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Research review paper

Production of bioelectricity, bio-hydrogen, high value chemicals and bioinspired nanomaterials by electrochemically active biofilms

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ABSTRACT

Microorganisms naturally form biofilms on solid surfaces for their mutual benefits including protection from environmental stresses caused by contaminants, nutritional depletion or imbalances. The biofilms are normally dangerous to human health due to their inherited robustness. On the other hand, a recent study suggested that electrochemically active biofilms (EABs) generated by electrically active microorganisms have properties that can be used to catalyze or control the electrochemical reactions in a range of fields, such as bioenergy production, bioremediation, chemical/biological synthesis, bio-corrosion mitigation and biosensor development. EABs have attracted considerable attraction in bioelectrochemical systems (BESs), such as microbial fuel cells and microbial electrolysis cells, where they act as living bioanode or biocathode catalysts. Recently, it was reported that EABs can be used to synthesize metal nanoparticles and metal nanocomposites. The EAB-mediated synthesis of metal and metal–semiconductor nanocomposites is expected to provide a new avenue for the greener synthesis of nanomaterials with high efficiency and speed than other synthetic methods. This review covers the general introduction of EABs, as well as the applications of EABs in BESs, and the production of bio-hydrogen, high value chemicals and bio-inspired nanomaterials.

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1. Introduction

Electrochemically active biofilms (EABs) generated by electrically active microorganisms have attracted significant attention for their potential applications in bioenergy and green chemical production (Borole et al., 2011; Erable et al., 2010). EABs can be found in a variety of natural sites, such as sea water sediments, soils and a range of wastewaters, such as municipal, dye, and swine wastewaters (Erable et al., 2010; Logan and Rabaey, 2012). Normally, dissimilatory

metal-reducing bacteria use iron or manganese oxides as electron acceptors for their respiration (El-Naggar et al., 2008; Lovley, 2011). Recent studies have shown that EABs can use a polarized electrode as the final electron acceptor by attaching to a conducting electrode (Logan and Rabaey, 2012; Pant et al., 2012). These findings allow EABs to function as living biocatalysts in bioelectrochemical systems (BESs). The transfer mechanism of electrons from EABs to the conducting electrode has been studied extensively but the actual mechanism is not completely understood (El-Naggar et al., 2008; Malvankar et al., 2011, 2012c; Polizzi et al., 2012; Reguera et al., 2005; Snider et al., 2012; Strycharz-Glaven and Tender, 2012; Strycharz-Glaven et al., 2011). Three major electron transport mechanisms have been used to

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explain electron transfer from a biofilm to an electrode surface. The first model proposes that electrons are transported using redox mediators, which can be produced by the bacteria themselves or added externally (Coursolle et al., 2010; Marsili et al., 2008b; Park and Zeikus, 2002). The second model proposes that electrons are transported by electron hopping between the c-type cytochromes in *Geobacter sulfurreducens* biofilms and pili or nanowires (Polizzi et al., 2012; Strycharz-Glaven et al., 2011). A recent model proposed that electrons are transported via pili, which possess delocalized electronic states to function as protein wires with metallic-like conductivity, without the involvement of c-type cytochromes (Lovley, 2011; Malvankar et al., 2011, 2012c). This live wire can provide direct extracellular electron exchange for bioenergy and the bioremediation of energy-related contamination (Lovley, 2011). Fig. 1a shows the potential applications of EABs in a range of fields.

2. Characterizations of EABs

EABs can be developed on an electrode surface of BESs. Four types of acclimatization procedures are normally used to populate EABs on an electrode surface (Babauta et al., 2012). EABs can be grown on an electrode surface in closed circuit mode by connecting the biofilm electrode with a supporting electrode through an external resistor. Here, the electrode acts as a sole electron acceptor for biofilm respiration and the EAB activity can be monitored directly from the observed current generation. The second method is to develop EABs on an electrode in open circuit mode in the presence of a soluble electron acceptor, such as fumarate, and the EAB can be switched to respire on the

biofilm electrode after achieving the desired state. The third acclimatization step uses a controlled cell potential by applying a constant potential between the biofilm electrode and supporting electrode. EABs can also be grown by applying a constant polarization potential between the biofilm electrode and reference electrode (Bonanni et al., 2012). EABs differ structurally according to the acclimatization procedures (Inoue et al., 2011; Nevin et al., 2009). Therefore, it is important to characterize EABs to better understand their behavior and efficiency. Rittmann et al. (2008) suggested that a pre-genomic, genomic and post-genomic study of microbial communities involved in EABs would be essential for understanding the structure and activity of these microbial communities.

Many methods available have been used to examine the performance of EABs in BESs. Confocal laser scanning microscopy can be used to visualize the growth and thickness of EABs on electrodes (Malvankar et al., 2011). Cyclic voltammetry (CV) is used to measure the catalytic activity of EABs but the application of CV to a living bacterial biofilm requires limiting the potential range to prevent harmful oxidation or reduction conditions with the selection of informative scan rates (Fricke et al., 2008; Marsili et al., 2008a). CV analysis at low scan rates revealed stable catalytic features of EABs. Under this condition, the rate of electron transfer from the EAB to the electrode was enhanced. The CV technique can also be used to examine the rate limiting steps in current generation by EABs and whether extracellular electron transfer occurs in EABs (Babauta et al., 2012; Katuri et al., 2012; Strycharz et al., 2011). Square wave voltammetry (SWV) is used to detect low concentrations of electrochemically active species not easily detectable by CV analysis (Babauta et al., 2012). Differential pulse voltammetry (DPV) analysis is normally used to better understand the redox behavior of EABs and is often used as a complementary technique to CV (Marsili et al., 2008a). Electrochemical impedance spectroscopy (EIS) is a solid analytical technique for examining biofilm growth, biofilm conductivity and electron transfer mechanisms (Dominguez-Benetton et al., 2012; Malvankar et al., 2012b). Electron transfer from a biofilm to an electrode surface can be examined by surface-enhanced resonance Raman spectroscopy in combination with CV (Millo et al., 2011). Confocal Raman microscopy is a non-invasive label-free and in vivo characterization method to analyze EABs. This method is based on the resonance Raman effect of the heme protein cytochrome C, which can provide characteristic spectral information of EABs at a pixel integration time and excitation wavelength of 0.2 s and 532 nm, respectively (Viridis et al., 2012). This will allow the monitoring of biofilm development at different growth stages without affecting its structural or metabolic activity. UV–vis spectroscopy can be used to analyze the redox state of c-type cytochromes on biofilms (Babauta et al., 2012).

