Engineering *Shewanella oneidensis* enables xylose-fed microbial fuel cell

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**Abstract**

**Background:** The microbial fuel cell (MFC) is a green and sustainable technology for electricity energy harvest from biomass, in which exoelectrogens use metabolism and extracellular electron transfer pathways for the conversion of chemical energy into electricity. However, *Shewanella oneidensis* MR-1, one of the most well-known exoelectrogens, could not use xylose (a key pentose derived from hydrolysis of lignocellulosic biomass) for cell growth and power generation, which limited greatly its practical applications.

**Results:** Herein, to enable *S. oneidensis* to directly utilize xylose as the sole carbon source for bioelectricity production in MFCs, we used synthetic biology strategies to successfully construct four genetically engineered *S. oneidensis* (namely XE, GE, XS, and GS) by assembling one of the xylose transporters (from *Candida intermedia* and *Clostridium acetobutylicum*) with one of intracellular xylose metabolic pathways (the isomerase pathway from *Escherichia coli* and the oxidoreductase pathway from *Scheffersomyces stipites*), respectively. We found that among these engineered *S. oneidensis* strains, the strain GS (i.e. harbouring *Gxf1* gene encoding the xylose facilitator from *C. intermedia* and *XYL1*, *XYL2*, and *XKS1* genes encoding the xylose oxidoreductase pathway from *S. stipites*) was able to generate the highest power density, enabling a maximum electricity power density of 2.1 ± 0.1 mW/m².

**Conclusion:** To the best of our knowledge, this was the first report on the rationally designed *Shewanella* that could use xylose as the sole carbon source and electron donor to produce electricity. The synthetic biology strategies developed in this study could be further extended to rationally engineer other exoelectrogens for lignocellulosic biomass utilization to generate electricity power.

**Keywords:** Microbial fuel cell, Synthetic biology, Xylose, *Shewanella oneidensis* MR-1

**Background**

Bio-electrochemical systems enabled many practical applications in environments and energy fields [1–7], including microbial fuel cell (MFC) for simultaneous organic wastes treatment and electricity harvest [8–12], microbial electrolysis cells for hydrogen production [13–16], and microbial electrosynthesis for production of valuable chemicals from CO₂ bioreduction [17–22]. Many mono-, di-saccharides as well as complex carbohydrates like starch and organics in wastewater and marine sediment have been used in MFCs for the production of electricity [8, 23, 24]. Xylose, one of primary ingredients from hydrolysis of lignocellulosic biomass, is the second most abundant carbohydrate after glucose in nature [25–27]. Conversion of xylose to electricity energy using MFC would thus provide a sustainable and green energy, which received increased attention in recent few years [24, 28–30]. However, xylose is hard to be effectively utilized by many microorganisms due to slow utilization rate and inefficient metabolic pathways of xylose [26, 31–35].

*Shewanella oneidensis*, one of the most well established metal-reducing exoelectrogens [36, 37], is capable of conducting extracellular electrons transfer (EET) through its metal-reducing (Mtr) pathway [38–42], being extensively studied for the optimization of MFC performance...
[40, 41, 43–47], MFC-based logic gate [48–50], bioremediation of toxic metals [51], etc., in recent decade. However, the wild-type (WT) S. oneidensis could only use three- (or two-) carbon substrates (e.g. lactate, pyruvate and acetate) as their carbon and energy sources, with an exception of N-acetyl-glucosamine (NAG) as a high-carbon carbohydrate [45, 52, 53], while common pentoses or hexoses (e.g. xylose and glucose), the most abundant composition of biomass, could not be utilized by the WT S. oneidensis owing to its incomplete sugar utilization pathways [36, 54, 55]. Such defect enormously restricted the wide applications of S. oneidensis.

