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

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23 **Abstract:**

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

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45 **Introduction**

46 Lignocellulose, which is produced through photosynthesis by plant, is a huge solar energy  
47 reservoir. As a green and sustainable alternative to fossil energy, it has attracted a broad  
48 interest to explore lignocellulose biomass for high added-value products and biofuel<sup>1, 2</sup>.  
49 Biological hydrolysis and fermentation process of lignocellulosic biorefinery are regarded as  
50 the most promise and eco-friendly approach compared to physical and chemical methods<sup>3, 4</sup>.  
51 As the most abundant component in lignocellulose, cellulose can be easily metabolized by  
52 most microorganism owing to only glucose units<sup>5</sup>. Whereas, hemicellulose, accounting for  
53 20-40% of lignocellulose, are hard to be effectively degraded and utilized by many  
54 microorganisms due to the heterogeneous structure of hemicellulose and the constraints of  
55 effective membrane transporters as well as the metabolic regulations *in vivo*<sup>6, 7</sup>. Thus, the low  
56 utilization efficiency of hemicellulose greatly affect lignocellulosic biorefinery<sup>8</sup>.

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

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

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

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

89 renewable biomass into electric energy and high-value chemical simultaneously in an  
90 environmentally-friendly way. The engineered *Escherichia coli* (*E. coli*) (up-stream pathway  
91 members) degrade xylan, the most abundant biopolymer in hemicellulose<sup>22</sup>, into  
92 monosaccharides, while the following recombinant *E. coli* (down-stream pathway members)  
93 oxidize monosaccharides into  $\alpha$ -ketoglutarate accompanied by generating the reduced form of  
94 nicotinamide adenine dinucleotide (NADH) (Fig. 1). Thus *denovo* MFC is designed to  
95 achieve one-pot efficient production of high added-value chemical of  $\alpha$ -ketoglutarate and  
96 powerful electricity from hemicellulose biomass in a “one-stone-two-birds” manner,  
97 demonstrating a model to efficiently utilize biomass in a sustainable way.

98

## 99 **Results and discussion**

### 100 **Bacterial surface displaying enzymes (engineered bacterial consortia) involving in the** 101 **saccharification of xylan (up-stream pathway)**

102 Herein, the utilization of xylan experienced two stages, firstly saccharified into  
103 monosaccharides (mainly D-xylose), which are then oxidized to produce  $\alpha$ -ketoglutarate and  
104 release electrons. For the initial phase, hemicellulases hydrolyze  $\beta$ -linkage in the xylan  
105 backbone to release monosaccharides. A marine symbiont *Teredinibacter turnerae* was  
106 selected as the bacterial endo- $\beta$ -1,4 xylanases (TtGH8) source. TtGH8 shows a wide variety  
107 of glycoside hydrolases activities including  $\beta$ -1,4 xylanases, especially the highest activity on  
108 mixed-linkage  $\beta$ -1,3 and  $\beta$ -1,4 xylanases<sup>23</sup>, which could expand the panel of substrate used in  
109 our system.  $\beta$ -D-xylosidase (SXA) from *Selenominas ruminantium* exhibits good  
110 thermo-stability as well as superior activity towards 1,4- $\beta$ -D-xylooligosaccharides<sup>24, 25</sup>.  
111 Besides, SXA also has  $\alpha$ -arabinofuranosidase activity towards arabino xylanases<sup>26</sup>. 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)<sup>27</sup>. 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<sup>28</sup>. 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<sup>29</sup>. 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-Pro)<sub>7</sub> between TtGH8 and SXA, respectively. Results  
125 show that the engineered strains harboring GS and GSGGSG linkers had similar activities.  
126 However, (AP)<sub>7</sub> 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

