

New perspectives in anaerobic digestion

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Abstract The IWA specialised group on anaerobic digestion (AD) is one of the oldest working groups of the former IAWQ organisation. Despite the fact that anaerobic technology dates back more than 100 years, the technology is still under development, adapting novel treatment systems to the modern requirements. In fact, most advances were achieved during the last three decades, when high-rate reactor systems were developed and a profound insight was obtained in the microbiology of the anaerobic communities. This insight led to a better understanding of anaerobic treatment and, subsequently, to a broader application potential.

The present "state-of-the-art" paper, which has been written by members of the AD management committee, reflects the latest achievements and sets future lines for further development.

Keywords Anaerobic; digestion; high-rate reactors; sludge bed; solid waste; wastewater treatment

Introduction

Anaerobic Digestion (AD) can be considered as one of the oldest technologies for stabilising waste(water)s. AD has been applied since the end of the 19th century, mainly for the treatment of household waste(water)s in septic tanks, treatment of slurries in digesters and for the treatment of sewage sludge in municipal treatment plants.

The request for more cost-effective treatment systems for the growing food industry, combined with the occurrence of an international oil crisis, was the driving force that stimulated the most important research achievements of the seventies in the field of AD. Particularly the introduction of the modern 'high-rate' reactors, in which hydraulic retention times (HRT) are uncoupled from the solids retention time (SRT), led to a world-wide acceptance of the anaerobic technology as a cost-effective alternative for conventional wastewater treatment systems. From the various systems which have been developed, the upflow anaerobic sludge blanket (UASB) reactors and/or related systems are mostly applied. One of the major features of the latter reactor concept is the spontaneous formation of granular conglomerates of the anaerobic organisms, leading to anaerobic sludge with an extremely low sludge volume index (SVI) and optimal settling properties.

Since the late seventies, anaerobic digestion has experienced an outstanding growth in research and full-scale application, particularly for the treatment of industrial effluents (Totzke, 1999) and to a lesser extent of municipal wastewater (mainly in tropical countries) (Hulshoff Pol *et al.*, 1997). Besides, a large number of large scale biogas plants have been established especially in Northern Europe which combine waste from agriculture, industry and households and produce both biogas and a liquid fertiliser which is re-circulated back on farmland. Several European cities have implemented a source-separation of household waste in two fractions; a green fraction containing food residues and in some cases garden waste and a rest-fraction containing all the material of non-biological nature. Anaerobic



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digestion is an obvious method for treatment of the green fraction and has several advantages compared to aerobic composting such as a net energy production and the conservation of ammonia in the digested material. The number of plants for the treatment of green waste is slowly increasing throughout Europe but composting is still the major choice and until now has been more successful on this market. Nonetheless, the present energy conservation policies as well as the strong demand for the reduction of atmospheric CO₂ emissions are in favour of the further development of advanced AD techniques.

In the following pages, the latest advances in anaerobic processes and related technologies will be briefly reviewed, and future developments will be discussed.

Pre-treatment

The methanogenic process is generally limited by the rate of hydrolysis of suspended matter and organic solids. This is of particular importance during the anaerobic treatment of solid wastes, slurries and manure, and wastewaters with a high concentration of suspended solids (SS), such as domestic sewage. By means of efficient pre-treatment the suspended substrate can be made better accessible for the anaerobic bacteria, optimising the methanogenic potential of the waste to be treated. The objective is to accelerate the digestion of solid waste and slurries such as sewage sludge, to raise the degree of degradation and thus to decrease the amount of sludge to be disposed.

Low biogas yield from excess activated sludge is caused by the low biodegradability of the cell walls and extracellular biopolymers formed in activated sludge. The enhancement of the biodegradability of particular substrate is mainly based on a better accessibility of the substrate for enzymes. There are several ways how this can be accomplished.

- *Mechanical methods – the disintegration and grinding of solid particles present in sludge:* releases cell compounds and creates new surface where biodegradation take place (Eastman and Ferguson, 1981; Dohányos M and Zábranská J, 1991; Baier and Schmidheiny, 1997; Dohányos et al., 1997; Kopp et al, 1997);
- *Ultrasonic disintegration:* (Tiehm et al., 1997)
- *Chemical methods:* the destruction of complex organic compounds by means of strong mineral acids or alkalis (Mukherjee and Levine 1992);
- *Thermal pretreatment:* thermal hydrolysis is able to split and decompose a significant part of the sludge solid fraction into soluble and less complex molecules (McCarty et al. 1976; Haug et al. 1983);
- *Enzymatic and microbial pre-treatment:* a very promising method for the future for some specific substrates (e.g. cellulose, lignin etc.) (Knapp and Howell 1978; Hakulinen 1988; Lagerkvist and Chen 1993);
- *Stimulation of anaerobic micro-organisms:* some organic compounds (e.g. amino acids, cofactors, cell content) act as a stimulating agent in bacteria growth and methane production (Gossett and Belser 1982).

Most of the above methods occur at the pre-methanation step and result in a better supply of methanogenic bacteria by suitable substrates. The exact composition of the substrate (solid waste, slurries) is of major importance for the selection of the most appropriate pre-treatment method.

Mechanical disintegration is a very promising method for various types of waste streams since it immediately accelerates the methanation step. This method creates new surface and releases cell compounds. The released content of bacteria cells into the bulk liquid after the destruction of cell walls has been known as cell lysate. Cell lysate represents not only better accessible and degradable organic compounds, but also contains enzymes, enzyme fractions and co-factors with still present remaining activity. Cell lysate can accelerate degradation reactions and consequently saves energy for biosynthesis. The presence of cell

lysate in sludges that have to be digested supports anaerobic bacteria growth and methane production. Thus far, various types of mechanical disintegration methods were developed and tested, such as ball mills (Baier and Schmidheiny, 1997; Kopp *et al.*, 1997), high-pressure homogenisers (Kopp *et al.*, 1997) and ultrasonic disintegration (Tiehm *et al.*, 1997). The main problems of the application of the mechanical pre-treatment into a full-scale methane fermentation are the costs of the cell disruption and the quality of the lysate produced. For a full-scale application a new method of cell disintegration by means of a lysis-thickening centrifuge was developed (Dohányos *et al.*, 1997). The objective of this method is a partial destruction of excess activated sludge cells during the thickening. A benefit of the procedure is that the cell destruction proceeds after the thickening in the thickened sludge flow only, which avoids additional water loads. A relatively small amount of the lysate present (4–10% related to incoming solids) is enough to cause a significant stimulation of the methane fermentation process.

Pilot scale research at the Prague Central Wastewater Treatment Plant demonstrated the advantages of activated sludge disintegration, which are: (i) the improvement of the anaerobic biodegradability, (ii) the acceleration of the degradation process, (iii) the increment of methane production, (iv) reduction of the amount of digested sludge, and (v) the improvement of the process energy balance. The improvement of methane yield from thickened activated sludge was on average 11.5–31.3% dependent on the sludge quality (Dohányos *et al.*, 1997; Kopp *et al.*, 1997; Tiehm *et al.*, 1997).

Optimization of sludge bed systems

The loading potentials of anaerobic reactors for wastewater treatment are determined by (i) the wastewater characteristics, (ii) the amount of viable biomass which can be retained by the system, and, (iii) the degree of mixing between the methanogenic sludge and the wastewater.

In the last decades the system-specific parameters of UASB reactors were modified to increase the loading potentials and/or to widen the applicability of anaerobic reactor systems for various types of wastewater. For a series of wastewaters, the conventional UASB reactors concept showed severe limitations mainly owing to problems related to mass transfer resistance and/or the appearance of concentration gradients inside the system. In case the biogas production rate drops, e.g. for low-strength and cold wastewaters, the degree of mixing must be brought about hydraulically to ensure the required mass transfer (Rebac *et al.*, 1999). Furthermore, the appearance of concentration gradients limits the treatment of protein rich wastewaters, and wastewaters containing long chain fatty acids (LCFA) (Rinzema *et al.*, 1989), or biodegradable toxic compounds, such as formaldehyde (Zoutberg and De Been, 1997). The latter type of waste waters can only be treated anaerobically at a high loading rate, when the incoming influent is sufficiently diluted, i.e. when the reactor content is well mixed (see also below).

