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USE OF METHANOGENIC ACTIVITY TESTS TO CHARACTERIZE ANAEROBIC SLUDGES, SCREEN FOR ANAEROBIC BIODEGRADABILITY AND DETERMINE TOXICITY THRESHOLDS AGAINST INDIVIDUAL ANAEROBIC TROPHIC GROUPS AND SPECIES

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ABSTRACT

A variety of test procedures have been reported in the literature for determination of the specific methanogenic activity profile of anaerobic sludges and for screening synthetic organic chemicals for anaerobic biodegradability. This paper reviews the methods developed and presents data obtained in the authors' laboratory using test procedures based on pressure transducer measurement of gas pressures in sealed anaerobic vials. Data on the feasibility of freeze-drying granular sludge are also presented as part of a wider study concerning the development of a standard reference sludge for anaerobic biodegradability testing. The use of the methanogenic activity test method to assess the relative toxicity of compounds, known to inhibit methanogenesis, against individual trophic groups involved in the overall digestion process is also described.

KEYWORDS

Anaerobic digestion; specific methanogenic activity; biodegradability, recalcitrance; anaerobic toxicity.

INTRODUCTION

Anaerobic digestion is now recognised as a complex process involving the coordinated activity of a number of different bacterial trophic groups (McInerney et al., 1980; Gujer and Zehnder, 1983; Zinder, 1984). Many of the species involved exhibit slow growth rates and require strict anaerobic conditions for cultivation or, as in the case of the syntrophic bacteria, cannot be cultivated in monoculture. The situation is further complicated by the fact that, in modern anaerobic digesters, anaerobic biomass is routinely developed either as a biofilm attached to an inert support or as discrete granules or flocs developed within the reactor as a result of the physico-chemical conditions applied.

Methodology for the characterisation of anaerobic sludges and for quantification of the relative proportions of the different trophic groups present is an increasingly important requirement for monitoring both the routine operation of sophisticated anaerobic digester designs and the initial development, during start-up, of biofilm and granular sludges (Iza et al., 1991). There is also a need for reliable methods for determination of the anaerobic biodegradability of xenobiotic chemicals (Holliger et al., 1988) and for assessment of the toxicity of such chemicals to the various subpopulations involved in the anaerobic digestion process (Iza et al., 1991).

MEASUREMENT OF SPECIFIC METHANOGENIC ACTIVITY

The start-up and operation of anaerobic digesters has, in the past, tended to be a rather empirical process in which the design loading rate was expressed in terms of reactor volume without reference to the quality or

quantity of either the seed sludge or the active biomass developed within the reactor during operation (Reynolds, 1986). Biomass quantification, when employed, was measured in terms of volatile suspended solids (VSS) or total suspended solids (TSS) and was mainly quantified for the purpose of estimating the amount of biological sludge requiring disposal. Although VSS measurements are easy to perform and give some indication of the quantity of biomass retained within a reactor, the assay does not distinguish between microbial biomass and any other particulate organic material that may be retained within the reactor, nor does it give any indication of the potential methanogenic activity of the microbial biomass present (Reynolds, 1986). Use of the most probable number viable counting technique for enumeration of the various sub-populations present in anaerobic sludge is not a practical proposition due to the long doubling times, the strict anaerobic conditions required and the difficulty experienced in cultivating some of the species involved. Consequently, interest has focussed on alternative methods based either on the quantification of specific molecular components exclusively associated with certain trophic groups or on determination of the specific biological activity of key reference groups.

Since coenzyme F_{420} is known to be exclusively present in methanogenic species (Cheeseman et al., 1972; Keltjens and Vogels, 1981), its quantification as an index of the specific methanogenic activity of sludges was suggested by Delafontaine et al. (1979) and by de Zeeuw and Lettinga (1980, 1983). However, due to the variability in F_{420} content of different methanogens and the effect of environmental conditions on the F_{420} level in individual species, it has not proved possible to use F_{420} determination as a reliable index of potential methanogenic activity (Dolfing and Mulder, 1985; Reynolds and Colleran, 1987). More meaningful data for prediction of methanogenic activity may be obtained by extraction and separate quantification of individual F_{420} types or other unique cofactors and coenzymes (Gorris and van der Drift, 1986a, b) but the techniques employed are too complex for routine analysis.

