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Volatile fatty acids produced by co-fermentation of waste activated sludge and henna plant biomass

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HIGHLIGHTS

- WAS and HPB were co-fermented for the enhanced VFAs production.
- VFAs accumulation was positively related to HPB proportion in mixed substrates.
- HPB played a dual role in adjusting the C/N ratio and shuttling electrons.

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ABSTRACT

Anaerobic co-fermentation of waste activated sludge (WAS) and henna plant biomass (HPB) for the enhanced production of volatile fatty acids (VFAs) was investigated. The results indicated that VFAs was the main constituents of the released organics; the accumulation of VFAs was much higher than that of soluble carbohydrates and proteins. HPB was an advantageous substrate compared to WAS for VFAs production; and the maximum VFAs concentration in an HPB mono-fermentation system was about 2.6-fold that in a WAS mono-fermentation system. In co-fermentation systems, VFAs accumulation was positively related to the proportion of HPB in the mixed substrate, and the accumulated VFAs concentrations doubled when HPB was increased from 25% to 75%. HPB not only adjust the C/N ratio; the associated and/or released lawsone might also have a positive electron-shuttling effect on VFAs production.

1. Introduction

During the past few decades, many wastewater treatment plants (WWTPs) have been constructed in China. At the end of 2014, there were more than 3700 WWTPs in operation treating ~160 million tonnes of municipal wastewater per year. Waste activated sludge (WAS) is the main solid by-product in WWTPs, and it is generated at a yield of 0.032 kg per m^3 of treated wastewater (Ng et al., 2014). To date, the production rate of WAS has surpassed 10% in China. Thus, WAS needs to be properly treated because of the vast quantities that are produced, which can cause secondary pollution and are highly costly. Anaerobic digestion (AD) is not only the most conventional but also an attractive way to treat WAS as it is a harmless process with high energy recovery. The AD process includes three steps: hydrolysis, acidification and methanogenesis. Among these, hydrolysis and/or acidification are the key steps due to the slow rate at which particle sludge is transformed to soluble organic compounds such as volatile fatty acids (VFAs) (Feng et al., 2014; Tiehm et al., 1997). Besides, insufficient carbohydrate content in the raw WAS results in a low carbon-to-nitrogen mass ratio (C/N), which also limits VFAs production from WAS fermentation. VFAs are very useful in applications such as biogas (methane or hydrogen) recovery, degradable plastic synthesis, electricity generation, and the enhanced removals of nitrate, phosphorus, sulfate, azo dyes and disinfection byproducts from wastewaters (Chen et al., 2014; Lee et al., 2014). Therefore, much research has been focused on the enhanced production of VFAs from WAS. Ultrasonic, thermal, hydrothermal, and added alkaline, surfactant or zero valent iron are the usual pretreatment methods used to promote the release of organic compounds from particle sludge and enhance the related enzymatic activities, with the aim of improving VFAs production (Feng et al., 2014; Tiehm et al., 1997; Yang et al., 2014; Yin et al., 2014; Zhou et al., 2015). Furthermore,

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carbohydrate-enriched wastes including primary sludge, food waste, agricultural and forestry residues, pulp and paper waste, organic-rich industrial wastewaters have been eco-friendly added to the raw WAS to adjust C/N ratio for the enhanced production of VFAs during the fermentation process (Bayr and Rintala, 2012; Chen et al., 2013; Feng et al., 2009; Jiang et al., 2013; Luste and Luostarinen, 2010; Zhou et al., 2014). This is referred to as co-fermentation process, which can be affected by pH, temperature, oxidation–reduction potential (ORP), feedstock proportion, sludge retention time (SRT) and so on. In this way, both WAS and organic wastes can be efficiently reduced due to the balanced C/N ratio and better pH buffering capacity (Wang et al., 2011).

Many natural plant biomass substances, such as rice straw, corn stover and bagasse, have been popularly used as co-substrates to adjust the C/N ratio during WAS digestion (Rughoonundun et al., 2012; Zhou et al., 2013). Plant biomass, which is an abundant carbohydrate sink, is mainly composed of cellulose, hemi-cellulose and lignocelluloses. Because of the complex structure and a caused enzymatic attack of these substances, the hydrolysis is quite slow and is the rate-limiting step of the overall fermentation process (Noike et al., 1985; Wang et al., 2014a). Therefore, natural plant biomass usually needs to undergo mechanical, thermal, and/or alkaline pretreatment before it is introduced to a co-fermentation system. However, the high energy consumption of these pretreatments currently restricts their broad application. As a solution, extracellular redox mediators (RMs), such as anthraquinone-2,6-disulfonate (AQDS) and humic acid (HA) associated with a quinone group, have been reported to act as electron-shuttling systems to improve the related enzymatic activities, and thus to enhance VFAs production during WAS fermentation (Liu et al., 2015; Yang et al., 2012). Henna (*Lawsonia inermis*) is a carbohydrate-enriched plant biomass containing abundant lawsone, which is a typical quinone group-based RM that has been reported to improve hydrogen production in a co-cultured fermentation system (Zhang et al., 2013). Therefore, using henna plant biomass (HPB) as a co-substrate during WAS fermentation could balance the C/N ratio, plus the associated and/or released lawsone might have a positive effect on VFAs production.

