Antonie van Leeuwenhoek **67**: 3–28, 1995.

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Anaerobic digestion and wastewater treatment systems

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Accepted 16 August 1994

Key words: UASB, anaerobic digestion, wastewater, EGSB

Abstract

Upflow Anaerobic Sludge Bed (UASB) wastewater (pre-)treatment systems represent a proven sustainable technology for a wide range of very different industrial effluents, including those containing toxic/inhibitory compounds. The process is also feasible for treatment of domestic wastewater with temperatures as low as 14–16° C and likely even lower. Compared to conventional aerobic treatment systems the anaerobic treatment process merely offers advantages. This especially is true for the rate of start-up. The available insight in anaerobic sludge immobilization (i.e. granulation) and growth of granular anaerobic sludge in many respects suffices for practice. In anaerobic treatment the immobilization of balanced microbial communities is essential, because the concentration of intermediates then can be kept sufficiently low.

So far ignored factors like the death and decay rate of organisms are of eminent importance for the quality of immobilized anaerobic sludge. Taking these factors into account, it can be shown that there does not exist any need for 'phase separation' when treating non- or slightly acidified wastewaters. Phase separation even is detrimental in case the acidogenic organisms are not removed from the effluent of the acidogenic reactor, because they deteriorate the settleability of granular sludge and also negatively affect the formation and growth of granular sludge. The growing insight in the role of factors like nutrients and trace elements, the effect of metabolic intermediates and end products opens excellent prospects for process control, e.g. for the anaerobic treatment of wastewaters containing mainly methanol.

Anaerobic wastewater treatment can also profitably be applied in the thermophilic and psychrophilic temperature range. Moreover, thermophilic anaerobic sludge can be used under mesophilic conditions.

The Expanded Granular Sludge Bed (EGSB) system particularly offers big practical potentials, e.g. for very low strength wastewaters (COD $<<$ 1 g/l) and at temperatures as low as 10° C. In EGSB-systems virtually all the retained sludge is employed, while compared to UASB-systems also a substantially bigger fraction of the immobilized organisms (inside the granules) participates in the process, because an extraordinary high substrate affinity prevails in these systems. It looks necessary to reconsider theories for mass transfer in immobilized anaerobic biomass.

Instead of phasing the digestion process, staging of the anaerobic reactors should be applied. In this way mixing up of the sludge can be significantly reduced and a plug flow is promoted. A staged process will provide a higher treatment efficiency and a higher process stability. This especially applies for thermophilic systems.

Introduction

'The anaerobic treatment process is in many ways ideal for wastewater treatment and it is almost certainly assured of increased usage in the future.' This statement of P.L. McCarty (1964) made in his inspiring review articles is still very relevant. He considered 'a lack of fundamental understanding of the process' as the primary obstacle for its broad implementation. Three decades have passed and a lot has been achieved. Anaerobic treatment method presently indeed enjoys an increasing popularity, although this still is not true worldwide. Particularly in the USA the very reluctant attitude of the established wastewater pollution control world persists. This negative attitude in the USA finds its ground primarily in the commercial disinterest at the established consultancies and contractors working in the field of conventional methods. They obviously refuse to advertise the merits and big potentials of anaerobic treatment and even are not prepared to spend time to understand the fundaments of the process. Rather than to implement low cost – sustainable – treatment systems, they continue to push expensive conventional systems wherever they are active in the world. However, regarding the increased insight of the microbiology and the technology of the anaerobic treatment process, and considering its enormous potentials and excellent performance of full scale installations, these established commercial groups ultimately will be unable to prevent the future rapid implementation of anaerobic treatment systems in their country and elsewhere in the world. At present meantime in various countries in Europe, South and South-East Asia and Latin America, anaerobic treatment is successfully implemented for various types of industrial wastewaters and for domestic wastewaters.

Compared to conventional aerobic methods, and in the light of the very much desirable development and implementation of sustainable methods and technologies, the anaerobic wastewater treatment concept indeed offers fundamental benefits, viz.:

- It can be employed at very low costs, because technically plain and relatively inexpensive reactors are used, and anaerobic treatment systems generally can be operated with little if any consumptive use of high grade energy.
- Instead of consuming energy, useful energy in the form of biogas is produced.
- It can be applied at practically any place and at any scale.
- Very high space loading rates can be applied in modern anaerobic wastewater treatment systems, so that the space requirements of the system are relatively small.
- The volume of excess sludge produced in anaerobic treatment generally is significantly lower as compared to aerobic systems; the absolute quantity in kg organic matter is low, and the dewatering capacity is very high.
- The excess sludge generally is well stabilized.
- Anaerobic organisms can be preserved unfed for long periods of time (exceeding one year) without any serious deterioration of their activity, while also other important characteristics of anaerobic sludge generally remain almost unaffected, e.g. the settleability of the sludge.
- It can be combined with post-treatment methods by which useful products like ammonia or sulfur can be recovered.

At the present state of knowledge – little if any serious drawbacks can be brought up against anaerobic treatment. Accepting that anaerobic digestion generally can not provide a complete treatment (viz. various mineralized compounds are left), previously mentioned drawbacks gradually will vanish, like:

- The relatively high susceptibility of methanogens and acetogens to a variety of xenobiotic compounds. Despite this, the situation is much less serious than presumed in the past. Significantly more is known about the extent of the toxicity, and gradually a better insight is gained in the countermeasures that can be taken.
- The presumed low stability of anaerobic treatment. Many upsets of anaerobic digestion systems in the past could be attributed to a lack of knowledge of the basic principles of the process. As a matter of fact, the anaerobic digestion process is highly stable, provided the system is operated in the proper way. This means that the process should be sufficiently understood by enginers and operators.
- The slow 'first' start-up as a drawback does not hold any more. A lot more is understood of the growth conditions of anaerobic organisms, and gradually large quantities of highly active anaerobic sludge from existing full scale installations are becoming available, so that the start-up of new full scale installations can be made within a few weeks, sometimes even a few days.
- The drawback, that anaerobic treatment would be accompanied with mal-odorous nuisance problems, belongs to the past. Any problems in this respect can be prevented using relatively simple means, i.e. physical-chemical and/or microaerophilic methods.

Anaerobic treatment technology

Reactor technology

To enable an anaerobic reactor system to accommodate high space loading rates for treating a specific wastewater the following five conditions should be met:

- *High retention of viable sludge in the reactor under operational conditions.* The higher the amount of sludge retained, the higher will be the loading potentials of the system. Therefore, it is necessary to cultivate a well settleable or immobilized biomass, and that the sludge will not deteriorate in this respect.
- *Sufficient contact between viable bacterial biomass and wastewater.* In case part of the sludge retained in the reactor would remain deprived of substrate, this sludge is of little if any value.
- *High reaction rates and absence of serious transport limitations.* It is clear that the kinetics of the degradation processes are a factor of great importance. It is essential that metabolic end-products can easily escape from the aggregate. The size of the biofilms should remain relatively small and the accessibility of the organisms inside the biofilm should be high.
- *The viable biomass should be sufficiently adapted and/or acclimatized.* For any wastewater subjected to treatment, the sludge should be enabled to adapt to the specific characteristics of the concerning wastewater.
- *Prevalence of favourable environmental conditions for all required organisms inside the reactor under all imposed operational conditions.* It should be emphasized here that this condition does not mean that the circumstances should be similar at any location within the reactor and at any instant. As a matter of fact even the contrary is true. Regarding the fact that a large variety of different organisms are involved in the degradation of more complex compounds, the existence of micro-niches within the system is an absolute pre-requisite. Only in this way the required flourishing growth of the very different organisms can be achieved. Once again this points to the extraordinary importance of immobilization of biomass. Particularly this is true for treatment processes in which interspecies electron transfer plays a crucial role, but it also will be true for possible future integrated aerobic-anaerobic treatment systems, where facultative organisms growing in the outer layers of an anaerobic sludge will protect methanogens present in deeper layers of the aggregate (Kato et al. 1993a, b). Very likely this also will be the case for many other possible integrated biological treatment systems e.g. those using sulfate reducers, denitrifiers, methanogens and acidogens.

Along with the improved understanding of the various specific reactor technological aspects and of microbial conversion processes in pure cultures, and new insights to be obtained from defined microbial communities the future presumably will bring interesting new treatment processes. In order to achieve this, microbiologists and biotechnologists should joint their research, and put more emphasis on studies of microbial communities in sludge aggregates.

Anaerobic reactor systems

'Sludge retention' and 'sludge wastewater contact' are directly related to the construction, design and mode of operation of the reactor (macro-aspects of the reactor system). Other factors mentioned in the preceding paragraph are more specifically dealing with the operational conditions imposed to the system and the related micro-aspects. An essential difference between anaerobic and aerobic treatment systems is that the loading rates of anaerobic reactors generally are not limited by the supply of any reagent, like oxygen in aerobic systems. The discussion concerning the anaerobic reactor technology will be mainly focused on Upflow Anaerobic Sludge Bed (*UASB*-) and Expanded Granular Sludge Bed (*EGSB*-) reactor concepts. Systems using membranes (Harada et al. 1994; Ross et al. 1994; Ahmadun 1994) for sludge retention will not be discussed, though they may become more popular when the membrane permeate flux can be kept high and the overall treatment costs of these systems are in the same range as those of other high rate systems.

The *anaerobic sludge bed reactor concept* is based on the idea that anaerobic sludge inherently exerts satisfactory settling properties, provided the sludge is not exposed to heavy mechanical agitation (Lettinga et al. 1980, 1983a). For this reason in UASB-reactors mechanical mixing is either omitted completely or applied at a relatively very low intensity and/or intermittently. For achieving the required sufficient contact between sludge and wastewater, the conventional UASB-system relies on the agitation brought about by the biogas production and an even feed distribution at the bottom of the reactor. Sometimes the hydraulic upflow velocity is raised by applying effluent recycling.