A variety of methods have been developed for direct measurement of the specific methanogenic activity of digester sludges. The majority of the test methods developed rely on measurement of the CH_4 produced rather than on depletion of the substrate supplied (Van den Berg et al., 1974; Owen et al., 1979; Valcke and Verstraete, 1983; Dolfing and Bloemen, 1985). Early methods relied either on quantification of the CH_4 produced by liquid displacement in external gas collectors (Valcke and Verstraete, 1983) or by displacement of the piston in glass syringes whose needles were inserted into the vial stoppers (Owen et al., 1979). Van den Berg and co-workers (1974) used Warburg manometric methods to follow methane production rates and this method has recently been re-evaluated by James et al. (1990) using a modified Warburg apparatus. The method developed by Dolfing and Bloemen (1985) was based on gas chromatographic analysis of the CH_4 content of the headspace gas in sealed activity test vials. This method required gas sampling with a pressure lock syringe which allowed quantification independent of the pressure prevailing in the vials. Since Dolfing and Bloemen (1985) utilised a range of direct and indirect methanogenic substrates, the assay procedure developed allowed estimation of the specific activities of different metabolic groups. By comparison with the specific activities of pure cultures, an estimate could also be obtained of the proportion of sludge biomass contributed by a particular trophic group.

Reynolds (1986) and Concannon et al. (1988a, b) modified the method developed by Shelton and Tiedje (1984) for anaerobic biodegradability testing and used electronic pressure transducer techniques to monitor the pressure increase produced in sealed test vials resulting from biogas release to the headspace during digestion of non-gaseous test substrates, such as acetate, butyrate, propionate, etc. A portable transducer system was initially developed which permitted frequent measurement of vial pressure with negligible loss of headspace gas during readings (Reynolds, 1986; Concannon et al., 1988a). The pressure transducer system was subsequently computerised using multiplexer chips and a BBC microcomputer (Concannon et al., 1988a). The latter system permitted continuous or discontinuous measurement of the pressure increase in a series of linked vials. Gas chromatographic analysis of samples removed from the headspace of test vials on completion of the test allowed correlation of the pressure developed with the millilitres of CH_4 produced, as described by Shelton and Tiedje (1984).

The test procedure was recently modified by Coates (1991) to allow measurement of the specific methanogenic activity of sludges against the gaseous substrate, H_2/CO_2. As noted by Dolfing and Bloemen (1985), transfer of H_2 from the gaseous to the liquid phase was found to be the rate-limiting factor at high specific activities for H_2 and even at quite low sludge concentrations in the test vial. The assay protocol developed suggests appropriate headspace to liquid volume ratios, mixing regimes and initial headspace gas pressures which ensure accurate determination of the specific hydrogenotrophic activity of the majority of test sludges. When the specific activity against H_2/CO_2 is extremely low, as with some sewage and animal manure sludges, measurement of the H_2/CO_2 depletion rate requires lengthy assay periods and is complicated by the growth of the H_2-utilising methanogens which exhibit relatively short doubling times. Coates (1991) showed that growth of these species occurred during the assay even when the assay medium contained none

of the normal inorganic nutrients required for growth. Presumably, sufficient nutrients were contributed to the assay mixture by the sludge inoculum.