3. Bioelectricity

EABs are used as living bioanode catalysts in microbial fuel cells (MFCs) to generate electricity (Kim et al., 2012; Logan and Rabaey, 2012; Pant et al., 2012). The EAB oxidizes organic substrates, such as acetate, to electrons, protons and CO₂ without combustion. The electrons produced are transferred through an external circuit, whereas the protons migrate to the cathode via a cation exchange membrane and react with oxygen to produce water (Fig. 1b). Many electron acceptors other than oxygen, such as permanganate (You et al., 2006), chromium hexacyanoferrate (Amutha et al., 2010) and azo dyes (Liu et al., 2009), are also used in MFC cathodes. The most striking feature of this technology is that a simultaneous wastewater treatment and bioelectricity generation can be achieved without the need for energy input. In addition, a wide range of wastewaters, such as industrial wastewater and dye wastewater, can be used readily as an anode fuel in MFCs (Pant et al., 2010a). Aromatic hydrocarbons, phenols and pyridines can also be used as substrates in a MFC anode suggesting the possibility of using a wide range of organic

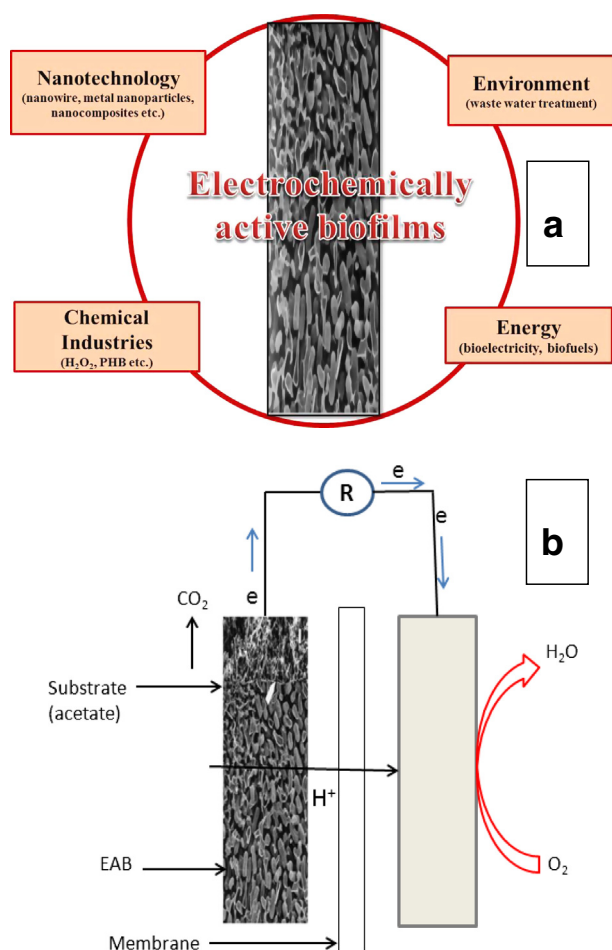


Fig. 1. (a) Applications of electrochemically active biofilms in various fields. (b) Typical microbial fuel cell diagram showing the anode and cathode reactions.

substrates to generate electricity (Lovley and Nevin, 2011). Electrically active microorganisms present in wastewater develop EABs on the anode surfaces, which in turn act as excellent biocatalysts. Both pure culture bacteria (Bond and Lovley, 2003; Chaudhuri and Lovley, 2003; Kim et al., 1999; Pham et al., 2003) and mixed culture bacteria can be used in MFC bioanodes to generate electricity. The use of a mixed culture, however, is more practical than a pure culture because in nature, microbial communities are mixed and the use of a pure culture is inconvenient (Kim et al., 2007; Rittmann et al., 2008). MFCs operated by a mixed culture generally show a higher power density than pure cultures, and pure cultures generally metabolize a limited range of organic compounds (Kim et al., 2007). Moreover, mixed culture bacteria can respond dynamically to changing conditions. MFCs can be used to sense the biological oxygen demand (Chang et al., 2004, 2005; Kim et al., 2003) and a MFC can be used as a tool for examining the microbiology of EABs.

The electrode support plays a critical role in the development and growth of EABs (Pant et al., 2011; Pham et al., 2009). High EAB formation was reported to result in higher power generation in MFCs (Malvankar et al., 2012b) and the characteristics of the anode material affects biofilm formation (Liu et al., 2007; Lowy et al., 2006). Therefore, it is better to use electrode supports with high surface areas and conductivity to design efficient bioanodes. Plain carbon and graphite materials are commonly used as bioanodes in MFCs (Pham et al., 2009; Wei et al., 2011). On the other hand, their poor conductivity compared to metals has restricted their applications. Thus, materials with high conductivity should be investigated (Pham et al., 2009). Carbon nanotubes (CNTs) are better candidates for bioanodes owing to their excellent conductivity and high surface area (Pham et al., 2009; Qiao et al., 2007). CNTs with conducting polymers, such as polyaniline (Qiao et al., 2007) or polypyrrole (Zou et al., 2008), also exhibit high power densities in MFCs. Qiao et al. (2008) proposed a nanostructured polyaniline/titanium dioxide composite as an anode material for high power generation in MFCs. Granular activated carbon (GAC) is also an effective bioanode in MFCs owing to its high surface area, which is beneficial for microbial attachment (Kalathil et al., 2012a). In addition, GAC exhibits high conductivity and an ability to absorb toxic organic compounds that may inhibit electrically active microorganisms (Liu et al., 2012). The bioanode performance can be enhanced by treating carbon materials with ammonia gas that can increase the bacterial attachment to a positively charged anode (Logan et al., 2008). An anaerobically-developed *Shewanella* biofilm on an electrode modified with iron oxide nano-colloids showed excellent bioanode performance in MFCs (Nakamura et al., 2009). Iron oxide nanoparticles can force the cells to self-assemble into an interconnected bacterial network that can serve as an electrical linkage owing to its n-type semiconductivity. *Shewanella oneidensis* MR-1 formed on a nanoparticle (Au or Pd) coated graphite anode in a MEC enhanced the current output and the Au coated electrode produced a 20-fold higher current density than the plain graphite anode (Fan et al., 2011). This enhanced performance of the bioanode might be due to the excellent bacteria–electrode interactions by the conducting nanoparticles. In addition, nanoparticles can alter the surface hydrophobicity and surface roughness, which will enhance bacterial attachment to the electrode surface. A bioanode with a hierarchical porous structure consisting of a graphite felt and polyaniline nanowire network in MFCs produced 10–100 fold improvements in current generation (Zhao et al., 2010). This might be due to the efficient electron collection from the biofilm to the anode electrode. The extension of this work on bioanodes modified with hydroxylated and aminated polyaniline nanowire networks largely improved the current generation in MFCs considerably (Zhao et al., 2011). The significant improvement in current generation might be due to the high specific surface area and improved electron-transfer kinetics. Recently, EABs formed on an ultra-microelectrode exhibited a high current density due to the improved electron transport network in the biofilm,

