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ANAEROBIC DEGRADATION OF BUTYRATE IN A UASB REACTOR

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Abstract

Wastewater with concentrated butyrate was treated in a 2·8 l UASB (Upflow Anaerobic Sludge Blanket) reactor at 37°C and pH 7·1–7·7. The process consistently removed 97–99% of COD for loading rates up to 31 g COD/l/day. Of all the COD removed, 94·5% was converted to methane; the average sludge yield was 0·037 g VSS/g COD. Conversion of acetate to methane appeared to be the rate-limiting step. Sludge granules had a maximum specific methane production rate of 1·57 g methane COD/g VSS/day, but were unable to degrade propionate. The granules were 1–2 mm in size and had a densely-packed skin layer which comprised two types of microcolony: one was composed of cocci with abundant extracellular polymer and the other was composed of two bacterial species in juxtapositioned syntrophic association. The interior was mainly composed of Methanothrix*-like bacteria, a large number of which were entwined into rope-shaped aggregates.*

Key words: Butyrate, degradation, granule, methanogenic activity, *Methanothrix*, syntrophic association.

INTRODUCTION

Under anaerobic conditions, complex organic pollutants are hydrolyzed and then converted by acidogens to volatile fatty acids (VFA), which are further converted to acetate and CO_2/H_2 by acetogens, and finally the acetate and CO_2/H_2 are converted by methanogens to methane. Butyrate is one of the major intermediates in the anaerobic degradation of complex organic pollutants (Gujer & Zehnder, 1983). On one hand, Harper (1989) estimated that in the anaerobic treatment of a soft-drink wastewater about 60% of the glucose was converted to methane through butyrate. On the other hand, Henson *et al.* (1986) reported that high concentrations of butyrate could inhibit methanogenesis.

Conversion of butyrate to acetate is thermodynamically unfavorable unless the acetate and hydrogen produced by the acetogens can be readily removed by acetotrophic and hydrogenotrophic bacteria respectively (Gujer & Zehnder, 1983). Of the 19 species of syntrophic acetogens identified so far (Li *et al.*, 1994), only five were found capable of converting butyrate, including *Syntrophomonas wolfei* (McInerney *et al.*, 1981), *Syntrophomonas sapovorans* (Roy *et al.*, 1986), *Syntrophospora bryantii* (Stieb & Schink, 1985; Zhao *et al.*, 1990), a thermophilic acetate-utilizing methanogenic bacterium (Ahring & Westermann, 1987*a*), and Strains SF-1 and NSF-2 (Shelton & Tiedje, 1984). Although research has been carried out to study the kinetics of butyrate degradation (Ahring & Westermann, 1988; Lawrence & McCarty, 1969; Massey & Pohland, 1978), and the population dynamics in a fluidized-bed reactor treating butyrate (Zellner *et al.*, 1991), little has been reported on the treatment of wastewater with concentrated butyrate in high-rate anaerobic reactors.

Among the high-rate anaerobic reactors for wastewater treatment, the upflow anaerobic sludge blanket (UASB) process (Lettinga *et al.*, 1980; Hulshoff Pol & Lettinga, 1986; Fang *et al.*, 1990) has gained popularity in recent years, with over 200 installations worldwide (Lettinga & Hulshoff Pol, 1991). The built-in gas–liquid–solid separator enables the bacteria to aggregate inside the UASB reactor while forming granules, which not only increase the overall density of the biomass but also improve the ability of the sludge to settle. Furthermore, a sludge granule forms an ecosystem which could facilitate syntrophic association of various bacteria (Fang *et al.*, 1994). This study was conducted to examine the efficacy of the UASB process treating wastewater with concentrated butyrate and to investigate the microstructure (MacLeod *et al.*, 1990; Grotenhuis *et al.*, 1991), the specific methanogenic activity (SMA) (Dolfing & Mulder, 1985; Dolfing & Bloeman, 1985) and the microbial composition of the UASB granules.

