Environmental Technology, Vol. 17. pp 283-291

YIELDS OF BIOMASS AND EXTRACELLULAR POLYMERS IN FOUR ANAEROBIC SLUDGES

X. S. JIA[1], H. FURUMAI[2], HERBERT H. P. FANG*[1]

[1] Environmental Engineering Research Centre, Department of Civil and Structural Engineering, The University of Hong Kong, Pokfulam Road, Hong Kong

[2] Department of Urban and Civil Engineering, Ibaraki University, Hitachi, Ibaraki 316, Japan

(Received 21 April 1995; Accepted 7 August 1995)

ABSTRACT

Extracellular polymers (ECP) are responsible for the flocculation of activated sludge as well as the granulation of anaerobic sludge. In this study, the biomass and ECP yields of four anaerobic sludge were examined. The sludges were enriched by using acetate, propionate, butyrate and glucose, respectively, as the sole substrate. Throughout the enrichment process, which lasted up to 73 cycles, the sludge samples were analyzed for their volatile suspended solids (VSS) as well as ECP's protein (ECP_p) and carbohydrate (ECP_c) contents. Under steady-state condition, the net yields of biomass, ECP_p and ECP_c were measured for the conversion of each individual substrate to methane and carbon dioxide. Furthermore, the corresponding yields for the acetogenesis of propionate and butyrate, and the acidogenesis of glucose, were also estimated, based on the stoichiometry of the degradation reactions. Results show that acidogenesis of glucose produced more ECP_p and ECP_c than acetogenesis and methanogenesis. This explains the reported observations that carbohydrate-degrading sludge produced better granules than the acid-degrading sludge.

Keywords: anaerobes, carbohydrate, ECP, protein, yield

INTRODUCTION

In biological wastewater treatment processes, bacteria tend to aggregate to form flocs, biofilms and even granules. This allows more biomass to accumulate in the reactor and also results in better separation of biomass from the effluent. Although the mechanisms involved in this bioflocculation process have not been fully understood, extracellular polymers (ECP) are believed to play a critical role. ECP are metabolic products of bacteria which accumulate on the cell surface(1). Interactions of these polymers between cells allow adjacent bacteria to aggregate (2). Mechanisms have been proposed for such a bioflocculation process (2, 3, 4, 5), and the chemical nature of sludge ECP has been characterized (1, 6). But, most of the studies on bioflocculation have been limited to aerobic processes.

Anaerobic technology, on the other hand, has matured in the past two decades as a viable alternative to the aerobic processes for the treatment of high-strength industrial wastewater. Anaerobic processes have two intrinsic advantages. They not only save the energy required for aeration but also convert wastes into a useful fuel, methane; furthermore, they produce less sludge than the aerobic processes and, thus, significantly reduce the cost related to sludge handling and disposal. Among the high-rate anaerobic reactors developed in recent years, the upflow anaerobic sludge blanket (UASB) reactor (7, 8, 9, 10) has probably received most commercial interests, especially in Europe and, more recently, in Asia. In the UASB reactor, bacteria aggregate to form biogranules which have high activity and also superb settleability. It has been demonstrated (8) in a 8.5 liter UASB reactor that over 94% of soluble COD (chemical oxygen demand) could be removed at loading rates up to 160 g-COD/(l• d).

Ross (1984) suggested that the bioflocculation mechanisms proposed for the aerobic processes may also be applicable to the formation of anaerobic granules in UASB reactors (11). Sam-Soon, *et al.*, (1987, 1991) speculated that high hydrogen pressure would induce hydrogen-utilizing methanogens to produce ECP, thus boosting sludge granulation(12, 13). Harada, *et al.* (1988) found that carbohydrate-degrading UASB granules were larger and having higher mechanical strength than those UASB granules degrading short chain fatty acids (14). This was believed resulting from higher yield of ECP by the former granules. Nevertheless understanding of the precise role of ECP in granulation of anaerobic sludge is still very limited.

This study was conducted to investigate the ECP yields of four anaerobic enrichment sludges respectively using glucose and three volatile fatty acids, key intermediates in

anaerobic degradation, as individual substrates. It is hoped that results from this study would lead to a better understanding of the granulation mechanism of anaerobic sludge.

