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Development of a new high-rate anaerobic process for the treatment of industrial and domestic wastewaters: the anaerobic migrating blanket reactor (AMBR)

Largus Theodora Angenent
Iowa State University

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Development of a new high-rate anaerobic process for the treatment of industrial and domestic wastewaters: The anaerobic migrating blanket reactor (AMBR)

by

Largus Theodora Angenent

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This is to certify that the Doctoral dissertation of

Largus Theodora Angenent

has met the dissertation requirements of Iowa State University

Signature was redacted for privacy.

Committee Member

Signature was redacted for privacy.

Committee Member

Signature was redacted for privacy.

Committee Member

Signature was redacted for privacy.

Committee Member

Signature was redacted for privacy.

Major Professor

Signature was redacted for privacy.

For the Major Program

Signature was redacted for privacy.

For the Graduate College

For my best friends: Bernadet, Dennis, Frans, Neelika, and Seda.

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Dr. Dague had worked together with several organizations and businesses to develop full-scale applications of the invented technologies. Intriguingly, some people in these organizations and businesses were not afraid in changing concepts into their "own" technologies shortly after Dr. Dague's death. Consequently, Dr. Shihwu Sung, who succeeded and continued Dr. Dague's work, was bound to have a hard time. Therefore, I want to thank him for becoming my major professor and supporting my research and ideas. Hopefully, we can work together some more in the future.

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Running anaerobic reactors is time-consuming and takes lots of baby-sitting skills. Lab visits become a way of living, which will ultimately not only influence your own life, but also your partner's life.

ABSTRACT

The anaerobic migrating blanket reactor (AMBR) was developed as a new high-rate system for the treatment of industrial and domestic wastewaters, at Iowa State University. A U. S. patent is pending for this new process. The AMBR, a continuously fed, compartmentalized reactor, required mechanical mixing to obtain a sufficient biomass/substrate contact. The formation of granular biomass was not dependent on a hydraulic upflow pattern in the reactor, but was dependent on biomass migration over the horizontal plane of the reactor and the settling characteristics of the final compartment. To prevent acclimation of biomass in the final compartment, the flow was reversed in a horizontal matter. Keeping the pH sufficiently high in the initial compartment without recycling effluent was another advantage of reversing the flow. This also prevented total phase separation of acidogenesis and methanogenesis in the AMBR.

Laboratory-scale AMBR systems have achieved high organic removal efficiencies when fed with non-acidified sucrose as a substrate at chemical oxygen demand (COD) loading rates up to 30 g/L/d. Furthermore, the AMBR was able to retain high levels of granular biomass at these loading rates. Due to moderate shear forces by mechanical mixing, the laboratory-scale AMBR was able to treat non-acidified sucrose at food to microorganism (F/M) ratios higher than found for other high rate systems. The AMBR out-competed the upflow anaerobic sludge blanket (UASB) and anaerobic sequencing batch reactor (ASBR) in a laboratory-scale comparison in terms of reactor performances and maximum organic loading rates.

A mature granular blanket was formed after four months of operating a 54-liter AMBR, seeded with flocculent primary digester sludge. This was accomplished with moderate hydraulic selection pressures at the start of the run, in which reactor performances were sufficient to build up an active biomass, without losing the selection mechanism for better settling biomass. In these studies, mixing of the final compartment and an effluent baffle system were required in selecting and growing a granular blanket.

A 20-liter AMBR was able to effectively remove organic material from dilute non-fat dry milk (NFDM) solution at a concentration of 600 mg/L under psychrophilic conditions. Moreover, this reactor was able to retain its granular biomass after the hydraulic retention time (HRT) was decreased from four to one hour during hydraulic shock load studies. Finally, staging or partial phase separation was found in the AMBR in which relatively more methanogens were present in the outer compartments.

CHAPTER 1. GENERAL INTRODUCTION

Introduction

The research, presented here, consisted of fundamental laboratory studies on a new high-rate anaerobic reactor for the biological conversion of organic liquid wastes to biogas (methane and carbon dioxide). The new reactor process is called the anaerobic migrating blanket reactor (AMBR). A formal application for a U.S. Patent was completed in May 1997. The objective of the laboratory research was to gather data and fundamental knowledge on the performance of the AMBR process that would lead to pilot-scale and proof-of-concept applications of the process. An extra focus was on finding a niche in which this new process could be beneficial compared with other high-rate anaerobic processes.

The AMBR was invented in August / October 1994. After operating an anaerobic sequencing batch reactor (ASBR) during the summer of 1994, the author and Dr. Dague discussed how the advantages of the ASBR system, such as a simple design and feast and famine alterations, could be combined in a continuous flow configuration. In addition, the absence of a hydraulic upflow pattern and the requirement of mechanical mixing had not prevented granular formation in ASBR systems, as shown by Wirtz and Dague (1994). With this knowledge, the AMBR was developed. To retain biomass in a continuous process other workers had invented a compartmentalized reactor, which showed promising results (Bachman et al., 1982). However, unidirectional compartmentalized reactors did not promote feast and famine conditions for the biomass. Also, effluent recycling at higher strength wastewaters was required to elevate the pH in the first compartments, which resulted in less plug-flow conditions. The key to eliminate these disadvantages was found in reversing the flow over the horizontal plane of the reactor. Furthermore, acclimation of biomass in the final compartment of a unidirectional reactor configuration, due to biomass migration, was prevented. Actually, granular formation in the AMBR was stimulated by migration of the sludge blanket through the reactor, which gave the system its name.

The overall hypothesis of the performed research was: The AMBR is a high-rate anaerobic system, which can compete with or out-compete other anaerobic systems on a laboratory-scale basis. The overall objectives, consisting of three separate objectives given in chapter 2-4, were to investigate this hypothesis in which principles of the AMBR and possible niches for full-scale AMBR systems were determined.

Dissertation Organization

The research was separated in three topics each with its own hypothesis, objectives, reactor operation and chapter in this dissertation. The results of these three topics will be published separately in papers which were included as Chapter 2, 3, and 4. Chapter 1 consists of a literature review which describes anaerobic fundamentals, common anaerobic reactor designs, compartmentalized anaerobic reactors, granulation, and staging or partial phase separation. Finally, Chapter 5 consists of general conclusions and recommendations.

The first research topic was included as a paper in Chapter 2, in which reactor performance and different reactor configurations of the anaerobic migrating blanket reactor (AMBR) were studied. Furthermore, the maximum COD loading rate and other performance parameters were compared with the upflow anaerobic sludge blanket (UASB) reactor and anaerobic sequencing batch reactor (ASBR). The postulated hypothesis for this research was: The laboratory-scale AMBR can out compete laboratory-scale ASBR and UASB systems when treating sucrose as a synthetic waste.

Chapter 3 consists of the second research paper, which describes studies to verify the following hypothesis: The AMBR can select for a granular biomass after seeding the reactor with flocculent digester sludge, without having a hydraulic upflow pattern in the reactor. In addition to the formation of granules, different substrates and hydraulic loading rates were studied to optimize and characterize the granular selection process.

The final research paper, which was included as Chapter 4, illustrates the applicability of the AMBR in treating low-strength wastewaters at psychrophilic conditions. The hypothesis for this research was: The AMBR is ideal for treating low-strength wastewaters at psychrophilic conditions and could as such be used for treatment of domestic wastewaters.

Literature Review

Advantages of anaerobic high-rate systems

Most wastewater treatment installations utilize aerobic biological processes in the treatment of large, relatively dilute combinations of domestic and industrial wastewater streams. If anaerobic treatment is used, it is in the form of digesters for final stabilization of the grown biomass. The treatment of high-strength industrial wastestreams with aerobic technologies can become costly, because elevated organic concentration results in increased aeration requirements, increased reactor size, and increased production of biomass. In addition, industrial wastestreams can vary greatly with respect to waste strength and quantity

of flow. These fluctuations can also result in large variations in the oxygen requirements and biomass production of the aerobic systems (Lettinga, 1995).

In some cases, biological treatment must be discarded due to the inhibitory or even toxic nature of wastes. In such cases, physical/chemical treatment methods may be used, but these can be quite costly to operate. Thus, many industries decide not to investigate industrial pre-treatment methods and pay sewage use fees. Therefore, many municipalities become responsible for stabilization of the wastewater from industries.

However, cost-effective wastewater treatment alternatives do exist in the form of anaerobic biological systems. These systems can achieve high organic loading rates without large increases in costs. In fact, higher strength wastewaters have more potential profit due to higher methane production per amount of wastewater treated. Notably, anaerobic pre-treatment systems have the potential to pay for themselves over a short period of time, especially in regions with high energy prices. In recent years, all presumed disadvantages of anaerobic high-rate systems, such as low stability of the digestion systems, slow speed of start-up, malodorous nuisance, and susceptibility to xenobiotic compounds have been overcome by increased amounts of research, operational know-how, and implementation of new techniques (Lettinga, 1995). High-rate systems, such as the widely used upflow anaerobic sludge blanket (UASB) (Lettinga et al., 1980) and more recently, the anaerobic sequencing batch reactor (ASBR) (Sung and Dague, 1992) along with others, were applied to different waste streams. These systems achieved high loading rates and high stability due to sufficient biomass retention. However, anaerobic high-rate systems are designed for pre-treatment of wastewaters and some form of post-treatment is required (Lettinga, 1995; Speece, 1988). Moreover, it must be realized that these methods are considered to be innovative technologies (Switzenbaum, 1995).

Fundamental knowledge of anaerobic treatment

Anaerobic biodegradation consists of several consecutive steps, each governed by a different trophic group of microorganism. First, hydrolysis of biopolymers by exo-enzymes takes place with the formation of less complex molecules. Next, these molecules are further fermented by acidogenic bacteria into simpler organic acids. These acids are then used by syntrophic acetogenic bacteria to form H_2, CO_2, acetate, and formate. Finally, methane is formed from H_2 and CO_2 via reductive methane formation, from acetate via a decarboxylation, or from formate degradation. Approximately 70% of the methane produced in anaerobic digesters originates from acetoclastic methanogenesis (Gujer and Zehnder,

1983). In cases where sulfate levels are high, sulfate reducing bacteria (SRB) can coexist with or out-compete the methanogens in the terminal reaction (Bhattacharya et al., 1996).

Hydrogen or formate interspecies transfer. Interspecies hydrogen transfer between hydrogen producing bacteria and hydrogen utilizing archae is a prerequisite for the oxidation of acids in anaerobic systems, since syntrophic relationships depend on low concentrations of hydrogen. More specifically, the anaerobic oxidation of acids which are carried out by acetogenic bacteria have a positive Gibbs free energy, and thus are only possible when the products are taken away (Stams, 1994). Thiele and Zeikus (1988) found the formate interspecies transfer to be more important compared to hydrogen interspecies transfer in flocculent biomass. However, Schmidt and Ahring (1995b) concluded that formate interspecies transfer might not be essential in degrading propionic acid and butyric acid in mesophilic granular biomass.

16S rRNA probes. Raskin et al. (1994a) designed hybridization probes for the study of communities of methanogens in anaerobic digesters. These probes were found to be very specific to the target methanogens and were not hybridized by the rRNA of non-target methanogens. Seven out of eight probes described methanogens which were represented in pure culture. With this technique the community structure of entire anaerobic reactors was studied. This was needed to establish the link between microbial function and structure. In addition, these taxon-specific probes were used to identify and quantify phylogenetic defined groups of methanogens in full-scale sewage sludge digesters. *Methanosaeta* (formally *Methanothrix*) species were the most abundant methanogens in these digesters (Raskin et al., 1994b).

High-rate anaerobic systems with self-immobilized biomass

Although high-rate anaerobic systems with carrier material for biofilms were developed, such as anaerobic filters (AF), anaerobic fluidized bed (AFB), anaerobic rotating biological contacter (ARBC), and hybrid reactors, only systems with a granular (self-immobilized) biomass were reviewed. One comment, that needs to be made before comparing several reactors in terms of loading rates, is that some workers do not include the volume of the acidification reactor for the total system volume. By doing that, systems which are fed a non-acidified complex organic substrate have a competitive disadvantage, as organic loading rates achieved will be lower.

Requisitions for high-rate systems. High-rate anaerobic systems are processes in which the hydraulic retention time (HRT) is uncoupled from the sludge retention time (SRT). Thus, these systems were required to maintain high concentrations of biomass while

obtaining high hydraulic loading rates. Among the factors that affected the treatment efficiency of high-rate anaerobic processes were the reactor type, the hydraulic regime, the kind and concentration of wastewater to be treated, the concentration of microbes, the type of microbes in the reactor, and the ability to achieve granulation (Jhung and Choi, 1995). To successfully create a new high-rate immobilized biomass reactor and provide an anaerobic treatment process which could handle high organic and hydraulic loading rates, the following conditions should be met. These conditions were:

1. Selection of a granular biomass: The selection for granular biomass was a condition that should be met to handle high volumetric loading rates. Granulation resulted in a better settleability of the biomass, which increased the retention of the biomass (Lettinga, 1995).

2. High retention of biomass: High levels of biomass were a requirement for a high loading potential of anaerobic processes. The process loading, based on food to microorganism (F/M) ratio, should be low to achieve efficient granular biomass formation and solids separation (Dague et al., 1966). A low F/M ratio at high loading rates (F) was achieved whenever the biomass concentration (M) was also high. Not only was the formation of granules responsible for retaining the biomass, but also the availability of an internal clarifier. Processes, such as the UASB and ASBR, made use of internal settling to keep high levels of biomass in the reactor (Lettinga et al., 1980; Sung and Dague, 1992).

3. Simple design: To become competitive, a new reactor system should be simple. For example, the absence of a hydraulic upflow pattern for the ASBR made an elaborate feed-distribution system and gas-solids-separator system unnecessary.

4. Sufficient biomass/substrate contact: A sufficient biomass/substrate contact in UASB reactors was maintained by a hydraulic upflow pattern and natural mixing by biogas production. However, when a hydraulic upflow pattern was absent, sufficient contact between the substrate and biomass was maintained by using intermittent, gentle mixing. Especially at high loading rates, biogas production itself increased mixing. But even then, artificial mixing was required to maintain sufficient biomass/substrate contact. Research by Dague et al. (1970) in the 1960s showed that mixing that was too intense could destroy the anaerobic bioflocs and result in poor solids separation.

5. Prevention of short circuiting: Short circuiting of substrate needed to be prevented to obtain low concentrations of substrate in the effluent.

6. Feast and famine conditions: Feast and famine conditions for the biomass showed advantages in the efficiencies of the ASBR. High substrate levels, just after feeding the batch reactor, created high substrate utilization rates by the biomass. This provided a

high driving force for metabolic activity, in accordance with Monod kinetics. However, the substrate concentration in the ASBR was low before decanting, which stimulated granulation and solids separation in the reactor (Sung and Dague, 1995).

7. High reaction rates and the absence of serious transportation limitations: Transportation limitations occurred whenever granules got too large. In this way, substrate was limited in transporting into the core of the granules. Eventually, hollow granules developed (Lettinga, 1995).

8. Sufficiently acclimated and adapted biomass: High organic loading rates were obtained after the biomass was sufficiently acclimated and adapted to the wastewater. In most cases, seed biomass for newly built anaerobic systems needed an acclimation period to the wastewater before high organic loading rates were applied (Lettinga, 1995).

Single vessel anaerobic systems. Two, single vessel systems with completely different reactor operations and designs were described in the following paragraphs:

1. The UASB Reactor: In 1997, the UASB reactor was the most popular high-rate anaerobic system in the world in which 61% of all anaerobic systems for the treatment of industrial wastewaters were UASB reactors (Hulshoff Pol et al., 1997). Unlike some other systems, such as the fluidized bed and biofilter systems, the UASB reactor did not require an attachment material. Microbial growth and hydraulic wash-out of poorer settling biomass selected for granular sludge. After the formation of the granules, the upflow velocity in the reactor was increased without excessive loss of biomass. Mixing in the UASB reactor was not necessary. A sufficient contact between the biomass and the substrate occurred because of the upflow velocity of the wastewater in the sludge blanket and biogas production. A good distribution of the substrate was obtained by having more inlet points at the bottom of the reactor. A gas solids separator (GSS) system was used in this type of reactor to collect and distribute the biogas and to separate the biomass from the effluent. Therefore, biomass fell back in the reactor because of a decreased upflow velocity in the settling section (Hulshoff Pol and Lettinga, 1986).

2. The ASBR: The UASB process was applied primarily to wastewaters that were low in suspended solids (SS). In contrast, the ASBR process was not only able to handle soluble influent streams but also those with higher SS. In studies by Dague and Pidaparti (1991) and Schmit and Dague (1993), diluted swine wastes with SS concentrations of 5% were successfully treated in the ASBR process. An intermittent feed and decant regime resulted in alternating high/low substrate (feast/famine) conditions in the reactor. The high substrate concentration just after feeding resulted in high rates of substrate conversion and biogas production. The low substrate concentration at the end of the cycle and the

resulting low gas production enabled efficient solids separation (Dague et al., 1992). The reactor sequenced through four steps; feed, react, settle, and decant. During feeding, substrate was added to the reactor. Normally, the volume of waste added to the reactor during feeding was the same as the volume decanted as effluent. At the end of feeding, the reactor was mixed to distribute the waste throughout the liquid volume. The substrate concentration was at its highest level just after feeding. The time of feeding was increased to obtain a lower concentration of feed in the reactor (Sung and Dague, 1992).

The second step in the cycle was the react step. The time required for the react step depended on several parameters, including substrate composition (for example, the amount of suspended solids in the influent), substrate strength, required effluent quality, biomass concentration, and waste temperature. Proper mixing of the biomass and substrate during the react step was found to be important. Gas production automatically led to mixing in the reactor, but mechanical mixing was required to create a good distribution of the substrate during the react step (Sung and Dague, 1992).

In the third step, mixing was stopped to settle the biomass. Mixing, before settling, was necessary to insure that entrapped biogas, which could inhibit settling of the sludge blanket, escaped. The time required for clarification varied, depending on biomass concentration and settleability, and ranged from few minutes to one hour. The settling time was found to be an important parameter and was changed during operation. The settling time needed to be short to wash out the poorly settling biomass, but not so short that granular biomass was washed out of the reactor. Following these concepts, an optimal settling time was found that selected for and enhanced granulation (Sung and Dague, 1992).

The last step was decanting of effluent out of the reactor. Since the used ASBR was a closed system, a reduced pressure resulted when effluent was withdrawn, unless a provision was made for biogas to backflow. To overcome this, a gas bag was installed to equalize the pressure. While decanting, the gas bag decreased in volume, refilling again during the feeding step. The time required for the decant step was governed by the total volume to be decanted during each cycle and the decanting rate. The total volume was dependent on the HRT and the volume of the reactor. After decanting, the reactor was ready to be fed another batch of influent (Sung and Dague, 1995).

Compartmentalized anaerobic reactor designs. Due to biogas production and mixing, compartmentalized anaerobic systems might be characterized as a series of completely mixed compartments, approaching a plug-flow system. It is well known that with chemical reactions of the first or higher order, reactors with a plug-flow pattern are more effective than completely mixed reactors from a strictly kinetic standpoint (Levenspiel,

1972). In a comparison between compartmentalized and completely-mixed aerobic biological wastewater treatment systems, the same conclusion was made. The superiority of the compartmentalized reactor was proven with regards to suspended solids (SS) in the effluent and the degradation of a toxic compound, such as phenol. The compartmentalized reactor produced a mixed culture which had higher maximum volumetric and specific rates of phenol removal than that of the completely mixed reactor (Chudoba et al., 1991). However, under normal operating conditions of an activated sludge system, both completely mixed and plug-flow systems yielded essentially identical removal efficiencies. Furthermore, Toerber et al. (1974) concluded that in response to a severe shock loading in an aerobic system, the completely-mixed system demonstrated higher overall removal efficiencies than the plug-flow system on the basis of BOD.

Compartmentalization was introduced in anaerobic reactors for retaining biomass at higher loading rates. Loss of biomass with the effluent due to excessive bed expansion or poor granulation posed problems to a non-compartmentalized reactor, such as the UASB process (Guiot et al., 1995). Compartmentalization in anaerobic reactors was first described by Bachman et al. (1982), who developed the anaerobic baffled reactor (ABR). In this reactor, the wastewater flowed under and over vertical baffles. The ABR was described as a number of UASB reactors in series, which indicated that a hydraulic upflow pattern was responsible for the contact between substrate and biomass. The ABR was able to treat a soluble substrate at high COD loading rates (36 g/L/d) with high stability and reliability (Bachman et al., 1985; Grobicki and Stuckey, 1992). In recent research, it was found that the ratio of acidogenic to methanogenic microorganism changes along the length of the reactor. The number of acidogenic bacteria in the granular biomass was highest in the initial compartment and decreased over the reactor length. Therefore, methanogens dominated the final compartments (Nachaiyasit and Stuckey, 1995).

Other compartmentalized reactor types have been developed. van Lier et al. (1994) and van Lier (1995) studied the thermophilic treatment of acidified and partially acidified wastewaters using upflow staged sludge bed (USSB) reactors. From results of this study, it was clear that the retention of biomass in a staged process was improved significantly, even under extreme loading conditions and short HRTs (100 gCOD/L/d and HRTs of two hours). After treating mixtures of sucrose and volatile fatty acids (VFAs) in the thermophilic USSB, it was found that sucrose was converted in the first compartment, followed in the next compartments by conversion and removal of butyrate and acetate. Propionate was only degraded in the last compartment. When treating a rather complex waste, the acidifying

stage of digestion was localized in the first compartment and, as a result, the specific methanogenic activity in this compartment remained relatively low.

Another vertical compartmentalized reactor described, was the multiplate anaerobic reactor (MPAR). A 450 m^3 reactor was started in 1992 for treating whey permeate and domestic wastewater in Quebec, Canada. The soluble COD removal efficiency was as high as 98% at a COD loading rate of 15 g/L/d. At these loadings, the reactor showed a high capacity for sludge retention, even with small granules (Guiot et al., 1995). The internal circulation (IC) reactor, is a system consisting of two UASB reactor compartments on top of each other. In this way, a two-stage process was created, in which one compartment had a high organic loading rate and one had a low organic loading rate. Biogas was collected in both stages by gas-solids-separator systems. The gas collected in the first stage was used to generate a gaslift and internal circulation (Hack et al., 1988). Full-scale IC reactors were able to handle both high COD and hydraulic loading rates for industrial wastewater. The IC reactor was able to treat potato processing wastewater at a COD loading rate of 40 g/L/d. In comparison with conventional UASB reactors, the two-stage process handled higher upflow velocities and biogas production rates. The second stage was more effective in biomass settling and retention. This made the treatment of low-strength wastewaters (high hydraulic loading rate) and high-strength wastewaters (high organic loading rate) more feasible (de Vegt and Yspeert, 1994). The final vertical compartmentalized reactor mentioned here, the biogas tower reactor, was developed by Reinhold et al. (1996). This reactor withdrew biogas production by gas-collecting devices at different levels along the height, preventing gas accumulation in the upper zones of the reactor and creating fluid circulation (mixing) around the baffles.

An upwards feeding and reversing flow pattern showed improved settling characteristics and granulation in the reversing anaerobic upflow system (RAUS). This system consisted of two anaerobic reactors connected in series. Both reactors were fed alternately at regular intervals of time. While wastewater was fed to one reactor, the other served as a settling tank. The system was batch (intermittently) fed in an upflow pattern. A pilot-scale RAUS reactor was built in Thailand and was able to treat distillery wastewater at a COD loading rate of 7 g/L/d. After 120 days of operation, each side of the system was fed every 16 hours for 10 minutes (Basu, 1995). A modification of the UASB process has been developed and introduced as the two-stage anaerobic unitank system (TSU-AN-system) (Beyen et al., 1988; Verstraete, 1991). The methane reactor, which was the second phase of the system, consisted of two compartments. The wastewater was introduced into one of the compartments, which created expansion of the biomass due to the hydraulic upflow and

higher biogas production. Next, the liquid and the lighter flocs flowed to the second more quiescent compartment in which biomass retention was high. The gas-solids-separator (GSS) system in the second compartment efficiently removed the remaining biomass from the effluent. After a regular interval of time, the flow was reversed. The length of this interval of time was limited by the hydraulic load and was found to be between 90 and 180 minutes. In the full-scale treatment of a brewery wastewater, the second-phase methane reactor was operated at an HRT of 12 hours and a COD loading rate of 6-9 g/L/d. The total COD removal efficiency was between 75% and 85% (Beyen et al., 1988). Finally, the periodic anaerobic baffled reactor (PABR) was operated with and without switching the feeding and effluent compartments. The PABR would behave as a ABR at a zero switching frequency, but would approach several UASB reactors in parallel at a infinite switching frequency (Skadias and Lyberatos, 1997).

Granulation

The UASB (and its derivatives), ABR, and IC reactors all depended on a hydraulic upflow pattern to select for a granular biomass. However, in the ASBR granulation did not depend on the upflow velocity of the wastewater in the reactor. The phenomenon of forming granules within a operation period of five months after seeding with non-granular anaerobic sludge was found in the ASBR process by Wirtz and Dague (1994). Adding a cationic polymer shortened this period of granule development to two months after seeding the reactors with primary anaerobic digester sludge. Vanderhaegen et al. (1992) also obtained granular methanogenic sludge without having a hydraulic upflow pattern. Granular sludge had several advantages over flocculent biomass. Three of these were:

1. Granular sludge was retained more efficiently in the reactor because of better settleability.
2. Granular sludge had a higher specific methanogenic activity than flocculent biomass (Lettinga et al., 1980; Hulshoff Pol et al., 1983; Dubourguier et al., 1988).
3. Due to a higher internal pH of the granules, the methanogenic activity was maintained at less favorable situations in the bulk fluid, e.g. lower pH levels or higher concentrations of unionized sulfide (H_2S) (de Beer et al., 1992).

The role of extracellular polymers and hydrophobicity on granulation. First, extracellular polymers (ECP) played a role in the formation of granular biomass. Several researchers showed a correlation between the production of ECP and the formation of granules (Dolfing et al., 1985; Grotenhuis et al., 1991). Furthermore, acidogenic populations had a greater influence on the production of ECP (Schmidt and Ahring, 1995a). ECP in anaerobic biomass consisted mainly of protein, polysaccharides, and lipids (Dolfing et al.,

1985; Schmidt and Ahring, 1994). A ratio of 2:1 for proteins and polysaccharides was found in granules grown on a complex carbohydrate substrate (Dolfing et al., 1985; Grotenhuis et al., 1991). There existed a positive of Fe and yeast extract on the production of extracellular carbohydrates by anaerobic bacteria and archae in granular biomass (Shen et al., 1993). Furthermore, these researchers found increased extracellular polysaccharide production in anaerobic reactors when feeding was omitted. That production of ECPs was manipulated at nutrient limiting conditions, was described by Wilkinson (1958). Research had shown that during growth-limiting nutrient concentrations, the amount of polysaccharide produced per cell rose to a maximum level. The reason for this behavior could be the aid of polysaccharides in the uptake of ions. In addition to the foregoing, Costerton et al. (1981) showed the relation of ECPs and adhesion of bacteria. Moreover, the chemical composition and the position of ECPs on the surfaces of cells affected the surface properties, as reported by Forster (1971). The large molecular structure of ECPs could also act as attachment matrices for bacteria to grow on (Wirtz and Dague, 1994).

Second, research by workers in Belgium found a correlation between the hydrophobicity of anaerobic biomass and granulation. This correlation was also found between hydrophobicity and sludge bed stability (Thaveesri et al., 1995). Grotenhuis et al. (1992) observed selection for hydrophobic bacteria and archae in anaerobic granular biomass. In particular, *Methanosaeta soehngenii*, a methanogen important in the granulation process, was highly hydrophobic. Research by van Loosdrecht et al. (1987a) showed that hydrophobic bacteria had increased adherence characteristics compared to hydrophilic bacteria. A more detailed study on cell hydrophobicity of granular biomass from UASB reactors revealed that abundant trophic groups of bacteria and archae in anaerobic biomass had different characteristics. Most acidogenic or fermentative bacteria were found to be hydrophilic but most acetogenic bacteria and methanogenic archae were found to be hydrophobic (Thaveesri et al., 1995). Since acidogenic bacteria contained large amounts of ECPs and were required for granulation to occur, it seems unclear, in terms of surface thermodynamics, why hydrophilic cells were needed and what the role of the ECPs were in this phenomenon. Moreover, variations in surface tension of the liquid in the anaerobic reactors showed differences in hydrophobicity of the selected bacteria. Reactor liquids with a high surface tension grew granules with hydrophobic surfaces. Conversely, sugar containing substrates selected for granules with hydrophilic surfaces but hydrophobic cores. Moreover, protein containing wastewater showed highly hydrophilic cells, which explained the formation of more fluffy bacteria (Thaveesri et al., 1995).

Floatation problems in high-rate anaerobic systems. Floatation of granular biomass after the introduction of a different substrate was described in the literature (Alphenaar, 1994). For practical reasons, the granules were crushed to eliminate floatation problems (Alphenaar, 1994; Yoda and Nishimura, 1997). Yoda and Nishimura (1997) found that after adding Fe to the reactors floatation was decreased. Thaveesri et al. (1995) discussed the hydrophobic surface of the granules to be susceptible to attachment of poorly water-soluble biogas, which created floatation.

The effect of high ammonia levels on the formation of granules. Research by Hulshoff Pol et al. (1983) revealed that ammonia concentrations in the wastewater had a negative effect on the granulation process. However, this research had no explanation for this feature. Furthermore, Bull et al. (1983) used the hypothesis of increased ECP production at increased C/N ratios of the waste, but did not test this. At low C/N ratios, thus high ammonia levels, decreased extracellular polymer production would explain the negative effects on granulation. Thaveesri et al. (1994) contributed negative effects of granulation on proteins rather than on the ammonia formed. Moreover, Grotenhuis et al. (1992) described that in wastewaters with a high ionic strength, such as high ammonia concentrations, the charge of the bacteria was of less importance in the adhesion process.

Staging in anaerobic systems

Research showed that acidogenic conditions in a two-phase treatment concept had negative effects on in-reactor granular growth (Vanderhaegen et al., 1992). Rather than phasing, partial phasing or staging kept all phases of anaerobic digestion present, but acidogenic activities to be higher in the initial compartments. The advantage of a slight pre-acidification in a staged process was postulated by Fox and Pohland (1994) and Lettinga (1995). Plugflow conditions, which promoted partial phase separation, was enhanced by the absence of both recycling and compartmentalization of gas production (Fox and Pohland, 1994). Also, compounds, such as the intermediate propionate, encountered an optimal environment for degradation in the final compartments. Furthermore, a staged process provided higher process stability, as was postulated by van Lier et al. (1994), especially at thermophilic conditions and with compartmentalized headspaces. Finally, compartmentalized headspaces could be beneficial regarding the stripping effect of intermediates (hydrogen sulfide and hydrogen) from the initial compartments, in which these intermediates could become very low in the final compartments (van Lier, 1995).

