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Enhanced anaerobic digestion of food waste by adding activated carbon: Fate of bacterial pathogens and antibiotic resistance genes

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ABSTRACT

Effects of activated carbon (AC) addition on biogas production, bacterial pathogens removal and antibiotic resistance genes (ARGs) during anaerobic digestion (AD) for food waste were investigated. Results indicate that anaerobic digesters with AC addition (R1) maintained stable operation with methane yield of 0.35 L CH_4/L/g VS at high organic loading rate of 4.38 g VS_{FW}/L/d while the control digesters displayed less stability and caused acidification. Some specific dominant bacteria were enriched in R1, resulting in a lower microbial biodiversity. A total of 11 bacterial pathogens and 12 ARGs was detected in the sludge samples. Compared to the control, adding AC enhanced the AD process by decreasing the relative abundance of bacterial pathogens by 18%. Moreover, AC supplementation also facilitated reduction of abundant ARGs, including *tetA*, *tetM*, *tetW*, *tetO*, *tetQ*, *sul2* and *tetX*. This study may provide an ecological treatment method for AD with the addition of AC.

1. Introduction

Anaerobic digestion (AD) is a promising technology for organic waste treatment and renewable energy recovery. During the AD process, the inoculum with a consortium of microorganisms is used for the degradation of complex organic substrates, with the major product being biogas (CH_4 and CO_2) [1]. The past several decades has met with an increasing demand for renewable energy. Hence, the research in AD is mainly focused on anaerobic enhancement technologies to convert waste to energy. Various strategies in enhancing the performance of AD, including (1) optimization of operating parameters, (2) physical, chemical, and biological pretreatment, (3) additives, (4) co-digestion, and (5) optimization of the anaerobic bioreactor, have been studied [2–6]. However, there are limited researches to investigate the enhancement effect of AD on the fate of emerging contaminants.

Antibiotics have been widely used as a type of antimicrobial drug to treat bacterial infections in the medical field and applied as live stock-food additives for anti-bacteria purposes [7]. Over the years, overuse and uncontrolled disposal has discharged large amounts of antibiotics into the environment and water body, causing some pathogenic bacteria to develop antibiotic resistance genes (ARGs) [8]. As of today, 271 subtypes of ARGs, originating from the 18 basic types, have been identified [9]. The rising number of ARGs worldwide can be seen as an emerging pollutant that poses as a threat to the environment and human health. To better address environmental and health concerns surfaced by the people in recent years, the performance of AD to remove ARGs is gaining momentum. AD has been reported to be an effective method in the removal of pathogenic bacteria and ARGs in the waste stream [10–14]. Thermophilic AD process can reduce some ARG fractions by 75% e.g. *tetA*, *tetO*, *tetX* and *intI*, due to a high temperature that is believed to contribute to the destruction of ARGs [15]. Moreover, two-stage AD technique (acidogenic/methanogenic phase) was also found to have the capacity to remove ARGs and inhibit the activation of bacterial pathogens which carry a high prevalence of ARGs [11,16].

Activated carbon is an amorphous carbonaceous material that exhibit a high degree of porosity and strong adsorptive property. Compared with other enhancing strategies, activated carbon (AC) as an additive has several advantages including low cost, easy accessibility and user-friendly control. These characteristics have made AC an attractive addition to the wastewater treatment process as it helps to remove the color, odor, taste, other organic and inorganic impurities including antibiotics and ARGs [17–19]. It has also been shown that AC was effective as electron acceptor and redox mediator during anaerobic biotransformation and could promote direct interspecies electron transfer between bacteria [20,21].

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It was anticipated that the latter interspecies electron transfer between bacteria and methanogens could stimulate metabolism in methanogenic digesters, resulting in enhanced anaerobic digestion performance. Moreover, amorphous and porous AC are good immobilization matrices for bacteria in anaerobic digesters [22]. Thus, AC could promote microorganisms' growth and enhance biological activity in anaerobic sludge, consequently improving the contact probability between functional microbes, nutrients and subsequent biological treatment efficiency [23–27]. The operational conditions such as AC supplementation during anaerobic digestion might serve to discourage selection of resistant bacteria, reduce horizontal transfer of ARGs, and aid in hydrolysis of DNA [28]. Therefore, adding AC into the sludge phase of AD could be a promising approach to enhance the waste-to-energy performance of AD on biogas production. However, to the best of our knowledge, there are no reports that investigate the migration and transformation of bacterial pathogens and ARGs during AD process by adding AC so far. The effects of AC addition on both the performance of AD on biogas production and the removal of bacterial pathogens and ARGs remain largely unclear.

Therefore, in this study, the main objective was to investigate the effects of AC addition on AD for biogas production with food waste (FW) as a substrate and removal of bacterial contaminants and ARGs. The potential value of AD with the addition of AC on the waste-to-energy performance and the contribution to environmental health were evaluated.

2. Materials and methods

2.1. Inocula and substrates

The seed sludge was collected from a large-scale anaerobic digester at Ulu Pandan Water Reclamation Plant in Singapore. The ratio of volatile suspended sludge (VS) to total suspended sludge (TS) was 0.69 with an initial TS of 14.5 g/L.

