Granulation and Sludge Bed Stability in Upflow Anaerobic Sludge Bed Reactors in Relation to Surface Thermodynamics

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Adhesion of bacteria involved in anaerobic consortia was investigated in upflow anaerobic sludge bed reactors and was related to surface thermodynamics. The adhesion of hydrophilic cells appeared to be enhanced at a low liquid surface tension (γ_{LV}), while the adhesion of hydrophobic cells was favored at a high γ_{LV} . Growth in protein-rich growth media resulted in low granular biomass yields; addition of polycations, such as poly-L-lysine and chitosan, increased the γ_{LV} and the granular biomass yield. On the basis of the results of activity tests and microbial counts with wash-out cells, we identified two types of structured granules that were related to the influence of γ_{LV} . In one type of granules, hydrophilic acidogens surrounded a more hydrophobic methanogenic association. These granules were selected at a low γ_{LV} provided that carbohydrates were available as substrates. The other type of granules was selected at a high γ_{LV} ; hydrophobic cells (i.e., methanogens) were predominant throughout these granules. The granules which had acidogens as solid-phase emulsifiers around a methanogenic association appeared to allow more stable reactor performance. Decreasing the γ_{LV} in the reactor by adding trace amounts of a surfactant also increased reactor stability.

Adhesion of bacteria is driven by decreases in free energy (2), which are due to changes in free energy at interfaces. There are many interactions, such as those based on electrostatic forces, as well as specific chemical, hydrophobic, and polymeric interactions. In the thermodynamic approach, in which the effects of electric charges, as well as specific biochemical interactions (e.g., receptor-ligand or steric forces), are neglected, an adhesion process is favored when the process itself causes the free energy to decrease. In such systems, the change in the free energy per unit of area (ΔG_{adh}) can be expressed as follows (1):

$$\Delta G_{adh} = \gamma_{BS} - \gamma_{BL} - \gamma_{SL} \quad (1)$$

where the subscripts B, L, and S stand for bacteria, liquid, and solid substratum, respectively, ΔG_{adh} is the free energy of adhesion, γ_{BS} is the bacterium-substratum interfacial tension, γ_{BL} is the bacterium-liquid interfacial tension, and γ_{SL} is the substratum-liquid interfacial tension.

Young's equation is:

$$\gamma_{SV} - \gamma_{SL} = \gamma_{LV} \cdot \cos\theta \quad (2)$$

where θ is the equilibrium contact angle of a liquid on a solid and γ_{SV} and γ_{LV} are the solid-vapor and liquid-vapor interfacial tensions, respectively. Of the parameters in this equation, only γ_{LV} and θ can be determined experimentally. By using thermodynamics it has been demonstrated that γ_{SL} is a function of γ_{SV} and γ_{LV} (28). Using experimental contact angle data and γ_{LV} values, Neumann et al. (16) developed the following equation of state:

$$\gamma_{SL} = (\sqrt{\gamma_{SV}} - \sqrt{\gamma_{LV}})^2 / (1 - 15\sqrt{\gamma_{SV} \cdot \gamma_{LV}}) \quad (3)$$

When the equation of Young mentioned above (equation 2) was applied to equation 3, then:

$$\cos\theta = [(15\gamma_{SV} - 2) \cdot \sqrt{\gamma_{SV} \cdot \gamma_{LV}} + \gamma_{LV}] / [\gamma_{LV} (15\sqrt{\gamma_{SV} \cdot \gamma_{LV}} - 1)] \quad (4)$$

With this equation, the unknown surface tension of a substratum or a bacterium (γ_{SV} or γ_{BV}) can be determined by measuring γ_{LV} and θ . Moreover, it has been proposed that equation 3 is generic and therefore can be used to calculate the interfacial tension γ_{12} from available interfacial tensions γ_{13} and γ_{23} , where the subscripts 1, 2, and 3 represent three different phases (16).

