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## Biological degradation of potato pulp waste and microbial community structure in microbial fuel cells

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The microbial electrochemical cell (MEC) is a promising waste treatment technology to accomplish simultaneous alternative energy production and degradation of organic matters. Potato pulp waste (PPW) from a potato processing plant contains a large amount of carbohydrates that need be degraded before discharge into the environment. Here, we describe electricity generation in single-chamber microbial fuel cells (MFCs) using PPW. Different organic loadings of PPW influenced obviously the power output of MFCs. The maximum power density of  $32.1 \pm 0.5 \text{ W m}^{-3}$  was obtained in a MFC fed with  $10 \text{ g L}^{-1}$  PPW. Microbial community analysis based on high-throughput sequencing of 16S rRNA gene indicated that the predominant populations were obviously different in the MFCs. The syntrophic interactions between polysaccharide-degrading bacteria and exoelectrogens in the anode biofilms drove the cascade utilization of potato pulp waste in MFCs.

## Introduction

As the largest potato producing country in the world, China produces more than 20 percent of the global yield. One-seventh of all the potatoes in China are consumed for the production of starch and starch-based products.<sup>1</sup> The extensive waste residues and wastewater produced by potato starch processing plants cause serious environmental problems.<sup>2</sup> The potato pulp is one of the by-products during potato starch production and their utilization is important for the starch industry.<sup>3</sup> The potato pulp waste contains a high ratio of starch, cellulose, hemicellulose and pectin but has low protein.<sup>4</sup> The traditional treatment technologies of potato pulp waste include landfill, incineration and animal feed, which are no longer preferred due to secondary pollution and energy recovery considerations.<sup>5,6</sup>

In recent years, the recovery of organic resources and energy from potato waste, such as potato pulp and peel, have been intensively investigated. Bioenergy (bioethanol and methane) and bioresource (lactic acid, single cell protein) could be generated using the potato waste through microbial fermentation or anaerobic digestion.<sup>7–10</sup> However, the high energy consumption is a major challenge for the cost reductions of the bioresource and bioenergy production processes.<sup>11</sup> Therefore, an environmentally friendly technology should be developed for the disposal and energy recovery of the potato waste.

Microbial fuel cell (MFC) is a sustainable waste treatment technology emerging in recent years,<sup>12–15</sup> which can convert chemical energy into electricity using the organic matters such as carbohydrates, cellulose and protein.<sup>16,17</sup> The exoelectrogenic bacteria attached on the anode surface of MFCs serves as the biocatalysts to oxidize the substrates and transfer extracellular electrons to the electrode.<sup>18,19</sup> In the previous studies on MFCs, glucose, acetate and sucrose or domestic and industry wastewater were most commonly used as the substrate.<sup>20–24</sup> Moreover, MFCs have been used in the bioenergy recovery from the solid waste such as cellulose biomass and waste sludge.<sup>25–31</sup> Solid waste disposal using MFCs is not common as MFC based wastewater treatment and it is not well studied. The performances of MFC with given configuration depend on the microbial community structure on the electrodes. Multiple syntrophic interactions drive the degradation of complex organics matters and bioenergy generation in microbial electrochemical cells.<sup>32–34</sup> In this study, the effect of organic loading on the performances of single-chamber microbial fuel cells fed with potato pulp waste was investigated. To probe the microbial interaction in the MFCs fed with potato pulps waste, microbial community structure of the anode biofilms was analyzed using 454 pyrosequencing of 16S rRNA gene amplicons.

## Materials and methods

### Potato pulps waste

Potato pulps waste (PPW) was obtained from a potato starch processing plant (Harbin, China). Prior to use, PPW was generally crushed using an electrical blender (starch factory) and stored in a refrigerator ( $4^\circ\text{C}$ ). The chemical oxygen demand

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(COD) and pH of pretreated potato pulps were 2000–25 000 mg L<sup>-1</sup> and 4.0–4.9, respectively. The PPW was diluted to 1, 5, 10 and 20 g L<sup>-1</sup> with 50 mM phosphate buffer solution (PBS) contained (per liter) 4.58 g Na<sub>2</sub>HPO<sub>4</sub>, 2.45 g NaH<sub>2</sub>PO<sub>4</sub>, 0.31 g NH<sub>4</sub>Cl and 0.13 g KCl.<sup>35</sup> Finally, pH of diluted PPW was neutralized to around 7.0 prior to feeding.

