtained by CV. In the case of the oxidation scan of the TC-MGF bioanode, a peculiar peak current of 0.25 mA was achieved at 0.021 V. The magnitude of this current was greater than the one recorded by the BGF bioanode. This would be due to the greater number of electrogens that adhered to the anode to catalyse the oxidation of glucose, and this was validated later through an FESEM analysis. Between the two types of modified and non-modified anode electrodes studied, the redox currents from the CV of the BGF anode were the lowest, and a slight oxidation peak current of 0.015 mA was recorded at -0.075 V. In addition, a CV scan of the TC-MGF bioanode showed two redox couples at 0.2 V and -0.25V. The presence of these redox couples suggests the presence of mediators (produced by the bacterial consortium in the biofilm) that would be reversibly oxidised and reduced during CV analysis. Therefore, it was concluded that the biofilm formation over the TC-MGF bioanode was more electrochemically active compared to that on the GF bioanode, and self-produced mediators by the biofilm could play a role in MFC performance (Sanchez et al. 2015).

An ATR-FTIR analysis spectrum (Fig. 7b) was performed to determine the loading of EPS by the NAR-2 bacterial consortia on the surface of the bioanodes. During the course of dye biodegradation, EPS was generated as a cellular by-product. The formation of a dense biofilm on the surface of the bioanode may assist the organism to utilise dye in MFC. The

IR spectra of EPS revealed the presence of a C=O band at 1648 to 1699.25 cm^{-1} and an OH band at 2800 to 3356 cm^{-1} ; thus indicating that the samples were EPS (Fig. 7b). The TC-MGF bioanode gave rise to a sharp band in the range 1699.25 – 1567.93 cm^{-1} in relation to the asymmetric vibration patterns of the C=O and C=C bonds found inside the EPS structure. On the other hand, the sharp and intense bands formed at 1124.80 and 1332.88 cm^{-1} were related to the stretching vibration modes of the S-O bond (Braissant et al. 2007). Moreover, the bands at 2854.22 and 2924.98 cm^{-1} related to the CH₂ symmetric and asymmetric stretching modes because of the greater leaching out of the biofilm and loss of alcohol and the stronger aliphatic structure of the EPS on the surface of the BGF bioanode (Braissant et al. 2007). Some peaks were found exclusively in the TC-MGF bioanode (i.e. 916 cm^{-1}), which did not appear in the BGF anode, thus validating that the TC-MGF bioanode had successfully loaded the sugar monomer structure (i.e. B-glycoside linkage) (Braissant et al. 2009). This result should be significant in the production of high-grade EPS formed due to the stable and thick biofilm developed on the TC-MGF bioanode compared to the BGF anode.

Analysis of dye degradation products

The intermediates produced in the liquid phase during the degradation and mineralization of the AR27 in the two different MFCs were identified using UV-Vis spectra and GC-MS analysis.

Wavelengths in the range of 200 to 800 nm were used to perform the spectral analysis of AR27 (λ_{max} 521nm). The degradation results showed that there was a gradual reduction in the dye peak in the visible region, which was eventually peak decreased with further retention time (Fig. 7c), indicating that the dye was rapidly decolorized. Complete decolorization of the dye with the dissociation of the chromophore group (breakage of the azo bond) was indicated by the intensity of the peak in the visible absorption spectrum (250 and 350 nm) (Li et al. 2010). It implied that the degradation of AR27 gave rise to new

1 metabolites. In addition, an examination of the UV-Vis spectra revealed that AR27 had two
2 major absorption peaks: i.e. 250 and 350 nm. There was a shift in the absorption peak present
3 in the visible region and the peak present in the UV region from 521 nm to 350nm and
4 250nm, respectively (i.e., the naphthalene rings and $-NH_2$ group on the aromatic rings). In
5 the case of the GF-MFC, the peak observed in the visible region (521nm) was not decreased
6 completely, thus indicating partial decolorization, and some dye was present in the anolyte
7 after 302 h of operation.
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10 A GC-MS analysis was carried out to identify the intermediate metabolites produced
11 from the degradation of AR27. Samples were extracted after 302 h, when decolorizations of
12 79 and 98% were recorded for the BGF-MFC and TC-MGF-MFC bioanodes, respectively.
13 During the analysis of the intermediates in the BGF-MFC, a hydrazo compound was
14 identified. Fig. 7d illustrates the suggested mechanism of biodegradation of AR27.
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16

