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SOLUBLE MICROBIAL PRODUCTS (SMP) OF ACETOTROPHIC METHANOGENESIS

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Abstract

Experiments on acetotrophic methanogenesis were conducted at 37°C in a 3·5 l chemostat reactor and ten 120 ml batch reactors. The degradation process produced substantial quantities of soluble microbial products (SMP). Converting 1 mg of acetate produced 0·0251 mg-C of SMP in the chemostat reactor, but 0·0150 mg-C of SMP in batch reactors. The reaction was not pH sensitive within the range of pH 6–8. At pH 5 and 9, methanogenesis and SMP production were both inhibited. The biomass inhibited at pH 9 was able to regain activities after adjusting the pH to neutrality, but that inhibited at pH 5 was not. The biomass yield was 0·0257 and 0·0297 mg-biomass/mg-acetate in chemostat and batch reactors, respectively. © *1998 Elsevier Science Ltd. All rights reserved*

Key words: acetate, batch, chemostat, degradation, methanogenesis, microbial, pH, SMP, yield.

INTRODUCTION

In a biological wastewater treatment process, biomass converts organic pollutants into harmless end products, such as water, carbon dioxide and methane. In the process, however, biomass also releases soluble microbial products (SMP), some of which are recalcitrant to biodegradation (Chudoba, 1967; Boero *et al.*, 1991). These SMP are composed of a wide variety of high molecular-weight organics, such as humic and fulvic acids, polysaccharides, proteins, fragments of DNA, antibiotics, steroids, enzymes, etc., (Rittmann *et al.*, 1987), products of cell metabolism and cell lysis (Noguera *et al.*, 1994).

The formation of SMP has two major impacts on the treatment process. The presence of SMP increases the organic content of the effluent, and thus adversely affects the effluent quality. Furthermore, SMP contain a large number of chelating functional groups, such as carboxyl, hydroxyl and amino, which could act as ligands forming complexes with metals in the wastewater. The overall impact of metal complexation by these microbial chelators is not clear. On one hand, it may alleviate the metal toxicity; on the other hand, it may reduce the amount of metal available to bacteria as micronutrient (Callander and Barford, 1983a,b).

Although the formation of SMP has been studied extensively for the aerobic treatment process, little information is available on its formation in the anaerobic treatment process. Anaerobic degradation of organic pollutants is a complex process involving a number of steps and microorganisms. Organic matters are first converted by fermentative bacteria to simple organic acids, which are then further converted by acetogens forming acetate and hydrogen. Finally, acetate and hydrogen/carbon dioxide are converted by two respective groups of methanogens forming the end-product methane. Even for a simple single-step reaction like acetotrophic methanogenesis, whether SMP is produced or not is still not clear. Noguera *et al.* (1994) reported that there was no SMP accumulation in this reaction, whereas Kuo *et al.* (1996) reported otherwise.

This study was conducted to investigate SMP formation during acetotrophic methanogenesis in both chemostat and batch reactors.

METHODS

Culture enrichment in a chemostat reactor

The acetotrophic methanogenic sludge was first enriched in a 3·5 l water-jacketed chemostat reactor at 37°C. Feed solution to the reactor contained acetate as the sole substrate at an average concen-

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tration of 9375 mg/l. In addition, the solution was also dosed with balanced nutrient, trace metals and bicarbonate following a previously established formula (Fang and Chui, 1993), except that sulfate was not added to the solution to avoid acetotrophic sulfidogenesis. The reactor was seeded with disintegrated granular sludge from a UASB (upflow anaerobic sludge blanket) reactor treating a wastewater composed of carbohydrate and protein. A Waring blender was used to disintegrate the granular sludge before seeding. The mixed liquor of the chemostat reactor was completely mixed by recirculating the biogas via a compressor.

The reactor was operated at an average retention time of 10 days. It reached steady-state conditions in a few weeks judging from the constant rate of gas production. During days 77–90, biogas production and its methane content were closely monitored along with the pH, concentrations of acetate and TOC (total organic carbon) in the mixed liquor.