MATERIAL AND METHODS

In this study, four series of culture enrichment experiments were conducted in 135 ml glass serum vials which were kept at a constant temperature of 35°C using in a water bath. Gentle mixing was provided by sitting the water bath on a reciprocal shaker table (35mm x 125 strokes/min). Each series was run in duplicate to demonstrate the reproducibility. A single substrate was used for each series: acetate (HAc) for Runs 1 and 1a, propionate (HPr) for Runs 2 and 2a, butyrate (HBu) for Runs 3 and 3a, and glucose (Glu) for Runs 4 and 4a. Each vial was seeded with 100 ml of sludge containing about 220 mg of VSS. The seed sludge was obtained from the blanket zone of a UASB reactor, which was operated using glucose as substrate at a loading rate of 5 g-COD/(l·d), corresponding to 2.5 g-COD/(g-VSS·d). Five ml of aqueous solution at pH 6.9-7.3 containing 50 mg-COD of substrate was added to each vial. The solution which also contained proper nutrients and trace metals, including K_2HPO_4 10000 mg/l, $NiCl_2 \cdot 6H_2O$ 500 mg/l, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 100 mg/l, $CuCl_2 \cdot 2H_2O$ 20 mg/l, $MgCl_2$ 1000 mg/l, H_3BO_3 100 mg/l, KI 100 mg/l, $CoCl_2 \cdot 6H_2O$ 4000 mg/l, $FeCl_2 \cdot 2H_2O$ 4000 mg/l, $AlCl_3 \cdot 6H_2O$ 100 mg/l, $MnCl_2$ 500 mg/l, $ZnCl_2$ 100 mg/l, EDTA 100 mg/l, and concentrated HCl 1 ml/l. After a few hours the substrate in each vial became depleted, as indicated by the cease of biogas production. Five ml of mixed liquor was then removed from the vial and replenished with an equal volume of fresh aqueous solution. Up to 73 batch cycles of such operation were repeated until steady-state condition was ensured, as indicated by the steady VSS content and constant methanogenic activity.

Samples taken from the vapour phase of each vial were periodically checked for the methane contents using a gas chromatograph (GC; Shimadzu GC-8APT) equipped with a thermal conductivity detector using nitrogen as the carrier gas. Mixed liquor samples were analyzed for volatile suspended solids (VSS), proteinaceous ECP (ECP_p) and carbohydrate ECP (ECP_c) contents. The rate of methane production during the batch operation was also closely monitored in a number of selected batches. In these batches, the residual vapour in each vial was flushed with nitrogen before a fresh substrate was injected. The volume of biogas produced was measured using a syringe, while the concentration of methane in the biogas was analyzed by GC analysis throughout the batch. Based on these data, the specific methanogenic activities (SMA) of the enriched cultures were estimated from the maximum rate of methane production, using the methodology developed by Owen *et al.*, 1979 (15).

The VSS contents were measured following the Standard Methods (16). ECP were extracted from the sludge samples using the cold aqueous extraction techniques (17, 18). Each sludge sample was washed twice with de-ionized water followed by low speed (3500 rpm) centrifugation. The centrifuged biomass was then re-suspended in a 5 ml 8.5% sodium chloride solution containing 0.22% formaldehyde. The solution was subsequently chilled in ice and mixed using an ultrasonic homogenizer at 40 watts for 3 minutes, during which the ECP of bacteria were extracted into the solution. After removing the residual solids by high speed centrifugation (12000 rpm for 30 minutes), the ECP_c in the extracted solution was measured using the phenol/sulphuric-acid method and the ECP_p using the folin method (19).

RESULTS AND DISCUSSION

The original biomass in the seed sludge was a mixed culture. As the enrichment batches proceeded, those biomass which could not degrade either the substrate or the degradation intermediates were slowly wasted. As a consequence, the biomass in each vial decreased initially, while the specific bioactivity of the enriched biomass increased gradually, until steady state was reached. Figures 1-4 illustrate the changes of VSS content and specific methanogenic activity (SMA) during culture enrichment using, respectively, acetate (Runs 1 and 1a), propionate (Runs 2 and 2a), butyrate (Runs 3 and 3a) and glucose (Runs 4 and 4a) as individual substrates. All series of experiments conducted in duplicate had satisfactory reproducibility on VSS and SMA measurements as illustrated in the figures. The enrichment experiments continued for about twenty batches under the steady state condition. Specific methanogenic activity of the biomass, and yields of VSS and ECP of the enriched culture were measured at the steady-state conditions.

1. Specific methanogenic activity and net yield of enriched sludges

As illustrated in Figure 1, for the experiments using acetate as sole substrate, steady state condition was reached after 40 batches having a constant VSS content of 610 mg/l and SMA of 1.39 mg-methane-COD/(g-VSS·d). The corresponding steady-state VSS and SMA values were 990 mg/l and 0.72 mg-methane-COD/(g-VSS·d) for propionate after 25 batches, 895 mg/l and 1.25 mg-methane-COD/(g-VSS·d) for butyrate after 40 batches, and 1450 mg/l and 0.51 mg-methane-COD/(g-VSS·d) for glucose after 30 batches.