Staging of biomass. Research with the USSB reactor showed that staged degradation of high-strength substrate in the separate compartments resulted in a staged biomass in which

relatively higher acetogenic and methanogenic activities were measured from biomass in the final compartments (van Lier, 1996). The same result was found in a staged reactor set-up of two EGSB in series treating partly acidified wastewater at psychrophilic conditions (van Lier et al., 1997). Furthermore, 16S rRNA probe techniques showed mainly *Methanosaeta* and *Methanobrevibacter* species as methanogens in the granular biomass and found the ratio of bacteria and methanoarchae hybridization signal to be three times higher in the first stage over the second stage.

Staging of sulfate. Hydrogen sulfide is produced by the reduction of sulfate in anaerobic systems, and could be toxic to the methanogenic and sulfate reducing consortia at levels higher than 100 mg/L. With single vessel systems, Rinzema and Lettinga (1988) discussed a COD to SO_4 ratio of 10 or higher at which anaerobic treatment proceeds without the toxicity difficulties. However, at lower COD to SO_4 ratios the COD of the wastewater should be lower than 15 g/L to ensure success. Problems related to the treatment of sulfate rich wastewaters are: corrosion from the H_2S in the biogas; lower COD removal and increased effluent odour due to sulfide in the effluent; toxicity of H_2S to the anaerobic consortia; and reduced methane production (Rinzema and Lettinga, 1988).

Two phase separation was discussed as a problem solver in which the H_2S could be stripped from the initial acidogenic phase (Rinzema and Lettinga, 1988). However, when this was tested, sulfate reduction seemed to be incomplete in the acidogenic phase. Better results were hypothesized for a staged reactor concept (van Lier et al., 1994). Moreover, Lens et al. (1998) stated that further research in anaerobic population dynamics between sulfate reducers and methanogens was required using specific analytical techniques, such as 16S rRNA probes. This technique proved to be successful in the evaluation of anaerobic systems (Raskin et al., 1994; Raskin et al., 1995).

References

Alphenaar, P. A. (1994) Anaerobic granular sludge: Characterization, and factors affecting its functioning, Ph.D. Dissertation, Wageningen Agricultural University, Wageningen, The Netherlands.

Bachman, A., Beard, V. L., and McCarty, P. L. (1982) Comparison of fixed-film reactors with a modified sludge blanket reactor. In: *Proceedings of the first international Conference on fixed-film Biological processes.* (Edited by Wu, Y. C., and Smith, E. D.), Arlington, VA, Noyes Publications, Park Ridge, N.Y., 1192.

Bachman, A., Beard, V. L., and McCarty, P. L. (1985) Performance characteristics of the anaerobic baffled reactor. *Water Research*, **19**, 99.

Basu, S. K. (1995) Innovative wastewater treatment using reversing anaerobic upflow system (RAUS). In: *Proceedings, 50th Purdue Industrial Waste Conference*, Ann Arbor Press, Chelsea, MI, 379.

de Beer, D., Huisman, J. W., van den Heuvel, J. C., and Ottengraf, S. P. (1992) The effect of pH profiles in methanogenic aggregates on the kinetics of acetate conversion. *Water Research.* **26**, 1329.

Beyen, G., Delaplace, P., Ghekiere, S., Mertens, M., and Vriens, L. (1988) The anaerobic unitank system for advanced wastewater treatment: pilot- and full-scale experience on brewery wastewater. *Mededelingen van de Faculteit Landbouwwetenschappen: Rijksuniversiteit Gent*, **53** , 1927.

Bhattacharya, S. K., Uberoi, V., and Dronamraju, M. M. (1996) Interaction between acetate fed sulfate reducers and methanogens. *Water Research*, **30**, 2239.

Bull, M. A., Sterritt, R. M., and Lester, J. N. (1983) An evaluation of four start-up regimes for anaerobic fluidized bed reactors. *Biotechnology Letters*, **5**, 333.

Costerton, J. W., Irving, R. T., and Cheng, K. J. (1981) The bacterial glycocalyx in nature and disease. *Annu. Rev. Microbiol.*, **35**, 299.

Chudoba, J., Straková, P., and Kondo, M. (1991) Compartmentalized versus completely-mixed biological wastewater treatment systems. *Water Research*, **25**, 973.

Dague, R. R., McKinney, R. E., and Pfeffer, J. T. (1966) Anaerobic Activated sludge. *Journal Water Pollution Control Federation*, **38** , 220.

Dague, R. R., McKinney, R. E., and Pfeffer, J. T. (1970) Solids retention in anaerobic waste treatment systems. *Journal Water Pollution Control Federation*, **42**, R29.

Dague, R. R., and Pidaparti, S. R. (1991) Anaerobic sequencing batch reactor treatment of swine wastes. In: *46th Purdue Industrial Waste Conference Proceedings*, Purdue University, West Lafayette, Indiana, Lewis Publishers, Inc., Chelsea, Michigan.

Dague, R. R., Habben, C. E., and Pidaparti, S. R. (1992) Initial studies on the anaerobic sequencing batch reactor. Water Science and Technology, **26**, 2429.

Dolfing, J. , Griffioen, A., van Neerven, A. R. W., and Zevenhuizen, L. P. T. M. (1985) Chemical and bacteriological composition of granular methanogenic sludge. *Can. J. Micrbiol.* **31**, 744.

Dubourguier, H. C., Prensier, G., and Albagnac, G. (1988) Structure and microbial activities of granular anaerobic sludge. In: *Granular Anaerobic Sludge; Microbiology and Technology, proceedings of the GASMAT-workshop*, (Edited by Lettinga, G, Zehnder, A. J. B., Grotenhuis, J. T. C., and Hulshoff Pol, L. W.), Wageningen, The Netherlands: Pudoc, 18.

Forster, C. F. (1971) Activated sludge surfaces in relation to the sludge volume index. *Wat. Res.*, **5**, 861.

Fox, P., and Pohland, F. G. (1994) Anaerobic treatment applications and fundamentals: substrate specificity during phase separation. *Water Environment Research*, **66**, 716.

Grobicki, A., and D. C. Stuckey, D. C. (1992) Hydrodynamic characteristics of the anaerobic baffled reactor.*Water Research*, **26**, 371.

Grotenhuis, J. T. C., van Lier, J. B., Plugge, C. M., Stams, A. J. M., and Zehnder, A. J. B. (1991) Effect of ethylene glycol-bis(ß-aminoethyl ether)-N,N-tetraacetic acid (EGTA) on stability and activity of methanogenic granular sludge. *Appl. Microbiol. Biotechnol.*, **36**, 109.

Grotenhuis, J. T. C., Plugge, C. M., Stams, A. J. M., and Zehnder, A. J. B. (1992) Hydrophobicities and electrophoretic mobilities of anaerobic bacterial isolates from methanogenic granular sludge. *Appl. Microbiol. Biotechnol.*, **58**, 1054.

Guiot, S. R., Safi, B, Frigon, J. C., Mercier, P., Mulligan, C., Tremblay, R., and R. Samson, R. (1995) Performances of a Full-Scale Novel Multiplate Anaerobic Reactor treating Cheese Whey Effluent. *Biotechnology and Bioengineering*, **45**, 398.

Gujer, W., and Zehnder, A. J. B. (1983) Conversion processes in anerobic digestion. *Wat. Sci. Tech.*, **15**, 127.

Hack, P. J. F. M, Vellinga, S. H. J., and Habets, L. H. A. (1988) Growth of granular sludge in the BIOPAQ IC-reactor. In: *Granular Anaerobic Sludge; Microbiology and Technology, proceedings of the GASMAT-workshop*, (Edited by Lettinga, G, Zehnder, A. J. B., Grotenhuis, J. T. C., and Hulshoff Pol, L. W.), Wageningen, The Netherlands: Pudoc, 211.

Hulshoff Pol, L. W., de Zeeuw, W. J., Velzeboer, C. T. M., and Lettinga, G. (1983) Granulation in UASB reactors. *Wat. Sci. Tech.*, **15**, 291.

Hulshoff Pol, L. W., and Lettinga, G. (1986) New technologies for anaerobic wastewater treatment. *Wat.Sci. Tech.*, **18**, 41.

Hulshoff, Pol L., Euler, H., Eitner, A., and Grohganz, D. (1997) GTZ sectoral project promotion of anaerobic technology for the treatment of municipal and industrial sewage and wastes. In: *Proc. 8th International Conf. on Anaerobic Digestion*, **2**, 285.

Jhung, J. K., and Choi, E. (1995) A comparative study of UASB and anaerobic fixed film reactors with development of sludge granulation. *Wat. Res.*, **29**, 271.

Lens P., van den Bosch M. C., Hulshoff Pol L. W., and Lettinga G. (1998) Effect of staging on volatile fatty acid degradation in a sulfidogenic granular sludge reactor. *Water Research*, (accepted for publication).

Lettinga, G., van Velsen, A. F. M., Hobma, S. W., de Zeeuw, W. J., and Klapwijk, A. (1980) Use of the Upflow Sludge Blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment. *Biotechnol. Bioeng.*, **22**, 699.

Lettinga, G. (1995) Anaerobic digestion and wastewater treatment systems. *Antonie van Leeuwenhoek*, **67**, 3.

Levenspiel, O. (1972) *Chemical Reaction Engineering*, 2nd ed., New York, Wiley.

van Lier, J. B., Boersma, F., Debets, M. M. W. H., and Lettinga, G. (1994) 'High rate' thermophilic anaerobic wastewater treatment in compartmentalized upflow reactors. In: *Proceedings, seventh international symposium on anaerobic digestion*, Cape Town, South Africa, 1994.

van Lier, J. B. (1995) Thermophilic Anaerobic Wastewater Treatment; Temperature Aspects and Process Stability. Ph.D. Dissertation, Wageningen Agricultural University, Wageningen, The Netherlands.

van Lier, J. B., Groeneveld, N., Lettinga, G. (1996) Development of thermophilic methanogenic sludge in compartmentalized upflow reactors. *Biotechnology and Bioengineering*, **50**, 115.

van Lier, J. B., Rebac, S., Lens, P., van Bijnen, F., Oude Elferink, S. J. W. H., Stams, A. J. M., and Lettinga, G. (1997) Anaerobic treatment of partly acidified wastewater in a two-stage expanded granular sludge bed (EGSB) system at 8°C. In: *Proc. 8th International Conf. on Anaerobic Digestion*, **2**, 86.

van Loosdrecht, M. C. M., Lyklema, J., Norde, W., Schraa, G., and Zehnder, A. J. B. (1987) The role of bacterial cell wall hydrophobicity in adhesion. *Applied and Environmental Microbiology*, **53**, 1893.

Nachaiyasit, S., and Stuckey, D. C. (1995) Microbial response to environmental changes in an Anaerobic Baffled Reactor (ABR). *Antonie van Leeuwenhoek*, **67**, 111.

Raskin, L., Stromley, J. M., Rittman, B. E., and Stahl, D. A. (1994a) Group-Specific 16S rRNA Hybridization Probes to Describe Natural Communities of Methanogens. *Applied and Environmental Microbiology*, **60**, 1232.

Raskin, L., Poulsen, L. K., Noguera, D. R., Rittman, B. E., and Stahl, D. A. (1994b) Quantification of Methanogenic Groups in Anaerobic Biological Reactors by Oligonucleotide Probe Hybridization. *Applied and Environmental Microbiology*, **60**, 1241.

Raskin, L., Zheng, D., Griffin, M. E., Stroot, P. G., and Misra, P. (1995) Characterization of microbial communities in anaerobic bioreactors using molecular probes. *Antonie van Leeuwenhoek*, **68**, 297.

Reinhold, G., Merrath, S., Lennemann, F., Märkl, H. (1996) Modelling the hydrodynamics and the liquid-mixing behaviour of a biogas tower reactor. *Chem. Eng. Sci.*, **51**, 4065.

Rinzema, A., and Lettinga, G. (1988) Anaerobic treatment of sulfate-containing waste water. In: Wise D. L. (Ed.) Biotreatment Systems, Volume III, 65, CRC Press, Boca Raton, Florida, USA.

Schmidt, J. E., and Ahring, B. K. (1994) Extracellular polymers in granular sludge from different upflow anaerobic sludge blanket (UASB) reactors. *Appl. Microbiol. Biotechnol.*, **42**, 457.

Schmidt, J. E., and Ahring, B. K. (1995a) Granular sludge formation in upflow anaerobic sludge blanket (UASB) reactors. *Biotechnology and Bioengineering*, **49**, 229.

Schmidt, J. E., and Ahring, B. K. (1995b) Interspecies electron transfer during propionate and butyrate degradation in mesophilic, granular sludge. *Appl. Environ. Microb.*, **61**, 2765.

Schmit, C. G., and Dague, R. R. (1993) Anaerobic Sequencing Batch Reactor Treatment of swine wastes at 20 C, 25 C, and 30 C. In: *48th Purdue Industrial Waste Conference Proceedings*, Purdue University, West Lafayette, Indiana, Lewis Publishers, Inc., Chelsea, Michigan.

Shen, C. F., Kosaric, N., and Blaszczyk, R. (1993) The effect of selected heavy metals (Ni, Co and Fe) on anaerobic granules and their extracellular polymeric substance (EPS). *Wat. Res.*, **27**, 25.

Skiadas, I. V., and Lyberatos, G. (1997) The periodic anaerobic baffled reactor: a novel design for anaerobic digesters. In: *Proc. 8th Internation Conf. on Anaerobic Digestion*, **3**, 240.

Speece, R. E. (1988) Advances in anaerobic biotechnology for industrial wastewater treatment. In: *Anaerobic Treatment of Industrial Wastewaters*, (Edited by Torpy, M. F), Noyes Data Corporation, Park Ridge, NJ, 1.

Stams, A. J. M. (1994) Metabolic interactions between anaerobic bacteria in methanogenic environments. *Antonie van Leeuwenhoek*, **66**, 271.

Sung, S., and Dague, R. R. (1992) Fundamental principles of the Anaerobic Sequencing Batch Reactor Process. In: *47th Purdue Industrial Waste Conference Proceedings*, Purdue University, West Lafayette, Indiana, Lewis Publishers, Inc., Chesea, Michigan.

Sung, S., and Dague, R. R. (1995) Laboratory studies on the Anaerobic Sequencing Batch Reactor. *Water Environmental Research*, **67**, 294.

Switzenbaum, M. S. (1995) Obstacles in the implementation of anaerobic treatment technology. *Bioresource Technology*, **53**, 255.

Thaveesri, J., Gernaey, K., Kaonga, B., Boucneau, G., and Verstraete, W. (1994) Organic and ammonioum noitrogen and oxygen in relation to granular sludge growth in lab-scale UASB reactors. *Wat. Sci. Tech.*, **30**, 43.

Thaveesri, J., Daffonchio, D., Liessens, B., Vandermeren, P., and Verstraete, W. (1995) Granulation and sludge bed stability in upflow anaerobic sludge bed reactors in relation to surface thermodynamics. *Applied and Environmental Microbiology*, **61**, 3681.

Thiele, J. H., and Zeikus, J. G. (1988) Control of interspecies electron flow during anaerobic digestion: significance of formate transfer versus hydrogen transfer during syntrophic methanogenesis in flocs. *Applied and Environmental Microbiology*, **54**, 20.

Toerber, E. D., Paulson, W. L, and Smith, H. S. (1974) Comparison of completely mixed and plug flow biological systems. *Journal Water Pollution Control Federation*, 46, 1995.

Vanderhaegen, B., Ysebaert, E, Favere, K., Van Wambeke, M, Peeters, T., Pánic, V., Vandenlangenbergh, V, and Verstraete, W. (1992) Acidogenesis in relation to in-reactor granule yield. *Wat. Sci. Tech.*, **25**, 21.

de Vegt, A., and Yspeert, P. (1994) Two stage UASB treatment of industrial wastewater with internal circulation (IC). In: *Proceedings, 49th Purdue Industrial Waste Conference*, Lewis Publishers, Chelsea, MI.

Verstraete, W. (1991) Anaerobic treatment of effluents: state of the art in Europe. *Mededelingen van de Faculteit Landbouwwetenschappen: Rijksuniversiteit Gent*, **56**, 97.

Wilkinson, J. F. (1958) The extracellular polysaccharides of bacteria. *Bact. Rev.*, **22**, 46.

Wirtz, R. A., and Dague, R. R. (1994) Enhancement of granulation and start-up in the anaerobic sequencing batch reactor. In: *Proc. 67th Annual Conference & Exposition: Weftec '94*, Chicago, Illinois, Water Environment Federation, Alexandria, VA, 241.

Yoda, M., and Nishimura, S. (1997) Controlling granular sludge floatation in UASB reactors. In: *Proc. 8th International Conf. on Anaerobic Digestion*, **3**, 579.

CHAPTER 2. THE ANAEROBIC MIGRATING BLANKET REACTOR: PRINCIPLES AND COMPARISON WITH UASB AND ASBR PROCESSES

A paper to be submitted to Water Research

Largus T. Angenent, Shihwu Sung and Richard R. Dague

Abstract-In this research, a 12-liter and a 54-liter anaerobic migrating blanket reactor (AMBR) were compared with 12-liter UASB and ASBR systems to study the performance and principles of the newly developed AMBR. A 12-liter AMBR was capable of achieving a maximum COD loading rate of 30 g/L/d at a 12 hour HRT, which resulted in a standard methane production rate (SMPR) of 7.0 L/L/d. In a 54-liter AMBR short-circuiting was prevented by placing baffles between the compartments instead of openings in the bottom of the inside walls, as was done for the 12-liter AMBR. This resulted in a soluble COD removal of 99% up to a COD loading rate of 23 g/L/d. Furthermore, the 54-liter AMBR was able to retain higher levels of biomass (40 gMLVSS/L) compared to the 12-liter AMBR at COD loading rates which exceeded 20 g/L/d. Although sucrose was fed as a synthetic substrate, no pre-acidification was required for the AMBR. On the contrary, in the upflow anaerobic sludge blanket (UASB) reactor the absence of pre-acidification created floating and bulking problems due to ingrowth of acidogenic bacteria. Laboratory-scale AMBRs were able to maintain and grow granular biomass, which resulted in an increase in the granule size over the operational period. A key element in the granular biomass formation of the AMBR was the migration of the biomass blanket through the reactor. Hence, flocculent biomass migrated faster and eventually washed out with the effluent. Furthermore, baffles in front of the effluent port and intermittent mixing of the final compartment increased the selection pressure for granules. Reversing the flow was required to prevent phase separation and accumulation of biomass in the final compartment. Compared with laboratory-scale ASBR and UASB reactors, the performance of the AMBR was found to be superior due to approached plug-flow conditions in the compartmentalized AMBR.

Key words-anaerobic, AMBR, UASB, ASBR, granulation, migrating blanket, methanogenesis, staging

INTRODUCTION

Anaerobic treatment of industrial and domestic wastewater proved over the last 20 years to be sustainable. Particularly, the upflow anaerobic sludge blanket (UASB) process, and its derivatives, showed good performance and stability in numerous full-scale operations

world-wide (Lettinga et al., 1980; Lettinga, 1995). However, for several reasons, other self-immobilized biomass processes were developed. For example, the loss of biomass with the effluent due to excessive bed expansion or poor granulation posed problems to non-compartmentalized reactors, such as the UASB process (Guiot et al., 1995).

Compartmentalization in anaerobic reactors was first described by Bachman et al. (1982), who developed the anaerobic baffled reactor (ABR). In this reactor, the wastewater flowed under and over vertical baffles. Since then, other compartmentalized reactors were developed, such as the horizontal-baffled anaerobic reactor (Yang and Chou, 1984), the internal circulation (IC) reactor (Hack et al., 1988), the multiplate anaerobic reactor (MPAR) (El-Mamouni et al., 1992), the "biogas turmreaktor" (Märkl and Reinhold, 1994), and the upflow staged sludge bed (USSB) reactor (van Lier, 1994). Furthermore, an upwards feeding and reversing flow pattern showed improved settling characteristics and granulation in the reversing anaerobic upflow system (RAUS). This system combined compartmentalization with a reversing flow pattern (Basu, 1995). The two-stage anaerobic unitank system (TSU-AN-system), a modification of the UASB process, combined the same characteristics (Beyen et al., 1988; Verstraete, 1991). In the above mentioned processes, a hydraulic upflow pattern was responsible for contact between substrate and biomass.

In addition to compartmentalization, a difference between the ABR and the UASB process is the absence of a special gas-solids-separator system, which simplifies the design (Bachman et al., 1985). Furthermore, the anaerobic sequencing batch reactor (ASBR) is a batch-fed process which does not rely on a hydraulic upflow pattern. This results in the absence of gas-solids-separator and feed-distribution systems (Sung and Dague, 1992; Angenent and Dague, 1995). Nevertheless, Wirtz and Dague (1994) developed a granular blanket with an ASBR in five months after seeding the reactor with non-granular primary digester sludge. This result indicated that granulation did not solely depend on a hydraulic upflow pattern. Vanderhaegen et al. (1992) also demonstrated granular formation in the absence of a hydraulic upflow pattern.

With this knowledge, a continuously fed, compartmentalized reactor that reverses its flow in a horizontal matter, was developed without the requirement of elaborate gas-solids-separator and feed-distribution systems. Effluent recycling was not required, but mixing was necessary to obtain a sufficient biomass/substrate contact. This process is known as the anaerobic migrating blanket reactor (AMBR). A key to the selection of a granular biomass in the AMBR process, and thus to the reactor performance, was found to be the migration of the biomass blanket through the reactor. A higher migration rate of flocculent biomass, compared with granular biomass, was responsible for the wash out of less settleable,

flocculent biomass. In this way, the formed aggregates were retained in the reactor and grew in size.

Research indicated that acidogenic conditions in a two-phase treatment concept showed negative effects on in-reactor granular growth (Vanderhaegen et al., 1992). Hence, the emphasis of this study was on staging, rather than on phasing of the acidogenesis and methanogenesis. In a staged process all phases of anaerobic digestion are present, but acidogenic activities will be higher in the initial compartments. Advantages of a slight pre-acidification in a staged process were postulated by Fox and Pohland (1992) and Lettinga (1995). However, the hydrogen gas partial pressure between the compartments was not uncoupled in the presented study, because the headspace was not compartmentalized. Nevertheless, plugflow conditions, which promoted partial phase separation (staging), were enhanced by compartmentalizing the mixing effects of gas production and the absence of recycling (Fox and Pohland, 1992). Notably, total phase separation in the AMBR process was prevented by reversing the flow over the horizontal plane of the reactor (Angenent and Dague, 1996).

In the presented study, the performances and principles of laboratory-scale AMBRs were studied by feeding them sucrose as a synthetic substrate. The obtained results were compared with the performances of laboratory-scale UASB and ASBR systems, which were operated under the same conditions. In doing so, a comparison between a plug-flow, a CSTR, and a batch-fed reactor system was made.

MATERIALS AND METHODS

Substrate

Sucrose was used as the main carbon and energy source in these studies. As sucrose did not contain nitrogen or essential nutrients and trace elements, additives were necessary. The nutrient stock solution consisted of 290 mL 29.4% NH_4OH/L and 68.75 g/L K_2HPO_4, and was supplied by the addition of 0.886 mL stock solution per gram of chemical oxygen demand (COD) fed. An excess of ammonium hydroxide provided an extra alkalinity source and buffering capacity. Trace-element stock solution was prepared by adding: 50 g $FeCl_2.4H_2O$; 1.25 g $ZnCl_2$; 12.5 g $MnCl_2.4H_2O$; 1.25 g $(NH_4)_6Mo_9O_{24}.4H_2O$; 3.75 $CoCl_2.6H_2O$; 2.5 g $NiCl_2.6H_2O$; 0.75 g $CuCl_2.2H_2O$; and 1.25 g H_3BO_3 into one liter tap water. This trace-element stock solution was added to the feed at a rate of 0.089 mL/gCOD fed. In addition, alkalinity was added to the sucrose solution in the form of sodium bicarbonate (0.45 g/gCOD), and yeast extract was added to provide for essential growth factors (1 mg/gCOD). The make-up water (City of Ames tap-water) contributed more

essential nutrients, such as calcium, magnesium, and sulfate. During the first two weeks of the start-up of the UASB and the ASBR processes, a solution of non-fat dry milk (NFDM) was used as the feed. However, due to foaming problems in the UASB reactor, the substrate was changed to sucrose.

Analysis

The composition of the biogas was measured using gas chromatography (GC; Gow Mac Model 350 with thermal conductivity detector; Column: 6'*1/8' stainless steel Poropack Q 80/100 mesh). The individual volatile fatty acids (VFAs) were measured by GC (HP 5730A with a flame ionization detector; Column: 6ft*2mm, silanized glass Carbopack C 60/80 mesh). The total alkalinity, total VFAs, total and soluble COD, sludge volume index (SVI), and total and volatile suspended solids (VSS) were performed according to procedures in *Standard Methods* (APHA, 1985). Effluent samples of the AMBR process were obtained at the midpoint of the time interval between two reversals of flow. At this point, the parameters were assumed to be representative of the overall performance. The Yield (Y) of biomass calculated in Table II, equaled the net biomass produced relative to the SCOD removed.

Biomass characteristics

The specific methanogenic activity (SMA) was assessed using the "headspace method" according to tests described by Rinzema et al. (1988). To analyze the sizes of the granules and any changes over time, the arithmetic mean diameter was calculated with automated image analysis (AIA). Samples of the mixed liquor of the reactor were mixed and diluted to obtain an overall distribution of clearly visible, non-overlapping biomass particles. Next, 1.75 mL was added to a AIA-glass, which consisted of two 3 mm thick glass sheets cemented together, with a one inch circle in the top sheet. This was further covered with a thin sheet, avoiding air bubbles. The AIA set-up contained a black and white video camera (Dage-MTI series 68), a microscope (Olympus SZH), and a PC with Quartz PCI Imaging software. Particles smaller than 0.1 mm were not included in the calculations of the size distribution (Grotenhuis et al., 1991).

Assessment of the standard methane production rate and calculated TCOD removal

The COD loading rate was the amount of COD that was fed into the system per reactor volume per day (g/L/d). The biogas production was corrected to standard temperature and pressure (STP) using the ideal gas law. Next, the standard methane production rate

(SMPR) was obtained after converting the biogas production with the wet volume of the reactor and the methane percentage that was present in the biogas. Therefore, the SMPR was expressed as liters of methane per reactor volume per day (L/L/d). The SMPR was a true measure of the COD that was being removed, because methane is the final product in the stabilization of COD (0.35 L methane/gCOD). However, COD removal by sulphate reducing bacteria (SRB) and methane loss due to its solubility in the effluent was not included in the following formula. Furthermore, COD removal due to biomass growth was not included because biomass wash-out was accounted for by the measured TCOD. To obtain the theoretical or calculated total COD removal efficiency (calculated TCOD removal) the following formula was used:

$$\text{Calculated TCOD removal, \%} = \frac{\text{SMPR}}{\text{COD loading rate} \cdot 0.35} \cdot 100$$

Laboratory-scale reactor studies

All systems were placed in a constant temperature room at 35°C (+/- 1°C). The concentrated substrate was stored in a refrigerator, to prevent pre-acidification, and was mixed to obtain a constant loading rate. Make-up water (35°C) was added to the substrate just before feeding to the reactors. To compare the different reactor systems, operational parameters for all reactors were maintained as close as possible. The hydraulic retention time (HRT), for example, was kept constant at 12 hours throughout all studies. The COD loading rate was increased in a stepwise manner, by increasing the sucrose feed concentration, as soon as the effluent VFA concentration, pH in the reactor, and calculated TCOD removal were lower than 0.3 g/L, higher than 6.5, and approximately 80%, respectively (without any other limiting factors). Therefore, the reactors seldom operated under steady-state conditions. After an increase in the COD loading rate was implemented, the systems were given time to adjust to the new conditions. All pumps used, were Masterflex pumps of Cole Parmer Instrument Co., Chicago, Illinois, USA. The gas collection systems consisted of an observation bottle, a bottle packed with steel wool to scrub hydrogen sulfide from the biogas, a gas sampling port, and a wet-test gas meter (GCA, Precision scientific, Chicago, Illinois, USA) or wet-tip gas meter (Rebel wet-tip gas meter company, Nashville, Tennessee, USA). Programmable timers (ChronTrol Corporation, San Diego, California, USA) were used to control the reactor operation. Table I shows the operational parameters of the 54-liter AMBR (AMBR54), the 12-liter AMBR (AMBR12), the 12-liter UASB reactor, and the 12-liter ASBR.

Studies with the 54-liter AMBR

The active volume of the laboratory-scale AMBR was 54 liters and was divided into three compartments, as illustrated in Figure 1. Substrate flowed horizontally into one end of the reactor and out the other end. Since the final compartment received the lowest substrate concentration, the activity of the microbes in this compartment was low. This resulted in low biogas production, which enabled the final compartment to serve as an internal clarifier and prevented biomass loss in the effluent. The biomass, illustrated in Figure 1 as shaded areas, tended to migrate into the final compartment. To prevent total phase separation and accumulation of biomass in the final compartment, the flow was reversed. The final compartment became the initial compartment and the process repeated itself. Two automatic ball valves, with an internal diameter of one inch, were used to open and close effluent ports (True blue electric actuator model EBV-6, Plast-o-matic valves Inc., Cedar Groove, New Jersey, USA). Three compartments were required in the AMBR to feed the middle compartment for a certain period of time before the flow was reversed. In this way, a break-through of substrate could be prevented. Thus, the middle compartment was fed for two hours between reversing the flow. Sufficient biomass/substrate contact was maintained using intermittent, gentle mixing. Research by Dague et al. (1970) showed that mixing that was too intense could destroy the anaerobic bioflocs. All three compartments were mixed equally for ten seconds every seven minutes (Mixers: Model 5vb, EMI Inc., Clinton, Connecticut, USA). These mixers, equipped with paddles, were able to start and operate at a slow speed to ensure gentle mixing. The biogas was directly discharged from the reactor to the gas collection system. A water lock was installed on the effluent tubes to prevent biogas from escaping through the effluent ports. Slanted baffles were placed in front of the effluent ports. The initial reactor set-up had vertical, movable walls between the compartments. In this way, the size of the opening in the bottom of the inside wall was variable. However, after 76 days of operation the openings in the inside walls were closed and baffles were placed between the compartments (see Figure 1). The pH was monitored by probes in the reactor (pH probe : Fermprobe pH-electrode (210 mm), Phoenix electrode Co., Houston, Texas, USA; pH-controller: Model PHCN-425, Omega engineering Inc., Stamford, Connecticut, USA).

The initial seed for the reactor was collected from the effluent out of the 12-liter AMBR and consisted of flocculent and granular biomass. This had been stored in a 4°C refrigerator for five months before it was seeded. The COD loading rate was kept constant at 10 g/L/d for the first 90 days to study different reactor configurations. After 90 days, the COD loading rate was increased.