Since acetate was the sole substrate in the feed solution, the only biochemical reaction taking place in the chemostat reactor was presumably acetotrophic methanogenesis. Thus, the organic components in the mixed liquor were residual acetate, dissolved methane and the SMP. The content of SMP, as expressed in mg-C/l, could be calculated from the difference between the soluble TOC and the corresponding carbon contents of acetate and dissolved methane. Each milligram of acetate and dissolved methane respectively correspond to 0·40 and 0·75 mg of organic carbon. The solubility of methane at 37°C is 17·76 mg/l (Liley and Gambill, 1973), equivalent to 13·32 mg-C/l, at atmospheric pressure. Assuming that methane in the mixed liquor was in equilibrium with that in the biogas, the quantity of dissolved methane could then be estimated, according to Henry's law, from the partial pressure.

Batch experiments

Two sets of experiments were conducted in 120 ml glass serum vials at 37°C. The first set was to investigate the pH effect by treating feed solutions at five levels of pH, i.e. 5, 6, 7, 8 and 9, while the acetate concentration was kept at a narrow range of 750–950 mg/l. The second set was to investigate the concentration effect by treating five feed solutions containing, respectively, 600, 1200, 1800, 3500 and 6000 mg/l of acetate, at pH 7·0–7·2.

An acetate-free stock solution was first prepared. It contained the essential nutrient, traced elements and bicarbonate (Jia *et al.*, 1991, 1996), but not sulfate. The solution was purged with nitrogen to remove any dissolved oxygen. Each batch reactor was seeded with acetotrophic methanogenic sludge enriched in the chemostat reactor for over 90 days. About 600 ml of sludge sampled from the chemostat reactor was first washed with the stock solution, followed by centrifugation. After decanting the supernatant, the sludge was then re-suspended in 600 ml of stock solution. About 60 ml of the mixed solution containing 25 mg of suspended sludge was transferred to each vial using a syringe. Acetate was then added to each vial to the pre-determined concentration, and the pH was adjusted by the addition of dilute hydrochloric acid or sodium hydroxide solution. All the sludge handling and feed solution transfer operations were conducted in an anaerobic-workstation (Forma Scientific, Model 1029).

All vials were submerged in a 37°C shaking water bath to ensure proper mixing. Biogas production and methane content, as well as pH, acetate and soluble TOC concentrations in the mixed liquor, were measured at given time intervals. The experiment was terminated when the reaction had been completed, as evidenced by the depletion of acetate in the mixed liquor and the cessation of methane production.

Analytical

The total protein content in solids suspended in the mixed liquor was measured following the folin method (Lowry *et al.*, 1951). The biogas was analyzed for methane, carbon dioxide and nitrogen content using a gas chromatograph (Hewlett-Packard, Model Series II) equipped with a thermal conductivity detector, and a CarboPLOT P7 column (25 m in length, 0·53 mm ID and 25 μm film thickness). Argon was used as the carrier gas at a flow-rate of 30 ml/min. The column was operated at a temperature program of 50°C for 2·5 min followed by 110°C for 1·3 min. The temperatures of the injection port and detector were both 180°C.

The concentration of acetate was determined by a second gas chromatograph of the same model equipped with a flame ionization detector and a capillary column (Alltech Econo-Cap FFAP; 30 m in length, 0·53 mm ID and 1·2 μm film thickness). The column was operated at a temperature program of 70°C for 4 min followed by 140°C for 3 min. The temperatures of the injection port and detector were both 200°C. Helium was used as the carrier gas at a flow-rate of 40 ml/min. The TOC content was analyzed using a TOC autoanalyzer (Shimadzu, TOC-5000A).

In wastewater engineering, the biomass content in a treatment system is often represented by the concentration of volatile suspended solids (VSS). However, the VSS contents in the batch reactors were very low, and could not be accurately measured. Thus, the biomass in this study was estimated indirectly from the protein concentration. Assuming that the biomass has a chemical formula of $C_5H_7O_2N$, each gram of biomass contains 0·124 g of N and 0·531 g of C. Because the average nitrogen content in protein is 16·0% (Speece and McCarty,

1964; Noguera *et al.*, 1994; Kuo *et al.*, 1996), each gram of protein in the mixed liquor represents 1·29 g (1·29 = 0·160/0·124) of biomass containing 0·685 g of C.