For the sake of simplicity, when only adhesion of two identical bacteria is considered, then $\gamma_{BS} = 0$ and $\gamma_{SL} = \gamma_{BL}$ and equation 1 becomes:

$$\Delta G_{adh} = -2 \cdot \gamma_{BL} \quad (5)$$

And the equation of state becomes:

$$\Delta G_{adh} = -2 \cdot (\sqrt{\gamma_{BV}} - \sqrt{\gamma_{LV}})^2 / (1 - 15\sqrt{\gamma_{BV} \cdot \gamma_{LV}}) \quad (6)$$

By varying the γ_{LV} and using discrete values for γ_{BV} , ΔG_{adh} values for identical bacteria under different γ_{LV} conditions (e.g., 0 to 73 mN/m) can be determined (Fig. 1). We found that there are two main regions where bacteria can obtain the maximum possible ΔG_{adh} ; these are the regions where γ_{LV} is sufficiently low or high. In the high- γ_{LV} region (zone B), low-energy surface types of bacteria (low- γ_{BV} or hydrophobic bacteria) can adhere in order to obtain minimal energy. On the other hand, in the low- γ_{LV} region (zone A), high-energy surface types of bacteria (high- γ_{BV} or hydrophilic bacteria) can adhere better. A third zone is arbitrarily defined between γ_{LV} values of 50 and 55 mN/m, and in this zone aggregation of neither hydrophobic nor hydrophilic cells is favored. If the rule "abioticum dictates bioticum" holds, operating a system at a high γ_{LV} should select for or favor aggregation of (rather) hydrophobic bacteria, and operating a system at a low γ_{LV} should select for or favor aggregation of (rather) hydrophilic bacteria.

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FIG. 1. Free energies of adhesion of two identical bacteria with different γ_{BV} values as a function of γ_{LV} . In zone A, hydrophilic cells aggregate better; in zone B, hydrophobic cells exhibit a greater decrease in free energy upon aggregation and thus are selected to compose aggregates. The zone from 50 to 55 mN/m has a low ΔG potential.

In most previous studies workers concentrated on how bacteria adhere to surfaces with different hydrophobicities. To our knowledge, there has been no research on how γ_{LV} affects bacterial adhesion under anaerobic conditions. It seems that surface thermodynamics play a role only in the first step of adhesion (so-called passive or spontaneous adhesion), but this may be important to methanogens, especially methanogens in anaerobic digesters, which have no apparent appendages for "active" adhesion (6). Most acidogens appear to be able to produce extracellular polymers and to effectively use their appendages (e.g., pili, fimbriae, and flagella) for adhesion. Although most bacteria are negatively charged, the charges of bacteria are considered less important in aqueous environments with relatively high ionic strengths (>10 mM), such as waste streams suitable for anaerobic digestion (24).

Bacterial cell surface hydrophobicity measured by the contact angle technique allows workers to estimate the surface energy of cells as described previously (7). It has been concluded that anaerobic microbes have a wide range of γ_{BV} values. If we arbitrarily assume that 45° is the cutoff contact angle between hydrophilicity and hydrophobicity, most of the methanogens and the acetogens can be considered rather hydrophobic (with low-energy surfaces and contact angles of $>45^\circ$), and most acidogens can be considered hydrophilic (with high-energy surfaces and contact angles of $<45^\circ$). If we assume that bacterial hydrophobicity is species specific and does not depend on γ_{LV} (7), previous surface thermodynamic considerations (equation 6) indicate that a low- γ_{LV} environment would be beneficial for aggregation of hydrophilic microorganisms (i.e., fermentative bacteria or acidogens). In the anaerobic digestion process, substrates such as carbohydrates, proteins, and lipids are fermented to methane in the following four stages involving three groups of microorganisms: hydrolysis (by acidogens), acidogenesis (by acidogens), acetogenesis (by acetogens), and methanogenesis (by methanogens). Therefore, this process intrinsically requires a good association among these types of bacteria. Granular sludge (e.g., sludge in upflow anaerobic sludge bed [UASB] reactors) is a bacterial consortium in which cells live in close proximity. Various authors have demonstrated that the presence of high-energy substrates (and consequently higher trophic acidogens) is essential for a well-granulated biomass (17, 19, 20, 22, 29). In addition, γ_{LV}

might be another crucial factor. Indeed, the system favors a surface-to-liquid configuration which has a minimum of free energy. Hence, in liquids with high surface tensions, hydrophobic cells are directly at the surfaces of the granules, but in liquids with low surface tensions the granules might be composed of an outer layer of hydrophilic acidogens and an inner layer of more hydrophobic methanogens. This model is consistent with the layered structure proposed by Guioit et al. (9) on the basis of activity measurements and corroborated by Lens et al. (12) on the basis of micro-pH profiles.

