Effect of manganese oxide-modified biochar addition on methane production and heavy metal speciation during the anaerobic digestion of sewage sludge

Jianhua Li, Min Zhang, Zhiyin Ye, Changming Yang

Key Laboratory of Yangtze River Water Environment of the Ministry of Education, Tongji University, Shanghai 200092, China

ARTICLE INFO

Article history:
Received 9 January 2018
Revised 3 May 2018
Accepted 10 May 2018
Available online 19 May 2018

Keywords:
Sewage sludge
Anaerobic digestion
Manganese oxide-modified biochar (MBC)
Methane production
Heavy metals
Chemical species

ABSTRACT

Low organic matter content and high heavy metal levels severely inhibit the anaerobic digestion (AD) of sewage sludge. In this study, the effect of added manganese oxide-modified biochar composite (MBC) on methane production and heavy metal fractionation during sewage sludge AD was examined. The MBC could increase the buffering capacity, enhance the methane production and degradation of intermediate acids, buffer the pH of the culture, and stabilize the sewage sludge AD process. The application of MBC positively impacted methane production and the cumulative methane yield increased up to 121.97%, as compared with the control. The MBC addition can improve metal stabilization in the digestate. An optimum MBC dose of 2.36 g was recommended, which would produce up to 121.1 L/kg volatile solids of methane. After the AD process, even though most of the metals accumulated in the residual solids, they could be transformation from the bio-available fractions to a more stable fraction. The total organic- and sulfide-bound and residual fraction content at a 3 g dose of MBC that is 0.12 g/g dry matter were 51.06% and 35.11% higher than the control, respectively. The results indicated that the application of MBC could improve the performance of AD and promote stabilization of heavy metals in sewage sludge post the AD process.

© 2018 The Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. Published by Elsevier B.V.

Introduction

The treatment of increasing volumes of municipal wastewater produces large amounts of sewage sludge, which pose an impending threat to human health and the environment in China. Over 25 million tons of sludge (with 80% water content) has been produced annually in recent years, which poses a threat to the environment if not disposed appropriately (Yang et al., 2010). Sewage sludge treatment and disposal has become one of the most critical environmental issues worldwide, with these processes accounting for up to 60% of the total cost of wastewater treatment (Appels et al., 2008). Therefore, it is imperative that more cost-effective technologies for sludge treatment are explored (Luo et al., 2011). Sewage sludge is rich in N, P, and other nutrients and is similar in composition to plant tissues. The application of sewage sludge in agriculture is becoming commonplace owing to the many benefits it provides, including increasing soil fertility and improving soil structure (Westerhoff et al., 2015). However, due to the high heavy metal, pathogen, and hazardous substance contents, untreated sewage sludge cannot be directly applied to land (Dąbrowska and Rosińska, 2012). Heavy metals pose a risk to
human health and organisms as they do not be biodegraded and can accumulate in tissues (Yuan et al., 2011; Zhao et al., 2014). Therefore, sludge should undergo chemical stabilization prior to application on land to reduce its bioavailability and prevent the uptake of heavy metals by crops (Singh and Agrawal, 2009; Walter et al., 2006). The sludge stabilization process has received considerable attention recently as, in it, the mobility of heavy metals can be minimized by the addition of various additives to ensure that the sludge meets the strict governmental regulations (Fang et al., 1999; Spinosa et al., 2004). Anaerobic digestion (AD) is a common technology for reducing the volume of sludge while simultaneously generating renewable energy for disposal (Choong et al., 2016; De et al., 2013). However, problems such as a low methane yield and process instability are often encountered due to the vulnerability of the anaerobic micro-ecology (Chen et al., 2008; Zhang and He, 2014). Previous studies have suggested that AD could increase the bioavailability of some heavy metals in sewage sludge, which may heighten ecological risk after land application (Dong et al., 2013). Considerable research has been conducted to enhance the activity of microorganisms and improve process stability, and the addition of biochar was found to be an effective immobilization method (Luo et al., 2015; Mumme et al., 2014). Porous biochar could provide a high surface area for the adhesion and growth of methanogenic bacteria and reduce inhibition by adsorbed inhibitors (Wang and Han, 2012).