Sludge aggregates will be dispersed under the influence of upflowing biogas. This mixing brought about by the biogas is very important for achieving the desired contact. Especially in tall reactors and at high(er) organic space loads this type of mixing may become rather intensive, though even then it is still a gentle mode of mixing. The dispersed sludge aggregates generally can be retained sufficiently well in the reactor by separating the biogas using a gas collector assembly, which is placed at the upper part of the reactor. The biogas is released from the system via this device. In this way a settler is created in the upper part of the reactor. Sludge particles can coalesce and settle out here, and then slide back into the digester compartment, despite the relatively high superficial liquid velocity (and liquid turbulence) prevailing in the aperture between the gas collectors. The Gas-Solids-Separator (GSS) constitutes an essential accessory of an UASB-reactor. The wash-out of floating sludge particles generally can be effectively prevented for medium and relatively low strength wastewaters by installing a baffle in front of the effluent weir. In cases where very high sludge loading rates are imposed to the system, the presence of gas within the sludge aggregates may cause heavy flotation of the sludge particles. In that case specific designs of gas solids separator may become necessary. Some commercial full scale UASB-reactors and IC (Internal circulation)-UASB-reactors employ rather sophisticated multistep gas separators (Vellinga et al. 1986). Also in the Biobed system of Biothane International (Versprille et al. 1994) special designs are used. For retaining floating granular sludge a kind of a sieve drum gas-solid separator might become attractive (Lettinga et al. 1983b; Hong Yucai et al. 1988; Rinzema 1988; Rinzema et al. 1993a, b). Especially for the treatment of very low strength wastewaters under very high hydraulic loading rates (HRT < 1–2 hrs), the retention of viable sludge should be extremely good. For domestic sewage treatment, where generally such exceptionally high hydraulic loads are not applied, the contact between wastewater and sludge can become the limiting factor, because the gas production remains too low and therefore a more even feed inlet is necessary than generally applied in industrial plants. In addition, also mechanical mixing or mixing on the basis of the gas-lift principle could be applied.

Some researchers suggest to replace the GSS-device by a packed bed in the upper part of the reactor. This recommendation is based on results obtained with such a hybrid of the UASB- and AF (Anaerobic Filter) system. Reynolds & Colleran (1986) found that reactors can maintain high treatment efficiencies at COD-loads considerably higher than those accommodated by upflow and downflow completely packed reactors (whey wastewater: 10 g COD/l). Guiot et al. (1986) compared upflow anaerobic sludge blanket and sludge bed-filter systems, and reported promising results. Despite these positive experiences it is quite doubtful whether these systems really are more advantageous. Results obtained in small scale hybrid AF-UASB-systems in experiments conducted with emulsions of triglycerides (Rinzema 1988; Rinzema et al. 1993b) did not show any benefit of these hybrid systems.

Anaerobic Fluidized Bed (*FB*) systems can be considered as a special variant of the sludge bed system. Although the FB-system was introduced as a more advanced anaerobic technology (Li & Sutton 1981; Heijnen 1983, 1988), this concept does not really fulfil the high expectations. The system relies on the formation of a more or less uniform (in thickness, density, strength) attached biofilm and/or particles. In order to maintain a stable situation with respect to the biomass film development, a high degree of pre-acidification is required (Heijnen 1983, 1988), while also dispersed matter should not be present in the influent (Ehlinger 1994). Despite these findings, it virtually is impossible to guarantee a stable process performance in practice. The complex dynamic process of film formation, viz. film attachment and release, is very difficult to control. Aggregates of very different size and density will occur always in the system, and consequently at a certain imposed specific superficial liquid velocity, segregation of sludge particles will occur. This means that in a FB-system bare carrier particles will accumulate in the lower part of the reactor as a stationary bed, while light fluffy aggregates (detached biofilms) will be present in the upper part, at least when they are retained. The latter only can be achieved when the superficial velocity remains relatively low. As a matter of fact the question is which comprehensive reasons exist to emulate a complete fluidization in an anaerobic (granular) sludge bed reactor. A slight sludge bed expansion already would suffice for the required proper contact between wastewater and sludge (Iza et al. 1987, 1988). This idea has been realized in the so-called Expanded Granular Sludge Bed (EGSB) and in the IC-UASB reactor concepts, which both use granular sludge. In the IC-UASB-reactor halfway the reactor gas is separated from the system using an in-built gas separator device. The lifting forces of the collected biogas are used to bring about a recirculation of granular sludge over the lower part of the reactor, which results in improved contact between sludge and wastewater. Also the so-called 'Anaerobic Fixed Film Expanded Bed' (AFFEB) reactor, developed by Jewell (1983) is based on a limited expansion of the biofilm aggregates.

In addition to the sludge bed systems, certainly an important place is left for *I*ntermittently *S*tirred *A*naerobic *S*ludge *T*ank (*ISAST*) systems. These systems can use both a granular sludge as well as a dense well settling flocculent type of sludge. The stirring assembly and stirring intensity should be adapted to the characteristics of the sludge, the loading rate applied (i.e. gas production achieved), and the required treatment efficiency. With respect to the hydraulic retention time applied such a reactor can be regarded as a 'completely mixed' reactor. Because of that, the feed can be introduced at any suitable place in the reactor. Since a well settling sludge is used, some kind of inbuilt settler could be installed for facilitating the sludge retention.

Fundamental aspects of anaerobic treatment technology

For the proper design and application of anaerobic treatment systems it is essential to know and understand the important process technological aspects of anaerobic reactor systems, and the (bio-)chemistry and the microbiology of anaerobic digestion. In the following, attention will be afforded to gained new insights in some of these aspects, viz.:

- Anaerobic sludge immobilization, i.e. start-up of anaerobic reactors.
- Kinetics of anaerobic conversion reactions in immobilized biomass.
- Process conditions imposed to the system.
- Environmental conditions in anaerobic treatment.

Bacterial sludge immobilization, granulation

The key for modern biotechnology is the *immobilization of proper bacteria*. The required high sludge retention in anaerobic treatment systems only can be accomplished using immobilized biomass. However, in anaerobic treatment it is not just a matter of immobilizing bacteria, but to develop and to immobilize well *balanced bacterial consortia*. This is needed because of the existence of various syntrophic conversion reactions in anaerobic digestion, the detrimental effect of higher concentrations of specific intermediates, and the strong effect of environmental factors like pH and redox potential. Therefore, a close cooperation between various scientific disciplines is a prerequisite for the further development and application of the anaerobic treatment system. It is astounding how poorly the anaerobic digestion process was understood by engineers working in this field. Due to the minor importance of anaerobic digestion in wastewater treatment in the past, also microbial disciplines put little attention to anaerobic digestion. Significant progress in the knowledge of the fundamentals of the process has been made only since the development and successful implementation of high rate anaerobic treatment in the early seventies.

The generally excellent sludge retention in Anaerobic Upflow Sludge Bed (UASB) reactors is based on *Bacterial sludge entrapment* in, on or between sludge particles, and *bacterial immobilization* by a mechanism of bacterial (self-)agglomeration and bacterial attachment to support material present in the (seed) sludge.

With respect to immobilization, particularly the phenomenon of granulation has puzzled many researchers from very different disciplines. Sludge granulation is important for the first start-up of an UASB-reactor, i.e. the start-up using a flocculent anaerobic sludge of relatively poor specific methanogenic activity. However, the formation of a granular sludge certainly is not a prerequisite for UASB-systems, because the formation of a well settling 'thick' flocculent sludge with a high methanogenic activity also suffices. Comprehensive investigations have been made on this phenomenon since it was observed for the first time in pilot UASB-plant treating sugar beet wastewater (Lettinga et al. 1972).

Granulation is a completely natural process, it will proceed in all systems where the basic conditions for its occurrence are met, i.e. on mainly soluble substrates and in reactors operated in an up-flow manner. This also is the case for the reversed flow Dorr Oliver Clarigesters applied in South Africa since the fifties. Indeed a granular sludge appears to be present in such a reactor. Surprisingly enough neither any engineer nor researcher in South Africa ever afforded attention to important sludge characteristics, like size, form and the mechanical strength, density and porosity of the sludge aggregates. Despite the excellent work on the bacterial and (bio)chemical composition of anaerobic sludges carried out in the sixties by a number of microbiologists in South Africa (e.g. Kotze et al. 1969), no attention was afforded to the presence of microbial consortia in anaerobic sludge. Since even presently emphasis seems to be put on the development of membrane type anaerobic reactors (Ross et al. 1994), apparently the importance of the phenomenon of granulation for sludge retention still is not really understood or accepted there.

As a matter of fact in their experiments with the anaerobic filter Young & McCarty (1969) already recognized the ability of anaerobic sludge to form very well settleable aggregates. They stated: 'The rolling action of the rising gas bubbles caused the sludge particle to take a granular form. These granules were as large as 3.1 mm in diameter and settle readily.' In AF-experiments with potato starch wastewater and methanol solutions we made similar observations (Lettinga et al. 1972, 1979). Like the researchers in South Africa, the group of McCarty was unable to continue the work on anaerobic wastewater treatment due to lack of funding.

In the meantime the insight in the mechanism of the sludge granulation process for mesophilic and thermophilic anaerobic treatment has been elucidated sufficiently for its practical application (e.g. De Zeeuw 1982, 1987; Hulshoff Pol & Lettinga 1986; Lettinga & Hulshoff Pol 1986; Hulshoff Pol et al. 1987; Dolfing 1987; Beeftink 1987; Beeftink & Staugaard 1986; Wiegant & De Man 1986; Lettinga et al. 1984; Grotenhuis 1992; Wu Wei-min 1987; Wu Wei-min et al. 1985, 1991; Van Lier et al. 1994a; Thaveesri et al. 1994; Fang et al. 1994). Wastewater engineers do not need to understand the complexity of the granulation phenomenon fully, but microbiologists endeavour to obtain a complete understanding of the phenomenon, including the processes involved in the built-up of balanced micro-ecosystems. More fundamental questions to be cleared are the role of exo-polymers, the structure and characteristics of carrier materials, the importance of the growth of mixed cultures. These questions remain for future research.

