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MICROBIOLOGICAL ASPECTS OF ANAEROBIC DIGESTION

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ABSTRACT

This paper reviews aspects of the microbiology of anaerobic digestion and focusses on the importance of interspecies reactions in the operation and control of anaerobic reactors. The potential role of micronutrient supplementation in the selection and optimization of digester populations is discussed. The application of specific methanogenic activity test procedures, phospholipid and archaebacterial lipid analyses and methanogen immunotyping to the characterization of digester biomass is reviewed. Current microbiological approaches to the study of methanogenic biofilm and granule formation are also discussed.

INTRODUCTION

The energy crisis of the seventies greatly stimulated engineering research on anaerobic digestion of industrial wastewaters, both in Europe and in North America, and resulted in the development of a new generation of anaerobic digester designs based on biomass recycle or on biomass retention independent of waste flow. This reduced reactor volume requirements and improved process stability and control, counteracting the early feelings of unreliability associated with anaerobic treatment. These advances stimulated adoption of the technology at full-scale for industrial wastewater treatment, as evidenced (Table 1) by the EEC-commissioned survey of biogas plants in Europe (1) and by the more recent review of biogas developments in North America by Pohland and Harper (2).

Major advances have also been made during the past decade, in our understanding of the microbiology of anaerobic digestion. Greater appreciation of the importance of bacterial interactions is already providing more informed guidelines for anaerobic digester operation and control. This paper reviews aspects of current microbiological research and indicates their potential impact on the continued development of anaerobic digestion technology.

Table 1. Biogas Plants Treating Industrial Wastes in Europe[a] and North America[b]

Reactor Type	Europe		North America	
	Pilot-Scale	Full-Scale	Pilot-Scale	Full-Scale
Anaerobic Contact	2	21	3	2
Anaerobic Filter	9	3	11	5
UASB	4	29	4	5
Fluidised Bed	0	0	6	0
Hybrid UASB/AF	0	0	0	1
Mixed Digester	2	12	4	1
Others	2	5	4	4
Total	19	70	32	18

[a]Data as of 1983 from the EEC biogas survey of Demuynck et al. (1) for Belgium, Denmark, France, West Germany, Greece, Ireland, Italy, The Netherlands, United Kingdom and Switzerland. The survey is currently being updated to December 1986 status.
[b]Data collected in 1985 by Pohland and Harper (2).

MICROBIAL INTERACTIONS

Anaerobic reactors present a unique ecosystem in which diverse groups of bacteria catalyze the conversion of complex organic compounds to methane and carbon dioxide in a highly controlled and coordinated fashion (3). Methanogenesis was initially considered to be a two-phase process in which the volatile fatty acid (VFA) and other fermentation end-products of hydrolytic/fermentative bacteria were directly converted to CH_4 and CO_2 by methanogenic species. The multiphase nature of the process was subsequently revealed by the discovery of hydrogen-producing acetogenic bacteria and by a better appreciation of the limited substrate capabilities of methanogens (Fig. 1).

Advances in our knowledge of the microbiology of methanogenesis have highlighted the role of interspecies H_2 transfer in anaerobic digestion. Obligate H_2-producing acetogens (OHPA species) oxidize VFA fermentation products, such as propionate, butyrate, etc., to acetate, CO_2 and H_2 (Fig. 1). Their importance in methanogenesis is evidenced by the finding

that propionate and butyrate are the primary precursors of acetate and H_2 in sewage sludge digesters (4). Acetogenic dehydrogenation of VFA is thermodynamically unfavorable when the H_2 partial pressure (P_{H_2}) exceeds 10^{-3} to 10^{-4} atm (5). VFA conversion in digesters is rendered feasible, however, through simultaneous removal of H_2 by H_2-utilizing species, such as the hydrogenotrophic methanogens (5,6). Until recently, OHPA isolates could only be maintained in the laboratory in syntrophic coculture with H_2-utilizing methanogens or sulphate-reducing Desulfovibrio spp. (which provide an alternative electron sink).

