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RECOVERY OF ANAEROBIC DIGESTION AFTER
EXPOSURE TO TOXICANTS

Final Report

MASTER

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December 1979

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U. S. DEPARTMENT OF ENERGY

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FINAL REPORT

Recovery of Anaerobic
Digestion After Exposure
to Toxicants

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ABSTRACT

Methane fermentation has excellent potential as an industrial wastewater treatment process. It offers a primary energy advantage of about 20×10^6 BTU per ton of chemical oxygen demand destroyed when compared with aerobic biological treatment. In addition, excess sludge production may be less than 10% of that for aerobic treatment. The concept that methane fermentation cannot tolerate chronic or slug doses of toxicants has almost totally precluded methane fermentation as a viable contender for the treatment of industrial wastewaters. This study assayed a wide variety of toxicants, heavy metals, inorganic salts, organic chemicals, solvents, and antibiotics which are used in industrial processes and, therefore, appear in the industrial wastewaters therefrom. Toxicity was related to the reduction in methane production of a control containing no toxicant. The response of methane fermentation after exposure to a toxicant was assayed with unacclimated cultures as well as cultures which had been acclimated to increasing concentrations of the toxicant over long periods of time. The reversible nature of the toxicants was assayed by adding slug doses to plug flow anaerobic filters and recording gas production prior to, during and after toxicant addition. Cyanide, chloroform, thiocyanate and Monensin resulted in 50% inhibition to unacclimated methanogens in batch culture at concentrations of 1.0, 0.5, 5 and 0.5 mg/l respectively. With acclimation, 50% inhibition did not occur until concentrations of 25, 45, 85 and 100 mg/l were exceeded. This demonstrated the exceptional acclimation potential of these compounds which were quite toxic to unacclimated methanogens. Copper, QAC, acrolein, acrylonitrile, anionic detergent, CNS and chloropropionate resulted in 50% inhibition in acclimated filters in the concentration range of 50 to 250 mg/l. Cr^{+++} , Ni^{++} , S^- , acrylic acid, formaldehyde, nitrobenzene, Bacitracin and oxytetracycline demonstrated 50% inhibition in acclimated filters in the concentration range of 250 to 1000 mg/l. SO_2 , acetaldehyde, acetone, CS_2 , chloropropandiol, lauric acid and penicillin inhibited acclimated filters in the concentration range of 1000 to 5000 mg/l. NH_4^+ , Na^+ , propanal and vinyl acetate had to be in excess of 5000 mg/l before 50% inhibition of acclimated filters resulted. When proper attention was paid to SRT and acclimation procedure, methane fermentation of acetate was demonstrated to be stable in the presence of relatively high concentrations of selected toxicants. Slug doses of a wide variety of toxicants to plug flow anaerobic filters demonstrated that most toxicants assayed were relatively reversible in that gas production resumed promptly after the toxicants passed through the system.

INTRODUCTION

Methane fermentation is an anaerobic, microbiological process which converts organic material to methane gas. The inherent advantages of methane fermentation over aerobic waste treatment processes are:

1. No energy consumption for oxygen transfer.
2. Energy is produced in the form of methane.
3. Excess sludge production is much reduced over aerobic processes.

The net energy differential in destruction of organic pollution is about 20×10^6 BTU per ton of COD in favor of methane fermentation over aerobic treatment. In some cases, less than one-tenth as much excess sludge is produced by methane fermentation as compared to aerobic processes. The operating cost differential is about \$90 per ton of COD in favor of methane fermentation over aerobic treatment. Generally, if an organic waste can be metabolized aerobically, it can also be metabolized anaerobically.

In view of these significant advantages, the question arises as to why methane fermentation has been so little exploited in the treatment of industrial wastewaters. There are a number of factors which are responsible for this situation.

A. Low cell yield. One of the major advantages of methane fermentation - low cell yield of excess sludge - becomes a major disadvantage by prolonging start-up of the process or recovery from a toxic dose which has killed a major fraction of the biomass. The role of solids retention time (SRT), therefore, requires considerably more attention than with aerobic processes. This has not been adequately appreciated by design and operational personnel.

B. Temperature Sensitivity. Cell yield and specific utilization rate mainly determine the minimum generation time, $\theta_c^{\min}$, of microbial systems.

$$\theta_c^{\min} = \frac{1}{Yk-b}$$

Since the cell yield, Y , is already relatively low, the specific utilization rate, k , must be relatively high to prevent intolerably long minimum generation times. The specific utilization rate, k , is sensitive to temperature. Below 20°C , the minimum generation time may exceed 30 days as compared approximately

0.5 days for an aerobic process. Therefore, with a safety factor of 5 for plant design, the SRT required would be 150 days. Cold, dilute wastes, therefore, require provision for maintenance of extended SRT and produce insufficient methane to heat the wastewater without supplementary fuel. This restriction does not apply with cold, concentrated wastewaters or warm, dilute wastewaters.

