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Performance and microbial community of carbon nanotube fixed-bed microbial fuel cell continuously fed with hydrothermal liquefied cornstalk biomass

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HIGHLIGHTS

- MFC was continuously fed with hydrothermal liquefied cornstalk biomass.
- 80% of COD and TOC was removed from cornstalk hydrolysate with low BOD/COD (0.16).
- Illumina MiSeq sequencing was used to analyze the microbial structure in MFC.
- Dominant bacteria was related to cellulose degradation, and was not *Proteobacteria*.

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ABSTRACT

Hydrothermal liquefaction (HTL) is a green technology for biomass pretreatment with the omission of hazardous chemicals. This study reports a novel integration of HTL and carbon nanotubes (CNTs) fixed-bed microbial fuel cell (FBMFC) for continuous electricity generation from cornstalk biomass. Two FBMFCs in parallel achieved similar performance fed with cornstalk hydrolysate at different organic loading rates (OLRs) (0.82–8.16 g/L/d). About 80% of Chemical oxygen demand (COD) and Total organic carbon (TOC) was removed from low-Biochemical oxygen demand (BOD)/COD (0.16) cornstalk hydrolysate at 8.16 g/L/d, whereas a maximum power density (680 mW/m^3) was obtained at 2.41 g/L/d, and a smallest internal resistance (R_{in}) (28 Ω) at 3.01 g/L/d. Illumina MiSeq sequencing reveals the diverse microbial structure induced by the complex composition of cornstalk hydrolysate. Distinguished from *Proteobacteria*, which a number of exoelectrogens belong to, the identified dominant genus *Rhizobium* in FBMFC was closely related to degradation of cellulosic biomass.

1. Introduction

Lignocellulose biomass, such as cornstalk, is one of the major renewable biomass resources. Hemicellulose and cellulose are two main components of lignocellulosic biomass and feedstock for downstream fermentation. However, it has a natural recalcitrance to degradation and utilization (Himmel, 2008). Hydrothermal liquefaction (HTL) is a thermochemical process where biomass is liquefied in a closed oxygen-free reactor at a certain temperature (100–500 °C) and pressure (5–30 MPa) using water as the solvent (Tian et al., 2014). In comparison to other pretreatment approaches, HTL has its specific characteristics, including the omission of hazardous chemicals and no need for solvents recycling (Nitsos et al., 2013). The challenge of HTL pretreatment is the difficulty in concise control of thermochemical reactions, which otherwise causes the formation of complex hydrolysate (Möller et al., 2011). The hydrolysate is composed of not only sugars, but also volatile fatty acids (VFAs), aldehydes, and phenols, which might inhibit the downstream fermentation (Möller et al., 2011).

Microbial fuel cell (MFC) allows a direct energy conversion into electricity from organic matter and has exhibited several potential applications in the fields of environment and energy (Logan and Rabaey, 2012). Different from ethanol fermentation, electricity generation through MFC has a broader feedstock spectrum (Pant et al., 2010), such as sugars, VFAs and even furan derivatives

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(Luo et al., 2010). Specifically, great efforts have been made to develop MFC based technology to deal with cellulosic biomass (Ahmad et al., 2013). However, exoelectrogens in cellulosic MFC alone may suffer from substrate accessibility and hydrolysis limitations (Ahmad et al., 2013).

There are currently two main approaches for electricity generation through cellulosic MFC. The first is based on completely biological conversion, for instance consolidated bioprocessing of cellulosic biomass (Lynd et al., 2005). This process could be catalyzed by artificially constructing a multi-functional microbial consortia (Ren et al., 2007), or employing an enriched microbial community alone (Rismani-Yazdi et al., 2007) or mixed with the cellulase (Rezaei et al., 2008). For instance, microbial consortia (Ren et al., 2007) were designed consisting of a cellulose-degrading bacteria *Clostridium cellulolyticum* and an exoelectrogen *Geobacter sulfurreducens*. Prominent synthetic effect of these two strains was observed in the designed MFC. By adding cellulase to a pre-acclimated MFC consisting of exoelectrogen, the cellulose was also rapidly converted to sugars and produce power in an MFC (Rezaei et al., 2008). Besides cellulose, power was also produced from more complex lignocellulosic biomass, such as rice straw (Hassan et al., 2014) and cornstalk (Wang et al., 2009). A key issue of these combined microbial consortia is how to make the consortia genetically stable (Elkins et al., 2010) and maintain the long-term efficient operation of MFC. Recently, solid lignocellulosic biomass, such as corncobs (Gregoire and Becker, 2012) and cow manure (Wang et al., 2014a) with a total solid (TS) over 15%, was directly used as feedstocks by combining the concept of leach-bed composing with MFC using natural mixed culture as inoculums. However, mass transfer resistance may be a great challenge for such MFC operation. The second approach is the combination of physico-chemical pretreatment with MFC. Steam-explosion was frequently used for the treatment of biomass (Liu et al., 2015). It was firstly introduced to treat cornstalk, which was used as the feedstock in a batch MFC study (Zuo et al., 2006). Wheat straw was pretreated with HTL and the hydrolysate was used for electricity generation in a batch two-chamber MFC. However, fermentation inhibitors, such as furan derivatives and phenols (Li and Chen, 2008; Wang et al., 2013) were released during pretreatment. Furan derivatives, including furfural (FUR) and 5-hydroxymethyl furfural (HMF), are notorious fermentation inhibitors through reducing cell growth rate and lowering cell membrane permeability (Wang et al., 2013). Therefore, the biggest challenge of MFC fed with biomass hydrolysate is to enrich robust exoelectrogens suitable to deal with the feedstock in a complex composition, including sugars, organic acids, furan derivatives, phenols and other components. Besides these, most reported cellulosic MFCs (Zuo et al., 2006; Wang et al., 2014a,b; Zhang et al., 2009) were operated in batch mode, depressing the application potential of such technology.