Recently, several strategies were developed to use xylose for electricity generation in Shewanella-inoculated MFCs. Firstly, an adaptive evolution approach was developed to activate an otherwise silent xylose metabolic pathway, i.e. oxidoreductase pathway in the WT S. oneidensis, thus generating a S. oneidensis mutant XM1 that could metabolize xylose as the sole carbon and energy source [56]. Secondly, microbial consortia including fermenters and exoelectrogens were developed to accomplish xylose-powered MFCs, in which the engineered Escherichia coli played as a fermenter to metabolize xylose for the synthesis of metabolites such as lactate and formate to feed the S. oneidensis as the carbon source and electron donor, thus enabling an indirect utilization of xylose by S. oneidensis for bioelectricity production [24].

Herein, we used synthetic biology strategy to rationally engineer S. oneidensis that could use xylose as the sole carbon source and electron donor for electricity generation in MFCs. To enable S. oneidensis to be able to use xylose, the xylose transporters (i.e. glucose/xylose facilitator encoded by gene Xgf1 from Candida intermedia [57, 58] and ω-xylose-proton symporter encoded by gene xylT from Clostridium acetobutylicum [59]), synthetic isomerase pathway (including the genes xylA and xylB from E. coli [60]), and oxidoreductase (including from Scheffersomyces stipites [61]) pathway for xylose metabolism were heterologously expressed in S. oneidensis in a combinatorial way. Thus, four recombinant S. oneidensis strains were synthesized (see Fig. 1). Xylose-fed MFCs experiments proved that these engineered S. oneidensis MR-1 strains were conferred with the ability of utilizing xylose to produce electricity, and the engineered S. oneidensis strain GS provided the highest electricity generation. Compared with the S. oneidensis strain XM1 previously evolved by an adaptive evolution strategy [56], our rationally engineered S. oneidensis strains GS and XS (bearing the oxidoreductase pathway from S. stipites) showed a higher xylose consumption rate and a superior growth rate. In addition, the relative higher electricity generation by the GS strain than other engineered strains can be attributed to the higher intracellular riboflavin level and reducing equivalents in the GS. To the best of our knowledge, this was the first report on the rationally designed Shewanella that gained the expanded metabolic capability of using xylose as sole carbon source and electron donor to produce electricity.

**Results and discussion**

**Engineered xylose-utilizing S. oneidensis strain via synthetic biology strategies**

A few xylose metabolic pathways in microorganisms were found, including the oxidoreductase, isomerase, and Weinberg–Dahms pathways [56]. For example, E. coli is a robust and well-studied xylose scavenger [56, 62], which could metabolize xylose by the isomerase pathway; however, S. stipites [56, 61] could utilize oxidoreductase pathway for the metabolism of xylose. In the xylose isomerase pathway of E. coli [56, 60, 63], xylose isomerase encoded by the gene xylA converts xylose to xylulose, which is then phosphorylated by xylulokinase encoded by the gene xylB to xylulose 5-phosphate (X-5-P), and then enters the pentose phosphate pathway (see Fig. 1). In the oxidoreductase pathway of S. stipites [26], NAD(P)H-dependent xylose reductase encoded by the gene XYL1 converts intracellular xylose to xylitol, which is then oxidized to xylose by xylitol dehydrogenase (XDH) encoded by the gene XYL2. Xylulose is then phosphorylated by xylulokinase encoded by the gene XKS1 to xylulose 5-phosphate (X-5-P), which enters the pentose phosphate pathway, similar to the isomerase pathway (see Fig. 1).