134 0.5 mM isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG) and 30 °C (Supplementary Fig. 3).  
135 However, when IPTG concentration was over 1.0 mM, the whole-cell activities of engineered  
136 strains were declined due to the imbalance of transcription and secretion<sup>30</sup>. According to  
137 results of Quantitative Immunoassay (Supplementary Fig. 4), the number of cell surface  
138 displayed proteins were estimated. Strains displayed different amounts of these enzymes,  
139 approximately 25560, 25300 and 18020 enzyme molecules for TtGH8, SXA and TtGH8-SXA,  
140 respectively. The fewer number of TtGH8-SXA displayed on the surface of per cell suggests  
141 the decreased display efficiency for protein with a larger molecule (108 kDa). On the basis of  
142 the number of enzymes displayed on cell surface, the effects of single display system and  
143 dual-display system on saccharification of hemicellulose were investigated. As can be seen  
144 from Supplementary Fig. 5, the strain displaying fusion protein TtGH8-SXA exhibited a  
145 30.37% lower activity towards xylan than the mixture of two strains displaying TtGH8 and  
146 SXA individually with molar ratio of 1:1 at the same levels of displayed enzyme molecules,  
147 probably due to the lower protein numbers of TtGH8-SXA on cell surface compared to the  
148 sum of TtGH8 and SXA single-displayed numbers. Then, the ratio of cell density of two  
149 engineered strains was optimized to efficiently hydrolyze xylan into monosaccharides. As  
150 indicated in Fig. 2a, different ratios of strain *E. coli*-TtGH8 to *E. coli*-SXA resulted in  
151 different hydrolysis efficiency with the best ratio of 3:7. TtGH8 and SXA possess different  
152 functions during the degradation process of xylan. TtGH8 is responsible for degrading xylan  
153 into xylooligosaccharide as well as a small amount of D-xylose and L-arabinose<sup>23</sup>. The  
154 xylooligosaccharide could be further hydrolyzed into D-xylose and L-arabinose under the  
155 action of SXA. So, the amounts of required SXA were higher than those of TtGH8 for full

156 hydrolyzation of xylan into monosaccharides. The hydrolysis efficiency of up-stream  
157 bacterial consortia including *E. coli*-TtGH8 and *E. coli*-SXA towards xylan at different  
158 concentrations were examined. To quantify the proportion and amounts of pentose in the  
159 hydrolysate after strains' treatment, the resultant D-xylose and L-arabinose were determined  
160 by HPLC method, accounting for 97.43% and 2.57%, respectively. Although the efficiency of  
161 saccharification was increased with the decreasing xylan concentration from 1 g/L to 0.1 g/L,  
162 the yields of pentose monomers also reduced accordingly (Supplementary Fig. 6). When  
163 corncob xylan was 1 g/L, the hydrolysis efficiency was 65.43% (w/w) after 6 h reaction  
164 (Supplementary Fig. 6), which was about 1.42-fold higher than *in vitro* process<sup>31</sup> and  
165 1.65-fold higher than *in vivo* process reported previously<sup>32</sup>. When the concentration of xylan  
166 continued to increase, the production of pentose failed to rise proportionally. Anyway, this  
167 simple bacterial consortium could generate soluble monosaccharides from xylan ready for the  
168 subsequent oxidative degradation.

169

#### 170 **Engineered bacterial consortia involving in oxidation of pentose monosaccharides** 171 **(down-stream pathway)**

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

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

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

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

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

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

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

244  
245

246 **The integration of the engineered bacterial consortia for producing  $\alpha$ -ketoglutarate**

247 To integrate the entire pathway for degrading xylan, according to above optimized  
248 enzyme-displaying strain ratios in up-stream pathway and down-stream pathway, these  
249 involved enzyme-displaying strains were mixed into bacterial consortia with different ratios  
250 of cell densities in the saccharification of xylan into pentose and the oxidation pentose into  
251  $\alpha$ -ketoglutarate. The most appropriate ratio was 3:7 for bacterial consortia in up-stream route  
252 to down-stream route with the total OD<sub>600</sub> of 10.0 taking the titer of  $\alpha$ -ketoglutarate as  
253 evaluation criterion (Fig. 2d). These results demonstrate that the imbalanced enzymes  
254 activities could be adjusted by optimizing strain ratios.

