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Efficient conversion of hemicellulose into high-value product and electric power by enzyme-engineered bacterial consortia

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Abstract:

As an abundant agricultural and forestry biomass resource, hemicelluloses are hard to be effectively degraded and utilized by microorganisms due to the constraints of membrane and metabolic regulations. Herein, we report a synthetic extracellular metabolic pathway with hemicellulose-degrading-enzymes controllably displayed on *Escherichia coli* surface as engineered bacterial consortia members for efficient utilization of xylan, the most abundant component in hemicellulose. Further, we develop a hemicellulose/O_2 microbial fuel cell (MFC) configuring of enzyme-engineered bacterial consortia based bioanode and bacterial-displayed laccase based biocathode. The optimized MFC exhibited an open-circuit voltage of 0.71 V and a maximum power density (P_{max}) of 174.33 ± 4.56 μW cm^{-2}. Meanwhile, 46.6% (w/w) α-ketoglutarate was produced in this hemicellulose fed-MFC. Besides, the MFC retained over 95% of the P_{max} during 6 days' operation. Therefore, this work establishes an effective and sustainable one-pot process for catalyzing renewable biomass into high-value product and powerful electricity in an environmentally-friendly way.

Introduction

Lignocellulose, which is produced through photosynthesis by plant, is a huge solar energy reservoir. As a green and sustainable alternative to fossil energy, it has attracted a broad interest to explore lignocellulose biomass for high added-value products and biofuel[1, 2]. Biological hydrolysis and fermentation process of lignocellulosic biorefinery are regarded as the most promise and eco-friendly approach compared to physical and chemical methods[3, 4]. As the most abundant component in lignocellulose, cellulose can be easily metabolized by most microorganism owing to only glucose units[5]. Whereas, hemicellulose, accounting for 20-40% of lignocellulose, are hard to be effectively degraded and utilized by many microorganisms due to the heterogeneous structure of hemicellulose and the constraints of effective membrane transporters as well as the metabolic regulations *in vivo*[6, 7]. Thus, the low utilization efficiency of hemicellulose greatly affect lignocellulosic biorefinery[8].

Currently, hemicelluloses have been used to produce valuable chemicals such as ethanol, furfural and xylitol[9]. Unfortunately, these processes still experience challenges of low conversion activity and poor stability of cells. For example, the yield of hemicelluloses-bioethanol is lower than that of bioethanol fermented from cellulose owing to the poor utilization efficiency of polysaccharide and pentose by microorganisms during fermentations[10]. Besides, bioelectric energy has been heralded as a one of the most potential renewable energies in the future[11, 12]. To realize the generation of energy from hemicelluloses biomass, great efforts via recruitment of microbial community (microbial fuel cells, MFCs) have been devoted[13]. However, diverse biological properties in one system result in the instability in bacterial community, which severely limits its large-scale application[14]. To

address these challenges, a strategy is highly desirable to realize high-efficient production of biofuels and biochemicals directly from hemicellulose instead of its monosaccharides.

As an efficient and green method, enzymatic catalysis has been employed to hydrolyze polysaccharides and oxidize monosaccharides. However, the high-cost and poor stability greatly limit their large-scale applications[15]. Meanwhile, microbial fermentation is another main biological approach to produce biofuels and biochemicals. The cell membrane barrier and intracellular metabolic regulation always restrict the highly efficient substrate utilization, especially polysaccharides[16]. Enzymes can be displayed on microbial surface and the resulted whole-cell biocatalyst was obtained by large-scale fermentation and directly applied in biocatalytic reactions without tedious protein purification process, which enables to implement complex biochemical reactions even metabolic pathways without the constraints of cell membrane and metabolic regulation[17, 18, 19]. Similar with secreted proteins, displayed enzymes could be expressed and folded in periplasm and exported directly across cell membrane of *E. coli* to the culture medium using Sec secretory system[20]. The displayed enzymes could be assembled and anchored onto outer membrane by fusing with anchoring motif, which could facilitate their abilities to be recycled and regenerated. In contrast, secreted proteins will be folded in cytoplasm or periplasm, depending on the sec or tat signal sequence used, and then secreted and dispersed in the culture media without geographical restrictions of cell surface, failing to be recycled or regenerated[21].

Herein, we report on an artificial hemicellulose degrading pathway catalyzed by the engineered bacterial consortia whose enzymes from various origins are recruited to achieve the optimal overall reaction rate in a controlled manner, for the high-efficiently converting

renewable biomass into electric energy and high-value chemical simultaneously in an environmentally-friendly way. The engineered *Escherichia coli* (*E. coli*) (up-stream pathway members) degrade xylan, the most abundant biopolymer in hemicellulose[22], into monosaccharides, while the following recombinant *E. coli* (down-stream pathway members) oxidize monosaccharides into α-ketoglutarate accompanied by generating the reduced form of nicotinamide adenine dinucleotide (NADH) (Fig. 1). Thus *denovo* MFC is designed to achieve one-pot efficient production of high added-value chemical of α-ketoglutarate and powerful electricity from hemicellulose biomass in a "one-stone-two-birds" manner, demonstrating a model to efficiently utilize biomass in a sustainable way.

Results and discussion

Bacterial surface displaying enzymes (engineered bacterial consortia) involving in the saccharification of xylan (up-stream pathway)

Herein, the utilization of xylan experienced two stages, firstly saccharified into monosaccharides (mainly D-xylose), which are then oxidized to produce α-ketoglutarate and release electrons. For the initial phase, hemicellulases hydrolyze β-linkage in the xylan backbone to release monosaccharides. A marine symbiont *Teredinibacter turnerae* was selected as the bacterial endo-β-1,4 xylanases (TtGH8) source. TtGH8 shows a wide variety of glycoside hydrolases activities including β-1,4 xylanases, especially the highest activity on mixed-linkage β-1,3 and β-1,4 xylanases[23], which could expand the panel of substrate used in our system. β-D-xylosidase (SXA) from *Selenominas ruminantium* exhibits good thermo-stability as well as superior activity towards 1,4-β-D-xylooligosaccharides[24, 25]. Besides, SXA also has α-arabinofuranosidase activity towards arabino xylanases[26]. The

112 synergy of these two hemicellulases could break down xylan into monosaccharides.
113 Unfortunately, *E. coli* BL21 (DE3) can metabolize xylose under the action of xylose
114 isomerase (XylA) and xylulose kinase (XylB)[27]. Therefore, in order to drive all of the
115 generated xylose by hemicellulase into the designed extracellular metabolic pathway and
116 avoid monosaccharides waste, *xylA* and *xylB* genes in *E. coli* BL21 (DE3) were knocked out.

117 During the first stage, when applying two types of hydrolase-displaying on the surface of
118 bacterial cell, polysaccharide generated from lignocellulose pretreatment could be
119 immediately degraded into monosaccharide by these displayed enzymes in a high efficient
120 way without passing through cell membrane[28]. TtGH8 and SXA were displayed on cell
121 surface separately or simultaneously as fusion protein using N-terminal region of ice nuclear
122 protein from *P. borealis* (InaPb) as anchoring motif[29]. The connection manner of cell surface
123 displayed TtGH8-SXA was optimized by introducing linkers Gly-Ser,
124 Gly-Ser-Gly-Gly-Ser-Gly and $(Ala\text{-}Pro)_7$ between TtGH8 and SXA, respectively. Results
125 show that the engineered strains harboring GS and GSGGSG linkers had similar activities.
126 However, $(AP)_7$ linker influenced the functions of cell surface displayed TtGH8-SXA, and the
127 whole-cell activity reduced 50% compared to that of strain displayed TtGH8-SXA with GS
128 linker. Therefore, Gly-Ser was employed as the linker between TtGH8 and SXA. Surface
129 displaying-enzymes demonstrated obvious expression levels, which were confirmed by
130 Western Blotting (Supplementary Fig. 1) and confocal imaging (Supplementary Fig. 2). Next,
131 biochemical activity assays were conducted to validate biological functions of
132 surface-displayed enzymes by using commercial xylan as substrate using 3,5-dinitrosalicylic
133 acid (DNS) method. The optimized protein expression conditions for displayed enzymes were