which allowed the EAB to grow to a greater thickness (Pocaznoi et al., 2012). Chen et al. (2011) reported that 3D-carbon fiber electrodes prepared by electrospinning and solution blowing are excellent candidates as anode materials in BESs. The modified anode produced a current density of up to 30 A/m², which is one of the highest values for EABs reported so far. Recently, carbonized stems derived from crop plant kenaf have been used as anode materials in MFCs (Chen et al., 2012a,b) and the current produced by the modified anode was comparable to that of other 3D anodes prepared using other methods. The anode material was derived from abundant natural resources, making it renewable and affordable for practical applications. Yu et al. (2011) designed a conducting artificial biofilm (CAB) using a conducting matrix interlocked with the conducting polymer chains of polypyrrole, within which *S. oneidensis* MR-1 was wrapped with micro-sized graphite. The CAB produced an eleven fold increase in power output compared to natural biofilms. Yong et al. (2012) reported that a dramatic enhancement of bioelectricity generation in MFCs can be achieved by increasing the intracellular releasable electrons with the abolishment of the lactate synthesis pathway, which can be transferred to the anode via a secreted diffusive electron shuttle. The cathodes are also considered one of the greatest stumbling blocks for MFC/MEC scale-up (Alvarez-Gallego et al., 2012; Pant et al., 2010b; Zhang et al., 2011). The activated carbon air cathode appears to be a suitable alternative for Pt based cathodes with long-term performance (Zhang et al., 2011). Gas porous electrodes can be utilized effectively as air cathodes in MFCs (Pant et al., 2010a,b).

Many inherent problems still hinder the large scale applications of MFCs, such as poor electricity generation, high internal resistance, complicated architecture and expensive materials (Kalathil et al., 2012a; Pant et al., 2011). Over the last few years, many attempts have been made to improve the MFC efficiency in terms of electricity generation and affordability. Recently, Kalathil et al. (2011a) proposed a GAC-based, two-chambered-MFC for the simultaneous decolorization of real dye wastewater and bioelectricity generation (Fig. 2a). The GAC-MFC produced a power density of 1.7 W/m³ with a total decolorization of 75% including the anode and cathode. This study was extended by modifying the MFC architecture to a single chamber GAC-biocathode MFC (Fig. 2b) for the large scale treatment of real dye wastewater (Kalathil et al., 2012a). The modified MFC architecture generated an enhanced power density of 8 W/m³ with efficient decolorization. Fig. 3 shows a photograph of the real dye wastewater before and after the MFC treatment. The proposed MFC (2.5 L, liquid volume) architecture was found to be suitable for the large scale treatment of dye wastewater with considerable bioelectricity generation, and is expected to revolutionize the bioenergy and bioremediation sectors. The bioelectricity produced by MFCs can be used as a source of energy to power electronics with low power consumption (Donovan et al., 2008; Shantaram et al., 2005). This type of MFC application has attracted considerable attention for practical applications of MFCs.

4. Bio-hydrogen

Hydrogen is a green fuel that is almost free of CO₂ and other pollutant emissions. Hydrogen can be produced from a wide range of resources including renewables, nuclear energy and fossil fuels. Currently, hydrogen is produced mostly from non-renewable fossil fuels, which is unsustainable in the long term. Water electrolysis is used for hydrogen production but the process is limited by the need to supply huge amounts of electricity. In this respect, the developments of advanced technologies for producing hydrogen from renewable resources that minimize the environmental impact have attracted widespread scientific attention.

Bio-hydrogen production from non-fossil fuels will provide a green chemical approach for the hydrogen economy. Fermentative

