Journal of Environmental Sciences Vol.16, No.3, pp.387—391, 2004

ISSN 1001—0742
CN11—2629/X

Article ID: 1001-0742(2004)03-0387-05 **CLC number**: X703 **Document code**: A

Conversion regular patterns of acetic acid, propionic acid and butyric acid in UASB reactor

LIU Min[1], REN Nan-qi[1], CHEN Ying[2], ZHU Wen-fang[1], DING Jie[1]

(1. School of Municipal & Environmental Engineering, Harbin Institute of Technology, Harbin 150090, China. E-mail: liumin72@sina.com; 2. College of Environmental & Energy Engineering, Beijing University of Technology, Beijing 100022, China)

Abstract: On the basis of continuous tests and batch tests, conversion regular patterns of acetate, propionate and butyrate in activated sludge at different heights of the UASB reactor were conducted. Results indicated that the conversion capacity of the microbe is decided by the substrate characteristic when sole VFA is used as the only substrate. But when mixed substrates are used, the conversion regulations would have changed accordingly. Relationships of different substrates vary according to their locations. In the whole reactor, propionate's conversion is restrained by acetate and butyrate of high concentration. On the top and at the bottom of the reactor, conversion of acetate, but butyrate, is restrained by propionate. And in the midst, acetate's conversion is accelerated by propionate while that of butyrate is restrained. It is proved, based on the analysis of specific conversion rate, that the space distribution of the microbe is the main factor that affects substrates' conversion. The ethanol-type fermentation of the acidogenic-phase is the optimal acid-type fermentation for the two-phase anaerobic process.

Keywords: conversion regular pattern; VFAs; specific conversion rate; UASB reactor

Introduction

As one of the high efficiency anaerobic bioreactors, the upflow anaerobic sludge blanket (UASB) reactor, for being could present low investment and running costs, operational simplicity, minimum mechanisation level and sustainability of the system as a whole, has been widely applied in wastewater treatment (Wang, 1999; Speece, 1996). There are many reports of such kind of reactors used in wastewater treatment, but almost all the reports focused only on the way of anaerobic digestion or the process of metabolism. And all the reports are based on the traditional researches of anaerobic filters. It is important to learn about the conversion regulations in the UASB reactor for the further improvement of its efficiency.

Acetic acid, propionic acid and butyric acid are the main intermediates of the anaerobic biological process. It has been proved that acetic acid can be used by methanogen directly. Propionic acid and butyric acid needed to be oxidized into acetate by H_2-producing acetogens, molecular hydrogen and carbon dioxide, firstly (Zhao, 1997). Traditionally it is regarded that propionic acid is one of the limited factors of the anaerobic biological process when its' concentration reaches a certain value. And some researches have shown that such limited role of propionic acid is a noncompetitive substrate inhibition one (McCarty, 1963; Honson, 1976). Under mesophilic conditions, the degradation of propionic acid is restrained by acetic acid and butyric acid (Fukuzaki, 1990; Gorris, 1989; Lin, 1986; Mawson, 1991; Nanba, 1983), inhibition of propionic acid degradation by acetic acid is a non-competitive product inhibition (Kus, 1995). But for the UASB reactor, more researches have to be done to study whether such conclusions can be used to explain its' substrate conversion regulations.

In this paper, acetate, propionate, butyrate and the mixtures of them have been used respectively as substrates of the UASB reactor. Their conversion regular patterns at different heights of the reactor have been researched accordingly to study propionate's effect on other kinds of organic VFAs, so as to provide theoretical bases for the improvement of the efficiency of the UASB reactor.

1 Materials and methods

1.1 Experimental set up

Fig.1 shows a schematic diagram of the continuous flow experimental set up with an effective volume of 62.7 L. The acidogenic fermentation production of molasses was used as substrate in the experiments. No other nutrient salt was added to this substrate except for small amounts of N and P that were added to maintain a ratio of COD : N : P = 1000 : 5 : 1. Batch tests were carried out after six months' operation of the UASB reactor and when it is kept in steady state. During batch tests the influent maintained at 50 L/d, the organic loading rate of volume was 6.05 kgCOD/(m^3·d).

