Surface properties of activated sludge-derived biochar determine the facilitating effects on Geobacter co-cultures

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Article info
Article history:
Received 5 February 2018
Received in revised form 19 May 2018
Accepted 30 May 2018
Available online 5 June 2018

Keywords:
Biochar
Geobacter
Surface functional group
Conductivity
Surface charge

Abstract
Biochar has been reported to facilitate direct interspecies electron transfer (DIET) in co-cultures between Geobacter metallireducens and Geobacter sulfurreducens, a model defined co-culture system. In this study, the biochar derived from the activated sludge with different pyrolysis temperature was added to the co-cultures, the ethanol metabolism rates (Re) and succinate production rates (Rs) of co-culture with biochar-800 were 1.05- and 1.42-fold higher than that without addition. The results suggested that the conductivity of the biochar did not correlate with the facilitating effect of the biochar on the co-culture metabolism. Furthermore, the surface functional group and surface charge of biochar may also influence the facilitating effect on the interspecies electron transfer between the two Geobacter cells. Based on these results, it supported that the electron transfer depending on the charging and discharging process of the surface functional groups might play a major role in facilitating the direct electron transfer process by the biochar derived from activated sludge here. This study could shed light on the better understanding of the bacteria-biochar electron transfer system and the potential utilization of the biochar in the environmental wastewater treatments.

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1. Introduction

Electrons can be transferred from electrochemically active bacteria to extracellular electron acceptors with the oxidation of organic matters, which plays important roles in areas of energy production, environmental remediation and geochemical cycle systems (Logan and Rabaey, 2013; Wang et al., 2009). The extracted electron can be transferred to extracellular electron acceptors, including electrode (Liu et al., 2014a), metal oxides (Byrne et al., 2015) and other bacteria (Nielsen et al., 2010). The manner of electron transfer from one kind of bacteria to another is known as the interspecies electron transfer (IET), which can be achieved through both direct and indirect pathways. Under anaerobic conditions, direct interspecies electron transfer (DIET) may be a more effective process than the mediated interspecies electron transfer (MIET) via molecules such as hydrogen or formate that rely on the diffusion of substrates (Liu et al., 2012, Lovley, 2011, 2012). The co-culture of Geobacter metallireducens and Geobacter sulfurreducens was first discovered to form electrically conductive cell aggregates, in which the two Geobacter species can transfer electrons to metabolize ethanol as the electron donor and fumarate as the electron acceptor (Summers et al., 2010).

Some conductive minerals and carbon materials can accelerate the rate of anaerobic metabolism by facilitating the DIET process (Chen et al., 2014a; Karn et al., 2009; Kato et al., 2012a; c; Liu et al., 2014b). The metabolism process was accelerated when granular activated carbon was added to the co-culture of G. metallireducens and G. sulfurreducens requiring DIET (Liu et al., 2012). Another study reported that the conductive magnetite can facilitate IET from G. sulfurreducens to Thiobacillus denitrificans, accomplishing acetate oxidation coupled to nitrate reduction (Kato et al., 2012c). Biochar...
was reported to be able to accelerate the IET rate with cells separately attached to the surface of biochar, suggesting that electrons were likely conducted through the biochar (Chen et al., 2014b). Many studies demonstrated that the methanogenesis process was significantly accelerated in the presence of iron oxide by promoting the DIET process (Cruz Viggi et al., 2014; Kato et al., 2012b; Li et al., 2015a; Ma et al., 2015). Beside iron oxide, carbon nanotubes (CNTs) in the methanogenic sludge of anaerobic wastewater treatment systems induced much faster methane production and substrate utilization rates (Li et al., 2015b). In a recent work, multiwalled CNTs (MWCNTs) was added in the anaerobic digestion reactor of beet sugar industrial wastewater (Ambuchi et al., 2017), which showed an obvious increase in the methane production, where MWCNTs act as electron transfer conduit for DIET.

As a primary method in wastewater treatment, the microorganism is used to degrade organic pollutants in wastewater and produces lots of surplus sludge as byproducts (Phuengprasop et al., 2011), which is composed of organic compounds, macro and micronutrients, trace elements, microorganisms and micro-pollutants (Hossain et al., 2011). However, the post-processing of the surplus sludge is a difficult issue in environmental area (Xiao et al., 2011; Zhang et al., 2012). Compared with the traditional methods of landfill and direct agriculture utilization (Hwang et al., 2007), conversion of wastewater sludge to biochar through pyrolysis process can potentially be a method of choice, for this process can reduce the volume of the solid residue and eliminate pathogens present in the sludge (Caballero et al., 1997; Koch and Kaminsky, 1993; Singh et al., 2010). However, less attention has been given to the effect of biochar derived from surplus sludge on the IET process. It is thought that further understanding of the biochar’s facilitating effect on the IET process will provide a basis for the development of better strategies for activated sludge biochar landfill and a better understanding of the biochar influence on biological systems.

