CHARACTERISTICS OF UASB GRANULES

PHYSICAL CHARACTERISTICS

Form of Granular Sludge

Granules are formed by the activities of various groups of microorganisms. Depending on size, shape, and composition various types of granular sludge can be distinguished as follows :

I. More or less spherical granules up to 1 to 5 mm in size. These granules may vary in colour from pale yellow to black, depending on the waste on which they were grown [Kosaric *et al.*, 1990b]. The VSS content of these granules may be up to 95 % of the total suspended solids. Based on the microscopic examination three very different types of spherical granules can be distinguished as under :

    Filamentous Granules: This granules mainly consists of long multicellular filaments of rod-shaped organisms, presumably *Methanothrix sohngenii*, and contain some kind of inert support originating from digested sewage sludge. The diameter of the granules ranges from 1 to 5 mm. These granules are observed on pure VFA substrates and are mechanically rather fragile, though fairly moldable [Hulshoff *et al.*, 1983c, Russo and Dold, 1989, Lettinga *et al.*, 1983c].

    Rod-type Granules : These granules mainly consists of smaller and denser fragments of *Methanothrix*. These rod-type granules are observed on sugarbeet wastewater, potato processing waste and under specific conditions also on VFA-substrates [Hulshoff *et al.*, 1983c]. These granules are mechanically more robust than the filamentous granules and are fairly moldable.

    Sarcina Granules : These granules are normally not found in UASB reactors. However, under certain conditions they may develop i.e., with a VFA-mixture as substrate at a low pH (6.0), under high acetate concentration in the feed, under constant overloading of the reactor, and also when ethanol is used as substrate [Grotenhuis *et al.*, 1991a, Weigant *et al.*, 1986a,b] or in pure cultures in laboratory reactors [Hulshoff *et al.*, 1983c, Dolfing and Mulder, 1985]. The predominant organisms in these granules are *Methanosarcina*. This kind of sludge granules are small in size and are loosely packed.

II. Rigid spiked-rod shaped granules which are very uniform in shape and size and contains up to 60 % $CaCO_3$. These granules are approximately one mm in length and less than 0.3 mm thick [Lettinga *et al.*, 1983c].

Thus, depending on the substrate on which granules are developed, different kinds of sludge granules have been reported. The importance of physical operating parameters on the size of the granules is not being addressed so far.

Surface Morphology

Most of the granules reported to have a compact surface with a few cavities, possibly for the release of biogas produced or to facilitate substrate diffusion [Kosaric *et al.*, 1990b]. Heterogenous population of bacteria is generally appear on the surface, this includes various types of rods, cocci and filaments similar to *Methanothrix* [Quarmby and Forster, 1995]. The external surface of the granules is normally exposed to the complex substrate and with some degree of acidification. Hence, external surface shoes the presence of acidogens in large number than methanogens.

Settleability

Granular sludge exhibits very good settleability. Buoyant densities of the granules have been estimated to be 1.00 - 1.05 g/cm^3 [Dolfing *et al.*, 1985, Lens *et. al.* 1998]. The density of granules depends upon starting inoculum material, feed composition, and operational conditions (i.e. pH, COD loading rate, VFA concentration) in the reactor. This buoyant density of granules is equal to the densities for dispersed bacterial cells, which shows that the excellent settling properties of the granules are mainly due to aggregation of the microorganisms [Dolfing *et al.*, 1985]. The settleability varies widely depending upon the size and shape of the granules. Settling velocity of UASB sludge varies in the range of 2 - 90 m/h [Lettinga *et al.*, 1983c], and for good granular sludge, settling velocity is in the range of 18 to 100 m/h [Dolfing and Mulder, 1985, Maaskant *et al.*, 1983, Thaveersri *et al.*, 1994, Park *et. al.* 1997, Lens *et. al.*, 1998]. It was reported that, granular sludge cultivated on wastewater with hydrolyzed substrate settles less well than sludge cultivated on wastewaters with carbohydrates [Fang *et al.*, 1994b].

