World Journal of Microbiology & Biotechnology 12, 221–238

Anaerobic bioprocessing of organic wastes

W. Verstraete,* D. de Beer, M. Pena, G. Lettinga and P. Lens

Anaerobic digestion of dissolved, suspended and solid organics has rapidly evolved in the last decades but nevertheless still faces several scientific unknowns. In this review, some fundamentals of bacterial conversions and adhesion are addressed initially. It is argued in the light of △G-values of reactions, and in view of the minimum energy quantum per mol, that anaerobic syntrophs must have special survival strategies in order to support their existence: redistributing the available energy between the partners, reduced end-product fermentation reactions and special cell-to-cell physiological interactions. In terms of kinetics, it appears that both reaction rates and residual substrate thresholds are strongly related to minimum △G-values. These new fundamental insights open perspectives for efficient design and operation of anaerobic bioprocesses. Subsequently, an overview is given of the current anaerobic biotechnology. For treating wastewaters, a novel and high performance new system has been introduced during the last decade; the upflow anaerobic sludge blanket system (UASB). This reactor concept requires anaerobic consortia to grow in a dense and eco-physiologically well-organized way. The microbial principles of such granular sludge growth are presented. Using a thermodynamic approach, the formation of different types of aggregates is explained. The application of this bioprocess in worldwide wastewater treatment is indicated. Due to the long retention times of the active biomass, the UASB is also suitable for the development of bacterial consortia capable of degrading xenobiotics. Operating granular sludge reactors at high upflow velocities (5-6 m/h) in expanded granular sludge bed (EGSB) systems enlarges the application field to very low strength wastewaters (chemical oxygen demand < 1 g/l) and psychrophilic temperatures (10°C). For the treatment of organic suspensions, there is currently a tendency to evolve from the conventional mesophilic continuously stirred tank system to the thermophilic configuration, as the latter permits higher conversion rates and easier sanitation. Integration of ultrafiltration in anaerobic slurry digestion facilitates operation at higher volumetric loading rates and at shorter residence times. With respect to organic solids, the recent trend in society towards source separated collection of biowaste has opened a broad range of new application areas for solid state anaerobic fermentation.

Keywords: Eco-engineering, expanded granular sludge blanket, granulation, methanogenesis, microbial consortia, sludge digestion, solid state fermentation, sulphate reduction, syntrophy, upflow anaerobic sludge blanket.

Several full-scale systems are currently in operation in Europe. This technology opens new perspectives for recycling various fractions of domestic wastes and furthermore has important implications for the producers of consumer goods.

In the last few decades, anaerobic bioconversion of environmental pollutants has become of prime interest. At present, anaerobic treatment is successfully implemented for various types of industrial as well as for domestic wastestreams all over the world. Compared to conventional aerobic methods, and in the light of the implementation of sustainable technologies, anaerobic processes solve waste problems in a much more holistic way (Verstraete & Top 1992; Lettinga 1995): a) instead of consuming energy, useful energy is produced in the form of biogas; b) only a few percent of the chemical oxygen demand (COD) is converted into new biomass so that the volume of surplus sludge produced is significantly lower. Moreover, it

W. Verstraete and D. de Beer are with the Center for Environmental Sanitation, University of Gent, Coupure L 653, B-9000 Gent, Belgium; fax: 329264 62 48. D. de Beer is also with the Max Plank Institut für Marine Mikrobiologie - Microzensor Group, Fahrenstrasse 1, 28359 Bremen, Germany. M. Pena is with the Grupo de Biotechnologia Ambiental, Departamento de Ingenieria Quimica, Universidad de Valladolid, Prado de la Magdalena, 47005 Valladolid, Spain. G. Lettinga is with the Department of Environmental Technology, Wageningen Agricultural University, Bomenweg 2, 6703 HD Wageningen, The Netherlands. P. Lens is with the Environmental Research Unit, Department of Microbiology, University College Galway, Galway, Ireland. * Corresponding author.

© 1996 Rapid Science Publishers

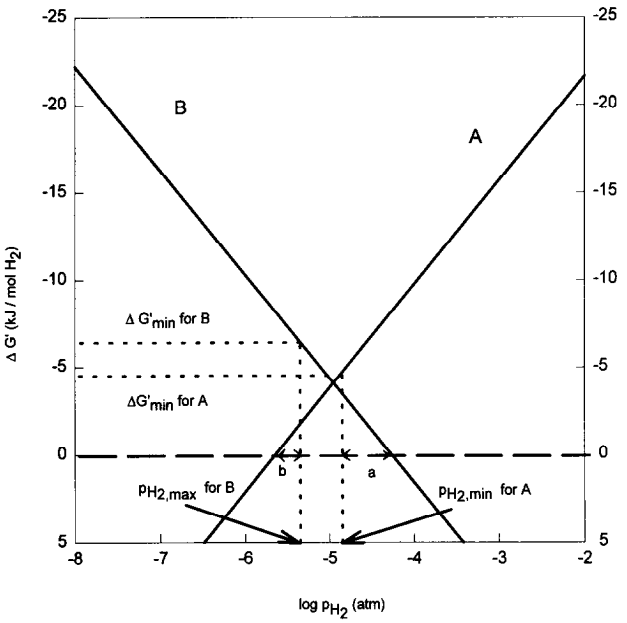


Figure 1. Effect of hydrogen partial pressure on free energy of conversion of propionate and hydrogen during methane fermentation. Reaction A: $H_2 + 1/4HCO_3^- + 1/4H^+ \rightarrow 1/4CH_4 + 3/4H_2O$ Reaction B: $1/3CH_3CH_2COO^- + H_2O \rightarrow 1/3CH_3COO^- + 1/3HCO_3^- + 1/3H^+ + H_2$ The p_{H_2} range for which reaction A is sufficient to support growth and H_2 production by reaction B is thermodynamically possible is indicated by <a>; the p_{H_2} range for which reaction B is generating the minimum energy quantum and removal of H_2 by reaction A is thermodynamically possible is indicated by <b>.

has a high dewatering capacity and is generally well-stabilized; c) they can be applied at practically any place and on any scale. Very high space loading rates can be applied in modern anaerobic treatment systems, so that the space requirements are relatively small; d) they can be employed at very low costs because technologically plain and relatively inexpensive reactors are used which can operate with little, if any, consumptive use of high grade energy and e) they can be combined with post-treatment methods by which useful products like ammonia or sulphur can be recovered.

The aim of this review is to illustrate how basic principles of anaerobic bioconversions can be engineered to full-scale environmental technology. The paper first examines a number of fundamental aspects of anaerobic waste treatment and subsequently, the current state of anaerobic process technology is described.

Fundamentals of Anaerobic Waste Treatment

Anaerobic Bioconversions

During methanogenesis, organic polymers are first hydrolysed and fermented by fermentative organisms to the methanogenic precursors H_2/CO_2, formate and acetate, and to reduced organic compounds such as propionate, butyrate, lactate, succinate and ethanol. These reduced compounds can be oxidized further to the methanogenic substrates by acetogenic bacteria (Stams 1994). About 76% of the organic matter is degraded via reduced organic intermediates (McCarty 1982). Hence, acetogenesis is a key process in the mineralisation of organic matter in methanogenic environments.

Syntrophic Degradation of Short Chain Fatty Acids. The understanding of syntrophism, where several anaerobic microorganisms can share the energy available in the bioconversion of a molecule to methane and carbon dioxide, and thus can achieve intermediate reactions which are endergonic under standard conditions, has been essential in the rather striking development of anaerobic digestion during the last decades. The effect of hydrogen partial pressure on the free energy of propionate conversion (via the succinate pathway) during methane fermentation is by now a text book figure (Dolfing 1988).

However, a number of challenging questions in this field urgently need further research. A first aspect relates to the minimum energy generated per mol of product formed or substrate converted. Schink & Friedrich (1994) postulated that the minimum energy quantum for life is some -21 kJ per mol product formed or substrate converted. Consequently, the reaction attaining that minimum determines the sharing of energy between the two syntrophic partners. If one implements this notion of minimal energy on the fermentation of propionate to methane, as depicted in Figure 1, it becomes clear that both syntrophs have to operate in a very narrow p_{H_2} region. The triangle indicates where both co-exist. However;

$\triangle G'$ min for A $= -5.25$ kJ/mol H_2 or
p_{H_2} min $= 10^{-4.83}$ atm. and

$\triangle G'$ min for B $= -7.0$ kJ/mol H_2 or
p_{H_2} max $= 10^{-5.45}$ atm.

where [4A ≡ 1 mol methane; 3B ≡ 1 mol propionic acid] ≥ - 21 kJ/reaction.

Strictly speaking, co-existence is not possible but the $\triangle G'$ values under practical conditions might be slightly different from the theoretical ones given. Clearly, co-existence based on co-operation on such a narrow energy basis appears unlikely. The fact that the reaction occurs suggests that the partner-organisms facilitate each other's lives and maintenance in more than one way. The understanding of the nature of their 'symbiosis' is a challenging task and essential to the optimisation of anaerobic biotechnology. In addition the syntrophic degradation of butyrate to methane and CO_2 (values relate to standard conditions and 37°C according to Thauer *et al.* (1977); under actual reactor conditions in which organics are of the order of 0.1 M rather than 1 M, the $\triangle G'$ values are slightly lower) has only a small thermodynamic niche:

	$\Delta G^{o\prime}$ (kJ/r)
A. $2CH_3CH_2CH_2COO^- + 4H_2O \rightarrow 4CH_3COO^- + 2H^+ + 4H_2$	+96
B. $4CH_3COO^- + 4H_2O \rightarrow 4CH_4 + 4HCO_3^-$	−124
C. $4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$	−136
D. $2CH_3CH_2CH_2COO^- + 5H_2O \rightarrow 5CH_4 + H^+ + 3HCO_3^-$	−164

The thermodynamic limiting unit reaction is A, which must run twice. However, the sharing of the energy is at the expense of the free energy available to the hydrogenotroph (reaction C). Indeed, in practice, the reactor partial pressure decreases to some $10^{-4.7}$ atm, which means that reaction C operates at a $\Delta G' \approx -23.4$ kJ/mol formed. The net result is a quite democratic redistribution of the available energy between the reactions and organisms involved:

	$\Delta G'$ (kJ/r)	Total
A:	2 (−23.4)	
B:	4 (−23.4)	
C:	1 (−23.4)	
		−164

Similar calculation can be made with formate instead of H_2 as the key species of reducing equivalents.

These calculations are done and validated for defined co-cultures or highly enriched syntrophic associations. When fatty acid metabolism is studied in sludges, other reactions can be observed as well. Reduced end-product fermentation reactions of propionate, where propionate is converted into higher volatile fatty acids or alcohols have been described (Smith & McCarty 1989; Tholozan *et al.* 1990). One of these reactions, the reductive carboxylation of propionate, has been studied in detail. *In vivo* NMR studies revealed that the propionate molecule is elongated by the addition of a carbon molecule at the carboxyl group of propionate, i.e. [3 − ^{13}C]propionate is converted into [4 − ^{13}C]butyrate. Although the exact redox equation of this reaction is not known, the proposed dismutation reactions have a $\Delta G'$ comparable to propionate degradation via the succinate pathway (Tholozan *et al.* 1988). Hence, the small amounts of free energy available in methanogenic fatty acid oxidation exerts a highly selective pressure on the organisms involved to operate with energy quanta in the range of fractions of an ATP. In this way, the occurrence of alternative reactions can support growth of consortia operating at the 'edge' of the thermodynamic feasibility. Such alternative reactions even exist for the direct methanogenic substrate acetate, which can also be oxidized syntrophically:

	$\Delta G^{o\prime}$ (kJ/r)
A. $2CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 4H_2 + H^+$	+104
B. $4H_2 + HCO_3^- \rightarrow CH_4 + 2H_2O + OH^-$	−135
C. $CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$	−31

Zinder & Koch (1984) postulated that the dismutation of acetate (reaction A) is brought about by a kind of inverse working acetogenic bacterium. Indeed at $p_{H_2} < 10^{-5}$ atm, reaction A becomes thermodynamically feasible. Ahring *et al.* (1993) argued that acetate oxidation is favoured above acetoclastic methanogenesis at very low acetate concentrations (< 10 mg/l) in very dense anaerobic associations, such as those present in granular sludge (see *Biomass Retention and Reactor Systems*). Under such conditions, the very little energy present in the overall reaction C would be shared by two organisms and clearly sharing cannot help them to attain each the quantum of 21 kJ/mol. Apparently in (microbial) ecology, the poorer the $\Delta G'$ conditions become and the more bacteria are restricted in their metabolism, the more they rely on co-operation and the more efficient they must become.

