Contact Angle Measurement and Cell Hydrophobicity of Granular Sludge from Upflow Anaerobic Sludge Bed Reactors

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The contact angle, which is generally used to evaluate the hydrophobicities of pure bacterial strains and solid surfaces, was used to study mixed cell cultures of bacteria involved in anaerobic digestion. Previously published data and data from this study showed that most acidogens are hydrophilic (contact angle, $<45^\circ$) but most of the acetogens and methanogens isolated from granular sludge are hydrophobic (contact angle, $>45^\circ$). The hydrophobicities of mixtures of hydrophilic and hydrophobic cells were found to be linearly correlated with the cell mixing ratio. The hydrophobicities of cells present in effluents from upflow anaerobic sludge bed reactors which were treating different types of substrates were different depending on the reactor conditions. When the reactor liquid had a high surface tension, cells sloughing off from sludge granules, as well as cells present on the outer surfaces of the granules, were hydrophobic. Short-term batch enrichment cultures revealed that proteins selected for highly hydrophilic cells. Long-term in-reactor enrichment cultures revealed that sugars selected for hydrophilic acidogens on the surfaces of the granules, while fatty acids tended to enrich for hydrophobic methanogens. When linear alkylbenzenesulfonate was added, the cells on the surfaces of granules became more hydrophilic. Control tests performed with pure cultures revealed that there was no change in the surface properties due to linear alkylbenzenesulfonate; hence, the changes in the wash-out observed probably reflect changes in the species composition of the microbial association. A surface layer with moderate hydrophobicity, a middle layer with extremely high hydrophobicity, and a core with high hydrophobicity could be distinguished in the grey granules which we studied.

Hydrophobicity of the bacterial cell surface is one of the most important factors which govern the mechanism of bacterial adhesion. Other well-known factors are electrostatic interaction (24), convective flow and diffusion in a medium (14), and the presence of extracellular polymers (20) and special cell appendages (13). Hydrophobic cellular interactions with various types of surfaces and interfaces play an important role in a great number of ecological systems. Techniques for determining cell hydrophobicity include adhesion of cells to hexadecane droplets (15), partitioning of cells in a two-phase system (e.g., polyethylene glycol-dextran) (3), the salt aggregation test (9), the internal hydrophobicity test (17), and measurement of the contact angle of apolar droplets (e.g., α -bromonaphthalene) or polar droplets (e.g., water) on a bacterial layer (1, 2, 23). The contact angle technique is used most frequently since it allows workers to estimate the surface energy (γ_{BV}) of a bacterial surface (1). In previous studies in which workers evaluated the hydrophobicity of aerobic bacteria typical of soil (23) and the hydrophobicity of anaerobic bacteria isolated from methanogenic granular sludge (4), it was found that most aerobes are hydrophilic (contact angle, $<45^\circ$) and that anaerobes, including strains isolated from methanogenic anaerobic granular sludge, are rather hydrophobic (contact angle, $>45^\circ$). When we deal with anaerobic degradation of organic wastes, there are generally three trophic groups of bacteria which contribute to the sequential substrate utilization process; these groups are the fermentative bacteria or acidogens, the oxidative acetogens which convert higher volatile fatty acids (VFA) to acetate and

hydrogen, and the methanogens. Therefore, we decided to examine the cell hydrophobicities of these different types of bacteria, not only as pure strains but also as mixed cultures, to better comprehend the behavior of methanogenic associations under various reactor conditions.

