Thermophilic hydrolysis and acidification of activated sludge with a low organic carbon content under different sludge concentrations

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Abstract

The effect of residue activated sludge concentration on characteristics thermophilic hydrolysis and acidification was investigated at the sludge concentrations of 10 g/L, 15 g/L and 20 g/L. Under steady state, the volatile suspended solids (VSS) removal percentage in reactors fed with 10g/L, 15 g/L and 20 g/L residue activated sludge was 10.8%, 10.1% and 10.7%, respectively. Soluble chemical oxygen demand (SCOD) was highest in the reactor fed with 20 g/L sludge, while the amount of SCOD contributed by specific VSS reached the peak when fed with 10 g/L sludge. Acidification rate was slightly decreased with increasing the sludge concentration, which accounted for 74%, 72% and 69%, respectively and the ratio of various VFAs was different. Ammonia and phosphate release was in proportion to the applied sludge concentration. At the phylum level, *Firmicutes* was the most dominant phylum in three reactors. The relative abundance of *Firmicutes* accounted for 19.6%, 21.2% and 23.5% fed with 10g/L, 15 g/L and 20 g/L sludge, respectively. At the genus level, *Caloramator* and *Fervidobacterium* were dominant in three reactors, who might contribute mostly to the hydrolysis and acidification process.

Keywords: Hydrolysis; Acidification; Sludge concentration; Microbial community; Thermophilic

Introduction

With the development of wastewater treatment, a large amount of residue activated sludge is produced and its treatment/disposal usually costs nearly 25%-60% of operation cost of wastewater treatment plant [1]. Nowadays, landfilling, incineration and fermentation are main sludge treatment technologies. Especially, anaerobic fermentation involves the degradation and stabilization of organic materials under anaerobic conditions, and energy would be produced. In addition, hydrolysis and acidification products from anaerobic digestion, especially soluble chemical oxygen demand (COD), can be used as organic carbon for enhancing nitrogen and phosphorus removal from main stream wastewater treatment processes. Usually, hydrolysis and acidification are limiting steps in anaerobic digestion of residue activated sludge [2]. Lots of efforts have been endeavored to enhance sludge hydrolysis and acidification efficiencies or rates, such as alkaline treatment [3], ultrasound treatment [4], and Fenton reagent reaction [5]. Physiochemical pretreatment processes costs too much compared with biological augmentation, especially thermo-pretreatment processes.

Anaerobic digestion of sludge is usually carried out under mesophilic or thermophilic conditions. Thermophilic anaerobic fermentation possesses high hydrolysis and acidification rate and efficiency, and also can inactivate pathogens efficiently. Liu et al. showed that sludge hydrolysis was enhanced at high temperatures [6]. For the dewatered sludge of 137400 mg/L total COD (TCOD), under mesophilic, thermophilic and extreme thermophilic conditions, soluble COD (SCOD) concentrations were 25000, 25400 and 31000 mg/L, and volatile fatty acids (VFAs) concentrations were 2850, 6128 and 5463 mg/L, respectively. Moreover, hydrolysis and acidification of sludge releases by-products of nitrogen and phosphorus, which can be recovered as fertilizer if applicable. Therefore, enhancing thermophilic hydrolysis and acidification of sludge is necessary to improve system performance through clarifying the possible underline mechanisms. Sludge loading is an important factor affecting hydrolysis and acidification of sludge, which can be achieved by adjusting sludge retention times (SRT) or sludge concentrations. Yuan et al. investigated the effect of SRT and biomass concentration on non-fermentation of waste activated sludge, showing that VFA yields increased with increasing SRTs and under the same SRT, VFA yields increased when the biomass concentration decreased [7].

During thermophilic digestion, thermophiles, which play an important role in thermophilic hydrolysis and acidification, may be influenced by operating conditions. Levén et al. found that the microbial commnuity structure at mesophilic and thermophilic temperatures was different. *Bacteroidetes* and *Chloroflexi* were predominant at the mesophilic temperature while at the thermophilic temperature, *Thermotogae* was the major group [8]. Yan et al. investigated the effect of heat-treatment for reduction of residue activated sludge on microbial population and found that *Bacilli*, which included most of thermophiles, became the dominant class in the community by the treatment [9]. Nevertheless, the effect of sludge concentration on microbial community at thermophilic temperature is less understood.