### MFC configuration and operation

Single-chamber MFCs with liquid volume of 25 mL were used as previously described.<sup>14</sup> The anode was made by using graphite brush with length of 3 cm and outer diameter of 3 cm.<sup>36</sup> Carbon cloth (30% wet proofed, E-Tek) air cathodes with a 0.5 mg cm<sup>-2</sup> Pt/C catalyst layer were fabricated as previously reported and the cathode was carbon cloth containing a 0.5 mg cm<sup>-2</sup> Pt catalyst (30% wet proofed, E-Tek).<sup>37</sup> The MFCs were operated in fed-batch mode at 25 °C in duplicate for each test.

### Chemical analyses and calculations

The chemical oxygen demand (COD) of the samples were measured according to standard method.<sup>38</sup> The estimated COD of the potato pulp waste was calculated based on the assumption that the potato pulp waste was only composed of starch with a molecular formula of (C<sub>6</sub>H<sub>10</sub>O<sub>5</sub>)<sub>n</sub> (molecular weight: 162 g mol<sup>-1</sup>). According to the assumption, the COD corresponded to 162 g (dry weight) potato pulp was 192 g, which means that the ratio COD/potato-weight equals to 1.185.

The voltage (V) over a high-precision resistor of 1000 Ω in the external circuit was measured at 30 min intervals using a multimeter data acquisition system (Model 2700, Keithly Instruments Inc, USA). Polarization curves were obtained by verifying external resistances from 2000 to 50 Ω. Power density, current density and coulombic efficiency (CE) were calculated as previously described.<sup>14</sup>

### DNA extraction, PCR amplification and 454 pyrosequencing

The anode biofilm samples for 454 GS FLX pyrosequencing analysis were obtained from the graphite fiber brush anodes of MFCs with satisfying performance after operated for two months. The graphite fiber brush was collected from respectively top, middle and bottom sections. Prior to DNA extraction, the samples were rinsed with sterile deionized water to remove the residual potato pulps.<sup>39</sup> Genomic DNA was extracted using a PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA). The V1–V3 region (~455 bp) of the bacterial 16S rRNA gene was amplified using a pair of bacterial universal primers as follows: 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 533R (5'-TTACCGCGGCTGCTGGCAC-3'). Individual samples were bar-coded for pooling multiple samples in each run of sequencing of PCR amplicon of 16S rRNA gene using 454 GS-FLX System (Roche).

The sequencing reads were analyzed using Quantitative Insights into Microbial Ecology (QIIME) software (<http://qiime.org>). Operational taxonomic units (OTUs) were determined using a 0.03 distance limit (97% similarity). A representative sequence from each OTU was selected for taxonomic identification using the Silva database (<http://www.arb-silva.de>)

and Ribosomal Database Project (RDP) classifier (<http://rdp.cme.msu.edu>). Principal component analysis (PCA) was conducted to visualize difference in community structure between different anode biofilms.

## Results and discussion

### Effect of PPW loading on electricity generation of MFCs

All MFCs fed with potato pulp waste immediately generated current after one week acclimation. The power densities of MFCs were measured after 30 days. The different organic loads of PPW obviously influenced the power output of MFCs. The power densities of MFCs improved with the increase of the organic load of PPW except 20 g L<sup>-1</sup> (28.7 ± 0.3 W m<sup>-3</sup>) (Fig. 1). The maximum power density of 32.1 ± 0.5 W m<sup>-3</sup> was obtained in MFC fed with 10 g L<sup>-1</sup> PPW, *versus* 26.4 ± 0.2 W m<sup>-3</sup> for MFCs fed with 5 g L<sup>-1</sup> PPW and 20.4 ± 0.3 W m<sup>-3</sup> for MFCs fed

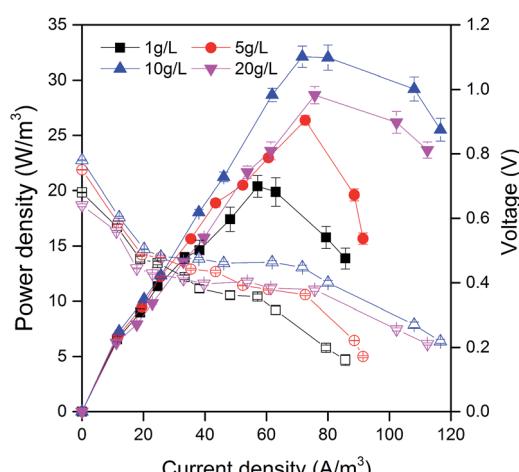


Fig. 1 Power density and polarization curves of MFCs fed with potato pulps waste of different organic loading rates. Error bars represent standard deviation based on measurements from duplicate reactors in at least three cycles.

