

A Study on Granulation Process inside a UASB Reactor

S.M.Suneeth Kumar¹, I.M. Mishra², G.Anusha³ and Vasudevan M⁴

¹Department of Civil Engineering, ATME College of Engineering, Mysore, Karnataka, India
Email: suneethsaragur@gmail.com

²Department of Chemical Engineering, IIT, Dhanbad, Jharkhand, India
Email: immishra49@gmail.com

³Department of Civil Engineering, KPR Institute of Technology Coimbatore, Tamil Nadu, India
Email: anushanivasbit@gmail.com

⁴Department of Civil Engineering, Bannari Amman Institute of Technology, Sathyamangalam, Tamil Nadu, India
Email: vasudevan@bitsathy.ac.in

Abstract—Advanced treatment of wastewater using anaerobic reactors has been versatile due to their simplicity of operation, minimum maintenance and high performance efficiency. The Up-flow Anaerobic Sludge Blanket (UASB) reactor is one of the best performing models having high retention record of viable microbial mass capable of degrading a wide range of organics. The success of this method largely depends on the development of compacted pellets (granules) owing to a complex but not well understood phenomenon of granulation. The theories of granulation proposed by several researchers have been reviewed in this study and an attempt has been made to propose a simplified theory analogous to crystallization. The hypotheses of this study were successfully verified with the experimental data to determine the growth rate of granules inside the reactor.

Index Terms— Anaerobic treatment; Biomass retention; Crystallization; Granulation; Organic loading; UASB reactor; Settling Velocity.

I. INTRODUCTION

In the last 20 years, over 150 up-flow anaerobic sludge blanket (UASB) reactor units have been built in the world for treating organic wastes. Nearly 35 plants exist in India, mostly on build, own, operate and transfer (BOOT) basis, covering various industries such as distilleries, dairies, pulp mills, pharmaceuticals, starch-maize units, textiles, tanneries and municipal sewage (Arceivala, 1998). The reason for the UASB process finding application at such a rapid pace is due its cost effectiveness with high loading capacity, simple design, low capital and operating cost, no loss of the reactor volume or filter/carrier material, no need for high rate effluent recirculation and thus excess pumping energy involved (Bal and Dhagat, 2001). The main advantage of the UASB reactor is the high retention of viable microbial mass in the form of compacted pellets (granules) owing to a complex but not well understood phenomenon of granulation. The granules are formed due to accumulation of high-density biomass inside the reactor under pressure due to a good water head thus leading to their high retention time inside the reactor resulting in minimal washout. Rich microbial diversity present within the granule helps in rapid degradation of organic wastes. It is the key factor for the successful operation of the UASB reactors. Lack of insight and quantification of operational and environmental parameters that have an influence on granule formation hinder a successful startup of an UASB reactor.

Weiland and Rozzi (1991) gave following factors that hinder startup of an UASB reactor: (i) Reasons for the lack of granule formation in distinct wastewater are unknown; (ii) Environmental factors which enhance the desegregation are mostly unknown; (iii) Stability of granules with changing wastewater properties cannot be predicted; (iv) Only few factors, stimulating granular formation are known; (v) Development of granular sludge from non-granular sludge is difficult; (vi) Granular size and density cannot be controlled exactly; (vii) Minimum amount of granules for enhancing reactor startup is unknown. Since the granule formation is the most important intrinsic parameter affecting the startup and subsequent operation of the reactor, various researchers have carried out extensive work to provide an insight on the granules formation in the reactor. However, there is a lack of simplified mechanistic model to represent the functional process of granulation at a meso-scale of operation to facilitate the upgradation of related process parameters of the reactor for better performance. The present paper critically examines the literature on granule formation in the UASB reactors and proposes a simple, yet a rational model on the pattern of crystallization, to explain the granulation process.

II. LITERATURE REVIEW

The phenomenon of granulation is vital in the successful start-up of a UASB reactor as the granulated sludge has shown higher cell density and rich microbial diversity as compared to ordinary digested sludge or anaerobic processing sludge, resulting in higher COD removal efficiency. Development of granulated sludge from digested sewage sludge is an art in itself. Sam-soon et al., (1987) postulated that: (i) High volatile suspended solids (VSS) yield in the UASB reactor is due to the generation of extra-cellular polymer, which enmeshed the organism mass into pellets; (ii) The polymer was composed predominantly of peptides and the generation of peptide polymer took place principally in the lower active (high pH) zone. From these, the conditions needed for pelletization can be summarized as (i) An environment with high pH; (ii) A nitrogen source, in the free and saline ammonia form, well in excess of the metabolic requirement of the organisms; and (iii) A limited source of cysteine either from the feed or the action of other organisms. They have also suggested the ratio of TKN (Total Kjeldahl Nitrogen) to COD (Chemical Oxygen Demand) to be greater than 0.017.