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

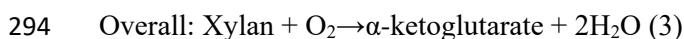
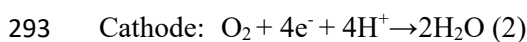
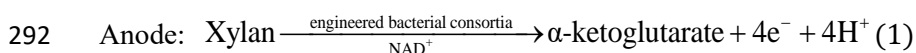
268 the engineered bacterial consortia saccharification and oxidation pathway. Our yield reaches  
269 the highest level within 6 h, which is significantly shorter than over 90 h for microbial cell  
270 factories using cost-ineffective glycerol and xylose as substrates (Table 1). It is worth noting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

277

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

279 Fig. 3 describes the mechanism of the two-compartment xylan/O<sub>2</sub> MFC separated by a  
280 Nafion® membrane. The mediator-less anodic compartment containing multi-walled carbon  
281 nanotubes (MWCNTs) covered carbon cloth (CC) electrode, which was modified with  
282 engineered bacterial consortia (CC/MWCNTs/bacterial-consortia) and fuel of xylan, while the  
283 cathodic compartment contained MWCNTs-coated CC electrode, which was modified with *E.*  
284 *coli*-displayed laccase (Lac) (CC/MWCNTs/*E.coli*-Lac) and 2,2'-azino-bis  
285 (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as the redox mediator. Lac was confirmed to  
286 display on the cell surface by SDS-PAGE, Western-Blot and confocal imaging  
287 (Supplementary Fig. 12) with good catalytic activity (Supplementary Fig. 13), which  
288 catalyzes the O<sub>2</sub> reduction at the cathode (Fig. 4a). *E.coli*-Lac exhibits the maximum activity  
289 at pH 5.0 (Supplementary Fig. 13c). The synthetic metabolic pathway on the anode

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



295 The performance of the biocathode and bioanode was examined by Cyclic voltammetry  
296 (CV). At the CC/MWCNTs/*E.coli*-Lac, ABTS exhibited a redox pair in the absence of oxygen  
297 (red line), while the cathodic specific current significantly enhanced in the presence of  $\text{O}_2$   
298 (Fig. 4a, light blue line), indicating that with ABTS as the electron transfer mediator, the  
299 bacteria-displayed laccase catalyzes the reduction of oxygen. For bioanode, as shown in Fig.  
300 4b, an onset potential at 0.13 V (vs. Ag/AgCl) for enzyme-displayed *E. coli* was observed (red  
301 line). Interestingly, the onset potential for CC/MWCNTs/bacterial-consortia was negatively  
302 shifted to 0 V (blue line), suggesting that the generated NADH from  $\text{NAD}^+$  was catalytically  
303 oxidized by MWCNTs<sup>43</sup>. 9,10-anthraquinone-2,7-disulfonic acid (AQDS) was used as  
304 electron mediator to investigate the impact of electron transfer mechanisms on the  
305 performance of bioanode. As shown in Fig. 4b, the two reduction peaks (green curves) at -0.6  
306 V and -0.9 V were the reduction peaks of AQDS with MWCNTs modified CC as electrode.  
307 The oxidation peak of AQDS was around -0.5 V on the purple curve, which was shifted to  
308 around -0.4 V due to the influence of CC modified with bacterial consortia. The oxidizing  
309 redox peak around -0.1 V on the purple curve was the oxidation peak of NADH produced by  
310 the catalysis of bacterial consortia in the presence of AQDS. AQDS can lower the onset  
311 potential of NADH which was produced by cell-surface displayed enzymes' catalysis. The  
312 onset potential was negatively shifted to -0.20 V (purple line). Therefore, this mediator was

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

314 Then, we studied the performance of MFCs composed of the above prepared bioanode  
315 and biocathode by varying the way of loading engineered strains. The current density ( $j_{\max}$ ) of  
316 the generated MFC was  $371.67 \pm 10.36 \mu\text{A}\cdot\text{cm}^{-2}$  when bacterial consortia were immobilized  
317 on electrode (Fig. 4c, light green line). However, the  $j_{\max}$  of MFC dropped to  $297.54 \pm 10.24$   
318  $\mu\text{A}\cdot\text{cm}^{-2}$  when bacterial consortia were dispersed in bioanode chamber (Fig. 4c, blue line).  
319 Then the effects of different concentrations of AQDS on the performance of xylan/O<sub>2</sub> MFC  
320 were investigated. The results show that the highest value of power was achieved when 10  
321 mM of AQDS was used (Fig. 4c, peach pink line). The similar trends were observed in Fig.  
322 4d, and the constructed MFC reached the maximum power output ( $P_{\max}$ ) of  $68.25 \pm 2.38$   
323  $\mu\text{W}\cdot\text{cm}^{-2}$  when bacterial consortia immobilized on bioanode in the presence of 10 mM of  
324 mediator AQDS.

