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Effect of inoculum to substrate ratio on the hydrolysis and acidification of food waste in leach bed reactor

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ABSTRACT

The aim of present study was to determine an appropriate ISR (inoculum to substrate ratio) to enhance the hydrolysis rate and reduce the solid retention time of food waste in hydrolytic-acidogenesis leach bed reactor (LBR). LBR 1–4 were inoculated with 0%, 5%, 20% and 80% (*w/w* basis) of anaerobically digested sludge, respectively, using artificial food waste as substrate. Experiments were conducted in batch mode at mesophilic condition (35 °C) for 17 days. Higher ISR resulted in 4.3-fold increase in protein hydrolysis; whereas, only a modest increase in the decomposition of carbohydrate. Two kinetic models for carbohydrate and protein degradation were proposed and evaluated. The differences among four ISRs in volatile solids removal efficiencies were marginal, i.e. 52.4%, 62.8%, 63.2% and 71.7% for LBR 1–4, respectively; indicating that higher ISR was insignificant in enhancing the overall hydrolysis rate in LBR. Therefore, a lower ISR of 20% was recommended in the hydrolytic-acidogenic process.

1. Introduction

The demands for reduction and effective utilization of municipal solid waste (MSW) have increased immensely for Hong Kong in recent years due to the limited landfill space. Anaerobic digestion of sorted organic fraction of MSW, especially food wastes, is the utmost attractive alternative and one of the most cost-effective technologies. Anaerobic digestion (AD) of organic matter is generally considered to be a two-step process in which the acidogenesis and methanogenesis are in dynamic equilibrium (Vavilin et al., 2001). Therefore, it is logical to develop a two-stage anaerobic digestion system, consisted of leach bed reactor (LBR) and upflow anaerobic sludge blanket (UASB) that can separate acidogens and methanogens spatially for the regulation of the two groups of microbial communities.

In general, the growth rates of acidogens ranged from 0.05 to 1.79 h^{-1}, which is about ten times higher than that of acetogens and methanogens ranging from 0.008 to 0.173 h^{-1} (Kalyuzhnyi, 1997; Vavilin et al., 2001). The imbalance between acid production by the acidogens and the acid consumption by the methanogens resulting from the different growth rate is the principle incentive and momentum of the stage separation. In the start-up of an anaerobic digester, an appropriate ratio of inoculum to substrate (ISR) should be added to enhance the hydrolysis of particular organic matter, accelerate biogas production rate, and consequently reduce the operation time (Raposo et al., 2009). About 80% of total biogas yield was achieved after the first 8 days of digestion for an ISR of 0.35 (*vs/vs* on the basis of volatile solid) with a single-stage batch AD of food and green wastes, where it was only 47% for ISR of 0.25 (*vs/vs*) (Liu et al., 2009).

However, due to the higher growth rate of acidogens than methanogens (Qi et al., 2003), it is reasonable to expect a lower ISR in the separated acidogenic reactor than methanogenic reactor. Nevertheless, previous studies on ISR were mostly investigated in the single-stage methanogenic reactor (Neves et al., 2004; Liu et al., 2009), and rarely on the startup of an acidogenic reactor. Furthermore, a wide range of ISR values were employed in existing studies and it is hard to make a comparison among the different substrates used. For example, an ISR of 1.0 (*vs/vs*) was suggested for the acidogenic fermentation of grass (Jagadabhi et al., 2010), whereas 0.02 (*vs/vs*) for manure (Demirer and Chen, 2008) and 0.13 (*vs/vs*) for food waste (Stabnikova et al., 2008) were reported.

Therefore, the objective of the present study was to determine the hydrolysis and acidogenesis rate of food waste under different ISR values, i.e. 0%, 5%, 20% and 80% (*w/w*) within lab scale LBR at 35 °C. The kinetics of hydrolysis rates of the particulate matters were then evaluated by the first-order equation and Contois equation.

2. Methods

2.1. Food waste and inoculum

Selected properties of the food waste and inoculum used in the study are described in Table 1. Synthetic food waste with a total

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Table 1
Characteristics of food waste and inoculum.

	Bread	Boiled rice	Cabbage	Pork	Food waste mixture	Anaerobically digested sludge
Proportion in food waste (%)	35	25	25	15	–	–
TS (%)	71.1	29.2	4.6	37.8	38.5	3.8
VS/TS (%)	97.3	99.3	88.1	97.6	97.1	85.2
Total organic carbon (%)	56.2	52.9	41.7	50.5	56.4	32.2
Total nitrogen (%)	2.49	1.16	3.99	8.71	4.5	6.6

solid (TS) content of 38.5% with volatile solid (VS) content of 97.1% (VS/TS) was used as the substrate. Freshly collected anaerobically digested sludge (ADS) from the digestion tank of Shek Wu Hui sewage treatment plant in Hong Kong was used as the inoculum, with TS of 3.8% and VS/TS of 85.2%.