Therefore, in this study, effect of sludge concentrations on hydrolysis and acidification under thermophilic conditions was investigated, including volatile suspended solids (VSS) degradation, VFAs production and release of protein, carbohydrate, nitrogen and phosphorus. In additions, effect of sludge concentration on microbial community structure was also examined.

Materials and Methods

Residue activated sludge

The residue activated sludge was collected from the secondary settling tank of municipal wastewater treatment plant in Kunming, China. The residue activated sludge was stored at $4^{o}C$ after concentration. The main characteristics of residue sludge were: pH of 6.8, SCOD of 83 mg/L, TCOD of 7400 mg/L, ammonia nitrogen (NH_4-N) of 16.7 mg/L, ortho-phosphate (PO_4-P) of 0.51 mg/L, VSS of 17.5 g/L, and soluble protein and carbohydrate of 236 mg/L and 5.2 mg/L, respectively.

Anaerobic sequencing batch reactors (ASBRs)

The effect of residue sludge concentrations on thermophilic hydrolysis and acidification was carried out in six 600 mL ASBRs with an effective volume of 500 mL. 10 g/L, 15g/L and 20 g/L residue activated sludge concentrations were applied. Each sludge concentration contained two parallel reactors. All reactors were incubated at $55^{o}C$ and stirred at a rate of 150 r/min. 250 mL sludge was decanted every day and meanwhile 250 mL fresh sludge was dosed into each reactor to maintain both SRT and hydraulic retention time (HRT) of 2 days.

Nitrogen gas (N_2) was purged to remove the oxygen from the headspace of the reactor during dosing residue sludge. 50 mL mix liquor was collected from the decanted samples. Before testing corresponding liquid parameters, mix liquor was centrifuged at 8000 r/min for 5 min and then filtered with a 0.45 μm membrane filter. Averaged results from the two parallel samples were presented.

Analytical methods

COD, NH_4-N, PO_4-P, suspended solids (SS) and VSS were measured according to standard methods [10] and pH were measured by a pH meter (pH 30d, HACH, USA). Protein was measured according to [11]. Carbohydrate was measured by the sulfuric-phenol method [12].

VFAs (including acetic acid, propionic acid, iso-butyric acid, butyric acid, iso-valeric acid and valeric acid) were measured by a gas chromatograph (GC-2014, Shimadzu, Japan) equipped with a flame ionization detector and a capillary column. The carrier gas was N_2 at a flow rate of 50 mL/min, with a split ratio of 20 at a flow rate of 1.1 mL/min in the column and a purge flow rate of 3.5 mL/min. The oven temperature was increased proportionally from 80^{o}C to 200^{o}C within 10 min and the final holding duration was 2 min. The temperatures of both injector and detector were 240^{o}C. The samples was dosed with 1 μL formic acid to adjusted pH to below 3.

DNA was extracted from the anaerobic sludge in the six reactors using a Fast DNA Spin Kit (Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instruction. After extraction, the DNA sample was amplified by polymerase chain reaction (PCR). 16S rRNA high-throughput sequencing method was applied to analyze microbial communities in all reactors.

Results and discussion

Effect of sludge concentration on VSS hydrolysis

Dynamics of VSS concentrations during the experiment are shown in **Fig. 1** After reaching steady state, the VSS removal percentage was 10.8%, 10.1% and 10.7% with sludge concentrations of 10 g/L, 15 g/L and 20 g/L, respectively. Therefore, the VSS removal percentage was not affected by the residue sludge concentrations. Banister et al. found that mass transfer efficiency could be enhanced at low sludge concentrations, thus improving the VSS removal performance [13]. In our study, the applied sludge concentration range did not affect system performance, and all condition might possess very good mixing conditions.