Fig. 2 shows the batch reactor set-up. A 250 ml Erlenmeyer flask fitted with a rubber stopper was used. A tube passing through the rubber stopper was used as the gas outlet. The gas evolved was collected in a collection bottle filled with 5% NaOH solution. The gas bubbled through the alkaline solution and collected at the top of the bottle. As the

Foundation item: The National High-Tech R&D Program(863) of China(No. 2001AA515030)

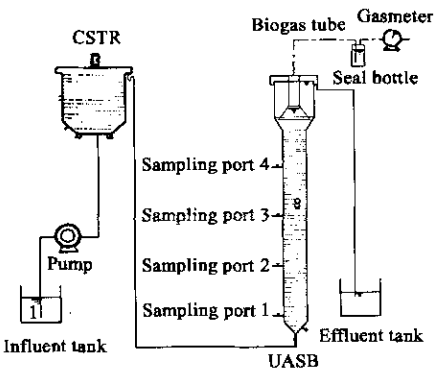

Fig.1 UASB reactor

gas collected in the bottle, the increased pressure resulted in NaOH being pushed out the exit tube in the bottle. The amount of NaOH solution collected was taken to be the amount of methane evolved. During experiment, took 150 ml sludge from the different sampling taps of methanogenic reactor, the height was 0.6 m, 1.1 m and 1.6 m, respectively. Added into organic acids and adjusted to the pH 6.5, 6.45 and 6.5 corresponding to sampling tap 2, 3 and 4. Shaken and taken the first water sample to mensurate concentration of the substrate. Then put through the gas collection system and cultured in water-bathing maintained at 35℃. During 6 h we took the sample every hour. The substrates used were acetic acid, propionic acid and butyric acid. During prepared process kept anaerobic condition by pure nitrogen gas sparging.

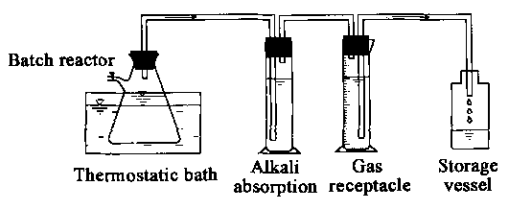

Fig.2 Batch tester apparatus

1.2 Analysis

The procedures described in Standard Methods(APHA, 1992) were used to determine COD, pH, MLSS and VSS. The liquid composition was analyzed by gas chromatography (GC-122, Shanghai, China) equipped with a flame ionization detector. Separation took place in a stainless steel column(2 m long by 5 mm diameter) and packed with GDX—103 60/80 mesh. Nitrogen was used as the carrier gas at a flow rate of 30 ml/min. The operational temperatures for the injection port, the oven and the FID were 220, 190 and 220℃, respectively. The water sample was centrifuged at 11000 r/min for 10 min to analyze ethanol, acetic acid, propionic acid, butyric acid, and so on.

2 Results and discussion

2.1 Conversion of sole organic acid at different sampling tap in methanogenic reactor

It is found that the VFAs do not degrade at sampling tap 1 in the prophase experiment. At the bottom of the UASB reactor, the pH and ORP is maintained about 5.0 and −200 mV, these will promote the fermentative bacteria and acetogens but not the methanogens. So the sampling port 1 is not considered in this research.

Conversation experiments of acetic acid, propionic acid and butyric acid in activated sludge of the different height of UASB reactor were conducted. The results indicated that the conversion rate of acetic acid and butyric acid were much higher than that of propionic acid. That is coincident with the character of organic compounds. As shown in Table 1, the free energy released from digestion of acetic acid, propionic acid and butyric acid to methane and carbonate was approximately the same. But more free energy is needed to oxide propionic acid to acetic acid in the first half-reaction, which limited the conversion of propionic acid. The main residual organic acids were acetic acid and propionic acid in the effluent of UASB reactor, while few butyrates appeared. All that is owe to the energy required in the first reaction. As other organic acids had to be converted to acetic acid firstly and then they were degraded, there was always some residual acetic acid in the effluent.