C. Concepts of Environmental Engineers. There is a vague, pervading concept among environmental engineers that methane fermentation requires very long hydraulic retention times (HRT) of 10 to 30 days and is incapable of treating many industrial wastes efficiently. Nemerow (1976) expresses the commonly held concept of environmental engineers that "generally, anaerobic processes are less effective than aerobic processes and that anaerobic processes are slow and require low daily loading rates and/or long detention times. Anaerobic digestion only has a definite advantage over aerobic processes where liquid waste volumes are small, contain no toxic matter and have high fractions of readily oxidized dissolved organic matter."

D. Toxicity. The general concept of environmental engineers that methane fermentation is a sensitive process with limited potential for the treatment of dilute industrial wastewaters containing sporadic or chronic levels of toxicity is not without some support in the literature. Indeed, there are many cases which demonstrate that methane production ceased due to the presence of a toxicant. The evidence of inhibition of methane production is valid, but the conclusion that methanogens are more sensitive to toxicity than aerobic processes is not necessarily valid. With similar biological safety factors, methanogens may be able to withstand toxicant concentrations equal to aerobic systems. However, due to the lack of attention to adequate SRT in design and operation of methane fermentation systems, the biological safety factor has been inadequate and the inhibition due to toxicity has been more pronounced.

Kugelman and Chin (1971) report that:

"Although this process (methane fermentation) has significant advantages over other methods of waste treatment, its use has been retarded because of a lack of understanding of toxicity phenomena.

The magnitude of the toxic effect generated by a substance can often be reduced significantly if the concentration is increased slowly. This is the phenomenon of acclimation which represents an adjustment of the biological population to the adverse effects of the toxin. In a waste treatment system, this does not usually represent either a mutation or a selection of the fittest because rarely is all of the toxic effect eliminated. Rather acclimation represents a rearrangement of the metabolic resources of the organism to overcome the metabolic block produced by the toxic substance.

In most toxicity studies, the concentration of the toxic substance is raised in one step to the level to be studied, leaving little chance for acclimation to take place. This experimental situation is not adequate for toxicity studies in waste treatment systems because in most of these systems, acclimation will probably occur. Adequate delineation of toxicity in waste treatment requires an allowance for acclimation.

Although there are good reasons why much of the data on toxicity in anaerobic systems are suspect, such data have not and cannot be interpreted properly. At best, most of these studies are useless: at worst, they are misleading.

It is significant that many of these early studies reported the divalent cations magnesium and calcium were less toxic or equal in toxicity on a molar basis to the monovalent cations sodium, potassium. This is at variance with all previous experience in toxicity studies in pure biology.Much of the deviation in previous studies was possibly caused by antagonism and synergism."

In view of the exceptional adaptation and acclimation characteristics of microorganisms as demonstrated by the extreme environmental conditions under which they propagate, it is apparent that "ideal" conditions are not required to achieve effective biological treatment as evidenced by the fact that aerobic and anaerobic processes have been treating municipal wastes satisfactorily for years and it is recognized that a host of toxicants is present in municipal wastes at various concentrations and for various periods. In fact, an underlying principle of biological wastewater treatment is that a correlation exists between SRT and toxicity levels, e.g., maintenance of higher SRT levels compensates for less ideal environmental conditions of wastewater treatment temperature, pH, toxicity, etc.

Environmental engineers should consider all published aerobic and anaerobic toxicity data (including that subsequently presented in this report) as being relative to the test conditions under which it was obtained. Too frequently, the published toxicity data are considered absolute and anaerobic digestion has been eliminated as a viable wastewater treatment option without consideration of the ability of the methanogenic bacteria to propagate under much higher concentrations of toxicants with proper acclimation procedures. As will be shown later, methanogenic bacteria can acclimate to toxicant concentrations which are, in some cases, 100 times greater than the concentrations which exhibit inhibition to unacclimated cultures.

Likewise environmental engineers have prematurely concluded that methanogenic bacteria were "dead" when methane production ceased. Methane production has been observed to abruptly resume at a rate much in excess of that which would be associated with bacterial regrowth. The effect of wastewater flow configuration on toxicity response has not been adequately addressed. A continuously stirred tank reactor (CSTR) exhibits a different toxicity response pattern from a "plug flow" anaerobic filter. This is related to the reversible or irreversible nature of the toxicity and the exposure time effect.

OBJECTIVE

Various substances demonstrate toxic inhibition to biological systems when present in adequate concentrations for sufficient exposure time. A number of questions must be addressed to characterize the toxicity response of a biological system to a particular toxicant:

1. What is concentration - inhibition relationship?
2. What is the exposure time - inhibition relationship?
3. What is the toxicant acclimation behavior pattern?
4. What is the recovery pattern in the presence of the toxicant?
5. What is the recovery pattern once the toxicant is removed?