Flux of Dihydrogen and Formate in Syntrophic Cultures. An element of discussion concerns the flux of substrate in methanogenic associations. The flux of substrate into a cell can be represented by the following equation (Rittmann 1982):

$$J_s = \frac{dM}{dt} \cdot \frac{1}{O} = D\frac{dS}{dx}$$

where M is mass (kg), t is time (s), O is surface (m^2), D is the diffusion coefficient (m^2/s), S is concentration (kg/m^3) and x is distance (m).

Fermenting cells producing H_2, a non-polar molecule, have a special problem. While the concentration of polar compounds in the cytoplasm is regulated by transport processes throughout the cell membrane, non-polar compounds can diffuse freely through the membrane. Consider the following well known fermentative reactions:

A. Glucose $\rightarrow 2Ac^- + HCO_3^- + 4H_2 + 4H^+ + 4ATP$
B. Glucose $\rightarrow 1But^- + 2HCO_3^- + 2H_2 + 3H^+ + 3ATP$

Surprisingly, in anaerobic reactor systems, the least ATP yielding reaction, the formation of butyrate, is predominant. Hence, it has been postulated that H_2 is a much too difficult an intermediate and that in microbial methanogenic associations, formate is the intermediate (Thiele & Zeikus 1988). Dong & Stams (1995) confirmed that formate is the intermediate for dispersed cells in co-cultures of acetogens and methanogens. The sharing of energy between the

partners places the appropriate concentration at around 50 μM for the methanogen, which is 1000 times higher than the corresponding concentration for H_2 (see above). The acetogen can make the formate intermediate at a concentration up to 300 μM but hydrogen only at a maximum of 1 μM. It follows that the diffusion gradient is at least a factor 100 times greater for formate. Since the diffusion coefficients for formate and hydrogen are of the same order of magnitude, the fluxes in dispersed cultures are 100 times higher for formate. Yet, cells in suspension are some 10 μm apart and in mixed cultures, lots of competitors (*e.g.* sulphate reducers) might interfere with this type of formate syntrophy. Hence, although a syntrophic association which exchanges formate might have high substrate fluxes, the concomitant high ambient formate concentrations deem this exchange to be prone to ecological disturbance. With regard to granules, Bleicher & Winter (1994) argued on the basis of selective inhibitor studies with bromoethanesulphonate, that H_2 is still the key intermediate and that this non-polar molecule is efficiently transported between acidogens and methanogens. Apparently, the transport problem is solved by a cell-to-cell contact between the microbial associates. As indicated before, the associates must exert some delicate form of physiological intercourse in more than one way.

Anaerobic Conversion of Nitrogenous Compounds. A rather adverse aspect of anaerobic digestion is the fact that proteins are incompletely broken down into a mixture of amines, thus giving rise to foul odours. Yet, mineralisation of amines is thermodynamically possible according to the following hydrogenase reaction:

	$\Delta G^{\circ\prime}$ (kJ/r)
$CH_3CH_2NH_3^+ + H_2 \rightarrow CH_3CH_3 + NH_4^+$	−192

Hence, the process could be enhanced in an advantageous way if a hydrogenase system could be found capable of using amines as H_2 acceptors. So far, these reactions have not yet been demonstrated. This illustrates that thermodynamic considerations only indicate the potential of a reaction to occur. Kinetics can, nevertheless, limit the reaction rate even under thermodynamically favourable conditions. Especially with biological systems, predictions based on thermodynamic considerations are uncertain because the biological pathway must exist, capable microorganisms must be present and environmental conditions must be suitable for the microorganisms.

Many examples can be given of theoretically feasible conversions that for unknown reasons do not take place. With respect to removal of nitrogenous compounds, the anaerobic fermentation of urea is quite puzzling:

	$\Delta G^{\circ\prime}$ (kJ/r)
$4CO(NH_2)_2 \rightarrow$ $3CH_4 + 4N_2 + HCO_3^- + H_2O + H^+$	−229

Bhadra *et al.* (1987) claimed that they observed such a conversion in their laboratory reactors; however no confirmation has been published since. Another controversial reaction is the anaerobic fermentation of ammonium and nitrate:

	$\Delta G^{\circ\prime}$ (kJ/r)
$5NH_4^+ + 3NO_3^- \rightarrow 4N_2 + 9H_2O + 2H^+$	−1483

This microbiological process has been proposed by Van der Graaf *et al.* (1995) and awaits further scale-up.

Conversion Kinetics and Threshold Values

In waste treatment technology, residual substrate concentrations are of direct practical importance because they determine the process efficiency. This has drawn attention to the question of higher threshold concentrations of anaerobes, a topic which can be addressed in several ways. The problem is quite interestingly presented in the work of Hopkins *et al.* (1995) who studied the syntrophic benzoate degradation threshold:

	$\Delta G^{\circ\prime}$ (kJ/r)
$Benzoate^- + 7H_2O \rightarrow$ $3\ acetate + 3H^+ + HCO_3^- + 3H_2$	−74

The authors isolated a co-culture of a benzoate degrader with an H_2 removing *Desulfovibrio* strain. They found that the culture removed benzoate according to first order kinetics but abruptly stopped at a residual benzoate concentration of 0.1 to 1 μM. The latter value was related to the concentration of acetate which was of the order of 1 to 10 mM. If acetate was added at the start of the experiment, the threshold reached for benzoate was higher. In fact, they found that under all circumstances, the growth stopped when the $\Delta G'$ of the reaction did not attain −54 kJ/r.

It can be calculated that the specific protein fraction of a critical enzyme in a cell is 0.01% of the cell dry weight. Based on the appropriate cell yield coefficient one can calculate the threshold concentration of a substrate in order to induce a microbial association to a specific bioconversion. Furthermore, once induced, there might be a minimal concentration at which the cells or the association maintains its action, hence a threshold of exhaustion of the substrate. In this respect, it is well known that aerobic organisms can thrive at concentrations of 1 μg/l (a trait used in drinking water biotechnology) but that anaerobic organisms appear only to have such low exhaustion levels for hydrogen but not for acetate. Indeed, one can distinguish between the substrate affinity constant (K_s) of hydrogenotrophic and acetotrophic methanogens. While the first exhibit quite high substrate affinities and remove hydrogen down to

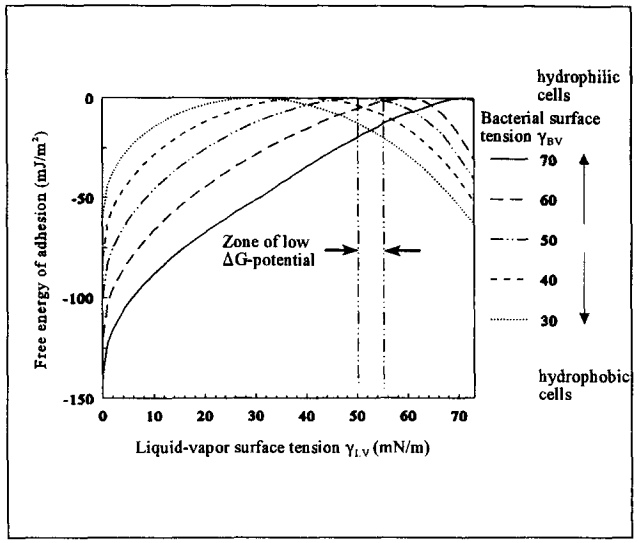


Figure 2. Free energy of bacterial adhesion (ΔG) as a function of the surface tension (in mN/m) of the liquid (γ_{LV}) and the bacteria (γ_{BV}) (After Thaveesri *et al.* 1995).

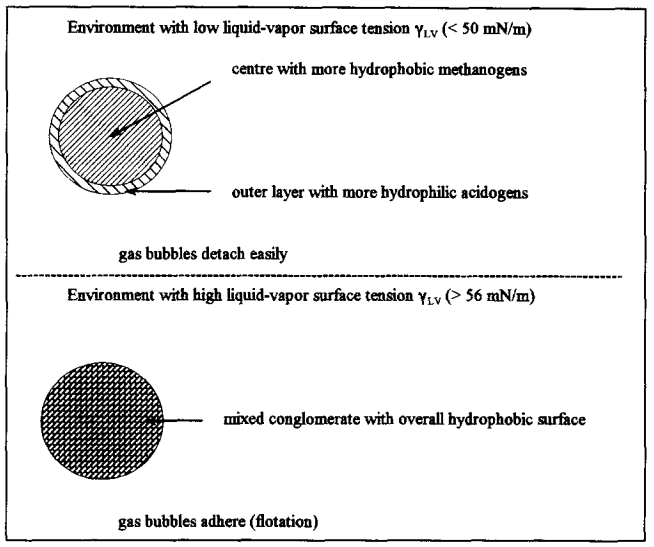


Figure 3. Concept of anaerobic cell aggregation, based on the thermodynamics of cell adhesion (After Thaveesri *et al.* 1995).

ppm levels, the second group seems to contain species with only low substrate affinities. For instance, *Methanosaeta* (formerly *Methanothrix*) sp. and *Methanosarcina barkeri* have K_s values for acetate of about 50 and 130 mg/l, respectively (Huser *et al.* 1982). Such high K_s values, obtained for mono cultures, could easily result in residual volatile fatty acid (VFA) concentrations comparable to the concentrations of the incoming waste water and thus implicate a low removal efficiency. However, in balanced methanogenic consortia growing in reactors, K_s values as low as 1–3 mg/l total acetate have been reported (Fukuzaki *et al.* 1990; Lettinga 1995). Consequently, anaerobic treatment is of interest only for relatively concentrated wastewaters (COD > 500 mg/l), unless highly adapted sludges can be used.

Although kinetics cannot be derived from ΔG-values, recent data suggest that most anaerobic bioconversions operate at low activation energy potentials and proceed according to the available change in free energy. For example if the minimum energy quantum of -21 kJ/mol is applied for the conversion of H_2 to methane, the minimum concentration for H_2 is $10^{-4.83}$ atm (corresponding to 0.01 μM H_2), while that for acetate is of the order of 10 mM (600 mg/l) and that of formate is of the order of 50 μM. The residual concentrations predicted by thermodynamics are observed in practice in reactor systems. Hence, engineers can with reasonable confidence select kinetic models and threshold levels based on ΔG-values. This opens perspectives for the more accurate design and operation of anaerobic processes.

Bacterial Adhesion

Bacterial Aggregation. Biomass retention is of particular importance for anaerobic processes, where the slow growth rate of anaerobic bacteria imposes the necessity to concentrate the methanogenic consortia in the reactor. In methanogenic biomass, the maximal growth rate of the slowest growing bacteria is about 0.08 to 0.15/d (de Zeeuw 1984), which means that a minimal cell residence time of 7 to 12 d is required.

Cell aggregation and biofilm formation have thus far been examined mainly from a mechanistic point of view (Costerton *et al.* 1987). In this approach, biofilm formation results from mechanical, electrostatical (*e.g.* Van der Waals forces) and biochemical (extra-cellular polymers, biopolymer bridges) binding forces and population dynamics (growth and decay of immobilized cells).