At each batch cycle, 5 ml of mixed liquor was removed from the serum vial while an equal volume of solution containing 50 mg-COD equivalent of substrate was added. Under steady-state condition the biomass produced equalled those removed. For batches using acetate as substrate, a total of 3.05 mg-VSS (5 ml x 0.610 mg-VSS/ml) was removed in each cycle for every 50 mg-COD of substrate converted to methane. Thus, the net yield for the acetate-degrading sludge was 0.061 mg-VSS/mg-COD. Likewise, the net yields for other

enriched sludges were calculated respectively as follows: 0.099 mg-VSS/mg-COD for propionate-degrading sludge, 0.090 mg-VSS/mg-COD for butyrate-degrading sludge and 0.145 mg-VSS/mg-COD for glucose-degrading sludge.

Table 1 shows that the SMA values found in this study for the four enriched sludges are, in general, in agreement with the corresponding data available in literature. Similarly, Table 2 shows that the net yields of four groups of biomass converting individual substrate into methane are also comparable to those in literature.

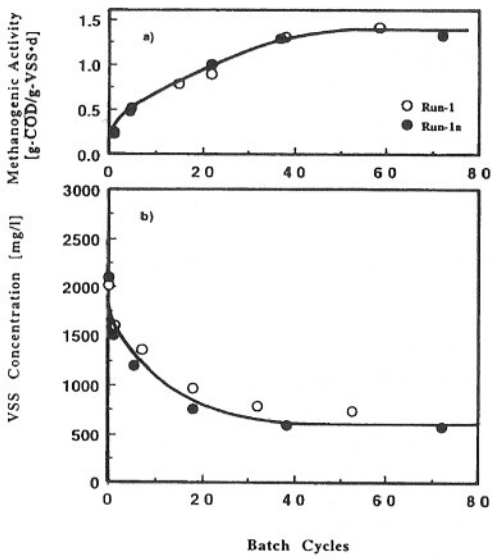

Figure 1: (a) Increase of SMA, and (b) reduction of VSS during the enrichment of acetate-degrading culture in Runs 1 and 1a.

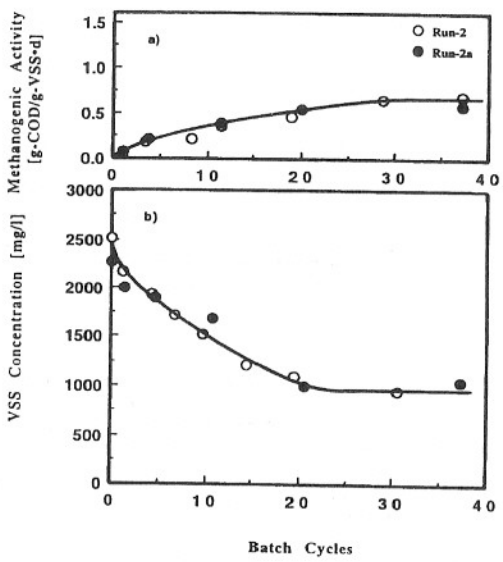

Figure 2: (a) Increase of SMA, and (b) reduction of VSS during the enrichment of propionate -degrading culture in Runs 2 and 2a.

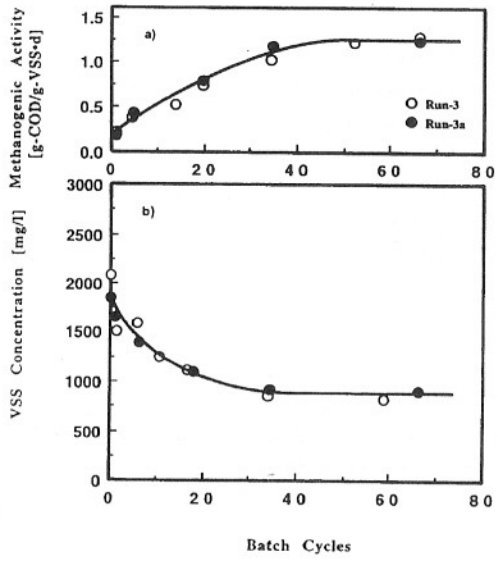

Figure 3: (a) Increase of SMA, and (b) reduction of VSS during the enrichment of butyrate-degrading culture in Runs 3 and 3a.

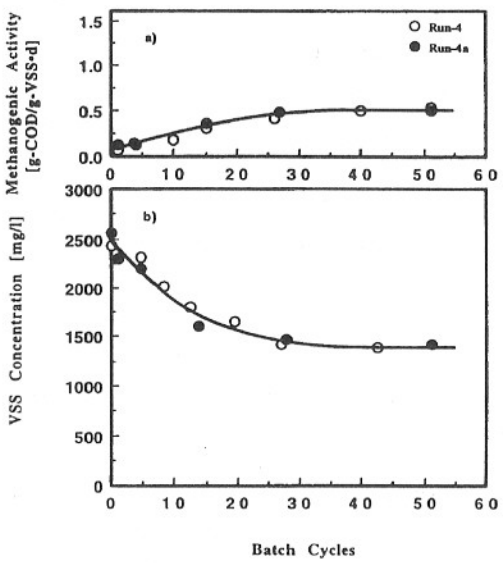

Figure 4: (a) Increase of SMA, and (b) reduction of VSS during the enrichment of glucose-degrading culture in Runs 4 and 4a.