In this study we focused on how the environmental factor γ_{LV} could affect the granulation process (especially the granular biomass yield [Y_{gran}]) and the stability of UASB reactors.

MATERIALS AND METHODS

Seed sludge. Methanogenic granular sludge obtained from the potato-processing treatment plant at S. A. Van Den Broeke, Leuze, Belgium, was used as the inoculum in this study, as described previously (12).

Batch reactors. The three batch reactors used consisted of 1-liter polyethylene Erlenmeyer flasks (22), and these reactors were designated reactors B1, B2, and B3. The reactors were initially operated at a volumetric loading rate (Bv) and a sludge loading rate of 2.0 g of chemical oxygen demand (COD) per liter per day and 0.5 g of COD per g of volatile suspended solids (VSS) per day, respectively. Each flask was connected to a liquid displacement gas collector and incubated on a shaker (160 to 170 rpm) at 30°C . Feed was prepared from 20% molasses and 80% peptone (in terms of COD) and then diluted to a concentration of ca. 7.5 g of COD per liter before it was used (i.e., the ratio of COD to N was 100:10). Every second day the shaker was stopped for 20 min, and 0.5 liter of reactor liquid was decanted as effluent. The amount of biogas produced and the reactor pH were recorded. Reactor B1 was the reference reactor for the batch experiment. Reactors B2 and B3 were supplemented with 100 mg of chitosan (molecular weight, 70,000; Fluka) per liter and 1 mg of poly-L-lysine (low molecular weight; Sigma) per liter, respectively. These polycations have been shown to increase both the ability of yeast to flocculate and cell hydrophobicity (8, 18). In particular, Goldberg et al. (8) suggested that the increased hydrophobicity of microbial cells that occurs in the presence of polycationic polymers can be attributed to a loss of surface electronegativity.

UASB reactors. Two laboratory-scale UASB reactors (volume, 2.3 liters), designated reactors S1 and S2, were set up and were initially operated at a Bv and a sludge loading rate of 2.5 g of COD per liter per day and 1.0 g of COD per g of VSS per day, respectively. These reactors were operated at $30 \pm 3^\circ\text{C}$ and were fed semicontinuously (2 min every hour). Feed was prepared by using sucrose and a synthetic medium; this feed was adapted from the feed described by Hulshoff Pol (10). The final amounts of nutrients (per liter of feed) were as follows: 0.5 mg of H_3BO_3 , 20 mg of $\text{FeCl}_3 \cdot 3\text{H}_2\text{O}$, 0.5 mg of ZnCl_2 , 5 mg of $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.5 mg of $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 0.5 mg of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 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 $(\text{NH}_4)_2\text{SO}_4$, 15 mg of KH_2PO_4 , and 5 mg of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$. In addition, the feed contained 0.01 ml of 36% HCl per liter. During the experiment, the influent COD concentration was adjusted by increasing the Bv from 2.5 to 10 g of COD per liter per day. The ratio of COD to N to P in the feed was maintained at 100:1.25:0.25 during the experiment by adding NH_4Cl and $(\text{NH}_4)_2\text{HPO}_4$. During the experiment, the feeding rate, upflow velocity, and hydraulic residence time were kept constant at 2.3 liters/day, 1 m/h, and 24 h, respectively. Linear alkylbenzenesulfonate (LABS) (C_{18} ; Aldrich) was added at a concentration of 2 mg/liter to reactor S2 in order to reduce the γ_{LV} in the reactor. Reactor S1 was the reference reactor used in the UASB experiment. LABS is not biodegradable under anaerobic conditions and has an inhibitory effect at concentrations of >10 mg/liter (26). The γ_{LV} of the prepared feed was reduced from 68 to 49 mN/m by the surfactant. In all of the experiments, a NaHCO_3 solution was used to keep the pH in the reactor above 7. To avoid bacterial growth on the walls of the feed container, the feed container was cleaned daily and the feed was stored at 4°C .