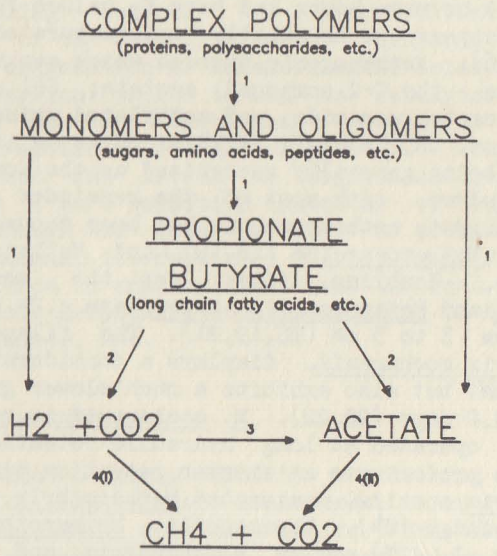

Figure 1. Multiphase Nature of Anaerobic Digestion

1: hydrolytic and non-hydrolytic fermentative bacteria
2: syntrophic acetogens (OHPA)
3: homoacetogens
4: (i) hydrogenotrophic methanogens
(ii) aceticlastic methanogens

To date, only a limited number of OHPA species has been isolated. These include the mesophilic species, Syntrophomonas wolfei (7) and Syntrophobacter wolinii (8), which oxidize butyrate and propionate, respectively. The generation times of these organisms are extremely long, with respective doubling times of 84 and 161 hours for S. wolfei and S. wolinii in methanogenic syntrophic coculture. The recent finding of Beaty and McInerney (9), that S. wolfei can be maintained in pure culture on a crotonate medium, may be expected to accelerate the biochemical study of this key digester group. OHPA species are also known to be involved in the β-oxidation of longer-chain fatty acids (stearate, oleate, etc.) arising

from lipid hydrolysis (10) and in the anaerobic degradation of aromatic compounds (11). The doubling time of the benzoate oxidizer isolated by Mountfort et al. (11) is 166 hours in methanogenic coculture. A thermophilic OHPA species which oxidizes butyrate in coculture with a H_2-utilizing methanogen has been isolated by Henson and Smith (12). Interspecies H_2 transfer is also involved in the oxidation of acetate to CH_4 and CO_2 by a thermophilic syntrophic coculture (13).

Maintenance of low P_{H_2} values in digesters is primarily dependent upon the activity of the H_2-utilizing methanogens. Almost all known methanogens convert H_2/CO_2 to CH_4 (14). Mesophilic species exhibit doubling times of the order of 3 or more hours and have K_s values for H_2 in the range 2.5-13 μM (15). They appear to be greatly undersaturated for H_2 in stably operating digesters (16). Methanogenic species which can utilize, as direct methanogenic substrates, the C-2 compound, acetate; C-1 compounds, such as formate, methanol and carbon monoxide, and methylated amines have also been isolated (14). Of these, only acetate is regarded as an important precursor of CH_4 in digesters, being generally recognized as the source of two-thirds or more of the CH_4 evolved, with most of the remainder coming from CO_2 reduction (17). Aceticlastic methanogenesis has been documented for only two methanogenic genera: Methanosarcina (18,19) and Methanothrix (20). With acetate as substrate, doubling times for the mesophilic species, Methanosarcina barkeri and Methanosarcina mazei, are c.24 hours and reported K_s values range from 3 to 5 mM (18,19,21). The filamentous mesophilic aceticlast, Methanothrix soehngenii, displays a considerably lower K_s value for acetate (0.5-0.7 mM) but also exhibits a much slower growth rate, with a doubling time of 3.5-9.0 days (20,22). M. soehngenii is reported to be more abundant in digesters operated at long hydraulic retention times whereas Methanosarcina tend to predominate at shorter retention times (23). A second mesophilic Methanothrix species, designated Methanothrix concilii, has been isolated from sewage sludge (24). Thermophilic Methanothrix spp. have been described by Zinder et al. (25) and by Nozhevnikova and Yagodina (26). A thermophilic Methanosarcina species, Methanosarcina thermophila, has also been isolated and characterized (27,28).

MONITORING AND PROCESS CONTROL

Parameters in current engineering use for routine monitoring of digester performance include organic/hydraulic loading rate, biogas/CH_4 productivity, COD/BOD reduction, alkalinity, pH and VFA concentrations (29). A fall in biogas production rate, an increase in VFA concentration or a change towards acidity are accepted indicators of potential digester failure. Appreciation of the central role of hydrogen in the overall process, however, suggests that these changes may be regarded as the result rather than the cause of digester imbalance. Monitoring of the partial pressure of H_2 is being considered as a potentially sensitive means of early detection of fermentation failure (5). Studies are currently in progress to develop reliable methods for estimation of H_2 levels in digester gases and mixed liquors (30) and to correlate fluctuations in H_2 concentration with changes in feed composition, loading rate and other process variables, for both soluble and particulate feedstocks (31).