MATERIALS AND METHODS

Strains and culture conditions. A total of 16 pure strains obtained from different sources were grown in axenic cultures and examined to determine their hydrophobicities. *Methanosaeta concillii* Opfikon (= DSM 2139), *Methanosarcina mazei* DSM 2907, *Selenomonas acidaminophila* DSM 3853^T (T = type strain), *Clostridium proteolyticum* DSM 3090^T, and *Desulfovibrio vulgaris* DSM 2119 were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Braunschweig, Germany, and were grown in DSM media 334, 318, 339, 339, and 1, respectively. *Methanobacterium formicicum* CLH1, *Streptococcus faecalis* SF6 (*Enterococcus faecalis*), *Streptococcus thermophilus* ST69 (*Streptococcus salivarius* subsp. *thermophilus*), *Lactobacillus bulgaricus* LB20, *Lactobacillus helveticus* LH7, *Lactobacillus helveticus* LH22, *Bacillus cereus* BC6, and *Propionibacterium acidipropionici* PA1 were obtained from the Department of Food Science and Microbiology, University of Milan, Milan, Italy, and were grown in DSM media 119, 53, 53, 11, 11, 11, 1, and 91, respectively. *Lactobacillus plantarum* WT1 and *Escherichia coli* CM120 were available at the Laboratory of Microbial Ecology, University of Ghent, Ghent, Belgium, and were grown in DSM media 11 and 1, respectively. *Veillonella* sp. strain S119 was isolated and grown in the medium described previously (17). Strains other than methanogens were harvested after at least 24 h of incubation at 30°C to eliminate possible variation in cell hydrophobicity during the exponential growth phase (24). Methanogenic bacteria were collected after at least 1 week of incubation.

Reactor conditions and performance. Cell wash-out (effluent biomass) and granules were harvested from five laboratory-scale upflow anaerobic sludge bed (UASB) reactors (volume, 2.3 liters) fed with synthetic wastewater; these reactors were designated reactors S1, S2, A1, A2, and A3. A stock solution of nutrients and trace elements was prepared and added as described previously (6). The final solution contained (per liter) 0.5 mg of H_3BO_3 , 20 mg of $FeCl_3 \cdot 3H_2O$, 0.5 mg of $ZnCl_2 \cdot 4H_2O$, 5 mg of $MnCl_2 \cdot 4H_2O$, 0.5 mg of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.5 mg of $CoCl_2 \cdot 6H_2O$, 0.5 mg of $NiCl_2$, 5 mg of disodium EDTA, 1 mg of Na_2SeO_3 , 0.5 mg of $AlCl_3$, 5 mg of resazurin, 100 mg of yeast extract, 60 mg of NH_4Cl , 15 mg of $(NH_4)_2SO_4$, 15 mg of KH_2PO_4 , and 5 mg of $MgCl_2 \cdot 6H_2O$; 5. This solution also contained 0.01 ml of 36% HCl per liter. The ratio of chemical oxygen demand (COD) to nitrogen to phosphorus in the feed solution was

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maintained at 100:1.25:0.25 by adjusting the NH_4Cl and $(\text{NH}_4)_2\text{HPO}_4$ concentrations. The feed used for reactors S1 and S2 contained sucrose as the sole carbon source, and these reactors were operated at a volumetric loading rate of 10 g of COD per liter per day with a COD removal efficiency of 90%. Reactor A3 was fed with a mixture of VFA (20% acetate, 40% propionate, 40% butyrate). Reactors A1 and A2 were fed with the same VFA mixture as reactor A3, but portions of the feed COD were replaced by sucrose (30 and 10%, respectively). Reactors A1, A2, and A3 were operated at a loading rate of 20 g of COD per liter per day with a COD removal efficiency of 90%. All of the reactors were operated at $30 \pm 3^\circ\text{C}$. In addition, the feed preparations used for all reactors except reactor S1 were supplemented with 2 mg of linear alkylbenzenesulfonate (LABS) (C_{18} ; Aldrich) per liter in order to decrease the feed liquid surface tension from 68 to 48 mN/m. LABS is considered nonbiodegradable under anaerobic conditions (25).

Enrichment for acidogens. A cell suspension from reactor S1 effluent was grown for 24 h at 30°C in medium containing either 5 g of glucose per liter or 5 g of peptone per liter with or without 2 mg of LABS per liter. To do this, 0.5-ml portions of effluent from reactor S1 were inoculated into 50-ml portions of medium in 120-ml serum bottles, each sealed with a butyl rubber septum and an aluminum cap. The bottles were flushed with nitrogen gas for 1 to 2 min to remove any oxygen. The resulting cultures were subcultured again (100-fold dilution) and grown under the same conditions.