Analytical techniques. Cell yields were calculated by determining the number of grams of VSS formed per gram of COD removed (COD_{rem}). Y_{gran} and Y_{floc} were the amounts of new granulated and fluffy biomass that accumulated in the reactor, respectively; $Y_{washout}$ was the total amount of wash-out cells found in the effluent; and Y_{total} was the total amount of biomass harvested, both in the reactor and in the reactor effluent, during the experimental period. The methane conversion coefficient ($Y_{methane}$) was the total amount of methane produced per gram of COD_{rem} . Methane and carbon dioxide contents were determined with an Intersmat model IGC 120MB gas chromatograph connected to a Hewlett-Packard model 3390A integrator. The gas chromatograph was equipped with dual columns consisting of a Porapak Q column (50 to 80 mesh) and a molecular sieve (pore size, 6 to 8 nm), as well as a thermal conductivity detector. The isothermal column temperature was 30°C , the carrier gas was argon, and the flow rate was 20 ml/min. Hydrogen sulfide contents were determined with a Gastec

TABLE 1. Influence of polycations on biomass yields and γ_{LV} in the batch experiment

Reactor	Additional compound	Methane productivity (liter of CH ₄ per liter per day)	Y_{gran} (g of VSS per g of COD _{rem})	Y_{floc} (g of VSS per g of COD _{rem})	$Y_{wash-out}$ (g of VSS per g of COD _{rem})	Y_{total} (g of VSS per g of COD _{rem})	Influent γ_{LV} (mN/m)	Effluent γ_{LV} (mN/m)
B1		0.57	0.012	0.015	0.049	0.076	47.5	54.0 ± 0.5 ^a
B2	Chitosan	0.65	0.019	0.008	0.047	0.074	47.2	56.0 ± 1.0
B3	Poly-L-lysine	0.59	0.021	0.014	0.037	0.072	46.0	57.3 ± 0.1

^a Mean ± standard deviation ($n = 4$).

multistroke gas sampling pump equipped with a hydrogen sulfide extra-high-range detector tube. Contact angles of bacterial cells were determined as follows. Cells were centrifuged and washed twice in 0.1 M phosphate-buffered saline. After the microorganisms were deposited on a Micropore filter (pore size, 0.45 μ m), the contact angle of 1 μ l of saline water (0.1 M NaCl) on the dry bacterial layer was determined at 20 ± 3°C (8). All of the contact angle values were obtained from at least six measurements.

The surface tensions of reactor liquids were determined at 20 ± 3°C by using the Wilhelmy plate method. Ammonium was distilled off with a Kjeltac System model 1026 distilling unit and captured in a boric acid solution, and then the amount of ammonium was determined by titration with 0.01 N sulfuric acid (12). COD values and values for other parameters were determined by standard methods (3). Routine analyses were performed every 3 or 4 days except when indicated otherwise.

Microbial metabolic activity assays. Microbial activity was measured in duplicate during flotation of the sludge bed by using harvested granules and wash-out cells (effluent). Specific activity was determined from the maximum slope of the graph of amount of product per gram of VSS versus time. The specific acidogenic activity of the biomass from each reactor was determined by using the technique described by Vanderhaegen et al. (22) and glucose as the substrate. The samples were centrifuged at 10,000 × g for 10 min and resuspended in distilled water that had been flushed with N₂ 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 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 KH₂PO₄, 1.0 g of K₂HPO₄, 1.0 g of NaCl, 0.1 g of MgCl₂, 0.1 g of Na₂S · 7H₂O, and 0.005 g of resazurin. The biomass loading values used were 0.25 to 2.0 g of COD per g of VSS in order to avoid any variation in methanogenic activity (15). 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 (12).

Size distribution of granules. The size distribution of about 2,000 granules obtained from a reactor was examined to determine the equivalent diameter at a cumulative frequency of 50% with an image analyzer when the experiment was terminated. The analyzer was preset to measure objects with diameters larger than 0.1 mm (which were arbitrarily classified as granules). The digital image-processing unit used consisted of a 80486 computer system with a maximum resolution of 712 × 512 pixels (three-color). A CCD video camera (model TK-1070E; JVC) was equipped with a video camera lens [model HZ-C6 11AF(u); JVC] which was placed 10 cm above the sample. The software used was the MicroScale software (Digithurst Ltd., Herts, United Kingdom).