0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and 30 °C (Supplementary Fig. 3). However, when IPTG concentration was over 1.0 mM, the whole-cell activities of engineered strains were declined due to the imbalance of transcription and secretion[30]. According to results of Quantitative Immunoassay (Supplementary Fig. 4), the number of cell surface displayed proteins were estimated. Strains displayed different amounts of these enzymes, approximately 25560, 25300 and 18020 enzyme molecules for TtGH8, SXA and TtGH8-SXA, respectively. The fewer number of TtGH8-SXA displayed on the surface of per cell suggests the decreased display efficiency for protein with a larger molecule (108 kDa). On the basis of the number of enzymes displayed on cell surface, the effects of single display system and dual-display system on saccharification of hemicellulose were investigated. As can be seen from Supplementary Fig. 5, the strain displaying fusion protein TtGH8-SXA exhibited a 30.37% lower activity towards xylan than the mixture of two strains displaying TtGH8 and SXA individually with molar ratio of 1:1 at the same levels of displayed enzyme molecules, probably due to the lower protein numbers of TtGH8-SXA on cell surface compared to the sum of TtGH8 and SXA single-displayed numbers. Then, the ratio of cell density of two engineered strains was optimized to efficiently hydrolyze xylan into monosaccharides. As indicated in Fig. 2a, different ratios of strain *E. coli*-TtGH8 to *E. coli*-SXA resulted in different hydrolysis efficiency with the best ratio of 3:7. TtGH8 and SXA possess different functions during the degradation process of xylan. TtGH8 is responsible for degrading xylan into xylooligosaccharide as well as a small amount of D-xylose and L-arabinose[23]. The xylooligosaccharide could be further hydrolyzed into D-xylose and L-arabinose under the action of SXA. So, the amounts of required SXA were higher than those of TtGH8 for full

hydrolyzation of xylan into monosaccharides. The hydrolysis efficiency of up-stream bacterial consortia including *E. coli*-TtGH8 and *E. coli*-SXA towards xylan at different concentrations were examined. To quantify the proportion and amounts of pentose in the hydrolysate after strains' treatment, the resultant D-xylose and L-arabinose were determined by HPLC method, accounting for 97.43% and 2.57%, respectively. Although the efficiency of saccharification was increased with the decreasing xylan concentration from 1 g/L to 0.1 g/L, the yields of pentose monomers also reduced accordingly (Supplementary Fig. 6). When corncob xylan was 1 g/L, the hydrolysis efficiency was 65.43% (w/w) after 6 h reaction (Supplementary Fig. 6), which was about 1.42-fold higher than *in vitro* process[31] and 1.65-fold higher than *in vivo* process reported previously[32]. When the concentration of xylan continued to increase, the production of pentose failed to rise proportionally. Anyway, this simple bacterial consortium could generate soluble monosaccharides from xylan ready for the subsequent oxidative degradation.

Engineered bacterial consortia involving in oxidation of pentose monosaccharides (down-stream pathway)

To date, D-xylose metabolism pathway has been found in a few microorganisms, which harbors three catabolic routes, including the Weimberg or Dahms pathway, the xylulose-1-phosphate or ribulose-1-phosphate pathway and the xylose isomerase or xylose reductase-xylitol dehydrogenase pathway[33]. *E. coli* can utilize xylose as a carbon source for growth through the native route mediated by XylA and XylB as well as pentose phosphate pathway and the glycolysis. However, the low efficiency limited the application of this pathway in metabolic engineering[34]. Weimberg route in *Caulobacter crescentus* involves

conversion of D-xylose to α-ketoglutarate by five steps successively catalyzed by XDH, XylC, XylD, XylX and KGSADH[35] (Fig. 1). This pathway has been proved to be an attractive route for biosynthesis of various chemicals from xylose[34, 36, 37]. In our current study, the oxidation of pentose and the transferring of electrons could be realized by employing this efficient pathway, during which 1 molecule of α-ketoglutarate and 4 electrons per pentose unit can be generated.

To identify the biological activity of each enzyme involved in Weimberg pathway, the *in vitro* activities of the purified enzymes were measured. Proteins expression conditions were optimized (Supplementary Fig. 7) and enzymes were purified to homogeneity through Ni^{2+} column affinity chromatography before SDS-PAGE analysis (Supplementary Fig. 8). In this first step of Weimberg pathway, D-xylose is oxidized into D-xylono-lactone and generates NADH catalyzed by XDH using NAD^+ as coenzyme. So, the activity of XDH was monitored by measuring the absorbance at 340 nm, typical absorption peak of NADH. As listed in Supplementary Table 3, the enzymatic activity of XDH was 1195.00 ± 8.60 U/mg. To examine the activity of XylC, D-xylono-lactone was used as substrate to produce D-xylonic acid. The enzymatic activity of XylC was 146.93 ± 2.16 U/mg, which was 7-fold lower than that of XDH (Supplementary Table 3). Subsequently, D-xylonic acid would be transformed into 2-keto-3-deoxyxylic acid by XylD with an activity of 77.00 ± 3.00 U/mg. The activity of KGSADH is 54.00 ± 6.00 U/mg, which was also monitored by measuring the generation of NADH using analogous glutaraldehyde as substrate. Finally, the enzymatic activity of XylX was determined only 35.65 ± 0.25 U/mg (Supplementary Table 3) by a coupled assay with XylD and KGSADH. Thus XylX possessed the lowest activity among these five enzymes

involved in Weimberg pathway. Therefore, all of the enzymes had biological functions, but the conversion of 2-keto-3-deoxyxylic acid to 2,5-dioxopentanoate catalyzed by XylX may be a limiting step in the overall route, as proposed in other literatures[37].

On the basis of the *in vitro* analysis of these five members in Weimberg pathway, cell-surface display systems were constructed. To confirm that the localization of enzymes on the surface of *E. coli* using InaPb anchoring motifs, Western-Blot analysis of outer membrane fraction and immunofluorescent labeling of cells were conducted. All the distinct bands of expected sizes from outer membrane fractions were found (Supplementary Fig. 9), confirming that the introduced genes in different recombinant plasmids were expressed obviously. Compared with the control strain, green fluorescence was visualized for strains samples by confocal microscope (Supplementary Fig. 10). To ascertain the displayed enzymes possessed functions on the surface of bacteria, the whole-cell was regarded as the catalyst in the enzymatic activity assay. After optimizing the protein expression conditions for displayed enzymes (Supplementary Fig. 11), the whole-cell activities were determined. As depicted in Tables S3 and S4, XDH-displaying *E. coli* exhibits the highest activity of 1.25 ± 0.04 U/OD_{600} with k_{cat} value of 11.34 ± 1.21 s^{-1}. The whole cell activity of *E. coli*-XylC was significantly lower than that of *E. coli*-XDH. So, the co-display of XDH and XylC would lead to the imbalance of cascade reaction. Therefore, the maximum cascade reaction rate could be achieved by separately displaying XDH and XylC on cell surface and regulating the ratio of strain *E. coli*-XDH and *E. coli*-XylC. On the contrary, XylX-displaying *E. coli* shows the lowest activity (0.044 ± 0.003 U/OD_{600}) with k_{cat} value of 0.07 ± 0.006 s^{-1}, which corresponds to the *in vitro* data. These results reveal that XylX was the rate-limiting enzyme in the whole

pathway, so the highest overall reaction rate could be realized by adjusting the level of *E. coli*-XylX in engineered bacterial consortia.