2. Materials and methods

2.1. Activated sludge biochar synthesis

The surplus sludge was collected from a local wastewater treatment plant (Majiaogu Wastewater Treatment Plant, Harbin, China), which was rich in microbial cells and inorganic minerals but no heavy metal ions. The activated sludge was dried in the ambient temperature to form dry granules, after that it was heated in a tubular furnace at specific pyrolysis temperatures for 1 h in N2 atmosphere at a heating rate of 5 °C min-1 (and the gases produced during the heating process were filtered by a KOH aqueous solution before it was released into the atmosphere). The pyrolysis temperatures were set at 700, 800, 900, 1000 and 1100 °C, respectively. The carbonized granules were ground to a state of powder in a mortar. These powders were washed with deionized water until the pH reached at 7. Finally, the carbon powder samples were dried in an oven at 90 °C for 24 h. The biochars were named after the corresponding pyrolysis temperature: biochar-700, biochar-800, biochar-900, biochar-1000, and biochar-1100.

2.2. Characterization of the biochar

The morphology of the samples was examined by scanning electron microscopy (SEM, Hitachi S-3500N, Tokyo, Japan) and high-resolution transmission electron microscopy (TEM, JEM1400, JEOL). X-ray photoelectron spectroscopy (XPS) analysis was performed to obtain information about the elemental composition of the prepared carbon. CasaXPS software was used to process the XPS data for the elemental analysis. The Brunauer–Emmett–Teller (BET) surface area was characterized with Micromeritics ASAP2020 by nitrogen adsorption at 77 K and Barrett-Joyner-Halenda method. X-ray diffraction (XRD) analysis was carried out to identify any crystallographic structure for the samples using a computer-controlled X-ray diffractometer (D8 ADVANCE, Bruker, Germany). The Fourier Transformation Infrared (FTIR) spectra of powdered samples were recorded in FTIR spectrometer (SPECTRUM ONE, PerkinElmer). The spectra were obtained by 32 scans of the sample at a resolution of 4 cm-1 and an interval of 1 cm-1. The resulting spectra were normalized to the highest peak in the fingerprint region between 4000 and 700 cm-1. The particle size distributions were determined and reported as cumulative volumetric percentage data (Mastersizer, 2000, Malvern, England). The pH of the biochar was measured by adding biochar to deionized water in a biochar concentration of 2 g/L. The solution was then hand-shaken and allowed to stand for 5 min before measuring the pH with a pH meter (InoLab pH 7110, China). To determine the zeta potential of the biochar, 0.020 g of the biochar sample was placed in a 10 ml deionized water and tested with the Zeta potential analyzer (Zetasizer nano, Malvern). The conductivity of the biochar particles was determined with the four-probe method by a powder re-sitivity tester (ST2722, Suzhou Jingge, China) (Zhang et al., 2015). A synthesized compound (10 mM) act as electron acceptor. Before the establishment of the co-cultures, G. metallireducens GS-15 and M. oceanica were established in strictly anaerobic NBF medium with fumarate (40 mM) as the electron donor and fumarate (40 mM) as the electron acceptor. Before the establishment of the co-cultures, G. metallireducens GS-15 and M. oceanica were established in strictly anaerobic NBF medium with fumarate (40 mM) as the electron acceptor. Before the establishment of the co-cultures, G. metallireducens GS-15 and M. oceanica were established in strictly anaerobic NBF medium with fumarate (40 mM) as the electron acceptor. Before the establishment of the co-cultures, G. metallireducens GS-15 and M. oceanica were established in strictly anaerobic NBF medium with fumarate (40 mM) as the electron acceptor. Before the establishment of the co-cultures, G. metallireducens GS-15 and M. oceanica were established in strictly anaerobic NBF medium with fumarate (40 mM) as the electron acceptor. 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performance liquid chromatography (HPLC) analysis as previously described (Zheng et al., 2015). The ethanol metabolism rates (Re) and succinate production rates (Rs) were calculated by the date during the linear phase of metabolism in the co-cultures. Quantitative analysis of the cells was conducted based on the protein mass on the surface of biochar and in the solution (Zhu et al., 2014). Briefly, after the co-culture experiment, the samples were soaked in 0.2 M KOH solution and heated for half an hour at 90 °C. The concentrations of the protein were determined by BCA method according to manufacturer’s protocol (Solarbio, China).

2.4. Morphology of the bacteria on the biochar

The morphology of cells on the biochar surface was examined by a scanning electron microscope (SEM). The pretreatment process of all the samples with microorganisms was as follows: samples were firstly fixed overnight in the fixative containing 2.5% glutaraldehyde at 4 °C, then washed with 0.1 M phosphate buffer solution for three times; then the samples were dehydrated in increasing concentrations of ethanol solution (50, 70, 90 and 100%); The samples were finally blown with nitrogen gas and dried with vacuum freeze-drying method; after that the samples were sputter coated with 10 nm of gold and imaged by a field emission SEM (FEI XL30 Sirion SEM) at an accelerating voltage of 5 kV.

3. Results

3.1. Physical properties of the biochar

SEM images in Fig. 1 showed that biochars at higher temperatures exhibited more porous structure with rough surface morphology. The TEM images showed that the edge of the carbon sheets become rough with higher pyrolysis temperature (Fig. 1). However, the edge of the carbon sheet with the pyrolysis temperature of 1100 °C became smooth, which should be due to the collapse of the carbon structure. These results confirmed that pyrolysis temperature could influence the particle morphology of the biochar.

The XRD patterns showed that biochar samples with various pyrolysis temperatures had similar diffraction peaks (Fig. 2a), which indicated the presence of same crystalline phase. By comparison, biochar with higher pyrolysis temperatures had a higher peak at the 2θ values of around 23°, indicating the development of atomic order in the increasingly carbonized material (Kelluweit et al., 2010). This peak comes from the formation and successive ordering of aromatic carbon (Tushar et al., 2012). The average size of the biochar decreased with pyrolysis temperature increasing (Fig. 2b and Fig. S1), indicating that the pyrolysis reaction could weaken the tensile strength of the raw materials and the biochar is more fragile and likely to be broken (Abdullah and Wu, 2009; Kim et al., 2012).

