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Enhanced depolluting capabilities of microbial bioelectrochemical systems by synthetic biology

Le Tao [a], Maoyong Song [a,b,c,*], Guibin Jiang [a,b]

[a] Key Laboratory of Environmental Nanotechnology and Health Effects, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, 100085, China
[b] State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Science, Beijing, 100085, China
[c] University of Chinese Academy of Sciences, Beijing, 100049, China

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ABSTRACT

Microbial bioelectrochemical system (BES) is a promising sustainable technology for the electrical energy recovery and the treatment of recalcitrant and toxic pollutants. In microbial BESs, the conversion of harmful pollutants into harmless products can be catalyzed by microorganisms at the anode (Type I BES), chemical catalysts at the cathode (Type II BES) or microorganisms at the cathode (Type III BES). The application of synthetic biology in microbial BES can improve its pollutant removing capability. Synthetic biology techniques can promote EET kinetics, which is helpful for microbial anodic electro-respiration, expediting pollutant removing not only at the anode but also at the cathode. They offer tools to promote biofilm development on the electrode, enabling more microorganisms residing on the electrode for subsequent catalytic reactions, and to overexpress the pollutant removing-related genes directly in microorganisms, contributing to the pollutant decomposition. In this work, based on the summarized aspects mentioned above, we describe the major synthetic biology strategies in designing and improving the pollutant removing capabilities of microbial BES. Lastly, we discuss challenges and perspectives for future studies in the area.

1. Introduction

Microbial bioelectrochemical system (BES) is widely considered as a promising sustainable technology for not only the electrical power recovery but also the wastewater treatment. In microbial BES, the microbial oxidation of organic matter takes place on the anode, yielding electrons and protons. The electrons released from electroactive microorganisms during metabolism are conveyed to the anode, which serves as the solid electron acceptor during the microbial electro-respiration process. Afterwards, these electrons are transported to the cathode through an external circuit, during which the chemical energy stored in the chemical bonds of organic matter is converted into the electrical energy [1,2]. In this process, the difference in redox potentials between the oxidation reaction in the anode and the reduction reaction in the cathode drives these electrons to flow spontaneously from the lower potential to the higher potential. In addition, when an external voltage is applied to that system, the electrons move in reverse order as compared with the direction mentioned-above and accordingly, the electricity is converted into the chemical energy (Fig. 1a). When microorganisms catalyze reactions on the anode, the electrode is called bioanode and when microorganisms catalyze reactions on the cathode, the electrode is then called biocathode [3,4].

Apart from the energy recovery, microbial BES are also able to be applied to the treatment of recalcitrant and toxic pollutants. Based on where the pollutant removing reaction occurs and who catalyzes the reaction accordingly, microbial BESs can be classified into three categories. Type I: The conversion of harmful pollutants into harmless products is microbially catalyzed by microorganisms at the anode (Fig. 1b). Type II: The conversion of harmful pollutants into harmless products is chemically catalyzed at the cathode (Fig. 1c). Type III: The conversion of harmful pollutants into harmless products is microbially catalyzed by microorganisms at the cathode (Fig. 1d). All these types

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* Corresponding author. Key Laboratory of Environmental Nanotechnology and Health Effects, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, 100085, China.
E-mail address: smsong@rcees.ac.cn (M. Song).

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share one common element: the bioanode. That is, the anodic substrate oxidation is catalyzed by microorganisms in all these types. Thus, by means of the electrochemical device, the microbial substrate metabolism and the electron generation processes are separated so as to enable the more flexible and well-controlled use of these electrons for subsequent application such as the pollutant removing.

2. Synthetic biology techniques to improve the performance of microbial BES on the pollutant removing

Conventional genetic engineering methods involving the cloning of individual genes from one microbe to another, which are usually template dependent and time-consuming. In contrast, to mimic biological traits or even create totally novel functionalities absent in nature, synthetic biology techniques assemble artificial cells from a series of standardized genetic building modules, with a special emphasis on the rational design and *de novo* synthesis of gene and genome by chemical methods that can expedite the process of genetic manipulation. Basic genetic engineering methods commonly used in synthetic biology include overexpressing key proteins, knocking out genes and genetically redirecting the metabolic flow, which play important roles in improving the performance of microbial BES on the pollutant removing. The principle for improving the efficiency of BES lies in the upregulation of electron flux between microbial catalysts and the electrode, which can be achieved largely through the optimization of microbial metabolism including reactant transport, uptake and oxidation, the enhancement of electron transfer rates within and between proteins as well as the improvement of cell adhesion and biofilm formation on the electrode surface. First, synthetic biology techniques offer the potential to promote extracellular electron transfer (EET) kinetics, which is helpful for microbial anodic electro-respiration, facilitating pollutant removing not only at the anode when the type I microbial BES device is used, but also at the cathode when the type II/III microbial BES devices are used. Further, direct overexpressing the pollutant removing-related enzymes in microorganisms by synthetic biology tools is conducive to the pollutant decomposition. Moreover, synthetic biology techniques offer opportunities to promote biofilm formation on the electrode, enabling more microorganisms (microbial catalysts) colonizing the electrode for subsequent catalytic reactions.

2.1. The mechanisms of EET

The EET between microorganisms and the electrode can be categorized into direct electron transfer (DET) and mediated electron transfer (MET). For instance, *Shewanella* spp. and *Geobacter* spp. are able to convey electrons directly to the electrode through physical contact between the electrode surface and outer-membrane c-type cytochrome (c-Cyts) as well as conductive nanowired pili, respectively [5]. Furthermore, *Shewanella* spp. can also transfer.

Electrons to the electrode by MET through self-secreted electron shuttles (ES) such as flavin mononucleotide (FMN) and riboflavin [6,7]. In addition, phenazine is another type of self-secreted ES used by *Pseudomonas aeruginosa* for EET.