In terms of sludge volume index (SVI) values, granular sludge SVI is about 10 to 20 mL/g [Maat and Habets, 1987, Yan and Tay, 1997], where as flocculent sludge SVI range from 20 to 40 mL/g [Lettinga *et al.*, 1980a]. The SVI of 5 mL/g for granular sludge has also been reported [Ciftci and Ozturk, 1993].

The VSS/SS ratio as high as 0.84 can be obtained in UASB reactor sludge [Yan and Tay, 1997]. In general, VSS/SS ratio for granular sludge was reported to be between 0.6 and 0.85 [Lin and Yang, 1991, Lens *et. al.,* 1998, Lin and Chui, 1999]. VSS to SS ratio as low as 0.21 was reported for UASB sludge treating leachate due to precipitation of inorganic materials [Kuttunen and Rintala, 1998].

It is reported that there exists relationship between the strength of granules, COD removal efficiency, and applied loading rate during start-up [Ghangrekar *et. al.*, 1996]. For higher strength of granules higher COD reduction was reported. Similar observation was reported by Pereboom [1997] stating that the strength of methanogenic granules is positively correlated with mean cell residence time. Loading rates applied during formation of granules are mainly responsible for strength of the granules.

CHEMICAL COMPOSITION

The chemical composition of granular sludge is comparable to that of bacteria in general. Protein, total carbohydrates, total organic carbon, kjeldhal nitrogen and ash content comprise about 35 to 60 %, 6 - 7 %, 41 - 47 %, 10 - 15 % and 10 - 23 % of the total dry weight, respectively [Lin and Yang, 1991]. Granules from reactors treating industrial wastewaters generally have higher ash content than those from synthetic wastes. the higher ash contents are due principally to the presence of higher concentrations of calcium and/ or iron salts and other inert suspended solids in the feed. About 30 % of the ash fraction consists of FeS, which may cause the black color of the granules and smell of H_2S [Dolfing *et al.*, 1985]. The other metals contributing the ash content are calcium, sodium, magnesium, potassium, phosphorus, and non metals in the form of clay [Bhatti *et al.*, 1995].

Sludge granules were also found to contain P and Si [Wu *et al.*, 1987]. In addition, 1 - 2 % of the extracellular polysaccharide fraction of the consortia compares well with data reported for aerobic activated sludge, but a great amount of inorganic, mineralized, or crystallized material is observed in anaerobic sludge [Vuoriranta *et al.*, 1985]. In general granular sludge has

VSS/SS ratio ranging from 0.60 to 0.85, and that the wet cake of the sludge after centrifugation had a moisture content of about 90% [Lin and Yang, 1991].

METABOLIC ACTIVITY

The aggregation of anaerobic microorganisms into granules optimizes the cooperation between the partner organisms, by reducing the diffusion distance for the transfer of metabolites and creating the close cell associations, which are obligatory for the degradation of propionate and butyrate. This is thermodynamically controlled by the H_2 partial pressure and could not occur unless the H_2 produced is scavenged by H_2 consuming organisms [Dwyer *et al.*, 1988]. Thus, aggregation of bacteria of different metabolic groups is of pivotal importance for the energetics and kinetics of the overall substrate conversion in anaerobic digestion and hence, for higher metabolic activity.

Metabolic activity of granules can be expressed in terms of specific methanogenic activity, specific COD removal rates, or specific substrate conversion rates. Granular sludges have higher volumetric activities than flocculent type sludges since; they are denser in structure and contain more biomass on a volumetric basis. Higher methanogenic activity is one of the important characteristics of the granular sludge. On a sludge cultivated with glucose at 35^{o}C and another cultivated with brewery wastewater, activity of 1.2 and 1.9 kg COD/ kg VSS.d respectively, was reported [Lin and Yang, 1991]. Using VFA as feed, activity at mesophilic temperature as 2.2 to 4.0 kg COD/ kg VSS.d [Hulshoff *et al.*, 1983b], and at thermophilic temperature as 4.2 to 7.3 kg COD/ kg VSS.d has been reported [Wiegant *et al.*, 1986a, Hickey *et al.*, 1991a]. In terms of specific methanogenic activity, using sucrose as feed substrate, activity as 1.7 g CH_4-COD/ g VSS.d has been reported [Fang and Chui, 1993].