Propionic acid may also be used as an indicator of digester balance. The P_{H_2} range in which acetogenic dehydrogenation of propionate and hydrogenotrophic generation of CH_4 can both proceed exergonically is extremely narrow (5,6). Any digester imbalance which results in H_2 partial pressures outside that range should, therefore, cause an immediate increase in propionate. This could be used to signal corrective measures before the microbial community becomes more drastically disturbed by acid accumulation and the consequent fall in pH.

Recent studies on the digestion of complex organic wastes have suggested that other process intermediates may also be used as indicators of the efficiency of coupling between the hydrolytic/acidogenic and the methanogenic phases of the overall process. Phenylacetic acid (PAA) is an intermediate of the degradation of aromatic amino acids and lignocellulosic materials. The conversion of PAA to CH_4 is catalyzed by the syntrophic activity of the OHPA species, Syntrophus buswellii, in association with hydrogenotrophic and aceticlastic methanogens (11). Iannotti et al. (32) reported that effluent PAA levels in digesters treating swine manure appeared to provide a more sensitive indicator of digester stress than volatile acids. Although the data reported is preliminary in nature, it implies that key process intermediates (other than VFA) may be used to monitor digester balance in applications where the feed is rich in lipids or more refractory organic compounds, such as aromatic or halogenated aliphatic compounds, lignocellulose, etc.

EFFECTIVE BIOMASS MEASUREMENT

The assay procedure most commonly used in engineering practice for seed sludge quantification and biomass measurement in operational digesters is the determination of volatile suspended solids (VSS). These analyses provide crude estimates of effective microbial biomass since they also measure non-microbial organic material contributed by wastewaters containing insoluble organics (e.g. animal manures, crop residues, starch wastewaters, etc.). Furthermore, these methods give no indication of the population density or functional activities of different trophic groups in seed sludges or in digester biomass. Direct enumeration techniques are not a viable alternative since they are tedious and time-consuming; require access to anaerobic culture facilities and may yield misleading data, as species of importance in digesters may not necessarily flourish under the diagnostic enrichment/isolation conditions used, and vice versa (33).

Since methanogenic bacteria exhibit a unique biochemistry, the possibility of quantifying the methanogenic biomass in digesters by measurement of unique methanogen components has been investigated by a number of researchers. Coenzyme F_{420} - a deazaflavin analogue which plays a key metabolic role in methanogens as an electron carrier - has received most attention. It is present in all methanogens tested to date and does not occur in other bacteria, with the exception of Streptomyces griseus and Anacystis nidulans. Although simple, reliable procedures are now available for F_{420} measurement (34,35), wide variation in individual methanogen F_{420} levels (Table 2) complicates interpretation of measured sludge F_{420} concentrations (35,36). Observations by van Bruggen et al. (37), that the

coccoid form of Methanosarcina mazei exhibits a weak F_{420} fluorescence compared to the strong fluorescence of the sarcinal aggregate, suggest that F_{420} levels may also vary with morphological form, thus further complicating interpretation of sludge F_{420} data.

Table 2. Coenzyme F_{420} Content of Mesophilic Methanogens[a]

Organism	F_{420} (nMole.gVSS^{-1})
H_2-utilizing methanogens	
Methanobacterium bryantii	1,400-2,400
Methanobrevibacter ruminantium	40
Methanobrevibacter arboriphilus	1,800
Methanospirillum hungatei	1,900
Formate-utilizing methanogens	
Methanobacterium formicicum	3,000-3,600
Acetate-utilizing methanogens	
Methanosarcina acetivorans	27
Methanosarcina barkeri, 277	9[b]
Methanosarcina barkeri, MS	19[c]
Methanothrix soehngenii	330

[a]Data adapted from Dolfing and Mulder (38).
[b]Expressed as nMole.g protein^{-1} (39).
[c]Expressed as nMole.g (wet wt) cells^{-1} (40).

Correlation of F_{420} concentrations with potential methanogenic activity for individual substrates is also extremely difficult, since the F_{420} levels maintained by acetate-utilizing methanogens are much lower than those of the H_2/CO_2 or formate-utilizing species (Table 2). A recent study by Dolfing and Mulder (38), using granular sludges cultivated on various direct and indirect methanogenic substrates (acetate, ethanol, propionate, etc.), revealed a good correlation only with formate as substrate. Measurement of sludge F_{420} levels may, however, be useful in determining the vertical

distribution of methanogenic bacteria in upflow or downflow fixed-bed reactors or the distribution of methanogens between the biofilm and mixed liquor in fixed-bed and hybrid reactors (41).

