# GRANULATION OF BIOMASS

## GRANULATION OF BIOMASS IN UASB REACTORS

A highly settleable and active sludge is generally considered to be essential for the UASB process in maintaining requisite solid retention time [Cail and Barford, 1985]. In most of the UASB reactors such kind of biomass is observed in the granular form [Hulshoff *et al.,* 1983a, Mahoney *et al*., 1987]. This methanogenic granular sludge developed in UASB reactor can be described as a spherical biofilm consisting of a densely packed anaerobic microbial consortium [Quarmby and Forster, 1995]. Granular sludge can be developed using non-granular inoculum materials at mesophilic, thermophilic as well as ambient temperature conditions for a number of wastewaters.

Granulation of sludge is an indication of the successful operation of the UASB reactor. It has been the point of investigation in the last two decades, and extensive studies have been done by various researchers. Bacteria get attached to the heavy sludge ingredients and growth will increasingly be concentrated at these particles, ultimately resulting in the formation of distinct granules, which has a better settling property than flocculent sludge [Hulshoff *et al*., 1983a].

Although, considerable efforts have been made on the study of the granulation process, the mechanisms involved in the formation of granular sludge are still not fully known, and the process has not been quantified. In some cases, other forms of sludge rather than granular sludge are obtained in the UASB reactor [Hulshoff *et al*., 1983a, Hickey *et al*., 1991a,b, Hamoda and Berg, 1884]. Such sludges still show good settleability and high methanogenic activity. Thus, even when granulation is not occurring, it may still be able to achieve good results at lower loading up to 5 kg COD/ $m^3$.d [Habets and Knelissen, 1985a, Maat and Habets, 1987]. From this point of view, it is not absolutely necessary to have a granular sludge configuration in UASB reactors, but when the sludge is in granular form organic loading more than 20 kg COD/ $m^3$.d are possible [Habets and Knelissen, 1985b, Jhung and Choi, 1995].

The granular form of sludge in UASB reactor has several engineering advantages. Microorganisms are usually densely packed and no space is lost for inert supports thus spherical granules provide a maximum microorganisms to space ratio [Guiot *et al.,* 1992a]. Granules show excellent settling properties due to higher buoyant densities and because of their large sizes (Stoke's law). This property is particularly important while dealing with low strength wastewater where adequate biomass retention can be possible even at higher upflow velocity. Due to higher specific activity of granular sludge, higher organic loading (> 30 kg COD/ $m^3$.d) could be possible [Maat and Habets, 1987]. Granular sludge also improves the ability of the reactor to sustain shocks induced by temperature, loading and inhibition [Morgan *et al.,* 1991a]. Due to its good settling ability and high specific activity, granular sludge fulfills the essential requirement of any high rate system by retaining a high concentration of active biomass in the reactor [Defour *et al*., 1994]. Typical values for the biomass concentrations (dry weight) that can be achieved with granular sludge range from 40 to 150 g/L [Morgan *et al*., 1991b]. Also, it is reported that, the granular sludge produce less biomass per gram COD removed than the diffused sludge [Dangcong *et al*., 1994].

Shut down periods of several days to many months do not harm the system, if granular sludge is present. The subsequent start-up reactivates the biomass within few hours, even if the granules have been frozen during reactor shutdowns in the winter [Maat and Habets, 1987, Ramjeawon *et al*., 1995].

Methanogens in granular sludge have higher tolerance to oxygen even if exposed to the wastewater with high dissolved oxygen levels [Kato *et al.*, 1993]. The reasons could be the presence of facultative bacteria metabolizing biodegradable substrates and secondly, granules are large excess of the diameter than that of the oxygen penetration depth (100 ③m) to maintain anaerobic zones.

## HYPOTHESIS FOR GRANULATION IN UASB REACTOR

Very little information is available on granulation mechanism in UASB reactor. A few researchers have given the hypothesis for granule formation in UASB reactor. Lettinga *et al.*, [1980a] have reported that in the first stage of the granulation process (in about 10 days) filamentous bacteria, which are almost absent in the original seed sludge, grew on the external surface of a separate sludge floc, but beyond 50 days increasing number of *Methanosarcina* were observed, which binds the different species into granular form.