Table 1 illustrates the specific methanogenic profiles obtained for a variety of different sludge samples using the pressure-transducer test method. In all cases tested, good agreement was obtained between the values derived by the pressure transducer method and those obtained using the headspace gas analysis method of Dolfing and Bloemen (1985). The pressure-transducer method, since it relies solely on the readings taken from the digital panel meter attached to the sensing element of the portable device and linked to the test vial by a needle inserted through the butyl rubber stopper, is extremely easy to perform, is far less labour-intensive than the Dolfing and Bloemen method and facilitates multiple replicate analysis. The assay also allows more accurate determination of initial rates than is obtainable with the GC based assay which is prone to analytical error at the very low concentrations of CH_4 in the headspace during the early stages of the assay.

TABLE 1 Specific Methanogenic Activity Profiles of Anaerobic Sludges Cultivated on Different Wastewaters

Wastewater used for sludge development	Specific Methanogenic Activity (μMole $CH_4.g^{-1}VSS.min^{-1}$)					
	Ac	Pro	Bu	MeOH	EtOH	H_2/CO_2
Sewage Sludge[a]						
Starch[b]	3.8	1.8	0.5	0.2	1.9	ND
Citric Acid[c]	4.5	0.5	0.6	1.4	0.5	2.7
Yeast[d]	2.8	1.5	1.6	7.4	3.2	ND
Brewery[b]	3.5	0.3	0.2	ND	5.1	ND
Whey[e]	3.7	2.2	2.2	0.3	8.2	3.1
Potato processing[e]	6.5	3.1	2.3	ND	7.0	15.2
Alcohol[e]	5.9	3.7	3.9	ND	7.8	21.0
Glucose/VFA[e]	10.1	10.5	5.0	2.4	ND	9.2
Glucose/VFA[b]	11.0	7.7	9.0	1.0	5.2	2.7

ND = not determined;
Ac = acetate; Pro = propionate; Bu = butyrate; MeOH = methanol; EtOH = ethanol.
a = mixed reactor; b = UASB; c = downflow fixed bed; d = upflow fixed bed; e = hybrid sludge bed/fixed bed.

ANAEROBIC BIODEGRADABILITY TESTING

The majority of procedures developed for anaerobic biodegradability testing rely on measurement of the CH_4 and CO_2 produced rather than on the disappearance of the test chemical. Use of Dissolved Organic Carbon (DOC), Total Organic Carbon (TOC) or COD assay methods to monitor substrate conversion have not proved suitable because of the complex nature of the test sludge and the tendency of some test chemicals to adsorb to sludge particulate material (Birch et al., 1989).

Shelton and Tiedje (1984) developed the first generalised protocol for anaerobic biodegradability testing based on earlier studies by Gledhill (1979), Healy and Young (1979) and Owen et al. (1979). The method relied on pressure transducer measurement of the biogas produced in sealed vials and degradation was expressed in terms of the percentage of theoretical gas production based on the stoichiometry of mineralization to $CH_4 + CO_2$ and correcting for gas solubilisation. This procedure was further refined by Battersby and Wilson (1988) and was also modified for use with the computerised pressure-transducer system developed in our laboratory (Geoghegan, 1989). A standard anaerobic biodegradability protocol based on this method has been published by the UK Department of the Environment under the title "The Assessment of Biodegradability in Anaerobic Digesting Sludge 1988". This method was used by Battersby and Wilson (1989) to analyse the anaerobic biodegradability of 77 organic chemicals.

Young (personal communication) recently developed an alternative procedure based on measurement of the biogas produced from test serum bottles in an external gas flow measuring cell. The device developed (the Challenge ANR-100 Anaerobic Respirometer) is linked to a computer for measurement of gas flow rates and

processing of the data obtained. The primary difference between this and the pressure transducer method is that the Challenge respirometer system allows the test vials to remain at atmospheric pressure throughout the assay. Consequently, calculation of the total $CH_4 + CO_2$ gas production in test vials is not complicated by changes in gas solubility resulting from pressure build-up in the vials.