One can also approach microbial adhesion in terms of thermodynamics. Adhesion phenomena are governed by electrostatic and hydrophobic forces, as classically described by the DLVO concept (Rutter & Vincent 1984). When one calculates the free energy of adhesion (ΔG_{adh}) for two identical bacteria as a function of the liquid surface tension (γ_{lv}) by means of the surface thermodynamic function of Neuman *et al.* (1974), one obtains lines as shown in Figure 2 for different bacterial surface properties. A remarkable feature is the impact of the liquid surface tension on the potential change of free energy of adhesion. At low surface tensions, hydrophilic bacteria in particular are favoured to adhere to one another, while at high surface tensions it is likely to be the hydrophobic species which adhere. In general, most acidogenic bacteria are hydrophilic, while methanogens appear to be hydrophobic (Daffonchio *et al.* 1995). Based on these thermodynamic considerations an overall concept of anaerobic cell aggregation has been proposed and is summarized in Figure 3. Depending on the surface tension of the water, cells are considered to grow,

respectively, in rather loose associations, in multilayered granules (γ_{lv} < 50 mN/m) or in mixed conglomerates (γ_{lv} > 56 mN/m). In wastewaters with an intermediate γ_{lv} granule formation is difficult (Thaveesri *et al.* 1995b). The latter does not exclude the applicability of sludge blanket systems because the formation of a well settling 'thick' flocculent sludge with a high methanogenic activity also suffices (Lettinga 1995).

Biomass Retention and Reactor Systems. The adherent properties of bacteria are employed in different reactor types, which can be roughly divided into reactors with biofilms attached to a support matrix and reactors that rely on the autoaggregation of the biomass. Anaerobic (up- or downflow) filters, fluid bed and gas-lift reactors belong to the first category. In these reactors, a large portion of the reactor volume is occupied by the support material. A biofilm can either be attached to a fixed support matrix (*e.g.* anaerobic filters) or to a matrix freely floating in the liquid (*e.g.* fluid bed and gas-lift reactors). Sand is the support material largely used, although it is not the best due to its high density and low porosity. More recently, other support materials such as natural or baked clay (sepiolite, kaolinite, Arlite, Argex and Biolite), granular activated carbon, pumice and reticulate polyurethane with less density and more porosity have been used. The latter supports favour biofilm development and decreases the energy cost during the bed expansion. Other reactor types use flocs (anaerobic contact process) or aggregates (anaerobic upflow sludge blanket (UASB) and expanded granular sludge blanket (EGSB) reactors) which develop mainly by mutual attachment of cells (see section below; *Initiation of Granular Anaerobic Sludge*).

Mass Transfer Aspects. The thickness of a biofilm is governed by different factors such as substrate concentration, temperature and shear forces (Hoehn & Ray 1973). Due to the immobilization of bacterial cells in a biofilm, mass transfer limitations can occur. For ideal conditions, it can be derived (Rittmann 1982):

$$L_{eff} = \left[\frac{2 \cdot D_f \cdot S_s}{k_v}\right]^{\frac{1}{2}}$$

where L_{eff} is the thickness of steady-state biofilm (m) at which the substrate concentration becomes ± zero, D_f is the molecular diffusivity of substrate within the biofilm (m^2/s), k_v is the maximum rate of substrate removal per volume biofilm ($kg/m^3.s$) and S_s is the substrate concentration at the liquid-biofilm interface (kg/m^3).

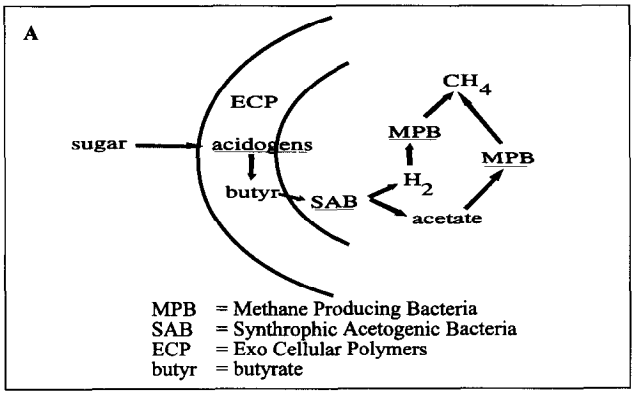
In general, aerobic and anaerobic bacteria metabolize substrates which have about the same order of D_f values in a biofilm. Furthermore, k_v values of both aerobic and anaerobic microorganisms are also of the same order of magnitude. Hence, L_{eff} is mainly governed by the substrate concentration S_s. For aerobic organisms, the limiting substrate is dissolved oxygen, with a concentration of a few g/m^3 at ambient temperatures. For most anaerobic organisms, the dissolved substrates are present at higher concentrations (up to kg/m^3). It follows that aerobic systems are restricted to thin biofilms (10-100 μm) and, consequently, to relatively low biomass densities (2 to 10 kg/m^3 reactor). In contrast, anaerobic systems, which rely on organisms which do not require a poorly soluble electron acceptor, can achieve high active biomass densities (20 to 100 kg/m^3 reactor).

Current Technology of Anaerobic Processing

Few, if any serious drawbacks can be identified for anaerobic treatment. Accepting that anaerobic digestion generally cannot provide a complete treatment (i.e. various minerals are left), an understanding of the fundamentals of the process has overcome previously mentioned drawbacks (Lettinga 1995) such as: a) The presumed low stability of anaerobic treatment, in fact anaerobic digestion systems are highly stable, provided they are designed, operated and controlled properly; b) The slow start-up of reactors has been overcome due to a better understanding of the growth conditions of anaerobic bacteria and the use of large quantities of highly active anaerobic sludge from existing full-scale installations as inoculum; Full-scale installations can now be started up within a few weeks, sometimes even days; c) Problems due to the production of malodours can be prevented by using relatively simple means, i.e. physical-chemical methods, or biofilters; and d) Despite the relatively high susceptibility of methanogens and acetogens to toxic compounds the potential of anaerobic consortia to adapt and to effect conversions of unwanted chemicals have been found to be much larger than previously perceived.

UASB Reactor Systems for Wastewater Treatment

Reactor Technology. One of the most important events in wastewater treatment during the last decades has been the development of the Upflow Anaerobic Sludge Blanket or UASB reactor. The principle of the reactor is quite straightforward: the wastewater is pumped upwards through a reactor under strictly anaerobic conditions at a rate between 0.5 and 1.5 m/h; inside the reactor a selection process occurs which can result in the growth of anaerobic microorganisms in a kind of conglomerate (granule) varying between 0.5 and 5 mm in diameter. These granules are powerful biocatalysts and convert the biodegradable organic matter in the influent in a rapid (space loadings varying from 10-20 kg COD per m^3 reactor per day) and complete way to biogas. Actually, the granular biomass is such a valuable biocatalyst that it is to our knowledge the only mixed culture which is at present commercially handled worldwide at a respectable price of the order about 1 US$ per kg dry weight.



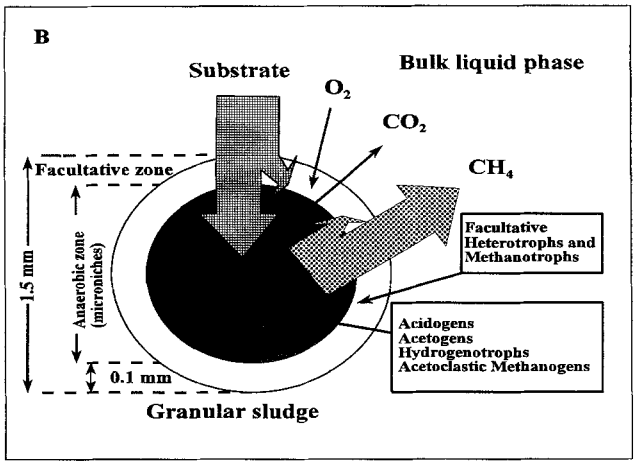


Figure 4. Schematic representation of multi-layered methanogenic granules. (A) Model for the distribution of fermentative and methanogenic bacteria in an anaerobic UASB granule (After Vanderhaegen *et al.* 1991). (B) Model for the distribution of anaerobic and facultative aerobic bacteria in an aerated UASB granule (After Kato 1994).

The principle of internal settling of suspended and granular sludge was initially reported in 1962 in South Africa (Hemens *et al.* 1962); the breakthrough in the technology occurred in the seventies in The Netherlands (Lettinga *et al.* 1980). At present, several hundred UASB reactors have been installed worldwide, in particular to treat different types of industrial wastewaters (Lettinga & Hulshoff Pol 1991; Lens & Verstraete 1992; Lettinga 1995).

Several modifications of the UASB reactor configuration have been proposed to optimize its treatment performance. A slight sludge bed expansion can improve the contact between the wastewater and the sludge. This idea has been realized in the Expanded Granular Sludge Blanket (EGSB) and in the Internal Circulation (IC) reactor concepts. Increasing the upflow velocity in the reactor to 5 to 6 m/h, as applied in EGSB systems, has been shown to expand the application field of UASB reactors. Rinzema *et al.* (1993) could treat solutions of sodium salts of higher fatty acids, such as lauric and capric acid, in EGSB reactors at space loading rates of up to 35 kg COD/m^3.day, whereas the UASB reactor failed at loading rates below 5 kg COD/m^3.day. In IC-UASB reactor systems, the gas is separated from the system halfway up the reactor by an in-built gas separator device. The lifting forces are used to bring about recirculation of the granular sludge within the lower part of the reactor, which results in improved contact between the sludge and the wastewater.

Some researchers have suggested that the gas-solid-separator device should be replaced by a packed bed in the upper part of the reactor (Coates & Colleran 1990; Borja *et al.* 1995). This recommendation is based on comparative studies of such a hybrid reactor and the anaerobic filter system. Despite the reported promising results, practical experience unfortunately indicates limitations due to clogging of the packed bed.

Other researchers have proposed combining UASB reactors with membrane reactors. Bailey *et al.* (1994) enhanced the performance of a UASB reactor treating synthetic wastewater by coupling a cross-flow micro-filtration unit to the UASB. Expansion of a UASB reactor system with membrane systems can be questioned (Lettinga 1995) and the main application field of membrane systems lies in the treatment of organic slurries (see section below; *Coupling Anaerobic Digestion to Ultrafiltration*).

Initiation of Granular Anaerobic Sludge. To present the problem in its proper perspective, a granular microbial consortium must be able to physically link the microbial species involved, some of which have to be in a nanometer proximity (Dolfing 1992), in order to achieve efficient H_2 transfer. Moreover, the granules have to achieve a mass flux in the order of 1 to 2 kg COD per kg VSS (Volatile Suspended Solids) per day of water-soluble polar substrates such as carbohydrates and to release the concomitant mass flux of gaseous metabolites, i.e. CO_2 and CH_4. Finally, the configuration of the granule must be such that it is retained in the reactor by settling (the law of Hazen and Newton) notwithstanding the upflow velocity of the water and the turbulence caused by the biogas bubbles produced.

Granulation proceeds well under all temperature conditions, *viz.* mesophilic (Vanderhaegen *et al.* 1991; Alphenaar *et al.* 1993), thermophilic (Wiegant & De Man 1986; Van Lier *et al.* 1995) and psychrophilic (Van der Last & Lettinga 1991). Moreover, different types of granules can be formed on the same substrate (Daffonchio *et al.* 1995; Thaveesri *et al.* 1995a), i.e. black granules mainly composed of methanogens, white granules of which the composition is not clear yet and grey granules of the multi-layer type (Figure 4A). Particularly the latter constitute the proper biocatalysts because they have a good strength and have a rather hydrophilic surface preventing adherence to gas bubbles and thus avoiding washout from the reactor.

UASB granule formation is mediated by auto-immobilization processes and several factors are involved in the granulation process. It has been shown that high levels of divalent cations (*e.g.* Ca^{2+} and Mg^{2+}) or organic and inorganic nuclei (*e.g.* clay minerals) can initiate granule formation. Microbial factors can mediate granule formation, *e.g.* extracellular polymer formation, the presence of *Methanosaeta* sp. microcolonies as microbial nuclei and the interactions between methanogens and syntrophic acetogens (Vanderhaegen *et al.* 1991). The preponderant role of acidogens in granule formation appears also valid in thermophilic UASB granules (Uemura & Harada 1993).