All the biochar samples with different pyrolysis temperatures exhibited type IV nitrogen adsorption/desorption isotherms with a clear H2-type hysteresis loop (Fig. 2c), indicating the formation of a mesoporous network (Zhou et al., 2014). The lowest BET surface area of biochar-700 sample was likely due to the insufficient carbonization process of the activated sludge (Table 1). And the surface area reached the maximum value for biochar-1000 and showed a slight decrease in higher pyrolysis temperature (biochar-1100), which due to the porous structures collapse.

The conductivity of the biochar powders increased as the pressure increased (Fig. 2d). However, under the same pressure, the conductivity of biochar with different pyrolysis temperature showed a significant difference. The conductivity of the biochar is greatly increased with the pyrolysis temperature increasing due to higher carbonization and the graphitization (Gabihi et al., 2017). Under the pressure of 28.5 MPa, the conductivity of biochar-700 is just 0.41 S m⁻¹, while the conductivity of biochar-800, biochar-900, biochar-1000, and biochar-1100 are 21.07 S m⁻¹, 86.51 S m⁻¹, 281.69 S m⁻¹, and 582.92 S m⁻¹. The conductivity of biochar-1100 is even 1433 times higher than the biochar-700.

3.2. Chemical properties of the biochar

The survey scan spectrum of the biochar samples with characteristic spectra of C1s, N1s, and O1s (Fig. 3a) indicated that the presence of carbon, nitrogen, and oxygen (no metal elements were identified). As shown in Fig. 3a, the height of peaks belong to N (around 400 eV) and O (around 532 eV) decreased with pyrolysis temperature increasing, indicating that the element contents of N and O in the biochar samples decreased. As shown in Table 2, from biochar-700 to biochar-1100, the carbon content increased from 72.90% to 81.98%, whereas the nitrogen and oxygen content decreased from 4.26% to 22.86%—11.4% and 16.88%, respectively. This result indicated that the increase of pyrolysis temperature increased the degree of the carbonization (Chen et al., 2012; Chun et al., 2004). Losses in nitrogen and oxygen content at higher pyrolysis temperature should be attributed to the cracking of corresponding weak bonds within the biochar structure (Demirbas, 2004; Kim et al., 2012).

The mean peak of the C1s spectra of all the biochar samples at the binding energy of 284.5 eV was due to graphitic carbon and the gradual increase of this peak with higher temperature indicated the content increase of the graphitic carbon structure (Fig. S2). The broad peak around the binding energy of 290 eV may be derived from many structures, including carbon present in quinone groups (287.4 eV), nitrogen-containing groups (288.7 eV), the (π-π*) shake-up satellite (290.4 eV) (Lee et al., 2001; Swiatkowski et al., 2004). The mean peak of the O1s spectra (Fig. S3), with a binding energy of about 533 eV, can be ascribed to O-H, C=O and C-N=O moieties in some surface oxygen- and nitrogen-containing
functional groups (Kapteijn et al., 1999; Pakula et al., 2002; Swiatkowski et al., 2002).

FTIR spectra of the biochars (Fig. 3b) revealed their chemical bond structure on the surface of the biochar. These spectra are similar to those reported of other carbons derived from a wide variety of sources (Burg et al., 2002; Gomezserrano et al., 1994; Moreno-Castilla et al., 1998). Moreover, the band at 1032 cm\(^{-1}\) was also observed to gradually decrease with higher temperature, indicating carboxylate groups on the surface were also gradually reduced. Aromatic and heteroaromatic compounds are also confirmed by C–H wagging vibrations in the region between 800 and 600 cm\(^{-1}\). The intensity of these peaks decreases with higher treated temperatures which indicates stability of the aromatic and heteroaromatic compounds and possible cyclisation of the biochar.

![Fig. 2. The XRD (a), particle size distribution (b), and BET surface area (c) of the biochar at different pyrolysis temperature; The biochar conductivity variation with the pressure at different pyrolysis temperature(d).](image)

### Table 1
The BET surface area, Zeta potential and pH of biochar with different pyrolysis temperature.

|                  | Biochar-700 | Biochar-800 | Biochar-900 | Biochar-1000 | Biochar-1100 |
|------------------|-------------|-------------|-------------|--------------|--------------|
| BET surface area (m\(^2\)/g) | 29.96       | 61.83       | 94.02       | 122.38       | 120.24       |
| Zeta potential (mV)    | -17.4 ± 0.70| 6.39 ± 2.07 | 10.55 ± 1.52| 7.15 ± 0.88  | -3.09 ± 1.19 |
| pH                 | 6.45 ± 0.16 | 4.99 ± 0.14 | 4.83 ± 0.13 | 5.32 ± 0.06  | 5.98 ± 0.06  |

![Fig. 3. The XPS (a) and FTIR (b) spectra of the biochar at different pyrolysis temperature.](image)
were destroyed (Hossain et al., 2011). The same reduction trend was also observed for C—N—C bending vibration at 1544 cm$^{-1}$ and the C—O stretching vibration located at 1620 cm$^{-1}$ (Chun et al., 2004).