In a comprehensive inter-laboratory evaluation of the Shelton and Tiedje procedure, Birch et al. (1989) concluded that the validity of the use of theoretical calculations based on application of the Buswell equation and correction for the solubilities of the gases is open to doubt. These authors developed a modified test procedure which includes measurement of dissolved inorganic carbon (DIC) in the test liquid phase in addition to pressure transducer measurement of the evolved biogas in the headspace (Birch et al., 1989). Separate determination of the liquid phase DIC eliminates the need for any correction for the solubility of CO_2 and obviates the need for knowledge of the chemical formula of the test compound or for use of the Buswell equation. The only requirement is for a knowledge of the carbon content of the test compound. Given the variety of screening methods now available, there is an evident need for a comprehensive comparative study of these methods using the same reference sludge and a representative range of potentially recalcitrant anaerobic chemicals.

Although Battersby and Wilson (1989) concluded that anaerobic biodegradability assessments made with sludge from one source can be extrapolated to sludge from another source with a reasonable degree of confidence, this was not found to be the case in our laboratory. Phenol has been reported by many authors to be readily degraded in anaerobic test systems and shown by Battersby and Wilson (1989) to be completely degraded after an initial lag period of 6 days. Using fresh sludge from an anaerobic sewage digester in Ireland as the test inoculum, no degradation of phenol was observed over a 50 day incubation period. The same sludge was used to seed a laboratory-scale hybrid reactor which was subsequently fed a simulated pharmaceutical wastewater containing a number of synthetic organic chemicals, including phenol at a concentration of 1mM. The reactor was operated at volumetric loading rates of 1.2 to 2.4 $kgCOD.m^{-3}.d^{-1}$ for a period of 200 days and the specific methanogenic activities against a variety of test substrates were determined with the results shown in Table 2.

TABLE 2 Specific Methanogenic Activity Profiles of Seed Sludge and Pharmaceutical Wastewater Adapted Sludge

Sludge	Specific Methanogenic Activity (μMole $CH_4.g^{-1}VSS.min^{-1}$)								
	Ac	Pro	Bu	H_2	Amyl Alcohol	Isoamyl Acetate	Benzoic Acid	Phenol	Monoethylene glycol
Seed sewage sludge	1.6	0.8	1.7	2.5	0.2	0.07	0.3	0	0.5
Acclimatized sludge	3.0	1.9	2.5	17.0	1.8	0.6	1.7	0.4	2.6

Ac = acetate; Pro = propionate; Bu = butyrate

It is evident from Table 2 that bacteria competent to degrade phenol had developed in the reactor over the period of operation. The initial degradability result, together with discrepancies between anaerobic biodegradability data reported in the literature for some synthetic chemicals, highlights the need for inter-laboratory collaboration and for use of different sludges and environmental samples in anaerobic biodegradability screening.

FREEZE-DRYING OF ANAEROBIC SLUDGES

Routine anaerobic biodegradability testing in different laboratories would clearly be greatly facilitated by the availability of a standard reference sludge against which other sludge types can be compared and contrasted. The provision of such a reference sludge requires that it should be capable of storage over time. Given the heterogeneous bacterial flora of anaerobic sludge and the necessity for coordinated sequential bacterial action during methanogenesis, any preservation or storage system applied must both maintain sludge activity over

the long term and prevent imbalance of the various syntrophic interrelationships involved in the digestion of complex substrates.

Very little data have been published in the literature on the preservation or storage of anaerobic sludges. de Zeeuw (1984) studied the effect of storage in the wet state without feeding at 4°C on the specific methanogenic activity of two different sewage sludges. For one sludge, the decrease in activity against a VFA mixture was negligible over an 18 month period, although the lag phase in the assay increased from 1.5 to 7.5 days. The second sludge exhibited a 57% decrease in specific activity against VFA over the same period, while the lag phase increased from 1.5 to 14.7 days. Cold storage of granular sludge without feeding is routinely practised but little published data are available on the effects of such storage. Hulshoff Pol (1989) cited unpublished results of Lettinga and Stellema on the storage of mesophilic granular sludge for several years without serious deterioration in sludge specific activity. Wu et al. (1985) also reported that the activity of granular sludge stored unfed at 15 - 28°C did not deteriorate significantly over lengthy storage.