Granulation in anaerobic treatment USB-systems proceeds well under all temperature conditions, viz. mesophilic (De Zeeuw 1982, 1987; Hulshoff Pol & Lettinga 1986; Hulshoff Pol et al. 1987; Dolfing 1987), thermophilic (Wiegant 1986; Van Lier et al. 1994) and psychrophilic as well (Van der Last 1991). Granulation in essence finds its ground in the fact that bacterial growth is restricted to a limited number of growth nuclei. For understanding the reason(s) why granulation occurs, the following should be considered:

- Proper growth nuclei, i.e. inert organic and inorganic bacterial carrier materials as well as bacterial aggregates, are already present in the seed sludge.
- Finely dispersed matter, including viable bacterial matter, will become decreasingly retained, once the superficial liquid and gas velocities increase. As a result film and/or aggregate formation then are reinforced.
- The size of the aggregates and/or biofilm thickness are limited, viz. it depends on the intrinsic strength (binding forces and the degree of bacterial intertwinement) and the external forces exerted on the particles/films (e.g. shear). Therefore at due time particles/films will fall apart, evolving a next generation. The first generation(s) of aggregates, indicated by Hulshoff Pol et al. (1983a, b) as 'filamentous' granules mainly consist of long multicellular rod shaped bacteria (when cultivated on VFA). They are quite voluminous and in fact more floc than granule.
- Retained secondary growth nuclei will grow in size again, but also in bacterial density. Growth is not restricted to the outskirts, but also proceeds inside the aggregates. At due time they will fall apart again, evolving a third generation, etc.
- The granules will gradually 'age' or 'mature'. As a result of this process of maturing the voluminous 'filamentous granules', predominating during the initial stages of the granulation process, will disappear and become displaced by dense 'rod' granules. In a matured granular sludge, filamentous granules generally will be absent.

With respect to the granulation process essentially there do not exist any principle differences between an UASB-reactor, seeded with digested sewage sludge, and a FB reactor which uses inert particles as carrier material for the in-growing biomass. In the FB-concept the inoculation is delegated to viable organisms present in the wastewater. Granulation indeed will proceed well in a FB-system, provided it is operated such that biofilms can grow sufficiently in thickness and/or different particles can grow together. This means that the idea of 'complete fluidization' should be dropped, which indeed was well recognized by Binot et al. (1981, 1983), Iza et al. (1987, 1988), and was confirmed by Van der Last (1991) in experiments with settled domestic sewage. In latter experiments it was found that the presence of 50–80 mg/l of finely dispersed organic matter (partially colloidal) does not hamper the granulation process significantly. This observation can become of big practical importance, and may speed up the acceptance of anaerobic treatment of sewage under lower ambient temperature conditions.

The granulation process can be significantly enhanced by the presence of a certain amount of non-acidified substrate in the wastewater. This especially is true for thermophilic systems. The understanding of the sludge granulation process under thermophilic conditions has been considerably improved recently (Van Lier 1994a, b). A definite breakthrough of thermophilic anaerobic treatment processes looks nearby now.

Despite the already available insight in sludge granulation, for practice the first start-up of an UASB-reactor remains a rather delicate matter and to some extent also rather time consuming. Regarding the complex character of the anaerobic treatment process, more particularly of the granulation process, it is desirable that the first start-up is made by operators having sufficient knowledge and experience. They should at least be enabled to follow the process closely during this phase. In many cases consultancies and contractors active in anaerobic treatment have available the required experts. For industries who still want to be self-sufficient in this respect, it is important that they follow the guidelines provided in Table 1.

Due to the slow growth rate of methanogenic organisms, a complete *first start-up* of an anaerobic reactor system needs a period of 3–5 months. From the side of scientists and engineers working in the field of conventional treatment, this frequently is brought in as one of the major drawbacks of anaerobic treatment. However, this drawback in fact belongs to the past. In this respect one should consider that along with the (first) start-up a rapidly increasing fraction of the wastewater will become purified. This obviously in many cases comprises already a considerable improvement of the situation compared to that prior to the installation of the anaerobic reactor. But there exists an even more important reason why this last argument against the use of the anaerobic treatment process soon will vanish. Along with the further employment of anaerobic treatment, big amounts of a high quality (granular) excess sludge will become available. This excess granular sludge comprises an almost *ideal seed* material; the start-up of new installations then can be completed within a few days. The main condition to be met is to keep sludge loading rates well below 50% of its maximum substrate utilization rate during the first weeks. The sludge then can adapt to the new situation, also when the wastewater to be treated differs significantly in composition and strength from the original wastewater. Since at an imposed sludge load of 50% of the maximum specific sludge activity, generally already high space loads can be accommodated by the new treatment systems, i.e. depending on the amount of seed sludge used, this restriction for practice does not represent any obstacle. An extremely important characteristic of methanogenic sludge obviously is its feature that it can be *preserved for long periods of time under unfed conditions.* Regarding the above, we can conclude that in fact no other biological treatment system can compete with the anaerobic process with respect to the speed of start-up. This is confirmed by the available practical experiences so far with this '*secondary start-up*'.

Although secondary start-up generally proceeds very satisfactory, in specific situations still some serious problems may manifest, such as *deterioration of the sludge granules, attachment of fast growing filamentous acidogenic bacteria, granular sludge flotation, $CaCO_3$-scaling.*

Regarding the practical importance of reactor start-up with granular sludge, the occurrence of any form of granular sludge deterioration has become a major field of research. Comprehensive investigations in this field have been made by Alphenaar (Alphenaar 1994; Alphenaar et al. 1992, 1993b), Fang et al. (1994) and Thaveesri et al. (1994). Alphenaar drew the following important conclusions from his investigations:

- Dispersed acidogenic organisms present in the feed solution can initiate flotation of granular sludge. This flotation is related to the breakdown of acidogenic sludge, but the real cause so far is unclear. According to Thaveesri et al. (1994) a minor alteration in the biomass composition can result in major changes of the sludge characteristics. Higher concentrations of finely dispersed acidogenic organisms in the feed are quite detrimental for the sludge retention of the system, because they can act as carrier material for in-growing methanogenic organisms. This was also observed for FB-systems (Ehlinger 1994). Since finely dispersed particles are hardly retained in the reactor, granular sludge growth is negatively affected.
- A non or only partially acidified sucrose feed (less than 70%) initiates the growth of a fragile type of granular sludge with a layered structure. This was also found by Fang (1994) and Thaveesri et al. (1994). The accumulation of biogas within and around the aggregates (e.g. between the layers) causes the flotation of the grains. Moreover, the growth of dispersed acidogenic bacteria enhances granular sludge flotation and also leads to an excessive expansion of the sludge bed, consequently to a poorer sludge retention in the system.

Table 1. Present understanding and guidelines for the first start-up of UASB-reactors.

I. *Seed sludge characteristics*

A variety of seed materials can be used, e.g. digested and undigested sewage sludges and different types of manure, aerobic activated sludge, and even inert organic and inorganic carrier particles provided anaerobic organisms are present in the wastewater. The presence of 'proper' carrier materials (e.g. size, gravity, surface properties) for bacterial attachment in the reactor is essential.

II. *Mode of operation*

It is essential to retain the heavier carrier ingredients of the seed material (i.e. the carrier materials), and to accomplish a complete and continuous removal of the lighter fractions of the seed sludge (by applying a sufficient selection pressure). In this respect the following recommendations can be made:

1. Finely dispersed sludge washed out from the systems should not be returned.
2. Effluent recycling should be applied in case the influent $COD_{biodegradable}$ exceeds 3 g/l to reinforce the selection pressure.
3. The organic loading rate should be increased stepwise, always after at least 80% of the $COD_{biodegradable}$ is eliminated. In this way the sludge loading rate is raised relatively rapidly. The acetate concentration should be maintained at a low level, e.g. below approximately 200 mg/l. In this way growth-in of organisms with a high substrate affinity is enhanced.
4. Some mechanical mixing in the reactor can be beneficial because it promotes sludge segregation.

III. *Wastewater characteristics*

1. The strength of the wastewater.

   Granulation proceeds faster at lower substrate influent levels, i.e. in the range 1–3 g $COD_{biodegradable}$/l.
2. Composition of the wastewater

   Granulation proceeds well on soluble proteins and slightly acidified carbohydrates, viz. substantially faster than on substrates merely consisting of VFA.

   High concentrations of Ca-salts of the VFA will lead to $CaCO_3$-scaling problems with granular sludge.
3. Nature of the pollutants

   Dispersed organic and inorganic matter retards granulation and therefore should not be present at concentrations exceeding appr. 100–200 mg/l (depending on the soluble COD_{biod}).

IV. *Environmental factors*

1. Temperature

   Granulation proceeds best at optimal mesophilic and thermophilic conditions.
2. *The pH* should be maintained at > 6.2.
3. All *essential growth factors*, N, P, S and trace elements should be present in sufficient amount and in available form.
4. *Toxic compounds* should be absent at inhibitory concentrations.

- Changes in the composition of the non-acidified fraction of the substrate may lead to granular sludge disintegration, especially when the acidogenic organisms are distributed more homogeneously over the granule.
- Contrary to carbohydrate substrates, an excellent quality of granular sludge always develops on non-acidified gelatine or other soluble proteins, as also found by Fang (1994).
- The size of the granular sludge is related to the imposed sludge loading rate. At lower loads a decrease of the strength of the granular sludge will occur.
- P-limitation results in a decrease of the methanogenic sludge activity. The effect is completely reversible. Phosphate is still well available for methanogenic granular sludge at phosphate concentrations < 0.1 mg P/l.

– Despite the presence of empty places (holes) in larger granules, the porosity of larger sludge granules is lower than that of smaller granules. Substrate transport in the granule seems not to be related directly with the porosity.

According to observations of Thaveesri et al. (1994) the settling properties of granular sludge deteriorate when non-polar compounds like napththalene are added as co-substrate. Also the presence of surfactant was found to be detrimental.

Sludge granulation certainly is not restricted to anaerobic treatment. It also will proceed in *other biological treatment processes*, provided the proper reactor system is used and the system is operated in the correct way. In some preliminary souring USB-experiments with beet sugar sap solutions indeed a good quality granular acidogenic sludge could be cultivated (Lettinga et al. 1980). As a result, in the concerning acidogenic reactors loads up to 70 $kg{\cdot}m^{-3}/day^{-1}$ (at 30° C) could be well accommodated. Comprehensive studies conducted by Zoetemeyer (1982) confirmed these observations, and recently also Van Lier (1994a, b) found that a mainly acidogenic thermophilic granular sludge develops in the bottom-compartment of a staged UASB-reactor treating a sugar containing wastewater.

