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Evaluating the effects of activated carbon on methane generation and the fate of antibiotic resistant genes and class I integrons during anaerobic digestion of solid organic wastes

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ABSTRACT

The effects of activated carbon (AC) on methane production and the fate of antibiotic resistance genes (ARGs) were evaluated through comparing the anaerobic digestion performance and transformation of ARGs among anaerobic mono-digestion of food waste, co-digestion of food waste and chicken manure, and co-digestion of food waste and waste activated sludge. Results showed that adding AC in anaerobic digesters improved methane yield by at least double through the enrichment of bacteria and archaea. Conventional digestion process showed ability in removing certain types of ARGs, such as *tet*A, *tet*X, *sul*1, *sul*2, *cml*A, *flo*R, and *intl*1. Supplementing AC in anaerobic digester enhanced the removal of most of the ARGs in mono-digestion of food waste. The effects tended to be minimal in co-digestion of co-substrates such as chicken manure and waste activated sludge, both of which contain a certain amount of antibiotics.

1. Introduction

Antibiotic resistance of pathogenic microorganisms is a growing environmental and health concern and antibiotic resistance genes (ARGs) have been regarded as emerging contaminants during the past decade (Pruden et al., 2006; Zhang et al., 2009). Current research has suggested that sewage treatment plants (STPs) are one of the major sources of ARGs (Tong et al., 2016). Hundreds of ARGs have been detected in activated sludge (Zhang et al., 2009); among them, resistance genes of tetracycline, sulfonamide, and chloramphenicol have been reported to be the most abundant in activated sludge (Yang et al., 2013; Zhang et al., 2009). ARGs are classified according to the four mechanisms: (1) efflux pump (e.g. *tet*A and *flo*R), (2) drug inactivation (e.g. *tet*X and *sul*2), (3) target modification (e.g. *tet*W and *tet*Q) and (4) target bypass (e.g. *sul*1). Apart from ARGs, another genetic element, the integrase gene (intI1) of class I integrons is believed to be involved with the evolution and proliferation of multiple antibiotic resistant bacteria.

The spread of antibiotic resistance from STPs is mainly attributed to the disposal of waste activated sludge (WAS) as the majority of antibiotic resistant bacteria (ARB) are found in municipal wastewaters (Diehl and LaPara, 2010). In the USA, WAS from STPs must meet certain federal and state standards to qualify for downstream uses such as fertilizers. Two regulatory limits with respect to pathogen densities – Class A and Class B, were established. Class A waste solids are typically dried and pasteurized, with a drastic reduction in pathogen level, whereas waste solids under Class B have less strict requirements for pathogen level but a similar degree of stabilization (US EPA, 1993).

Generally, WAS from STPs are treated by the various processes, such as AD, composting and incineration (Diehl and LaPara, 2010). Among them, AD has been widely applied as a cost-effective technology to convert WAS to methane and subsequently reduce the number of solids and remove pathogens (Wang et al., 2014). It is believed that removal of ARGs could be an additional benefit for AD, although the effects of digestion conditions remain unclear (Zhang et al., 2015b). Therefore, it is essential to determine the fate of ARGs in AD process so that the design could be optimized to suppress the proliferation of antibiotic resistance accordingly. Various attempts have been made to enhance the removal efficiency of ARGs during AD. Some studies showed that

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temperature had a great impact on ARG removal during AD process (Diehl and LaPara, 2010; Ghosh et al., 2009). However, the results were inconsistent. Some researchers reported that higher temperature favored the removal of certain types of ARGs, whereas others found that ARGs were enriched during thermophilic digestion process (Beneragama et al., 2013; Ghosh et al., 2009). Besides, different pretreatments were also explored, such as thermal hydrolysis (Ma et al., 2011), alkaline pretreatment (Calabrò et al., 2015) and ultrasonic/microwave pretreatment (Tong et al., 2016). Though these pretreatments showed satisfactory results, there were concerns with rebounding of ARGs during subsequent anaerobic/aerobic digestion process or with economic feasibility of the processes (Ma et al., 2011; Zhang et al., 2015a). In addition, with respect to the effects of different substrates in AD process, current studies focus more on the performance of the process (Bouallagui et al., 2009), with less information on the removal of ARGs.

Activated carbon (AC) is a form of an amorphous carbonaceous material exhibiting a high degree of porosity and high surface area. Due to its high adsorptive capacities, AC is extensively employed for the removal of color, odor, taste, and even antibiotics from wastewater by physical adsorption (Yu et al., 2016). In addition, AC can help to enhance the AD performance by reducing the impact of organic shock loading on the stability (Aktaş and Çeçen, 2007) and facilitating microorganism enrichment (Kaarela et al., 2015). Regarding ARG removal, physical sorption was proven to be responsible for removing ARGs in the constructed wetland (Chen et al., 2016a). Sorption could also be important for the biodegradation process by providing retention sites and increasing microbial activities (Chen et al., 2016b; Liu et al., 2013). Since AD is considered an important technique in limiting the dissemination of antibiotic resistance (Zhang et al., 2015b), multiple strategies which could ensure the performance of AD process and simultaneously enhance the removal of ARGs in a cost-effective manner are needed. Hitherto, removal of ARGs by adding AC during AD process has rarely been reported. Thus, we hypothesized that adding AC into anaerobic digester will not only enhance the AD process for methane production but can also physically adsorb ARGs from water phase, potentially enhancing the biodegradation of ARGs.

