The phenomenon of granulation of anaerobic sludge

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Aan Jane, Jasper en Tessel

STELLINGEN

1. De veronderstelling dat Methanobacterium strain AZ een belangrijke rol speelt bij de korrelvorming is niet juist.

   Sam-Soon, P.A.L.N.S., R.E. Loewenthal, P.L. Dold and G.R. Marais, 1987. Hypothesis for pelletisation in the Upflow Anaerobic Sludge Bed reactor. Water SA, 13, pp. 69-80.

2. De vorming in een maand van oranje korrels met een diameter van 5-6 mm uit slijkgistingsslib met molasse als substraat moet als een klein wonder worden beschouwd.

   Adebowale, O. and R. Kiff, 1988. Operational trends in UASB reactor bed stability and during initiation of granulation. In: (A. Tilche and A. Rozzi eds.) Poster Papers of the Fifth International Symposium on Anaerobic Digestion, Monduzzi Editore, Bologna , Italy, pp. 99-103.

3. Het maximaliseren van de aktiviteit van de biomassa in de de methaanreactor van het "substrate shuttle process" zal ten koste gaan van het gebruikelijke doel van ieder waterzuiveringproces: minimaliseren van de effluent CZV.

   Thiele, J.H. and J.G. Zeikus, 1988. Substrate shuttle process for high rate biomethanation. In: Anaerobic Digestion 1988. (E.R. Hall and P.N. Hobson eds.), Pergamon Press, pp. 165-173.

4. Het is onjuist de UASB-reactor als voorbeeld te noemen van een anaeroob systeem met een propstroom model.

   o.a. Sam-Soon, P.A.L.N.S., R.E. Loewenthal, P.L. Dold and G.R. Marais, 1988. Pelletisation in Upflow Anaerobic Sludge Bed reactors. In: Anaerobic Digestion 1988. (E.R. Hall and P.N. Hobson eds.). Pergamon Press. pp. 55-60.

5. De bewering van Dolfing, dat de zgn "fluffy pellets", die op mengsels van vluchtige vetzuren kunnen worden gekweekt bij ongevoed bewaren snel desintegreren, wordt niet door de feiten gestaafd.

   Dolfing, J., 1987. Microbiological aspects of granular methanogenic sludge. PhD-thesis, Agricultural University Wageningen.

6. Het belang van de selektieve uitspoeling van vlokkig entslib voor de korrelvorming van anaëroob slib wordt onvoldoende erkend.

   Dit proefschrift

7. Het op grote schaal verharden van wegen in de provincie Overijssel met asbestcement is een ernstig milieuschandaal.

8. De keerzijde van milieutechnologie is, dat het terugschroeven van ons verspillende produktie- en consumptiepatroon er minder noodzakelijk door wordt geacht.

9. Door overconsumptie van zuivel hebben Nederlanders zich ontwikkeld tot onnatuurlijk lange mensen. Het is daarom niet terecht de groei van Nederlandse baby's als norm te beschouwen voor onderzoek naar het effekt van macrobiotische voeding op de groei van baby's.

   Dagnelie, P.C., 1988. Nutritional status and growth of children on macrobiotic diets: a population based study. PhD-thesis, Agricultural University, Wageningen.

10. De vorming van anaëroob korrelslib kan vergeleken worden met het eeuwenoude Chinese spel go, beiden zijn door hun boeiende veelzijdigheid niet zomaar in een model of een computerprogramma te "stoppen".

Stellingen behorende bij het proefschrift "The Phenomenon of Granulation of Anaerobic Sludge" van L.W. Hulshoff Pol.

Wageningen, 6 december 1989

Dankwoord

Het tot stand komen van dit proefschrift was niet mogelijk geweest zonder de medewerking van en de samenwerking met vele personen.

Alleerst wil ik graag de studenten danken, die aan dit onderzoek hebben meegewerkt. Dit waren Ger-Jan Goedvriend, Chris Velzeboer, Karin van Straten, Johan van Groenesteijn, Harry Webers, Jan van Est, Erik ten Brummeler, Kees Heijnekamp, Albertien Paardekoper en Jacques van de Worp.

Voorts wil ik graag de prettige samenwerking memomeren met de Vakgroepen Levensmiddelentechnologie, Sectie Proceskunde en de vakgroep Microbiologie. De samenwerking met de laatstgenoemde vakgroep is zelfs uitgemond in de organisatie van een internationale workshop over het thema korrelvorming in oktober 1987 (GASMAT-workshop).

Binnen het Biotechnion wil ik de medewerkers van de technische dienst, de tekenkamer, de fotografische dienst, de electronische dienst en de automatiseringsdienst bedanken voor hun onmisbare ondersteuning.

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Veel dank ben ik verschuldigd aan mijn vrouw Jane, voor het corrigeren van het Engels. You have been a big support, Janie!

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CONTENTS

CHAPTER 1

GRANULATION OF ANAEROBIC SLUDGE - A REVIEW OF THE STATE OF THE ART AND PRESENTATION OF A HYPOTHESIS OF THE GRANULATION PROCESS

Introduction

Interest in anaerobic digestion processes for wastewater treatment has recently increased. Prior to the 1973 energy crisis anaerobic digestion was almost exclusively employed for sewage sludge and manure digestion. Anaerobic wastewater treatment was not considered to be a feasible alternative to aerobic treatment. The process was presumed to be inherently unstable and due to the low growth rate of anaerobic bacteria, it was not considered possible to apply substantial loading rates. A breakthrough came however, with the pioneering work of Young and McCarty (1969), developers of the Anaerobic Filter system. With this system they could apply space loading rates of up to 3.4 kg $COD.m^{-3}.day^{-1}$ at a removal efficiency of 87% with VFA substrates. Their research initiated the development of a number of new reactor systems for anaerobic wastewater treatment. Reviews of these systems are presented by Henze and Harremoes (1983), Speece (1984), van den Berg (1983) and Switzenbaum (1983).

Work on the development of new reactor systems focused on improving the retention of active biomass in the reactor; consequently the uncoupling of solids retention from the liquid retention. The treatment capacity of anaerobic reactors is dictated by:

1. the amount of active biomass that can be retained in the system;
2. the amount of contact between sludge and wastewater.

Previous investigations revealed that anaerobic sludge easily attaches to a wide variety of carrier and support materials. These observations resulted in the development of anaerobic treatment systems based on attachment of biomass to either fixed or mobile surfaces (downflow fixed film reactors, expanded and fluidized bed reactors and the anaerobic gas lift reactor).

The system with the widest application and recognition is undoubtedly the Upflow Anaerobic Sludge Bed (UASB) process (Lettinga, 1980, 1983), a considerable improvement over the reversed flow Dorr Oliver clarigester which was applied in South Africa (Cillie et al, 1969; Pretorius, 1971). The successful full-scale implementation of the UASB system for various types of wastewaters can be attributed to the fast and successful upscaling of the process made in cooperation with the CSM, a Dutch sugar beet company, and with considerable financial support from the Ministery of Public Health and Environment (presently the Ministery of Housing, Physical Planning and Environment). One of the reasons for the excellent reactor performance on pilot and demonstration scale, was the gradual conversion of the flocculent seed sludge into a well settleable highly active granular sludge. The phenomenon of granulation was observed for the first time in 1973-1974 in the 6 m^3 pilot reactor operated at the CSM factory in Breda. In this reactor, space loading rates up to 32 kg $COD.m^{-3}.d^{-1}$ could be accommodated at treatment efficiencies of 80-98% (Lettinga, 1977). A few years later the same sludge granulation phenomenon was observed in a 6 m^3 pilot reactor installed at a potato processing industry (CAB in Wezep) where loading rates up to 45 kg $COD.m^{-3}.d^{-1}$ could be accommodated at efficiencies over 90% (Versprille, 1978).

After these first pilot and demonstration scale experiences, full-scale UASB reactors were constructed for a variety of different wastewaters. From these experiences we know that the granulation of anaerobic sludge takes place on many different types of wastewaters including cornstarch wastewater (Ross and Smollen, 1981; Zeevalkink and Maaskant, 1983; Ross, 1984), sugar beet wastewater (Lettinga et al, 1980, 1984 and 1985), potato starch wastewater (van Campen et al, 1985; Paris et al, 1988; Wijbenga and Bos, 1988), starch wastewater (Wu et al, 1985), pulp and paper wastewater (Habets and Knelissen; 1985; Vuoriranta et al, 1985; Chen et al, 1988), distillery wastewater or molasses (Wu et al, 1987; Adebowale and Kiff, 1988; Harada et al, 1988; Xiushan et al, 1988), whey (Clark, 1988; Killilia and Colleran, 1988), apple juicing wastewater (Dold et al, 1987), brewery wastewater (Novaes, 1986; Wu et al, 1985; Lui and Hu, 1988), slaughterhouse wastewater (Wu et al, 1985), citrate wastewater (Wu et al, 1987) and even domestic sewage (Vieira, 1984; Vieira and Souza, 1986; Novaes, 1988; Orozco, 1988; van der Last, 1989).

Granulation has also been observed on a variety of synthetic substrates such as ethanol and propionate (de Zeeuw, 1984; Dolfing, 1987; Grotenhuis, 1986), acetate (de Zeeuw, 1984; Harada et al, 1988), mixtures of volatile fatty acids (de Zeeuw, 1984; Harada et al, 1988), sucrose/glucose (Ross and Smollen, 1981; Wu et al, 1985; Wiegant and de Man, 1986; Harada et al, 1988) and gelatine (Schulze et al, 1988). Grotenhuis et al (1988a) also observed initial granulation on propionate in specific recycling systems designed for aggregation of biomass with a low growth rate.

Bacterial adhesion

From experiences with conventional aerobic wastewater treatment it is well-known that micro-organisms easily form flocs and tend to adhere to solid surfaces. The adhering qualities of bacterial matter are successfully employed in oxidation beds, submerged filters, activated carbon filters and in biodiscs.

In high-rate anaerobic treatment processes bacterial adhesion is the major mechanism for achieving the required high sludge retention. In the most prominent representatives of these high-rate processes such as anaerobic expanded bed reactors, fluidized bed reactors, anaerobic filters and up- or downflow fixed film reactors, the surface of inert carrier materials is utilized for bacterial attachment. The UASB process does not require the addition of such a material.

On the other hand however, bacterial adhesion plays an eminent role in the UASB process because of the mutual attachment of micro-organisms as well as their easy attachment to inert inorganic and organic support particles present in the seed sludge. Therefore, to understand the granulation process of anaerobic sludge it is important to know the factors that play a role in bacterial attachment.

Factors governing the initial bacterial adhesion have been extensively described in the literature, e.g. by Atkinson et al (1979 and 1980); Heijnen (1984); Ho (1986); Costerton et al (1987) and Bryers (1987). The following three factors can be distinguished:

1. The nature of the substratum (support particle), including its porosity, pore structure and dimension (Messing et al, 1979a and 1979b), charge of the surface, density of the particles (in case of fluidized and expanded bed reactors), specific surface area and surface tension.
2. Environmental factors such as the pH, temperature, ionic strength, composition and concentration of the organic compounds in the solution and flow regime.
3. The properties of the involved micro-organisms such as the surface characteristics including hydrophobicity and electrophoretic mobility (van Loosdrecht et al, 1987a and 1987b), the bacterial physiology (e.g. the production of extra cellular polymers) and the bacterial morphology.

What are the advantages of granular sludge aggregates over flocculent sludge?

Since flocculent sludge can also be regarded as aggregated sludge it should be pointed out that the most important difference between granular sludge and flocculent sludge is that flocculent sludge will easily fall apart under conditions of mild mixing, while granular sludge stays intact even under fairly extreme cases of hydraulic stress. There is a "grey area" however, between sludge aggregates to be designated as flocs and as granules.

There are several ideas regarding the practical benefits of the formation of aggregates. McCarty and Smith (1986) calculated that at a given H_2 turnover rate (320 sec^{-1}) the distance between acetogenic and hydrogenotrophic bacteria under steady state conditions is 5-11 m, while Stams et al (1989) calculated maximum distances of 4, 9.3 and 660 for propionate, butyrate and ethanol respectively. This indicates that for this high conversion rate it is favorable that the organisms are very close to each other. This idea is supported by various researchers; Calleja et al (1984), Harada et al (1988) and Dubourguier et al (1988a and 1988b). Dubourguier et al (1988a) observed a decrease in propionate degrading activities after disintegration of granular sludge. This is supported by observations we made in own experiments. Thiele et al (1988) concluded that syntrophic relations in flocs (or granules) are not only of importance for interspecies hydrogen transfer, but also for interspecies formate transfer. They suggest that formate plays an important role as intermediate in the anaerobic degradation process. According to Fitzpatrick et al (1989) there are several possible advantageous reasons for bacteria to form aggregates:

1. Aggregation of bacteria leads to heterogeneous ordered populations of syntrophic microorganisms in the form of multicellular associations under favorable physiological conditions.
2. Symbiosis i.e. interactions between adjacent organisms and genetic exchange is facilitated.
3. Growth within a granule unlike freely suspended cells may increase the nutrient uptake.
4. Granulation may protect cells from predators, e.g anaerobic ciliates.
5. The diffusion distance for fermentation intermediates is minimized in granules; this is a very efficient means to conserve every fraction of energy available in a complex degradation system.
6. Under conditions where the composition of the bulk solution is unfavorable for growth (e.g. extreme pH values), a more favorable microenvironment can be created within the aggregate so that metabolism is still possible.

Cultivation of granules

The mechanism of granulation is presently being explored by an increasing number of investigators. Frequently however, their insights are inconsistent such as on the subject of the role of Ca^{2+} ions, and the predominant micro-organisms in the granulation process. According to Dubourguier et al (1988b) FeS plays an important role in the granulation process. He observed that by firmly attaching to the sheath of *Methanothrix*, FeS stabilizes the bacterial aggregates (Dubourguier, 1985). Dubourguier et al (1988b) further suggest that larger granules (>200 m) originate from microflocs of 50 m consisting of *Methanothrix* covered by rod and cocci. These microflocs, which were also described by Macario and Conway de Macario (1988), form conglomerates through bridging of *Methanothrix* filaments.

Additional views are presented by Guiot et al (1988a) and de Zeeuw (1984). Guiot described the hypothesis of granules formed by the colonization of acetoclastic organisms on small aggregates of mainly acidogenic organisms. De Zeeuw presented the idea of hollow *Methanosarcina*-clumps acting as granule precursors.

According to Guiot et al (1988b), preferential growth of acetoclastic organisms induced by trace elements and/or ethanol would promote sludge granulation. This idea is based on the observation that in the digestion of carbohydrate containing substrates, acetoclastic organisms tend to accumulate in biofilms or granules rather than in dispersed bacterial growth. Dispersed bacterial matter was found to contain a larger fraction of acidogenic organisms (Murray, 1984; Dolfing et al 1985 and Guiot et al 1986). Guiot et al (1988a) attribute the observed increased metabolic activity to an improved synthesis of extracellular polysaccharides (EPS). The synthesis of EPS would result in the induction of bacterial aggregation and consequently, the granulation process.

Guiot (1988c) also found a correlation between upflow velocity and size of the granular sludge particles formed. At an upflow velocity of 1 m/hr he found the standard size for granules to be 1-3 mm, whereas at a velocity of 2 m/hr granule size increased.

Sam-Soon et al (1987, 1988) concluded that pellet formation is caused by *Methanobacterium strain AZ*, an organism that utilizes hydrogen as the sole energy source and generates all essential amino acids except cysteine. Under high H_2 partial pressure (substrate) conditions and with an adequate supply of ammonia, a high intra-cellular ATP/ADP ratio would be generated. The growth of the organism however, is still limited by the availability of cysteine. The high ATP/ADP ratio would induce an over-production of amino acids, the excess of which would be secreted as an extra-cellular polypeptide promoting the formation of biopellets. The addition of cysteine in the feed was found to decrease both polymer production and pellet formation. The polymer also entraps other organisms, forcing them to form aggregates. At lower partial H_2 pressures, Sam-Soon and co-workers expect cessation of pellet formation because of the lower ATP/ADP ratios under these conditions.

Mulder et al (1988) obtained evidence that in anaerobic gas-lift reactors operating as acidifying reactors, lysed cells play the role of nuclei for the formation of bacterial granules of *Selemonomas ruminantium*.

Wu and co-workers (1987) describe the granulation process as follows: initially a flocculent type of sludge is formed. Upon increasing the space load a considerable part of bulking sludge flocs are washed out resulting in a decrease in the total amount of sludge in the reactor. Granular sludge develops starting from the bottom part of the reactor, described by Hulshoff Pol et al (1983).

Harada and co-workers (1988) suggest the following granulation mechanism: hydrolytic bacteria attach to solid insoluble substrate ingredients with pili-like appendages and hydrolyze them with exo-enzymes. These pili may also serve as a cell to cell attachment mechanism through bridge formation. They state that solid particles (starch) are entrapped in the granule under conditions of overloading. These entrapped starch particles may be the cause of very low local pH values.

Wiegant and de Man (1986) do not attribute any significant effect on the nature of inoculum nor to the addition of inert support particles in granulation under thermophilic conditions. They do however, consider the selection of *Methanothrix* of importance. Under thermophilic conditions, the sludge selection process is not necessarily accompanied by a massive sludge wash-out.

According to Yoda and co-workers (1989) aggregates of *Methanothrix* serve as secondary nuclei for granulation. Wiegant (1985, 1988) emphasizes the role of superficial biogas loading rates. At the moment that granulation becomes apparent, these superficial velocities are in the range of 6.9 to 11.5 m/day. Other parameters vary over a much wider range, i.e. the liquid superficial loading rate from 1.1-6.1 m/day and the organic loading rate from 4-223 kg COD/m^3.day. Any correlation with sludge granulation could therefore not be assessed.

Wiegant (1988) presented a theory for the granulation of *Methanothrix* dominated granules: the "Spaghetti Theory". According to Wiegant, filamentous *Methanothrix* bacteria may become entangled in microscopic knots which may or may not contain inert support particles. Following a proper step-up routine these knots will grow out to granules. Thereafter, the more compact rod-type granules (granules dominated by *Methanothrix* growing in short chains of 4-6 cells) will develop from the filamentous granules.

Alibhai and Forster (1986a) also argue that *Methanothrix* plays a key role in the granulation process. They found the presence of elements such as Al and Si in the granules when the wastewater contained clay particles (Alibhai and Forster, 1986b).

The role of extracellular polymers

The role of extra cellular polymers (ECP) in the granulation process is still not sufficiently understood. Relatively little is known about the nature and chemical structure of ECP's, as well as the operational conditions that might enhance their formation. It is generally accepted however, that they play an important role in the formation of a supporting matrix (or glycocalix) for the micro-organisms.

According to Alibhai and Forster (1986b) ECP's improve the long-term stability of granules. The production of ECP is affected by the nutritional balance and/or the diversity of the granule micro-flora. According to experimental data of Dolfing (1985) the extracellular polysaccharides contributed to the granules only 1-2% on a dry-weight basis. Although ECP structures can be clearly observed with scanning electron microscopy (SEM), transmission electron microscopy (TEM) seems to indicate that ECP's do not play a major role in the granule matrix (Dolfing, 1986). Ross (1984) found that ECP accumulation plays a major role in the "clumping of bacteria" comparable to the role of microbiological agglutination in the flocculation of aerobic sludge (Brown and Lester, 1979). The polymers can be present in the substrate as well as produced by the polymerization of simpler precursor molecules as a result of metabolism. Ross (1984) found that 4% of the dry biomass consisted of ECP, and he observed filamentous-like extracellular structures composed of long-chain macropolymers of carbohydrates and protein. According to the findings of Roam (1989), 60-90 % of the ECP's produced by anaerobic granules in the conversion of various substrates find their way into the solution phase. The assessed weight percentage of ECP's in a microbial granule therefore, would be an underestimation of the total amount of ECP's produced, and consequently the importance of ECP's in the aggregation process could be underestimated as well. Mass balances of ECP in the solution and granule come to ECP yields (total ECP produced/COD removed*100) of 20% on sucrose and 2-5% on VFA feed. Harada and co-workers (1988) concluded on the basis of observations with SEM and TEM, that ECP's excreted by acidogenic bacteria appeared to assist with cell to cell attachment and the enhancement of mechanical strength and structural stability. The main constituents of ECP's in order of importance are: glucose, rhamnose galactose, mannose and ribose. Galactouronic acid is responsible for the polyanionic nature of ECP, as was determined by anion exchange chromatography.

Through scanning electron microscopy, evidence was obtained indicating that cells often are abundantly covered by polymers (Dubourguier et al, 1985; Dolfing et al, 1985 and Ross, 1984).

Sam-Soon et al (1987,1988) determined on the basis of COD/VSS and TKN/COD ratios of the granular sludge, the disappearance of free and saline ammonia and the generation of organic N. Because of the high yield of VSS obtained (0.36 mg VSS/mg COD) and the effect of supplementary cysteine added to the system, evidence was obtained indicating that *Methanobacterium strain AZ* produces exo-polypeptides under conditions of cysteine shortage. They hypothesized that these exo-polypeptides play a major role in the granulation process.

According to Harada et al (1988), biopolymer production on acetate is limited and therefore, ECP's are not a prerequisite for granulation. A completely opposite conclusion is presented by de Zeeuw (1984) who observed high-growth yield factors in batch-fed reactors and UASB reactors using acetate as a single substrate. These yield factors amounted to 0.064 gVSS/gCOD and 0.17 gVSS/gCOD respectively. De Zeeuw explained these high yield factors by presuming that most of the growth took place in the form of ECP production. This conclusion was supported by the observation that extra ammonia fixation could not be detected.

Effect of calcium

Considerable attention has been focused on the effects of calcium on granule properties and on the granulation process. It is well-known that divalent cations have a positive effect on the flocculation of dispersed sludges, which is explained by the assumption that the divalent cations condense the diffusive double-layers, resulting in a relatively stronger effect of "van der Waals attractive forces" (DLVO theory). Calcium can also lead to the formation of "calcium bridges" as a result of the formation of strong interactions with anionic groups present in the organic matrix (Brunetti, 1984; Verrier and Albagnac, 1985; Guiot et al, 1988b). In addition, Ca^{2+} ions affect the methanogenic activity of the anaerobic sludge. According to McCarty, Ca^{2+} ions stimulate at concentrations of 100-200 mg/l, and become inhibitory at concentrations exceeding 2500 mg/l. A complicating factor here is the synergistic and antagonistic relationship between Ca^{2+} and other cations (Kugelman and Chin, 1971).

Start-up experiences in pilot-scale UASB reactors in The Netherlands showed the positive effect of Ca^{2+} on sludge settleability and sludge granulation after replacing $NaHCO_3$ by $Ca(OH)_2$ as neutralizing agent (Versprille, 1978; van der Vlugt, 1980).

Verrier and Albagnac (1985) found that Ca^{2+} concentrations, as well as sodium concentrations have a positive effect on the microbial attachment at concentrations up to 4 meq. In addition to the factors mentioned above, they suggest the possibility that divalent cations indirectly promote bacterial adhesion by increasing the hydrophobicity.

A positive effect of Ca^{2+} has also been observed on sludge granulation (de Zeeuw and Lettinga, 1980; Cail and Barford, 1985; Mahoney et al, 1987).

There is presently general consensus that higher Ca^{2+} concentrations are detrimental to the development of a good quality granular sludge. Wu et al (1987) found that at 800 mg Ca^{2+}/l granulation will not proceed satisfactorily. The granules show a high ash-content (36%) and exert a lower methanogenic activity. Guiot et al (1988b) did not find a significant positive effect on the granulation at a Ca^{2+} concentration of 640 mg/l . According to their research the expected positive effect of Ca was absent because of two reasons: 1) the formation of $CaCO_3$ precipitates, assuming that the precipitates are not effective in granule nucleation and 2) the competition with sodium for binding sites. An experiment performed to check the second hypothesis however, indicated that there were no benefits from reduced monovalent cation concentrations (Guiot et al 1988a).

With granulated sludge, problems with $CaCO_3$ scaling around granules may occur at concentrations above 1000 mg/l (Pereboom, 1984), or problems may arise from the gradual accumulation of $CaCO_3$ precipitates. These precipitates replace the active (granular) biomass (Tschersich and Zoetemeijer, 1984) and form a thick, mostly inorganic sludge bed.

Wu et al (1987) observed that crystals containing Ca, as well as Fe, P or Si, were found embedded in the granules. They found a correlation between size of crystals and Ca^{2+} concentration. On citrate waste with 800-900 mg Ca/l and glucose molasses (Ca < 25 mg/l) precipitates of 100 m and 30 m were obtained.

Contacting granular sludge with a Ca^{2+} chelating agent (EGTA) showed that the granules disintegrate and become weaker (Grotenhuis et al, 1988b). Based on this, Grotenhuis et al concluded that Ca may play an important role in the stability of granules in two ways:

1. inorganic Ca precipitates might serve as surface for adhesion of anaerobic bacteria;
2. Ca may be a constituent of the extracellular polysaccharides and/or proteins which are present as sticking material.

Seed sludge

Although digested sewage sludge is usually used for the start-up of a UASB reactor, various other types of seed sludge can be successfully utilized when granular sludge for seeding is unavailable. Wu et al (1987) utilized aerobic activated sludge from a sewage treatment plant and primary sludge from an aerobic plant treating textile dyeing wastewater. Apparently, sufficient anaerobic nuclei were present in the aerobic flocs. Using a MPN technique for counting the methanogens, it was found that aerobic activated sludge contains 10^8 methanogens/gSS. For digested sludge Zeikus (1979) found a number of 10^8/ml, giving for a 4% (w/v) sludge a figure of 2.5 10^{10}/gSS. All the important methanogens seem to be present in aerobic activated sludge. Other seed sludges that have been applied are lotus pond mud (Oi et al, 1985), cow manure (Wiegant, 1986) and primary sewage sludge (Ross, 1984).

With respect to the use of digested sewage sludge, de Zeeuw (1984) observed that heavy, relatively inactive sludge is preferred over lighter, more active sludge because of expected differences in wash-out. He distinguished two types of sludge wash-out; erosion wash-out and sludge bed wash-out. Sludge bed erosion wash-out represents the selective wash-out on the basis of differences in settleability. Sludge bed expansion wash-out predominately occurs when using a diluted digested sewage sludge in the treatment of a medium strength wastewater. It is caused by the expansion of the sludge bed as a result of the increased hydraulic and gas loading rates and involves little selection between sludge particles with a difference in settleability. By choosing a concentrated digested sewage sludge as seed the latter type of sludge wash-out can be avoided.

Characterization of granules

General observations

The variety in quality and characteristics of granules originating from different sources is great. It is therefore sometimes difficult to differentiate between flocs and granules in sludge agglomerates. Dolfing (1987) used the term "fluffy pellets" to refer to agglomerates that lack the strength of granules in full-scale installations, while still possessing a greater consistency than flocs. At this point, a clear definition for granular sludge does not exist. Based on UASB start-up experiments on laboratory-scale with VFA mixtures as substrate, de Zeeuw (1984, 1988) came to describe three types of granules:

- Type A granules. These are compact spherical granules which consist mainly of rod-shaped bacteria resembling *Methanothrix soehngenii.*
- Type B granules. These are more or less spherical, but consist of loosely intertwined filamentous bacteria usually attached to an inert particle. The prevailing organism again resembles *Methanothrix soehngenii.*
- Type C granules. The granules of this type are spherical and composed of *Methanosarcina*-like bacteria. These granules are smaller (D_p < 0.5 mm).

Stereo microscopic observation at low magnification (up to 40x) of a number of "industrial" granules, revealed a large variety of granule "morphologies". Some granules were composed of smaller aggregates which appeared to be glued together, generally showing an infra-structure of bigger and smaller pores presumably serving for the transport of substrate, degradation intermediates and end products, e.g. the biogas, and are usually irregularly shaped. The sludge produced in the UASB reactor of the sugar beet factory of CSM BV in Breda is an example of this type of sludge.

In other cases the granular sludge is smooth and spherical in shape, as in the case of sludge grown on potato processing wastewater at AVIKO, Steenderen, The Netherlands. Sometimes these smooth granules show large cavities through which gas can escape. Wiegant and de Man (1985) found such cavities in thermophilically cultivated granules. We observed that these granules are often composed of several layers like growth rings in trees. It seems reasonable to assume that these rings are the result of changing conditions in the UASB reactor over time, affecting bacterial growth within the granules. Growth rings were also observed by Yoda et al (1989). Larger granules of this smooth spherically-shaped type have a strong tendency toward flotation at higher loading rates due to problematic gas release from the interior to the outside of the granule. Moreover, many of the larger granules are hollow; the reason for this phenomenon may be the insufficient supply of substrate to the granule's center.

A small, spiky-shaped granular sludge developed on a maize starch wastewater at ZBB maize starch factory, Koog-aan-de-Zaan. This wastewater contained high calcium concentrations (up to 800 mg Ca^{2+}/l), and the granular sludge consisted of about 60% of $CaCO_3$.

Chemical composition of granules

The mineral content of granular sludge is greatly dictated by factors such as:

- the occurrence of precipitation, e.g. of $CaCO_3$, $MgNH_4PO_4$, sulphides, and consequently the composition of the wastewater;
- the presence of inorganic dispersed matter in the wastewater and the incorporation of this suspended matter into the granular sludge;
- the age of the sludge.

As a consequence, the ash-(mineral) content varies significantly (values varying from 8-65% have been assessed in our laboratory).

Various investigators attempted to characterize their granular sludge on the basis of the mineral composition, including Ross (1984) who observed that the granules cultivated in his study had a VSS content of up to 90%. This organic matter contained 11-12.5% crude protein and 10-12% total carbohydrates.

To date, too little relevant information with respect to wastewater composition, process conditions, etc. has been presented. Along with the scarcity of available information in general, no conclusions can yet be drawn.

Some indications have though, been obtained on the role of FeS precipitates: Dolfing et al (1985) found that 30% of the ash fraction of granular sludge cultivated on beet sugar wastewater (CSM) consists of FeS. FeS precipitates presumably onto the slightly lipophilic surface of bacteria. It is believed that the combination of the higher surface tension of FeS and the lipophicity of bacterial surfaces might stabilize the bacterial aggregates in aquatic systems (Grotenhuis, 1986).

In general, granular methanogenic sludge cultivated on true wastewater is black. As anaerobic sludge cultivated on synthetic VFA mixtures is pale yellow, it is presumed that the black color is caused by the presence of precipitated Fe, Ni and Co sulfides.

Microbial characterization of granular sludge - morphology.

Several researchers (Dolfing et al, 1985; Hodalic, 1985; Dubourguier et al, 1988b and Prensier et al, 1988) have attempted to assess the microbial composition of granular sludges using transmission electron microscopy (TEM). According to their observations a significant percentage of the bacterial matter consists of *Methanothrix*-like organisms. In addition, a wide variety of different bacterial morphotypes are present in the granules frequently as micro-colonies, distributed randomly throughout the granules. Using a modified MPN technique (Dubourguier et al; 1985, 1988a) and with immunofluorescence techniques (Prensier et al, 1988) it has been shown that these micro-colonies consist of *Methanothrix* and syntrophic organisms

(*Syntrophobacter* and/or *Syntrophomonas*) associated with *Methanobrevibacter*. As *Methanobrevibacter* will grow faster than other hydrogenotrophic methanogens, this organism will very likely be the major hydrogenotrophic methanogen present in the sludge. The matrix in which the micro-colonies are embedded frequently contains a large amount of empty cells.

According to microscopic data observations made by Thiele et al (1988), acetogens and methanogens are not localized as separate micro-colonies but are positioned in a "lattice" type of arrangement. In granules cultivated on carbohydrate wastewaters, Harada et al (1988) found a fairly distinct localization of various specific groups of organisms (hydrolytic and acidogenic organisms) on the outskirts of the granule, and *Methanothrix*-like organisms dominating in the inner part. In granular sludge grown on VFA mixtures he observed intertwined spherical clumps of *Methanothrix*-filaments of 100-300 m in size sticking out of the granules.

Although *Methanothrix* is mostly referred to as the predominant organism in anaerobic granules, there have been some other observations. Liu and Hu (1988) observed that granules cultivated on diluted malt juice and brewery wastewater consisted mostly of *Methanosarcina*, with filamentous bacteria (presumably *Methanothrix soehngenii*) present on the exterior of the granules. Sam-Soon et al (1988) regard *Methanobacterium Strain AZ* as the key bacteria in granules.

Microbial characterization of granular sludge - physiology.