At University College Galway, granular sludge is routinely stored unfed at 4°C. We have noted, however, that the specific activity profile of such sludges changes with both the length of storage time and the sludge type (Table 3).

TABLE 3 Effect of Cold Storage on the Specific Methanogenic Activity of Anaerobic Granular Sludge

Sludge Source	Storage Time (d)	Activity (μMole $CH_4.g^{-1}VSS.min^{-1}$) Acetate	Butyrate	Ethanol
Cerestar(a)	0	14.57	5.64	3.01
	65	13.51	2.45	1.79
	121	17.38	1.34	*
Carbery(b)	0	6.43	5.24	7.16
	110	2.55	3.89	3.75

a = sludge developed on starch production wastewater; b = sludge developed on wastewater from an alcohol from whey fermentation plant.
* Biphasic pattern of gas production.

No alternative method to cold storage in the wet state has been described in the literature for anaerobic sludge preservation. Freeze-drying is the most commonly used procedure for long-term preservation of aerobic microorganisms. It has also been successfully applied to anaerobic bacteria in general (Impey and Phillips, 1984) and to some methanogenic bacteria (Hippe, 1984). Since freeze-drying can be scaled up to batch sizes of 1 tonne or more, its potential application for preservation of anaerobic sludge appeared to merit investigation.

Coates (1991) initially developed a routine protocol for sample preparation, freezing, drying and reconstitution which would allow assessment of the benefits, if any, of cryoprotectant usage and which would permit valid estimation of the effect of long-term storage on the activity of the freeze-dried sludge samples. Granular sludges developed either on starch production wastewater or on the effluent from a plant producing alcohol from whey were used as the test sludges. The effect of freeze-drying on the viability of a bacterial culture is usually monitored by comparing the number of colony-forming units before and after freeze-drying. Due to the slow growth rates of many of the anaerobic species present in the granular sludges under test, the specific methanogenic activity against H_2/CO_2, acetate, butyrate and ethanol before and after freeze-drying was used as an index of the success or failure of the freeze-drying process (Coates, 1991). The stability of methanogenic activity against propionate was not investigated in this study since both sludge types exhibited a very low specific activity against propionate in the wet state.

The effect of inclusion of a range of cryoprotectants on the recovery of methanogenic activity after immediate reconstitution was initially investigated. The cryoprotectants used were examined at different concentrations and were representative of the compounds or compound mixtures used in commercial lyophilisation practice - e.g. sugars such as glucose, sucrose or trehalose (Fry, 1951; Heckly, 1984); proteinaceous solutions such as

blood serum, skim milk or whey (Greaves, 1956; Lapage et al., 1970) or antifreeze/anti-oxidant compounds such as glycerol and dimethyl sulfoxide (DMSO) (Polge et al., 1949; Lovelock and Bishop, 1959; Gekko and Timasheff, 1981). In general, cryoprotectants were found to enhance the stability of sludge methanogenic species during the freeze-drying process, although the effect on individual trophic groups varied with both the nature and concentration of the individual cryoprotectants used (Coates, 1991).

Table 4. The Effect of Cryoprotectants on the Stability of Freeze-dried Sludge Stored at 4°C under N_2.*

Cryoprotectant	Specific Activity Against Acetate (μMole $CH_4.g^{-1}VSS.min^{-1}$)		
	Wet sludge	Immediate Reconstitution	Reconstitution after 7 months
No Cryoprotectant	2.54	1.45(57.1%)	1.04(40.9%)
10% Trehalose	2.54	2.15(84.6%)	1.46(57.5%)
10% Glucose	2.54	1.31(51.6%)	1.41(55.5%)
25% Horse Serum	2.54	1.56(61.4%)	1.40(55.1%)
10% DMSO	2.54	1.79(70.5%)	1.16(45.7%)
10% Glycerol	2.54	2.19(86.2%)	1.40(55.1%)