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METHODS

The UASB reactor was 2·8 l in volume with an internal diameter of 84 mm and a height of 500 mm, as illustrated in Fig. 1. Five evenly distributed sampling ports were installed over the height of the column. Total biomass in the reactor was estimated from the profile of the volatile suspended solids (VSS) of the samples taken from these ports. On top of the reactor was a gas–liquid–solid separator with an internal diameter of 114 mm and a height of 250 mm, making a filled volume of 2·0 l. Volumetric loadings were calculated based on the reactor volume alone, excluding the volume of the separator. The reactor was water-jacketed and operated at a constant temperature of 37°C.

Prior to this study, flocculent sludge from an anaerobic sludge digester of the Shatin Wastewater Treatment Works, Hong Kong, was partially granulated in a 65 l UASB reactor for 2 months, using sucrose as the organic substrate. About 1·5 l of this partially-granulated sludge was used to seed the experimental reactor. The reactor was continuously fed with synthetic wastewater by a peristaltic pump. The wastewater was composed of butyrate as the sole organic substrate, plus trace metals and balanced nutrients using a formulation of a previous report (Fang & Chui, 1993*a*). The pH of the wastewater was kept in the range of 6·0–6·5 by dosing with sodium bicarbonate. The sampling strategies and the analytical procedures, such as the methane content in biogas, the VFA levels in effluent, etc., also followed those reported previously (Fang & Chui, 1993*a*).

The initial COD loading rate to the reactor was 4 g/l/day, corresponding to 2100 mg/l of COD in the wastewater and a hydraulic retention time (HRT) of 12·5 h. The loading rate was increased stepwise only when the COD removal efficiency reached over 80%. The loading was increased initially by increasing the butyrate concentration and later by reducing the HRT. The concentrations of VFA (from acetate to valerate) were monitored by gas chromatography (HP 5890-II). Granule samples were taken for analyses of the microstructure, SMA and bacterial composition on day 190, when the reactor had been operated at 10 g COD/l/day for over 5 months. The microstructure of the UASB granules was examined not only by optical microscopy, but also by scanning and transmission electron microscopies (SEM and TEM). The instruments and the sample preparation procedures were as reported previously (Fang & Chui, 1993*b*). The SMA of the granules were measured in duplicate in serum vials based on the method (Dolfing & Mulder, 1985; Hwang & Cheng, 1990) adapted from Owen *et al.* (1979). The individual substrate used for the SMA measurements was either formate, acetate, propionate, or butyrate. The bacterial population of each trophic group in the granules was enumerated, after being dispersed by a blender and an ultrasonic homogenizer, using the most probable number (MPN) method (Chartrain & Zeikus, 1986; Li & Noike, 1992).

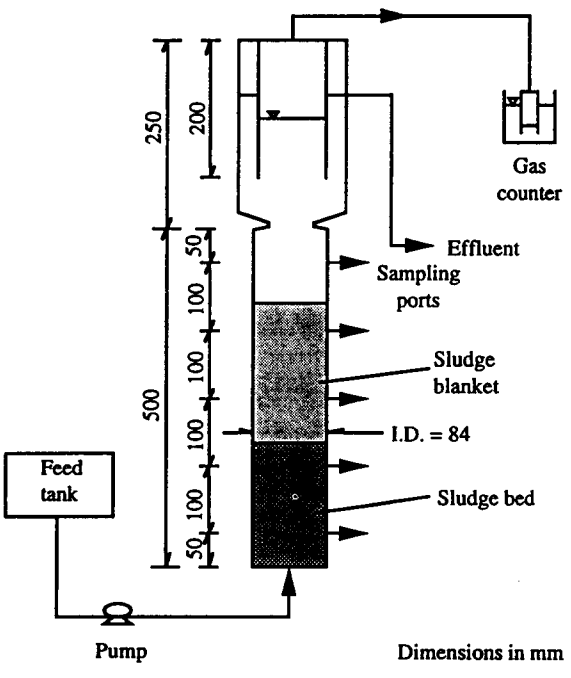

Fig. 1. The UASB reactor set-up.