Microbial aggregation is considered to be beneficial to the interspecies hydrogen transfer between juxtapositioned acetogenic and hydrogenotrophic methanogenic organisms (see also Ferry and Wolfe, 1981; Jones et al, 1984; Conrad et al, 1985; McCarty and Smith, 1986; Schink and Thauer, 1988). According to Thiele and co-workers (1988) however, less than 5% of the syntrophic conversion of ethanol is directly a result of interspecies hydrogen transfer, while the rest can be attributed to interspecies electron flow in which formate plays a key role (Thiele and Zeikus, 1988).

Methanogenic activities of granules

The specific methanogenic activity of granular sludge depends strongly on operational conditions and substrate composition. The more complex the wastewater is, the higher the fraction of acidifying organisms in the granules will be, resulting in a lower specific methanogenic activity of the granular sludge. Under mesophilic conditions we usually find methanogenic activities at 30 °C up to 1.0 kg COD/kg VSS.d for non-acidified substrates and up to 2.5 kg COD/kg VSS.d for acidified substrates. In the literature much higher values have been reported. Guiot et al (1988a) found at 27-29 °C for granules grown on sucrose as substrate, activities ranging from 1.3 to 2.6 kg COD/kg VSS.d, depending on the presence and availability of trace elements, whereas Wiegant (1986) reported activities up to 7.3 kg COD/kg VSS.day for thermophilic "*Methanothrix*-granules" cultivated on an acetate and butyrate mixture at 55 °C.

Assuming *Methanothrix* is the predominant organism in the granule and taking for its growth yield a value of 0.05 kg VSS/kg removed COD, we can estimate the specific methanogenic (acetoclastic) activities of some different *Methanothrix* strains (Table 1.1).

In the many activity test performed in our laboratory we never found an activity at 38 °C exceeding 4.0 kg COD/ kg VSS.day for granules cultivated on a mixture of acetate and propionate. It should therefore be understood that the data of Table 1.1 represents the theoretical upper limits of the activity of granular sludge. The activity of granules fed with real wastewater will always be lower than the values found for the *Methanothrix* isolations calculated in Table 1.1, since a considerable part of the granular sludge usually consists of non-methanogenic biomass, extra cellular polymers, biologically inert VSS and dead organisms. Some reported methanogenic activities of granular sludge are listed in Table 1.2.

TABLE 1.1 Calculated doubling times and calculated specific methanogenic activities of some isolated *Methanothrix* strains.

strain	optimum temp. (^oC)	doubling time (*) (hours)	specific activity (**)	reference
Methanothrix soehngenii (strain Opfikon)	37	82	4.1	1,2
Methanothrix soehngenii (strain VNBF)	40	23-29	11.5-14.5	3
Methanothrix concilii (strain GPG)	35-40	24-29	11.5-13.9	5
Methanothrix sp.	60	31.5	10.6	6
Methanothrix sp.	60	72	6.6	7

* The doubling time (t_d) was calculated from the specific growth rate using the equation $t_d = \ln 2/\mu$

** The specific activities were calculated with the equation: $A = \mu/Y$ in which A is the specific methanogenic activity (kg COD/kg VSS.day), μ is the growth rate (day^{-1}) and Y is the growth-yield factor (kg VSS/kg removed COD), assuming a constant growth-yield factor of 0.05 for all cases.

1, Zehnder et al, 1980; 2, Huser, 1982; 3, Fathepure, 1983, 4, Touzel et al, 1988; 5, Patel, 1985; 6, Zinder et al, 1984; 7, Ahring and Westermann, 1985.

TABLE 1.2 Specific methanogenic activities of granular sludges cultivated on true wastewaters.

type of wastewater	temp. (°C)	spec. methanogenic activity (kgCOD/kgVSS.d)	reference
diluted malt juice	25	0.85	1
glucose solution	35	1.2	2
brewery wastewater	35	1.9	2
domestic sewage	-	0.15	3
domestic sewage	30	0.02-0.04	4
wheat starch wastewater	35	0.55	5
distillery	32	0.60	5
brewery wastewater	20	0.40	5
paper wastewater	27-30	0.45	5
slaughterhouse wastew.	30	0.34	6
potato wastewater	30	1.2	7
beet sugar wastewater	30	1.2	7
paper wastewater	30	0.19-0.62	7
wheat starch wastewater	30	0.70	7
distillery wastewater	30	0.99	7
brewery wastewater	30	0.45	7
animal carrion waste	30	0.75	8

1, Lui and Hu, 1988; 2, Wu et al, 1985; 3, Novaes, 1988; 4, de Man et al, 1988; 5, Rijs, 1988; 6, Sayed, 1987; 7, own determinations, de Zeeuw, 1982.

The very low specific activities found for sludges cultivated on domestic sewage suggest the presence of a large fraction of non-methanogenic organisms, non viable biomass as well as inert organic matter within the granules.

Dolfing and Bloemen (1985) used activity tests as a tool to characterize the microbial composition of granules. The methanogenic activity on hydrogen was 26-60 kg COD-CH_4/kg cells.day whereas on acetate this was 2.4 kg COD-CH4/kg cells.day. On the basis of these values, the part of the biomass consisting of acetoclastic methanogens was estimated. On a mixture of acetate and propionate this was 60%. The addition of sucrose reduced the methanogenic activity by 30-70%, reflecting the increase of sucrose converting bacteria.

A number of researchers tried to characterize the sludge activity by measuring the F420 content (Hulshoff Pol, 1979; de Zeeuw, 1984; Dolfing and Mulder, 1985; Wu et al, 1985; Sayed, 1987; Harada et al, 1988; Roam and FitzPatrick, 1989) The assessed values for the amount of F420 ranged from 0.04 - 0.77 M/g VSS. Since F420 mainly plays the role of an electron carrier in the reduction process of CO_2 by hydrogenotrophic methanogens, it is clear that the F420 content of this group of methanogens will be appreciably higher than that of the acetoclastic methanogens. However, by employing only one extraction procedure F420 can be a good indicator of the specific methanogenic activity of anaerobic sludge (de Zeeuw, 1984) although this correlation is not always clear (Dolfing and Mulder, 1985).

Storage of granules

In storage experiments conducted in our laboratory we observed that anaerobic granular sludge can be stored unfed for several years (Lettinga and Stellema, 1974; unpublished results) without any serious deterioration provided that the storage temperature is maintained below 15 - 20 °C. These observations were later confirmed by various other investigators such as Wu et al (1985) who stored granules at 15-28°C and Liu and Hu (1988) who stored granular sludge unfed for more than six months. They observed that little if any deterioration of granular sludge occurred. This high resistance can be attributed to the low decay rate of methanogens, especially at lower temperatures, and to the relatively high strength of the granular sludge matrix. It will be evident that this high resistance of anaerobic granular sludge is of eminent practical importance, particularly for campaign industries such as the sugar beet and potato starch industries.

Mechanical characteristics of granules

Important physical and mechanical properties of granular sludge are size of the granules, size distribution, shape, settleability, density, strength, porosity, pore size distribution, and structure.

The size of granular types of sludges cultivated in full-scale and pilot plant and laboratory reactors varies widely. As the mechanical strength of any agglomerate, whatever its nature or origin, will decrease with increased agglomerate size, it is clear that there is a maximum size above which they will fall apart. This maximum size will depend on 1) the various external forces exerted on the agglomerate and 2) its inherent strength. Both factors depend on a large number of factors. Insight into this matter is presently fairly limited.

With respect to granule size, a wide span of granule diameters is reported, ranging from 0.2 to 7 mm (Ross, 1984; Lui and Ho, 1988). Granules obtained on non-acidified wastes are generally bigger than granules cultivated on acidified substrates (Hulshoff Pol et al, 1986; Harada et al, 1988; Yoda et al, 1989).

As far as the substrate utilization rate of granules is concerned, a decreasing specific methanogenic activity would be expected with an increased size of the sludge granules due to the final rate of intra-particle diffusion of substrate ingredients as well as the end products. Surprisingly enough, comparative rate experiments conducted with granular sludge and gently degraded granules showed a higher substrate utilization rate for the non-degraded granules. A likely explanation for this phenomenon (Lettinga et al, 1983b) is the existence of a well-balanced microbial micro-ecosystem within the granules. This ecosystem of organisms presumably enhances very significantly the degradation of a VFA mixture over dispersed microbial growth systems of the same microbial composition. This may be the case for the degradation of more complex substrates as well. The increased methanogenic activities found by Pereboom (1988) for increased granule sizes are in accordance with these observations.

According to extensive measurements performed at our laboratory on a variety of granular sludges since 1982, we have found the density to be in the range of 1040-1080 kg/m^3. A clear relationship between size and density has not yet been established. According to Roam and FitzPatrick (1989) density is independent of size, while according to Beeftink and van der Heuvel (1988) granule density diminishes with increasing diameters. This decreased density was attributed to substrate limitations in the center of large aggregates and subsequent autolysis of organisms in the center of the granules. As a result, hollow aggregates will be formed with a lower density and lower mechanical strength.

According to our determinations (Hulshoff Pol et al, 1986) as well as experiments performed by Pereboom et al (1988), there is a positive correlation between the ash-content of the granular sludge and the density.

Alibhai and Forster (1986) investigated various sludge characteristics including granule sizes, surface charge, particle stability and specific surface area of different types of granular sludge. Although they reported wide variation between the different granules studied, they found a relationship between the size and the surface charge. According to their findings, the surface charge gives information about the compactness of the granules; the higher the charge the more compact the granule.

The settleability of granular sludge has been quantified with methods such as the solid flux method, assessment of settling velocities and the sludge volume index (SVI). Ross (1984) measured settling properties using the solid flux method and recorded a maximum value of 4500 $kg/m^2.d$, which will lead to a potential thickening of 9% (w/v). With a granular sludge of 60g SS/l he found a SVI of 11 ml/g. With respect to the settling velocities he found that 50% of the pellets settle faster than 27 m/h. Liu and Hu (1988) found a SVI of 15 ml/g and settling velocities for the granules varying from 20.9 to 45.6 m/h. Wu et al (1985) reported values for the SVI of 21 ml/g and less. According to our observations it is difficult to obtain accurate indication of the settleability with the SVI method; we recommend therefore use of a sedimentation balance (see Chapter 6). We did find values of the SVI for "beet sugar and potato processing granules" of around 12 ml/g, with settling velocities as high as 60 m/h for large granules (Dp > 2 mm)

We determined the granule strength by measuring the resistance against compression in a tension and compression test apparatus (see Chapter 6) and found that granules cultivated on true wastewaters disintegrated at pressures ranging from 0.26×10^5 to 1.51×10^5 N/m^{-2}, whereas the granules cultivated in the laboratory on a VFA mixture already disintegrated at a pressure of 0.1×10^5 N/m^{-2}. This method proves to be an informative tool for the determination of granule strength.

Other strength determination methods employed are a Rushton Turbine Mixer (Roam and FitzPatrick, 1989), a Couette vessel (own unpublished data, Tramper et al, 1984) and a stirred CSTR (Tramper et al, 1984). With these methods the granule resistance against eroding hydraulic conditions is measured.

Kinetics

With respect to the substrate utilization rate of granular sludges, the prevalence of mass transfer resistance is of great importance. Mass transfer will depend greatly on the granule morphology, granule size, the substrate concentration and the degree of turbulence in the fluidum. As an indicator for mass transfer resistance Dolfing (1985) and Tramper et al (1984) used apparent Km values according to Ngian et al (1977), and concluded that mass transfer becomes significant only at low substrate concentrations and in thick biolayers in the case of high intrinsic activities of the bacterial biomass, e.g. for granular sludge cultivated on acetate, ethanol and/or formate. With industrial granules and granules grown on propionate, Dolfing presumes that there are no serious problems with mass transfer resistance because of the relatively low activities. According to Grotenhuis (1986) mass transfer resistance may already prevail with granule sizes of 1.5 mm using formate, acetate and ethanol as substrate, whereas with propionate as substrate such a resistance was not observed. The latter phenomenon was explained by the low conversion rate of propionate (q_{max} of propionate is 0.83 $mmol.g^{-1}.hr^{-1}$). Alibhai and Forster (1986) state that the mass transfer of nutrients is probably rate limiting at very low substrate concentrations while not at higher concentrations due to the higher activities of granular sludge compared to non-granular sludge.

In our laboratory we found conversion rates of 1.1 g acetate COD/g VSS.day and 0.5 g propionate COD/g VSS.day for sugar beet cultivated granules fed with acetate and propionate, also indicating that diffusional problems can be sooner expected with acetate than with propionate.

It will be clear that the specific micro-environments existing in the granules will increase the conversion rates of the degradation steps in which syntrophic organisms are involved.

Models

Although the granulation process can be regarded as an extremely complex phenomenon, some recent attempts have been made to develop a model for granule growth (Beeftink, 1987; Kissel et al, 1988; FitzPatrick et al, 1989). Beeftink (1987) gave a model for granulation of acidogenic organisms in an anaerobic gas-lift reactor based on continuous growth of organisms and the presumption that the specific density is size-dependent due to lysis and disintegration of large aggregates.

Kissel et al (1988) presented a model for granulation with non-uniformly sized granules using the reaction rate, diffusion rate and the precipitation of inorganic matter as main parameters. Fitzpatrick et al (1989) presented a granule growth model based on three sub-models:

- a sub-model describing the relationship between the inter-granule diffusion and the simultaneous reaction of substrate;
- a sub-model describing fluid flow and mixing in the sludge bed;

- a granule growth sub-model describing the growth in relation to the substrate level incorporating the decay rate.

In the process of anaerobic degradation by sludge granules, too many factors are involved including granule form, pore dimension and distribution, compactness, gas evolution and release, and accumulation of substrate intermediates, to be well incorporated into a model. We believe therefore, that the models presented so far, are inadequate in describing the very complex dynamics in granular sludge formation in the start-up of UASB reactors. Nevertheless, they can give us valuable insight into some aspects of the overall process.

Granulation in other reactor systems and in other microbial processes

Other high-rate methanization systems

Granulation of anaerobic sludge is not an exclusive feature of UASB reactors. There are a number of other high-rate anaerobic treatment systems where granulation has been observed including fluidized bed systems, anaerobic filters, anaerobic gas-lift reactors, the so-called anaerobic baffled reactors and the upflow sludge bed filters. Iza et al (1988) operated fluidized bed reactors using PVC particles and natural and calcinated sepiolite as support and observed the development of an excellent type of granular sludge on beet sugar wastewater. A considerable fraction (40%) of the granules contained no support particles. In some of these granules however, precipitated $CaCO_3$ was found which we observed in granules formed on maize starch wastewater in a full-scale UASB reactor. Beeftink and Staugaard (1986) studied the glucose acidification in anaerobic gas-lift reactors (AGLR) and observed that the sand particles, utilized as carrier material, eventually disappeared from the reactor while granules of pure biomass were retained.

Dubourguier et al (1988) observed that a significant amount of the retained biomass in upflow anaerobic filters was found as bacterial conglomerates at the bottom of the reactor. Young and Dahab (1983) reported the occurrence of granules in the interstices of the media of anaerobic filters. Guiot et al (1988) studied the granulation process in an upflow anaerobic sludge bed filter (UBF) reactor using sucrose and ethanol as carbon sources. They observed a strong positive effect on granulation from trace metals (mainly Fe, Co, Ni and Mn), and concluded that these metals positively affect the amount of viable cells per unit of biomass. Such a distinct positive effect of trace elements was previously observed in experiments conducted in our laboratory with potato starch wastewater in UASB reactors (van Campen et al, 1985).

Granulation will also proceed well in anaerobic baffled reactors (Bachmann et al, 1985). Orozco (1988) cultivated granules of 2-5 mm in size in a hybrid anaerobic baffled reactor (RAP = Reactor Anaerobio a Piston), consisting of 11 open chambers filled with a filter medium.

Xiushan et al (1988) and Tilche and Yang (1988) observed granulation in a hybrid anaerobic baffled reactor and in an anaerobic sludge bed filter reactor. The granules in the sludge bed filter were larger (0.5-3 mm) and consisted mainly of *Methanothrix*-like organisms. The granules in the anaerobic baffled reactor contained more *Methanosarcina* cysts, especially in the first compartment.

Other substrates and conditions

Aggregate formation has been observed on different substrates and under different conditions, including the so-called pellets of molds (Metz and Kossen, 1977), denitrifying granules cultivated on methanol as carbon source in an upflow sludge bed reactor (Klapwijk et al, 1981; van der Hoek, 1988) and *Methanosarcina*-aggregates (Zhilina and Zavarzin, 1979; Bochem et al, 1982), which develop even in stirred cultures. The pellet formation (pellet-diameter up to 5 mm) reported by Klapwijk et al (1981) occurred in an upflow sludge bed (USB) reactor serving for denitrifying nitrified final effluent using the BOD from settled domestic sewage as C source. The pellets developed only at high surface loads (8 m/h); they contained up to 50% $CaCO_3$ and were rather fragile.

Zoetemeijer et al (1981) observed granulation in the acidification of glucose using USB reactors, provided the residence time was maintained below 1 hour for glucose concentrations of 10 kg/m^3 and below 6 hours for glucose concentrations of 50 kg $glucose/m^3$. The granules formed were about 1 mm in diameter; they appeared to be unstable under unfed conditions. The fact that 'methanogenic' granulation takes place at higher residence times, far more stable granules form, and methanogenic granular sludge doesn't deteriorate under unfed conditions, gives evidence that different factors may be involved in the formation of methanogenic granules than for denitrifying and acidifying aggregates.

It has been shown (Wiegant and Lettinga, 1982; Wiegant and de Man, 1986) that granulation also proceeds under thermophilic conditions.

In general it can be concluded that reactor systems utilizing an upward liquid flow are favorable for granulation, provided the conditions for selection and the cultivation of sludge agglomerates are appropriate.

Hypothesis for granulation and motivation for the selection of subjects

Nowadays in The Netherlands, an increasing pool of excess granular sludge is available for seeding new UASB reactors. In other countries, especially outside of Europe it is still difficult to acquire good quality seed sludge; even digested sewage sludge is often unavailable as seed material. For these situations it is important to develop procedures to accomplish a sufficiently rapid start-up of UASB reactors in order to apply the desired loading rates in the shortest possible time.

This study will focus on the more practical aspects of the initial start-up, and particularly on the phenomenon of granulation of anaerobic sludge. More fundamental (microbiological) aspects have been studied by Dolfing (Dolfing, 1987).

Factors affecting the granulation process

As bacterial granulation must be primarily governed by bacterial growth, the granulation process will be affected by:

Environmental conditions including:

- the availability of essential nutrients for optimal growth conditions;
- the temperature, since the specific activity of methanogenic sludge is highly temperature dependent;
- the pH, which should be in the optimal range (6,5-7,8);
- the type of wastewater with respect to composition of the waste, biodegradability of the organic matter, the presence of finely dispersed non-biodegradable organic and inorganic matter, the ionic composition (concentration of mono- and divalent cations) and the presence of inhibitory compounds.

The type of seed sludge, i.e. with respect to its specific activity, settleability and the nature of the inert fraction.

The process conditions applied during the start-up including:

- the procedure followed in increasing the loading rate, e.g. the extent of overloading and the allowed wash-out of suspended solids;
- the amount of seed sludge used.

Hypothesis of the granulation mechanism

We are convinced that the crucial factor in the granulation process is the sludge selection pressure imposed on the system. During the early phase of start-up, growth of the desired acidogenic, acetogenic and methanogenic organisms will take place both as attached and dispersed biomass. By applying a gradually increasing selection pressure, the dispersed-growing organisms and the voluminous poorly settling flocculent will be washed out from the reactor, while heavier biomass aggregates, which consist of organisms either attached to each other or attached to support particles and potential carrier material, are retained in the reactor. The selection is based on the minor differences in the settling properties (density) of free organisms and bacterial agglomerates. The selection pressure originates from both the hydraulic loading rate (or superficial upflow velocity) and the gas loading rate. In the first week of an initial start-up the upflow velocity is generally very low (0,1 to 1,0 m/d). However, once higher loads can be imposed the upflow velocity will gradually increase, ultimately to values of up to 30 to 50 m/d, which are frequently applied in full-scale UASB installations. The upward moving fluid and the mixing brought about by the production of gas bubbles (both gradually increase once higher loadings can be accommodated and are imposed) also cause a distinct expansion of the sludge bed, which in fact commences directly after the start-up (de Zeeuw, 1984). Poorly settling light material (colloids, dispersed growing biomass) start to be rinsed out from the system from the very beginning. The sludge bed TSS profile, as a result of these phenomena, will show a gradual change in the course of the process. Heavy sludge ingredients tend to

increasingly concentrate in the lower part of the reactor, while more voluminous sludge will accumulate in the upper part. Obviously bacterial growth will increasingly concentrate on the sludge ingredients present in the lower part of the reactor as the substrate load is highest there. Gradually therefore, more and more active biomass in the form of attached bacterial matter (and in due time bacterial aggregates as well) will be present there. As a result of the wash-out of dispersed and voluminous bacterial matter and the stimulated growth of aggregates and biofilms, a granular type of sludge will develop in the reactor.
From the above it is clear that granulation in UASB reactors in fact, originates from the fact that bacterial growth in these reactors is delegated to a relatively limited number of growth-nuclei. These nuclei can consist, in our opinion, of both inert organic and inorganic carrier material as well as small, well settling bacterial aggregates present in the seed sludge. As finely dispersed bacterial matter has little if any chance of being retained in the reactor, and depending on the conditions imposed on the system, film and aggregate formation is greatly enhanced. As the dimension and thickness of aggregates are limited, (dictated by internal binding forces and the degree of intertwinement) in due time a new generation of growth nuclei (secondary nuclei) will be generated from detached films and fragments of disrupted granules. These growth nuclei will grow in size and eventually produce a third generation, etc. The first generation, which consists of relatively voluminous aggregates, will also gradually become dense as bacterial growth is not only limited to the outside of the granule but also extends to inside the aggregates. This will particularly be the case for voluminous aggregates because:

- substrate diffusion limitation will be less than with dense bacterial aggregates;
- substrates can more deeply penetrate the aggregates in view of the lower volumetric bacterial activity inside the granule. The decrease of substrate concentration in the granule (or biofilm) with the distance will be related to the density of the aggregates. The denser, the sharper the drop.

Maturation is therefore, in our opinion, one of the reasons for the disappearance of "filamentous granules" which predominate during the initial stages of the granulation process.

Motivation for the selection of subjects

Granulation of biomass is a versatile subject with many aspects, such as the fundamentals of bacterial attachment, the production of extra cellular polymers, kinetics and diffusional resistance.
This study has focused on the more practical aspects of the phenomenon, its objectives being:

- to gain a deeper understanding of the mechanisms of granulation;
- to assess the process conditions that influence the granulation process;
- to study the possibilities of shortening the time required for granulation;
- to offer the UASB operator useful guidelines for the reactor start-up and operation.

The investigations of Dolfing were continued by Grotenhuis; the results to be published in the near future.

The investigations on reactor start-up and granulation in our laboratory were started in 1978 by de Zeeuw who focused mainly on the acclimatization of digested sewage sludge. Our research on granulation of anaerobic sludge began in 1979 and was made possible by a grant from the Ministery of Housing, Physical Planning and the Environment of The Netherlands and the potato starch industry (AVEBE BA, in Veendam, The Netherlands). Presently, studies dealing with the factors affecting erosion of granular sludge are performed by Alphenaar. This research is financially supported by various Dutch Ministries.

Many of the results discussed in this dissertation have been presented elsewhere (Hulshoff Pol et al, 1982; Hulshoff Pol et al, 1983a; Hulshoff Pol et al, 1983b; Hulshoff Pol et al, 1984; Tramper et al, 1984; ten Brummeler et al, 1985, Hulshoff Pol et al, 1986; Hulshoff Pol et al, 1988; Sierra et al, 1988).

The materials and methods used in the investigations presented in Chapters 3, 4 and 5 are described in Chapter 2 where a summary of the operational conditions of the different start-up and granulation experiments can also be found.

Chapter 3 discusses all the experiments performed with digested sewage sludge as seed sludge and VFA mixtures as the feed solutions. The effects of a number of relevant factors on the granulation process were subjects of investigation.

Chapter 4 deals with experiments using digested sewage sludge as basic seed sludge together with additional seed materials. In these experiments VFA mixtures were also used as substrate.

Chapter 5 describes the granulation experiments on more complex substrates (VFA sucrose mixtures) using, once again, digested sewage sludge as inoculum.

Chapter 6 is an overview of our work on physical characterization of granular sludge. A number of new methods for characterizing granular sludge are presented there.

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CHAPTER 2

MATERIALS AND METHODS

General

The experiments performed are summarized in Tables 2.1, 2.2 and 2.3. In these tables the experimental conditions are presented for the different research items including granulation with digested sewage sludge on VFA mixtures and sucrose/VFA mixtures (Tables 2.1 and 2.3) and granulation with digested sewage sludge plus additional carrier material on VFA mixtures (Table 2.2).

Reactors

All granulation experiments were performed in perspex laboratory-scale UASB reactors ranging in size from 2.5 to 120 liters. Figure 2.1 shows a schematic diagram of the 2.5 l UASB, and Figure 2.2 represents a schematic diagram of the design

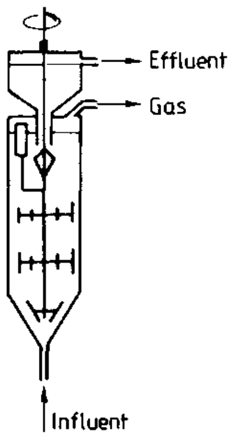


Figure 2.1 Schematic diagram of the 2.5 l reactors used in the experiments.

of the other UASB reactors. All reactors were equipped with sample ports over the height of the reactor in order to be able to make sludge profiles. The biogas produced in the reactors passed a column of soda lime pellets and an alkaline liquid (3 N NaOH) displacement system to pressurize the gas and to remove the CO_2 from the gas. The (wet) methane was measured in a wet-test gas meter (Meterfabriek Schlummbergen, Dordrecht).

The reactors were equipped with a central axis with stirring blades. To prevent channeling of the medium in the sludge bed during the initial stages of start-up, the reactors were mechanically stirred (30 rpm) every 30 min. for 5 s. Once the methane production exceeded 1 liter per reactor volume a day, stirring was terminated because beyond that point the

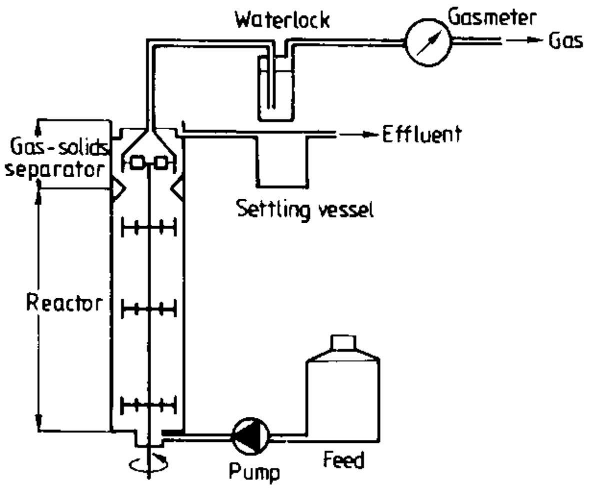


Figure 2.2 *Schematic diagram of the other UASB reactors (10 to 120 l) used in the experiments.*

natural mixing of the system by the gas production appeared to be sufficient.
The effluent was directed to a sludge collection vessel to separate the rinsed out suspended particles from the effluent stream. The design of this vessel is represented in Figure 2.3

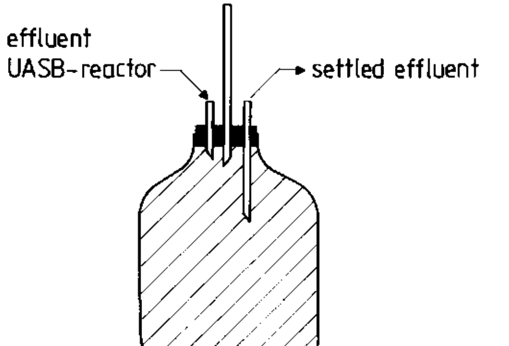


Figure 2.3 *Design of post-settler for removal of suspended solids from the effluent.*

Media and seed sludge

The UASB reactors were treating synthetic solutions. The composition of the substrates for the different experiments are listed in Tables 2.1, 2.2 and 2.3. To each medium a nutrient solution was added in the following concentrations (in milligrams per liter): H_3BO_3 (0.5), $FeCl_3$. $3H_2O$ (20), $ZnCl_2$ (0.5), $MnCl_2$. $4H_2O$ (5), NH_4Mo_7 . $4H_2O$ (0.5), $CoCl_2$. $6H_2O$ (0.5), NiCl (0.5), EDTA (5), $NaSeO_3$ (1), $AlCl_3$ (0.5), resazurin (5), HCL (36% solution, 10^{-2} ml/liter of medium), yeast extract (100), NH_4Cl (60), $(NH_4)_2SO_4$ (15), KH_2PO_4 (15). The addition of N.P and S was based on an assumed growth yield of 5% (on COD basis) for VFA substrates and 20% for the substrate of experiment 5-II which consists of 95% sucrose.

The VFA mixtures were neutralized up to a pH-value of 6.5 with NaOH. The reactors fed with a VFA/sucrose mixture were buffered at a pH of 6.5 with $NaHCO_3$.

The seed sludge was obtained from the municipal sewage sludge digester in Ede, The Netherlands. The volatile suspended solids (VSS) content of the sludge was ± 60% and the maximum specific activity, as determined in a batch-fed experiment, amounted to 0.10 - 0.15 kg CH_4-COD/kg VSS.day. The amount of seed sludge added to the reactors was 6 - 10 kg VSS/m^3, unless otherwise stated.

Analysis

VFA analyses

Samples (one sample per day per reactor) were taken from the settler compartment of the reactors and analyzed for acetate, propionate or butyrate with a gas chromatograph (model 417; Becker; flame ionization detector [FID] 200°C chromosorb column [200 by 0.2 cm]; carrier gas: N_2 equipped with a computing integrator (Spectra Physics 4100). Peak areas were measured and compared with a standard volatile fatty acid (VFA) mixture (precision, ± 3%).

Methane measurement

The total volumetric methane production was measured every 24 hours after the biogas (CH_4-CO_2 mixture) was put through a 3 N NaOH solution to scrub the CO_2 with a wet-test gas meter (Dordrecht Meterfabrieken, The Netherlands).

The methane concentration was determined with a gas chromatograph (Packard-Becker 406) equipped with a thermal conductivity detector and a molecular sieve column operated at 50°C. The carrier gas was argon and was used at a flow rate of 20 ml/min.

Sludge characteristics

1. Activity tests.

The specific methanogenic activities were determined in 2.5 and 5 l batch digesters as well as in serum flasks.

The 2.5 and 5 l digesters (Figure 2.4) were intermittently

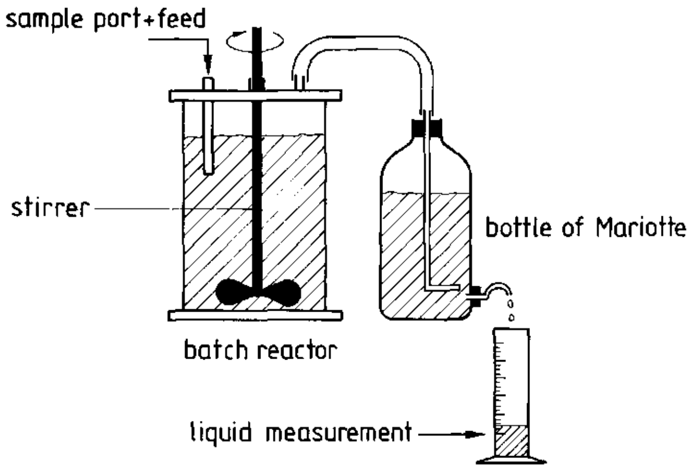


Figure 2.4 *Set-up with batch fed digesters (2.5 and/or 5 l) for the determination of the specific methanogenic activity of the sludge.*

stirred (15 seconds at 140 rpm every 10 minutes) and fed with a VFA mixture of acetate, propionate and butyrate up to a concentration of 600 mg/l for each VFA after flushing with nitrogen to remove oxygen. The VFA mixture was neutralized with NaOH. To each batch digester 1 ml of a nutrient solution containing 174 g NH_4Cl, 36 g K_2HPO_4 and 265 g $Na_2SO_4.10H_2O$ per liter and 2.5 ml of a trace element solution according to Zehnder et al (1980) was added. The gas production was measured with a Mariotte bottle using the liquid displacement technique.