Studies with the 12-liter AMBR

The active volume of this laboratory-scale AMBR was 12 liters. Two openings, with a diameter of one inch, were placed on the bottom of each wall between the compartments. These openings were placed in a way to create good biomass/substrate contact, to ensure migration of biomass, and to reduce short-circuiting of substrate. Impeller mixers were installed in all three compartments (Mixers: model 5vb EMI, Inc., Clinton, CT, USA; Lightning A-310 axial flow impeller). The effluent ports of the reactor were connected to a gas-liquid-separation tank. Biogas was discharged at the top of this tank to a gas collection system. The liquid flowed out of the separation tank through a water lock into a settling tank. Baffles before the effluent ports were glued in the reactor after 30 days of operation.

The initial seed for the 12-liter AMBR was collected from the 12-liter ASBR. The biomass had been stored in a 4°C refrigerator for four months before it was seeded. The COD loading rate at the start-up was 8 g/L/d.

Studies with the 12-liter UASB reactor

For the UASB reactor, a Plexiglas column was utilized with a height of one meter and an inside diameter of 14 cm. One inlet point for the feed was located in the bottom center. Walls slanted from the bottom inlet point at an angle of approximately 45 degrees. The bottom 10 cm of the reactor was filled with marbles to achieve a good distribution of the feed and an equal upflow velocity in the reactor. Recycling was used to create a sufficient upflow velocity in the reactor. During start-up of the UASB reactor, the upflow velocity was set at 0.7 meter per hour. This had to be increased to one meter per hour to avoid trapping of biogas in the sludge blanket at the higher COD loading rates. An inverted funnel (outside diameter of 13 cm) was installed at about 3/4 of the reactor height above a rim (inside diameter of 11 cm) to create the gas-solids-separator system, preventing the escape of gas between the reactor wall and the funnel. The funnel was connected to a foam separation and observation bottle. The pressure, and thus the height of the water surface in the funnel, was easily manipulated by changing the water level in the observation bottle. A recycling tube was placed at about the 1/3 depth point of the settling section of the UASB reactor. Above this point, the hydraulic upflow velocity in the settling section resulted only from the amount of feed pumped into the reactor, which was low enough for internal settling of biomass. Gravity was used as the force to discharge effluent.

Seed biomass was collected from three different sources to be sure of getting a balanced microbial population. Two-thirds of the biological seed was granular sludge from a full-scale UASB reactor (G. Heileman Brewery, La Crosse, Wisconsin, USA); one sixth were

granules collected from a laboratory-scale ASBR system using NFDM as a substrate; and the rest of the biomass originated from a pilot-plant ASBR (Penford; a starch producing factory, Cedar Rapids, Iowa, USA). The COD loading rate at the start-up of the UASB was 6 g/L/d.

Studies with the 12-liter ASBR

For the ASBR, a Plexiglas column with a volume of 13 liters and an inside diameter of 14 cm. The one liter headspace was connected to the gas collection system. Additions to the regular gas collection system were an aspirator bottle to collect and to distribute foam, and a gas bag (ball) to prevent a pressure drop in the headspace during decanting of effluent. A pump was used to intermittently recirculate biogas from the aspirator bottle through the diffuser ring in the bottom of the reactor to provide mixing of the water contents. The ASBR was mixed for two minutes every half hour. The ASBR sequenced through four steps; feed, react, settle, and decant, as described by Sung and Dague (1992). The cycle time for the 12-liter ASBR was four hours, resulting in six sequences per day. The settling time before the decant step was found to be very important. This time was chosen too long at the start-up of the 12-liter ASBR and caused severe wash-out of biomass, because the entire blanket rose during the decant step due to the formed biogas in this time period. Next, biomass that was collected in the settling tank of the ASBR had to be reseeded after one week of operation. At the same time, the length of the settling time was shortened to two minutes. The time required for settling varied, depending on biomass concentration, settleability of the biomass, and reactor height and ranged from a few minutes to an hour. Thus, the settling time had to be short to wash out the poorly settling biomass, but not so short that granular biomass was washed out. Following these concepts, an optimal settling time was found that selected for and enhanced granulation. The biomass seed was the same as used for the start-up of the 12-liter UASB and the COD loading rate at the start-up period was 6 g/L/d.

RESULTS

Studies with the AMBR

The performance of the 54-liter AMBR is shown in Figure 2. Baffles between the compartments, instead of openings in the bottom of the inside walls, were placed in the 54-liter AMBR at day 76. This reduced short-circuiting and thus increased the soluble COD removal efficiency (SCOD removal) to 99% up to a loading of 23 gCOD/L/d. The data for the measured total COD removal efficiencies (measured TCOD removals) was obtained with COD tests. During most of the operational time, the calculated TCOD removal followed the same trend as the measured TCOD removal, as it should. However, placing baffles between

the compartments decreased migration of biomass, which resulted in biomass accumulation and higher measured TCOD removals for about two weeks. At a COD loading rate of 23 g/L/d the calculated TCOD removal was around 80% and the SMPR was 6.0 L/L/d. Maximum COD loading rates were reached, since further increase in the COD loading rate showed a severe decrease in TCOD and SCOD removals. To prevent these unstable performances, the COD loading rate was decreased to 20 g/L/d at day 136 and the number of reversals of flow was increased to three times per day. After calculated TCOD removals were exceeding 70% again, the reactor was shut down. Figure 3 shows the increase in suspended solids levels over the operational time. Although the mixed liquor volatile suspended solids (MLVSS) is not a true measure of the biological active mass in the reactor but rather an indication, it is clear that biomass levels were increasing over time. At the end of operation, the MLVSS in the reactor was 40 g/L and the AMBR was retaining the granular biomass. To examine the individual VFAs concentrations in the compartments of the 54-liter AMBR during a reverse in flow, the VFAs were measured at a COD loading rate of 17 g/L/d which are shown in Figure 4. In Figure 4, the right compartment was fed for the first half hour, but it became the final compartment after subsequent feeding the middle compartment for two hours. After feeding ceased in the right compartment, VFA concentrations decreased. On the contrary, the VFA concentrations increased rapidly after the left compartment became the initial compartment. This shows VFA gradients over the horizontal plane of the AMBR. Moreover, propionic acid concentrations were high in the initial compartment but leveled off in the final compartments. The pH inside the initial compartment was always higher than 6.2 to prevent total phase separation. However, pH levels inside the final compartment were approximately seven. The SMA of the biomass in the 54-liter AMBR decreased over the operational time, as seen in Figure 5. The seed biomass which originated from the 12-liter AMBR, had a higher SMA because it was developed at a sludge loading rate (SLR) of 1.9 gCOD/gVSS/d (Figure 5). However, the SLR was only around 0.5 gCOD/gVSS/d for the 54-liter AMBR because of lower COD loading rates and higher biomass concentrations, as seen by comparing Figure 3 and 7. Because of a decrease in SLR of the biomass, the SMA decreased over time. To prevent the decrease of the SMA and thus promote the ability to increase the loading rate faster, the SLR should be increased by increasing the COD loading rate from the beginning or wasting biomass periodically. Significant differences in SMA of biomass between the compartments were not detected.

The performance of the 12-liter AMBR is shown in Figure 6. Over the operational time, the COD loading rate was increased to 30 g/L/d. At this COD loading rate, the SCOD

removal decreased to 90%, which showed that maximum COD loading rates were reached for these operational conditions. In addition, the calculated TCOD removal was around 70% and the SMPR of the 12-liter AMBR was 7.0 L/L/d, as seen in Figure 7. Because of a high migration of the granular biomass in the 12-liter AMBR the flow had to be reversed three times a day in which the MLVSS did not exceed 16 g/L, as seen in Figure 7. Consequently, the 12-liter AMBR was operated at SLRs which exceeded 1.5 gCOD/gVSS/d.

Both laboratory-scale AMBRs were capable of maintaining and growing a highly settleable granular biomass, which resulted in an increase in the granule size over the operational time, as seen in Figure 8. At the end of operation, the arithmetic mean diameter of the granules in the 12-liter and 54-liter AMBR were 0.74 and 0.82 mm, respectively. At the start of operation of the 12-liter AMBR, it was noticed that flocculent biomass accumulated in the final compartment whenever the final compartment was not mixed. After starting intermittent mixing of the final compartment, the flocculent biomass washed out of the AMBR with the effluent, slowly increasing the arithmetic mean diameter of the biomass. Moreover, placing baffles in front of the effluent ports prevented floating granules of washing out the AMBR. The SVI of the granular biomass of the 54-liter AMBR was 16.3 mL/gVSS at the end of the operation.

Comparison of the AMBR, UASB reactor, and the ASBR

All laboratory-scale reactors were operated in the same way to compare the reactor performances. Figures 9 and 10 show reactor performance of the UASB reactor and ASBR, respectively. For the UASB reactor, the SCOD removal exceeded 95% at a COD loading rate of 20 g/L/d. However, due to formation of a fluffy granular biomass in the UASB reactor, at these COD loading rates, rising of the entire blanket prevented an increase in the load. Moreover, unstable conditions were noticed after the synthetic waste of the 12-liter UASB reactor was changed from NFDM to sucrose at day 14, but the performance improved again at day 40. Deterioration of the performance due to this change in synthetic waste was not noticed for the 12-liter ASBR. At the end of the operational time, the calculated TCOD removal of the ASBR decreased to 60% at a COD loading rate of 19 g/L/d. Therefore, it was concluded that the maximum COD loading rate was achieved at these operational conditions. The 12-liter UASB and ASBR processes achieved lower maximum COD loading rates compared with the 12-liter AMBR, which achieved a COD loading rate of 30 g/L/d.

To make a comparison, the reactor performances of the AMBR, UASB reactor, and ASBR at a COD loading rate of approximately 20 g/L/d are shown in Table II. Calculated TCOD removals were 76% and 78% for the AMBR, compared with 70% and 59% for the

UASB reactor and ASBR, respectively. The SCOD removal of 99% for the 54-liter AMBR was competitive compared with the UASB process (97%). The SCOD removal of the 54-liter AMBR was higher than for the 12-liter AMBR as a result of less short-circuiting. Due to a high retention of biomass in the UASB reactor, surplus biomass had to be removed on day 62, 77, and 85, not to plug the gas-solids-separator system. However, surplus biomass was automatically washed out of the AMBR and ASBR systems. This was done to compare the measured and calculated TCOD removal. However for practical operation of the ASBR or AMBR, surplus biomass should be wasted out of the reactor periodically to decrease the effluent solids concentration. The arithmetic mean diameter of the biomass in the UASB reactor was much higher than in the AMBR and ASBR systems. However, granules in the AMBR and ASBR systems tended to be more dense than granules in the UASB reactor, which were visually more fluffy. Also, granules in the UASB reactor were gray, indicating a higher association with the acidogens (Daffonchio et al., 1995).

DISCUSSION

Due to mechanical mixing, compartmentalized biogas production, and the absence of recycling, the AMBR might be characterized as a series of completely mixed compartments, which approached plug-flow conditions. VFA concentrations in the compartments (Figure 4) and the pH gradient over the length of the reactor confirmed these plug-flow conditions. Moreover, the feast and famine conditions for the biomass in the AMBR process resulted in high substrate utilization rates in the initial compartment followed by low substrate utilization rates in the final compartment. Low substrate concentrations in the final compartment were responsible for enhanced internal settling of the granular biomass and for a high treatment efficiency. Conversely, due to recycling and biogas production in one vessel, the UASB reactor approached CSTR conditions (Guiot and van den Berg, 1985). Moreover, the ASBR is a batch-fed system with the same advantages as a plug-flow reactor, but approached CSTR conditions during the feed sequence, as described by Sung and Dague (1995). Therefore, from a strictly kinetic standpoint, the AMBR performance was superior to both the UASB reactor and ASBR, because with chemical reactions of the first or higher order, reactors with a plug-flow pattern were more effective than completely mixed reactors (Levenspiel, 1972). However, a very good biomass/substrate contact due to gentle, continuous mixing conditions in the UASB reactor could be the explanation for the comparable performance of the UASB reactor. The performance of the ASBR was limited by high VFA concentrations and resulting low pH values just after feeding the substrate.

Therefore, shorter feed/decant cycles and a longer feeding period per cycle could have resulted in more favourable conditions in the reactor.

Methanogenesis inside the initial compartment of the AMBRs was maintained by keeping the pH higher than 6.2 due to reversing the flow. In this way, recycling and addition of enormous amounts of buffer were prevented. Consequently, ideal conditions for the methanogens were created and fluffy acidogens were quickly washed out of the system after the initial compartment became the final compartment. Granules in the UASB-reactor tended to be gray and fluffy, while granules in the AMBR and ASBR seemed to be black, small, and dense due to higher shear forces and grazing of the acidogens. Resulting wash out of the filamentous acidogens for the AMBR and ASBR could be the explanation for the bigger difference between SCOD and measured TCOD removal compared with the UASB-reactor, even before surplus biomass was formed in the AMBR and ASBR systems, as seen in Figures 2, 6, 9, and 10 (Zilverentant, 1996). The fluffy granules in the UASB reactor created problems, such as bulking and rising of the blanket. Alphenaar (1994) mentioned that to avoid these problems in the UASB process, pre-acidification of sucrose is necessary. Furthermore, Alphenaar (1994) found that for non-acidified sucrose a maximum SLR of 0.6 gCOD/gVSS/d could be applied. Not surprisingly, fluffy biomass was found in the UASB reactor which was operated at a SLR of 1.6 gCOD/gVSS/d. Next, a SLR of only 0.4 gCOD/gVSS/d in the ASBR could be responsible for not having biomass floatation problems. However, no bulking or biomass floatation due to acidogenic bacteria was found in the 12-liter AMBR with a SLR as high as 1.6 gCOD/gVSS/d. It is likely that higher shear force and biomass wash-out in the AMBR and ASBR were responsible for the absence of problems associated with acidogenic bacteria. Therefore, the AMBR and ASBR systems are not dependent on a pre-acidification step. In addition to the foregoing, Fox and Pohland (1995) postulated that different wastewaters had different needs for pre-acidification. Consequently, the change from NFDM to sucrose for both the UASB reactor and ASBR, deteriorated the performance of the UASB reactor only. This occurred due to a change in the microbial population towards the growth of filamentous acidogens and the formation of a more fluffy granular biomass in the UASB reactor.

At high COD or hydraulic loading rates (HLRs), the migration of the biomass in the AMBR needed to be controlled to limit the frequency of reversing the flow. High COD loading rates increased turbulence due to biogas production in the initial compartments, and subsequently increased the BMR. Increasing the size of openings in the bottom of the inside walls or placement of baffles between compartments reduced the BMR. Openings in the bottom of the walls between the compartments could be used for systems with a long HRT.

At short HRTs, baffles should be used to reduce migration and to prevent the substrate from short-circuiting in the reactor. It should be realized that the BMR should be high enough to wash out flocculent biomass and select for a granular biomass. However, migration of the granules is not totally necessary. This could ultimately result in staging of the biomass in which relatively more acidogens are present in the outer compartments. Staging of biomass was not found in the AMBR of this study, because migration of biomass and reversing the flow prevented this. Even at a low BMR in the AMBR, the extent of staging of biomass will probably be not as extensive as in unidirectional, compartmentalized systems, such as the ABR and USSB systems. This could be an advantage when maintaining a correct balance of bacterial populations in all compartments (Flamming et al., 1997). It needs to be noticed that when wastewater contains solids, a higher BMR is required for separating and washing out these solids to prevent accumulation of refractory solids.

The maximum COD loading rate for the 54-liter AMBR was found to be lower compared with the 12-liter AMBR. This could have been the result of increasing the COD loading rate too fast, for the biomass was used to be fed only at a SLR of 0.5 gCOD/gVSS/d. Also, a longer interval time between reversing the flow for the 54-liter AMBR could have been a factor, because the flow was reversed once and three times per day for the 54-liter and the 12-liter AMBR, respectively. Moreover, reversing the flow was probably responsible for higher removal efficiencies of the AMBR compared with the compartmentalized ABR. As Bachman et al. (1985) found a SMPR exceeding 6 L/L/d at a COD loading rate of 36 g/L/d for the ABR treating sucrose, while the 12-liter AMBR achieved a SMPR of 7 L/L/d at a lower COD loading rate of 30 g/L/d. The reversing flow cycle length of the AMBR is regulated by either the HLR or the COD loading rate. For the HLR, biomass levels in the initial compartment will be the regulating factor, especially at a low HRT for low-strength wastewater. At higher COD loading rates, the pH and the VFA concentration in the initial compartment will be the regulating factor, since the VFA production will take place mainly in the initial compartment. This indicates that biomass levels or the pH in the initial compartment could be used to determine the cycle length of time between the reversals of flow to obtain optimal operating conditions. The length of the interval of time for the TSU-AN system was found to be limited by the HLR and was normally between 90 and 180 minutes (Beyen et al. 1988).

If operated semi-continuously, the AMBR system could consist of a minimum of two compartments. However, if plugflow conditions are desired, three, four or even five compartments could imply more favourable conditions for operation of the AMBR process. The choice of the design for this new reactor type will be heavily dependent on the

wastewater conditions and cost factors. Possible advantages of more than three compartments includes smaller BMRs, less chance of short-circuiting, and operation in a step feed mode for high strength wastewaters during shock loadings. In addition, more difficult compounds, such as the intermediate propionate, could find a more optimal environment for degradation. Therefore, a staged process could provide higher process stability, as was postulated by Van Lier et al. (1994), especially at thermophilic conditions and with compartmentalized headspaces. Flamming et al. (1997) found high stability of the AMBR during a shock-load in which the reactor maintained plugflow conditions. Research with a sulfate rich wastewater showed a hydrogen sulfide gradient over the horizontal plane of the AMBR mainly due to a pH gradient. However, a compartmentalized headspace in the AMBR could be more beneficial regarding the stripping effect of intermediates (hydrogen sulfide and hydrogen) in the initial compartments (Flamming et al., 1997).

Ongoing research with the AMBR showed granulation after seeding the reactor with flocculent digester sludge (Angenent et al., 1997). Furthermore, compartmentalization of the headspace increased reactor stability and performance (not yet published data). Future research will include low strength wastewater, thermophilic conditions, shock-loading, and bacterial composition over the length of the reactor (staging of the biomass). Scale-up factors and future research will probably change the optimum design for a full-scale AMBR.

CONCLUSIONS

Based on laboratory studies with anaerobic systems fed non-acidified sucrose as a substrate at mesophilic conditions, the next conclusions were drawn:

In terms of stabilization of organic matter, the laboratory-scale AMBR was highly efficient with SCOD removals of 99% up to loadings of 23 gCOD/L/d at an HRT of 12 hours for a 54-liter AMBR, which resulted in a SMPR of 6.0 L/L/d. A SMPR of 7.0 L/L/d was found for a 12-liter AMBR at a COD loading rate of 30 g/L/d after 110 days of operation with sucrose as a synthetic waste. However, SCOD removals were 95% for the 12-liter AMBR. Higher SCOD removals were found for the 54-liter AMBR because of baffles between the compartments, which prevented short-circuiting and slowed the migration of biomass in the reactor. In addition, the performance of the AMBR was superior to both the UASB reactor and ASBR with regard to maximum COD loading rates, SMPR, and SCOD removals.

The AMBR was capable of maintaining and growing a highly settleable granular biomass, which resulted in an increase in the arithmetic mean diameter of the granules over the operational time. Both intermittent mixing of the final compartment and baffles in front

of the effluent port, had a positive effect on the selection pressure. Granules in the AMBR and ASBR systems tended to be darker in colour, smaller, and more dense than granules in the UASB reactor, which were light gray and fluffy due to the presence of filamentuous acidogens. Problems related to the fluffy biomass, such as bulking and biomass floatation, were noticed in the UASB-reactor. However, the absence of these problems in the ASBR and AMBR made pre-acidification superfluous for these systems. Moreover, the AMBR was able to maintain high levels of biomass (40 gMLVSS/L) even at high COD loading rates up to 23 g/L/d for the 54-liter AMBR.

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REFERENCES

Alphenaar P. A. (1994) Anaerobic granular sludge: Characterization and factors its functioning. Ph.D. dissertation, Wageningen Agricultural University, Wageningen, The Netherlands.

Angenent L. T. and Dague R. R. (1995) A laboratory-scale comparison of the UASB and ASBR processes. In: *Proc. of the 50th Purdue Industrial Waste Conference* (Edited by Dalton C. S. and Wukasch R. F.), pp. 365-377, Ann Arbor Press, Chelsea, Michigan, USA.

Angenent L. T. and Dague R. R. (1996) Initial studies on the anaerobic migrating blanket reactor. In: *Proc. of the 51st Purdue Industrial Waste Conference* (Edited by Dalton C. S. and Wukasch R. F.), pp. 271-288, Ann Arbor Press, Chelsea, Michigan, USA.

Angenent L. T., Sung S. and Dague R. R. (1997). Start-up and granulation of the anaerobic migrating blanket reactor (AMBR). In: *Proc. of the WEFTEC'97*, pp. 113-124, Water Environment Federation, Alexandria, Virginia, USA.

APHA (1985) *Standard Methods for the Examination of Water and Wastewater* , 16th edition. American Public Health Association, Washington, D. C., USA.

Bachman A., Beard V. L. and McCarty P. L. (1982) Comparison of fixed-film reactors with a modified sludge blanket reactor. In: *Proc. of the first international Conference on fixed-film Biological processes* (Edited by Wu Y. C. and Smith E. D.), pp. 1192-1211, Noyes Publications, Park Ridge, NJ, USA.

Bachman A., Beard V. L. and McCarthy P. (1985) Performance characteristics of the anaerobic baffled reactor. Water Res. **19**, 99-106.

Basu S. K. (1995) Innovative wastewater treatment using reversing anaerobic upflow system (RAUS). In: *Proc. of the 50th Purdue Industrial Waste Conference* (Edited by Dalton C. S. and Wukasch R. F.), pp. 379-383, Ann Arbor Press, Chelsea, Michigan, USA.

Beyen G., Delaplace P., Ghekiere S., Mertens M. and Vriens L. (1988) The anaerobic unitank system for advanced wastewater treatment: pilot- and full-scale experience on brewery wastewater. *Mededelingen van de Faculteit Landbouwwetenschappen: Rijksuniversiteit Gent* **53**, 1927-1938.

Daffonchio D., Thaveesri J. and Verstraete W. (1995) Contact angle measurement and cell hydrophobicity of granular sludge form upflow anaerobic sludge bed reactors. *Applied and Environmental Microbiology*, **61**, pp. 3676-3680.

Dague R. R., McKinney R. E. and Pfeffer J. T. (1970) Solids retention in anaerobic waste treatment systems. *J. Water Pollut. Control Fed.* **42**, Part 2, R29-R45.

El-Manouni R., Rouleau D., Mayer R., Guiot S. R. and Samson R. (1992) Comparison of the novel multiplate anaerobic reactor with the upflow anaerobic sludge blanket reactor. In: *Proc. of the 46th Industrial Waste Conference* (Edited by Dalton C. S. and R. F. Wukasch), pp. 681-687, Lewis Publishers, Chelsea, Michigan, USA.

Flamming J. J., Angenent L. T., Sung S. and Dague R. R. (1997) Treatability of two different industrial wastewater streams using the anaerobic migrating blanket reactor. In: *Proc. of the 52nd Industrial Waste Conference.* Ann Arbor Press, Chelsea, Michigan, USA.

Fox P. and Pohland F. G. (1994) Anaerobic treatment applications and fundamentals: substrate specificity during phase separation. *Water Environ. Res.* **66**, 716-724.

Grotenhuis J. T. C., Kissel J. C., Plugge C. M., Stams A. J. M. and Zehnder A. J. B. (1991) Role of substrate concentration in particle size distribution of methanogenic granular sludge in UASB reactors. *Wat. Res.* **25**, 21-27.

Guiot S. R. and van den Berg L. (1985) Performance of an upflow anearobic reactor combining sludge blanket and a filter treating sugar waste. *Biotechn. Bioeng.* **27**, 800-806.

Guiot S. R., Safi B., Frigon J. C., Mercier P., Mulligan C., Tremblay R. and Samson R. (1995) Performances of a full-scale novel multiplate anaerobic reactor treating cheese whey effluent. *Biotechnol. Bioeng.* **45**, 398-405.

Hack P. J. F. M., Vellinga S. H. J. and Habets L. H. A. (1988) Growth of granular sludge in the BIOPAQ IC-reactor. In: *Proc. GASMAT Workshop* (Edited by Lettinga G., Zehnder A. J. B., Grotenhuis J. T. C. and Hulshoff Pol L. W.), pp. 211-215, Pudoc, Wageningen, The Netherlands.

Lettinga G., van Velsen A. F. M., Hobma S. W., de Zeeuw W. J. and Klapwijk A. (1980) Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment. *Biotechnol. Bioeng.* **22**, 699-734.

Lettinga G. (1995) Anaerobic digestion and wastewater treatment systems. *Antonie van Leeuwenhoek* **67**, 3-28.

Levenspiel O. (1972) *Chemical Reaction Engineering*. 2nd ed., New York, Wiley

Märkl H. and Reinhold G. (1994) Der Biogas-Turmreaktors, ein neues reaktorkonzept für die anaeroben abwasserreinigung. *Chem.-Ing.-Tech.* **66**, 534-536. In German.

Rinzema A., van Lier J. B. and Lettinga G. (1988) Sodium inhibition of acetoclastic methanogens in granular sludge from a UASB reactor. *Enzyme and Microb. Technol.* **10**, 24-32.

Sung S. and Dague R. R. (1992) Fundamental principles of the Anaerobic Sequencing Batch Reactor Process. In: *Proc. of the 47th Purdue Industrial Waste Conference* (Edited by Dalton C. S. and Wukasch R. F.), pp. 393-407, Lewis Publishers, Chelsea, Michigan, USA.

Sung S. and Dague R. R. (1995) Laboratory studies on the anaerobic sequencing batch reactor. *Water Environ. Res.* **67**, 294-310.

Vanderhaegen B., Ysebaert E., Favere K., van Wambeke M., Peeters T., Pánic V., Vandenlangenbergh V. and Verstraete W. (1992) Acidogenesis in relation to in-reactor granule yield. *Wat. Sci. Tech.* **25**, 21-30.

Van Lier J. B., Boersma F., Debets M. M. W. H. and Lettinga G. (1994) 'High-rate' thermophilic anaerobic wastewater treatment in compartmentalized upflow reactors. In: *Proc. seventh international symposium on anaerobic digestion*, Cape Town, South Africa, pp. 338-347. *Wat. Sci. Technol.* **30**, 251-261.

Van Lier J. B. (1996) Limitations of thermophilic anaerobic wastewater treatment and the consequences for process design. *Antonie van Leeuwenhoek*, **69**, 1-14.

Verstraete W. (1991) Anaerobic treatment of effluents: state of the art in Europe. *Mededelingen van de Faculteit Landbouwwetenschappen: Rijksuniversiteit Gent*, **56**, 97-111.

Wirtz R. A. , Dague R. R. (1994) Enhancement of granulation and start-up in the anaerobic sequencing batch reactor. In: *Proc. 67th Annual Conference of the WEF*, Chicago, Illinois, USA, pp. 241-252.

Yang P. Y. and Chou C. Y. (1984) Horizontal-baffled anaerobic reactor for treating diluted swine wastewater. In: *Proc. of the 39th Purdue Industrial Waste Conference* (Edited by Bell J. M.), pp. 585-597, Ann Arbor Science Book, Butterworth publishers, Boston, USA.

Zilverentant A. G. (1996) Personal communication.

Table I. Operational parameters

Operational parameters	(.)	AMBR54	AMBR12	UASB	ASBR
Temperature	(°C)	35	35	35	35
pH minimum	units	6.2	6.2	6.5	6.5
HRT	(d)	0.5	0.5	0.5	0.5
Volume	(L)	54	12	12	12
Flow of influent	(L/d)	108	24	24	24
Concentrated substrate flow	(L/d)	10.8	2.1	1.8	1.8
Dilution (make-up water)	(-)	10	11	13	13
Upflow velocity	(m/h)	0	0	0.7-1	0
Recycle	(L/d	0	0	240	0
No. of reversals in flow	(1/d)	1-3	3	0	0
COD loading rate at start	(g/L/d)	10	8	6	6
COD concentration influent*	(g/L)	5-12.5	4-15.5	3-11.5	3-9.5

* Concentration of influent after dilution with make-up water, but without recycling.

Table II. Comparison of the AMBR, UASB reactor, and ASBR at a COD loading rate of approximately 20 g/L/d

Parameters of performance	(.)	AMBR54	AMBR12	UASB	ASBR
at a COD loading rate of	(g/L/d)	21	21	19.5	18.9
HRT	(d)	0.5	0.5	0.5	0.5
MLVSS	(g/L)	40	13	12	30
VSS of effluent	(g/d)	142.6	35.3	4.3	34.7
Sludge retention time (SRT)	(d)	15	5	NA	10
Yield	(gVSS/gCOD)	0.15	0.16	NA	0.16
SLR	(gCOD/gVSS/d)	0.5	1.6	1.6	0.4
Effluent VFA (as acetic acid)	(g/L)	0.075	0.19	0.120	0.360
SCOD removal	(%)	99	95	97	94
Measured TCOD removal	(%)	80	78	96	80
Calculated TCOD removal	(%)	76	78	70	59
SMPR	(LCH_4/L/d)	5.6	5.7	4.8	3.9
Arithmetic mean diameter	(mm)	0.82	0.74	2.9	0.8
Maximum COD loading rate	(g/L/d)	23	30	21	19
BMR	(gVSS/L/d)	6	30	NA	NA

Table III. Reactor characteristics

Characteristics	AMBR	UASB	ASBR
Reactor type	plug-flow	CSTR	batch-fed
Mixing	mechanical	recycling effluent	mechanical
Pre-acidification required	no	yes	no
Surplus biomass	washed out	manually removed	washed out
Short-circuiting	possible	possible	not possible
Staging	possible	not possible	not possible
Granules (non acidified)	black; small; dense	grey; big; fluffy	black; small; dense

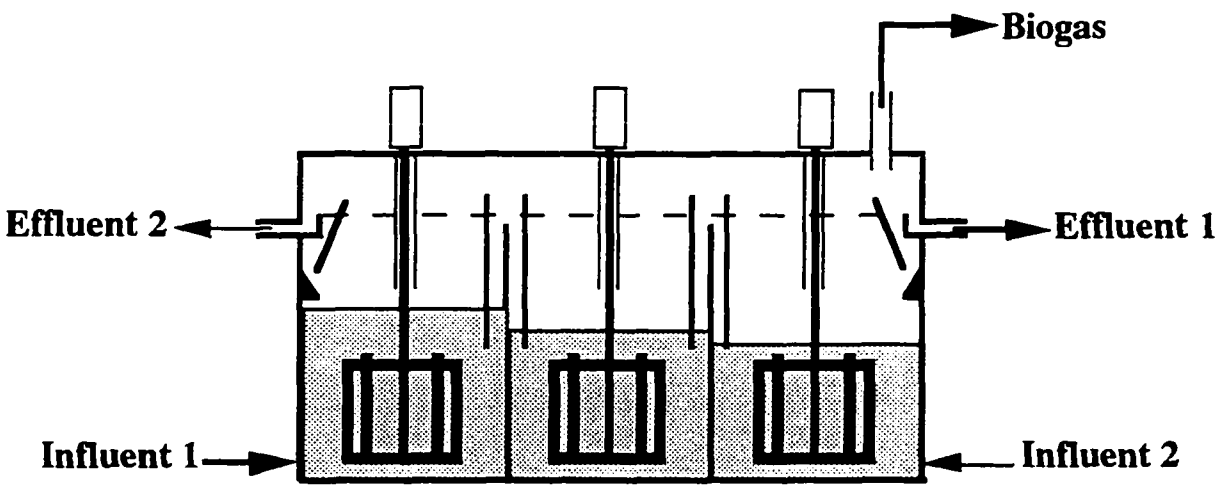

Fig. 1 Schematic diagram of the anaerobic migrating blanket reactor (AMBR).