Application of a fluidized bed (FB) reactor in principle overcomes mass transfer limitations but these systems are difficult to manage because of problems of biofilm stability, due to shear stresses or to bed segregation from the inert support material. Moreover, in order to obtain complete fluidization, the energy requirement of FB reactors is relatively high. By making use of the high settleability of the methanogenic sludge granules ($40\text{--}60\text{ m}\cdot\text{h}^{-1}$), expanded granular sludge bed (EGSB) systems were developed, which are operated at upflow velocities exceeding $8\text{ m}\cdot\text{h}^{-1}$, brought about by an increased height-diameter ratio and an external recirculation pump. In contrast to the conventional UASB reactor, the EGSB systems are not equipped with an internal settler but with an advanced (gas-)liquid-solids separation device. Such device may consist of screens (Rebac *et al.*, 1998) or a modified internal lamella separator (Zoutberg and Eker, 1999). The internal circulation (IC)

reactor is an expanded bed system based on the gas lift concept. The reactor is equipped with two gas-solids separator, of which one is placed halfway along the reactor and the second is placed at the top. The gas-water mixture collected halfway is transported to the top where the liquid is degassed. Hereafter, the liquid is guided to the bottom of the reactor where it is mixed with the influent. The height of the increased upflow depends on the biogas production rate and may reach values of 25–30 m/h, creating a completely mixed compartment below the first solids separator (Driessen *et al.*, 1996; Yspeert *et al.*, 1993).

The main features of the EGSB and IC reactors are: (i) high design organic loading rates: 20–40 kg · m⁻³ · day⁻¹; (ii) very small surface area; (iii) tall reactor systems: h = 12–20 m; (iv) high upflow velocity: 8–30 m · h⁻¹.

So far, expanded sludge bed reactors are profitably used for:

- cold wastewaters (< 20°C) (Rebac *et al.*, 1999)
- dilute wastewaters (< 1 g COD · l⁻¹) (Kato *et al.*, 1997)
- chemical wastewaters containing toxic degradable compounds (e.g. formaldehyde) (Zoutberg and De Been, 1997)
- wastewaters leading to foaming problems in UASB systems (fats, lipids, proteins)
- effluents containing fats and long chain fatty acids (LCFA). Fatty effluents generally lead to (sludge bed) clogging problems in UASB reactor (Rinzema *et al.*, 1989; Rinzema *et al.*, 1993).

The above types of wastewaters generally lead to operational problems when UASB reactors are applied.

Satisfactory operation of sludge bed reactors may also be limited by inadequate retention of viable biomass. This is particularly the case during the treatment of specific types of wastewaters, i.e., when the system is not able to cultivate granular sludge. Examples are wastewaters with a high concentration of suspended solids (SS), such as alcohol distillery wastewater and domestic sewage (see also below). In such cases hybrid reactor systems might be more advantageous. In addition, also in granular sludge bed reactors, problems with biomass retention may occur (Alphenaar, 1994), resulting from (i) granule disintegration, (ii) wash-out of hollow granules, (iii) occurrence of fluffy granules, (iv) scaling by inorganic precipitates (van Langerak *et al.*, 1998), etc. Modifications in the process layout and/or instalment of appropriate post treatment systems will generally result in the improvement of the granular sludge bed.

Novel reactor systems: membrane bioreactors

Efficient liquid-solids separation is the basis of any anaerobic high-rate reactor system for wastewater treatment. Solids separation may be improved distinctly by the combination of a digester with a membrane process, where the separated solids (biological and non-biological) are continuously recycled. The sludge retention time can be easily adjusted through the amount of waste sludge withdrawal, and is highly dependent on the amount of inert solids loaded to the reactor. With a low inert solid loading, these systems can be operated even at approximately infinite SRT, thus allowing them to reach very low effluent substrate concentrations. In addition, allowing the growth of slow-growing micro-organisms, this reactor concept could be particularly suited for the treatment of recalcitrant compounds. However, one of the big drawbacks of the high pressure physical separation device is the disruption of the microbial conglomerates needed for the efficient conversion of complex organic matter (Brockmann and Seyfried, 1997). The latter study shows higher loading rates and better removal efficiencies with UASB reactors compared to the anaerobic membrane bio-reactors. Nonetheless, considering their potential, anaerobic membrane bio-reactors may be very beneficial for specific applications, e.g. when biomass granulation proceeds with great difficulty. Also with respect to slurry digestion where a solids liq-

uid separation step is needed after digestion, membrane bio-reactors show interesting perspectives. Another application is the treatment of wastewaters with high concentrations of suspended solids (Nagano *et al.*, 1992). Successful industrial feasibility studies were performed in Japan and South Africa. Results show good fluxes with membrane systems, well optimised for duration, robustness and resistance to fouling. Future enhancements can be expected from the extension to anaerobic treatment of in-reactor membrane systems developed for aerobic MBRs. These systems (like Zenon or Kubota) make use of flat microfiltration membranes placed inside the bio-reactor, through which effluent is withdrawn by extraction pumps or by the hydraulic pressure. In the aerobic set-up, the membrane surface is continuously cleaned by intense air bubbling. In an anaerobic configuration, the air could be substituted by biogas recycling. The advantage of this configuration can be the lower energy need. The drawback for an anaerobic reactor could be the difficulty of maintaining the membranes, which can be subjected to fouling and scaling with e.g. typical precipitates such as calcium carbonate.

Extreme conditions

During the last decades numerous studies explored the potential of anaerobic treatment under extreme conditions, such as low and high temperatures, low and high pH values, saline conditions and/or the presence of toxic compounds.

Temperature

Anaerobic reactors are normally operated at mesophilic (30 to 40°C) or at moderate thermophilic (50 to 60°C) temperatures in accordance with the optimal temperature range for the groups of anaerobic microorganisms performing the whole digestion process (Ahring 1994, 1995, Van Lier, 1996). However, recent research has demonstrated that anaerobic digestion is possible at temperatures up to 80°C (Lepistö and Rintala, 1996). Even though methanogenic conversion can occur at very high temperatures, a temperature of 50–60°C is generally more applicable for thermophilic treatment as higher temperatures can result in instability of the treatment process (Van Lier *et al.*, 1993). At 50–60°C anaerobic digestion will be just as stable and well performing as found for mesophilic digestion. With regard to manure digestion, ammonia concentrations higher than 4 g·l⁻¹, may limit the thermophilic anaerobic treatability, owing to toxicity problems (Angelidaki and Ahring 1993, Ahring 1994, Zeeman *et al.*, 1985). Slurries and wastes with a high concentration of ammonia such as swine manure can still be digested with a stable but low gas yield. However, it will be possible to increase this yield using different additions and operation schemes (Hansen *et al.*, 1999) and allowing sufficient adaptation time for the anaerobic biomass (Zeeman *et al.*, 1985). Experiments with thermophilic digestion did, however, often lead to poorer performance than that found in mesophilic digestion. It is obvious that under thermophilic conditions the concentration of free NH₃ is somewhat higher than under mesophilic conditions and, therefore, toxicity problems could be more pronounced. On the other hand, many of these literature experiments were done with unstable reactors without a stable population of thermophilic microorganisms. The need for an active, acclimatised, thermophilic inoculum, or for a controlled start-up strategy if no or only limited amount of inoculum is available, has now been fully accepted (Ahring 1994) and has resulted in a faster and more reliable performance of thermophilic reactors. Increasing the temperature to more than 60°C will often lead to an increased concentration of volatile fatty acids in the effluent especially when treating manure or solid waste in completely mixed tank reactors. Despite a high hydrolytic activity at higher temperatures, the activity of other groups of bacteria such as the propionate and acetate degrading bacteria has been shown to decrease when the temperature increases to more than 60°C. Therefore, higher temperatures are more applicable

for immobilized systems, where the lower rate of specific groups of bacteria can be circumvented by a higher bacterial number (Angelidaki and Ahring 1993, Ahring 1994, 1995, Van Lier 1996, Van Lier *et al.*, 1996). Temperature strongly affects the activity of microorganisms and, therefore, the bioconversion rate of anaerobic organisms. Therefore, a smaller reactor volume will be sufficient at thermophilic temperatures compared to mesophilic conditions. Comparative results between thermophilic and mesophilic sewage sludge digesters showed a considerably lower solids residence time (SRT) at high temperatures (Wiegant, 1986). Another major advantage of thermophilic treatment is a much higher reduction of pathogens compared to mesophilic digestion (Buhr and Andrews, 1977). Inactivation in the thermophilic biogas process will be of importance for both agricultural pests and parasites and the effect of thermophilic anaerobic treatment will be of greater magnitude than can be expected from the increased temperature alone (Lund *et al.* 1996). When using common large-scale biogas plants for treatment of waste from several farms or from households and some industries, a proper sanitation of the organic waste is of major importance for the reuse of the digested material as a fertilizer on agricultural land (Aitken and Mullennix, 1992, Bendixen 1994, Lund *et al.* 1996). This can only be ensured by thermophilic anaerobic treatment with a controlled and well defined holding period between in- and out-flow of material from the anaerobic reactor (Bendixen 1994).