Serum bottles with a volume of 130 ml were also used for the activity measurements. The bottles were made anaerobic by flushing with O_2-free nitrogen gas, and subsequently filled with 40 ml of an anaerobic buffer solution. The nitrogen gas was made free from residual O_2 by passing it over hot copper coils. The buffer was made anaerobic by boiling and then by cooling to room temperature under continuous gassing with O_2-free nitrogen. The buffer solution (pH values as indicated) was a 0.2 M KH_2PO_4-K_2HPO_4 buffer containing 0.5 g of NH_4Cl per liter of demineralized water.
Sludge was anaerobically distributed over the vials in portions of 100 to 1,000 mg. Substrate was added to the mixed liquor from concentrated stock solutions to reach final concentrations of 20 to 50 mM. The vials were closed with serum bottle caps and incubated in a shaking water bath at 30°C. The sludge was stored at 4°C and was reacclimatized by incubating it overnight in the presence of small amounts of substrate. After an overnight reacclimatization, the head space of the vials was flushed with O_2-free nitrogen before the methane production rate was determined.

2. Settleability

Sedimentation characteristics of the sludge were determined with a sedimentation balance (*Sartorius 4620*).

3. Microscopic observations.

Sludge was examined with epifluorescence and phase-contrast microscopy. The macroscopic morphology of Kaiser's glycerin-gelatin immobilized granules was investigated with a stereo-microscope.

Start-up procedure

The start-up procedure applied was identical to that described previously by de Zeeuw (1984). The space loading rate (in terms of kg COD/m^3.day) was increased by 75% once the COD reduction of the system exceeded 85% of the influent concentration. In several of the experiments (4-V, 4-VII and 4-VIII) the start-up procedure was changed in an attempt to reduce start-up time by allowing lower treatment efficiencies when the loading rates were increased.

Frequency of sampling

Flow rate, pH, gas production, sludge wash-out and VFA concentrations in the effluent were monitored in the reactor each day.

Sludge profiles and sludge activities were measured several times during all of the experiments except for those in the 2.5 l reactors which would require too much sludge.

Temperature

The experiments were performed at 30°C (± 1°C) except for experiment 3-XII which was operated at 38 °C in a temperature-controlled room with insulated and heated columns.

Literature

Zeeuw, W.J. de, 1984. Acclimatization of anaerobic sludge for UASB reactor start-up. PhD-thesis, Wageningen Agricultural University, Wageningen, The Netherlands.

TABLE 2.1. Overview of the various reactors, substrates and environmental conditions of the different experiments performed to investigate the granulation on VFA and digested sewage sludge (Chapter 3). For reference with the text the codes for each experiment are indicated.

investigated effect	reactor volume (l)	medium composition (mg/l)	environmental conditions	experimental code
effect of the sludge loading rate	30	C_2: 44000 C3: 3520	LR = ± 0.3 kgCOD/kgVSS.day	3-I
	30	C2: 4490 C3: 3520	OLR = ± 0.9 kgCOD/kgVSS.day	3-II
effect of the concentration of ammonium	2.5	C2: 600 C3: 600 C4: 600	40 mg NH_4^+-N/l	3-III
	2.5	C2: 600 C3: 600 C4: 600	400 mg NH_4^+-N/l	3-IV
	2.5	C2: 600 C3: 600 C4: 600	1000 mg NH_4+-N/l	3-V
effect of the concentration of calcium	2.5	C2: 600 C3: 600 C4: 600	6 mg Ca^{2+}/l	3-IV
	2.5	C2: 600 C3: 600 C4: 600	150 mg Ca^{2+}/l	3-VI
	2.5	C2: 600 C3: 600 C4: 600	450 mg Ca^{2+}/l	3-VII
effect of the influent concentration	10	C2: 208 C3: 162	influent-COD = 500 mgCOD/l	3-VIII
	10	C2: 417 C3: 333	influent-COD = 1000 mgCOD/l	3-IX
	10	C2: 4160 C3: 3330	influent-COD = 10,000 mgCOD/l	3-X
effect of the temperature	2.5	C2: 1250 C3: 1000	temperature = 30 °C	3-XI
	2.5	C2: 1250 C3: 1000	temperature = 38 °C	3-XII
effect of the pH	2.5	C2: 1250 C3: 1000	pH = 7.0	3-XI
	2.5	C2: 1250 C3: 1000	pH = 6.0	3-XIII

TABLE 2.2. Overview of the various reactors, substrates and environmental conditions of the different experiments performed to investigate the granulation on a VFA mixture (1250 mg C2/l and 1000 mg C3/l) and digested sewage sludge and additional carrier or seed material (Chapter 4). For reference with the text the codes for each experiment are indicated.

investigated effect	reactor volume (l)	amount of seed sludge (gVSS/l)	environmental conditions	experi-mental code
effect of the addition of	1201	3.3	no extra addition crushed granular sludge	4-I
	120	13.3	1.9% crushed granular sludge added	4-II
	120	13.3	3.8% crushed granular sludge added	4-III
	10	6	no extra addition slow start-up	4-IV
	10	6	2% crushed granular sludge added quick start-up	4-V
	10	6	8% crushed granular sludge added slow start-up	4-VI
	10	6	8% crushed granular sludge added quick start-up	4-VII
	10	6	15% crushed granular sludge added quick start-up	4-VIII
effect of the addition of carrier material	2.5	9.4	sieved seed sludge (100 μm)	4-X
	2.4	9.2	sieved seed sludge (100 μm) + 385 g hydroanthracite	4-XI
	2.5	9.25	sieved seed sludge (44 μm)	4-XII

TABLE 2.3. Overview of the various reactors, substrates and environmental conditions of the different experiments performed to investigate the granulation on sucrose/VFA mixtures and milk-powder and digested sewage sludge (Chapter 5). For reference with the text the codes for each experiment are indicated.

investigated effect	reactor volume (l)	medium composition (mg/l)	environmental conditions	experi-mental code
granulation on VFA/sucrose mixtures	23.5	10% sucrose 90% C2/C3	molar ratio C2/C3 = 1.5 COD = 3,000 mg/l	5-I
	23.5	95% sucrose 5% C2/C3	molar ratio C2/C3 = 1.5 COD = 3,000 mg/l	5-II
granulation on VFA/sucrose mixtures	23.5	95% sucrose 5% C2/C3	COD = 3,000 mg/l (period a) COD = 1,500 mg/l (period b) COD = 3,000 mg/l (period c)	5-III

CHAPTER 3

GRANULATION ON VFA AND DIGESTED SEWAGE SLUDGE

Introduction

This chapter describes the granulation experiments performed on mixtures of volatile fatty acids (VFA's) as carbon and energy sources. VFA's were selected as substrates because of the following considerations:

- methanogenic and acetogenic organisms have low growth rates. Since these growth rates are significantly lower than the faster growing acidogenic organisms, they play a decisive role in the overall anaerobic digestion process;
- as a result of the complexity of the phenomenon of granulation it seemed important to start working with a simple substrate of mixtures of volatile fatty acids to study only the granulation of methanogenic and acetogenic organisms;
- the work of Zoetemeijer (1982) and Cohen (1982) showed that granulation on easy hydrolyzable carbohydrates (i.e. glucose) can proceed well, therefore it was of interest to concentrate on the granulation of the methanogens and acetogens;
- to be able to compare the results of these investigations with the studies of de Zeeuw (1984) on the UASB reactor start-up and the acclimatization of digested sewage sludge, we considered it appropriate to utilize feed solutions similar to those used in de Zeeuw's studies.

We concentrated this study on sludge granulation with digested sewage sludge as seed. Digested sewage sludge was chosen for two reasons. Firstly, this type of seed is usually available in most industrialized countries in quantities sufficient enough for inoculating full-scale anaerobic reactors up to several 1000 m^3 in volume. Secondly, it has a relatively high specific methanogenic activity (0.05-0.25 kg COD/kg VSS.d). The effects of different process conditions on the granulation speed were investigated. The conditions that were selected are of particular relevance for the application of the process on the anaerobic treatment of industrial effluent. The investigations were not aimed at the presentation of a clue to "the fundamentals" of granulation, but rather were directed to the anaerobic wastewater treatment practice of industrial effluent using UASB reactors.

Of all the different process conditions possible, those selected were found to be the most relevant ones for supplying useful information for reactor start-up. We made this selection in the years 1979 - 1983 on the basis of available full-scale experience with UASB technology. The following factors were investigated:

- factors related to the substrate composition

  These include the pH, NH_4^+ concentration, Ca^{2+} concentration, and influent concentration.

- factors related to the reactor operation

  The sludge loading rate, temperature, and the influent concentration since the hydraulic loading rate changes considerably at different influent concentrations at a fixed space loading rate.

Table 2.1 gives a summary of the different experiments conducted. Data are presented on the reactor volumes, reactor heights, substrate composition and the various environmental conditions.

A description of materials and methods is presented in Chapter 2.

Results

One of the great difficulties with this type of research is the difficulty in quantifying the granulation process, especially its "exact" beginning. Granulation is a dynamic process without a clear starting point. Nevertheless, the beginning of the granulation process was identified quite clearly under laboratory conditions with the appearance of macroscopic, distinctly different colored sludge agglomerates, which generally occurs within a period of a few days. However, it should be kept in mind that at the first stage of the granulation process the main part of the sludge is still very similar in appearance to the initial seed sludge, although the sludge is generally more voluminous than the original seed sludge due to the development of filamentous methanogenic organisms.

In almost all the experiments, a very similar characteristic pattern was observed regarding the development of sludge retention and wash-out. This is illustrated in Figure 3.1 which presents the results obtained in Experiment 4-II.

Roughly three characteristic phases can be distinguished in the granulation process:

- Phase I (organic loading rate < 2 kg $COD.m^{-3}.day^{-1}$). In this phase the sludge bed markedly expands as a result of the initiation of the gas evolution and the stepwise increase of the surface load imposed on the reactor. Growth of filamentous organisms causes a deterioration in the sludge settleability.
- Phase II (organic loading rate: 2-5 kg $COD.m^{-3}.day^{-1}$). This is the wash-out phase of fine suspended solids due to the further increase of the loading rate and the consequently increased gas production. The more voluminous part of the sludge or the expanded sludge bed is forced out of the reactor and the heavier fraction of the sludge is concentrated in the lower part of the reactor. Due to the strong wash-out and the unchanged or increased imposed organic space loading rate, a sharp increase of the sludge loading rate exceeding

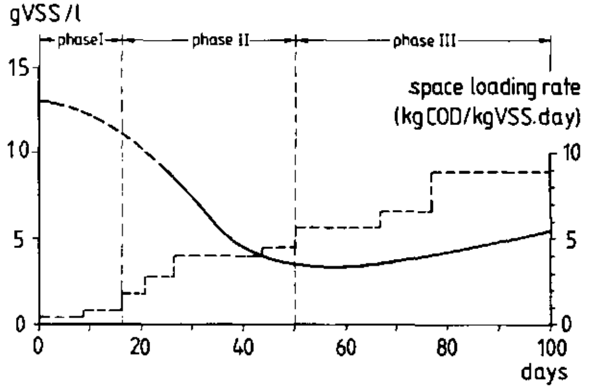


Figure 3.1 *Development of the sludge concentration and organic space loading rate during the granulation process in Experiment 4-II.*
———— VSS concentration
-------- organic space loading rate
(The transition from phase I to phase II was marked by the formation of a flotation layer. During that stage, an exact wash-out rate was not determined).

approximately 1 kg COD.kg VSS^{-1}.day^{-1} will occur during this phase.

- Phase III (organic loading rate > 3-5 kg COD.m^{-3}.day^{-1}). This phase starts at the point beyond which a net increase in the total amount of sludge in the reactor occurs. The concentrated granular growth now exceeds the sludge wash-out. After a period of stagnation caused by the loss of active biomass through sludge wash-out, the organic space loading rate can be further rapidly increased to its maximum values, which may be as high as 50 kg COD.m^{-3}.day^{-1} at 30 ^{o}C. Because the sludge settleability has dramatically improved, wash-out will be reduced to very low values.

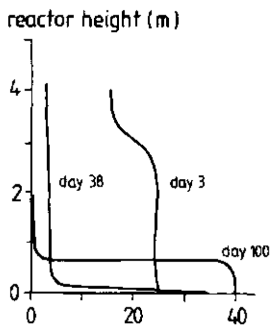


Figure 3.2 *Sludge profiles over the reactor height in reactor 4-II at day 3, 38 and 100.*

Sludge development during the three phases is illustrated in Figure 3.2 where for each phase a representative sludge profile over the reactor height is given.

1. Effect of the sludge loading rate (Experiments 3-I and 3-II).

Granulation is usually observed once the sludge loading rates exceed 0.6 kg COD.kg $VSS^{-1}.day^{-1}$. In order to establish the effect of the sludge load on the granulation process, two parallel identical 30 l UASB reactors were operated; one reactor was run at a maximum sludge loading rate of 0.3 kg COD.kg $VSS^{-1}.day^{-1}$ (reactor 3-I), and the other loaded at values above 0.9 kg COD.kg $VSS^{-1}.day^{-1}$ (reactor 3-II). The reactors were inoculated with 11.8 and 7.3 g VSS/l of digested sewage sludge. In reactor 3-II, phase III started around day 63 at a minimal VSS concentration of 3.0 g/l. At day 100 the VSS concentration had increased to 4.6 g/l and a clear granular sludge bed was apparent. The objective of the experiment was to keep the other reactor at a sludge loading rate of approximately 0.3 kg COD.kg $VSS^{-1}.day^{-1}$ until the same total amount of VFA COD was converted as in reactor 3-II. However, after 13 weeks of operation the sludge became extremely voluminous (bulking) and most of it was forced out of the reactor within a period of a few days. After returning the sludge to the reactor and operating it at a 50% lower organic loading rate it appeared to still be impossible to retain the sludge, i.e. it was washed out at about the same speed as before. Microscopic examination revealed that the sludge consisted almost completely of long filamentous bacteria, presumably Methanothrix soehngenii as described by Huser (1981). Significant granulation had still not occurred at this stage.

Figures 3.3 and 3.4 show the imposed space loading rates and sludge loading rates, the removal efficiencies, the concentration of acetate and propionate in the effluent, the total amount of biomass present in the reactor, the accumulated amount of sludge wash-out and the calculated sludge yield which was calculated on the basis of a sludge balance.
Both reactors were started up very carefully with low loading rates; the initial sludge loading rates were 0.03-0.04 kg COD/kg VSS.d., making it possible to achieve a good removal of the influent COD within 2 weeks. As shown in Figures 3.3 and 3.4, after 2 weeks the loading rates could be increased without seriously affecting the degradation of the volatile fatty acids in the feed. In Experiment 3-I the space loading rate was increased up to a value of 2.7 kg COD/m^3.d and a sludge loading rate of ± 0.3 kg COD/kg VSS.d.. At that moment the corresponding upflow velocity was 0.29 m/d. From then onward the space loading rate was unchanged, while in the other reactor (Experiment 3-II) the space loading rate was further increased to values of 4 - 6 kg COD/m^3.d three weeks after the start-up, which corresponds to upflow velocities of 0.42-0.63 m/d. and HRT's of 1.7 and 2.5 days. Under these conditions the sludge loading rate drastically increased to a value of almost 2.0 kg COD/kg VSS.d., mainly as a result of sludge wash-out.

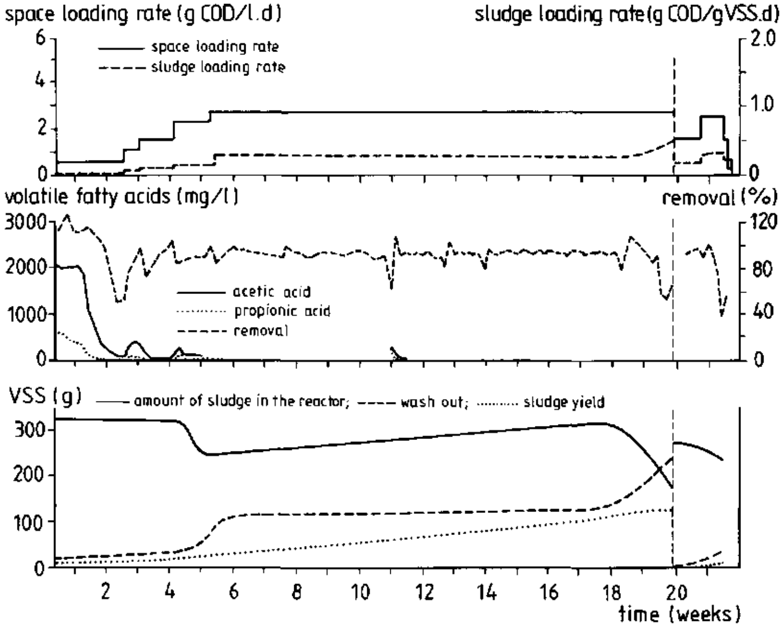


Figure 3.3 *Operation and results of Experiment 3-I started up under a continuous low organic loading rate of ± 0.3 kg COD/kg VSS.d.*

As the acetate and propionate concentration in the effluent increased at this high sludge loading rate, we decided to lower the space loading rate to 4.2 kg COD/m^3.d, which corresponded to a sludge loading rate of 1.85 kg COD/kg VSS.d. The slow increase in the total amount of biomass in the reactor beyond week 15 resulted in a further gradual decrease in the sludge loading rate to 1.0 kg COD/kg VSS.d at the termination of the experiment (week 15). The sludge wash-out from reactor 3-I was relatively insignificant except during the period in which the loading rate was increased when considerable wash-out (0.35 kg VSS/m^3.d) temporarily (during a few days) occurred. Once the desired sludge loading rate of 0.3 kg COD/kg VSS.d. was reached, the wash-out rate became insignificant. This situation remained unchanged until a massive flotation of the sludge suddenly occurred during week 18; the sludge then once again started to wash out at a high rate (0.26 kg VSS/m^3.d). The washed-out sludge was collected in a settling tank and returned to the reactor. However, the same phenomenon reoccurred immediately after the sludge was returned. The distribution of the sludge over the height of the reactor in Experiment 3-I was very unfavorable (shown in Figure 3.5) as the majority of the sludge appeared to accumulate in the settler compartment.

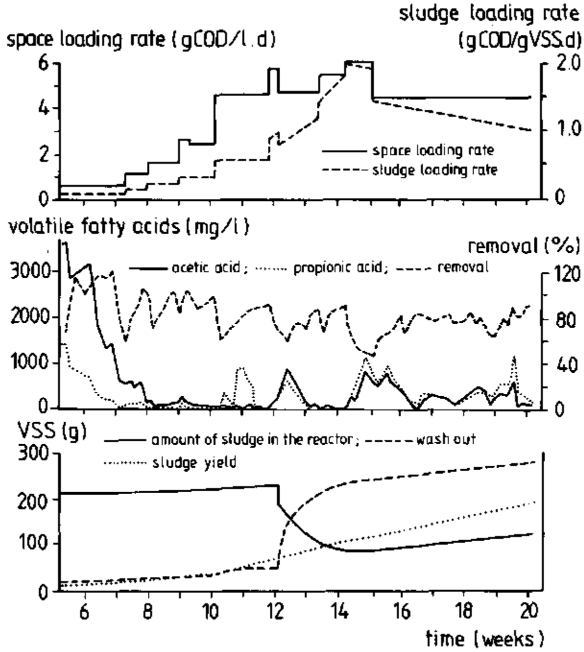


Figure 3.4 *Operation and results of Experiment 3-II, started up at an organic loading rate of over 0.9 kg COD/kg VSS.d.*

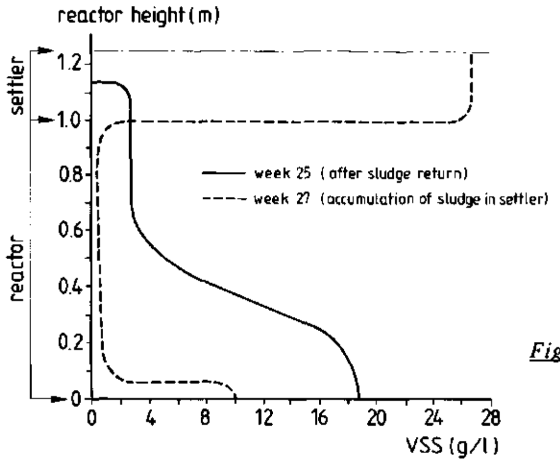


Figure 3.5 *Sludge profile in Experiment 3-I after the occurrence of massive flotation.*

This sludge has a strong tendency toward flotation. Clearly it is impossible to achieve an effective sludge retention in the system with this type of sludge.

Microscopic examination of the sludge revealed that a major part of the biomass in reactor 3-I consisted of long filaments, presumably *Methanothrix soehngenii*. These filaments form voluminous floc structures which easily float due to the attachment of gas bubbles.

Contrary to reactor 3-I, in reactor 3-II a quite satisfactory type of sludge bed developed with a gradually increasing bed thickness. Microscopic examination revealed that filamentous granules were also present in the sludge bed, while after 15 weeks of operation the major part of the sludge was still flocculent in nature.

2. The effect of the NH_4^+ concentration (Experiments 3-III, 3-IV and 3-V).

The effect of the NH_4^+ concentration has become a point of investigation due to the fact that in large (30 m^3) pilot plant studies concerning the treatment of a high NH_4^+-N industrial waste it was repeatedly shown that the sludge obtained a severely bulky appearance as a result of the development of a filamentous bacterial culture.

The digestion process developed fairly similarly in reactors 3-III and 3-IV fed with 40 and 400 mg NH_4^+-N/l respectively. In both reactors the beginning of phase III could be assessed around day 55 at a space loading rate of approximately 3.2 kg $COD.m^{-3}.day^{-1}$. However, regarding the sludge wash-out, reactor 3-IV lost less sludge than reactor 3-III; the minimum sludge concentrations for reactor 3-III and reactor 3-IV were 2.4 g VSS/l and 3.2 g VSS/l respectively. In both reactors the first granules were observed around day 42. All of the granules formed were filamentous in appearance. The operation of reactors 3-III and 3-IV was terminated at day 96 at an organic loading rate of 6.5 kg $COD.m^{-3}.day^{-1}$.
The experiment conducted at a high NH_4^+-N concentration (reactor 4-V, 1000 mg NH_4^+-N/l) showed a serious inhibition, particularly with respect to the breakdown of propionic acid. After 150 days of continuous operation the experiment was terminated. Phase III was not reached, while an organic loading rate of 5 kg $COD.m^{-3}.day^{-1}$ could still not be accommodated. Moreover, there lacked clear evidence of granulation at that stage.

3. Effect of Ca^{2+} (Experiments 3-IV, 3-VII and 3-VIII)

As already indicated in Chapter 1, it is a well known fact that divalent cations have a positive effect on the flocculation of (anaerobic) sludge. In earlier experiments conducted in our laboratory we also obtained evidence that the wash-out of sludge during the initial phase of the start-up could be reduced by increasing the Ca^{2+} concentration of the feed solution (de Zeeuw and Lettinga, 1980). For the above reasons we decided to study in more detail the effects of the Ca^{2+} concentration.

According to the results of reactor 3-IV, the minimum sludge concentration was reached in the low Ca^{2+} experiment (reactor 3-IV; 6 mg/l Ca^{2+}) after approximately 55 days. The minimum value was 3.2 g VSS/l. An improved sludge settleability was obtained in reactor 3-

VII with a VFA solution containing 150 mg Ca^{2+}/l. The minimal sludge concentration in this reactor was relatively high: 4.5 g VSS/l. Due to the slower wash-out rate it lasted 80 days before a clear net sludge increase could be established. After 88 days of continuous operation, an organic loading rate of 8 kg $COD.m^{-3}.day^{-1}$ could be applied. However, at a Ca^{2+} level of 450 mg/l in the influent (reactor 3-VIII) the sludge retention was clearly less than in reactor 3-VII. Phase III started at approximately day 75 at a minimum sludge concentration of 2.8 g VSS/l. However, once phase III was reached the loading rates could be increased relatively quickly, from 3.5 to 8.5 kg COD/m^3.day within 20 days. In all of these reactors granulation could be detected at approximately the same time (day 42-50). The granules found in reactors 3-VII and 3-VIII differed slightly from those formed at a low Ca^{2+} level (reactor 3-IV); more sarcina-type and coc-type bacteria were found in the 'Ca-granules'.

4. Effect of the influent concentration (Experiment 3-VIII, 3-IX and 3-X).

Presumably, one of the crucial factors dictating the granulation process is the selection pressure.

Given the methanogenic capacity of the sludge present in the anaerobic reactor a specific space loading rate can be applied which will not cause overloading of the system. It is clear that with different influent concentrations the hydraulic conditions will also be different at similar loading rates. Therefore, it is evident that the influent concentration will have an effect on the selection pressure, since the hydraulic conditions in the reactor (the superficial upflow velocity) directly correlate to it.

For these reasons we studied the effects of the substrate (waste) concentration to obtain further insight into the significance of the superficial upflow velocity as a selection force in the granulation process.

The results of Experiments 3-VIII and 3-X are presented in Figures 3.6 and 3.7 together with the applied loading rate regimes. Experiment 3-VIII shows quick expansion of the sludge bed immediately after start-up. Sludge began to wash out almost immediately at an initial rate of 0.4 kg VSS per day, which is 2.7% of the total amount of seed sludge. However, within the first week this wash-out rate decreased to 0.03 kg VSS/d. At day 69 the minimum amount of retained sludge was 6.8 kg VSS/m^3, i.e. 46.6% of the sludge amount at the start. The granules formed in the reactor were of the filamentous type. In most cases these organisms were attached to inert support particles.

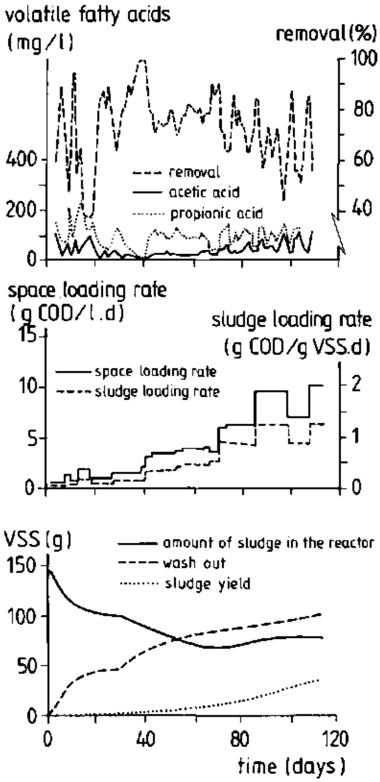


Figure 3.6 *Operating conditions and results of the UASB reactor started up with an influent COD of 500 mg/l (Experiment 3-VIII).*

The reactor, operated at an influent COD of 10 kg COD/m^3, started up very slowly. After day 30 a rare situation occurred; propionic acid was further degraded than acetic acid. During approximately 50 days of operation at a space loading rate of 2 - 4 kg COD/m^3.d the acetate concentration in the reactor amounted to 2 g/l or even higher. Only beyond day 80 did this situation end when the effluent acetate concentration dropped suddenly to a value below 1 g/l. From that time onward the loading rate could be increased stepwise to an ultimate value of 50 kg COD/m^3.d. without any further problems of acetate buildup. The sludge loss through wash-out due to sludge bed expansion was very limited during the first 80 days of the experiment. Then a mild sludge wash-out occurred: the minimum sludge hold-up was reached 35 days later when 68% of the initial quantity of seed sludge was still present. Granulation was observed at around day 50; the granules were formed mainly from the growth of very small Sarcina-aggregates and not by Methanothrix.

5. Effect of the temperature (Experiment 3-XI and 3-XII).

Methanothrix soenghenii is frequently the predominant organism in all granules obtained on VFA feedstocks. The optimal temperature according to Huser (1981 and 1982) is 38 °C, when the activity is about 3 times higher than at 30 °C. It can therefore be expected that the

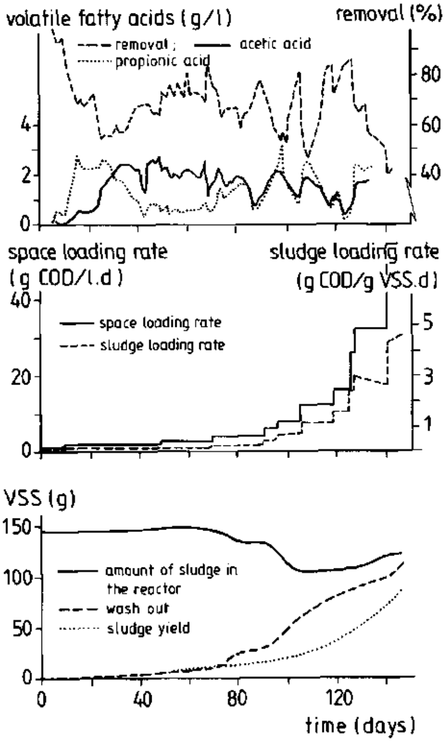


Figure 3.7 *Operating conditions and results of the UASB reactor started up with an influent COD of 10.000 mg/l. (Experiment 3-X)*

start-up period is significantly shorter at 38 °C than at 30 °C. Experiment 3-XII, a start-up experiment conducted at 38 °C, was performed to assess the effect of a higher temperature on the start-up period and the granulation process. Experiment 3-XI was operated as a control.

The start-up of Experiment 3-XI proceeded slowly, particularly with respect to the degradation of propionic acid. This poor breakdown was accompanied by a rather low pH in the reactor. The pH of the influent (5.8) was therefore increased to 6.5 at day 55. A significant sludge wash-out occurred in this experiment at loading rates of 2 - 3 kg COD/m^3.day. As a result of this slow start-up, the minimum in the amount of sludge retained was reached only at day 105. Only 12% of the initial amount of sludge remained. Only filamentous types of granules were formed; most granules observed were generated by attachment to inert particles.

The results of Experiment 3-XII (start-up at 38 °C) revealed a rapid start-up compared to the control experiment (Experiment 3-XI), as well as the other experiments performed at 30 °C. Moreover, there was no strong sludge wash-out. After this initial rapid start-up however, it appeared that the sludge was very vulnerable to non-optimal growth conditions. A temporary "shock" of a temperature drop to 35 °C and a simultaneous pH drop from 7.1 to 6.3 for 1 day resulted in a significant reduction in the breakdown rate of propionic acid. It took therefore a very long time before a sufficient amount of propionic degrading acetogens was established in the sludge.

The experiments were terminated after 150 days of operation. The sludge in both reactors was predominately flocculent, with grey-white filamentous granules distributed over the total height of the sludge bed. As shown in Figure 3.8, the settling characteristics of the sludge significantly worsened, compared to those of the seed sludge.

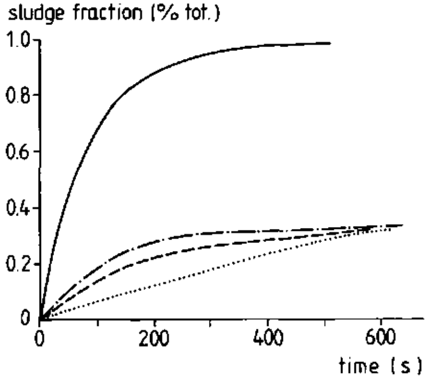


Figure 3.8 *Settling characteristics of the final sludges of Experiments 3-XI (········), 3-XII (- - - -) and 3-XIII (-·-·-·-) and the digested sewage sludge (———) utilized as seed in these experiments.*

The sludge still contained predominately filamentous organisms (presumably Methanothrix).

6. Effect of pH (Experiments 3-XI and 3-XIII).

Reactor run at pH 7.5.

The results of Experiment 3-XI conducted at a pH of 7.5 appear in Figures 3.9, showing the concentrations of acetate and propionate in the effluent solution, the course of the gas production rate and the reactor pH in relation to the space loading rate that was applied.

The conversion of propionate was poor up to day 50. Granules of 0.5 to 1 mm were detected in the sludge bed after 80 days of operation. The predominant organism in the washed out sludge was similar to Methanothrix soehngenii. The mean VSS content of the reactor decreased from 10 g/l (day 0) to 1.1 g/l at day 80 and then gradually increased to 1.8 g/l at day 150.

Reactor run at pH 6.