RESULTS

Batch reactors and biomass yields. Table 1 shows the performance of the batch reactors and the biomass yields obtained during the batch experiment, which lasted 62 days. The reactors supplemented with polycations, reactors B2 and B3, produced Y_{gran} values that were about 60 and 75% higher, respectively, than the value produced by reference reactor B1. The reactors supplemented with polycations exhibited slightly better biogas productivity. Table 1 also shows that the γ_{LV} increased during digestion. The final values reached were in the low ΔG potential region (Fig. 1).

UASB reactors and biomass yields. Figure 2 shows the efficiency of COD removal and the Bv used during the 72-day experiment with reactors S1 and S2. The reactors functioned successfully with efficiencies greater than 90% with different Bv (2.5 to 10 g of COD per liter per day); a Bv of 10 g of COD per liter per day was used from day 35 on. On day 42, we observed that in reference reactor S1 individual granules became at-

tached to small biogas bubbles, floated to the top of the reactor, where the bubbles detached, and returned to the sludge bed. We noticed that as the efficiency of UASB reactor S1 approached 95%, the γ_{LV} gradually increased from 50 to more than 55 mN/m (Table 2). Concomitantly with the increase in γ_{LV} , the bubbles adhered more strongly to the granular sludge in reactor S1 and carried clumps of granules (diameter, >10 cm) to the top of the reactor. This pronounced clumping and flotation of granules resulted in a significant loss of active biomass. As a result, the efficiency of reactor S1 gradually decreased, and finally the reactor could not function; the efficiency became less than 50% (Fig. 2). This phenomenon did not occur in the reactor treated with LABS (reactor S2), in which γ_{LV} was quite stable at values around 46 to 48 mN/m throughout the experiment (Table 2).

Table 2 shows the reactor performance results and the various biomass yields observed. The two reactors had about the same Y_{total} , 0.09 g of VSS per g of COD_{rem}. Y_{gran} was particularly important; the Y_{gran} in reactor S1 during the experiment (0.009 g of VSS per g of COD_{rem}) was one-half the Y_{gran} in reactor S2 (0.019 g of VSS per g of COD_{rem}). On the other hand, the $Y_{wash-out}$ and Y_{floc} values for reactor S1 were higher than the corresponding values for reactor S2.

Metabolic activity. To verify any differences in microbial composition when the reactors operated at different γ_{LV} values (at a Bv of 10 g of COD per liter per day), samples of wash-out cells (effluent) and granular sludge were used for microbiological tests. Table 3 shows the specific acidogenic and specific methanogenic activities of the samples obtained from reactors S1 and S2. Acidogens were more prevalent in the wash-out cells of reactor S2, and methanogens appeared to be more prevalent in the reactor S1 effluent biomass.

Size distribution. To detect physical differences in the gran-

FIG. 2. Efficiency of COD removal and Bv used in the UASB experiment. Symbols: +, reactor S1 (control); Δ, reactor S2 containing LABS. The dotted line indicates the Bv.

TABLE 2. Performance and biomass yields in UASB reactors operating at different γ_{LV} values

Reactor ^a	γ_{LV} (mN/m)	Y_{gran} (g of VSS per g of COD _{rem})	Ratio of VSS to suspended solids in granules	Y_{floc} (g of VSS per g of COD _{rem})	$Y_{wash-out}$ (g of VSS per g of COD _{rem})	Y_{total} (g of VSS per g of COD _{rem})	Avg % of H ₂ S in biogas	$Y_{methane}$ (liter of meth- ane per g of COD _{rem})	pH in reactor	pH of effluent	NH ₄ -N (mg of N per liter)	Overall efficiency (% COD removal)
S1	55.2 ± 3.6 ^b	0.009	0.59	0.008	0.076	0.093	0.1	0.305	7.21 ± 0.18 ^b	7.88 ± 0.31 ^b	72.3 ± 29.3 ^b	92.5
S2	47.8 ± 1.5	0.019	0.59	0.006	0.067	0.092	0.1	0.300	7.22 ± 0.09	7.97 ± 0.29	65.7 ± 21.0	96.4

^a The γ_{LV} of reactor S2 was decreased by adding LABS as described in Materials and Methods.^b Mean ± standard deviation.