A. Factors favoring Granule Formation

Kennedy and Guiot (1986) have given the following conditions for achieving a fast growth of the start-up culture: Temperature (33-37° C or 50-55°C), pH (7.2 – 7.6), COD: N: P (100 : (10:1): (5:1)), addition of nutrients (as necessary), and Iron, Nickel, Cobalt and Molybdenum as essential for methanogenic growth. Quarmby and Forster (1995) as well as Bastone and Keller (2001), in their studies have shown that the presence of carbohydrates promotes the production of extra cellular polysaccharides, which enhance bacterial agglomeration. Adequate amounts of essential nutrients must be available in the UASB system so that sufficient build-up of biomass can take place. Nutrient deficient anaerobic systems often face the problem of sludge bulking due to filamentous growth. Granular seed sludge favors the phenomenon of granulation compared to that of non-granular seed sludge even though various reactors are successfully running by taking non-granular seeds sludge as the inoculums. Along with that, if the source wastewater from which the granular seed sludge has been taken matches the nature of the wastewater to be treated, then it will be easy for the seed microbes to adapt themselves to new climate. Studies of Wu et al. (1985, 1987) indicate that high specific COD loading rate tends to enhance the process of granulation. They emphasized the application of relatively high specific COD loading rate (>0.3 to $0.5 \text{ kg COD (kgVSS)}^{-1}\text{d}^{-1}$) whereas Takashima and Speece (1988) proposed the use of trace elements (such as Ni, Co, Mo) to stimulate granulation process. According to Lettinga et al. (1980), a low concentration ($80\text{-}200 \text{ mg L}^{-1}$) of calcium appears to be beneficial for the development of granules in an UASB reactor. Laboratory scale experiments by Brummeler et al. (1985) demonstrated that granular sludge can be obtained in the UASB reactor over a pH range of 6.5 – 7.5. Small amounts of suspended solids have been reported to enhance the attachment of *Methanosaeta* (formerly known as *Methanotrix*) and to favour the granulation (Wiegant, 1987). Alphenar et al. (1993) observed that the granulation in UASB reactor is favoured by a combination of high liquid up-flow velocity and short hydraulic retention time (HRT). El-Mamouni et al. (1998) have shown that some additives like activated carbon and especially the natural one, enhances sludge granulation. Tay and Fang (2000) reported that addition of Fe has positive influence on sludge granulation but has no effect on the COD removal efficiency.

B. Factors inhibiting Granular Formation

Hulshoff Pol et al. (1983) observed that high concentration of ammoniacal nitrogen ($>1000 \text{ mg L}^{-1} \text{ NH}_4\text{-N}$) inhibits granulation in the UASB reactors. According to Liu and Tay (2002), recirculation of activated sludge to UASB reactors can result in the de granulation of the sludge. A sudden temperature shock (increase or decrease)

resulted in poor COD removal and biomass washout in thermophile UASB reactors. Further, growth and activities of bio-granules were adversely affected more by the temperature increase than by decrease (Ivan et al., 1997; Suneeth Kumar, 1998).

C. Mechanistic Models of Granulation

The review of literature shows that there are several factors which seem to affect the granulation phenomenon. Based on these factors and taking into account the microbiological considerations, various authors have proposed different mechanisms of granulation. Yu et al. (2003) have presented a detailed review on the mechanisms and models for anaerobic granulation in the UASB reactor. These models were categorized into physic-chemical, structural, proton translocation – dehydration, cellular automation and cell to cell communication models. They explained that the individual models do not fully explain the granulation process and proposed a general four – step model for anaerobic granulation as shown here.

Step1: Physical movement to initiate bacterium – to – bacterium contact or bacterium attachment onto nuclei.

Step 2: Initial attractive forces to keep stable multicellular contacts

Step 3: Microbial forces to make cell aggregation mature.

Step 4: Steady state three – dimensional structure of microbial aggregates shaped by hydrodynamic shear forces.

These steps may be considered as analogous to the steps given by Yan and Tay (1997) for the granular growth. They had developed the model equations for the granule growth based on the assumptions that the growth of the granules occurs in three phases namely: (i) Acclimation (very startup to granule initiation), (ii) Granulation (when initial granules are developed at the bottom of the reactor), and (iii) Maturation. Researchers have been working on the anaerobic treatment of various high strength wastes in different reactors and found the use of UASB reactors with certain modifications / hybridization to be very advantageous, particularly when the primary aim is energy recovery. During this research work on crystallization, it is felt that the process of granulation can be explained on the basis of the phenomenon of crystallization. In the following paragraphs the salient features of these models of the crystallization phenomenon, their relevance and analogy to granulation are discussed.