For maintaining a pH of about 6 in the reactor while feeding the system with a VFA feed, it is necessary to apply low influent pH values so that part of the VFA's are present in

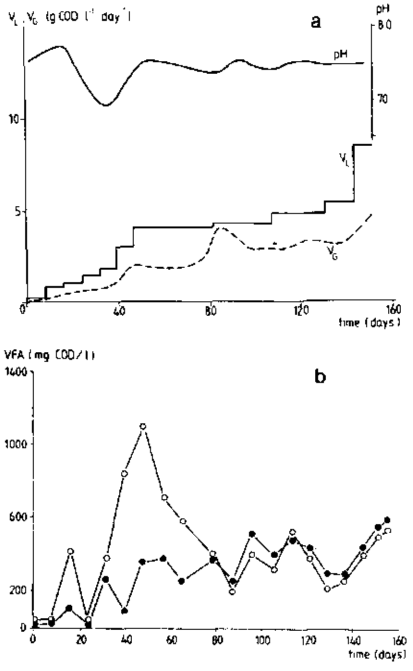


Figure 3.9 *a) Course of gas production rate (V_G), space loading rate (V_L) and pH during the start-up experiment with the UASB reactor operated at pH 7.0. b) Levels of acetate (o) and propionate (•) expressed as milligrams of COD per liter in the effluent of the UASB reactor operated at pH 7.0. (Experiment 3-XI).*

undissociated form. This is due to the fact that methanogenesis, at neutral pH, is accompanied by a net elimination of protons:

$$CH_3COO^- + H_3O^+ \longrightarrow CH_4 + CO_2$$

$$CO_2 + H_2O \longrightarrow H_2CO_3$$

$$H_2CO_3 + H_2O \longrightarrow H_3O^+ + HCO_3^-$$

A relatively strong acid (VFA) is replaced by a considerably weaker acid (H_2CO_3), whose concentration is determined by the solubility of CO_2, which in turn is dictated by the partial pressure of CO_2 in the biogas and the amount of cations (e.g. Na^+) which have to be neutralized. Maintaining a pH of 6 in the reactor under conditions of, for example, 90% removal of the VFA therefore sets some limitations on the fraction of the VFA being neutralized in the feed, as this neutralized fraction primarily determines the concentration of HCO_3^-.

The results of the reactor run at pH 6 are shown in Figure 3.10.

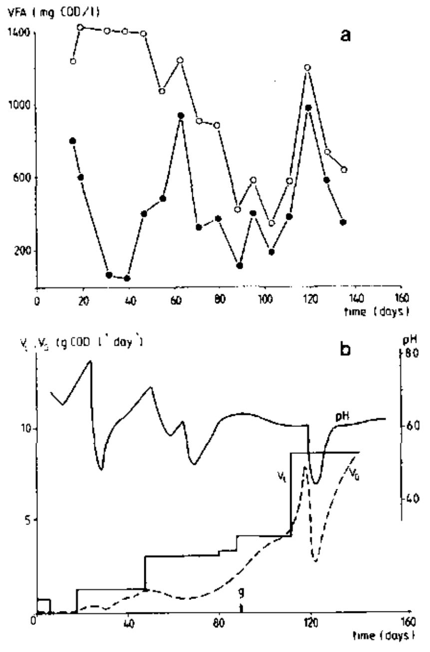
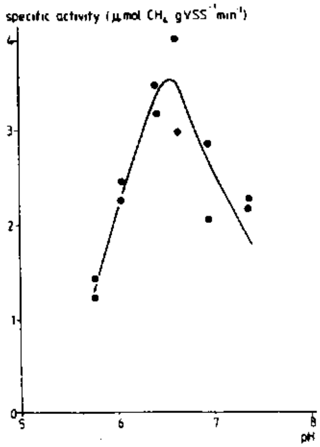


Figure 3.10 *a) Levels of acetate (●) and propionate (o) expressed as milligrams of COD per liter in the effluent of the UASB reactor operated at pH 6. b) Course of gas production rate (V_G), space loading rate (V_L) and pH during the start-up experiment with the UASB reactor operated at pH 6. (Experiment 3-XIII).*

The reactor feed was interrupted 7 days after the start of the experiment because gas was not detected. However, during the feed interruption a slow but distinct increase in gas production occurred. This was noticed by the observation of the evolution of small gas bubbles. Therefore, at day 17 we decided to resume the feeding of the reactor. During the experiment it appeared difficult to maintain the pH constant at 6 due to the low influent pH and consequently the absence of a sufficient buffering capacity. This resulted in considerable pH fluctuation between 4.9 and 7.8, and a drop of the pH to 4.5 on day 120 causing a serious upset of the process. We decided to double the flow rate to operate the system with some extra selection force, despite the fact that only 85 to 90% of the acetate was converted and the propionate conversion was negligible. Beyond day 50 a distinct attachment of biomass to the reactor wall became visible. This process of attachment and subsequent growth of the attached biomass continued until the termination of the experiment on day 140. At that time roughly 50% of the biomass was attached to the reactor wall and 50% was present in the sludge bed. The attached biomass was grey in color, while the dispersed aggregates (ca. 0.5 mm) were white. The propionate conversion only started beyond day 50 which coincided with an evident increase in wall growth. The sludge washed out from the reactor during days 0 to 50 consisted mainly of Methanosarcina-like organisms; beyond day 50 filamentous organisms predominated in the biomass present in the effluent. Granulation of the sludge became apparent 80 days after the start of the experiment. The VSS content of the reactor decreased from 10 g liter^{-1} on day 0 to 2.0 g liter^{-1} on day 80, but then gradually increased to 3.5 g liter^{-1} on day 140. The main characteristics of the sludge after termination of the experiments are shown in Table 3.1. It appeared that the biomass on the reactor wall was very loosely attached, so it was impossible to analyze it independently of the biomass of the sludge bed. The predominant organism in the cultivated sludge was a filamentous bacterium. Only a few granules consisted of *Methanosarcina*-like organisms.

To assess the effect of pH on the acetate and propionate conversion rates of the sludge, the specific activity for acetate and propionate degradation was measured at several pH's in batch experiments. The results are shown in Figure 3.11 and 3.12.



<u>Figure 3.11</u> *Specific activity on acetate in relation to the pH of the sludge cultivated at pH 6 (sludge from Experiment 3-XIII).*

For acetate degradation an optimum was found at pH 6.6 to 6.8. The sludge continued to show a slight but distinct activity at pH 5. For propionate degradation one distinct optimum was found. Activity measurements at pH 6.6 indicated that the sludge might have a second optimum in this range (Figure 3.12).

TABLE 3.1. Characteristics of sludges obtained in UASB reactors run at pH 7.0 (Experiment 3-XI) and 6.0 (Experiment 3-XIII) after 150 days of operation.

Reactor pH	Biomass conc (g of VSS liter^{-1})	activity CH_4 COD g of VSS^{-1} day^{-1})	Yield (g of VSS g of COD^{-1})[a]	Predominant organism morphology[b]	Avg granule diameter (mm)
7.5	1.8	1.8	0.031	Filaments	1.5
6	3.5	1.3	0.034	Filaments	1.0

[a] Calculated from the total converted COD during the experiment.
[b] Organisms resembling Methanothrix soehngenii.

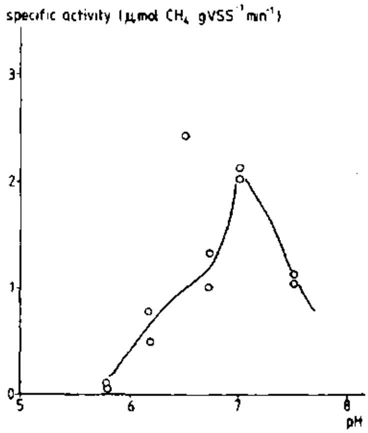


Figure 3.12 Specific activity on propionate in relation to the pH of the sludge cultivated at pH 6 (sludge from Experiment 3-XIII).

Discussion

Granulation in UASB reactors principally occurs due to the fact that bacterial growth in these reactors is delegated to a limited number of (growth) nuclei. These nuclei can consist, in our opinion, of both inert organic and inorganic carrier materials as well as small bacterial aggregates already present in the seed sludge. As finely dispersed bacterial matter has little if any chance of being retained in the reactor (except at very low hydraulic loading conditions), film and aggregate formation is greatly enhanced. As the dimensions of aggregates and the thickness are limited (i.e. dictated by internal binding forces and the degree of intertwinement), at due time a new generation of growth nuclei (secondary nuclei) will be generated from detached films and fragments of disrupted granules. These growth nuclei will grow in size and eventually they will produce a third generation, etc. The first generation consists of relatively voluminous aggregates which will gradually become dense as bacterial growth will not only be limited to the outside of the granule but will occur inside the aggregates as well. This will particularly be the case for the voluminous aggregates because:

- substrate diffusion limitation will be less than with dense bacterial aggregates;
- substrate can penetrate deeper in the aggregates in view of the lower volumetric bacterial activity inside the granule. The decrease of substrate concentration in the granule (or biofilm) with the distance will be related to the density of the aggregates. The denser, the sharper the drop.

Aging is therefore, in our opinion, one of the reasons for the disappearance of "filamentous granules" which predominate during the initial stages of the granulation process.

The results of all UASB start-up experiments carried out so far reveal a very significant wash-out of sludge during the initial phase of the process, resulting in a deep depression in the retained amount of seed sludge. This confirms the principle idea that a strong initial wash-out of finely dispersed sludge is an essential part in the granulation process as new growth should be concentrated as "attached growth" rather than growth of dispersed biomass. Concerning the minimal amount of retained sludge, significant and characteristic differences exist between the various experiments. These differences are determined by a number of factors including:

- the hydraulic loading rate. A comparison of Experiments 3-VIII, 3-IX and 3-X shows that the influent COD concentration at a given space loading rate (and the hydraulic loading rate) has a strong effect on the wash-out of the finely dispersed sludge fraction. As is clear from Figures 3.6 and 3.7 the sludge minimum with influent concentrations of 500 mg COD/l and 10,000 mg COD/l was reached at day 60 and day 110 respectively.
- the applicable loading rate. Under conditions resulting in an inhibition of the methanogenic activity of the sludge, the loading rate has to be more slowly increased. It is clear that this will directly effect the wash-out pattern of the start-up process: wash-out will proceed slower.

The specific activity of the sludge lost from the reactor is generally close to that of the sludge retained in the reactor. This signifies that a considerable fraction of the net bacterial growth occurring during the initial phases of the start-up will also be lost with the effluent. However, as growth gradually concentrates more and more in or on the heavier (retained) part of the sludge, the specific activity of this sludge will improve after the initial start-up phase. It will become obvious that an unlimited wash-out of sludge cannot be tolerated: the deeper the depression in the retained amount of sludge the longer will be the stagnation in the increase of the gas production. Therefore, the development of an excessive depression in the retained amount of sludge should be prevented. This can be achieved by:

a) selecting the right type of seed sludge. As mentioned in Chapter 1, there is evidence that a thick, relatively inactive digested sewage sludge as seed is preferable to thinner types with a higher activity. This was investigated by de Zeeuw in 1983 in start-up experiments with a 30 liter UASB reactor. He used a concentrated digested sewage sludge (40 g VSS/l and 47% ash) with a low specific activity (0.04 kg COD.kg VSS^{-1}. day^{-1}) and observed a relatively good sludge retention. The minimum sludge concentration during the depression was 4.9 g VSS/l. In all experiments presented in this chapter we used a less concentrated digested sewage sludge (20 g VSS/l; 35% ash) exerting a relatively high specific activity (0.15 kg COD.kg VSS^{-1}. day^{-1});
b) adjusting the wastewater composition, such as by the addition of lacking nutrients, vitamins or trace elements to assure optimal growth conditions, the removal of inhibitory compounds and the addition of agents that improve the settling characteristics of the sludge, i.e. polyelectrolytes (Cail and Barford, 1985).
c) proper operation of the system. During start-up, long periods of over- and underloading must be avoided. As observed in Experiment 3-I, underloading leads to the development of a

voluminous type of sludge. Overloading is detrimental because it causes gas production in the gas-solids-separator (GSS) which will hamper the settlement of the sludge aggregates in the GSS due to continuing gas evolution in the aggregates.

d) maintaining sufficient space above the sludge bed. Sludge bed expansion into the settler compartment should be avoided. For a good segregation of sludge particles it is important to maintain a free zone between the bottom of the gas collector and the top of the sludge bed in the expanded state.

We are dealing with a dynamic process involving a variety of factors (characteristics of the seed sludge, the wastewater) in the granulation process. It is impossible therefore, to present exact quantitative figures with respect to the recommended sludge loading and hydraulic loading regime. However, from available experience and insight it can be concluded that the imposed sludge loading rate should closely follow the specific activity of the retained sludge so that the treatment efficiency of biodegradable COD remains well above approximately 80%. This means that once the major part of the sludge growth remains in the system, the space loading rate can be gradually increased more frequently.

Experience to date indicates that a surface load of approximately 1.0 $m^3.m^{-2}$. day^{-1} and a sludge loading rate of more than 0.6 kg COD.kg $VSS^{-1}.day^{-1}$ should be possible during the second phase (the initial phase of granulation) of the start-up.

1. Effect of the sludge loading rate (Experiments 3-I and 3-II).

Experiment 3-I shows that under conditions of low selection pressures (the sum of the hydraulic and the gas loading rate) growth will occur predominately as dispersed (filamentous) biomass. Growth of attached biomass will be limited, if at all, as the major part of the substrate will be degraded by the voluminous sludge, resulting in particularly dispersed growth. Microscopic investigation of the sludge showed neither granules nor denser flocs. The filamentous biomass in fact gave rise to the formation of a bulking type of sludge. The problem of bulking sludge is obviously not limited to aerobic wastewater treatment, however, there is a major difference in the cause of the bulking sludge problem in aerobic systems (Carousel, Pasveer-ditch) and the UASB reactor. In aerobic systems bulking sludge is reported to be caused by nitrogen and/or phosphorus deficiency and the feed pattern of most aerobic activated sludge systems (Rensink et al, 1982). Complete mixing of aerobic reactors in CSTR systems tends to promote the formation of bulking sludge, whereas high loaded plug flow systems have little problem. In UASB reactors bulking sludge formation appears to be the result of a lack of selection pressure on the system, which may also play an important role in the occurrence of bulking sludge in aerobic activated sludge systems. Based on our experience the selection pressure in these systems is small if existing at all. In both aerobic and anaerobic systems we are generally dealing with a predominance of filamentous organisms. In aerobic systems *Sphaerotilus natans* and *Microthrix parvicella* are frequently found, while in anaerobic reactors the predominant organism is *Methanothrix* in the filamentous form. Methanothrix filaments can grow quite long (200 - 300 m), and when they grow without attaching to a solid support particle a loosely intertwined structure of filaments occurs. A sludge with very poor settling properties will result. Through attachment of gas bubbles to these loosely intertwined filaments the sludge may even show a strong tendency toward flotation. The results of Experiment 3-I show that this is a gradual process in which an increasing part of the original seed sludge (partially consisting of heavier ingredients) is incorporated in the voluminous structures. Experiment 3-I also shows the uselessness of returning the sludge into the reactor. These results indicate therefore that once this type of sludge is obtained the best thing to do is to discharge it and to re-inoculate the reactor with new sludge. The importance of a selective wash-out of dispersed sludge during start-up cannot be better illustrated. The lack of selection pressure in Experiment 3-I is clearly reflected in the low average wash-out value during the experiment which was 0.022 kg VSS/m^3.

The start-up of reactor 3-II (start-up with a sludge loading rate of > 0.9 kg COD/kg VSS.d) shows that after 7 weeks the sludge bed expanded into the settling compartment. Compared to results of start-up experiments using lower influent COD levels (ten Brummeler et al, 1985), it is evident that sludge bed expansion and consequently expansion wash-out (de Zeeuw, 1984) is significantly delayed at high influent COD levels. This is a result of the lower superficial velocities under those conditions.
In Experiment 3-II there was a short period (2 weeks) with a high wash-out during which the first granules became macroscopically visible. Beyond the 10th week of operation of this experiment a gradual increase of the total amount of sludge occurred. However, at termination of the experiment there was not a clear separation between the granular and the remaining flocculent sludge. The granules, which consisted predominately of filamentous *Methanothrix*-like organisms, were entrapped in the flocculent sludge bed. As a result, the granules did not sufficiently settle to the bottom of the reactor.

2. The effect of the NH_4^+ concentration (Experiments 3-III, 3-IV and 3-V).

The results of the experiments with varying NH_4^+ concentrations demonstrate the adverse effects of non-optimal growth conditions. After 150 days of continuous operation at an average organic loading rate of 2.5 kg COD.kg VSS^{-1}.day^{-1} (after 635 g COD had been converted), there was no real granulation in the high NH_4^+ experiment (reactor 3-V). In the comparative Experiments 3-III and 3-IV the granulation process was proceeding well when the same amount

of COD had been converted (day 93 in Experiment 3-III and day 92 in Experiment 3-IV). Except for inhibition of part of the methanogenic bacteria, the absence of a clear granulation could be attributed to the presence of a high concentration of mono-valent cations which might increase the electrical charge of the "bio-colloids" in the sludge framework.
Ammonium represents the main nitrogen source for anaerobic bacteria, as it is an end product of the anaerobic fermentation of proteins and other N-containing compounds. Approximately 14% of the dry weight of a cell is nitrogen. This means that for the formation of new cell material considerable amounts of N are required. The need for N is usually expressed as a ratio of the biodegradable COD. For methanogens this ratio COD/N is about 70/1, whereas for acidifying organisms it is about 100/1. There is however evidence that at higher ammonium concentrations than those required for the growth with no N limitation, the specific methanogenic activity is even higher (Dolfing, 1987)

3. Effect of Ca^{2+} (Experiments 3-IV, 3-VII and 3-VIII).

The experiments with varying Ca^{2+} concentrations indicate that in the lower concentration range Ca^{2+} ions improve sludge retention ability of the system, i.e. the depression in the retained amount of sludge is less deep than very low and too high Ca^{2+} concentrations. As a result more viable bacterial matter and proper germs for the granules are retained and consequently the granulation process will be enhanced. The relatively high wash-out of sludge at higher (but not exceptionally high) Ca^{2+} concentrations (Experiment 3-VII) should presumably be attributed to the formation of a large number of small $CaCO_3$ crystals. These crystals also act as a carrier for bacterial attachment and therefore stimulate more dispersed growth and consequently an increased wash-out of the sludge. Evidence of this phenomenon was also indicated in a full-scale UASB installation treating cornstarch waste which contained more than 700 mg Ca^{2+}/l.

In connection with the effect of a high Ca^{2+} concentration, a few comments should be made about the treatment of liquid wastes containing a high fraction of finely dispersed suspended solids. Through attachment of bacterial matter to the dispersed particles, the wash-out of viable biomass will presumably be seriously stimulated. Microscopic examinations of the washed-out sludge in the experiments discussed in this paper revealed that a significant fraction of this sludge consisted of small fibrous ingredients originating from the seed sludge with filamentous bacterial growth attached to the surface. The same wash-out problem will be encountered with dispersed fibrous solids supplied with the wastewater.

4. Effect of the influent concentration (Experiment 3-VIII, 3-IX and 3-X).

The results of Experiment 3-VIII show that with a VFA mixture of 500 mg COD/l the formation of granular sludge may indeed be quite well possible. Under these low influent conditions light sludge ingredients are washed out right from the beginning of the experiment, while the minimum amount in the sludge remains well above 5 kg VSS/m^3.

The start-up of reactor 3-X (influent COD = 10.000 mg/l) proceeded very slowly. There was no expansion wash-out during the first 80 days of the start-up. Although overloading is generally reflected by elevated concentrations of propionic acid in the reactor, in this case acetic acid accumulation occurred. We don't have a clear explanation for this phenomenon. Since there was abundant growth of *Methanosarcina* in this reactor acetate consumption was expected. However, the acetoclastic biomass apparently did not develop quickly enough. As there were no toxicants present in the feed, and the reactor pH was around 7.0 no other inhibitory factors were causing this acetate accumulation.

During the start-up of reactor 3-X, small granules with diameters of up to ± 0.5 mm were gradually formed. These granules consisted mainly of *Methanosarcina*'s. Considering the high acetate concentrations in the reactor this is not surprising. At acetate concentrations of more than 200 mg/l the kinetic conditions for growth of *Methanosarcina* are more favorable than the growth of *Methanothrix*. The *Sarcina* granules however, were too small to be retained in the reactor at higher hydraulic loading rates. It can therefore be concluded that it is unstrategic to select for *Methanosarcina* during a UASB start-up: *Methanothrix* granules will grow 4 to 6 times as big as *Sarcina* granules and will therefore be more easily retained in the reactor at high selection pressures.

5. Effect of the temperature (Experiments 3-XI and 3-XII).

Start-up at 38°C clearly proceeds faster than at 30 °C. Therefore, especially for the early usually time-consuming period of the start-up, it is beneficial to operate the system at a temperature of 38 °C. However, a drawback of operating at this elevated temperature is, according to the performance of reactor 3-XII, that under conditions that negatively effect growth (by pH and/or temperature fluctuations or other causes), there is an immediate and relatively strong effect on the reactor performance: increased temperatures lead to increased reactor instabilities.

6. Effect of pH (Experiments 3-XI and 3-XIII).

The predominance of a filamentous bacterium in the sludge cultivated at pH 6 indicates that *Methanosarcina sp.* cannot successfully compete with this organism for acetate. Consequently, the wash-out during the start-up period, as was originally intended, cannot be significantly minimized, in comparison to a start-up period at pH 7.5. The difference that was found (i.e. the difference in the lowest calculated VSS content during the experiments of 0.9 g liter^{-1} in favor of the reactor operated at pH 6) can be explained by an improved attachment of biomass in the reactor operated at pH 6. This phenomenon was not observed in the reactor run at pH 7.5. During the period of day 0 to 50 there was no substantial acetoclastic activity. As a result, the acetate concentration prevailing in the reactor was high (1,000 to 1,200 mg of COD liter^{-1}) and consequently, preferential growth of *Methanosarcina sp.* occurred. Once the acetate degradation proceeded satisfactorily, the kinetic circumstances seemed to gradually change in favour of the filamentous organisms.

Indeed, beyond day 50 a gradual shift occurred in the bacterial composition of the sludge, i.e. the filamentous organisms predominated over *Methanosarcina sp.* Apart from the kinetic advantage of the filamentous organisms, another mechanism favored the population shift. *Methanosarcina sp.* tend to form smaller aggregates (less than 0.5 mm) than the sludge aggregates in which the filamentous organisms dominate (1.0 to 1.5 mm). These small *Methanosarcina sp.* aggregates wash out more readily from the reactor at comparable hydraulic retention times than the conglomerates formed by the filamentous organisms. The *Methanothrix*-like organisms show a strong tendency to attach to either the reactor wall or the inert particles that originate in the inoculum. This phenomenon leads to improved retention of this type of sludge, and consequently *Methanosarcina sp.* is increasingly outcompeted. The low methanogenic activity at pH 6 during day 0 to day 50 of the experiment indicates that both *Methanosarcina* sp. and the filamentous organism were present in the inoculum in relatively low numbers.

Two possible explanations for the development of the filamentous organisms at this pH level can be given: 1) the bacteria were already present in the inoculum in very low numbers and represent a *Methanothrix* strain with a lower pH optimum on acetate; and 2) *Methanothrix* soehngenii adapted to the lower pH. Van den Berg et al. (1976) have described an acetate-utilizing methanogenic culture with a similar optimal pH range for the specific activity at pH 6.6 to 6.9. The predominant organism in this culture also appeared to be a filamentous bacterium (filaments of 100 to 200 m) which is possibly the same organism described in our experiments.

The results of the experiment at pH 6 showed that propionate degradation is possible in a UASB reactor operated at relatively low pHs. This may be a result of the formation of micro-environments in which higher pH's might exist within the granules or the biofilm attached to the reactor wall. According to the investigations of Arvin and Kristensen (1982), higher pH values, relative to the pH in bulk solutions, prevail in denitrifying biofilms. The maximum difference measured amounted to 0.5 to 2 pH units. Arvin and Kristensen assume that this phenomenon is a result of lower diffusion coefficients of H^+ and HCO_3^- ions inside the biofilm. In the case of methanogenic biofilms the existence of higher pH values inside the biofilm (granules) is fairly likely because VFA's are being degraded here as a result of the high bioactivity which necessarily causes a rise in the pH of the entrapped solution. According to Dolfing (1987) a high pH gap is not likely as mass transfer resistance in the granules is very limited.

Another possible explanation for the propionate degradation at pH 6 may be the existence of a second group of propionate utilizers, as suggested by Heyes and Hall (6), which is faster growing and less sensitive to pH shocks in comparison to the propionate degraders normally found in anaerobic digesters.

From our experiments it can be concluded that a high rate of anaerobic digestion in a UASB reactor in which an acetate-propionate mixture is treated is possible at pH 6. During a period of 4 months of continuous operation at pH 6, a space load of almost 10 kg $CODm^{-3}$ day^{-1} could be reached. The start-up period of a pH 6 reactor can possibly be shortened by applying a more proper seed material, i.e., a sludge adapted to low pH conditions. As previously reported by Williams and Crawford (1984), methanogenic activity in some acid peatlands is found to be optimal at pH 6. For the treatment of acid wastewater in UASB reactors, complete neutralization of the influent is not a prerequisite. This has economic implications as fewer chemicals for neutralization are needed. The importance and the effect of different pH's in micro-environments and bulk solutions require further investigation.

Conclusions

The following conclusions can be drawn from the results of the experiments presented in this chapter:

1. It is possible to cultivate anaerobic granular sludge using a mixture of acetate and propionate as feed solutions;
2. The granulation process is accompanied by a strong wash-out of seed sludge. After granulation becomes apparent, the sludge concentration in the reactor slowly starts to increase again. This wash-out pattern is typical for most of the start-up experiments performed;
3. Prolonged periods of underloading will lead to the formation of an anaerobic bulking type of sludge consisting of filamentous bacteria (presumably Methanothrix soehngenii), indicating

the importance of higher loading rates for a proper selection of lighter and heavier sludge components;

4. NH_4^+ concentrations of 1000 mg/l will have a negative effect on the start-up speed and the granulation process;
5. Ca^{2+} ions have a positive influence at a concentration of 150 mg/l. However, at increased concentrations (> 450 mg/l) a positive influence was no longer observed;
6. It has been demonstrated that granulation is possible on very dilute VFA solutions (500 mg COD/l). At this concentration granulation proceeds faster than at higher feed concentrations;
7. At a feed concentration of 10.000 mg COD/l, *Methanosarcina* granules were formed. These granules remained small (Dp < 0.5 mm) and were easily washed out of the reactor. Therefore, it is concluded that *Methanothrix* granules are preferred over the sarcina granules;
8. An increase in temperature will have a positive effect on start-up speed and granulation. However, the process will also become more vulnerable to changes in the reactor operation such as pH and short temperature shocks;
9. It appeared to be possible to perform a start-up at decreased pH levels. The organisms, including a *Methanothrix*-like organism, show lower pH optima than would normally be expected.

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CHAPTER 4

GRANULATION ON VFA WITH DIGESTED SEWAGE SLUDGE PLUS ADDITIONAL CARRIER MATERIAL

Introduction

The initial start-up of a UASB reactor using digested sewage sludge as an inoculum may last several months before a distinct granular sludge bed develops. This relatively slow start-up of a UASB reactor system can, apart from the low growth rate of methanogenic bacteria, be attributed to the low specific activity of the seed sludge and the relatively strong wash-out of sludge during the initial phase of the start-up of the reactor.

Two strategies can be applied to improve the quality of the seed sludge. These are:

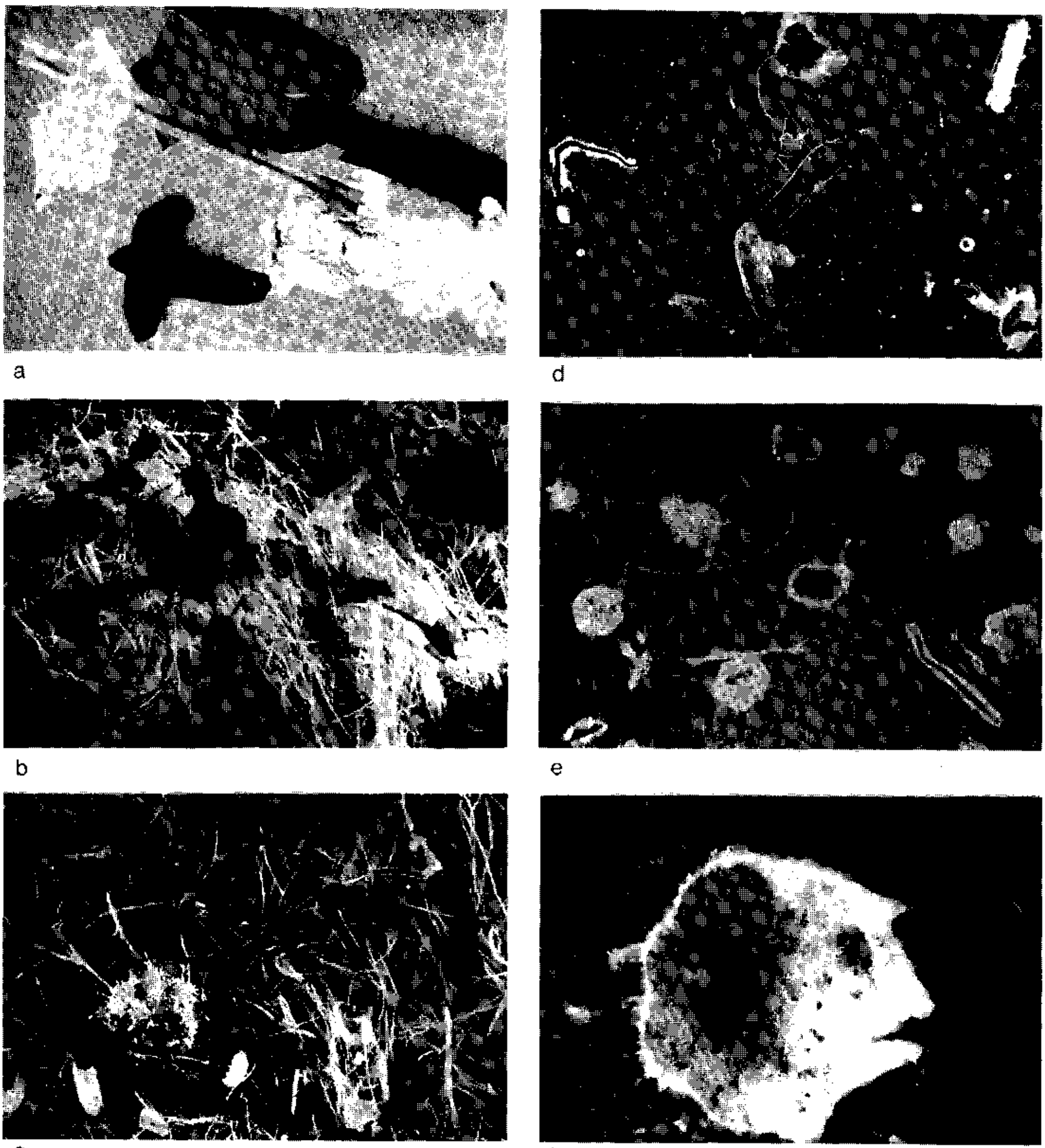
- the addition of small amounts of granular sludge. Since there is already a small, gradually increasing pool of excess granular sludge from existing full-scale UASB reactors, it is of great practical importance to investigate the effect of the addition of small quantities of granular sludge to low quality flocculent inocula for UASB reactors, i.e. digested sewage sludge or manure. The addition of small amounts of granular sludge can be advantageous for at least two reasons:

  1. As granular sludge largely consists of active methanogenic biomass, and digested sewage sludge consists only of approximately 2% methanogenic sludge VSS (estimated from the specific activity), the addition of a relatively small amount of granular sludge will result in a relatively sharp increase in the methanogenic activity.
  2. It is likely that granular sludge will act as a precursor for new granules. By "crushing" the granules into a large number of small granule fragments, a large quantity of growth nuclei for new granules will be offered.

- the addition of inert carrier material.

  Stereomicroscopic investigations of anaerobic sludges during the early phase of start-up clearly show that initial granulation takes place through attachment of bacteria to inert particles present in the digested sewage sludge. The photographs presented in Figure 4.1 show different size fractions of sieved digested sewage sludge and the granulation process at different periods after the start-up. These pictures illustrate the role of the inert particles as initial precursors for the granulation process. Other investigators also acknowledge the importance of carrier materials for the granulation process (Yoda et al, 1989).

