Supporting Information (SI)

**Engineering microbial consortia for high-performance cellulosic hydrolyzates-fed microbial fuel cells**

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**Optimization of the seeding OD600 of *K. pneumoniae* and *S. oneidensis* in the glucose and xylose co-fed MFCs**

In order to maintain a stable MFC system inoculated with the K. pneumoniae and S. oneidensis co-culture, we optimize the initial co-cultivation of the two microorganisms by achieving a maximum voltage output via multimeter. We initially chosed 20 mM glucose and 20 mM xylose as substrate, four different proportions of seeding ratios were selected, such as OD600 KG-3: CP-S1-C5 =0.5:0.1; 0.5:0.5; 0.1:0.5; and 0.01:0.5. When the each microorganism grew up to OD600=2.0, the calculated cell suspensions were harvested by centrifugation with 5000 for 8 min at 4°C. Subsequently, we adjusted different OD600 of the seeding K. pneumoniae and S. oneidensis to obtain the optimal seeding ratio for maximized output voltage, when cell pellets were washed three times with M9 fresh buffer. The seeding OD600 ratio of 0.1:0.5 resulted in the hightest electricity ouput in MFCs (Figure S1).



**Figure S1**. Optimization of the initial seeding ratio between *K. pneumoniae* and *S. oneidensis* in MFCs. With the substrate concentration were 20 mM glucose and 20 mM xylose, the output voltages of MFCs with different OD600of seeding ratios were measured. Three independent replicates of MFC experimentswere calculate



**Figure S2.** The proportion of each bacterium on the anode-attached biofilm of MFCs. Statistics were calculated from three independent replicates in this experiment.



**Figure S3.** Electrochemical analyses of microbial consortia in non-sterile cellulose hydrolyzates-fed MFCs. (a) Time course of output voltage profiles produced by KG-3: CP-S1-C5under non-sterile cellulose hydrolyzates feedstock anolyte. (b) Turnover cyclic voltammetry (CV) curves at a scan rate of 1 mV/s. (c) MFC polarization curves obtained by linear sweep voltammetry (LSV) with a slow scan rate of 0.1 mV/s. (d) MFC power density output curves calculated on the basis of the corresponding polarization curves.