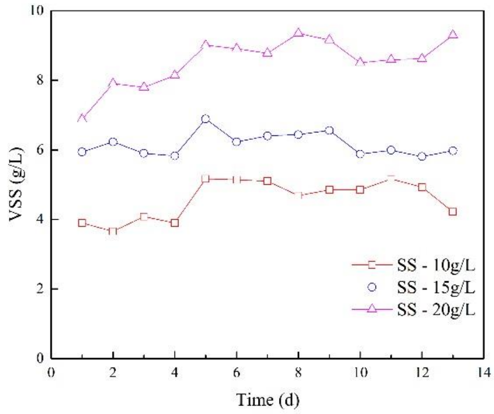

Fig. 1 Dynamics of VSS concentrations with different sludge concentrations

Organic matters in residue activated sludge mainly exists in microbial cell and extracellular polymeric substances. These organic matters are hydrolyzed into small molecules substances, thereby increasing SCOD concentrations. Dynamics of SCOD corresponding to VSS degradation is shown in **Fig. 2** Under steady state, SCOD concentrations were 1053 mg/L, 1293 mg/L and 1903 mg/L at 10 g/L, 15 g/L and 20 g/L, respectively. SCOD increased with increasing residue activated sludge concentrations. Bouzas et al. found that with a SRT of 6 d at 20^{o}C, a high concentration of residue activated sludge led to a high SCOD [14]. Xiong et al. also obtained that the highest SCOD was produced when feeding the highest VSS concentration in the range of 4.57-23.78 g/L during hydrolysis of waste activated sludge [15]. Although increased sludge concentrations resulted in high SCOD concentrations, the specific yield coefficients of SCOD showed a different trend. The specific yield coefficients of SCOD were 262, 213 and 216 mg/g at 10 g/L, 15 g/L and 20 g/L, respectively. The highest specific yield of SCOD was obtained at 10 g/L, which was 23% and 21.3% higher than at 15 g/L and 20 g/L. Yuan et al. reported that longer SRTs resulted in higher solubilization of activated sludge and suggested that longer SRT enhanced the degradation of biomass [7]. According to the solubilization results obtained from this experiment, lower excess slduge concerntration might also enhanced the degradation of biomass. The hydrolysis rates at 10 g/L, 15 g/L and 20 g/L conditions were 18.5%, 15% and 15.2%, respectively. These results were comparable with primary sludge fermentation results reported by Ucisik and Henze [16], where values of 15.9%-17.9% were obtained using an SRT of 6 d.

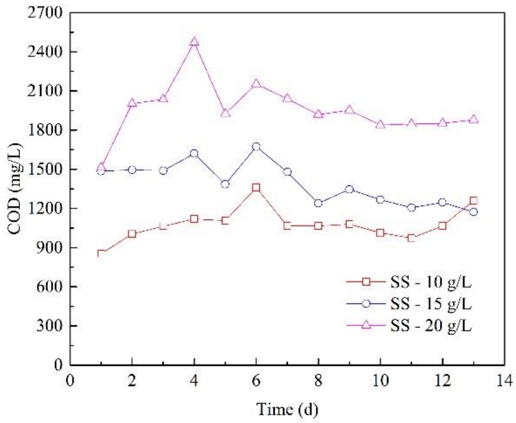

Fig. 2 Dynamics of SCOD with different sludge concentrations

Protein and carbohydrate accounted for more than 60% of the total organic matters in sludge [17]. During the hydrolysis, protein and carbohydrate were released. **Fig. 3** shows the effect of sludge concentrations on released soluble protein and carbohydrate concentrations. Under steady state, protein and carbohydrate in each reactor remained relatively stable, with protein concentrations of 119.9 mg/L, 142.9 mg/L and 185.3 mg/L, and carbohydrate concentrations of 36.6 mg/L, 52.4 mg/L and 67.4 mg/L in reactors fed with 10 g/L, 15 g/L and 20 g/L residue activated sludge, respectively. Chen et al. reported that protein and carbohydrate concentrations from 10.8 g/L waste activated sludge on the second day were 112.25 mg/L and 30.11 mg/L at pH 7.0 [2], which were similar to our results. Sludge concentration had almost the same effect on protein and carbohydrate concentrations as on SCOD. Both protein and carbohydrate were increased with increasing sludge concentrations. When the sludge concentration increased from 10 g/L to 20 g/L, concentrations of soluble protein and carbohydrate increased by 54.5% and 84.2%, respectively.

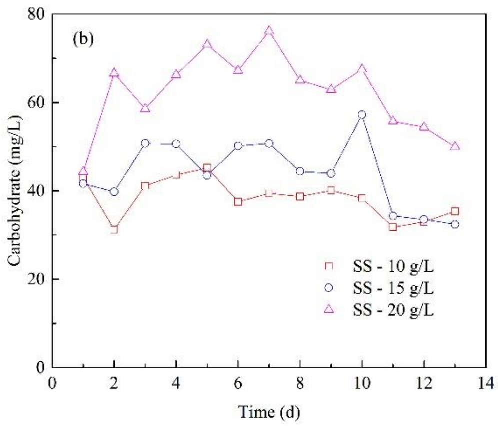

Fig. 3 Soluble protein (a) and carbohydrate (b) concentrations with different sludge concentrations