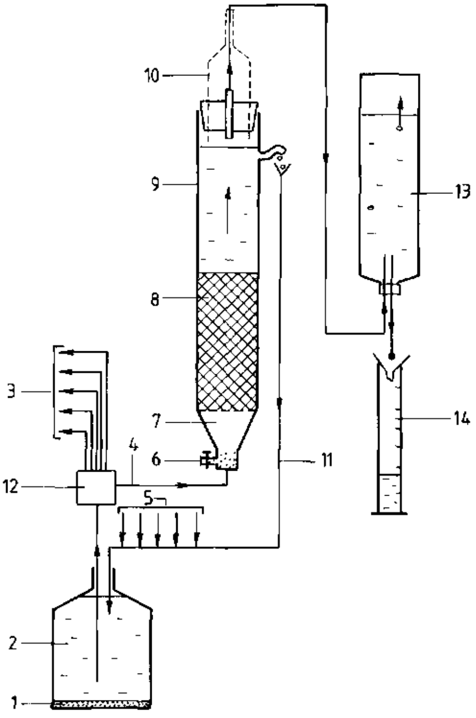
We decided therefore, to study the role of carrier materials on the start-up and granulation in more detail.



<u>*Figure 4.1*</u> *Photos of different sized fractions of typical inert particles present in digested sewage sludge (a, b and c; a = fraction above 1 mm, b = fraction between 0.5 and 1 mm, c = fraction below 0.5 mm) and development of granular sludge by attachment of biomass on inert particles (d and e represent early stages of granulation, f is a granule obtained in Experiment 4-I after 10 weeks of operation; enlargements 20x).*

Bacterial adhesion and/or attachment depends on a number of factors including the specific surface of the support particle, the critical surface tension (Dexter, 1979, Fletcher, 1987, Switzenbaum, 1988) and the hydrophobicity of the surface of the carrier material (van Loosdrecht, 1987).

To select the proper carrier material, introductory experiments were performed with 0.125 l anaerobic filters (see Figure 4.2) filled with different carrier materials. The concentrations of VFA's were kept at constant levels (acetate 1250 mg/l; propionate 1000 mg/l) and the substrate was recirculated over the filter with a flow rate of 150 ml/hour. The hydraulic residence time over the filters was 50 minutes. Homogenized granular sludge was added to the feedstock. The following carrier materials were tested: resin, hydro-anthracite, PVC rings, glassbeads, pumice and clay. As shown in Figure 4.3, the anaerobic filter with hydro-anthracite showed the fastest increase in methane production.



<u>Figure 4.2</u> *Experimental set-up of the anaerobic filter test for selection of filter media. (1 = settled sludge; 2 = feedstock; 3 = tubes to the other test columns; 4 = influent; 5 = effluent recycle of the other test columns; 6 = port for sludge withdrawal; 7 = conically shaped reactor bottom for improved substrate sludge contact; 8 = anaerobic filter; 9 = anaerobic filter column with a diameter of 33 mm and a volume of 125 ml; 10 = modified gas collection system; 11 = effluent recycle; 12 = peristaltic pump; 13 = Mariotte bottle; 14 = measuring cylinder).*

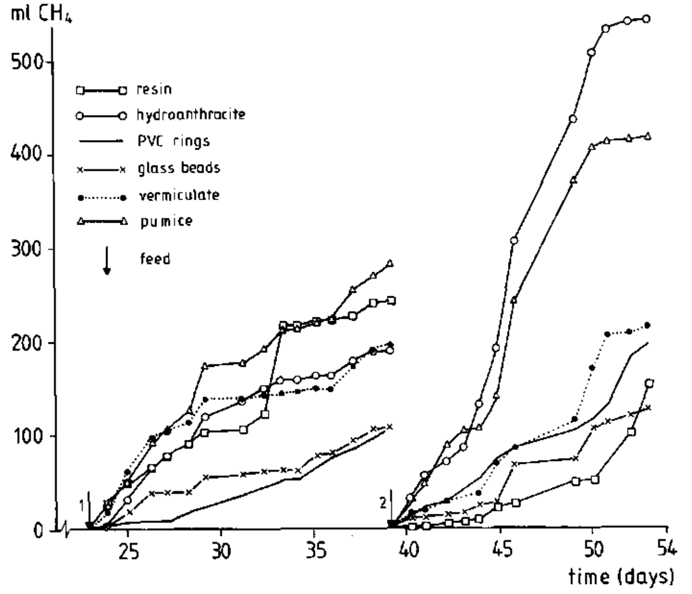


Figure 4.3 *Increase in gas production after two feedings in anaerobic filters with 6 different filter media.*

The tests with the anaerobic filters showed that the start-up rate with hydro-anthracite as carrier proceeded the fastest. Therefore, this material was selected as the supplementary carrier material to be added to the seed sludge.

Microscopic examination of sludge washed out from the reactor indicated that the form of the support particles should also be considered. Small spherical particles are better retained in the reactor, while long fibrous-like carriers including the early bacterial attachments, tend to be more easily washed out.

Materials and methods

1. The addition of crushed anaerobic granules to digested sewage sludge as a UASB reactor inoculum.

The supplementary granular sludge was cultivated on beet sugar waste from the CSM beet sugar factory. The supplied amount of treated (gently crushed) granular sludge (TGS) expressed as % VSS of the total amount of VSS in the reactor in the various experiments was 0%, 1,9% and

3,8% respectively. It was sonificated into smaller fragments to increase the amount of possible granule precursors. Activity tests on both the crushed and the uncrushed granular sludge revealed that sonification did not result in a significant loss in methanogenic activity.
The different experiments, applied reactor volumes, amount of initial seed sludge, carriers and experimental codes are listed in Table 2.2. of Chapter 2.

2. The effect of the addition of crushed granular sludge to digested sewage sludge as UASB reactor inoculum under quick and slow start-up regimes.

The supplied amount of treated (crushed) granular sludge (TGS) expressed as % VSS of the total amount of VSS in the reactor in the various experiments was 2%, 8% and 15% respectively. The experiments carried out with 2% and 8% TGS were terminated after almost 50 days and a new experiment with 0% and 8% TGS was started in which a more moderate start-up was tested in order to obtain information on the effect of the start-up regime with respect to wash-out of sludge. The reactor with 15% TGS was kept in operation during 88 days to assess the further development in reactor performance and in granulation process. Table 4.1 summarizes these experiments.

TABLE 4.1 Experiments performed for evaluating the effect of the addition of extra granular inoculum at different start-up speeds.

experimental code	quick start-up		moderate start-up	
	amount of TGS[1] (as % VSS of total inoculum)	duration of start-up	amount of TGS[1] (as % VSS of total inoculum)	duration of experiment (days)
4-IV	2%	47		
4-V			0%	40
4-VI	8%	47		
4-VII			8%	40
4-VIII	15%	88		

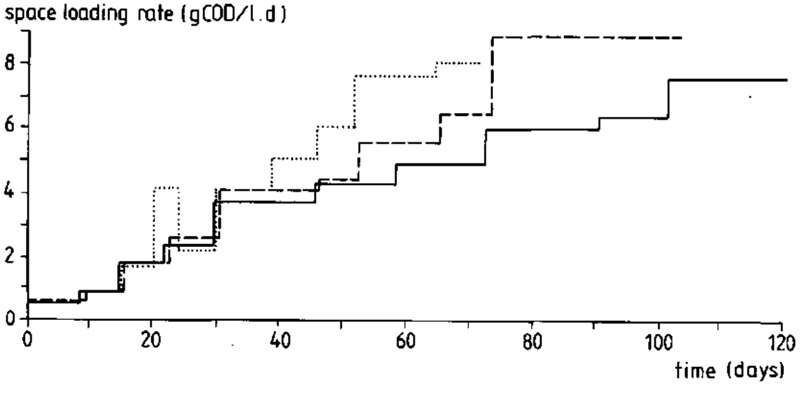
[1]TGS = Treated Granular Sludge

3. The role of inert support particles during the start-up of a UASB reactor.

The support particles present in digested sewage sludge were removed from the sludge using 44 and 100μm sieves. The sieved sludge served as the basic inoculum.
Start-up experiments were performed with the sieved sludges as such and with sieved sludge (100μm) with hydroanthracite as an additional support medium.

1. The addition of crushed anaerobic granules to digested sewage sludge as a UASB reactor inoculum.

The results obtained in the start-up of the three 120 l UASB reactors (Experiments 4-I, 4-II and 4-III) are summarized in Figure 4.4 loading rates were increased as soon as treatment efficiencies of approximately 80% were reached.



<u>*Figure 4.4*</u> *The effect of the addition of granular sludge to digested sewage sludge with respect to the maximum loading potentials (at a minimal COD reduction of 80%). (Experiment 4-I:——; Experiment 4-II:– – –; Experiment 4-III:······)*

As shown in Figure 4.4, the loading rates in reactors 4-II and 4-III could be increased according to the criteria set for the load increase, slightly faster than in reactor 4-III. However, granulation was observed in all three reactors after the same period, i.e. after approximately 55 days when the imposed organic loading rates were respectively 4.3, 4.5 and 6.0 kg $COD.m^{-3}.day^{-1}$ for reactor 4-I, 4-II and 4-III respectively. Reactor 4-I showed the highest wash-out and its minimal sludge concentration was about 2 g VSS/l (at day 65). The minimal sludge concentration in reactors 4-II and 4-III appeared to be slightly higher, i.e. 2.7 g VSS/l in both reactors; these values were reached at day 55 and 60 respectively. Microscopic examination revealed a remarkable difference between the granules found in the reactors. This is illustrated by the scanning electron micrographs shown in Figure 4.5 a-d.

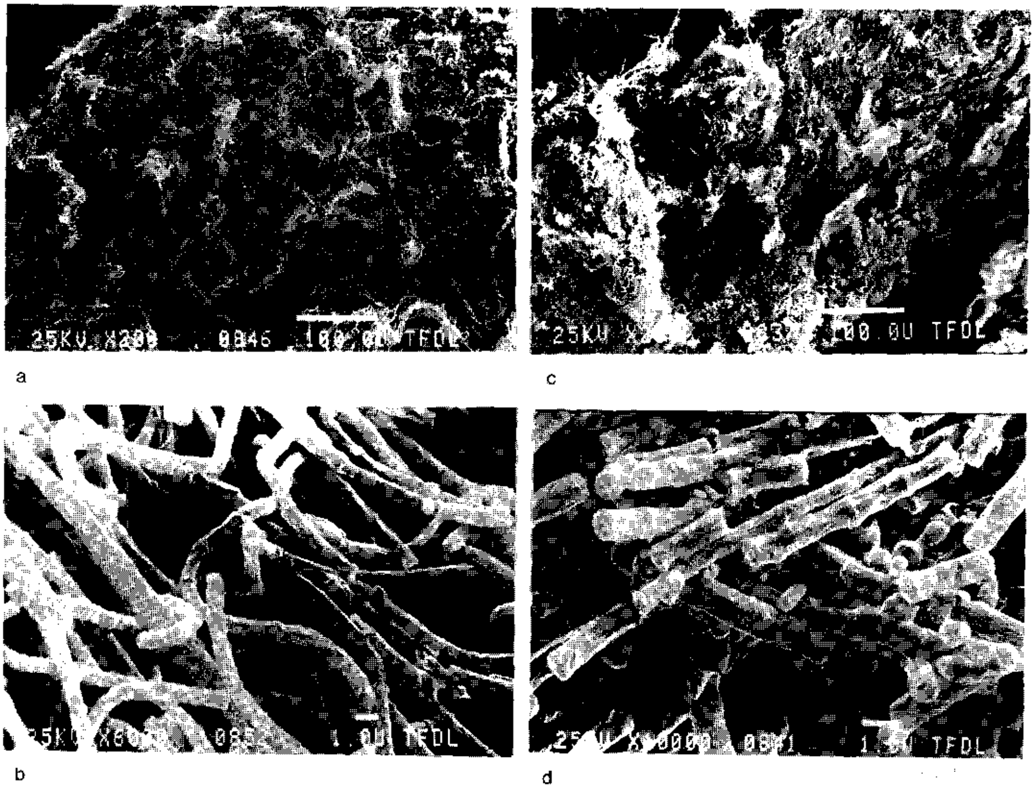


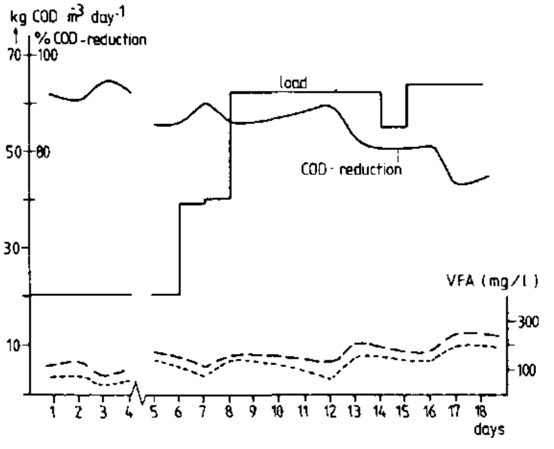
Figure 4.5 Scanning Electron Micrographs of the outside of a "filamentous granule" of Experiment 4-I, presumably consisting of filamentous Methanothrix soehngenii cells (a and b) and a "rod granule" of Experiment 4-II, presumably consisting of Methanothrix cells in short chains (c and d). Bar inidicates 0.1 mm for a and c and 1 m for b and d. (SEM-pictures taken by TFDL, Wageningen, The Netherlands).

The granules cultivated in reactor 4-I were composed mainly of long multicellular filaments of rod-shaped organisms, presumably *Methanothrix soehngenii*. These granules will be referred to as **filamentous granules**. Microscopic examination of the sludge during the experiment revealed that all these granules contain some kind of inert support material originating from the digested sewage sludge. The nature of these inert particles is very diverse and presumably varies considerably in chemical composition and nature. Chitin containing insect parts, as well as poorly degradable lignocellulitic plant materials of different size fractions could be distinguished. The maximum diameter of the granules was approximately 5 mm.

The granules cultivated in reactors 4-II and 4-III consisted of short multicellular fragments composed of up to about 4 cells (**rod-type granules**), presumably also *Methanothrix*. The granules of reactor 4-II and 4-III were smaller (diameter 2 mm) and more densely packed than the more voluminous, filamentous granules present in reactor 4-I. There was no clear evidence of the presence of any inert support material in former granules. In almost none of the granules could *Methanosarcinas* be detected. Both types of granules exerted excellent settling characteristics, i.e. the settling velocity of the average sized granules was about 0.5 m/min. Moreover, both types of granules consisted predominantly of viable bacterial matter (VSS = approximately 90% of DSS).

The maximal achievable loading rates were assessed using 30 l UASB reactors filled with 440 g VSS and 370 g VSS of granular sludge of the filamentous type of granules and the rod-type granules respectively. Again VFA feed solutions composed of 1250 ppm acetic acid and 1000 ppm propionic acid were used in these experiments.

The results obtained are shown in Figures 4.6 and 4.7.



<u>*Figure 4.6*</u> *Capacity test of filamentous granules cultivated in Experiment 4-I. Assessment of upper loading limits at 30 ^{o}C in a 10 liter UASB-reactor.*

From the results it appears that the rod-type granules exert slightly lower activity than the filamentous granules, particularly with respect to the degradation of propionic acid. At high organic space loads the degradation of propionic acid is much less complete with the rod-type granular sludge than the filamentous sludge granules. The specific activities calculated from the results were 2.3 kg COD.kg VSS^{-1}.day^{-1} and 2.2 kg COD.kg VSS^{-1}.day^{-1} for the filamentous and rod-type granules respectively (at 30 ^{o}C). Additional batch experiments with the rod-type granules revealed a very strong temperature dependency of the specific activity of the sludge.

A maximum specific activity of 4.0 kg COD.kg VSS^{-1}. day^{-1} was found at 38 ^{o}C. (See Figure 4.8).

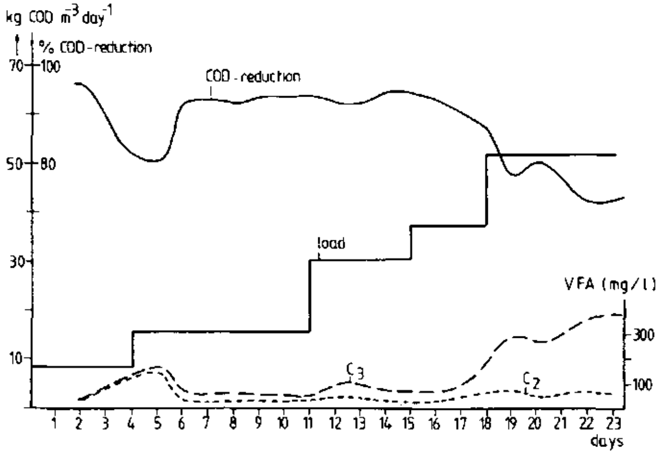


Figure 4.7 *Capacity test of rod granules cultivated in Experiment 4-II. Assessment of upper loading limits at 30 °C in a 10 liter UASB reactor.*

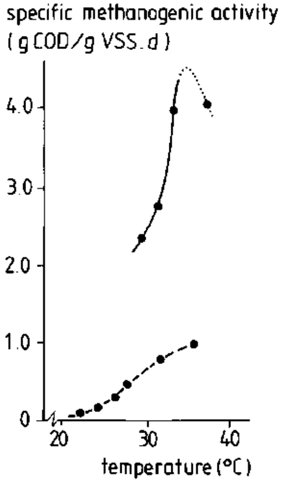


Figure 4.8 *Effect of temperature on specific methanogenic activity of granular sludge cultivated in Experiment 4-I (————) and anaerobic sludge (partly granular) adapted to potato starch wastewater (— — — —).*

2. The effect of crushed granular sludge on digested sewage sludge as UASB reactor inoculum under conditions of quick and slow start-up regimes.

The main results of these experiments are presented in Figures 4.9, 4.10 and 4.11. Reactors 4-IV, 4-V, 4-VI, 4-VII and 4-VIII contained respectively 2%, 0%, 8%, 8% and 15% additional granular sludge as indicated in Table 2.2.

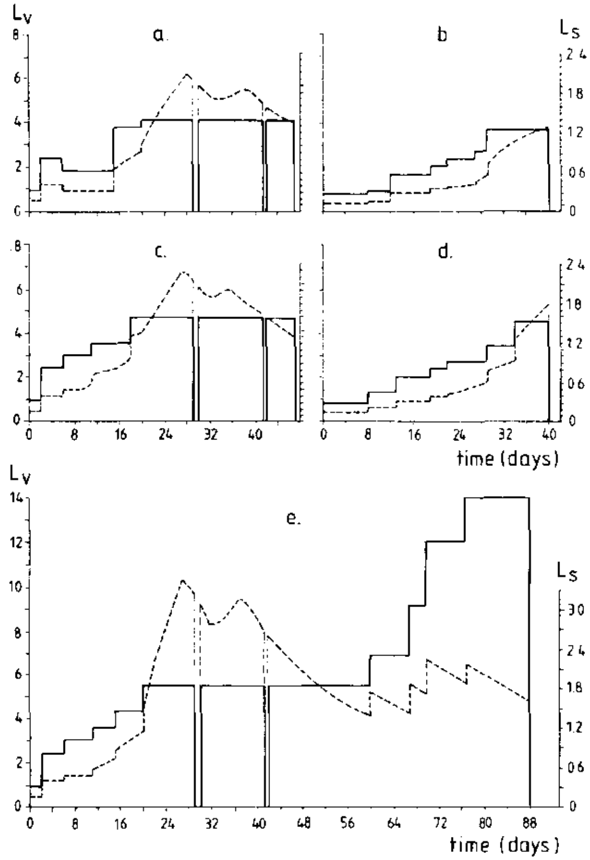


Figure 4.9 *Applied space loading rates (L_V) and sludge loading rates (L_S), as respectively kg COD/m^3.d and kg COD/kg VSS.d, in the Experiments 4-IV (= a), 4-V (= b), 4-VI (= c), 4-VII (= d) and 4-VIII (= e).*
(L_V:————; L_S:— — — —)

Figure 4.9 shows the imposed space loading rates and sludge loading rates. The concentration of acetate and propionate in the effluent solution is shown in Figure 4.10, while the data obtained for sludge retention, sludge growth and sludge wash-out are presented in Figure 4.11. In the first run (Experiments a., c. and e.) relatively high loading rates were applied directly from the start of the experiments. This resulted in overloading in all three of the reactors leading to an accumulation of acetate and especially propionate. Appearing from the results in Figure 4.10 there was a distinct difference in the rate of recovery between the reactors depending on the amount of TGS supplied. The loading rate of the reactor in Experiment a. (2% TGS) had to be diminished since the system was unable to degrade the propionate. The reactors in Experiments c. and e. (8% and 15% TGS) showed little difference in this early stage of the start-up. In both these reactors the first granules were observed around day 18, about 1 week sooner then in Experiment a. As mentioned above, the formation of a granular sludge bed is

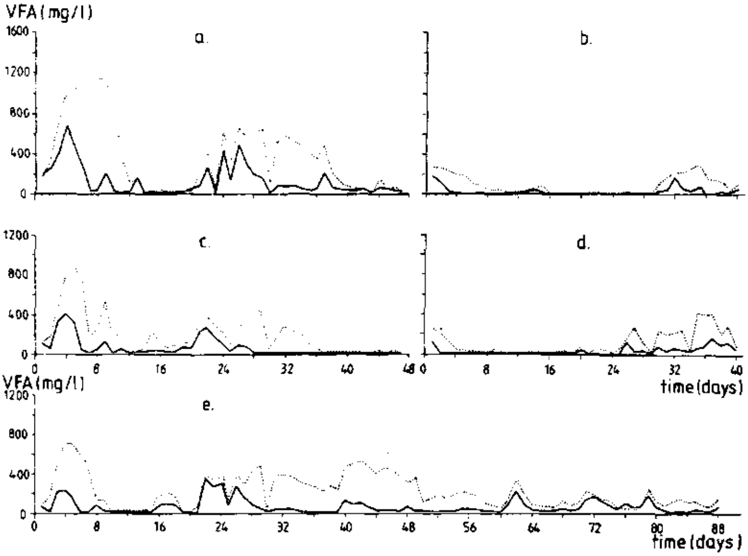


Figure 4.10 *Effluent acetate and propionate concentrations during start-up in the Experiments 4-IV (= a), 4-V (= b), 4-VI (= c), 4-VII (= d) and 4-VIII (= e). (acetate: ——— ; propionate: ……..)*

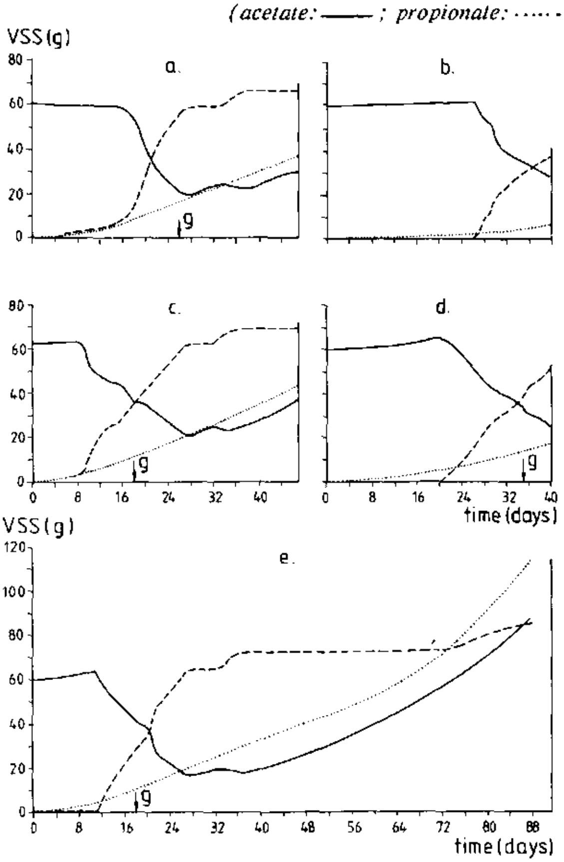


Figure 4.11

Development of sludge wash-out, retained sludge amount and sludge increase during the investigation periods of Experiments 4-IV (= a), 4-V (= b), 4-VI (= c), 4-VII (= d) and 4-VIII (= e). (T(Total amount of VSS in the reactor: ——— ; amount of washed out VSS: – – – ; growth as VSS: …….)

preceded by a considerable wash-out of the finer ingredients of the seed sludge. This wash-out of sludge leads to a sharp increase in the sludge load and frequently also temporarily in certain overloading of the system. This is also the case in the present experiments as is reflected in the increased acetate and propionate concentrations in the effluent (Figure 4.10). In Experiment e. a space loading rate of 5.5 kg COD/m^3.day could be applied already 20 days after the start. Although the acetate was almost completely removed within 10 days, it took 40 days for propionate to be satisfactorily degraded. A very similar pattern was found in Experiments a. and c. However, as a result of the lower loading conditions applied in these reactors (respectively 4.2 and 4.8 kg COD/m^3.day) the formation of an adequate propionate degrading capacity (PDC) was established earlier on. Once a good PDC was built up, subsequent increments in the loading rates (Figure 4.9, Experiment e.) were well accommodated. Moreover, the increased loads were accompanied by a steady increase in the granular sludge bed.

The granules formed in Experiment a. contained inert particles originating from the digested sewage sludge. These granules contained both filamentous and rod-shaped organisms. On the other hand, the granules developed in Experiments c. and e. consisted almost completely of rod-shaped organisms. Macroscopic examination of the granules revealed that fragments of CSM granules were often incorporated in the newly formed granules.

As a result of the more moderate start-up regime applied in the experiments with 0% and 8% TGS (Experiments b. and d.), the wash-out of the finer sludge ingredients occurred only after 4 and 3 weeks respectively, i.e. significantly later than in Experiments a., c. and e. Furthermore, in the case of 8% TGS (Experiment d.) it took almost twice as long before any granulation became apparent.

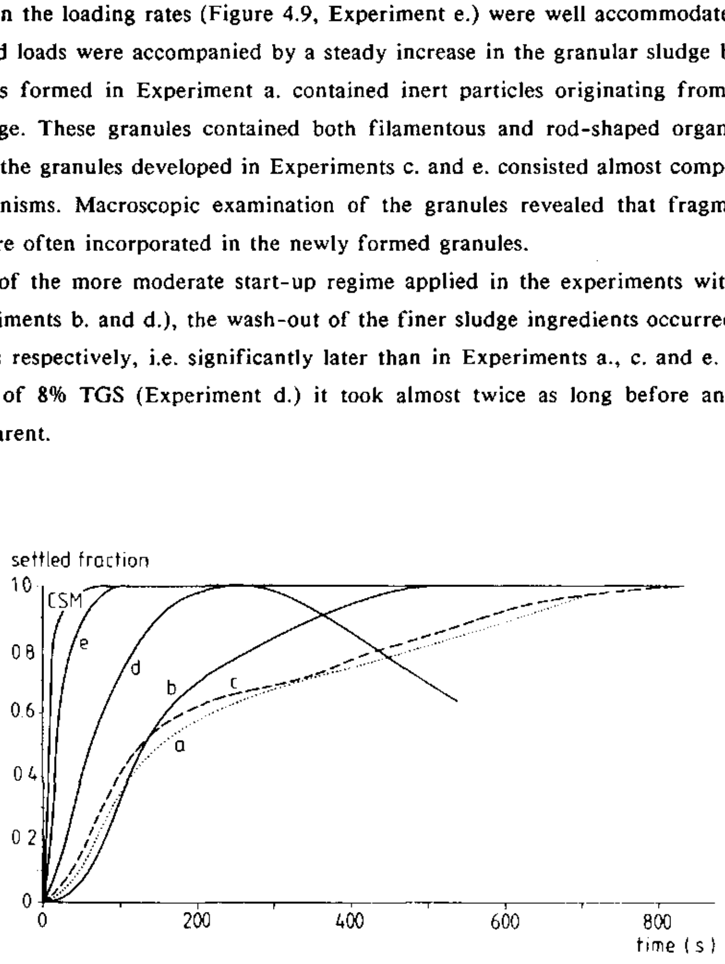


Figure 4.12 *Settled sludge fractions of the final sludges of Experiments 4-IV (= a), 4-V (= b), 4-VI (= c), 4-VII (= d) and 4-VIII (= e). CSM sludge was used as a reference.*

Figure 4.12 shows the settling properties of the sludges cultivated in the various experiments, together with that of the granular CSM sludge as a reference determined with a sedimentation balance. The results show an improved settleability with increasing amounts of supplied TGS. The settling pattern of the sludges from reactors a., b. and c. is dominated by the presence of flocculent sludge.

3. The role of inert support particles during the start-up of a UASB reactor.

The results of the start-up experiments are presented in Figures 4.13, 4.14 and 4.15.

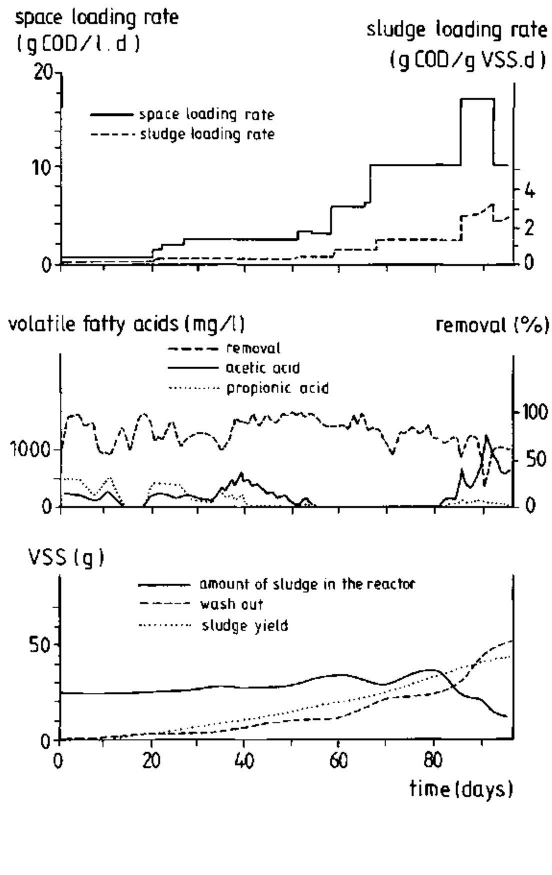


Figure 4.13 *Performance of the reactor seeded with digested sewage sludge sieved with a 100μm sieve (Experiment 4-X).*

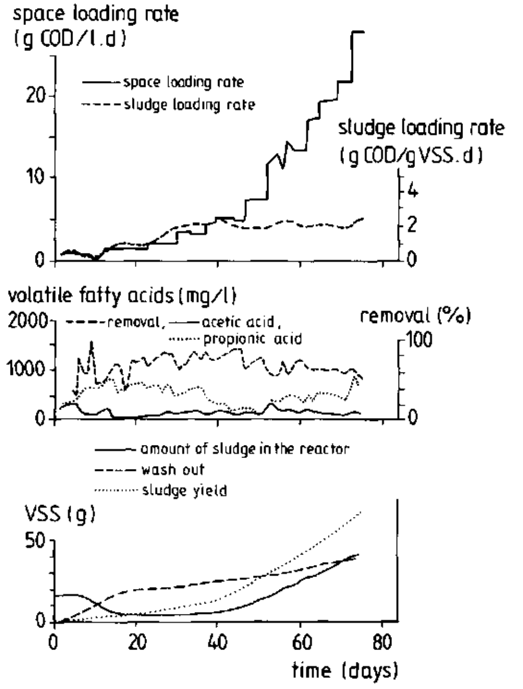


Figure 4.14 *Performance of the reactor seeded with digested sewage sludge sieved with a 100 μm sieve to which 154 g/l hydroanthracite particles with a diameter of approximately 100 m were added (Experiment 4-XI).*

There was no clear evidence of granulation in the reactor inoculated with digested sewage sludge sieved over a 44 μm sieve, whereas the addition of hydro-anthracite clearly enhanced the granulation process as well as the rate of start-up. The loading rate could be increased significantly faster than in the other experiments with the same substrates and the same amounts of seed sludge, and within 80 days values of more then 20 kg COD/m^3.day could be applied.

The sludge treated with a 100 μm sieve still contained small inert particles and as a result granulation could take place during the start-up of this reactor.

