



## Aged refuse enhances anaerobic digestion of waste activated sludge



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### ABSTRACT

In this work, a low-cost alternative approach (i.e., adding aged refuse (AR) into waste activated sludge) to significantly enhance anaerobic digestion of sludge was reported. Experimental results showed that with the addition dosage of AR increasing from 0 to 400 mg/g dry sludge soluble chemical oxygen demand (COD) increased from 1150 to 5240 mg/L at the digestion time of 5 d, while the maximal production of volatile fatty acids (VFA) increased from 82.6 to 183.9 mg COD/g volatile suspended solids. Although further increase of AR addition decreased the concentrations of both soluble COD and VFA, their contents in these systems with AR addition at any concentration investigated were still higher than those in the blank, which resulted in higher methane yields in these systems. Mechanism studies revealed that pertinent addition of AR promoted solubilization, hydrolysis, and acidogenesis processes and did not affect methanogenesis significantly. It was found that varieties of enzymes and anaerobes in AR were primary reason for the enhancement of anaerobic digestion. Humic substances in AR benefited hydrolysis and acidogenesis but inhibited methanogenesis. The effect of heavy metals in AR on sludge anaerobic digestion was dosage dependent. Sludge anaerobic digestion was enhanced by appropriate amounts of heavy metals but inhibited by excessive amounts of heavy metals. The relative abundances of microorganisms responsible for sludge hydrolysis and acidogenesis were also observed to be improved in the system with AR addition, which was consistent with the performance of anaerobic digestion.

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## 1. Introduction

Waste activated sludge (WAS), which is the primary byproduct of wastewater treatment plants (WWTPs), is daily produced with huge quantities (Hao et al., 2011; Ni and Yu, 2008; Chen et al., 2016). WAS contains pathogens and heavy metals, which would cause potential risk to the ecological environment if it is not treated properly (Zhao et al., 2015a; Wang et al., 2016). The treatment and disposal of WAS is costly, accounting for up to 60% of the total operation cost of a WWTP (Appels et al., 2008; Luo et al., 2011). On

the other hand, WAS contains high concentrations of organic matter such as protein and carbohydrate, which makes WAS a renewable bioenergy resource (Wang et al., 2015a; Zhao et al., 2015a, b). However, most of the energy involved in WAS is often squandered in many WWTPs via e.g., burying WAS in landfill. With the increasing world's population, more energy is needed, which makes WWTPs growingly considered as facilities for energy recovery rather than merely for waste removal (Li et al., 2014a, 2015; Wang et al., 2016, 2017a, b). Thus, recovering bio-energy from WAS treatment attracts great interest in the past few decades (Zhao et al., 2016a; Wang et al., 2015b, 2017c). Among the available sludge treatment technologies, anaerobic digestion is widely accepted as the most promising technology due to its low cost input and high energy output (Wang et al., 2013a; Lee et al., 2014; Zhao et al., 2017). Moreover, anaerobic digestion could largely reduce sludge amounts and effectively kill pathogenic microorganisms, benefiting the final disposal of sludge (Guo et al., 2014).

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In sludge anaerobic digestion process, the following four stages: solubilization, hydrolysis, acidogenesis, and methanogenesis are generally included. Among them, solubilization is widely accepted as the rate-limiting steps due to the poor biodegradability caused by the protection of extracellular polymeric substances (EPS) and cell wall/membrane (Zhao et al., 2015a; Peng et al., 2016). In the past few decades, therefore, extensive efforts have been dedicated to accelerating the rate of solubilization process through thermal, mechanical, chemical, or biological methods (Wang et al., 2013b; Li et al., 2016;; Lee et al., 2014). Alkaline, ultrasonic, and biological enzyme treatments are widely applied in previous studies (Kim et al., 2003; Xu et al., 2010; Luo et al., 2011). Although these approaches could effectively disintegrate sludge and promote the accumulation of VFA, almost all of them required large consumption of either chemical reagents or energy, which was cost-intensive in application (Alvira et al., 2010; Zhao et al., 2015a). Thus, low-cost and efficient methods to stimulate solubilization process in anaerobic digestion of WAS are urgently needed.

Aged refuse (AR), which contains high concentrations of enzymes and functional microorganisms, is produced massively in landfills. The easily degradable organic matters in AR are almost depleted after the closure of landfills for several years (e.g., 8 years). The special formation conditions and heterogeneous architectures make AR enriching different microbial communities, particularly the microorganisms that could degrade recalcitrant pollutants (Zhao et al., 2007a). Additionally, AR has high specific surface areas and porous structures, as well as excellent physical-chemical properties and hydraulic properties (Li et al., 2008; Zhu et al., 2012). Those special characteristics provide very favorable conditions for the utilization of AR. For example, previous studies showed that AR could be utilized for the enhancement of high loads of livestock wastewater, landfill leachate, and coking wastewater treatments (Zhu et al., 2012). AR was also applied to improve biohydrogen yield from anaerobic digestion of food waste (Li et al., 2008). Due to the merits of AR, it is assumed that the addition of AR might accelerate the solubilization, hydrolysis, and acidogenesis processes of anaerobic sludge digestion. However, this hypothesis has never been tested so far.