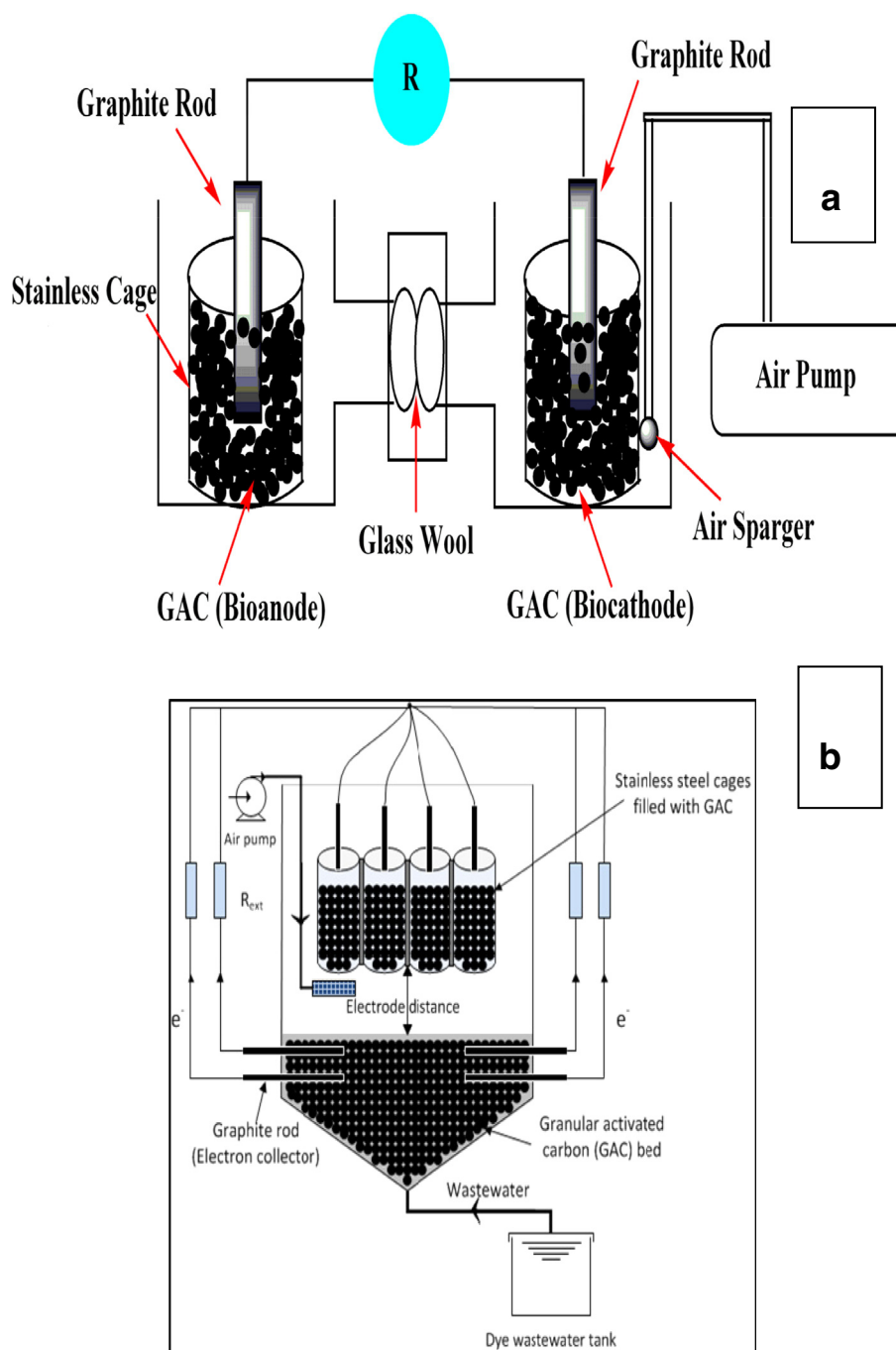


Fig. 2. (a) Schematic diagram of GAC-based two-chambered microbial fuel cell for the simultaneous decolorization of real dye wastewater and bioelectricity generation (Kalathil et al., 2011a). (b) Schematic diagram of a GAC-biocathode single-chamber microbial fuel cell for the simultaneous decolorization of real dye wastewater and bioelectricity generation (Kalathil et al., 2012a).

hydrogen production appears to be a promising technology but the low hydrogen yield hinders its practical applications (Geelhoed et al., 2010). Recently, bio-hydrogen production by EAB without fermentation was reported which was catalyzed by positively charged gold nanoparticles (Khan et al., 2013a). In recent past, microbial electrolysis cells (MECs) have emerged as a potential technology for efficient hydrogen production from renewable resources (Liu et al., 2005, 2010; Logan et al., 2008). The anode part of a MEC is similar to a MFC, where the EAB growing on the anode surfaces breaks down the organic substrates to CO_2 , electrons and protons. The electrons and protons travel through the external circuit and membrane, respectively, and combine at the cathode to generate hydrogen using a small

amount of external electricity. Hence, the cathode part is similar to a water electrolyzer. On the other hand, the external electricity required for MECs to produce hydrogen is much lower than the theoretical value of 1.23 V that is required for water electrolysis (Liu et al., 2010). In MECs, electrically active microorganisms can utilize a range of organic substrates, such as complex mixtures of biomasses including municipal, animal and dye wastewaters. This is unlike fermentative hydrogen production, which is limited to pure compounds of sugars, alcohols and carboxylic acids.

Considerable research effort has been undertaken to produce hydrogen in MECs by developing a variety of MEC architectures and electrode materials (Liu et al., 2010). Normally, chemical catalysts,

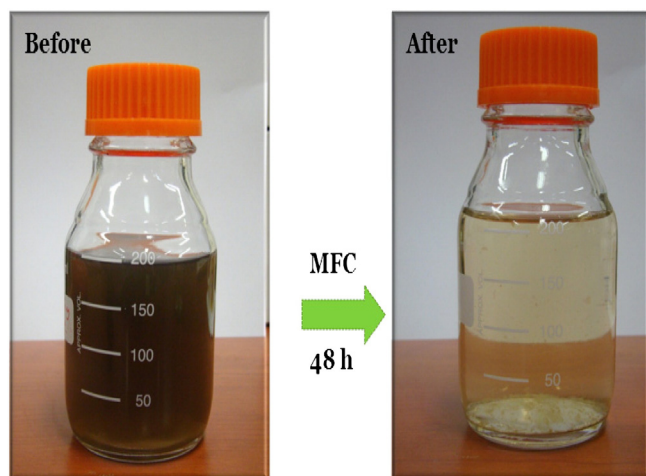


Fig. 3. Photograph of real dye wastewater before and after the microbial fuel cell treatment (48 h).