Granular sludge preparation. The different layers of harvested granules were separated arbitrarily under the magnifying lens of a colony counter (Spiral System MV) with a flush of phosphate-buffered saline generated with a micropipette fitted with a long metal needle. About 20 to 40 granules from each sample were peeled layer by layer. During the peeling procedure, the outer surface was easier to distinguish and easier to remove than the inner layers. Before the hydrophobicity was measured, each fresh or treated cell suspension was disintegrated by passing it through the needle (diameter, <0.1 mm) of a syringe three times in order to break down persistent conglomerates or flocs. We found that this simple procedure was more efficient than other treatments (e.g., sonication).

Contact angle and liquid surface tension measurements. Cells were centrifuged and washed twice in 0.1 M phosphate-buffered saline. Bacterial hydrophobicity was measured on a Micropore filter (pore size, $0.45 \mu\text{m}$) by determining the contact angle of 1 μl of saline water (0.1 M NaCl) on a dry bacterial layer at $20 \pm 3^\circ\text{C}$ (23). For a mixed culture, in order to prevent adsorption of hydrophobic cells at the air-liquid interface and separation of the species, the length of the filtration period was decreased by reducing the volume of the cell suspension. The following procedure was used: a 20-ml cell mixture was centrifuged at $10,000 \times g$ for 10 min, resuspended in 1 ml of phosphate-buffered saline, and vortexed before filtration. All contact angle values were obtained from at least six measurements. Liquid surface tension (γ_{LV}) was measured at $20 \pm 3^\circ\text{C}$ by the Wilhelmy plate method (12).

Combination of hydrophilic and hydrophobic strains. *E. coli* CM120 and *L. helveticus* LH22, a hydrophilic strain and a hydrophobic strain, respectively, were used in an experiment in which we examined a combination of hydrophilic and hydrophobic strains. Pure cultures (250 ml) of the two strains were harvested, washed as described above, and diluted with phosphate-buffered saline to obtain identical cell concentrations, as determined spectrophotometrically at 600 nm. A series of combinations of the two strains were prepared by mixing different volumes of the cell suspensions.

Microbial metabolic activity. The specific metabolic activity of a culture was determined from the maximum slope of the graph of amount of product per gram of volatile suspended solids versus time. The specific acidogenic activity of granules obtained from each reactor was determined by a previously described technique (22) by using glucose as the substrate. The samples were centrifuged at $10,000 \times g$ for 10 min and resuspended in distilled water that had been flushed with N_2 for 10 min. The final pellets were ground in a mortar to a fine amorphous paste, 2 g of glucose per liter, was added, and the pH was maintained at a set value by using an automatic titrator and 0.1 M NaOH. The maximum specific methanogenic activity test was performed with intact granules by the pressure bottle technique by using acetate as the substrate in an anaerobic dilution medium (21). The prepared medium had a final pH of 7.2 and contained (per liter) 4 g of sodium acetate, 2.5 g of KH_2PO_4 , 1.0 g of K_2HPO_4 , 1.0 g of NaCl, 0.1 g of MgCl_2 , 0.1 g of $\text{Na}_2\text{S} \cdot 7\text{H}_2\text{O}$, and 0.005 g of resazurin. The biomass loading value used was 0.25 to 2.0 g of COD per g of volatile suspended solids (11). The bottles were incubated at 33°C , and gas production was measured regularly by recording the pressure in each bottle with a tensimeter. The biogas volume was calculated by using the ideal gas law.