Thermophilic wastewater treatment offers the possibility of closing the water cycles in industrial processes avoiding the need for cooling and heating of process water before the biological treatment of the water. At present, the water cycles of paper mills are being researched for loop closure, in which a thermophilic treatment process is being implemented (Vogelaar *et al.*, 2000; Sipma *et al.*, 2000).

Acidic and alkaline conditions

AD is generally applied under neutral pH conditions (pH 6.5–8). The observed toxicity under low pH conditions is associated with the presence of undissociated volatile fatty acids (VFA). Recent research has demonstrated that the anaerobic process proceeds well at pH levels as low as 4.5–5, provided no VFA is present (Florencio *et al.*, 1993). The phenomenon was found using methanol as the sole carbon source during anaerobic treatment.

Regulation of pH in industrial applications can be a costly process when treating large amounts of wastewaters. A study using fish industry wastewater with a high pH showed that it was possible to have a steady and stable performance of a UASB reactor with a high degree of COD reduction when operating the reactor at a pH of 9.0 (Sandberg *et al.*, 1992). Granular sludge from this reactor was found to have an increased activity at higher pH compared to a control reactor operated at neutral conditions. Treatment at a high pH was important for the viability of the wastewater process as a whole as the anaerobic step was to be followed by ammonia stripping which only proceeds at a high pH.

Anaerobic treatment under acidic and alkaline conditions may play an important future role when in line – or side stream – treatment processes in industries call for a higher tolerance for extreme conditions.

Toxic, xenobiotic and recalcitrant compounds

Treatment of toxic, and/or recalcitrant chemical waste(water) by anaerobic methods is a relatively new area. However, anaerobic treatment has a major potential for the elimination of several groups of xenobiotics such as halogenated organics. The latter is due to unique processes such as reductive dehalogenation, an energy yielding process only occurring under anaerobic conditions. Several anaerobic microorganisms possessing degrading capabilities have been isolated during the last ten years and the numbers are steadily increasing. An important study was done in 1992 showing the potential for implementation

of new capabilities into granular sludge for degradation of specific xenobiotics by using pure cultures of a xenobiot-degrading microbe (Ahring *et al.*, 1992). Recently, this finding has been repeated with different anaerobic microbes possessing different capabilities and these studies have all pointed to the major potential which eco-engineering of sludge can have for anaerobic removal of xenobiotic compounds (Christiansen and Ahring 1994, Christiansen *et al.*, 1995; Horber *et al.*, 1998). For instance, while natural sludge often degrades the target compound via a less favourable pathway leading to toxic intermediates, a controlled implementation of specific microorganisms can result in optimization of the degradation rate and the degradation pathway (Horber *et al.*, 1998).

Several kinds of chemical wastewaters which were believed to be unsuitable for anaerobic treatment are currently treated with advanced reactor systems (Frankin *et al.*, 1992; Frankin *et al.*, 1994; Tseng and Yang, 1994; Narayanan *et al.*, 1993a, 1993b). Actually, while only 19 anaerobic plants were treating chemical waste until 1990, at least 80 plants are now in operation in the world (Macarie, 2000). This results in part from the discovery that contrarily to the common idea, methanogenic bacteria are not more sensitive to toxicants than aerobic ones (Blum and Speece, 1991). In addition, recent research shows that various recalcitrant compounds, like chlorinated aliphates, chlorinated aromates, nitroaromates, can be degraded under either anaerobic conditions or in aerobic-anaerobic sequences (Christiansen *et al.*, 1995, Pavlostathis, 1994; Field *et al.*, 1995, Horber *et al.* 1998). Recently it was shown that azo dyes can be completely removed in anaerobic-aerobic sequenced reactor systems (e.g. Tan *et al.*, 1999).

Another breakthrough, which has allowed the application of anaerobic digestion to the treatment of some chemical wastewaters, corresponds to the development or use of particular reactor design or arrangements. For instance, thanks to their high recirculation rates, EGSB reactors can be used for the treatment of toxic but biodegradable compounds such as formaldehyde. Presently, this concept is used at least in two full scale plants (reactor volume of 275 and 550 m³) treating wastewaters containing up to 3.8 and 10 g formaldehyde/l (Zoutberg and de Been, 1997; Constable and Kras, 1998). In these plants, effluent recycling dilutes the raw wastewater by a factor of 10 to 30, which lowers formaldehyde concentration below 0.5 g·l⁻¹ at the entrance points of the reactors. At this concentration the rate of formaldehyde degradation is higher than that of biomass decay allowing to maintain in the system a net growth rate. Both reactors show very stable treatment performances at organic loading rates of 10 kg COD·m⁻³·day⁻¹ and removal efficiencies of 90–98%, with effluent formaldehyde concentrations below 20 mg·l⁻¹. From other side, lab scale experiments have shown the importance of staged reactor systems for the treatment of wastewaters generated during the production of terephthalic acids, a petrochemical compound used in the synthesis of polyesters (Fajardo *et al.*, 1997; Kleerebezem *et al.*, 1999). These wastewaters contain both easy (acetic and benzoic acids) and more difficult to degrade (terephthalic and p-toluic acids) organic compounds. The methanization of the second group of molecules is inhibited by the first. Therefore, the conversion of terephthalic and p-toluic acids to CH₄ requires a very low concentration of acetic and benzoic acids. High rate conversion of such complex mixtures can be achieved in reactors operated in a plug flow mode since these reactors are characterized by the formation of gradients. A pseudo-plug flow mode can however also be achieved with two completely stirred reactors operated in series. It should be noted that for this last class of wastewaters, 14 full scale anaerobic plants are already operating in the world, though at a relatively low loading rate (Kleerebezem, 1999).

Domestic and municipal wastewater

Domestic wastewater can be considered as a low-strength, complex type of wastewater, characterized by: (i) low COD concentrations; (ii) high fractions of suspended solids; (iii)

relatively low temperatures; (iv) strong fluctuations in hydraulic and organic loading rates. These characteristics are particularly relevant to the anaerobic treatment process, generally having a negative impact on the process performance and/or the costs. Nonetheless, by taking into account the limitations caused by the above characteristics, the anaerobic reactor technology can be profitably applied for the treatment of domestic wastewater under a wide range of conditions.

The breakthrough for anaerobic sewage treatment only recently occurred by using UASB reactor systems, which were originally developed for industrial wastewater treatment (Lettinga *et al.*, 1980, 1983). In view of the above mentioned limitations, the UASB reactor design required several adaptations, both in the field of process technology as well as in the field of practical application. For instance, due to the low gas production, adequate gas withdrawal is of much less importance. On the other hand, efficient solids separation determines the maximum liquid velocities and thus allowable surface areas inside the reactor. Also, working with sewage requires adequate pre-treatment and easily accessible inlet points. The present 'state of the art' with regard to the application of sludge bed systems for sewage treatment has been recently reviewed by Segghezo *et al.* (1998). Full scale applications showed excellent results of anaerobic sewage treatment under tropical conditions, viz. temperatures $> 20^{\circ}\text{C}$ with COD removal efficiencies of 75% at 6 h HRT (van Haandel and Lettinga, 1994, Draaijer *et al.*, 1992, Schellinkhout and Osorio, 1994). The system tolerates strong fluctuations in flow, composition and temperatures as long the temperature does not drop below $18\text{--}20^{\circ}\text{C}$.

Generally, 50% of the COD in domestic sewage consists of suspended solids. Particularly at low temperatures, this fraction gives rise to a deterioration of the methanogenic sludge bed. However, the soluble COD can be efficiently converted to methane at temperatures as low as 5°C (see also Collins *et al.*, 1998; de Man *et al.*, 1988; van der Last and Lettinga, 1992). For a successful application of anaerobic treatment of raw domestic sewage under low temperature conditions, the incoming suspended solids must be separated from the waste stream before the sewage enters the methanogenic reactor. The removal of suspended solids from the raw sewage can be achieved by means of a purely physical pre-treatment, i.e., primary clarifier (Collins *et al.*, 1998; de Man *et al.*, 1988; van der last and Lettinga, 1992), or by applying a sequence of 2 anaerobic reactors in series. In the latter set-up, the first anaerobic step is designed for either: (i) solely entrapping the incoming SS (Zeeman *et al.*, 1997), (ii) entrapping and (partly) hydrolysis/acidification of the solids (Wang, 1994), or (iii) pre-digestion of the solids including methanogenesis (El-Gohary and Nasr, 1999).