The distinctive archaebacterial membrane lipids present in all methanogens contain unique phytanyl glycerol ethers which can be extracted, purified and quantified by HPLC procedures (42). Consequently, measurement of the phytanyl glycerol ether content of anaerobic sludges has potential as a technique for estimating methanogenic biomass content. For non-methanogenic trophic groups, measurement of membrane phospholipid esters has been shown to provide a good estimate of microbial biomass and to correlate well with extractable ATP measurements (43). In addition, analysis of individual phospholipid fatty acids may permit more detailed characterization of microbial community structure since some fatty acids are unique to individual bacteria, or to groups of bacteria, and may function as molecular signatures for these species (44). Measurement of both phytanyl glycerol ethers and membrane phospholipids and identification of individual phospholipid fatty acids, in anaerobic sludges, may prove useful in determining the relative contents of methanogenic and non-methanogenic biomass; in monitoring changes in the ratio of the two populations and in assessing individual species distribution in response to changes in loading rate, presence of toxicants, etc. Lipid analyses may also be used to distinguish between microbial and non-microbial VSS in digesters treating plant biomass or animal waste feedstocks (43).

BIOMASS ACTIVITY MEASUREMENTS

Detailed knowledge of the activities of different trophic groups in seed sludges, mixed liquors, biofilm and granule samples should assist in defining start-up conditions and assessing maximum loading capacities for operational reactors. Earlier methods, which were confined to estimating methanogenic activity levels with acetate as substrate, required large amounts of sludge and were both time-consuming and error-prone (35). Routine test procedures have now been developed, based on gas chromatography and pressure-lock syringe sampling (45) or pressure-transducer monitoring (35) of the headspace in test vials. These methods are sensitive, rapid and reliable, and can be utilized to determine the potential methanogenic activities of digester sludges against the full range of direct and indirect methanogenic substrates. Activity test procedures may also be used to assess the inhibitory effect of toxicants on individual methanogenic and OHPA populations in crude sludges; to study the effect of changes in process parameters on the distribution and activity of these populations and to characterize the microbiological composition of biofilms and granules developed in reactors under different loading rates and with varying feed compositions.

ANAEROBIC BIOFILM FORMATION

Start-up and long-term operation of fixed-bed reactors, particularly if operated in downflow mode with modular or channelled support materials, is dependent upon the initial development of a competent biofilm on the support

surface and upon its continued maintenance in a form which ensures efficient contact and reaction of the influent substrate with the retained flora. It is important, therefore, to determine the influence of the support material on the attachment of different trophic groups and of individual species within these groups. Such studies have already been initiated (46,47) and may be expected to provide a more valid basis for choice of support material type and packing arrangement. For example, efficient biofilm retention of slow-growing organisms, such as the OHPA bacteria or the aceticlast, Methanothrix soehngenii, could accelerate start-up and ensure more stable operation and more efficient COD removal at possibly higher loading rates. Ongoing studies have shown that Methanosarcina mazei MC3 does not attach to a wide range of plastic and clay supports at the initial adhesion stage of biofilm formation (48). Methanosarcina spp. have, however, been reported to play a dominant role in anaerobic biofilms during the start-up phase (35,49). The possibility that the presence of Methanosarcina spp. results from passive entrapment, rather than active involvement in biofilm formation, merits further study. Studies of this kind should assist in the definition of start-up regimes and could be economically beneficial if they result in significantly shorter start-up times for fixed-film reactors.

SLUDGE GRANULATION

Studies on mesophilic granule formation have shown that varied granular forms may be cultivated on different wastewaters and under different start-up conditions (50,51). Granules developed on mainly VFA feeds tend to be of the "filamentous" type, up to 5 mm in size and mechanically fragile. They are reported to contain inert carrier material and to be dominated by a highly filamentous form of Methanothrix, presumed to be M. soehngenii. More robust, "rod"-type granules develop on sugar beet or potato processing wastewaters. These are generally smaller (up to 3 mm in size), contain no detectable inert carrier and are again dominated by M. soehngenii-like species, but in a much shorter chain-length form (50,51). Granules containing Methanosarcina spp. as the dominant aceticlast can also be cultivated under high VFA concentrations and low pH (50,52). However, Lettinga et al. (52) consider their development in reactors to be undesirable since they exhibit poor activity at low acetate concentrations and are prone to washout because of their small size (< 0.5 mm). A fourth type of granule has been reported from a full-scale plant treating maize starch production wastewater which contained an exceptionally high Ca^{++} content (> 700 $mg.L^{-1}$). These "spiky" granules (c. 1 mm in length) were dominated by filamentous organisms and contained up to 60% by weight of $CaCO_3$ (50,51).