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

### 335 **The optimization of MFC performance**

336 To boost the power output of MFC, effects of several parameters on electricity generation  
337 were investigated. First, the loading amounts of bacterial consortia on the bioanode was  
338 optimized. The  $j_{\max}$  of MFCs was  $671.33 \pm 26.21 \mu\text{A}\cdot\text{cm}^{-2}$ , corresponding  $P_{\max}$  of  $112.87 \pm$   
339  $7.83 \mu\text{W}\cdot\text{cm}^{-2}$  when loading 10 OD<sub>600</sub> bacterial consortia (Fig. 5a, peach pink line). The  
340 results indicate that the bacterial cell loading exerted effects on power output since the  
341 insulated nature of concentrated cells would cause ohmic losses<sup>47</sup>. Then, MFCs performance  
342 fueled by commercial xylan from corncob was tested under the optimal conditions established  
343 according to above experiments. As shown in Fig. 5b, when the concentrations of corncob  
344 xylan increased from 0.5 g/L to 1 g/L, the  $P_{\max}$  boosted from  $111.82 \pm 5.35 \mu\text{W}\cdot\text{cm}^{-2}$  (green  
345 line) to  $174.33 \pm 4.56 \mu\text{W}\cdot\text{cm}^{-2}$  (peach pink line). However, further increase in concentrations  
346 of xylan resulted in 12.22% decrease in power density (blue line), probably due to the poor  
347 solubility and high viscosities of commercial xylan prepared in buffer solution. The  $P_{\max}$  of  
348 our MFC fueled by 1 g/L xylan is  $174.33 \pm 4.56 \mu\text{W}\cdot\text{cm}^{-2}$ , which is significantly higher than  
349 those for xylan-fueled MFCs inoculated with activated sludge ( $0.609 \mu\text{W}\cdot\text{cm}^{-2}$ ) or rumen  
350 microorganisms ( $40.5 \mu\text{W}\cdot\text{cm}^{-2}$ )<sup>13,48</sup>.

351 In order to investigate the release and transfer of electrons in bioelectrochemical system,  
352 Faraday efficiency ( $\eta_F$ ) was determined (Supplementary Fig. 14). The current peaked at 2.07  
353 mA at the onset time and then declined over time. In a batch reaction of 35 h, the cumulative  
354 electric charges generated from 50 mL of reaction solution were 58.34 C. The concentration  
355 of product  $\alpha$ -ketoglutarate determined by HPLC was 3.19 mM, convertible to the yield of  
356 46.6%, suggesting that the same amount of D-xylose was consumed and corresponding to

357 61.58 C of electricity. In consideration of a conversion efficiency of 97% from NADH to  
358 electrons determined in a previous study<sup>49</sup>, the  $\eta_F$  value was calculated to be 97.7%  
359 ( $58.34 \div 61.58 \div 0.97 \times 100\%$ ).

360

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

362 As xylan is a predominant component of hemicellulose, we next examined the performance of  
363 MFCs powered by real hemicellulose. Biomass corncob pretreated by heating in alkaline  
364 solution after neutralizing was used as fuels for power generation<sup>50</sup>. 1.06 g/L xylan from the  
365 pretreated hydrolysates of corncob was fueled, in which D-xylose was not detectable by  
366 HPLC. To supply constant fuels to MFC, the above pretreated hemicellulose was  
367 supplemented once a day to roughly maintain the same level. Then we examined the  
368 performance of the MFC using enzyme-engineered-bacterial-consortia-modified-bioanode. As  
369 shown in Fig. 6, the  $P_{max}$  of enzyme-bacterial-consortia-bioanode based MFC was  $162.73 \pm$   
370  $3.69 \mu\text{W}\cdot\text{cm}^{-2}$  (Fig. 6, black line). The remarkable power output of the  
371 enzyme-bacterial-consortia-bioanode based MFC is much excelled over microbial community  
372 based MFC<sup>13</sup>, possibly because diverse biological properties of various microorganisms in  
373 one system may result in the instability in bacterial community. In addition, the long-term  
374 generation of electricity was also evaluated. The bacterial-consortia-bioanode based MFC  
375 maintained more than 95% of the  $P_{max}$  after 6 days' operation. On the contrary, the poor  
376 stability was observed in other biomass-based sugar-fed enzymatic fuel cells<sup>51</sup>. Besides, the  
377 conversion of pretreated hemicellulose from corncob to  $\alpha$ -ketoglutarate was also monitored  
378 during 6 days. The yield of  $\alpha$ -ketoglutarate was 44.3% during the 6 days' long-term electricity  
379 generation of MFC fueled by the pretreated hydrolysates of corncob (Fig. 6, red line).