RESULTS

Hydrophobicities of pure cultures. Table 1 shows the contact angles of facultatively and strictly anaerobic bacteria together with the estimated γ_{BV} values of these bacteria. Our data and previously published data indicate that many facultative anaerobes (acidogens) are hydrophilic (facultative anaerobes with contact angles less than 45°); some exceptions are *Streptococcus mitior* T6, *Veillonella* sp. strain S119, and *L. helveticus*

TABLE 1. Water contact angles with saline and γ_{BV} values of bacteria

Strain or species	Contact angle ($^\circ$) ^a	γ_{BV} (mN/m) ^b
<i>Bacillus cereus</i> 6 ^c	8.1 ± 0.8	76
<i>Lactobacillus plantarum</i> ^c	11.4 ± 3.4	74
<i>Streptococcus faecalis</i> ATCC 6055 ^c	12.7 ± 1.1	73
<i>Escherichia coli</i> NCTC 9002 ^d	15.7 ± 1.2	71
<i>Lactobacillus bulgaricus</i> LB20 ^e	16.6 ± 2.6	71
<i>Escherichia coli</i> 055 ^e	16.7 ± 1.0	70
<i>Staphylococcus aureus</i> 049 ^e	18.5 ± 1.2	69
<i>Veillonella alcalescens</i> V1 ^f	20.0	68
<i>Escherichia coli</i> 2627 ^e	21.2 ± 0.7	68
<i>Escherichia coli</i> CM120 ^c	22.2 ± 0.6	68
<i>Staphylococcus epidermidis</i> 047 ^e	23.4 ± 0.5	67
<i>Escherichia coli</i> K-12 ^d	24.7 ± 0.4	66
<i>Streptococcus salivarius</i> HB ^f	26.0	66
<i>Listeria monocytogenes</i> ^e	26.1 ± 1.2	66
<i>Lactobacillus helveticus</i> LH7 ^e	26.5 ± 4.4	65
<i>Streptococcus thermophilus</i> ST69 ^c	28.0 ± 1.5	64
<i>Methanospirillum hungatei</i> JF1 ^g	31.8 ± 1.4	62
<i>Acinetobacter</i> sp. strain 210A ^d	32.6 ± 0.5	62
<i>Propionibacterium acidipropionici</i> PA1 ^c	34.4 ± 1.0	61
<i>Methanobacterium formicicum</i> CLH1 ^c	35.7 ± 4.0	60
<i>Streptococcus sanguis</i> CH3 ^f	42.0	56
<i>Desulfovibrio vulgaris</i> DSM 2119 ^c	45.5 ± 1.9	54
<i>Veillonella</i> sp. strain S119 ^c	47.7 ± 1.6	53
<i>Pelobacter propionicus</i> ^g	49.0 ± 1.4	52
<i>Methanobrevibacter arborophilicus</i> AZ ^g	53.8 ± 1.4	49
<i>Streptococcus mitior</i> T6 ^f	55.0	49
<i>Methanotherx soehngenii</i> 2139 ^{Tc}	58.7 ± 1.2	47
Acetogenic strain EF174 ^g	68.4 ± 1.4	41
Acetogenic strain EF173 ^g	69.3 ± 1.4	40
<i>Methanosaeta soehngenii</i> ^g	69.4 ± 1.4	40
<i>Pelobacter carbinolicus</i> RE1 ^g	70.0 ± 1.4	40
<i>Methanosarcina mazei</i> 2907 ^{Tc}	70.5 ± 5.5	40
<i>Methanosarcina barkeri</i> ^g	71.4 ± 1.4	39
<i>Selenomonas acidaminophila</i> DSM 3853 ^{Tc}	72.3 ± 0.4	39
<i>Methanobrevibacter</i> sp. strain PHC 2511 ^g	72.5 ± 1.4	38
<i>Methanobrevibacter</i> sp. strain PHC 259-3 ^g	73.0 ± 1.4	38
<i>Methanobrevibacter</i> sp. strain PHC 254 ^g	73.6 ± 1.4	38
<i>Methanobrevibacter</i> sp. strain PHC 259-1 ^g	73.8 ± 1.4	38
<i>Methanobrevibacter</i> sp. strain PHC 256-1 ^g	75.5 ± 1.4	37
<i>Methanobrevibacter</i> sp. strain PHC 256-2 ^g	76.5 ± 1.4	36
Acetogenic strain EF175 ^g	78.5 ± 1.4	35
Anaerobic strain EE121 ^g	82.0 ± 1.4	33
<i>Pelobacter carbinolicus</i> BE4 ^g	86.1 ± 1.4	31
<i>Clostridium proteolyticum</i> DSM 3090 ^{Tc}	94.4 ± 0.9	26
<i>Lactobacillus helveticus</i> LH22 ^c	123 ± 2.9	10