Microbial composition:

Direct count of cells using microscopy indicate that granular sludges contain between 1 x 10^{12} and 4 % 10^{12} cells per gram of VSS. Granular sludges, developed either on complex substrates, or VFA mixtures, have been observed to contain essentially the same microbial tropic groups typically found in anaerobic digesters (i.e. hydrolytic fermentative, syntrophic acetogenic, methanogenic and sulphate reducing bacteria). For the granules developed on hydrolyzed proteins Fang *et al.*, [1994b] have reported that, each gram of the granule biomass the populations of bacteria capable of using H_2/CO_2, acetate, propionate, butyrate, and hydrolyzed proteins, individually, as a sole substrate were 3.4 % 10^{7}, 1.3 % 10^{8}, 3.4 % 10^{7}, 6.1 % 10^{7}, 1.3 % 10^{8}, respectively. There is great diversity of hydrogen-utilizing methanogens in the mesophilic granules than in those grown under thermophilic conditions [Visser *et al.*, 1991]. Methanogens particularly acetate utilizers, are found to be a major microbial group [Hickey *et al.*, 1991a]. *Methanothrix* and *Methanosarcina* are the main groups of methanogens, which favors granulation. *Methanothrix* are found to be almost universal feature of granules, and strong population of this methanogens may be an essential feature of a well formed granule [Morgan *et al.*, 1991a, Fang *et al.*, 1994b]. This sludge is characterized by small rod-shaped fluorescent bacteria sludge and the structure of the granule is very dense [Dolfing and Mulder, 1985].

TOLERANCE TO OXYGEN

Methanogens located in granular sludge have a high tolerance for oxygen. The most important factor contributing to the tolerance is the oxygen consumption by facultative bacteria metabolizing biodegradable substrates. Uptake of oxygen by these bacteria creates anaerobic microenvironments where the methanogenic bacteria are protected. However, the methanogens particularly *Methanothrix* in sludge consortia still have some tolerance to oxygen, even in the absence of facultative substrate for oxygen respiration. The other reason could be that the most of the granules are large excess of the diameter than that of the oxygen penetration depth (100μm) to maintain anaerobic zones [Kato *et al.*, 1993].

LOSS OF ACTIVITY DUE TO LACK OF FEEDING

UASB reactors are able to maintain sludge viability without feeding [Souza, 1986]. The granular form of sludge could be observed even after being stored for one year at 15 to 28°C under anaerobic conditions [Lin and Yang, 1991, Maat and Habets, 1987]. The methanogenic activity easily could be reestablished after a reactor shut off for some months [Ciftci and Ozturk, 1993]. In the experiment to study acetic acid utilization activity after no-feed conditions at 55°C, it was observed that 30 % of the sludge activity was lost within one week, and the decrease continued afterwards. On the other hand in 30°C environment no loss of activity was reported [Ohtsuki *et al.*, 1992]. Wiegant *et al.* (1983), reported that the methanogenic activity of thermophilic granular sludge (55°C) was reduced to 50% after storage at 30°C for 50 days. This states that the thermophilic granule looses its activity much faster than mesophilic granules even for storage under mesophilic conditions. Thus, the loss of activity is less for storing at mesophilic or psycrophilic temperature than the thermophilic temperature.

In separate experiment Wu *et al.*, [1995], have reported that during storage at 22°C the degradation rate for all the three acids viz., acetate, propionate and butyrate decreased gradually. At low temperature (4°C), reduction in degradation rates of acetate and propionate was relatively slower than that at 22°C. Reduction in butyrate degradation rate was faster during the first month of storage at 4°C, but the rate declined afterwards. Nevertheless, the granules maintained, their metabolic activities for all three VFAs even after storage of 18 months at reduced level. For relatively short period one to five months, granules can be stored at ambient temperature with limited loss in their VFA degradation rates. However, higher VFA degradation rates can be maintained for storage at 4°C. Also, the granules can be retained even for the storage as long as three years.