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

386

387 In summary, a synthetic pathway that can generate high value-added chemical of  
388  $\alpha$ -ketoglutarate and 4 electrons from hemicellulose in a controllable manner was successfully  
389 constructed by integrating six enzyme-displayed strains into a high-efficient bacterial  
390 consortia through tuning the expressed enzyme molecules and adjusting ratios of consortia  
391 members. The production of  $\alpha$ -ketoglutarate with excellent yield of 0.47 g/g was realized  
392 within 6 h by the enzyme-engineered bacterial consortia surface-displayed saccharification  
393 and oxidation pathway of xylan, the most abundant hemicellulose type. Then a  
394 two-compartment xylan/O<sub>2</sub> MFC was assembled using enzyme-bacterial consortia modified  
395 bioanodes and a laccase-displayed strain modified biocathode, realizing the direct conversion  
396 of biomass into electricity and  $\alpha$ -ketoglutarate. The optimized MFC realized 46.6% (w/w)  
397 yield of  $\alpha$ -ketoglutarate, the highest OCV and the largest  $P_{max}$ . The Faraday efficiency was  
398 97.7%. Furthermore, this MFC exhibited a considerable power output and long-term stability  
399 towards real biomass samples. To the best of our knowledge, this is the first example to use  
400 engineered bacteria consortia as biocatalyst for efficient utilization of hemicellulose,  
401 representing an important step toward further improving conversion of biomass to high  
402 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* DH5 $\alpha$  was used for gene recombinant  
411 manipulation and *E. coli* BL21 (DE3)  $\Delta xyLAB$  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  $\mu$ g/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- $\beta$ -D-xylopyranoside (PNPX), ABTS, AQDS, D-xylose,  
419 L-arabinose, xylono-1,4-lactone, D-xylonic acid lithium salt and  $\alpha$ -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- $\Delta$ *xyLAB*, approximately 862 bp upstream and 857 bp  
430 downstream fragments of the *xyIA* and *xyIB* 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- $\Delta$ *xyLAB* were transferred from *E. coli*  $\chi$ 7213 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*- $\Delta$ *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<sup>35</sup> 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,

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

492 Kit).

### 493 **Construction of lac-expressing plasmid**

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

### 497 **Purification of cytoplasmic enzymes**

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

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

515 For displaying enzymes on cell surface of *E. coli*, recombinant strains were cultured in LB  
516 medium to an OD<sub>600</sub> of 0.6, 0.5 mM of IPTG was used to induce protein expression. For  
517 laccase, cells were further cultured overnight at 16 °C, collected by centrifugation and  
518 incubated in a buffer of Tris-HCl (50 mM, pH 7.0). The other cells were further cultured at  
519 30 °C overnight, which were collected by centrifugation and incubated in Tris-HCl buffer (50  
520 mM, pH 7.0). To determine the successful display of enzymes on the cell surface, outer  
521 membrane proteins of recombinant strains and negative control strain were isolated<sup>53</sup>. The  
522 outer membrane protein fractions of related strains were analyzed by 10% (wt/vol)  
523 SDS-PAGE.

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

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

536 rinsed three times with PBST. It was then incubated with goat anti-mouse IRDye 800CW  
537 fluorescent secondary antibodies (catalog number 926-32210, LI-COR, Inc., Lincoln, NE) for  
538 1 h at room temperature, and then washed with PBST, air dried, wrapped in aluminum foil,  
539 and stored at 4 °C. LI-COR Odyssey CLx imaging system was used to detect bands on  
540 membrane. The standard curve from known concentrations of purified recombinant XDH run  
541 on the same blot was obtained. Specific protein bands are quantified by fluorescent signals  
542 (excitation wavelength, 785 nm; emission wavelength, 820 nm) and a linear regression  
543 equation generated from recombinant XDH to estimate concentrations of proteins.