Biochar, a carbon-rich biomass, is produced by thermal decomposition or pyrolysis with little or no oxygen. Biochar exhibits large physicochemical heterogeneity as well as diversity in its chemistry after combustion. Biochar was investigated as an AD additive for its ability to facilitate enzyme immobilization (Luo et al., 2015; Mumme et al., 2014; Torri and Fabbri, 2014), improve the methane yield (Zhao et al., 2015), and increase methane content (Shen et al., 2015) during AD. The severe thermal treatment during pyrolysis decreases the iron exchange groups on the surface of the biochar, which is predominantly negatively charged. Metal ions in sewage sludge mainly exist as cations, which limits the adsorption ability of biochar (Yu et al., 2016a, 2016b). Therefore, some efforts have been made to develop a modified biochar with more binding sites for heavy metals, which would enhance the adsorption capacity. Zhang et al. (2013) observed that a biochar/γ-Fe2O3 composite had excellent ferromagnetic properties and a strong sorption ability towards aqueous arsenic, and that the arsenic-laden biochar/γ-Fe2O3 could be easily separated from the contaminant by a magnet. Zhou et al. (2014) reported that an nZVI-biochar composite exhibited relatively strong sorption capacities for heavy metals and anionic contaminants (As (V) and P) from a foul solution. Therefore, introducing metal oxides to biochar to increase surface hydroxylation is more effective for binding heavy metals in sewage sludge (Wang et al., 2015). Manganese-based materials, which have a positively charged mineral surface, can remove or oxidize metal ions (Lafferty et al., 2010; Lenoble et al., 2004; Ying et al., 2012). Manganese oxide-modified biochar (MBC) can immobilize arsenic (As) and can be applied to As-contaminated paddy soil in order to reduce the concentration of As in rice (Yu et al., 2016a, 2016b). Therefore, utilizing MBC to assist the immobilization of heavy metals in sewage sludge could be a suitable, as Mn oxides can decrease the mobility of heavy metals via anion exchange (Yu et al., 2015). Applying MBC during AD is an alternative approach towards stabilizing sewage sludge. The objectives of this study were as follows: (1) to study the performance and types of main volatile fatty acid (VFA) inhibition during AD; (2) to determine the optimum dosage of MBC for hydro-acidification and methane production during AD; and (3) to investigate the influence of added MBC on the mobility and bioavailability of heavy metals in the end product of AD.

1. Materials and methods

1.1. Synthesis of MnO-loaded biochar

The pristine biochar was produced through the slow pyrolysis of corn straws at a maximum temperature of 600°C for 120 min in a tube furnace under constant N2 gas protection, with a heating rate of 25 K/min. The obtained biochar was ground so it could pass through a 0.15-mm sieve and soaked with a KMnO4 solution; the mass ratio of biochar to KMnO4 was 10:1. The obtained suspension was vigorously agitated with a magnetic stirrer and continuously purged with N2 gas, and then vacuum dried at a low temperature. The dried composite was heated at 600°C for 1 hr under N2 to produce MnO-loaded biochar (BC). The obtained samples were washed with deionized water to remove impurities and then dried at 80°C.

1.2. Characterization of MnO-loaded BC

The total C, H, O and N contents in the MBC composite were analyzed using an Elemental Analyzer LECO CHNS-932 (Leco Corporation, St. Joseph, MI, USA). The total amount of Mn in the BC composite was determined by inductively-coupled plasma atomic emission spectrometry (ICP-AES) (Perkin Elmer Optima 2100 DV ICP-AES). The ash contents of the samples were defined as the remaining mass after pyrolysis at 600°C in a crucible until a constant level was obtained. The surface morphology was scanned with a scanning electron microscope (SEM, Hitachi 4700 microscope, Hitachi). The X-ray powder diffraction (XRD) patterns of the BC and MBCs were obtained on an X-ray diffractometer (XRD, D8 Advance X-ray diffractometer) equipped with a stepping monitor and graphite crystal monochromator. The XRD patterns were recorded by step scanning from 10° to 90° with the sample rotating at 2 r/sec. The Brunauer–Emmet–Teller (BET) surface areas (SBETs) of BC and MBC were measured through nitrogen adsorption using a gas sorption analyzer (Quan tahrome, USA). The surface chemistry of the composite was determined by X-ray photoelectron spectroscopy (XPS, ESCALAB 250Xi).