Zeta potential variation with the pyrolysis temperature was shown in Table 1. With the pyrolysis temperature increased from 700 °C to 900 °C, the zeta potential increased from $-17.4$ to $-10.55$ mV. However, when the pyrolysis temperature increased to 1000 and 1100, the zeta potential decreased to $7.15$ mV (biochar-1000) and $-3.09$ mV (biochar-1100). The zeta potential variation of the biochar may be influenced by many factors, including particle size and surface functional groups. The pH variation of the biochar with the pyrolysis temperature (Table 1) showed the corresponding trend with the zeta potential variation. The biochar-900 showed the lowest pH of 4.83.

### 3.3. Metabolism facilitation of biochar on the Geobacter co-cultures

As previously reported, *G. metallireducens* can directly transfer electrons to *G. sulfurreducens* in a defined medium with ethanol as electron donor and fumarate as the electron acceptor (Summers et al., 2010). Biochar was reported to be able to accelerate the DIET process between *G. metallireducens* and *G. sulfurreducens* (Chen et al., 2014b). However, the feasibility of facilitating the DIET by activated sludge derived biochar has not been investigated before. Thus, firstly, biochar-700 was chosen to test its possibility of promoting metabolism of the *Geobacter* co-culture. As showed in Fig. 4a and b, the result showed that the biochar-700 addition accelerated the ethanol metabolism with a coincident accumulation of succinate. Without biochar addition, the amounts of ethanol oxidation and succinate production were slightly low over the experiment period. The facilitating effect of the biochar increased with more biochar-700 addition. The corresponding ethanol metabolism rates (Re) and succinate production rates (Rs) during the linear phase of metabolism in the co-cultures were listed in Table 3. The Re and Rs gradually increased from 0.11 ± 0.03 mM d$^{-1}$ and 0.66 ± 0.01 mM d$^{-1}$ (Control) to 0.23 ± 0.05 mM d$^{-1}$ and 2.37 ± 0.18 mM d$^{-1}$ (2 g L$^{-1}$, biochar-700). The results indicated that biochar can effectively facilitate the syntrophic growth of *Geobacter* co-cultures.

The total biomass concentration was gradually increased with higher biochar-700 dosage (Fig. 4c). The biomass of the co-culture with biochar-700 dosage of 2.0 g L$^{-1}$ was 22.36 mg L$^{-1}$, which was 2.94-fold higher than without biochar (5.68 mg L$^{-1}$). More biochar addition can induce the faster metabolism of the co-cultures. Cells in the co-cultures exist in one of two types: planktonic, freely existing in solution, and sessile, attached to the surface of the biochar (Garrett et al., 2008). It was also observed that the ratio of the biomass on the biochar surface to the total biomass increased with the dosage increasing (Fig. 4d). For the co-culture with biochar-700 dosage, 71.5% of the cells were found on the surface of the biochar of 2.0 g L$^{-1}$ dosage, while only 56.11% of the cells attached on the biochar surface of 0.5 g L$^{-1}$. Therefore, the majority of biomass in co-cultures was attached to the surface of the biochar, which facilitated IET in the two *Geobacter* co-cultures.

As showed in Fig. 5, the co-culture metabolism with biochar of different pyrolysis temperature showed different metabolism rate. It exhibited the lowest metabolism rate in co-cultures without biochar addition and the Rs was just 0.48 ± 0.05 mM d$^{-1}$ (Table 4). While the biochar addition greatly promoted the metabolism rate of the co-cultures. The addition of biochar-700, biochar-800 and biochar-900 showed the metabolism rates at the same level. The Re and Rs analysis showed that the co-culture with biochar-800 exhibited the highest metabolism rate, with Re and Rs being 0.16 ± 0.04 mM d$^{-1}$ and 1.15 ± 0.06 mM d$^{-1}$ respectively. The Re and Rs of co-culture with biochar-800 were 1.05 and 1.42 times higher than without condition (Re, 0.08 ± 0.01 mM d$^{-1}$; Rs, 0.48 ± 0.05 mM d$^{-1}$). However, the facilitating effect of biochar reduced with higher pyrolysis temperature. The Re and Rs of the co-cultures with biochar-1000 were 0.11 ± 0.03 mM d$^{-1}$ and 0.89 ± 0.04 mM d$^{-1}$, which were 0.35 and 0.87 times higher than without addition. The Re and Rs of the co-cultures with biochar-1100 were 0.09 ± 0.01 mM d$^{-1}$ and 0.69 ± 0.05 mM d$^{-1}$, which were just 0.10 and 0.44 time higher than without addition.

The protein concentration test showed that the biochar addition with different pyrolysis temperature all promoted the co-culture growth (Fig. 5c). The total protein concentrations with the addition of biochar-700, biochar-800, biochar-900, biochar-1000 and biochar-1100 were 21.39 mg L$^{-1}$, 21.53 mg L$^{-1}$, 22.37 mg L$^{-1}$, 19.10 mg L$^{-1}$ and 17.99 mg L$^{-1}$, which were 1.07, 1.08, 1.16, 0.84 and 0.73 times higher than without addition (10.34 mg L$^{-1}$). However, the cells in the solution were greatly reduced with the biochar addition, which may be due to the adhesion of the cells on the biochar surface. The cells in the co-culture solution with biochar-700, biochar-800 and biochar-900 were 2.36 mg L$^{-1}$, 0.90 mg L$^{-1}$ and 0.28 mg L$^{-1}$, which were just 22.82%, 9.48% and 2.71% of the control one. The ratio of the cells on the biochar surface to the total cells increased with the pyrolysis temperature increased from 700 to 900 °C, with the ratio increasing from 66.86% (biochar-700) and 87.41% (biochar-800) to 96.27% (biochar-900) (Fig. 5d). However, this ratio decreased with higher pyrolysis temperatures was applied; the ratio with the addition of biochar-1000 and biochar-1100 slightly decreased to 96.07% and 92.40%. The slight decrease of this ratio should be attributed to the more negative surface potential and less surface functional groups.