544 For fluorescence imaging, the induced cells were washed with PBS and blocked in PBS  
545 buffer containing 1% bovine serum protein (BSA) for 30 min at room temperature. Next  
546 anti-6xHis tag mouse monoclonal antibody was added (1:100) after blocking, incubated  
547 overnight at 4 °C, and incubated for 1 h the next day at room temperature. After three washes  
548 with PBS, the cells were incubated with FITC-conjugated Donkey anti-Mouse IgG (1:100) for  
549 2 h at room temperature. The cells after PBS washing were observed with Laser Scanning  
550 Confocal Microscope (TCSsp5 II 03040101, Agilent, USA).

#### 551 **Enzymatic activity assay**

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

558 using pNPX as the substrate at 37 °C. The product *p*-nitrophenol shows typical peak at 405  
559 nm, which can be monitored by a spectrophotometer<sup>26</sup>. Enzymatic activity of XDH was  
560 assayed by detecting the generation of NADH at 340 nm and 37 °C using 10 mM D-xylose as  
561 substrate as well as 1 mM NAD<sup>+</sup> as coenzyme. For XylC, it can accelerate the hydrolysis of  
562 xylono-1,4-lactone into D-xylonic acid to generate D-xylonic acid, which can reduce the  
563 absorbance at 405 nm of *p*-nitrophenol. Enzyme activity was quantified by measuring the  
564 decrease in the absorbance at 405 nm<sup>55</sup>. Spontaneous hydrolysis of xylono-1,4-lactone was  
565 analyzed without catalysts. Enzymatic activity of XylD was assayed according to a modified  
566 procedure. In a typical assay, the reaction cocktail contained a certain amount of purified or  
567 cells and D-xylonic acid. After incubation at 37 °C for a certain period, the samples were  
568 mixed with solution containing 1% semi-carbazide hydrochloride and 1.5% sodium acetate.  
569 Finally after incubation at 30 °C for 10 min, the 2-keto-3-deoxyxylic acid produced was  
570 quantified by detection of the absorbance at 250 nm typical of semicarbazone<sup>37</sup>. Enzymatic  
571 activity of XylX was assayed spectrophotometrically in a coupled assay with the  
572 corresponding previous dehydratase XylD and KGSADH.<sup>37, 56</sup> The assay was performed in 50  
573 mM Tris-HCl buffer (pH 7.0) with 10 mM MgCl<sub>2</sub> containing 20 mM D-xylonic acid and 1  
574 mM NAD<sup>+</sup>. After the addition of purified enzymes or enzyme-surface displayed strains, the  
575 mixture was incubated at 37 °C. The increasing absorbance at 340 nm caused by NADH  
576 produced in the reaction was monitored. Glutaraldehyde was used as the substitute substrate  
577 to measure the activity of KGSADH by following the rate of NAD<sup>+</sup> reduction by measuring  
578 the optical density at 340 nm<sup>57</sup>. To determine kinetic constants of cell surface-displayed  
579 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<sub>2</sub>HPO<sub>4</sub>–NaH<sub>2</sub>PO<sub>4</sub> 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 ( $\epsilon=36 \text{ mM}^{-1}\text{cm}^{-1}$ , 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<sub>600</sub> 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<sub>600</sub> 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 $\alpha$ -ketoglutarate**

598 Recombinant strains were cultured in LB medium to an OD<sub>600</sub> 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  $\alpha$ -ketoglutarate, 100 mL of 50 mM Tris–HCl buffer (pH 7.0) with 10 mM MgCl<sub>2</sub>, 10 mM

602 CaCl<sub>2</sub>, 1.0 g/L commercial corncob xylan and 4 mM NAD<sup>+</sup> were incubated with artificial  
603 bacterial consortia (OD<sub>600</sub>=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<sup>2</sup> and washed by sonication in water and anhydrous ethanol, respectively. A 100  
608 μL of poly (acrylic acid)-MWCNTs dispersion (0.089 mg/cm<sup>2</sup>) 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<sub>600</sub>=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<sub>600</sub>=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 μA·cm<sup>-2</sup>.