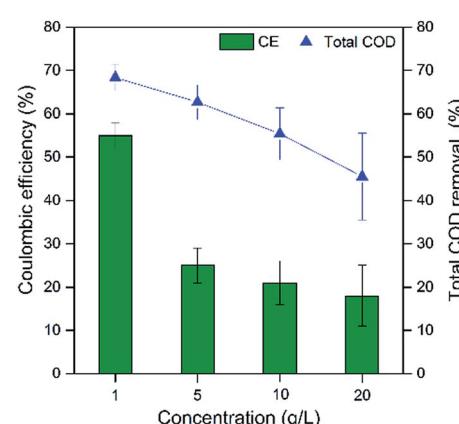


Fig. 2 Coulomb efficiency and total chemical oxygen demand (TCOD) removal of MFCs fed with potato pulps waste of different organic loading rates.



with  $1 \text{ g L}^{-1}$  PPW. The optimal organic loading of PPW may result in the accessibility of the soluble organic substances for exoelectrogens of the anode biofilm. The maximum power density of MFCs fed with PPW was higher than that obtained from MFCs with starch processing wastewater ( $9.6 \text{ W m}^{-3}$ ),<sup>40</sup> which was also acceptable compared with previous reports on MFCs using food waste, beer brewery wastewater and corn stover.<sup>14,41,42</sup>

### Potato waste residue treatment efficiency

The removal of total chemical oxygen demand (TCOD) in MFCs decreased with the increase of potato pulps organic loading (Fig. 2). The maximum TCOD removal of 68.4% was obtained in MFCs fed with  $1 \text{ g L}^{-1}$  PPW, with 62.7% of MFCs fed with  $5 \text{ g L}^{-1}$  PPW and 55.4% of MFCs fed with  $10 \text{ g L}^{-1}$  and  $20 \text{ g L}^{-1}$  PPW. A maximum total suspended solids (TSS) removal of 56.8% was obtained in MFCs fed with  $20 \text{ g L}^{-1}$  PPW, with 53.6% of MFCs fed with  $10 \text{ g L}^{-1}$ . MFCs fed with  $1 \text{ g L}^{-1}$  and  $5 \text{ g L}^{-1}$  PPW obtained slightly lower solid removal of 50.6% and 50.4%, respectively ( $5 \text{ g L}^{-1}$ ) (Fig. 3). The coulombic efficiencies (CEs) of the MFCs fed with PPW were 18–56%. TSS removal of 50.4–56.8% indicated that the residues of starch, cellulose and hemicellulose occurred in the effluent, suggesting to enhance the hydrolysis is important to improve PPW degradation.<sup>29–31,43</sup>

### Microbial community structure in the anode biofilms of MFCs

Total 4419, 2672, 4096 and 4710 high-quality reads were obtained from the anode biofilms of MFCs fed with PPW of  $1 \text{ g L}^{-1}$ ,  $5 \text{ g L}^{-1}$ ,  $10 \text{ g L}^{-1}$  and  $20 \text{ g L}^{-1}$ , respectively, which corresponded to the operational taxonomic units (OTUs) of 640, 525, 624 and 812. The Shannon index of MFCs fed with  $5 \text{ g L}^{-1}$  PPW was highest 5.11 and lowest was 4.39 for MFC fed with  $10 \text{ g L}^{-1}$  PPW. The diversity indices of MFCs fed with  $1 \text{ g L}^{-1}$  PPW and  $20 \text{ g L}^{-1}$  PPW were similar (4.74 and 4.63). The three predominant phyla in all MFCs were *Bacteroidetes*, *Proteobacteria* and