Successful exploitation of the low net sludge production and energy production potential of anaerobic digestion as an alternative for treatment of industrial wastewaters inherently requires that the response characteristics of methane fermentation to various toxicants be evaluated. This study was directed toward assay of a number of potential toxicants with the goal of determining the methane production recovery pattern after exposure to various toxicant concentrations.

The toxicant concentration - inhibition relationship was determined for unacclimated methanogens in a CSTR with no wasting of contents. The exposure time - inhibition relationship was evaluated by administering the toxicant at various concentrations for various exposure times in anaerobic filters. Long term acclimation studies with anaerobic filters were conducted to determine allowable toxicant concentrations which could be tolerated without reduction of the acetate metabolism rate. The effect of one day slug additions of toxicants to anaerobic filters evaluated the gas production recovery pattern with increased toxicant concentrations being administered after normal gas production resumed.

Toxicants Assayed

Representative toxicants were selected from the following categories:

Heavy Metals

Inorganic Salts

Organic Chemicals

Antibiotics

Table 1 lists the toxicants which were assayed.

Table I

TOXICITY CATEGORIES ASSAYED

Heavy Metals

Copper

Chromium

Nickel

Inorganic Chemicals

Na^+

NH_4^+

Sulphide

Sodium Fluoride

Iodide

Table I (con'd.)

TOXICITY CATEGORIES ASSAYEDOrganic Chemicals

Cyanide
 Carbon Disulfide
 Formaldehyde
 Acrolein
 Acrylic Acid
 Hydroquinone
 Chloroform
 Phenol
 2-Chloropropionic
 Nitrobenzene
 Dinitrophenol
 Acrylonitrile
 Vinyl Acetate
 3 Cl 1,2 Propandiol
 Propanal

Miscellaneous Organics

Lauric Acid
 Quarternary Ammonium Compound (QAC)
 Detergent-Anionic
 Hydroxylamine

Pharmaceuticals

Monensin
 Penicillin
 Bacitracin
 Oxytetracycline

Literature Review

The literature contains many references to toxicity in anaerobic digestion. The majority of these references relate to anaerobic digesters treating municipal sludges. Relatively few literature references cite toxicity problems specifically related to the treatment of industrial wastewaters. It is significant to note that almost without exception, the literature references to toxicity describe only the initial period of toxicity. Little if any is indicated about the eventual recovery characteristics.

British Notes on Water Pollution (1971) reports on a study of anaerobic digestion in Britain. Persistent difficulties with heated digesters were reported by works serving less than 4% of the population sampled, although those experiencing temporary difficulties served nearly 52%. Inadequate design or operation appeared to be the main cause of poor performance. The chief problems specifically cited being stratification and loss of solids, overloading and inadequate heating capacity in cold weather. At works experiencing inhibition, the most common single cause was said to be heavy metals. Inhibition has almost certainly

been the most intractable although not the most frequent cause of difficulty and there is an urgent need for systematic procedures for controlling the problem.

Heavy Metals

Barth et al (1965) found metal concentrations in solution were reduced by a factor of over 100 by complexing reactions.

Total and Soluble Heavy Metal Content of Digesters

<u>Metal</u>	<u>Total Conc. mg/l</u>	<u>Soluble Conc. mg/l</u>
Chromium (VI)	420	3.0
Copper	196	0.7
Nickel	70	1.6
Zinc	341	0.1

The most important complex-type reaction for controlling toxicity in anaerobic waste treatment was the precipitation of heavy metals by sulfides.

Lawrence and McCarty (1965) demonstrated that heavy metal toxicity could be eliminated by sulfide precipitation of the metals. Based on the volatile acids concentrations, they concluded that heavy metals affected the acid-forming bacteria at least as much as the methane bacteria.

Regan and Peters (1970) reported the failure of municipal digesters because of high concentrations of iron, zinc, chromium, nickel, lead and cadmium exceeding 9800, 4300, 2100, 1200, 790, 650, and 290 mg/l respectively. Some relief from the toxicity could be obtained by adding 20-50 pounds of sodium sulfide and lime.

British Water Pollution Notes (1971) state that it has also been shown that protection from cadmium toxicity can be achieved by raising the pH value of the digester contents and that the degree of protection is similar to that which would be expected from the precipitation of soluble cadmium by the free carbonate ion in equilibrium with the dissolved carbon dioxide at different pH values of the digester contents. Although the optimum range of pH values of digester contents is said to be between 6.8 and 7.2, the controlled elevation of pH above this range might offer a form of treatment more suitable than the addition of sulfide. Although the efficacy of the method involving the adjustment of pH appears to decrease in the order of $Cd > Cu > Ni > Zn$. When cadmium and other metals were tested individually under identical conditions in batch digesters, a 20% reduction in gas production resulted from the presence of nickel, cadmium, copper and zinc at concentrations in the digester contents of 2000, 2200, 2700 and 3400 mg/kg of solid respectively. Typical concentrations observed in sewage works were 500, 100, 900, and 2000 mg/l.