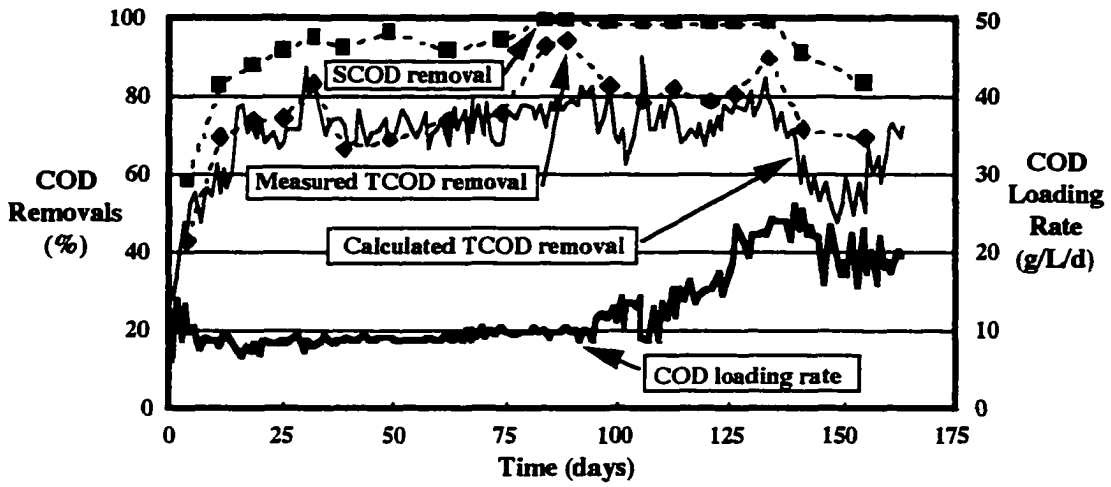

Figure 2. COD removal efficiencies and loading rates for the 54-liter AMBR.

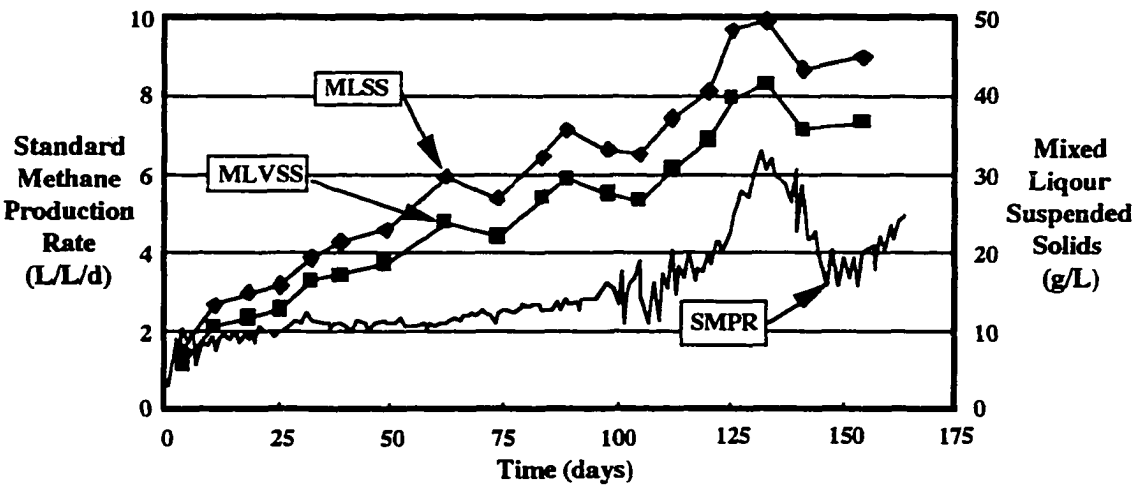

Figure 3. Standard methane production rate and mixed liqour suspended solids for the 54-liter AMBR.

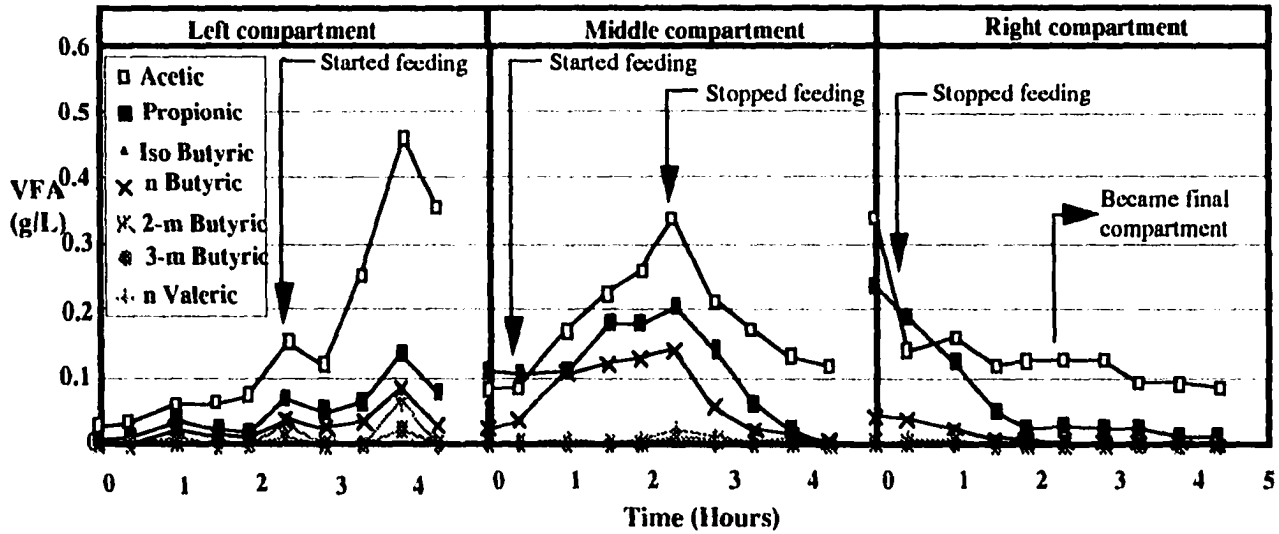

Figure 4. Change of VFAs in the compartments of the 54-liter AMBR due to reversing the flow at a COD loading rate of 17 g/L/d.

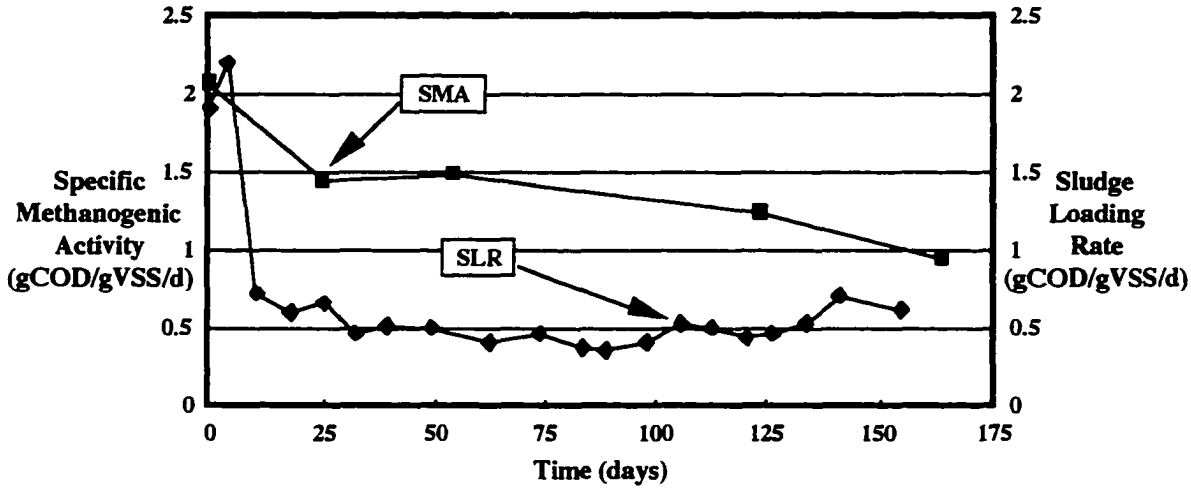

Figure 5. Specific methanogenic activity of the biomass and sludge loading rate for the 54-liter AMBR.

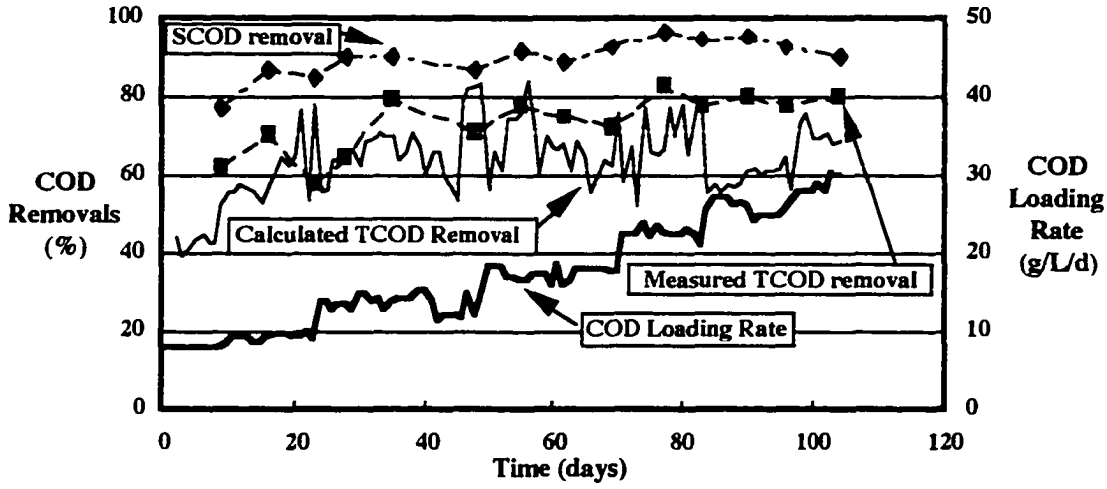

Figure 6. COD removal efficiencies and loading rates for the 12-liter AMBR.

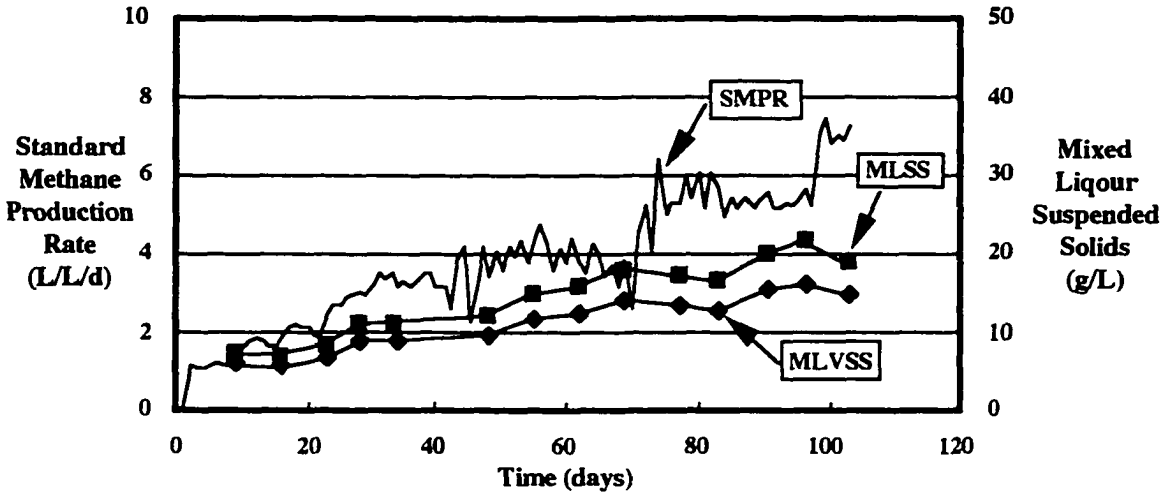

Figure 7. Standard methane production rate and mixed liqour suspended solids for the 12-liter AMBR.

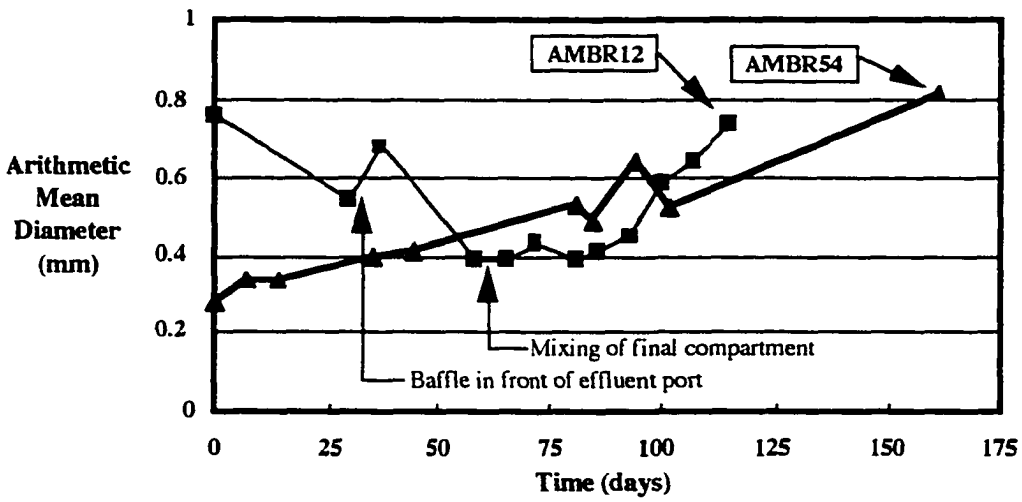

Figure 8. Aritmetic mean diameter of the granules for the AMBR systems.

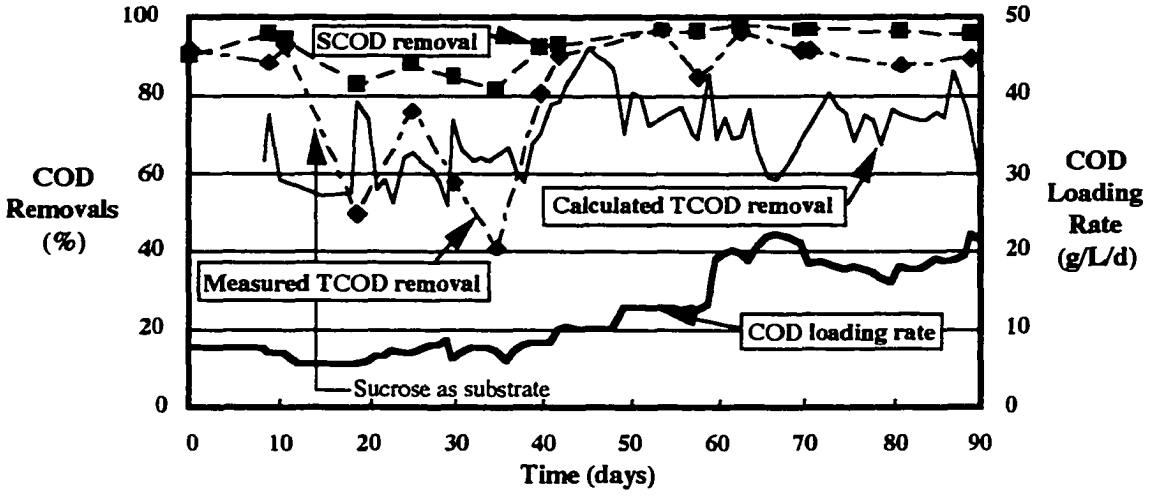

Figure 9. COD removal efficiencies and loading rates for the 12-liter UASB reactor.

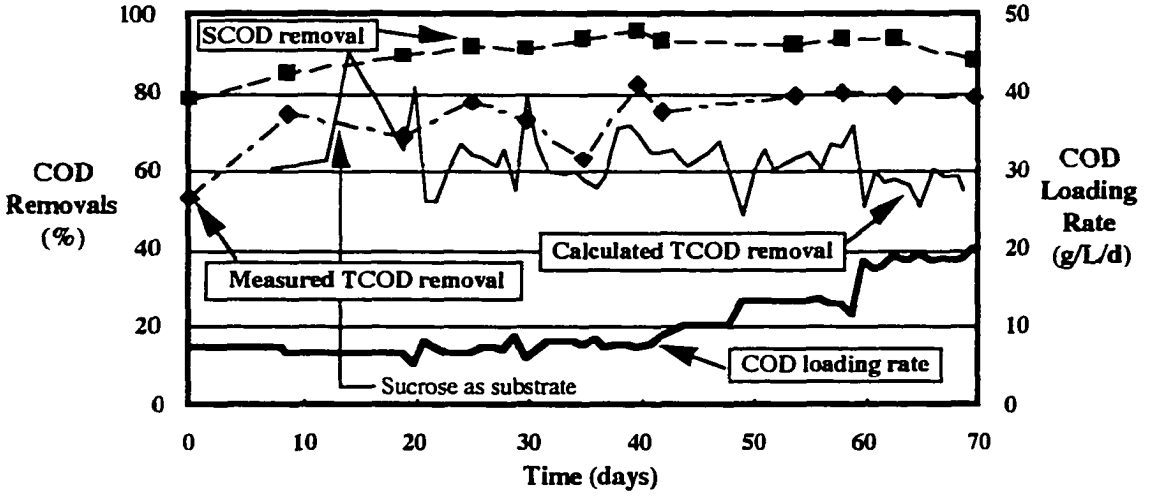

Figure 10. COD removal efficiencies and loading rates for the 12-liter ASBR.

CHAPTER 3. PROCEDURES TO ENHANCE GRANULATION IN THE ANAEROBIC MIGRATING BLANKET REACTOR (AMBR)

A paper submitted to Water Environment Research

Largus T. Angenent, Shihwu Sung, Richard R. Dague

ABSTRACT: In this exercise, three runs were performed with different start-up procedures to study the enhancement of granular biomass formation after seeding the anaerobic migrating blanket reactor (AMBR) with flocculent digester sludge. Shear forces induced by mechanical mixing, and separation of the less settleable biomass from the better settling biomass, accomplished through migration of biomass and mixing of the final compartment, led to granulation. Granules were formed in the AMBR system without relying on a hydraulic upflow pattern such as in the upflow anaerobic sludge blanket (UASB) reactor and its derivatives. When moderate initial selection pressures were maintained and a substrate of acetic acid: propionic acid: butyric acid: sucrose (1:1:1:1 based on COD) in a concentration of 10 gCOD/L was fed to the AMBR, white granules were formed within two months of operation. After an additional two months of maturation of the granular blanket, the COD loading rate could be increased. Eventually, the COD loading rate was increased to 11 g/L/d with SCOD removals exceeding 98% and a standard methane production rate (SMPR) of 3.6 L/L/d. To enhance the formation of granules in the AMBR, the hydraulic selection pressure needed to be moderate at the start of operation.

KEYWORDS: anaerobic, AMBR, granulation, start-up, biomass migration, staging, hydraulic selection pressure

Introduction

The anaerobic migrating blanket reactor (AMBR) was developed as a new high-rate wastewater treatment system by the authors and coworkers at Iowa State University (Angenent and Dague, 1996). It featured compartmentalization, mechanical mixing, staging, reversing flow, and a simple design, without the need for a feed distribution system, gas-solids-separation system, and effluent recycle. Plug-flow conditions for this continuously fed system were enhanced by the absence of recycling and by the mixing effects of gas production in a compartmentalized reactor (Fox and Pohland, 1994). The plug-flow conditions of the AMBR created a substrate gradient over the horizontal plane of the reactor with high substrate concentrations in the initial compartments and low substrate concentration in the final compartment. This not only resulted in high removal efficiencies,

but also created an ideal solid/liquid clarification zone in the final compartment where low gas production was observed. Moreover, plugflow conditions promoted phase separation, as documented by Fox and Pohland (1994). However, total phase separation in the AMBR process was prevented by reversing the flow over the horizontal plane of the reactor (Angenent and Dague, 1996). Advantages of using a slight pre-acidification, as seen in this staged process, over a two-phase treatment concept were already postulated by Lettinga (1995).

Studies showed that the AMBR was able to maintain and grow a granular blanket after the reactor was seeded with granular biomass. This was due to the migration of biomass over the horizontal plane of the reactor in which any flocculent biomass migrated faster than the granular biomass. Eventually, this less settleable biomass washed out with the effluent thus selecting for the better settling granular biomass. In this way, the formed aggregates were retained in the reactor and were able to grow in size. However, reversing the flow was required to prevent this biomass from accumulating in the final compartment due to migration of the blanket (Angenent and Dague, 1996).

The granulation process was a result of microbial and hydraulic selection processes. First, substrate concentration, substrate type, and environmental factors affected the microbial selection (Hulshoff Pol, 1983; Grotenhuis et al. 1991a; Tay and Yan, 1996). Hulshoff Pol et al. (1988) found large granules consisting mainly of *methanosaeta* spp. (formerly *methanothrix*) in laboratory-scale upflow anaerobic sludge blanket (UASB) reactors which were fed a low concentration influent (500 mg/L of a volatile fatty acid; VFA-mixture). However, small *methanosarcina* spp.-granules were found when a high concentration influent (10 g/L) was fed. Furthermore, Morvai et al. (1992) revealed microbial selection of *methanosaeta* spp. at low acetic acid levels (0-0.2 g/L) and at much higher acetic acid levels. However, granules consisting of layers of different trophic groups were found in UASB reactors when developed on a carbohydrate substrate, such as sucrose (Guiot et al., 1992). In addition to the foregoing, surface thermodynamics, Ca^{2+}, filamentous bacteria, and extracellular polymers were postulated to play an important role in the granulation process (Thaveesri et al., 1995; Grotenhuis et al., 1991b; Wiegant, 1988; Allison and Sutherland, 1987). Sam-Soon et al. (1987) described favourable granule formation in a plugflow reactor configuration, which consisted of zones with high and low hydrogen partial pressures at neutral pHs. This was accomplished when the feed consisted a substrate that produced hydrogen as an intermediate and had non-limiting ammonia sources.

The hydraulic selection depended on physically separating any dispersed bacteria from the aggregate forming bacteria by using shear forces (mixing) and differences in

settleability (Vanderhaegen et al., 1992; Lettinga, 1995). Subsequently, better settling biomass was utilized as initial nuclei for new bacterial growth and granule formation (Tay and Yan, 1996). Schmidt and Ahring (1995) reviewed the initiation and development of granulation in the UASB reactor. From an operational standpoint, high biomass retention occurred at the initial stages of start-up studies with the UASB reactor. Next, the selection pressures were increased by gradually increasing the food to microorganism (F/M) ratio. This increased the upflow velocity in the UASB reactor, which determined the hydraulic selection pressure (Hulshoff Pol et al., 1988). The same research showed that at low hydraulic selection pressures, the growth of bacteria occurred mainly as filamentous biomass. Recently, the specific methanogenic activity (SMA) and the microbial load index (MLI), which is the F/M ratio divided by the SMA, were used to determine the loading rate during the start-up (Ince et al., 1995; Tay and Yan, 1996). Tay and Yan (1996) initiated granulation in laboratory-scale UASB reactors within one month by maintaining a MLI of 0.8.

Laboratory-scale AMBRs achieved soluble COD removal efficiencies (SCOD removals) of 95% at COD loading rates up to 30 g/L/d, when fed non-acidified sucrose (Angenent and Dague, 1996). However, SCOD removals increased to 99% with a COD loading rate of up to 23 g/L/d after placing baffles between the compartments. Treatability studies with a wastewater from a paper recycling company showed SCOD removals of 80% at a hydraulic retention time (HRT) of eight hours (Flamming et al., 1997). It must be realized that these COD loading rates in the AMBR were achieved after seeding with and maintaining a granular biomass. The faster settleability of granules, which increased the retention of the biomass, and higher methanogenic activity, due to favourable conditions for the methanogens inside the granules, were reasons for higher loading rates (Lettinga et al., 1980; Schink and Thauer, 1988; Pauss et al., 1990; de Beer et al., 1992).

In places where granular biomass is not available, alternative biomass sources, such as flocculent digester sludge, could be used as a seed to develop granules. Furthermore, granulation studies with the AMBR would gain insight into the selection process, which could lead to shorter start-up periods. Therefore, the objective of this study was to shorten and enhance granulation in laboratory-scale AMBRs seeded with flocculent primary digester sludge.

Methodology

Analysis. The composition of the biogas was measured using gas chromatography (GC; Gow Mac Model 350 with thermal conductivity detector; Column: 6'*1/8' stainless steel Poropack Q 80/100 mesh). The total alkalinity, total VFAs, total and soluble COD, sludge

volume index (SVI), and total and volatile suspended solids (VSS) were performed according to procedures in *Standard Methods* (APHA, 1985). Effluent samples of the AMBR processes were taken at the midpoint of the time interval between two reversals of flow.

Biomass characteristics. The specific methanogenic activity (SMA) was assessed with the "headspace method" according to tests described by Rinzema et al. (1988). To analyze the sizes of the granules and their change in time, the arithmetic mean diameter (Sum(d)/n) and area-weighted mean diameter ($Sum(d^3)/Sum(d^2)$) were calculated with automated image analysis (AIA). Samples of the mixed liquor of the reactor were mixed and diluted to obtain an overall distribution of clearly visible and non-overlapping biomass particles. Next, 1.75 mL was added to a special slide, which consisted of two, three mm thick glass sheets cemented together, with a one inch diameter hole in the top sheet. This in turn was covered with another thin sheet. The AIA set-up contained a black and white video camera (Dage-MTI series 68), a microscope (Olympus SZH), and a PC with Quartz PCI Imaging software. Some manual editing of the image was necessary to separate adjacent granules. Particles smaller than 0.1 mm were not included in the calculations of the size distribution (Grotenhuis et al., 1991a).

Sample preparation for scanning electron microscopy (SEM) involved fixation over night at 4°C by placing the granules in 2% paraformaldehyde, 2% glutaraldehyde, and anaerobic 0.05 M cacodylate buffer. The fixed granules were then washed with the same buffer three times and again fixed with 1% osmium tetroxide for one hour. Next, the granules were dehydrated with a graded series of ethanol in distilled water from 50% to 100% (v/v). Then the specimens were placed in 100% ethanol and critical point dried in CO_2. The prepared specimens were mounted on aluminum stubs and were sputter coated in a Polaron E5100, USA, with platinum/palladium target (60:40). A Jeol JSM-5800LV SEM, Japan was used for the analysis.

Assessment of the SMPR and calculated TCOD removal. The COD loading rate was defined as the amount of COD that was fed into the reactor per reactor volume per day (g/L/d). The biogas production was corrected to standard temperature and pressure (STP) using the ideal gas law. Next, the standard methane production rate (SMPR) was obtained after converting the biogas production with the wet volume of the reactor and the methane percentage that was present in the biogas. Therefore, the SMPR was expressed as liters of methane per reactor volume per day (L/L/d). The SMPR is a true measure of the COD that was being removed, because methane was the final product in the stabilization of COD (0.35 L methane/gCOD). However, utilization by sulfate reducing bacteria and soluble methane washed out with the effluent also accounted for COD removal, which was not included in

Equation 1. Furthermore, COD removal due to biomass growth was not included because biomass wash out is part of the measured total COD. To obtain the theoretical or calculated total COD removal efficiency (calculated TCOD removal) Equation 1 was used:

$$\text{Calculated TCOD removal, \%} = \frac{\text{SMPR}}{\text{COD loading rate} \cdot 0.35} \cdot 100 \quad (1)$$

Assessment of the BMR and BMI. The biomass migration rate (BMR) was calculated by the decrease of mixed liquor volatile suspended solids (MLVSS) in the initial compartment over a time period (t_1 to t_2) in which the direction of flow was not reversed. Solids in the influent were not accounted for because the synthetic substrate in this study did not contain solids. The BMR was expressed as gVSS/L/d. To create an operational parameter in which different situations could be compared, the BMR was corrected for the amount and settleability of biomass by the MLVSS and SVI in the denominator, respectively (Equation 2). The formed biomass migration index (BMI) is an empirical parameter.

$$\text{BMI, gVSS/mL/d} = 1000 \cdot \frac{(MLVSS_1 - MLVSS_2)}{\frac{(MLVSS_1 + MLVSS_2)}{2} \cdot SVI \cdot (t_2 - t_1)} \quad (2)$$

Biomass seed. The seed for Run 1-3 was obtained from the primary digesters of the wastewater pollution control plant of the city of Ames, Iowa, USA. This sludge was screened through a 1.25 mm sieve before addition to the reactor.

Substrate. Concentrated substrate, consisting of sucrose plus essential nutrients (C/N ratio of 16), alkalinity, yeast extract, and trace-elements (Zehnder et al., 1980; van Lier, 1995), was stored in a refrigerator to prevent pre-acidification, and was mixed to obtain a constant loading rate (see Table I). Furthermore, make-up water (35°C) was added to the substrate before feeding to the reactor. This 100% sucrose substrate was used in Run 1 and 2. The substrate in Run 3 consisted of a mixture of acetic acid, propionic acid, butyric acid, and sucrose at a 1:1:1:1 ratio based on COD. Instead of 624 mg bicarbonate/gCOD, 100 mg bicarbonate/gCOD was added, and sodium hydroxide was used to correct the pH of the concentrated substrate to approximately 6.75.

Laboratory-scale AMBR. A laboratory-scale AMBR was placed in a constant temperature room at 35°C (+/- 1°C) and was used for all runs. The active volume of the AMBR was 54 liters and was divided into three compartments, as illustrated in Figure 1. A minimum of three compartments was required for the AMBR to feed the middle compartment for a certain amount of time before the flow was reversed. In this way, a break-

through of substrate was prevented. Therefore, the middle compartment was fed for two hours between reversing the flow. During all runs, the flow was reversed three times per day. Two automatic ball valves, with an internal diameter of one inch, were used to open and close effluent ports (True blue electric actuator model EBV-6, Plast-o-matic valves Inc., Cedar Groove, New Jersey, USA). The pH was monitored by probes in the reactor (pH-probe: Fermprobe pH-electrode (210 mm), Phoenix electrode Co., Houston, Texas, USA; pH-controller: Model PHCN-425, Omega engineering Inc., Stamford, Connecticut, USA). Sufficient biomass/substrate contact was maintained using intermittent mixing. Research by Dague et al. (1970) showed that mixing that was too intense could destroy the anaerobic bioflocs. Mixers (Model 5vb, EMI Inc., Clinton, Connecticut, USA) were able to start and operate at a slow speed (30 rotations per minute; rpm) and the use of paddles further enhanced gentle mixing. All pumps used, were Masterflex pumps of Cole Parmer Instrument Co., Chicago, Illinois, USA. The gas collection systems consisted of an observation bottle, a bottle packed with steel wool to scrub hydrogen sulfide from the biogas, a gas sampling port, and a wet-test gas meter (GCA, Precision scientific, Chicago, Illinois, USA). The biogas was directly discharged from the reactor to the gas collection system. A water head was installed on the effluent tubes to prevent biogas from escaping through the effluent ports. Timers (ChronTrol Corporation, San Diego, California, USA) regulated the operation.