#### 618 **Fabrication of MFC**

619 The dual-chamber hemicellulose/O<sub>2</sub> 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<sub>2</sub>, 10

624 mM CaCl<sub>2</sub>, 4 mM NAD<sup>+</sup> and xylan. 10 mM AQDS was used as mediator when necessary.  
625 The cathodic electrolyte consisted of 100 mM Na<sub>2</sub>HPO<sub>4</sub>-citric acid buffer (pH 5.0) containing  
626 0.5 mM ABTS. Polarization curves were obtained by performing linear sweep voltammetry  
627 (LSV) at the scan rate of 1 mV s<sup>-1</sup> and 37 °C. The specific current (I) was recorded in real  
628 time. The voltage (V) between the anode and the cathode was set as the Y-axis of polarization  
629 curves. The output power (P) was derived via the relationship: P = V × I. The specific current  
630 and power were normalized to the geometric area of the anode (1.5 cm × 1.5 cm = 2.25 cm<sup>2</sup>)  
631 to obtain the current density and power density, respectively. The calculations of current  
632 density and power density refer to Eq.(1) and Eq.(2), respectively:

$$633 \quad j_{\max} = I/2.25 \quad (1)$$

$$634 \quad P_{\max} = V \times I/2.25 \quad (2)$$

### 635 **Faradaic efficiency ( $\eta_F$ ) assay**

636 The  $\eta_F$  was determined through amperometry at 0.45 V. The generation of current was  
637 monitored during a reaction time in a volume of 50 mL at 37 °C. The reaction system  
638 composed of 100 mM NaCl, 10 mM MgCl<sub>2</sub>, 10 mM CaCl<sub>2</sub> and 4 mM NAD<sup>+</sup>, 1 g/L  
639 commercial corncob xylan in 100 mM Tris-HCl buffer (pH 7.0). The production of  
640  $\alpha$ -ketoglutarate was detected by HPLC. The total charge (C) was calculated according to the  
641 generated current during the whole time. The  $\eta_F$  was calculated using the equation as  
642 follows<sup>58</sup>:

$$643 \quad \eta_F = \int I \times dt / (C_{\alpha\text{-KG}} \times V \times n \times F) \quad (3)$$

644 where I is the current generated, dt is the time to produce current, C <sub>$\alpha$ -KG</sub> is the concentration of  
645 produced  $\alpha$ -ketoglutarate during the whole time, V is the reaction volume, n is the number of

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

#### 648 **Long-term electricity generation of MFCs powered by pretreated hemicellulose**

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

#### 660 **Morphology observation of the bio-nanocomposite modified CC**

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

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

## 672 **Analytical methods**

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

681

## 682 **Statistics and reproducibility**

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

690

### 691 **Data availability**

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

695

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899 **Author contributions**

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

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907 **Competing interests**

908 The authors declare no competing interest.

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921 **Table 1 The production of  $\alpha$ -ketoglutarate by microbial cell factories or *in vitro* one-pot**  
 922 **reaction using different substrates.**

Organism	Substrate	Time (h)	Yield (g/g)	Reference
<i>Y. lipolytica</i> H355A (PYC1-IDP1)	glycerol	117	40%	41
<i>Y. lipolytica</i> WSH-Z06	glycerol	204	47%	39
<i>C. glutamicum</i> WMB2 <sub>evo</sub>	xylose	90	0.55%	42
<i>E. coli</i> consortia	xylan	6	47%	this study

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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  $\alpha$ -ketoglutarate. TtGH8,  $\beta$ -1,4 xylanase; SXA,  $\beta$ -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<sub>2</sub> and 1.0 g/L commercial corncob xylan were incubated  
950 with artificial bacterial consortia (OD<sub>600</sub>=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*-XylDXX, respectively. The total OD<sub>600</sub> 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<sub>2</sub>, 20 mM D-xylonic acid and 1 mM NAD<sup>+</sup> were  
959 incubated with artificial bacterial consortia (OD<sub>600</sub>=10) at 37 °C (*n* = 3 biologically  
960 independent experiments). Strain *E. coli*-XylDXX 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  $\alpha$ -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<sub>2</sub>, 10 mM D-xylose and 1 mM NAD<sup>+</sup> were incubated with artificial bacterial  
967 consortia (OD<sub>600</sub>=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  $\alpha$ -ketoglutarate compared  
969 to those with other ratios. **d** Relative production of  $\alpha$ -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. coli*-XDH, *E. coli*-XylC, *E. coli*-XylDK and *E. coli*-XylX with the optimal ratio  
974 of 1:5:20:25. The total OD<sub>600</sub>=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  $\alpha$ -ketoglutarate compared to those with other ratios. Data are presented as mean  $\pm$  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<sub>2</sub> 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<sub>2</sub>HPO<sub>4</sub>-citric acid buffer (pH 5.0) under N<sub>2</sub>-saturated atmosphere without ABTS (black  
985 line), and in the presence of 0.5 mM ABTS under N<sub>2</sub>-saturated (red line) and under  
986 oxygen-saturated atmosphere (blue line). Scan rate: 10 mV s<sup>-1</sup>. **b** CVs of CC in electrolyte