In the last decade, the mechanisms of sludge granulation have been elucidated sufficiently for their practical application (Lettinga 1995). For understanding the reason(s) why granulation occurs, intensive research on the ultrastructure of granules has been performed. MacLeod *et al.* (1990) were the first to propose a structured model of the anaerobic granule consortium; from electron microscopy they proposed the hypothesis that the granule was composed of different layers with the acidogenic bacteria primarily at the outside. The work of Lens *et al.* (1993) with microelectrodes confirmed that methanogenic aggregates have a heterogeneous activity distribution. It was clearly demonstrated that acidogenic activity was situated in the outer 200 μm while methanogenic activity was located inside. This does not exclude the fact that the filamentous and hydrophobic properties of *Methanosaeta* sp. play an important role in giving cohesion to the granule, as the death and decay rates of acidogens are much higher compared to those of the methanogens (Visser *et al.* 1993; Alphenaar *et al.* 1994). Recent work incorporated surface thermodynamics in an explanation of the granulation phenomena (Daffonchio *et al.* 1995; Thaveesri *et al.* 1995a). Thermodynamic calculations revealed that, particularly at a low surface tension of the wastewater, the hydrophilic acidogens can gain an important decrease in free energy by adhering to one another. Hence, addition of certain surfactants was found to be instrumental in obtaining good granules (Thaveesri *et al.* 1995b).

An overall concept of the auto-immobilization processes in granules is now becoming clear. Yet, it is still another step before one can achieve controlled in-reactor granular sludge growth during the first start-up. The preponderant role of the acidogens indicates that a fraction or minimum 10% of the COD must be high energy substrates for the acidogens, and this is not always the case in practice. In this respect, it is feasible to design concentrated substrates which can be supplemented to wastewaters in order to attain or maintain the granular sludge conditions. Moreover, the type of acidogens with the correct surface characteristics need to be enriched for, and in this respect, few tools are available to the biotechnologist. Addition of selected strains to the feed appears inadvisable, as the presence of acidogenic organisms in the feed have been shown to initiate granular sludge flotation (Alphenaar 1994). At present there are indications that surfactants could be of specific use in the induction of granulation during the first start-up, as they lower the surface tension without having a negative impact on the overall functionality of the association (Thaveesri *et al.* 1995a).

With the further employment of UASB systems, large amounts of high quality granular sludge will become available, which can be used as seed material for new installations, thus reducing the start-up period to a few days. The main condition to be met is to keep the sludge loading rate well below 50% of its maximum substrate utilisation rate during the first weeks (Lettinga 1995). Although secondary start-up generally proceeds very satisfactorily, specific problems may manifest themselves such as deterioration of the sludge granules, attachment of fast growing filamentous acidogenic bacteria, granular sludge flotation and $CaCO_3$-scaling (Table 1). An understanding of these problems will extend the applicability of anaerobic wastewater treatment to other types of wastewaters which, until now, have been considered unsuitable for anaerobic treatment.

Treatment of Low Strength Wastewaters. In general, anaerobic treatment systems have been applied to industrial wastewaters with a COD exceeding 2 g/l. Some industries produce dilute effluents of less than 2 g/l, (*e.g.* fruit and vegetable canneries, malting and brewing processes and soft-drink-bottling industries) and the COD of domestic wastewater typically varies between 0.3 and 1 g/l. The problems of such dilute wastewaters are low substrate concentrations occurring in the reactor and the possible presence of dissolved oxygen. Several technological approaches have been developed for the treatment of such wastewaters.

A first approach is to create a high level of hydraulic turbulence and good sludge bed expansion to provide an adequate wastewater-biomass contact. This can be achieved by increasing the upflow velocity in UASB reactors. EGSB systems successfully treated low strength wastewaters of dilute ethanol solutions (influent COD 10 to 300 mg/l) at 30°C (Kato 1994) and dilute VFA-solutions (influent COD < 1 g/l) at temperatures as low as 10°C (Rebac *et al.* 1995). Thus, the EGSB concept will very likely substantially reinforce the application of anaerobic treatment under psychrophilic conditions. The potential of the latter system can be attributed mainly to surprisingly low K_s values which the micro-organisms appear to acquire under those conditions (Kato 1994).

Secondly, anaerobic treatment reactors have been combined with aerobic systems, such as trickling filters or rotating biological contactors (Lens & Verstraete 1992). Combined anaerobic/aerobic conditions in one reactor are also feasible, as dissolved O_2 concentrations can reach up

Table 1. Schematic overview of potential problems in the effectiveness of granular sludge in UASB reactors (after Alphenaar 1994).

	Problem		Cause		Solution
1	Insufficient sludge growth	1.1a	Trace-element- or nutrient limitation.	1.2a	Raising the nutrient- and/or trace-element concentration in the UASB influent
		1.1b	Too high a degree of influent pre-acidification	1.2b	Reducing the degree of pre-acidification.
		1.1c	Too low a sludge loading rate	1.2c	Increasing the loading rate (removing sludge)
		1.1d	Granular sludge wash-out (see 4, 5)		
		1.1e	Wash-out flocculent sludge, granule disintegration (see 6)		
2	Insufficient methanogenic capacity, (reactor overloaded).	2.1a	Not enough sludge in the reactor	2.2a	Reducing the loading rate. Raising the amount of sludge. Using external seed sludge. Promoting sludge growth (see 1), reducing wash-out (see 3-6)
		2.1b	Insufficient methanogenic activity (see 3).	2.2b	Decreasing sludge loading rate, increasing (methanogenic) activity of the sludge (see 3.2)
3	Insufficient methanogenic activity	3.1a	Nutrient- or trace-element deficiency (see 1.1a)		
		3.1b	An abundant growth of acidogenic bacteria	3.2b	Increasing the degree of wastewater pre-acidification. Reducing the loading rate.
		3.1c	Accumulation of organic suspended material in the sludge bed	3.2c	Ensuring that the influent does not contain suspended material.
		3.1d	A too low process temperature	3.2d	Increasing temperature
		3.1e	Toxic compounds in the wastewater fed or activity inhibiting conditions (see 6.1d)		
4	Granule wash-out.	4.1a	Gas trapped in hollow granules. Formation of too big granules due to insufficient forces: low temperature, low loading rate, low influent concentration (see also 6.1a,b).	4.2a	Increasing forces on granules, reducing the granule size.
		4.1b	Gas entrapment due to the formation of a layered structure, covering the granules with (acidogenic) biomass.	4.2b	Applying more stable process conditions, increasing the degree of wastewater pre-acidification.
5	Sludge wash-out, formation of bulking sludge and fluffy granules.	5.1a	The conglomeration of individual sludge granules, related to suspended acidogenic bacteria in the influent.	5.2a	Withdrawing suspended matter from the influent. Diminishing the degree of pre-acidification.
		5.1b	Extensive growth of suspended or more or less at the granule surface attached acidogenic bacteria.	5.2b	Increasing the degree of pre-acidification. Intensifying the mixing applied.
		5.1c	Formation of very fluffy granules, strong growth of attached acidogenic bacteria.	5.2c	Increasing the degree of pre-acidification. Decreasing the sludge loading rate.
6	Granule disintegration.	6.1a	'Delayed' start-up problems, see 6.1 b-d	6.2a	Applying other start-up strategy (faster increase of sludge loading rate), choosing another type of seed sludge.
		6.1b	Sudden variations in loading rate and/or influent concentration	6.2b	Applying more stable process conditions.
		6.1c	Sudden increase in the degree of pre-acidification. Starvation of acidogenic bacteria.	6.2c	Applying a more constant pre-acidification; (at start-up: choosing another type of seed sludge).
		6.1d	(Periodically) exposure to toxic compounds and harmful conditions	6.2d	Removing or detoxifying the toxic compound. Keeping longer adaptation periods. Using a larger hydraulic buffer.
		6.1e	Too strong mechanical forces	6.2e	Preventing too strong mechanical forces, decreasing sludge loading rate.
		6.1f	Formation flocculant sludge due to an insufficient selection pressure.	6.2f	No problem if the process is stable. Otherwise increasing the selection pressure (effluent recirculation).

to 10 mg/l in very low strength wastewaters (Figure 4B). Recent investigations have shown that methanogenesis can still occur in such reactor configurations (Kato *et al.* 1993). Deterioration of methanogenesis upon O_2 exposure is apparently not due to a direct toxic effect of oxygen on anaerobes, but due to the competition of methane producing bacteria (MPB) and (facultative) aerobes for reducing equivalents. An additional negative effect of O_2 might be the deterioration of the granular sludge due to growth and attachment of filamentous (facultative) aerobic microorganisms. However, introducing oxygen into a UASB reactor has been shown to improve the reactor efficiency, either by improving granular sludge yield (Thaveesri *et al.* 1994) or by allowing complete mineralisation of xenobiotics (Field *et al.* 1995).

Thirdly, an approach in which the initial organic load is concentrated before anaerobic treatment, can also be applied. An interesting example of such a process is the CSIRO magnetite process (Priestley 1990). Fine magnetite particles (size = 5 to 20 μm; 1% concentration) are mixed for 5 to 7 minutes with raw sewage. During this period coagulants such as ferric sulphate and/or polyelectrolytes are added. Thus, the magnetite particles and the sewage organics flocculate. The flocs are separated from the sewage water by settling and magnetic flocculation. They are passed through a washing stage where the sewage organics are concentrated by a factor 30 relative to the original raw sewage. The most important advantages of the process are the short retention time of the wastewater in the system (15 to 20 min) and the insensitiveness to shock loads.

Treatment of Sulphate Rich Wastewaters. Molasses-based fermentation industries, *e.g.* alcohol, citric acid and monosodium glutamate production, typically generate effluents with both high COD (30 to 95 g/l) and high sulphate (2.5 to 9 g/l) concentrations (Colleran *et al.* 1995). Wastewaters from the paper board industries typically contain 1 to 2 g sulphate/l. The highest waterwater sulphate concentrations are associated with the industrial production of fatty acids, where edible oil refinery effluents can contain up to 40–50 g sulphate/l and a COD/sulphate ratio of ≤ 1.

During the methanogenic treatment of sulphate-rich wastewaters, the interactions between sulphate-reducing bacteria (SRB) and MPB can result in failure of methanogenesis. The SRB may compete with MPB for available electrons, resulting in a decreased methane yield. This is mainly due to the higher affinity of the SRB for common energy substrates like H_2 and acetate and the greater trophic flexibility of SRB, allowing them to scavenge electrons directly from acetate precursors (*e.g.* propionate and butyrate). In addition, sulphate reduction results in the production of sulphide, which is corrosive, malodorous and can inhibit methanogenic bacteria.

Despite the theoretical competitive advantages of the SRB, methanogenesis can prevail in reactors treating sulphate-rich wastewaters. In general, immobilized cell reactors appear to be more favourable environments for methanogens. Moreover, complete suppression of methanogenic activity is difficult when optimizing sulphate reduction in granular sludge reactors. Occurrence of methanogenic activity in the presence of high sulphate concentrations has been attributed to different substrate uptake kinetics of SRB and MPB, a more effective adhesion of MPB to the carrier material, lack of a suitable energy-rich organic substrate for the SRB to outcompete the MPB, or mass transfer limitation of sulphate (Overmeire *et al.* 1994).

Recently, there has been a growing interest in depolluting wastewaters by the activity of SRB only (Visser *et al.* 1993). This can be accomplished in UASB reactors treating wastewaters with a $COD/SO_4^{2-} > 1$. Sulphidogenic UASB reactors operate at a pH as a high as 8 to reduce the sulphide toxicity and at upflow velocities of 2 m/h. The latter is to compensate for inferior mass transfer rates due to a lack of gaseous end products (biogas) in sulphidogenic conversions.

Eco-engineering of Anaerobic Breakdown of Xenobiotics. In view of the fact that wastewaters contain a wide range of chemical substances that must be degraded, it is of major importance to have a diverse collection of metabolic pathways available in the microbial community. In particular, the removal of xenobiotic compounds, such as synthetic polymers, aromatic compounds, haloaromatic compounds, phosphonates and sulphonates has become a major issue in biological wastewater treatment during recent years (Clark *et al.* 1991). In this context, aerobic microorganisms generally have more powerful enzymes than anaerobic microorganisms. Enzymes such as oxygenases and ligninases are very important in the biodegradation of natural and xenobiotic compounds and remain a unique feature of aerobes. Moreover anaerobic organisms, in particular methanogens, are quite susceptible to a large variety of compounds (Field *et al.* 1995). Anaerobic consortia are, nevertheless, able to degrade severely inhibitory compounds like chloroform once they have become adapted. The key factor in the application of anaerobic treatment of 'toxic' wastewaters is indeed adaptation.