such as platinum, nickel, or tungsten carbides, are used as cathode electrodes in MECs (Geelhoed et al., 2010). Hu et al. (2009) designed a single chamber tubular MEC using NiMo as the cathode material for the production of hydrogen. The MEC produced a hydrogen production rate of $2 \text{ m}^3/\text{day}/\text{m}^3$ at an applied voltage of 0.6 V, which was comparable to expensive Pt catalysts. Recently, CNT-based cathode materials have been used in MECs for the production of hydrogen (Wang et al., 2012). Sun et al. (2008) designed a MEC–MFC coupled system for bio-hydrogen production from acetate. In this system, the MFC acted as an external energy source to produce hydrogen at the MEC cathode. A solar-powered MEC with a Pt catalyst-free cathode was reported for the production of hydrogen (Chae et al., 2009). Because both the MFC and solar cell use renewable energy, the application of them as the external energy sources for MEC operation can extend this technology in practical fields. Interestingly, some microorganisms were reported to also catalyze hydrogen production in MEC cathodes (Rozendal et al., 2008). Croese et al. (2011) examined the microbial community of the biocathode responsible for hydrogen production in MECs and reported *Proteobacteria* to be the most populated community in the biocathode. The biocathode produced $0.63 \text{ m}^3 \text{ H}_2/\text{m}^3$ cathodic liquid volume per day. A MEC with an anion exchange membrane produced hydrogen from acetic acid with an energy efficiency of 82% at an applied voltage of 0.6 V (Cheng and Logan, 2007). Recently, a modified MEC using the energy derived from the salinity gradient between seawater and river water produced hydrogen at a rate of 0.8 mL/min (Kim and Logan, 2011). Nam et al. (2012) reported hydrogen generation in microbial reverse-electrodialysis electrolysis cells (MRECs) using a heat generated salt solution. The MRECs used the current derived from organic matter and salinity-gradient energy, such as river water and seawater solutions, for the generation of hydrogen. The system produced hydrogen at a maximum rate of $1.6 \text{ m}^3 \text{ H}_2/\text{m}^3/\text{day}$ and a salinity ratio of infinity. Zhang and Angelidaki (2012) proposed a novel self-powered submersible MEC for bio-hydrogen production from anaerobic reactors with a maximum rate of 32.2 mL/L/day. Lu et al. (2012) reported that syntrophic interactions combining glucose fermentation with the oxidation of fermentation products by exoelectrogens can lead to the production of bio-hydrogen from glucose at low temperatures in MECs. This finding showed that low temperature MECs can be used for the production of bio-hydrogen from the abundant biomass of carbohydrates. Recently, Kalathil et al. (2012b) reported hydrogen production in a MFC cathode via the quantized capacitance charging effect of gold nanoparticles by maintaining an acidic cathode. The MFC showed a hydrogen production rate of 1.5 mL/h with a hydrogen yield of 0.69 mL H_2 /mg COD. The hydrogen produced by the MEC was four times more efficient than the classical systems based on

water electrolysis (Erable et al., 2010; Halan et al., 2012). On the other hand, MEC technology is still in its infancy due to several inherent drawbacks, such as poor anode–cathode performance and high internal resistance. In addition, the requirement of an external energy supply is the main challenge for the practical applications of a MEC system.

5. High-value chemicals

BES technology is used to generate many high-value chemicals from inexpensive substrates and is expected to be a key process in future bioproduction (Logan and Rabaey, 2012; Rabaey and Rozendal, 2010). Srikanth et al. (2012) observed the production of polyhydroxyalkanoates (PHA) at the biocathode of a BES under a microaerophilic microenvironment. The low dissolved oxygen level at the biocathode initiated PHA synthesis as an alternative pathway to re-oxidize the NADH. The EABs formed on the bioanode can produce polyhydroxybutyrate (PHB) or C3 and C4 chemicals, such as amino acids and lactic acids from organic substrates (Pham et al., 2009). The EABs formed by *Pseudomonas* species can produce phenazine, which is a quorum-sensing biomolecule that acts as an electron shuttling agent from the biofilm to the anode (Rabaey et al., 2005). In addition, phenazine is an excellent antibacterial agent (Pham et al., 2009). *Shewanella* secretes flavin, which is a redox-active molecule that transfers electrons to the electrode surface (Marsili et al., 2008a,b). Kuntke et al. (2012) reported that MFCs can be used as a novel approach for ammonium recovery from urine. Recently, ethanol was reported to be produced by the reduction of acetate with methyl viologen as a mediator at the MEC cathode (Steinbusch et al., 2010). On the other hand, the use of a mediator is unsustainable, and the non-mediated reduction of acetate by growing microorganisms at the electrode surface should be developed (Pant et al., 2012). Cathodic hydrogen peroxide (H_2O_2) production in MECs appears to be promising for practical applications by offering a more sustainable approach than existing methods (Rabaey and

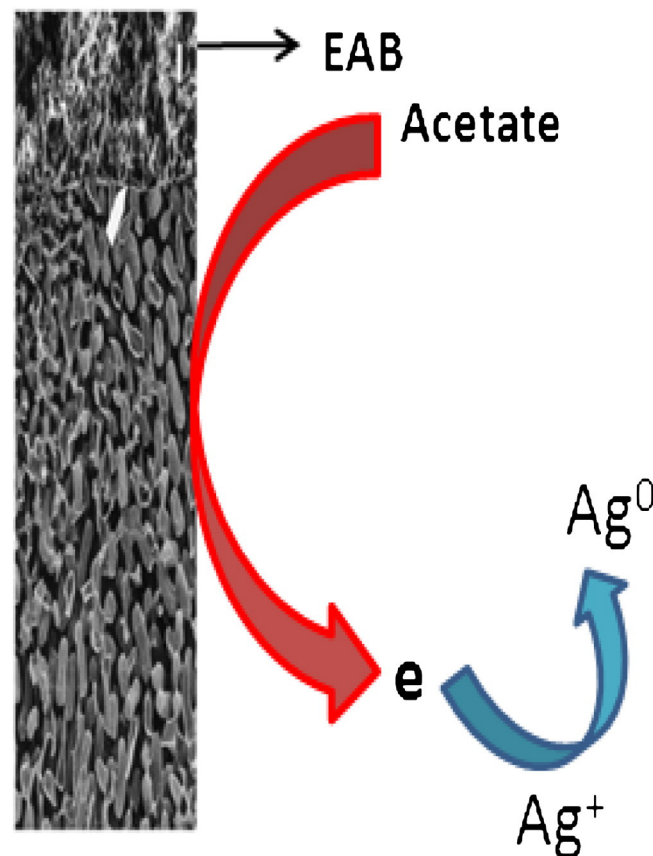


Fig. 4. Mechanism involved in the synthesis of silver nanoparticles by electrochemically active biofilm.