To facilitate convenient and fast multigene assembly in S. oneidensis, a Biobrick compatible vector named pYDTr including an IPTG-inducible promoter Placlq-Iaclq-Ppac was well developed in our laboratory (Additional file 1: Figure S1B) [64]. Furthermore, to avoid the codon usage bias and prevent blocked translation due to shortage of tRNAs for rare codons between S. oneidensis and other bacteria, in vitro chemical synthesis of codon-optimized genes instead of direct cloning from other bacteria was used. The xylose metabolic pathway was then assembled by several routines of Biobrick ligation steps of the relevant genes. With the combinations of the two xylose transporters and the two xylose-utilizing metabolic pathways (the isomerase and the oxidoreductase pathways), four recombinant S. oneidensis strains harbouring engineered gene assembly (plasmid) for enhanced xylose transport and metabolism were synthesized, respectively, which were XC (including the gene xylT for xylose symporter, xylA and xylB for the xylose isomerase pathway), GE (including Xgf1 for the xylose facilitator, xylA and xylB for the xylose isomerase pathway), XS (including xylT for xylose symporter, and XYL1, XYL2, and XKS1 for the xylose oxidoreductase pathway), and GS (including Xgf1 for the xylose facilitator, and XYL1, XYL2, and XKS1 for the xylose oxidoreductase pathway).
Evaluation of xylose utilization and cell growth of the recombinant *S. oneidensis*

The cell growth and xylose consumption by the wild-type (WT, harbouring the pYYDT empty vector) and four genetically engineered *S. oneidensis* strains (i.e. harbouring XE, GE, XS, and GS, respectively) were evaluated in SBM supplemented with 5 mM xylose as the sole carbon source.

Under aerobic conditions, the WT *S. oneidensis* strain showed almost no growth and xylose consumption, while the four engineered *S. oneidensis* strains showed a superior growth over the WT *S. oneidensis* strain. In addition, the growth rate of the engineered strains XS and GS (harbouring the oxidoreductase pathway) was faster than that of the strains XE and GE (harbouring the isomerase pathway) (Fig. 2a). The engineered strain XS and GS consumed xylose at a rate of ~28.1 and ~35.2 μM/h, which was faster than that of the engineered strains XE and GE (~11.2 and ~20.3 μM/h) (Fig. 2b). Thus, the rate of xylose consumption of these engineered strains was in good agreement with that of the growth rate, respectively.

The anaerobic respiratory capabilities of the WT and the recombinant *S. oneidensis* were also determined under anaerobic conditions with xylose as the sole electron donor and fumarate as the electron acceptor. Similar to the aerobic conditions, the four genetically engineered strains grew faster than that of the WT strain. The recombinant strains XS and GS (harbouring the oxidoreductase pathway) consumed xylose at a rate of ~14.8, and ~17.2 μM/h, respectively, which had a faster xylose consumption rate than that of the strains XE and GE (harbouring the isomerase pathway, ~6.3 and ~9.7 μM/h, respectively) (Fig. 2c, d). Furthermore, the engineered strain GS could intake xylose faster than XS, which indicated that the glucose/xylose facilitator Gxf1 enabled a higher xylose transportation than that of the D-xylose-proton symporter XylT. It was revealed that sugar uptake via facilitated diffusion by Gxf1 required less energy.
(ATP) than proton symport XylT, and thus the facilita-
tor protein would probably be more efficient with higher
substrate affinity under oxygen-limited or anaerobic con-
ditions where ATP production is restricted in our MFC
conditions [58, 65]. In addition, all recombinant S. onei-
densis strains were able to utilize lactate as the sole carbon
source at a rate similar to that of the WT strain, suggest-
ing that the lactate metabolism of S. oneidensis was not
altered by such engineering efforts (data not shown).
Thus, our results indicated that the introduction of one
of the synthetic xylose transporters (the d-xylose-proton
symporter from C. acetobutylicum and the glucose/xylose
facilitator from C. intermedia) and one of the metabolic
pathways (i.e. the isomerase pathway from E. coli and the
oxidoreductase pathways from S. stipites) could success-
fully confer Shewanella strains with the ability of utiliz-
ing xylose as the sole carbon source for the cell growth.
Especially, our rationally designed S. oneidensis strains
XS and GS (bearing the oxidoreductase pathway from
S. stipites) showed a higher consumption of xylose and a
superior growth rate than that of the S. oneidensis strain
XM1 (that was recently developed through an adaptive
evolution strategy) [56]. Escherichia coli (the BL21 strain)
harbouring those genes related to xylose transport and
metabolism exhibited a superior xylose consumption rate
(~455 μM/h), i.e. ~12 times faster than that of the engi-
enneered S. oneidensis GS (~35.2 μM/h) (Additional file 1:
Figure S2). This result indicated that although the engi-
enneered S. oneidensis was enabled the capability of xylose
utilization, there was much room to further improve its
xylose consumption rate by synthetic biology endeavours.