Considering the imbalance of activities between *E. coli* displaying enzymes, the dual-display and tri-display systems were developed to accelerate the overall reaction rate of the latter half of the pathway. The Western Blotting analysis and confocal imaging micrographs indicate the successful expression and display of fusion enzymes on cell surface (Supplementary Fig. 9 and Supplementary Fig. 10). Unfortunately, the degradation of triple fusion protein XylX-XylD-KGSADH was observed, implying the unstable presentation of much larger protein (157 kDa) on cell surface. The overall reaction rates from D-xylonic acid to α-ketoglutarate were varied using different display systems as biocatalysts. Among them, the bacterial consortia containing XylD-KGSADH-displaying *E. coli* (*E. coli*-XylDK) and XylX-displaying *E. coli* (*E. coli*-XylX) exhibited the best catalytic efficiency when using D-xylonic acid as substrate and NADH as indicator (Fig. 2b). Then, the cell density ratios of down-stream pathway were optimized to realize the maximum overall reaction rate from D-xylose to α-ketoglutarate. Considering the highest activity of *E. coli*-XDH in the five whole-cell catalysts, the ratio of *E. coli*-XDH in the prepared bacterial consortia was controlled at a lower level. The ratios of other three strains, including *E. coli*-XylC, *E. coli*-XylDK and *E. coli*-XylX, were varied to realize the highest overall reaction rate from D-xylose to α-ketoglutarate. As shown in Fig. 2c, the ratios of *E. coli*-XylDK and *E. coli*-XylX had obvious synergistic effect on the generation of the final product α-ketoglutarate. Thus, the Weimberg pathway can function in the engineered bacterial consortia.

The integration of the engineered bacterial consortia for producing α-ketoglutarate

To integrate the entire pathway for degrading xylan, according to above optimized enzyme-displaying strain ratios in up-stream pathway and down-stream pathway, these involved enzyme-displaying strains were mixed into bacterial consortia with different ratios of cell densities in the saccharification of xylan into pentose and the oxidation pentose into α-ketoglutarate. The most appropriate ratio was 3:7 for bacterial consortia in up-stream route to down-stream route with the total OD_{600} of 10.0 taking the titer of α-ketoglutarate as evaluation criterion (Fig. 2d). These results demonstrate that the imbalanced enzymes activities could be adjusted by optimizing strain ratios.

α-Ketoglutarate is not only an important intermediate metabolite in cells, but also is widely used as antioxidant and nutrient supplements in food and pharmaceutical fields[38]. Due to the low efficiency and complex process of chemical synthetic route[39], α-ketoglutarate is 10 times more expensive than other common organic acids such as citric acid and lactic acid. Moreover, the usage of toxic chemical reagent cyanide in chemical reaction process has aroused the concerns of environmental pollution. The microbial synthesis method is characterized as cost-effective, high efficient and eco-friendly[40]. Until now, *Yarrowia lipolytica* and *Corynebacterium glutamicum* have been engineered as microbial cell factories to produce α-ketoglutarate using various substrates, such as raw glycerol and xylose (Table 1). Compared with these substrates, hemicellulose is considered to be the most promising feedstock due to its global availability and cost-effective benefits. Herein, to the best of our knowledge, the employment of renewable hemicellulose to produce α-ketoglutarate was realized for the first time with the yield of 47% (g/g) within 6 h *in vitro* one-pot reaction by

the engineered bacterial consortia saccharification and oxidation pathway. Our yield reaches the highest level within 6 h, which is significantly shorter than over 90 h for microbial cell factories using cost-ineffective glycerol and xylose as substrates (Table 1). It is worth noting that, as the pivot metabolites among tricarboxylic acid cycle (TCA cycle), glyoxylate cycle and amino acid metabolism, the generation of α-ketoglutarate would be impacted by complex metabolic regulation *in vivo*. In this study, extracellular pathway circumvented this bottleneck and improved the synthetic efficiency. Thus, the bioproduction of α-ketoglutarate from hemicellulose in this work realized the efficient conversion of agricultural and forestry residues to high-added value compound.

The integration of the engineered bacterial consortia for generating electricity in MFC

Fig. 3 describes the mechanism of the two-compartment xylan/O_2 MFC separated by a Nafion® membrane. The mediator-less anodic compartment containing multi-walled carbon nanotubes (MWCNTs) covered carbon cloth (CC) electrode, which was modified with engineered bacterial consortia (CC/MWCNTs/bacterial-consortia) and fuel of xylan, while the cathodic compartment contained MWCNTs-coated CC electrode, which was modified with *E. coli*-displayed laccase (Lac) (CC/MWCNTs/*E.coli*-Lac) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as the redox mediator. Lac was confirmed to display on the cell surface by SDS-PAGE, Western-Blot and confocal imaging (Supplementary Fig. 12) with good catalytic activity (Supplementary Fig. 13), which catalyzes the O_2 reduction at the cathode (Fig. 4a). *E.coli*-Lac exhibits the maximum activity at pH 5.0 (Supplementary Fig. 13c). The synthetic metabolic pathway on the anode

compartment was designed to degrade xylan into α-ketoglutarate, generating 4 electrons per pentose unit. The reactions can be expressed as:

Anode: $\text{Xylan} \xrightarrow[\text{NAD}^+]{\text{engineered bacterial consortia}} \alpha\text{-ketoglutarate} + 4e^- + 4H^+$ (1)

Cathode: $O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$ (2)

Overall: Xylan + $O_2 \rightarrow$ α-ketoglutarate + $2H_2O$ (3)

The performance of the biocathode and bioanode was examined by Cyclic voltammetry (CV). At the CC/MWCNTs/*E.coli*-Lac, ABTS exhibited a redox pair in the absence of oxygen (red line), while the cathodic specific current significantly enhanced in the presence of O_2 (Fig. 4a, light blue line), indicating that with ABTS as the electron transfer mediator, the bacteria-displayed laccase catalyzes the reduction of oxygen. For bioanode, as shown in Fig. 4b, an onset potential at 0.13 V (vs. Ag/AgCl) for enzyme-displayed *E. coli* was observed (red line). Interestingly, the onset potential for CC/MWCNTs/bacterial-consortia was negatively shifted to 0 V (blue line), suggesting that the generated NADH from NAD^+ was catalytically oxidized by MWCNTs[43]. 9,10-anthraquinone-2,7-disulfonic acid (AQDS) was used as electron mediator to investigate the impact of electron transfer mechanisms on the performance of bioanode. As shown in Fig. 4b, the two reduction peaks (green curves) at -0.6 V and -0.9 V were the reduction peaks of AQDS with MWCNTs modified CC as electrode. The oxidation peak of AQDS was around -0.5 V on the purple curve, which was shifted to around -0.4 V due to the influence of CC modified with bacterial consortia. The oxidizing redox peak around -0.1 V on the purple curve was the oxidation peak of NADH produced by the catalysis of bacterial consortia in the presence of AQDS. AQDS can lower the onset potential of NADH which was produced by cell-surface displayed enzymes' catalysis. The onset potential was negatively shifted to -0.20 V (purple line). Therefore, this mediator was

implemented in the electrolyte of anodic chamber in the following studies.

Then, we studied the performance of MFCs composed of the above prepared bioanode and biocathode by varying the way of loading engineered strains. The current density (j_{max}) of the generated MFC was 371.67 ± 10.36 μA·cm^{-2} when bacterial consortia were immobilized on electrode (Fig. 4c, light green line). However, the j_{max} of MFC dropped to 297.54 ± 10.24 μA·cm^{-2} when bacterial consortia were dispersed in bioanode chamber (Fig. 4c, blue line). Then the effects of different concentrations of AQDS on the performance of xylan/O_2 MFC were investigated. The results show that the highest value of power was achieved when 10 mM of AQDS was used (Fig. 4c, peach pink line). The similar trends were observed in Fig. 4d, and the constructed MFC reached the maximum power output (P_{max}) of 68.25 ± 2.38 μW·cm^{-2} when bacterial consortia immobilized on bioanode in the presence of 10 mM of mediator AQDS.

Shewanella oneidensis is one of the most well-known electricigen in nature[44]. The attempt to engineer *S. oneidensis* MR-1 using D-xylose as fuel to generate bioelectricity in MFC by introducing D-xylose metabolic route shows restricted power owing to poor efficiency of this exogenous pathway[45]. Herein, we reconstituted a D-xylose oxidative pathway and electron transfer chain on cell surface by displaying Weimberg pathway members. There are two steps can produce NADH for generating power, which were catalyzed by XDH and KGSADH. Finally, the oxidation of one D-xylose can yield two NADH and four electrons during the whole process, and the final metabolite α-ketoglutarate can be used as the key precursor of several medicine and nutrient substance in food as well as feed[46].