Some researchers propose to increase the enzyme potential in a reactor by introducing new species or DNA fragments in existing systems. Genetic engineering or molecular breeding has been considered as a future technology in order to enhance the degradation rate of xenobiotics (Fujita *et al.* 1991). Although considerable progress has been made in the isolation, selection and manipulation of bacteria capable of degrading specific target pollutants, application of this knowledge to practical wastewater treatment is still in its infancy (McClure *et al.* 1991). The potential and problems associated with the enhancement of

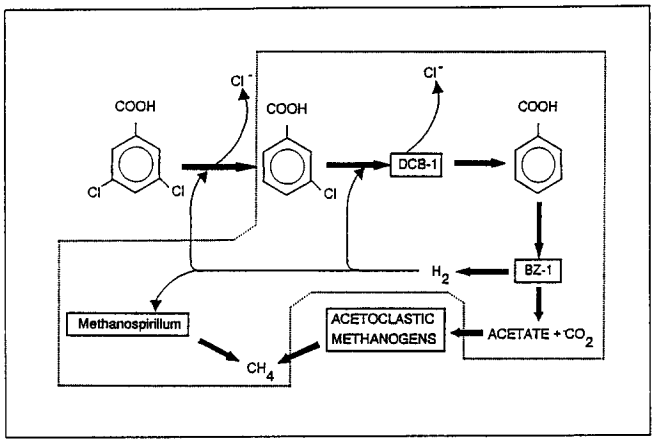


Figure 5. Anaerobic degradation of 3,5-dichlorobenzoate (After Dolfing & Tiedje 1986 (within dotted line) and Sulfita *et al.* 1982).

anaerobic wastewater treatment systems by the addition of (genetically engineered) microorganisms have been widely discussed for the degradation of halogenated compounds. Halogenated compounds are used for a variety of applications, such as plasticizers, cleaning products, wood preservatives, herbicides and insecticides. Also, the compounds may be formed as by-products, *e.g.* during bleaching in paper manufacturing or incineration of wastes. Since wastewater treatment plants have input from a variety of municipal, industrial and agricultural sources, halogenated compounds often enter these systems.

Highly halogenated compounds are resistant to aerobic degradation. In contrast, many types of chlorinated compounds can be degraded anaerobically, either to a lower halogenation level, that is suitable for aerobic degradation, or completely to Cl^-, methane and CO_2 as shown in Figure 5. Complete anaerobic degradation of halogenated compounds involves dehalogenation by a consortium of respiring anaerobes and breakdown of the dehalogenated intermediates by fermentative bacteria and methanogens. Complete or partial degradation has been shown for a wide range of compounds such as chlorinated phenols, benzenes, alkyls and even PCBs (Poly Chlorinated Biphenyls). However, some of these (PCB) are degraded too slowly (in the order of weeks) to consider reactor processing as an option.

Anaerobic dechlorination, the first step in anaerobic degradation, is a form of anaerobic respiration in which halogenated substrates are used as terminal electron acceptors. Consequently, dechlorination is strongly dependent on the redox potential (Dolfing & Harrison 1993). The presence of other electron acceptors, such as O_2, nitrate, nitrite, sulphate, sulphite or thiosulphate strongly inhibits or blocks the dechlorination (Allard *et al.* 1992; Hendriksen & Ahring 1992; Morris *et al.* 1992; Haggblom *et al.* 1993; Ramanand *et al.* 1993). Reported electron donors include H_2, acetate, glucose, maltose, pyruvate, lactate, formate, *p*-cresol, propionate, ethanol and butyrate. Dehalogenation is an exergonic process with ΔG values of -150 to -190 kJ/reaction, with H_2 as the electron-donor (Dolfing & Janssen 1994). Mohn & Tiedje (1991) showed that dehalogenation is coupled to ATP formation and Holliger *et al.* (1993) showed that it leads to cell growth. Many species are able to dehalogenate, these include *Pseudomonas* sp. (Fulthorpe *et al.* 1993), *Clostridium* sp. (Madsen & Licht 1992) and sulphate reducing bacteria (Mohn & Tiedje 1991; Pavlostathis & Zuang 1991).

Dechlorination of compounds with more than one chloro group takes place in steps. For example, Mohn & Kennedy (1992) described a single species able to degrade pentachlorophenol to 3-chlorophenol, while an anaerobic association completely degraded pentachlorophenol to methane (Hendriksen *et al.* 1992). This shows that full degradation to harmless products is a complex, multi-step process and often requires the concerted action of a group of organisms. The complexity of these processes is illustrated in Figure 5, which schematically shows the degradation of 3,5-dichlorobenzoate by a defined mixed culture of three organisms; a sulphate reducer (DCB-1), a benzoate fermenter (BZ-1) and a *Methanospirillum*. Dehalogenation takes place in two steps both requiring H_2. The further breakdown of the intermediate benzoate to acetate, carbonate and H_2 can only take place under very low p_{H_2}. Although a portion of the H_2 produced is recycled in the dehalogenation, the surplus must be consumed by the methanogens to keep the p_{H_2} sufficiently low to facilitate the process. This is in agreement with Ramanand *et al.* (1993) who reported a decrease in the dehalogenation rate upon inhibition of methanogenesis. Another type of multi-species interaction was described by Distefano *et al.* (1991). An anaerobic mixed culture was reported to dehalogenate tetrachloroethene, although, after a few weeks the culture declined. Extended activity required addition of filtered supernatant from a methanol-fed culture. This shows nutritional dependency on substrates produced by other species.

It may take a community of microorganisms, obtained from sewage sludge or soil, weeks or even months to adapt to a new compound before optimal conversion takes place (Govind *et al.* 1991; Morris *et al.* 1992; Nicholson *et al.* 1992; Ramanand *et al.* 1993; Enzien *et al* 1994) or even years (Fahmy *et al.* 1994). This time is needed for development or invasion of all species necessary to completely degrade the substrates as well as for their colonization of the reactor system. Important for the understanding of the long start-up times is the finding that the ability of a bacterial population to dehalogenate is associated with elevated amounts of plasmids (Fulthorpe *et al.* 1993). Adaptation of the population (proliferation of the right plasmids and of the desired species) takes many generation times.

Only a few studies have reported on the degradation of halogenated compounds in operating methanogenic reactor systems. Removal of adsorbable organic halogen (AOX) by a

UASB treating kraft-mill bleach wastewater was 27 to 65%, depending on the residence time (Parker *et al.* 1993). Mohn & Kennedy (1992) reported incomplete degradation of tri- and dichlorophenols by anaerobic sludge. The findings of Govind *et al.* (1991) were not very promising: during anaerobic digestion of sewage sludge, degradation of a mix of twenty different compounds was tested. All compounds were degraded, although only eight completely, the others partially, and some only up to 10%. The sludge retention time was only 30 days, probably too short for complete adaptation. Hendriksen & Ahring (1992) reported the UASB degradation of pentachlorophenol to mainly di- and monochlorophenols. However, shortly after this, Hendriksen *et al.* (1992) reported almost complete (94%) dehalogenation of pentachlorophenol in an UASB. Glucose addition was necessary for sludge growth and increased the total dechlorination by a factor of 5, possibly by acting as an electron donor. Comparison of UASB reactors with anaerobic fixed film reactors showed that the former had higher conversion rates and a more complete degradation (Hendriksen & Ahring 1993). In the fixed film reactor the dechlorination decreased after 15 months, while it remained stable in the UASB.

Spontaneous adaption of an UASB reactor to a new xenobiotic compound is a slow process, although it can be accelerated. Ahring *et al.* (1992) inoculated UASB reactors with either a mono-culture of *Desulfomonile tiedjei*, able to dechlorinate 3-chlorobenzoate (3-CB), or a three-membered consortium consisting of *D. tiedjei*, a benzoate degrader and a H_2-utilizing methanogen. No degradation occurred in a non-inoculated control reactor started-up with the same granular sludge, but inoculated UASB reactors rapidly transformed 3-CB. The degradation was stable for several months and with antibody staining, it was shown that *D. tiedjei* was incorporated in the methanogenic granules. This demonstrates that inoculation of UASB reactors with specific degraders can be an effective means of establishing the consortium needed for degradation.

Several compounds cannot be completely dehalogenated under anaerobic conditions. For example, tetrachloroethylene is converted to dichloroethylene, while multiple chlorinated benzenes are degraded to trichlorobenzenes. These reduced compounds can then be further degraded aerobically. Two-stage process anaerobic/aerobic biofilm reactors have been designed to degrade highly chlorinated hydrocarbons (Fathepure & Vogel 1991; Field *et al.* 1995).

In conclusion, anaerobic dehalogenation is a necessary step in the microbial degradation of highly halogenated compounds. Mineralisation of halogenated compounds is often possible but it requires a fine-tuned and specialized consortium of microorganisms. Development of such a consortium takes time, sometimes years, and seeding with the desired consortium may accelerate reactor start-up. Reactors with stability over long time periods are needed and UASB systems appear to be the most promising.

Digestion of Organic Slurries

Effective treatment of organic slurries containing particulate organic matter, i.e. animal slurries or primary and secondary sludges, requires adapted reactor systems. The problem with these types of waste streams is that the particulate matter must be solubilized before it can be subjected to anaerobic conversions. It has been well established that the rate of hydrolysis is the limiting factor (Pavlosthatis & Gossett 1986). The result of this is that, in terms of digestion of slurries and suspensions, volumetric loading rates are generally low, i.e. 1 to 3 kg COD/m^3.d. Little increase in rate has been achieved over the last decades.

Although particulate organic matter can be degraded to some extent in granular-sludge-based reactor configurations, slow digestion rates result in particle build-up in the reactor, thereby taking up the space normally occupied by the granular sludge. As a rule, the suspended solids of a wastestream fed to an UASB reactor should not be more than 10% of the total COD (Rozzi & Verstraete 1981). To give the particulate matter a more complete degradation without extending the hydraulic residence time, two approaches have been explored during the last decade.

Thermophilic Digestion. At temperatures of 55 to 70°C, cellulases and other polymer hydrolases have been reported to achieve maximal rates (Vandevoorde *et al*, 1988). Notwithstanding its potential high conversion rates (Mackie & Bryant 1995), thermophilic digestion has thus far not been popular in environmental biotechnology. A first problem is related to the difficulty of start up. In the past, this was initiated by adding a small amount of inoculum to a large amount of feed maintained at the proper temperature, a procedure which usually failed. By starting up with a sufficiently large amount of thermophilic biomass and supplying the latter with feed at an appropriate rate, reactors of several thousands of m^3 can be fully functional within a few months.

Additional drawbacks of thermophilic digestion of slurries appear to be the sensitivity of the microbial consortium to minor temperature changes (Buhr & Andrews 1977) and also to certain stress factors such a NH_3 and H_2S. With respect to temperature sensitivity, no breakthrough has been made on the microbial front, but practical engineering has shown that by working in parallel digesters (minimum three) a reactor can be reinstalled by heavy (minimum 30 to 40% volume) inoculation with good mixed liquor from the others. In terms of NH_3 inhibition, good results have been obtained by the addition of bentonite clay which apparently adsorbs the ammonia and thus lowers the concentration of NH_3 affecting the cells (Angelidaki & Ahring 1993). *In situ* control of sulphide emissions during the thermophilic anaerobic digestion process can be achieved by dosing with $FePO_4$ at a ratio of one to one to

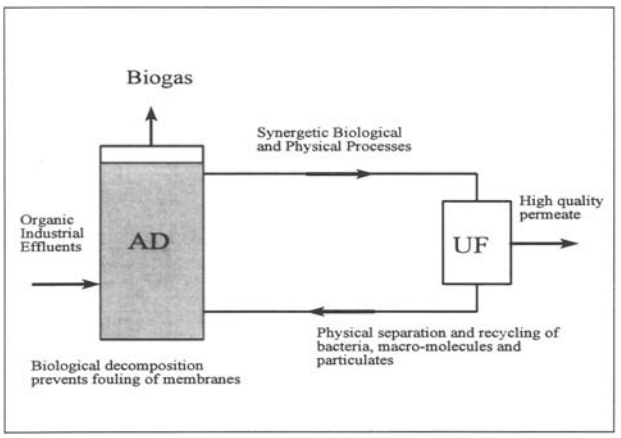


Figure 6. Schematic diagram of the Anaerobic Digestion-Ultrafiltration (ADUF) process.

the amount of sulphate in the feed (McFarland & Jewell 1989).