An excellent granular type of sludge can develop with denitrifying organisms (Lettinga et al. 1980) on an alcohol substrate within 6–8 weeks. In order to prevent channelling in the sludge bed it appeared necessary to apply a moderate – intermittent or continuous – mechanical agitation in the sludge bed compartment, especially at high hydraulic loading rates and at low gas production rates. The performance of denitrification systems using a mixture of alcohols as substrate remained excellent (90% COD-reduction) at COD-loading rates up to 20 $kg{\cdot}m^{-3}{\cdot}day^{-1}$, at hydraulic residence times as low as 15 min and upflow liquid velocities exceeding 4 m/h. This also appeared to be possible with sewage (Klapwijk 1978; Van der Hoek 1988). Recently it was found that also sulfate reducing (Visser et al. 1993a) and nitrifying (Heijnen et al. 1992; Tijhuis et al. 1992) organisms can produce a granular type of sludge in USB-type reactor-systems. It therefore can be concluded that a large variety of organisms can produce granular aggregates. However, the course and speed of the granulation process, consequently the ultimate characteristics of the granular sludge formed, will depend distinctly on specific properties of the organisms involved in the process. Important factors to be considered are *growth rate* and *death and decay rate* of the organisms, the *kinetics* of the degradation process in immobilized consortia, the *prevalence of syntrophic relationships* in the degradation of polluting compounds, and certain *characteristics of the organisms*, like morphology, substrate specificity, hydrophobicity, surface charge, ability to produce specific polymers, and sensitivity to environmental parameters.

The insight in the effect of most of the above mentioned specific bacterial properties is far from complete yet, such as the effect of the *growth rate*. It is well known that the slowly growing methanogenic and acetogenic organisms give an excellent compact granular sludge. The granulation process then proceeds well, though rather slowly. Results obtained with the significantly faster growing acidogenic organisms are less positive, but a granular acidogenic sludge can develop. However, the risk of formation of a voluminous flocculent type of sludge in that case presumably is rather big. This particularly will be true when filamentous organisms are involved at relatively low substrate concentration in the bulk of the liquid, because growth then will be restricted mainly to the outskirts of the aggregates. The formation of a granular sludge under these circumstances can be enhanced by reinforcing the selection pressure on the system, e.g. by applying intermittent or continuous mechanical mixing as was observed in earlier denitrification experiments.

Along with the growth rate, the *death and decay rate* of the organisms participating in the granulation process are factors of crucial importance. In systems operated at a solids retention time (SRT) significantly higher than the death and decay rate of some of in-growing organisms, a sludge will develop with a relatively low content of the concerning organisms, even when the growth rate of these organisms is high compared to that of the other organisms participating in the degradation process. A typical and very relevant example in the anaerobic wastewater technology concerns the treatment of a non- or partially acidified substrate. Because of the high growth rate of acidogenic organisms, a number of researchers (Pohland & Ghosh 1971; Cohen et al. 1982; Zoetemeyer 1982; Breure 1986; Breure & Van Andel 1984; Breure et al. 1985) proposed to apply a *two-step treatment process* in which the acidogenic and methanogenic phases are spatially separated. A high specific loading rate in the methanogenic reactor and a high process stability were reported as main benefits for the two-step treatment concept (Cohen et al. 1979, 1985; Komatsu et al. 1991). They presume(d) that the rapidly grow-

Table 2. Growth parameters for anaerobic bacteria under mesophilic conditions (from Alphenaar 1994).

System/population	Substrate	k[a] g·(g·day)$^{-1}$	Yield[b] g·g^{-1}	Decay d^{-1}
Acid producing systems				
Acid producing sludge	Glucose		0.34	0.43
Acid producing sludge	Glucose	9.5	0.40	0.79
Mixed culture	Glucose	2.5	0.54	0.87
Mixed culture	Glucose	179	0.17	6.1
Dispersed culture CSTR	Glucose	63	0.10–0.18	
Dispersed culture UASB, HRT < 81 min	Glucose	24–33	0.12–0.17	
Aggregates UASB, HRT > 26 min	Glucose	11–19	0.06–0.08	
Aggregates Gaslift	Glucose	74	0.13	0.96
Disperse culture CSTR	Gelatin	81	0.085–0.14	
Disperse culture UASB	Gelatin	10.5	0.094	
Methane producing systems				
Methanothrix pure culture	Acetate	3.3	0.018–0.023	
Methanothrix pure culture	Acetate	2.3		
Methanothrix pure culture	Acetate	2.3	0.023	
Sarcina pure culture	Acetate	14–20	0.035	
Sarcina pure culture	H_2		0.136	
	Acetate	5.1	0.054	0.037
Mixed culture CSTR				
Mixed culture CSTR	Acetate	8.5	0.04	0.036
Granular sludge UASB	Acetate	3.1	0.013	
Mixed culture CSTR	VFA mix	17	0.03	0.099
Granular sludge UASB	VFA mix		0.015–0.025	0.02
Granular sludge UASB	VFA mix	2.85	0.064	0.06
Granular sludge UASB	Sucrose		0.12–0.15	
Granular sludge 1 step UASB	Glucose	0.4		
Granular sludge 2 step UASB	Glucose	1.6–2.2	0.11	
	Glucose	0.16	0.21	0.03
Granular sludge full-scale UASB	Brewery	0.45–1.9		
	Papermill	0.2–0.6		
	Sugar beet	1.2		
Thermophilic granular sludge at 30° C			0.011	

[a] gCOD·(gVSS·d)$^{-1}$
[b] gVSS·gCOD^{-1}
[c] Cited by Pavlostathis et al. 1990
[d] Cited by Henze & Harremoës 1982

ing acidogenic organisms would give a sludge of poor methanogenic activity in one-step process configurations. In two step treatment systems growth-in of acidogenic organisms in the methanogenic reactor can be kept at a minimum, so that a sludge with a high methanogenic activity will develop here. It is clear that the effect of possible big differences in death and decay rate between acidogenic and methanogenic organisms were completely ignored. Information of growth parameters available in literature is summarized in Table 2 (from Alphenaar 1994).

Regarding the comparatively high death and decay rate of acidogenic organisms, the growth-in of acidogenic organisms in modern high rate treatment systems in fact hardly can be detrimental with respect to the development of a sludge with a high specific methanogenic activity. Although acidogenic bacteria will grow in rapidly when fed with non-acidified wastewater, the major part will die and degrade due to the high sludge age prevailing in these high rate anaerobic reactor systems. The situation obviously would be more serious when after death the remaining biomass would not decay. In that case no space is set free for consortia consisting of acetogens and methanogens.

Apart from these kinetic considerations there exist two other important reasons to reject the two-phase treatment concept. First of all dispersed acidogenic bacteria when present in the feed of the methanogenic reactor stimulate sludge floatation of the sludge, and therefore negatively affect the sludge retention of the methanogenic reactor. Therefore acidogenic bacteria should be absent, or at least the biomass concentration should remain below 300–400 mg/l (Alphenaar 1994). Earlier results of Hulshoff Pol (1989) and Vanderhaegen et al. (1992) led to the same conclusion. Regarding further the fact that the investment and operating costs of two-step systems are significantly higher (Lettinga & Hulshoff Pol 1991), it is clear that in most situations a one-step treatment system is to be preferred.

Kinetics of anaerobic conversion reaction in immobilized biomass

Mathematical descriptions of the kinetics of anaerobic treatment so far in fact merely exist for dispersed cultures. Useful kinetic models for immobilized biomass have not been developed. For the degradation of substrate in immobilized biomass external and internal mass transfer processes may play an important role in addition to the factors dictating the rate of conversion in dispersed types of biomass. Both for the substrate(s) and end-product(s) there generally exist concentration gradients in immobilized biomass. Mass transport inside biological matrices generally is attributed merely to diffusion according to Fick's law (Pavlosathis & Geraldo-Gomez 1991; Arcand et al. 1994; Wijffels 1994). Diffusion coefficients for solutes inside biofilms were found to be lower than those in clean water. So in anaerobic matrices the diffusion coefficient for ethanol, acetate and hydrogen seems to amount only 12%, 12% and 27% of the value for an aqueous solution, respectively. Such low values obviously would result in very low values for mass transfer in immobilized biomass. Nevertheless, whatever exact values are used for the diffusion coefficient, the diffusion based mathematical models can provide useful qualitative information. However, in reality the situation is more complex, but very likely also more favourable, than follows from diffusion considerations. There exist at least two reasons to speculate that the situation with respect to the rate of transport in immobilized systems is distinctly better than predicted from these diffusion considerations. First it looks reasonable to assume that in addition to diffusion a *convective type of mass transfer* will occur in the anaerobic aggregates, particularly in its macropores. A reason for the occurrence of these convection flows here could be found in the formation and release of gas bubbles. According to Kato (1994) convective mass transport in the biofilm (or in the pores of the film/aggregate) can be created by intensifying the hydraulic turbulence in the bulk of the liquid by increasing the superficial liquid velocity in the reactor. Secondly, along with the rate of mass transfer considerations, the possibly positive effect of the existence of *syntrophic processes* should be taken into account in the degradation (rate) of the polluting compounds. So far this has not been the case.

In order to enable the application of high space loading rates it is clear that the *process conditions* prevailing in the reactor should be as optimal as possible. One of these factors is the *substrate level*. According to Monod kinetics the maximum specific substrate utilization values for dispersed cultures are found at substrate levels $>>$ K_m, i.e. the substrate affinity. Due to that the loading potentials of a treatment system become limited. Since a high treatment efficiency is required in practice, it is inevitable to maintain the substrate concentration of the reactor contents at a very low level. The loading potentials therefore theoretically depend strongly on the value of K_m. Moreover, since the rate of substrate transport in the biomass-aggregates (biofilms) according to prevailing theories is restricted by diffusion, one would expect distinctly lower intrinsic values of this parameter than for immobilized biomass (apparent K_m). The intrinsic K_m-values apply for dispersed bacteria. Substrate gradients only exist here in the water film, surrounding the organisms. Due to substrate transport limitation, inside the immobilized biomass the substrate level is lower than in the bulk of the liquid. Even part of the viable biomass may remain deprived of substrate, consequently will not take part in the process. This particularly will be true for very low strength wastewaters and for higher operational temperatures. In order to lower the apparent K_m, sufficient mixing should be applied, e.g. using high superficial upflow velocities, like in EGSB-reactors.

Besides the detrimental effect of biomass-immobilization with respect to the rate of substrate transport inside bacterial aggregates, biomass-immobilization on the other hand may enhance the rate of conversion reactions, i.e. in fact lower the K_m-values. This particularly will be the case when

Table 3. The theoretical lowest COD_{in} which can be applied to UASB and EGSB reactors for the treatment of wastewaters containing simple substrates, assuming the parameters outlined in [a] (from Kato 1994).