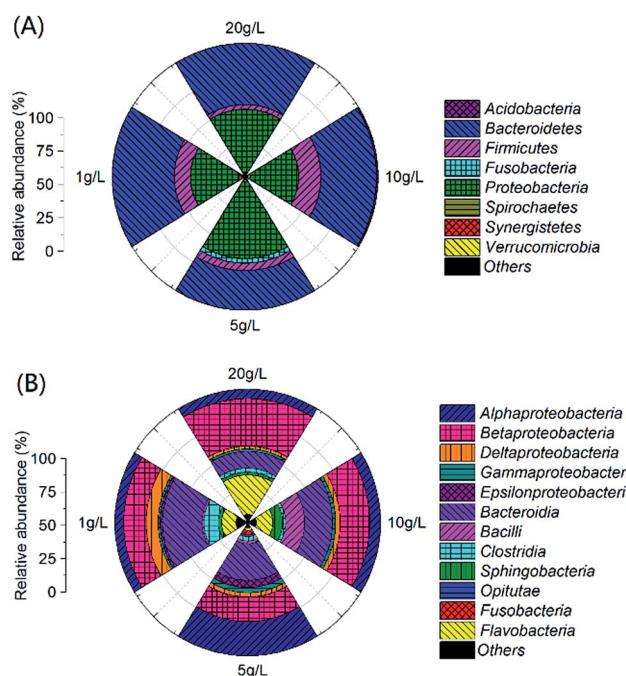


Fig. 4 Taxonomic classification of 16S rRNA gene amplicons for 454 pyrosequencing at the (A) phyla and (B) class. Relative abundance was defined as the number of sequences affiliated with that class or genus divided by the total number of sequences per sample. Phyla and classes making up less than 1% of total composition in all three libraries were classified as "others".

*Firmicutes* (Fig. 4A). The relative abundance of *Firmicutes* in MFCs fed with  $10 \text{ g L}^{-1}$  PPW accounted for 12.2%, which might facilitate hydrolysis of PPW and provide the electron donors for exoelectrogens.<sup>39</sup> The predominate classes belonged to *Alphaproteobacteria*, *Bacteroidia*, *Bacilli*, *Betaproteobacteria*, *Flavobacteriia* and *Clostridia* (Fig. 4B). The relative abundance of *Clostridia* (10.8%) in the MFCs fed with  $10 \text{ g L}^{-1}$  PPW were higher than that in the other MFCs (2.2–3.4%).

The majority of predominant populations were affiliated with *Bacteroides*, *Flavobacterium*, *Geobacter* and *Dysgonomonas* (Fig. 5). The relative abundance of *Bacteroides* was 7.1% ( $20 \text{ g L}^{-1}$ ), 9.5% ( $1 \text{ g L}^{-1}$ ) and 10.6% ( $5 \text{ g L}^{-1}$ ), respectively. *Flavobacterium* showed the most dominant portion in  $20 \text{ g L}^{-1}$  (21.4%), followed by  $1 \text{ g L}^{-1}$  (12.1%),  $10 \text{ g L}^{-1}$  (8.4%) and  $5 \text{ g L}^{-1}$  (1.9%). *Bacteroides* and *Flavobacterium* were reported to be related to starch degradation.<sup>44,45</sup> *Ruminococcus* capable of cellulose-degradation, took up 6.8% in MFCs fed with  $10 \text{ g L}^{-1}$  PPW,<sup>46</sup> implying that both starch and cellulose were degraded and utilized, which led to an easier taken substrates background for exoelectrogens and end up with better electricity production. Open-circuit MFCs also showed TSS removal, implying that both planktonic bacteria and biofilms on the electrodes contributed to the degradation of starch and cellulose or TSS removal of PPW. There are difference in the predominate populations between planktonic bacteria and anode biofilms in MFCs.<sup>32</sup> To enhance the hydrolysis of starch and cellulose, optimization of biofilm populations and planktonic community in MFCs need be further investigated in the future study.