D. Crystallization v/s Granulation

Crystallization phenomenon may be considered akin to microbial cell granulation /pelletization. It is proposed that the granulation process may be postulated to actually mimic and imitates the crystallization process. Crystallization process occurs in three sequential steps: Nucleation, ii) Crystal growth and iii) Aging of crystals, respectively. These three steps resemble the steps of acclimation or nucleation, granulation and maturation as observed in anaerobic granulation. Since the nucleation itself can occur in many ways and several theories are propounded for crystal growth therefore it is imperative to review the crystallization phenomena. As defined by Nyvlt et al., (1985), crystallization is a phenomenon in which an ordered solid phase is precipitated from a gaseous, liquid or solid phase. The liquid phase may be either a melt or a solution. For crystallization to proceed, the concentration of the dissolved component should be greater than its equilibrium concentration i.e. the solution should be supersaturated with the solute to be crystallized. The kinetics of crystallization usually can be divided into two stages: (i) Formation of crystal nuclei or nucleation, and (ii) Crystal growth which are explained below.

Nucleation

The formation of crystal nuclei or nucleation can be considered as a process that decides the size of product crystals and thus also plays a substantial role in determining a number of their physical properties and their purity. As studied by Nyvlt et al. (1985), the primary requirement in control of the crystallization process is the number of crystals formed, i.e. control of the nucleation rate. It has been shown that the nucleation rate is very low at low super saturations and only at large super saturations, does it begin to increase. This can be compared with the growth of micro-organisms inside the reactor which are actually acting as nuclei for further attachment and compaction of cells to form granules. The growth of microorganisms is also dependent upon the food that is supplied to them and the number of microbial cells increases with time, if food supply is sufficient. Depending upon the mechanism involved, the formation of nuclei can be subdivided according to following scheme (Figure 1).

The basic criteria for distinction between primary and secondary nucleation is the presence or absence of solid phase. While primary nucleation occurs in the absence of solid particles, secondary crystallization is dependent on the presence of crystals. For homogenous nucleation, no solid phase is required, while heterogeneous

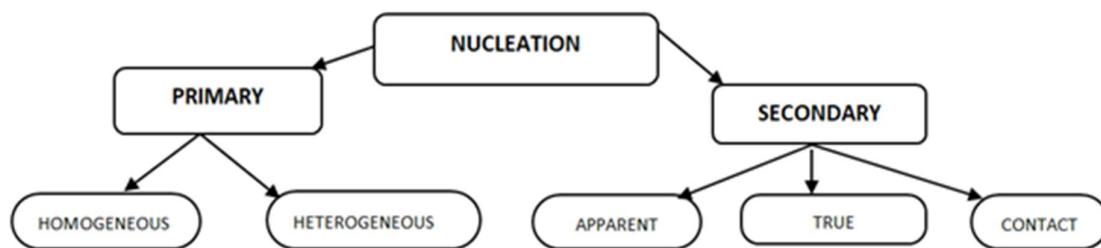


Figure 1: Mechanisms of nuclei formation during crystallization

nucleation is initiated by any foreign surface (vessel wall, dust, colloids, etc.). In the case of anaerobic microbial granulation, the heterogeneous nucleation and secondary nucleation shall predominate.

Primary nucleation

During primary nucleation, small regions are formed within the homogenous phase, consisting of variable numbers of ordered atoms or molecules with different properties from those of the original phase, termed clusters or embryos. Some of these clusters disintegrate in further process, while others grow until they attain a size in equilibrium with the mother liquor. This cluster, termed as critical nucleus, is converted during further growth into a macro species forming the new phase. For stable nucleus to be formed in solution, a certain degree of super saturation must be exceeded. Similarly for microbial granulation, the primary heterogeneous nucleation shall occur by bacteria only when enough food in the form of Chemical Oxygen Demand (COD) is available in the reactor.

Secondary nucleation

The nucleation resulting from the presence of crystals in supersaturated solutions is generally termed secondary nucleation. Nyvlt (1973, 1978) has classified the mechanism of secondary nucleation as (i) Apparent secondary nucleation, (ii) True secondary nucleation and (iii) Contact nucleation. These three categories differ in the sources of crystal nuclei. In the first group of mechanisms, the crystals introduced into the system are the source of the nuclei and the formation of completely new nuclei does not really occur. In the second group, crystal nuclei are formed by interaction of the crystals and the solution. In the third group, interactions between the crystals and other solid surface is necessary, e.g. with a crystal, the stirrer or the walls of the crystallizer. In the UASB reactor, the substrate (food) is initially seeded with viable microbial cell obtained from digested sewage sludge. These act as nuclei for granular formation and propagation of microbial cells. The newly born cells behave as new nuclei for further granule formation. From the above discussion it can be postulated that the acclimatization (very startup of granule initiation) is the special case of simultaneous occurrence of primary, apparent secondary and true secondary nucleation. The recent work of Mahoney et al. (1987), Schimdt and Ahring (1993) and that of Yu et al. (2003) show that the presence of di- and multivalent- metals enhance granulation.