Reactor Apparent	K_m (mg COD/l)	OLR (g COD/l·day)	COD_{in} (mg/l)	COD_{ef}[b] (mg/l)	Yield of methane [gas/(liquid + gas)][c]
UASB	145[d]	5	187	28	0.51
		10	483	73	0.81
		15	967	145	0.91
		20	1933	290	0.95
		25	4833	725	0.98
EGSB	10e	5	13	2	0
		10	33	5	0
		15	67	10	0
		20	133	20	0.31
		25	333	50	0.73

[a] Assuming VSS = 20 g/l; V_{max} = 1.5 g COD/g VSS·day; T = 30° C; and COD removal efficiency = 85%.
[b] Calculated using Monod kinetics, assuming effluent COD as the reactor substrate concentration.
[c] Assuming 75 mg COD/l as methane dissolved in the effluent and (0.96·0.85·COD_{in}) as total methane produced (gas phase + liquid phase); 0.96 refers to total methane yield from COD eliminated; 0.85 refers to COD removal efficiency.
[d] Average value found in literature of UASB granular sludge.
[e] Value observed in EGSB reactors of study of Kato.

well balanced microbial communities have developed within the aggregates. It is obvious that for anaerobic digestion this especially is important. Even the apparent K_m-values may become lower than the intrinsic values. Which of the phenomena will dominate, the detrimental or the favourable, depends on a variety of factors, such as the size and porosity of the biofilm, the constitution of the micro-ecosystem, the extent of adaptation of the consortium, the substrate level, the loading rate, the mixing conditions, etc.

The big importance of the value of K_m for the feasibility of anaerobic treatment for low strength wastewater is illustrated in Table 3 (Kato 1994). This table provides theoretical acceptable COD_{in}-values at different organic loading rates (OLR) as calculated on the basis of Monod kinetics, using average intrinsic K_m-values from literature and apparent K_m-values, as assessed in EGSB-experiments conducted with dilute ethanol solutions at 30° C (Kato 1994). The calculations are based on a required minimum COD-treatment efficiency of 85%.

In EGSB-experiments recently conducted with dilute VFA-solutions (COD, 1000 mg/l) at temperatures as low as 10° C (Rebac et al. 1994), the big practical potentials of EGSB-systems compared to conventional UASB-systems were demonstrated. The EGSB-concept very likely will reinforce the application of anaerobic treatment under psychrophilic conditions very substantially. The big potentials of the latter system can be attributed mainly to surprisingly low K_m-values prevailing in these systems. As found earlier by Rinzema (Rinzema 1988; Rinzema & Lettinga 1993a) these features apply for the treatment of solutions containing sodium salts of higher fatty acids, like lauric acid and capric acid. In EGSB-systems space loading rates could be accommodated up to 35 kg COD/m^3·day, whereas the UASB-reactor already failed at loading rates below 5 kg COD/m^3·day. The significantly better performance of EGSB here can be attributed to the much better sludge water contact. According to the investigations of Kato (1994), the optimal superficial velocity (v_f) for EGSB-systems is in the range of 2.5 to 5 m/h. At higher values of v_f the hydrodynamic conditions do not further improve significantly. These findings are of big importance for practice, because at higher values of v_f the sludge hold-up of the system will decrease due to the higher expansion of the sludge bed.

Immobilization of viable biomass is an important factor for the *temperature susceptibility of thermophilic sludge* (Van Lier et al. 1994c). Thermophilic granular sludges degrading acetate and butyrate were found to be little temperature sensitive. The explanation for this unexpected behaviour lies in the fact that the rate of the degradation process for immobilized thermophilic sludge is determined by diffusion and the substrate level in the reactor medium. At a specific substrate level in the bulk of the solution, the substrate will penetrate deeper into the aggregate at lower temperatures, because at lower operational temperatures the specific activity of thermophilic organisms drops down substantially, i.e. relatively much more than the rate of mass transfer (i.e. diffusion coefficient). Depending on the substrate level and on the size and density of the aggregate, a bigger fraction of the biomass may become deprived from substrate at higher temperatures, resulting in a relatively low average specific activity of the sludge. Despite the fact that the intrinsic maximum specific activity of the organisms will drop down with the temperature, this for immobilized biomass also is the case for the apparent K_m, and therefore a clear temperature effect will be absent for these types of sludges, at least in case of acetate and butyrate as substrate. A temperature effect will only manifest under conditions where the substrate indeed can penetrate into the entire aggregate. It will be obvious that this will occur later at lower substrate levels. Similar observations were reported recently by Wijffels et al. (1991) for nitrifying organisms immobilized in carrageen.

Contrary to the degradation of butyrate and acetate, for the degradation of propionate a relatively high temperature dependence was found. The reason for this discrepancy in the breakdown of these acids lies in the fact that the maximum substrate utilization rate for propionate is substantially lower than that for acetate and butyrate. As a result even at high temperatures and relatively low substrate levels, propionate will penetrate up to the core of the aggregate. Consequently, crushing of the granules does not result in higher specific substrate utilization rate of propionate, contrary to what was found for acetate and butyrate degradation. In fact crushing even has a detrimental effect on propionate conversion. This can be attributed to the disturbance of tight and balanced associations of acetogenic and methanogenic organisms present in the uncrushed aggregate. Disruption of these associations leads to a lower conversion rate of propionate (Van Lier et al. 1994c), which is in agreement with the results of other authors (Grotenhuis et al. 1991; Schmidt & Ahring 1993).

Process-conditions imposed on the reactor system

The loading potentials of an anaerobic treatment system for a specific wastewater are determined by process conditions imposed on the systems, i.e. *the substrate level maintained in the reactor, the mixing conditions, the temperature*.

The kinetic effect of the required low substrate concentration has been explained above. Another factor to be considered in relation to the substrate concentration, concerns the occurrence of substrate inhibition. At high concentration the substrate will become inhibitory. The extent to which this will be the case depends on the type of substrate. This matter is of particular importance for thermophilic systems. One of the more serious disadvantages of thermophilic treatment systems (Wiegant et al. 1986) is that frequently the effluent VFA-concentration remains relatively high. This can be due to the inhibitory effect of low concentrations of intermediates on thermophilic acetogenic and methanogenic transformations. Propionate and butyrate oxidation, and the degradation of acetate as well, are seriously affected at high temperatures by the acetate pool in the medium (Ahring & Westermann 1988; Van Lier et al. 1993b, 1994b). Hydrogen was found to have a strong effect on the hydrogen producing acetogenic reactions (Ahring & Westermann 1988; Schmidt & Ahring 1993; Wiegant et al. 1986), despite the slightly more favourable thermodynamic situation at high temperatures for the acetogenic conversions (Thauer et al. 1977). The practical consequences of the occurrence of substrate inhibition will be discussed below.

The importance of *the mixing conditions* has been explained before. The amount of mixing prevailing in the treatment system greatly depends on the specific features of the system and the loading conditions. In mechanically stirred reactors the extent of mixing can be controlled rather well.

The process temperature. Within certain limits, depending on the micro-organisms involved, the conversion rate will increase with the temperature. In order to save reactor volume, it therefore can be profitable to raise the temperature of the wastewater, i.e. to apply thermophilic conditions. However, unless cheap waste heat is available, such a procedure is not recommended for practice, because it would lead to a waste of high grade energy.

Environmental conditions in anaerobic treatment

The environmental conditions prevailing in an anaerobic reactor partially depend on the imposed process conditions. Generally they are dictated by the wastewater characteristics, and specific measures taken, such as the supply of chemicals. Important environmental factors to be considered are toxicity, and the presence of nutrients and trace elements.

Toxicity

Anaerobic organisms, particularly methanogens, are quite susceptible to a large variety of components. However, the situation is much less dramatic than presumed in the recent past, the more so as it gradually becomes clear that even severely inhibitory compounds like chloroform can be degraded by anaerobic consortia once they have become adapted. The key factor in the application of anaerobic treatment of 'toxic' wastewaters indeed is adaptation. As a result of the rapidly increasing insight in this matter, anaerobic treatment is becoming increasingly applicable for this category of 'difficult wastewaters'.

A presumed toxic/inhibitory compound for methanogens and acetogens, and of very specific interest for treating very low strength wastewaters by anaerobic processes concerns *dissolved oxygen.* Dissolved oxygen concentration can come up to 10 mg/l in very low strength wastewaters. In case oxygen indeed would be very detrimental for methanogenesis, its presence might preclude the application of anaerobic treatment for very low strength wastewaters. An additional negative effect of oxygen might be a deterioration of the granular sludge due to growth and attachment of filamentous aerobic or facultative organisms. So far little information is available about the occurrence of such filamentous growth in anaerobic granular sludge bed reactors. With respect to the inhibitory effect of oxygen, recent results of Kato (Kato et al. 1993a, b; Kato 1994) reveal that there does not exist any risk for a serious upset. Generally facultative organisms will grow in sufficiently rapidly and consume the dissolved oxygen, protecting the methanogens from the detrimental effect of oxygen. Once again this particularly will be the case for immobilized anaerobic sludge. However, according to Kato (1994) methanogens also can stand oxygen, they remain active or at least will survive.

Nutrients and trace elements

Organisms need all essential ingredients for their growth, viz. macro-nutrients and trace elements (Speece 1987). If this condition is not met, growth will not proceed satisfactorily. However, even in absence of growth, still a substantial degradation of the polluting compounds will occur. The latter was found in anaerobic digestion experiments conducted with dry solids of potato (Zeeman 1978), with diluted solutions of potato juice (i.e. the wastewater of the potato starch industry) and with composite solutions as well (Alphenaar 1994). These observations open interesting perspectives for diminishing the excess sludge production.

A rough estimate of the required amount of the *macro-nutrients N, P and S* in principle can be obtained from the bacterial composition and the bacterial growth of the concerning anaerobic sludge. Values mentioned in literature for the phosphorous content of viable bacterial biomass vary significantly. Scherer et al. (1983) reported C:P ratios for acetate utilizing methanosarcina varying from 16:1 to 75:1 (% wt/wt), which give 9.5 mg/g VSS. Speece & McCarty (1964) gave an empirical microbial formula for the anaerobic population in digesters, viz. $C_5H_7O_2NP_{0.06}$, which gives a P-content of 10.5 mg/g VSS.