The major advantage of a two-module system is the higher upflow velocities applicable in the methanogenic stage, leading to an enhanced liquid-biomass contact. The latter is a prerequisite for the anaerobic treatment of low temperature wastewater, since natural mixing by biogas production is nearly absent. In fact, the second module can be operated as an expanded sludge bed, taking advantage of improved hydraulics and a low percentage of channeling (Collins *et al.*, 1998; Van der Last and Lettinga, 1992; Wang, 1994).

Solid wastes

Anaerobic digestion of solid waste has become a mature technology, well developed both at the research and the industrial level. At present, world wide some 1 million tons of organic wastes (wet weight) are digested per year (De Baere, 1999). These are converted to biogas on the one hand, and a stabilised residual matter on the other hand.

Several types of digesters are successfully operational at large scale (10,000 to 100,000 tons per year) in different countries treating different types of solid wastes (source separated bio-wastes, mixed (grey) wastes). Overall, the investment costs for anaerobic digestion

are a factor 1.2–1.5 higher than for aerobic composting. The net costs per ton waste treated, taking into account the recovery of biogas energy, are also 1.2–1.5 higher than that of conventional aerobic composting (Genon, 1999). However, this figure can change with new restrictions on emissions from waste treatment facilities. The prediction is that in the near future anaerobic digestion will keep a position of being more costly than aerobic composting. World wide, the major amount of municipal wastes is destined for landfilling; incineration ranks second, aerobic composting third and anaerobic digestion a very modest fourth.

This makes industrial anaerobic digestion at present a technology of only limited quantitative impact. Therefore, the evolution of regulations, particularly in Europe, will strongly limit in the near future the possibility of landfilling of organic wastes, thus pushing towards a growing market for anaerobic digestion.

The future of anaerobic digestion of solid wastes has to be sought in the integration of this unique unit process in overall sustainable waste treatment. Indeed, anaerobic digestion, when considered in the context of Life Cycle Analysis (LCA) offers a number of interesting features. The recovery of energy (100–150 m³ biogas per ton bio-wastes) is an important factor. Moreover, aerobic treatment of solids is inevitably giving rise to extensive emission of undesired volatiles such as ketones, aldehydes, ammonia and even methane (several kg per ton waste treated). Landfill gas extraction, that today represents the larger biogas generation technology at global scale, can only partially (maximum ~60%) recover the generated gases, giving rise to considerable methane emissions in the atmosphere. In anaerobic treatment, all gases are contained and, via the use of the biogas, incinerated. Indications are that in terms of the contribution to global warming anaerobic digestion therefore scores considerably better than other treatments (Klüber and Rumphorst, 1999). A number of aspects require further optimisation such as e.g. the temporary emission of methane at the transition from anaerobic digested to aerobic after treatment (Edelmann *et al.*, 1999). One aspect that particularly deserves to be further explored is the capacity of anaerobic digestion to decompose chlorinated organics and thus achieve a putative decontamination of organochlorines (Christiansen *et al.*, 1995). Indeed, the problems concerning the fate of micro-pollutants (nonylphenol, heavy metals, PCBs, dioxins) and the overall end-product quality have become a major factor for all types of waste treatments and anaerobic digestion offers specific potentials in this respect. The overall organic matter stabilisation can be improved through the improvement of pre-treatments (see above); the study of the literature on rumen physiology and microbiology could be beneficial to anaerobic digestion process engineers for finding the right blend of mechanical, chemical and enzymatic/biological pre-treatment options.

It may be also that the normative evolution will favour a broader application of co-digestion. Co-digestion is the combined treatment of different kinds of solid and semi-solid biodegradable organic wastes, that can range from the organic fraction of municipal solid wastes, to animal wastes, to municipal sludges, to concentrated organic wastes from industrial and agro-industrial processes. The final objective of the co-digestion treatment would be to produce a compost to be recycled as a soil conditioner. However, an increase in the gas production from the higher gas yield obtained by many organic industrial wastes compared to sewage sludge or manure will also drive the initiative for combining different waste types (Ahring, 1992; Tafdrup, 1994). Combining wastes will further leave the possibility of treating waste, which cannot be successfully treated alone such as fatty wastes or waste with a very high protein content such as size wastewater (Ahring *et al.*, 1992, 1995). Thermophilic processes seems to be better suited for handling co-digestion with fatty wastes in addition to manure, a combination used in most of the 22 centralized biogas plants constructed throughout the countryside of Denmark (Tafdrup, 1994).

Modeling and process control

The development of biological processes modelling has increased the capabilities of representing – in mathematical terms – the AD process. However, the absence of a common platform for modelling – like ASM1 for aerobic treatment – has limited the developments in this field. So far, many researchers independently developed different modelling strategies. Since there was no communication with other research groups these models are destined to be abandoned in the near future and a huge amount of effort will be lost. For this reason, the AD Specialist Group has established a Task Group that is trying to set up a common platform on which to base the future developments. The Task Group firstly unifies the various notations and symbols which are applied worldwide and sets the bases for a common kinetic model. An AD model is a valuable tool for design and process operation purposes.

Monitoring and process control of anaerobic wastewater treatment could be manual fully automatic. In the past decades, a large development is going on concerning the latter approach. The crucial question in this development was to identify the most important control parameters. For monitoring, values of parameters could be measured in solid, liquid or gaseous phases (Switzenbaum *et al.*, 1990). For automatic monitoring and control, parameters in the solid phase are not often used (since they usually need manual operations). The parameters mostly used in the liquid phase are: pH, Volatile Fatty Acids (VFA), alkalinity, and COD concentrations for example (e.g. Pretorius, 1994). In the gaseous phase they are: carbon dioxide, methane and hydrogen contents and gas production mainly.

The fully automatic approach in industrial applications diminishes the quantity of parameters available because there are only few industrial sensors. New sensors development (Hawkes *et al.*, 1993; Rozzi *et al.*, 1997) opened new possibilities for on-line monitoring of various process parameters, such as bicarbonate alkalinity and VFA. This development could lead to a broader application of industrial automatic process control devices. The infrared approach is a promising technique in this field. Generally the controlled variable on the process is the feed pump speed. Guwy *et al.* (1997) adjusted the digester buffering by addition of NaHCO_3 . Other strategies can be used as addition of acid or soda, or to increase the amount of micro-organisms in anaerobic contact for example. Steyer and others (1999) proposed the monitoring of gas production rate after a disturbance on the input flow rate as a way to control the anaerobic digesters.

If modelling is essential for process design, it is also important for process control, even if in this field the most recent advances (adaptive command, fuzzy logic, neural networks, set of rules etc.) can generate new controllers. Empirical or black box models are also used. Adaptive command, fuzzy logic, neural network controllers have been successfully applied (Dochain and Bastin, 1997, Wilcox *et al.*, 1997; Tay and Zhang, 2000), allowing users to reduce drastically start-up time and improving overall plant performances.

Related bio-technologies

The combination of anaerobic digestion with other biological or physico-chemical processes has led to the development of optimised processes for the combined removal of organic matter, sulphur and nutrients.

Application of the sulphur cycle

In anaerobic wastewater treatment research, sulphate reduction has been for many years an important topic, since many waste streams contain substantial amounts of oxidised sulphur compounds, which are reduced into hydrogen sulfide. As the production of H_2S causes a multitude of problems, such as toxicity, corrosion, odour, increase of the liquid effluent COD, reduced biogas quality and amount, emphasis of the research in the past has been mainly on the prevention or minimisation of sulphate reduction (Isa *et al.*, 1986, Rinzema

and Lettinga, 1988; Oude Elferink *et al.*, 1994; Colleran *et al.*, 1995; Bhattacharya *et al.*, 1996; Lens *et al.*, 1998). Presently, it can be concluded that treatment of sulphate-rich wastewater is quite possible by applying adequate measures allowing an integration of sulphate reduction with methanogenesis (Hulshoff Pol *et al.*, 1998).