Experimental approach. Three runs were performed in series. In this way, knowledge about granulation in the AMBR was used to design the consecutive run, such that a more optimal operation was examined to speed up granular formation. The initial COD loading rate of all runs was chosen to achieve high COD removals and low VFA concentrations in a one week period. During the operational time, the COD loading rate was increased after the calculated TCOD removals were exceeding 70% and the VFA concentration of the effluent was lower than 0.3 g/L. Conversely, the COD loading rate was decreased whenever these two parameters were not satisfactory. The pH of the initial compartment was maintained between 6.5 and 6.8 over the entire operational period. Reversing the flow three times per day corresponded to favourable pH levels in the initial compartment, without having to add enormous amounts of alkalinity to the non-acidified substrate. At these pH levels, methanogenic activities prevailed in all compartments. The operational parameters for the three runs are shown in Table 2.

Run 1. Run 1 was performed with an influent concentration of 2 gCOD/L (sucrose as a substrate) and a high initial hydraulic selection pressure. This high selection pressure was established by mixing the final compartment every 15 minutes for 15 seconds and

maintaining a relatively low HRT of three days initially. Baffles were used between the compartments to limit the BMR at these high hydraulic pressures.

During the first 75 days of operation of Run 1, the HRT was slowly decreased from three to 0.75 days, as seen in Figure 2. Consequently, the COD loading rate increased from one to 2.75 g/L/d. However, the COD loading rate needed to be decreased at day 75 because of a high VFA concentration of 0.4 g/L in the effluent, and thus decreased removal efficiencies. The reactor was apparently unstable because the MLVSS had decreased from 10 g/L to 3 g/L in the first 20 days, as seen in Figure 2.

Run 2. For the second run, the AMBR was fed with an influent concentration of 10 gCOD/L (sucrose as a substrate), resulting in initial HRTs of approximately 20 days and thus a lower hydraulic selection pressure compared to Run 1. Furthermore, during the first 104 days of operation, an even lower hydraulic selection pressure was maintained by omitting the mixing of the final compartment. At day 104 of the operational time, the hydraulic selection pressure was increased by mixing the final compartment every half hour for 15 seconds, because the flocculent biomass was not sufficiently separated from the better settling biomass. Again at day 145, the hydraulic selection pressure was increased by decreasing the influent concentration from 10 gCOD/L to 2 gCOD/L and consequently decreasing the HRT five times (see Figure 3). This was done because it was realized that the baffle arrangement could not provide a sufficient BMR at an HRT of 2.5 days, and thus the feed concentration needed to be lowered. Indeed, the BMR and BMI after the change in HRT, at a COD loading rate of 3.5 g/L/d, changed from 3.4 and 5 to 23.5 gVSS/L/d and 51 gVSS/mL/d, respectively. At day 170, to even further escalate the selection pressure, mixing of the final compartment was increased to every 15 minutes. Simultaneously, the initial compartments were mixed for 10 seconds at intervals of seven minutes. Finally, at day 200, to prevent further attachment of fluffy acidogenic bacteria to the granules, the mixing speed was increased from 30 to 54 rpm. At day 228 of the operational time, the BMR and BMI were measured at a COD loading rate of 3.5 g/L/d and were found to be 1.3 gVSS/L/d and 31.8 gVSS/mL/d, respectively.

Figure 3 shows the operational parameters for Run 2. An increase in the MLVSS of up to 16 g/L was seen in the initial period of this run. Simultaneously, the COD loading rate was increased to 5.5 g/L/d over a 90 day period by decreasing the HRT to 2.1 days. This was possible because of low F/M ratios and high COD removal efficiencies. After increasing the hydraulic selection pressure at day 104, the sludge retention time (SRT) decreased from 124 to 12 days and the VSS of the effluent increased from 6.6 to 48.2 g/d, decreasing the MLVSS from 15 to 11 g/L in 10 days. Consequently, the F/M ratio increased from 0.3 to 0.5

gCOD/gVSS/d, which mandated a decrease in the COD loading rate, especially, after a pump failure and the subsequent shock-load at day 115 had further decreased the MLVSS to 6 g/L. At day 115 of the operational period the HRT was increased to 2.5 days, subsequently decreasing the COD loading rate to 3.5 g/L/d. The loading rate had to be further decreased to 2.6 g/l/d after VFA concentrations exceeded 0.4 g/L at day 160. However, at day 175 the reactor was stabilized due to granular formation and increasing MLVSS levels of up to 5.3 g/L at day 195, making it possible to increase the COD loading rate to 6 g/L/d. Next, washing out of the granules necessitated a decrease in the COD loading rate to 3.5 g/L/d.

Run 3. To speed up the start-up and granulation time and to prevent growth of vast amounts of acidogenic bacteria, a mixture of sucrose:acetic acid:propionic acid:butyric acid (1:1:1:1 based on COD) was used as a synthetic substrate at an influent concentration of 10 gCOD/L. The initial hydraulic selection pressure was chosen to be moderate by mixing the final compartment once every hour for 10 seconds at 30 rpm. Simultaneously, the initial compartments were mixed at 15 minute intervals for 10 seconds. Furthermore, a seven cm opening over the bottom length of the walls between the compartments was made instead of the baffles. In this way, the BMR was supposed to increase, as Run 2 had shown insufficient migration of biomass with the maintained feed concentration of 10 g/L. At day 63 of the operational period, the mixing frequency of the final compartment was increased to once every half hour to increase the selection pressure. At day 75, the mixing frequency of the initial compartments was increased to once every 10 minutes, because the BMI was found to be 4.0 gVSS/mL/d, which was insufficient for washing out all flocculent biomass. Simultaneously, the final compartment was mixed every 15 minutes. At day 100, the openings in the bottom of the reactor were lowered to a 0.5 cm height, which increased the BMI from 1.8 at day 92 to 13.3 at day 112 and 20.2 gVSS/mL/d at day 120. The mixing intensity had to be increased from 30 to 45 rpm at day 128 to ensure sufficient mixing, because the granular blanket had built up. At the end of the operational period the openings in the bottom of the walls were increased to a height of one cm to decrease the BMR, because the BMI and BMR was found to be 62.4 gVSS/mL/d and 13 gVSS/L/d, respectively.

Although the MLVSS concentration in the reactor decreased from 11 to 5 g/L in the first month of operation, the wash out of biomass was moderate and stable during the rest of the operation. This is illustrated in Figure 4, which shows VSS concentrations in the effluent of less than 20 g/d for the next 3.5 months. Consequently, the HRT could be decreased to 3 days resulting in an increase of the COD loading rate from 0.5 to 5 g/L/d within 65 days of the operational period. However, around day 90 the COD loading rate had to be decreased to 3 g/L/d due to VFA concentrations of up to 2 g/L in the effluent, as seen in Figure 4. After

the reactor became stable again, the COD loading rate was increased up to 11 g/L/d over a period of two months.

Results

Run 1. The reactor performance of the AMBR in this run was not very satisfactory in that the measured and calculated TCOD removals were decreasing during the operational period to less than 60%. Apparently, an increase of the VFA concentration and subsequent decrease of the SCOD removal decreased the performance, as seen in Figure 2. Because of a high hydraulic pressure, too much biomass was washed out in the initial stages of the run, preventing a build up of active biomass.

Table 3 shows that the SMA of the biomass had increased over the first 66 days to 0.85 gCOD/gVSS/d, but this decreased again at the end of the operational time. Although the MLI at day 81 was 0.79, which should be ideal for granulation to occur, no granules were detected. In contrast, a low concentration biomass developed with a very poor settleability (SVI was 276 mL/gVSS at the end of the operational period). This "fluffy" biomass was light-gray in colour and was hard to dewater. Moreover, the biomass tended to float when taken out the reactor and could be determined as bulking sludge. At these unfavourable conditions Run 1 was stopped and the reactor was reseeded.

Run 2. The reactor performance of the AMBR in Run 2 was satisfactory for the first 100 days of operation in which the measured TCOD removal exceeded 90%, because of the accumulation of solids in the reactor. After mixing of the final compartment began at day 104, the measured TCOD removal (74%) approached the calculated TCOD removal, as it should, because solids were prevented from accumulating in the reactor. Moreover, the SCOD removal and SMPR were 97% and 1.5 L/L/d, respectively, which still showed favourable conditions. Nevertheless, the reactor performance deteriorated soon after, because the MLVSS decreased from 6 to 2.6 g/L and the F/M ratio increased from 0.6 to 1.7 over the next 50 days. At day 170 of the operational period, the TCOD and SCOD removal, and VFA in the effluent were 48%, 76%, and 0.41 g/L, respectively, as seen in Figure 4. Indeed, a MLI of 1.36 indicated unstable conditions, as illustrated in Figure 5. This shows that the F/M ratio was higher than the SMA, in other words the biomass was fed more substrate than it maximally could utilize to form methane. Meanwhile, granules were detected, which prevented further destabilization of the reactor. Reactor performance improved quickly as the SMPR increased to almost 1.5 L/L/d, however, this decreased again due to the wash-out of small granules and, hence, an increase of the VSS in the effluent.

After 170 days of operating the AMBR, the biomass consisted of clearly distinguishable granules, which were white, gray, and amber in colour. Granules might have been in the reactor before that day, but they became more notable after most flocculent biomass was washed out. Figure 5 shows an increase of the area-weighted mean diameter and arithmetic mean diameter between day 151 and 170 of the operational time. Also, Figure 5 shows that the SVI of the biomass before and after granular formation was 90 and 38 mL/gVSS, respectively. Between day 170 and day 195 of the operational period, the F/M ratio and MLI were decreased to 0.9 gCOD/gVSS/d and one, respectively, because of an increase in the MLVSS and SMA. A blanket of good settleable, small, black granules was formed in the AMBR. Unfortunately, after 200 days these small granules became less settleable due to the formation of acidogenic biomass around the granules. Consequently, these granules increasingly were washed out, decreasing the MLVSS of the AMBR to 1.3 g/L.

Even after granules were formed the hydraulic selection pressure and mixing scheme needed to be as high, not to build up acidogens. Difficulties with separation and retention of biomass were a result of high F/M ratios of approximately 2.5 gCOD/gVSS/d of non-acidified sucrose fed. Consequently, the SRT, MLVSS, and COD loading rate stayed low at the end of the operational period. Eventually, the granular biomass SMA and MLI were 2.5 gCOD/gVSS and one, respectively. This high SMA shows that the AMBR was finally able to separate highly settleable biomass, consisting of high levels of methanogenic consortia, from acidogenic biomass. After most small black granules were washed out, the granular blanket consisted of the one that was seen at day 170. Figure 7 shows the granular size distribution of the biomass which was sampled at the end of the operation.

SEM views of sliced white granules did not show layers of different bacteria or archae. On the contrary, *Methanosaeta*-like rods were uniform throughout the granule. SEM of the surface of a small gray granule showed rods and cocci, but this granule was not sliced open (Angenent et al., 1997).

Run 3. Run 3 showed superior reactor performances compared to Run 1 and 2. Except for a period in which flocculent biomass had to be washed out, SCOD removals were always exceeding 98%. At the last two months of operation the TCOD removals were exceeding 90%.

This shows that the AMBR was able to maintain a sufficient SRT of the granules such that the MLVSS was increased at COD loading rates of up to 11 g/L/d and SMPRs of up to 3.6 L/L/d (300 L/d of biogas production). Very stable reactor performances were apparent at the end of the operational time, because the F/M ratio was stable at 1.6 gCOD/gVSS/d and

the SMA increased to 3 gCOD/gVSS/d. This lowered the MLI and showed that COD loading rate of the AMBR could have been increased at a faster rate.

White granules were detected in the reactor within two months of operation, as seen in Figure 6 by the decrease of the SVI at day 60. Further maturing of the granular blanket continued for the next two months, after which the MLVSS, and the SCOD and TCOD removal increased. The mature granular blanket mainly consisted of small, light-gray granules. At the end of the operational time, the arithmetic and area-weighted mean diameter increased up to 0.32 and 0.54 mm, respectively, as seen in Figure 6. Furthermore, Figure 7 shows the granular size distribution of this biomass, which consisted of relative more smaller size granules compared to the biomass sampled at the end of Run 2. Although, the accumulation of flocculent biomass was expected to be lower in Run 3 compared to Run 1 and 2, due to smaller amounts of sucrose in the feed, less settleable biomass with a SVI of 260 mL/gVSS was found at day 55 of operation. However, no wash-out of vast amounts of granules occurred due to the growth of acidogens around the small granules, as occurred in Run 2.

Figure 8 shows SEM views of the shape and surface of a granule that was sampled at the end of the operational time of Run 3. Very long bundles of *Methanosaeta* are apparent on the surface of the granule (100 times magnification). Eventually, numerous one cm long fibers were found in the AMBR consisting of only *Methanosaeta* (published elsewhere). The surface of this granule consisted of rods and cocci, as seen in Figure 8b (6000 times magnification).

Discussion

Hydraulic selection pressure. This research showed that selection and formation of granules in the AMBR was possible without having a hydraulic upflow pattern in the reactor such as the UASB reactor. Research by Vanderhaegen et al. (1992); Sung and Dague (1992); and Wirtz and Dague (1994) supported this finding. More specifically, any anaerobic system that combines shear force with a way of separating flocculent from better settling biomass has the potential of forming and growing granules. Table 4 gives the utilization of shear force and hydraulic selection pressure for the UASB reactor, the ASBR, and the AMBR. Moreover, research by Morgenroth et al. (1997) found aerobic granules after utilizing these pre-requisitions in the aerobic sequencing batch reactor (SBR).

Research that was presented here, shows that manipulation of the hydraulic selection pressure can have a big impact on the speed of granulation to occur. Too high hydraulic selection pressures at the start of the operation prevented sufficient reactor performance and

granular formation in Run 1. At the start of Run 2, no separation of flocculent and better settling biomass resulted in adequate reactor performances, but slowed the speed of granular formation. A moderate selection pressure at the start of Run 3 established a sufficient reactor performance in which a balanced consortia was built up without losing the selection mechanism for better settling biomass. The hydraulic selection pressure in the AMBR was manipulated by changing the biomass migration rate (BMR) and mixing of the final compartment. In addition, COD loading rate (mixing by biogas production), mixing scheme in initial compartments, reactor configuration, and hydraulic loading rate determined the BMR over the horizontal plane of the AMBR.

Granular blanket maturation in Run 2 and 3 was accomplished after initiating a heavy selection pressure on the system after a build up of a balanced consortia. In both runs this resulted in increased flocculent biomass wash-out, and thus increased F/M ratios, which deteriorated a stable reactor performance to insufficient levels. However, this seemed to stimulate the granulation process. After the granular blanket matured, the MLVSS increased again which quickly reversed the reactor performance and made increasing the COD loading rate possible. The period in which the biomass levels reached minimum levels is also referred to as "the valley of death" in which stable conditions are followed by unstable ones that are reversed again by granular formation. Wirtz and Dague (1994) also found this phenomenon in the ASBR.

The BMI was found to be helpful in deterring if the migration of biomass was sufficient to promote granulation. At a COD influent concentration of 10 g/L and a COD loading rate of 3.5 g/L/d in Run 2, the BMI was insufficient according to previous experiences. As research showed that the BMI should be between 10 and 100 gVSS/mL/d, but that the BMR should be smaller than the MLVSS times the amount of reversals per day (unpublished data). The BMI was increased 10 fold after increasing the hydraulic flow five times in Run 2. At the end of operating Run 2, the BMI was exceeding 30 gVSS/mL/d at a COD loading rate of 3.5 g/L/d and a COD concentration of 2 g/L. Alternatively, openings in the bottom of the inside walls, instead of baffles between the compartments, have ensured a BMI of 13.3 gVSS/mL/d at a COD loading rate of 3.8 g/L/d and a substrate COD concentration of 10 g/L in Run 3.

In-growth of acidogenic bacteria. Start-up of Run 1 and 2 formed a biomass which was less settleable and could be characterized as being "fluffy" and "bulky" due to the high growth rate of acidogenic bacteria. Start-up studies with UASB reactors which were fed non-acidified sucrose showed the same problems whenever the hydraulic selection was not adequate to separate bulking sludge from heavier biomass (Sierra-Alverez, 1988; Hulshoff

Pol, 1988). These authors postulated selection pressures high enough in the initial stages not to build up a flocculent acidogenic biomass. Alphenaar (1994) concluded that only moderate F/M ratios up to 0.5 gCOD/gVSS/d could be applied in one step UASB reactors for the treatment of non-acidified sucrose influent. Experimental results at higher F/M ratios showed an abundant growth of acidogenic bacteria and consequently problems with sludge retention. To avoid these problems in the UASB process, pre-acidification of sucrose is necessary. Problems with settleability of small granules surrounded by flocculent biomass were encountered in Run 2, which resulted in the loss of most of the granular blanket. Previous studies with laboratory-scale AMBRs showed that higher shear forces and biomass wash-out were responsible for the absence of problems associated with acidogenic bacteria. Therefore, the AMBRs seeded with granules, were not dependable on a pre-acidification step (Angenent and Dague, 1996). However, pre-acidification of sucrose-containing wastewaters could have positive effects on the granulation process in AMBRs seeded with flocculent sludges. Indeed, in Run 3 the better settling biomass was easier separated from the flocculent acidogenic biomass.

Determination of the COD loading rate. A MLI of 0.8, as postulated by Tay and Yan (1996), was found to be a good indication for deterring the F/M ratio, and thus the COD loading rate, for Run 3 to enhance granulation. In contrast, Figure 5 shows that the MLI was 1-1.36 during the end of Run 2, because biomass wash-out required to be higher to select for the better settling biomass, when fed a 100% sucrose substrate. Finally, a MLI of 0.8 showed no granular formation for Run 1 in which a build up of a balanced consortia was absent.

Compartmentalization. Due to a plug-flow configuration of the compartmentalized AMBR, hydrogen partial pressure in the granules and VFA concentration of the water contents were higher in the initial compartment compared to the final compartment. In this way, the AMBR had the same characteristics of favouring granulation, as was seen in UASB reactors with a lower and upper active zone (Sam-Soon et al., 1987).

Conclusions

Based on laboratory studies with the AMBR, which was seeded with flocculent primary digester sludge, the following conclusions were drawn:

Granules with an area-weighted mean diameter of one mm were formed after 170 days of operating an AMBR fed with 100% sucrose as a substrate (Run 2). This was accomplished without having a hydraulic upflow pattern in the reactor. The hydraulic selection pressure was established by a migration pattern of biomass over the horizontal plane of the reactor and mixing of the final compartment. Moreover, shear forces were

applied by intermittently mixing the biomass. After active biomass had built up in the initial stages of Run 2, the hydraulic loading rate and mixing intensity had to be increased to separate the fast growing "fluffy" acidogenic bacteria from the better settling biomass. In this way, the less settleable biomass was separated from the better settling biomass and subsequently selection of heavier biomass in the AMBR occurred.

White granules were formed within two months of operation of Run 3, where acetic acid: propionic acid: butyric acid: sucrose (1:1:1:1 based on COD) in a concentration of 10 gCOD/L was fed. After a two month maturation period, the granular blanket consisted of small, light-gray granules. At the end of Run 3, the COD loading rate was increased to 11 g/L/d with SCOD removals exceeding 98% and a SMPR of 3.6 L/L/d. Furthermore, the area-weighted diameter of the granules increased to 0.6 mm. Granulation was enhanced by establishing a moderate hydraulic selection pressure at the start of the operation. Reactor performances were sufficient to build up a balanced consortia, without losing the selection mechanism for better settling biomass. The formation of a granular blanket was only initiated after hydraulic selection pressures were increased and flocculent biomass was washed out. Separation of flocculent acidogenic and better settling biomass was enhanced in Run 3 by using a VFA/sucrose over a 100% sucrose substrate.

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Authors. Dr. Dague passed away in October 1996. Unfortunately, he did not see the formed granules in the AMBR. However, his positive input and belief in this system was a requisition for its success. At the time this study was conducted, Largus T. Angenent was a graduate research assistant, Shihwu Sung was an assistant professor, and Richard R. Dague was professor of environmental engineering, Department of Civil and Construction Engineering, Iowa State University, Iowa, USA. Correspondence should be addressed to Shihwu Sung, 394 Town Engineering Building, Ames, IA 50011, USA.

References

Allison, D. G., and Sutherland, I. W. (1987) The role of exopolysaccharides in adhesion of freshwater bacteria. *J. Gen. Microbiol.*, **133**, 1319.

Alphenaar, P. A. (1994) Anaerobic granular sludge: Characterization, and factors affecting its functioning, Ph.D. Thesis, Wageningen Agricultural University, Wageningen, The Netherlands.

Angenent, L. T., and Dague, R. R. (1996) Initial studies on the anaerobic migrating blanket reactor. *Proc. of 51st Purdue Industrial Waste Conference* (Edited by Dalton, C. S., and Wukasch, R. F.), 271, Ann Arbor Press, Chelsea, Michigan, USA.

Angenent, L. T., Sung, S., and Dague, R. R. (1997) Start-up and granulation of the anaerobic migrating blanket reactor (AMBR). *Proc. of the WEFTEC'97*, **1**, 113, Water Environment Federation, Alexandria, Virginia, USA.

APHA (1985) *Standard Methods for the Examination of Water and Wastewater* , 16th edition. American Public Health Association, Washington, D. C., USA.

de Beer, D., Huisman, J. W., van den Heuvel, J. C., and Ottengraf, S. P. P. (1992) The effect of pH profiles in methanogenic aggregates on the kinetics of acetate conversion. *Wat. Res.*, **26**, 1329.

Dague, R. R., McKinney, R. E., and Pfeffer, J. T. (1970) Solids retention in anaerobic waste treatment systems. *J. Water Pollut. Control Fed.* **42**, Part 2, R29.

Flamming, J. J., Angenent, L. T., Sung, S., and Dague, R. R. (1997) Treatability studies of a combined industrial wastewater stream using the anaerobic migrating blanket reactor. *Proc. of 52nd Purdue Industrial Waste Conference* , (accepted for proceedings).

Fox, P., and Pohland, F. G. (1994) Anaerobic treatment applications and fundamentals: substrate specificity during phase separation. *Water Environ. Res.*, **66**, 716.

Grotenhuis, J. T. C., Kissel, J. C., Plugge, C. M., Stams, A. J. M., and Zehnder, A. J. B. (1991a) Role of substrate concentration in particle size distribution of methanogenic granular sludge in UASB reactors. *Wat. Res.*, **25**, 21.

Grotenhuis, J. T. C., van Lier, J. B., Plugge, C. M., Stams, A. J. M., and Zehnder, A. J. B. (1991b) Effect of ethylene glycol-bis(ß-aminoethyl ether)-N,N-tetra acetic acid (EGTA) on stability and activity of methanogenic granular sludge. *Appl. Microbiol. Biotechnol.*, **36**, 109.

Guiot, S. R., Pauss, A., and Costerton, J. W. (1992) A structured model of the anaerobic granule consortium. *Wat. Sci. Tech.*, **25**, 1.

Hulshoff Pol, L. W., de Zeeuw, W. J., Velzeboer, C. T. M., and Lettinga, G. (1983) Granulation in UASB-reactors. *Wat. Sci. tech.*, **15**, 291.

Hulshoff Pol, L. W., Heijnenkamp, K., and Lettinga, G. (1988) The selection pressure as a driving force behind the granulation of anaerobic sludge. *Proc. GASMAT Workshop* (Edited by Lettinga, G., Zehnder, A. J. B., Grotenhuis, J. T. C., and Hulshoff Pol, L. W.), 153, Pudoc, Wageningen, The Netherlands.

Ince, O., Anderson, G. K., and Kasapgil, B. (1995) Control of organic loading rate using the specific methanogenic activity test during start-up of an anaerobic digestion system. *Wat. Res.*, **29**, 349.

Lettinga, G., van Velsen, A. F. M., Hobma, S. W., de Zeeuw, W. J., and Klapwijk A. (1980) Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment. *Biotechnol. Bioeng.*, **22**, 699.

Lettinga, G. (1995) Anaerobic digestion and wastewater treatment systems. *Antonie van Leeuwenhoek*, **67**, 3.

van Lier, J. B. (1995) Thermophilic anaerobic wastewater treatment; temperature aspects and process stability, Ph.D. Thesis, Wageningen Agricultural University, Wageningen, The Netherlands.

Morgenroth, E., Sherden, T., van Loosdrecht, M. C. M., Heijnen, J. J., and Wilderer, P. A. (1997) Aerobic granular sludge in a sequencing batch reactor. *Wat. Res.*, **31**, 3191.

Morvai, L., Miháltz, P., and Czakó, L. (1992) The kinetic basis of a new start-up method to ensure the rapid granulation of anaerobic sludge. *Wat. Sci. Tech.*, **25**, 113.

Sam-Soon, P. A. L. N. S., Loewenthal, R. E., Dold, P. L., and Marais, G. v. R. (1987) Hypothesis for pelletisation in the Upflow Anaerobic Sludge Bed Reactor. *Water SA*, **13**, 69.

Pauss, A., Samson, R., and Guiot, S. (1990) Thermodynamic evidence of trophic microniches in methanogenic granular sludge-bed reactors. *Appl. Microbiol. Biotechnol.*, **33**, 88.

Rinzema, A., Van Lier, J. B., and Lettinga, G. (1988) Sodium inhibition of acetoclastic methanogens in granular sludge from a UASB reactor. *Enzyme and Microb. Technol.*, **10**, 24.

Schink, B., and Thauer, R. K. (1988) Energetics of syntrophic methane formation and the influence of aggregation. *Proc. GASMAT Workshop* (Edited by Lettinga, G., Zehnder, A. J. B., Grotenhuis, J. T. C., and Hulshoff Pol, L. W.), 5, Pudoc, Wageningen, The Netherlands.

Schmidt, J. E., and Ahring, B. K. (1995) Granular sludge formation in upflow anaerobic sludge blanket (UASB) reactors. *Biotechnol. Bioeng.*, **49**, 229.

Sierra-Alverez, R., Hulshoff Pol, L. W., and Lettinga, G. (1988) Start-up of a UASB reactor on a carbohydrate substrate. *Proc. GASMAT Workshop* (Edited by Lettinga, G.,

Zehnder, A. J. B., Grotenhuis, J. T. C., and Hulshoff Pol, L. W.), 223, Pudoc, Wageningen, The Netherlands.

Sung, S., and Dague, R. R. (1992) Fundamental principles of the anaerobic sequencing batch reactor process. *Proc. of 47th Purdue Industrial Waste Conference* (Edited by Dalton, C. S., and Wukasch, R. F.), 393, Lewis Publishers, Chelsea, Michigan, USA.

Tay, J., and Yan, Y. (1996) Influence of substrate concentration on microbial selection and granulation during start-up of upflow anaerobic sludge blanket reactors. *Wat. Environ. Res.*, **68**, 1140.

Thaveesri, J., Daffonchio, D., Liessens, B., Vandermeren, P., and Verstraete, W. (1995) Granulation and sludge bed stability in upflow anaerobic sludge bed reactors in relation to surface thermodynamics. *Appl. Environ. Microbiol.*, **61**, 3681.

Vanderhaegen, B., Ysebaert, E., Favere, K., van Wambeke, M., Peeters, T., Pánic, V., Vandenlangenbergh, V., and Verstraete, W. (1992) Acidogenesis in relation to in-reactor granule yield. *Wat. Sci. Tech.*, **25**, 21.

Wiegant, W. M. (1988) The 'spagetti theory' on anaerobic sludge formation, or the inevitability of granulation. *Proc. GASMAT Workshop* (Edited by Lettinga, G., Zehnder, A. J. B., Grotenhuis, J. T. C., and Hulshoff Pol, L. W.), 146, Pudoc, Wageningen, The Netherlands.

Wirtz, R. A., and Dague, R. R. (1994) Enhancement of granulation and start-up in the anaerobic sequencing batch reactor. *Proc. of the WEFTEC'94*, 241, Water Environment Federation, Alexandria, Virginia, USA.

Zehnder, A. J. B., Huser, B. A. , Brock, T. D., and Wuhrman, K. (1980) Characterization of an acetate-decarboxylating, non-hydrogen-oxidizing methane bacteria. *Arch. Microbiol.*, **124**, 1.

Table 1 - Sucrose substrate mixture.

Component	mg added (per g of COD)
Sucrose	960
Bicarbonate, as $NaHCO_3$	624
Yeast extract	3
NH_4Cl	100
K_2HPO_4	20
$NaH_2PO_4.H_2O$	17
$FeCl_2.4H_2O$	10
$CoCl_2.6H_2O$	2
EDTA	1
$MnCl_2.4H_2O$	0.5
Resazurin	0.2
$NiCl_2.6H_2O$	0.142
Na_2SeO_3	0.123
$AlCl_3.6H_2O$	0.090
H_3BO_3	0.050
$ZnCl_2$	0.050
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.050
$CuCl_2.2H_2O$	0.038
HCl (37.7% solution)	0.001 mL

Table 2 - Operational parameters for Run 1, 2, and 3.

Operational parameters	(.)	Run 1	Run 2	Run 3
Substrate	(-)	Sucrose	Sucrose	VFA/sucrose mixture
Reactor configuration	(-)	Baffles	Baffles	Openings in bottom
Mixing final compartment at start	(1/hour)	4	0	1
Initial hydraulic pressure	(-)	High	Low	Moderate
Volume	(L)	54	54	54
No. of reversals in flow	(1/d)	3	3	3
Temperature	(^oC)	35	35	35
pH minimum	units	6.5	6.5	6.5
HRT at start	(d)	3	20	11
COD loading rate at start	(g/L/d)	1	0.5	1
COD concentration influent	(g/L)	2	10 / 2	10

Table 3 - Change in biomass characteristics over the operational period of Run 1.

Time	SMA	MLI	SVI
days	gCOD/ gVSS/d	-	mL/ gVSS
0	0.19	0.84	115
66	0.85	0.94	NA
81	0.77	0.79	276

Table 4 - Granulation in the UASB reactor, ASBR, and AMBR.