### 4. Discussion

Previous studies reported that the material’s conductivity played an important role in its facilitating effect on the DIET process in natural environments (Kato et al., 2010; Li et al., 2015a). Biochar was reported to be able to accelerate the IET process between *Geobacter* species with electrons likely transferred through the biochar in laboratory experiments (Chen et al., 2014b). Another study suggests that biochar can influence soil biogeochemistry by directly mediating electron transfer processes, i.e., by functioning as an electron shuttle between bacteria and Fe (III) minerals (Kappler et al., 2014). Otherwise, a non-conductive material, glass beads, did not promote DIET under similar conditions (Liu et al., 2012). However, in this study, the facilitating effect of co-culture
metabolism did not increase with the conductivity. The correlation is negative with the succinate production rate and biochar conductivity (Fig. 6a). It is generally known that a higher pyrolysis temperature of the biochar would result in a greater degree of carbonization and higher conductivity (Gabhi et al., 2017). As shown in Fig. 6b, the conductivity of the biochar had a negative correlation with O content of the biochar (R² = 0.88). The co-culture with biochar-700, biochar-800, and biochar-900 exhibited similar metabolism rates. However, the conductivity of biochar-800 and biochar-900 are 50.84 and 212.80 times higher than that of biochar-700, indicating that the conductivity of the biochar is not the critical factor for promoting the co-culture metabolism in this study. Although, the conductivity of the biochar-1000 and biochar-1100 were greatly higher than that of the others; the metabolism facilitation effect of biochar-1000 and biochar-1100 were even lower than the other. Biochar is a complex material with properties other than conductivity (Chen et al., 2014b). Thus, besides the conductivity, other properties of the biochar should also play important roles in the facilitating effect on the co-culture metabolism.

In previous studies, activated carbon (Van der Zee et al., 2003) and solid humic substances (Roden et al., 2010) were reported to have the electron accepting function. Quinone moieties, phenolic moieties and arene rings in biochar are the possible redox-active moieties responsible for its electron accepting ability (Kluepfel et al., 2014; Peiris et al., 2017; Tong et al., 2014). The biochar with lower pyrolysis temperature has a higher content of the redox-active moieties, which may participate in the electron transfer process. The biochar in the reductive forms may further serve as an electron donor for other microorganisms. The charging and discharging cycles of biochar surface functional groups have been shown to reversibly accept and donate electrons (Klupfel et al., 2014; Lovley et al., 1996). The direct electron transfer across the interface of biochar matrices and external electron donors and acceptors is different from the established electron transfer through carbon matrices due to electrical conductivity (Xu et al., 2013). In a recent study, it was reported that, besides the direct electron transfer through the biochar, the electron transfer through the charging and discharging cycles of the surface functional groups had been proposed to play important roles in the electron transfer process of the biochar (Sun et al., 2017). It has been demonstrated that the biochar with O/C ratio of less than 0.09 can transfer electrons by the charging and discharging cycles of the surface functional groups about one-third of the direct electron transfer by conductivity. In this study, the O/C ratios of all the biochar were much higher than 0.09 (Table 2), indicating that the surface functional groups may play a major role in the electron transfer through the biochar. And this analysis could explain the phenomenon that the great improvement of the biochar

Table 3
The ethanol metabolism rates (Re) and succinate production rates (Rs) in the co-culture with different biochar-700 dosage.

| Sample (g/L) | Ethanol metabolism rates (Re, mM/d) | Succinate production rates (Rs, mM/d) |
|-------------|-----------------------------------|-------------------------------------|
| Control     | 0.111 ± 0.025                     | 0.66 ± 0.012                        |
| 0.5         | 0.133 ± 0.010                     | 1.048 ± 0.021                       |
| 1           | 0.132 ± 0.016                     | 1.182 ± 0.053                       |
| 1.5         | 0.224 ± 0.029                     | 1.298 ± 0.056                       |
| 2           | 0.226 ± 0.045                     | 2.365 ± 0.183                       |

Fig. 4. Concentrations of succinate (a) and ethanol (b) as a function of time in the G. metallireducens/G. sulfurreducens co-cultures amended with biochar-700 of different concentration. The protein concentration variation (c) and the ratios of cells on the biochar surface to the total cells (d) with biochar-700 of different concentration.
conductivity with higher pyrolysis temperature did not promote the accelerating effect of biochar on DIET process. The surface functional groups were decreased with higher pyrolysis temperature, which can explain the phenomenon that the facilitating effect of biochar-1000 and biochar-1100 was lower. There was a high positive correlation (R² = 0.90) between the succinate production rate and the O content of the biochar in Fig. 6c, indicating that the oxygenic functional groups may deeply participate into the facilitating effect of biochar. The previously reported redox moieties, including quinone moieties and phenolic moieties, are oxygenic functional groups. However, the weak relevance between the succinate production rate and the N content of the biochar (Fig. 6d, R² = 0.40) implied low engagement of the nitrogen-containing structures in the electron transfer accelerating process.