The objective of this study was to employ AC as an *in situ* strategy to enhance AD performance for methane production and further evaluate the fate of ARGs during AD process. However, most studies focus only on one of the two aspects. Therefore, the contribution of this work was in investigating the effects of AC in methane production and ARGs removal during AD process. Additionally, the effects of different substrates on ARGs removal during AD process were also discussed. To gain insights into potential mechanisms driving the methane production and the fate of ARGs in AD reactors, the metabolic pathways and population structure of microbial communities were analyzed.

2. Materials and methods

2.1. Inoculum and substrates

The seed sludge and WAS were collected from Ulu Pandan Water Reclamation Plant (UPWRP) in Singapore. The ratio between volatile suspended solids (VSS) to total suspended solids (TSS) in seed sludge was 0.71 with initial TSS of 17.80 g/L. Food waste (FW) was obtained from a canteen at National University of Singapore (NUS), which mainly consisted of rice, noodles, meat, vegetables, and condiments. After removing bones and non-biodegradable waste like plastic bags, FW was homogenized by a blender and then stored at −20 °C in the dark. The chicken manure (CM) used in this study was collected from Chew's Group Limited, Singapore. After collection, CM was homogenized by a blender. The detailed characteristics of FW, CM, and WAS are listed in Table 1. The methanogenic activities of seed sludge for the substrates of FW, FW and WAS, and FW and CM were 0.2 ± 0.02 L CH_4/g VS/d, 0.15 ± 0.01 L CH_4/g VS/d, and 0.17 ± 0.02 L CH_4/g VS/d, respectively.

Table 1
Characteristics of food waste, chicken manure and waste activated sludge.

Element/Property	Food waste	Chicken manure	Waste activated sludge
Non-Metals (w/w, %)			
C	53.4 ± 0.8	28 ± 2	34.1 ± 0.3
N	3.7 ± 0.5	2.8 ± 0.2	5.7 ± 0.3
H	7.5 ± 0.1	4.4 ± 0.1	5.5 ± 0.1
S	< 0.50	0.7 ± 0.2	1.9 ± 0.2
Metals (w/w, %)			
Na^+	1.4 ± 0.2	0.5 ± 0.3	0.12 ± 0.1
K^+	0.59 ± 0.8	3.3 ± 0.5	0.21 ± 0.02
Ca^{2+}	0.3 ± 0.2	15 ± 6	2.2 ± 0.5
Mg^{2+}	< 0.10	1.5 ± 0.3	0.33 ± 0.02
Fe^{3+}	< 0.10	0.16 ± 0.03	1.90 ± 0.09
Cu^{2+}	< 0.10	< 0.10	< 0.10
Zn^{2+}	< 0.10	< 0.10	< 0.10
Al^{3+}	< 0.10	0.14 ± 0.04	1.3 ± 0.2
Mn^{2+}	< 0.10	< 0.10	< 0.01
Cr^{3+}	–	< 0.10	< 0.10
Ni^{2+}	–	< 0.10	< 0.10
Antibiotics (ng/g dry weight)			
Tetracycline	< LOD	0.281 ± 0.047	0.046 ± 0.017
Sulfamethazine	< LOD	0.015 ± 0.002	0.025 ± 0.017
Sulfamethoxazole	< LOD	< LOD	0.327 ± 0.048
Chloramphenicol	< LOD	0.027 ± 0.02	< LOD

Values are expressed as mean ± standard deviations of triplicate tests of three samples.

2.2. Reactor specification and operation

Three glass anaerobic digesters (Φ150 mm × 390 mm) were operated with the addition of 75 g powder AC (hereafter referred to as R2, R4, and R6). The pore volume and surface area of AC (100–400 mesh) were 0.30 cc/g and 385 m^2/g, respectively. The control reactors were the same as these three digesters but without the addition of AC (hereafter referred to as R1, R3, and R5). The working volume of all the digesters was 5 L, leading to an AC dosage of 15 g/L. The AC dosage was supplemented in accordance with a previous study (Hansen et al., 1999). After being seeded with seed sludge, the digesters R1 and R2 were operated for anaerobic mono-digestion of FW in a semi-continuous mode (feeding the reactor once a day) with a gradual increase in the organic loading rate (OLR) and reached a final OLR of 3.8 g VSS/L/d. The digesters R3 and R4 were operated for anaerobic co-digestion of FW and WAS (wet mass ratio 1:1) in a semi-continuous mode (feeding the reactor once a day) with a gradual increase in the OLR and reached a final OLR of 5.2 g VSS/L/d. The digesters R5 and R6 were operated for anaerobic co-digestion of FW and CM (wet mass ratio 1:1) in a semi-continuous mode (feeding the reactor once a day) with a gradual increase in the OLR and reached a final OLR of 7.2 g VSS/L/d. These digesters were operated at 35 °C in parallel. The feed was mixed with certain amount of water to load into the digesters once a day. The sludge retention time was 30 d. All the experiments were conducted in triplicate. When the methane yield was over 300 ml/g VS, the performance of the anaerobic digester was defined as “normal operation with high efficiency” with the label of “+”. If the methane yield was less than 200 ml/g VS wastes, the performance of the anaerobic digester was defined as “unstable operation with low efficiency” with the label of “0″. If the digester produced very low methane or even stopped producing methane, it was defined as “failed operation” with the label of “−”.

2.3. Analytical methods

Samples collected from the reactors were subjected to pH measurements using pH analyzer (Agilent 3200M, USA) before centrifugation. Total suspended solids (TSS) and volatile suspended solids (VSS) were determined based on the gravity method after the suspension was dried at 103–105 °C and burnt to ash at 550 °C. Methane gas collected in