**Table S1.** Original sequences of genes in this study

|  |  |
| --- | --- |
| *ldhD* (1002bp) | atgactaaaatttttgcttacgcaattcgtgaagatgaaaagccattcttgaaggaatgggaagacgctcacaaggacgtcgaagttgaatacactgacaagcttttgaccccagaaactgttgctttggcaaagggtgctgacggtgttgttgtttaccaacaacttgactacaccgctgaaactctgcaagctttggcagacaacggcatcactaagatgagcctgcgtaacgttggtgttgacaacatcgacatggctaaggctaaggaacttggcttccaaatcaccaacgttccagtttactcaccaaacgccatcgcagaacacgctgctatccaagctgcccgcatcctgcgtcaagacaaggctatggacgaaaaggttgcccgtcacgacttgcgttgggcaccaactatcggccgtgaagttcgcgaccaagttgttggtgttataggtactggccacatcggtcaagtcttcatgcaaatcatggaaggcttcggcgctaaggttatcgcttacgacatcttccgcaacccagaattggaaaagaagggctactacgtagactcacttgacgacctgtacaagcaagctgacgttatttccctgcacgttcctgacgttccagctaacgttcacatgatcaacgacgagtcaatcgctaaaatgaagcaagacgtagttatcgttaacgtatcacgtggtccattggttgacactgacgcggttatccgtggtttggactcaggcaagatcttcggttacgcaatggacgtttacgaaggtgaagttggcatcttcaacgaagactgggaaggcaaggaattcccagacgcacgtttagctgacttaatcgctcgtccaaacgttctggtaactccacacactgctttctacactactcacgctgttcgcaacatggtagttaaggccttcgacaacaaccttgaattggttgaaggcaaggaagctgaaactccagttaaggttggctaa |
| *lldP* (1656bp) | atgaatctctggcaacaaaactacgatcccgccgggaatatctggctttccagtctgatagcatcgcttcccatcctgtttttcttctttgcgctgattaagctcaaactgaaaggatacgtcgccgcctcgtggacggtggcaatcgcccttgccgtggctttgctgttctataaaatgccggtcgctaacgcgctggcctcggtggtttatggtttcttctacgggttgtggcccatcgcgtggatcattattgcagcggtgttcgtctataagatctcggtgaaaaccgggcagtttgacatcattcgctcgtctattctttcgataacccctgaccagcgtctgcaaatgctgatcgtcggtttctgtttcggcgcgttccttgaaggagccgcaggctttggcgcaccggtagcaattaccgccgcattgctggtcggcctgggttttaaaccgctgtacgccgccgggctgtgcctgattgttaacaccgcgccagtggcatttggtgcgatgggcattccaatcctggttgccggacaggtaacaggtatcgacagctttgagattggtcagatggtggggcggcagctaccgtttatgaccattatcgtgctgttctggatcatggcgattatggacggctggcgcggtatcaaagagacgtggcctgcggtcgtggttgcgggcggctcgtttgccatcgctcagtaccttagctctaacttcattgggccggagctgccggacattatctcttcgctggtatcactgctctgcctgacgctgttcctcaaacgctggcagccagtgcgtgtattccgttttggtgatttgggggcgtcacaggttgatatgacgctggcccacaccggttacactgcgggtcaggtgttacgtgcctggacaccgttcctgttcctgacagctaccgtaacactgtggagtatcccgccgtttaaagccctgttcgcatcgggtggcgcgctgtatgagtgggtgatcaatattccggtgccgtacctcgataaactggttgcccgtatgccgccagtggtcagcgaggctacagcctatgccgccgtgtttaagtttgactggttctctgccaccggcaccgccattctgtttgctgcactgctctcgattgtctggctgaagatgaaaccgtctgacgctatcagcaccttcggcagcacgctgaaagaactggctctgcccatctactccatcggtatggtgctggcattcgcctttatttcgaactattccggactgtcatcaacactggcgctggcactggcgcacaccggtcatgcattcaccttcttctcgccgttcctcggctggctgggggtattcctgaccgggtcggatacctcatctaacgccctgttcgccgcgctgcaagccaccgcagcacaacaaattggcgtctctgatctgttgctggttgccgccaataccaccggtggcgtcaccggtaagatgatctccccgcaatctatcgctatcgcctgtgcggcggtaggcctggtgggcaaagagtctgatttgttccgctttactgtcaaacacagcctgatcttcacctgtatagtgggcgtgatcaccacgcttcaggcttatgtcttaacgtggatgattccttaa |

**Table S2.** Main Ingredients of the M9 minimal medium

|  |  |
| --- | --- |
| Main Ingredients  | Concentration  |
| Na2HPO4 | 6 g/l |
| KH2PO4 | 3 g/l |
| NaCl | 0.5 g/l |
| NH4Cl | 1 g/l |
| MgSO4 | 1 mM |
| CaCl2 | 0.1 mM |

**Table S3.** Analyses of remaining substrate level at 24 hours under different feed-in concentrations of glucose and xylose

|  |  |  |  |
| --- | --- | --- | --- |
|  | KG-1: WT | KG-2: CP-S1 | KG-3: CP-S1-C5 |
| Glucose | Xylose | Glucose | Xylose | Glucose | Xylose |
| 10 mM glucose+30 mM xylose | 7.2±1.2 | 19.3±0.8 | 3.4±0.4 | 10.7±0.9 | 0.9±0.3 | 6.1±0.9 |
| 20 mM glucose+20 mM xylose | 12.1±0.4 | 14.8±1.1 | 6.2±0.8 | 8.4±1.2 | 1.5±0.7 | 4.8±0.4 |
| 30 mM glucose+10 mM xylose | 18.3±0.7 | 8.1±0.5 | 9.3±1.3 | 5.3±0.7 | 2.1±1.0 | 2.4±0.8 |