E. Crystal growth

Crystal growth is the second step during crystallization kinetics. Phenomenon of crystal growth is of great importance. By knowing the laws that govern the crystal growth, we can achieve the desired properties of the crystalline product, with L as length:

$$L = dL/dt \dots\dots\dots(1)$$

For practical reasons, the characteristic dimension is frequently chosen as the second largest dimension of the crystal as this value can best be correlated with the results of sieve analysis (Mullin, 1992). Crystallization is a complicated phenomenon as it involves phase change. This complexity has led to ever-evolving discussions on crystal growth in literature both from the thermodynamic and kinetic angles. The theories of crystal growth can be divided into two broad categories as theoretical studies and kinetic studies. Theories dealing with crystal growth from a purely thermodynamic point of view. These theories consider the dependence of the final crystal shape on the energy conditions prevailing in the crystal-mother phase system. This group includes the theories such as: (i) The theory of limiting faces; (ii) The Kossel and Stranski theory and (iii) The Solid on Solid (SOS) model.

Theories dealing with the actual kinetics of crystal growth that attempt to describes the effects of external parameters (such as concentration, temperature, pressure) on the final crystal shape and also to determine the effect of these parameters on the rate of growth of the individual crystal faces. They thus permit optimal conditions to be found for the production of a crystal with defined properties. This group includes the theories

such as (i) The boundary layer theory; (ii) The BCF surface diffusion model; (iii) The volume diffusion model; (iv) Combined surface-volume diffusion; (v) The diffusion model; (vi) The kinematic theory and (vii) The poly-nuclear (“birth and spread”) or “nuclei above nuclei” (NAN) model.

III. MATERIALS AND METHODS

A. Model Development

Since the process of granulation involves the microbial growth over already present seed sludge particles in a specific orderly manner and this orderly manner is dependent upon the external condition such as pH, hydraulics, and concentration of essential nutrients and the microbial diversity prevailing in the UASB reactor, the dependence of growth of granules on the external factors in the UASB reactor permits us to treat the granulation phenomenon analogous to that of kinetic theories of crystallization. Among the kinetic theories, it is the theory of the poly-nuclear growth (“birth and spread”) model that closely resembles the phenomenon of granulation. The “birth and spread model” considers the formation of nuclei and their subsequent growth assuming that: (i) There is no internal growth between nuclei; (ii) The rate of growth of individual faces is constant; and (iii) The nucleus may be formed anywhere, either on an unfinished layer or on an earlier nucleus that is now growing, i.e. a nucleus forms on a nucleus (“top on top”).

In the granule formation nuclei is a microbial cell that is excreting ECPs (extra cellular polymers). These ECPs will then enhance the attachment of one nucleus (bacterial cell) with the other nucleus present in the bed. Once they attach one another, there will be no internal growth in between them i.e., although ECPs will be generated, they will form on the outer surface of attached nuclei (microbial cell). The second assumption that the growth of an individual face is constant is similar to the growth of granules where rate of growth is constant because the total Kjeldahl nitrogen (required for ECPs generation) is present, well in excess of that required for metabolic activity of the bacterial cells, thus allowing them to excrete the ECPs at constant rate. The third assumption propounds that the generation of new cells that will take part in the growth of granules can come from anywhere and attaches themselves to already growing granule, so this can be called as cell on cell. It may be argued that the seed sludge, having microbial diversity, will have uptake of feed (nutrients and micronutrients) from a given substrate in such a manner that its initial diverse nature is altered in specific manner. Therefore, the cell on cell growth is not a mono- or uni-culture growth, but may be multicultural growth having specificity in relation to a given substrate. Each substrate will, then, have a unique granule structure, thriving on the synergistic metabolism in a unique manner. The above theory has been strengthened by the experimental study conducted during the start-up of a UASB reactor, which has been explained below.

B. Materials

Microbial seed culture

Digested sewage sludge was used as the starting inoculums. The anaerobic sludge was acquired from the anaerobic reactors of Okhla Sewage Works, New Delhi, which are used for the treatment of municipal wastewaters. The sludge was sieved through an ASTM 16 sieve to remove floating and large suspended solids and then washed with tap water to remove undesired substances as well as low-density flocs in the sludge. This sludge was then used for seeding the reactor. The features of the seed sludge used in this study are given in Table I.

TABLE I: FEATURES OF DIGESTED SEWAGE SLUDGE

Sl. No.	Parameter	Value
1	Total Solids (TS)	150.2 g/l
2	Total Suspended Solids (TSS)	91.1 g/l
3	Volatile Suspended Solids (VSS)	32.0 g/l
4	Colour	Dark Grey
5	Height of Sludge Bed	0.45 m
6	Volume of Sludge used	3.5 liters
7	Sludge Occupancy in the reactor	31.25 l/ g VSS

Substrate

Aqueous solution of glucose and sugarcane molasses obtained from a local sugar factory, has been used as substrate to supplement carbon and the nutrients for the growth of the anaerobic microorganism. In order to

develop well acclimatized microbial mass culture for the UASB process, molasses solution was used as the substrate during the startup period and then during steady state operation of the reactor. Fresh molasses was procured from a nearby sugar mill and stored at low temperature (3-4 °C) in a deep freezer. The chemical composition was determined by methods suggested in APHA, AWCA, WPCA (1992). The characteristics of molasses are given in Table II.