2.2. Experimental set-up

Four LBRs with working volume of 4.6 L were used in this study. Each LBR was provided with a percolating plate at the bottom of reactor for leachate collection. Four volumes of inoculum, i.e. 0, 50, 200 and 800 g, were tested and each was mixed with 75 g wood chips (as bulking agent) and 1.0 kg food waste and loaded into each LBR. In this way, the initial ISR achieved were 0%, 5%, 20% and 80% (*w/w* basis), representing LBR1, LBR2, LBR3 and LBR4, respectively, excluding the weight of wood chips (Table 2). On *vs/vs* basis, these ISR ratios correspond to 0, 0.004, 0.017 and 0.069, respectively. Then 1.0 L of tap water was added into each LBR on day 0. Leaching occurred naturally and the leachate was collected at the bottom chamber of the LBR daily. Exactly 50% of the collected leachate was adjusted to pH 6.0 using sodium carbonate and recycled back to LBR as the pH adjustment enhanced the chemical oxygen demand (COD) yield in our previous study (Xu et al., 2011). Remaining 50% of the leachates were taken out from LBRs and used for analysis. The pH, COD, volatile fatty acids (VFA), ammoniacal nitrogen (NH_4^+-N) and total Kjeldahl nitrogen (TKN) of leachate were determined to evaluate the performance of acidogenic LBRs.

2.3. Analyses

The physicochemical properties including pH, COD, VFA, NH_4^+-N and TKN of aqueous and solid samples collected were determined following the Standard Methods of APHA (2005). Due to the filtering effect of sand bed placed at the bottom of LBR, COD of soluble organic matter represented more than 90% of the total COD in the collected leachate. Thus, the hydrolyzed product was calculated based on the total COD of leachate. Experiments were run for 17 days under controlled mesophilic temperature (35 °C) with two replicates for each ISR. Therefore the reported results are mean ± standard deviation of two independent analyses. SigmaPlot software (version 11.0) was used to create graphs and to perform the statistical analysis.

2.4. Kinetics study

First-order kinetics and Contois equation were used in the present study to evaluate the hydrolysis rate of food waste in LBR with varied ISRs. The effects of different processes on the hydrolysis have traditionally been simplified to the first-order kinetics for the substrate biodegradation (Vavilin et al., 2008). In the First-order kinetics,

$$\frac{dS}{dt} = -k_h S \tag{1}$$

S is the concentration of particulate substrate; k_h represents the hydrolysis constant (d^{-1} basis). However, a relatively high hydrolysis rate was obtained in the anaerobic biodegradability tests with a high ISR (Fernández et al., 2001), showing some degree of dependence of hydrolysis on biomass concentration or activity. Consequently, the first-order kinetics may not be applicable in all circumstances.

In the Contois kinetics, hydrolysis is considered to be a surface reaction occurring when the particulate substrate is in close contact with microorganisms that provide the hydrolytic enzymes (Tomei et al., 2008). According to the Contois equation, the substrate degradation rate is assumed to be regulated by the relationship of substrate and inoculum, which will be appropriate to describe the kinetics of hydrolysis of food waste in the LBR with different ISRs in the present study. Thus, the hydrolysis rates are evaluated from the mass balance Eq. (2) for substrate and biomass, respectively,

$$r_h = -\frac{dS}{dt} = \frac{k_h S}{(K_X X + S)} X = X \frac{k_h \cdot S/X}{(K_X + S/X)} \tag{2}$$

where r_h is hydrolysis rate (g COD_S d^{-1}); k_h is hydrolysis constant (g COD_S g $COD_B{}^{-1}$ d^{-1}); K_X is Contois constant (g COD_S g $COD_B{}^{-1}$ d^{-1}); X (g L^{-1}) is the concentration of acidogens (responsible for hydrolysis of particulate substrate) and S is the particulate substrate; X/S is the ratio between acidogens concentration (X) and particular substrate (S); COD_B and COD_S represent the COD content of acidogens (B) and substrate (S), respectively.

3. Results and discussion

3.1. pH and volume of leachate

pH is an important parameter that can affect the bacterial activity; and metabolic pathways with a pH of 4.0–6.0 is more favorable for the hydrolysis and acidogenesis (Li and Fang, 2007). Addition of inoculum at >20% increased the initial pH of the feedstock to ∼4.5; whereas, an initial pH of around 3.8 was observed in ⩽5% inoculation. Daily pH adjustment with sodium carbonate increased the pH of LBRs gradually from 3.8–4.5 to pH higher than 5.0 after 8 days (Fig. 1a) as also observed in our previous experiment (Selvam et al., 2010; Xu et al., 2011). Similar pH changes were noted in LBR 2–4, with LBR1 exhibited a lower pH in the range of 3.8–5.0. The lower pH of 3.8 found in LBR1 and LBR2 might be attributable to the production of formic acid and lactic acid due to their lower pK_a values, i.e. 3.77 and 3.86, respectively. The mixture of acetate, lactate, formate, succinate and ethanol are the common end products of mixed acid fermentation, which could be mediated by the members of the family Enterobacteriaceae, Clostridia and lactic acid bacteria (LAB) (Haruta et al., 2005). LAB were found to be the preferential bacteria in the kitchen waste that was stored for 24 h at 37 °C (Wang et al., 2001). Meanwhile, the continuous pH adjustment during the treatment led to the selective reduction of lactic acid bacteria and increased the coliform bacteria and Clostridia (Sakai et al., 2000).

The quantities of leachates collected daily from the LBRs were similar, ranging from 900 to 1100 mL. However, a decrease in leachate production was observed in the LBR4 after 13 days of digestion, which would be unfavorable for the soluble organic