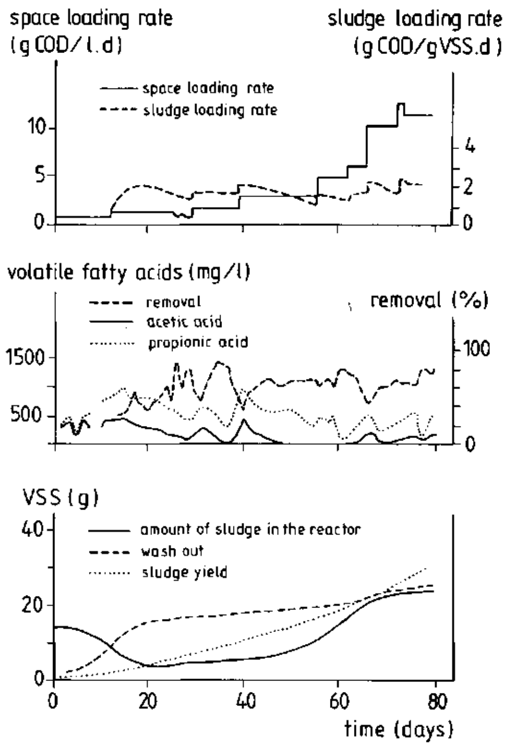


Figure 4.15 *Performance of the reactor seeded with digested sewage sludge sieved with a 44μm sieve (Experiment 4-XII).*

Discussion

The addition of TGS clearly enhances the granulation process as well as the start-up speed. In the absence of TGS, granules will not form within 30 days. In supplying 2%, 8% and 15% TGS the first granulation can be observed 26 days after the start for the 2% TGS experiment and as early as 18 days after the start for both the 8% and 15% TGS experiments. It is clear that the rapid granulation is a result of the presence of large quantities of granule fragments, which serve as granule precursors; growth increasingly occurs in and on these precursors.

The loading regime applied during the start-up has a pronounced effect on the granulation process. In applying a more moderate start-up regime, sludge wash-out as well as granulation commences later. A slight overloading during the initial phases of the start-up appears to be beneficial. The reason for this can be attributed to the higher concentrations of VFA present in the reactor during the slight overloading leading to increased growth rates and thus an increased formation of bacterial agglomerates. If the overloading is too great however, the

situation alters; the acetate concentration will be so high that growth of *Methanosarcinas* will stifle the growth of *Methanothrix*. This should be avoided as *Methanosarcina* granules are inferior to *Methanothrix* granules due to the smaller granule sizes (danger of wash-out) and the higher substrate affinity (lower efficiencies).
As all the present experiments were inoculated with ± 6 g VSS/l, they can be compared with the results of earlier investigations (de Zeeuw and Lettinga, 1980; Hulshoff Pol et al, 1983) in which 10-15 g VSS/l as seed sludge was used. In doing this it appears that the minimal retained amount of VSS in the reactor is approximately equal to that in the previously conducted experiments. This indicates that the required amount of seed sludge can be reduced significantly to a value of 6 g VSS/l because the rate of start-up will be almost equal.
The presence of the DSS from the digested sewage sludge is still of importance due to the fact that TGS is more evenly distributed in the sludge bed, although the specific methanogenic activity is low compared to the activity of the TGS. As a result, a better contact between sludge and wastewater is obtained. Inoculating the UASB reactor with just TGS when large quantities of it are not available is therefore not recommended. However, if there is a large quantity of granular sludge available (more than 6 kg VSS.m^{-3}) for the reactor, then digested sewage DSS is not required.
Although the granulation process is enhanced by increasing the supply of TGS from 2% to 8%, this is not the case when the TGS supply is increased from 8% to 15%. So according to the results, and in fact our expectations, obtained in these experiments, it is clear that the first start-up of new UASB reactors can be speeded up significantly by enriching the digested sewage sludge with a relatively small amount of granular sludge. An additional beneficial effect of the addition of TGS is its tendency to decrease the rate of wash-out of sludge (Figures 4.11a, 4.11c and 4.11e), although the extent of sludge wash-out remains unaffected by the supply of TGS. For Experiments a., c. and e. this was ± 70% of the original VSS amount.
Confirming the results of Experiments 4-I, 4-II and 4-III, as well as Experiments 4-V, 4-VI, 4-VII and 4-VIII, rod-type granules are cultivated when TGS is added, although in reactors 4-V to 4-VIII a fraction of filamentous organisms were also present. The size of the cultivated granules (d = 1-2 mm) correspond well with the size of the granules cultivated in full-scale reactors.

Why two types of granules (filamentous and rod-type) can develop is difficult to answer at this stage. Huser (1981) could not provide guidelines for the forced growth of *Methanothrix* into one of the two morphological types. As the additional granular inoculum in reactors 4-II and 4-III consisted of rod-type granules, it became clear that a small addition of rod-type granules already promotes the formation of a complete rod-type granular sludge bed, whereas under a very similar loading regime and without this extra inoculum, only a filamentous granular sludge bed develops. An explanation for these phenomena is that, of both morphological forms occurring in the digested sewage sludge, only the filamentous organisms, as a result of their greater affinity to the more dense inert support material present in the seed sludge, manage to be retained within the system despite the relatively high gas and liquid loading rates that prevailed in this experiment. Microscopic examination of the granules support this hypothesis. In all the experiments with only DSS from digested sewage sludge, filamentous granules developed. In our opinion, these type of granules will ultimately evolve into rod-type granules due to a process of erosion and compaction. It is possible to distinguish different generations of granules as new granules will be continuously formed from eroded parts of full-grown granules. Each generation of granules will be more compact and contain shorter chains of *Methanothrix* cells.

The fact that the so-called "rod-type granules" develop from the seed sludge inoculated with only a small percentage (on VSS basis) of TGS, is a result of the presence of a large quantity of small "rod-type" granule fragments. These fragments, which are very densely packed, promote the growth of short chained *Methanothrix* cells, since the dense infrastructure of the granule prohibits the growth of long filaments; there is no space available. The opposite applies to the voluminous open structure of the "filamentous granules". In a highly loaded large-scale UASB reactor, weaker and less compact granules are gradually replaced by the more compact granules with shorter *Methanothrix* chains. The long filaments containing granules will disappear because of lower resistance to the eroding forces brought about by the continuous high hydraulic and gas loading rates.

The experiments with the sieved digested sludge clearly show that the inert support particles indeed exert an important role in early granulation. The fact that sieving over a 100 μm screen was insufficient in avoiding the growth of sludge agglomerates, and that granulation could not be detected in sludge sieved over a 44 μm sieve indicates that the size range of inert particles originating from the digested sewage sludge with a clear positive effect on granulation is in between 44 and 100 μm. These experiments also indicate that the inert particles also play an important role in the initial granulation process. The digested sewage sludge that was sieved over a 44 μm screen showed no granulation indicating the importance of the size of the inert particles. Inert particles of less than 44 μm are apparently too small to have a clear contribution in the granulation process. This can be due to the low settleability of these small particles; they will be washed out of the system at a comparable speed to the wash-out of the dispersed growing biomass.

The positive effect of the presence of support particles of a specific size was also demonstrated in the experiment with the additional support particles. After the introductory experiments with the anaerobic filters, hydro-anthracite particles of 100 μm were selected because of their good "adhesion properties", compared to the other tested support particles. The results of Experiment 4-X show that the implementation of the additional support is beneficial to the start-up speed as well as the granulation process. These results support the hypothesis that a good granulation will be obtained if attached biomass is retained in the reactor while dispersed growing biomass is forced to gradually wash-out. As a result, growth will concentrate on the retained part of the sludge. The selection of the sludge fractions takes place on the basis of differences in density.

It is possible to inoculate reactors with only support material similar to the start-up regime for the fluidized bed reactors as employed by Heijnen (1984). This would only be possible in UASB systems if the reactor is operated more as a fluidized bed during the initial start-up, with high up-flow velocities to provide optimal contact between the wastewater and the support particles. After biomass present in the waste-stream has the opportunity to attach to the support particles, the operation of the reactor could be adjusted by reducing the upflow velocity, and the reactor would be operating once again as a UASB system.

Conclusions

The results of the experiments described in this chapter allow us to draw the following conclusions:

1. The addition of a small fraction of gently crushed granular sludge to the seed sludge clearly enhances start-up speed and the granulation process;
2. A slight overloading of the anaerobic reactor reduces the time necessary for start-up, with growing conditions still in favour of *Methanothrix* rather than *Methanosarcina*;
3. Heavy seeding of UASB reactors appears to be unnecessary; inoculation with 6 g VSS/l will give results comparable to an inoculum of 10-15 g VSS/l. With heavy seeding a large fraction of the seed sludge will be useless due to the resulting strong wash-out;
4. Two major types of granules were found:

   - granules consisting of *Methanothrix*-like organisms growing in long filaments, the so-called filamentous granules. These granule types have an open structure and a diameter of up to 5 mm and develop by attachment to inert particles present in the seed sludge;

   - granules consisting of *Methanothrix*-like organisms growing as short chains of 4-6 cells, the so-called rod-type granules. These granules were weaker and more compact than the filamentous granules (diameter up to 2 mm) with hardly any attachment to inert particles taking place.

   Filamentous granules developed without the addition of gently crushed granular sludge to the seed, while rod-type granules were cultivated with additional crushed granular sludge;
5. The addition of extra carrier material to the seed sludge enhances the granulation process and start-up speed. Hydro-anthracite particles of $100 \mu m$ were very effective in this respect;
6. The crucial role of the inert particles in the seed sludge for development of (filamentous) sludge granules was demonstrated by removing these particles from the seed sludge through sieving. When the inert particle fraction of $44 \mu m$ to $100 \mu m$ is removed, granulation does not occur within the period of time that is usually required for the formation of anaerobic sludge granules (50-90 days).

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CHAPTER 5

GRANULATION OF ANAEROBIC SLUDGE ON SUCROSE-CONTAINING SUBSTRATES

Introduction

Based on the observation that granulation is not restricted to mesophilic methanogenic processes but also occurs in upflow sludge bed reactors with denitrification (Klapwijk et al, 1981a and 1981b, van der Hoek, 1988), acidification (Zoetemeijer et al, 1981), and under thermophilic conditions (Wiegant and Lettinga, 1982; Wiegant and de Man, 1985), it is now generally believed that in any bio-reactor in which the substrate solution is fed through the reactor in an upflow mode, the biomass will grow in a granular form. Each specific bioconversion process however, will have its own set of conditions necessary for granulation. In several instances granulation will proceed poorly, if at all. Generally, in these cases the wastewater has a high content of suspended solids (e.g., slaughterhouse waste), or contain inhibiting or toxic compounds. However, these wastewaters can generally be efficiently treated in flocculent sludge bed UASB reactors, although at lower maximum loading rates than with granular sludge bed systems. In addition to the comprehensive granulation experiments conducted on VFA mixtures, we also decided to study more complex wastewaters in order to assess the granulation speed and type of granules formed under these conditions, and to compare the results with those found with pure VFA mixtures. Sucrose was selected as substrate since it can serve as a representative model substrate for polysaccharides, a wastewater component frequently encountered in practice. Since the yield of acidogenic organisms is considerably higher than that of methanogens ($Y_{acidogens}$ = ± 0.15; $Y_{methanogens}$ = ± 0,03), it can be expected that the speed of granulation on sucrose will proceed faster than granulation on VFA mixtures.

Materials and methods

The granulation experiments were, as indicated in Table 2.3 in Chapter 2, performed in 23.5 liter UASB reactors. The reactors were inoculated with 300 g of sludge volatile suspended solids (VSS) in the form of digested sewage sludge obtained from the municipal sewage treatment plant in Ede, The Netherlands. Two sucrose-volatile fatty acid (VFA) combinations were tested, both with a total chemical oxygen demand (COD) of ± 3,000 mg of O_2 per liter (Table 3.3).

The addition of other nutrients (N, P, and S) was based on a presumed growth yield of 5% and 20% (as COD and based on growth yields of methanogenic and acidogenic organisms of 0.035 and 0.15 kg VSS/ kg removed COD) for Experiments 6-I and 6-II, respectively, so biomass growth would be carbon limited. Yeast extract (100 mg/liter) was added to enrich the substrate in Experiment 6-I, and 4 ml of a trace element solution, described by Zehnder et al (1980), was added to both substrates. To maintain the pH in the reactor of Experiment 6-II beyond 6.5, the substrate was buffered with a $NaHCO_3$ solution.

The flow rate, pH, gas production (using a wet test gas meter), and VFA concentrations in the effluent (by gas-liquid chromatography) were monitored daily. When the treatment efficiency reached 85% (Experiment 6-I) and 75% (Experiment 6-II), the loading rate was increased. The amount of VSS present in the reactor was calculated from sludge VSS profiles assessed from the samples collected from five sampling ports situated over the height of the reactor. The amount of washed out solids was determined with the aid of an overdesigned external settler in which scum layers were broken down with an antifoam agent (Structol no. 20548/9, Schill & Seilacher, Chemische Fabrik). The biomass yield (Y) was calculated on the basis of equation:

$$Y = \frac{1.4 * (VSS_{sludge})_{produced}}{(\text{amount of COD})_{converted}}$$

The amount of biomass produced over a specific period of time was obtained by the difference of the assessed total amount of biomass present in the reactor at time t_1 and t_2 (using the sludge profiles at t_1 and t_2) and the total amount of sludge washed out between t_1 and t_2. The amount of biomass was multiplied by 1.4 to calculate the sludge COD.

Sedimentation characteristics of the sludge were determined with a sedimentation balance (Sartorius 4620). Sludge was examined with epifluorescence and phase-contrast microscopy. The macroscopic morphology of Kaiser's glycerin-gelatin immobilized granules was investigated with a stereomicroscope.

The specific methanogenic activity of the sludge was determined according to Dolfing and Bloemen (1985), by suspending small amounts of sludge (0.1 to 0.5 g) in a phosphate-bicarbonate buffer (pH 7) in 130 ml serum vials under CO_2 atmosphere and using H_2, formate, acetate and propionate as substrates. Methane was collected in a pressure-lock syringe and determined by gas chromato-graphy.

Results

Experiment 5-I. The data obtained for the most important process parameters are graphically presented in Figure 5.1. In the 95 days of continuous operation of the experiment, the space loading rate could be increased from 2.1 to 16.0 g of COD liter $^{-1}$ day^{-1}. The specific sludge substrate utilization rate increased sharply around day 30. After this sharp increase, the imposed sludge loading rate remained fairly constant during the rest of the experiment at approximately 1.6 g of COD per g of VSS per day. The assessed biomass activity amounted to about 1.45 g of COD per g of VSS per day during this period. The results indicate that almost each imposed increase in the organic load is accompanied with an increased propionate and acetate concentration in the effluent. The sharp increase in sludge load occurred at a space loading rate of 5.5 g of COD liter^{-1} day^{-1} and a hydraulic retention time (HRT) of 13 hours. The propionate concentration remained high for 2 weeks but the acetate concentration remained low during this period. After completion of the adaption of this system to propionic acid, subsequent periods of occasional overloading were accompanied by a temporarily incomplete degradation of both acetate and propionate. In the present experiment this is particularly the case during day 75 to day 85.

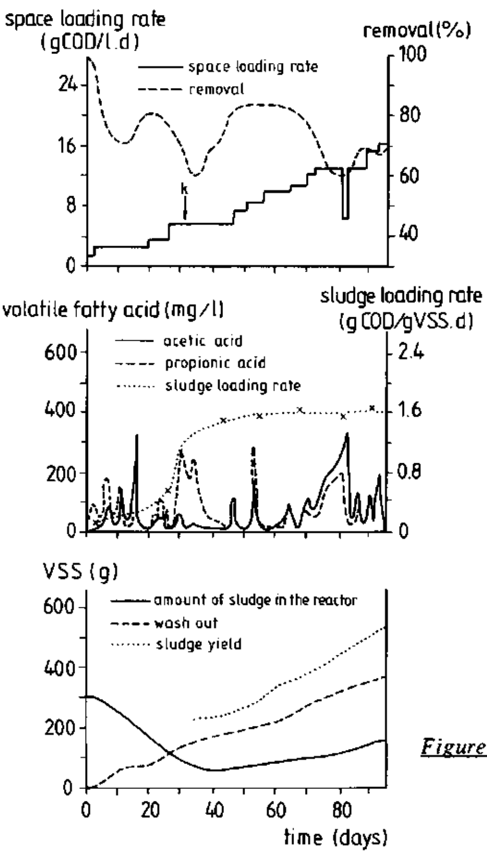


Figure 5.1 *Results obtained with start-up of the reactor with 10% sucrose COD plus 90% VFA COD as feed (Experiment 5-I; k indicates the day the first granulation was observed).*

The total amount of biomass retained in the reactor decreased during the first 30 days from 12.8 to 3.2 g of VSS per liter reactor volume but then immediately after the first granules could be visually observed in the sludge bed it started to slowly increase again. At the termination of the experiment the biomass concentration amounted to 6.3 g of VSS per liter reactor volume. Beyond day 30, biomass growth exceeded the wash-out, although a significant fraction of the retained sludge still consisted of a rather flocculent sludge. Moreover, the settleability of the granules formed was rather poor. The granules were composed mainly of filamentous bacteria. In fact, they did not constitute a separate granular sludge bed as we observed in similar experiments with merely acetate and propionate as substrate (see Chapter 4, Experiments 4-I, 4-II and 4-III). Most of the granules formed consisted of filamentous bacteria attached to biologically inert (support) particles originating from the seed sludge. The "loose" structure of the granules, which had a diameter of approximately 3 mm, remained unchanged during the process. The maximal specific methane production rate was 0.59 liter of CH_4 per g of VSS per day at a dilution rate of 5.33 day^{-1}. The assessed biomass yield (Y) amounted to approximately 0.039 g VSS COD per g of COD removed.

Experiment 5-II. The start-up in Experiment 5-II proceeded more slowly than in Experiment 5-I (Figure 5.2). The space loading rate could only be increased from 1.1 to 5 g of COD $liter^{-1}$ day^{-1} within 30 days. Beyond day 30 the space load could not be substantially increased because repeatedly higher C_2 and C_3 concentrations were observed in the effluent solution. The amount of retained biomass in the reactor gradually dropped from 12.5 g to 8.3 g of VSS per liter on day 55, but from then onwards the biomass concentration in the reactor increased until day 95 when 19.6 g VSS/l was reached. This improved sludge retention could be attributed to the formation of a granular type of sludge. Granules were initially observed on day 28 when the imposed space loading rate amounted to 4 g of $COD.liter^{-1}.day^{-1}$ at a hydraulic retention time of 18 hours, while the sludge load amounted to 0.3 g COD/g VSS.day. The pH value of the mixed liquor in the reactor during the experiment could be maintained at approximately 6.7. Although there were periods of slight overloading, particularly between days 30 and 70, the effluent COD consisted mainly of acetate and propionate. Sucrose could not be detected in the effluent during this period. The imposed sludge load did not increase as sharply as in the previous experiment (5-I) however, in contrast to Experiment 5-I the specific activity further improved beyond the day when the total amount of biomass in the reactor reached its minimum value. As a result, the sludge load could be increased from approximately 0.45 kg COD/kg VSS.day on day 40 to 0.62 kg COD/kg VSS.day on day 95. The assessed maximal specific CH_4 production rate amounted to 0.5 g CH_4-COD/g VSS.day. The assessed maximum activity in 0.5 liter serum bottle assays using a mixture of 600 ppm of C_2, 600 ppm of C_3, and 600 ppm of C_4, were significantly higher, i.e. values up to 0.95 g $COD_{converted}$/g VSS.day. The sludge yield, estimated on the basis of sludge concentration measurements over the height of the reactor

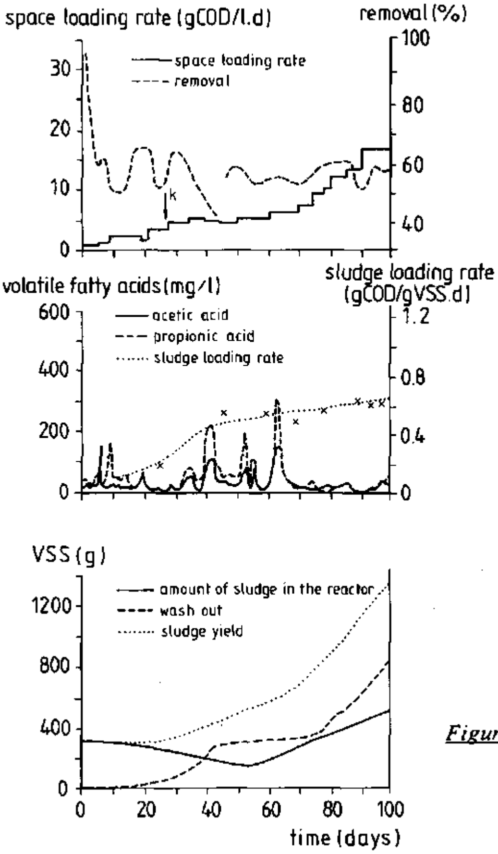


Figure 5.2 *Results obtained with start-up of the reactor with 95% sucrose COD plus 5% VFA COD as feed (Experiment 5-II; k indicates the day the first granulation was observed).*

amounted to 0.13 g VSS-COD/g COD-removed. The granules formed were up to 5 mm in diameter and were pale yellow in color. They consisted mainly of long filaments and small numbers of cocci and diplococci. The mechanical strength of the granules was fairly satisfactory, although the largest granules tended to fall apart into smaller particles approximately 1 mm in diameter. During the course of the experiment a distinct shift was observed in the composition of the granules. The number of filaments diminished, whereas the number of mobile rods remarkably increased. Moreover, contrary to the granules formed in Experiment 5-I, no inert support particles could be detected within the granules. At the termination of the experiment, relatively stable granules prevailed, which remained almost unchanged (unhydrolyzed) after a storage period of 6 weeks at 4, 20, and 30°C.

The data concerning the methanogenic activity of the sludges from Experiments 5-I and 5-II are presented in Table 5.1.

TABLE 5.1. Methanogenic activity of the sludges of Experiments 5-I and 5-II on various substrates.

Experiment	Substrate	Activity (g CH_4-COD.g $VSS^{-1}.d^{-1}$)			
		day 40	day 70	day 88	day 96
5-I	H_2			1.34	0.82
	Formate	0.33	1.27	0.90	1.0
	Acetate	0.84	0.83	0.90	0.68
	Propionate	0.19	0.68	0.45	0.41
5-II	H_2			1.17	1.62
	Formate	0.22	0.85	0.86	0.71
	Acetate	0.27	0.57	0.76	0.59
	Propionate	0.07	0.34	0.30	0.13

An explanation for the distinct drop in specific activity found on acetate in Experiment 5-I and on formate, acetate and propionate in Experiment 5-II is difficult to provide at this stage. The data in Table 5.1 however, clearly demonstrate that the sludge from reactor 5-I exerts a higher specific methanogenic activity than sludge from reactor 5-II. This difference in activity was expected on the basis of the fraction of VFA-COD in the feed solutions of the reactors; the higher this fraction is, the higher the specific activity of the cultivated granules will be.
Beyond day 70 methanogenic activity on the acetate and formate for the sludge cultivated on the 10% sucrose solution was about 20% higher than on the 90% sucrose-grown sludge.
Results of the settling experiments with the sludges produced in Experiments 5-I and 5-II, the seed sludge and a granular sludge from the CSM beetsugar factory in Breda, are summarized in Figure 5.3.

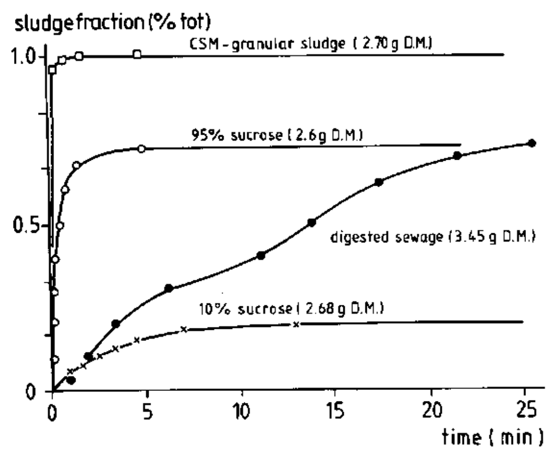
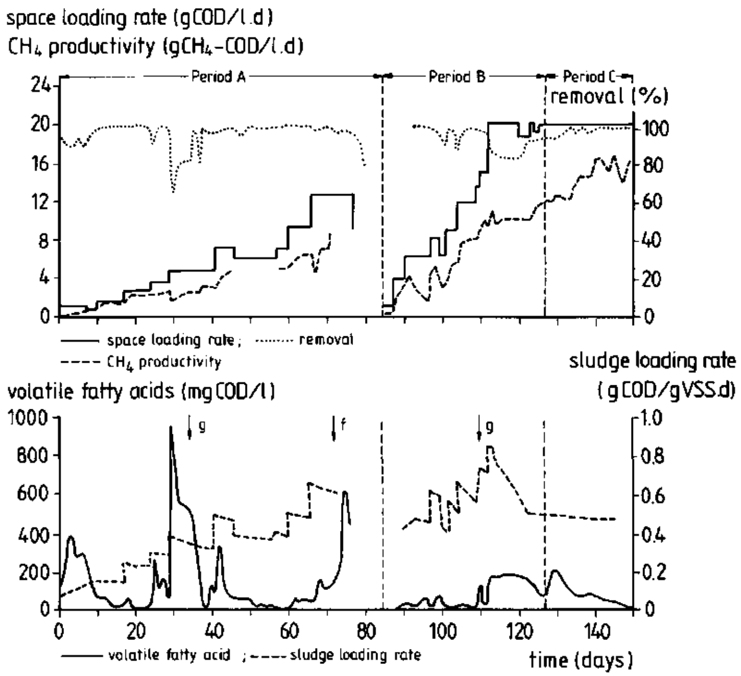


Figure 5.3 *Settling properties of the seed sludge (digested sewage sludge). CSM granular sludge and the sludge cultivated in Experiments 5-I (10% sucrose) and 5-II (95% sucrose).*

Additional experiments have been conducted also using digested sewage sludge form the municipal sewage treatment plant in Ede, and as substrate a sucrose solution. Although the results of these experiments have been published elsewhere (Lam Min Triet, 1985; Mendez Pampin et al, 1986; Sierra Alvarez et al, 1988), they form an integral part of our studies. The observations made in these experiments therefore, will be discussed in the context of the present study. The results are graphically presented in Figure 5.4.



<u>*Figure 5.4*</u> *Additional granulation experiment (Experiment 5-III) on sucrose solution under changing hydraulic conditions. The periods indicate the different feed concentrations applied. Period a: 3000 mg sucrose COD/l; period b: 1500 mg sucrose COD/l; period c: again 3000 mg sucrose COD/l. g = granulation observed; f = formation of flotation.*

In this additional experiment it appeared that granulation was not proceeding as distinctly as in Experiments 5-I and 5-II. Although we initially obtained a satisfactory granulation in this experiment, i.e. sludge aggregates were clearly visible after 35 days of operation, the quality of the sludge deteriorated after imposing higher loading rates (a space loading rate up to 12 kg COD/m^3.day). A rather filamentous sludge developed in the reactor. Voluminous layers of filamentous sludge grew on and around the granules, ultimately leading to their incorporation into a filamentous matrix which showed a strong tendency toward flotation, resulting in severe wash-out of sludge. We then adapted the operation of the reactor. The feed concentration was reduced from 3000 mg COD/l to 1500 mg COD/l, leaving the space loading rates unchanged, resulting in a doubling of the superficial upflow velocities in the reactor. As a second action we intermittently applied stirring in the reactor in order to improve the segregation of the granules from the filamentous matrix. From then onwards the space loading rate indeed could be rapidly increased and, as expected, the required segregation of the sludge fractions proceeded satisfactorily. However, the granules were smaller than those cultivated earlier; 1 mm in diameter instead of 2-5 mm. When a good quality granular sludge bed was established the feed concentration was increased from 1500 to 3000 mg COD/l at the same space load. Under these conditions high loading rates could be accommodated and problems with granule entrapment in accumulated filamentous sludge did not occur.

Discussion

According to the ideas of several researchers (Pohland and Ghosh, 1971; Cohen et al, 1979; Verstraete et al, 1981; Zoetemeijer et al, 1981; Kunst, 1982) the practice of separating the acidogenesis from the methanogenesis by using separate reactors for both steps would be profitable. Such a phase separation would result in a more stable process, even for easily hydrolyzable soluble substrates. According to the views of these researchers, a constant composition of the effluent from the acidification step would lead to better formation of the desired bacterial population in the methane reactor and consequently to a more stable methanogenic treatment step. Therefore, as a result of phase separation, a significantly higher maximal space loading capacity of the methane reactor might be achievable. Although true on theoretical rather than academic grounds, it should be noted that:

1. the maximum loading potentials do not solely depend on the specific methanogenic activity of the sludge, but also on the total amount of sludge that can be retained in the reactor under high loading conditions;
2. the construction of a separate (acidification) reactor increases investment costs considerably;
3. generally, the acidification of soluble carbohydrates present in wastewater proceeds so rapidly that often a significant fraction of these ingredients will already be converted into VFA-COD in the pipelines and/or an equalization tank or buffer tank when present;
4. the sludge growth yield on VFA substrates is significantly lower than on partially non-acidified substrates and consequently, much more time may be involved in increasing the amount of granular sludge. By using non- or less-acidified substrates, provided of course a granular sludge of satisfactory quality will grow on partially acidified substrates, time required for the increase of the amount of granular sludge could be considerably decreased;

5. a major part of the acidogenic sludge present in the effluent of the acidogenic reactor has to be removed from this solution (particularly at VSS concentrations exceeding approximately 1000 mg/l) prior to introducing it into the methanogenic reactor;
6. the best guarantee for a high process stability of any biological process is to avoid severe overloading, independent of the type of treatment system applied, e.g. a one-step or two-step process;
7. under practical conditions the wastewater composition and strength often strongly fluctuate and consequently it is impossible to guarantee a "constant" effluent composition from the acidogenic reactor.

For the above reasons we do not support phase separation. Based on some of the above points, studies have been conducted in recent years to assess the effect of partial acidification on the performance of the methane reactor. Cohen (1982) conducted such experiments using a glucose VFA mixture as feed, and found that the specific methanogenic activity of the sludge improved significantly by replacing only 13% of the glucose COD by VFA COD. Moreover, by using mixtures of VFA and glucose as substrate, the granular seed sludge in the methane reactor could be better preserved than by feeding the sludge (one phase treatment) with pure glucose solutions. On the other hand, he found that at high sludge loading rates a rather gelatinous sludge formed with the partially acidified glucose solution (Cohen, 1982) leading to the formation of voluminous layers surrounding the original granules. Under such conditions of sludge deterioration, a sharp increase in sludge wash-out was observed. These findings correspond well with observations made at our laboratory (unpublished data) in experiments with a dilute (1,000 mg of COD per liter) sucrose solution and a granular sludge bed reactor using a granular seed sludge cultivated on cornstarch waste. At high loading rates we observed a strong increase in the sludge wash-out, which could be mainly attributed to the formation of distinct voluminous layers of newly produced bacterial biomass attached to the granules.

In both examples cited here, the reactor was inoculated with granular sludge. As each substrate will lead to the formation of a specific composition of bacterial populations, it is quite clear that a sudden change in substrate composition cannot always be fully accommodated. This seems to be particularly true for granules cultivated on mainly acidified effluents. The growth in or on the granules from a mainly carbohydrate substrate will consist predominantly of acidogenic organisms. This newly formed attached bacterial matter is rather voluminous and consists mainly of filamentous bacteria. In addition, considerable amounts of gelatinous exopolymers may be produced. Both of these phenomena lead to a severe decrease in sludge settleability and sometimes (at high sludge loads) to serious deterioration of the sludge granules and consequently an increased wash-out of bacterial sludge. This increased wash-out can also be partially due to the fact that gas cannot sufficiently escape from the granules. In light of the above, the results of the present experiments are clearly of particular practical interest.

The results of Experiment 5-II conducted with a 95% sucrose COD plus 5% VFA COD solution and digested sewage sludge as inoculum, clearly demonstrate that a highly active and granular sludge can be cultivated on a mainly carbohydrate waste. This granular sludge consisted apparently of a balanced micro-environment containing all the microorganisms required for the conversion of the substrate into methane. No severe sludge wash-out was observed even at high loading rates, and once the granules were developed significant changes in granule morphology did not occur.