TABLE II: FEATURES OF THE MOLASSES

Sl. No.	Parameter	Value
1	Chemical Oxygen Demand (COD)	900 g/l
2	Total Solids (TS)	1200 g/l
3	Sulphate (as BaSO ₄)	48.88 g/l
4	Calcium (as CaO)	8.43 g/l
5	Magnesium (as MgO)	9.67 /l

Chemicals

The chemicals used as secondary supplements, such as urea, potassium di-hydrogen orthophosphate, sodium bicarbonate etc. were of commercial grade and obtained from local vendors. The micronutrients were of analytical grade and obtained from M/s.S.D. fine chemicals, Mumbai.

C. Experimental Set-up

The lab-reactor is made of plexiglass and has an effective volume of 9.75 L. It comprised of two modules each of 600 mm length and 100 mm diameter made up of plexiglass pipes and sheets. The modules are fitted one above the other with the help of flanges made by using 12.5 mm plexiglass sheets to have the reactor of 1200 mm height. Neoprene pads are provided between the flanges to make the joints leak-proof. Sampling ports of 10 mm size, made from brass tubes have been provided along the height of the reactor, at a distance of 150 mm from each other. Provision is made at the bottom to have multiple entry of the influent into the reactor. This inlet system was provided with four, 10 mm size brass nozzles around the circumference of the reactor at a height of 50 mm from the reactor bottom. The gas was collected in the sealed inner concentric pipe, which in turn was connected to a gas meter as shown. The gas was measured by liquid displacement method. The reactor was placed in a constant temperature, environmental chamber maintained at 35 + / - 0.5 °C and was fed with aqueous molasses solution as the substrate (with predetermined COD and pH) with the help of a peristaltic pump. Figure 2 shows the schematic diagram of the experimental set-up.

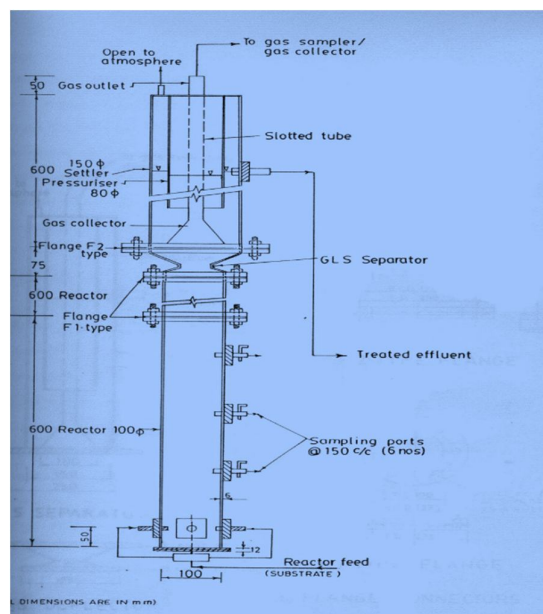


Figure 2: Fabrication details of UASB Reactor

D. Studies on the UASB reactor

The reactor was seeded with 3.5 L of screened and washed digested sewage sludge using the peristaltic pump with minimum dilution with tap water required for pumping. The feed solution (Glucose to begin with) was fed into the reactor at a rate of 1.2 L h^{-1} in an upflow mode at an Organic Loading Rate (OLR) of $1.5 \text{ g COD L}^{-1} \text{ d}^{-1}$ with a HRT of 8 h and the wastewater COD concentration of 0.5 g L^{-1} . To begin with, for an initial period for thirteen days, the required OLR was supplemented with solution of both molasses and glucose in order to enhance microbial activity. To prepare the feed solution equal amount of glucose and molasses was dissolved in 20L of tap water in clean borosil-make glass container to get the required organic loading in terms of COD concentration. The corresponding sludge loading rate (SLR) or conventional Food: Microbial mass ratio was $0.129 \text{ g COD (g VSS)}^{-1} \text{ d}^{-1}$. After filling up of the reactor with feed, nitrogen and carbon dioxide were bubbled through the reactor for 1-2 minutes at low flow rate once every day for three days to reduce oxygen toxicity, as suggested by Suneeth Kumar (1998). Urea and potassium di-hydrogen phosphate were added to maintain desired COD: N: P (micronutrients) ratio of 300:10:1 to have balanced nutrients in the feed for the growth of micro organisms. In order to improve the settling characteristics of the sludge, trace amounts of micro nutrients such as cobalt chloride, nickel chloride, zinc chloride, barium chloride, strontium chloride, all of analytical grade were added once a week as suggested by Henze and Harromoes (1983) and Suneeth Kumar (1998). After reaching the steady state, the OLR was increased to $3.0 \text{ g COD L}^{-1} \text{ d}^{-1}$ with close monitoring of pH, temperature and the flow rate to maintain the conditions for good anaerobic activity. The OLR was increased later in the steps of $3.0 \text{ g COD L}^{-1} \text{ d}^{-1}$, until it reached $9 \text{ g COD L}^{-1} \text{ d}^{-1}$, the steady state OLR as suggested by Suneeth Kumar (1998). The initial start-up period at the optimum OLR was 74 days by which time, stable sludge granules were obtained. After the acclimatization period, the growth of granules in size was noted. The pH of feed was adjusted to 6.8 – 7.0 (optimum range for methanogenesis) with the help aqueous solution of sodium bicarbonate. Since the best performance of the reactor with respect to COD removal and gas production was at an OLR of $15 \text{ g COD L}^{-1} \text{ d}^{-1}$ (Suneeth Kumar, 1998), the reactor was operated at this OLR for about 40 days.