Apart from the microbial EET system pumping electrons from the intracellular microbial metabolism to the electrode, which is called outward EET, several types of microorganisms possess the EET system capable of receiving electrons from the external solid electron donor such as the electrode. This sort of EET system can be named inward EET. For instance, the *P. aeruginosa* biofilm can be developed on the cathode of the BES reactor to catalyze nitrate reduction using electrons provided by the electrode [8]. Moreover, sulfate-reducing bacterium (SRB) is able to corrode the extracellular Fe (0), indicating SRB can transfer electrons from the oxidation of Fe (0), the external solid electron donor, to the cytoplasm through the inward EET system [9].

2.2. Synthetic biology techniques to accelerate EET kinetics

2.2.1. Synthetic biology techniques to enhance DET

The EET pathway of many electroactive bacteria such as *S. oneidensis* MR-1 is composed of a wide range of c-Cyts and oxidoreductases

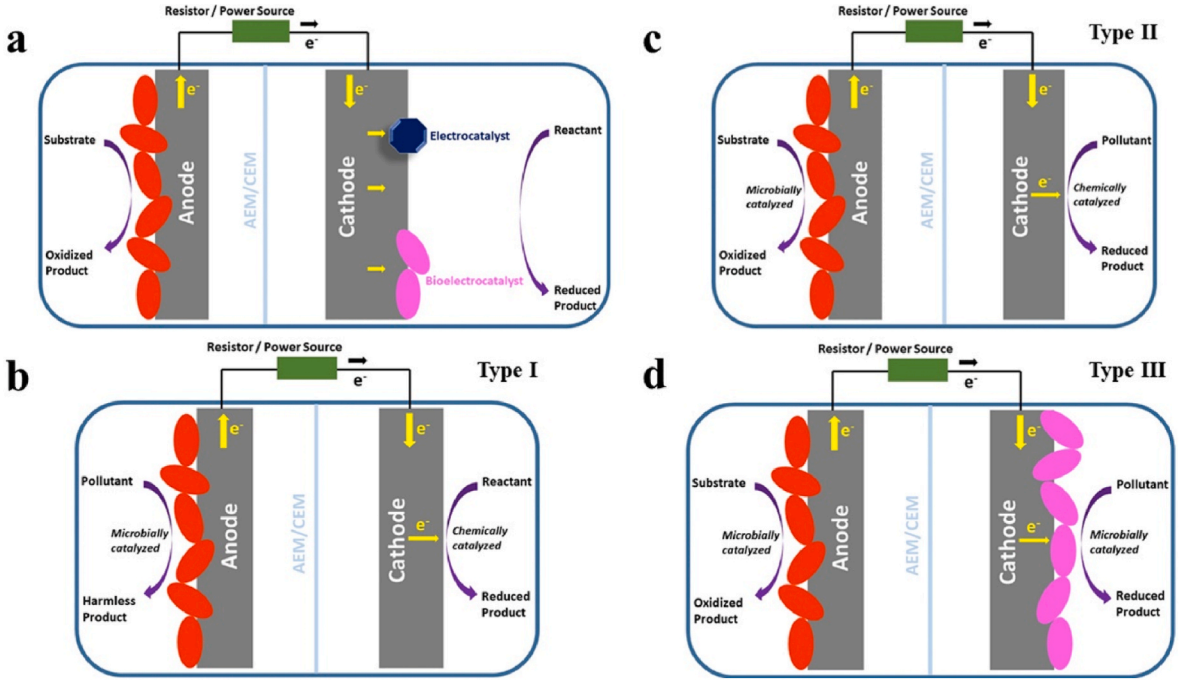

Fig. 1. (a) Schematic diagram of a typical microbial BES showing catalytic processes at the anode and the cathode surfaces and corresponding catalysts involved. The electricity either can be harvested through the external circuit or should be input into the system for the reaction to proceed. AEM, anion exchange membrane; CEM, cation exchange membrane. (b) Type I microbial BES device: The conversion of harmful pollutants into harmless products is microbially catalyzed by microorganisms at the anode. (c) Type II microbial BES device: The conversion of harmful pollutants into harmless products is chemically catalyzed at the cathode. (d) Type III microbial BES device: The conversion of harmful pollutants into harmless products is microbially catalyzed by microorganisms at the cathode.

localized at the cell membrane or in the periplasm. Electrons produced by the oxidation of the carbon source (electron donor) in the cytoplasm are transported to the quinol pool in the inner membrane, where the c-Cyts CymA, anchored at the inner membrane and facing the periplasm, acts as the quinol oxidase to transfer electrons from the quinol pool to a large number of oxidoreductases and electron carrier proteins stored in the periplasm [10]. Afterwards, electrons are further conveyed by these oxidoreductases and electron carriers to MtrCAB and OmcA system, the c-Cyts protein complex located in the outer membrane. Then, electrons can be delivered, directly or through self-secreted ES, to the electrode eventually [5,11].

According to previous investigations of the EET pathway, the CymA is overexpressed in the *S. oneidensis* MR-1, so that the mutant strain overexpressing CymA produces a higher maximum output power density of 0.13 W m^{-2} than that of the wild-type *S. oneidensis* MR-1 (0.11 W m^{-2}), suggesting that more respiratory electrons can be pumped into the EET through the overexpression of CymA (Fig. 2a) [10]. Furthermore, oxidoreductases and electron carrier proteins in the periplasm can be engineered too. For instance, in the periplasm, CctA, NapB and TsdB function as the electron carrier, one electron transfer subunit of nitrate reductase and one electron transfer subunit of thiosulfate oxidase, respectively. In addition, FccA functions both as the fumarate reductase and electron carrier protein (Fig. 2b). A mutant strain depleted of *FccA*, *NapB* and *TsdB* while overexpressing *CctA* is constructed and produces the maximum output power density of 0.4365 W m^{-2}, which is approximately 3.62-fold higher than that of the wild-type *S. oneidensis* MR-1, indicating that optimizing the composition of oxidoreductases and electron carrier proteins in the periplasm contributes to the improved efficiency of bringing electrons to the outer membrane MtrCAB and OmcA complex and consequently leads to the enhanced EET [12]. Moreover, the genetically engineered *Escherichia coli* strain heterogeneously overexpressing the outer membrane OmcA from *S. oneidensis* MR-1 in *E. coli* BL21 (DE3) is added in the anode of mixed bacteria-preinoculated microbial BES during operation, which produces the maximum output power density of 1.98 W m^{-2}, more than 2-fold higher than that of the control (Fig. 2c and d). A more compact biofilm is created after the addition of the mutant strain, reinforcing the electrocatalytic activity of the amended bioanode [13].