The optimization of MFC performance

To boost the power output of MFC, effects of several parameters on electricity generation were investigated. First, the loading amounts of bacterial consortia on the bioanode was optimized. The j_{max} of MFCs was 671.33 ± 26.21 μA·cm^{-2}, corresponding P_{max} of 112.87 ± 7.83 μW·cm^{-2} when loading 10 OD_{600} bacterial consortia (Fig. 5a, peach pink line). The results indicate that the bacterial cell loading exerted effects on power output since the insulated nature of concentrated cells would cause ohmic losses[47]. Then, MFCs performance fueled by commercial xylan from corncob was tested under the optimal conditions established according to above experiments. As shown in Fig. 5b, when the concentrations of corncob xylan increased from 0.5 g/L to 1 g/L, the P_{max} boosted from 111.82 ± 5.35 μW·cm^{-2} (green line) to 174.33 ± 4.56 μW·cm^{-2} (peach pink line). However, further increase in concentrations of xylan resulted in 12.22% decrease in power density (blue line), probably due to the poor solubility and high viscosities of commercial xylan prepared in buffer solution. The P_{max} of our MFC fueled by 1 g/L xylan is 174.33 ± 4.56 μW·cm^{-2}, which is significantly higher than those for xylan-fueled MFCs inoculated with activated sludge (0.609 μW·cm^{-2}) or rumen microorganisms (40.5 μW·cm^{-2})[13, 48].

In order to investigate the release and transfer of electrons in bioelectrochemical system, Faraday efficiency (η_F) was determined (Supplementary Fig. 14). The current peaked at 2.07 mA at the onset time and then declined over time. In a batch reaction of 35 h, the cumulative electric charges generated from 50 mL of reaction solution were 58.34 C. The concentration of product α-ketoglutarate determined by HPLC was 3.19 mM, convertible to the yield of 46.6%, suggesting that the same amount of D-xylose was consumed and corresponding to

61.58 C of electricity. In consideration of a conversion efficiency of 97% from NADH to electrons determined in a previous study[49], the η_F value was calculated to be 97.7% (58.34÷61.58÷0.97×100%).

Long-term electricity generation of MFCs fueled by hemicellulose fractions of corncob

As xylan is a predominant component of hemicellulose, we next examined the performance of MFCs powered by real hemicellulose. Biomass corncob pretreated by heating in alkaline solution after neutralizing was used as fuels for power generation[50]. 1.06 g/L xylan from the pretreated hydrolysates of corncob was fueled, in which D-xylose was not detectable by HPLC. To supply constant fuels to MFC, the above pretreated hemicellulose was supplemented once a day to roughly maintain the same level. Then we examined the performance of the MFC using enzyme-engineered-bacterial-consortia-modified-bioanode. As shown in Fig. 6, the P_{max} of enzyme-bacterial-consortia-bioanode based MFC was 162.73 ± 3.69 $\mu W \cdot cm^{-2}$ (Fig. 6, black line). The remarkable power output of the enzyme-bacterial-consortia-bioanode based MFC is much excelled over microbial community based MFC[13], possibly because diverse biological properties of various microorganisms in one system may result in the instability in bacterial community. In addition, the long-term generation of electricity was also evaluated. The bacterial-consortia-bioanode based MFC maintained more than 95% of the P_{max} after 6 days' operation. On the contrary, the poor stability was observed in other biomass-based sugar-fed enzymatic fuel cells[51]. Besides, the conversion of pretreated hemicellulose from corncob to α-ketoglutarate was also monitored during 6 days. The yield of α-ketoglutarate was 44.3% during the 6 days' long-term electricity generation of MFC fueled by the pretreated hydrolysates of corncob (Fig. 6, red line).

Moreover, the morphology of the bacterial-consortia-bioanode was observed by scanning electron microscope (SEM). After generating power for 6 days, the *E. coli* consortia and MWCNTs were still closely bound to the CC, while the *E. coli* were intact (Supplementary Fig. 15). These results indicate that the cells survived and stayed stable on CC during 6 days' operation. These results suggest that the capabilities of engineered bacterial consortia can efficiently convert hemicellulose biomass into high-value products and electric power.

In summary, a synthetic pathway that can generate high value-added chemical of α-ketoglutarate and 4 electrons from hemicellulose in a controllable manner was successfully constructed by integrating six enzyme-displayed strains into a high-efficient bacterial consortia through tuning the expressed enzyme molecules and adjusting ratios of consortia members. The production of α-ketoglutarate with excellent yield of 0.47 g/g was realized within 6 h by the enzyme-engineered bacterial consortia surface-displayed saccharification and oxidation pathway of xylan, the most abundant hemicellulose type. Then a two-compartment xylan/O_2 MFC was assembled using enzyme-bacterial consortia modified bioanodes and a laccase-displayed strain modified biocathode, realizing the direct conversion of biomass into electricity and α-ketoglutarate. The optimized MFC realized 46.6% (w/w) yield of α-ketoglutarate, the highest OCV and the largest P_{max}. The Faraday efficiency was 97.7%. Furthermore, this MFC exhibited a considerable power output and long-term stability towards real biomass samples. To the best of our knowledge, this is the first example to use engineered bacteria consortia as biocatalyst for efficient utilization of hemicellulose, representing an important step toward further improving conversion of biomass to high value-added chemical and powerful electric energy. Based on these results, we envision that

403 controllably enzyme-engineered bacterial consortia would find a wide range of applications in
404 the fields of metabolic engineering, synthetic biology, enzyme engineering, bioenergy and
405 enzymatic electrosynthesis.
406
407 **Methods**
408 **Strains and chemicals**
409 All strains and plasmids used in this study are listed in Supplementary Table 1. The primers
410 used are listed in Supplementary Table 2. *E. coli* DH5α was used for gene recombinant
411 manipulation and *E. coli* BL21 (DE3) Δ*xylAB* was used as host to express recombinant
412 proteins. Strains of *E. coli* carrying recombinant plasmids were routinely grown in
413 Luria-Bertani (LB) medium (10 g/L tryptone, 10 g/L NaCl, 5 g/L yeast extract) at 37 °C and
414 200 rpm. Whenever necessary, antibiotic (kanamycin, 50 μg/mL) was added. Taq DNA
415 Polymerase and all restriction endonucleases were purchased from Fermentas (St. Leon Rot,
416 Germany). Kits used for molecular cloning were obtained from Sparkjade Biotech Co., Ltd
417 (Qingdao, China). Corncob xylan to represent hemicellulose were purchased from Meryer Co.
418 Ltd (Shanghai, China). 4-nitrophenyl-β-D-xylopyranoside (PNPX), ABTS, AQDS, D-xylose,
419 L-arabinose, xylono-1,4-lactone, D-xylonic acid lithium salt and α-ketoglutarate were
420 purchased from Sigma-Aldrich (St. Louis, MO, USA). Platinum (Pt) wire, Ag/AgCl electrode
421 and Nafion 117 membrane (N117) were purchased from Incole Union Technology Co. Ltd
422 (Tianjin, China). Multi-walled carbon nanotubes (MWCNTs) were purchased from Macklin
423 Biochem Technology Co. Ltd (Shanghai, China). Corncob was obtained from Corn Research
424 Center of Qingdao Agriculture University. Other chemicals were purchased from Solarbio
425 Science & Technology Co., Ltd (Beijing, China).

426 **Plasmids construction**

427 **Construction of the knockout plasmids**

428 For genetic operations into the genome of *E. coli* BL21 (DE3), the pRE112 suicide vector was
429 utilized. To construct plasmids pRE112- Δ*xylAB*, approximately 862 bp upstream and 857 bp
430 downstream fragments of the *xylA* and *xylB* genes were amplified using the upstream primers
431 xylAB-Up-F/R and downstream primers xylAB-Down-F/R, respectively, and inserted into the
432 Kpn I site of pRE112. The plasmid pRE112-Δ*xylAB* were transferred from *E. coli* χ7213 to *E.*
433 *coli* BL21(DE3) by conjugation. Suitable recombinants were identified by antibiotic
434 resistance screening and sucrose reverse screening. The resulting strain *E. coli*-Δ*xylAB* was
435 identified by PCR via the primers xylAB-1-F/R.