New insights obtained during the last decade in thermophilic digestion resulted in the construction in Denmark of five large scale thermophilic digesters to treat farm manure. The digesters co-treat certain organic wastes from the agricultural and food industries and ensure a good stabilization and concomitantly a fair degree of sanitation. Normally, a reduction in fecal enterococci of 3 to 4 orders of magnitude is achieved (Bendixen 1994). This is the most important factor as it is by no other means economically feasible to provide a fair sanitation for farm wastes.

The developments in thermophilic slurry digestion open new possibilities in the field of sewage sludge digestion. Conventionally, sanitary engineers preferred mesophilic digestion for reasons of reliability. Yet, regulations in terms of pathogen reduction become more stringent. In the USA, for example, biosolids destined for land should have $< 2 \times 10^6$ faecal coliforms per gram total solids. Most USA mesophilic digesters achieve the standard (97%), although usually just (Stukenberg *et al.* 1994). In the near future, it should be possible, both in terms of thermal and microbial engineering, to re-examine the digesters to operate in a thermophilic mode and thus achieve higher sanitation standards. It should be noted that recent work by Ahring (1994) indicates that digestion at 70°C can proceed efficiently; clearly this permits production of a very sanitized product.

Coupling Anaerobic Digestion to Ultrafiltration. Anaerobic sludge digestion is an important step in the wastewater treatment process. It is economically advantageous due to the biogas production, increased dewaterability and volume reduction of the produced solids. The data in Table 2 indicate that for most particulates present in primary sewage sludge, 0.5 to 1.0 day residence times will effect 90% hydrolysis. Yet, microbial cells present in waste activated sludge (biosolids) are much more resistant to hydrolysis. Hence, in sewage treatment plants, sludge digesters are generally operated at long residence times (minimum 15 to 20 days) and corresponding low volumetric loading rates of a few kg COD/m^3.d.

The conventional digester for organic slurries is a completely mixed reactor, with no solids recycle. Consequently, the solids residence time (SRT) is identical to the hydraulic residence time (HRT). This limits the volumetric capacity of the digester, since a relatively long SRT (15 to 20 days) is required for effective destruction of solids. Improvements to the conventional reactor design generally involve methods to selectively retain the solids in the digester. Solids-liquid separation can be effectively done by membrane separation techniques. Subsequent recycling of the solids into the digester enables operation at a higher active biomass concentration, and hence the loading of organics can be increased. Since the HRT is decoupled from the SRT, the volumetric throughput of the digester can be increased. It also allows the maintenance of a constant sludge age in the digester.

The ADUF process (anaerobic digestion - ultrafiltration) comprises two main unit operations: an anaerobic digester (AD) coupled with an external-pressure-driven ultrafiltration (UF) unit (Figure 6). The UF permeate constitutes the final effluent, while the retained sludge concentrate (containing both the bacteria and solids) is rapidly recycled into the AD. The performance of the AD is further improved by better mixing of the reactor contents. Influent macromolecules and particulate organics are also rejected at the membrane surface and are selectively retained in the AD by the UF unit until complete metabolization to the molecular mass cut-off of the membrane. A technological problem common to many membrane separation systems is the long-term decline in permeate production, due to irreversible fouling of the membrane pores, an intrinsic aspect of all membrane operations. The membrane can be restored by chemical, physical or biological cleaning. Anaerobic digestion decomposes organics which would otherwise foul the membrane filters and thus bacterial cleaning minimizes chemical cleaning of the membrane (Ross *et al.* 1994).

Nagano *et al.* (1992) and Harada *et al.* (1994) successfully applied ADUF to wastewaters containing high strength particulate organics. Pillay *et al.* (1994) improved the performance of anaerobic sludge digesters using the coupled cross-flow micro-filtration/digester process. In an economic evaluation of the process, they demonstrated that the coupled system gave a saving of 17% compared to a conventional system. In anaerobic sludge digestion, the biomass concentration in conventional digesters is relatively low (1.5 to 3%), giving a low biomass loading per unit digester volume (Pillay *et al.* 1994). The advantage of the coupled process is that the solids loading and the volumetric throughput of existing digesters may be significantly

Table 2. Biodegradability and rate of hydrolysis (k_h) of particulate organic matter in mesophilic anaerobic digestion.

Substrate	Biodegradable fraction (%)	K_h (d^{-1})	DT_{90}	Reference
Domestic primary sludge	75 of total COD 50 of non-lipid COD	3	0.76	Eastman & Ferguson (1981)
Domestic primary sludge	50	4	0.57	Rozzi & Verstraete (1981)
Surplus activated sludge	50 to 60	0.15	15	Pavlosthathis & Gosset (1986)

DT_{90} = time (d) in order to degrade 90%.

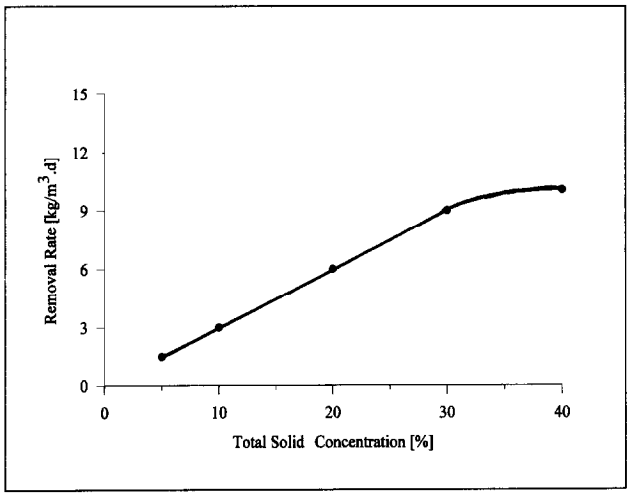


Figure 7. Removal rate of polymeric substrate by solid state anaerobic digestion as a function of % of total solids in the substrate.

increased, while still ensuring the necessary sludge stabilization. Furthermore, there are indirect advantages for other process steps, for example the volumetric loading of downstream sludge-dewatering units will be decreased. The permeate of the membrane filtration cannot be disposed of without further treatment, which is facilitated by its negligible solids content.

ADUF has also been successfully applied to the anaerobic treatment of several industrial effluents, *e.g.* wine distillery, malting, egg, brewery and maize-processing effluents (Ross *et al.* 1994). The effluents in these cases were typically soluble or colloidal in nature with high COD concentrations in the range 3.5 to 37 kg/m³. COD removal efficiencies in the range 77 to 97% were obtained when operated at mean volumetric loading rates of 3 to 15 kg COD/m³.d and 35°C. These corresponded to equivalent sludge loading rates in the range of 0.24 to 0.7 kg COD/kg $VSS^1.d^1$ and HRTs in the range 0.8 to 3.3 days. Final mixed liquor suspended solids (MLSS) values were in the range 10 to 50 kg/m^{-3} and the permissible concentration is governed by its influence on the membrane flux. The membrane flux attained on the various ADUF systems varied in the range 8 to 83 l/m².h. The lower flux values were generally due to poor operational conditions such as low linear tube velocity or high MLSS concentration.

Solid State Anaerobic Fermentation

For the fermentation of solid wastes, i.e. domestic household waste, most of the COD is in the form of insoluble volatile solids (VS) at a concentration of at least 150 kg COD per ton. Hence, an intensive hydrolysis/acidification phase is required in solid state fermentation (20–40% total solids) where the solid waste is converted into methanogenic substrates. This process can become inhibited by the high acid concentrations. The rate of conversion of polymers can be calculated according to the Monod model. A completely mixed fermentor operates according to the following equation:

$$R = \frac{S_0(\mu\theta - 1) - K_s}{\theta(\mu\theta - 1)}$$

where R is the removal rate of substrate (kg VS/m³.d), θ is the hydraulic retention time (d), S_0 is the influent substrate concentration (kg/m³), K_s is the affinity constant (kg/m³) and μ is the maximum specific growth rate (d^{-1}). If it is assumed that the solid state fermentation can be operated completely in a mixed mode (by gas mixing or by creating movement in the reactor), the rate of hydrolysis at various solid concentrations in the fermentor can be calculated. The kinetic constants for the hydrolysis of cellulose in anaerobic mesophilic digestion were determined by Ghosh & Lall (1981): μ = 1.7 d^{-1} and K_s = 36.8 kg/m³. Figure 7 gives the rate of hydrolysis as a function of the solid concentration in the fermentor, with a retention time of 21 days and a VS concentration of 65% of the dry matter of the substrate. With 30% total solids, a rate of hydrolysis of 19.2 kg VS per m³ reactor per day is obtained, which could

Table 3. Performance of various reactor configurations for anaerobic digestion of solid waste (after Baeten & Verstraete 1993).

		Reactor types			
		VALORGA	**BIOCEL**	**SEBAC**	**DRANCO**
TS feed	%	20–30	30	20	30–40
SRT	d	15	9	42	15–20
B_v	kg VSS/m³.d	14	18–20	3.2	15–20
Temperature	°C	37	60	55	55
Methane yield	1l/kg¹ VSS	230	220	200	235

TS: total solids; SRT: solids residence times; B_v: volumetric loading rate; VSS: volatile suspended solids.

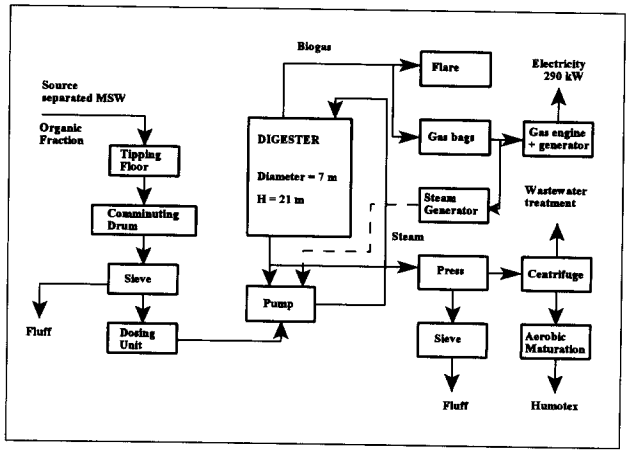


Figure 8. Flow sheet of the full scale Dry Anaerobic Conversion (DRANCO) plant in Brecht, Belgium, treating source-separated municipal solid waste (MSW).

give a gas production rate of about 3.5 m³ methane or 6.4 m³ of biogas per m³ reactor per day. At higher solid concentrations, the volume of available water limits further improvement of the hydrolysis rate.

The anaerobic fermentation of domestic refuse has been of interest in the United States since the mid-seventies (Pfeffer 1974; Wujich & Jewell 1980). However, effective full-scale technical developments in solid state fermentation (20–40% TS) were realized only in recent years in Europe (Baeten & Verstraete 1993). A variety of reactor designs, which vary considerably in terms of operational temperature and residence time (Table 3), have come into practice in recent years. Figure 8 gives a typical flow scheme of a full-scale solid state digester and shows that anaerobic digestion can be a very valuable technology to convert source separated municipal solid waste into two valuable end products, *i.e.* energy (biogas) and a peat like compost (so-called humotex in this process). By this method, a final product is obtained which has been subjected to both anaerobic and aerobic microbial biodegradation processes; the resulting so-called double processed (DP) compost has improved hygienic qualities relative to conventional single processed (SP) compost (Gellens *et al.* 1995).