1.3. Substrates and sludge inoculum

Dewatered sewage sludge collected from a municipal wastewater treatment plant in Shanghai, China, was used as the substrate in this study. The sludge characteristics were as follows: pH 6.5, total solids (TS) 11.3% ± 1.3 (W/W), and volatile solids (VS) 57.6% of the TS. The C/N ratio of the substrate was 4.6 ± 1.0. The total contents of Cr, Ni, Cd, Cu, and Zn in the sludge were 226.9, 163.1, 2.9, 186.9, and 2011.5 mg/kg, respectively. The inoculum (inoculated sludge) was collected from a long-term continuous lab-scale
anaerobic digester in the National Engineering Research Center for Urban Pollution Control of Tongji University. The TS and VS of the inoculated sludge were 9.2% ± 0.32% (W/W) and 49.7% ± 1.6% of the TS, respectively. The main characteristics of the substrates and seed sludge are listed in Table 1.

1.4. Experimental design and analytical methods

Anaerobic batch experiments were conducted in 500-mL serum bottles. Mesophilic AD experiments were performed using substrate with sludge inoculums without BC (R0), and with 0.75 g corn straw BC (0.03 g/g dry matter) (R1), 0.75 g MBC (0.03 g/g dry matter) (R2), 1.50 g MBC (0.06 g/g dry matter) (R3), 3.00 g MBC (0.12 g/g dry matter) (R4), 4.00 g MBC (0.16 g/g dry matter) (R5), and 5.00 g MBC (0.20 g/g dry matter) (R6). All the experiments were conducted in batch mode, and each set of experiments was conducted in triplicate. Mixtures were homogenized and diluted to 10% of the TS with distilled water and then sonicated for 10 min. After feeding the reactors, all bottles were sparged with high-purity nitrogen (>99.99%) for 10 min to create an anaerobic environment, and then sealed with rubber plugs and secured with aluminum caps (Koch et al., 2015). The bottles were then kept in thermostatic shakers under mesophilic temperature conditions (35°C) until gas production ceased.

The biogas volumes were recorded daily using a gas-tight syringe, and the methane content was analyzed every three days using a gas chromatograph (GC, Agilent Technologies 6890N, CA, USA) with a thermal conductivity detector. VFAs including acetic acid, propionic acid, and butyric acid were analyzed by a gas chromatograph (GC, Agilent Technologies 6890N, CA, USA) with a FID detector. 20 g of the sludge samples were collected every three days and divided into two 10 g samples. 10 g was centrifuged and passed through a microfiber filter (0.22 μm), and the filtrate was physiochemically analyzed. The pH, TS, VS, alkalinity, and ammonia contents were determined following standard methods (APHA, 1995). The other 10 g of the sample was freeze-dried, mashed and evenly mixed, and then sieved through a 0.25-mm mesh to determine their heavy metal contents and speciation.

1.5. Fractionation procedure of heavy metals

To determine the total heavy metal contents in the sludge, 0.5 g of the dry sludge samples were digested with aqua regia (3 mL 65% HNO₃ + 9 mL 37% HCl) in a Teflon tube. The suspension was then shaken for 16 hr at room temperature in an air-bath shaker. After the mixture was centrifuged (5000 r/min) for 30 min, the supernatant was filtered through a 0.22 μm membrane filter and the filtrate was stored at 4°C for analysis. The remaining steps followed Tessier’s four-step extraction procedure. The recovery (%) of heavy metals was investigated by comparing the sum of the six fractions with the total heavy metal concentrations.

1.6. Risk assessment code

In this study, the risk assessment code (RAC) was applied to evaluate the environmental eco-toxicity risk of the heavy metals in the sludge (Leng et al., 2016). The RAC assesses the ecological risk of heavy metals from the percentage of metals present in water-soluble, exchangeable, and carbonate-bound fractions. The classification of RAC was defined as RAC ≤ 1% as no risk (NR), 1% < RAC ≤ 10% low risk (LR), 10% < RAC ≤ 30% middle risk (MR), 30% < RAC ≤ 50% high risk (HR), and 50% < RAC very high risk (VHR) according to Zhai et al. (2014).