MFC performance and bio-electrochemical analyses
MFC was used to examine the extracellular electron
transfer and power generation by the engineered S. onei-
densis MR-1 using xylose as the sole carbon source. The
WT and the engineered S. oneidensis strains were inoc-
ulated into the anodic chamber of MFCs, respectively,
with a 2 kΩ external resistor, across which the voltage
output was recorded.
Initially, 18 mM lactate was used (as the favourable car-
bon source of Shewanella) to feed the engineered S. onei-
densis strains in MFCs to verify the capacity of power
output of each strain (Fig. 3). After the output voltage
decreased to baseline levels (indicating the depletion of
lactate), 18 mM xylose was added into the anodic cham-
ber as the carbon source. Obviously, the output voltages
of these engineered S. oneidensis strains with xylose as the
carbon source were lower than those of lactate as the car-
bon source, because lactate is the favourable carbon source
When lactate was used as the carbon source, the maximum output voltage could not be utilized by the xylose transporter and metabolic pathway into Shewanella species. However, when xylose was used as the carbon source, the wild-type Shewanella oneidensis could generate any voltage output when xylose was used as the carbon source, with maximum output voltages of \(~205\) mV (Fig. 3). Notably, the dropping slope of the polarization curve obtained from the engineered Shewanella oneidensis strain GS (harbouring the xylose facilitator and the xylose oxidoreductase pathway) was smaller than those obtained from the other three engineered Shewanella oneidensis strains (i.e. XE, GE, XS), implying that the internal charge transfer resistance of the MFC inoculated with GS was relatively smaller (Fig. 4c). The power density was calculated, which showed that the engineered Shewanella oneidensis strain GS obtained a maximum power density of \(~2.1\pm0.1\) mW/m² \((n=3)\), which was \(~0.3\), \(~0.9\), and \(~1.1\) times higher than that of XE, GE, and XS, respectively (Fig. 4c). Previous xylose-fed MFCs generally used sludge, natural or synthetic microbial consortia, the power generation of which were in the range of \(6.3\)–\(2330\) mW/m² (as shown in Additional file 2: Table S1), higher than that of our recombinant Shewanella oneidensis strain. Thus, future engineering of Shewanella oneidensis to enable higher output electricity remained of paramount importance.

Biochemical characterizations showed that the engineered strains GS and XS had a higher utilization efficiency of xylose and higher growth rate, and a more efficient formation of biofilm attached on the anodes (Fig. 5a). Meanwhile, the engineered strains GS and XS could also generate higher intracellular reducing equivalents (i.e. NADH/NAD⁺, Fig. 5b). Such a high intracellular releasable electron pool (i.e. NADH) had resulted from the oxidative reaction of xylitol to xylulose, mediated by the reduction of NAD⁺ to NADH in the oxidoreductase pathway [65, 67–69]. Both the efficient biofilm formation on the anodes [24, 70] and higher intracellular reducing equivalents [71, 72] in the engineered Shewanella oneidensis strains GS and XS synergistically enabled an enhanced EET efficiency and electricity generation. In addition, an increase in the secretion of riboflavin in the recombinant strains also enabled an increase in the output voltage of MFCs. The increased biosynthesis of riboflavin would be attributed to the biosynthesis of xylulose 5-phosphate (X-5-P) owing to the heterologously introduced xylose metabolism pathway (i.e. the oxidoreductase pathway). X-5-P, as a metabolic product of the oxidoreductase pathway, was converted to ribulose-5-P, a crucial precursor for the biosynthesis of riboflavin,
by ribulose-phosphate 3-epimerase encoded by the rpe gene in the pentose phosphate pathway. Subsequently, ribulose-5-P and guanosine triphosphate (GTP) were converted to riboflavin via the riboflavin biosynthesis pathway (Additional file 1: Figure S3) [24, 36, 53].