Table 1. Specific methanogenic activities (SMA)

Substrate	SMA ($g\text{-}CH_4\text{-}COD/g\text{-}VSS \bullet d$)	References
Acetate	1.39	Present study
Propionate	0.72	Present study
	0.49	Fang *et al.* (1995b, 10)
	0.52	Dolfing and Bloeman (1985, 20)
	1.78	Grotenhuis *et al.* (1991, 21)
Butyrate	1.25	Present study
	1.31	Fang *et al.* (1995a, 9)
Glucose	0.51	Present study

Table 2. Net yeilds of enriched sludge for methane production

Substrate	Net yield of enriched sludge (g-VSS/g-COD)	References
Acetate	0.061	Present study
	0.041	Lawrence and McCarty (1969, 22)
	0.04	Smith and Mah (1978, 23)
	0.05	Smith and Mah (1980, 24)
	0.108	Chang *et al.* (1983, 25)
	0.04	Frostell (1985, 26)
	0.041	Furumai *et al.* (1991, 27)
Propionate	0.099	Present study
	0.042	Lawrence and McCarty (1969, 22)
	0.12	Frostell (1985, 26)
	0.040	Fang *et al.* (1995b, 10)
Butyrate	0.090	Present study
	0.047	Lawrence and McCarty (1969, 22)
	0.16	Frostell (1985, 26)
	0.037	Fang *et al.* (1995a, 9)
Glucose	0.145	Present study
	0.22	Frostell (1985, 26)

2. Estimated yields of acetogens and glucose-utilizing acidogens

Anaerobic degradation of organic matters is a complex process. Polymeric organics, such as proteins, lipids and polysaccharides, are first hydrolyzed by enzymes forming amino acids, fatty acids and sugars. These hydrolyzed products are then degraded by acidogenic bacteria forming VFA, which are further degraded by acetogenic bacteria forming acetate, carbon dioxide and hydrogen. Finally, both acetate and H_2/CO_2 are converted to methane by the methanogenic bacteria (28, 29). The biochemical reactions involved in the degradation of substrates used in this study are as follows (30, 31, 32, 33, 9, 10):

Methanogenesis

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad (1)$$

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (2)$$

Acetogenesis

$$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + 3H_2 + CO_2 \quad (3)$$

$$CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2 \quad (4)$$

Acidogenesis

$$4C_6H_{12}O_6 + 2H_2O \rightarrow 3CH_3COOH + CH_3CH_2COOH + 2CH_3CH_2CH_2COOH + 7CO_2 + 9H_2O \quad (5)$$

Methanogenesis of either H_2/CO_2 or acetate is a one-step process, as shown in Reactions 1 and 2. On the other hand, conversion of either propionate or butyrate to methane is a two-step process; the acids are first converted to acetate by acetogens, as shown respectively in Reactions 3 and 4, before methanogenesis can take place (22, 34). Furthermore, degradation of glucose requires another step, acidogenesis, in which glucose is first converted into a mixture of HAc, HPr and HBu, as shown in Reaction 5, prior to acetogenesis and the subsequent methanogenesis (30, 32).

Since hydrogen and fatty acids exhibit oxygen demand, their concentrations can be expressed in terms of COD-equivalent: 1 g-H_2 equals 8 g-COD; 1 g-HAc, 1.067 g-COD; 1 g-HPr, 1.514 g-COD; 1 g-HBu, 1.818 g-COD and 1 g-Glu, 1.067 g-COD. The net yield for hydrogenotrophic methanogens in Reaction 1 was recently reported as 0.046 mg-VSS/mg-H_2-COD (35), whereas that of acetotrophic methanogens in Reaction 2 was 0.061 mg-VSS/mg-HAc-COD, as discussed in the previous section. According to Reaction 3, the enriched HPr-degrading sludge was composed of a mixture of three trophic groups of bacteria, i.e. HPr-degrading acetogen, plus acetotrophic and hydrogenotrophic methanogens. The observed net yield of 0.099 mg-VSS/mg-HPr-COD, therefore, represented the sum of the yields of HPr-degrading acetogen and the two methanogens. Reaction 3 shows that one g-COD of propionate is converted to 0.571 g-HAc-COD and 0.429 g-H_2-COD. Since yields of the two methanogens are known, the net yield of HPr-degrading acetogen was estimated as 0.047 mg-VSS/mg-HPr-COD, based on the mass balance and the stoichiometry of the reaction.