The purposes of this study were to (1) investigate the feasibility of continuous conversion and power generation from HTL treated cornstalk biomass through carbon nanotubes (CNTs) based FBMFC under different organic loading rates (OLRs); (2) study microbial structure and evolution of exoelectrogen using next generation sequencing on the Illumina MiSeq platform.

2. Methods

2.1. Cornstalk and HTL treatment

Cornstalk was collected from Golden-sun farm (Beijing, China). It was dried, milled and screened through 10–40 mesh sieves. As shown in Table 1, the dry biomass mainly consisted of cellulose (45.06%), hemicellulose (29.68%), and lignin (5.65%). HTL of cornstalk was performed in a 1.8 L batch reactor (Model 4578, Parr Instrument Company, USA), similar to the procedure described previously (Li et al., 2014). The cornstalk was mixed with water to achieve a TS of 20% and then put into the reactor. After the reactor was sealed, pure nitrogen gas was flowed to exclude oxygen and reach a set initial pressure as 2.5 MPa for the experiments. The retention time started when the reactor reached the set temperature (312 °C), and was set as 0 min with a mixing speed of 400 rpm. All cellulose and hemicellulose were liquefied through HTL and the solid residue contained 22.08% of lignin (Table 1). The liquefaction yield of cornstalk was 59%. The cornstalk hydrolysate contained 64.59 g/L total VFAs (TVFAs), 5.4 g/L reducing sugars, 0.67 g/L HMF and 0.74 g/L FUR with a pH of 3.34. In addition, the analysis of Gas Chromatograph–Mass Spectrometer (GC–MS) revealed the complexity of cornstalk hydrolysate, and many other organic chemicals were included, such as phenols, ketones and esters (Fig. S1). Chemical oxygen demand (COD), Total organic carbon (TOC), Biochemical oxygen demand (BOD) and IC (Inorganic carbon) of hydrolysate were 44.94 g/L, 18.51 g/L, 7.37 g/L and 0.06 g/L, respectively. Most measurements were performed in triplicates, and the mean values were presented. The cornstalk hydrolysate had a BOD/COD of 0.16, indicating its characteristics of poor biodegradability. The detailed analysis method was described in Section 2.4.

2.2. Inoculum and electrolyte

The inoculum was collected from an anaerobic digester of Xiaohongmen Municipal Wastewater Treatment Plant (Beijing, China). Cornstalk hydrolysate was diluted for the desired experiment, amended with trace elements (10 mL/L) and minerals solutions (10 mL/L) and adjusted to pH 7.0 prior to use. The catholyte consisted of a 50 mM phosphate buffer (0.31 g NH_4Cl/L, 0.13 g KCl/L, 2.45 g $NaH_2PO_4{\cdot}H_2O$/L, 4.58 g Na_2HPO_4/L, pH 6.8–7.1).

2.3. MFC structure and experimental procedure

FBMFC consisted of two chambers as previously described (He et al., 2015). The anode chamber had a working volume of 290 ml and was packed with multi-walled CNTs (Cnano Technology Company, Beijing, China) with a packing density of 0.13 g/cm^3. Carbon fiber felt with a projected area of 515 cm^2 was placed around the CNTs as the current collector. The CNTs

Table 1
Characterization of cornstalk before and after HTL.

Components	Cornstalk before HTL	Cornstalk after HTL
Solid contents (%)		
Cellulose	45.06 ± 0.70	N.D.
Hemicellulose	29.68 ± 0.31	N.D.
Lignin	5.65 ± 0.27	22.08 ± 2.74
Ash content	3.98 ± 0.05	4.06 ± 0.24
Others	10.26 ± 0.34	73.86 ± 2.51
Liquid information		
pH	–	46.60
Reducing sugars (g/L)	–	4.99
HMF (g/L)	–	0.79
FUR (g/L)	–	0.74
COD (g/L)	–	44.94 ± 0.18
BOD (g/L)	–	7.37 ± 0.09
BOD/COD	–	0.16
TOC (g/L)	–	18.51
IC (g/L)	–	0.06
TVFAs (g/L)	–	64.59
Formic acid (g/L)	–	21.86
Lactic acid (g/L)	–	13.62
Acetic acid (g/L)	–	11.20
Succinic acid (g/L)	–	10.40
Propionic acid (g/L)	–	2.45
Butyric acid (g/L)	–	5.06

N.D., not detected.