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Long-term performance of anaerobic digestion for crop residues containing heavy metals and response of microbial communities

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ABSTRACT

In order to investigate the long-term stability on the performance of the anaerobic digestion process, a laboratory-scale continuous stirred-tank reactor (CSTR) was operated for 1100 days with sunflower harvested in a heavy metal contaminated site. Changes of microbial communities during digestion were identified using pyrosequencing. According to the results, soluble heavy metal concentrations were lower than the reported inhibitory level and the reactor performance remained stable up to OLR of 2.0 g-VS/L/day at HRT of 20 days. Microbial communities commonly found in anaerobic digestion for cellulosic biomass were observed and stably established with respect to the substrate. Thus, the balance of microbial metabolism was maintained appropriately and anaerobic digestion seems to be feasible for disposal of heavy metal-containing crop residues from phytoremediation sites.

1. Introduction

Anaerobic digestion is a biological process in which microorganisms degrade organic matter and convert it into biogas as the end product. Agricultural crop residues represent an important source of biomass that can be utilized as a substrate in anaerobic digestion (Prabhudessai et al., 2013). Anaerobic digestion for crop residues has been applied as an effective technology in terms of renewable energy production, byproduct utilization, and agricultural waste reduction (Zhang et al., 2013). Cuetos et al. (2011) conducted semi-continuous reactor to determine the methane yields for crop residues, and the results were 0.30 ± 0.01, 0.34 ± 0.03, and 0.26 ± 0.02 L CH_4/g-VS for maize, rapeseed, and sunflower residues, respectively. In addition, methane production of crop residues has been found to range between 0.19 ± 0.01 and 0.31 ± 0.01 L CH_4/g-VS for leaves of cauliflower (*Brassica oleracea* var. *botrytis*) and cabbage (*Brassica oleracea* var. *capitata*), respectively (Gunaseelan, 2004). It has been proposed that crop residues harvested in heavy metal contaminated sites may have the potential advantage of becoming economically and environmentally attractive in terms of efficient land utilization and soil remediation (Evangelou et al., 2012). Studies on anaerobic digestion have been only focused on the energy crop residues (*e.g.*, clover, wheat straw, corn stalks, and rice straw) as the substrates to produce biogas through anaerobic digestion (Agneessens et al., 2014), and the application of anaerobic digestion for crop residues containing heavy metals from phytoremediation sites has not been investigated.

Although anaerobic digestion may be an attractive disposal method for crop residues, it may be an improper consideration in the case of cultivating crop residues from heavy metal phytoremediation sites due to the endogenous heavy metals in crop residues. The existence of heavy metals in the anaerobic digestion process may cause adverse effects, resulting in toxicity to microbial activities and causing possible process upset or failure. The adverse effect (*i.e.*, toxicity) of heavy metals is attributed to the interruption of enzyme function and structure by the forming of metal complex with thiol and other groups on protein molecules or by replacing naturally occurring metals in enzyme prosthetic groups (Vallee and Ulmer, 1972). Previous studies have reported that various factors such as soluble metal concentrations (*i.e.*, ionic form in the solution), type of metal species, and amount/distribution of biomass in the digester can cause metal inhibition (Bertin et al., 2012; Fang and Chan, 1997). Among various factors, existing forms of heavy metals and concentrations of soluble heavy metals are known to be significant factors. Previous studies have confirmed the inhibitory ranges of heavy metal concentrations in anaerobic digestion process to be >20 mg/L for Cd^{2+} (Yu and Fang, 2001), >1 mg/L for Cu^{2+}, >10 mg/L for Ni^{2+}, >4 mg/L for Zn^{2+} (Kouzeli-Katsiri and

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Kartsonas, 1986; Yenigün et al., 1996; Zayed and Winter, 2000), and >30 mg/L for Pb^{2+} (Kouzeli-Katsiri and Kartsonas, 1986). Most studies are exclusively focused on the inhibition of soluble heavy metal to anaerobic digestion and there are a few studies on the effects of endogenous heavy metals within substrate. The effects of heavy metals in crop residues on anaerobic digestion process should be studied to secure sustainable application of anaerobic digestion for heavy metal-containing crop residues.

The objective of this research was to investigate the long-term stability on the performance of anaerobic digestion for the treatment of crop residues harvested in heavy metal phytoremediation sites. In order to achieve this goal a laboratory-scale reactor was operated under anaerobic condition with sunflower harvested from heavy metal contaminated site. The effects of endogenous heavy metals on the reactor performance were investigated. Additionally, the microbial communities were identified by using pyrosequencing to investigate the heavy metal effects on the structure and diversity of bacterial and archaeal communities.