FW was obtained from a canteen of the National University of Singapore, which mainly consisted of rice, noodles, meat, vegetables, and condiments. After removing any bones and non-biodegradable waste like plastic bags, FW was homogenized by a blender and then stored in a −20 °C freezer. The detailed characteristics of FW are listed in Table S1 (see Supplementary Material).

2.2. Reactor specification and operation

A glass anaerobic digester (Φ150 mm × 390 mm) was fabricated and operated with the addition of 75 g AC (hereafter referred to as R1). The working volume of R1 was 5 L. The control reactor was the same as R1 but without the addition of AC (hereafter referred to as R2). AC was supplemented in the form of powdered activated carbon (100–400 mesh). The AC was only added once in digester R1 at the beginning of the anaerobic digestion process. The pore volume and surface area of AC are 0.30 cc/g and 385 m^2/g, respectively. After being seeded with seed sludge, the two anaerobic digesters (R1 and R2) were operated for AD of FW in a semi-continuous mode (feeding every day with the respective feedstocks) with a gradual increase in the organic loading rate (OLR) from 1.5 to 4.4 g VS_{FW}/L/d. The two reactors were operated at 35 °C in parallel with mixing at 80 rpm. The sludge retention time was 30 days. All the experiments were conducted in triplicate at the same experimental conditions.

2.3. Analytical methods

COD were determined using HACH color meter (DR900, USA) according to the manufacturer's instructions. The pH was recorded using a pH analyzer (Agilent 3200 M, USA). TS and VS were determined based on the weighing method after being dried at 103–105 °C and burnt to ash at 550 °C. The CH_4 production was determined using a gas chromatograph (Clarus 580 Arnel, PerkinElmer, USA) equipped with a thermal conductivity detector. C and N elemental analyses in FW were determined using the vario MICRO cube (Elementar, HANAU, Germany). The abundance of bacteria and archaea were determined by real-time PCR according to the method described in the Ref. [29]. Main volatile fatty acids (VFA) such as acetic acid, propionic acid, and butyric acid were determined by a gas chromatograph (Clarus 580GC, PerkinElmer, USA) equipped with a flame ionization detector. The concentration of total VFA (TVFA) was accounted using COD-equivalent concentration of main VFA. The equivalent relationship between COD and substrates are as follows: 1.06 g-COD/g carbohydrate, 1.07 g-COD/g acetate, 1.51 g-COD/g propionate and 1.82 g-COD/g butyrate [30]. The one way analysis of variance (ANOVA) was used to analyze the data.

2.4. Quantification of ARGs by real-time PCR analysis

A total of 11 ARGs – seven tetracycline resistance genes (*tetA*, *tetW*, *tetO*, *tetX*, *tetB*, *tetM*, *tetQ*), two sulfonamide resistance genes (*sul1*, *sul2*), one chloramphenicol resistance gene *cmlA*, one florfenicol resistance gene *floR*, and the integrase gene *intI1* of class I integrons were detected and quantified using a PCR Thermal Cycler Dice Real Time System by Sangon Biotech Shanghai Co., Ltd. Primers, the annealing temperature and amplification size are listed in Table S2 (see Supplementary Material) [11]. Each sample was analyzed in triplicate, which means that there were three separate AD runs for both R1 and R2.

2.5. DNA extraction and high-throughput 16S rDNA gene pyrosequencing

The genomic DNA of the sample was extracted using an extraction kit (MO BIO Laboratories, Inc. Carlsbad, USA) according to the manufacturer's instructions. The quality of the extracted DNA was checked by determining its absorbance at 260 nm and 280 nm.

The diversity of microbial communities was examined by IIIumina Hiseq 2000 technology. A set of bacterial primers 341F (5′ – CCTACGGGNGGCWGCAG- 3′) and 805R (5′ – GACTACHVGGGTATCTAATCC – 3′) was used to amplify the hypervariable V3–V4 region of bacterial 16S rRNA gene. After being purified and quantified, the PCR products of the V3-V4 region of 16S rRNA gene was determined by pyrosequencing using the IIIumina Hiseq 2000 sequencer (Sangon Biotech Shanghai Co., Ltd.). To obtain the effective sequencing data, raw pyrosequencing results were processed as follows: 1) check the completeness of the barcodes and the adapter; 2) remove sequences containing ambiguities ("Ns"); 3) remove sequences shorter than 200 bps. 4) remove low – quality sequence i.e. a sequencing quality value lower than 20. Subsequently, effective sequences were clustered into operation taxonomic unit (OTUs) by a 3% or 5% level. Rarefaction curves, Shannon diversity index, species richness estimator of Chao1 and coverage index were conducted by MOTHUR to identify the species diversity for each sample. The OTUs defined by a 3% distance level were classified using the RDP-II classifier at a 50% confidence threshold. Bacterial pathogen database used in this study includes 538 pathogenic species, covering most of the pathogens in the environment [31].

3. Results and discussion

3.1. Waste-to-energy performance of AD of FW by adding AC

To investigate the effects of AC on the waste-to-energy performance of AD, an AD reactor with the addition of AC (R1) and a control reactor (R2) were operated in parallel for FW treatment at