Impact of recycled effluent on the hydrolysis during anaerobic digestion of vegetable and flower waste

F. Lü, P. J. He, L. P. Hao and L. M. Shao

ABSTRACT

Two trials were established to investigate the effect of recycled effluent on hydrolysis during anaerobic co-digestion of vegetable and flower waste. Trial I evaluated the effect by regulating the flow rate of recycled effluent, while Trial II regulated the ratio of hydrolytic effluent to methanogenic effluent, which were recycled to hydrolysis reactor. Results showed that the recirculation of methanogenic effluent could enhance the buffer capability and operation stability of hydrolysis reactor. Higher recycled flow rate was favourable for microbial anabolism and further promoted hydrolysis. After 9 days of hydrolysis, the cumulative SCOD in the hydrolytic effluent reached 334, 407, 413, 581 mg/g at recycled flow rates of 0.1, 0.5, 1.0, 2.0 $m^3/(m^3 \cdot d)$, respectively. It was feasible to recycling a mixture of hydrolytic and methanogenic effluent to the hydrolysis reactor. This research showed that partially introducing hydrolytic effluent into the recycled liquid could enhance hydrolysis, while excessive recirculation of hydrolytic effluent will inhibit the hydrolysis. The flow ratio 1:3 of hydrolytic to methanogenic effluent was found to provide the highest hydrolysis efficiency and degradation rate of lignocelluloses-type biomass, among four ratios of 0:1, 1:3, 1:1 and 3:1. Under this regime, after 9 days of hydrolysis, the cumulative TOC and TN in the hydrolytic effluent reached 162 mg/g and 15 mg/g, the removal efficiency of TS, VS, C and cellulose in the solid phase were 60.66%, 62.88%, 58.35% and 49.12%, respectively. The flow ratio affected fermentation pathways, i.e. lower ratio favoured propionic acid fermentation and the generation of lactic acid while higher ratio promoted butyric acid fermentation.

Key words | flower waste, hydrolysis, methanogenesis, recycled effluent, two-phase anaerobic digestion, vegetable waste

F. Lü
P. J. He
L. P. Hao
L. M. Shao
State Key Laboratory of Pollution Control and Resource Reuse, Key Laboratory of Yangtze River Water Environment,
College of Environmental Science and Engineering,
Tongji University,
1239 Siping Road,
Shanghai 200092, P.R.China
E-mail: *solidwaste@mail.tongji.edu.cn*

INTRODUCTION

In recent years, there are an increasing development of large-scale commercial farms which specialize in vegetable and flower planting and processing in China, resulting in a large amount of waste production. The vegetable waste belongs to readily biodegradable organic waste, while flower waste contains more lignocelluloses and belongs to hardly biodegradable organics (Zhang *et al.* 2007). The biodegradation efficiency of flower waste is expected to be improved by anaerobic co-digestion with vegetable waste, since the low pH and acids produced from vegetables are reported able to loose the physio-chemical structure of lignocelluloses (Zhang *et al.* 2008).

Two-phase anaerobic digestion process can separately optimize methanogenesis and hydrolysis, with the latter normally a rate-limiting step if the substrate is in particulate form, and especially for the lignocelluloses-rich matter (Mata-Alvarez *et al.* 2000). In the two-phase solid-liquid anaerobic digestion process(Xu *et al.* 2002; Wang *et al.* 2006; Chen *et al.* 2007; Cirne *et al.* 2007; Zhang *et al.* 2007), the effluents from hydrolysis reactor and/or methanogenesis

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reactor can be recycled to hydrolysis reactor to accelerate disintegration of particulates, because liquid recirculation can increase the moisture content, promote mass transportation, redistribute the enzymes and microbes, minimize local shortages of nutrients and dilute potential toxins. On the other hand, the acid accumulation from long term effluent recirculation easily leads to the inhibition of anaerobic digestion. Hence, the operation of liquid recirculation should be optimized, in order to enhance the co-digestion efficiency of vegetable and flower waste.

In this study, two trials were established to investigate the effect of recycled effluent on hydrolysis during anaerobic co-digestion of vegetable and flower waste. Trial I evaluated the effect by regulating the flow rate of recycled effluent, while Trial II regulated the ratio of hydrolytic effluent to methanogenic effluent, which were recycled to hydrolysis reactor.