RESULTS

Chemostat reactor in steady state

During days 77–90, the effluent contained, on average, 2124 mg/l of residual acetate and 1043 mg/l of soluble TOC. The concentration of dissolved methane was estimated as 15·0 mg/l, based on its partial pressure being 85·6 kPa. The corresponding carbon contents of acetate and dissolved methane were 850 and 11 mg-C/l, respectively. The SMP content in the effluent was estimated, accordingly, as 182 mg-C/l. The suspended solids in the chemostat effluent contained an average of 144 mg/l of protein, equivalent to 186 mg/l of biomass containing 108·4 mg/l of C.

Based on these results, each gram of acetate (containing 0·400 g of C) in the chemostat reactor was converted into 0·227 g of methane, 0·0484 g of biomass and 0·0251 g-C of SMP. Each acetate molecule is stoichiometrically converted to one molecule each of methane and carbon dioxide. Accordingly, the 0·400 g of C in each gram of acetate was converted to 0·170 g-C each of methane and carbon dioxide, plus 0·0257 g-C of biomass and 0·0251 g-C of SMP. The mass balance of C was 97·7%.

SMP production of various initial pH and acetate concentrations

Figure 1 illustrates that there was little difference in the three batch experiments treating feed solutions containing 850–950 mg/l of acetate at initial pH values of 6·0, 7·0 and 8·0. Acetate degraded linearly at an average rate of 11·3 mg/(l·h) initially in the three experiments. It became depleted after 65–107 hours. In all batches, production of methane reached a maximum of 400 ml (Fig. 1b), whereas the SMP reached a maximum concentration of 11·1 mg-C/l (Fig. 1c). There was little pH variation in the reactor started at pH 8, but pH increased slightly in the other two reactors, from pH 6·0 to 7·1 and from pH 7·0 to 7·4 at the end, respectively. Slight changes in pH near neutrality did not appear to affect acetate degradation.

Two additional experiments were conducted treating wastewater containing 750 mg/l of acetate, but at the initial pH of 5 and 9. As illustrated in Fig. 2, acetotrophic methanogenesis and SMP production did not take place in either reactor. The pH values in both reactors were adjusted to neutrality on hour-135. Figure 2 illustrates that the reactor which was initially at pH 5 was still unable to degrade acetate; whereas the other reactor, which was initially at pH 9, was able to convert acetate to methane, and to produce SMP. After a short adjustment period, acetate began to degrade but at a rate considerably lower than those batches with the initial pH values of 6·0–8·0. Figure 2a illustrates that it took 260 h for the complete depletion of acetate, as compared to 65–107 h illustrated in Fig. 1a. Figure 2b and c illustrates that the methane production and SMP production were in correspondence to the acetate depletion, as in Fig. 1.

These results show that acetotrophic methanogens were sensitive to pH (Mah and Smith, 1981). Methanogenic activities were completely inhibited at pH 5 and 9, but the inhibition at pH 5 was persistent long after the pH was returned to neutrality, whereas the inhibition at pH 9 was not.

Figure 3 illustrates the results of six batch experiments at initial pH values of 7·0–7·2, including one batch containing 950 mg/l of acetate which was