The procedure commonly used in practice for assessing the nutrient requirements are based on growth yield and biomass composition. However, very likely considerably smaller amounts will be required for an optimal performance of the process. In fact relatively very little reliable and useful quantitative information is available about this matter. According to Alphenaar (1994) the P-content of granular sludge from full scale UASB-reactors range from 6.5 to 37 mgP/gVSS. The higher values generally can be attributed to the presence of phosphate precipitates. Phosphorous deficiency reduces methanogenic activity to 50% of the control, but the activity can be completely recovered by supplying a small amount of phosphate, i.e. even at 0.1 g PO_4-$P \cdot l^{-1}$, the methanogenic activity of the sludge could be recovered substantially. Interesting is also the observation that inorganic phosphate precipitates can serve as internal P-source. Phosphorous limitation will lead to a very low P-content of the biomass, i.e. around 6 mg/g VSS. Once phosphate is present again, the P-content of the biomass immediately will come up to higher values, i.e. 10 mgP/gVSS, which is the value found in the control reactor. Of practical importance is the observation that the treatment efficiency of the reactor remains unaffected under phosphorous-limiting conditions, provided the applied sludge loading rate remains below the methanogenic activity of the sludge. The sludge growth then is slightly lower. The possibility of using nutrient addition for control of sludge growth obviously could become an interesting tool in practice for reducing the excess sludge production. Uncoupling of growth and methane formation was reported for *Methanobacterium thermoautotrophicum* (Schönheit et al. 1980). For *Methanosarcina* cultures, Powell et al. (1983)

and Archer (1985) found a reversible uncoupling of growth and substrate utilization due to phosphorous limitation. For growth of methanogenic organisms De Zeeuw (1984) found that a sufficient amount of sulphide should be present, although its concentration always should remain well below its inhibitory value. The toxicity value of sulphide depends on the pH of the reaction medium because undissociated H_2S is the most toxic form (Visser et al. 1993b; Rinzema 1988; Koster et al. 1986).

Relatively much is known about the *trace nutrient requirements* of aceticlastic methanogens. Several cases are reported in the literature where addition of trace elements resulted in an increased treatment efficiency. Speece at al. (1983, 1986) and Takashima & Speece (1989) conducted experiments on the stimulation of methane production from acetate by trace nutrients. Among the trace elements, Fe, Ni and Co were essential to achieve a high acetate conversion rate into methane (Takashima & Speece 1989). Until recently very little was known about the trace element requirements for a mixed population degrading methanol in wastewater. More light has been brought in this matter recently (Florencio 1994; Florencio et al. 1994; Florencio et al. 1993a, b). Of the trace elements tested (Co, Ni, Fe, Zn, Mn, Al, Se, B, Mo, Cu), Co was the only one affecting methanogenesis from methanol. It enhances the process significantly. Cobalt stimulates merely those trophic groups which directly utilise methanol, methylotrophic methanogens and acetogens. Methanogens using acetate or H_2/CO_2 remain largely unaffected. At low Co-concentrations methylotrophic methanogens and acetogens have a comparable activity and growth rate. At optimal Co-concentrations the acetogens exert a slightly higher growth rate and specific activity. The optimum Co-concentration found was around 0.05 mg Co/l for both trophic groups. The relatively high Co-requirements of the organisms is thought to be caused by the production of corrinoid (i.e. vitamin B_{12}).

In the few studies previously conducted on nutrient stimulation of methylotrophic methanogenesis during the anaerobic wastewater treatment, no decisive conclusions could be drawn (Lettinga et al. 1979; Norrman 1983). Only some information was available on the trace element requirements for pure cultures of methanogens grown on methanol. Calcium (Murray & Zinder 1985), cobalt (Lin et al. 1989; Mah et al. 1978; Scherer & Sahm 1981), iron (Lin et al. 1989), molybdenum (Scherer 1989; Scherer & Sahm 1981, 1989), nickel (Diekert et al. 1981; Lin et al. 1989; Scherer & Sahm 1981), sodium (Blaut et al. 1985; Bock et al. 1994), selenium (Scherer et al. 1981), vanadium (Scherer 1989) and mineral components of yeast extract (Mah et al. 1978) have been identified as essential nutrients for the growth of methanogenic bacteria on methanol. Table 4 summarizes the trace element composition and trace element requirements for *Methanosarcina barkeri* grown on methanol (Nishio et al. 1992; Scherer et al. 1983).

Table 4. Trace element composition and nutrient requirement of *Methanosarcina barkeri* Fusaro (DSM 804) grown on methanol (from Florencio 1994).

Trace element	*Methanosarcina barkeri* Fusaro (DSM 804)	
	Cell content[a] ($mg \cdot g^{-1}$ cell)	Experimental nutrient requirement[b]
Na	9200	8756
K	2500	16131
S	11000	9245
P	12000	12806
Ca	3800	3616
Mg	1700	1614
Fe	2150	230
Ni	135	71
Co	60	57
Mo	60	nr[c]
Zn	130	nr
Mn	5	nr
Cu	10	nr

[a] From Scherer et al. (1981).
[b] From Nishio et al. (1992).
[c] nr, not reported.

Nothing is known in the literature about the trace element requirements of methylotrophic acetogens, although it looks likely that Co plays a key role in both trophic groups due to the involvement of cobalt containing corrinoids of methyltransferases in the initial step of methanol conversion.

The crucial role of trace elements for practical application also was experienced in laboratory and small pilot plant experiments dealing with the treatment of potato starch wastewater. Although the organic pollutants present in this wastewater could be degraded quite well, clear growth did not occur until a cocktail of trace elements was supplied to the wastewater. Based on these results a good performance could be obtained in a full scale installation treating this type of wastewater.

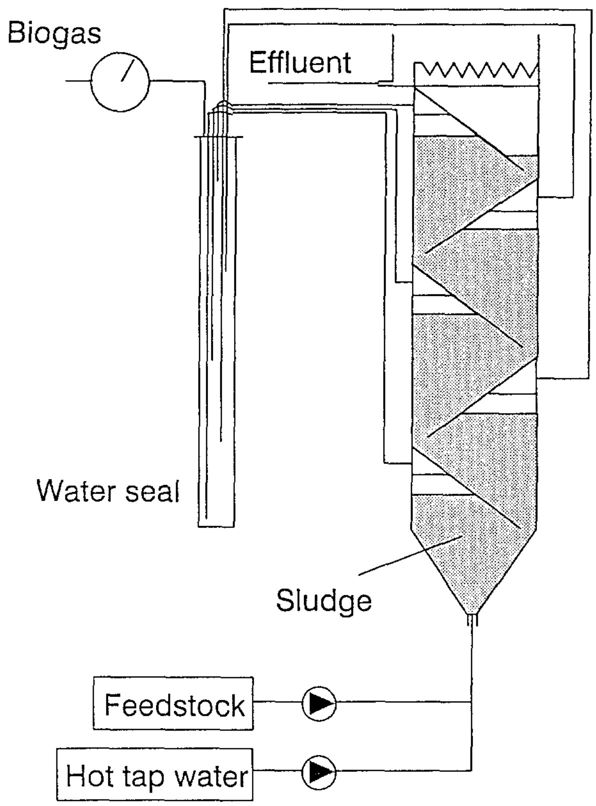

Fig. 1. A compartmentalized anaerobic reactor in which mixing of the sludge is kept at a minimum level and the liquid flow pattern approaches that of a plug flow system.

Phase separation and reactor staging in anaerobic treatment

A matter discussed extensively in the past concerns the need of *phase separation* in anaerobic treatment. Based on available insight it can be stated that a complete or almost complete pre-acidification is undesirable, but a slight pre-acidification of the wastewater generally is beneficial. For achieving this, the installation of a separate reactor is not needed.

An important development in anaerobic treatment undoubtedly will become the application of *staged anaerobic reactor systems*, which comprise a kind of anaerobic plug flow treatment system (Van Lier et al. 1994a). Process staging can be accomplished by using a number of sequentially operated reactors, or an upflow compartmentalized reactor system as shown in Fig. 1. At high loading rates the liquid hydraulics of an UASB-reactor approaches that of a CSTR-system.

In the various modules of a staged reactor system in principle still all phases of the anaerobic degradation process can proceed simultaneously. Consequently, for a non-acidified soluble wastewater the acidogenic flora will develop mainly in the first module(s), but very likely still acetogenic and methanogenic organisms will be present there. When treating a partially soluble wastewater, the first module will serve for hydrolysis and partially also for acidification. Along with the degradation process in the next modules of the system, a sludge with a higher methanogenic activity will develop. The sludge in each compartment will differ depending on the specific environmental conditions prevailing there and the remaining compounds/intermediates to be degraded. Since mixing of the sludge over the total reactor system is prevented, from module to module a specific sludge will develop. In case phase separation would occur in a moduled reactor, this will be the result of a kind of self-selection process.

A staged reactor system will provide a higher treatment efficiency, because also more difficult compounds like intermediates such as propionate will find a more optimal environment for degradation. Moreover also the process stability of such a system will be higher. This is true for mesophilic systems, but particularly for the thermophilic systems (Van Lier et al. 1994a). Compared with mesophilic digestion, thermophilic digestion suffers from a higher degree of product and/or substrate inhibition. A satisfactory treatment of concentrated wastewaters under high loading conditions therefore generally can not be accomplished in a single stage thermophilic reactor.

The importance of staging for the degradation of propionate was already suggested by Wiegant & De Man (1986), Wiegant et al. (1986), but the need for staging was only clearly demonstrated by Van Lier et al. (1994a) in experiments conducted in a compartmentalized reactor with a sucrose-VFA mixture. They found a high treatment efficiency (effluent VFA-COD < 300 mg/l) under loading conditions up to 120 kg COD/m^3·day. Sucrose was converted in the first compartment, followed by the conversion of butyrate and acetate in the next compartments, leaving the elimination of propionate to the last compartments.

The use of a sequentially operated moduled type of reactor certainly also is useful for mesophilic treatment.