MATERIALS AND METHODS

Materials and inoculums

Fresh vegetable and flower wastes were collected from a vegetable and flower market located in Shanghai, China. The collected vegetable and flower wastes were mixed in the ratio 4:1 (wet weight) according to a survey on their generation data. The vegetable and flower wastes were homogenized in a blender to an average size of below 3 cm. Table 1 shows the physicochemical characteristics and the biochemical compositions of the sampled mixed wastes. The materials for two trials were collected at different time, and their characteristics only differed in the total solid (TS) content of flower waste (Table 1). No inoculums were added, since the original microbes in the solid waste could play a substantial role in the hydrolysis and acidogenesis.

Table 1 | Physiochemical characteristics of vegetable and flower wastes

Items	Vegetable waste		Flower waste		Mixture of vegetable and flower wastes (4:1 in wet weight)	
	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II
TS (%)	4.55	4.50	12.94	22.88	6.23	9.50
VS (%TS)	81.94	83.50	89.49	91.90	85.08	85.20
N (%VS)	4.54	4.86	2.80	2.66	3.81	3.63
C (%VS)	38.25	37.95	42.92	43.46	40.19	41.03
S (%VS)	0.61	0.71	0.33	0.43	0.49	0.55
H (%VS)	5.68	5.57	6.16	6.14	5.88	5.91
C/N	8.43	7.81	15.35	16.34	10.54	11.31

Experimental setup

The process scheme of the reactors for two-phase anaerobic digestion is shown in Figure 1. Part of the hydrolytic effluent was recycled back to the hydrolysis reactor at a flow rate of Q_h, the remainder was pumped into the methanogenesis reactor. Part of the methanogenic effluent was recycled back to the hydrolysis reactor at a flow rate of Q_m. Hence, the liquid was recycled into the hydrolysis reactor at a flow rate of Q_r ($Q_r = Q_h + Q_m$). In Trial I, no hydrolytic effluent was recycled, i.e. $Q_h = 0$ and $Q_r = Q_m$. Four parallel-operated hydrolysis reactors were established with Q_r kept at 0.1, 0.5, 1.0 and 2.0 $m^3/(m^3 \cdot d)$, respectively. (Note: Q_r was accounted as the volume of recycled effluent to that of hydrolysis reactor per day.) In Trial II, Q_r was controlled at 2.0 $m^3/(m^3 \cdot d)$. Four parallel-operated hydrolysis reactors were established with the ratio of Q_h to Q_m (Q_h/Q_m) kept at 0:1, 1:3, 1:1 and 3:1, respectively. The volumes of the

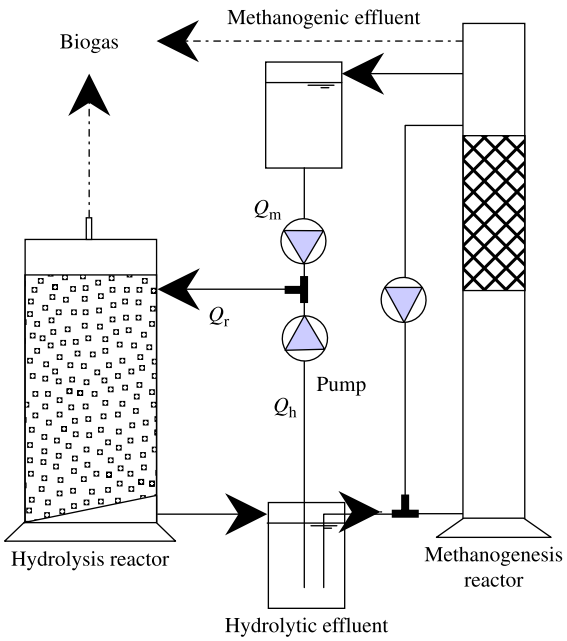

Figure 1 | Scheme of the reactors for two-phase anaerobic digestion.

methonogenesis reactor and each hydrolysis reactor were 15 L and 1.4 L, respectively. In each hydrolysis reactor, 500 g mixture of the vegetable and flower waste was loaded. The characteristics of the methanogenic effluent were listed as follows, pH 7.58 ± 0.30, ORP −190 ± 50 mV, $CaCO_3$ alkalinity 3,251 ± 100 mg/L, SCOD 580 ~ 800 mg/L, TOC 150.5 ~ 250.8 mg/L, NH_4^+—N 130.0 ± 10.0 mg/L and TN 230.2 ± 20.0 mg/L.