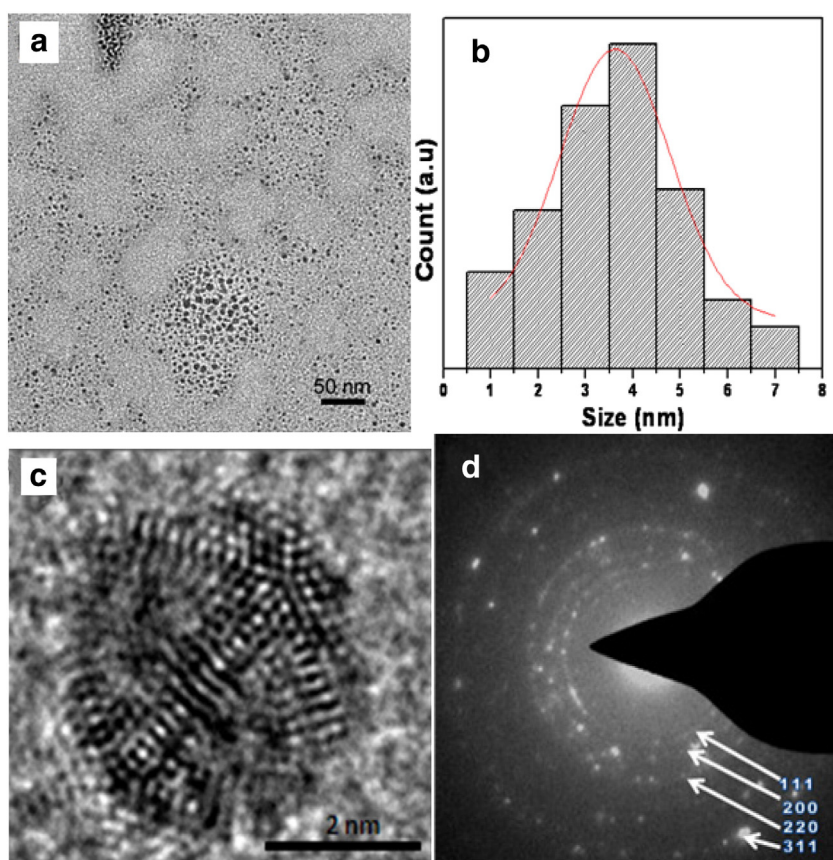


Fig. 5. (a) TEM image of as-synthesized silver nanoparticles. (b) The particle size distribution of the as-synthesized silver nanoparticles. (c) High resolution TEM image of an individual silver nanoparticle. (d) Selected area diffraction (SAED) pattern (figure drawn with modifications after Kalathil et al., 2011b).

Rozendal, 2010). Cathodic H_2O_2 production can be achieved without the use of expensive catalysts (Rabaey and Rozendal, 2010; Rozendal et al., 2009). H_2O_2 is a strong oxidizing agent that is used in the bleaching industry and for chemical syntheses. Rozendal et al. (2009) reported a H_2O_2 production rate of $\sim 1.9 \pm 0.2 \text{ kg H}_2\text{O}_2/\text{m}^3/\text{day}$ from acetate at an applied voltage of 0.5 V in a MEC. The external electricity required for H_2O_2 production in a MEC is much lower than that for conventional electrochemical systems (Ando and Tanaka, 2004; Foller and Bombard, 1995). Rabaey et al. (2010) reported that BES can be used to produce caustic soda at the cathode. Recently, a microbial electrolysis desalination and chemical production cell were used for desalination as well as for the production of HCl and NaOH in the cell (Chen et al., 2012a,b).

6. Bioinspired nanomaterials

The studies on *Shewanella* nanowires showed non-linear transport behavior indicating discrete energy levels with a higher electronic

density of states on the bacterial nanowires (El-Naggar et al., 2008). These surprising nonlinear electrical properties discovered for bacterial nanowires might be fascinating for applications in molecular bioelectronics. Recently it was reported that pili nanofilaments, known as microbial nanowires, extracted from *G. sulfurreducens* exhibit comparable metallic-like conductivity to synthetic metallic nanostructures, such as polyaniline and polyacetylene (Malvankar et al., 2011). The nanowires showed similar structural features to synthetic organic metals. X-ray diffraction (XRD) revealed a highly ordered structure of pili filaments as well as π stacking, which explains the metallic conductivity. This may have applications in nanobioelectronics in the near future. Malvankar et al. (2012a) reported that c-type cytochromes present in the conducting matrix of *G. sulfurreducens* biofilms impart large capacitance to the biofilms that is comparable to synthetic supercapacitors, exhibiting the behavior of a living supercapacitor. This finding highlights the new possibilities for the design of capacitors that are inexpensive and

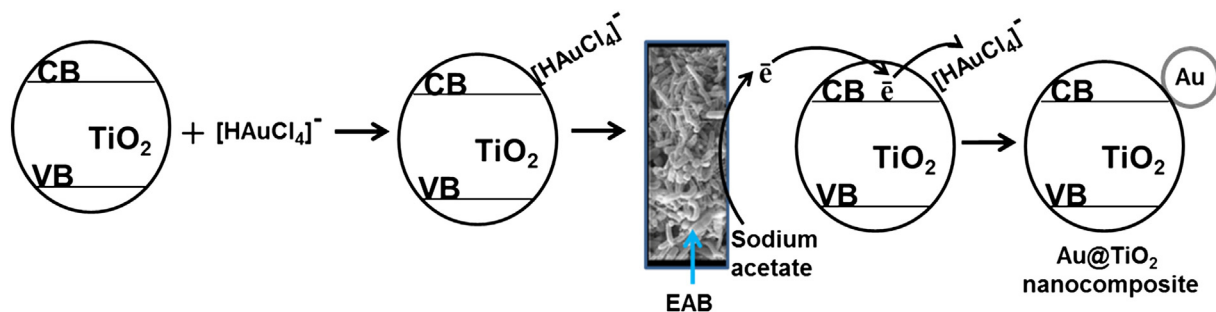


Fig. 6. Schematic diagram of the proposed synthesis mechanism for Au@TiO₂ nanocomposite by the electrochemically active biofilms (figure drawn with modifications after Kalathil et al., 2012b).

environmentally-sustainable. Another striking feature is that they can work under normal aqueous and acidic environments.