2. Materials and methods

2.1. Substrate and inoculum

In this study, sunflower (*i.e.*, *Helianthus annuus*) containing heavy metals from a phytoremediation site was used as the substrate. Sunflower is the most frequently used biomass for remediation of heavy metal contaminated site due to its high heavy metal accumulating capacity (Lee et al., 2013; Lone et al., 2008) and its relatively high biomass production compared to other plants (Zhuang et al., 2005). In addition, sunflower can easily be cultivated in various soil textures of the Republic of Korea. The sunflower used in this study was grown for 120 days in a heavy metal contaminated site near abandoned mine at Jecheon-si, Chungcheongbuk-do, Republic of Korea. This site was contaminated with 3.98 mg-Cd, 29.17 mg-Cu, 28.65 mg-Ni, 155.13 mg-Pb, and 236.25 mg-Zn/kg-soil, respectively. Physicochemical properties of soils in site showed organic matter content of 2.51% by dry wt., pH of 6.9, and texture of silt loam (sand 27, silt 55.6, and clay 17.4% by dry wt.). Since the sunflower used in this study was cultivated from aforementioned heavy metal contaminated site, the sunflower contained 3.21 mg-Cd, 26.3 mg-Cu, 1.45 mg-Ni, 13.1 mg-Pb and 56.0 mg-Zn/kg-sunflower, respectively. All parts of harvested sunflowers (*i.e.*, stem, leaf, and flower) were ground into fine particles using a blender. Ground substrate was mixed in order to apply homogeneity and to facilitate injection.

Sewage sludge was obtained from a waste water treatment plant in Seoul, Republic of Korea and used as the inoculum. Acquired inoculum was pretreated using a sieve with a pore size of 500 μm for the purpose of removing impurities. No additional alkalinity, or buffer, was introduced into the inoculum. The characterizations of sunflower and seeding sludge are summarized in Table 1.

Table 1
Characterization of sunflower and seeding sludge used in this study.

	Sunflower (substrate)	Seeding sludge (inoculum)	Unit
Moisture	60.0	96.4	wt.% (wet basis)
VS	33.6	2.19	
FS	6.40	1.41	
C	41.9	25.2	wt.% (dry basis)
H	5.40	3.83	
N	2.06	3.06	
O	42.9	17.8	
S	0.22	1.11	
Ash	7.52	49.0	
Cellulose	65.0	N.A.	wt.% (dry basis)
Hemicellulose	24.3	N.A.	
Lignin	10.7	N.A.	

N.A.: Not analyzed.

2.2. CSTR operation

A continuous stirred-tank reactor (CSTR) with an effective volume of 5 L (total volume of 8 L) was used in this study. The CSTR was operated with less than 5% by wt. of TS. Anaerobic condition was achieved by purging the reactor with nitrogen gas before the digestion process and the reactor was operated under mesophilic condition at 35 ± 1 °C. Consistent stirring was managed using an electrical motor attached to the reactor. The substrate was fed once a day with pulse feeding method using 50 mL plastic syringe. The initial organic loading rate (OLR) of reactor was set as 0.5 g-VS of sunflower/L/day daily feeding rate for three months to acclimate with the sunflower substrate. After three months of acclimation period, the OLR was increased stepwise from 1.0 to 2.0 g-VS of sunflower/L/day (Table 2). In order to allow acclimation period for the anaerobic microorganisms within reactor, gradual and careful changes in the environment was employed. In order to investigate the effects of heavy metals on anaerobic digestion, OLR was gradually increased. When heavy metal-containing substrate is fed to the reactor, changes in OLR and HRT can indicate changes in absolute heavy metal concentrations within the reactor. The anaerobic reactor operation can be summarized into three different phases: Phase I, II, and III. Phase I had a hydraulic retention time (HRT) of 30 days with an OLR of 1.0 g-VS/L/day for 103 days, and Phase II had an HRT of 24 days with an OLR of 1.25 g-VS/L/day for the next 175 days. Phase III had a constant HRT of 20 days, but the OLR was sequentially increased from 1.5 (Phase III-1) to 1.75 (Phase III-2) and 2.0 g-VS/L/day (Phase III-3). Phase III-1 had operated for 622 days, and the remaining phases (Phases III-2 and 3) had operated for 100 days each. The raised OLR up to 2.0 g-VS/L/day can be considered relatively high as the reported OLR for anaerobic digestion of crop residues ranges from 1.3 to 2.3 g-VS/L/day (Stewart et al., 1984; Wilkie et al., 1986).

2.3. Analytical methods

For heavy metal analysis, the leachate was oven-dried to remove moisture completely until constant weight of leachate was maintained. Oven-dried leachate was ground into fine powder and then digested with a solution of HNO_3, H_2O_2, and distilled water (9:1:1, v/v/v) using a microwave digester (MSP1000, CEM, USA) according to the US Environmental Protection Agency (US EPA) 3052 method for heavy metal analysis. After digestion, the

Table 2
Summary of reactor operating condition.

	Phase I	Phase II	Phase III-1	Phase III-2	Phase III-3
HRT (days)	30	24	20	20	20
OLR (g-VS/L/day)	1.0	1.25	1.5	1.75	2.0
Operating period (days)	103	175	622	100	100