Analytic parameters and methods

The hydrolytic and methanogenic effluents were sampled every day to measure pH, oxidation and reduction potential (ORP). After filtrated through 0.45-μm polyester filters, the effluents were tested for total carbon (TC), total nitrogen (TN), total organic carbon (TOC), soluble chemical oxygen demand (SCOD), ammonia, reducing sugar and organic acids. The solid residues after hydrolysis for 9 days were measured for total solid (TS), volatile solid (VS), lignocelluloses and C/H/N/S element contents.

pH, ORP, ammonia, reducing sugar, TS and VS were analyzed according to the standard methods (APHA *et al.* 1998). TC, TN and TOC were measured by a TC/TN analyzer (Multi N/C 3000, Analytik JenaAG, German). SCOD was measured by a COD analyzer (45600-DR/890, Hach Company, USA). Organic acids (including lactic acid and C1-C6 volatile fatty acids (VFA)) were measured by HPLC (LC-20AD, Shimadzu, Japan). The C/H/N/S element contents were measured by an elemental determinator (varioEL, Germany). The measurement of cellulose, hemicellulose and lignin was based on the analysis of neutral detergent fiber (NDF), acid detergent fiber (ADF) and ash contents of the materials (Faithfull 2002).

RESULTS AND DISCUSSION

Trial I. Optimal flow rate of recycled effluent

pH, ORP and alkalinity in the hydrolytic effluent

As shown in Figure 2a, at low flow rate Q_r, pH declined from the initial 7.66 to 6.47, 6.46, 7.04 respectively for Q_r 0.1, 0.5 and 1.0 $m^3/(m^3 \cdot d)$ in the first 3 days, and then gradually climbed up. As for the highest Q_r 2.0 $m^3/(m^3 \cdot d)$, the pH gradually ascended and maintained at 7.25 ~ 7.95. Figure 2b showed that the alkalinity of the batch Q_r 0.1 $m^3/(m^3 \cdot d)$ declined quickly from 3,465 mg/L to 2,054 mg/L in day 3, and then gradually ascended to 3,116 mg/L until in day 7. Contrariwise, the alkalinity at Q_r 0.5, 1.0 and 2.0 $m^3/(m^3 \cdot d)$ was relatively stable at 3,200 ± 50 mg/L. The ORP in four batches declined gradually with fermentation time, and decreased with increasing Q_r (Figure 2c). Compared to other 3 batches, the ORP of the batch Q_r 0.1 $m^3/(m^3 \cdot d)$ fluctuated. Figure 2 indicated that the recirculation of methanogenic effluent introduced alkalinity into hydrolysis reactor, avoided the pH descending during the initial acidogenesis. Hence, the higher Q_r was, the higher the pH buffer ability and pH value was.

Ammonia, reducing sugar and cumulative SCOD

Ammonia and reducing sugar were the fermentative products of protein and carbohydrate, respectively. Figure 3 showed