More recently, interest has grown in applying sulphate reduction for the treatment of specific waste streams containing no or very little organic matter. Examples of such waste streams are from mining and mineral processing industries, metallurgical and chemical industry as well as sulphite liquor from flue gas scrubbing (van Houten and Lettinga, 1996). Under these conditions it is necessary to supply an appropriate electron donor and carbon source to the wastewater. It has been demonstrated that a successful reactor performance can be obtained with H₂ and CO₂ (van Houten *et al.*, 1994), synthesis gas (du Preez *et al.*, 1992; van Houten *et al.*, 1996), ethanol (Scheeren *et al.*, 1991), methanol (Weijma *et al.*, 1999) and digested solid waste (Hilligsmann *et al.*, 1998). Combination of sulphate reduction with biological or physico-chemical sulphide oxidation allows a complete removal of sulphur from these wastewaters by its conversion into insoluble elemental sulphur (Janssen *et al.*, 1997). The produced sulphur can be separated from the liquid stream and re-utilised as fertiliser or raw material for sulphuric acid production.

New promising potentials for the use of sulphate reduction in anaerobic reactors are the enhanced organic matter removal (Omil *et al.*, 1996; Dries *et al.*, 1998), degradation of xenobiotics (Boopathy *et al.*, 1998) and the elimination of heavy metals (Hao *et al.*, 1996). It can be concluded therefore that apart from the known disadvantages, sulphate reduction offers also new promising tools for anaerobic waste and wastewater treatment.

Removal and recovery of nutrients from anaerobic effluents

Because anaerobic digestion removes mainly carbon, the traditional processes should be sometimes completed for nitrogen and phosphorus removal according to regulations. New processes (with oxic phase) have been integrated with that aim in view. They mainly use the potentiality of micro-organisms and sometimes physico-chemical processes.

For the treatment of municipal wastewater, the ANANOX process (Garuti *et al.*, 1992) takes advantage of sulphate reduction to sulphide to provide an electron donor for the denitrification process.

The integration of the nitrogen cycle in anaerobic digestion could be maximised with the application of the ANAMMOX process (Jetten *et al.*, 1999), that makes use of particular micro-organisms that are able to oxidise ammonium to di-nitrogen gas with nitrite as electron acceptor. The coupling of this process for nitrogen removal from anaerobic digestion effluents seems to be very promising concerning the low energy needs by the partial oxidation to nitrite of only a portion of the ammonia-rich and COD-poor effluent.

After the anaerobic digestion of pig manure (in sequencing batch reactor mode), ammonium of the effluent could be oxidised to nitrate or nitrite; part of it is recirculated in the anaerobic digester where it is mixed with the organic matter of the influent which plays the role of electron donor. The over quantity of organic matter is methanised (Bernet *et al.*, 2000).

Biological removal of phosphorus is carried out in integrated processes where the micro-organisms are exposed to alternated anaerobic and aerobic (or sometimes anoxic) conditions, that stimulate the growth of a microbial community that is able to accumulate polyphosphates, thus removing phosphorus from the water phase. In the DEPHANOX process (Bortone *et al.*, 1998), which makes use of a particular configuration for combining P-uptake and denitrification, an anaerobic-derived technology has been used for the design of the first anaerobic step. This is carried out in an upflow sludge bed reactor that separates a clarified ammonia rich supernatant (that goes to a biofilm nitrification reactor) from the COD-rich sludge that goes directly to the denitrification/P-uptake reactor.

The level of integration of the biological nutrient removal in the process could be very high as in the AF-BNR-SCP process (Llabres *et al.*, 1999). In this strategy, the organic fraction of municipal solid waste is used for production of easily biodegradable carbon (as volatile fatty acid) for a biological nutrient removal of a sewage process. In this approach, struvite is precipitated and allows recovery of ammonium and phosphate ions.

Struvite is a crystal which is formed in anaerobic digesters. It is an ammonium magnesium phosphate that presents a very low solubility even at pH slightly over the neutrality (Maqueda *et al.*, 1994). The precipitation can be controlled in crystallisation reactors where a small amount of crystal primer is added while inducing a small but sharp pH increase via aeration (CO₂ stripping), allowing the recovery of the salt and resulting in a nitrogen and phosphorus removal from waste water (Battistoni *et al.*, 1998, 2000).

Future developments

Optimisation of reactor systems

Various constructors improved granular sludge bed reactors in recent years aiming at lowering mass transfer resistance and therewith achieving higher organic loading rates (see above). Further improvement might be expected in the field of staging sludge bed systems for the treatment of specific wastewaters, such as wastewaters coming from (petro-)chemical industries. In addition, if auto-immobilisation of specific bacteria is very difficult, further development of combination of complementary anaerobic systems, such as hybrid systems, is foreseen.

Interesting developments are expected for anaerobic reactors that cannot rely on the development of granular conglomerates or formation of biofilms, e.g. anaerobic treatment under extreme thermophilic conditions. Also in such systems, adequate sludge retention is essential for successful treatment. The latter might be achieved by enhanced physical (or physico-chemical) separation of the viable biomass from the treated water. Potential systems are hybrid systems and/or membrane bioreactors as discussed above.

With respect to the treatment of domestic water attention must be paid to the development of the second generation of anaerobic reactor systems. The major bottle-necks are (again) the relatively high wash-out of suspended solids and the low rate of hydrolysis under low temperature conditions in the conventional first generation UASB reactors. Therefore, the improvement of hydrolysis of complex organic matter is of fundamental importance, being the limiting step for the treatment of complex substrates both in sewage as well as in (semi-)solid wastes and slurries. Improved retention of suspended solids in the reactor system will lead to higher sludge retention times, subsequently leading to improved treatment efficiencies. Moreover, a decreased solids load in the effluent will minimise the requirements of the post treatment step.

Conventional anaerobic digestion of slurries and solids often makes use of a continuous stirred tank reactor which needs a long retention time to complete the reaction. Lowering this reaction time is and will be a driving force for novel developments. An already proven approach is the use of a controlled gas collection system in connection with the holding tank for the treated material. Full scale systems in Denmark show that up to 30% of the produced gas comes from the holding tank with the digested effluent. The overall required treatment time is considerably reduced.

Optimisation of the reactor configuration can involve staging of the process into separate tanks whereby the conditions for the specific groups of bacteria involved can be optimal. Hydrolysis is greatly improved at high temperatures of 70°C or more and a two phase operation scheme whereby the initial treatment occurs at a very high temperature followed by a methanogenic phase at either mesophilic or thermophilic temperatures could be an interesting future development. Results of Japanese research groups already confirmed this approach.

Further developments in modelling and process control will lead to a wider acceptance of AD in the established wastewater engineering world. Obviously, this accounts for any anaerobic system which will be developed in the near future.

Specific features of anaerobic conversions

The great capability of anaerobic digestion to efficiently treat several organic micro-pollutants, particularly halogenated compounds, substituted aromatics and azo-linkages makes anaerobic/aerobic processes more and more attractive for industrial effluents or for municipal effluents containing industrial loads. The AD-specific conversions determine the unique position of AD amongst other treatment methods.

An example where the specific features of anaerobic conversions must be exploited further is the treatment of sludges and slurries aiming at the production of safe end products. AD of sewage sludge followed by recycling on agricultural land is the largest world-wide application of anaerobic processes. More stringent regulations on heavy metals and "rest-organic" pollutants may make an end to the normal practice of applying digested sludge on land for food production. With respect to these "rest-organics", it is important to design the anaerobic digestion process to handle organic pollutants and to make new treatment concepts involving both anaerobic and aerobic treatment to clean the sludge for any rest compounds of concern.

Anaerobic treatment as core technology in recycling processes

Major future process developments will come from the enhancement of pre- and post-treatment processes, implying physical, chemical and biological processes, for the reclamation of the products from the waste(water) treatment system (including the treated water). Wastewater treatment for reuse will emphasise the central role of AD as the most sustainable treatment method for mineralising organic matter. Hence, AD has the potential to play a major role in closing water, raw materials, and nutrient cycles in industrial processes as well as agro-communal activities. With respect to the latter, further development is required on the community on-site treatment of domestic sewage under a wide range of conditions, opting for the reuse of the treated water in agriculture, making use of the mineralised nutrients for fertilisation purposes (Van Lier and Lettinga, 1999).

An upstream integration of the anaerobic process with industrial primary production processes also calls for further research under extreme conditions (temperature, pH, salinity, toxic and recalcitrant compounds, etc.). Together with appropriate pre- and post treatment systems the role of AD in closed circuit and/or side stream treatment will increase in the near future. The foreseen application also calls for novel reactor systems and treatment approaches.