1.7. Statistical analysis

In this study, all analyses were conducted in triplicate and the results were expressed as the mean ± standard error (n = 3). Duncan’s new multiple range test (DMRT) was used to assess the differences between the treatment means. The differences were declared as significant at 5% probability level (p < 0.05). One-way ANOVA was implemented to compare the mean results of different treatments. The above statistical analyses were conducted using SPSS18.0 and Origin 8.5 for Windows.

2. Results and discussion

2.1. Properties of BC and MBC

The elemental contents and surface area analysis of BC and MBC are listed in Table 2. The C and H contents of the MBC composite decreased from 83.2% and 1.25% (BC) to 75.6% and 0.37%, respectively, while the O content increased from 6.89% (BC) to 9.92% (MBC). The Mn content in MBC was 4.96%, and no manganese was detected in the BC. The specific surface area significantly decreased from 59.8 m²/g BC to 9.32 m²/g (MBC). The results indicated that MnO particles were successfully modified to the pores of the BC in the MBC. To further examine the surface structure properties of the MBC composite, SEM, XRD, and XPS analyses were conducted. The SEM images (Fig. 1) show that the surface of the BC consists of porous structures,

Table 1 – Characteristics of the substrates and seed sludge.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Substrates</th>
<th>Seed sludge</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.5 ± 0.2</td>
<td>6.9 ± 0.1</td>
</tr>
<tr>
<td>TS (mg/L)</td>
<td>38,762 ± 972.6</td>
<td>69,057 ± 1207.3</td>
</tr>
<tr>
<td>SCOD (mg/L)</td>
<td>775 ± 22.9</td>
<td>7906 ± 126.7</td>
</tr>
<tr>
<td>TCOD (mg/L)</td>
<td>37,309 ± 598.9</td>
<td>80,529 ± 1176.9</td>
</tr>
<tr>
<td>Soluble proteins (mg/L)</td>
<td>9.7 ± 0.2</td>
<td>257.6 ± 9.6</td>
</tr>
<tr>
<td>Soluble carbohydrates (mg/L)</td>
<td>70.5 ± 2.9</td>
<td>306.2 ± 7.9</td>
</tr>
</tbody>
</table>

TS: total solid; SCOD: soluble chemical oxygen demand; TCOD: total chemical oxygen demand. The data are expressed as the mean value ± standard deviation (n = 3).
and is smoother than that of MBC. As shown by XRD patterns of MBC composite (Fig. 2), the $2\theta$ diffraction peaks at 34.9°, 40.6°, 58.8°, 70.2°, and 73.96° can be assigned to the cubic phase of MnO (Zhang et al., 2015). The XPS spectra confirm the presence of C, N, O, and Mn (Fig. 3). The Mn (2p) binding energy exhibits two peaks at 641.8 and 653.2 eV for Mn2p3/2 and Mn2p1/2, respectively, which was consistent with the results of Gu et al. (2014). The N (1s) binding energy is approximately 400 eV, which is well matched with the pyridinic (398.4 eV) and pyrrolic carbon peaks (Xiao et al., 2014).

### 2.2. Methane production at different dosages

During the AD experiment for 34 days, all reactors continuously produced methane. The daily and cumulative methane production volumes are presented in Fig. 4. The operation of all reactors was stable during the first three days, however, this changed after the introduction of different dosages of MBC on the fourth day. At the mesophilic temperature, the addition of BC and MBC increased the methane yield by 59.91% and 76.64% beyond that of the control (R0) from the digesters with 0.75 g of BC (R1) and MBC (R2), respectively. The addition of MBC improved the methanogenesis, and methane production increased as the dosage of MBC increased until the addition dosage up to 0.12 g/g dry matter. The methane yields of digesters with 0.06 g/g (R3), 0.12 g/g (R4), and 0.16 g/g (R5) of added MBC increased by 103.12%, 121.97%, and 71.08%, as compared with no BC or MBC treatment, respectively. The highest methane production of 118.29 ± 4.35 L/kg VS was obtained in R4, which received with 3.0 g of MBC added. However, the culture with 5.0 g of MBC (0.20 g/g, R6) exhibited the lowest cumulative methane production, which was 30.74% lower than that of the control (R0). This suggests that microbial activity could be inhibited by high dosages of MBC.