Matsumoto and Noike (1978) added heavy metal salts to digesters being fed raw sludge. The concentrations of heavy metals to reduce gas production by 10% were as follows:

Cu	13.48×10^{-3} g/gm VS
Zn	3.37×10^{-3} g/gm VS
Cr	3.37×10^{-3} g/gm VS
Ni	7.27×10^{-3} g/gm VS
Cd	9.00×10^{-3} g/gm VS

Heavy metal toxicity to methane bacteria decreased in the order of $\text{Cr}^{+6} > \text{Cu} > \text{Zn} > \text{Cd} > \text{Ni}$.

Mosey, et al (1971) suggested that the inhibitory effects of heavy metals could be countered by heavy concentrations of soluble sulfide, ferric sulfide, carbonate ions and chloride ions.

Hobson and Shaw (1976) found heavy metals ions are generally inhibitory to all bacterial activity and all the heavy metals with the exception of chromium form insoluble sulfides and are, therefore, likely to be removed from solution. Inactivation of heavy metal inhibition by sulfides present in the digesting sludge is possible. In the case of fattening pigs, it is now standard practice to include 200 mg/l of copper in cereal diets to increase food conversion efficiency and this copper accumulates in the waste from the pigs. Ammonia was not inhibitory at 2000 mg/l but there was some inhibition at 3000 mg/l and complete inhibition at 4000 mg/l. Copper increased the lag in gas production at the lower concentration and a concentration of 300 mg/l total copper was almost completely inhibitory. It is evident that acetate or butyrate at concentrations up to at least 10,000 mg/l do not inhibit Methanobacterium formicicum which is the principal hydrogen utilizing bacterium in piggery waste digesters.

Both Jorgensen (1971) and Goebgen and Brockmann (1971) studied the disturbing effects of various metals including copper, chromium, iron, zinc, and nickel and concluded that it is essential to remove these elements from industrial wastewaters before their introduction to an anaerobic sludge digester.

McBride and Wolfe (1972) reported that arsenate, tellurate, and selenate inhibited methane formation by serving as methyl traps. In the pathway arsenate was reduced to arsenite which was methylated to methyl arsinic acid. Reductive methylation of methyl arsinic acid lead to the formation of dimethylarsinic acid, which was finally reduced to dimethylarsine.

Koenigfeld (1973) observed satisfactory anaerobic digestion of tannery waste-waters that contained chromium hydroxide sludge, Chromosal B, chrome alum and a chrome leather dust. The presence of trivalent chromium in concentrations of 500 mg/l did not seem to effect the biological activity adversely at pH 7-8 and a digestion temperature of 30-32°C.

Rudolfs (1937) reported gas production was not effected by quantities of copper sulphate less than .03% on the basis of volatile matter in the fresh solids and only slightly effected by additions of .04% or in a concentration of .025% of the total mixture of the domestic sewage. During the first part of the digestion period, gas production was actually stimulated. Larger additions of copper sulphate progressively retarded volatile matter reduction and gas production. Quantities of .075% of copper sulphate suppressed gas production while volatile matter reduction was only slightly retarded. With still greater amounts of copper sulphate no gas was produced but volatile matter reduction was still evident. Comparing the results with non-acclimated cultures receiving .08% copper sulfate, gas production was much greater in an acclimated system so that after 60 days digestion time the gas produced from the mixtures was 5 times the amount when copper sulfate was nearly double that of the mixture with the lower copper sulfate concentration which was not acclimated.

Rudolfs (1937) reported on a study in which sodium arsenate was added to solids in quantities varying from 1 to 16 mg/l on the basis of sewage of average strength assuming that this toxicant would be present in the fresh settled solids. Even the small quantity of 2 mg/l of sodium arsenate caused some retardation in gas production. With larger quantities digestion and gas production were greatly retarded. With 4 mg/l digestion after 60 days was equal only to the control after about three days. This was true for both gas production and volatile matter destruction. Repeated use of seed produced by the acclimation of sodium arsenate increased gas production only slightly but appeared to have less and less effect on volatile matter reduction. As a matter of fact, it seemed gradually to aid liquifaction as measured by the standard method of volatile matter determination. Part of this increase in liquifaction may have been due to an accumulation of liquified material in the seed sludge but calculations show that the entire increase could not be accounted for by the actual accumulation. It is probable that certain arsenate tolerant bacteria increase gradually. It is also a well-known fact that specific organisms can decompose arsenates.

Hayes and Theis (1978) found rather large accumulation of metals in the intracellular fraction of the digested sludge. Depending on the metal, between 30 and 60% of the total was associated with the biomass. It appears that microbial uptake actively competes with precipitation in the removal of heavy metals from digester supernatant. This phenomenon is undoubtedly aided by the demonstrated ability of bacteria to concentrate metal ions around the cell wall through complexation with protein and acid groupings that serve as binding sites. The inability to release these metals in the presence of an EDTA wash further suggests the active transport of these metals to the cell interior.