^a The values are means $\pm$ standard deviations ($n = 6$).

^b Values were estimated by the method of Neumann et al. (12).

^c Data from this study.

^d Data from reference 23.

^e Data from reference 1.

^f Data from reference 2.

^g Data from reference 4.

LH22. The high hydrophobicity of *L. helveticus* LH22 appears to be a characteristic of the strain and not of the species. In fact, the other *Lactobacillus* strains tested (*L. plantarum* WT1 and *L. bulgaricus* LB20) exhibited low contact angles, and another strain of *L. helveticus*, strain LH7, was also hydrophilic. Strains EF173, EF174, EF175 are reductive acetogens (4). These acetogens and methanogens appear to be more hydrophobic (contact angles, $>45^\circ$) than the acidogens.

Hydrophobicities of binary mixtures. The relationships observed when we used different proportions of *E. coli* CM120 (a hydrophilic strain) and *L. helveticus* LH22 (a hydrophobic strain) and their water contact angles are shown in Fig. 1. The

FIG. 1. Relationship between the water contact angles of binary mixtures of *E. coli* CM120 (a hydrophilic organism) and *L. helveticus* LH22 (a hydrophobic organism) and the ratio of the two organisms.

hydrophobicity increased in proportion to the number of hydrophobic (*L. helveticus* LH22) cells added. Hence, the hydrophobicity of a mixed culture is apparently an average of the hydrophobicities of its components.

Hydrophobicities of pure and mixed cultures in batch tests.

The effects of different growth conditions and the presence of 2 mg of LABS per liter on cell hydrophobicity are shown in Table 2. The hydrophobicities of three pure strains (*C. proteolyticum* DSM 3090^T, *E. coli* CM120, and *Selenomonas acidaminophila* DSM 3853^T) were not significantly different in the absence and in the presence of LABS. During short-term enrichment of wash-out cells from reactor S1, LABS did not significantly alter the overall cell hydrophobicity. However, wash-out cells grown in protein-rich medium appeared to be more hydrophilic than cells grown in medium containing glucose (Table 2). Apparently, during short-term enrichment lasting a few days, the surface behavior of the microbial associa-

tion changed under the influence of the substrate, probably because of the selection of different bacterial populations.

Hydrophobicities of mixed cultures from the UASB reactors.

The wash-out cells (Table 3) in the effluent of reactor S1 (contact angle, $72.7 \pm 0.9^\circ$) appeared to be significantly more hydrophobic than the wash-out cells in the effluent of reactor S2 (contact angle, $58.5 \pm 1.1^\circ$), which was supplemented with LABS and thus had a lower γ_{LV} . This suggests that LABS influenced the species composition during growth of the microbial association in the reactor. Three types of granules (grey, white, and black) were distinguished visually. Large grey granules were found mainly in reactors S1 and S2, which were fed with the same principal substrate (100% sucrose). These granules had three layers with different hydrophobicities, as follows (Table 3): the outer layer exhibited the lowest overall hydrophobicity; the middle layer was very hydrophobic; and the inner layer or core was moderately hydrophobic. The outer cells of the grey granules obtained from reactor S2 appeared to be more hydrophilic than the outer cells of the grey granules obtained from reactor S1. This is consistent with the data obtained for the wash-out cells from reactors S1 and S2.