RESULTS AND DISCUSSION

COD Removal efficiency

Fang and Chui (1993*a*) reported that the COD removal efficiency of a UASB reactor was, in general, dependent on the COD loading rate and was insensitive to either the HRT or the COD level in the wastewater individually. In this study, COD loading rate was increased either by increasing the butyrate concentration in the wastewater or by decreasing the HRT, as summarized in Table 1.

The pH of the mixed liquor in the reactor was kept at a constant value of 7·1–7·7. Figure 2 illustrates the COD loading rate and the performance of the UASB reactor throughout this study. The COD removal efficiencies were based on the soluble COD in the effluent and the total COD in the wastewater, which was attri-

Table 1. Wastewater COD, HRT and COD-loading-rates

Day	Wastewater COD (mg/l)	HRT (h)	COD-loading-rate (g/l/day)
1–10	2100	12·5	4
11–17	4500	12·5	9
18–32	6600	12·5	13
33–228	5300	12·5	10
229–235	7700	12·5	15
236–250	10000	12·5	20
251–262	13000	13·0	24
263–270	15500	12·0	31
271–299	15500	10·0	37
300–332	15500	7·5	50
333–341	15500	5·5	68
342–348	15500	4·5	83

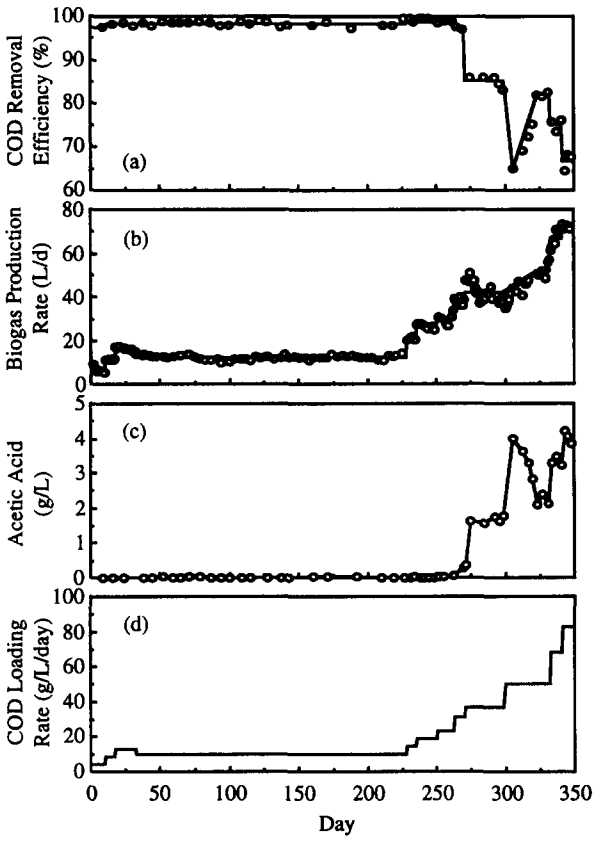

Fig. 2. Conditions and performance of the UASB reactor throughout the experiment: (a) COD removal efficiency; (b) biogas production rate; (c) residual acetate in effluent; (d) COD-loading rate.