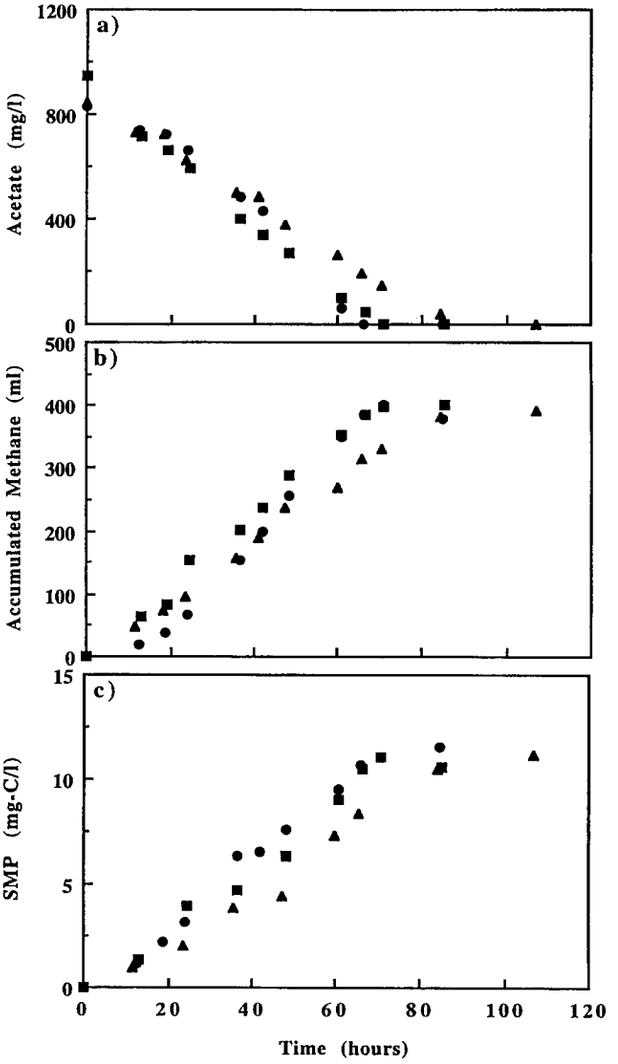

Fig. 1. Batch degradation of acetate ranging from 840 to 950 mg/l at pH 6–8: (a) residual acetate in mixed liquor; (b) methane in biogas; and (c) SMP produced (● pH 6; ■ pH 7; ▲ pH 8).

plotted in Fig. 1, and five additional batches of which the feed solutions respectively contained 600, 1200, 1800, 3500 and 6000 mg/l of acetate. The final pH at the end of these batch experiments remained within pH 7·2–7·9. Figure 3 shows that, as one would expect, acetate degradation (Fig. 3a), methane production (Fig. 3b) and SMP production (Fig. 3c) increased with acetate concentrations.

SMP, methane and protein productions versus acetate consumption

Figure 4 illustrates the linear relationships between acetate consumption and productions of SMP, methane and biomass in the eight batch experiments previously discussed at pH values ranging from 6 to 8. Slopes in Fig. 4 illustrates that for each milligram of acetate degraded, 0·015 mg-C of SMP (Fig. 4a), 0·392 ml (i.e. 0·247 mg) of methane (Fig. 4b) and 0·0297 mg of biomass (Fig. 4c) were produced. This means that 0·400 mg of C in each gram of acetate was converted to 0·015 mg-C of SMP, 0·185 mg-C each of methane and carbon dioxide, and 0·016 mg-C of biomass. The mass balance on C is 100·3%.

Yield of SMP

Results of this study show that SMP were produced during acetotrophic methanogenesis, as reported by Kuo *et al.* (1996). However, the SMP yield was dependent upon the reactor operation. Converting each milligram of acetate to methane produced 0·0251 mg-C of SMP in the chemostat reactor, but only 0·0150 mg-C of SMP in batch reactors. There were no yield data available in the literature for comparison.