Similarly, the enriched HBu-degrading sludge was composed of a mixture of HBu-degrading acetogen and the two methanogens, according to Reaction 4. The net yield of the HBu-degrading acetogen could thus be estimated based on Reaction 4 as 0.033 mg-VSS/mg-HBu-COD. Furthermore, the enriched Glu-degrading sludge was composed of Glu-degrading acidogens, plus HPr- and HBu-degrading acetogens and the two methanogens, according to Reaction 5. Using the estimated yield values for the two acetogens and two methanogens, the net yield of Glu-degrading acidogens could also be estimated as 0.105 mg-VSS/mg-Glu-COD.

Table 3 summarizes the net yield values for various trophic groups of bacteria estimated from this study and the corresponding values reported in literature. They are in general comparable.

3. Formation of ECP by each substrate utilizing anaerobes

The glucose-degrading seed sludge in all reactors were obtained from a UASB reactor operated at a volumetric loading rate of 5 g-COD/(l•d) and a specific loading rate of 2.5 g-COD/(g-VSS•d). Each gram of seed sludge contained 30-35 mg of ECP_p and 15-19 mg of ECP_c. Figures 5-8 illustrate that in all runs both ECP_p and ECP_c contents decreased from the initial values as the enrichment experiment proceeded. The decrease of ECP contents was probably due to the very low loading rate, ranging 0.075-0.43 g-COD/(g-VSS•d), operated in the batch reactions. In each batch, the ECP content in the biomass levelled off to a constant value after reaching steady state. Under steady state condition, each gram of HAc-degrading sludge contained 17.5 mg-ECP_p and 7.5 mg-ECP_c (Figure 5). The corresponding ECP contents for HPr- , HBu- and Glu-degrading sludges, 15.4 mg-ECP_p and 5.5 mg-ECP_c for HPr-degrading sludge (Figure 6), 22.3 mg-ECP_p and 6.6 mg-ECP_c for HBu-degrading sludge (Figure 7) and 16.5 mg-ECP_p and 6.8 mg-ECP_c for Glu-degrading sludge (Figure 8).

Table 3. Net yields of microorganisms for acetogenesis and acidogenesis.

Reaction	Net yield (g-VSS/g-COD)	References
Actogenesis of propionate	0.047	Present study
(Reaction 3)	0.043	Chang *et al.* (1983, 25)
	0.041	Furumai *et al.* (1991, 27)
Acetogenesis of butyrate	0.033	Present study
(Reaction 4)	0.020	Lin *et al.* (1989, 38)
	0.041	Furumai *et al.* (1991, 27)
Acidogenesis of glucose	0.105	Present study
(Reaction 5)	0.115	Hill and Barth (1977, 39)
	0.082	Zoetemeyer *et al.* (1982, 40)

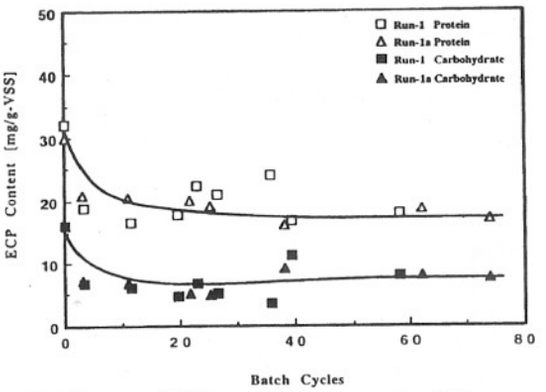

Figure 5: Changes of ECP_p and ECP_c content in VSS during the enrichment of acetate-degrading culture in Runs 1 and 1a.

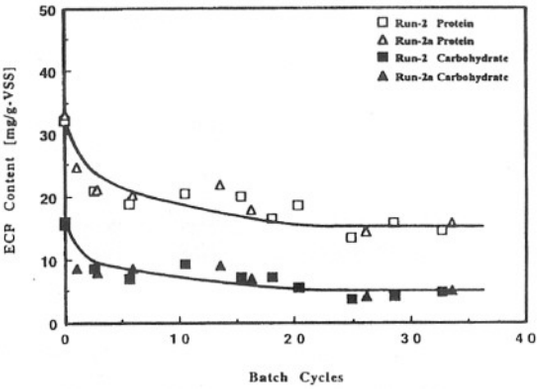

Figure 6: Changes of ECP_p and ECP_c content in VSS during the enrichment of propionate-degrading culture in Runs 2 and 2a.

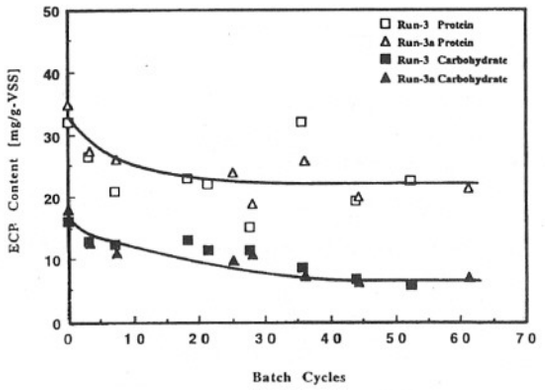

Figure 7: Changes of ECP_p and ECP_c content in VSS during the enrichment of butyrate-degrading culture in Runs 3 and 3a.