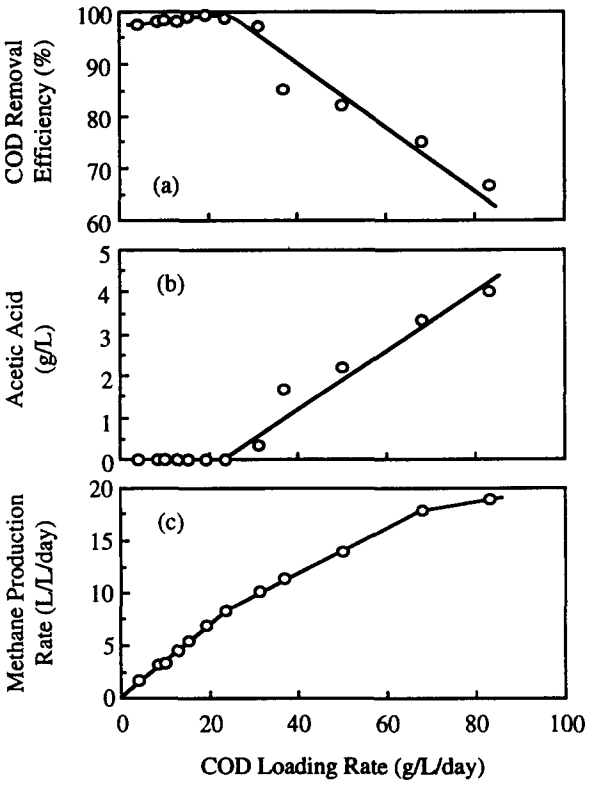

Fig. 3. Performance of the UASB reactor at various COD loading rates: (a) COD removal efficiency; (b) acetate concentration in effluent; (c) methane production rate.

buted solely to the butyrate. As illustrated in Fig. 2(a), the COD removal efficiency was consistently 97–99%, up to day 270 when the loading rate was 31 g COD/l/day [Fig. 2(d)]. Starting from day 271, when the loading rate was increased to 37 g COD/l/day and higher, the removal efficiency was sharply reduced. On day 300, when the loading rate was increased to 50 g COD/l/day, the removal efficiency was reduced to 65% initially but it gradually recovered and levelled off to 82% in 20 days. After the removal efficiency had become steady the loading rate was further increased. The experiment was terminated on day 348 at 83 g COD/l/day, when the COD removal efficiency was reduced to 67%. Figure 2(b) shows that the biogas production rate increased with the COD loading rate, as expected, and Fig. 2(c) illustrates increased acetate concentration in the effluent at high loading rates.

Figure 3 shows the performance of the reactor at various COD loading rates. At loading rates of 31 g COD/l/day or higher, the COD removal efficiency declined linearly with the increase of loading rate [Fig. 3(a)]. The decline was mainly due to the accumulation of acetate in the effluent [Fig. 3(b)]. Under the anaerobic conditions and in the absence of sulfate and nitrate, butyrate was converted to acetate according to the following equation (Thiele & Zeikus, 1988):

$$\text{butyrate}^- + 2H_2O \rightarrow 2\,\text{acetate}^- + 2H_2 + H^+. \quad (1)$$

There was no residual butyrate, nor other VFA, detected in the effluent throughout the experiment. A similar observation of acetate accumulation in the effluent was also reported by Henson *et al.* (1986) on the degradation of butyrate in a thermophilic digester. The high concentrations of acetate and the absence of residual butyrate in the effluent seems to indicate that, in the degradation of butyrate, conversion of acetate to methane is the rate-limiting step. A similar observation was reported by Ahring and Westermann (1987*b*) for a continuous culture.

Methane production and COD balance

The methane content of the biogas was in the range of 70–80% throughout the study. Figure 3(c) shows that the volumetric methane production rate increased linearly with the COD loading rate up to 31 g COD/l/day. Since each gram of methane is equivalent to 4 grams of COD, the specific methane production rate (SMPR), expressed as grams of methane COD produced daily by each gram of VSS, can thus be calculated. Figure 4 shows that the SMPR increased linearly with the specific substrate utilization rate (SSUR) with a slope of 0·945. The slope indicates that, of all the COD removed, about 94·5% was converted to methane. The rest was converted to biomass with an average yield of 0·037 g VSS/g COD, which is comparable to the 0·047 g VSS/g COD for butyrate utilization reported by Lawrence and McCarty (1969).