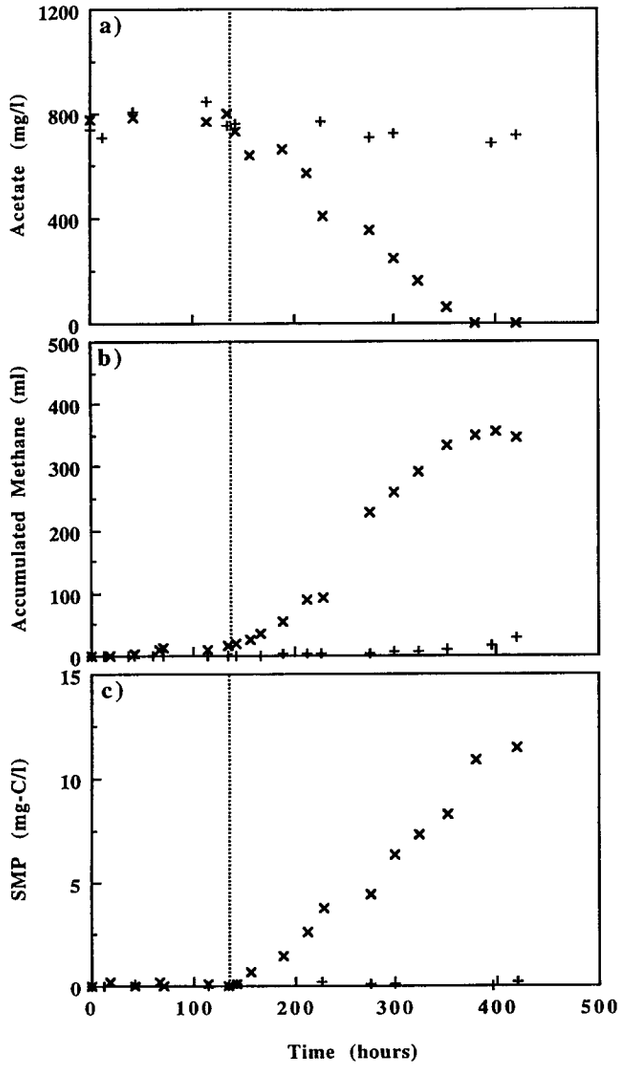

Fig. 2. Batch degradation of acetate ranging from 740 to 760 mg/l initially at pH 5 and 9 but adjusted to pH 7 after hour-135: (a) residual acetate in mixed liquor; (b) methane in biogas; and (c) SMP produced (+ pH 5; × pH 9).

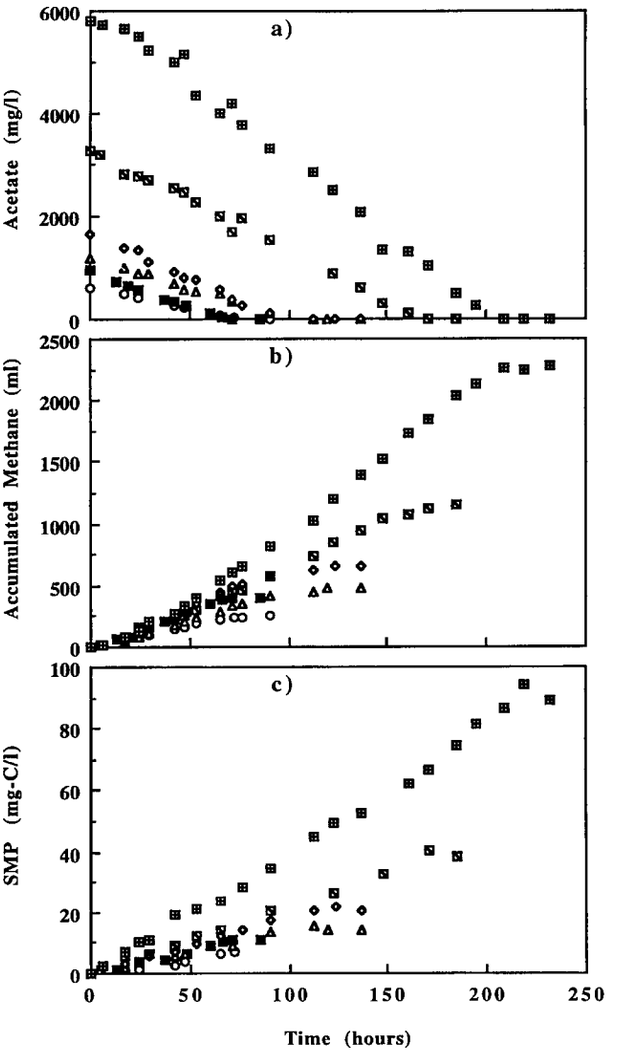

Fig. 3. Batch degradation of acetate at neutral pH and various initial concentrations: (a) residual acetate in mixed liquor; (b) methane in biogas; and (c) SMP produced (○ 600 mg/l; ■ 950 mg/l; △ 1200 mg/l; ◇ 1800 mg/l; □ 3500 mg/l; and ⊞ 6000 mg/l).