However, Figure 8 also indicates a rather complex scheme where the anaerobic digester has to be complemented by various physical and biological treatments to completely process the solid state waste. This is necessary as the digested anaerobic mash has, upon leaving the reactor, a low content of labile organics although it contains a large amount of amines, ammonium and sulphides. Moreover, its water content has risen to some 80-85% and it therefore needs dewatering. The separation of the residual solids and the subsequent treatment of the wastewater are of particular concern because these are rather odorous materials. Rechanneling anaerobic digestion pathways so that malodorous compounds can be avoided would be a major step forward. This can be done by redirection of metabolic fluxes by introduction of genetically manipulated organisms, as illustrated for the aerobic metabolism of aromatic products (Lee *et al.* 1994). In the present operational mode, the odorous compounds are removed by a final aerobic maturation period which is in the order of 1 to 2 weeks, but this still involves considerable investment and operation costs. The material leaving the thermophilic reactor is virtually pasteurized in terms of its aerobic microbiota. Subsequently, it is recolonized, particularly by microbes in the seed aerobic compost. It remains a challenge to append to the anaerobic process a specifically engineered aerobic process by which not only a soil conditioner is produced but an end product with a higher value. It would be of particular interest to use this material to produce large amounts of inocula with specific microbiological characteristics which could be used in the bioremediation of soils or dredged sediments.

Acknowledgements

Part of the research was financially supported by the EU Human Capital and Mobility network 'Improved Applicability of Anaerobic Digestion Technology' (ERBCHRXCT930262).

References

Alphenaar, P.A. 1994 Anaerobic granular sludge: Characterization and factors affecting its performance. Ph.D thesis, p.209. Wageningen Agricultural University, The Netherlands.

Alphenaar, P.A., Lettinga, G. & Visser, A. 1993 The effect of liquid upward velocity and hydraulic retention time on granulation in UASB reactors treating waste water with a high sulphate content. *Bioresearch Technology* **43**, 249–258.

Ahring, B.K. 1994 Status on science and application of thermophilic anaerobic digestion. *Water Science and Technology* **30**, 241–249.

Ahring, B.K., Christiansen, N., Mathrani, I., Hendriksen, H.V., Macario, A.J.L. & Conway de Macario, E. 1992 Introduction of a *de novo* bioremediation ability, aryl reductive dechlorination, into anaerobic granular sludge by inoculation of sludge with *Desulfomonile tiedjei*. *Applied and Environmental Microbiology* **58**, 3677–3682.

Ahring, B.K., Schmidt, J.E., Winther-Nielsen, M., Macario, A.J.L. & Conway de Macario, E. 1993 Effect of medium composition and sludge removal on the production, composition and architecture of thermophilic (55°C) acetate-utilizing granules from an upflow anaerobic sludge blanket reactor. *Applied and Environmental Microbiology* **59**, 2538–2545.

Allard, A.S, Hynning, P.A., Remberger, M. & Neilson, A.H. 1992 Role of sulphate concentration in the dechlorination of coumarin and flavanone glycones and aglycones. *Applied and Environmental Microbiology* **58**, 961–968.

Angelidaki, I. & Ahring, B.K. 1993 Effect of the clay mineral bentonite on ammonia inhibition of anaerobic thermophilic reactors degrading animal waste. *Biodegradation* **3**, 409–414.

Baeten, D. & Verstraete, W. 1993 In-reactor anaerobic digestion of MSW organics. In *Science and engineering of compost - Design, environmental, microbiological and utilization aspects*, eds Hoitink, H.A.J. & Keener, H.M. pp. 111–130, Ohio State University.

Bailey, A.D., Hansford, G. S. & Dold, P.L. 1994 The enhancement of upflow anaerobic sludge bed reactor performance using crossflow microfiltration. *Water Research* **28**, 291–295.

Bendixen, H.J. 1994 Safeguards against pathogens in Danish biogas plants. *Water Science and Technology* **30**, 171–180.

Bhadra, A., Scharer, J.M. & Moo-Young, M. 1987 Methanogenesis from volatile fatty acids in a down-flow stationary fixed-film reactor. *Biotechnology and Bioengineering* **30**, 314–319.

Bleicher, K. & Winter, J. 1994 Formate production and utilization by methanogens and by sewage sludge consortia. Interference with the concept of interspecies formate transfer. *Applied Microbiology and Biotechnology* **40**, 910–915.

Borja, R., Banks, C.J. & Wang, Z. 1995 Performance of a hybrid anaerobic reactor, combining a sludge blanket and a filter, treating slaughterhouse wastewater. *Applied Microbiology and Biotechnology* **43**, 351–357.

Buhr, H.O. & Andrews, J.F. 1977 The thermophilic anaerobic digestion process. *Water Research* **11**, 129–143.

Clark, L.B., Rosen, R.T., Hartman, T.G., Alaimo, L.H., Louis, J.B., Hertz, C., Ho, C.-T. & Rosen, J.D. 1991 Determination of nonregulated pollutants in three New Jersey publicly owned treatment works (POTWs). *Journal of Water Pollution and Control Federation* **63**, 104–113.

Coates, J. & Colleran, E. 1990 Effect of initial agitation on the start-up and operational performance of anaerobic hybrid reactors treating a synthetic feed. *Process Biochemistry* **25**, 162–171.

Colleran, E., Finnegan, S. & Lens, P. 1995 Anaerobic treatment of sulphate-containing waste streams. *Antonie van Leeuwenhoek* **67**, 29–46.

Costerton, J.W., Cheng, K.-J., Geesey, G.G., Ladd, T.I., Nickel, J.C., Dasgupta, M. & Marrie, T.J. 1987 Bacterial biofilms in nature and disease. *Annual Review of Microbiology* **41**, 435–464.

Daffonchio, D., Thaveesri, J. & Verstraete, W. 1995 Contact angle measurement and cell hydrophobicity of granular sludge from UASB reactors. *Applied and Environmental Microbiology* **61**. In press.

De Zeeuw, W. 1984 Acclimatization of anaerobic sludge for UASB-reactor start-up. Ph.D. thesis. p.157. Wageningen Agricultural University, The Netherlands.

Distefano, T.D., Gosset, J.M. & Zinder, S.H. 1991 Reductive dechlorination of high concentrations of tetrachloroethene to ethene by an anaerobic enrichment culture in the absence of methanogenesis. *Applied and Environmental Microbiology* **57**, 2287–2292.

Dolfing, J. 1988 Acetogenic dehydrogenations. In *Biology of Anaerobic Microorganisms*, ed Zehnder, A.J.B. pp. 417–468, Wiley and Sons: New York.

Dolfing, J. 1992 The energetic consequences of hydrogen gradients in methanogenic ecosystems. *FEMS Microbiology Ecology* **101**, 183–187.

Dolfing, J. & Harrison, B.K. 1993 Redox and reduction potentials as parameters to predict the degradation pathway of chlorinated benzenes in anaerobic environments. *FEMS Microbiology Ecology* **13**, 23–29.

Dolfing, J. & Janssen, D.B. 1994 Estimates of Gibbs free energy of formation of chlorinated aliphatic compounds. *Biodegradation* **5**, 21–28.

Dolfing, J. & Tiedje, J.M. 1986 Hydrogen cycling in a three-tiered food web growing on the methanogenic conversion of 3-chlorobenzoate. *FEMS Microbiology Ecology* **38**, 293–298.

Dong, X. & Stams, A.J.M. 1995 Evidence for H_2 and formate during syntrophic propionate and butyrate degradation. *Anaerobe* **1**, 35–39.

Eastman, J.A. & Ferguson, J.F. 1981 Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. *Journal of Water Pollution and Control Federation* **53**, 352–366.

Enzien, M.V., Picardal, F., Hazen, T.C., Arnold, R.G. & Fliermanns, C.B. 1994 Reductive dechlorination of trichloroethylene and tetrachloroethylene under aerobic conditions in a sediment column. *Applied and Environmental Microbiology* **60**, 2200–2204.

Fahmy, M., Kut, O.M. & Heinzle, E. 1994 Anaerobic-aerobic fluidized bed biotreatment of the sulfite pulp bleaching effluents- II Fate of individual chlorophenolic compounds. *Water Research* **28**, 1997–2010.

Fathepure, B.Z., & Vogel, T.Y.M. 1991 Complete degradation of polychlorinated hydrocarbons by a two-stage biofilm reactor. *Applied and Environmental Microbiology* **57**, 3418–3422.

Field, J.A., Stams, A.J.M., Kato, M. & Schraa, G. 1995 Enhanced biodegradation of aromatic pollutants in cocultures of anaerobic and aerobic consortia. *Antonie van Leeuwenhoek* **67**, 47–77.

Fujita M., Ike, M. & Hashimoto, S. 1991 Feasibility of wastewater treatment using genetically engineered microorganisms. *Water Research* **25**, 979–984.

Fukuzaki, S., Nishio, N. & Nagai, S. 1990 Kinetics of the methanogenic fermentation of acetate. *Applied and Environmental Microbiology* **56**, 3158–3163.

Fulthorpe, R.R., Liss, S.N. & Allen, D.G. 1993 Characterization of bacteria isolated from a bleached pulp mill wastewater treatment system. *Canadian Journal of Microbiology* **39**, 13–24.

Gellens, V., Boelens, J. & Verstraete, W. 1995 Source separation, selective collection and in reactor digestion of biowaste. *Antonie van Leeuwenhoek* **67**, 90–97.

Ghosh, S. & Lall, U. 1981 Kinetics of anaerobic digestion of solid substrates. In *Anaerobic digestion. Proceedings of the 5th Inter-*

national Symposium on Anaerobic Digestion, eds Hall, E.R. & Hobson, P.N. pp. 365–373. Oxford: Pergamon Press.

Govind, R., Flaherty, P.A. & Dobbs, R.A. 1991 Fate and effects of semivolatile organic pollutants during anaerobic digestion of sludge. *Water Research* **25**, 547–556.

Haggblom, M.M., Rivera, M.D. & Young, L.Y. 1993 Effects of auxiliary carbon sources and electron acceptors on methanogenic degradation of chlorinated phenols. *Environmental Toxicology and Chemistry* **12**, 1395–1403.

Harada, H., Momomoi, K., Yamazaki, S. & Takizawa, S. 1994 Application of anaerobic-uf membrane reactor for treatment of a wastewater containing high strength particulate organics. *Water Science and Technology* **30**, 307–319.

Hemens, J., Meiving, P.J.G. & Stander, G.J. 1962 Fullscale anaerobic digestion of effluents from the production of maize-starch. *Water Waste Treatment Journal* **9**, 16–18.

Hendriksen, H.V. & Ahring, B.K. 1992 Metabolism and kinetics of pentachlorophenol transformation in anaerobic granular sludge. *Applied Microbiology and Biotechnology* **37**, 662–666.

Hendriksen, H.V. & Ahring, B.K. 1993 Anaerobic dechlorination of pentachlorophenol in fixed-film and upflow anaerobic sludge reactors using different inocula. *Biodegradation* **3**, 399–408.

Hendriksen, H.V., Larsen, S. & Ahring, B.K. 1992 Influence of supplemental carbon source on anaerobic dechlorination of pentachlorophenol in granular sludge. *Applied and Environmental Microbiology* **58**, 365–370.

Hoehn, R.C. & Ray, A.D. 1973 Effects of thickness on bacterial film. *Journal of Water Pollution and Control Federation* **45**, 2302–2320.

Holliger, C., Schraa, G., Stams, A.J.M. & Zehnder, A.J.B. 1993 A highly purified enrichment culture couples the reductive dechlorination of tetra chloroethene to growth. *Applied and Environmental Microbiology* **59**, 2991–2997.

Hopkins, B.T., McInerney, M.J. & Warikoo, V. 1995 Evidence for an anaerobic syntrophic benzoate degradation threshold and isolation of the syntrophic benzoate degrader. *Applied and Environmental Microbiology* **61**, 526–530.

Huser, B.A., Wuhrmann, K. & Zehnder, A.J.B. 1982 *Methanothrix soehngenii* gen, nov. sp. nov., a new acetotrophic non-hydrogen-oxidizing methane bacterium. *Archives of Microbiology* **132**, 1–9.

Kato, M. 1994 The anaerobic treatment of low strength soluble wastewaters, Ph.D thesis, p. 159. Wageningen Agricultural University, The Netherlands.

Kato, M., Field, J.A. & Lettinga, G. 1993 Methanogenesis in granular sludge exposed to oxygen. *FEMS Microbiology Letters* **114**, 317–324.