2.2.2. Synthetic biology techniques to enhance MET

P. aeruginosa PAO1 is known to secrete phenazines as soluble ES to enhance EET. The biosynthesis of phenazine is upregulated by the *P. aeruginosa* quinolone signal (PQS) system. However, the PQS signal suppresses the growth of *P. aeruginosa* PAO1 in the anodic compartment under anaerobic condition and accordingly, will do harm to the electrocatalytic activity of the bioanode. Since PqsE is able to strengthen phenazine biosynthesis in the absence of the PQS signaling system, an engineered strain overexpressing PqsE in the PQS signaling-abolished mutant strain is constructed. The genetically engineered strain yields pyocyanin, one type of phenazine, at a concentration of 165 nM which is more than 2-fold higher than that of the wild-type *P. aeruginosa* PAO1 (72 nM) and consequently, generates the maximum output power density of about 0.3 mW m^{-2}, which is nearly 5-fold higher than that of the wild-type strain (0.06 mW m^{-2}) [14]. Further, another.

Mutant strain overexpressing *phzM*, encoding the S-adenosylmethionine-dependent methyltransferase that catalyzes the conversion of phenazine-1-carboxylic acid to pyocyanin, is also constructed. The mutant strain produces 41.23 μg/mg of pyocyanin (wet-weight) which is 1.6-fold higher than that of the wild-type strain (15.89 μg/mg) and consequently, generates the maximum output power density of about 1.67 W m^{-2}, which is nearly 4-fold as much as that of the wild-type strain (0.43 W m^{-2}) [15].

Besides, the recombinant strain can also be utilized as a biocatalyst without occupying the anode surface. The *ribA, ribB, ribD,*

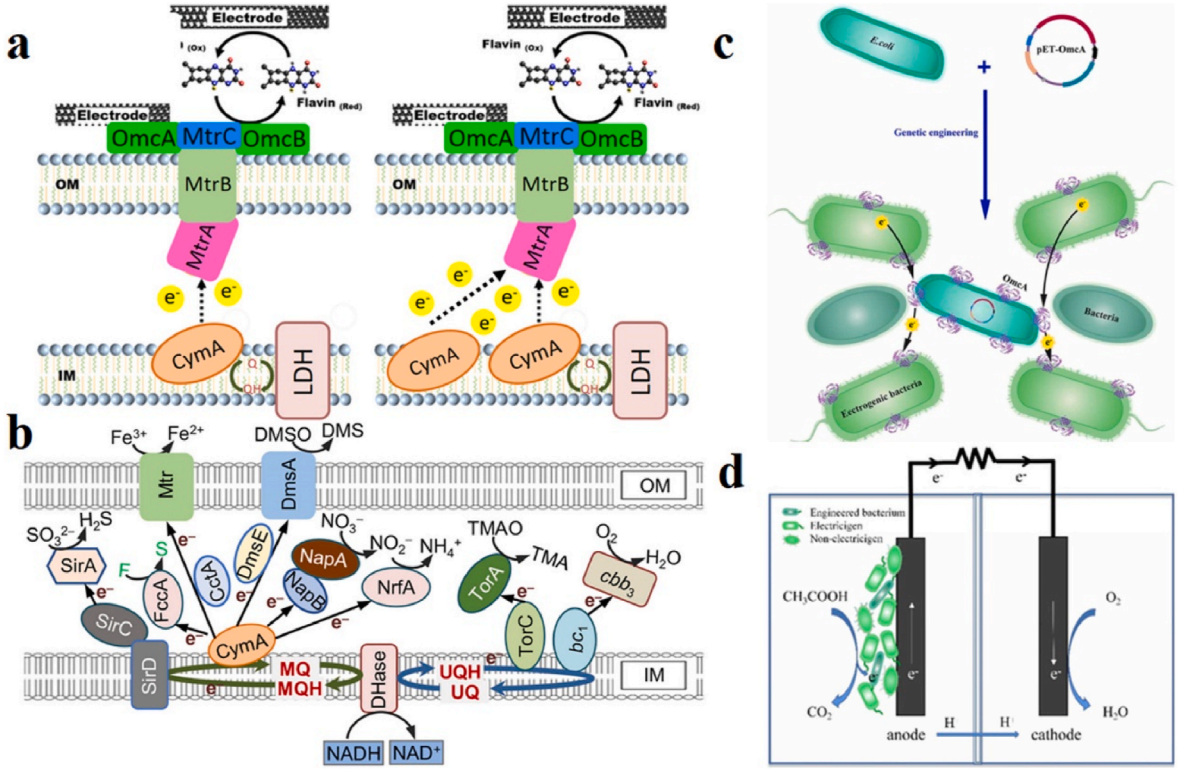

Fig. 2. Schematic diagram of (a) EET pathways in the wild-type *S. oneidensis* MR-1 and the mutant overexpressing CymA (reproduced with permission from John Wiley and Sons) [10]. (b) c-Cyts involved in EET pathways of *S. oneidensis* MR-1. Proteins written in black indicate c-Cyts while those in white are not (reproduced with permission from Frontiers in Microbiology) [12]. (c) Genetically engineered *E. coli* BL21 (DE3) overexpressing the OmcA and (d) engineered bacteria are added in the anode of mixed bacteria-preinoculated BES during operation to increase the power output (reproduced with permission from Elsevier) [13].

ribC and *ribE* genes of riboflavin biosynthesis are cloned and introduced into *E. coli* BL21 (DE3). The genetically engineered strain is then immobilized as alginate beads and placed at the bottom of the microbial BES reactor to anaerobically produce riboflavin without occupying the anode (Fig. 3). The *S. oneidensis* MR-1 bioanode loaded with these riboflavin-secreting bio-cocatalyst beads generates the maximum output power density of approximately 1.08 W m^{-2}, which is nearly 9.6-fold as much as that of the control bioanode without these bio-cocatalyst beads (0.11 W m^{-2}) [6]. A mutant *E. coli* strain overexpressing the glycerol dehydrogenase GldA also displays the production of soluble ES in the reator and generates the maximum output power density of approximately 0.64 W m^{-2}, which is more than 2-fold higher than that of the wild-type strain (0.27 W m^{-2}) [16].