As shown in Fig.3, 4 and 5, the conversion rate varied with different sampling port when any sole organic acid is

Table 1 Bio-chemical reaction and values of released free energy of organic compound

Compound	Microbe	Half-reactions	$\Delta G^{0'}$, kJ	$\Delta G^{0'}$, kJ/molCH_4
Ethanol	"Sorganism"	$2ethanol + H_2O \rightarrow 2acetate^- + 4H_2 + 2H^+$	+19.3	
	methanogenesis	$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$	−135.6	
	reaction	$2ethanol + HCO_3^- \rightarrow 2acetate^- + H^+ + CH_4 + H_2O$	−116.3	−116.3
Acetate	AOR[b]	$Acetate^- + 4H_2O \rightarrow 2HCO_3^- + 4H_2 + H^+$	+104.6	
	methanogenesis	$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$	−135.6	
	reaction	$Acetate^- + H_2O \rightarrow HCO_3^- + CH_4$	−31.0	−31.0
Propionate	*Syntrophobacter Wolinii*	$4propionate^- + 12H_2O \rightarrow 4acetate^- + 4HCO_3^- + 12H_2 + 12H^+$	+304.6	
	methanogenesis	$12H_2 + 3HCO_3^- + 3H^+ \rightarrow 3CH_4 + 9H_2O$	−406.6	
	reaction	$4propionate^- + 3H_2O \rightarrow 4acetate^- + HCO_3^- + H^+ + 3CH_4$	−102.0	−34.0
Butyrate	*Syntrophomonas Wolfei*	$2butyrate^- + 4H_2O \rightarrow 4acetate^- + 4H_2 + H^+$	+96.2	
	methanogenesis	$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$	−135.6	
	reaction	$2butyrate^- + HCO_3^- + H_2O \rightarrow 4acetate^- + CH_4 + H^+$	−39.4	−39.4

used as the substrate. The specific conversion rates are shown in Table 2. The results indicated that the specific conversion rates of acetic acid, propionic acid and butyric acid were approximately constant in sampling port 2 and 3, while the specific conversion rates at sampling port 4 had a marked improvement. It was mainly because of the different composing of microbe in the activated sludge. In the sludge of sampling port 2 and 3, the proportion of fermentative bacteria, H_2-producing acetogens and methanogens kept in relatively stabilization. There was no preponderant population and the matter transfer process was slow in granules sludge. While the H_2-producing acetogens and methanogens was predominant in the flocculation sludge of sampling port 4. There it was easier to gain substrate, so microbes behaved more active when converted the three organic acids. At the same sampling port, the specific conversion rates were: butyric acid > acetic acid > propionic acid and matched with the theory analysis. The results indicated that the character of substrates were decisive for the conversion capacity of bacteria.

Table 2 Speiflc conversion rate of single substrate by bacteria at the different height of UASB reactor(mg/(g TSS·h))

Sampling port	Acetic acid	Propionic acid	Butyric acid
2#	2.26	1.05	4.05
3#	2.19	1.17	6.01
4#	8.11	2.19	15.27

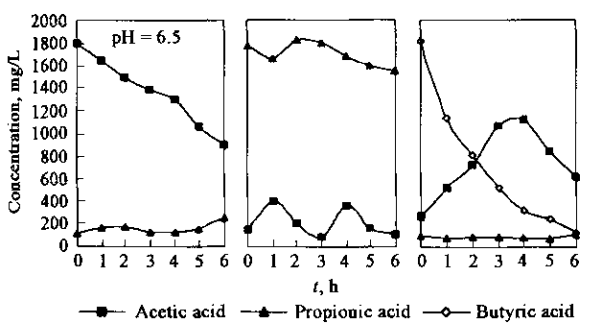

Fig.3 Sole substrate conversion curve by bacteria at sampling port 2

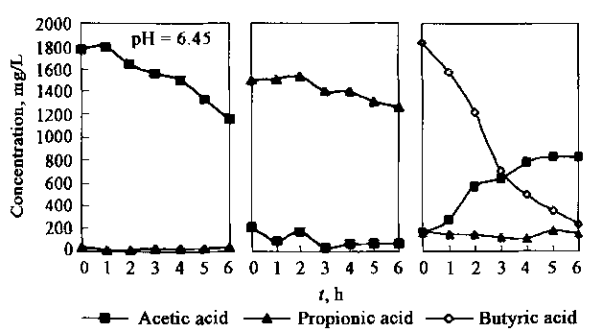

Fig.4 Sole substrate conversion curve by bacteria at sampling port 3

During the organic acids conversion experiments at sampling port 4, the butyric acid has a lag phase(about 1 h) then with a relatively high speed to convert, when the concentration reduced to 500 mg/L, the conversion rate