References

- Ahring, B.K. (1994). Status on science and application of thermophilic anaerobic digestion. *Wat. Sci. Tech.*, **30**(12), 241-249.
- Ahring, B.K. (1995). Methanogenesis in thermophilic biogas reactors. *Antonie van Leeuwenhoek*, **67**, 91-102.
- Ahring, B.K., Angelidaki, I. and Johansen, K. (1992). Anaerobic treatment of manure together with industrial waste. *Wat. Sci. Tech.*, **25**(7), 311-318.
- Ahring, B.K., Christiansen, N., Mathrani, I., Hendriksen, H.V., Macario, A.J.L. and Conway de Macario, E. (1992). Introduction of a *de novo* bioremediation ability, aryl reductive dechlorination, into granular sludge by inoculation of sludge with *Desulfomonile tiedjei*. *Appl. Environ. Microbiol.*, **58**, 3677-3682.
- Aitken M.D. and Mullenix R.W. (1992). Another look at thermophilic anaerobic digestion of wastewater sludge. *Wat. Environ. Res.*, **64**, 915-919.
- Alphenaar, A. (1994). Anaerobic granular sludge: characterization, and factors affecting its functioning. *PhD. Thesis*. Department of Environmental Technology, Agricultural University, Wageningen, The Netherlands.

- Angelidaki, I. and Ahring, B.K. (1993). Anaerobic digestion of manure at different ammonia loads: effect of temperature. *Wat. Res.*, **28**, 727-731.
- Baier, U. and Schmidheiny, P. (1997). Enhanced anaerobic degradation of mechanically disintegrated sludge. *Wat. Sci. Tech.*, **36**(11), 137-143.
- Battistoni, P., Pavan, P., Cecchi, F. and Mata Alvarez, J. (1998). Phosphate removal in real anaerobic supernatants: modelling and performance of a fluidized bed reactor. *Wat. Sci. Tech.*, **38**(1), 275-283.
- Battistoni, P., Pavan, P., Prisciandaro, M. and Cecchi, F. (2000). Struvite crystallization: a feasible and reliable way to fix phosphorus in anaerobic supernatants. *Wat. Res.*, **34**, 3033-3041.
- Bendixen, H.J. (1994). Safeguards against pathogens in Danish Biogas Plants. *Wat. Sci. Tech.* **30** (12) 171-180.
- Bernet, N., Delgenes, N., Akunna, J.C., Delgenes, J.P. and Moletta, R. (2000). Combined anaerobic-aerobic SBR for the treatment of piggery wastewater. *Wat. Res.*, **34**(2), 611-619.
- Bhattacharya, S.K., Uberoi, V. and Dronamraju M.M. (1996). Interaction between acetate fed sulphate reducers and methanogens. *Wat. Res.* **30**, 2239-2246.
- Blum, D.J.W. and Speece, R.E. (1991). A data base of chemical toxicity to environmental bacteria and its use in interspecies comparisons and correlations. *Res. J. WPCF*, **63**(3), 199-207.
- Boopathy, R., Kulpa, C.F. and Manning, J. (1998). Anaerobic biodegradation of explosives and related compounds by sulfate-reducing and methanogenic bacteria: a review. *Bioresource Technol.*, **63**(1), 81-89.
- Bortone, G., Alonso, V., Spagni, A., Stante, L., Wanner, J., and Tilche, A. (1998). *European conference on new advances in biological nitrogen and phosphorus removal for municipal or industrial wastewaters*. October 12-14, 1998, Narbonne France.
- Buhr, H.O. and Andrews, J.F. (1977). The thermophilic anaerobic digestion process. *Wat. Res.*, **11**, 129-143.
- Brockmann, M. and Seyfried, C.F. (1997). Sludge activity under the conditions of crossflow-microfiltration. *Wat. Sci. Tech.* **35**(10) 173-181.
- Christiansen, N. and Ahring, B.K. (1994). Introduction of bioremediation ability into granular sludge. *Wat. Sci. Tech.*, **29**(5-6).
- Christiansen, N., Hendriksen, H.V., Jarvinen, K.T. and Ahring, B.K. (1995). Degradation of chlorinated aromatic compounds in UASB reactors. *Wat. Sci. Tech.*, **31**(1), 249-259.
- Colleran, E., Finnegan, S. and Lens, P. (1995). Anaerobic treatment of sulphate-containing waste streams. *Antonie van Leeuwenhoek*, **67**, 29-46.
- Collins, A.G., Theis, T.L., Kilambi, S., He, L., and Pavlostathis, S.G. (1998). Anaerobic treatment of low-strength domestic wastewater using an anaerobic expanded bed reactor. *J. Environm. Eng.*, (July) 1998, 652-659.
- Constable, S.W.C. and Kras, R. (1998). Selection, start-up and operation of an anaerobic pretreatment system for wastewater from a thermoplastic production facility. *Proc. WEFTEC*, October 3-7, 1999, Orlando, USA, 10 pages.
- De Man, A.W.A., van der Last, A.R.M. and Lettinga, G. (1988). The use of EGSB and UASB anaerobic systems on low strength soluble and complex wastewaters at temperatures ranging from 8 to 30°C. *Proc. of the 5th International Symposium on Anaerobic Digestion*. Bologna, Italy. Hall ER, and Hobson PN, Eds. p 197-208.
- Dohányos, M. and Záborská, J. (1991) Intensification and stimulation of the anaerobic stabilisation of sludges. *Sci. Papers of PICT*, **F 28**, 159-167.
- Doheanyos, M., Záborská, J. and Jeníček, P. (1997). Enhancement of sludge anaerobic digestion by using of a special thickening centrifuge. *Wat. Sci. Tech.* **36**(11), 145-153.
- Dries, J., De Smul, A., Goethals, L., Grootaerd, H. and Verstraete, W. (1998). High rate treatment of sulfate-rich wastewater in an acetate-fed EGSB reactor. *Biodegradation*, **9**, 103-111.
- Driessen, W.J.B.M., Habets, L.H.A. and Groeneveld, N. (1996). New developments in the design of Upflow Anaerobic Sludge Bed reactors. *2nd Specialised IAWQ conference on Pretreatment of Industrial Wastewaters*, October 16-18, 1996.
- Eastman, J.A. and Ferguson, J.F. (1981). Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. *JWPCF*, **53**, 352-366.
- Edelmann, W., Schleiss, K. and Joss, A. (2000). Ecological, energetic and economic comparison of anaerobic digestion with different competing technologies to treat biogenic wastes. *Wat. Sci. Tech.*, **41**(3), 263-273.
- El-Gohary, F.A. and Nasr, F.A. (1999). Cost-effective pre-treatment of wastewater. *Wat. Sci. Tech.*, **39**(5), 97-103.