Cryoprotectant	Specific Activity Against Butyrate (μMole $CH_4.g^{-1}VSS.min^{-1}$)		
	Wet sludge	Immediate Reconstitution	Reconstitution after 7 months
No Cryoprotectant	3.46	1.60(46.2%)	1.26(36.4%)
10% Trehalose	3.46	2.65(76.6%)	1.70(49.1%)
10% Glucose	3.46	1.70(49.1%)	1.14(32.9%)
25% Horse Serum	3.46	2.67(77.2%)	1.87(54.0%)
10% DMSO	3.46	1.95(56.3%)	BP
10% Glycerol	3.46	2.61(75.4%)	2.99(72.0%)

Cryoprotectant	Specific Activity Against H_2/CO_2 (μMole $CH_4.g^{-1}VSS.min^{-1}$)		
	Wet sludge	Immediate Reconstitution	Reconstitution after 7 months
No Cryoprotectant	12.40	1.92(15.5%)	0
10% Trehalose	12.40	8.44(68.1%)	7.37(59.4%)
10% Glucose	12.40	6.03(48.6%)	6.01(48.5%)
25% Horse Serum	12.40	2.90(23.4%)	0
10% DMSO	12.40	1.68(13.5%)	0
10% Glycerol	12.40	7.94(64.0%)	0

* = granular sludge developed in a hybrid reactor treating effluent from an alcohol from whey fermentation plant.
B.P. = biphasic curve obtained

Based on the results obtained, a large sample of fresh granular sludge was freeze-dried in the presence and absence of a representative range of individual cryoprotectants at selected concentrations (Table 4). Portions of the dried sludge were immediately reconstituted and assayed for methanogenic activity against acetate, butyrate and H_2/CO_2. The remaining dried sludge was stored at 4°C under N_2 for seven months and then

reconstituted and assayed as above. As is evident from Table 4, cryoprotectant addition, in virtually all cases, resulted in an enhancement of the recovery of activity against all three substrates on immediate reconstitution. Typically, losses of the order of one log unit are regarded as acceptable (Owen et al., 1989). Consequently, the recovery values obtained (from 15 to 86% of initial specific methanogenic activities) indicate that granular sludge can be subjected to the lyophilisation process. The freeze-dried sludge also retained activity in most samples during long-term storage (7 months) at 4°C under N_2. Of the various subpopulations under test, the hydrogenophilic methanogens appeared to be the least stable during storage (Table 4). Significant recovery of the hydrogenophilic activity of stored, dried sludges was only obtained with samples dried in the presence of sugar cryoprotectants. Continued refeeding with H_2/CO_2 in the presence of a growth-supporting nutrient medium was, however, shown to restore hydrogenophilic activity in the sludge samples due to the relatively rapid doubling time of the majority of H_2-utilising methanogens.

It was noted by Coates (1991) that the age of the sludge prior to freeze-drying affected the activity patterns obtained on immediate reconstitution after freeze-drying. Sludges that were stored for lengthy periods at 4°C prior to freeze-drying tended to exhibit lag phases of variable duration on immediate reconstitution. As a general rule, the longer the period of cold storage prior to freeze-drying, the longer the lag phase obtained in activity tests with reconstituted dried sludges. Lag phases of up to 40 hours and, in extreme cases, up to 200 hours were obtained. Freeze-dried sludges that were stored at room temperature, rather than at 4°C, also exhibited lag phases in activity tests, with a lag of approx. 180 - 200 hours being obtained against acetate, butyrate and ethanol as substrates.