2.3. Synthetic biology to promote biofilm formation on the electrode

The formation and functioning of biofilm on the electrode is also crucial to the performance of microbial BES. The mutant *S. oneidensis* MR-1 strain with the deletion of *SO3177* gene, encoding the formyltransferase that takes part in the biosynthesis of cell surface polysaccharides (CPS), exhibits more hydrophobic cell surface and enhanced bacterial attachment to the electrode and consequently, generates the maximum output power density of approximately 65.3 mW m^{-2}, which is nearly 50% higher than that of the wild-type strain (43.4 W m^{-2}) [17]. Furthermore, the mutant *S. oneidensis* MR-1 strain with the disrupted *uvrY* gene, encoding the DNA-binding response element of the BarA/UvrY signal system, displays a reduced transcription level of genes involved in the CPS biosynthesis and generates the maximum output power density of approximately 87.4 mW m^{-2}, which is nearly 1.5-fold as much as that of the wild-type strain (64.6 W m^{-2}), indicating that CPS may negatively affect the bioelectrocatalytic activity [18]. Moreover, the genetically engineered strain overexpressing the diguanylate cyclase dgcS promotes the formation of *S. oneidensis* biofilm on the electrode through the cyclic diguanosine monophosphate-dependent signal pathway [19]. Besides, the mutant *S. oneidensis* MR-1 strain with the deletion of *SO3350* gene, involved in the pilus synthesis, displays a better bacterial adhesion to the electrode and accordingly, generates the maximum output power density of more than 100 mW m^{-2}, which is higher than that of the wild-type strain (80 W m^{-2}) [20].

The mutant *P. aeruginosa* PAO1 strain with the deletion of an ATPase *pilT* not only increases the quantity of conductive type IV pili, facilitating the.

Electron transfer to the electrode, but also leads to the suppression of twitching motility which reinforces the cell-to-cell adhesion and the electrode attachment and accordingly, the improved biofilm formation on the electrode. Thus, this mutant strain generates the maximum output power density of about 54.2 mW m^{-2}, which is 2.7-fold as much as that of the wild-type strain (20 W m^{-2}) [21,22]. For *Geobacter sulfurreducens*, the mutant strain with the deletion of *GSU1240* gene, encoding the protein with a PilZ domain, results in the enhanced formation of pili and biofilm. The mutant strain forms a smooth and homogenous biofilm on the electrode while the wild-type strain are not (Fig. 4a–c). The attachment assay of the mutant strain shows 8-fold more biomass attached to the surface than that of the wild-type (Fig. 4d) and the biofilm of the mutant strain is highly cohesive (Fig. 4e). Thus, the mutant strain generates the maximum output power density of approximately 1.3 W m^{-2}, which is more than 60% higher than that of the wild-type strain (about 0.8 W m^{-2}) [23].

2.4. Synthetic biology to optimize the intracellular cofactor NADH

The intracellular nicotinamide adenine dinucleotide (NADH) is regarded as the reducing power and most of the intracellular metabolic energy is converted into NADH, acting as the primary electron donor, whose oxidation reduces the quinone pool in the membrane, from which electrons enter into the EET chain. Thus, the ratio of NADH/NAD^+ reflects the redox and energetic state of the bacterial cell. The heterologous expression of NAD^+-dependent formate dehydrogenase FDH from *Moraxella* spp. in *S. oneidensis* MR-1 strain results in the increased intracellular NADH level, promoting anaerobic electro-respiration and accordingly, current density production [24].

The overexpression of the NAD synthetase *nadE* in *P. aeruginosa*, catalyzing the final step for NADH *de novo* biosynthesis, leads to the increased pool size of NADH/NAD^+ by 1.36-fold. The mutant strain

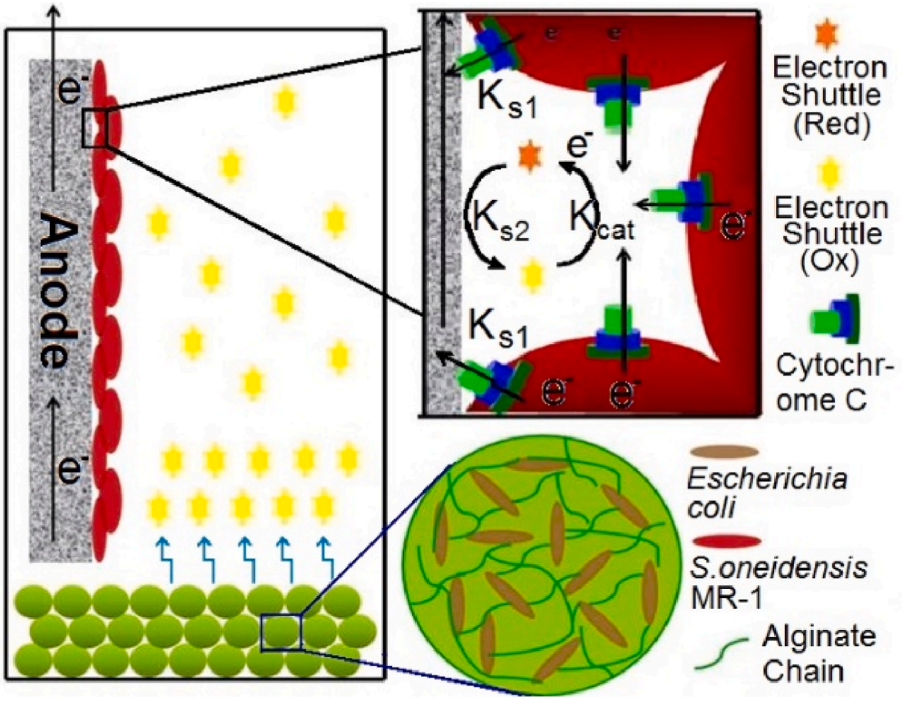

Fig. 3. Schematic diagram of the mechanism of BES anodic electrocatalyzed process accelerated by bio-cocatalyst alginate beads in which riboflavin-secreting *E. coli* cells are immobilized (reproduced with permission from RSC Publishing) [16].