Results in this study show that toxic effects occur when a large build-up of metals exist in close association to the cell mass.

The order of decreasing toxicity on a weight-weight or molar basis was $Ni > Cu > Pb > Cr > Zn$. Cadmium in the dosages used, produced no toxic effects.

Inorganic Salts - Cations

McCarty and McKinney (1961) demonstrated that the cations were toxic instead of the volatile acid anions. They assayed sodium, potassium, calcium, magnesium and ammonium. They reported that pH played a significant role in the toxicity of ammonium ion. They deduced that when the free ammonia concentration exceeded 150 mg/l that severe toxicity resulted.

Kugelman and Chin (1971) investigated the action of cations individually and in combinations. They found both stimulation and toxicity could be demonstrated by the addition of light metal cations depending on the concentration range. They found that antagonism and synergism were mutually exclusive. The concentration of the synergist at which the synergistic effect began was below the concentrations at which the synergistic cation would produce inhibition when present alone. They found antagonism of toxicity by multiple cations was superior to that achieved by a single antagonist. Stimulation of metabolism to a rate higher than the control was achieved by multiple cation antagonism even in the presence of a very high concentration of a toxic cation. The concentration of cations which produce peak antagonism, 0.01 m for monovalent cations and 0.005 m for divalent cations, also ensure optimum metabolic activity even when toxic conditions do not exist. The upper limit of cation concentration which would result in only 10% inhibition in anaerobic waste treatment was reported to be:

<u>Slug Feed</u>			<u>Daily Feed</u>	
<u>Cation</u>	<u>Single Cation</u>	<u>Antagonists Present</u>	<u>Single Cation</u>	<u>Antagonists Present</u>
Sodium	0.2	0.3 - 0.35	0.3	0.35
Potassium	0.09	0.15 - 0.2	0.13	0.35
Calcium	0.07	0.125 - 0.15	0.15	0.20
Magnesium	0.05	0.125	0.065	0.14
Ammonium	0.1	0.25	not measured	not measured

Van Velsen (1979) investigated the influence of ammonia-nitrogen on methane fermentation. In an experiment with digested sewage sludge, methane production took place at ammonia-nitrogen concentrations as high as 5000 mg/l. However, an increasing lag phase was observed. The maximum methane production rate slowly decreased with increasing ammonia-nitrogen concentrations. Acclimation was considered to be an important factor.

Inorganic Salts - Anions

Lawrence, et al (1964) found 200, 400, and 800 mg/l of sulfide addition gave 96, 94 and 88% of gas production, respectively, as the control. The soluble sulfide measured in the unit fed 800 mg/l S^{2-} was 200 mg/l. This large reduction in soluble sulfides is almost entirely due to the sulfide lost by daily expulsion of gas.

The decrease in gas production was not significant (< 12%) in the digesters having equilibrium soluble sulfide concentrations up to 200 mg/l. Stripping of H_2S in the digester gas was demonstrated to be a significant loss.

Rudolfs and Amberg (1952) found that the addition of 200 mg/l of sulfides decreased gas production by 70% and volatile matter reduction by 50%. From a slug feed study of acetate fermentation, no methane was formed with sulfide additions above 165 mg/l.

Noordam-Goldewegen, et al (1949) reported that methane fermentation of sodium and calcium acetates was strongly inhibited by sulphite at a pH lower than 6.5.

Rudolfs (1937) noted that certain industrial wastes contain considerable quantities of sulphuric acid or larger quantities of sulphates in an acid medium. Quantities of sulphuric acid varying from .25 to 1.0% on a volume basis were added to fresh solids. The addition of sulphuric acid effected the initial reaction of the mixtures materially but at the end of 35 days all mixtures except the one receiving the highest quantity was slightly above the neutral level.

The percentage solids reduction was in all instances about the same as would be expected from well digested mixtures. The mixture to which .25% sulphuric acid was added, produced somewhat more gas in 35 days but the time required to reach the peak of gas production was increased. As could be expected the results obtained with repeated use of digested sludge with seed material which had received sulfuric acid were not materially different from those obtained when acid was added previously. At thermophilic temperatures the digestion was more sensitive to toxicant.

Shea, et al (1974) investigated the anaerobic digestion of rum distillery slops. Sulfate concentrations were in the range of 3,000 to 5,000 mg/l. They reported that high volumetric loadings were possible if the waste is diluted.

Tenney and Budzin (1972) determined that a fluoride concentration of about 1000 mg/l was toxic to a South Bend, Indiana field digester. They noted, however, that fluoride toxicity would be of little concern in plant operation, however, because the concentration of this ion was usually about 1 mg/l in the primary digester.

Balderston and Payne (1976) reported that nitrate and nitrite stopped or decreased hydrogen dependent methane fermentation. Sulfite also stopped or decreased hydrogen dependent methane fermentation. They noted that observed inhibition was not caused by redox problems or competition.