The aim of this study was to investigate the feasibility of using WAS and HPB as substrates in a co-fermentation process to enhance VFAs production. Under different feedstock proportions of WAS to HPB, the concentrations of various soluble organics and key enzymatic activities in the fermentation systems were evaluated to aid in understanding the mechanism of improved VFAs production.

2. Materials and methods

2.1. Sources of WAS and HPB

The WAS was obtained from a secondary sedimentation tank of a WWTP in Hangzhou, China. After sampling, the sludge was firstly screened with a 1 mm sieve to remove the impurities, and then was washed with deionized water for three times. After pretreatment, the WAS was concentrated by settling at 4 °C for 24 h before use. The main characteristics of the concentrated WAS were shown in Table 1. Commercial HPB (leaves powder) was purchased from Kaihangzhongyi Trading Co. Ltd, in Xinjiang, China. HPB powder was dried at 45 °C for 8 h before usage. The HPB particle size distributed between 0.02 and 355 μm, while around 80% of them were in the range of 3–25 μm (Fig. S1, Supplementary Materials). To analyze the functional group of HPB, FTIR spectrum was recorded, showing two specific bands of transmission at 1625 cm^{-1} and 1725 cm^{-1}, respectively (Fig. S2, Supplementary Materials). This functional group characteristic indicates the presence of lawsone (Nik et al., 2012).

Table 1
Main characteristics of concentrated WAS.

Parameters	Value[a,b]
pH	7.12 ± 0.11
Total solids (TS)	31,680 ± 1040
Volatile suspended solids (VSS)	20,592 ± 844
Soluble chemical oxygen demand (SCOD)	76 ± 15
Soluble carbohydrate (as COD)	22 ± 3
Soluble protein (as COD)	18 ± 2
VFAs in the suspended solution (as COD)	ud.[c]
Soluble lawsone	ud.

[a] All values are expressed in mg L^{-1} expect pH.
[b] Average value ± one standard deviation.
[c] ud. represents undetectable.

2.2. Experimental set-up

The fermentation experiments were conducted in plexiglass anaerobic reactors with a working volume of 5.0 L. The water seals were located at the top of each reactor to ensure that the reactors were airtight. During the fermentation process, the reactors were mechanically stirred (120 rpm) using a power-driven force mixer (HD 2004W, Sile Co., China), and feedstock was mesophilically fermented in a water bath at 35 ± 1 °C.

To investigate the effect of feedstock proportion (TS_{WAS}: TS_{HPB}) on the fermentation process, five sets of reactors (R1, R2, R3, R4 and R5), each with a duplication, were established in this study. The concentrated WAS and dried HPB were added to the reactors to achieve a total solids (TS) content (WAS + HPB) of 30 g L^{-1}. In these reactors, the HPB proportions in the mixed substrate were as follows: R1 (0%), R2 (25%), R3 (50%), R4 (75%) and R5 (100%). R1 and R5 were the control systems (mono-fermentation), in which a mono-substrate, i.e., only WAS or HPB, was supplied; while R2, R3 and R4 were the co-fermentation systems with different feedstock proportions (TS_{WAS}: TS_{HPB}). The inoculum in the above systems was originally obtained from an anaerobic reactor in a starch WWTP in Hangzhou, China. During this study, 50 mL of the inoculated sludge was supplemented, resulting in a final inoculum concentration in each reactor of 1.0 g VSS L^{-1}. The initial pH was adjusted to 8.0 using a stocked $NaHCO_3$ solution (50 g L^{-1}). NH_4Cl (80 mg L^{-1}), KH_2PO_4 (20 mg L^{-1}), $MgCl_2{\cdot}6H_2O$ (20.3 mg L^{-1}), $CaCl_2{\cdot}2H_2O$ (14.7 mg L^{-1}), $FeSO_4{\cdot}7H_2O$ (2.8 mg L^{-1}), and stocked trace metals and vitamins (1.0 mL/L) were supplied as the previous study (Huang et al., 2015). After preparation, the reactors ran continuously for 21 days. During the entire operating period, pH and ORP in the systems were measured daily. Mixture samples were withdrawn from each reactor at appropriate time intervals (1–3 days) for the following chemical and enzymatic activity analysis.

2.3. Chemical analysis

pH and ORP in the reactors were measured by a portable pH/ORP meter (SANXIN, SX751, China). Suspended mixture samples, withdrawn at days 1, 2, 4, 6, 8, 10, 12, 15, 18 and 21 from each reactor, were firstly centrifuged at 8000×*g* for 20 min, and then be filtered through 0.45 μm membrane filters (Anpel Co., Shanghai, China). The filtered supernatant was analyzed for soluble chemical oxygen demand (SCOD), soluble carbohydrate, soluble protein, VFAs and the released lawsone.

Soluble carbohydrate was measured by the anthrone–sulfuric acid colorimetric method with glucose as standard. Soluble protein was determined according to the Bradford method with bovine serum albumin as the standard. VFAs were composed of acetate,