Poorly granulated sludge looses its activity faster than properly granulated sludge. UASB reactor granule sludge can be stored at 4 °C without loosing mush of its activity up to nine months. Even the granular sludge store for more than this duration can be preferred as inoculum, because its activity is more than the digested sewage sludge up to about one and half year of cold storage. Poorly granulated sludge stored at 4 °C for less than six months can be used for reactor start-up. The activity of the nongranular sludge stored beyond this duration is comparable with activity of digested sewage sludge [Ghangrekar, 1997]. UASB reactor granular sludge can even be stored at ambient temperatures for few months, without loosing much of its activity.

INTERNAL ARCHITECTURE OF UASB GRANULES

The anaerobic granules found in UASB reactors are highly structured consortium. Two types of granule structure have been reported so far by various investigators, viz., layered structure and non-layered structure. This difference in the structure of granules was mainly attributed to the substrate on which the granules were developed.

Layered Structure

In sucrose fed wastewater [Guiot *et al.*, 1992a, Fang *et al.*, 1994b] three-layered structure of the granules was reported. Each layer of granule was reported to have different distinguishing bacterial morphotypes. Briefly, the central layer i.e., the granule core, consisted of cavities encased by rod shaped bacteria which possessed flat ends. These bacteria were predominantly *Methanothrix* species. The middle layer consists of large number of cocci and rod shaped bacteria. With transmission electron microscopy it was observed that these rods included numerous very electrondence organisms which resemble a *Methanobrevibacter* species, and which were juxtapositioned to syntrophobacter like organisms. This arrangement was particularly predominant in the middle layer of the granule. The external layer contained a variety of organisms, including acidogen morphotypes, *Methanococcales* like organisms, chain forming cocci, large long rods, smaller coccoid organisms, and *Methanospirillum* like filaments. Similar stratified appearance of the granules was also observed by MacLeod *et al*, [1990] for sucrose fed granules, for VFA fed granules [Kosaric *et al*., 1990a], and for sugar wastewater fed granules [Guiot *et al*., 1992c].

The substrate fed to the reactor moves from the bulk liquid to the granule surface by mass transport, and then diffuses in to the granule, where it is transformed in to propionate, acetate, and hydrogen. In response to gradients H_2, propionate, and acetate are transported back to the bulk liquid and diffuse decreasingly to the granule center. This selects for specific active zones, schematically as acidogens, in the bulk liquid and in the outer layer, low affinity high-rate H_2 consumers in the surface and high affinity low rate H_2 consumers in the mid space, low affinity acetoclastic in the periphery and high affinity acetoclasts in the mid space. The proposed structure of the granule population arrangement, related to a substrate and product diffusion model for layered granules is shown in Figure 2.1 [Guiot *et al*., 1992a and Fang *et al*., 1994b].

The pH profile inside the granule was studied by Vanderhaegen *et al*. [1992] for glucose degrading granules. Their study also showed the stratified arrangement inside the granule. The pH of outer layer of the granule was observed to be 1.0 to 1.5 units lower than that of the outer medium. The pH at the center of the granule was 0.5 to 0.1 unit lower than outside medium. The pH profile shows that fermentative bacteria are present at the outside, and possibly also at the inside of the granules with less amount. The pH at the center was slightly higher than that of outer layer, this shows active acetate use at the center which can be attributed to the predominance of *Methanothrix*.

Three-layered structure of the granules was also reported by Quarmby and Forster [1995] for potato, liquorice, cannery, and molasses wastewater treatment. The outer layer mainly consist of gram positive bacteria was observed. In the middle layer increased number of gram negative bacteria was observed. Predominance of this gram negative bacteria was observed at the center. The amount of carbohydrate was observed to be decreasing towards center of the granules with extremely small amount of carbohydrate in the center. In another study for determining the bacterial population in the different layers of the granules treating sucrose as substrate, spirochetes and *Methanosarcina* were found only near the surface [Fang et al., 1994a], while *Methanothrix* were the predominant bacteria in the interior.