Organics - Chlorinated Hydrocarbons

British Notes on Water Pollution (1971) reported that chlorinated hydrocarbons are widely used as solvents; for example, in the pharmaceutical industry, for degreasing mechanical and electrical components, and for dry cleaning clothes. Some are extremely toxic to the digestive process and have led to inhibition at a number of works. The effect of chloroform which is the most toxic material depends on a number of factors. For example, when a number of other variables were excluded inhibition depended both on the solids content of the sludge and on the concentration of the chloroform in the wet sludge. This depended solely on concentration expressed in terms of mg of chloroform per kg of dry solids. When digesters were acclimated to different loadings within the limits of stability, the tolerance of chloroform increased with loading. However, at still higher loads simulated in a batch digester with a mixture of between 8.5 and 60% raw sludge, the reverse effect was observed. As the loading on a partially inhibited digester is reduced, the content of undigested sludge can rise between similar limits and the system is likely to become increasingly sensitive to chloroform. Examination of the distribution equilibrium for chloroform in sewage showed that the concentration in sludge was little greater than in settled sewage and was completely degraded in the digester in 7 days.

Lumb, et al (1977) reported that an Imperial Chemicals Industry laboratory discharged small proportions of chloroform which was used as a solvent in the laboratory. Discussions indicated that no substitute was practical and that despite good housekeeping small losses of chloroform were unavoidable because of the appreciable water solubility. Accordingly, provisions had to be made to accept a normal concentration in the Imperial Chemicals Industry's effluent of up to 10 mg/l with occasional peaks of up to 20 mg/l. Consequent upon the known severely inhibitory effect of chloroform on the sludge digestion process the need for a full investigation of the problem arose. Air stripping of the sludge was used to remove the chloroform. For a given air supply the rate of chloroform stripping was markedly reduced by an increase in sludge solid. For instance, an increase in sludge solids from 2-4% resulted in a stripping rate nearly three times slower. Increased temperature increased the stripping rate. Sludge from Great Warford which had initially inhibited digestion did digest satisfactorily after air stripping. The initial concentration of chloroform in the wet sludge was 5 mg/l. This was equivalent to an initial chloroform content based on dissolved solids of 125 mg/kg of solid. The final chloroform content of the wet sludge to give 10 mg/kg was .4 mg/l. This required a reduction factor of 12.5.

Prins, et al (1972) showed that halogenated methane analogs were inhibitory to the methane bacteria at micromolar concentrations. Unsaturated long-chain fatty acids and sulfites could inhibit methane production by 50% when present in millimolar concentrations. It was further discovered that the presence of a free carboxyl group was obligatory for the creation of inhibition. Substitution of the carboxyl group of oleate by a hydroxyl group as in oleyl alcohol, for the introduction of a second alkyl chain as in oleon, lessens the inhibitory effect of methane production. Furthermore, with these long-chain fatty acids, toxicity was increased as the degree of unsaturation of these fatty acid increased. A number of inhibitors were tested including chloroform, carbon tetrachloride, chloralhydrate, linseed oil, hydrolyzate, linolate, oleate, oleyl alcohol, and sodium sulfite.

Sykes and Kirsch (1972) reported that complete inhibition of methanogenesis by anaerobic wastewater sludge digester cultures could be obtained with a carbon tetrachloride dosage of 16 mg/l. Inhibition of methanogenesis was accompanied by accumulation of hydrogen, and a decrease in the production of propionate and stimulation of the formation of valerate and caproate by these mixed cultures.

McBride and Wolfe (1972) found that 10^{-6} molar trichloroethane inhibited reduction of carbon dioxide to methane by 75%. They postulated that the inhibitory site was centered in the tetrachloroethane portion of the molecule.

Bauchop (1967) also noted that chlorinated hydrocarbons inhibited methane production.

Wood, et al (1968) found chloroform, carbon tetrachloride and methylchloride were competitive inhibitors of methane formation.

Wolin, et al (1968) concluded that benzyl viologen is a potent inhibitor of methane formation. It is believed that these dyes inhibit a specific site in the electron transport support system by an irreversible reaction. It is postulated that in the enzyme system the chlorinated hydrocarbons react with the cobamide protein to inhibit methane formation. Inhibition is reversed by light.