436 **Construction of anchoring motif expressing plasmid**

437 The gene encoding N-terminal ice nucleation protein originating from *Pseudomonas borealis*
438 was amplified from vector pTInaPbN-Xdh by PCR using primers INP-F/INP-R, and inserted
439 into the *Nco* I/*Nde* I site of pET-28a (+) to generate plasmid pYJ-00.

440 **Constructions of hemicellulose hydrolytic enzymes expressing plasmids**

441 The gene encoding TtGH8 from *Teredinibacter turnerae* (GenBank No. CP001614.2), SXA
442 from *Selenominas ruminantium* (GenBank No. WP_026766185) were codon-optimized for *E.*
443 *coli* and synthesized by BGI Co., Ltd (Shenzhen, China). To construct intracellular protein
444 expression vectors pYJ-01 and pYJ-02, TtGH8 and SXA encoding gene and corresponding
445 cloning vector gene were amplified from above cloning plasmids and pET-28a by PCR
446 method using primers pET-28a-F(TtGH8)/pET-28a-R(TtGH8) & TtGH8-1-F/TtGH8-1-R,
447 pET-28a-F (SXA)/pET-28a-R (SXA) & SXA-1-F/SXA-1-R, respectively, and using the

448 ClonExpress Ultra One Step Cloning Kit (Vazyme Biotechnology, Nanjing, China). To
449 construct intracellular protein expression vectors pYJ-03, pYJ-01 and pYJ-02 vectors were
450 used as PCR templates and pET-28a-TtGH8-F/pET-28a-TtGH8-R & SXA-2-F/SXA-2-R as
451 primers, and two amplified gene fragments were ligated by in-fusion method using the
452 ClonExpress Ultra One Step Cloning Kit. For the construction of plasmids overexpressing
453 enzymes displayed on cell surface, TtGH8 and SXA encoding gene and corresponding
454 cloning vector gene were amplified from above cloning plasmids and pYJ-00 by PCR using
455 primers pET-28a-INP-F (TtGH8)/pET-28a-INP-R (TtGH8) & TtGH8-2-F/TtGH8-2-R,
456 pET-28a-INP-F(SXA)/pET-28a-INP-R(SXA) & SXA-3-F/SXA-3-R, respectively, and using
457 the ClonExpress Ultra One Step Cloning Kit to generate pYJ-04 and pYJ-05. Plasmid pYJ-06
458 was constructed using pYJ-04 and pYJ-05 as PCR templates and
459 pET-28a-INP-TtGH8-F/pET-28a-INP-TtGH8-R & SXA-4-F/SXA-4-R as primers by fusion
460 PCR strategy.

461 **Construction of pentose oxidative enzymes expressing plasmids**

462 To construct intracellular protein expression vectors pYJ-07, pYJ-08, pYJ-09, pYJ-10 and
463 pYJ-11, the genes encoding XDH, XylC, XylD, XylX, KGSADH were amplified from the
464 genomic DNA of *Caulobacter crescentus* NA1000[35] and corresponding cloning vector gene
465 was amplified from pET-28a by PCR method using primers pET-28a-F
466 (XDH)/pET-28a-R(XDH) & XDH-1-F/XDH-1-R, pET-28a-F (XylC)/PET-28a-R (XylC) &
467 XylC-1-F/XylC-1-R, pET-28a-F(XylD)/pET-28a-R(XylD) & XylD-1-F/XylD-1-R,
468 pET-28a-F(XylX)/pET-28a-R(XylX) & XylX-1-F/XylX-1-R,
469 pET-28a-F(KGSADH)/pET-28a-R(KGSADH) & KGSADH-1-F/KGSADH-1-R, respectively,

and using the ClonExpress Ultra One Step Cloning Kit. To construct intracellular protein expression vectors pYJ-12, pYJ-09 and pYJ-11 vectors were used as templates and pET-28a-XylD-F/pET-28a-XylD-R & KGSADH-2-F/KGSADH-2-R as primers, and two amplified gene fragments were ligated by in-fusion method (the ClonExpress Ultra One Step Cloning Kit). For the construction of plasmids overexpressing enzymes displayed on cell surface, XDH, XylC, XylD, XylX, KGSADH encoding genes was amplified from above cloning plasmids and corresponding cloning vector gene was amplified from pYJ-00 by PCR method using primers pET-28a-INP-F(XDH)/pET-28a-INP-R(XDH) &XDH-2-F/XDH-2-R, pET-28a-INP-F(XylC)/pET-28a-INP-R(XylC) & XylC-2-F/XylC-2-R, pET-28a-INP-F(XylD)/pET-28a-INP-R(XylD) & XylD-2-F/XylD-2-R, pET-28a-INP-F(XylX)/pET-28a-INP-R(XylX) & XylX-2-F/XylX-2-R, pET-28a-INP-F(KGSADH)/pET-28a-INP-R(KGSADH) & KGSADH-2-F/KGSADH-2-R, respectively, and using the ClonExpress Ultra One Step Cloning Kit to generate plasmids pYJ-13, pYJ-14, pYJ-15, pYJ-16 and pYJ-17. Plasmid pYJ-18 was constructed using pYJ-15 and pYJ-16 as PCR templates and pET-28a-INP-XylD-F(XylX)/pET-28a-INP-XylD-R(XylX) & XylX-3-F/XylX-3-R primers by fusion PCR strategy (ClonExpress Ultra One Step Cloning Kit). Plasmid pYJ-19 was constructed using pYJ-15 and pYJ-17 as PCR templates and pET-28a-INP-XylD-F(KGSADH)/pET-28a-INP-XylD-R(KGSADH) & KGSADH-3-F/KGSADH-3-R primers by fusion PCR strategy (ClonExpress Ultra One Step Cloning Kit). Plasmid pYJ-20 was constructed using pYJ-19 and pYJ-16 as PCR templates and pET-28a-INP-XylD-KGSADH-F/pET-28a-INP-XylD-KGSADH-R & XylX-4-F/XylX-4-R primers by fusion PCR strategy (ClonExpress Ultra One Step Cloning

Kit).

Construction of lac-expressing plasmid

For the construction of plasmids overexpressing lac- displayed on cell surface, the gene encoding laccase from *Bacillus subtilis* [52] was subcloned into pYJ-00 to generate pYJ-21 by BGI Co., Ltd (Shenzhen, China).

Purification of cytoplasmic enzymes

Cells expressing seven intracellular enzymes, including TtGH8, SXA, TtGH8-SXA, XDH, XylC, XylD, XylX, KGSADH and XylDK (strain TtGH8, SXA, TtGH8-SXA, XDH, XylC, XylD, XylX, KGSADH and XylDK), were separately grown overnight in LB medium with kanamycin, and induced with 0.5 mM IPTG when cells had reached an OD_{600} of 0.6. After overnight growth at 16 °C, cells were harvested by centrifugation, resuspended in 40 mL 50 mM Tris–HCl (pH 7.0) buffer, broken by ultrasonication and centrifuged at 12000 g for 40 min at 4 °C to remove cell debris and unbroken cells. The soluble extract was applied to a 5 mL Ni-NTA column that had been equilibrated with 50 mM Tris–HCl (pH 7.0). Then the column was washed consecutively with resuspended buffer and several washing buffer (20 mM Tris–HCl pH 7.0, 300 mM NaCl, 1 mM β-mercaptoethanol) containing increasing amounts of imidazole (10, 20, 30 and 50 mM). The bound protein was eluted with elution buffer (20 mM Tris–HCl pH 7.0, 300 mM NaCl, 200 mM imidazole, 1 mM β-mercaptoethanol). Protein dialysis was conducted in a buffer (10 mM Tris–HCl pH 7.0 containing 100 mM NaCl, 1 mM β-mercaptoethanol) at 4 °C. Protein purity was analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and protein concentration was determined by Bradford assay.