As shown in Fig. 4a, methane production began immediately on the first day of fermentation, and there were three peaks in the methane production rate of all cultures. The first methane production peaks appearing on days from five to seven could correspond to the initial transformation of the available soluble organic substrates, while the second peaks on days 14–19 could be related to easily degraded compounds, and the third peaks on day 24–25 could be attributed to the non-easily degraded compounds utilized by microorganisms and methane release (Motte et al., 2013). Thereafter, methane production in all cultures gradually stopped. However, a significant ($p < 0.05$) decrease from 13.02 L/kg VS on day 5 to 1.92 L/kg VS on day 12 in the rates of methane production was observed after the first peak, indicating that metabolic intermediates, such as long-chain fatty acids, ammonia, and ammonium, could inhibit microorganism activity. According to Sung and Liu (2003), ammonia can be toxic to methanogenic bacteria as it can penetrate cell membranes. The inhibition of methanogenesis decreased with the addition of MBC. During sludge digestion without MBC (R1), the second methane production rate peak appeared on day 19 as opposed to day 14–15 for digestion with MBC. The biochar composite could have provided a surface that the microbial cells could colonize, and the immobilization of microbial cells has been reported to reduce the distance between syntrophic bacteria and methanogens, which increases the oxidation of volatile fatty acids and hydrogen production (Fagbohungbe et al., 2016). The methane conversion efficiency for sludge digestion without MBC was lower than that of the other treatments, but excluding R6.

The above results indicate that the AD of sewage sludge was improved by adding moderate doses of MBC, which increased the cumulative methane yield and shortened the lag phase. During methane production, MBC could have acted as a suitable microbial carrier and promoted methanogenic biofilm.
formation, enhancing microbial activity and ultimately increasing methane production (Cooney et al., 2016; Sunyoto et al., 2016).

As shown in Fig. 5, there is a strong correlation between the increment of cumulative methane production ($\Delta$ cumulative methane production) and MBC dosages under the test conditions ($R^2 = 0.9825$). The peak value (121.2 L/kg VS) was reached when 2.36 g of MBC was added to the AD system. The results showed that the $\Delta$ cumulative methane production was positively correlated with the amount of MBC at doses below 2.36 g, while the $\Delta$ cumulative methane production decreased as doses increased beyond this. Therefore, the optimum dose of MBC was 2.36 g, which would promote microorganism activity and hydrolysis-acidification during AD.

2.3. Temporal changes in pH and VFA at different dosages of MBC

To evaluate the process stability, the pH and VFA accumulation were measured (Fig. 6). As expected, the digester pH decreased during the AD start-up phase as hydrolysis and acidification occurred due to the accumulation of VFAs. After the addition of different MBC dosages, there was a turning point due to the alkaline nature of the composite samples, especially in R5 and R6. The pH in R4 increased significantly to 7.43, and then remained within a range of 7.45–7.72, which was within the optimum range for high-solid AD (6.6–7.8). There was a pH buffer period in R2 that prevented the pH from increasing during days 6–9, which was attributed to the accumulation of VFAs. At the end of the experiment, the pH in R1 was 7.42, while that in the cultures with 0.03 g/g (R2), 0.06 g/g (R3) and 0.12 g/g (R4) treatment was 7.66, 7.78 and 7.72 under the test condition, respectively. It has been reported that the buffering capacity of an AD process could be maintained or increased by the addition of some alkali compounds (Ward et al., 2008). The MBC amended digesters prevents the pH in cultures from significantly decreasing due to the VFA accumulation, which inhibits the AD (Torri and Fabbri, 2014). The pH of R5 and R6, which had MBC doses of 4.00 and 5.00 g, increased substantially on day 23 to 7.82 and 7.95, respectively, indicating that the collapse of the AD system was caused by excessive MBC dosage, resulting in low methane production. These results indicated that appropriate dosages of MBC promoted pH stability, alleviated inhibition by excessive VFAs, and enhanced the activity of microorganisms. As shown in Fig. 6a, the cultures with and without MBC all generated VFAs during the initial six days and then reached peak levels.