Non Layer Structure

Non layer homogeneous structure of granules has also been observed for the granules degrading some wastewaters. In the study with paper mill wastewater and sugar refinery wastewater [Morgan *et al*., 1991a] the granules developed reported to have randomly oriented filaments of *Methanothrix*, which was tightly packed with little space between individual filament sheaths, revealing a 'honeycomb' appearance. Towards the center the degree of compaction was observed to be increased. The non layer structure of the granules was also reported by Quarmby and Forster [1995] for paper mill and wheat-starch wastewater. In laboratory scale UASB reactor treating propionate rich wastewater, the granules developed reported to have non layered structure [Fang *et al*., 1995]. These granules were composed of microcolonies of *Methanothrix* and several other microcolonies with juxtapositioned syntrophic associations between the H_2-producing acetogens and H_2-consuming methanogens. Juxtapositioned syntrophic associations between these bacteria makes the rapid removal of hydrogen, which would otherwise hinder the propionate degradation, as elucidated from thermodynamic analysis. Similar granule structure was reported for the granules treating hydrolyzed proteins in UASB reactor [Fang *et al*., 1994a]. In this case, spirochetes, *Methanosarcina* and *Methanothrix* were distributed throughout the granule. Spirochetes were probably responsible for the degradation of amino acids and fatty acids, whereas *Methanosarcina* converts H_2/CO_2, methanol, methylamine, and acetate to methane and *Methanothrix* converts acetate only at low concentration.

The non layered structure of granules was also reported by Grotenhuis *et al*. [1991a] for UASB granules treating propionate, ethanol, and sugar refinery wastewaters. The glutamate degrading granules also showed the non layered structure [Fang *et al*., 1994b]. The network of *Methanothrix* with packets of non-methanogenic bacilli throughout the granules was observed in this case.

Role of *Methanothrix* and Syntrophic Microcolonies in Granules

Based on SEM and TEM studies different researchers have reported that *Methanothrix* is a key structural element in all the granules observed. In three layered structure of granules, *Methanothrix* are observed in predominance at the center, and the different anaerobic microorganisms essential for anaerobic digestion are situated at the outer layers. For non-layered structure of the granules, *Methanothrix* network throughout the granule was reported.

Propionate and butyrate are among the major intermediates in the anaerobic degradation. The ultimate degradation of propionate and butyrate involves three different groups of bacteria, i.e., syntrophic acetogens, hydrogen-consuming methanogens, and acetate-consuming methanogens. However, the degradation of propionate and butyrate are thermodynamically unfavorable, unless the concentrations of the two metabolites, hydrogen and acetate, are being kept at very low levels. The syntrophic acetogens thus, have to grow in the vicinity of the hydrogen-consuming and acetate-consuming methanogens. Such a syntrophic relationship could be enhanced if the syntrophic bacteria are in juxtaposition so that the diffusion distance for the metabolites is minimized. Observations of similar syntrophic microcolonies were reported by number of investigators [MacLeod *et al*., 1990, Grotenhuis *et al*., 1991a, Fang *et al*., 1994a, 1994b, 1995].

Clearly, the aggregation of anaerobic microorganisms in to granules either layered or non layered structure optimizes the cooperation between the partner organisms, by reducing the diffusion distance for the transfer of metabolites and creating the close cell associations which are obligatory for the degradation of propionate and butyrate [Thiele et al., 1990]. Aggregation of bacteria possessing different metabolic pathways is of pivotal importance, for the energetics and kinetics of the overall substrate conversion, in granulation.

Thus, the structure of the granule is mainly assumed to be influenced by the substrate on which the granules are developed. The layered structure seems to be limited to granules treating soluble carbohydrates as substrate, such as those in the sucrose, glucose, and brewery wastewaters. In case of paper mill wastewater, glutamate degrading granules, propionate degrading granule, and hydrolyzed protein treatment non layered structure was reported. Although, bacteriological composition, and thus, the microstructure of a granule was reported to be dependent on the substrate, this statement is oversimplified and there are other factors such as operating conditions and hydrodynamics inside the reactor which may have some effect on the structure of the granules. Further research is needed in this direction to arrive at any rigid conclusion.

The loading rates applied during formation of granules mainly responsible for the strength of granules. For getting good strength of the granules developed, it is advisable to start the reactor in the loading range SLR=0.15-0.24kgCOD/kgVSS.d. and OLR=2-3.6kgCOD/m^3.d. also for the higher strength of granules developed, higher COD removal efficiency can be obtained [Ghangrekar, 1997].