Conclusions
To the best of our knowledge, this research is the first to use synthetic biology strategy to rationally engineer S. oneidensis MR-1 to enable direct utilization of xylose as the sole carbon source and electron donor for bioelectricity production in MFCs. The efficient xylose metabolic pathways (the isomerase pathway or the oxidoreductase pathway) combined with two different xylose transporters were heterologously expressed in S. oneidensis MR-1 to construct four engineered S. oneidensis strains (namely XE, GE, XS, and GS), which could successfully utilize xylose under anaerobic and aerobic conditions. These recombinant S. oneidensis strains could generate bioelectricity in MFCs with xylose as the sole carbon source and electron donor. The maximum power density of the MFC inoculated with the engineered S. oneidensis strain GS (harbouring the xylose facilitator and the xylose oxidoreductase pathway) could reach \( \sim 2.1 \pm 0.1 \text{ mW/m}^2 \). This rationally engineered xylose transport and metabolic pathway significantly expanded the spectrum of carbon source that could be used by S. oneidensis. In the foreseeable future, with continuous development of synthetic biology strategies [73–75] to engineer exoelectrogens, a diverse array of organics such as lignocellulosic biomass and recalcitrant wastes may be more efficiently converted to electricity power.

Methods
In vitro gene synthesis
The information and coding sequences of the genes (Additional file 2: Tables S2 and S3) were extracted from the NCBI database and adapted for optimal expression in S. oneidensis MR-1 by a Java codon adaption tool (JCAT) in order to prevent blocked translation due to shortage of tRNAs for rare codons [49]. Each gene component was synthesized as a Biobrick [76, 77], and restriction enzyme sites of EcoRI, XbaI, SpeI, and SbfI were avoided in the codon-optimized sequences. The optimized gene sequence was flanked by an upstream prefix (containing EcoRI and XbaI), a RBS site (BBa_B0034, iGEM) located at 6 bp ahead of the start codon, and a downstream suffix (containing SpeI and SbfI) (Additional file 1: Figure S1A). The designed gene sequences were synthesized in vitro, verified by Sanger sequencing (AuGCT, China).

Plasmid construction, transformation, and culture conditions
All plasmid constructions were performed in E. coli Trans T1. The E. coli strains were cultured in the LB (Luria–Bertani) medium at 37 °C with 200 rpm. The plasmid to be transformed into S. oneidensis MR-1 (ATCC 700550) was firstly transformed into the plasmid donor strain E. coli WM3064 (auxotroph), and then transferred into S. oneidensis by conjugation. Then, 100 μg/ml 2,
6-diaminopimelic acid (DAP) was added for the growth of *E. coli* WM3064. Whenever needed, 50 μg/ml kanamycin was added in the culture medium for plasmid maintenance. All the strains and plasmids used in this study are listed in Table (Additional file 2: Table S4).

### Determination of cell growth and xylose utilization

To determine cell growth and xylose utilization under both aerobic and anaerobic conditions, 0.5 ml of the wild-type (WT) or engineered xylose-utilizing *S. oneidensis* strain culture suspension was inoculated into 15 ml *Shewanella* basal medium (SBM) [53] (Additional file 2: Table S5), supplemented with 5 mM xylose as the electron donor and carbon source in the test tube. When needed, 10 mM sodium fumarate was supplemented as the electron acceptor, which was stoichiometrically sufficient from both theoretical calculations and experimental validations (Additional file 1: Figure S4). The cell cultures were incubated at 30 °C, and samples were withdrawn periodically for the determination of cell density (optical density at 600 nm, i.e. OD600) and xylose consumption. The OD600 was measured by an ultraviolet and visible spectrophotometer (TU-1810, Beijing, China).