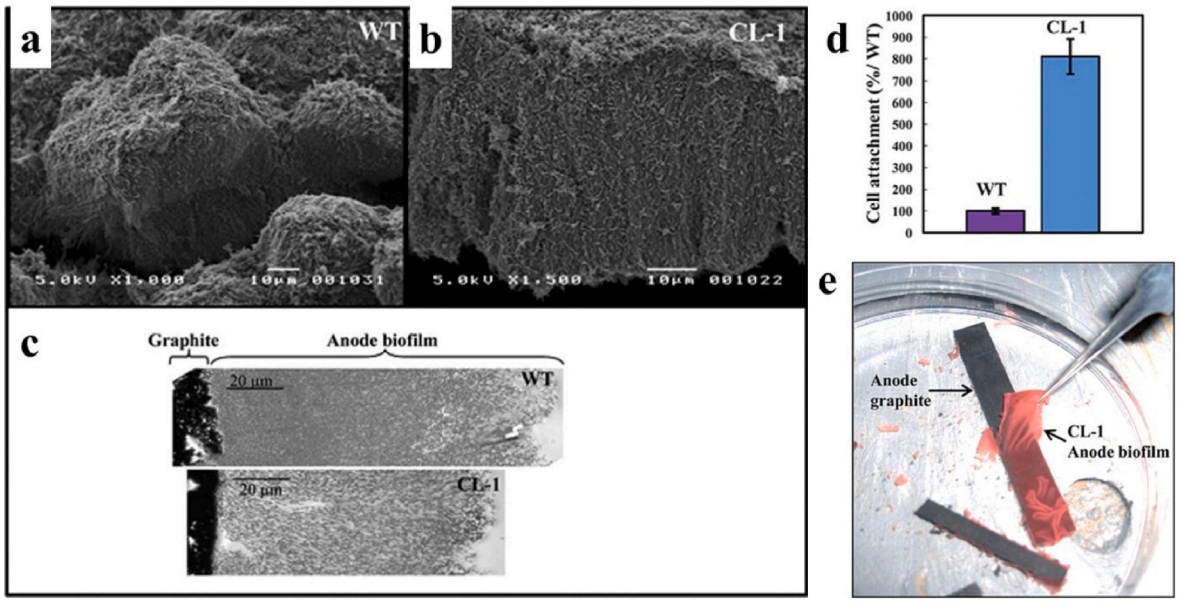

Fig. 4. (a) The wild-type *Geobacter sulfurreducens* biofilms and (b) CL-1 mutant strain biofilms growing on the graphite anodes. (c) Their light microscope images. (d) Comparison between the attached biomass of wild-type *G. sulfurreducens* and that of CL-1 strain. (e) Cohesive feature of CL-1 biofilms on the graphite anode (reproduced with permission from RSC Publishing) [23].

produces the pyocyanin at a concentration of 2.17 μg/ml, which is 1.5-fold higher than that of the wild-type strain and generates the maximum output power density of approximately 0.401 W m^{-2}, which is 2.7-fold as much as that of the wild-type strain (about 0.109 W m^{-2}) [25]. Moreover, the overexpression of nicotinic acid mononucleotide adenyltransferase nadD and quinolic acid phosphoribosyltransferase nadC in *P. aeruginosa* PAO strain simultaneously leads to the increased activity of NAD synthetase, further promoting pyocyanin secretion as well as electricity generation [26]. Besides, the heterologous expression of type II NADH dehydrogenase from *S. loihica* PV-4 in the *S. oneidensis* MR-1 strain accelerates the use of intracellular NADH, meaning more electrons can be pumped into the EET chain (Fig. 5a). Thus, the mutant strain generates the maximum output power density.

Of approximately 0.372 W m^{-2}, which is 3.3-fold as much as that of the wild-type strain (about 0.112 W m^{-2}) [27]. Also, the heterologous expression of formate dehydrogenase FDH from *E. coli* in *Clostridium ljungdahlii* results in a 4.3-fold increase in the concentration of NADH compared with the wild-type strain (Fig. 5b and c). Thus, the mutant