Multiphasic patterns of biogas evolution were obtained by Coates (1991) in activity tests against ethanol and butyrate when using freeze-dried granular sludge from different sources and of different ages. Such patterns were never observed with either acetate or H_2/CO_2 (i.e. direct methanogenic substrates) and were regarded by Coates (1991) as indicative of imbalance between the various subpopulations needed to convert indirect substrates to CH_4 and CO_2. Such imbalances as were observed were recorded primarily with sludges that either had low levels initially of ethanol or butyrate utilisers or suffered losses of the H_2/CO_2 population during the freeze-drying step. With a properly balanced fresh granular sludge and with appropriate use of suitable cryoprotectants, Coates (1991) concluded that freeze-drying could be validly used as a preservation method for a reference sludge and that the dried sample would both retain a balance of syntrophic and methanogenic activities and also be stable to longterm storage at 4°C.

TOXICITY TESTING AGAINST SLUDGE SUBPOPULATIONS

Although major advances have been made in recent years in our knowledge of the degradative pathways involved in anaerobic metabolism of halogenated hydrocarbons and homocyclic and heterocyclic aromatic compounds (Holliger et al., 1988; Reineke and Knackmuss, 1988), considerably less information has been accumulated on the toxicity of the majority of these xenobiotic chemicals to individual trophic groups involved in methanogenesis. Most of the studies to date have focussed on determination of toxicity thresholds against the overall digestion process in laboratory-scale digesters treating defined chemical mixtures or natural wastewaters (Chou et al., 1978; Speece and Parkin, 1983; Parkin et al., 1983). Since inhibition of any one of the individual stages catalysed by different trophic groups will have consequent adverse effects on other stages of the overall coordinated process of methanogenesis, it is of interest to determine toxicity thresholds for the individual subpopulations involved and also to investigate the feasibility of acclimatisation of these groups to particular toxicants. Studies at University College Galway are currently focussed on examining the effect of representative organic compounds on the specific activities of granular sludge against a range of direct and indirect substrates using the pressure-transducer methodology described above.

Initial investigations concentrated on determining the potential toxicity of low molecular weight chlorinated and fluorinated aliphatic and aromatic compounds against the specific hydrogenophilic and acetoclastic activities of granular sludge cultivated on a starch processing wastewater. Inhibition thresholds determined against chlorinated and fluorinated aliphatics were very similar for both populations, with the acetoclasts showing slightly lower values than the hydrogenophilic species against the majority of the compounds tested. This is demonstrated in Table 5 for three chlorinated aliphatic compounds. Halogenated aromatic compounds were significantly more inhibitory against the acetoclastic than the hydrogenophilic activity ot the test sludge. Although the data are still incomplete, the toxicity thresholds for the syntrophic populations involved in butyrate and ethanol conversion appear to be similar to the values determined for acetoclastic species, at least for the range of chemicals evaluated to date.

Speece & Parkin (1983) studied the effect of molecular structure on the toxicity of 52 petrochemicals to the acetoclastic population of unacclimatised sludge. The results obtained showed a definite correlation between molecular structure and toxicity, particularly with respect to chloro substitution, chain length and the position of double bonds and particular functional groups. With an enriched acetate utilising culture, acclimatisation of the acetoclastic population was shown to occur on continued exposure to individual toxicants with a resultant increase in the toxicity thresholds for the compounds tested (Parkin et al., 1983). Ongoing studies in the authors' laboratory are designed to evaluate the feasibility of toxicant acclimatisation of the hydrogenophilic methanogens in pure culture and as members of granule consortia.

TABLE 5 Percentage Inhibition of the Specific Acetoclastic Activities of Granular Sludge by Chlorinated Hydrocarbons

Inhibitor	Concentration (ppm)	% Inhibition	
		Acetoclastic	Hydrogenophilic
Methylene Chloride	500	100	100
	100	96	94
	50	94	86
	10	56	28
	5	46	14
	0.5	26	12
Ethylene Chloride	500	97	96
	100	30	88
	50	25	44
	10	20	18
	5	15	10
	0.5	13	6
Carbon Tetrachloride	500	100	100
	100	100	72
	50	53	33
	10	41	30
	5	30	8
	0.5	20	0

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