Applicability of anaerobic sludge bed reactor systems

Application of under extreme temperature conditions

Modern high rate anaerobic systems are successfully applied since the mid seventies for the *mesophilic treatment* of less complex wastewaters, i.e. mainly soluble and non-toxic wastewaters, e.g. those from agro-industries. Anaerobic treatment is increasingly employed for the treatment of effluents from non-food industries, like from the paper industry. On the available high rate systems, the UASB-complex so far is most widely applied. Soon after its implementation for the treatment of sugar beet wastewater, the feasibility of the system was demonstrated for more difficult wastewaters, viz. for partially soluble and very low strength wastewaters, like slaughterhouse wastewater and raw sewage. Anaerobic sludge bed processes in many cases are quite well feasible under *psychrophilic* temperature conditions, viz. at temperatures as low as 10° C, although the latter only has been demonstrated for mainly soluble wastewaters consisting of VFA in laboratory scale EGSB-reactors and in small (200 litre) EGSB-pilot scale reactors using diluted brewery wastewater and malting wastewater (Lettinga et al. 1983b; Rebac et al. 1993). In treating very low strength wastewaters, e.g. raw sewage (De Man et al. 1988; Van der Last 1991; Van der Last & Lettinga 1991), little if any wash-out of viable biomass can be accepted. The design of the system needs some specific adjustments to improve the sludge retention.

Results of comprehensive investigations learn that *thermophilic anaerobic digestion* may become attractive for treating warm industrial effluents and specific types of slurries (Zinder 1986; Wiegant 1986). Thermophilic systems can accommodate very high loading rates at a reasonable treatment efficiency (Schraa & Jewell 1984; Wiegant & Lettinga 1985; Van Lier et al. 1994a). On the other hand, there exists some evidence that thermophilic processes are less stable than mesophilic processes and that high concentrations of volatile fatty acids (VFA) generally occur in the effluents of thermophilic reactors (Fernandez & Forster 1993; Rudd et al. 1985; Soto et al. 1992; Wiegant & De Man 1986; Wiegant et al. 1986). For these reasons thermophilic treatment is still not really an accepted technology, although a satisfactory full scale performance has been reported (Chin & Wong 1983; Cail & Barford 1985; Yeoh 1986). Thermophilic treatment systems make use of the high growth rates of the bacteria involved (Fig. 2).

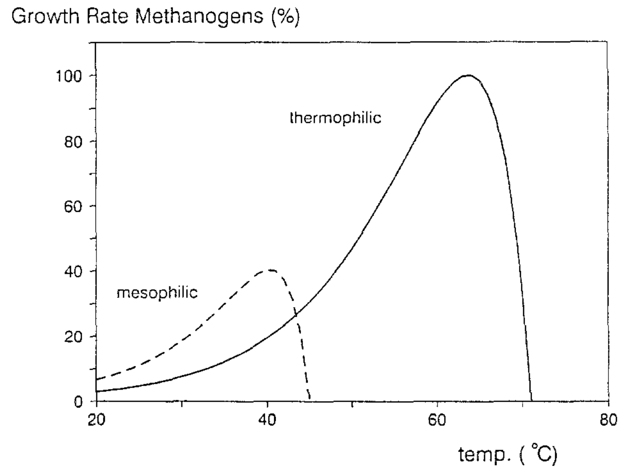

Fig. 2. Relative growth rate of mesophilic and thermophilic methanogens.

High rate thermophilic treatment systems so far hardly have been installed, despite the very promising results achieved in bench-scale studies (Schraa & Jewell 1984; Rintala 1992; Wiegant 1986; Wiegant et al. 1986; Van Lier et al. 1994a). A first 75 m^3 thermophilic UASB reactor for the treatment of vinasse has been started up only recently (Souza et al. 1992). The performance of this reactor is satisfactory.

The lack in popularity of thermophilic systems can partially be attributed to the high susceptibility for temperature fluctuations. This indeed is true for completely mixed reactor systems (Garber et al. 1975; Pohland & Bloodgood 1963; Zinder et al. 1984). A 5° C temperature increase in a 58° C-completely stirred tank reactor (CSTR) according to Zinder et al. (1984) will lead to a complete reactor failure. In contrast, thermophilic sludge cultivated in UASB reactors has a high thermostability (Van Lier et al. 1992, 1993a, c). This sludge has a single temperature optima for acetate and propionate conversion irrespective of the operational temperature in the range 46–64° C (Van Lier et al. 1992, 1993a). Very likely in sludge bed reactors some other selection criteria prevail than merely the specific growth rate (Van Lier et al. 1992, 1993a). Differences in adherence properties of thermophilic methanogens and their kinetic characteristics presumably determine the bacterial composition of the UASB-sludge. As explained before, the formation of biofilms and/or granular aggregates even will improve the thermostability of the sludge (Van Lier et al. 1994c).

Table 5. Treatment efficiencies during anaerobic-aerobic treatment of hemp stem wood black liquor (12 g COD l^{-1}) with application of upfront dilution.

Parameter		Anaerobic	Anaerobic + aerobic
COD removal	(% COD_{in})	43	55
Methanogenesis	(% COD_{in})	35	
BOD removal	(% Bod_{in})	83	99

Ratio influent:effluent aerobic post-treatment = 1:3.
OLR_{UASB} = 20 g COD $l_{UASB}{}^{-1}$ day^{-1}. $OLR_{UASB+AER}$ = 3.3 g COD $L_{UASB+AER}{}^{-1}$ d^{-1}.

A very interesting feature of thermophilic cultures for practice undoubtedly is that they can also be used under mesophilic conditions, because still considerable activity is left under mesophilic temperature conditions, while the loss in thermophilic methanogenic activity as a result of a 3-months exposure to 30–35° C is small (Wiegant 1986; Van Lier unpublished results). On the other hand after returning the temperature to 55° C, an adaptation period of about 5 days is needed. It also should be noted that temperature fluctuations have a relatively high impact on thermophilic biomass under mesophilic conditions due to the fact that at low temperatures conversion rates are not limited any more by substrate diffusion limitation.

The earlier mentioned drawback of the relatively high VFA-effluent concentration can be overcome by applying staged anaerobic reactor systems (Van Lier et al. 1994a).

Anaerobic treatment of specific compounds and wastewaters

The potentials of anaerobic wastewater treatment reach much farther than soluble and non-complex wastewaters. Results of laboratory studies and some full scale applications, reveal that the process in many cases represents the proper treatment for *inhibitory types of wastewaters*. Examples are those from paper pulp industry and some specific food industries. E.g. potato starch wastewater may also contain toxic compounds (Field et al. 1987). The presence of toxic, poorly and/or non-biodegradable compounds in the wastewater of chemical, semi-chemical and chemical-thermo-mechanical *pulping processes*, and in the effluent of bleaching and from debarking processes, seriously hampered the application of the anaerobic technology (Sierra-Alvarez 1994; Sierra-Alvarez et al. 1990; Sierra-Alvarez & Lettinga 1990; Field & Lettinga 1987, 1988, 1990, 1991; Field et al. 1987, 1988, 1990a, b). Chemo-thermo-mechanical pulping (CTMP) effluents and black liquors are highly toxic wastewaters. Wood extractives are the main source of methanogenic toxicity in soda black liquors (Sierra-Alvarez et al. 1991, 1994) and CTMP-wastewater (McCarthy et al. 1991; Richardson et al. 1991; Welander & Andersson 1985). Anaerobically they are not degraded (Sierra-Alvarez & Lettinga 1990), but they can be eliminated in aerobic treatment (Mueller et al. 1976; Easty et al. 1978; Servizi & Gordon 1986). Combined anaerobic-aerobic treatment can be used to facilitate detoxification of the wastewater, like was shown by Kortekaas et al. (1994) with black liquors (see Table 5). The detoxification mainly occurs in the aerobic treatment step. Anaerobic treatment then can be used for these toxic types of wastewater by recycling part of the aerobically post-treated effluent, because the incoming wastewater in this way can be sufficiently diluted preventing toxicity. This method of 'upfront dilution' (Habets & De Vegt 1991) is becoming increasingly popular. Anaerobic treatment of toxic wood industry wastewaters, e.g. those containing bark extracts, can be accomplished after a process of autoxidation has been applied (Field et al. 1990b, 1991; Field & Lettinga 1990, 1991; Lettinga et al. 1991).

The principal of combined *anaerobic-aerobic* process offers excellent prospects for treatment of numerous other inhibitory and complex wastewaters, such as those from the coal processing industry, petrochemical wastewater and textile dye wastewater. By combining anaerobic digestion with other biological processes in integrated treatment schemes, biological methods gradually will become better applicable for wastewaters containing refractory and inhibitory compounds, like polychlorinated compounds. Interesting in this context is the observation of Kato et al. (1993a, b) that obligatory aerobic and anaerobic can co-exist in the same mixed culture.

A very special type of wastewater is that generated by production plants of purified *terephthalic acid* (PTA), a compound produced on massive scale. The wastewater of a PTA production plant contains terephthalic acid, benzoic acid, p-toluic acid, acetic acid as main components. It is a high strength wastewater (COD = 6–13 g/l). The results of anaerobic treatment experiments with PTA wastewater on laboratory scale (Ely & Olsen 1989; Guyot et al. 1990; Noyola et al. 1990; Macarie et al. 1992; Peereboom et al. 1994) and pilot scale (Wang unpublished) and experiences obtained in full scale installations (Vanduffel 1993; Peereboom et al. 1994), show that the adaptation of anaerobic seed sludge proceeds extremely slowly and that the process is highly susceptible for fluctuations in the environmental conditions. Results of experiments conducted at our laboratory using 2.5 l UASB-reactors, show that anaerobic degradation of terephthalate only starts after a lag phase of 3.5 months, while the conversion rate then still was low. The conversion rate of PTA presumably is limited by slow transport of undissociated terephthalic acid through the cell membrane due to the low solubility of terephthalic acid.

A very important category of more complex (difficult) wastewaters are those containing higher concentrations of *fats and/or higher fatty acids*. Comprehensive laboratory and pilot plant investigations using UASB and EGSB-reactors have been made by Rinzema (Rinzema 1988; Rinzema et al. 1993a, b). For treating such complex wastewaters, particularly under psychrophilic conditions, the design of the system needs specific improvements, as also was found in investigations conducted with slaughterhouse wastewater (Sayed 1987; Sayed et al. 1988).