Hulshoff Pol *et al.*, [1982,83c] indicated that when digested sewage sludge was used as inoculum; and fatty acid mixture was used for feed (acetate plus propionate), three different phases could be distinguished during the granulation process. Each phase was characterized by different sludge concentration profiles in the reactors and differences in the nature of the sludge itself. In phase-I, the sludge bed expanded and sludge concentration in sludge blanket increased. During the Phase-II, the sludge concentration in the blanket continued to increase and wash out of the inoculated sludge began, consequently the total amount of the sludge in the reactor dropped to a minimum level. At the same time, granules began to form in the sludge bed and gradually grow. In Phase-III, the growth of granules exceeds the sludge wash-out rate and the total biomass in the reactor again increased. The higher COD loading rates tended to enhance the process of granulation and, therefore, emphasized that a relatively high specific COD loading rate (>0.3 to 0.5 kg COD/kg VSS-d) should be applied as soon as possible to accelerate granule formation.

Samsoon *et al.*, [1987] proposed an hypothesis to describe pellet or granule formation. According to them the sludge bed can be divided into three parts. A lower active zone, is the bottom most zone in which concentration of short chain fatty acids increased to maximum because of acidification. Hydrogen is generated in this zone at such a rate that high hydrogen partial pressure is created. In the second zone, called upper active zone, the partial hydrogen pressure is reduced to minimum by the action of hydrogenotrophs. In the third zone, called upper inactive zone, no observable biological activity takes place. The granulation takes place mainly in the lower active zone and granule breakup in the higher zones [Wentzel *et al.*, 1994].

Thus, the reactor operation is such that there is partial phase separation of acidogenic and methanogenic phase in the lower active zone. Therefore, high Hydrogen pressure is created in this zone because of acidification. Under such high Hydrogen partial pressure region a methanogens presumably *Methanobacterium* strain which uses hydrogen only as its energy source, can produce all its amino acids for cell synthesis except cysteine. Amino acids (except cysteine) are produced at higher rate but the growth is limited by availability of cysteine. The excess production of aminoacids induces the organisms to form extra cellular long chain polypeptides, which bind the species and others into granules. Hence, for granulation, the substrate must yield $H_2$ and opportunity should be there for high $H_2$ partial pressure build up in bottom zone.

Russo and Dold [1989] identified that requirement of a high $H_2$ partial pressure region to induce granule formation was very significant in the treatment of wastes from pulp and paper industry with very high sulphate concentration.  Since, sulphate reducing organisms have a high affinity for hydrogen than the methanogens, the generated hydrogen was utilized in reduction of sulphate ion to sulphide, and reduction in the $H_2$ partial pressure took place. Hence, the granulation might have been inhibited due to non availability of high $H_2$ partial pressure zone, due to presence of high sulphate concentration in the wastewater.

Morgan *et al.*, [1991b] gave suggestion for possible granulation in UASB reactor.  According to them precursors of granules are bundles of entangled *Methanothrix* filaments that are selected during reactor start-up.  Initially, granules develop from a precursor, which consists of a small aggregate of *Methanothrix* and other microorganisms.  With time, the *Methanothrix* filaments grow, forming characteristic bundles separated by the surrounding matrix in which other methanogenic and non-methanogenic bacteria are embedded.  As the bundles increase in size the surrounding matrix becomes excluded, producing a region towards the center of the granule, which consists exclusively of compact filaments of *Methanothrix*.  Within this region, the discrete bundles can no longer be seen.  These observations were made on well formed anaerobic granular sludge from paper mill effluent and sugar refinery waste treatment by using scanning electron microscopy and light microscopy of thin section.

Carefully analyzing the effects of factors on sludge on sludge granulation and on bacterial adhesion in UASB reactor, a conclusion can be drawn that the upflow hydraulic stress including the shear stress and the wastewater composition (i.e. the substrate) are the most important factors in microbial granulation. The hydraulic stress is a physical effect that selects for biomass with a high settling velocity under pre-determined physical conditions rather than biological controlling factors [Tay *et. al.*, 2000]. The composition of wastewater is directly related to the success of sludge granulation, therefore it is probably very important stimulative factor.