Table 3 also shows the hydrophobicities of wash-out cells and granulated biomass samples obtained from reactors fed with different amounts of VFA and supplemented with LABS (i.e., reactors S2, A1, A2, and A3). The hydrophobicities of the wash-out cells were in the following order: S2 $\approx$ A1 < A3 < A2. A tendency to have more hydrophobic cells (i.e., acetogens and methanogens) on the outside of the granules was observed when the concentration of VFA in the feed was high (Table 3). Actually, the specific activities of the acidogens and methanogens in the granules (Table 4) are consistent with the hypothesis that when high levels of VFA are present in the feed, acidogens are scarce compared with methanogens.

DISCUSSION

Acidogens are primary degraders in the anaerobic food chain, while acetogens and methanogens (Table 1) are intermediate and ultimate degraders, respectively. According to the surface thermodynamics approach (1), when interactions such as steric and electrostatic forces are neglected, hydrophilic cells have a high γ_{BV} and, in contrast, hydrophobic cells have a low γ_{BV} . Previously published data and our experimental data indicate that acidogens have a tendency to be rather hydrophilic, while acetogens (such as reductive acetogenic strains EF173, EF174, and EF175) and methanogens are more hydrophobic. It should be interesting to confirm and support this hypothesis by analyzing the hydrophobicities of more anaerobic microorganisms with different types of metabolism (saccharolytic and proteolytic acidogens, oxidative and reductive acetogens, hydrogenotrophic and acetotrophic methano-

TABLE 2. Water contact angles of pure and mixed cultures grown in the batch tests and γ_{LV} values during enrichment

Prepn	Pure culture		Wash-out cells	
	γ_{LV} (mN/m)	Contact angle (°) ^a	Culture	Contact angle (°) ^a
<i>Clostridium proteolyticum</i> DSM 3090 ^T	52–54	94.4 $\pm$ 0.9	S1 effluent (100% sucrose)	
<i>Clostridium proteolyticum</i> DSM 3090 ^T + LABS	46–47	94.5 $\pm$ 3.0	Glucose enriched	53–55
<i>Escherichia coli</i> CM120	51–53	22.2 $\pm$ 0.6	Glucose enriched + LABS	46–48
<i>Escherichia coli</i> CM120 + LABS	46–47	23.2 $\pm$ 0.6	Peptone enriched	50–53
<i>Selenomonas acidaminophila</i> DSM 3853 ^T	53–54	72.3 $\pm$ 0.4	Peptone enriched + LABS	46–48
<i>Selenomonas acidaminophila</i> DSM 3853 ^T	46–47	72.5 $\pm$ 1.5		

^a Means $\pm$ standard deviations ($n \geq 6$).

TABLE 3. Water contact angles of mixed cultures from UASB reactors and reactor γ_{LV} values

Reactor	γ_{LV} (mN/m)	Mean contact angle (°)								
		Wash-out cells	White granules			Grey granules				Whole black granules
			Whole	Surface	Core	Whole	Surface	Middle	Core	
S1 (100% sucrose)	55–58	72.7 (0.9) ^a	NF ^b	NF	NF	ND ^c	71.4 (2.7)	81.2 (1.3)	77.8 (0.2)	NF
S2 (100% sucrose + LABS)	46–49	58.5 (1.1)	NF	NF	NF	ND	61.7 (0.5)	94.3 (2.2)	77.0 (1.1)	NF
A1 (30% sucrose + 70% VFA + LABS)	43–45	55.4 (0.4)	ND	ND	ND	53.9 (0.6)	ND	98.2 (2.3)	ND	NF
A2 (10% sucrose + 90% VFA + LABS)	44–46	80.6 (1.9)	72.1 (2.5)	75.0 (2.1)	86.6 (2.0)	89.2 (1.4)	89.1 (2.1)	99.3 (1.2)	ND	92.4 (1.2)
A3 (100% VFA + LABS)	43–44	68.9 (2.0)	71.9 (1.1)	71.4 (2.4)	82.2 (1.0)	NF	NF	NF	NF	ND

^a Values in parentheses are standard deviations ($n \geq 6$).^b NF, not found in reactor operated under defined conditions.^c ND, not determined or could not be determined because of cracking of the bacterial layer.

gens). Interactions of bacterial surfaces with different γ_{BV} values in the aqueous environment result in biomass structures which have practical importance (e.g., the granules in anaerobic wastewater reactors).