Cell-surface display of enzymes and cell-surface location analysis

For displaying enzymes on cell surface of *E. coli*, recombinant strains were cultured in LB medium to an OD_{600} of 0.6, 0.5 mM of IPTG was used to induce protein expression. For laccase, cells were further cultured overnight at 16 °C, collected by centrifugation and incubated in a buffer of Tris–HCl (50 mM, pH 7.0). The other cells were further cultured at 30 °C overnight, which were collected by centrifugation and incubated in Tris–HCl buffer (50 mM, pH 7.0). To determine the successful display of enzymes on the cell surface, outer membrane proteins of recombinant strains and negative control strain were isolated[53]. The outer membrane protein fractions of related strains were analyzed by 10% (wt/vol) SDS-PAGE.

For Western-Blot assay, samples were transferred to PVDF membranes at 100 mA for 10 min. Anti-6×HisTag mouse monoclonal antibody (catalog number D191001, Sangon Biotechnology, Shanghai, China) at 1:1000 was added and incubated for 3 h at room temperature, then rinsed three times with phosphate buffered saline (PBS) with 0.05%(v/v) Tween-20 (PBST). It was then incubated with goat anti-mouse immunoglobulin G (IgG)-horseradish peroxidase (HRP) conjugate (catalog number D110068, Sangon Biotechnology, Shanghai, China) for 1 h at room temperature, washed three times with PBST and then once with PBS. The conjugation of antigen and antibody was detected with W-TMB chromogenic kit (Sangon Biotechnology, Shanghai, China).

For Quantitative Immunoassay, outer membrane proteins of recombinant strains were transferred to low fluorescent background PVDF membranes. Anti-6×HisTag mouse monoclonal antibody at 1:1000 was added and incubated for 3 h at room temperature, then

rinsed three times with PBST. It was then incubated with goat anti-mouse IRDye 800CW fluorescent secondary antibodies (catalog number 926-32210, LI-COR, Inc., Lincoln, NE) for 1 h at room temperature, and then washed with PBST, air dried, wrapped in aluminum foil, and stored at 4 °C. LI-COR Odyssey CLx imaging system was used to detect bands on membrane. The standard curve from known concentrations of purified recombinant XDH run on the same blot was obtained. Specific protein bands are quantified by fluorescent signals (excitation wavelength, 785 nm; emission wavelength, 820 nm) and a linear regression equation generated from recombinant XDH to estimate concentrations of proteins.

For fluorescence imaging, the induced cells were washed with PBS and blocked in PBS buffer containing 1% bovine serum protein (BSA) for 30 min at room temperature. Next anti-6xHis tag mouse monoclonal antibody was added (1:100) after blocking, incubated overnight at 4 °C, and incubated for 1 h the next day at room temperature. After three washes with PBS, the cells were incubated with FITC-conjugated Donkey anti-Mouse IgG (1:100) for 2 h at room temperature. The cells after PBS washing were observed with Laser Scanning Confocal Microscope (TCSsp5 Ⅱ 03040101, Agilent, USA).

Enzymatic activity assay

Enzymatic activity was monitored by measuring the optical density at 600 nm (OD_{600}) for cells using a Cary-60 UV-VIS spectrophotometer (Agilent Technologies, Inc., Santa Clara, CA). Soluble commercial corncob xylan was prepared by water dissolution. TtGH8 activity was assessed on 1 g/L soluble commercial corncob xylan in 50 mM Tris–HCl buffer pH 7.0 with 10 mM $CaCl_2$ at 37 °C. The reducing sugars released by TtGH8 hydrolysis reaction was measured by DNS method with xylose as standard[54]. SXA enzymatic activity was detected

using pNPX as the substrate at 37 °C. The product *p*-nitrophenol shows typical peak at 405 nm, which can be monitored by a spectrophotometer[26]. Enzymatic activity of XDH was assayed by detecting the generation of NADH at 340 nm and 37 °C using 10 mM D-xylose as substrate as well as 1 mM NAD^+ as coenzyme. For XylC, it can accelerate the hydrolysis of xylono-1,4-lactone into D-xylonic acid to generate D-xylonic acid, which can reduce the absorbance at 405 nm of *p*-nitrophenol. Enzyme activity was quantified by measuring the decrease in the absorbance at 405 nm[55]. Spontaneous hydrolysis of xylono-1,4-lactone was analyzed without catalysts. Enzymatic activity of XylD was assayed according to a modified procedure. In a typical assay, the reaction cocktail contained a certain amount of purified or cells and D-xylonic acid. After incubation at 37 °C for a certain period, the samples were mixed with solution containing 1% semi-carbazide hydrochloride and 1.5% sodium acetate. Finally after incubation at 30 °C for 10 min, the 2-keto-3-deoxyxylic acid produced was quantified by detection of the absorbance at 250 nm typical of semicarbazone[37]. Enzymatic activity of XylX was assayed spectrophotometrically in a coupled assay with the corresponding previous dehydratase XylD and KGSADH.[37, 56] The assay was performed in 50 mM Tris–HCl buffer (pH 7.0) with 10 mM $MgCl_2$ containing 20 mM D-xylonic acid and 1 mM NAD^+. After the addition of purified enzymes or enzyme-surface displayed strains, the mixture was incubated at 37 °C. The increasing absorbance at 340 nm caused by NADH produced in the reaction was monitored. Glutaraldehyde was used as the substitute substrate to measure the activity of KGSADH by following the rate of NAD^+ reduction by measuring the optical density at 340 nm[57]. To determine kinetic constants of cell surface-displayed enzymes, different amounts substrate was used to initiate a series of enzymatic assays. The

580 data were applied to the Michaelis-Menten kinetic model using Graph-Pad Prism 5 software
581 (www.graphpad.com). To determine the optimum pH for the *E. coli*-Lac, all enzymatic
582 activities of the whole-cell catalyst were measured by adding ABTS to the final concentration
583 of 5 mM at 37 °C by varying the pH values of the buffer solution (50 mM), including HAc–
584 NaAc buffer (pH 3–5.5), Na_2HPO_4–NaH_2PO_4 buffer (pH 6) and Tris–HCl buffer (pH 7–9).
585 The oxidation activity of *E. coli*-Lac towards ABTS was calibrated by measuring the
586 absorbance of the supernatant at 420 nm (ε=36 $mM^{-1}cm^{-1}$, referred to ABTS concentration).

587 **Optimization of protein induced expression conditions**

588 Recombinant strains were cultured in LB liquid medium at 37 °C and 200 rpm. To optimize
589 inducer concentration for overexpressing INP-fused proteins, IPTG at different concentrations
590 of 0.1 mM, 0.5 mM, 1 mM and 1.5 mM were used when cells grew to an OD_{600} value of 0.6.
591 Protein expression was induced overnight, and the activities of whole cell catalyst were
592 determined as above described. In order to investigate the effects of induction temperatures on
593 intracellular and displayed proteins, 0.5 mM of IPTG were added in to cultures when cells
594 grew to an OD_{600} value of 0.6. Protein expression was induced overnight at different
595 temperatures of 16 °C, 25 °C, 30 °C and 37 °C, and the activities of crude extract or whole
596 cell catalyst was determined as above described.

597 **Bioproduction of α-ketoglutarate**

598 Recombinant strains were cultured in LB medium to an OD_{600} of 0.6, 0.5 mM of IPTG was
599 used to induce protein expression. Cells were further cultured at 30 °C overnight, which were
600 collected by centrifugation and incubated in Tris–HCl buffer (50 mM, pH 7.0). To produce
601 α-ketoglutarate, 100 mL of 50 mM Tris–HCl buffer (pH 7.0) with 10 mM $MgCl_2$, 10 mM

602 $CaCl_2$, 1.0 g/L commercial corncob xylan and 4 mM NAD^+ were incubated with artificial
603 bacterial consortia (OD_{600}=10) in a flask, which was shaken in an incubator at 150 rpm and
604 37 °C for 6 h. The produced α-ketoglutarate was determined by HPLC.