In R1, VFAs began to accumulate immediately and the peak level was reached on day 9 (6837.8 ± 216.59 mg COD/L). After this point, the total concentrations of TVFA ranged from 5238.6 ± 115.69 to 6021.8 ± 199.06 mg COD/L. In R3 and R4, with MBC doses of 1.5 and 3.0 g, the maximum VFA concentrations were reached on day 6 and day 9, respectively. After this, the concentrations significantly decreased to residual values of 4927.6 ± 169.99 and 4016.8 ± 196.18 mg COD/L on day 15 and 18, respectively. As compared with the control (R1), the addition of MBC (R2, R3, and R4) enhanced the degradation of intermediate VFA products, and the adaption and recovery of *Methanobacter* then balanced VFA generation and consumption in the reactors. However, as the dosage reached 0.16 g/g dry matter in reactors R5, a relatively high VFA concentration was obtained after the initial rapid increase during the digestion start-up phase.

The temporal changes in the concentrations of the main VFAs (acetic, propionic, and butyric acids) in all the reactors
are shown in Fig. 6b, c and d. Following the trend of the total VFA, rapid accumulation occurred in the initial digestion phase, and the content of acetic acid was higher than that of propionic and butyric acids. The concentrations of acetic acid on day 6 were as follows: R1 (3062.8 mg COD/L) > R2 (2991.5 mg COD/L) > R3 (2852.6 mg COD/L) > R4 (2791.8 mg COD/L) > R5 (2308.7 mg COD/L) > R6 (2290.2 mg COD/L). Meanwhile, a sharp decrease in the acid concentrations was observed in R3 and R4 during day 9–12. Acetic intermediates may have been utilized by the methanogens and converted into methane, which was consistent with the increased methane accumulation in R3 and R4. The above results also indicated that a moderate dosage of MBC would contribute to the generation and utilization of acetic acid. However, towards the end of the experiment, the concentration of propionic acid fluctuated less than acetic acid in all reactors. This could be because the conversion of propionic to acetic acid requires heat, and because propionic acid is rarely utilized by methanic bacteria (Yu et al., 2016a, 2016b). The content of butyric acid in R4 was the lowest at the end of the experiment, which indicated that butyric acid was effectively converted during AD.

2.4. Migration behavior of heavy metals in sewage sludge

2.4.1. Distributions of heavy metal in the raw sludge

The total metal content and species distributions of the initial sludge are shown in Table 3. The content of target heavy metals (Cr, Ni, Cd, Cu, and Zn) in the samples were within the maximum permitted levels for sludge reuse in agriculture (CJ/T309–2009), excluding Ni and Zn. The recovery of analysis reached 101.92%, 90.02%, 103.21%, 96.23%, and 93.11% for Cr, Ni, Cd, Cu, and Zn, respectively, which demonstrated the validity of the sequential extraction method and reliability of the results. The eco-toxicity and bioavailability of heavy metals depend on their chemical speciation (Huang and Yuan, 2016). Therefore, it is necessary to apply speciation techniques to obtain meaningful data on both mobility and bioavailability.

The dominant binding phase for Cd was the residual fraction (46.7%), which consisted of similar levels of the carbon-bound (13.8%), Fe-Mn oxide-bound (16.8%), and organic- and sulfide-bound (19.6%) fractions. It was also found in previous publications (Fuentes et al., 2004; Lasheen and Ammar, 2009) that the fraction of Cd in sludge is widely distributed between various chemical species without species dominating. Cu was mainly present in the organic- and sulfide-bound fraction (45.9%), which was attributed to the high stability of copper complexes with organic matter. Cu has been demonstrated to have a high affinity for organic compounds in previous studies (Alonso et al., 2006; Lasheen and Ammar, 2009). Ni contained large exchangeable, carbonate-bound, and Fe-Mn oxide-bound fractions, and the sum of these three fractions reached 37.4% of the total concentration. High concentrations of Ni existing in unstable fractions were also reported by Fuentes et al. (2008).
The highest concentration of zinc was found in the Fe-Mn oxide-bound and organic- and sulfide-bound fractions. The Fe-Mn oxide bound fraction is sensitive to redox change, while metals bound to organic compounds are more easily released during substrate mineralization (Dąbrowska and Rosińska, 2012).