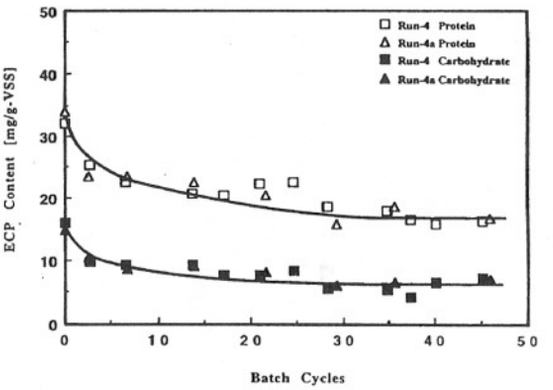

Figure 8: Changes of ECP_p and ECP_c content in VSS during the enrichment of glucose-degrading culture in Runs 4 and 4a.

4. Yields of ECP_P and ECP_c

The yield of ECP_P and ECP_c could, thus, be calculated based on steady-state data. For each gram of HAc-COD converted to methane, the HAc-degrading sludge produced 1.07 mg-ECP_P and 0.46 mg-ECP_c. The corresponding yields for the conversion of other substrates to methane were 1.52 mg-ECP_P and 0.54 mg-ECP_c for HPr-degrading sludge, 2.00 mg-ECP_P and 0.59 mg-ECP_c for HBu-degrading sludge, and 2.39 mg-ECP_P and 0.99 mg-ECP_c for Glu-degrading sludge.

In all batches, more ECPp were produced than ECP_c regardless of substrates, as reported for digester sludge (1, 6). Jia, et al. (1991) suggested that more ECP_P was accumulated than ECP_c in the biomass of a UASB reactor treating wastewater containing glucose (18). A recent study also reported that more ECP_P was produced than ECP_c in the hydrogenotrophic methanogenesis process (35).

The aforementioned ECP_P and ECP_c yields were the overall yields for converting each individual substrate into methane and carbon dioxide. The yields for hydrogenotrophic methanogens in Reaction 1 were reported as 1.06 mg-ECP_P and 0.64 mg-ECP_c for 1 g-H_2-COD removed (35), whereas those for acetotrophic methanogens in Reaction 2 were 1.07 mg-ECP_P and 0.46 mg-ECP_c for 1 g-HAc-COD removed. Similar to the estimations of biomass yields for acidogenesis and acetogenesis as demonstrated in previous sections, the ECP_P and ECP_c yields for the acidogenesis and acetogenesis could also be estimated based on Reactions 1-5 and material balances. The resulting estimated yields were 0.53 mg-ECP_P/g-HPr-COD and 0.04 ECP_c/g-HPr-COD for the HPr-degrading acetogens, 0.99 mg-ECP_P/g-HBu-COD and 0.12 ECP_c/g-HBu-COD for the HBu-degrading acetogens, and 1.52 mg-ECP_P/g-Glu-COD and 0.72 ECP_P/g-Glu-COD for the Glu-degrading acidogens.

Results of many previous researches seem to suggest that anaerobic sludge could form granules without much difficulties for wastewaters containing carbohydrates, such as glucose, sugar, etc. (8, 11, 14, 18, 36, 37, 41). In addition, Harada, et al. (1988) found that carbohydrate-degrading granules were larger and having higher mechanical strength than those degrading short chain fatty acids (14). On the other hand, results of this study show that acidogens have higher ECP yields than acetogens and methanogens, as illustrated in Figure 9. Since acetogens and methanogens

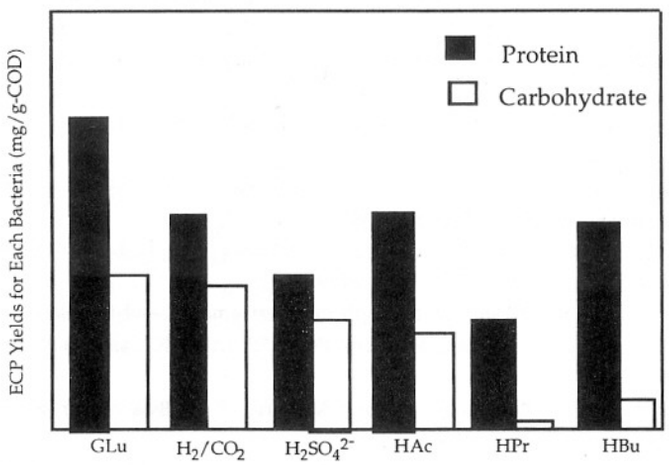

Figure 9. Yields of ECPp and ECPc for various trophic groups of bacteria.

were present in both carbohydrate-degrading and short-chain-fatty-acids-degrading granules, whereas acidogens were present only in the former granules, the higher ECP content produced by acidogens could result in the superior quality of the former granules. One may thus draw a conclusion that ECP have a positive effect on the granulation of anaerobic sludge.