17 On the other hand, the major intermediates identified by the GC-MS analysis of the
18 initial analyte after 302 h of successful operation using the TC-MGF-MFC included 1-
19 aminonaphthalene-2-hydroxy-3,6-disulphonic acid. However, 3-phenyl-1propanol was found
20 in abundance after 368 h of operation using the TC-MGF-MFC. The detection of these
21 organic compounds showed that breakage of the naphthalene ring took place on the TC-
22 MGF-MFC under microaerophilic conditions. This means aromatic organic compounds were
23 broken down to aliphatic acids.
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46 **Fig. 7**
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51 **Discussion** 52

53 The results in this study indicate that the positive charge of the HDTMA-Br had promoted
54 interaction with the negative charge of the NAR-2 immobilized bacterial consortium on the
55 TC-MFG bioanode. On the other hand, the tail of the HDTMA-Br provided hydrophobicity
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for the adhesion of the bacterial consortium. It can be deduced that the treatment of clinoptilolite with a cationic surfactant led to a high immobilization potential by the NAR-2 bacterial consortium.

It was found that a greater SSA was available for the adhesion of bacteria in the case of the TC-MGF bioanode. This modified anode also demonstrated a superior electrochemical activity and semi-hydrophilicity, which complemented the microbial affinity for adhesion with the anode. Clinoptilolite is a microporous substance that has a pore size of around 0.74 nm. Considering the size of the majority of bacterial cells (i.e. 0.5–3.0 μm), the surface area offered by the S-TC fine powders could be accessed by bacteria through the polar glucose molecules, which serve as the organic substrate for the access of electrogens on the surface area of the bioanode. This phenomenon may boost the rate of the redox reactions on the surface of bioanode. These factors, the wettability and electrochemical activity of the anode, would play a significant role in enhancement of efficiency of the MFC equipped with the modified anode. On the other hand, the low conductivity and hydrophobicity of the graphite felt anode are the main factors behind the poor efficiency of the MFC with the BGF anode (Song et al. 2015; Huang et al. 2004; Ke et al. 2001).

The TC-MGF bioanode consistently produced higher decolorization rates and voltage generation than did the BGF bioanode in all the feeding cycles. This finding would be based on the improved activity of NAR-2 bacterial consortium on the TC-MGF bioanode. The bioanode and dye acted as electron acceptors and glucose as electron donor (Fang et al. 2015). This result may suggest that the glucose and bacteria attached themselves to the hydrophobic tail of the modified anode, which was required for hydrophobic interaction efficiency and desired contact conditions between bacteria and glucose. It could accelerate the digestion of the glucose to produce more electrons for azo dye reduction. Therefore, the rate of color removal was increased.

The differences in the polarisation curves of the two bioanodes indicated that the TC-MGF-MFC consistently produced more power than the BGF-MFC. The high OCV was followed by a much lower initial drop in voltage with external resistance in the TC-MGF anode. The low drop in voltage in this high current region points to lower activation losses in the TC-MGF-MFC. The differences in power observed here would be due to the biofilm efficiency and internal resistances. The power density increased as the internal resistance of the MFCs decreased. The difference in the internal resistance from the TC-MGF-MFC compared to the BGF-MFC is most likely due to the interaction between the NAR-2 microbial communities with the anodes.

The results obtained in this study showed also that the NAR-2 bacterial consortium was able to form a stable biofilm over the surface of the TC-MGF bioanode, and this biofilm network was able to act as an active site for the transfer of electrons to the anode. The current generated by the MFC would be expected to increase with the formation of more stable and efficient biofilms (Chen et al. 2011; Eaktasang et al. 2013; Champavert et al. 2015). In addition, the generation of electricity in an MFC can be facilitated by the EPS anodic biofilm, to transfer electrons to the TC-MGF anode, resulting in the generation of electricity (Wang et al. 2013).

Additionally, the FESEM results showed continuous layers, thick biofilms and a slimy nature of the TC-MGF bioanode surface due to the bacterial cells which formed EPS on the TC-MGF anode after 16 days of operation. It is supposed that the hydrophobic (hexadecyl) tail and a cavity of the S-TC fine powders on the graphite felt may be localised not only for both the dye and the bacterial consortium but also for the nutrients due to their hydrophobic properties. This should be significant in the production of high-grade EPS of the

biofilm formed on the TC-MGF anode which was validated latter through an ATR-FTIR analysis.

Although bacterial cells were wrapped around the large-sized carbon fibres in the BGF anode, they did not demonstrate the formation of continuous layers of biofilm spreading over the entire BGF anode. This was because of the large pore size in the BGF rendered by the large diameter of the fibres. Moreover, the detachment area (pitting) induced by the NAR-2 was readily observed on the BGF anode when the biofilm was removed after 16 days of operation of the BGF-MFC. In the biofilm detachment area, the microbial enzymes destroyed the EPS matrix and released the cells, which then regained mobility and were able to colonise new surfaces (Lembre et al. 2012). Therefore, it was concluded that the formation of continuous and thick biofilms would offer a suitable anode structure to allow the generation of high current densities.