Specific methanogenic activity and microbial composition

Table 2 shows the SMA of the butyrate-degrading granules using individual fatty acids as the sole organic substrate. Corresponding SMA data for UASB granules treating different wastewaters are also listed for comparison. The SMA in the present experiments using butyrate as the substrate was 1·31 g methane COD/g VSS/day, which was comparable with those using acetate and formate as substrates; this seems to be supporting evidence that acetogenesis is probably not the rate-limiting step in butyrate degradation. Table 2 also shows that the butyrate-degrading granules did not exhibit any SMA when using propionate as the substrate, this is also in accordance with the studies on pure cultures (Li *et al.*, 1994) that none of the butyrate-degrading acetogens could degrade propionate. However, it is of interest to note that, in two previous independent studies (Grotenhuis *et al.*, 1991; Fang *et al.*, in press) propionate-degrading granules were found capable of degrading butyrate, as also shown in Table 2.

Using the MPN method, each milligram of granules was found to have $4{\cdot}6 \times 10^6$ hydrogenotrophic methanogens, $1{\cdot}4 \times 10^7$ formate-consuming methanogens, $2{\cdot}9 \times 10^7$ acetotrophic methanogens and $1{\cdot}4 \times 10^8$ butyrate-degrading bacteria. The population of propionate-degrading bacteria was not measured.

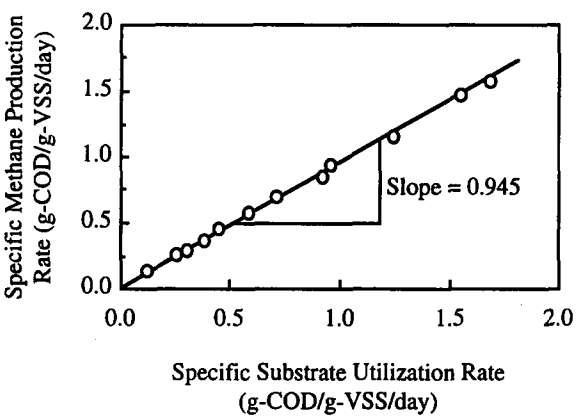

Fig. 4. Specific methane production rate at various substrate utilization rates.

Microstructure of granules

Butyrate-degrading granules were 1–2 mm in size. They had a densely-packed skin layer and exhibited satisfactory ability to settle. However, unlike any other UASB granules ever reported, the skin layers of a large number of butyrate-degrading granules were ruptured for no obvious reason, exposing the granule interior, which was mainly composed of *Methanothrix*-like bacteria, as illustrated in Fig. 5. *Methanothrix* is a strict acetotrophic methanogen, which can be identified by, among other features (Zehnder *et al.*, 1980), its size (about 0·6 μm) and the bamboo-shaped morphology when observed by TEM, as illustrated in Fig. 6. It is of interest to note that many of these *Methanothrix*-like filaments entwined into rope-shaped aggregates, as illustrated in Fig. 7. *Methanothrix*-like bacteria have also been found as the predominant species in the interior of many other UASB granules (Fang & Chui, 1993*a*; Fang *et al.*, 1993; Fang *et al.*, 1994). With a very small half-rate constant of 30 mg COD/l (Gujer & Zehnder, 1983), it outcompetes other methanogenic bacteria when the acetate concentration is low, as in the interior of a granule.