At concentrations below 200-300 ppm, Ca^{++} has been shown to exert a positive effect on granule formation (53). This has been attributed to the chelating effect of Ca^{++} bridges and to the increased density arising from $CaCO_3$ precipitation (54). Physicochemical studies by Mahoney et al. (55) also provide further evidence of the importance of divalent cation bridging in the initial stages of granulation. Higher concentrations of Ca^{++} adversely affect granule formation due to excessive precipitation of $CaCO_3$ and $(Ca)_3(PO_4)_2$ (51). This causes scaling of the outer surfaces of pre-formed granules, increasing granule density and decreasing sludge activity. The

high sludge concentrations which result at the base of the reactor make efficient contacting of the wastewater with the sludge impossible (52).

Formation of thermophilic granules has also been investigated. Wiegant et al. (56) initially failed to obtain granulation during a 300 day study with pure VFA mixtures. Granulation occurred rapidly, however, with glucose or sucrose feedstocks. The granules obtained differed from the common mesophilic forms, being smaller in size (c. 1.3 mm) and consisting mainly of thermophilic Methanosarcina spp. (56). Successful formation of very small thermophilic granules (c. 0.2 mm), on a mixture of acetic, propionic and lactic acids, has recently been reported by Endo and Watanabe (57). Much larger aggregates (c. 3.0 mm) have been obtained by Bochem et al. (58) in chemostat studies of acetate enrichments. These granules consisted of densely-packed Methanosarcina clusters surrounding a more loosely-packed central area which contained at least two non-methanogenic species.

Adaptation of granular sludge, cultivated initially on one type of industrial wastewater, to a different waste stream has not always been successful at laboratory or at full-scale. Early studies by Lettinga et al. (59) showed that granules with high specific activity and excellent settling properties form readily on sugar beet processing wastewater. Transfer of granules, developed on sugar beet or corn starch wastewaters, to a sucrose feedstock resulted in the development of voluminous layers of bacterial biomass and gelatinous exopolymers on the granule surfaces, causing a severe decrease in sludge settleability and ultimate washout from the reactor (60). These authors concluded that a sudden change in feed composition cannot always be accommodated by granular sludge developed initially on mainly acidified effluents. Granules with excellent settling properties could, however, be formed ab initio with sucrose-containing feedstocks (60).

Similarly, initiation of granulation with more recalcitrant wastewaters has not always been successful, as shown by pilot-scale studies on sludge conditioning liquor in Canada (61). Granulation was not obtained during 360 days of operation, despite repeated re-seeding, modification of the gas-liquid-solids separator and eventual operation in anaerobic contact mode with effluent solids settlement and recycle. Change to a brewery wastewater, however, resulted in granulation within six weeks (61).

Although granulation occurs with a variety of wastewaters, further studies are required in order to ascertain the roles of individual bacterial groups or species and of insoluble inorganic materials (e.g. calcium) in effective granule formation. Identification and study of the Methanothrix species which dominate mesophilic "filamentous" or "rod"-type granules is of particular interest, in view of reported differences in doubling times and K_s values for acetate between M. soehngenii and M. concilii (20,24). The possible involvement of carbohydrate decomposing bacteria, as suggested by Dolfing and Bloemen (62), also merits investigation.

MICRONUTRIENT EFFECTS

An increasing number of digester studies indicates considerable stimulation of methanogenesis by supplementation with trace elements (63,64,65).

Bioavailability of minerals to digester microorganisms is difficult to measure and what appears to be a sufficient concentration of individual micronutrients in a feedstock may not equate with adequate bioavailability (64).