### Table 3 – Chemical fractionations of heavy metals in the sewage sludge before anaerobic digestion and their threshold values in China’s standards for sludge disposal.

<table>
<thead>
<tr>
<th>Heavy metals (mg/kg)</th>
<th>Cr</th>
<th>Ni</th>
<th>Cd</th>
<th>Cu</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total content</td>
<td>226.89 ± 10.21</td>
<td>163.12 ± 6.12</td>
<td>2.93 ± 0.11</td>
<td>186.89 ± 7.59</td>
<td>2011.52 ± 70.63</td>
</tr>
<tr>
<td>Water-soluble</td>
<td>4.39 ± 0.15</td>
<td>5.72 ± 0.19</td>
<td>nd</td>
<td>7.01 ± 0.21</td>
<td>54.36 ± 2.07</td>
</tr>
<tr>
<td>Exchangeable</td>
<td>12.25 ± 0.29</td>
<td>7.19 ± 0.22</td>
<td>nd</td>
<td>6.47 ± 0.19</td>
<td>305.54 ± 11.29</td>
</tr>
<tr>
<td>Carbonate-bound</td>
<td>8.55 ± 0.22</td>
<td>23.19 ± 0.92</td>
<td>0.42 ± 0.01</td>
<td>24.82 ± 0.79</td>
<td>50.61 ± 1.79</td>
</tr>
<tr>
<td>Fe-Mn oxide-bound</td>
<td>23.81 ± 0.96</td>
<td>24.51 ± 1.05</td>
<td>0.51 ± 0.01</td>
<td>30.75 ± 1.02</td>
<td>665.45 ± 26.71</td>
</tr>
<tr>
<td>Organic-and-sulfide-bound</td>
<td>106.58 ± 2.37</td>
<td>62.81 ± 2.52</td>
<td>0.59 ± 0.02</td>
<td>82.55 ± 2.96</td>
<td>631.71 ± 21.92</td>
</tr>
<tr>
<td>Residual</td>
<td>75.60 ± 1.92</td>
<td>23.33 ± 0.72</td>
<td>1.41 ± 0.03</td>
<td>28.18 ± 0.92</td>
<td>166.83 ± 7.09</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>101.92</td>
<td>90.02</td>
<td>103.21</td>
<td>96.23</td>
<td>93.11</td>
</tr>
<tr>
<td>Threshold valuesb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard A</td>
<td>500</td>
<td>100</td>
<td>3</td>
<td>500</td>
<td>1500</td>
</tr>
<tr>
<td>Standard B</td>
<td>1000</td>
<td>200</td>
<td>15</td>
<td>1500</td>
<td>3000</td>
</tr>
</tbody>
</table>

* Concentrations of the target heavy metals is the mean value ± standard deviation (n = 3).
* According to “Standards on Disposal of Sludge From Municipal Wastewater Treatment Plant for Agricultural Use” (CJ/T309-2009).
* nd means not detectable.

Fig. 6 – Variations of total volatile fatty acid (a); acetic acid (b); propionic acid (c); and butyric acid (d) concentrations at different dosages of manganese oxide-modified biochar composite (MBC) (R0, no BC and MBC addition; R1, 0.03 g BC/g dry matte; R2, 0.03 g MBC/g dry matter; R3, 0.06 g MBC/g dry matter; R4, 0.12 g MBC/g dry matter; R5, 0.16 g MBC/g dry matter; R6, 0.20 g MBC/g dry matter).
2.4.2. Migration behavior of the target heavy metals during AD process

The results show that the target heavy metals were mainly distributed in the residual digestate, and less than 6.5% were released into the water solution (Fig. 7). Most of the heavy metals accumulated in the digestate after AD. Cu and Ni exhibited relatively high release ability, followed by Cr and Zn. The release of metals into the solution could be attributed to the decomposition of organic matter in the sludge (Zhu et al., 2014).