Organics - Synthetic Detergents

British Notes on Water Pollution (1971) reported on a detailed study at the laboratory which led to the conclusion that detergents were inhibiting anaerobic digestion at a few works in their country. It was shown that because of the absorption by the solid phase thickened sludge contained very high levels of detergent, e.g., 1400 mg/l in the wet sludge. In order to interpret sensibly the likely affect on digestion the detergent concentration was better expressed as a proportion of dry solids. Laboratory studies showed that gas production was inhibited when the feed sludge contained a concentration of about 1.5% of dry solids as alkyl benzene sulphonates of the type used in Great Britain. At that concentration, however, acclimation occurred fairly readily, but at levels above 2% the effect was likely to be sustained. The test revealed no significant difference in toxicity between hard and soft ABS and in neither case was any significant breakdown of detergent observed. The toxic effect of detergents in admixture with chloroform and with zinc appeared to be additive. One affect of detergents is to reduce the initial rate of gasification without necessarily reducing the total amount produced over the whole period between feeds. This effect is the key to the present situation because although on the basis of daily gas production a digester may appear to be functioning normally, its tolerance of temporary overloading and of other adverse conditions may be greatly reduced. The distribution of detergent between the solid and liquid phases in digested sludge showed that about 98% of the surface active material remained absorbed on the solid phase -- implying that after release by crude solids during digestion the detergent was then reabsorbed by the remaining solids.

Swanwick and Shurben (1969) found that the detergent concentration in sewage was about 16.4 mg/l or 1/5% of dry sludge solids. Amine materials which are cationic in character were used to neutralize anionic detergents. Stearylamine, is a hydrogenated tallow amine. N-stearyl propylene diamine was also used to neutralize anionic detergents. Stearylamine was toxic in itself if added in excess of the amount of anionic detergents to be neutralized.

Pitter, et al (1972) revealed that meophilic wastewater sludge digestion is not impaired by linear ABS in concentrations up to 1% of the dry solids. Higher concentrations inhibited digestion 11-15% when the ABS was added. It was found that 11-15% of the ABS was digested and 2% was found in the effluent.

Woodworth, et al (1975) found that hard anionic, soft anionic and cationic syndets caused inhibition at 500, 750 and 100 mg/l respectively. Nonionic detergents caused little inhibition.

Organics - Miscellaneous

Fermentation in the presence of four known methane inhibitors was investigated by Marty and Demeyer (1973) in rumen contents from sheep given molasses and showing two different fermentation patterns. The average hydrogen obtained with the high carbohydrate and high propionate patterns were 93 and 95% respectively. Sodium sulfite, chloral hydrate and a hemiacetal of chloral and starch xanthate all inhibited methane production and were associated with an accumulation of hydrogen and lactate in rumen contents showing the high butyrate fermentation pattern. Propionate production was slightly stimulated. In rumen content showing the high propionate fermentation pattern, linseed oil hydrolysate depressed methane production at high levels only, increased hydrogen and lactate production but depressed all other products and xanthate depressed methane production and slightly increased propionate production. Low hydrogen recoveries in the presence of the inhibitors were probably associated with the utilization of metabolic hydrogen in reactions not accounted for in the scheme under investigation.

Bannink and Muller (1951) found that methane fermentation of calcium acetate was about as sensitive to phenylhydrazine, hydroxylamine, cyanide and azide as it was to sulphite.

Another chemical, acrylonitrile, which is a basic monomer for the production of many synthetic fibers, was found to be harmless in anaerobic digesters at a concentration of 22 mg/l according to Lank and Wallace (1970). This observation was not in agreement with earlier reports that showed that even 10 mg/l of acrylonitrile could be significantly toxic to digester organisms.

Rudolfs (1937) investigated the addition of gasoline on the basis of volatile matter in fresh solids which varied from .5 to 10 ml/l of sludge or from 1 to 20 ml/l of fresh solids. Gasoline appears to have attached itself readily to the solid particles and was carried down into the sludge. There was no effect on gas production when .02% by volume on the basis of fresh solids was added, while 1% gasoline had a strongly retarding effect. Repeated addition of gasoline to sludge appeared to reduce the toxic action of gasoline to some extent. Eighty days were required to produce 500 ml of gas per gram volatile matter in the 1% gasoline mixture without acclimated seed, but only 50 days were required to produce the same amount of gas when 1.25% of gasoline was added to the mixtures containing seed previously produced in the acclimated sludge. Nevertheless, the amount of gasoline added, retarded gasification materially as compared with the controls which required only 19 days to produce the same quantity of gas.

Rudolfs (1937) also reported that quantities of waste crank case oil were added equivalent to 40 and 100 gallons of waste oil per million gallons of sewage of average strength and it was also assumed that all oil would be carried down into the sludge. Based on the volume of fresh sludge the quantity of oil added was equivalent to 1 and 2.5%. The results indicate that the addition of waste crank case oil in these quantities had no detrimental effect on gas production. More gas was produced per gram of fresh solids when oil was present. The volatile matter destruction decreased.

Digestion was retarded considerably with quantities of oil above 25 ml/l or rather gas production continued for a much longer time. The quantity of gas produced per gram of fresh solids added after different lengths of time can be shown by comparing the gas production after 35 days. With no oil added 592 ml of gas were produced in the control and 750 ml were produced in the system containing 100 ml of oil per liter of sludge. In every instance, the peak gas production of the sludge-oil mixtures came a few days later than the control but gas production was sustained longer at a higher level.