- Fajardo, C., Guyot, J. P., Macarie, H. and Monroy, O. (1997). Inhibition of anaerobic digestion of terephthalic acid and its aromatic byproducts. *Wat. Sci. Tech.*, **36** (6-7), 83-90.
- Field, J.A., Stams, A.J.M., Kato, M. and Schraa, G. (1995). Enhanced biodegradation of aromatic pollutants in cocultures of anaerobic and aerobic bacterial consortia. *Antonie van Leeuwenhoek*, **67**, 47-77.
- Florencio, L., Nozhevnikova, A., van Langerak, A., Stams, A.J.M., Field, J.A. and Lettinga, G. (1993). Acidophilic degradation of methanol by a methanogenic enrichment culture. *FEMS Microbiol. Lett.*, **109**, 1-6.
- Frankin, R.J., Koevoets, W.A.A., Van Gils, W.M.A. and Van der Pas, A. (1992). Application of the biobedR upflow fluidized bed process for anaerobic waste water treatment. *Wat. Sci. Tech.*, **25**(7), 373-382.
- Frankin, R.J., Koevoets, W.A.A. and Versprille, A.I. (1994). Application of the BiobedR system for formaldehyde containing dimethyl-terephthalate (=DMT) waste water. *Poster Paper Pre-prints of the Seventh International Symposium on Anaerobic Digestion*, Cape Town, January 23-27, 1994, South Africa, pp. 244-247.
- Garuti, G., Dohanyos, M. and Tilche A. (1992). Anaerobic-aerobic wastewater treatment system suitable for variable population in coastal area: the ANANOX process. *Wat. Sci. Tech.*, **25**(12), 185-195.
- Genon, G. (1999). Economic assessment of MSW anaerobic digestion in comparison with composting plants. *Proc. of II Int. IAWQ Symp. on Anaerobic Digestion of Solid Waste*, Barcelona 15-17 June, 1999, page: 282-289.
- Gossett, J.M. and Belser, R.L. (1982). Anaerobic digestion of waste activated sludge. *J. Environ. Eng. Division ASCE*, **108**, 1101-1120.
- Hakulinen, R. (1988). The use of enzymes for wastewater treatment in the pulp and paper industry - a new possibility. *Wat. Sci. Tech.*, **20**(1), 251-262.
- Hansen, K.H., Angelidaki, I. and Ahring, B.K. (1999). Improving thermophilic anaerobic digestion of swine manure. *Wat. Res.*, **33**, 1805-1810.
- Hao, O.J., Chen, J.M., Huang, L. and Buglass, R.L. (1996). Sulfate-reducing bacteria. *Crit. Rev. Environ. Sci. Tech.*, **26**, 155-187.
- Haug, R.T., LeBrun, T.J. and Tortorici, L.D. (1983). Thermal pretreatment of sludges - a field demonstration. *JWPCF*, **55**, 23-34.
- Hilligsmann, S., Jacques, P. and P. Thonart (1998). Isolation of highly performant sulphate reducers from sulphate rich environments. *Biodegradation*, **9**, 285-292.
- Horber, C., Christiansen, N., Arvin, E. and Ahring, B.K. (1998). Improved dechlorination performance of upflow anaerobic sludge blanket (UASB) reactors by incorporation of *Dehalospirillum multivorans* into granular sludge. *Appl. Environ. Microbiol.*, **64**, 1860-1863.
- Houten, R.T. van and Lettinga, G. (1996). Biological sulphate reduction with synthesis gas: microbiology and technology, pp. 793-799. R.H. Wijffels, R.M. Buitelaar, C. Bucke and J. Tramper (eds.) *Progress in Biotechnology*, **11**, Elsevier, Amsterdam, The Netherlands.
- Houten, R.T. van, Hulshoff Pol, L.W. and Lettinga, G. (1994). Biotechnical sulphate reduction using gas-lift reactors fed with hydrogen and carbon dioxide as energy and carbon source. *Biotech. Bioeng.*, **44**, 586-594.
- Houten, R.T. van, van der Spoel, H., van Aelst, A.C., Hulshoff Pol, L.W. and Lettinga, G. (1996). Biological sulfate reduction using synthesis gas as energy and carbon source. *Biotech. Bioeng.*, **50**, 136-144.
- Hulshoff Pol, L.W., Lens, P., Stams, A.J.M. and Lettinga, G. (1998). Anaerobic treatment of sulphate-rich wastewaters: microbial and process technological aspects. *Biodegradation*, **9**, 213-224.
- Hulshoff Pol., Euler, H., Eitner, A. and Grohganz, D. (1997). GTZ sectoral project, promotion of anaerobic technology for the treatment of municipal and industrial sewage and wastes. *Proc. 8th Int. Conf. Anaerobic Digestion*, **2**, May 25-29, 1997, Sendai, Japan, pp. 285-292.
- Isa, Z., Grusenmeyer, S. and Verstraete, W. (1986). Sulfate reduction relative to methane production in high-rate anaerobic digestion: Technical aspects. *Appl. Environ. Microbiol.*, **51**, 572-579.
- Janssen, A.J.H., Ma S, C., Lens, P. and Lettinga, G. (1997). Performance of a sulphide oxidizing expanded bed reactor with a spatially separated aeration unit. *Biotech. Bioeng.*, **53**, 32-40.
- Jetten, M.S.M., Strous, M., Van de Pas-Schoonen, K.T., Schalk, J., Van Dongen, U.G.J.M., Van De Graaf, A.A., Logemann, S., Muyzer, G., Van Loosdrecht, M.C.M., and Kuenen, J.G. (1999). The anaerobic oxidation of ammonium, *FEMS Microbiology Reviews*, **22**, 421-437.
- Kato, M.T., Field, J.A. and Lettinga, G. (1997). The anaerobic treatment of low strength wastewaters in UASB and EGSB reactors. *Wat. Sci. Tech.*, **36**(6-7), 375-382.

- Kleerebezem, R., Hulshoff Pol, L.W. and Lettinga, G. (1999). The role of benzoate in anaerobic degradation of terephthalate. *Appl. Environ. Microbiol.*, **65**(3), 1161–1167.
- Knapp, J.S. and Howell, J.A. (1978). Treatment of primary sewage sludge with enzymes. *Biotechnol. Bioeng.*, **20**, 1221–1234.
- Kopp, J., Müller, J., Dichtl, N. and Schwedes J. (1997). Anaerobic digestion and dewatering characteristic of mechanically disintegrated excess sludge. *Wat.Sci.Tech.*, **36**(11), 129–136.
- Kübler, H. and Rumphorst, M. (1999). Evaluation of processes for treatment of biowaste under the aspects of energy balance and CO₂ emission. *Proc. of II Int. IAWQ Symp. on Anaerobic Digestion of Solid Waste*, Barcelona 15–17 June, 1999, page: 405–410.
- Lagerkvist, A. and Chen, H. (1993). Control of two step anaerobic degradation of municipal solid waste (MSW) by enzyme addition. *Wat.Sci.Tech.*, **27**(2), 47–56.
- Lens, P., Visser, A., Janssen, A.J.H., Hulshoff Pol, L.W. and Lettinga, G. (1998). Biotechnological treatment of sulfate rich wastewater. *Crit. Rev. Env. Sci. Technol.*, **28**, 41–88.
- Lepistö, R. and Rintala, J. (1996). Conversion of volatile fatty acids in extreme thermophilic (76–80°C) upflow anaerobic sludge blanket reactors. *Bioresource Technol.*, **56**, 221–227.
- Lettinga, G., van Nelsen, A.F.M., Hobma, S.W., de Zeeuw, W. and Klapwijk, A. (1980). Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment. *Biotech. Bioeng.*, **22**, 699–734.
- Lettinga, G., Roersma, R. and Grin, P. (1983). Anaerobic treatment of raw domestic sewage at ambient temperatures using a granular bed UASB reactor. *Biotech. Bioeng.*, **25**, 1701–1723.
- Llabres, P., Pavan, P., Battistoni, P., Cecchi, F. and Mata-Alvarez, J. (1999). The use of organic fraction of municipal solid waste hydrolysis for biological nutrient removal in waste water treatment plant. *Wat. Res.*, **33**, 214–222.
- Lund, B., Jensen, V.F., Have, P. and Ahring, B.K. (1996). Inactivation of virus during anaerobic digestion of manure in laboratory scale biogas reactors. *Antonie van Leeuwenhoek*, **69**, 25–31.
- Macarie, H. (2000). Overview of the application of anaerobic treatment to chemical and petrochemical wastewaters. *Wat.Sci.Tech.* **42**(5–6) 201–213.
- Maqueda, C., Perez Rodriguez, J.L. and Lebrato, J. (1994). Study of struvite precipitation in anaerobic digesters. *Wat. Res.*, **28**, 411–416.
- McCarty, P.L., Young, L.Y., Gossett, J.M., Stuckey, D.C. and Healy Jr, J.B. (1976). Heat treatment for increasing yields from organic materials. H.G. Schlegel and J. Barnen (eds.). *Microbial Energy Conversion*. (179–199) Göttingen.
- Mukherjee, S.R. and Levine, A.D. (1992). Chemical solubilization of particulate organics as a pretreatment approach. *Wat.Sci.Tech.*, **26**(9–11), 2289–2292.
- Nagano, A., Arikawa, E. and Kobayashi, H. (1992). Treatment of liquor wastewater containing high-strength suspended solids by membrane bioreactor system. *Wat. Sci. Tech.*, **26**(3–4), 887–895.
- Narayanan, B., Suidan, M.T., Gelderloos, A.B. and Brenner, R.C. (1993a). Treatment of semivolatle compounds in high strength wastes using an anaerobic expanded-bed GAC reactor. *Wat. Res.*, **27**, 171–180.
- Narayanan, B., Suidan, M.T., Gelderloos, A.B. and Brenner, R.C. (1993b). Treatment of VOCs in high strength wastes using an anaerobic expanded-bed GAC reactor. *Wat. Res.*, **27**, 181–194.
- Omil, F., Lens, P., Hulshoff Pol, L. and Lettinga, G. (1996). Effect of upward velocity and sulfide concentration on volatile fatty acid degradation in a sulphidogenic granular sludge reactor. *Process Biochem.*, **31**, 699–710.
- Oude Elferink, S.J.W.H., Visser, A., Hulshoff Pol, L.W. and Stams, A.J.M. (1994). Sulfate reduction in methanogenic bioreactors. *FEMS Microbiology Reviews*, **15**, 119–136.
- Pavlostathis, S.G. (1994). Anaerobic processes, the 1994 literature review. *Wat. Environ. Res.*, **66**(4), 342–356.
- Preez, L.A. du., Odendaal, J.P., Maree, J.P. and Ponsonby M. (1992). Biological removal of sulphate from industrial effluents using producer gas as energy source. *Environ. Technol.*, **13**, 875–882.
- Pretorius, W.A. (1994). pH-controlled feed-on-demand for high rate anaerobic systems. *Wat. Sci. Tech.*, **30**(8), 1–8.
- Rebac, S., Van Lier, J.B., Lens, P., Van Cappellen, J., Vermeulen, M., Stams, A.J.M., Dekkers, F., Swinkels, K.T.M. and Lettinga, G. (1998). Psychrophilic (6–15°C) high-rate anaerobic treatment of malting wastewater in a two-module EGSB system. *Biotechnol. Progress*, **14**, 856–864.
- Rebac, S., van Lier, J.B., Lens, P.N.L., Stams, A.J.M., Dekkers, F., Swinkels, K.T.M. and Lettinga, G. (1999). Psychrophilic anaerobic treatment of low strength wastewaters, *Wat. Sci. Tech.*, **39**(5), 203–210.