Selection pressure	UASB reactor	ASBR	AMBR
Shear force	Hydraulic upflow pattern	Mechanical mixing	Mechanical mixing
Separation principle Mechanism to utilize settleability	Settleability Hydraulic upflow pattern	Settleability Settling time before decanting	Settleability Horizontal migration and settling final compartment

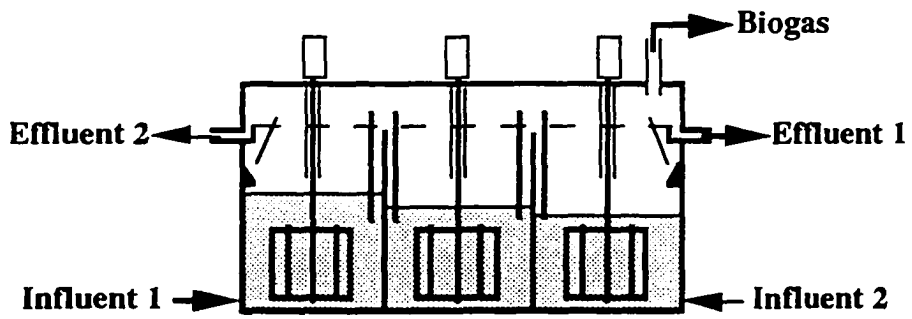


Figure 1 - Schematic diagram of the anaerobic migrating blanket reactor (AMBR) with a baffle configuration.

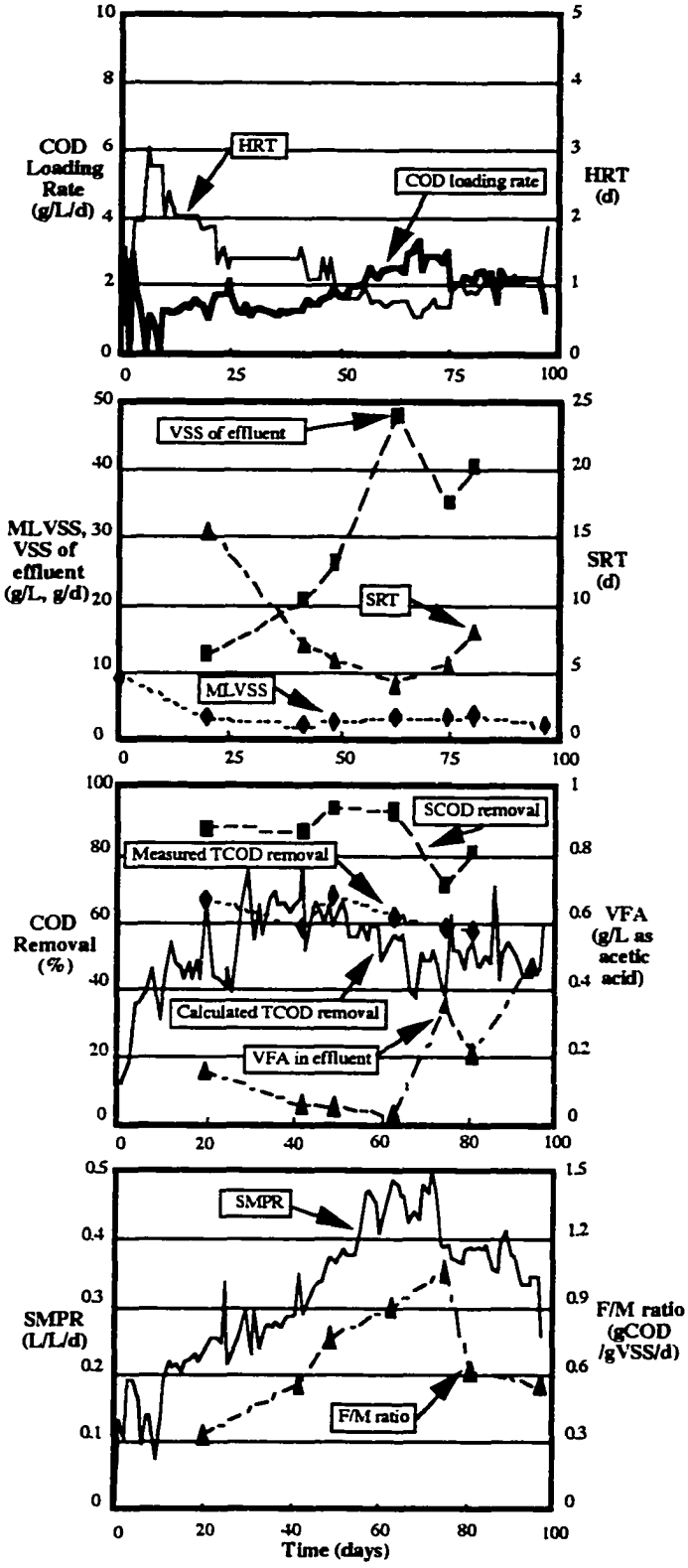


Figure 2 - Operational conditions and results with a high initial hydraulic pressure in Run 1.

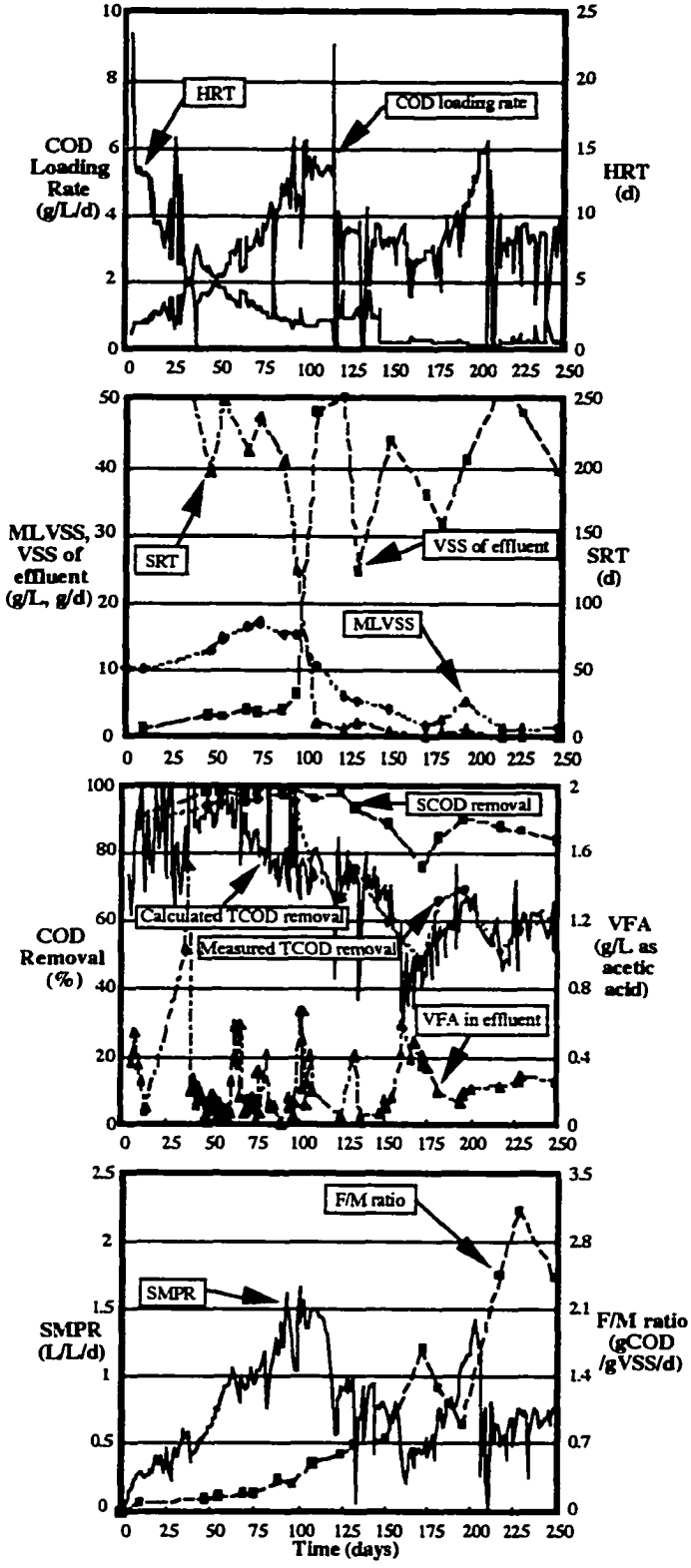


Figure 3 - Operational conditions and results with a low initial hydraulic pressure in Run 2.

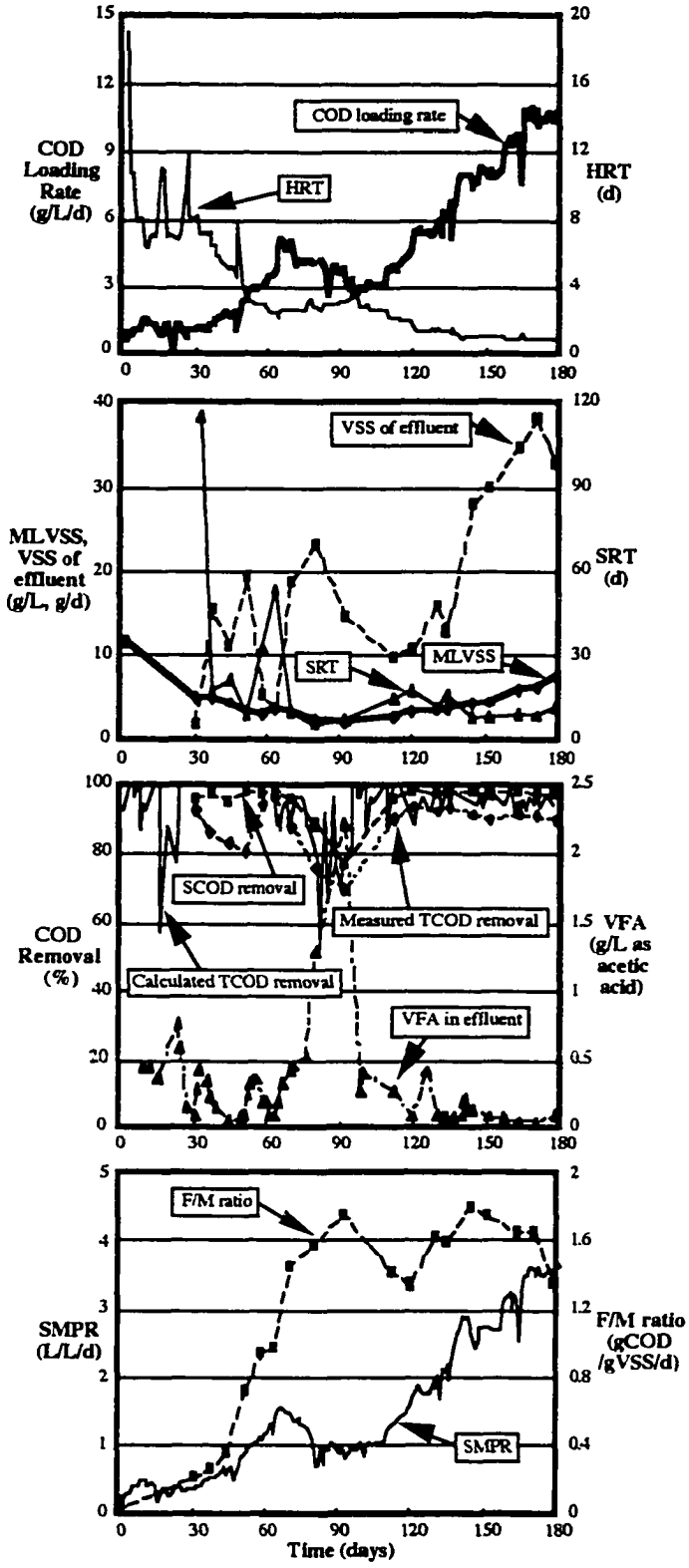


Figure 4 - Operational conditions and results with a moderate initial hydraulic pressure in Run 3.

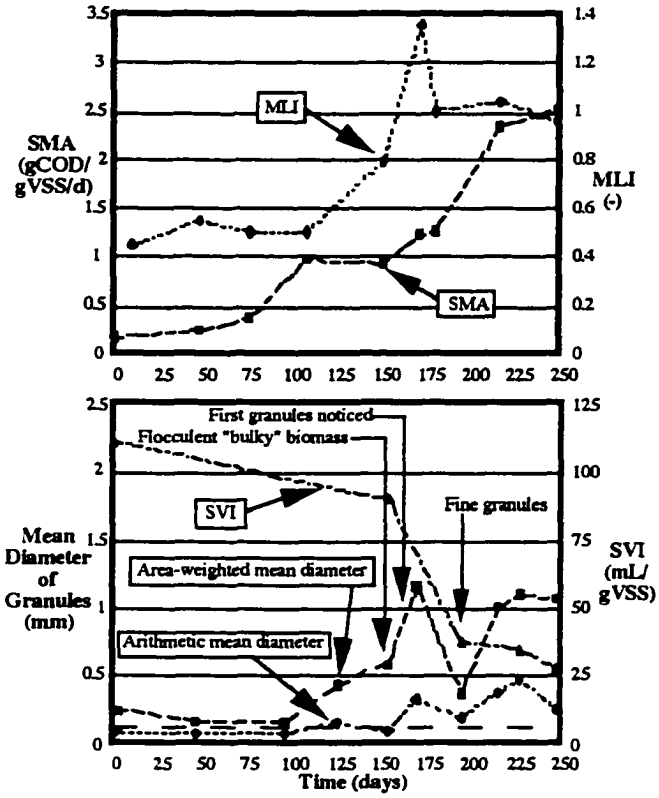


Figure 5 - Change in biomass characteristics over the operational period of Run 2.

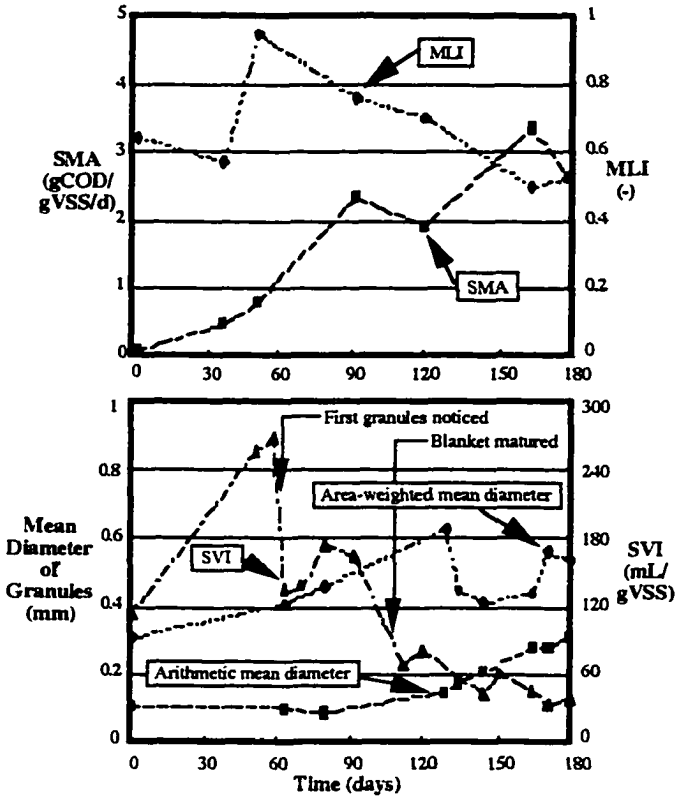


Figure 6 - Change in biomass characteristics over the operational period of Run 3.

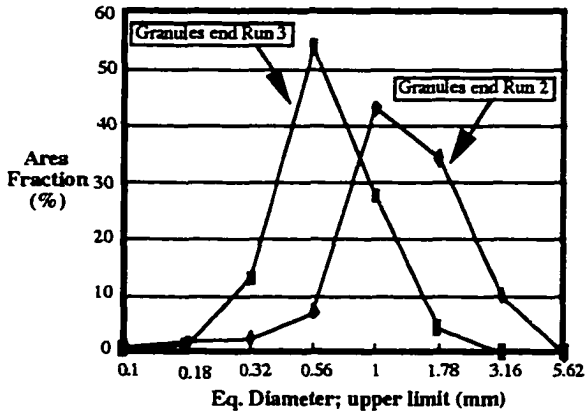
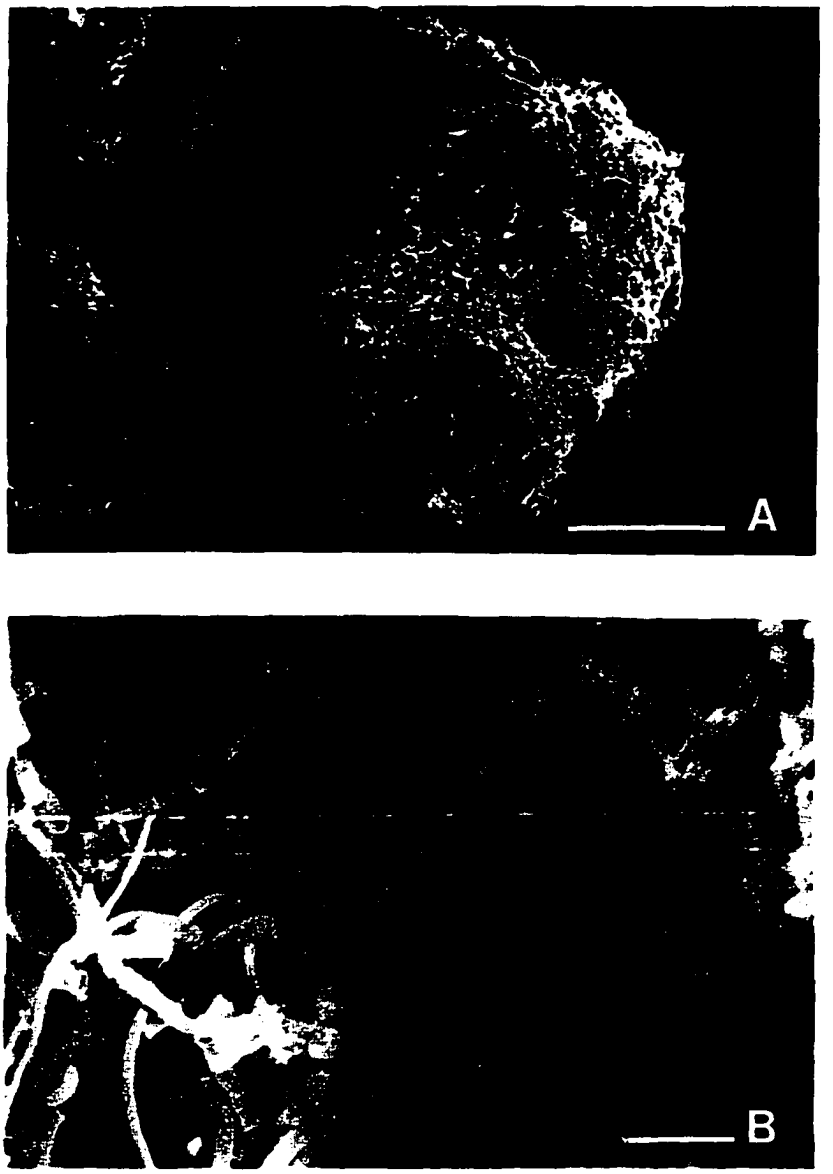


Figure 7 - Granular size distribution by area at the end of Run 2 and 3.

Figure 8 - SEM views of a granule at the end of Run 3. (a) Granule showing long *Methanosaeta* filaments on the surface (bar indicates 200 μm); (b) surface of same granule (bar indicates 2 μm).


A
B

CHAPTER 4. PSYCHROPHILIC ANAEROBIC DIGESTION OF LOW-STRENGTH WASTEWATER USING THE ANAEROBIC MIGRATING BLANKET REACTOR (AMBR)

A paper to be submitted to Water Environment Research

Largus T. Angenent, Gouranga C. Banik, and Shihwu Sung

ABSTRACT: The applicability of the anaerobic migrating blanket reactor (AMBR) was studied, for the treatment of low-strength wastewater at psychrophilic conditions, by operating a 20-liter AMBR fed non-fat dry milk (NFDM) as a substrate at a temperature of 15°C. The concentration of the influent was 600 mgCOD/L during the entire study with a five day biological oxygen demand (BOD_5) to chemical oxygen demand (COD) ratio of 0.48. The soluble chemical oxygen demand removal efficiency (SCOD removal) was 74% at the end of the operation, in which the hydraulic retention time (HRT) was decreased to four hours. Moreover, measured and calculated total COD removal efficiencies (TCOD removals) were 58% and 45%, respectively. The specific methanogenic activity (SMA) of the granules increased up to the end, illustrating a slow in-growth of methanogens and improving reactor performances. Thus, an active and acclimated granular biomass was needed for successful treatment of low-strength wastewater at psychrophilic conditions. Finally, the AMBR was able to retain its granular biomass at a hydraulic shock load in which the system HRT was decreased from four hours to one hour.

KEYWORDS: anaerobic, low-strength, psychrophilic, shock load, compartmentalized, granules, staging, AMBR, ambient temperatures, methanogenesis

Introduction

The anaerobic migrating blanket reactor (AMBR) was developed as a new high-rate anaerobic process for the treatment of municipal and industrial wastewaters by the authors and coworkers at Iowa State University (Angenent et al., 1997). Several other high-rate anaerobic processes proved to be sustainable over the last 20 years. Notably, the upflow anaerobic sludge blanket (UASB) reactor showed good performances and stability in numerous full-scale operations world-wide. Furthermore, low-strength wastewaters, such as domestic and food processing wastewaters, were successfully treated with the UASB reactor (Lettinga et al., 1993; Hulshoff Pol et al., 1997).

However, Kato (1994) showed that the expanded granular sludge blanket (EGSB) reactor was more efficient because of a higher mixing intensity, which decreased transport

limitations of substrate into the granules. Challenges for treating low-strength wastewaters are reactor related, because a good substrate/biomass contact is required without losing too many solids in the effluent. The AMBR system showed high retention of biomass due to a compartmentalized design. In addition, mechanical mixing provided a sufficient contact between the substrate and biomass (Angenent and Dague, 1996). Because of the absence of a hydraulic upflow pattern, no feed distribution system and gas solids separation (GSS) system were needed, which accomplished a simpler design. Another concern in treating these wastewaters are the low substrate levels in the reactor, but higher concentrations of substrate could be established in initial compartments of a plug-flow approaching reactor, such as the compartmentalized AMBR. Moreover, the AMBR was able to develop and grow granular biomass (Angenent et al., 1997), which protected the strict methanogens from oxygen toxicity (Kato, 1994).

Advantages of anaerobic pre-treatment of low-strength wastewaters are less sludge production and less energy requirements (Mergaert et al., 1992). However, since most low-strength wastewaters are discharged at ambient temperatures, maintaining the reactor under mesophilic conditions would increase the energy requirements and operational costs severely. Thus, pre-treatment of low-strength wastewaters is more attractive under psychrophilic conditions. Recently, pilot-scale studies of an EGSB system, revealed promising results of high-rate anaerobic treatment of malting waste at 13 to 20°C (Rebac et al., 1997). Also, Dague et al. (1998) found soluble chemical oxygen demand removal efficiencies (SCOD removals) of 70% at a six-hour hydraulic retention time (HRT) and 5°C in a laboratory-scale ASBR fed non-fat dry milk (NFDM).

Partial separation of acidogenesis and methanogenesis or staging in the AMBR was found when fed sucrose as a substrate at high COD loading rates and at mesophilic conditions, due to approached plug-flow conditions of the compartmentalized design (not yet published results). Similar results were obtained by van Lier et al. (1995) using the compartmentalized upflow staged sludge blanket (USSB) reactor, and by Nachaiyasit and Stuckey (1995) using the anaerobic baffled reactor (ABR). These workers found low hydrogen partial pressures in the headspaces of the final compartments, which enhanced acetogenesis. Thus, stimulating higher populations of acetogens and methanogens in the final compartments. Although, the headspace in the 20-liter AMBR was not divided per compartment, hydrogen concentrations of the liquid phase could have been relatively higher in the initial compartment, stimulating staging. Also, different concentrations of formic acid per compartment might be of even greater importance for the syntrophic relationships, which depend on interspecies hydrogen or formate transfer (Thiele and Zeikus, 1988; Stams, 1994).

Although COD loading rates of 44 g/L/d were efficiently applied to the AMBR (not yet published results), the system could also be ideal for treating low-strength wastewaters. Consequently, the applicability of the AMBR system for the anaerobic digestion of low-strength wastewater was evaluated under psychrophilic conditions at different HRTs. Furthermore, a hydraulic shock load was applied to study the behavior of the AMBR under peak flows which are common for low-strength wastewaters, such as sewage.

Methodology

Analysis. The composition of the biogas was measured using gas chromatography (GC; Gow Mac Model 350 with thermal conductivity detector; column: 6'*1/8' stainless steel Poropack Q 80/100 mesh; carrier gas: helium). The individual volatile fatty acids (VFA) were measured with ion chromatography (IC; Dionex DX-500 with CD 20 conductivity detector and anion micromembrane suppresser; column: Ion Pac ICE-As1; eluent: 0.8-1.0 mM heptafluorobutyric acid). IC samples were first acidified with HCl. The total alkalinity, total VFAs, total and soluble COD, five day biological oxygen demand (BOD_5), sludge volume index (SVI), and total and volatile suspended solids (VSS) were performed according to procedures in *Standard Methods* (APHA, 1995). Effluent samples of the AMBR processes were taken at the midpoint of the time interval between two reversals of flow.

Biomass characteristics. The specific methanogenic activity (SMA) was assessed with the "headspace method" according to tests described by Rinzema et al. (1988) The SMA of the biomass was determined at 35°C in a constant temperature room. Next, the dimensionless microbial load index (MLI) was calculated by dividing the food to microorganism (F/M) ratio by the SMA, which indicated the relative substrate utilization adequacy of the biomass in terms of methane production (Tay and Yan, 1996). However, to use the MLI at these conditions, the SMA needed to be corrected to 15°C by assuming a four time decrease of the biomass activity at a temperature decrease of 20°C, according to the van 't Hoff rule. To analyze the sizes of the granules and any change over time, the arithmetic mean diameter ($Sum(d)/n$) and area-weighted mean diameter ($Sum(d^3)/Sum(d^2)$) were calculated with automated image analysis (AIA). Samples of the mixed liquor of the reactor were mixed and diluted to obtain an overall distribution of clearly visible and non-overlapping biomass particles. Next, 1.75 mL was added to a special slide, which consisted of two, three mm thick glass sheets cemented together, with a one inch diameter hole in the top sheet. This in turn was covered with another thin sheet. The AIA set-up contained a black and white video camera (Dage-MTI series 68), a microscope (Olympus SZH), and a PC with Quartz PCI Imaging software. Some manual editing of the image was necessary to

separate adjacent granules. Particles smaller than 0.1 mm were not included in the calculations of the size distribution (Grotenhuis et al., 1991). The light microscopy views in Figure 6 were taken with a Pixera digital camera mounted on an Olympus microscope. The biomass migration rate (BMR) was the amount of biomass decreased over a period of time in which the flow was not reversed. The BMR and biomass migration index (BMI) and the utilized techniques to process the granular samples for scanning electron microscopy (SEM) were explained elsewhere (Angenent et al., 1997).

Assessment of the SMPR and calculated TCOD removal. The COD loading rate was defined as the amount of COD that was fed into the reactor per reactor volume per day (g/L/d). The biogas production was corrected to standard temperature and pressure (STP) using the ideal gas law. Next, the standard methane production rate (SMPR) was obtained after converting the biogas production with the wet volume of the reactor and the methane percentage that was present in the biogas. Therefore, the SMPR was expressed as liters of methane per reactor volume per day (L/L/d). The SMPR was a true measure of the COD that was removed, because methane was the final product in the stabilization of COD (0.35 L methane/gCOD). For the calculated TCOD removal, soluble methane washed out with the effluent was accounted for by adding its equivalent methane loss to the SMPR (SMPR of effluent in Figure 3). In addition, COD removal by sulfate reducing bacteria (SRB) was added to the calculated TCOD removal (on average 100 mg/L sulfate was removed; SRB COD removal calculated by COD of sulfide formed). The COD removal due to biomass growth was not included because biomass wash-out was part of the measured total COD. To obtain the theoretical or calculated total COD removal efficiency (calculated TCOD removal) the next equation was used:

$$\text{Calculated TCOD removal, \%} = 100 \cdot \frac{\text{total SMPR}}{\text{COD loading rate} \cdot 0.35} + \text{SRB COD removal}$$

Laboratory-scale AMBR. The temperature of the laboratory-scale AMBR was kept constant at 20°C (+/- 1°C) in the initial stages of the operational period. At day 88, the temperature of the incubator was decreased to 15°C (+/- 1°C) and pre-cooling of the influent was required. The active volume of the AMBR was 20 liters and was divided into four compartments, as illustrated in Figure 1. Baffles were placed between the compartments to reduce short-circuiting. The space between the baffle and the inside wall was one cm to prevent clogging problems in the laboratory-scale reactor. The flow over the horizontal plane of the reactor was reversed once a day to prevent accumulation of biomass into the final compartment due to migration. The second compartment was fed for four hours, before the

flow was reversed, to prevent a break-through of substrate. Sufficient biomass/substrate contact was maintained using intermittent, gentle mixing. Research by Dague et al. (1970) showed that mixing that was too intense could destroy the anaerobic bioflocs. Mixers (Model 5vb, EMI Inc., Clinton, Connecticut, USA) were able to start and operate at a slow speed and the use of paddles further enhanced gentle mixing. The compartments were mixed equally for ten seconds every four minutes at 60 rotations per minute (rpm) for the first 156 days of operation. At day 156, the mixing frequency was doubled to once every two minutes in the three initial compartments. Simultaneously, the final compartment was mixed every four minutes to prevent excessive biomass loss. All pumps used, were Masterflex pumps of Cole Parmer Instrument Co., Chicago, Illinois, USA. The gas collection systems consisted of an observation bottle, a gas sampling port, and a wet-test gas meter (GCA, Precision scientific, Chicago, Illinois, USA). The biogas was directly discharged from the reactor to the gas collection system. A water head was installed on the effluent tubes to prevent biogas from escaping through the effluent ports. Timers (ChronTrol Corporation, San Diego, California, USA) regulated the operation. An effluent baffle system (EBS) was placed in front of the effluent ports to prevent floating granules from washing out with the effluent.

Substrate. The concentrated substrate, consisting of non-fat dry milk (NFDM), sodium bicarbonate, and trace-elements, was stored in a refrigerator and was mixed to obtain a constant loading rate (Table 1). The same substrate was used for studies by Dague et al. (1998) and Banik et al. (1997). Make-up water was added to the substrate before feeding to the reactors. The sulfate concentration of the influent was on average 110 mg/L (COD/sulfate ratio was 5.5) mainly from Ames tap water, and the BOD_5 to COD ratio was 0.48 (Dague et al., 1998). Moreover, the SCOD concentration of the influent was on average 8.4% smaller than the TCOD concentration.

Seed. The seeded granules were obtained from three laboratory-scale ASBRs and were stored for three months at 5°C and one month at 20°C. These granules were grown on the same synthetic substrate as in the presented study and were acclimated at psychrophilic temperatures down to 5°C. Moreover, *Methanosaeta*-like microorganism were apparent throughout the granular structure (Dague et al., 1998; Banik et al., 1997). At the start-up, the mixed liquor volatile suspended solids (MLVSS), which is an indication of the amount of viable biomass in the reactor, was 20 g/L.