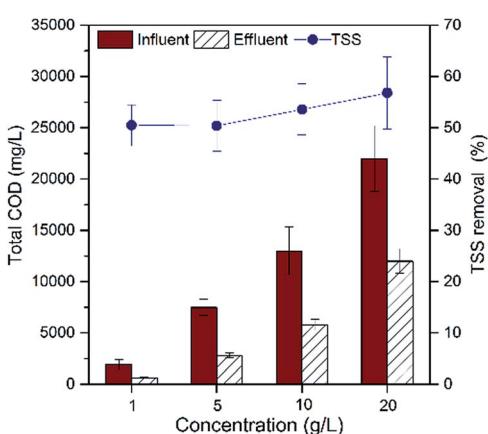


Fig. 3 Removal efficiency of total chemical oxygen demand (TCOD) and total solid suspended (TSS) removal of rMFCs fed with potato pulps waste of different organic loading rates. Error bars represent maximum deviation based on measurements from duplicate reactors in at least one month.



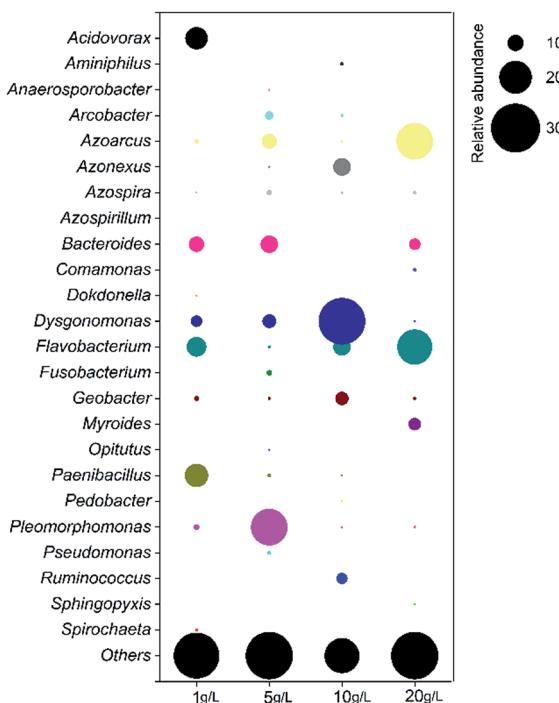


Fig. 5 The relative abundance of predominant genera in microbial communities of MFC fed with PPW of different concentrations. "Others" represents genera which relative abundance are less than 1% of total composition.

The predominant exoelectrogens in MFCs were affiliated with *Geobacter* and *Dysgonomonas*. Both of them took up the highest portion in MFCs fed with  $10\text{ g L}^{-1}$  PPW, which was 8.2% (*Geobacter*) and 28.5% (*Dysgonomonas*). The percentage *Geobacter* represented among the other three organic loading groups were 3.1% ( $1\text{ g L}^{-1}$ ), 1.8% ( $5\text{ g L}^{-1}$ ) and 2.2% ( $20\text{ g L}^{-1}$ ), while that of *Dysgonomonas* were 7.1% ( $1\text{ g L}^{-1}$ ), 8.4% ( $5\text{ g L}^{-1}$ ) and 1.6% ( $20\text{ g L}^{-1}$ ). *Dysgonomonas* sp. was proved to be related to electricity generation in previous researches.<sup>47-49</sup> Previous investigations on the degradation of cellulose, semicellulose and starch using MFC were focused on electricity generation, few studies monitored the community structures using conventional technology denaturing gradient gel electrophoresis (DGGE) of 16S rRNA gene.<sup>24</sup> Community analyses based on 454 pyrosequencing shows that diverse the cellulose- or starch-degrading bacteria and exoelectrogens (*Geobacter* and *Dysgonomonas*) are present in the anode biofilms, hence, to get insight into the syntrophic interaction between these populations is important to recover bioenergy from PPW in microbial electrochemical cells.

## Conclusions

Simultaneous degradation of the potato pulps waste (PPW) and electricity generation were implemented using microbial fuel cells. The organic loading of PPW influenced obviously the performances of MFCs, the maximum power density of  $32.1\text{ W m}^{-3}$  was obtained in MFCs fed with  $10\text{ g L}^{-1}$  PPW. The 454 pyrosequencing of 16S rRNA gene amplicons indicated that the

difference in the microbial community occurred between the anode biofilms of MFCs fed with different organic loading of PPW. The predominant populations in the anode biofilms were affiliated with exoelectrogenic *Geobacter* and *Dysgonomonas*, and polysaccharide-degrading bacteria. The syntrophic interaction between exoelectrogenic and fermentative bacteria drove the electricity generation and degradation of PPW. MFCs provides another approach for potato pulps waste treatment and bioenergy generation.

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