CONCLUSIONS

Four series of batch experiments were conducted in duplicate for the enrichment of sludge degrading acetate, propionate, butyrate and glucose, individually, as the sole substrate. The net yields of biomass, ECP_p and ECP_c were measured from the steady-state data for the conversion of each individual substrate to methane and carbon dioxide. These net yields were also estimated, based on the stoichiometry of degradation reactions, for the acetogenesis of propionate and butyrate, and the acidogenesis of glucose. Results show that acidogenesis of glucose produced more ECP_p and ECP_c than acetogenesis and methanogenesis. The high ECP yield of acidogenesis could be the reason for the better granulation of carbohydrate-degrading sludge.

ACKNOWLEDGMENT

The writers would like to thank the Hong Kong Research Grants Council for the partial financial support of this study.

REFERENCES

1. Morgan, J. W., Forster, C. F. and Evison, L., A comparative study of the nature of biopolymers extracted from anaerobic and activated sludge. *Wat. Res.*, **24**(6), 743-750, (1990).
2. Tenney, M. W. and Stumm, W. J., Chemical flocculation of microorganisms in biological waste treatment. *Jour. Water. Pollut. Control Fed.*, **39**, 1370-1388, (1968).
3. Busch, P. L. and Stumm, W., Chemical interactions in the aggregation of bacteria Bioflocculation in waste treatment. *Environ. Sci. Tech.*, **2**, 49-53, (1968).
4. Ryssov-Nielson, H., The role of natural extracellular polymers in the bioflocculation and dewatering of sludge. *Vatten*, **31**, 33-39, (1975).
5. Sutherland, I. W., Biosynthesis and composition of gram negative bacterial extracellular and wall polysaccharides. *A. Rev. Microbiol.*, **39**, 243-270, (1985).
6. Forster, C. F. and Clarke, A. R., The production of polymer from activated sludge by ethanolic extraction and its relation to treatment plant operation. *Wat. Pollut. Control*, **82**, 430-433, (1983).
7. Lettinga, G., van Velsen, A. F. M., Hobma, S. M., de Zeeuw, W. and Klapwijk, A., Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment. *Biotech. Bioengrg.* **22**, 699-734, (1980).
8. Fang, H. H. P. and Chui, H. K., Maximum COD loading capacity in UASB reactors at 37°C. *J. Environ. Engrg.*, **119**(1), 103-119, (1993).