Experimental approach. The reactor was started at a 12-hour HRT and a COD loading rate of 1.25 g/L/d. Relative high loading rates were possible, because the seed biomass was already acclimated to similar environmental conditions. The concentration of the influent was kept constant at 600 mg COD/L (as TCOD) and a decrease of the HRT

resulted in an increase of the COD loading rate. During more than six months the applicability of the AMBR was evaluated for different HRTs. At the end of operational time, the HRT was four hours, which resulted in a COD loading rate of 3.5 g/l/d, as seen in Figure 2. The length of the periods of applying an HRT of 12, eight, six, and four hours were chosen as to reach pseudo steady-state conditions in which the reactor performance of two or more data points was equal. Thus, the period of time to reach the ultimate HRT could have been shorter if taking data points was not required. Finally, the HRT was decreased from four to one hour for one day to study a hydraulic shock load. The one-hour HRT (480 L/d) was applied to both flow directions for 12 hours each.

Results

Operational conditions. The operational parameters of the 20-liter AMBR are given in Figure 2. The top bar of this figure shows the HRT at which the system was operated. Biomass levels in the reactor were constant during the operational period in which the MLVSS oscillated around 23 g/L. The biomass levels in the effluent showed increases just after the temperature decrease on day 88, and any decrease in HRT. However, after some acclimation time the VSS levels in the effluent decreased after relative smaller particles were washed out. Consequently, this oscillating pattern is also found for the sludge retention time (SRT), which showed its highest levels on day 43, 108, and 164, just before the HRT was decreased. Furthermore, an increase in mixing intensity showed a small decrease in the SRT at day 156. Despite this oscillating pattern, the SRT for most of the operational time was exceeding 100 days, which showed that the AMBR was able to retain the granular biomass under these conditions. Notably, this is important for the treatment of low-strength wastewater at psychrophilic conditions because of the slow growth rate of biomass.

The F/M ratio was increased over the operational time up to 0.18 gCOD/gVSS/d. However, this showed no decrease in pH of the initial compartment or alkalinity of the effluent, which also indicates that stable conditions prevailed in the reactor. The pH in the reactor always exceeded 6.5 and was found to be approximately 6.75.

Reactor performances. Figure 3 illustrates reactor performances over the operational time of the AMBR. First, the COD removals are given in Figure 3a. All removals decreased over time due to a temperature decrease on day 88 and due to decreasing HRTs and subsequently increasing COD loading rates . The SCOD removal, calculated and measured TCOD removal were 93%, 73% and 84%, respectively, at a 12-hour HRT (20°C). At an HRT of four hours at 15°C, SCOD, calculated TCOD, and measured TCOD removals were 74%, 45%, and 58%, respectively. This differed not much from removals found at a

six-hour HRT, indicating that the system was increasing in performance at that period of time. During the six-hour HRT period the importance of mixing frequency was demonstrated, as doubling mixing frequencies in the initial compartments showed an increase in SCOD removal from 71% to 75%. The measured and calculated TCOD removals should have been the same for the entire run, but this never was the case, which could indicate biomass accumulation or methane loss due to an unknown reason. Actually, the measured biogas production and SMPR (Figure 3b), and thus the calculated TCOD removal, were severely decreased due to the temperature decrease at day 88 without decreasing the measured TCOD removal as much. The soluble methane in the effluent, which was calculated with the Henry constant (SMPR of effluent), did not entirely correct for this, but still accounted for one third of the total SMPR at a temperature of 15°C.

The results illustrated stable reactor performances throughout the entire run, with SCOD removals exceeding 70%. Also, the VFAs of the effluent were constant despite a small increase at an HRT of four hours. Actually, the reactor still showed slow improvement at the end of the four-hour HRT period, since the total SMPR and SMA were increasing up to the end of operation, as illustrated in Figure 3b. In-growth of methanogens occurred very slowly at these conditions, and a prolonged operation possibly could have shown an increased SMA of the granules and increased reactor performances. Especially, since the system was overloaded, as illustrated by a corrected MLI higher than 1 in Figure 3c. Also, the elevated total VFAs showed that reactor performances could be improved. Thus, longer operation of the AMBR would possibly have increased the SMA of the granules some more, which would have decreased the corrected MLI to less than one and would have decreased the VFA concentration of the effluent. This in turn would have resulted in slightly higher SCOD removals. Its needs to be noticed that the corrected MLI higher than one indicated that the F/M ratio was higher than the SMA of the biomass at 15°C, which means that the biomass was fed more substrate than it could maximally utilize to form methane.

Figure 5a shows VFA and SCOD concentrations of the individual compartments at the midpoint between reversals of the flow at day 184. Plug-flow conditions are apparent from this figure with relative high concentrations of acetic acid and SCOD in the initial compartment and low concentrations in the final compartments. Furthermore, the appearance of formic and propionic acid in the initial compartment shows staging of the substrate in the AMBR. This figure was used as a baseline to compare reactor performances during and after the shock load.

Biomass characteristics. The size of the granules increased over time, as illustrated in Figure 3d. At the end of the operational period, the arithmetic and area-weighted mean

diameter were 1.1 and 3.0 mm, respectively. Also, the area distribution of the granules in Figure 4 shows this phenomenon in which the graph at day 185 had slid to the right compared to the graph of the seed granules and the graph at day 105. A wash-out of smaller biomass particles and a size increase of granules, due to growth, were responsible for the increase of the mean diameter. At day 185, 40% of the projected granular area was due to particles between 3.16 and 5.62 mm in diameter, but this was zero for the seed sludge.

Staging of biomass was detected by SMA in which biomass of the initial compartment had a significant lower activity compared to the second compartment at the end of the operational period (Student t-test: 95% significance level). The SMA of the initial compartment was found to be 0.53 gCOD/gVSS/d (+/- 0.01; n=2) and the SMA of the second compartment was 0.58 gCOD/gVSS/d (+/- 0.01; n=3). Furthermore, the biomass in the outside compartments had white dots on the black surface, which gave them a lighter-coloured appearance compared to the biomass in the middle compartments, which did not lose a smooth black surface. Indeed, light microscopic views show that small white/gray colonies were growing on the surface of the granules in the initial compartments (as seen in Figure 6a), and much less on the granules in the second compartments (as seen in Figure 6b). SEM views of granules out of the initial compartments show differences in phenotype between microorganism in the colonies and microorganism on the granular surface (as seen in Figure 7a and 7b). In addition, storage of these granules at 4°C over a three month period did not deteriorate the structure of the white/gray colonies.

Shock load. The COD loading rate during the hydraulic shock load was increased from 3.5 to approximately 15 g/L/d with an HRT of one hour, as shown in Figure 2a. Simultaneously, the F/M ratio was increased from 0.18 to 0.72 gCOD/gVSS/d. Consequently, the SCOD removal decreased to 39%, the measured TCOD removal decreased to 30%, and the VFA concentration of the effluent increased to 0.1 g/L, as seen in Figure 3a. However, the hydraulic shock load did not upset the reactor in terms of a severe pH drop, as Figure 2d shows a pH level exceeding 6.5 during the shock load. Approached plug-flow conditions, seen at an HRT of four hours, were lost at day 185, as Figure 5b only shows a small decrease of the SCOD concentration and an increase of the VFA concentration over the horizontal plane of the reactor. Clearly, acidogenesis of the NFDM substrate shifted from mainly the first compartment to all compartments.

The BMR and BMI during the shock load were 13.2 gVSS/L/d and 30.7 gVSS/mL/d, respectively. This shows that the granules with a SVI of 21.4 mL/gVSS were migrating, but within the range of possible MLVSS loss in the first compartment. Clearly, the migration of granules had gone up during the shock load, because the granules were not migrating much

before the shock-load. Also, the wash out of biomass increased from 8.7 to 35 g/d and the SRT decreased from 50.6 to 12.6 days (Figure 2c), but this had decreased the MLVSS only 1 g/L. Indeed, the reactor performances one day after the hydraulic shock load (t = 186 days) were almost similar as one day before the shock load (Figure 3). Figure 5d illustrates that the AMBR at 186 days approached plug-flow conditions as it did before the shock load, which indicated that not much biomass could have been washed out. One difference was that propionic acid was noticed in all compartments, which showed a small upset.

Discussion

Applicability of the AMBR. These results, presented here, showed that the 20-liter AMBR was able to effectively remove organic material from dilute NFDM at a concentration of 600 mg COD/L under psychrophilic conditions. Furthermore, the reactor performance was found to be stable over a six month operational period, which illustrated the ability of the AMBR to retain biomass. Mixing was found to be very important to promote substrate/biomass contact and to prevent short-circuiting in the laboratory-scale AMBR. Compared to an ASBR fed the same influent at an HRT of six hours and a temperature of 15^{o}C (Dague et al., 1998), the AMBR was less efficient at these conditions. A better SCOD removal in the ASBR could be explained by the absence of short-circuiting in a batch fed system. Thus, SCOD removals could have been higher for a full-scale AMBR, because a better baffle arrangement between the compartments and scale factors would reduce chances for short-circuiting. As for the 20-liter AMBR the baffles had to be placed at a certain distance to prevent clogging problems by the relative big granules. Although the ASBR was performing well with the low-strength wastewater, physical problems would limit the flow rate into the system in which HRTs of one hour would be impossible without losing biomass. Making continuous flow system, such as the AMBR, advantageous in that regard.

The successful pre-treatment of sewage by anaerobic high rate systems at ambient temperatures was studied by several workers (Kaijun et al., 1997; Singh et al., 1997; Barbosa and Sant' Anna, 1989). The results, presented here, suggests that the AMBR could achieve similar efficiencies and loading rates when treating sewage, and that staging could be an advantage in that regard. However, treatability studies with the AMBR fed sewage are required.

Shock load. Studies with the ABR found this high-rate compartmentalized system to be very stable to large changes in flow (Nachaiyasit and Stuckey, 1997). The ABR recovered back to its baseline performance shortly after a period of shock-load flow ended, as was

found for the AMBR. Hence, the stability to hydraulic shock loads made these reactor types potentially favourable for treating domestic and industrial wastewaters.

Staging. Feeding non-acidified NFDM to the compartmentalized AMBR resulted in staging of the substrate and biomass. During an HRT of four hours, plug-flow conditions were approached, as seen in Figure 5a, in which acidogenesis took place mainly in the initial compartment. Notably, high formic acid (or hydrogen) concentrations in the initial compartment and low concentrations in the final compartments probably stimulated differences in the syntrophic relationships of biomass in which acetogenic and methanogenic activities would be higher in the final compartments. As lower levels of formic acid (or hydrogen) can only favour acetogenic reactions (Stams, 1994). Therefore, differences in the methanogenic activities between the outside and inside compartments were probably the result of staging of biomass, due to differences in environmental conditions between the initial and second compartment. Indeed, staging in two EGSB reactors in series, fed partly acidified substrate, was found by van Lier et al. (1997) at temperatures as low as 8°C, in which the acidogenic population was dominant in the first stage and acetogenic and methanogenic populations were dominant in the second stage. However, it must be realized that levels of oxygen were also higher in the initial compartment compared to the final compartments of the AMBR, making it possible that staging of biomass resulted from increased growth of facultative oxygen consuming bacteria on the surface of the outside compartments. Also, SRB could have grown mainly in the initial compartment, as sulfate would be used as an electron acceptor first.

Conclusions

Based on laboratory studies with a 20-liter AMBR, which consisted of four compartments and was fed NFDM in concentrations of 600 mgCOD/L at psychrophilic conditions, the following conclusions were drawn:

The AMBR was able to achieve SCOD, measured and calculated TCOD removals of 74%, 58% and 45%, respectively, at an HRT of four hours. Mixing was found to be important to achieve sufficient biomass/substrate contact and to prevent short circuiting of substrate in the laboratory-scale reactor. The reactor performance was elevated over time in which granular size and SMA of the granules increased. To sufficiently treat low-strength wastewater at these conditions an active and acclimated granular biomass was required.

A hydraulic shock load, in which the HRT was decreased from four to one hour, did not upset the AMBR in terms of biomass loss and reactor performances, illustrating high retention of biomass and stability of the system.

Staging of the biomass was found in the compartmentalized reactor due to approach plug-flow conditions in which the SMA of the granules in the outside compartments was lower over granules in the inside compartments. This showed higher methanogenic levels of granules in the compartments which received relative more acidified substrate. In addition, light microscopic views showed growth of small white/gray micro colonies on the black surface of the granules in the outside compartments only.

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Authors. At the time this study was conducted, Largus T. Angenent and Gouranga C. Banik were graduate research assistants, and Shihwu Sung was an assistant professor of environmental engineering, Department of Civil and Construction Engineering, Iowa State University, Iowa, USA. Correspondence should be addressed to Shihwu Sung, 394 Town Engineering Building, Ames, IA 50011, USA.

References

Angenent, L. T., and Dague, R. R. (1996) Initial studies on the anaerobic migrating blanket reactor. In: *Proc. of 51st Purdue Industrial Waste Conference* (Edited by Dalton C. S. and Wukasch R. F.), 271, Ann Arbor Press, Chelsea, Michigan, USA.

Angenent, L. T., Sung, S., and Dague, R. R. (1997). Start-up and granulation of the anaerobic migrating blanket reactor (AMBR). In: *Proc. of the WEFTEC'97*, **1**, 113, Water Environment Federation, Alexandria, Virginia.

APHA (1995) *Standard Methods for the Examination of Water and Wastewater* , 19th edition. American Public Health Association, Washington, D. C., USA.

Banik, G. C., Ellis, T. G., and Dague, R. R. (1997) Structure and methanogenic activity of granules from an ASBR treating dilute wastewater at low temperatures. In: *Proc. 8th International Conf. on Anaerobic Digestion*, **2**, 548.

Brabosa, R. A., and Sant' Anna, G. L. (1989) Treatment of raw domestic sewage in an UASB reactor. *Wat. Res.*, **23**, 1483.

Dague, R. R., McKinney, R. E., and Pfeffer, J. T. (1970) Solids retention in anaerobic waste treatment systems. *J. Water Pollut. Control Fed.*, **42**, Part 2, R29.

Dague, R. R., Banik, G. C., and Ellis, T. G. (1998) Anaerobic sequencing batch treatment of dilute wastewater at psychrophilic temperatures. *Wat. Environ. Res.*, **70**, 155.

Grotenhuis, J. T. C., Kissel, J. C., Plugge, C. M., Stams, A. J. M., and Zehnder, A. J. B. (1991) Role of substrate concentration in particle size distribution of methanogenic granular sludge in UASB reactors. *Wat. Res.*, **25**, 21.

Hulshoff Pol, L., Euler, H., Eitner, A., and Grohganz, D. (1997) GTZ sectoral project promotion of anaerobic technology for the treatment of municipal and industrial sewage and wastes. In: *Proc. 8th International Conf. on Anaerobic Digestion*, **2**, 285.

Kaijun, W., van der Last, A. R. M., and Lettinga, G. (1997) The hydrolysis upflow sludge bed (HUSB) and the expanded granular sludge blanket (EGSB) reactor processes for sewage treatment. In: *Proc. 8th International Conf. on Anaerobic Digestion*, **3**, 301.

Kato, M. T. (1994) The anaerobic treatment of low strength soluble wastewaters. Ph.D. dissertation, Wageningen Agricultural University, Wageningen, The Netherlands.

Lettinga, G., de Man, A., van der Last, A. R. M., Wiegant, W., van Knippenberg, K., Frijns, J., and van Buuren, J. C. L. (1993) Anaerobic treatment of domestic sewage and wastewater. *Wat. Sci. Tech.*, **27**, 67.

van Lier, J. B., Groeneveld, N., and Lettinga, G. (1995) Development of thermophilic methanogenic sludge in compartmentalized upflow reactors. *Biotech. Bioeng.*, **50**, 115.

van Lier, J. B., Rebac, S., Lens, P., van Bijnen, F., Oude Elferink, S. J. W. H., Stams, A. J. M., Lettinga, G. (1997). Anaerobic treatment of partly acidified wastewater in a two-stage expanded granular sludge bed (EGSB) system at 8°C. In: *Proc. 8th International Conf. on Anaerobic Digestion*, **2**, 86.

Mergaert, K., Vanderhaegen, B., and Verstraete, W. (1992) Applicability and trends of anaerobic pre-treatment of municipal wastewater. *Wat. Res.*, **26**, 1025.

Nachaiyasit, S., and Stuckey, D. C. (1995) Microbial response to environmental changes in an anaerobic baffled reactor (ABR). *Antonie van Leeuwenhoek*, **67**, 111.

Nachaiyasit, S., and Stuckey, D. C. (1997) The effect of shock loads on the perfromance of an anaerobic baffled reactor (ABR). 2. Step and transient hydraulic shocks at constant feed strength. *Wat. Res.*, **31**, 2747.

Rebac, S., van Lier, J., Janssen, M. G. J., Dekkers, F., Swinkels, K. Th. M., and Lettinga, G. (1997) High-rate anaerobic treatment of malting waste water in a pilot-scale EGSB system under psychrophilic conditions. *J. Chem. Tech. Biotechnol.*, **68**, 135.

Rinzema, A., Van Lier, J. B., and Lettinga, G. (1988) Sodium inhibition of acetoclastic methanogens in granular sludge from a UASB reactor. *Enzyme and Microb. Technol.*, **10**, 24.

Singh, K. S., Viraraghavan, T., Karthikeyan, S., and Caldwell, D. E. (1997) Low temperature start-up of UASB reactors for municipal wastewater treatment. In: *Proc. 8th International Conf. on Anaerobic Digestion*, **3**, 192.

Stams, A. J. M. (1994) Metabolic interactions between anaerobic bacteria in methanogenic environments. *Antonie van Leeuwenhoek*, **66**, 217.

Tay, J, and Yan, Y. (1996) Influence of substrate concentration on microbial selection and granulation during start-up of upflow anaerobic sludge blanket reactors. *Wat. Envron. Res.*, **68**, 1140.

Thiele, J. H., and Zeikus, J. G. (1988) Control of interspecies electron flow during anaerobic digestion: significance of formate transfer versus hydrogen transfer during syntrophic methanogenesis in flocs. *Appl. Environ. Microbiol.*, **54**, 20.

Table 1 - NFDM substrate recipe.

Component	mg added (per g of COD)	
non-fat dry milk (NFDM)		962
Bicarbonate, as $NaHCO_3$	962	
$FeCl_2.4H_2O$	17.1	
$NiCl_2.6H_2O$	1.9	
$CoCl_2.6H_2O$	1.9	
$MnCl_2.4H_2O$	1.7	
$ZnCl_2$	1.3	

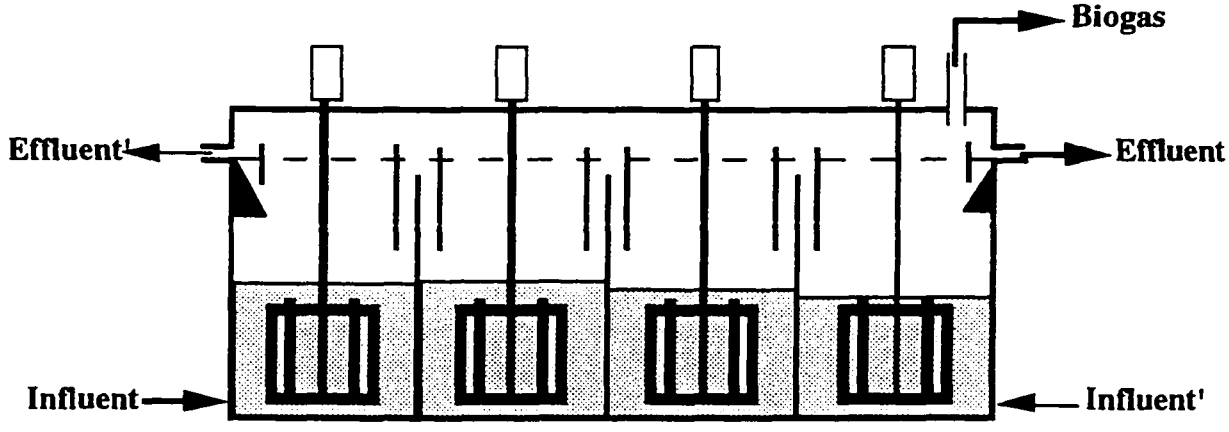


Figure 1 - Schematic diagram of the 20-liter AMBR.

Figure 2 - Operational conditions of the 20-liter AMBR: (a) loading conditions; (b) biomass levels in reactor and effluent; (c) SRT and F/M ratios; (d) pH and alkalinity over time.

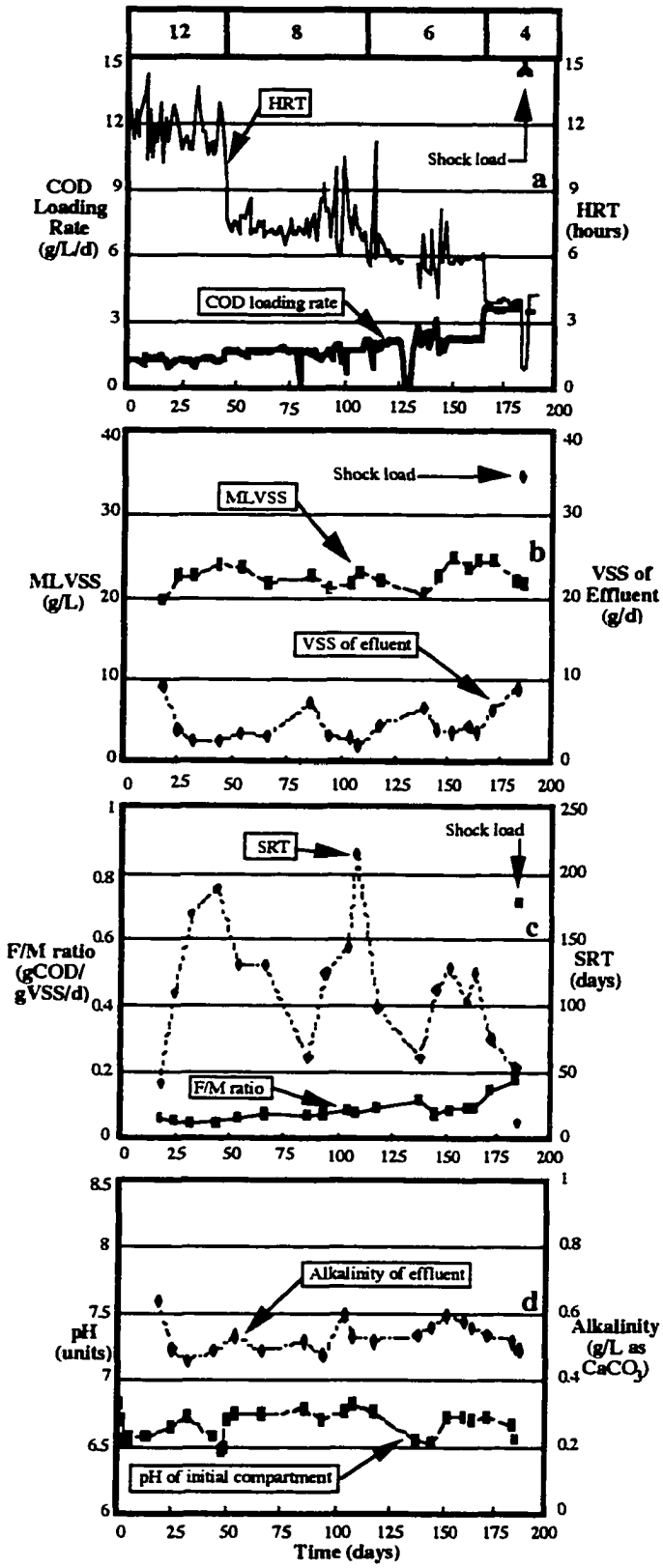
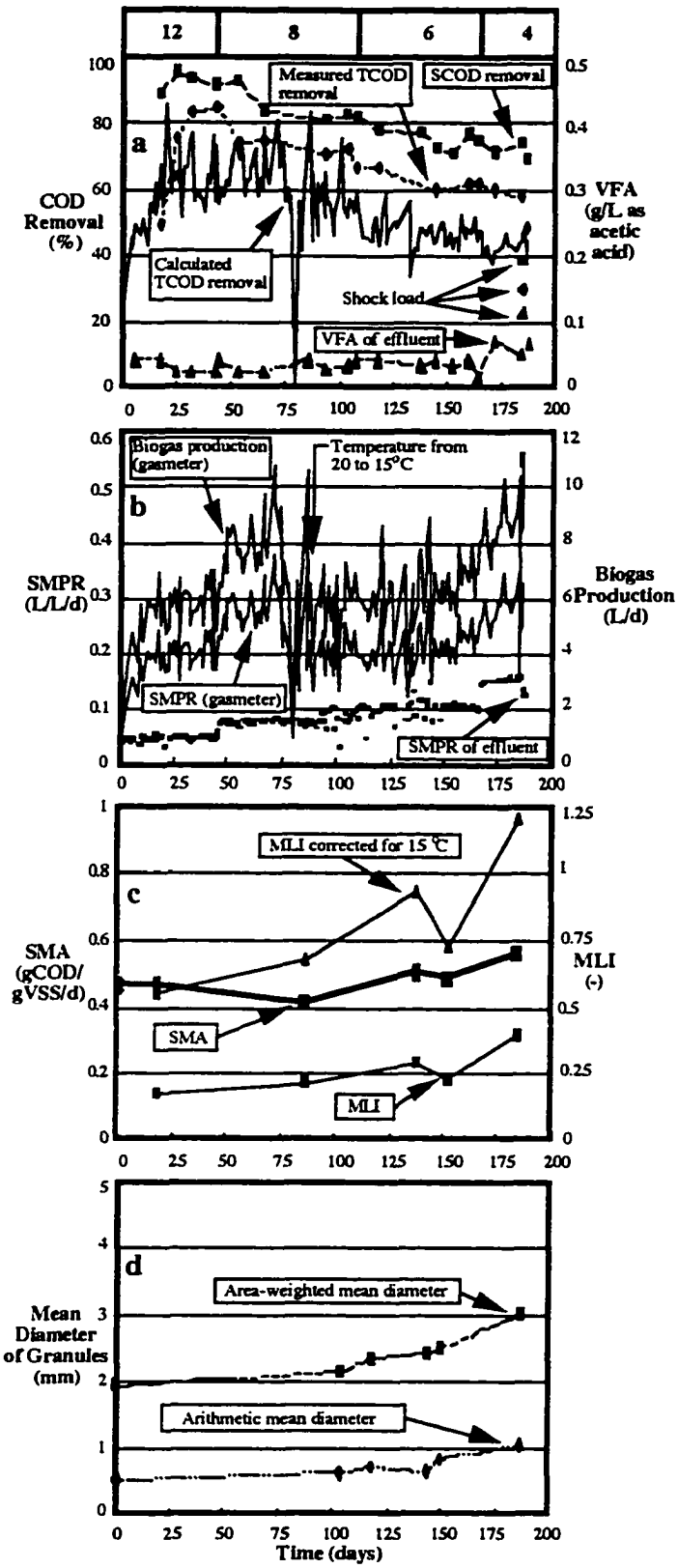

12
8
6
4
HRT
Shock load
a
COD Loading Rate (g/L/d)
HRT (hours)
COD loading rate
Shock load
MLVSS
b
MLVSS (g/L)
VSS of Effluent (g/d)
VSS of effluent
SRT
Shock load
c
F/M ratio (gCOD/ gVSS/d)
SRT (days)
F/M ratio
Alkalinity of effluent
d
pH (units)
Alkalinity (g/L as $CaCO_3$)
pH of initial compartment
Time (days)

Figure 3 - Reactor performances of the 20-liter AMBR: (a) removals; (b) produced biogas and methane; (c) biomass activity; (d) granular sizes over time.


12
8
6
4
a
Measured TCOD removal
SCOD removal
Calculated TCOD removal
Shock load
VFA of effluent
COD Removal (%)
VFA (g/L as acetic acid)
b
Biogas production (gasmeter)
Temperature from 20 to 15°C
SMPR (gasmeter)
SMPR of effluent
SMPR (L/L/d)
Biogas Production (L/d)
c
MLI corrected for 15 °C
SMA
MLI
SMA (gCOD/ gVSS/d)
MLI (-)
d
Area-weighted mean diameter
Arithmetic mean diameter
Mean Diameter of Granules (mm)
Time (days)

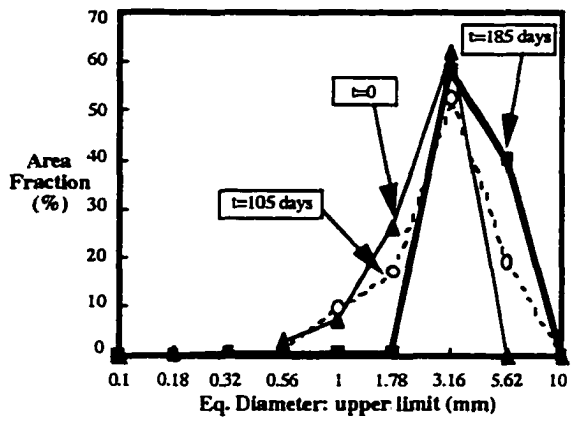


Figure 4 - Area distribution of granules from the start, middle, and end of the operational time.

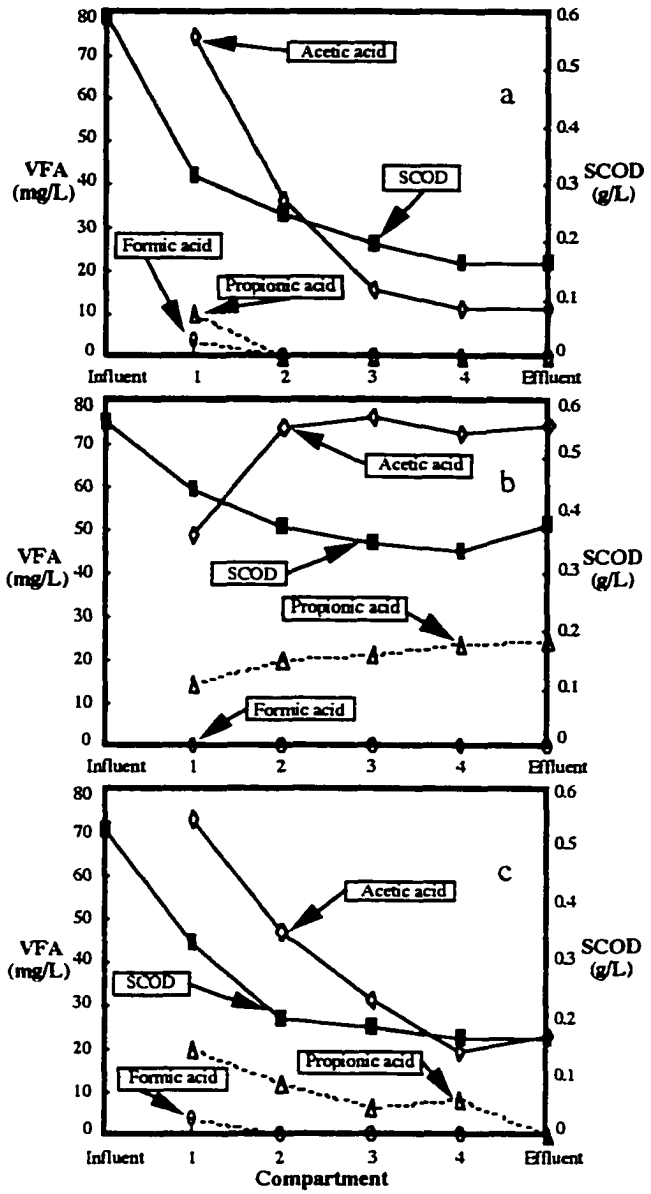


Figure 5 - VFA and SCOD concentration at the midpoint between intervals of flow per compartment: (a) t=184 days: baseline at an HRT of four hours; (b) t=185 days: hydraulic shock load at an HRT of one hour; (c) t=186 days: next day at an HRT of four hours again.