Effect of sludge concentrations on acidification processes

Fig. 4a shows dynamics of VFAs concentrations during the study period. The acidification rate was consistent with sludge concentrations. The steady state VFAs concentrations were 780 mg/L, 935 mg/L and 1309 mg/L in reactors fed with 10 g/L, 15 g/L and 20 g/L residue activated sludge, respectively. The specific VFAs yield coefficients were 132 mg/g, 137 mg/g and 133 mg/g respectively, indicating that high sludge concentration didn't inhibit acidification. Yuan et al. found that VFAs yield varied under different SRTs and the VFAs yield increased with a decrease in sludge concentration for the sludge concentration range of 4.3-13 g/L [7]. For example, at SRTs of 7 and 5 d, the VFAs yield increased by 19% and 16% when sludge concentration decreased from 12.3 g/L to 4.8 g/L and from 12.4 g/L to 4.3 g/L, respectively. Skalsky et al. found that when the solid concentration decreased from 2.6% to 0.43%, the VFAs yield increased by 50% [18]. In this study, the acidification ratio (the proportion of VFAs to SCOD) was 74%, 72% and 69% at 10 g/L, 15 g/L and 20 g/L, respectively. Therefore, only 5% acidification ratio increasing was obtained when the sludge concentration decreased from 20 g/L to 10 g/L. These might be due to the high temperature applied in this study. Compared to room temperature [18], thermophilic conditions might enhance mass transfer and improve the acidification efficiency, thus reducing the inhibition of high sludge concentrations.

Fig. 4b shows components of VFAs in three reactors. Acetic acid, propionic acid, butyric acid, iso-butyric acid, valeric acid and caproic acid were main VFAs generated by acidification. Acetic acid was dominant among all VFAs, accounted for 35%, 41%, and 42% in reactor fed with 10 g/L, 15 g/L and 20 g/L sludge, respectively. Liu et al. also reported that acetic acid was the dominant VFA under thermophilic conditions which followed by propionic acid and butyric acid [6]. Propionic acid and butyric acid, on the other hand, accounted for 16%, 16% and 14%, and 18%, 13% and 15%, respectively. A low concentration of acetic acid was generated by the reactor fed with 10 g/L sludge, while the maximum acetic acid was obtained by the reactor fed with 20 g/L. Therefore, the produced VFA concentration could be affected by sludge concentration. Bouzas et al. found that a high sludge concentration led to an increase of acetic acid, while propionic acid decreased [14]. Together, it could be suggested that hydrolysis and acidification were both affected by residue activated sludge concentration, and these two processes might be influenced by each other.

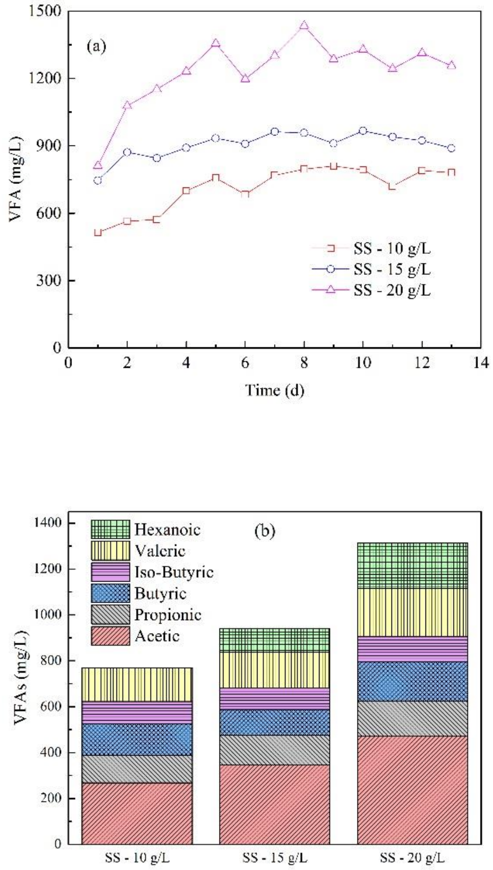

Fig. 4 Dynamics of VFAs concentrations (a) and components of VFAs (b) with different sludge concentrations