Generally, anaerobic digestion of WAS is implemented in WWTPs while AR is produced in landfills. In this situation, transportation of AR to WWTPs is required, which is not economical. In many developing countries such as China, however, digesting WAS directly in WWTPs is not executed. In such WWTPs, WAS is often dewatered and then transported to landfills for further treatment. As thus, WAS and AR can be available in landfills in situ. With the developing high-solid anaerobic digestion technology and the increasing worldwide energy crisis, this strategy attracts more and more attention even in developed countries, because high-solid anaerobic digestion has several advantages such as smaller reactor volume, lower energy supplies for heating, and less material handling, as compared with traditional low-solid anaerobic digestion (Guendouz et al., 2008; Dai et al., 2013). If AR can accelerate the solubilization, hydrolysis, and acidogenesis processes, a low-cost alternative method to enhance sludge digestion efficiency and methane production might be developed. Considering the huge quantity of WAS produced daily, this method should have great economic and ecological benefits. To date, however, no information is available on the potential role of AR in anaerobic digestion of sludge.

The aim of this work is therefore to examine whether the presence of AR benefits the processes involved in anaerobic digestion of sludge and to explore the underlying mechanism of how AR accelerates these processes. Firstly, the effect of different dosages of AR on anaerobic digestion of sludge was investigated. Then, mechanisms of how AR addition affects the processes in

anaerobic digestion of sludge were explored.

## 2. Materials and methods

### 2.1. Source of WAS, inoculum, and AR

WAS used in this work was collected from the secondary sedimentation tank of a WWTP located in Changsha, China. The WAS obtained was first filtered with 1.0 mm mesh to remove the large debris, and then transferred to a 4°C-controlled refrigerator for 24 h before use. The main characteristics of the sludge used are as follows: pH  $6.8 \pm 0.1$ , total COD  $15630 \pm 385$  mg/L, total suspended solids (TSS)  $14500 \pm 260$  mg/L, volatile suspended solids (VSS)  $10082 \pm 190$  mg/L, total protein  $7520 \pm 220$  mg COD/L, total carbohydrate  $1380 \pm 160$  mg COD/L, and total lipids  $284 \pm 12$  mg/L.

Inoculum used in this study was collected from an anaerobic sludge digester in our laboratory. The main characteristics of the inoculum sludge are as follows: pH  $7.2 \pm 0.1$ , TSS  $10250 \pm 210$  mg/L, VSS  $7380 \pm 160$  mg/L, and total COD  $12560 \pm 320$  mg/L.

The AR used in this study was obtained from a 8-year-old closed landfill compartment in Taiyuan, China. Before use, large inorganic substances such as stones, sticks, and glass, were manually removed. Afterwards, AR was screened by a 1.0 mm mechanical screener to ensure the granule size less than 1.0 mm. The main characteristics of AR are shown in Supporting Information.

### 2.2. Effect of AR on anaerobic digestion of WAS

The test was performed in eight replicate anaerobic reactors with a working volume of 1.0 L each. Reactor-1 to reactor-6 were first fed with 900 mL of WAS and 100 mL of inoculum, and then received different amounts of AR to control the AR concentration of 0, 200, 400, 600, 800, and 1200 mg/g dry sludge (DS), respectively. Reactor-7 was fed with 4 g AR, 100 mL identical inoculum, and 900 mL tap water and used to test the intermediate and final products from the anaerobic digestion of AR alone. Reactor-8 (the blank reactor) consisted of 100 mL of inoculum and 900 mL of tap water without WAS. The pH in all reactors was controlled at  $7.0 \pm 0.1$  by adding 3.0 M hydrochloric acid (HCl) or sodium hydroxide (NaOH) to eliminate the influence of pH. The reactors were purged with nitrogen gas for about 5 min and then capped, sealed, and placed in an air-bath shaker (150 rpm). The temperature was controlled at  $35 \pm 1$  °C. The collection and calculation of methane produced were detailed in Supporting Information. In this work, all tests, unless otherwise described, were performed in triplicate with a blank reactor containing 100 mL of inoculum and 900 mL of tap water, and the data reported below are net values with the values measured in the blank having already been subtracted.

### 2.3. Mechanism of AR affecting anaerobic digestion of WAS

#### 2.3.1. Effect of AR on each process of anaerobic digestion

The following batch test in nine replicate serum bottles with working volume of 1.0 L each containing synthetic wastewater was performed. Nine serum bottles were first divided into three groups (Group-I, Group-II, and Group-III) with three in each. Group -I was conducted to evaluate the presence of AR on the process of hydrolysis, while Group-II and Group-III were used to assess the effect of AR on the processes of acidogenesis and methanogenesis, respectively. The synthetic wastewater used in Group-I consisted of 5.1 g bovine serum albumin (BSA, average molecular weight 67000, a model protein compound)/L and 1.3 g dextran (average molecular weight 23800, a model polysaccharide compound)/L. The synthetic wastewater used in Group-II contained 5.1 g L-alanine/L and 1.3 g glucose/L while Group-III received synthetic wastewater consisting

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