Recent studies by Speece et al. (65), on methane production from acetate in mixed reactors receiving unlimited substrate and operated at 10 or 20 day HRT/SRT, highlighted interesting effects of nutrient supplementation. The enrichment culture, which had been maintained in the laboratory for eight years on a synthetic medium containing a variety of inorganic nutrients, appeared to be dominated by Methanothrix-like rods. Additional supplementation with 13 $mg.L^{-1}$ Fe^{++} above the basal level was shown to change the microbiological dominance of the enrichment within 10 days to a Methanosarcina mazei-like organism and to increase the maximum acetate utilization rate from 3-4 to 30-40 g $acetate.L^{-1}.d^{-1}$. This change was inhibited if either phosphate or sulfide anions were added with the extra Fe^{++}, indicating that bioavailability of the iron was essential for the population change (65). The higher acetate utilization rates were sustainable for only a short period (c. 7 days), during which the Methanosarcina-like cells were shown to become associated in large aggregate form. The acetate utilization rate then dropped abruptly to c. 5 $g.L^{-1}.d^{-1}$. This was shown to correlate with a break-up of the aggregates to individual coccoid cells. Maintenance of Ca^{++} and Mg^{++} levels above 200 $mg.L^{-1}$ prevented de-aggregation and maintained high acetate utilization rates for prolonged periods (65).

Pure culture studies by Scherer et al. (66) have yielded conflicting results. Omission of Ca^{++} from the growth medium resulted in flocculant growth of Methanosarcina barkeri strain Fusaro (flocs of 1 mm size), whereas addition of 2 mM $CaCl_2$ caused an immediate change to a dispersed growth form. By contrast, M. barkeri strain Julich grew in large aggregate form (c. 3 mm) in the presence of Ca^{++}. Ability to aggregate appears to be a strain-specific property of strain Julich and to be associated with a significantly higher polysaccharide content, rich in glucuronic acid residues (66).

These, and other studies, suggest that the bioavailability of a variety of trace elements, vitamins and other nutrients may have dramatic effects on the microbial composition of digester biomass. A better understanding of the microbial requirements for, and the bioavailability of, trace elements and other nutrients in anaerobic reactors may permit greater control of both the composition and the activity of the microbial populations involved.

BIOMASS CHARACTERIZATION

The immunological procedures developed by Conway de Macario and Macario (67,68) appear to offer most promise for the rapid identification of methanogenic species active in anaerobic digesters. These authors have assembled a comprehensive bank of antisera against 30 reference methanogens available in pure culture (69) and have developed a series of calibrated polyclonal antibody probes from the antisera bank. Testing of individual methanogens against all of the available probes has provided a sequence of

data, distinctive for each methanogen and referred to as its antigenic fingerprint. Arrangement of the fingerprint data in a matrix formed by the phylogenetic organization of methanogens clearly showed that antigenic relatedness coincides with phylogenetic kinship (67). Arrangement of the data in this way permitted selection of representative methanogens for development of panels of monoclonal antibodies using mouse hybridoma techniques (68). Many of the monoclonal antibodies studied to date are monospecific, reacting exclusively with a single antigenic determinant on a particular methanogen immunotype (69).

The availability of polyclonal and monoclonal antibody probes will permit precise identification and quantification of individual methanogens - not only at the species or strain level but at the immunotype level - in microlitre samples of digester mixed liquor, biofilm and granule preparations. In particular, this will permit identification of immunotypes active under start-up conditions; assessment of immunotype changes in response to duration of operation, alteration of HRT or increase in loading rate; correlation of immunotypes with wastewater composition; identification of predominant immunotypes in granules and in biofilms developed on different support materials, etc. (70). Ultimately, such information should prove invaluable for definition of seeding, start-up and operational regimes and for optimization of reactor design with respect to individual wastewater types.

CONCLUSION

Developments in reactor design and operation have established anaerobic digestion as an accepted process for industrial wastewater treatment. Parallel advances in our understanding of the complex microbiology of the process are providing new insights into microbial interactions and into factors governing the dominance, activity and maintenance of individual species in digester mixed liquors, biofilms and granules. This knowledge will contribute significantly to improved design, start-up, process control and operation of anaerobic digesters, with corresponding effects on the efficiency, stability and cost effectiveness of full-scale anaerobic waste treatment applications.

Use of monoclonal antibody probes may be expected, in the long term, to identify the methanogen immunotypes most promising for genetic engineering manipulation (70). The discovery of methanogen plasmids (71), the identification of a phage specific for a *Methanobrevibacter* species (72) and the more recent isolation and characterization of a *Methanothrix* phage (73) have increased the possibility of developing genetic exchange systems for methanogens. In addition, cloning studies have been successful in obtaining expression of functional methanogen gene products in *Escherichia coli* and *Bacillus subtilis* (74,75). These developments suggest that future microbiological research will continue to complement process and engineering studies, expanding the application of anaerobic digestion technology in the areas of wastewater treatment and renewable energy production.

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