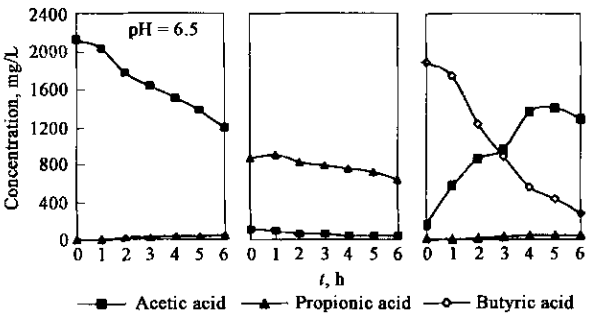

Fig.5 Sole substrate conversion curve by bacteria at sampling port 4

decreased and a great deal acetic acid appeared. The conversion rate of acetic acid was rather steady going. The change of propionic acid was very little. In the good performance reactor, the microbe can only get little acetic acid and propionic acid at sampling port 4, few butyric acids could get there, so the lag phase was the necessary adapting process of bacteria.

Most organic matters were degraded between sampling port 1 and 2, but the specific conversion rate was not the maximum because of the effect of sludge activity. The sludge activity of sampling 1 and 2 was about 60%(calculated with VSS:TSS), while it was 80%—90% at sampling point 3 and 4. The TSS was 80—100 g/L at sampling port 1 and 2 while it was about 40 g/L and 15 g/L at sampling 3 and 4 respectively. The microbe at the bottom could gain food easily because that the influent of UASB was at the bottom, when the influent up to sampling point 3, there was little organic acid maintained. So the degradation process occurred mainly between sampling point 1 and 2, the function of sludge near sampling point 3 and 4 was degraded residual organic acids and prepared for the impact organic loading.

2.2 Conversion of mixed organic acids at different sampling points in methanogenic reactor

When the mixed organic acids such as acetic acid and propionic acid, propionic acid and butyric acid were used as substrates, the results are shown in Fig.6 and Table 3. As Table 3 shown, the specific conversion rate of propionic acid was slower than that of the sole substrate(Table 2). The concentration of propionic acid was 550 mg/L which cannot restrained the activity of microbe (Lin, 1986), so the decrease of specific conversion rate mainly owed to the bacteria's selected ingestion of substrate. Another reason is the increase of hydrogen partial pressure when bacteria digested others organic acids such as butyric acid, acetogenesis bacteria utilized propionic acid was affected strongly by hydrogen partial pressure, which could not happened spontaneously only when the hydrogen partial pressure was lesser than 0.01 kPa(Ren,1993).

At the different sampling port, the specific conversion rates of acetic acid increased along with the increasing height of UASB reactor, while butyric acid kept stability at sampling port 2 and 3 but increased sharply at sampling port 4. The

Table 3 Specific conversion rates of propionic acid and acetate or propionate and butyrate (mg/(gTSS·h))

Sampling port	Specific conversion rate of acetic acid(propionic acid concentration: 450—600 mg/L)	Specific conversion rate of propionic acid(acetic acid concentration: 1400—1800 mg/L)	Specific conversion rate of butyric acid(propionic acid concentration: 450—600 mg/L)	Specific conversion rate of propionic acid (butyric acid concentration: 1400—1800 mg/L)
2#	1.98	0.29	4.04	0.19
3#	5.98	0.91	3.93	0.26
4#	7.60	0.11	15.84	0.75