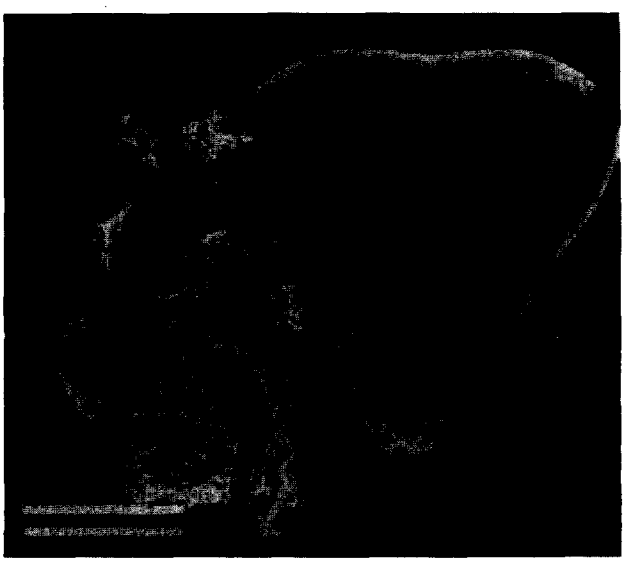

Fig. 5. A butyrate-degrading granule under SEM (bar = 1 mm).

Table 2. Specific methanogenic activities (SMA) of granules treating various wastewaters and using fatty acids as substrates

Wastewater	Substrate SMA (g-methane-COD/g-VSS/day)				Source
	Formate	Acetate	Propionate	Butyrate	
Butyrate	0·96	1·51	n/d	1·31	This study
Propionate	1·17	1·02	0·49	0·58	Fang *et al.* (in press)
Propionate	n/a	1·89	1·78	1·32	Grotenhuis *et al.* (1991)
Propionate	n/a	0·55	0·52	n/a	Dolfing and Bloeman (1985)
Ethanol	n/a	2·60	0·43	0·16	Grotenhuis *et al.* (1991)
Sugar refinery	n/a	0·30	0·31	0·28	Grotenhuis *et al.* (1991)
Brewery	1·26	0·49	0·13	0·12	Fang *et al.* (1993)

n/a = Not available.
n/d = Not detectable.

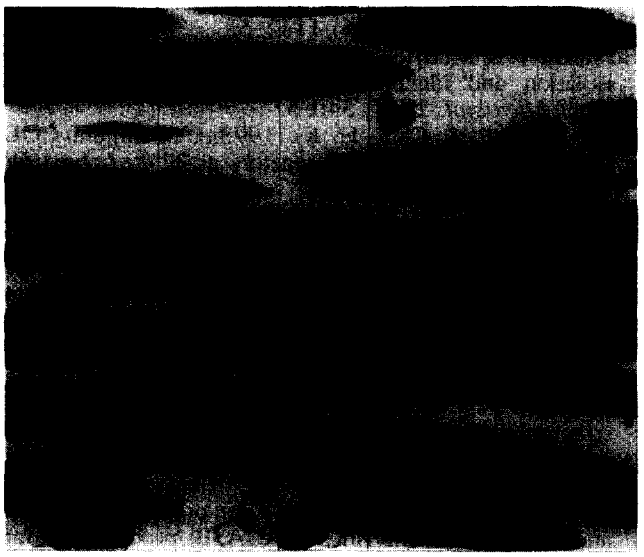

Fig. 6. *Methanothrix*-like filaments under TEM (bar = 2 μm).

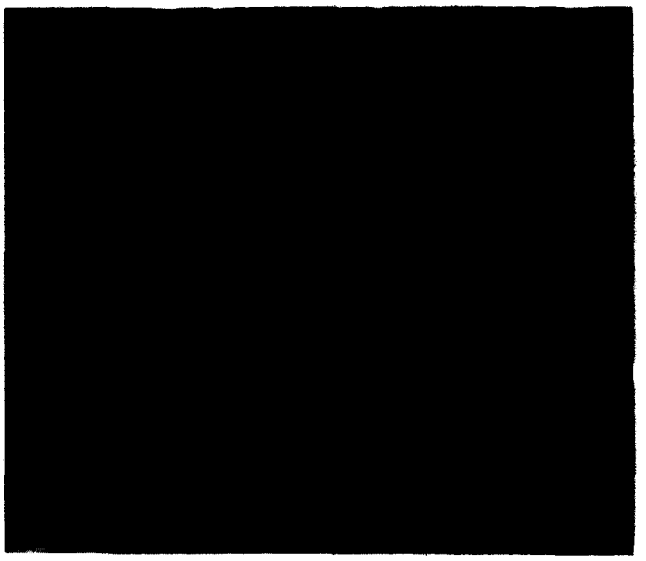

Fig. 8. A section of granule observed by optical microscopy under epi-fluorescent excitation (bar = 100 μm).