605 **Preparation of bioanode and biocathode**

606 The thickness of CC (CeTech, Taiwan, China) is 0.32 ± 0.02 mm, which was cut into pieces
607 of 1.5×1.5 cm^2 and washed by sonication in water and anhydrous ethanol, respectively. A 100
608 μL of poly (acrylic acid)-MWCNTs dispersion (0.089 mg/cm^2) was cast and dried in air to
609 acquire modified CC/MWCNTs. For the preparation of bioanode, 100 μL of the prepared cells
610 (OD_{600}=5.0) were dropped onto the CC/MWCNTs and dried at room temperature to obtain
611 CC/MWCNTs/cells modified electrodes. Then 50 μL of Nafion solution (0.1 wt%) was
612 syringed to the electrode surface to cover the electrode. For the modification of biocathode,
613 100 μL *E. coli*-Lac aqueous dispersion (OD_{600}=10.0) was coated on the CC/MWCNTs, and
614 subsequently, 50 μL of Nafion solution (0.1 wt%) was dropped onto the surface of the
615 resulting electrode, then dried at room temperature. The thus-prepared electrode was denoted
616 as CC/MWCNTs/*E.coli*-Lac. The onset potential is defined as the potential at which the
617 current or current density goes above 1 $\mu A \cdot cm^{-2}$.

618 **Fabrication of MFC**

619 The dual-chamber hemicellulose/O_2 MFC was assembled with anodic compartment
620 containing the artificial bacterial consortia modified CC/MWCNTs/cells and cathodic
621 compartment containing CC/MWCNTs/*E.coli*-Lac, separated by a Nafion 117 proton
622 exchange membrane (DuPont, USA) with a diameter of 1.6 cm. The anodic electrolyte
623 consisted of 100 mM Tris–HCl buffer (pH 7.0) containing 100 mM NaCl, 10 mM $MgCl_2$, 10

mM $CaCl_2$, 4 mM NAD^+ and xylan. 10 mM AQDS was used as mediator when necessary. The cathodic electrolyte consisted of 100 mM Na_2HPO_4–citric acid buffer (pH 5.0) containing 0.5 mM ABTS. Polarization curves were obtained by performing linear sweep voltammetry (LSV) at the scan rate of 1 mV s^{-1} and 37 °C. The specific current (I) was recorded in real time. The voltage (V) between the anode and the cathode was set as the Y-axis of polarization curves. The output power (P) was derived via the relationship: P = V × I. The specific current and power were normalized to the geometric area of the anode (1.5 cm × 1.5 cm = 2.25 cm^2) to obtain the current density and power density, respectively. The calculations of current density and power density refer to Eq.(1) and Eq.(2), respectively:

$j_{max=}$ I/2.25 (1)

$P_{max=}$ V ×I/2.25 (2)

Faradaic efficiency (η_F) assay

The η_F was determined through amperometry at 0.45 V. The generation of current was monitored during a reaction time in a volume of 50 mL at 37 °C. The reaction system composed of 100 mM NaCl, 10 mM $MgCl_2$, 10 mM $CaCl_2$ and 4 mM NAD^+, 1 g/L commercial corncob xylan in 100 mM Tris–HCl buffer (pH 7.0). The production of α-ketoglutarate was detected by HPLC. The total charge (C) was calculated according to the generated current during the whole time. The η_F was calculated using the equation as follows[58]:

$\eta_F = \int I \times dt/(C_{\alpha\text{-}KG} \times V \times n \times F)$ (3)

where I is the current generated, dt is the time to produce current, $C_{\alpha\text{-}KG}$ is the concentration of produced α-ketoglutarate during the whole time, V is the reaction volume, n is the number of

electrons generated per D-xylose consumed, and F is Faraday constant=96,485 C per mole electron.

Long-term electricity generation of MFCs powered by pretreated hemicellulose

To obtain the hemicellulose from lignocellulose, biomass sample corncob was pretreated[50]. The milled corncob was pretreated by 2.5 M NaOH with a solid to liquid ratio of 1:30 (g/mL) at 115 °C for 1 h. After filtration, the filtrate was neutralized with 1 M acetic acid for use. The amounts of xylan were determined by gradient precipitation with ethanol and then freeze drying[6]. This pretreated hemicellulose was used as fuels to power MFC for monitoring electricity and α-ketoglutarate generation. 100 μL of the prepared cells (OD_{600}=10.0) was dropped onto the 2 mg/mL of MWCNTs coated CC surface (2.25 cm^2) and dried at room temperature. The pretreated corncob was supplemented into anodic electrolyte daily to maintain the constant concentration according to that of α-ketoglutarate. LSV was performed every 12 h incubating at 37 °C during 6 days. The produced α-ketoglutarate was determined by HPLC.

Morphology observation of the bio-nanocomposite modified CC

To determine whether the *E. coli* consortia and MWCNT were successfully attached to the CC surface, scanning electron microscopic images of the modified CC were recorded using JSM-7500F scanning electron microscopy (JEOL, Tokyo, Japan). The CC was first washed three times with PBS, soaked with 2.5% glutaraldehyde, and fixed at 4 ℃ for 12 h. Next, the fixed CC was cleaned three times with PBS for 5 min each time. Then the cleaned CC was dehydrated with 30%, 50%, 70%, 90% and 100% ethanol for 5 min each time. Then the dehydrated CC was further dehydrated with 30%, 50%, 70%, 90%, 100% tert-butanol/ethanol

for 5 min each time. After dehydration, the CC was frozen at -20 °C. Next, the frozen CC was put into the freeze-drying machine (Songyuan Huaxing Biotechnology Co., Ltd, Beijing, China) for freeze-drying. Then, the CC was pasted to the copper platform with conductive glue, followed by gold spraying.

Analytical methods

Cell growth was monitored by measuring the optical density at 600 nm (OD_{600}) for cells using a Cary-60 UV-VIS spectrophotometer. Concentrations of D-xylose, L-arabinose, α-ketoglutarate were detected via Ultimate 3000 HPLC (ThermoFisher, USA) using an Aminex HPX87H column. The mobile phase was 0.05 M H_2SO_4, and the flow rate was 0.6 mL min^{-1} at a refractive-index detector at 50 °C[59]. Cyclic voltammetry (CV) was performed in a three-electrode configuration with CC/MWCNTs/cells as working electrode, an Ag/AgCl reference electrode and Pt wire as auxiliary electrode connecting to a CHI 1000C potentiostat (CH Instrument, Shanghai, China). The electrochemical reactions were performed at 37 °C.

Statistics and reproducibility

Statistical analyses were mainly performed using Microsoft Excel software (version 2021). Double-tailed t test or one-way ANOVA and a posteriori test were used for variance analysis. The data were expressed as mean ± standard deviation (SD). Each group included at least three independent biological samples. Compared to a reference sample, significance was established with a *P*-value less than 0.05. No statistical method was used to predetermine sample size. No data were excluded from the analyses. The experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment.

Data availability

Data supporting the findings of this work are available within the paper and Supplementary Information files. A reporting summary for this Article is available as a Supplementary Information file. Source data are provided with this paper.

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Author contributions

B.L., S.C., A.H.L., and J.M.Y. conceived and coordinated the study. B.L., A.H.L., S.C. and J.M.Y. wrote the article with input from all other co-authors. B.L., J.Y., C.F.M., and L.W. constructed the engineered strains, tested enzymatic activity and MFCs. Y.R.Z. and Z.C.L. performed electrochemical testing. J.Y. conducted SEM characterizations. L.Z. and J.L. performed biomass pretreatment. B.L., J.Y., C.F.M., and L.W. analyzed the experimental data. All authors contributed to the writing of the manuscript.

Competing interests

The authors declare no competing interest.

Table 1 The production of α-ketoglutarate by microbial cell factories or *in vitro* one-pot reaction using different substrates.