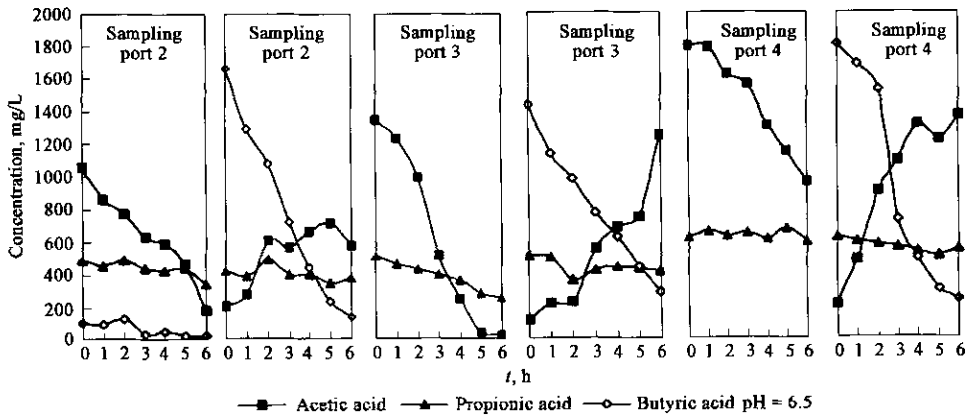

Fig.6 Conversion curves of acetic acid and propionic acid, propionic acid and butyric acid at different sampling port

specific conversion rates of propionic acid were different when digested with acetic acid or butyric acid. When acetic acid was exist, specific conversion rates of propionic acid increased at sampling port 2 and 3, decreased sharply at sampling port 4. When butyric acid was exist, specific conversion rates of propionic acid increased along with the increasing height of UASB reactor.

2.3 Mutual effects of propionic acid on acetic acid and butyric acid

Compared Table 2 with Table 3, the specific conversion rates of acetic acid was depressed but butyric acid kept relative stabilization at sampling ports 2 and 4 by the existing of propionic acid, while the specific conversion rate of acetic acid increased and butyric acid depressed at sampling port 3. This indicated that propionic acid could restrain the conversion of acetic acid at sampling port 2 and 4, but promote it at sampling port 3; propionic acid has no effect on the conversion of butyric acid at sampling port 2 and 4, but restrain it at sampling 3. On the other hand, acetic acid and butyric acid restrained the digestion of propionic acid, the effect of butyric acid on propionic acid conversion was stronger than acetic acid at sampling ports 2 and 3. It was on the contrary at sampling port 4. It was different compared with the research of Jules *et al*. (Jules, 1993), their research showed that propionic acid conversion was inhibited severely by acetic acid and was not affected by the addition of butyric acid.

When acetic acid or butyric acid was in existence, the specific conversion rates of propionic acid at sampling point 4 were 0.11 mg/(gTSS · h) and 0.75 mg/(gTSS · h), respectively. For the microbe of sampling port 4, acetic acid was the most favorable substrate for methanogens in these three kinds of organic acids, propionic acid and butyric acid must be converted into acetic acid, hydrogen and carbon dioxide by H_2-producing acetogens firstly. As shown in Table 1, butyric acid was converted easy than propionic acid in acetogenisis process, when propionic acid and butyric acid exist at the same time, the products of butyrate will restrain the conversion process of propionic acid, too. These indicated that acetic acid would restrain acetogenisis process of propionic acid directly while the effect of butyric acid on propionic acid conversion is indirectly. So the effect of acetic acid on propionic acid conversion was larger than butyric.

The results indicated that bacteria composing and the dominance bacteria were variety at the different profiles of UASB reactor (Zhu, 1990), the conversion of the same substrate will variety even at the same conditions, this variety was much more distinctness when mixed acids was the substrate. Studied of this variety tendency could direct us to consider the configuration fully in order to improve the system buffering capacity and space utilize efficiency when designed a reactor.

2.4 Effects of propionic acid on ethanol

The conversion experiment of propionic acid mixed with ethanol at sampling point 4 was investigated, the results indicated that the conversion of ethanol was not inhibited by propionic acid, but propionic acid conversion was inhibited badly by ethanol. As Table 1 shown, the free energy needed by acetogenisis of ethanol was the least. This bio-chemical reaction can take place easily and produce hydrogen at the same time. For thermodynamic reasons, propionic acid cannot be converted effectively, and the effect of its intermediate products on ethanol conversion could be neglected.