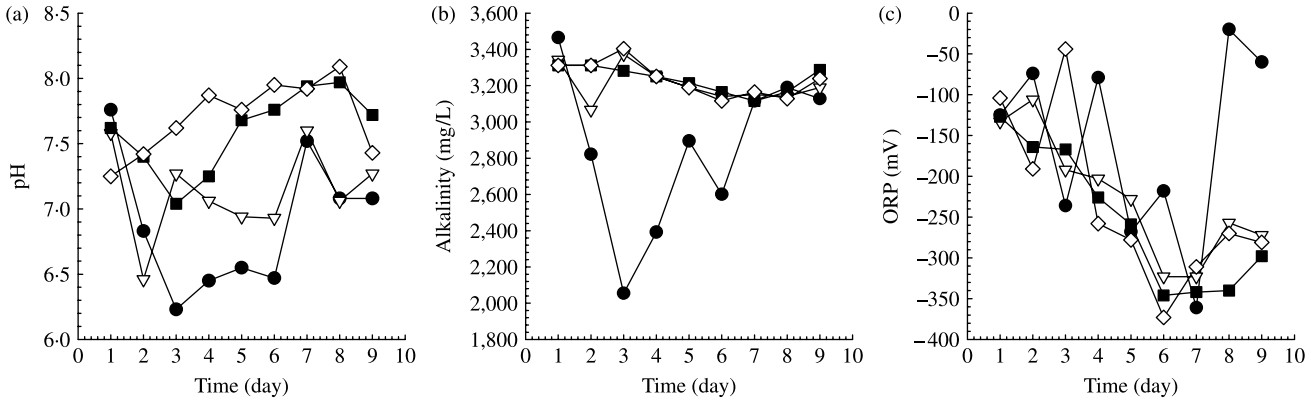

Figure 2 | Temporal evolution of pH, alkalinity and ORP. (a) pH; (b) Alkalinity; (c) ORP. ● Q_r 0.1 $m^3/(m^3 \cdot d)$; ▽ Q_r 0.5 $m^3/(m^3 \cdot d)$; ■ Q_r 1.0 $m^3/(m^3 \cdot d)$; ◇ Q_r 2.0 $m^3/(m^3 \cdot d)$.

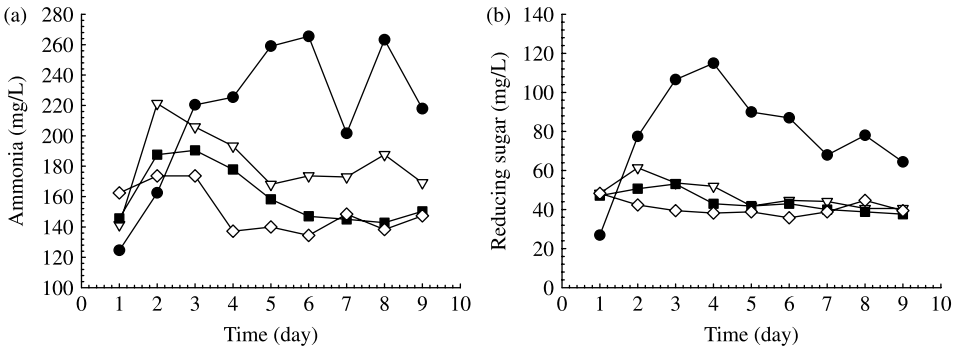

Figure 3 | Temporal evolution of ammonia and reducing sugar. (a) Ammonia; (b) Reducing sugar. ● Q_r 0.1 $m^3/(m^3 \cdot d)$; ▽ Q_r 0.5 $m^3/(m^3 \cdot d)$; ■ Q_r 1.0 $m^3/(m^3 \cdot d)$; ◇ Q_r 2.0 $m^3/(m^3 \cdot d)$.

that both the concentration of ammonia and reducing sugar followed the descending sequence of Q_r 0.1 > Q_r 0.5 > Q_r 1.0 > Q_r 2.0 $m^3/(m^3 \cdot d)$ from day 3 on. Contrariwise, the cumulative quantity of ammonia and reducing sugar followed the descending sequence of Q_r 2.0 > Q_r 1.0 > Q_r 0.5 > Q_r 0.1 $m^3/(m^3 \cdot d)$. In day 9, the cumulative reducing sugar was 3.7, 6.0, 6.9 and 13.6 mg/g, respectively for Q_r 0.1, 0.5, 1.0 and 2.0 $m^3/(m^3 \cdot d)$. The cumulative SCOD at day 9 was 334, 407, 413 and 581 mg/g, respectively for Q_r 0.1, 0.5, 1.0 and 2.0 $m^3/(m^3 \cdot d)$. It suggested that the recirculation of methanogenic effluent alleviated the accumulation of fermentative products in the hydrolysis environment. The higher the flow rate Q_r was, the lower the concentration of fermentative products were. Higher Q_r corresponded to higher hydrolysis efficiency of both carbohydrate and protein.