Additional results (see Figure 5.4) clearly demonstrate that sludge selection is a truly delicate matter. It will become clear that the amount of selection pressure to be imposed on the system greatly depends on the type of organisms involved; their growth rate, morphology and affinity for attachment. Acidogenic organisms exert a significantly higher growth rate than methanogens, they are partly filamentous and presumably they have a strong affinity for aggregation. For these reasons, in treating mainly carbohydrate wastes a stronger selection pressure than that for acidified wastes has to be imposed on the system. Nevertheless, the performance data of the present experiments clearly show that sludge granulation on sucrose will proceed well at dilution rates (D) below the maximum specific growth rate of the involved organisms. According to Heijnen's insights (Heijnen, 1984), the dilution rate should exceed the maximum growth rate of the organisms in order to obtain a successful start-up and immobilization of biomass on the support particles in fluidized bed systems. In this way all the suspended organisms will be washed out, so that only the attached organism will be able to survive and grow in the system. According to his experience a D of more than 14.5 days^{-1} is required for a proper start-up with unacidified wastewater. Our results clearly demonstrate that in UASB systems at dilution rates of half the value of the maximum specific growth rates, sludge granulation proceeds well. In the last experiment the D was 6.6 days^{-1}, while the $_{max}$ for acidifying organisms is approximately 7-12 days^{-1} average.
The smaller granule size obtained during the period with the higher hydraulic surface loads can be attributed to the higher erosion forces prevailing in the reactor due to the mechanical mixing and the higher upflow velocity.
According to our insights, a one-step treatment of the sucrose solution of the type investigated here needs a stronger "selection pressure" than do VFA substrates on which bacterial growth proceeds significantly slower. The required segregation between the dispersed filamentous sludge and the sludge granules can only be achieved at a sufficiently high selection pressure.

The granules formed in the experiments with the 10% sucrose COD plus 90% VFA COD substrate resembled the filamentous granules previously cultivated (see Chapter 4) on 100% VFA solutions. However, unlike the experiments performed on pure VFA solutions under similar process conditions, a less satisfactory segregation between the granular and flocculent sludge could be accomplished, despite the fact that the granules appeared approximately 10 days sooner than with the pure VFA solutions. The difference in settleability between the dispersed flocculent and the filamentous granular sludge was too small for an effective segregation under the loading conditions applied. As a result, growth could not be sufficiently delegated to the granular sludge. As growth of the flocculent filamentous sludge is presumably kinetically in favour of the growth of the organisms present in the granules, it will become clear that selective removal of flocculent and dispersed sludge from the system is a prerequisite to granulation.

The results of Experiment 5-II clearly demonstrate that, provided a proper start-up regime is followed, the conditions for granulation are excellent for mainly sucrose waste. Compared to Experiment 5-I, granulation visually occurred sooner, although the start-up period was distinctly longer. Apparently more time is required for the formation of the appropriate flora in the case of mainly carbohydrate wastewaters. The granules formed were up to 5 mm in size and exhibited excellent settling characteristics. At the termination of the experiments hardly any flocculent sludge was left in the reactor. The available reactor volume was almost completely filled with the granular sludge. The methanogenic activity of the sludge was approximately 0.75 g COD/g VSS.day, which corresponds well with the values reported by Cohen (1982) for the one-step digestion of a glucose VFA mixture (0.5 to 1.0 g COD/g VSS.day). According to Zoetemeyer et al. (1981), hydraulic retention times of 1 and 6 hours respectively would be required for cultivating granules of acid-forming sludge on 1% and 5% glucose-monohydrate solutions. However, the results of Experiment 5-II demonstrate that granulation with a very similar substrate in a one step digestion process proceeds well at considerably longer hydraulic retention times (<18 h).

The sludge retention figures were similar to those with pure VFA substrates although there was a clear difference between Experiments 5-I and 5-II with respect to the minimum values for the amount of retained sludge at the end of the "wash-out phase". In Experiment 5-II, the minimum amount of sludge retained was 8.3 g of VSS per liter, whereas in Experiment 6-I it was only 3.2 g of VSS per liter. The latter value corresponds closely to that found with pure VFA feeds. The relatively high minimum value in Experiment 5-II can be attributed to the excellent granulation occurring in that experiment, also reflected in the total amount of solids retained in the reactor at the termination of the experiment. The measured sludge profiles in both reactors revealed that the biomass concentrations at the end of the investigations were approximately 9 g and 40 g VSS/l (at day 130) for Experiments 5-I and 5-II, respectively. In experiments with 100% VFA mixtures as feed conducted under similar conditions, biomass concentrations exceeding 30 g VSS/liter in the granular sludge beds were never found. Thus, an obvious advantage of the use of unacidified soluble carbohydrates as substrate is that a high sludge retention can be obtained. Assuming the sludge retention will not change and the specific activity will not further increase at increased loading rates, the maximal achievable space load for a feed solution containing 95% sucrose and 5% VFA can be estimated at approximately 35 kg COD/m^3.day[1].

The excellent settling characteristics of the sludge formed in Experiment 5-II were reflected in the sedimentation data. The settling results of the sludge from Experiment 5-I were however, remarkably poor. Even deterioration of this sludge with respect to the initial settling properties was observed. This finding is consistent with the observations of sludges cultivated on VFA mixtures where reduced settleabilities were also generally found due to the entrapment of newly formed granules in a "matrix" of poorly settling flocculent sludge. We also found that after extended periods of operation at higher space loading rates (> 10 g COD/l.day), the flocculent sludge separates more and more from the aggregated biomass, ultimately leading to a sharp increase in sludge settleability.

The results of the activity tests indicate that the period for cultivating a sludge with a higher specific activity lasts at least 40 days. The composition of the microflora as reflected in its methanogenic activity on different substrates apparently remains fairly constant beyond 70 days of continuous operation. Microscopic observation of the sludge of reactor 5-II revealed microflora consisting mainly of autofluorescing, hydrogen-consuming methanogens and long filaments of bacteria which resembled Methanothrix soehngenii as described by Huser et al (1982). These observations, together with unpublished results of activity assays, indicate that such specific activity tests can be a useful tool in the characterization of anaerobic sludge. The maximal methanogenic activity on a range of substrates provides useful information on the composition and the condition of the sludge examined.

Conclusions

From the experimental results discussed in the present study we can draw the following conclusions:

1. An excellent quality granular sludge with a high settleability and relatively high specific methanogenic activity can be cultivated on a mainly carbohydrate containing substrate (95% sucrose COD and 5% VFA-COD) using digested sewage sludge as seed, provided a proper start-up regime is applied. The time needed for granulation was shorter, and larger granules (Dp < 5 mm), were formed than on VFA substrates. As a result, the amount of retained granular sludge in the reactor was significantly higher.
2. Compared to substrates consisting of VFA COD or mainly VFA COD, the granulation process proceeds faster on substrates consisting of mainly carbohydrate COD. The main reason for this is the considerably higher growth-yield on carbohydrate substrates than on VFA substrates.
3. Granulation still proceeds quite satisfactorily at dilution rates below the maximum growth rates of the organisms. Granulation occurred at much longer hydraulic retention times than found with separate acidification of glucose solutions.

4. Microscopic observation revealed a shift in the bacterial population of the granules formed. Gradually, filamentous organisms were replaced by rod-type bacteria. This phenomenon was also observed on the granules cultivated on VFA substrates and can be interpreted as the maturation effect mentioned in the hypothesis for granulation in Chapter 1.
5. With a substrate containing 10% sucrose and 90% VFA mixture (acetate plus propionate), granular and flocculent sludge cannot be effectively separated. The granules contained a high fraction of filamentous organisms which were mainly attached to inert support particles.
6. A feed change from a VFA mixture to a carbohydrate solution may lead to problems of flotation and formation of a rather voluminous type of sludge if the granules are cultivated on acidified wastewaters.
7. The additional experiments with changing hydraulic loading rates indicate that particular attention should be directed to the selection pressure during start-up. The selection pressure is the driving force behind the selective wash-out of dispersed flocculent sludge to promote the bacterial growth in and on biomass aggregates. Main components of this driving force are the hydraulic loading rate and the gas loading rate.

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CHAPTER 6

PHYSICAL CHARACTERIZATION OF ANAEROBIC GRANULAR SLUDGE

Introduction

It is becoming more and more practice to inoculate newly built full-scale Upflow Anaerobic Sludge Blanket (UASB) reactors with anaerobic granular sludge cultivated in existing reactors treating wastewaters with a different composition of pollutants. For proper application of the UASB process it is important to have adequate knowledge of the effects of environmental conditions on the quality of granular sludge. Some possible problems that might manifest are:

- a reduction in the specific methanogenic activity of the sludge;
- a serious reduction in granule strength which will lead to increased erosion and wash-out of sludge;
- gas release problems leading to flotation and sludge wash-out.

Important characteristics of granular sludge to be determined are:

- specific methanogenic activity;
- strength of the granules;
- settling characteristics;
- *density and granule porosity;*
- particle size distribution;
- bacterial composition and amount of exo-polymers;
- ash-content and mineral composition.

The specific methanogenic activity can be easily measured in the laboratory on a mixture of volatile fatty acids (VFA's) with batch-fed assays. For mesophilic digestion this value should be in the range of 0.6 - 2.5 kg CH4-COD.kg $VSS^{-1}.d^{-1}$ depending on the amount of acidified COD in the wastewater. The activity measurement however, gives no information on the physical condition of the sludge. In the past therefore, research was conducted with emphasis on the determination of the **granule strength** (Tramper et al, 1984). Tramper presented the results of experiments in which the effects of shear forces on granule erosion were tested. Working with a continuously fed and stirred vessel and a Couette vessel they measured the abrasion as a function of the stirrer speed and the rotation speed of the other cylinder of the Couette vessel. The Couette vessel employed was similar to the one described by Leentvaar and IJwema (1980) and was equipped with a fixed inner cylinder and rotating outer cylinder. The main finding of their investigation was that the mechanical strength was rather low and that the abrasion strongly increased above a certain critical shear force. For the experiments with the Couette vessel the critical shear stress was around 7.10^{-2} $N.m^{-2}$. This sharp increase of the abrasion at a critical shear stress indicates that the tensile strength with which the organisms are attached to each other is fairly constant. In our efforts to develop a reliable method for the determination of the granule strength, initially we used a Couette vessel with a fixed outer cylinder and a rotating inner cylinder. Elutriated granular sludge was used in the experiments. After the test, the COD of the liquid obtained after sieving over a 0.5 mm sieve was used as a parameter for the erosion of the granules. It appeared that the strongest erosion occurred in the first 30 minutes. However, the precision of this procedure was rather poor; the mean standard deviation for the various duplo's was 18%. Therefore we developed another method, which will be discussed in this Chapter.

We also attempted to develop a rapid and convenient method for the determination of the **particle size distribution**. The application of the sieving procedure to granular sludge proposed by Leschber and Haacke (1974) is unattractive because of the occurrence of erosion of the granules in the sieves and the fact that fairly large samples were needed. Therefore, we immobilized a limited amount of granules (100-300) in Kaiser's gelatin glycerin in a petri dish. The size measurement of the granules could be performed with a binocular equipped with a Porton graticule in the ocular. For sludge samples merely consisting of granules (and no other large particles as is always the case with digested sewage sludge) a more sophisticated method can be applied: a photograph of the immobilized sludge in the petri dish is taken and the negative is analyzed with a Quantimet for automatic determination of the particle size distribution.

The **settling properties** of granular sludge can be assessed by determining the sludge volume index (SVI). However, this method has little value for granular sludge because of its high settling velocities. For granular sludge, SVI values of 12-20 ml/g are normal. Ross (1984) determined the settling characteristics using the solids flux method. He found a maximum solids flux value of 4500 kg. $m^{-2}.d^{-1}$ and a potential sludge thickening concentration of 90 $kg.m^{-3}$ suspended solids for granules cultivated on maize processing wastewater. We used an electronic sedimentation balance for the determination of the settling properties of a number of granular sludges. This method was an improvement over the SVI test, but the results were still fairly inaccurate as a result of the high settling velocities of the granules (20 - 80 $m.h^{-1}$, depending on granule size and density). In this Chapter a modified sedimentation balance will be presented, which enables the combined determination of the settling properties of the sludge and the assessment of the particle size distribution.

Materials and methods

The granule strength, i.e. the resistance of the granular sludge against compression forces. This compression resistance factor has been measured with a tension and compression test apparatus (Overload Dynamics S900, Overload Dynamics BV, Schiedam, The Netherlands), an apparatus (see Figure 6.1) used to determine the rheological properties of food products (Bourne, 1976). The modified compression test apparatus consists of a piston connected to a downward moving pressing bar. The piston fits tightly in a measuring cell filled with a granular sludge sample (see Figure 6.1). At a certain pressure the granules disintegrate and flow through the opening between the cell wall and the piston. The resistent and breakage point is measured and recorded.

Klein and Washausen (1979) described the use of the compression apparatus for strength determinations of immobilized biocatalysts. They found that the critical pressure increased when the size of the spheres increased.

Yusa and Igarashi (1984) used a compression test to determine the effect of chemical flocculation on the sludge density.

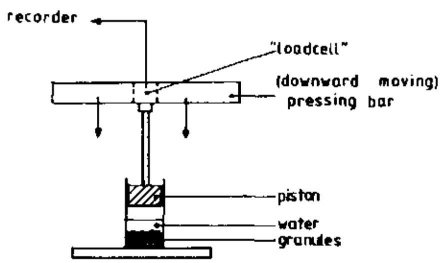

Figure 6.1 *Schematic diagram of the test apparatus for the compression measurements.*

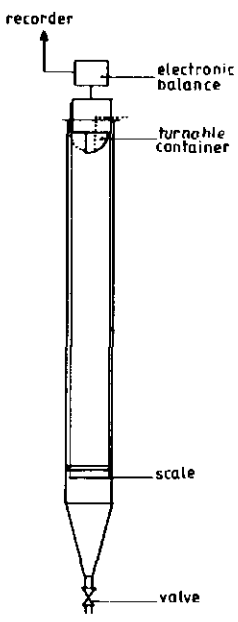

Figure 6.2 *Schematic diagram of the modified sedimentation balance. The container in the upper part of the column is filled with granular sludge. After turning the container, the weight increase on the scale at the bottom of the column (which is connected to the electronic balance) is followed on a recorder. The height of the column is about 2.5 m.*

For the determination of the settling characteristics of the sludge we used a modified sedimentation balance developed by Paques BV, Balk, The Netherlands. The apparatus is shown

in Figure 6.2. Apart from measuring the sedimentation characteristics, this apparatus also allows for the determination of the particle size distribution and the density of the granule.
The settling characteristics are obtained by plotting the weight fraction of the sedimentated sludge of the total weight of settled sludge, against the sedimentation time. The contribution of very small particles will be overlooked, which is acceptable because their weight fraction is very small. The S-curve which is obtained can be linearized with the following equation:

$$\log(-\log M) = n \log t_m/t + \log\log e$$

in which
M = weight fraction of settled sludge ($M_{at\ t}/M_{total}$)
n = slope
t = time (s)
t_m= the average settling time (s)

The particle size distribution cannot be calculated with Stoke's Law for the sedimentation of spheres, as this law is only valid under conditions of laminar flow (Re > 1). The flow pattern for the sedimentation of granules is in between laminar and turbulent flow (1 > Re > 1000). Under these conditions Galileo's number (N_{Ga}) is recommended for the calculations:

$$N_{Ga} = \frac{g.Dp^3.d_w.(d_s - d_w)}{\mu^2}$$

in which
g = gravitational acceleration ($m.s^{-2}$)
Dp = granule diameter (m)
d_w = the density of water ($kg.m^{-3}$)
d_s = the density of the granules ($kg.m^{-3}$)
μ = the dynamic viscosity ($Ns.m^{-2}$)

For the intermediate flow range between laminar and turbulent flow the following relation between Re and N_{Ga} can be given:

$$Re = 0.153.\ N_{Ga}^{0.71}$$

Assuming that granules can be considered as spheres, the following relationship between the granule diameter and the settling velocity can be derived:

$$Dp = 5.26 \frac{\mu^{0.372} . d_w^{0.257} . v^{0.885}}{g^{0.628} . (d_s - d_w)^{0.628}}$$

in which v = the sedimentation velocity (m/s)

The relationship between M and v can be obtained from the S-curve or its linearized form; the S-curve representing a plot of M versus t. Each point on the curve represents a certain v, which is determined in the sedimentation balance. In this way, a relationship can be derived between M and v. By substituting various values for v, a direct relationship between M and Dp can be obtained, which represents the particle size distribution and can be transferred into a histogram.

The density of the granules can be determined by measuring the weight of "kleenex-dried" granules (M) and the weight of the same amount of granules under water; the weight measured after sedimentation of all the sludge (M_w). Drying of the granules using a kleenex tissue is a proper method for removing extra-granular water. The granule density can be calculated with the equation:

$$d_s = \frac{M . d_w}{M - M_w}$$

The granule density was also determined with a picnometer according to the method of Mahling (1965). This density measurement was performed at 30 °C using 25 ml flasks with glass stoppers with capillars. The density was calculated according to the equation:

$$d_s = (d_w - d_l) \frac{m_3 - m_1}{(m_2 - m_1) - (m_4 - m_3)} + d_l$$

in which d_l = the density of the air ($kg.m^{-3}$), which is 1.165 at 30 °C and a P_{atm} of 760 mm

m_1 = weight of the empty picnometer (kg)

m_2 = weight of the picnometer filled with water (kg)

m_3 = weight of the picnometer filled with sludge (kg)

m_4 = weight of the picnometer filled with water and sludge (kg)

d_w at 30 °C = 995.65 $kg.m^{-3}$.

Based on the available information, we selected for the physical characterization of granular sludge:

- granule density;
- settling characteristics;
- granule strength;
- mineral content of the sludge;
- particle size distribution.

In the present investigations, we determined and compared the physical properties of five anaerobic granular sludges cultivated on different types of wastewater. The granular sludge samples tested were:

- **Nedalco granules**; granular sludge cultivated on distillery wastewater (Nedalco, Bergen op Zoom, The Netherlands); reactor size: 700 m^3; the influent COD: 4000 - 5000 $mg.l^{-1}$ and a space loading rate of 11.5 - 14.5 kg $COD.m^{-3}.d^{-1}$.
- **CSM granules**; granular sludge cultivated on the wastewater of a beet sugar factory (Centrale Suiker Maatschappij, Breda, The Netherlands); reactor size: 30 m^3; influent COD: in-between 2000 and 17000 $mg.l^{-1}$ and a space loading rate of 15-20 kg $COD.m^{-3}.d^{-1}$.
- **AVIKO granules**; granular sludge cultivated on the wastewater of a potato processing plant (Aviko, Steenderen, The Netherlands); reactor size: 1165 m^3 (excluding the gas solids separator); influent COD: 5000-8000 $mg.l^{-1}$ and a space loading rate of 8-11 $kgCOD.m^{-3}.d^{-1}$.
- **BT granules**; granular sludge cultivated on wastewater of a wastepaper processing plant (Papierfabriek Roermond, member of the Buhrmann-Tetterode group, Roermond, The Netherlands); reactor size: 1000 m^3; wastewater concentration: about 4000 mg $COD.l^{-1}$ and a loading rate of 10 kg $COD.m^{-3}.d^{-1}$.
- **SU granules**; granular sludge cultivated on the wastewater of a beet sugar factory (Suiker Unie, Roosendaal, The Netherlands); reactor size: 1000 m^3; wastewater concentration: 3000-4000 $mg.l^{-1}$ and applied space loading rate: 11.5 - 15.5 kg $COD.m^{-3}.d^{-1}$.

In addition, we attempted to assess the changes in the physical properties of granular sludge after exposing this sludge to substrates different in composition than the original substrate using 10 liter UASB reactors. The seed sludge used was cultivated on partially acidified (30-60%) potato processing wastewater. Three reactors were operated under similar conditions. The synthetic substrate used during the first 17 days consisted of 1250 $mg.l^{-1}$ acetate and 1000 $mg.l^{-1}$ propionate. The applied space loading rate was 22-26 $kg.m^{-3}.d^{-1}$. After 17 days of operation the substrate in 2 reactors was changed. In one of the reactors the VFA feed was replaced by a sucrose solution of the same COD in order to assess the effect of the extra growth of acidifying biomass on the granule properties.
The other reactor was kept on the same substrate but the ionic strength of the medium was increased. This was done by raising the Na^+ concentration from approximately 600 to 3200 $mg.l^{-1}$. Such Na^+ concentrations can be found in various wastewaters including edible oil wastewater, sauerkraut wastewater and shellfish wastewater. The reactors were operated for 59 days at space loading rates of 22-26 kg $COD.m^{-3}.d^{-1}$. After termination of the experiments the granule properties were determined.

Before conducting the measurements, all sludges were elutriated to remove the flocculent part of the sludge.

DSS (dry suspended solids) and VSS (volatile suspended solids) were determined according to Standard Methods.

In addition to the assessment of these physical/mechanical characteristics it should be noted that it is very important to determine the specific methanogenic activity and the ratio of flocculent/granular sludge.

Results

The compression tests with granular sludge revealed that granules cultivated in full-scale UASB reactors were significantly stronger (the critical pressure was $0.26x10^5$ - $1.51x10^5$ $N.m^{-2}$) than those cultivated in laboratory scale reactors in a mixture of acetate and propionate (Hulshoff Pol et al, 1983). Latter granules break up at a critical pressure of $0.07x10^5$ - $0.13x10^5$ $N.m^{-2}$.

The results of the tests on the different types of sludge are presented in Table 6.1 and Figures 6.3 and 6.4. Data on the changes in sludge properties in the three 10 liter laboratory reactors are given in Table 6.2 and Figures 6.5 and 6.6.

TABLE 6.1. Density, ash-content, strength, mean settling velocity and granule diameter of five types of granular sludge.

	sludge type				
	NEDALCO	CSM	AVIKO	BT	SU
density[1] ($kg.m^{-3}$)	1028	1036	1049	1036	1068
density[2] ($kg.m^{-3}$)	1039	1038	1057	1042	1082
ash-content (%)	17.5	11.8	28.9	18.5	55.0
strength ($10^5.N.m^{-2}$)	1.18	0.82	1.21	1.46	2.50
mean settling velocity ($m.h^{-1}$)	52.9	83.3	97.8	98.9	53.6
mean granule diameter (mm)[3]	1.50	1.89	1.76	2.20	0.80

1. Determined with the modified sedimentation balance
2. Determined with the picnometer
3. Determined by weight and not by number

TABLE 6.2. Density, ash-content, strength, mean settling velocity and mean granule diameter of AVIKO granules after having been exposed to different feeding conditions in a 10 liter UASB reactor at a space loading rate of 22 - 26 kg $COD.m^{-3}.day^{-1}$ during 59 days (I = original granules, II = control, III = granules fed with sucrose solution with a COD of 3000 $mg.l^{-1}$, IV = granules fed with a VFA solution containing 3200 mg $Na^{+}.l^{-1}$).

	Sludge			
	I	II	III	IV
density[1] ($kg.m^3$)	1049	1039	1029	1037
density[2] ($kg.m^3$)	1057	1048	1032	1049
ash-content (%)	28,9	19,6	15,3	26,9
strength ($10^5.N.m^{-2}$)	1,21	1,32	0,38	0,82
mean settling velocity ($m.h^{-1}$)	94.7	84.9	91.8	85.7
mean granule diameter (mm)[3]	1.76	1.87	2.34	1.89

1. Determined with the modified sedimentation balance.
2. Determined with the picnometer.
3. Determined by weight and not by number.

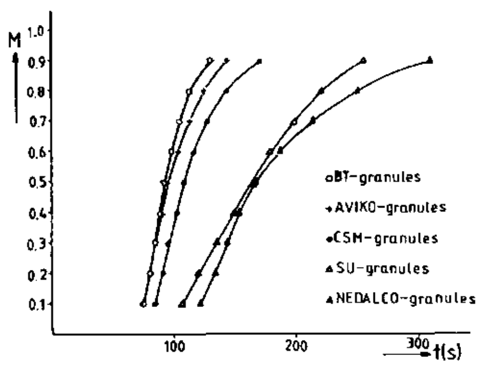

Figure 6.3 *Settling properties of five types of granular sludges. M. is the weight fraction of the sludge settled at time = t.*

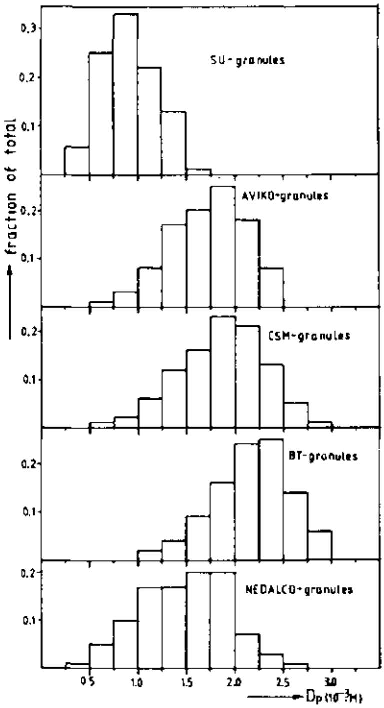

Figure 6.4 *Particle size distribution of five different granular sludges. The weight fraction is presented as a function of the particle diameter (Dp).*

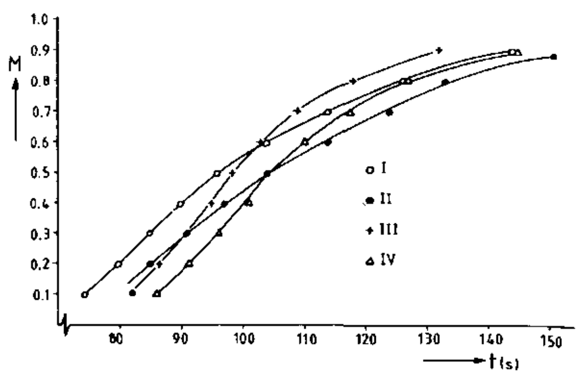

Figure 6.5 *Settling properties of AVIKO granular sludge exposed to different feeds in 10 liter UASB reactors. I = original sludge; II = sludge fed with acetate and propionate; III = sludge fed with sucrose; IV = sludge fed with acetate, propionate and 3200 mg $Na^{+}.l^{-1}$ (see also Table 6.2).*

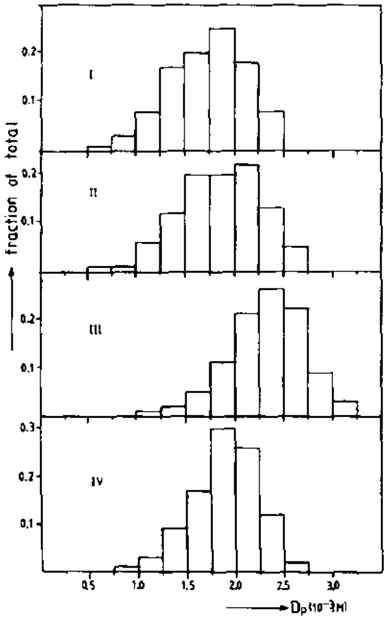

Figure 6.6 *Particle size distribution of AVIKO granular sludges exposed to different feeds in 10 liter UASB reactors. I = original sludge; II = sludge fed with acetate and propionate; III = sludge fed with sucrose; IV = sludge fed with acetate, propionate and 3200 mg $Na^{+}.l^{-1}$. The weight fraction is presented as a function of the particle diameter (Dp).*

Discussion

Comparison of the properties of five different types of granular sludge.

Table 6.1 and Figures 6.3 and 6.4 show distinct differences between the various sludges investigated. SU granules differ strongly from the rest; their ash-content is much higher, the granules are clearly stronger and they are smaller. The BT granules possess the most desirable qualities; they are relatively strong, have a low ash-content (which means more active biomass per kg sludge DSS) and have the best settling properties. Regarding particle size distribution, the BT granules are the largest. Despite the higher density of the SU granules, they settle more slowly, demonstrating the importance of granule diameter on the settling velocity of the granules. This is supported by Stoke's Law for the unhindered sedimentation of spheres in which the settling velocity is a function of the second power of the particle diameter.

According to representation of the particle size distribution (Figure 6.4) NEDALCO granules are unevenly distributed; the relative fraction of larger granules (Dp > 2 mm) is low.

Comparison of the sludges from the experiments in the 10 liter UASB laboratory reactors.

Results showed a considerable change in the sucrose-fed sludge. The granules became bigger and weaker, attributed to the growth of acidogenic organisms on the original granules. The sharp drop in the granule strength suggests that one should be cautious in applying granules that have been cultivated on partly acidified waste to the treatment of completely unacidified wastewaters (see also Chapter 5). A major advantage of using unacidified solutions like sucrose however, is the higher sludge yield, compared to VFA substrates (e.g. in Experiment II a yield of 0.185 kg DS.kg COD^{-1} was found). This higher yield value may enhance the formation of new granular sludge, provided the process is properly operated (see Chapter 5). The decreased strength of the "Na^+ granules" might indicate that divalent cations play a role in keeping the granule matrix together. High Na^+ concentrations in the wastewater may lead to an exchange with Ca^{2+} ions from the granules which can result in a weakening of the structures.

In addition to the above changes, the macroscopic morphology of the granules also changed. The original AVIKO granules were spheres built up by different layers of sludge, possibly as a result of fluctuating conditions for growth in the full-scale reactor. After feeding these granules with a 100% VFA solution almost all the granules split in half, indicating that this phenomenon is related to problems of gas diffusion from the center to the outskirts of the granule. Larger AVIKO granules often contain a gas bubble which can lead to either splitting of the granule (which can be demonstrated by bringing the granules under vacuum) or to flotation when the gas production rate exceeds the gas diffusion rate.

The sucrose-fed granules changed in color from black to pale white by the attachment of acidogens on the outside.

The results in Tables 6.1 and 6.2 suggest a good correlation between the density and ash-content of the granules (r^2 ash/d picn = 0.943), which is in fact not unlikely. A correlation does not exist between the ash-content and the granule strength (r^2 ash/ strength = 0.676), although SU granules with the highest ash-content (55%) are the strongest. However, these granules are much smaller than those of the other samples. As the strength of particles is very likely inversely proportional to the diameter of the particles, this factor is presumably much more important than the ash-content of the sludge. Generally the size of granules does not exceed 3 mm in sludges present in full-scale installations. The absence of larger granules in sludge from full-scale reactors can probably be attributed to the increased granule erosion prevailing in large reactors, while a major part of the sludge granules consists of matured "rod-type" granules which have a high bacterial density.

It would be useful to follow the development of various relevant properties of the granular sludge over a period of time, particularly in those cases where the wastewater strongly fluctuates in composition and strength. Changes in the relevant characteristics of the sludge could indicate incorrect operation of the process.

The present investigations should be viewed as preliminary. Due to the importance of characterization of granular sludge, it is highly recommendable to reinforce research in this specific field. Except for measurement of the mechanical strength (standard deviation 10%), the test methods presented here can be easily reproduced. Except for the strength measurement (which had a standard deviation of about 10%), the reproducability of the tests was good. Determination of density with the picnometer is more accurate than the method of determining the density out of the weight differences between granules under water and "kleenex" dried granules.

Characterization methods presently under investigation are the determination of granule porosity and the specific surface. We are also investigating the effects of various process conditions on the properties of granular sludge.

Conclusions

This research should be viewed as a preliminary investigation; further research in this field is indicated. On the basis of the results the following conclusions can be drawn:

1. The measurements of density, strength, settling properties and particle size distribution offer a set of useful tests for characterizing granular sludge.
2. The modified sedimentation balance investigated is a useful apparatus to assess both the settling characteristics as well as the particle size distribution of the granules.
3. Clear evidence has been obtained indicating that granular sludge characteristics dramatically change when the feed composition is changed.
4. The results demonstrate that the sludge characterization methods investigated here are useful, e.g. for studying the effects of various process conditions on important sludge characteristics.

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CHAPTER 7

7A. SUMMARY AND CONCLUSIONS

Successful high-rate anaerobic wastewater treatment can only be accomplished when the slow-growing anaerobic biomass is efficiently held back in the anaerobic treatment system. This biomass retention can be achieved in various ways including immobilization of the organisms on fixed materials and immobilization on mobile support materials: in the latter case, combined with settling of the anaerobic sludge aggregates.
This dissertation focuses on the phenomenon of flocculent anaerobic sludge transferring into highly active well-settling sludge granules under specific conditions. A high level of sludge retention can be achieved with granular anaerobic sludge under conditions of high gas production and turbulence in the digester, and consequently, the anaerobic treatment system accommodating high loading rates. This sludge granulation phenomenon proceeds in treatment systems operated in an upflow mode; the Upflow Anaerobic Sludge Bed (UASB) reactor being the main reactor system supporting this phenomenon. The UASB system is in fact nowadays, the most widely applied high-rate anaerobic treatment system in the world. One of the reasons for the UASB system's popularity is undoubtedly this granulation phenomenon.