The addition of MBC appeared to stabilize the target heavy metals in the digesters, and less heavy metal content was detected in the water solution. The increase in the MBC addition dosage reduced the release of target heavy metals into the liquid phase. The decrease in the metal concentration of the solution could further indicate that MBC may decrease their leaching ability and stabilize heavy metals in sludge during AD. MBC has a large surface area and could absorb and create complexes with heavy metals owing to the presence of negatively charged functional groups on the biochar’s surface (Yu et al., 2015). Moreover, manganese oxides have a high affinity with Cu²⁺ in aqueous solutions. Biochar has a higher surface area and could provide more sites for manganese oxides loading, which leads to stronger bonding with heavy metals (Song et al., 2014).

The above results suggest that the target heavy metals can migrate from the bio-available fractions (F1, F2, and F3) into the more stable fractions (F5 and F6) with the addition of MBC, indicating that surface complexation (Fagbohungbe et al., 2016; Pignatello, 2011), adsorption (Tan et al., 2015), and precipitation (Fagbohungbe et al., 2016) might occur between MBC and the target heavy metals in sewage sludge. This could be explained by the decomposition of the organic matter in sewage sludge, causing the released metals to be adsorbed by MBC and concentrated in the organic and residual fractions. There are negatively charged organic functional groups on the surface of BC, and manganese oxides have powerful oxidation ability. Furthermore, the increased stability of heavy metals in the MBC-added digesters could be also attributed to the enhanced stability of the AD process due to the buffering capacity and surface complexation of MBC (Shen et al., 2016).

2.5. Ecological risk assessment of metals after anaerobic digestion

To better evaluate the ecological risk of heavy metals in sludge, their risk assessment codes (RACs) before and after AD (with and without MBC) were compared, and are listed in
Table 4 - Risk assessment code (RAC) for the initial sludge and anaerobically digested sludge.

<table>
<thead>
<tr>
<th>Metals</th>
<th>Before AD a (%)</th>
<th>After AD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RAC (R1)</td>
<td>RAC (R2)</td>
</tr>
</tbody>
</table>

a AD means anaerobic digestion.
b NR, LR, MR, HR, and VHR indicate no risk, low risk, middle risk, high risk, and very high risk, respectively.

The results show that the heavy metals exhibited moderate ecological risk after AD without MBC (R1) and moderate environmental toxicity. However, the sewage sludge in the AD with MBC exhibited significantly (p < 0.05) lower RAC values for the investigated heavy metals, indicating that the metals were better stabilized in the sludge and could pose a low environmental risk.

The RAC values of the heavy metals in R5 and R6 were significantly larger than those in R4 (p < 0.05), especially those of Zn and Cd, though there was little difference in the RAC index between the two digesters. This could be due to the possible inhibition of microbial activity by high dosages of MBC, which would eliminate the desirable AD process stability. Furthermore, the carbonate-bound and exchangeable fractions of Zn and Cd in the initial sludge were higher. The alkaline environment was unfavorable for translation from exchangeable and carbonate-bound fractions to organic and residual fractions.

Previous studies have demonstrated that heavy metals could be adsorbed to plant-derived biochars through cation-π bonding with electron-rich areas on aromatic structures (Harvey et al., 2011). It has also been reported that the adsorption of Cu(II) on residual crop biochar was through hydroxylic and carboxylic groups (Tong et al., 2011). The biochar used in this study was produced at 600°C from corn straw, which may contain abundant cation-π and hydroxyl and carboxylic groups on its surface (Keiluweit et al., 2010). The increased heavy metal stabilization was mainly attributed to the formation of surface complexes with MnO and O-containing groups. Meanwhile, other mechanisms, including cation-π bonding, oxidation-reduction, and cation-exchange may also occur between the heavy metals and MBC, which may make contribution to the reduction of heavy metal ecological risk in the AD process with MBC addition.

Acknowledgment

This work was supported by the Foundation of National Special Item on Water Resource and Environment (No. 2014ZX07303-003 and 2017ZX07603003). We also would like to thank Mr. Ci, the chief engineer of the Hefei Drainage Management Office, for his assistance in collecting the sewage sludge samples from the Shiwiulhe Municipal Wastewater Treatment Plant.

REFERENCES