987 solution (black line); CC/bacterial consortia in electrolyte solution containing xylan (1 g/L)  
988 and  $\text{NAD}^+$  (4 mM) (red line); CC/MWCNTs/bacterial-consortia in electrolyte solution  
989 containing xylan (1 g/L) and  $\text{NAD}^+$  (4 mM) (blue line); CC/MWCNTs in electrolyte solution  
990 containing AQDS (10 mM) (green line). CC/MWCNTs/bacterial-consortia in electrolyte  
991 solution containing xylan (1 g/L),  $\text{NAD}^+$  (4 mM) and AQDS (10 mM) (purple line). The  
992 electrolyte solution is 100 mM Tris-HCl buffer (pH 7.0) containing 100 mM NaCl, 10 mM  
993  $\text{MgCl}_2$  and 10 mM  $\text{CaCl}_2$ . Scan rate:  $10 \text{ mV s}^{-1}$ . **c** Profiles of potential versus current density  
994 (j). MFC consisting of CC/MWCNTs/bacterial-consortia bioanode and  
995 CC/MWCNTs/*E.coli*-Lac cathode, which contain 10 mM of AQDS in the anodic chamber  
996 (peach pink line). MFC consisting of CC/MWCNTs bioanode and CC/MWCNTs/*E.coli*-Lac  
997 cathode, which contained bacterial consortia and 1 mM of AQDS in the anodic chamber (blue  
998 line). MFC consisting of CC/MWCNTs/bacterial-consortia and CC/MWCNTs/*E.coli*-Lac  
999 biocathode, which contained 1 mM of AQDS in the anodic chamber (light green line). **d**  
1000 Profiles of power density dependent on different bioanodes, which are the same as c. Scan  
1001 rate:  $1 \text{ mV s}^{-1}$ . Abbreviation: CC: carbon cloth. Source data are provided as a Source Data  
1002 file.

1003 **Fig. 5 The optimization of MFC performance. a** Effect of loading amounts of bacterial  
1004 consortia onto the CC/MWCNTs/bacterial-consortia bioanode on the power output. The  
1005 bioanodes were prepared by dropping 100  $\mu\text{L}$  of the prepared cells with different  $\text{OD}_{600}$   
1006 values onto the CC/MWCNTs and dried at room temperature. The xylan concentration is 1.0  
1007 g/L. The amounts of bacterial consortia were 5 OD (blue line), 10 OD (pink line) and 20 OD  
1008 (green line), respectively. **b** Effect of concentrations of commercial corn cob xylan on the

1009 power output. The bioanodes were prepared by dropping 100  $\mu\text{L}$  of the prepared cells with  
1010 10.0 OD<sub>600</sub>. The concentrations of commercial corncob xylan were 0.5 g/L (blue line), 1.0 g/L  
1011 (pink line) and 2.0 g/L (green line), respectively. Abbreviation: CC: carbon cloth. Source data  
1012 are provided as a Source Data file.

1013 **Fig. 6 Long-term operation stability of MFC fueled by hemicellulose fractions of**  
1014 **corncob.** The time-dependent power density curve (black line) and  $\alpha$ -ketoglutarate titers (red  
1015 line) of enzyme-bacterial-consortia-modified-bioanode based MFC. Electrical outputs (power  
1016 density) from engineered bacterial consortia were shown. The production of  $\alpha$ -ketoglutarate  
1017 in MFC was measured every 12 h during 6 days. LSV was recorded every 12 h during 6 days.  
1018 The systems absence of pretreated hemicellulose from corncob were used as negative controls  
1019 (blue line representing power density curve and green line representing  $\alpha$ -ketoglutarate titers).  
1020  $n = 3$  biologically independent experiments. Data are presented as mean  $\pm$  SD. Source data  
1021 are provided as a Source Data file.

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