Numbers of microorganism on the surface of solid waste

The microorganism numbers at day 9 were counted to be 353 ± 24, 217 ± 63, 282 ± 37 and 607 ± 174 × 10^8cells/g. The change in microorganism numbers was a balanced result of microbial growth and liquor flushing. When the flow rate Q_r was low, the effect of microbial growth surpassed liquor flushing, so that the microbes retention time was longer and the microorganism number increased. The effect of liquor flushing intensified with increased Q_r. Correspondingly, the increased Q_r diluted the inhibitory factors in the hydrolysis environment, so that the microbial growth increased too. In the present study, at a Q_r 0.5 ~ 2.0 $m^3/(m^3 \cdot d)$, the microorganism number increased with Q_r.

Effect of the flow rate Q_r on hydrolysis

The flow rate of recycled methanogenic effluent regulated the hydrolysis environment by influencing pH, alkalinity, ORP, acidogenic products, and then affected the metabolism and growth of fermentative microorganisms, resulting in the change of hydrolysis efficiency. The alkalinity introduced by methanogenic effluent ensured a neutral pH environment, promoting the development of bacterial diversity and enrichment (Ye *et al.* 2007). Methanogenic effluent could dilute the inhibitory effects of hydrolysates and organic acids (Veeken *et al.* 2000; He *et al.* 2006; Lü *et al.* 2008; Vavilin *et al.* 2008). Higher Q_r made the role of pH buffer and dilution more significant. Besides, the nitrogen, phosphorus and micronutrients in the methanogenic effluent were also favourable for the microbial growth. Hydrolysis was a surface-dependent reaction (Vavilin *et al.* 2008). Liquid flow enhanced the mass transfer around the substrate surface, so as to reduce the inhibitor accumulation in heterogenous zones, improve the mobility of enzymes and increase the probability of enzymatic accessibility to substrate surface (O'Dwyer *et al.* 2007; Zhang *et al.* 2007). Therefore, higher flow rate Q_r of methanogenic effluent promoted the hydrolysis of solid waste.

Nevertheless, there existed the risk that too intense liquid flushing destroyed the absorbance of enzymes onto substrate surface and washed out the enzymes by liquid refreshment. Zhang *et al.* (2007) observed that the enzyme activities on substrate surface were higher than in liquors, especially for cellulase. In fact, the hydrolysis of lignocelluloses under anaerobic environment was realized by cellulosome which bound both to the surfaces of cell and

substrates (Leschine 1995; Schwarz 2001; Bayer *et al.* 2006). In the present study, a flow rate of 2.0 $m^3/(m^3 \cdot d)$ seemed to have weak flushing on enzymes, or the enzyme synthesis was superior to enzyme washing out. When the flow rate was below 2.0 $m^3/(m^3 \cdot d)$, the hydrolysis efficiency increased with Q_r.

Trial II. Optimal ratio of hydrolytic effluent to methanogenic effluent

pH and ORP in the hydrolytic effluent

The pH of Batch Q_h/Q_m 3:1 fluctuated from 7.70 to 6.79, while the pH of other 3 batches kept stable in 7.10 ± 0.20. It indicated the methanogenic effluent played a role of pH buffer, while higher ratio of hydrolytic effluent was inclined to reduce pH value. The ORP of four batches ranged from −500 to −200 mV, all lower than methanogenic effluent. Besides, ORP decreased with increasing Q_h/Q_m, suggesting the recirculation of hydrolytic effluent could reduce ORP, because the acidogenesis of easily degradable organics in the hydrolytic effluent would reduce pH, consume oxygen and produce hydrogen.

TOC and TN in the hydrolytic effluent

As shown in Figure 4, the cumulative TOC increased quickly in day 1–3 and slowly from day 4 on. The cumulative TOC was the highest at Q_h/Q_m 1:3 in day 9 (162 mg/g), followed by Q_h/Q_m 0:1 (119 mg/g), 1:1 (84 mg/g) and 3:1 (82 mg/g). The cumulative TN increased in day 1–3, and kept constant or slowly decreased from day 4 on. In day 9, the cumulative TN was similarly in the batches Q_h/Q_m 0:1(14.5 mg/g), 1:3(15.2 mg/g) and 1:1(13.2 mg/g), while it was the lowest at Q_h/Q_m 3:1 (4.4 mg/g). The first-order hydrolysis rate constants K_{HC} calculated on TOC data were 0.50, 0.61, 0.40 and 0.25 d^{-1}, respectively for Q_h/Q_m 0:1, 1:3, 1:1 and 3:1. Therefore, the hydrolysis efficiency was the highest at Q_h/Q_m 1:3 and the lowest at Q_h/Q_m 3:1 evaluated by TOC or TN. Furthermore, higher ratio of hydrolytic effluent depressed the protein hydrolysis greatly.