- Rinzema, A., Alphenaar, A. and Lettinga, G. (1989). The effect of lauric acid shock loads on the biological and physical performance of granular sludge UASB reactors digesting acetate. *J. Chem. Technol. Biotechnol.*, **46**, 257–266.
- Rinzema, A., Alphenaar, P.A. and Lettinga, G. (1993). Anaerobic digestion of long chain fatty acids in UASB-reactors and expanded granular sludge bed reactors. *Process Biochem.*, **28**, 527–537.
- Rinzema, A. and Lettinga, G. (1988) Anaerobic treatment of sulfate containing waste water. In *Biotreatment systems*, D.L. Wise (ed.), **III**, 65–109, CRC Press Inc., Boca Raton, USA.
- Sandberg, M. and Ahring, B.K. (1992). Anaerobic treatment of fish condensate in a UASB reactor at high pH. *Appl. Microbiol. Biotechnol.*, **36**, 805–809.
- Seghezzo, L., Zeeman, G., van Lier, J.B., Hameler, H.V.M. and Lettinga, G. (1998). A review: The anaerobic treatment of sewage in UASB and EGSB reactors. *Bioresource Technol.*, **65**, 175–190.
- Sipma, J., Lens P., Vieira A., Miron Y., Van Lier J.B., Hulshoff Pol, L.W. and Lettinga G. (2000). Thermophilic sulphate reduction in upflow anaerobic sludge bed reactors under acidifying conditions. *Process Biochem.* **35**, 509–522.
- Scheeren, P.J.H., Koch, R.O., Buisman, C.J.N., Barnes, L.J. and Versteegh, J.H. (1991). New biological treatment plant for heavy metal contaminated groundwater. *Trans. Instn Min. Metall.* (Sect. C: Mineral Process. Extr. Metall.) **101**, C190–C199.
- Tafdrup, S. (1994). Centralized biogas plants combine agricultural and environmental benefits with energy production. *Wat. Sci. Tech* **30**(12) 133–4.
- Tan, N.C.G., Prenafeta-Boldu, F.X., Opsteeg, J.L., Lettinga, G., and Field J.A. (1999) Biodegradation of azo dyes in cocultures of anaerobic granular sludge with aerobic aromatic amine degrading enrichment cultures. *Appl. Microbiol. Biotechnol.*, **51**, 865–871.
- Thay J-H. and Zhang X. (2000). A fast predicting neural fuzzy model for high-rate anaerobic wastewater treatment systems. *Wat. Res.*, **34**, 2849–2860.
- Tiehm, A., Nickel, K. and Neis U. (1997). The use of ultrasound to accelerate the anaerobic digestion of sewage sludge. *Wat.Sci.Tech.*, **36**(11), 121–128.
- Totzke, D.E. (1999). 1999 anaerobic treatment overview. Anaerobic Treatment of High Strength Agricultural and Industrial Wastes. Lecture organized by the University of Wisconsin, March 11–12, 1999, San Francisco, California, USA, 14 pages.
- Tseng, S-K. and Yang, C-J. (1994). The reaction characteristics of treatment of wastewater containing nitrophenol by anaerobic biological fluidized bed. *Wat. Sci. Tech.*, **30**(12), 233–240.
- Van der Last, A.R.M. and Lettinga, G. (1992). Anaerobic treatment of domestic sewage under moderate climatic (Dutch) conditions using upflow reactors at increased superficial velocities. *Wat.Sci.Tech.*, **25**(7), 167–178.
- Van Haandel, A.C. and Lettinga, G. (1994). *Anaerobic sewage treatment. A practical guide for regions with a hot climate*. Chichester, England. John Wiley and Sons Ltd. 226 p.
- Van Langerak, E.P.A., Gonzalis Gil, G., Aelst, A., van Lier, J.B., Hamelers, H.V.M., and Lettinga, G. (1998). Effects of high calcium concentrations on the development of methanogenic sludge in UASB reactors. *Wat. Res.*, **32**, 1255–1263.
- Van Lier, J.B. (1996). Limitations of thermophilic anaerobic waste water treatment and the consequences for process design. *Antonie van Leeuwenhoek*, **69**, 1–14.
- Van Lier, J.B., Hulsbeek, J., Stams, A.J.M. and Lettinga, G. (1993). Temperature susceptibility of thermophilic methanogenic sludge: implications for reactor start-up and operation. *Bioresource Technology*, **43**, 227–235.
- Van Lier, J.B., Sanz Martin, J.L. and Lettinga, G. (1996). Effect of temperature on the anaerobic thermophilic conversion of volatile fatty acids by dispersed and granular sludge. *Wat. Res.*, **30**, 199–207.
- Van Lier, J.B. and Lettinga, G. (1999). Appropriate technologies for effective management of industrial and domestic wastewaters: the decentralised approach. *Wat. Sci. Tech.*, **40** (7), 171–183.
- Vogelaar, J.C.T., Klapwijk A., Van Lier J.B. and Rulkens W. (2000). Temperature effects on the oxygen transfer rate between 20 and 55°C. *Wat. Res.*, **34**, 1037–1041.
- Wang, K. (1994). Integrated anaerobic and aerobic treatment of sewage. PhD Thesis. Wageningen Agricultural University. Wageningen, The Netherlands. 145 p.
- Weijma, J., Stams, A. Hulshoff Pol, L. and Lettinga, G. (1999), Thermophilic (65°C) methanogenesis and sulfate reduction with methanol in a high-rate anaerobic reactor. *Biotech. Biotechnol.* In Press.
- Wiegant, W.M. (1986). Thermophilic anaerobic digestion for waste and wastewater treatment. *PhD. Thesis*. Department of Environmental Technology, Agricultural University, Wageningen, The Netherlands.
- Yspeert, P., Vereijken, T., Vellinga, S., De Vegt, A. (1993). The IC reactor for anaerobic treatment of

- industrial wastewater. Proc. of "1993 Food Industry Environmental Conference", Atlanta Nov. 14-16, 1993.
- Zeeman, G., Wiegant, W.M., Koster-Treffer, M.E. and Lettinga, G. (1985). The influence of the total ammonia concentration on the thermophilic digestion of cow manure. *Agr Wastes*, **14**, 19-35.
- Zeeman, G., Sanders, W.T.M., Wang, K.Y., and Lettinga, G. (1997). Anaerobic treatment of complex wastewater and waste activated sludge. Application of an Upflow Anaerobic Solids Removal (UASR) reactor for the removal and pre-hydrolysis of suspended COD. *Wat. Sci. Tech.*, **35**(10), 121-128.
- Zoutberg, G.R. and de Been, P. (1997). The biobed EGSB (Expanded Granular Sludge Bed) systems covers short comings of the upflow anaerobic sludge blanket reactors in the chemical industry. *Wat. Sci. Tech.*, **35**(10), 183-188.
- Zoutberg, G.R. and Eker, Z. (1999). Anaerobic treatment of potato processing wastewater. *Wat.Sci.Tech* , **40**(1), 297-304.