IV. RESULTS AND DISCUSSION

A. Startup of the Reactor

As mentioned earlier, the reactor was fed with the solution of molasses and glucose (50 % each) in tap water as substrate for cultivation of the biomass in the reactor. The reactor is operated for 13 days, initially with this substrate. The COD: N: P was maintained as 300:10:1. Micronutrients were also added. After 13 days, the reactor was fed with only molasses as the substrate. The reactor was operated and monitored continuously for a period of 94 days.

B. Sludge Granules Development

Granules are marked by their high density or high VSS. Therefore, the VSS of the reactor substrate reaction mixture is determined under reactor steady state condition at each OLR. As the reactor operation proceeded, total solids (TS) and VSS and pH are determined at each OLR and at each sampling point (port) along the height of the reactor. The variation of TS and VSS and the VSS:TS ratio as a function of OLR is shown in Figure 3.

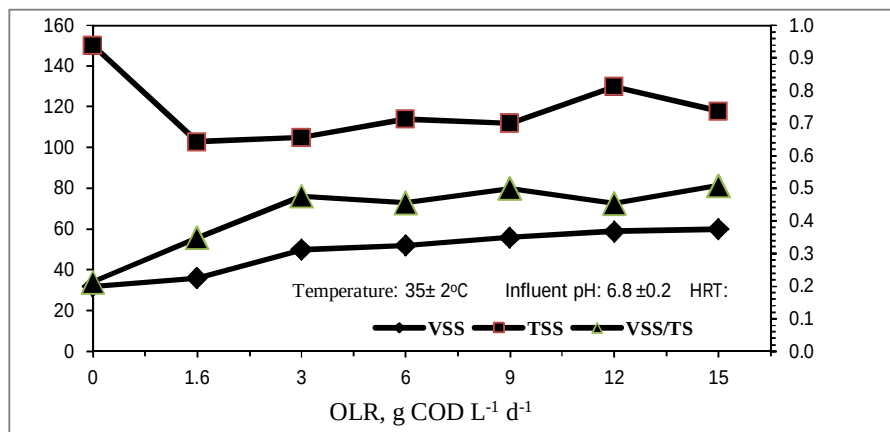


Figure 3: Variation of bio-solids inside reactor with OLR

C. Granular Growth with OLR

Sludge growth that was almost negligible initially, showed progress with time and increasing OLR. The granulation process could be taken to have started after 65 days when the granules having size ranging from 0.3 mm to 1.2 mm with average size of 1.4 mm were observed in the reactor. Some of these granules were flaky while others were dense and angular in shape. The average size of granules got increased to about 1.9 mm at an OLR of 15 g COD L⁻¹d⁻¹. The sludge profile along the height of the reactor indicated that the maximum concentrations of the granules are between 200 mm and 500 mm of the reactor height. The large size and higher density of granules were found to be much lower up to 200 mm from the bottom. This is due to the fact that up flow velocity and the bubble buoyancy creates turbulence in the upper part and accelerates the sludge granule formation. These results are in consonance with the observations made by Suneeth Kumar (1998) while working with distillery spent wash. It is observed that there is a distribution of sludge granules and particles due to disintegration at higher OLR (higher upflow velocity and gas bubble coalescence) along the reactor height. The maximum VSS at the optimum OLR of 15 g COD L⁻¹d⁻¹ was found to be 59.12 g VSS L⁻¹. The VSS concentration at the interface between the bed and blanket was in the range of 18-29 g VSS L⁻¹. The average size of the granules increases with time at the optimum OLR of 15 g COD L⁻¹d⁻¹, as shown in Figure 4.