Nanomaterial synthesis is a very exciting field because of its potential applications in bioenergy, catalysis (Lewis, 1993), electronics (Li et al., 2005), optics (Murphy et al., 2005), medicine (Salata, 2004) and environmental remediation (Gupta et al., 2011). A range of methods (chemical, physical and biological) are available for the synthesis of metal nanoparticles and metal-semiconductor

nanocomposites (Kalathil et al., 2011b, in press; Khan et al., 2012, 2013a,b). Chemical methods normally used for the synthesis of nanoparticles utilize strong reducing agents, such as NaBH_4 or citrate, as well as large quantities of chemicals that can contaminate the nanoparticles (Dorjnamjin et al., 2008). A large number of microorganisms, such as bacteria (Kalimuthu et al., 2008; Klaus et al., 1999; Nair and Pradeep, 2002; Zhang et al., 2005), fungi (Mukharjee et al., 2001; Vigneshwaran et al., 2007), and yeast (Kwowschik et al.,

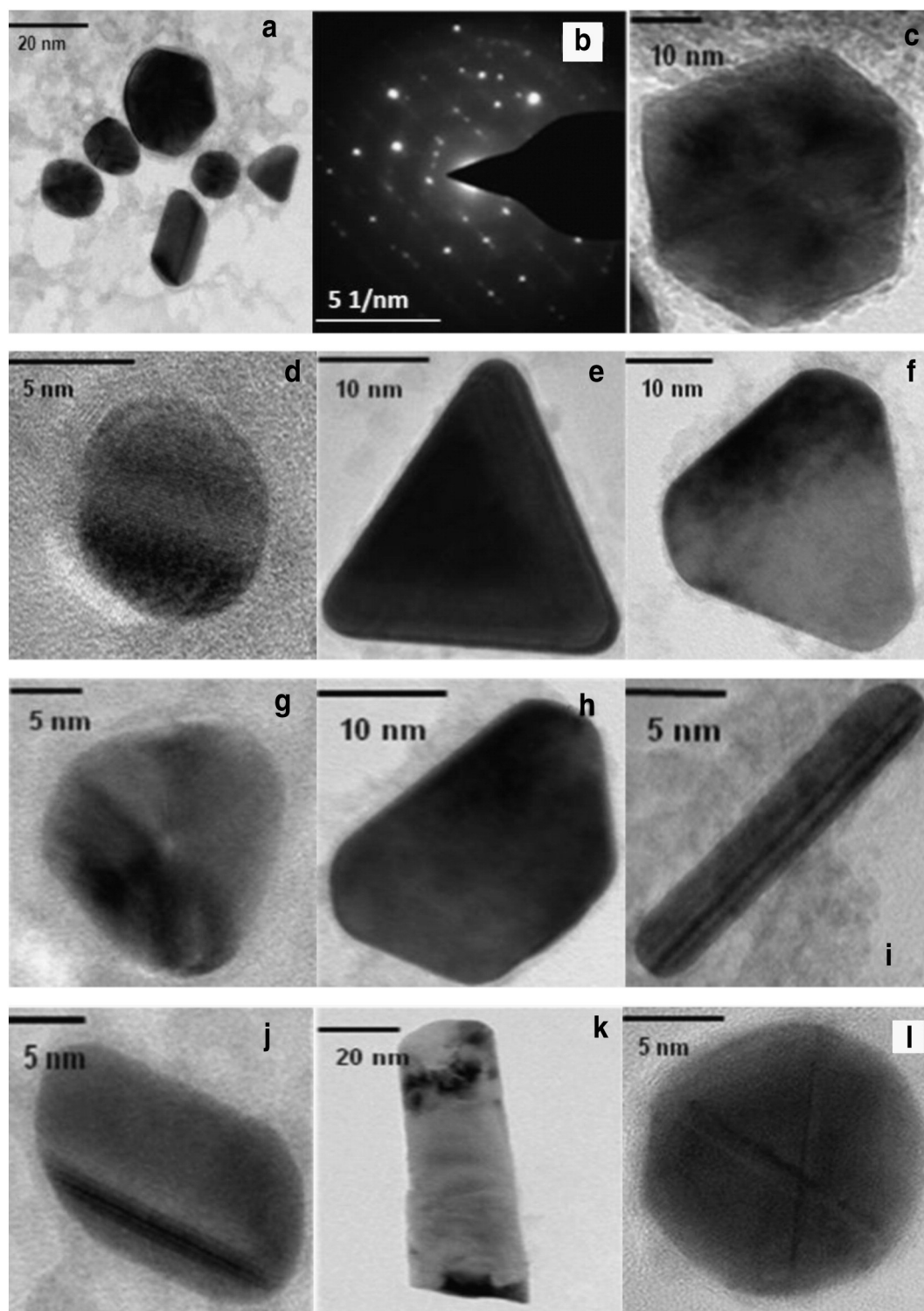


Fig. 7. TEM images of as-produced gold nanoparticles at microbial fuel cell-cathode with various exotic shapes. (a) Shows a low magnification TEM image of the as-produced gold nanoparticles, (b) SAED pattern of as-produced gold nanoparticles, (c) hexagonal, (d) geode, (e) triangle, (f) truncated triangle, (g) pyramidal, (h) boat, (i) pentagonal rod, (j) sand-wich, (k) and finger like structures of as-produced gold nanoparticles. (l) Spherical multiple twinned (figure drawn with modifications after Kalathil et al., 2013).

2003), have been used to synthesize nanoparticles, which suggest that these microorganisms can act as nanofactories.