To demonstrate the potential of biochar to function as electron donor and acceptor, the pure culture experiments were carried out. Pure culture of G. metallireducens GS-15 was added in strictly anaerobic NBF medium with the biochars. As shown in Fig. 7b, the obvious succinate production was detected in the cultures with biochars, in which the succinate concentration increased over time. The succinate production rate with biochar-700 addition was much higher than that with other biochars (Table S1). The result of ethanol adsorption experiment showed that the ethanol concentration did not decreased over time (Fig. S4), indicating that the biochar adsorption effect can be neglected. These results proved that the biochars can accept electrons from G. metallireducens GS-15 and transfer electrons to G. sulfurreducens.

Table 4

| Sample (pyrolysis temperature, °C) | Ethanol metabolism rates (Re, mM/d) | Succinate production rates (Rs, mM/d) |
|-----------------------------------|-----------------------------------|-------------------------------------|
| Control                           | 0.079 ± 0.007                     | 0.475 ± 0.048                       |
| 700                               | 0.157 ± 0.019                     | 1.088 ± 0.071                       |
| 800                               | 0.162 ± 0.038                     | 1.151 ± 0.058                       |
| 900                               | 0.122 ± 0.034                     | 1.110 ± 0.080                       |
| 1000                              | 0.107 ± 0.026                     | 0.892 ± 0.042                       |
| 1100                              | 0.087 ± 0.013                     | 0.685 ± 0.046                       |

P. Zhang et al. / Water Research 142 (2018) 441–451
Fig. 6. Correlation between the succinate production rates (Rs) and the conductivity of the biochar (a); Correlation between the conductivity and oxygen element content of biochar (b); Correlation between the succinate production rates (Rs) and the oxygen element content (c) and nitrogen element content (d) of the biochar.

Fig. 7. Concentrations of ethanol (a) as a function of time in the G. metallireducens pure culture with biochars. Concentrations of succinate (b) as a function of time in the G. sulfurreducens pure culture with biochars; Correlation between the electron accepting capacities of the biochars and the ethanol metabolism rates of pure cultures G. sulfurreducens PCA with biochars (c); Correlation between the electron accepting capacities of the biochars and the ethanol metabolism rates of pure cultures G. metallireducens GS-15 with biochars (d).
The surface charge of the samples was determined by measuring the zeta potential ($\zeta$) of the biochar. The surface of charcoals (biochar, activated carbon) is often negatively charged, which makes them unlikely to adsorb negatively charged bacteria (Yao et al., 2011). Biochar-900 ($10.55 \pm 1.52$ mV) has the most positive surface charge, which could benefit the initial attachment of cells on the biochar surface. The surface charge of biochar-700 ($-17.4 \pm 0.70$ mV) was negatively charged, inhibiting the initial attachment of cells. While the surface charge of biochar-800 ($6.39 \pm 2.07$ mV) was between biochar-700 and biochar-900. As shown in Fig. 8a, there was a positive correlation ($R^2 = 0.73$) between the cell attachment ratio and the Zeta potential of the biochar, confirming that the surface charge could actually influence the bacteria adhesion on biochar surface. However, unlike the $\zeta$ element content, there is no obvious linear relationship between $R_s$ and Zeta potential (Fig. 8b), indicating that more complicated relationship between EECs and $O$ content of the biochars should exist. Otherwise, no obvious linear relationship was observed between $R_s$ (co-culture) and the EEC (Fig. S7b). Thus, it was believed that, besides the electron transfer property of the biochar, other properties may also influence the stimulatory effect on the co-culture metabolism. The adhesion of microorganisms on a surface was also an important process for the bacteria metabolism and can be divided into three stages: (1) reversible adhesion on the surface due to long-range forces; (2) consolidation between microorganisms and the surface; (3) colonization and growth of organisms on the surface. And the surface physical and chemical properties, including surface charge and chemical functionalities, dominate the initial attachment of the cells (Garrett et al., 2008; Rattier et al., 1997).

Overall, the result showed that the biochar derived from activated sludge promoted the metabolism process of the co-cultures. Higher dosage of the biochar can induce much faster metabolism and the biochar with different pyrolysis temperatures all promoted the co-culture metabolism. However, the conductivity did not play a decisive role in the facilitating effect, which did not increase with the higher pyrolysis temperature. Beside conductivity, the electron transfer depending on the charging and discharging cycles of the surface functional groups played a major role in the electron transfer process through the biochar. Otherwise, the biochar surface charge and porosity may also influence the adhesion of cells on the biochar surface and the accelerating effect of biochar on DIET process.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.watres.2018.05.058.

Acknowledgements

This work was supported by the National Key R&D Program of China (Grant No. 2016YFC0401010), National Natural Science Fund of China (Grant No. 51408156), and the International Cooperating Project between China and European Union (Grant No. 2014DFE90110). This work was also supported by the Major Research plan (91751112) and the General Programme (41573071) of the National Natural Science Foundation of China. We sincerely thank Prof. D. R. Lovley at the University of Massachusetts for kindly providing the bacterial strains – Geobacter sulfurreducens PCA and Geobacter metallireducens GS-15.