Organic acids in the hydrolytic effluent

Figure 5 showed the distribution of organic acids at day 9. As for the batches Q_h/Q_m 1:1 and 3:1, the organic acids were mainly composed of acetate, butyrate and propionate. The butyrate proportion of Q_h/Q_m 3:1 was higher than that of Q_h/Q_m 1:1. As for the batches Q_h/Q_m 0:1 and 1:3, the organic acids were mainly composed of acetate, propionate and lactate. Higher Q_h/Q_m enriched the catalogues of organic acids, promoted the production of butyrate. Lower Q_h/Q_m promoted the production of propionate and lactate.

Degradation of organics in the solid phase

As shown in Figure 6, the reduction rates of TS (60.7%), VS (62.9%) and carbon (58.4%) were the highest at Q_h/Q_m 1:3, and followed by Q_h/Q_m 1:1, 0:1, 3:1. The reduction rates of cellulose (49.1%) were also the highest at Q_h/Q_m 1:3, indicating that the ratio of Q_h/Q_m 1:3 was favourable for the hydrolysis of recalcitrant organics like lignocelluloses.

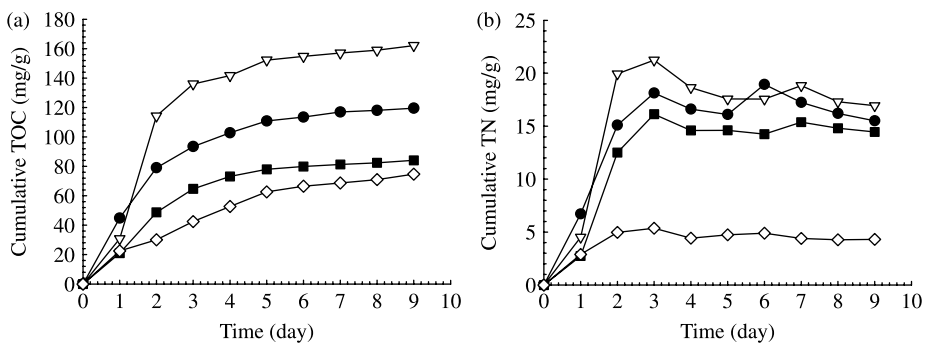

Figure 4 | Temporal evolution of cumulative TOC and TN. (a) Cumulative TOC; (b) Cumulative TN. ● Q_h/Q_m 0:1; ▽ Q_h/Q_m 1:3; ■ Q_h/Q_m 1:1; ◇ Q_h/Q_m 3:1.

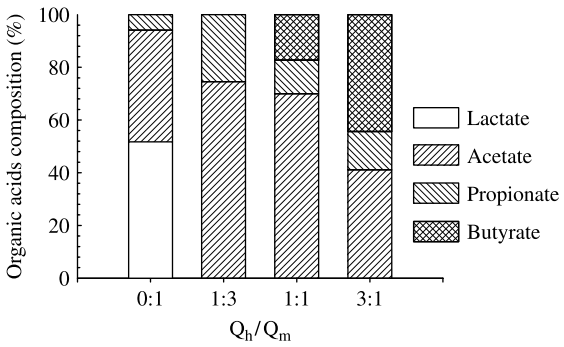

Figure 5 | Speciation of organic acids in the hydrolytic effluents.

Effect of the ratio Q_h/Q_m on hydrolysis

Q_h/Q_m could regulate the hydraulic retention time of hydrolysate, acidogenic products and microorganisms in the hydrolysis reactor, resulting in the change of hydrolysis environment. Partial recirculation of hydrolytic effluent prolonged the retention time of fermentative microorganisms and ensured abundant nutrients (Vavilin *et al.* 2003). It was known that the hydrolysis of lignocelluloses in anaerobic environment required the action of cellulosomes, which bound both to the lignocelluloses and anaerobic microorganisms (Leschine 1995; Schwarz 2001; Bayer *et al.* 2006). It was found that the activity of hydrolytic enzymes was higher on solid surface than in liquids, especially for the cellulose (Zhang *et al.* 2007). Organic acids, to some extent, could loose the structure of lignocelluloses, reduce the degree of crystallization and improve the biodegradability (Xiao & Clarkson 1997; Yu *et al.* 2004; Sun *et al.* 2007). Thereby, partial recirculation of hydrolytic effluent was favorable for the hydrolysis of recalcitrant biomass.