Wu et al. (2011) reported that a sulfate-reducing bacterium, *Desulfovibrio desulfuricans*, produced Pd nanoparticles on the microbial surfaces when the bacterium was exposed to Pd^{II} cations. These biologically produced Pd nanoparticles bound to the cell membrane of the microbe participated in the direct extracellular electron pathways as well as electrocatalysis on an electrode surface. Recently Ag nanoparticles (AgNPs) were synthesized by an EAB using an AgNO₃ solution as a precursor and sodium acetate as the electron donor (Kalathil et al., 2011b). Fig. 4 shows a schematic diagram of AgNP production by the EAB. The EAB oxidized sodium acetate to produce electrons, which were used to reduce Ag⁺ ions. Transmission electron microscopy (TEM) showed that the as-synthesized AgNPs were extremely small with spherical morphologies and a mean size of 3.6 nm (Fig. 5c). XRD confirmed the high crystallinity of the as-synthesized nanoparticles. This synthesis protocol is far superior to most microbial synthesis methods. AgNPs production was rapid (2 h) compared to other sluggish microbial methods (consuming 24–96 h) (Kalimuthu et al., 2008; Klaus et al., 1999; Kwowshik et al., 2003; Mukharjee et al., 2001; Nair and Pradeep, 2002; Vigneshwaran et al., 2007; Zhang et al., 2005). In addition, the EAB produced nanoparticles with an extremely small size (3.6 nm) compared to other biological methods (10–50 nm) (Kalimuthu et al., 2008; Klaus et al., 1999; Vigneshwaran et al., 2007; Zhang et al., 2005). Nanoparticles within the range of 1–5 nm exhibit tremendous catalytic activity (Kamat, 2007; Primo et al., 2011; Tsunoyama et al., 2009). Moreover, unlike other intracellular productions of nanoparticles by microorganisms, the nanoparticles were produced extracellularly. The EAB-mediated synthesis of nanoparticles was extended to the synthesis of cysteine-capped AgNPs to protect the nanoparticles from aggregation (Khan et al., 2012). The as-synthesized cysteine-capped AgNPs showed excellent antibacterial properties against pathogenic bacteria, *Escherichia coli* (O157:H7) and *Pseudomonas aeruginosa* (PAO1). Au nanoparticles were also synthesized by EAB. TEM revealed the as-synthesized nanoparticles to have spherical morphologies (Kalathil et al., 2012b; Khan et al., 2013c). The as-synthesized Au nanoparticles were used as anode materials in dye sensitized solar cells (DSSC) by mixing with TiO₂ nanoparticles to enhance the electron transfer kinetics in the anode. The resulting DSSCs showed an improved efficiency of 13.5% (unpublished data). More recently, Au@TiO₂ and Ag@TiO₂ nanocomposites were synthesized by EABs (Kalathil et al., 2012b; Khan et al., 2013b). Fig. 6 presents the proposed mechanism for Au@TiO₂ synthesis by EABs. Initially, Au³⁺ ions are adsorbed on the TiO₂ surfaces during magnetic stirring and an as-prepared EAB is dipped into the solution with sodium acetate as the electron donor. The electrons produced by the oxidation of acetate are injected into the conduction band (CB) of the TiO₂ nanoparticles. The electrons accumulated in the CB of the TiO₂ nanoparticles are transferred to the Au³⁺ ions adsorbed on the TiO₂ surfaces and are reduced to metallic Au nanoparticles. TEM showed that the TiO₂ surfaces were coated uniformly with ~8 nm diameter Au nanoparticles. The as-synthesized Au@TiO₂ showed excellent visible light active photocatalysis upon dye degradation. Recently Au nanoparticles with exotic structures, such as triangle, rod, spherical, hexagonal and pyramidal (Fig. 7) were produced at the cathode of an MFC (Kalathil et al., 2013). The anisotropic growth of the Au nanoparticles at the cathode was attributed to the difference in the partial pressure of hydrogen adjacent to the Au nuclei produced at the cathode. The SAED pattern (Fig. 7b) confirmed that as-produced Au nanoparticles were single crystalline in nature and the Au nanoparticles showed excellent quantized capacitance charging effects.

7. Future perspectives

In conclusion, EABs can be used as living biocatalysts in BESs for the production of bioelectricity, bio-hydrogen and value-added

chemicals. The EAB appears to act as a nano-factory to synthesize metal nanoparticles and nanocomposites. The EAB-mediated synthesis of nanoparticles paves a green chemistry approach for the efficient synthesis of a range of nanoparticles. The most fascinating feature of the EAB is that it can be found easily in a variety of natural sites, such as wastewater, and shows enhanced tolerance to environmental stresses, which is a beneficial characteristic for practical applications.

More research will be needed to explore the real potential of EABs. Although many studies have examined the electron transfer mechanism from EABs to the electrode surfaces, concrete evidence is still lacking. A molecular level understanding of the electron transfer mechanism is needed to achieve a better utilization of EABs in many applications. Density functional theory might be a suitable option for understanding the density of states on the microbial nanowires to substantiate the recent findings of the metallic-like conductivity of biofilms. In addition, further studies should examine how EABs interact with the metal nanoparticles that they come in contact with. As microbial nanowires are supposed to exhibit metallic-like conductivity, the metal nanoparticles may develop a nanowire–metal nanoparticle linkage that appears to act as a bi-metallic system. Bi-metallic systems have many potential benefits, such as excellent conductivity on a single-metallic system. Au nanoparticles can stimulate hydrogen-producing microorganisms via a quantum size effect, indicating the possible role of nanoparticles on the bioactivity of microorganisms (Zhang and Shen, 2007). Although this study was on hydrogen producing bacteria, similar behavior can be expected on electrically-active microorganisms. The formation of EABs on advanced materials, such as nanoparticles, has been neglected. Therefore, more study will be needed to determine how biofilms develop on advanced materials as an electrode surface. Moreover, the effect of external electricity on the EABs formed on the bioanode of MECs needs to be addressed in detail to better understand the physiological and microbial community changes in the EAB under an applied electric current.

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