Fig. 7. Bundles of *Methanothrix*-like filaments under SEM (bar = 40μm).

Fig. 9. Microcolony of an unknown bacterium in the skin layer of the granule (bar = 2 μm).

Unlike the three-layered structure observed for granules treating sucrose (MacLeod *et al.*, 1990) and brewery wastewater (Fang *et al.*, 1993), SEM micrographs show that butyrate-degrading granules exhibited a simpler structure with a skin layer and an interior mainly composed of *Methanothrix*-like bacteria. The skin layer was also observed by optical microscopy. All methanogens have F_{350} and F_{420} co-factors, which have not been found in any other bacteria. Methanogens, then, can be readily identified by their emitted fluorescence under epi-fluorescent excitation at both 350 and 420 nm (Edwards & McBride, 1975). The distribution of methanogens of a butyrate-degrading UASB granule was examined using a recently developed technique (Fang & Chui, 1993*b*). As illustrated in Fig. 8, the granule had a skin layer of 40–60 μm, which exhibited a high fluorescence intensity, probably attributable to the hydrogenotrophic methanogens. Distribution of *Methanothrix*-like bacteria, which emitted much dimmer fluorescence (Zehnder *et al.*, 1980), beneath the skin layer was rather uniform.

During degradation, butyrate in the bulk solution was first converted by syntrophic acetogens, such as *Syntrophomonas wolfei* (McInerney *et al.*, 1981; Li *et al.*, 1994), into acetate in the skin layer near the surface of the granule. The change of standard Gibbs free-energy for the reaction at neutral pH is 48·1 kJ. Thus, the reaction is thermodynamically unfavorable unless the two products, acetate and hydrogen, are removed either by diffusion or by syntrophic reactions. It is therefore reasonable to expect that the skin layer was composed of a variety of bacteria, including not only

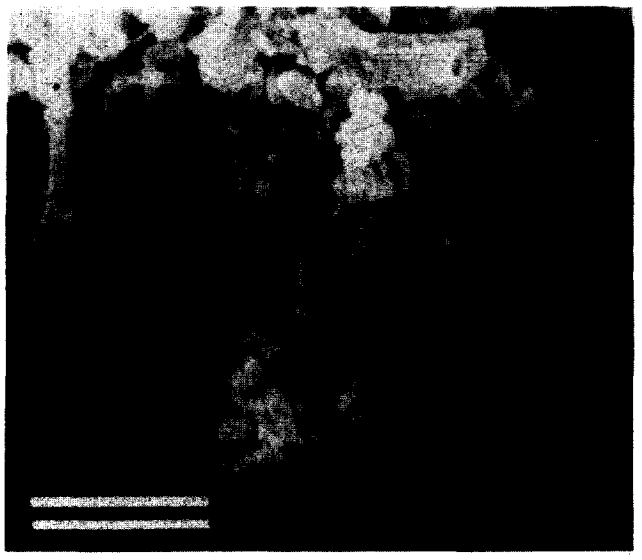

Fig. 10. A microcolony in the skin layer showing bacteria in juxtapositioned syntrophic association (bar = 4 μm).

syntrophic acetogens, but also acetotrophic and hydrogenotrophic methanogens. These bacteria could not be identified simply by morphology alone. However, SEM and TEM micrographs did show two types of microcolony in the skin layer: one was composed of cocci with abundant extracellular polymer, as illustrated in Fig. 9; and the other was composed of two species of bacteria which appeared to be juxtaposed, as illustrated in Fig. 10, for syntrophic association.

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