References

Abdullah, H., Wu, H.W., 2009. Biochar as a fuel: 1. Properties and grindability of biochars produced from the pyrolysis of mallee wood under slow-heating conditions. Energy Fuels 23 (8), 4174–4181.
Ambuchi, J.J., Zhang, Z., Shan, L., Liang, D., Zhang, P., Feng, Y., 2017. Response of anaerobic granular sludge to iron oxide nanoparticles and multi-wall carbon nanotubes during beet sugar industrial wastewater treatment. Water Res. 117, 87–94.
Burg, P., Fydrych, P., Cagniant, D., Namse, G., Bimer, J., Jankowska, A., 2002. The characterization of nitrogen-enriched activated carbons by IR, XPS and LSER
methods. Carbon 40 (9), 1521–1531.
Byrne, J.M., Kluegel, N., Pearce, C., Rosso, K.M., Appel, E., Kapper, A., 2015. Redox cycling of Fe(II) and Fe(III) in magnetite by Fe-metabolizing bacteria. Science 347 (6229), 1473–1476.
Caballero, J.A., Front, R., Marcilla, A., Conesa, J.A., 1997. Characterization of sewage sludges by primary and secondary pyrolysis. J. Anal. Appl. Pyrol. 40–41, 1–150.
Chen, Y.Q., Yang, H.P., Wang, X.H., Zhang, S.H., Chen, H.P., 2012. Biomass-based pyrolytic polymerization system on cotton stalk pyrolysis: influence of temperature. Bioresour. Technol. 107, 411–418.
Chen, S., Rotaru, A.E., Liu, F., Philips, J., Woodard, T.L., Nevin, K.P., Lovley, D.R., 2014a. Carbon cloth stimulates direct interspecies electron transfer in syntrophic cultures. Bioresour. Technol. 173, 82–86.
Chen, S., Rotaru, A.E., Shrestha, P.M., Malvankar, N.S., Liu, F., Fan, W., Nevin, K.P., Lovley, D.R., 2014b. Promoting interspecies electron transfer with biochar. Sci. Rep. 4, 5019.
Chun, Y., Shi, O.G., Chio, C.T., Xing, B.S., 2004. Compositions and sorptive properties of crop residue-derived chars. Environ. Sci. Technol. 38 (17), 4649–4656.
Coppi, M.V., Leang, C., Sandler, S.J., Lovley, D.R., 2001. Development of a genetic system for Geobacter sulfurreducens. Appl. Environ. Microbiol. 67 (7), 3180–3187.
Cruz Viggi, C., Rossetti, S., Fazi, S., Paano, P., Majone, M., Aulenta, F., 2014. Magnetite particles triggering a faster and more robust syntrophic pathway of methano- genesis from propionate degradation. Environ. Sci. Technol. 48 (13), 7536–7543.
Demirbas, A., 2007. Effects of temperature and particle size on bio-char yield from pyrolysis of agricultural residues. J. Anal. Appl. Pyrol. 72 (2), 243–248.
Gabski, R.S., Kim, D.W., Jia, C.Q., 2017. Preliminary investigation of electrical conductivity of metallic biomass. Carbon 116, 435–442.
Garete, L., Thakore, A., Zhu, W., Zhang, J., 2008. Bacterial adhesion and biofilm formation on carbon surfaces. Progress Nat. Sci. Mater. Int. 18 (9), 1049–1056.
Gomezrezano, V., Acedoramos, M., Lopezpeinado, A.J., Valenzuelacalahorro, C., 1994. Oxidation of activated carbon by hydrogen-peroxide - study of surface oxygen complexes. Colloid. Surface. Physicochem. Eng. Aspect. 208 (1), 325–331.
Huang, J.H., Ouchi, Y., Matsuo, T., 2007. Characteristics of leachate from pyrolysis residue of sewage sludge biomass. Chemosphere 68 (10), 1913–1919.
Kappler, A., Wuesten, M.L., Ruecker, A., Harter, J., Halama, M., Behrens, S., 2014. Biochar as an electron shuttle between bacteria and Fe(II) minerals. Environ. Microbiol. 16 (1), 312–320.
Kapteijn, F., Mouljin, J.A., Matzner, S., Boehm, H.P., 1999. The development of nitrogen functionality in model chars during gasification of coal and the bioremediation of energy-related contamination. Energy Environ. Sci. 4 (12), 4896–4906.
Kapteijn, F., Moulijn, J.A., Matzner, S., Boehm, H.P., 1999. The development of nitrogen functionality in model chars during gasification of coal and the bioremediation of energy-related contamination. Energy Environ. Sci. 4 (12), 4896–4906.
Kapteijn, F., Moulijn, J.A., Matzner, S., Boehm, H.P., 1999. The development of nitrogen functionality in model chars during gasification of coal and the bioremediation of energy-related contamination. Energy Environ. Sci. 4 (12), 4896–4906.
Kapteijn, F., Moulijn, J.A., Matzner, S., Boehm, H.P., 1999. The development of nitrogen functionality in model chars during gasification of coal and the bioremediation of energy-related contamination. Energy Environ. Sci. 4 (12), 4896–4906.
Kapteijn, F., Moulijn, J.A., Matzner, S., Boehm, H.P., 1999. The development of nitrogen functionality in model chars during gasification of coal and the bioremediation of energy-related contamination. Energy Environ. Sci. 4 (12), 4896–4906.
Kapteijn, F., Moulijn, J.A., Matzner, S., Boehm, H.P., 1999. The development of nitrogen functionality in model chars during gasification of coal and the bioremediation of energy-related contamination. Energy Environ. Sci. 4 (12), 4896–4906.