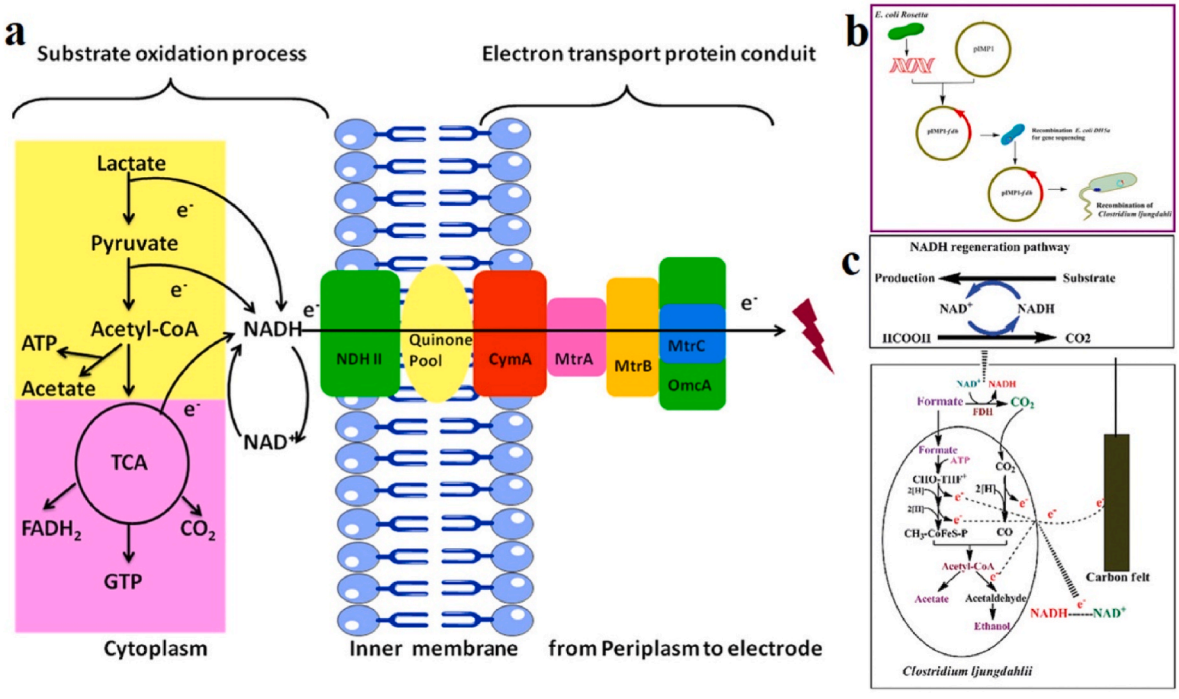

Fig. 5. Schematic diagram of (a) The NDH II recombinant strain overexpressing type II NADH dehydrogenase NDH II in the inner membrane of *S. oneidensis* MR-1 whose microaerobic and anaerobic catabolism are in violet and yellow, respectively (reproduced with permission from RSC Publishing) [27]. (b) Genetically engineered *Clostridium ljungdahlii* overexpressing the formate dehydrogenase fdh and (c) the corresponding metabolic pathway and NADH regenerating system of *C. ljungdahlii* (reproduced with permission from RSC Publishing) [28].

strain generates the maximum output power density of approximately 35 mW m^{-2}, which is 2.3-fold as much as that of the wild-type strain (about 15 mW m^{-2}) [28].

2.5. Pollutant removing by synthetic biology

2.5.1. Pollutant removing in type I BES device

In type I microbial BES device, the catalytic conversion of harmful pollutants into harmless products occurs at the anode with the help of microorganisms. Azo dyes are widely used synthetic dyes in the textile industry. For biological removal of azo dyes such as Congo red, the azo bond has to be cleaved under anaerobic conditions, which is called azo decolourisation. Biological azo decolourisation is reported to involve electron transfer through a series of c-Cyts and the membrane-associated azoreductase with the azo dye as the terminal electron acceptor and can be coupled to the bacterial respiration and cell growth. Thus, the heterologous overexpression of MtrCAB protein complex, the EET components from *S. oneidensis* MR-1, in *E. coli* K-12 generates a mutant strain with a higher decolourisation efficiency. When it is inoculated in the BES device, the Congo red decolourisation for the mutant strain is 82% compared with 55.4% for the wild-type strain [29]. Furthermore, the addition of riboflavin, one type of ES capable of promoting EET, in the BES device enhances the decolourisation of Congo red dye by *S. oneidensis* MR-1 [30].

Besides, for the treatment of terephthalic acid, one type of the petrochemical product, the BES device is fed with terephthalic acid as the carbon source and the overexpression of the corresponding membrane proteins is suggested to improve the electron transfer kinetics for better electricity recovery as well as waste treatment [31]. In addition, for the removal of petroleum hydrocarbon contaminants, the BES device is fed with diesel hydrocarbons as the carbon source and the overexpression of biodegradative alkane hydroxylase alkB is suggested to enhance the remediation of diesel hydrocarbon contaminated groundwater (Fig. 6) [32].

2.5.2. Pollutant removing in type II BES device

In type II microbial BES device, the conversion of harmful pollutants into harmless products occurs at the chemical cathode, at which the contaminants such as heavy metals can be reduced and removed, using electrons produced by the oxidation of organic matter at the anode. Based on whether the extra external voltage is applied to the BES device when it's used to remove heavy metals from wastewater, these metals can be divided into two major categories: The metals which can be spontaneously reduced by the microbial BES device include Cu(II), Cr (VI), Ag(I) and Au(III); The metals which cannot be spontaneously reduced by the microbial BES device include Cd(II), Pb(II), Zn(II) and Ni (II) [33]. Extra electricity is needed to drive the reduction of heavy metals in the latter system.

A three-chamber BES for the removal of copper from soil combined with electricity generation is shown in Fig. 7a. This BES device consists of an anodic compartment, a soil compartment and a cathodic compartment separated by the cation exchange membrane. Copper ions move from the anode, under the electric field, to the cathode, at which they are reduced to the elemental Cu and deposited as nanoparticles on the cathode surface [34]. Cadmium removal in BES device is operated under applied voltages of 0.4–1.0 V via cathodic reduction. Apart from the reduction mechanism, cadmium can be removed by precipitation as $Cd(OH)_2$ and $CdCO_3$ when the local pH near the cathode rises during BES operation too (Fig. 7b). Carbonate ions may come from the substrate oxidation at the bioanode [35].

As shown in Fig. 7c, in a dual-chamber BES, electrons are generated by electricigens on the anode and transferred to the cathode, during which hexavalent chromiums are reduced to nontoxic trivalent chromiums in the cathode. After the reduction, $Cr(OH)_3$ with small solubility is deposited on the cathode surface as precipitation [36]. Since Cr(VI) has a redox potential of 1.33 V (vs. standard hydrogen electrode, SHE) while Pb(II) has a redox potential of −0.126 V (vs. SHE), the electricity generated from hexavalent chromium reduction in one BES can be utilized to drive divalent lead reduction in the other BES (Fig. 7d). Cr(VI) and Pb(II) can be removed simultaneously in this coupling system. During the operation, Pb(0) is deposited on the cathode surface.