Effect of sludge concentration on ammonia nitrogen and phosphate release

Fig. 5a and **b** show the release of NH_4-N and PO_4-P under different sludge concentrations. NH_4-N and PO_4-P both increased at the beginning. After three reaction cycles, concentration of NH_4-N released was 114.2 mg/L, 148.6 mg/L and 213.4 mg/L in reactors fed with 10 g/L, 15 g/L and 20 g/L sludge, respectively. Concentration of PO_4-P was 5.8 mg/L, 8.3 mg/L and 10.6 mg/L, respectively. NH_4-N and PO_4-P concentrations were in proportion to residue activated sludge concentrations. By increasing residue activated sludge concentrations from 10 g/L to 15 g/L and then to 20 g/L, concentration of NH_4-N increased by 30.1% and 86.9%. The corresponding PO_4-P concentration increased was 43.1% and 82.8%, respectively. Pitman et al. found that the PO_4-P release potential was consistent with SS concentrations [19]. Moreover, concentrations of NH_4-N and VFAs (**Fig. 5c**) had a good linear relationship (R^2=0.94), which might be used to reflect the acidification process. This might be due to that protein was the dominating organic carbon in sludge, which released nitrogen during acidification.

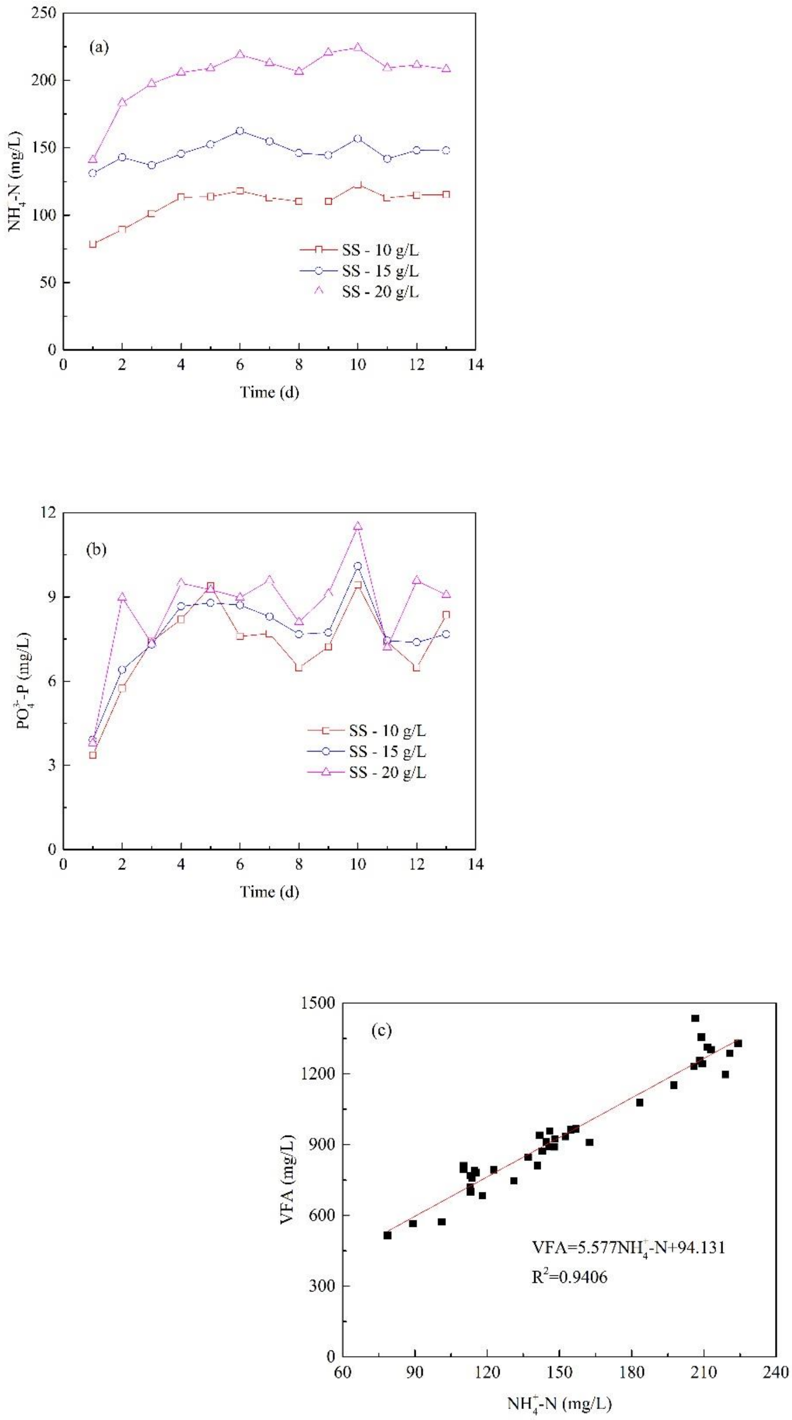

Fig. 5 The release of NH_4-N (a) and PO_4-P (b) with different sludge concentrations and relationship between NH_4-N and VFA (c)