### BES setup

To evaluate the efficiency of extracellular electron transfer (EET), the overnight *Shewanella* culture suspension (1.5 ml) was inoculated into 150 ml fresh LB broth at 30 °C with shaking (200 rpm) till the OD600 reached 0.6–0.8. Then, the cells were harvested by centrifugation and washed 3 times with fresh M9 buffer (Additional file 2: Table S6). The cell pellets were subsequently re-suspended in 140 ml electrolyte (5% LB broth plus 95% M9 buffer supplemented with 18 mM lactate or xylose). 50 μg/ml kanamycin was added to ensure consistent culture condition. The medium was supplemented with 0.1 mM IPTG as the inducer of the tac promoter. Our previous experiments proved that IPTG had no effect on the cell physiology and EET of *Shewanella* [70]. The dual-chamber MFCs were used in this study, namely the anodic and cathodic chambers (140 ml working volume) separated by the nafion 117 membrane (DuPont Inc., USA), were the same as those used in the previous study. Carbon cloth was used as the electrodes for both the anode (2.5 cm × 2.5 cm, i.e. the geometric area is 6.25 cm²) and the cathode (2.5 cm × 3 cm). The cathodic electrolyte consisted of 50 mM K₃[Fe(CN)₆] in 50 mM K₂HPO₄ and 50 mM KH₂PO₄ solution. To measure the voltage generation, a 2 kΩ external resistor was connected into the external circuit of MFCs, and the output voltage (V) across the external loading resistor (R) was measured by a digital multimeter (DT9205A).

### Electrochemical analyses

Cyclic voltammetry (CV) was performed in a three-electrode configuration with an Ag/AgCl reference electrode on a CHI 1000C multichannel potentiostat (CH Instrument, Shanghai, China). At the pseudo-steady state of MFCs, the polarization curves were obtained by varying the external resistor. Current density (I) was calculated as

\[ I = \frac{V}{R} \]

and power density (P) was calculated as

\[ P = I \times V \]

Then, the I and P were normalized to the projected geometric area of the anode to obtain the current density and power density, respectively [78].

### Quantification of metabolites

For the quantification of riboflavin, the samples in the MFC supernatant were firstly centrifuged (35,000 rpm for 20 min) and filtered (0.22 μm), and then, the eluted media were detected by a liquid chromatograph-tandem mass spectrometer (LC–MS) (Agilent LCMS-1290-6460) in a positive ion mode using a Waters XBridge C8 column (2.1 × 100 mm; particle size: 3.5 μm). Xylose in the
analyses were analysed using a high-performance liquid chromatography (HPLC) system equipped with a diode array detector. Sulphuric acid (5 mM) was used as the mobile phase flowing at 0.6 ml/min through the Aminex HPX-87H column (Bio-Rad, USA), which was incubated at 50 °C. Signals at 190 nm were used to quantify xylose.

Quantification of intracellular NADH/NAD⁺
Cells (10 ml) were collected by centrifugation (10,000 rpm at 4 °C for 5 min) and immediately re-suspended in 300 μl of 0.2 M HCl (for NAD⁺) or 0.2 M NaOH (for NADH). The suspensions were boiled for 7 min, rapidly quenched in an ice bath, and added with 300 μl of 0.1 M NaOH (for NAD⁺) or 0.1 M HCl (for NADH). Cell debris was removed by centrifugation at 10,000 rpm for 10 min, and the supernatant was used in a cycling assay to determine the amounts of NAD⁺ and NADH [79, 80]. Meanwhile, the cell concentration for the detection of NAD⁺ and NADH concentration was detected by plate counts on LB agar.