9. Fang, H. H. P., Li, Y. Y. and Chui, H. K., Anaerobic degradation of butyrate in a UASB reactor. *Bioresource Technology*, **51**, 75-81(1995a).
10. Fang, H. H. P., Li, Y. Y. and Chui, H. K., Performance and sludge characteristics of UASB process treating propionate-rich wastewater. *Wat. Res.*, **29(3)**, 895-898, (1995b).
11. Ross, W. R., The phenomenon of sludge pelletisation in the anaerobic treatment of a maize processing waste. *Water SA*, **10**, 197-203, (1984).
12. Sam-Soon, P. A. L. N. S., Loewenthal, R. E., Dold, P. L., and Marais, G. v. R., Hypothesis for pelletisation in the upflow anaerobic sludge bed reactor. *Water SA*, **13**(2), 69-80, (1987).
13. Sam-Soon, P. A. L. N. S., Loewenthal, R. E., Wentzel, M. C., and Marais, G. v. R., Effect of Sulfate on pelletisation in UASB system with glucose as substrate. *Water SA*, **17**(1), 47-56, (1991).
14. Harada, H., Endo, G., Tohya, Y. and Momonoi, K., High rate performance and its related characteristics of granulated sludges in UASB reactors treatment various waste waters. *Procs. 5th IAWPRC Symposium on Anaerobic Digestion, Bologna.* 1011-1019, (1988).
15. Owen, W. F., Stuckey, D. C., Healy, J. B., Young. L. Y., and McCarty, P. L., Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Wat. Res.*, **13**, 485-492, (1979).
16. APHA, *Standard Methods for the Examination of Water and Wastewater*, 16th edition. American Public Health Association, Washington, D. C., (1985).
17. Sutherland, I. W. and Wilkinson, J. F., Chemical extraction methods of microbial cells. In: *Methods in Microbiology*. Vol. 5B, Chapter 5, Academic Press, London and New York, (1971).
18. Jia, X., Furumai, H. and Kusuda, T., Change of hydrogen partial pressure and the formation of extracellular polymer during start-up of upflow anaerobic sludge bed reactor. *Jour. Japan Sewage Works Association Research*, **28**, 83-93, (1991).
19. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**, 265-275, (1951).
20. Dolfing, J. and Bloeman, W. G. B. M., Activity measurements as a tool to characterize the microbial composition of methanogenic environment. *J. Microbiol. Methods*, **4**, 1-12, (1985).
21. Grotenhuis, J. T. C., Smit, M., Plugge, C. M., Xu, Y., van Lammeren, A. A. M., Stams, A. J. M. and Zehnder, A. J. B., Bacteriological composition and structure of granular sludge adapted to different substrates. *Appl. Environ. Microbiol.* **57**(7), 1942-1949, (1991).
22. Lawrence, A. W. and McCarty, P. L., Kinetic of methane fermentation in anaerobic treatment. *Jour. Water Pollut. Control Fed.*, **41**(2), R1-R17, (1969).
23. Smith, M. R. and Mah, R. A., Growth and methanogenesis by *Methanosarcina* strain 227 on acetate and methanol. *Appl. Environ. Microbiol.*, **36**(6), 870, (1978).
24. Smith, M. R. and Mah, R. A., Acetate as sole carbon and energy source for growth of *Methanosarcina* strain 227. *Appl. Environ. Microbiol.*, **39**, 993, (1980).
25. Chang, J. E., Noike, T. and Matsumoto, J., Effect of pH on methanogenesis in anaerobic digestion. *Proc. of JSCE*, **335**, 79-87, (1983).
26. Frostell, B., Process control in anaerobic wastewater treatment. *Wat. Sci. Tech.*, **17**, Tampere, 173-189, (1985).
27. Furumai, H., Kuba, T., Imai, T. and Kusuda, T., Transient responses of wastewater treatment and biomass development in a methanogenic fluidized bed. *Wat. Sci. Tech.*, **23**, 1327-1336, (1991).
28. Speece, R. E., Anaerobic biotechnology for the industrial wastewater treatment. *Envir. Sci. Tech.*, **17**(9), 416A-427A, (1983).
29. Vogels, G. D., Keltjens, J. T., and van der Drift, C., Biochemistry of methane production. In: *Biology of Anaerobic Microorganisms*. Zehnder, A. J. B. (ed.) Wiley-Liss, New York, (1988).
30. Thauer, R. K., Jungermann, K. and Decker, K., Energy conservation in chemotrophic anaerobic bacteria. *Bacteriological Review*, **41**(March), 100-180, (1977).
31. Gujer, W. and Zehnder, A. J. B., Conversion processes in anaerobic digestion. *Wat. Sci. Tech.*, **15**(8/9), 127-167, (1983).
32. Mosey, F. E., Mathematical modelling of the anaerobic digestion process: regulatory mechanisms for the formation of short-chain volatile acids from glucose. *Wat. Sci. Tech.*, **15**, 209-232, (1983).
33. Harper, S. R. and Pohland, F. G., Recent developments in hydrogen management during anaerobic biological wastewater treatment. *Biotech. Bioengrg.* **28**, 585-602, (1986).
34. McCarty, P. L. and Smith, D. P., Anaerobic wastewater treatment. *Environ. Sci. Tech.* **20**(12), 1200-1206, (1986).
35. Jia, X., Furumai, H. and Fang, H. H. P., Extracellular polymers of hydrogen-utilizing methanogenic and sulfate-reducing sludges. *Wat. Res.*, (in press, 1996)
36. Hulshoff Pol, L. W., de Zeeuw, W. J., Velzeboer, C. T. M. and Lettinga, G., Granulation in UASB reactors. *Wat. Sci. Tech.*, **15**, 291-304, (1983a).

37. Hulshoff Pol, L. W., Dolfing, J, van Straten, K., de Zeeuw, W. J. and Lettinga, G., Pelletization of anaerobic sludge in upflow anaerobic sludge bed reactor on sucrose-containing substrates. In: *Current Perspectives in Microbial Ecology*, American Society for Microbiology, Washington, DC., 636-642, (1983b).
38. Lin, C. Y., Noike, T., Furumai, H. and Matsumoto, J., A kinetic study on the methanogenesis process in anaerobic digestion. *Wat. Sci. Tech.*, **21**, 175-186, (1989).
39. Hill, D. T. and Barth, C. L., A dynamic model for simulation of animal waste digestion. *Jour. Water Pollut. Control Fed.*, **49**(10), 2129-2143, (1977).
40. Zoetemeyer, R. J., van den Heuvel, J. C. and Cohen, A., pH influence on acidogenic dissimilation of glucose in an anaerobic digestor. *Wat. Res.*, **16**, 303-311, (1982).
41. Wu, W., Hu, J., Gu, X., Zhao, Y., Zhang, H. and Gu, G., Cultivation of anaerobic granular sludge in UASB reactors with aerobic activated sludge as seed. *Wat. Res.*, **21**(7), 789-799, (1987).