Organism	Substrate	Time (h)	Yield (g/g)	Reference
Y. lipolytica H355A (PYC1-IDP1)	glycerol	117	40%	[41]
Y. lipolytica WSH-Z06	glycerol	204	47%	[39]
C. glutamicum $WMB2_{evo}$	xylose	90	0.55%	[42]
E. coli consortia	xylan	6	47%	this study

939 **Figure Legends**

940 **Fig. 1 The saccharification and oxidative pathway of xylan catalyzed by bacterial**
941 **surface displayed enzymes using N-terminal region of ice nuclear protein as anchoring**
942 **motif.** The final product is α-ketoglutarate. TtGH8, β-1,4 xylanase; SXA, β-D-xylosidase;
943 XDH, D-xylose dehydrogenase; XylC, xylonolactonase; XylD, xylonate dehydratase; XylX,
944 2-keto-3-deoxy-D-xylonate dehydratase; KGSADH, 2,5-dioxopentanoate dehydrogenase.

945 **Fig. 2 The optimization of ratios and constituents of engineered bacterial consortia. a**
946 Relative production of reducing sugars using corncob xylan (1g/L) as substrate catalyzed by
947 engineered bacterial consortia involving in the saccharification of xylan (up-stream pathway)
948 with different cell density ratios of *E. coli*-TtGH8 to *E. coli*-SXA. 1 mL of 50 mM Tris–HCl
949 buffer (pH 7.0) with 10 mM $CaCl_2$ and 1.0 g/L commercial corncob xylan were incubated
950 with artificial bacterial consortia (OD_{600}=10) at 37 °C (n = 3 biologically independent
951 experiments). The bacterial consortia with the ratio of 3:7 produced the highest level of
952 reducing sugars compared to those with other ratios. **b** Relative production of NADH
953 catalyzed by different bacterial consortia using D-xylonic acid as substrate. When different
954 fusion proteins of XylD-KGSADH, XylD-XylX and XylD-XylX-KGSADH were separately
955 displayed on cell surface, the resultant strains were named as *E. coli*-XylDK, *E. coli*-XylDX
956 and *E. coli*-XylDXK, respectively. The total OD_{600} of these four systems were the same and
957 the cell density ratios of different strains were the same in one system. 1 mL of 50 mM Tris–
958 HCl buffer (pH 7.0) with 10 mM $MgCl_2$, 20 mM D-xylonic acid and 1 mM NAD^+ were
959 incubated with artificial bacterial consortia (OD_{600}=10) at 37 °C (n = 3 biologically
960 independent experiments). Strain *E. coli*-XylDXK as the control. The bacterial consortia
961 system composed of *E. coli*-XylDK and *E. coli*-XylX produced the highest level of NADH
962 compared to those with other system. **c** Relative production of α-ketoglutarate catalyzed by

963 different bacterial consortia involving in oxidation of pentose monosaccharides (down-stream
964 pathway) with various cell density ratios among *E. coli*-XDH, *E. coli*-XylC, *E. coli*-XylDK
965 and *E. coli*-XylX using D-xylose as substrate. 1 mL of 50 mM Tris–HCl buffer (pH 7.0) with
966 10 mM $MgCl_2$, 10 mM D-xylose and 1 mM NAD^+ were incubated with artificial bacterial
967 consortia (OD_{600}=10) at 37 °C (n = 3 biologically independent experiments). The bacterial
968 consortia with the ratio of 1:5:20:25 produced the highest level of α-ketoglutarate compared
969 to those with other ratios. **d** Relative production of α-ketoglutarate catalyzed by different
970 bacterial consortia with various cell density ratios of up-stream pathway to down-stream
971 using corncob xylan (1g/L) as substrate at 37 °C for 6 h. The up-stream pathway included *E.*
972 *coli*-TtGH8 and *E. coli*-SXA with the optimal ratio of 3:7. The down-stream pathway
973 included *E. col*i-XDH, *E. coli*-XylC, *E. col*i-XylDK and *E. coli*-XylX with the optimal ratio
974 of 1:5:20:25. The total OD_{6000}=10 of the bacterial consortia was applied. n = 3 biologically
975 independent experiments. The bacterial consortia with the ratio of 3:7 produced the highest
976 level of α-ketoglutarate compared to those with other ratios. Data are presented as mean ± SD.
977 The statistical significance is determined by a two-sided t test, and ***, **, * indicate
978 P<0.001, 0.01, and 0.05, respectively. Source data are provided as a Source Data file.

979 **Fig. 3 Schematic drawing of electron transfer route and catalytic reactions in the**
980 **proposed two-compartment xylan/O_2 MFC.** The system was composed of the
981 enzyme-engineered bacterial consortia based bioanode and *E. coli*-Lac based biocathode.

982 **Fig. 4 The integration of the enzyme-engineered bacterial consortia for generating**
983 **electricity in MFC. a** CVs of the CC/MWCNTs/*E.coli*-Lac biocathode in 100 mM
984 Na_2HPO_4-citric acid buffer (pH 5.0) under N_2-saturated atmosphere without ABTS (black
985 line), and in the presence of 0.5 mM ABTS under N_2-saturated (red line) and under
986 oxygen-saturated atmosphere (blue line). Scan rate: 10 mV s^{-1}. **b** CVs of CC in electrolyte

solution (black line); CC/bacterial consortia in electrolyte solution containing xylan (1 g/L) and NAD^+ (4 mM) (red line); CC/MWCNTs/bacterial-consortia in electrolyte solution containing xylan (1 g/L) and NAD^+ (4 mM) (blue line); CC/MWCNTs in electrolyte solution containing AQDS (10 mM) (green line). CC/MWCNTs/bacterial-consortia in electrolyte solution containing xylan (1 g/L), NAD^+ (4 mM) and AQDS (10 mM) (purple line). The electrolyte solution is 100 mM Tris-HCl buffer (pH 7.0) containing 100 mM NaCl, 10 mM $MgCl_2$ and 10 mM $CaCl_2$. Scan rate: 10 mV s^{-1}. **c** Profiles of potential versus current density (*j*). MFC consisting of CC/MWCNTs/bacterial-consortia bioanode and CC/MWCNTs/*E.coli*-Lac cathode, which contain 10 mM of AQDS in the anodic chamber (peach pink line). MFC consisting of CC/MWCNTs bioanode and CC/MWCNTs/*E.coli*-Lac cathode, which contained bacterial consortia and 1 mM of AQDS in the anodic chamber (blue line). MFC consisting of CC/MWCNTs/bacterial-consortia and CC/MWCNTs/*E.coli*-Lac biocathode, which contained 1 mM of AQDS in the anodic chamber (light green line). **d** Profiles of power density dependent on different bioanodes, which are the same as c. Scan rate: 1 mV s^{-1}. Abbreviation: CC: carbon cloth. Source data are provided as a Source Data file.

Fig. 5 The optimization of MFC performance. a Effect of loading amounts of bacterial consortia onto the CC/MWCNTs/bacterial-consortia bioanode on the power output. The bioanodes were prepared by dropping 100 μL of the prepared cells with different OD_{600} values onto the CC/MWCNTs and dried at room temperature. The xylan concentration is 1.0 g/L. The amounts of bacterial consortia were 5 OD (blue line), 10 OD (pink line) and 20 OD (green line), respectively. **b** Effect of concentrations of commercial corncob xylan on the

power output. The bioanodes were prepared by dropping 100 μL of the prepared cells with 10.0 OD_{600}. The concentrations of commercial corncob xylan were 0.5 g/L (blue line), 1.0 g/L (pink line) and 2.0 g/L (green line), respectively. Abbreviation: CC: carbon cloth. Source data are provided as a Source Data file.

Fig. 6 Long-term operation stability of MFC fueled by hemicellulose fractions of corncob. The time-dependent power density curve (black line) and α-ketoglutarate titers (red line) of enzyme-bacterial-consortia-modified-bioanode based MFC. Electrical outputs (power density) from engineered bacterial consortia were shown. The production of α-ketoglutarate in MFC was measured every 12 h during 6 days. LSV was recorded every 12 h during 6 days. The systems absence of pretreated hemicellulose from corncob were used as negative controls (blue line representing power density curve and green line representing α-ketoglutarate titers). n = 3 biologically independent experiments. Data are presented as mean ± SD. Source data are provided as a Source Data file.