TABLE 3. Specific bacterial metabolic activities of wash-out cells and granules harvested during the flotation period

Reactor	Biomass type	Specific acidogenic activity ^a	Specific methanogenic activity ^b
S1	Wash-out cells	2.90 ± 0.10 ^c	0.063 ± 0.002 ^c
	Granules	2.05 ± 0.09	0.092 ± 0.002
S2	Wash-out cells	4.02 ± 0.10	0.040 ± 0.001
	Granules	2.54 ± 0.08	0.077 ± 0.001

^a Expressed in milliequivalents of H⁺ per gram of VSS per hour.^b Expressed in liter of CH₄ per gram of VSS per day.^c Mean ± standard deviation.

ular sludges in reactors S1 and S2, granules were collected from each reactor, and size distributions were determined at the end of the experiment. Figure 3 shows the size distributions of granules with diameters of >0.1 mm. This figure shows that the equivalent diameter at a cumulative frequency of 50% for granules harvested from reactor S2 (2.1 mm) was slightly larger than the equivalent diameter of granules harvested from reactor S1 (1.7 mm).

DISCUSSION

As determined by surface thermodynamics, hydrophilic and hydrophobic bacteria obtain their maximal free energies at different γ_{LV} values (i.e., zones A and B, respectively). There is also a theoretical zone in which aggregation of both hydrophilic and hydrophobic cells is not favored, at γ_{LV} values between 50 and 55 mN/m (Fig. 1).

The adverse effect of substrates rich in proteins on Y_{gran} has been reported previously, and it has been demonstrated that the final product of proteins (i.e., NH₄⁺) does not counteract the formation of granules (19, 22). Indeed, it was observed that the γ_{LV} of a reactor fed with 100% molasses decreased from 57 to <55 mN/m when 80% of the feed COD was replaced by proteins, and it was proposed that this decrease in γ_{LV} was the cause of poor granulation (19). In this study we found that aggregation of cells cultivated in protein-rich feed can be enhanced by adding the polycations chitosan and poly-L-lysine (Table 1). These two compounds have been shown to increase cell hydrophobicity (8, 18). Acidogenic cells grown in peptone medium are quite hydrophilic (contact angle, ≈20°) (7). Thus, these cells have high-energy surfaces, and they also exhibit a low potential for decreases in free energy by adhering, as shown in Fig. 1. Thermodynamic considerations clearly demonstrated that for hydrophobic cells (cells with lower surface energy), aggregation is favored. The reason why the γ_{LV} values of the reactors increased during digestion is not clear; probably some surface-active intermediates were effectively removed by anaerobic conversions.

The Y_{gran} of the reactor operated at a constantly low γ_{LV} (reactor S2) was twice the Y_{gran} of the reactor operated at a high γ_{LV} (reactor S1). There was more wash-out of cells from reactor S1, in which strong adhesion of biogas bubbles to aggregate surfaces was observed (Table 2). The metabolic activity test results and microbial counts for wash-out cells also indicated that the cells on the surfaces of granules cultivated at a low γ_{LV} were predominantly hydrophilic (i.e., acidogenic) compared with cells grown in a high- γ_{LV} environment. On the other hand, high- γ_{LV} conditions are favorable for hydrophobic (i.e., methanogenic) cells and enable such cells to efficiently aggregate at the interface with the liquid phase. As a result, cells with hydrophobic surfaces adhere strongly to biogas bub-

FIG. 3. Size distribution of granules harvested from UASB reactors. —, reactor S1 (control); ---, reactor S2 containing LABS.

bles and are susceptible to wash-out. This explains the differences found in the hydrophobicities of the wash-out cells obtained from the reactors operated at high and low γ_{LV} values (7). The activity test results and the size distribution in granular sludge showed that the granules from the reactor operated at a low γ_{LV} (reactor S2) had more acidogens on their surfaces, which resulted in slightly larger granules than the granules obtained from the reactor operated at a high γ_{LV} . This implies that low- γ_{LV} environments favor the formation of larger aggregates when substrates for acidogens are provided.