In a word, under mesophilic condition, the conversion of propionic acid was restrained by acetic acid, butyric acid and ethanol. Propionic acid is an unfavourable substrate for anaerobic bacteria and easily accumulated in the reactor. Increasing of propionic acid concentration will decrease the pH value and sometimes make the anaerobic reactor failed. In a two-phase anaerobic treatment system, maintained ethanol-type fermentation in the acidogenic reactor could produce few propionic acid, so the possibility of reactor fail, which caused by propionic acid accumulation, would decreased. For the maintaining of operational stability and improving of treatment efficiency of the methanogenic reactor, the ethanol-type fermentation is superior to propionate or butyric type(Ren, 1997).

3 Conclusions

It can be deduced that when sole VFA is used as the substrate of the UASB reactor, degradation capabilities of the microbes are decided mainly by the characteristic of the substrate. But when mixed organic acids are used and if locations of the substrates change, mutual effects of the substrates and so the conversion regulations would have changed accordingly. Results showed that in the whole reactor propionate's conversion is restrained by the existing of acetates or butyrate. At the bottom or on the top of the reactor, acetate's conversion, not that of butyrate, is limited by propionate's existing. At the midst of the reactor, acetate's conversion is promoted by the existing of propionate, and butyrate's conversion is restrained.

The microbes' spatial distribution is the main effect factor for the change of specific conversion rates. The ethanol-type fermentation of the acidogenic-phase is an optimal acidogenic one for the two-phase anaerobic process.

References:

Fukuzaki S, Nishio N, Shobayashi M *et al*., 1990. Inhibition of the fermentation of propionate to methane by hydrogen, acetate, and propionate[J]. Appl Environ Microbial, 56: 719—723.

Honson P N, Shaw B G, 1976. Inhibition of methane production methanobacterium formicicum[J]. Wat Res, 10: 849—852.

Gorris L G M, J M A van Deursen, C van der Drift *et al*., 1989. Inhibition of propionate degradation by acetate in methanogenic fluidized bed reactors[J]. Biotechnol Lett, 11: 61—66.

Jules van, Lier B *et al*., 1993. Effects of acetate, propionate, and butyrate on the thermophilic anaerobic degradation of propionate by methenogenic sludge and defined cultures[J]. Appl Environmental Microbiology, 4: 1003—1011.

Lin C Y, Sato K, Noike T *et al*., 1986. Methanogenic digestion using mixed substrate of acetic, propionic, and butyric acids[J]. Water Res, 20: 385—394.

Lin C Y, Kazuaki Sato, Tatsuya Noike *et al*., 1986. Methanogenic digestion using mixed substrate of acetic, propionic and butyric acids[J]. Wat Res, 20: 385—394.

Kus F, Wiesmann U, 1995. Degradation kinetics of acetate and propionate by immobilized anaerobic mixed cultures[J]. Wat Res, 29: 1437—1443.

Mawson A J, Earle R L, Larsen V F, 1991. Degradation of acetic and propionic acids in the methane fermentation[J]. Water Res, 25: 1549—1554.

McCarty P L, Jeris J S, Mrudoch W, 1963. Individual volatile acids in anaerobic treatment[J]. J Wat Pollut Control Fed, 35: 1501—1516.

Nanba A, Nukada R, Nagai S, 1983. Inhibition by acetic and propionic acids of the growth of propionibacterium shermanii[J]. J Ferment Technol, 61: 551—556.

Ren N Q, Zhou D S, Ma F, 1993. Wastewater treatment microbiology[M]. Harbin: Scince and Technology Press of Heilongjiang. 228.

Ren N Q, Wang B Z, 1997. Ethanol-type fermentation form carbohydrate in high rate acidogenic reactor[J]. Biotechnology and Bioengineering, 54(5): 428—433.

Speece R E, 1996. Anaerobic biotechnology[M]. Published by Archae Press. 1—2.

Wang Q H, Masaaki Kuninobu, Hiroaki I O *et al*., 1999. Degradation of volatile fatty acids in highly efficient anaerobic digestion[J]. Biomass & Bioenergy, 16: 407—416.

Zhao Y Z, 1997. Methanogenic bacteria and methodology[M]. Chengdu: Chengdu Acdamic University Press. 36.

Zhu J R, 1990. Microbiological research of two-stage upflow anaerobic sludge blanket process[M]. Beijing: Tsinghua University. 1: 35—37.

(Received for review April 4, 2003. Accepted July 22, 2003)