Microbial community for systems with different sludge concentrations

Microbial community structure of three reactors fed with different sludge concentration was identified (**Fig. 6**). At the phylum level, *Firmicutes* was the dominant phylum, accounting to 19.6%, 21.2% and 23.5% in reactors fed with 10 g/L, 15 g/L and 20 g/L sludge, respectively. *Proteobacteria* was the followed dominant phylum, accounting to 16.0%, 14.6% and 16.8%, respectively. *Bacteroidetes*, the third dominant phylum, accounted to 10.6%, 9.2% and 9.3%, respectively. At the genus level, *Caloramator* (belonged to *Firmicutes* phylum) and *Fervidobacterium* were dominant in three reactors. The relative abundance of *Caloramator* increased with increasing sludge concentrations, accounting to 9.8%, 11.0% and 13.1% when fed with 10g/L, 15 g/L and 20 g/L residue activated sludge, respectively.

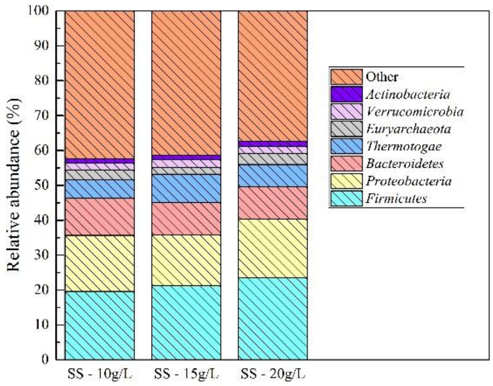

Fig. 6 Microbial community structure at phylum level with different sludge concentrations

Microorganisms within *Firmicutes*, *Proteobacteria* and *Bacteroidetes* were widely distributed throughout not only mesophilic conditions but also thermophilic conditions [8, 20]. Digestion at thermophilic temperature provides higher efficiency in the degradation of organic matters [21, 22]. Yan et al. reported that thermophilic proteases which contribute to the hydrolysis of residue activated sludge could be secreted by thermophiles and the overall protease activities measured at thermophilic temperature was significantly higher than that at 28℃ [9]. Tan et al. reported that *Firmicutes* and *Proteobacteria* were found when sludge was treated at 60℃ [20], which indicated that these bacteria might be heat-resistant. *Firmicutes* have the ability to metabolize substrates such as proteins, lipids, celluloses, sugars and amino acids by producing extracellular enzymes [8]. *Firmicutes* was the typical phylum in all reactors, indicating that it was probably responsible for organic carbon and proteins reduction. What's more, the relative abundance of *Firmicutes* increased with increasing sludge concentrations, suggesting that it could adapt well to the high organic loading applied in this study. *Proteobacteria* was typical phylum in hydrolysis and acidification of ultrasonic-pretreated wasted activated sludge [23]. *Bacteroidetes* could convert proteins and carbohydrates to propionic acid and acetic acid as primary products during anaerobic digestion of sludge [24]. Therefore, *Bacteroidetes* might be also involved in the fermentation process of soluble proteins and carbohydrates.

Conclusions

Effect of sludge concentrations on thermophilic hydrolysis and acidification of sludge was investigated. Sludge concentration had little effect on VSS removal percentage. Soluble COD, protein and carbohydrate concentrations increased with increasing the sludge concentration. The acidification was consistent with the sludge concentration and the highest VFAs yield was obtained in reactor fed 20 g/L residue activated sludge. Thermophiles were found in all reactors and at the phylum level, *Firmicutes* was the most dominant phylum in three reactors, whose relative abundance increased with increasing sludge concentrations.

Acknowledgements

This research was supported by the Major Science and Technology Program for Water Pollution Control and Treatment of China (No. 2011ZX07317-001) and Kunming Science and Technology Plan (2014-04-A-S-01-3065).

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