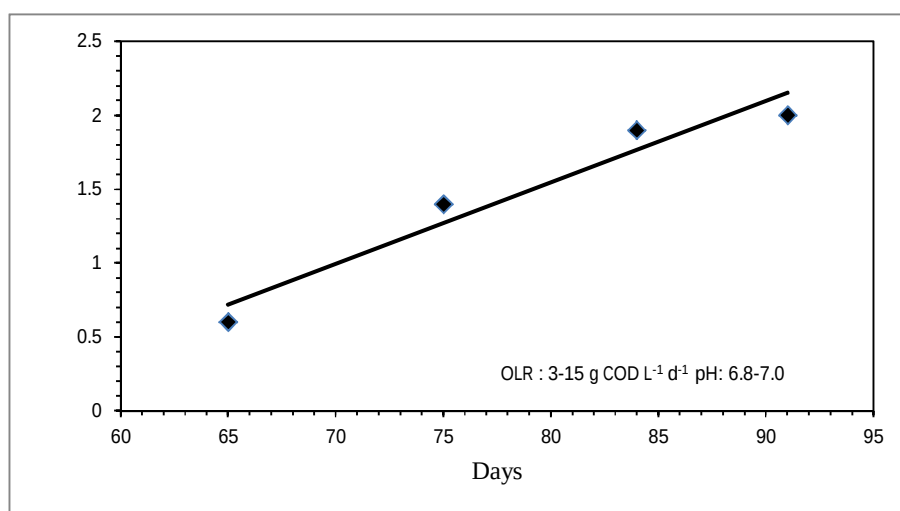


Figure 4: Granular Growth with time at constant OLR

D. Variation in Settling Velocity

The average settling velocity, as determined by the jar test, rises linearly as the average size of the granules increases. This is shown in Fig. 5. Granules, which were noticed after 65 days at an OLR of 12 g COD L⁻¹d⁻¹ have size varying from 0.5 mm to 1.2 mm and settling velocity ranging from 0.514 to 0.625 m/min. The rate of change of average size granules between 65th and 74th day was found to be 0.061 mm/day. The ratio of N: COD at this OLR is 0.033. On the 75th day when the OLR is changed to 15 g COD L⁻¹d⁻¹, the amount of urea added to the influent solution is kept constant so as to make N:COD ratio equal to 0.0264. Since the TKN of influent molasses solution is very small, the total TKN is attributed to the urea solution added to the influent. The average granule size on 75th day was found to be 1.4 mm and the settling velocity of granules ranged from 0.816 m min⁻¹ to 1.8 m min⁻¹. The average size increases with time and on 84th day it was 1.9 mm and it was 2.05 mm on 91st day of operation.

The rate of change in granular dimension at this OLR was found to be 0.0382 mm d⁻¹. The lowering of granular growth at a lower value of TKN: COD ratio proves the dependence of granular growth on the amounts of total nitrogen into the sludge bed fluid substrate. Sam-soon et al., (1990) have suggested that the ratio of TKN to COD should be greater than 0.017, for the hydrogen utilizing bacteria to secrete more amino acids. The over produced amino acids would then induce the ECPs formation, and then Methanobacterium stain and other bacteria would be enmeshed in the ECPs matrix. Suneeth Kumar (1998) has found that the nitrogen to carbon ratio should be greater than 0.02 to have unhindered granule growth in molasses solution and distillery spent-wash. These ratios are considered well enough to cause super saturations of TKN in the reactor.