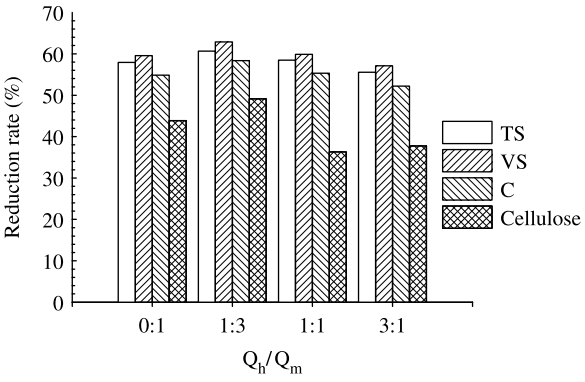

Figure 6 | Reduction rate of VS, TS, carbon and cellulose.

However, excessive recirculation of hydrolytic effluent led to the accumulation of organic acids and reduced pH. Not only acid pH inhibited hydrolysis and acidogenesis (Veeken *et al.* 2000; Vavilin *et al.* 2008), but also the organic acids themselves were toxic to fermentative pathways. Acetate and lactate were observed to severely inhibit the hydrolysis of carbohydrate(He *et al.* 2006; Lü *et al.* 2008), because high concentration of acetate or lactate influenced intracellular pH, ATPase and fatty acids composition of cell membrane, and so inhibited the growth of bacteria (Narendranath *et al.* 2001). Figure 4 indicated that the inhibition of hydrolytic effluents on carbohydrate was higher than on protein, consistent with He *et al.* (2006)'s results, where the inhibitory effects of pH and acetate were more significant on starch than protein. Furthermore, the viscosity of hydrolysates reduced the mobility and transfer of enzyme in liquors, the hydrolysates could introduce product inhibition, resulting in the depressed enzymatic reaction rate (O'Dwyer *et al.* 2007). Therefore, excessive recirculation of hydrolytic effluent was unfavorable for hydrolysis.

The recirculation of methanogenic effluent into hydrolysis reactor could introduce alkalinity and ammonia, buffer the pH fluctuation, dilute the inhibitors(Vavilin *et al.* 2003; Zhang *et al.* 2007), and increase the total enzyme activities in hydrolysis environment(Zhang *et al.* 2007). High ratio of Q_h/Q_m meant that less methanogenic effluent was recycled, resulting in pH decrease and fluctuation, and the accumulation of hydrolysates or acidogenic products. Therefore, partial recirculation of methanogenic effluent was required to promote hydrolysis. The respective roles of hydrolytic and methanogenic effluents indicated that the ratio of Q_h/Q_m should be controlled at a reasonable value, i.e. Q_h/Q_m 1:3 among four batches of Q_h/Q_m 0:1, 1:3, 1:1 and 3:1.

CONCLUSION

This work comes to the conclusions as follows. 1) Recycling methanogenic effluent into the hydrolysis reactor can enhance its buffer capability and operation stability. Higher recycled flow rate is favourable for microbial anabolism and further promotes hydrolysis. After 9 days of hydrolysis, the

cumulative SCOD in the hydrolytic effluent reached 334, 407, 413, 581 mg/g for the flow rates of 0.1, 0.5, 1.0, 2.0 $m^3/(m^3 \cdot d)$, respectively. 2) There existed an optimal ratio of hydrolytic effluent to methanogenic effluent. In this study the optimal ratio is determined to be 1:3 among 0:1, 1:3, 1:1 and 3:1. The research indicated that recycling a small part of hydrolytic effluent was favourable for hydrolysis, while too much hydrolytic effluent will inhibit the hydrolysis.

ACKNOWLEDGEMENTS

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