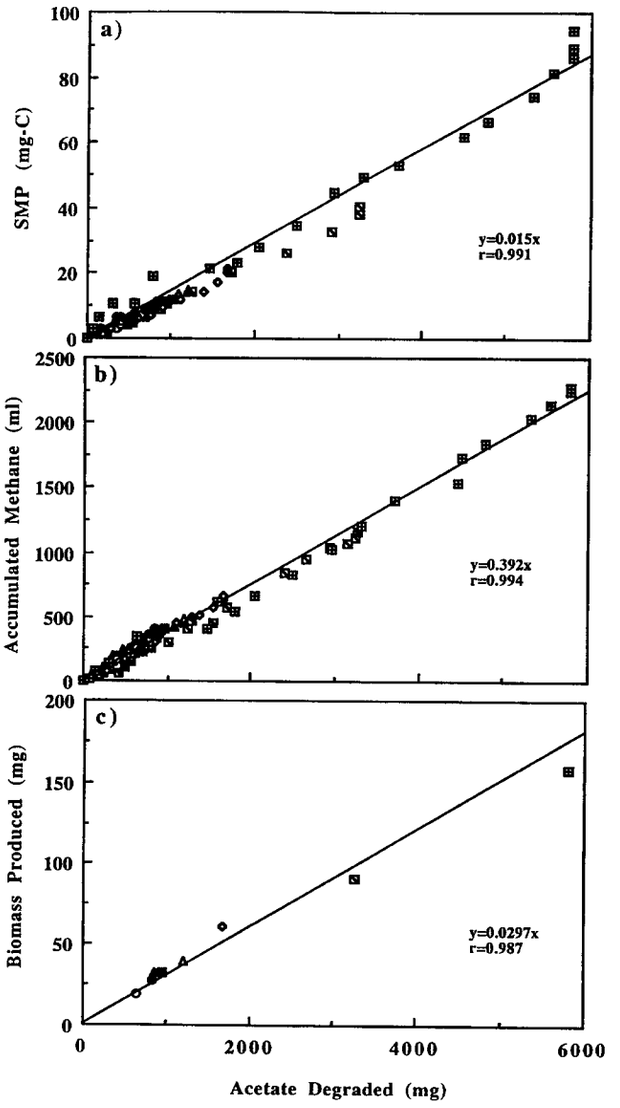

Fig. 4. Correlations between consumed acetate versus (a) SMP, (b) methane production, and (c) biomass production.

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REFERENCES

Boero, V. J., Eckenfelder, W. W. & Bowers, A. R. (1991). Soluble microbial product formation in biological systems. *Wat. Sci. Tech.*, **23**, 1067–1076.

Callander, I. J. & Barford, J. P. (1983a). Precipitation, chelation, and the availability of metals as nutrients in anaerobic digestion. I. Methodology. *Biotechnol. Bioengr.*, **25**, 1947-1958.

Callander, I. J. & Barford, J. P. (1983b) Precipitation, chelation, and the availability of metals as nutrients in anaerobic digestion. II. Applications. *Biotechnol. Bioengr.*, **25**, 1959–1970.

Chudoba, J. (1967). Residual organic matter in activated sludge process effluents — I. Degradation of saccharides, fatty acids, and amino acids under batch conditions. *Technol. Wat.*, **12**, 39–75.

Fang, H. H. P. & Chui, H. K. (1993). Maximum COD loading capacity in UASB reactors at 37°C. *J. Environ. Engr.*, **119**, 1 103–119.

Jia, X., Furumai, H. & Kusuda, T. (1991). Change of hydrogen partial pressure and the formation of extracellular polymer during start-up of upflow anaerobic sludge bed reactor. *J. Japan Sewage Works Assoc. Res.*, **28**, 334 83–93.

Jia, X., Furumai, H. & Fang, H. H. P. (1996). Extracellular polymers of hydrogen-utilizing methanogenic and sulfate-reducing sludges. *Wat. Res.*, **30**, 1439–1444.

Kuo, W. C., Sneve, M. A. & Parkin, G. F. (1996). Formation of soluble microbial products during anaerobic treatment. *Wat. Environ. Res.*, **68**, 3 279–285.

Liley, P. E. & Gambill, W. R. (1973). Physical and chemical data. In: *Chemical Engineers' Handbook*, ed. Perry, R. H. and Chilton, C. H., 5th edition, Vol. 3. McGraw-Hill, New York, p. 98.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.

Mah, R. A. & Smith, M. R. (1981). The methanogenic bacteria. In: *The Prokaryotes*, ed. Starr, M. P., Stolp, H., Turper, H. G., Balows, A. and Schlegel, H. G. Springer-Verlag, Berlin, pp. 948–977.

Noguera, D. R., Araki, N. & Rittmann, B. E. (1994). Soluble microbial products (SMP) in anaerobic chemostats. *Biotechnol. Bioengr.*, **44**, 1040–1047.

Rittmann, B. E., Bea, W., Namkung, E. & Lu, C. J. (1987). A critical evaluation of microbial product formation in biological processes. *Wat. Sci. Tech.*, **19**, 517–528.

Speece, R. E. & McCarty, P. L. (1964). Nutrient requirements and biological solids accumulation in anaerobic digestion. *Proc. of 1st Int. Conf. on Wat. Poll. Res.* Vol. 2, pp. 305–322.