Growth of pure strains at high and also low γ_{LV} values does not result in changes in the cell surface contact angles (Table 2). The results obtained with short-term enrichment cultures of mixed populations did not reflect the initial γ_{LV} , but rather reflected the predominant influence of the substrate used (Table 2). Under the enrichment conditions which we used, *r* strategists were selected; these organisms were predominantly glucose- and protein-fermenting bacteria. The latter are mainly hydrophilic acidogens. It is noteworthy that when protein was the substrate, highly hydrophilic cells were selected; this is consistent with the fact that protein-rich wastewaters tend to produce fluffy flocs rather than granules (7, 18, 22).

Although the presence of LABS did not result in changes in the overall surface tension of bacterial cells in short-term cultures (cultures grown for a few days), the γ_{LV} had a striking influence on long-term enrichment cultures (Table 3). The wash-out cells from reactor S1 were much more hydrophobic than the wash-out cells from reactor S2. The surfaces of the grey granules were also more hydrophobic in reactor S1 with a high γ_{LV} than in LABS-supplemented reactor S2. Apparently, in the long-term reactor enrichment culture the microbial community on the granule surface adapted itself, probably by a shift in composition, to the γ_{LV} of the reactor liquid.

The contact angles of different layers of the sludge granules from the UASB experiment revealed that the granules consisted of layers with different hydrophobicities. These results are consistent with the layered structure model of sucrose-fed granular sludge proposed previously (5, 8, 10, 22) and suggest that the highly hydrophobic layers might be the sites where hydrogenotrophs or acetogens occur. The fact that layers with different hydrophobicities exist raises a question about the

interface between layers. From a functional point of view, the critical factor is the existence of a rather robust outer layer which protects each granule against fluid and particle shear. Determining to what extent and how the layers are connected will require further study. The presence of different types of granules, as found in reactors A1, A2, and A3, has been reported elsewhere (19). The different types of granules were different colors and had different metabolic activities. Indeed, we found that the grey granules were associated more with the acidogens than with the other bacteria. The white and black granules exhibited high levels of methanogenic activity. The contact angles of wash-out cells obtained from reactors containing different types of granules were due to the contributions of mixtures of granules.

On the basis of our results we propose that contact angle measurement can be a useful technique for evaluating the presence or proportions of hydrophilic cells (acidogens) and hydrophobic cells (acetogens and methanogens) in anaerobic consortia. It has been well documented that in ore flotation solid surfaces with contact angles of at least 50 to 75° are required to remove particles from the liquid phase to the air-water interface (16). In anaerobic reactors in which poorly water-soluble biogases (e.g., CO₂ and CH₄) are produced, granular sludge with a very hydrophobic surface is obviously susceptible to flotation. The latter phenomenon is considered detrimental to biomass retention in self-immobilized cell systems, such as UASB reactors. Therefore, the effects of LABS and other substances which can promote the formation of consortia with more hydrophilic cells in the outer layer should be studied further.

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TABLE 4. Specific acidogenic activities and specific methanogenic activities of mixtures of granular sludge collected from reactors A1, A2, and A3

Reactor	Specific acidogenic activity ^a	Specific methanogenic activity ^b
A1	1.97 ± 0.07 ^c	0.28 ± 0.00
A2	1.32 ± 0.07	0.44 ± 0.03
A3	0.16 ± 0.01	0.55 ± 0.03

^a Expressed in milliequivalents of H⁺ per gram of volatile suspended solids per hour.^b Expressed in liters of CH₄ per gram of volatile suspended solids per day.^c Mean ± standard deviation ($n = 2$).

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