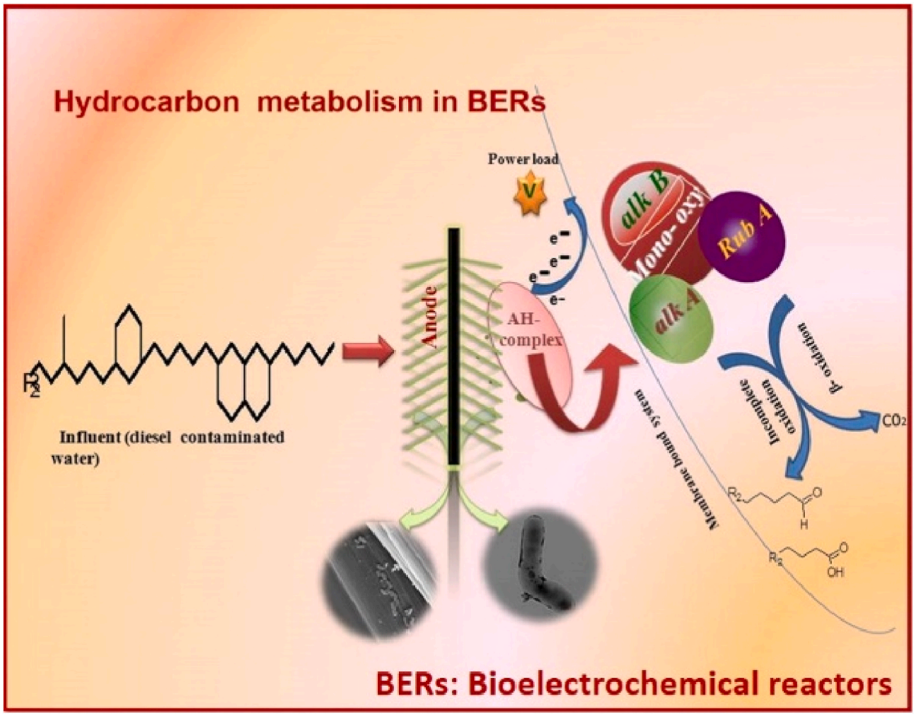

Fig. 6. Schematic diagram of the proposed overexpression of alkane hydroxylase gene *alkB* in genetically engineering bacteria inoculated in the anodic chamber of BES might enhance the removal of diesel range hydrocarbons (reproduced with permission from Elsevier) [32].

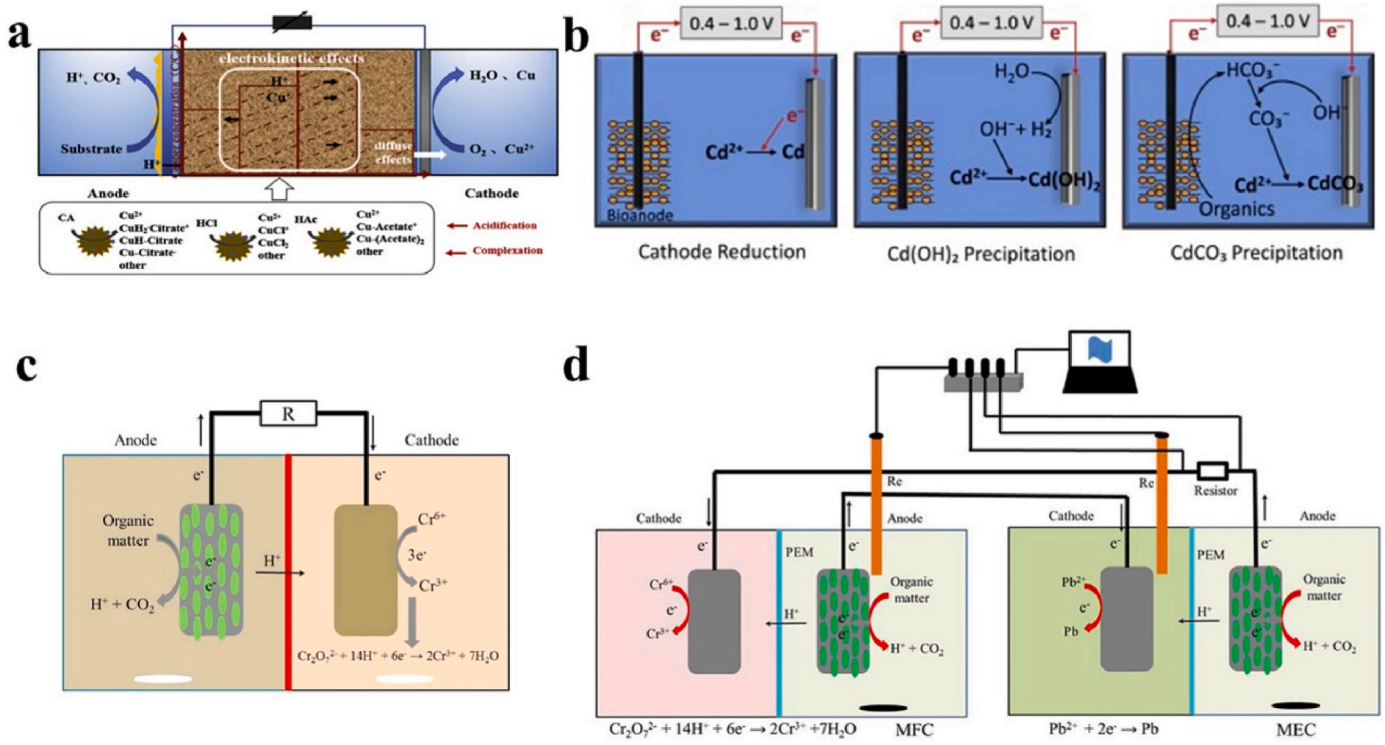

Fig. 7. (a) Schematic diagram of a three-chamber BES for the removal of copper from soil combined with electricity generation. Copper ions move from anode to cathode under the electric field (reproduced with permission from Elsevier) [34]. (b) BES reactors operated under applied voltages of 0.4–1.0 V in which cadmium is removed by cathodic reduction, $Cd(OH)_2$ precipitation and $CdCO_3$ precipitation (reproduced with permission from Elsevier) [35]. (c) a dual-chamber BES in which electricity is generated by electricigens on the anode while hexavalent chromium is reduced to nontoxic trivalent chromium in the cathode (reproduced with permission from Elsevier) [36]. (d) a two-BES coupling system in which the electricity generated from hexavalent chromium reduction in one BES is utilized to drive divalent lead reduction in the other BES (reproduced with permission from Elsevier) [33].