Lee, J.Y., Roh, J.R. & Kim, H.S. 1994 Metabolic engineering of *Pseudomonas putida* for the simultaneous biodegradation of benzene, toluene and *p*-xylene. *Biotechnology and Bioengineering* **43**, 1146–1152.

Lens, P.N., De Beer, D., Cronenberg, C.H., Houwen, F.P., Ottengraf, S.P.P. & Verstraete, W.H. 1993 Heterogeneous distribution of microbial activity in methanogenic aggregates: pH and glucose microprofiles. *Applied and Environmental Microbiology* **59**, 3803–3815.

Lens, P. & Verstraete, W. 1992 Aerobic and anaerobic treatment of municipal wastewater. In *Profiles on biotechnology*, eds Villa, T.G. & Abalde, J. pp. 333–356. Universidade di Santiago de Compostela.

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

Lettinga, G. & Hulshoff Pol, L.W. 1991 UASB Process design for various types of wastewaters. *Water Science and Technology* **24**, 87–107.

Lettinga, G., Van Velsen, A.F.M., Hobma, W., de Zeeuw, J. & Klapwijk, A. 1980 Use of the upflow sludge blanket (USB) reactor concept for biological waste water treatment, especially for anaerobic treatment. *Biotechnology and Bioengineering* **22**, 699–734.

Mackie, R.I. & Bryant, M.P. 1995 Anaerobic digestion of cattle waste at mesophilic and thermophilic temperatures. *Applied Microbiology and Biotechnology* **43**, 346–350.

MacLeod, F.A., Guiot, S.R. & Costerton, J.W. 1990 Layered structure of bacterial aggregates produced in an upflow anaerobic sludge bed and filter reactor. *Applied and Environmental Microbiology* **56**, 1598–1607.

Madsen, T,. & Licht, D. 1992 Isolation and characterization of an anaerobic chlorophenol transforming bacterium. *Applied and Environmental Microbiology* **58**, 2874–2878.

McCarty, P.L. 1982 One hundred years of anaerobic treatment. In *Anaerobic digestion 1981*, eds Hughes, D.E., Stafford, D.A., Wheatly, B.I., Baader, W., Lettinga, G., Nyns, E.J. & Verstraete, W. pp. 3–22. Amsterdam: Elsevier.

McClure, N.C., Fry, J.C. & Weightman, J. 1991 Survival and catabolic activity of natural and genetically engineered bacteria in a laboratory-scale activated sludge unit. *Applied and Environmental Microbiology* **57**, 366–373.

McFarland, M.J. & Jewell, W.J. 1989 *In situ* control of sulfide emissions during the thermophilic anaerobic digestion process. *Water Research* **23**, 1571–1577.

Mohn, W.W. & Kennedy, K.J. 1992 Limited degradation of chlorophenols by anaerobic sludge granules. *Applied and Environmental Microbiology* **58**, 2131–2136.

Mohn, W.W. & Tiedje, J.M. 1991 Evidence for chemiosmotic coupling of reductive dechlorination and ATP synthesis in *Desulfomonile tiedjei*. *Archives of Microbiology* **157**, 1–6.

Morris, P.J., Mohn, W.W., Quensen, J.F. Tiedje, J.M. & Boyd, S.A. 1992 Establishment of a polychlorinated biphenyl-degrading enrichment culture with predominantly meta dechlorination. *Applied and Environmental Microbiology* **58**, 3088–3094.

Nagano, A., Arikawa, E. & Kobayashi, H. 1992 The treatment of liquor wastewater containing high-strength suspended solids by membrane bioreactor systems. *Water Science and Technology* **26**, 887–895.

Neuman, A.W., Good, R.J., Hope, C.J. & Sejpal, M. 1974 An equation-of-state approach to determine surface tensions of low-energy solids from contact angles. *Journal of Colloid and Interface Science* **49**, 291–304.

Nicholson, D.K., Woods, S.L., Istok, J.D. & Peek, D.C. 1992 Reductive dechlorination of chlorophenols by a pentachlorophenol acclimated methanogenic consortium. *Applied and Environmental Microbiology* **58**, 2280–2286.

Overmeire, A., Lens, P. & Verstraete, W. 1994 Mass transfer limitation of sulphate in methanogenic aggregates. *Biotechnology and Bioengineering* **44**, 387–391.

Parker, W.J., Hall, E.R. & Farquhar, G.J. 1993 Assessment of design and operating parameters for high rate anaerobic dechlorination of segregated kraft mill bleach plant effluents. *Water Environment Research* **65**, 264–270.

Pavlosthatis, J.G. & Gossett, J.M. 1986 Kinetic model for anaerobic digestion of biological sludge. *Biotechnology and Bioengineering* **28**, 1519–1530.

Pavlostathis, S.G. & Zuang, P. 1991 Transformation of trichloroethylene by sulphate-reducing cultures enriched from contaminated subsurface soil. *Applied Microbiology and Biotechnology* **36**, 416–420.

Pfeffer, J. 1974 Anaerobic digestion processes. In *Anaerobic*

Digestion, eds Stafford, D.A. Wheatley, B.I. & Hughes, D.E., pp. 15–35. London: Applied Science Publishers.

Pillay, V.L., Townsend, B. & Buckley, C.A. 1994 Improving the performance of anaerobic digesters at wastewater treatment works: the coupled cross-flow microfiltration/digester process. *Water Science and Technology* **30**, 329–337.

Priestley, A. 1990 Sewage treatment using magnetite particles. *Water and Wastewater* **5**, 31–34.

Ramanand, K., Nagarajan, A. & Suflita, J.M. 1993 Reductive dechlorination of the nitrogen heterocyclic herbicide picloram. *Applied and Environmental Microbiology* **59**, 2251–2256.

Rebac, S., Ruskova, J., Gerbens, S., van Lier, J.B., Stams, A.J.M. & Lettinga, G. 1995 High-rate anaerobic treatment of wastewater under psychrophilic conditions. *Journal of Fermentation and Bioengineering* **80**, 499–506.

Rinzema, A., Alphenaar, A. & Lettinga, G. 1993 Anaerobic digestion of long chain fatty acids in UASB-reactors and Expanded Granular Sludge Bed reactors. *Process Biochemistry* **28**, 527–537.

Rittmann, B.E. 1982 Comparative performance of biofilm reactor types. *Biotechnology and Bioengineering* **24**, 1341–1370.

Ross, W.R., Strohwald, N.K.H., Gobler, C.J. & Sanetra, J. 1994 Membrane-assisted anaerobic treatment of industrial effluents: the South African ADUF process. In *Proceedings of the 7th International Symposium on Anaerobic Digestion*, pp. 550–559. IAWQ, RSA Litho Ltd., South Africa.

Rozzi, A. & Verstraete, W. 1981 Calculation of active biomass versus waste composition in anaerobic contact processes. *Tribune de Cebedeau* **455**, 421–427.

Rutter, P.R. & Vincent, B. 1984 Physicochemical interactions of the substratum, microorganisms, and the fluid phase. In *Microbial adhesion and aggregation*, ed Marshal, K.C. pp. 21–38. Dahlem Konferenzen, Berlin, Heidelberg, New York: Springer-Verlag.

Schink, B. & Friedrich, M. 1994 Energetics of syntrophic fatty acid oxidation. *FEMS Microbiological Reviews* **15**, 85–94.

Smith, D.P. & McCarty, P.L. 1989 Reduced product formation following perturbation of ethanol- and propionate-fed methanogenic CSTRs. *Biotechnology and Bioengineering* **34**, 885–895.

Stams, A.J.M. 1994 Metabolic interactions between anaerobic bacteria in methanogenic environments. *Antonie van Leeuwenhoek* **66**, 271–294.

Stukenberg, J.R., Shimp, G., Sandino, J., Clark, J.H. & Crosse, J.T. 1994 Compliance outlook: meeting 40 CFT part 503 class B pathogen reduction criteria with anaerobic digestion. *Water Environment Research* **66**, 255–263.

Thauer, R.K., Decker, K. & Jungermann, K. 1977 Energy conservation in chemotrophic anaerobic bacteria. *Bacteriological Review* **41**, 100–180.

Thaveesri, J., Daffonchio, D., Liessens, B., Vandemeren, P. & Verstraete, W. 1995a Granulation and sludge bed stability in UASB reactors in relation to surface thermodynamics. *Applied and Environmental Microbiology* **61**, 3681–3686.

Thaveesri, J., Gernaey, K., Kaonga, B., Boucneau, G. & Verstraete, W. 1994 Organic and ammonium nitrogen in relation to granular sludge growth. *Water Science and Technology* **30**, 43–53.

Thaveesri, J., Liessens, B. & Verstraete, W. 1995b Granular sludge growth under different reactor liquid surface tensions in lab-scale upflow anaerobic sludge blanket reactors treating wastewater from sugar-beet processing. *Applied Microbiology and Biotechnology* **43**, 1122–1127.

Thiele, J.H. & Zeikus, J.G. 1988 Control of interspecies electron flow during anaerobic digestion: the role of formate versus hydrogen transfer during syntrophic methanogenesis in flocs. *Applied and Environmental Microbiology* **54**, 20–29.

Tholozan, J.L., Samain, E., Grivet, J.P. & Moletta, R., Dubourguier, H.C. & Albagnac, G. 1988 Reductive carboxylation of propionate to butyrate in methanogenic ecosystems. *Applied and Environmental Microbiology* **54**, 441–445.

Tholozan, J.L., Samain, E., Grivet, J.P. & Albagnac, G. 1990 Propionate metabolism in a methanogenic enrichment culture. Direct reductive carboxylation and acetogenesis pathways. *FEMS Microbiology Ecology* **73**, 291–298.

Uemura, S. & Harada, H. 1993 Microbial characteristics of methanogenic sludge consortia developed in thermophilic UASB reactors. *Applied Microbiology and Biotechnology* **39**, 654–661.

Van de Graaf, A.A., Mulder, A., de Bruijn, P., Jetten, M.S.M., Robertson, L.A. & Kuenen, J.G. 1995 Anaerobic oxidation of ammonium is a biologically mediated process. *Applied and Environmental Microbiology* **61**, 1246–1251.

Van der Last, A.R.M. & Lettinga, G. 1991 Anaerobic treatment of domestic sewage under moderate climatic conditions using upflow reactors at increased superficial velocities. In *Proceeding of the 6th International Symposium on Anaerobic Digestion*. Sao Paulo, 12–16 May.

Van Lier, J.B., Sanz Martin, J.L. & Lettinga, G. 1995 Effect of temperature on the anaerobic thermophilic conversion of volatile fatty acids by dispersed and granular sludge. *Water Research* **30**, 199–207.

Vanderhaegen, B., Ysebaert, E., Favere, K., Van Wambeke, M., Peeters, T., Panic, V., Vandenlangenbergh, V. & Verstraete, W. 1991 Acidogenesis in relation to in-reactor granule yield. *Water Science and Technology* **25**, 21–30.

Vandevoorde, L., Bruyneel, B. & Verstraete, W. 1988 Inreactor cellulose conversion. *Animal Feed Science and Technology* **21**, 99–125.

Verstraete, W. & Top E. 1992 Holistic environmental biotechnology. In *Microbial Control of Pollution*, eds Fry, J., Gadd, G., Herbert, R., Jones, C. & Watson-Craik, I.A. pp. 1–18. Cambridge: Cambridge University Press.

Visser, A., Alphenaar, P.A., Gao, Y., Van Rossum, G. & Lettinga, G. 1993 Granulation and immobilization of methanogenic and sulphate reducing bacteria in high-rate anaerobic reactors. *Applied Microbiology and Biotechnology* **40**, 575–581.

Wiegant, W.M. & De Man, A.W.A. 1986 Granulation of biomass in thermophilic anaerobic sludge blanket reactors. *Biotechnology and Bioengineering* **28**, 718–727.

Wujich, W.J. & Jewell, W.J. 1980 Dry anaerobic fermentation. *Biotechnology and Bioengineering* **10**, 43–65.

Zinder, S.H. & Koch, M. 1984 Non-acetoclastic methanogenesis from acetate-acetate oxidation by thermophilic syntrophic coculture. *Archives in Microbiology* **138**, 263–272.

(Received in revised form 10 January 1996; accepted 30 January 1996)