Previous studies have reported the mechanism for the mineralization of AR27 in an aerobic biofilm, according to which the azo bond was reduced, followed by the degradation of the aromatic amine that was formed due to reduction of organic matter (Coughlin et al. 2002). In the current research, under the microaerophilic condition of the TC-MGF-MFC, the mineralization of the dye was successfully observed and resembled the degradation of the aromatic amine produced from the decolorization of AR27. Therefore, the aromatic amine could undergo advanced degradation under microaerophilic conditions by the stable and thick biofilms along with the formation of EPS on the NAR-2 bacterial consortium. In fact, individual strains may attack the dye molecule at different sites and at the same time may consume the metabolites produced by the co-existing strains for further degradation on the TC-MGF anode (Chan et al. 2012a). Alves and co-workers reported a faster degradation rate in thick biofilms because of the dense population of the bacterial consortium in relation to formation of EPS (Alves et al. 2002). In this study, a relatively high rate of degradation was

observed on the TC-MGF anode, which might be due to the formation of EPS, assisting the NAR-2 bacterial consortium in forming a dense biofilm on the modified anode surface.

Conclusion

This study has demonstrated that the generation of electricity and degradation of the dye can be improved through the modification of the anode using 25 wt. % S-TC fine powders. The enhanced efficiency in the performance of the TC-MGF-MFC could be due to the simultaneous execution of the phenomena: i.e. continuous and thick biofilms of NAR-2 with the stable formation of an EPS matrix built on the TC-MGF bioanode, and the ability of the EPS to transfer electrons along with the mediator products formed by the biofilm on the TC-MGF bioanode. Considering the cost-effectiveness of the treatment of clinoptilolite with surfactant employed in the modification of the anode, this substance may prove to be valuable for enhanced modified anode implemented MFCs.

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List of Figures:

Fig. 1 Schematic diagram of experimental system. (a) Close loop immobilization reactor, with fibrous matrix as working electrode. Arrows depict direction of phosphate buffer solution and bacteria, (b) Schematic representation of MFC and connection of multimeter to MFC for (i) open circuit voltage and (ii) close circuit voltage, (c) three-electrode electrochemical system.

Fig. 2 (a) CVs of different amount of the S-TC fine powders on the graphite felt, FESEM images of the (b) S-TC fine powders, (c) BGF and (d) TC-MGF anodes. The area in Figs. 2c and 2d (square) are magnified and shown in Figs. 2e and 2f.

Fig. 3 EDX analysis or spectra of (a) S-TC fine powders, (b) BGF(c) TC-MGF anodes and (d) ATR-FTIR analysis spectra of S-TC fine powders, BGF and TC-MGF anodes, (e) CVs of the BGF and TC-MGF anodes. CVs were scanned from -0.6 V to $+0.6$ V vs. SCE at 0.02 V/s.

Fig. 4 FESEM images of biofilm on the anode electrode surfaces after 6 day operation in the bioreactor (a) BGF bioanode, (b) TC-MGF bioanode, (c) and (d) the area (square) are magnified at 25000, and 6000, respectively.

Fig. 5 (a) The voltage and green arrow represents the beginning of one complete cycle, (b) color removal and leached out of the NAR-2 bacterial consortium [(■)TC-MGF bioanode; (▲) BGF bioanode; solid line, decolorization; dotted line, leached out, and (c) polarisation curves of the BGF and TC-MGF bioanodes at various resistances (300,000-10Ω) after 368h.

Fig. 6 FESEM images of the bioanode electrode surfaces after 16 days operation in MFCs (a) TC-MGF bioanode, (b) BGF bioanode, (c) and (d) the attachment of sessile cells was observed within the EPS on the TC-MGF bioanode (squares) are magnified, and (e) the area on the BGF bioanode (square) is magnified.

Fig. 7 (a) CVs BGF and TC-MGF bioanodes, (b) ATR-FTIR of BGF and TC-MGF bioanodes surfaces after 16 days operation in MFCs, (c) UV-vis spectral change of AR 27 in BGF and TC-MGF-MFCs at four cycles operating times, and (e) Proposed degradation mechanism of AR27 on the BGF and TC-MGF-MFCs.

List of Table:

Table 1. Compression of the characteristics of the BGF and TC-MGF anodes

Figure

b)

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Fig. 7

Table 1

Anode material	BET SSA (m ² g ⁻¹)	Resistance (Ω)	Weight (g m ⁻¹)	Thickness
BGF	1.2	0.7	0.11	0.3mm
TC-MGF	9.8	1.0	0.18	0.5mm