Kapteijn, F., Moulijn, J.A., Matzner, S., Boehm, H.P., 1999. The development of nitrogen functionality in model chars during gasification of coal and the bioremediation of energy-related contamination. Energy Environ. Sci. 4 (12), 4896–4906.
Kapteijn, F., Moulijn, J.A., Matzner, S., Boehm, H.P., 1999. The development of nitrogen functionality in model chars during gasification of coal and the bioremediation of energy-related contamination. Energy Environ. Sci. 4 (12), 4896–4906.
Kapteijn, F., Moulijn, J.A., Matzner, S., Boehm, H.P., 1999. The development of nitrogen functionality in model chars during gasification of coal and the bioremediation of energy-related contamination. Energy Environ. Sci. 4 (12), 4896–4906.
Kapteijn, F., Moulijn, J.A., Matzner, S., Boehm, H.P., 1999. The development of nitrogen functionality in model chars during gasification of coal and the bioremediation of energy-related contamination. Energy Environ. Sci. 4 (12), 4896–4906.
Kapteijn, F., Moulijn, J.A., Matzner, S., Boehm, H.P., 1999. The development of nitrogen functionality in model chars during gasification of coal and the bioremediation of energy-related contamination. Energy Environ. Sci. 4 (12), 4896–4906.
Kapteijn, F., Moulijn, J.A., Matzner, S., Boehm, H.P., 1999. The development of nitrogen functionality in model chars during gasification of coal and the bioremediation of energy-related contamination. Energy Environ. Sci. 4 (12), 4896–4906.
Kapteijn, F., Moulijn, J.A., Matzner, S., Boehm, H.P., 1999. The development of nitrogen functionality in model chars during gasification of coal and the bioremediation of energy-related contamination. Energy Environ. Sci. 4 (12), 4896–4906.
Kapteijn, F., Moulijn, J.A., Matzner, S., Boehm, H.P., 1999. The development of nitrogen functionality in model chars during gasification of coal and the bioremediation of energy-related contamination. Energy Environ. Sci. 4 (12), 4896–4906.
Kapteijn, F., Moulijn, J.A., Matzner, S., Boehm, H.P., 1999. The development of nitrogen functionality in model chars during gasification of coal and the bioremediation of energy-related contamination. Energy Environ. Sci. 4 (12), 4896–4906.
Kapteijn, F., Moulijn, J.A., Matzner, S., Boehm, H.P., 1999. The development of nitrogen functionality in model chars during gasification of coal and the bioremediation of energy-related contamination. Energy Environ. Sci. 4 (12), 4896–4906.
Kapteijn, F., Moulijn, J.A., Matzner, S., Boehm, H.P., 1999. The development of nitrogen functionality in model chars during gasification of coal and the bioremediation of energy-related contamination. Energy Environ. Sci. 4 (12), 4896–4906.
Kapteijn, F., Moulijn, J.A., Matzner, S., Boehm, H.P., 1999. The development of nitrogen functionality in model chars during gasification of coal and the bioremediation of energy-related contamination. Energy Environ. Sci. 4 (12), 4896–4906.
Kapteijn, F., Moulijn, J.A., Matzner, S., Boehm, H.P., 1999. The development of nitrogen functionality in model chars during gasification of coal and the bioremediation of energy-related contamination. Energy Environ. Sci. 4 (12), 4896–4906.
Kapteijn, F., Moulijn, J.A., Matzner, S., Boehm, H.P., 1999. The development of nitrogen functionality in model chars during gasification of coal and the bioremediation of energy-related contamination. Energy Environ. Sci. 4 (12), 4896–4906.
Kapteijn, F., Moulijn, J.A., Matzner, S., Boehm, H.P., 1999. The development of nitrogen functionality in model chars during gasification of coal and the bioremediation of energy-related contamination. Energy Environ. Sci. 4 (12), 4896–4906.
electricity generation from sewage sludge using biocathode microbial fuel cell.

Zhang, C.K., Song, H.Q., Liu, C.F., Liu, Y.G., Zhang, C.P., Nan, X.H., Cao, G.Z., 2015. Fast and reversible Li ion insertion in carbon-encapsulated Li3VO4 as anode for lithium-ion battery. Adv. Funct. Mater. 25 (23), 3497–3504.

Zheng, S.L., Zhang, H.X., Li, Y., Zhang, H., Wang, O.M., Zhang, J., Liu, F.H., 2015. Co-occurrence of Methanosarcina mazei and Geobacteraceae in an iron (III)-reducing enrichment culture. Front. Microbiol. 6.

Zhou, K., Zhou, W., Liu, X., Wang, Y., Wan, J., Chen, S., 2014. Nitrogen self-doped porous carbon from surplus sludge as metal-free electrocatalysts for oxygen reduction reactions. ACS Appl. Mater. Interfaces 6 (17), 14911–14918.

Zhu, X.P., Yates, M.D., Hatzell, M.C., Rao, H.A., Saikaly, P.E., Logan, B.E., 2014. Microbial community composition is unaffected by anode potential. Environ. Sci. Technol. 48 (2), 1352–1358.