And no extra energy supply is needed for lead reduction [33].

2.5.3. *Pollutant removing in type III BES device*

In type III microbial BES device, the conversion of harmful pollutants into harmless products is microbially catalyzed by microorganisms at the cathode. For instance, in the denitrifying BES, Chemo-lithoautotrophic denitrifying microorganisms residing on the cathode dominate the denitrification process when organic matter is absent in the catholyte. Denitrification in the cathodic compartment is an anaerobic respiration process, in which either nitrate or nitrite is used as the electron acceptor. The overexpression of a cytochrome cd_1-type dissimilatory nitrite reductase nirS is suggested to enhance the nitrate reduction at the cathode [8].

The reductive anaerobic dechlorination of chlorinated aliphatic

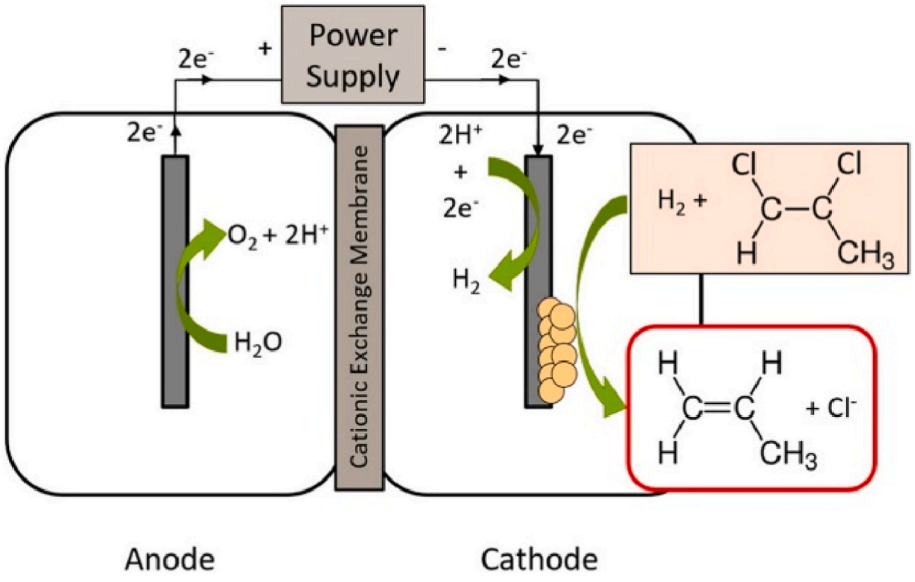

Fig. 8. Schematic diagram of BES catalyzing dechlorination reactions in which H_2 generated in the cathode drives the growth of *Dehalogenimonas alkenigignens* and its dechlorination of 1, 2-dichloropropane to non-toxic propene (reproduced with permission from Elsevier) [37].

hydrocarbons (CAH) such as 1, 2-dichloropropane can also be catalyzed by the microbial BES, in which organohalide-respiring microorganisms residing on the cathode use CAH as the terminal electron acceptor for metabolism. The reductive dechlorination reaction takes place at the cathode utilizing electrons coming either directly from the electrode or indirectly through soluble redox mediators like H_2. For instance, H_2 generated in the cathode drives the growth of *Dehalogenimonas alkenigignens* and its dechlorination of 1, 2-dichloropropane to non-toxic propene (Fig. 8) [37]. Furthermore, overexpressing the EET-related omcX is suggested to more easily receive the electrical stimulation from the electrode. Moreover, the overexpression of the reductive dehalogenases pceA and tceA is suggested to further enhance the dechlorinating ability of the corresponding biofilm [38].

3. Conclusions and perspectives

Microbial BES possesses great promise for the efficient treatment of recalcitrant and toxic pollutants. The application of synthetic biology in microbial BES can improve its pollutant removing capability. Synthetic biology techniques can promote EET kinetics, which is helpful for microbial anodic electro-respiration, expediting pollutant removing not only at the anode but also at the cathode. Furthermore, synthetic biology techniques offer opportunities to promote biofilm development on the electrode, enabling more microorganisms residing on the electrode for subsequent catalytic reactions. Moreover, the direct overexpression of the pollutant removing-related enzymes in microorganisms by synthetic biology tools contributes to the pollutant decomposition.

However, there exist a series of challenges to be addressed before the practical application of synthetic biology techniques in microbial BES. For instance, the bidirectional EET kinetics of bioelectrodes are still too sluggish and need to be further improved. The.

Pollutant degradation pathway should be rationally designed and the intracellular metabolism ought to be systematically optimized. In addition, developing more efficient synthetic biology tools to genetically engineer gene targets in those non-model microbes is still a challenging work. Moreover, for those electro-inert microorganisms, they cannot directly convey electrons to or uptake electrons from the electrode owing to the lack of EET system, which constrains their application in microbial BES accordingly. Besides, given that the treatment of contaminants by microbial BES may eventually be carried out in an open environment, the spread of artificially engineered microorganisms into the natural ecology and the corresponding horizontal gene transfer into the environment must be considered seriously. In future, synthetic biology may work with other techniques such as materials science to solve problems mentioned above. For instance, novel materials can be developed to immobilize the genetically engineered microorganism in microbial BES to prevent them from leaking into the environment.

CRediT authorship contribution statement

Le Tao: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing. **Maoyong Song:** Funding acquisition, Resources, Writing – review & editing. **Guibin Jiang:** Resources, Writing – review & editing.

Declaration of competing interest

The authors indicate that they have no conflict of interest.

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