According to Rinzema et al. (1993a) even exceptionally high loading rates can be applied for wastewaters containing quite inhibitory compounds like lauric acid, although this merely is true for treatment systems where an excellent contact between biomass and wastewater prevails, such as EGSB-reactors. Also gently (or intermittently) stirred tank reactors using granular or well flocculated sludge could be proper systems. However, conventional UASB-reactors can not accommodate high loading rates due to the rather poor contact between viable biomass and the polluted water.

The main reasons for the lack in popularity of modern high-rate digesters for treating fat-containing wastes are: (a) problems in biomass retention due to the presence of readily floatable fats (De Zeeuw 1982; Spies 1985; Rinzema et al. 1989), (b) inhibition of the digestion process by specific long chain fatty acids (Hanaki et al. 1981; Koster & Cramer 1987; Rinzema 1988; Rinzema et al. 1989).

In UASB-experiments conducted with sodium caprate solutions (influent concentration 0.57 kg $C_{10:0}{\cdot}m^{-3}$), Rinzema (1988) found that the space loading rate only could be increased to 4.2 kg $COD{\cdot}m^{-3}{\cdot}day^{-1}$ over a 70 day period (sludge loading rate 0.1 kg $COD{\cdot}kg^{-1}$ $VSS{\cdot}day^{-1}$), but even then the stability of the processes was poor due to problems with precipitation of the capric acid.

The solution was found by using significantly higher superficial velocities (i.e. 7.2–7.7 $m{\cdot}h^{-1}$), consequently by using an EGSB-system. In this way a highly improved mass transfer from the liquid to the granules could be achieved, while also the occurrence of 'dead spaces' in the sludge bed could be prevented. It turned out that space loading rates up to 31.5 kg $COD{\cdot}m^{-3}{\cdot}day^{-1}$ (sludge loading rate ca. 1 kg $COD{\cdot}kg^{-1}$ $VSS{\cdot}day^{-1}$) could be accommodated. Moreover neither foaming nor formation of a floating layer occurred. Very similar results were found for lauric acid, which is quite inhibitory for acetogens and methanogens. Space load rates up to 31 kg $COD{\cdot}m^{-3}{\cdot}day^{-1}$ were very well accommodated in EGSB-reactors.

From the results obtained it is clear that sufficient mixing of the reactor contents and efficient contact between substrate and all biomass are absolute prerequisites for the proper performance of a high-rate anaerobic digestion system treating compounds like LCFA. If one of these conditions is not met, local overloading will occur, even at moderate or low overall organic loading rates. As the sodium salts of these acids are virtually insoluble at neutral pH, accumulation inevitably will result in precipitation, and in a sharp drop in the conversion rate of the LCFA due to physical limitations, and as a result in heavy sludge floatation, and ultimately also in sludge wash-out.

Little if any problems with anaerobic treatment would be expected at first sight with wastewaters containing exclusively methanogenic substrates. This indeed is true for acetate and hydrogen. However, the situation is much more complex for *methanol*. A variety of serious problems may manifest with methanol containing wastewaters, which comprise an important category of wastewaters in practice. Consequently a thorough knowledge of the possible biodegradation routes of this compound under anaerobic conditions is of big practical importance. But insight in this matter certainly is of big scientific interest.

Methanol can be utilized under anaerobic conditions by methanogenic and also by acetogenic organisms in case CO_2 is available. The latter will always be the case to a limited extent as a result of the methanogenesis of methanol, because this reaction proceeds as follows:

$$4\ CH_3OH \rightarrow 3\ CH_4 + HCO_3^- + H^+ + H_2O$$

Acetogenic organisms can produce acetate according to the reaction:

$$4\ CH_3OH + 2\ HCO_3^- \rightarrow 3\ CH_3COO^- + H^+ + 4\ H_2O$$

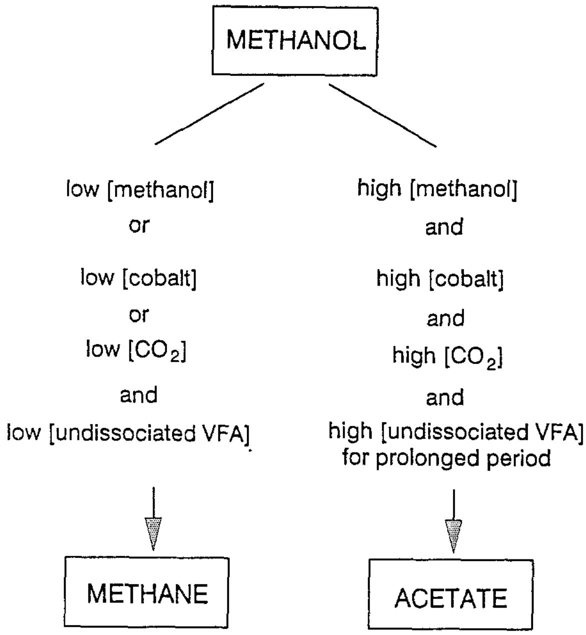

Fig. 3. Potential end products and trophic groups involved in the anaerobic conversion of methanol.

Since the digestion of VFA will pass into a severe upset when the pH would drop below 6 due to VFA-formation and a lack of buffer capacity in the solution, it is clear that latter reaction can not be tolerated. Regarding the requirement of CO_2 for the acetogenic reaction with methanol, it will be obvious that the supply of bicarbonate will act counter productive. Therefore some other solution has to be found. Although it is known from previous research that methanol can be converted into methane at pH-values as low as 4 (Lettinga et al. 1979), only very recently sufficient insight has been gained to guarantee a really stable system (Florencio et al. 1993a, b; Florencio 1994). Figure 3 summarizes the results of these investigation in comprised form.

Methanogens will predominate if either the reactor methanol concentration, the inorganic carbon content and/or the cobalt concentration (< 0.0001 pm Co) remain at a (very) low level. Methanol then is directly converted into methane by methylotrophic methanogens. Significant acetogenesis can predominate only when the reactor methanol concentration is high (i.e. at low treatment efficiency), when exogenous CO_2 is supplied, when cobalt is available and when methanogens are inhibited, e.g. by undissociated VFA. All these four conditions should be met.

Conclusions

Anaerobic wastewater treatment based on the upflow sludge bed principle like the UASB-system belongs to the category 'grown-up environmental technologies'. The UASB-process can be applied at high space loading rates (> 25 kg COD/m^3·day) for very different industrial effluents varying in COD from approximately 1.5 kg/m^3 to over 100 kg/m^3. The process is feasible for soluble and partially soluble wastewaters, for high strength and very low strength, e.g. domestic sewage.

Considering (and accepting!) that anaerobic treatment is a pre-treatment method, the process merely offers advantages over conventional aerobic systems and no drawbacks. Even with respect to the rate of start-up, anaerobic treatment is superior to aerobic treatment. Using a high quality seed material (e.g. granular sludge) a new system can be started up within a few days. Since anaerobic sludge can be preserved unfed for long periods of time, the method is ideal for campaign industries.

For practical application presently sufficient insight is available in the sludge immobilization (i.e. granulation) process. The understanding of the factors controlling granular sludge growth and/or deterioration is growing rapidly. In anaerobic treatment the immobilization of balanced bacterial consortia is essential. Such balanced bacterial communities enhance the rate of degradation of acetogenic and acidogenic organic substrates very significantly, because the concentration of intermediates is kept sufficiently low and the environmental conditions optimal. In addition to well known factors, like seed sludge characteristics, process and environmental conditions, the morphology and growth rate of the organisms, other factors of big importance with respect to the ultimate characteristics of sludge aggregates comprise the death and decay rate of the organisms. These factors have been almost completely ignored in all contemplations of high rate treatment systems. Regarding their high sludge hold-up, death and decay rate should be considered. Organisms with a relatively high death and decay rate, such as acidogenic bacteria, will constitute only a minor fraction of the sludge, even despite their relatively high growth rate. Therefore for cultivating a sludge with a high specific methanogenic activity on non- or slightly acidified wastewaters there does not exist any need for 'phase separation'. Phase separation also should be abandoned because of the detrimental effect of dispersed acidogenic organisms on the settleability of granular sludge and on its growth and formation.

A stable performance of the anaerobic sludge bed systems, including their start-up, can be guaranteed for a wide range of wastewaters, including toxic wastewaters, e.g. containing chlorinated compounds, higher fatty acids, tannins, wood resins, terephthalic acid, oxygen, etc. The role of nutrients and trace elements, and of environmental factors like pH, buffer capacity and redox potential, as well as the effect of metabolic intermediates and end products like CO_2, NH_3, H_2S is becoming 'well' understood. These growing insights in many cases open excellent prospects for process control. This for instance will be the case for the anaerobic degradation of methanol. Until recently a stable performance of the anaerobic treatment in fact could not be guaranteed for this compound, but presently things have been sufficiently elucidated.

Although so far mainly mesophilic anaerobic wastewater treatment is applied, recent results reveal the big potentials of the system in the thermophilic range and in psychrophilic and sub-mesophilic range. A matter of particular practical interest of thermophilic anaerobic sludge comprises its feature that it can be used under mesophilic conditions, because still sufficient activity is left.

The most recent variant of the sludge bed concept, the EGSB-system, offers big practical prospects, particularly for very low strength wastewaters (COD $<<$ 1000 mg/l) and for lower temperatures, i.e. as low as 10° C. In the EGSB-system not merely all the retained sludge is contacted with the wastewater, but also a substantially bigger fraction of the immobilized viable biomass participates in the process, as a result of the prevailing extraordinary low values of the apparent substrate affinity for the sludge. The relatively very high substrate affinity of the granular sludge in EGSB-systems may lead to a reconsideration of the theories developed for mass transfer in immobilized biomass. It looks necessary to account for the positive effect of the presence of balanced bacterial ecosystems in anaerobic granular sludge, and the existence of convection flows in the pores which may greatly enhance the rate of mass transfer in the immobilized biomass. Convection flows may be caused by gas bubble formation. A positive consequence of the prevailing high substrate affinity in EGSB-reactors, is that significantly higher loading rates can be accommodated in these systems with wastewaters containing lipids and/or higher fatty acids, as compared to conventional UASB-reactors.

A distinct improvement in the anaerobic reactor technology can be obtained by staging the reactor, so that mixing up of the sludge will not occur and a plug flow pattern is approached. A staged process will provide a higher treatment efficiency and a higher process stability. This particularly will be the case for thermophilic systems, because substrate and product inhibition is relatively more important here than for mesophilic processes.

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