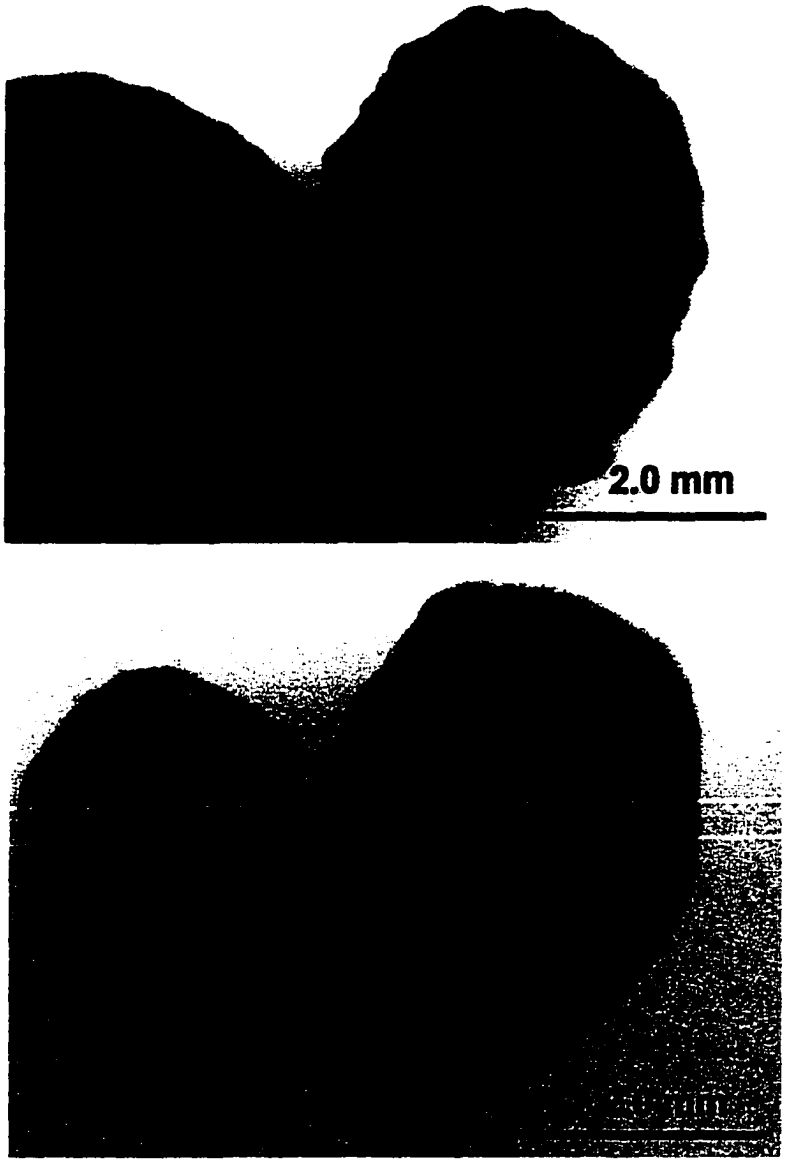


Figure 6 - Light microscopic views (10 times magnification) of granules: (a) from outside compartements; (b) from inside compartments.

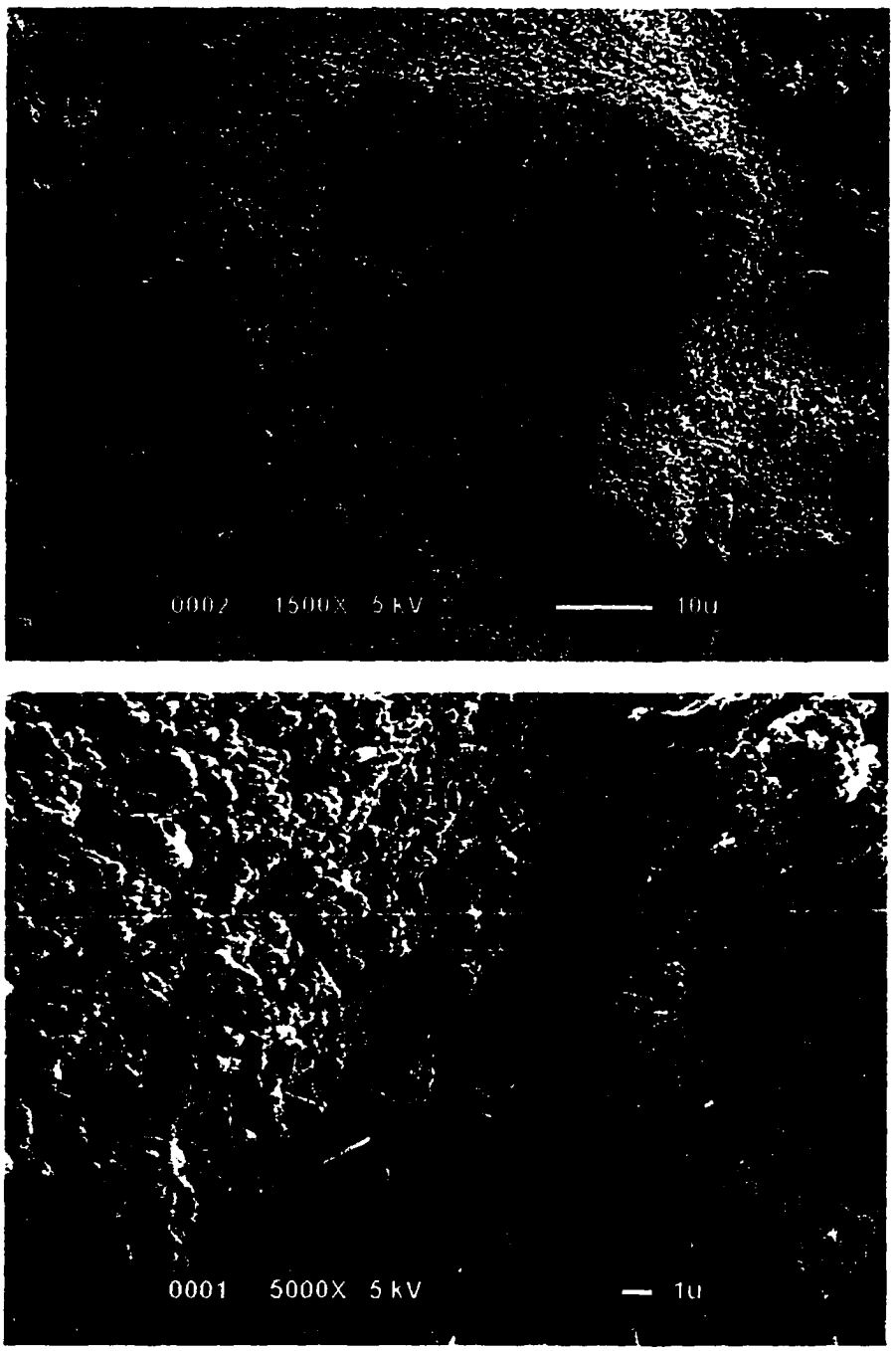


Figure - 7- SEM views of white/gray colonies on the surface of granules found in the outside compartments only. (2) broken up colony (1500 times magnification); (1) microorganism of the colony seen on the left (5000 times magnification).

CHAPTER 5. GENERAL CONCLUSIONS

General Discussion

The data collected over the past four years showed that the AMBR is an outstanding process that has capabilities for waste treatment that exceed those of other technologies available in the field for similar applications, including the ASBR and UASB processes. Also, several professors, who are working in the same field world-wide, published about the advantages of having a compartmentalized reactor configuration (such as the AMBR) in terms of organic loading rates and stability. Treatability studies with a paper recycling wastewater showed promising reactor performances of a laboratory-scale AMBR (Flamming et al, 1997). Furthermore ongoing research with a five compartment AMBR and divided headspaces showed high efficiencies at higher organic loading rates than found in the literature, when treating non-acidified sucrose as a substrate.

Therefore, the authors feel that the AMBR could become the major applied compartmentalized design, since it is simple and it does not have the disadvantages of other compartmentalized processes. Furthermore, a possible niche for the AMBR could be the treatment of low-strength wastewater, such as domestic wastewater. This could be of a major importance since low-strength wastewater contributes to the bulk of wastewater in this country in the form of sewage. However, treatment of this wastewater will probably only be accepted in regions were energy is valued. Currently sewage is treated aerobically, which costs energy. Conversely, anaerobic processes like the AMBR can produce energy by converting the formed methane to electricity or heat.

The formation of a granular blanket was achieved within four months of operating a 54-liter AMBR, which was seeded with flocculent digester sludge. This result disproved the theory that granulation can only be found in anaerobic reactors with a hydraulic upflow pattern. Thus simpler reactor configurations could evolve, such as the AMBR, in which food-distribution and gas-solid-separator systems are absent.

Scale-up and cost factors will probably change the reactor design of full-scale AMBR systems. Probably, migration of the blanket will be slower and short-circuiting will be less pronounced in a full-scale reactor, which could make openings in the walls between the compartments sufficient.

Recommendations for Future Research

Scale-up factors will have an impact on the design of the AMBR. Therefore, more research is needed in how biomass will behave in larger scale operations. Two questions will

have to be answered before a successful transition from laboratory- to full-scale systems can be made: 1. How can sufficient mixing be provided to the granular blanket? 2. And, what is the biomass migration rate and where is the biomass located during the operation of the AMBR? Consequently, pilot-scale studies are required to answer these question.

In addition, laboratory-scale studies should be run in finding more niches in which the AMBR could be competitive over other high-rate systems such as the UASB reactor. Therefore, the following research topics are given in separate paragraphs:

Thermophilic conditions. As van Lier (1994) proved, compartmentalized anaerobic systems with separated headspaces per compartment were able to provide efficient treatment of substrates during thermophilic conditions. The partial hydrogen pressure in the final compartments was as low as to successfully degrade all volatile fatty acids (VFA) in the reactor. Therefore, an AMBR with separate headspaces and a sufficiently acclimated granular biomass should be operated to show if this system could be advantageous in that regard.

Sulfate-rich wastewaters. Food, agricultural, and pulp and paper industries often produce wastewaters with high levels of sulfate. Although anaerobic treatment of these wastewaters is very attractive, problems develop because sulfate is reduced in anaerobic environments to sulfide, which can become toxic to the microbial community. Fortunately, new compartmentalized reactors were developed, such as the anaerobic migrating blanket reactor (AMBR), in which hydrogen sulfide can be stripped out of the initial compartments. This develops a less toxic environment in the final compartments where by, the methanogens can fully degrade the organic matter into methane. Subsequently, methane can be used as an energy source for the heating of water or the production of electricity.

However, before this new technology, can be applied to sulfate-rich wastewaters, more needs to be known about the maximum amount of sulfate that can be tolerated, the impact on the microbial community, and the overall reactor performance. Knowledge of the microbial community is critical to fully understand the processes that are going on and to be able to optimize the system. Therefore, research is required to study the AMBR fed with sulfate-rich wastewater. The reactor performance should be compared to an upflow anaerobic sludge blanket (UASB) reactor, which is a continuously-fed reactor based on a single vessel design such that no capacity to lessen the toxicity to the anaerobic community is present.

High solids content wastewater. The ASBR technology proved to stabilize wastewaters with a high solids content, such as diluted pig manure. However, a single vessel reactor configuration has disadvantages in terms of biomass retention and stability, as seen

with slaughterhouse wastewater. Therefore, research should be proposed in which treatability studies would be performed with an AMBR fed living-stock wastewater, such as diluted pig manure or slaughterhouse wastewater rich in solids. Notably, the AMBR showed retention of most flocculent biomass up to a organic loading rate of 5.5 gCOD/L/d and only flocculent biomass is capable of solids destruction (Angenent et al., 1998).

References

Angenent L. T., Sung, S. and Dague, R. R. (1998). Start-up of the anaerobic migrating blanket reactor (AMBR) seeded with primary digester sludge. *Separation and filtration.* (Accepted for publication).

Flamming, J. J., Angenent, L. T., Sung, S. and Dague, R. R. (1997) Treatability of two different industrial wastewater streams using the anaerobic migrating blanket reactor. In: *Proc. of the 52nd Industrial Waste Conference.* Ann Arbor Press, Chelsea, Michigan, USA.

van Lier, J. B. (1995) Thermophilic Anaerobic Wastewater Treatment; Temperature Aspects and Process Stability. Ph.D. Dissertation, Wageningen Agricultural University, Wageningen, The Netherlands.

APPENDIX A. SPECIFIC METHANOGENIC ACTIVITY TEST

(Rinzema et al., 1988)

Introduction

In small serum bottles the increase of methane in the headspace over time is measured by GC. During the test all parameters like temperature, pH, diffusion limitations, and concentration of the feed are chosen as favourable for the methanogens as possible. This is done by putting the batches on a shaker table in a 35°C room, setting the pH at 7, and adding acetate at a concentration of 2 g/L in which the maximum possible methane production is achieved. Thus, the acetoclastic methanogenic activity is measured. Furthermore, nutrients, trace-elements, yeast-extract, and a buffer are available in the batch stock solution. The amount of biomass added needs to be manipulated as not to get higher methane percentages as 3 % in the headspace of the serum bottle. Otherwise there will be a pressure build up which will inhibit the methane production. Regression will be used to calculate the increase in methane over time. For this reason 5 points are needed to create a perfect straight line. For statistical reasons use two or better three serumbottles per sample. Of course oxygen needs to be minimized, so bottles will be flushed with nitrogen gas and sodium sulfide will be added to create a reducing environment. But, think anaerobically which means open the bottles as short as possible!! To remind you of oxygen, resazurin is added to the batch medium. This will colour pink whenever oxygen is dissolved in the solution,

Materials

weigh dishes (porcelain)
103°C dryer oven
syringes and needles
pH-meter
acetate solution (1M)
sodium sulfide solution (0.25 M)
incubator
550°C muffler
250 mL serum bottle with septa
sodium hydroxide solution (3%)
nitrogen gas

Trace element stock solution[1] (Zehnder *et al.*, 1980)

	Compound	Concentration		Add mg to 7 liter
1.	$FeCl_2.4H_2O$	10000	mg/L*	70000
2.	$CoCl_2.6H_2O$	2000	mg/L*	14000
3.	EDTA	1000	mg/L*	7000
4.	$MnCl_2.4H_2O$	500	mg/L	3500
5.	Resazurin	200	mg/L*	1400
6.	$NiCl_2.6H_2O$	142	mg/L*	994
7.	Na_2SeO_3	123	mg/L	861
8.	$AlCl_3.6H_2O$	90	mg/L	630
9.	H_3BO_3	50	mg/L	350
10.	$ZnCl_2$	50	mg/L	350
11.	$(NH_4)_6Mo_7O_{24}.4H_2O$	50	mg/L	350
12.	$CuCl_2.2H_2O$	38	mg/L	266
13.	HCl (37.7% solution)	1	mL/L	7 mL

* Changed over time (van Lier, 1995; Angenent et al, 1997)

Nutrient stock solution for batch tests[2] (van Lier, 1995)

	Compound	Concentration	
1.	$NaH_2PO_4.H_2O$	7.95	mg/L
2.	K_2HPO_4	6.0	mg/L
3.	NH_4Cl	2.8	mg/L
4.	$MgSO_4.7H_2O$	1.0	mg/L
5.	Yeast extract	1.0	mg/L
6.	$CaCl_2.2H_2O$	0.1	mg/L
7.	Trace element solution[1]	10	mL/L

[2]Will be diluted ten times with anaerobic water and biomass.

Methods

Two days are used for this test. The first day the serum bottles are prepared and are incubated over night. The next day acetate is added and the actual test can take place.

Day 1:

1. Weigh the empty serum bottle
2. Weigh the bottle when filled with nanopure water to the top
3. Make anaerobic water by flushing tap water with nitrogen gas
4. Add 15 mL of the batchmedium to the bottles (10% of wet volume)
5. Add 5 mL of 1M acetic acid (conc. will be 2 g/L)
6. Add anaerobic water until volume will be around 140 mL (including the biomass)
7. Correct the pH to 6.85-6.9 by adding NaOH (flushing with N_2 will further increase the pH to 7)
8. Add the biomass: f.e. add 1 or 2 mL of active granules
   f.e. add 15-30 mL of MLVSS of digester sludge
9. Flush with nitrogen gas for 15 seconds when bottle is open (high flow)
10. Close the bottle and flush with two needles for a couple of minutes
11. Add 0.5 mL of 0.25 M Na_2S
12. Put on shakertable and leave overnight. The solution should be white in colour. When the solution is still pink after half an hour add a little more Na_2S or flush more (you have kept the bottle too long open to the atmosphere).

Day 2:

1. Take a VFA sample and check how much to add to achieve an acetate concentration of 2 g/L (or add 2.5 mL of 1M acetate solution)
2. Correct pH to 6.85-6.9 and write down the pH
3. Flush headspace with N_2 (as yesterday)
4. Put in the shaker for an hour
5. Measure the methane conc. in the headspace five times in a row (f. e. every 15-30 minutes)
6. Measure the pH and measure the weight of the bottle with solution (this is done to calculate the volume of the headspace)
7. Measure the VSS of all the granules in the bottle. However, these need to be rinsed three times with nanopure water (VSS). But, for flocculent sludges 5 mL can be taken and a filter can be used for the VSS measurement.
8. Plot the increase in methane percentage over time and calculate the %CH4/d with regression. Correct this with the volume of the headspace, the VSS, and a factor 0.388 to yield the SMA (gCOD-CH4/gVSS/d at STP).

References

Angenent, L. T., Sung, S., and Dague, R. R. (1997) Start-up and granulation of the anaerobic migrating blanket reactor. In: Proceedings of the WEFTEC'97, October 18-22, Chicago, Illinois.

van Lier, J. B. (1995). Thermophilic Anaerobic Wastewater Treatment; Temperature Aspects and Process Stability, Doctoral dissertation, Agricultural University Wageningen, Wageningen, The Netherlands.

Rinzema, A., van Lier, J. B., and Lettinga, G. (1988) Sodium inhibition of acetoclastic methanogens in granular sludge from a UASB reactor. *Enzyme and Microb. Technol.* **10**, 24-32.

Zehnder, A. J. B., Huser, B. A., Brock, T. D., and Wuhrman, K. (1980). Characterization of an acetate-decarboxylating, non-hydrogen-oxidizing methane bacteria, *Arch. Microbiol.*, 124: 1-11.

APPENDIX B. RECIPES FOR SYNTHETIC SUBSTRATE

Addition to sucrose
For the nutrients it is assumed that 5% of the COD will be needed for the grow of biomass (Biomass contains of 14% N and 2% P). Magnesium (20-30 mg/L), sulfate (70-130 mg/L), and calcium (130-140 mg/L) are available in Ames' tap water.

Nutrient stock solution for sucrose feed[3]

	Compound	Concentration		Add g to 7 liter
1.	NH_4CL	113	g/L	791
2.	K_2HPO_4	22.6	g/L	158.2
3.	$NaH_2PO_4.H_2O$	19.2	g/L	134.4

Sucrose feed (per gram of sucrose = per gram of COD)

	Compound	Amount per gram of sucrose
1.	Sucrose	1 gram
2.	Bicarbonate ($NaHCO_3$)	0.55-0.75 gram (add more at very low loadings)
3.	Nutrient stock solution[3]	0.886 mL (mimimum 0.3 % (v/v))
4.	Trace element solution[1]	0.07 mL (mimimum 0.1 % (v/v))
5.	Yeast extract	0.003 g

[1] See Appendix A

APPENDIX C. REACTOR CONFIGURATIONS

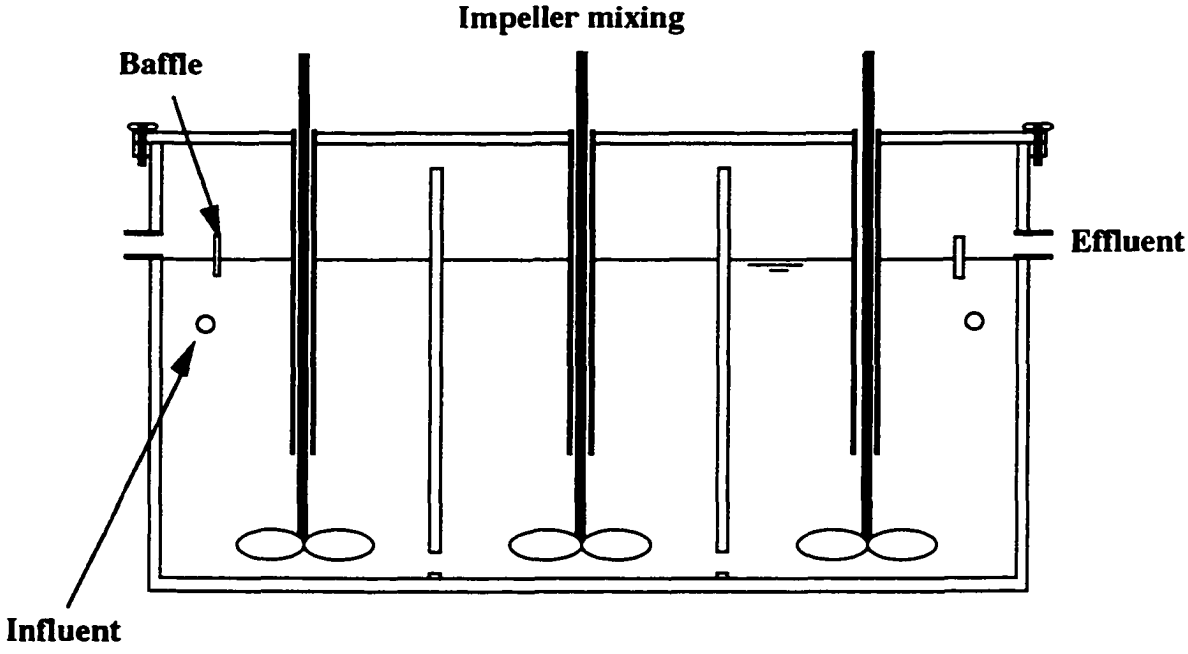


Figure 1. 12-liter AMBR

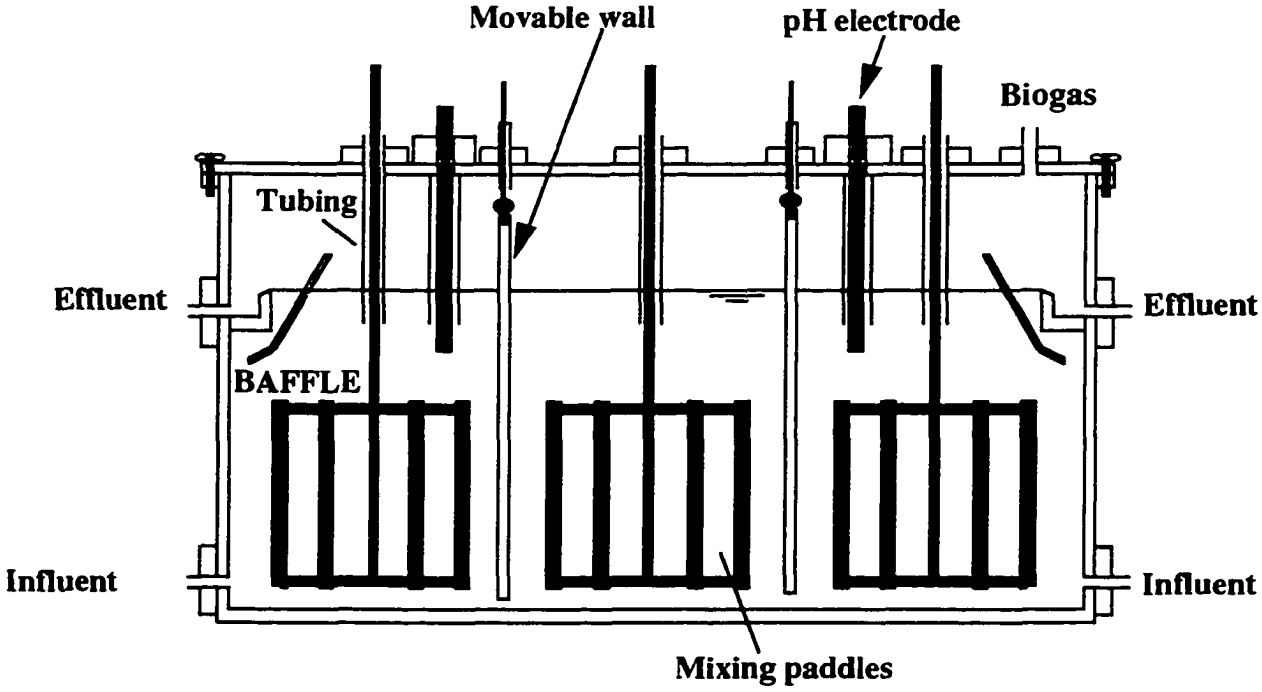
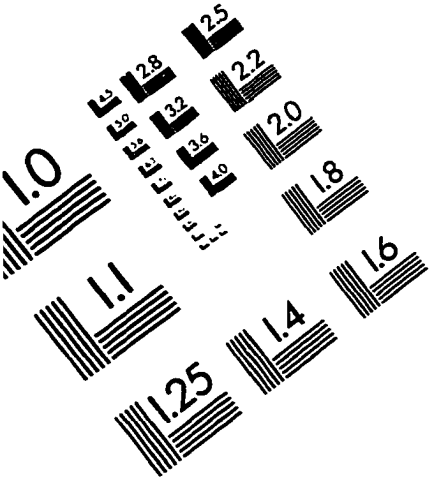


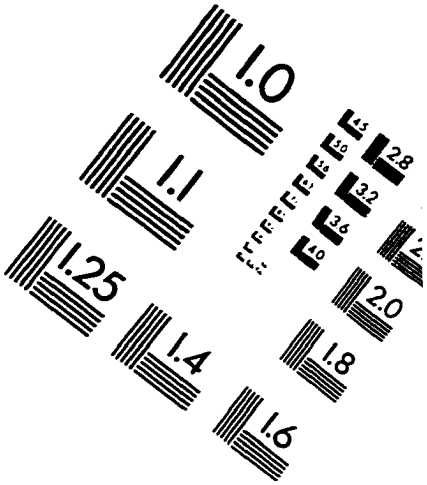
Figure 2. 54-liter AMBR with openings in bottom of walls between the compartments

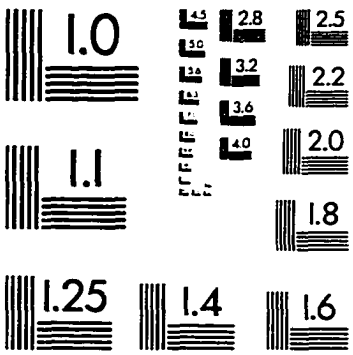
BIOGRAPHICAL SKETCH

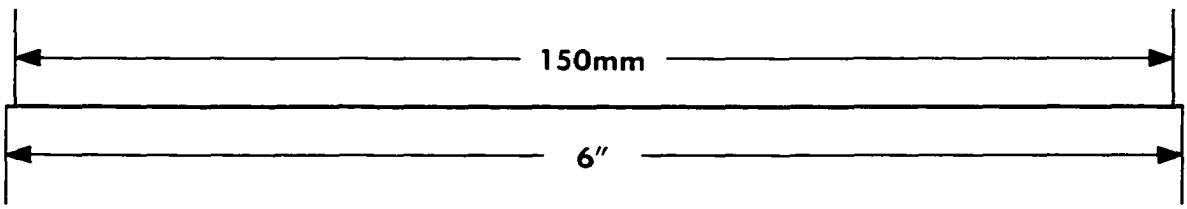
Lars Angenent was born July 31, 1969 in Beek (Bergh), The Netherlands. He received the Bachelor of Science in Environmental Science in 1992 and the Master of Science in Environmental Technology in 1994 from Wageningen Agricultural University, Wageningen, The Netherlands. In this university he completed two projects in the field of anaerobic microbiology. First, he performed enrichments experiments with anaerobic, monochlorophenol degrading bacteria at the Department of Microbiology. Second, he studied the influence of pH on the competition between sulfate reducing bacteria (SRB) and methane producing microorganism (MPM) in laboratory-scale upflow anaerobic sludge blanket (UASB) reactors at the Department of Environmental Technology. He served as a Graduate Research Assistant in the Department of Civil and Construction Engineering at Iowa State University from 1994 to 1998, where he developed the anaerobic migrating blanket reactor (AMBR). Part of his duties were to supervise research of Master's students.

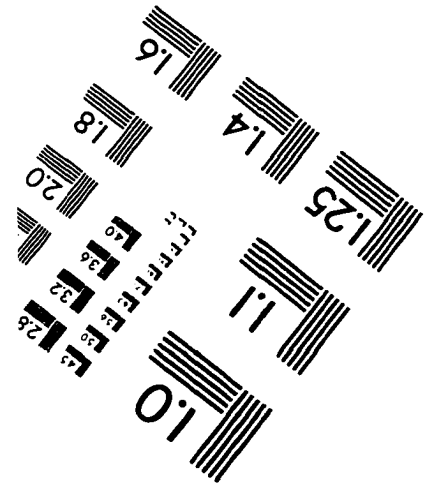
IMAGE EVALUATION
TEST TARGET (QA-3)

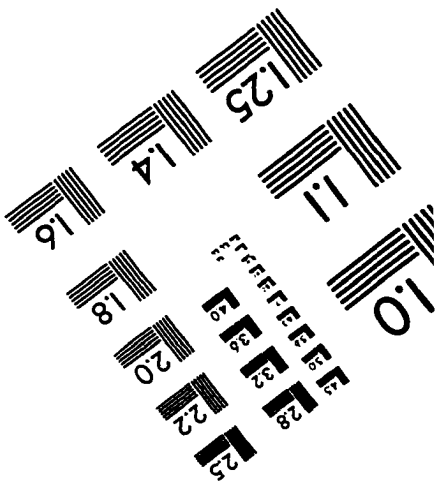












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1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989