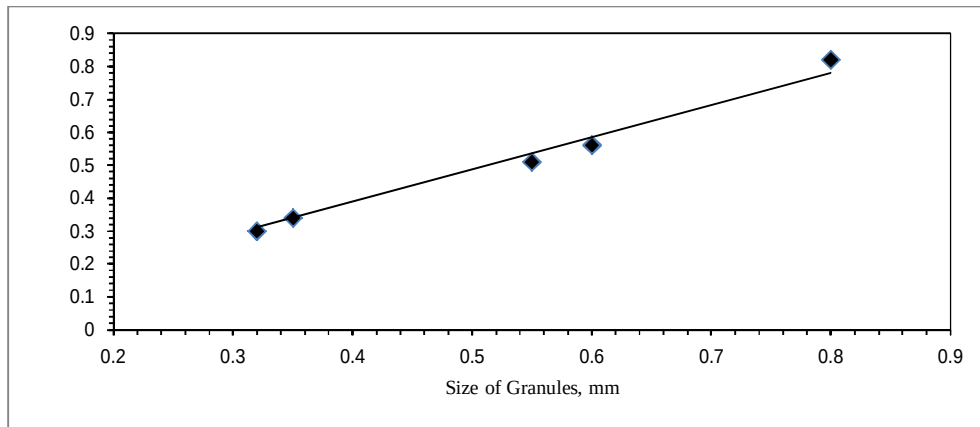


Figure 5: Variation of settling velocity with granule size

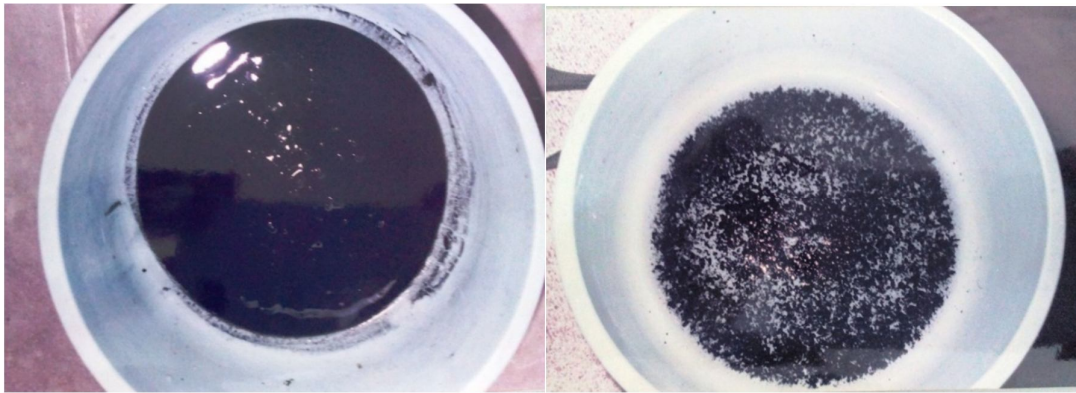


Figure 6: Bacterial sludge before and after granulation

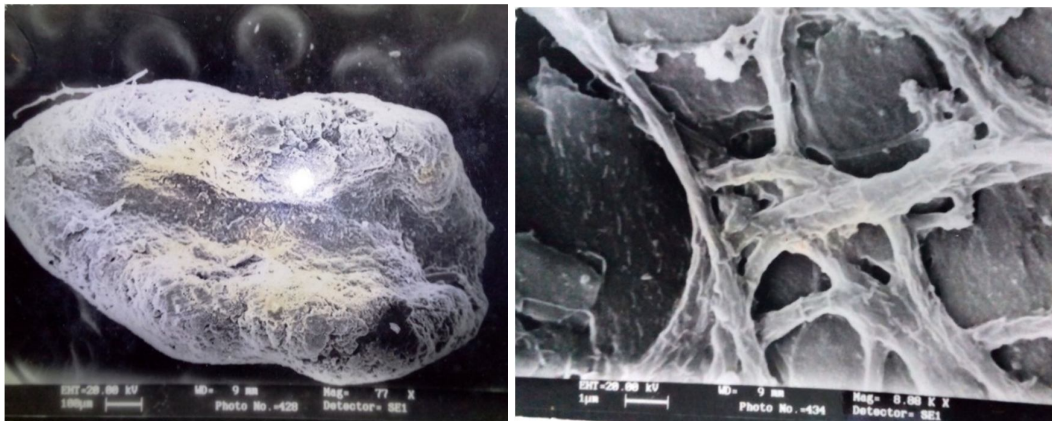


Figure 7: SEM of Single enlarged granule along with rod shaped methanotrix matrix

The bacterial seed sludge, which was buttery and slimy to begin-with, got transformed to discrete granulated sludge particles as shown in Figure 6. The SEM of the enlarged single granule along with the entangled methanotrix matrix is shown in Figure 7.

V. CONCLUSIONS

The following conclusions may be drawn with reference to the granulation hypothesis:

- The granulation mechanism could be treated analogously with the crystallization kinetics. The birth and spread model of crystallization can be used to explain granular formation and their growth.

- As in crystallization, the crystal growth occurs with local super saturation of the solute.
- The granulation is initiated at around neutral pH, VFA ($<500\text{mg L}^{-1}$) and TKN: COD ratio >0.017 .
- At any OLR, under stable operation of the reactor, granular growth rate and the size of the granules are affected by TKN: COD ratio.
- The granulation in the UASB starts at an OLR of about $8\text{ g COD L}^{-1}\text{d}^{-1}$ or Chemical oxygen demand of 2600 mg L^{-1} , when sufficient macronutrients are added as mentioned by many authors. This may be due to enough generation of ECPs, along with the growth of microorganisms due to sufficient food available. Thus this theory is applicable for systems having fed with COD greater than 2600 mg L^{-1} .
- For high COD wastes, like distillery spent wash as substrate, the optimum value of OLR equal to $15\text{ g COD L}^{-1}\text{d}^{-1}$ or COD of 5000 mg L^{-1} could be taken, as suggested by Suneeth Kumar (1998). At higher OLRs, the reactor upsets, become frequent due to accumulation of VFA and lowering of pH. This leads to disintegration of the granules. Thus granulation range limits itself to $\text{COD}<5000\text{mgL}^{-1}$.
- Hulshoff Pol et al. (1983) have suggested that the value of nitrogen ammonium in saline form should not be greater than 1000 mg L^{-1} as it abnegates the phenomenon of granulation. Thus TKN should not be greater than 1000 mg L^{-1} .
- TKN to COD ratio should be greater than 0.017 as reported by Sam-soon et al., (1990).
- The maximum size of a granule can be of 5 mm as the granules greater than this size will get disintegrated due to the hydrodynamic shear force and bubble movement inside the sludge bed.

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