Measurement of electrode-attached biomass
The electrode was placed in a 50-ml tube containing 5 ml of 0.2 mol/l NaOH, then vortexed for 2 min, and incubated in a water bath to lyse cells at 96 °C for 30 min. The extracts were tested by bicinchoninic acid protein assay kit (Solarbio, China) after being cooled to room temperature.

Additional files

Additional file 1: Figure S1. Construction of synthetic xylose metabolic pathways in Shewanella oneidensis MR-1. (A) Schematic of the plasmid with a synthesized functional fragment of genes. The restriction sites EcoRI and XbaI with the ribosome binding site (RBS) are located upstream of each codon-optimized gene sequence, while the restrictions SpeI and PstI are located downstream of the gene. (B) Four plasmid constructs with xylose utilization pathways. To construct the multiple plasmid in S. oneidensis, a BioBrick compatible expression vector pYYDT was adopted, which was previously constructed in our laboratory. Layout of the four plasmid constructs containing gene components in the xylose pathway examined in this study. Figure S2. Xylose consumption rate by E. coli (BL21) and by the recombinant S. oneidensis strain. The error bars were calculated from triplicate experiments. Figure S3. Metabolic pathway of riboflavin synthesis from xylose fermentation in S. oneidensis. A synthetic intracellular xylose metabolic pathway, i.e., the oxidoreductase pathway including genes XYL1, XYL2 and XKS1 from S. stipitis, is incorporated into S. oneidensis MR-1 to enable the direct utilization of xylose. Xylosulose 5-phosphate, as a metabolite in the oxidoreductase pathway, was converted to ribulose-5-P by ribulose-phosphate 3-epimerase (encoded by theyps gene) in the pentose phosphate pathway, which was a crucial precursor for the biosynthesis of riboflavin via the riboflavin synthesis pathway. Figure S4. Xylose consumption under anaerobic conditions with 10 mM and 50 mM fumarate. The error bars were calculated from triplicate experiments.

Additional file 2: Table S1. Summary of the reported energy output of Xylose-Fed MFCs. Table S2. Genes used in this study. Table S3. Synthesized sequences of genes in this study. Table S4. Strains and plasmids used in this study. Table S5. Main constituents for S. oneidensis basal medium (SBM). Table S6. Main constituents for M9 buffer.

Abbreviations
ETET: extracellular electron transfer; MQ: methyl naphthoquinone; IM: inner membrane; OM: outer membrane; Cytc: inner membrane tetraheme c-type cytochromes; Mtr: metal-reducing; MtrA: periplasmic decarboxylase; MtrB: β-barrel trans-OM protein; MtrC and OmcA: two OM decarheme c-type cytochromes; TCA: tricarboxylic acid cycle; ndhII: NADH dehydrogenase; P: phosphatase; PEP: phosphoenolpyruvate; NAD+: nicotinamide adenine dinucleotide; NADH: reduced nicotinamide adenine dinucleotide; NADP+: nicotinamide adenine dinucleotide phosphate; NADPH: reduced nicotinamide adenine dinucleotide phosphate; ATP: adenosine triphosphate; ADP: adenosine diphosphate; X-5-P: xylulose-5-phosphate; GTP: guanosine triphosphate; IPTG: isopropyl β-D-1-thiogalactopyranoside; DAP: 2,6-diaminopimelic acid, MFC: microbial fuel cell; OD: optical density, CV: cyclic voltammetry; XDH: xylitol dehydrogenase; NAG: N-acetyl-glucosamine.

Authors’ contributions
FL designed the project, performed experiments, analysed data, and drafted the manuscript. YL, LS and XL performed some experiments, collected data, analysed data, and drafted the manuscript. CY, XA, XC and YT provided some reagents, helped design the experiment and drafted the manuscript; HS supervised the project, analysed the data, and critically revised the manuscript. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

Availability of supporting data
All data supporting the conclusions of this article are included within the manuscript and Additional files 1 and 2.

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