Many researchers have focused on bacterial attachment to solid surfaces with different hydrophobicities (14, 25, 27). From these studies the following observations can be made: (i) methanogens alone can attach to surfaces but to a lesser extent than when they are associated with other bacteria (e.g., acidogens and acetogens that convert the higher fatty acids to acetate); (ii) consortia appear to stick better to (low-energy) hydrophobic surfaces than to (high-energy) hydrophilic surfaces; and (iii) in water, hydrophobic cells tend to stick first to one another and by associating with more hydrophilic organisms can form aggregates that exhibit hydrophilic behavior.

Previously published data and our contact angle measurement results (7) clearly demonstrated that the surfaces of sludge granules in a low- γ_{LV} environment were enriched with acidogens (hydrophilic bacteria), which made these methanogenic granules less susceptible to wash-out by biogas bubbles that adhered to their surfaces. Consequently, stable performance of UASB reactors could be achieved. On the other hand, the granules in a high- γ_{LV} environment had rather hydrophobic surfaces (i.e., surfaces enriched with methanogens) to which biogas bubbles could adhere strongly, and this led to reactor instability. Indeed, the latter phenomenon has been observed frequently in many laboratory- and plant-scale reactors in which clumps of granular sludge adhering to biogas bubbles floated to the top of reactors and left the reactors via effluent ports. Granule wash-out reportedly has been decreased by adding antifoaming agents, which have surface-active properties and subsequently reduce the γ_{LV} (4, 10, 13, 23).

In wastewater purification, bacterial adhesion is essential for biomass retention in systems such as UASB reactors. Changing the γ_{LV} is practical and simple. γ_{LV} can be decreased to the minimum value obtainable (high ΔG potential, zone A) (Fig. 1) by adding small amounts of effective surfactants. Increasing the γ_{LV} so that it is close to the γ_{LV} of pure water (ca. 73 mN/m) is also possible, but is much more difficult and costly;

this can be done by dilution or removal of surface-active agents with activated carbon.

In practice, surface-active agents, such as proteins and detergents, are a nuisance (e.g., they produce foaming), especially in anaerobic reactors in which poorly soluble gases (e.g., methane) are produced in the bulk liquid. However, very low concentrations of surfactants, such as some nonionic detergents, can effectively decrease the γ_{LV} with less foaming (5). Moreover, some nonionic detergents (e.g., Afranil, Mogul DX-236, Nalco, Sedipol) can function as antifoaming agents and at the same time promote bubble coalescence in bulk liquid. Addition of foam destabilizers at parts-per-million levels is also practical and effective (4, 5, 23). Manipulating γ_{LV} in order to obtain the optimal bacterial adhesion by adding such surfactants seems possible and promising, provided that adsorption of the surfactant does not significantly alter the in situ hydrophobicity of the bacterial surface. Such changes have not been observed in previous experiments performed with pure and mixed cultures (7) or in our laboratory-scale reactor systems.

In aqueous environments, another inherent characteristic of solid surfaces is gas bubble attachment. Gas bubbles minimize their contact area with hydrophilic (high- γ_{SV} and high-energy) surfaces; therefore, they tend to leave the surface. On the other hand, gas bubbles tend to stick effectively to hydrophobic surfaces (11). This indicates that a (rather) hydrophilic bacterial community is indeed preferable in anaerobic processes in which a biogas (e.g., methane) is produced. Loss of (suspended or aggregated) cells would be minimized compared with (rather) hydrophobic consortia. On the basis of thermodynamic considerations and the experimental results obtained in this study and another study (7), two structural models for granular sludge in relation to γ_{LV} regimes can be proposed, as shown in Fig. 4. We postulate that the composition of microbial granules

1. Environment with low γ_{LV} (< 50 mN/m)

2. Environment with high γ_{LV} (> 55 mN/m)

3. Environment with intermediate γ_{LV} low ΔG -potential, difficult formation of granules

FIG. 4. Configurations of UASB reactor granules based on thermodynamic considerations.

is strongly influenced by γ_{LV} . At a high γ_{LV} the generally hydrophobic methanogens can exist as cell aggregates in direct contact with the water, and the acidogens are dispersed throughout the granules. At a low γ_{LV} thermodynamic considerations suggest that there is a layered granular structure, with mainly hydrophilic cells on the outside and mainly hydrophobic cells on the inside. In this case, the acidogens are important not only because they supply substrates to the methanogens, but also because they act as "solid-phase emulsifiers."

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