The work presented in this thesis is focused on the study of the various operational factors that effect the granulation process. This study was conducted in small-scale UASB reactors using well defined feed stocks. The main objective of this work was to develop useful guidelines for operators of full-scale UASB reactors for cultivating granular sludge in their systems.

Chapter 1 reviews the present knowledge on relevant factors related to the granulation of anaerobic sludge. Important factors discussed regarding the formation of granular sludge include: the mechanisms governing bacterial adhesion and the production and role of extracellular polymers as "sticking" agents, the role of Ca^{2+}, and the effect of the seed sludge. The ideas of the various researchers are often contradictory, emphasizing the fact that the fundamental mechanism of granulation is a very complex and still only partially understood process.
Considerable emphasis has recently been directed to the characterization of granular sludge, particularly with respect to the chemical and microbiological composition of the granules. The chemical composition varies considerably and depends on the composition of the wastewater. As far as the microbiological composition is concerned, it is commonly agreed that the acetate utilizing methanogen *Methanothrix soehngenii* is the predominant organism, although various

other methanogenic and acetogenic organisms are always present, as well as, depending on the composition of the wastewater, a certain population of acidogenic bacteria. The specific methanogenic activity of the granular sludge is a useful tool for characterizing the sludge. The high methanogenic activities generally found with granular sludges demonstrate their ability to be excellent micro-ecosystems with a good micro-environment for all kinds of organisms, including the syntrophically-growing organisms involved in the anaerobic degradation process.
For the physical characterization of granules, factors of specific importance are size, size distribution, density, settleability and granule strength.
And finally, presented in Chapter 1 is our hypothesis of the granulation mechanism. In our opinion, the selection pressure plays a key role in the granulation process. It is a result of the hydraulic surface-load and the gas surface-load, and is required for accomplishing the necessary separation of lighter and heavier sludge fractions. Almost all growth should eventually concentrate on or in the heavier particles which can be accomplished by allowing all finely dispersed sludge to wash out of the system. The first generations of granules will be fairly open and fluffy in nature, but as a result of a maturation process the granules will gradually become more compact, and filamentous *Methanothrix* bacteria will be overgrown by the short-chained *Methanothrix* cells.
For the proper separation of dispersed sludge and agglomerated sludge, it is important that the sludge bed is kept at a level that leaves an area of free space between the sludge bed and the gas collector.

Chapter 3 describes the experimental results concerning granulation on volatile fatty acid (VFA) mixtures (acetate, propionate and butyrate) with digested sewage sludge as seed material. Various relevant parameters were investigated such as the sludge loading rate, substrate concentration, the effect of Ca^{2+} and NH_4^+, pH and temperature. The results demonstrate that granulation with a simple VFA substrate proceeds very well. The granules developed on VFA substrates with VFA concentrations below 150 mg/l consisted mainly of filamentous *Methanothrix*.
In all the granulation experiments, including those discussed in Chapters 4 and 5, independent of the type of substrate used, a very similar pattern of retained sludge amounts in relation to the imposed space load could be observed. Initially, the amount of sludge diminishes as a result of wash-out of finely dispersed sludge ingredients from the system. In this initial phase of the start-up process, to prevent overloading of the system, the space loading rates should not be further increased. As a result of wash-out of inert organic sludge ingredients and the continuous accumulation of new bacterial matter, the specific methanogenic degradation capacity of the sludge (based on overall VSS) will show a distinct continuous increase until a steady state with respect to sludge composition is established.

Generally after a period of 40 to 50 days, sludge granules up to a size of 0.5 mm can be observed in the sludge bed. From then onward, the total amount of sludge in the reactor will again gradually increase, while the space loading rate can usually be increased without further stagnation in the digestion process.

With increasing loading rates, the fraction of granular sludge will increase in the sludge bed.

The importance of imposing and maintaining a proper selection pressure has been demonstrated in the following ways:

- if the system remains underloaded for a long period of time, a bulking type of anaerobic sludge, which is very hard to retain in the reactor, will develop;
- at a given space loading rate, which is determined by the degradation capacity of the sludge present in the reactor, granulation proceeds clearly faster with less concentrated wastewaters.

The influent VFA concentration is also of importance with respect to the ultimate bacterial composition of the granular sludge. When using a concentrated VFA solution as feed, i.e. 10.000 mg VFA-COD/l, a granular sludge will develop consisting mainly of *Methanosarcina* rather than *Methanothrix*. With lower substrate concentrations, *Methanosarcina* may develop under conditions of continuous overloading. Fluidized bed systems are usually started up under such conditions, however they are kinetically unfavorable to growth of the desired well-attaching *Methanothrix* organism, and may therefore lead to an unwanted accumulation of less well-attaching *Methanosarcina* sludge in the system.

The *Methanosarcina* granules remain very small (Dp < 0.5 mm) and consequently are easily washed out of the reactor. Based on kinetical reasons we recommend maintaining the acetate concentration in the reactor below 150 mg acetate/l, in order to promote the development of *Methanothrix* granules rather than *Methanosarcina* granules.

The granulation process can be enhanced significantly by increasing the temperature in the mesophilic range to values near 40 °C. At this temperature however, the process will also become more unstable.

We have found clear evidence indicating that granulation also proceeds well at reduced pH values. At a pH level of 6, start-up and granulation proceeded surprisingly well. Quite interestingly, we also found that the *Methanothrix*-like organism cultivated in the sludge had a much lower pH optimum than that of the *Methanothrix* strains described in the literature so far.

Chapter 4 describes the results of experiments investigating the effects of specific additives to the seed sludge. Again, digested sewage sludge was utilized as seed sludge.

In a number of experiments (gently) crushed granular sludge was introduced to the reactor, adding a large number of small granular nuclei to the seed. We observed that with this addition (only a few percent on VSS basis), a distinct enhancement of the granulation occurred.

Moreover, and of great importance, we also observed a clear difference in the type of granule cultivated. On mere digested sewage sludge, granules consist mainly of *Methanothrix*-like organisms in a filamentous state, whereas in the experiment with digested sewage sludge enriched with a small amount of crushed granular sludge, all of the granules consisted of *Methanothrix*-like organisms present as short chains of 4 to 6 cells. Both granules exert excellent settling properties and are highly active. Organic loading rates up to 50 kg COD/m^3.day could be very well accommodated in reactors containing 20-25 kg VSS/m^3 . The so-called "filamentous" granules have a more open structure, while the first generation granules contain inert support particles originating from the seed sludge. The so-called "rod type" granules are significantly more compact and generally do not contain inert support particles.
Experiments with hydro-anthracite as an additional inert support particle reveal its presence to have a positive effect on the granulation speed. Removal of inert particles from the digested sewage sludge contrarily results in poor granulation. The results of these experiments clearly demonstrate the importance of inert support particles generally present in digested sewage sludge.
We also observed that inert particles with a filamentous nature have a negative effect on the sludge characteristics by reducing the sludge settleability and promoting excessive wash-out of sludge. Seed sludges with a high fibrous material content are therefore not recommended for inoculating the anaerobic system.

The granulation process was also studied on a more complex substrate (Chapter 5). Sucrose was selected for these experiments; its investigations indicating that a stable granular type of sludge could be cultivated on this carbohydrate, and at a faster rate than on VFA substrates.
The experimental results also revealed the importance of imposing proper selection pressure with more complex soluble substrates, in order to avoid the accumulation of fast-growing voluminous and dispersed acidogenic biomass. We wish to emphasize however, that it is not necessary to apply hydraulic retention times lower than the maximum specific growth rate, sometimes considered a prerequisite for the successful start-up of a fluidized bed system.

In Chapter 6 the results of preliminary work on the physical characterization of granular sludge is discussed. Tools for characterization used were:

- a modificated sedimentation balance for the determination of the settling characteristics and particle size distribution;
- an apparatus to measure the resistance against compression forces.

Both methods, combined with a picnometric density measurement, are considered useful tools for the characterization of granular sludge. With the aid of these techniques we were able to demonstrate that a substrate shift from a VFA mixture to a sucrose solution will lead to the formation of weaker and bigger granules. Furthermore, a positive correlation between ash content and granule density could also be demonstrated.

Based on the insights obtained from our investigations, we can conclude that the sludge granulation process, as it occurs in upflow reactors such as the UASB system, originates from:

- the strong tendency of anaerobic organisms to stick together;
- the fact that a complex community is required for the anaerobic degradation of organic substrates, which forces the system to grow in the form of a micro-ecosystem;
- the fact that growth of bacterial matter is stimulated to take place on and in immobilized aggregates (nuclei) present and formed in the sludge, by imposing the proper selection pressure to the system.

We believe that the results of the experiments presented in this thesis provide strong evidence in support of our hypothesis of the granulation phenomenon.

Summarizing the results of the present investigations, the following practical guidelines can be provided for the start-up of full-scale reactors.

I Seed sludge

1. The presence of "proper" carrier materials for bacterial attachment is important for the initiation and stimulation of bacterial aggregation.
2. The specific methanogenic activity of the seed sludge is not the only factor of importance. Thicker types of digested sewage sludge, i.e. > 60 kg TSS/m^3, are preferred over thinner types, despite their lower methanogenic activity.
3. The addition of a small amount of (crushed) granular sludge to seed sludge enhances the granulation process.

II The mode of operation of the process

It is essential to sufficiently and continuously remove the lighter sludge fractions from the reactor to retain the heavier sludge ingredients and to promote bacterial growth in/on the heavier sludge ingredients. To achieve this, we recommend the following:

1. Washed out dispersed sludge should not be returned.
2. Apply effluent recycle or dilution at an influent COD of more than 5,000 mg/l.
3. Increase the organic loading rate step-wise, always after at least an 80% reduction in the biodegradable COD has been achieved.
4. Maintain a low acetate concentration (< 200 mg/l).
5. Start with 12-15 kg sludge VSS/m^3 with thick seed (> 60 kg TSS/m^3) and approximately 6 kg sludge VSS/m^3 with thin seed sludge (< 40 kg TSS/m^3).

III Wastewater characteristics

1. A general observation is that the lower the strength of the wastewater the faster the granulation will proceed. The strength however, should be high enough to maintain good

conditions for bacterial growth. The minimum COD level is presumably approximately 500 mg/l.

2. Dispersed solids, such as acidogenic biomass and fibrous matter, retard or may even prevent granulation.
3. Granulation will more quickly occur on mainly soluble unacidified wastewaters than on acidified wastewaters.
4. High ion concentrations (e.g. Ca^{2+}, Mg^{2+}) will lead to chemical precipitation ($CaCO_3$, $CaHPO_4$, $MgNH_4PO_4$) resulting in the formation of a granular sludge with a high ash-content.

IV <u>Environmental factors</u>

1. The optimal temperature for mesophilic treatment is in the range of 30-38 °C and for thermophilic treatment in the range of 50-60 °C.
2. The pH should always be maintained above 6.
3. All essential growth factors such as N, P, S and trace elements (Fe, Ni, Co) should be present in sufficient amounts and in available form.
4. Toxic compounds should not be present at inhibitory concentrations. If they are present, sufficient time should be allowed for bacterial acclimatization.

7B. SAMENVATTING EN CONCLUSIES

Anaërobe afvalwaterzuiveringsinstallaties kunnen hoog worden belast, wanneer de traag groeiende anaërobe bacteriën goed kunnen worden achtergehouden in het anaërobe systeem. Deze zogenaamde retentie van biomassa kan op verschillende manieren worden verkregen. Meestal gebeurt dit door hechting (immobilisatie) van micro-organismes op vast of bewegend drager materiaal, in het tweede geval gecombineerd met de bezinking van de gevormde anaërobe slib-aggregaten.
In dit proefschrift wordt aandacht besteed aan het verschijnsel, dat vlokkig anaëroob slib onder bepaalde omstandigheden langzaam over gaat in zeer aktief goed bezinkend korrelvormig slib. Met dit type slib kan ook bij hoge gasproduktie snelheden en onder turbulente hydraulische omstandigheden, een goede slibretentie worden verkregen. Hierdoor worden hoge volumebelastingen mogelijk. Het verschijnsel korrelvorming wordt waargenomen in systemen die opwaarts doorstroomd worden, waarbij de Upflow Anaerobic Sludge Bed (UASB) reactor het belangrijkste systeem is, dat gebruik maakt van dit verschijnsel. Het UASB systeem is momenteel het meest toegepaste hoogbelaste anaërobe zuiveringssysteem ter wereld en één van de belangrijkste oorzaken van de populariteit van dit systeem is ongetwijfeld het verschijnsel van de korrelvorming.

In het onderzoek, dat in dit proefschrift wordt beschreven, is aandacht besteed aan verschillende procesomstandigheden, die een rol spelen bij de korrelvorming. Het onderzoek werd uitgevoerd in kleinschalige UASB-reactoren, met goed gedefiniëerde voedingsoplossingen. Een belangrijke doelstelling van dit onderzoek was het verkrijgen van een aantal richtlijnen voor de procesvoering van UASB-installaties op praktijkschaal gericht op de vorming van korrelvormig slib.

In Hoofdstuk 1 wordt het huidige inzicht in belangrijke factoren, die verband houden met het korrelvormingsproces, beschreven. Deze factoren zijn: de mechanismen, die een rol spelen bij de hechting van bacteriën, de produktie en funktie van extracellulaire polymeren als "kit-stoffen", de rol van Ca^{2+} en de invloed van de samenstelling van het entslib. De verklaringen van de verschillende onderzoekers zijn vaak tegenstrijdig, hetgeen duidelijk maakt dat het fundamentele mechanisme van de korrelvorming zeer complex is en nog steeds slechts gedeeltelijk begrepen.
Veel aandacht is er de laatste tijd gericht op de karakterisering van korrelslib, speciaal voor wat betreft de chemische en microbiologische samenstelling van de korrels. De chemische samenstelling variëert aanzienlijk en hangt af van de samenstelling van het afvalwater. Voor wat betreft de microbiologische samenstelling, is men het er in het algemeen mee eens, dat de acetaat gebruikende methanogen bacterie *Methanothrix soehngenii* de overheersende bacteriesoort is, hoewel verschillende andere methanogene en acetogene organismen altijd aanwezig zijn, evenals, afhankelijk van de samenstelling van het afvalwater, een bepaalde populatie van

zuurvormende micro-organismen. De specifieke methanogene aktiviteit van het korrelslib is een bruikbare parameter voor de slib karakterisering. De hoge methanogene aktiviteiten, die normaal worden gevonden bij verschillende korrelslibsoorten, laten zien, dat korrels goede micro ecosystemen bieden voor verschillende organismen, inclusief de syntrofisch groeiende bacteriën, die een belangrijke rol spelen bij het anaërobe zuiveringsproces.
Voor wat betreft de fysische karakterisering van korrelslib zijn belangrijke kenmerken de korrelgrootte, de korrelgrootte-verdeling, de dichtheid, de bezinkingseigenschappen en de korrelsterkte.
Aan het eind van Hoofdstuk 1 is onze visie op het mechanisme van het korrelvormingsproces vastgelegd. Volgens deze visie speelt de selektiedruk een zeer belangrijke rol. Deze selektiedruk wordt verkregen door de hydraulische belasting an de gasbelasting en is noodzakelijk voor het verkrijgen van een goede scheiding tussen lichtere en zwaardere fracties van het slib. Bijna alle groei van biomassa moet uiteindelijk plaatsvinden op of in de zwaardere deeltjes, hetgeen mogelijk wordt wanneer alle fijn gedispergeerde slibdeeltjes uit het systeem worden gespoeld. De eerste generatie korrels zullen nogal open en vlokkig van struktuur zijn, maar als gevolg van een soort "rijpingsproces" worden de korrels geleidelijk kompakter en zullen de draadvormige *Methanothrix* bacteriën overgroeid worden door de *Methanothrix* cellen in korte ketens. Om een goede scheiding te verkrijgen tussen het disperse slib en het geagglomoreerde slib is het belangrijk, dat er een vrije zone is tussen het slibbed en de onderkant van de 3 fasen scheider.

In Hoofdstuk 3 worden de resultaten beschreven van (korrelvormings-) experimenten met vluchtige vetzuur (VVZ) mengsels (acetaat, propionaat en butyraat) met uitgegist slijkgistingsslib als entmateriaal. Er werden verschillende belangrijke procesomstandigheden onderzocht, zoals de slibbelasting, de substraatconcentratie, het effect van Ca^{2+} en NH_4^+, de pH en de temperatuur. De resulaten van deze proeven laten zien, dat op een eenvoudig VVZ-substraat korrelvorming goed mogelijk is. De korrels, die werden verkregen bij VVZ-concentraties onder de 150 mg/l bestonden hoofdzakelijk uit draadvormige *Methanothrix* cellen.
In alle korrelvormingsexperimenten, inclusief de experimenten die worden beschreven in Hoofdstuk 4 en 5, wordt onafhankelijk van het type substraat een overeenkomstig patroon in slibuitspoeling en slibretentie in relatie met de de volumebelasting waargenomen. Aanvankelijk neemt de slibhoeveelheid in de reactor af als gevolg van de uitspoeling van fijn gedispergeerd slib uit het systeem. In deze eerste opstart-fase is het van belang, dat de volumebelasting niet te snel wordt opgevoerd om overbelasting te voorkomen. Ten gevolge van de uitspoeling van inerte organische slibbestanddelen en de voortdurende accumulatie van nieuw bacteriemateriaal zal de specifieke methanogene aktiviteit van het slib (gebaseerd op de totale hoeveelheid organische stof) een duidelijke en continue toename vertonen, totdat zich er een steady state heeft ingesteld voor wat betreft de samenstelling van het slib. In het algemeen kunnen na een periode van 40 tot 50 dagen slibkorrels met een grootte tot 0,5 mm worden aangetroffen in het

slibbed. Vanaf die tijd zal de totale hoeveelheid slib in de reactor geleidelijk toenemen zonder verdere stagnatie in het opstartproces. Met het toenemen van de volumebelasting zal de fraktie korrelslib in het slibbed stijgen. Het belang van het opleggen van een juiste selektiedruk is experimenteel bevestigd:

- als het systeem voor een lange tijd wordt onderbelast, ontwikkelt zich een anaëroob licht slib, dat moeilijk in de reactor kan worden gehouden;
- bij een bepaalde volumebelasting, die wordt ingesteld aan de hand van de afbraakcapaciteit van het slib in de reactor, neemt de korrelvormingssnelheid toe bij verlagen van de influent concentratie en dus verhoging van de hydraulische belasting.

De influent concentratie is ook van belang voor wat betreft de tot standkoming van de uiteindelijke samenstelling van het korrelslib. Indien een geconcentreerde VVZ-oplossing (1000 mg VVZ-CZV/l) als substraat wordt gebruikt zal er een type korrelslib ontwikkelen met vooral *Methanosarcina's* en minder *Methanothrix* organismen. Bij lagere VVZ-concentraties zullen bij overbelasting van het systeem ook vooral *Methanosarcina* organismen tot ontwikkeling komen. Fluidized bed systemen worden meestal onder dergelijke omstandigheden opgestart, die echter kinetisch ongunstig zijn voor de groei van de gewenste goed hechtende *Methanothrix* bacteriën en derhalve leiden tot de ongewenste accumulatie van minder goed hechtend *Methanosarcina* slib in het systeem. De *Methanosarcina* korrels blijven klein (D < 0,5 mm) en zullen daarom gemakkelijk uit de reactor worden gespoeld. Op grond van kinetische overwegingen bevelen wij daarom aan de acetaat concentratie onder 150 mg/l te houden, met het doel de vorming van *Methanothrix*-korrels te bevorderen in plaats van *Methanosarcina*-korrels.

Het korrelvormingsproces kan aanzienlijk worden versneld door binnen het mesofiele temperatuursgebied de temperatuur te verhogen van 30 °C naar waarden tot 40 °C. Bij deze temperatuur zal het zuiveringsproces echter minder stabiel zijn.

We hebben duidelijke aanwijzingen gevonden, dat korrelvorming ook goed verloopt bij lage pH-waarden. Bij een pH van 6 verlopen de opstart en de korrelvorming verassend voorspoedig. In dit opzicht is het interessant, dat de *Methanothrix*-achtige bacterie die werd gevonden in het slib, een veel lager pH-optimum had dan de *Methanothrix*-stammen, die tot dusverre in de literatuur zijn beschreven.

In Hoofdstuk 4 worden de resultaten beschreven van de experimenten, waarmee de invloed van extra toevoegingen aan het entslib zijn onderzocht. Ook hier werd vergiste slijkgistingsslib weer als entslib gebruikt.

In een aantal experimenten werd (rustig) vermalen korrelslib toegevoegd aan de reactor, waardoor een groot aantal kleine "korrelkernen" aan het entslib werden toegevoegd. Wij vonden dat de toevoeging van een kleine hoeveelheid vermalen korrelslib (slechts een paar procenten op basis van de hoeveelheid organische stof), al een duidelijke versnelling van de korrelvorming tot gevolg had.

Bovendien konden wij een groot verschil waarnemen in het korreltype, dat werd gevormd. Met alleen slijkgistingsslib als ent worden korrels verkregen, die voornamelijk bestaan uit *Methanothrix* in de "draderige" vorm, terwijl in de experimenten met slijkgistingsslib verrijkt met kleine hoeveelheden vermalen korrelslib, alle verkregen korrels zijn opgebouwd uit *Methanothrix*-achtige organismen in korte ketens van 4 to 6 cellen. Beide korrelsoorten hebben uitstekende bezinkingseigenschappen en zijn zeer aktief. Volumebelastingen tot 50 kg CZV/m^3.dag konden goed worden behandeld in reactor gevuld met 20 - 25 kg organische stof per liter.

De zogenaamde "draadkorrels" hebben een struktuur die meer open is, terwijl de eerste generatie korrels inerte dragerdeeltjes kunnen bevatten, die afkomstig zijn uit het slijkgistingsslib. De zogenaamde "staafkorrels" zijn veel kompakter van struktuur en bevatten over het algemeen geen inerte dragerdeeltjes.

Proeven met hydro-anthraciet als extra inerte dragerdeeltjes laten een duidelijke versnelling van het korrelvormingsproces zien. Verwijdering van inerte deeltjes uit het slijkgistingsslib leidt daarentegen tot een verslechterde korrelvorming. De resultaten van deze experimenten laten duidelijk het belang van de aanwezigheid van inerte deeltjes in het entslib zien.

Verder is ook waargenomen, dat vezelige inerte deeltjes een negatief effekt hebben op de slibeigenschappen, omdat de bezinkbaarheid verslechtert en daardoor buitensporige slibuitspoeling in de hand wordt gewerkt. Het wordt daarom afgeraden UASB-reactoren te enten met entslib met een hoog gehalte aan vezelig materiaal.

Het korrelvormingsproces is ook bestudeerd met een meer complex substraat (Hoofdstuk 5). Bij deze proeven werd gebruik gemaakt van sucrose en er werd aangetoond, dat het mogelijk is op dit substraat een stabiel korrelslibbed te kweken, dat bovendien sneller wordt verkregen dan het korrelslib gevormd met zuivere VVZ-oplossingen als voedings.

De experimenten met dit type substraat laten het belang zien van het aanhouden van de juiste selektiedruk, om te voorkomen, dat er zich snel groeiend dispers zuurvormend slib in de reactor kan ophopen. Wij willen er echter de nadruk op leggen, dat het niet nodig is hydraulische verblijftijden toe te passen, die lager zijn dan de specifieke groeisnelheid van de betreffende bacteriën, hetgeen vaak als een voorwaarde wordt beschouwd voor de succesvolle opstart van een fluidized bed systeem.

In Hoofdstuk 6 worden inleidende experimenten op het gebied van de physische karakterisering van korrelslib behandeld. De volgende apparatuur werd hierbij gebruikt:

- een aangepaste sedimentatiebalans voor de bepaling van de bezinkingseigenschappen en de deeltjesgrootteverdeling;
- een apparaat voor het meten van de weerstand tegen compressiekrachten.

Beide methodes, samen met de picnometrische bepaling van de dichtheid, kunnen worden beschouwd als bruikbare hulpmiddelen bij de karakterisering van korrelslib. Met behulp van deze technieken was het mogelijk aan te tonen, dat een verandering in substraat van een mengsel van vluchtige vetzuren naar een sucrose oplossing zal leiden tot de ontwikkeling van grotere en zwakkere korrels. Voorts kon een positief verband gedemonstreerd worden tussen het asgehalte en de korreldichtheid.

Uitgaande van de inzichten, die zijn verkregen met onze korrelvormingsexperimenten, kunnen we concluderen, dat het korrelvormingsproces, zoals het zich manifesteert in het UASB-systeem, een gevolg is van:

- de sterke neiging van anaërobe organismen zich te hechten;
- het feit dat een complexe bacteriële gemeenschap vereist is voor de anaërobe afbraak van organische stof, waardoor de vorming van micro-ecosystemen wordt gestimuleerd;
- het feit dat bacteriële groei wordt gestimuleerd op of in geïmmobiliseerde aggregaten (kernen), die aanwezig zijn of gevormd worden in het entslib, door het aanhouden van een juiste selektiedruk in het systeem.

We zijn van mening, dat de resultaten van de proeven, die in dit proefschrift zijn beschreven, sterke aanwijzingen geven voor de juistheid van de hypothese van het korrelvormingsproces, zoals die aan het eind van het eerste hoofdstuk is gepresenteerd.

Wanneer de resultaten van het onderzoek worden samengevat, dan kunnen de volgende praktische richtlijnen worden geformuleerd voor de opstart van grootschalige UASB-installaties, geënt met slijkgistingsslib:

I <u>Entslib</u>

1. De aanwezigheid van "geschikt" dragermateriaal is van belang voor het op gang brengen en versnellen van de aggregatie van bacteriën.
2. De specifieke methanogene aktiviteit van he entslib is niet de enige faktor van belang. Zware soorten slijkgistingsslib (> 60 kg TSS/m^3) dienen de voorkeur te krijgen boven lichtere soorten, ondanks een eventuele lagere methanogene aktiviteit.
3. De toevoeging van kleine hoeveelheid van (vermalen) korrelslib versnelt het korrelvormingsproces.

II <u>De procesvoering</u>

Het is van essentiëel belang, dat er een voldoende en continue verwijdering plaatsvindt van de lichtere fraktie van het entslib met het doel de zwaardere slibingrediënten selektief achter te houden en om bacteriële groei op en in deze zwaardere slibdeeltjes te doen plaatsvinden. Om dit te bereiken, raden we het volgende aan:

1. Uitgespoeld slib moet niet worden teruggevoerd.
2. Pas recirculatie van effluent of verdunning toe bij een influent-CZV van meer dan 5000 mg/l.

3. Verhoog de volumebelasting stapsgewijs, steeds als tenminste een zuiveringspercentage van 80% van de afbreekbare CZV is bereikt.

4. Handhaaf een lage acetaat concentratie (< 200 mg/l).

5. Begin met 12-15 kg slib-organische stof/m^3 met zwaar entslib (> 60 kg TSS/m^3) en ongeveer 6 kg slib-organische stof/l voor dun entslib (< 40 kg TSS/m^3).

III Eigenschappen van het afvalwater

1. Een algemene waarneming is, dat hoe lager de concentratie van het afvalwater is, hoe sneller de korrelvorming zich zal ontwikkelen. De concentratie zal echter nog wel voldoende hoog moeten zijn om goede groeiomstandigheden voor bacteriële groei in het systeem te behouden. De minimale CZV-concentratie is waarschijnlijk ongeveer 500 mg/l.

2. Fijn gedispergeerde vaste stoffen, zoals verzuringsslib en vezelig materiaal, hebben een negatief effect op de korrelvorming.

3. Korrelvorming zal vlotter verlopen op hoofdzakelijk opgeloste onverzuurde afvalwaters dan op volledig of bijna volledig verzuurde afvalwaters.

4. Hoge ion-concentraties (b.v. Ca^{2+}, Mg^{2+}) zullen aanleiding geven tot chemische precipitatie ($CaCO_3$, $CaHPO_4$, $MgNH_4PO_4$), hetgeen de vorming van een korrelslib met een hoog asgehalte tot gevolg heeft.

IV Omgevingsfaktoren

1. De optimale temperatuur voor mesofiele zuivering ligt in de range 30-38 °C en voor thermofiele behandeling in het gebied 50-60 °C.

2. De pH moet altijd boven 6 worden gehouden.

3. Alle belangrijke groeifaktoren zoals N, P en S en spore-elementen moeten in voldoende hoeveelheid en in een voor de bacteriën goed opneembare vorm aanwezig zijn.

4. Toxische verbindingen moeten niet aanwezig zijn in remmende hoeveelheden. Indien ze echter wel aanwezig zijn, dan moet er voldoende tijd uitgetrokken worden voor het plaats laten vinden van een eventueel adaptatieproces.

Abstract

Hulshoff Pol, L.W. (1989) The phenomenon of granulation of anaerobic sludge. Doctoral Thesis, Wageningen Agricultural University, Wageningen, The Netherlands.

Over the past two decades anaerobic wastewater treatment has become an accepted technology for the purification of a large variety of industrial wastewater. An important factor in this recent development is the successful upscaling and application of the Upflow Anaerobic Sludge Blanket (UASB) system. This success can for a large part be attributed to the development of a highly active well-settling granular type of sludge. Consequently, an excellent retention of the anaerobic sludge can be obtained, and high organic loading rates can be applied to the treatment system.

This thesis focuses on the granulation phenomenon. Present knowledge of the granulation process is reviewed and a hypothesis of the granulation mechanism is presented. According to this hypothesis the selection pressure, the combined upflow hydraulic load and the gas load occurring in the system, is the driving force behind the granulation process. The selection pressure leads to the required selection of heavier and lighter sludge components; light dispersed sludge washes out of the system while the heavier parts remain in the reactor. The granules ultimately develop on and in these heavier parts.

According to start-up experiments with digested seed sludge as inoculum and VFA mixtures as substrate, the lower the substrate concentrations were, the better granulation proceeded. Prolonged periods of underloading lead to the formation of a bulking type of sludge. Underloading should therefore be avoided. Acetate concentrations should be kept below 150-200 mg/l to promote the selective growth of *Methanothrix* which is found to be the predominant bacterium in the granules. High acetate concentrations lead to the formation of small unstable and therefore undesirable *Methanosarcina* granules.

The addition of (gently) crushed granular sludge from full-scale installations promotes granulation speed as well as the formation of a different granule type. Without this addition, granules mainly consist of filamentous *Methanothrix* cell-chains, rather than *Methanothrix* in rods of 4 to 6 cells which develop in the presence of the additional crushed granular sludge. "Filamentous" granules develop by attaching to inert particles present in the seed sludge. These inert particles play an important role in the granulation process; their removal by sieving severely decreases the granulation speed, whereas the addition of hydro-anthracite particles of 100 m in size is very beneficial to granulation.

Granulation also occurs on soluble carbohydrates (sucrose) containing substrates. Due to the higher growth rates of acidogenic organisms extra attention should be given to the installment of the proper selection pressure for an effective separation of flocculent and granular sludge.

The characterization of sludge is important for a proper operation and control of UASB systems. The determination of settling characteristics with a modified sedimentation balance and the assessment of granule strength are presented in this work.

Curriculum vitae

De auteur van dit proefschrift is op 31 oktober 1947 geboren in Hengelo (Ov.). In 1965 behaalde hij het diploma HBS-b aan de gemeentelijke HBS in Hengelo. Daarna begon hij met de studie aan de Landbouwhogeschool in Wageningen. In 1975 legde hij het doktoraalexamen af in de studierichting Milieuhygiëne met als hoofdvakken waterzuivering en natuurbeheer. Van 1976 tot 1977 werkte hij bij de vakgoep Natuurbeheer van de Landbouwhogeschool aan een studie voor de Stichting Toekomstbeeld der Techniek over milieuinvloeden bij houtproduktie en houtverwerking. Vanaf 1978 tot nu is hij in dienst bij de vakgroep Waterzuivering van de Landbouwuniversiteit, met een korte onderbreking in 1985 voor een verblijf aan de Universiteit van Paraiba in Campina Grande, Brazilië. Na het onderzoek naar de korrelvorming van anaëroob slib, dat grotendeels plaats vond in de jaren 1979 tot 1985 heeft hij zich bezig gehouden met de begeleiding van vele onderzoeksprojekten op het gebied van de anaërobe waterzuivering in zowel binnen- als buitenland. Sinds september 1988 is hij aangesteld als universitair docent bij de vakgroep Waterzuivering van de Landbouwuniversiteit.