EXPERIMENTAL PROCEDURES

Innoculum Source

Methanogenic cultures enriched on acetate as the sole organic carbon source (exclusive of 10 mg/l of cysteine) were used in each phase of this study. An inoculum digester was maintained as the source of all methanogenic cultures. Originally, digested sludge from a municipal wastewater treatment plant was used to seed the inoculum digester. Prior to commencement of this study, the inoculum digester had been maintained for 3 years and over 20 detention times with acetate as the organic carbon source. The inoculum digester was a CSTR maintained at a 50 day hydraulic retention time and fed 1 gm/l-day of acetic acid. Gas production from

this innoculum digester was quite stable. The background nutrient salt solution fed to the innoculum digester and all related systems is shown on Table II.

Table II

NUTRIENT SALT SOLUTION

1. Nutrient I Constituents	Conc. (mg/l)
NH_4Cl	400
KCl	400
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	400
$\text{FeCl}_2 \cdot 6\text{H}_2\text{O}$	40
CoCl_2	4
$(\text{NH}_4)_2\text{HPO}_4$	80
2. Nutrient II Constituents	
Cysteine	10
KI	10
Na Hexametaphosphate	10
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	0.5
NH_4VO_3	0.5
Nickel	0.5
ZnCl_2	0.5
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.5
H_3BO_3	0.5
$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$	0.5
NaHCO_3	6000

Anaerobic Toxicity Assay (ATA)

Owens, et al (1979) have proposed an anaerobic toxicity assay to determine the toxicity of substances to unacclimated methane bacteria using a modification of the Hungate serum bottle technique. In this study, a modification of Owens, et al ATA was utilized. Fifty ml of an innoculum from the methane culture grown on acetate as the carbon source and described above was anaerobically transferred to a 125 ml serum bottle which had been purged with a gas mixture of 75% N_2 and 25% CO_2 . Initially, 100 μl of glacial acetic acid was injected with a microliter syringe to yield an initial acetate concentration of 2000 mg/l. This resulted in

the immediate production of about 40 ml of CO_2 due to the neutralization of the glacial acetic acid. Since methanogenic activity is related only to the production of methane gas, only gas production in excess of the 40 ml of CO_2 produced was considered to be indicative of metabolism. In some of the plots total gas production is shown while in other graphs only methane production is plotted.

The desire to increase the acetate concentration without overcoming the buffer capacity led to the addition of calcium acetate at concentrations of 8,000 and 16,000 mg/l as acetate. In this case, only methane gas is evolved because the calcium acetate salt is neutral. The calcium precipitates out as calcium bicarbonate and does not result in any ion toxicity to the methanogens. The pH is maintained between 6.5 and 7.7.

Anaerobic Filter Assays

Anaerobic filters were constructed of 5 cm I.D. transparent plexiglas tubes one meter high and packed with 1 cm nominal diameter gravel. The void volume was 1 liter. The filters were fed 1 liter per day of the feed solution shown in Table II to yield a one day nominal hydraulic detention time. The toxicants were added at the desired concentration in the daily feed. Gas production from the filters was measured by liquid displacement and the effluent was collected to determine COD reduction in the filter. The daily feed was administered by positive displacement pumps on automatic timers which injected approximately 20 ml each 30 minutes to approximate continuous feeding. The concentration of acetate in the daily feed was 2000 mg/l (133 lbs/1000ft³-day) for part of the study. In the remainder of the study, the acetate concentration in the feed was 3300 mg/l (200 lbs/1000ft³-day).

RESULTS

Chromium - Trivalent

Trivalent chromium precipitates as the hydroxide and the digester environment contains 3500 mg/l of alkalinity. Therefore, high concentrations of trivalent chromium can be tolerated in the feed without inhibition of methane fermentation.

As shown in Figure 1, there was very little inhibition of unacclimated methanogens up to concentrations of 250 mg/l of chromium. The gas production rate was progressively inhibited up to 1500 mg/l. No inhibition was noted for Day 1 at any concentration indicating a lag before toxicity became apparent.

In a filter receiving 2000 mg/l of acetate in the feed, progressive increases in chromium from 100 to 300 mg/l showed no decrease in gas production when fed continuously for 20 consecutive days. Figure 2. However, in an unacclimated filter a one day slug addition of 250 mg/l caused sharp decrease in gas production as shown in Figure 3.

Copper

Copper showed no inhibition to unacclimated methanogens at 10 mg/l. However, at 20 mg/l, the gas production rate was only 25% of the control for Day 1. Figure 4. Acclimation was then noted on each successive day by an increase in the gas production rate. At 40 mg/l, there was not the same acclimation pattern as at 20 mg/l. Instead there was a rather constant rate of gas production without an acclimation trend.

A filter was fed 50 mg/l of copper as the chloride salt in the daily feed for 1, 2, 4 and 7 consecutive day periods with no adverse effect as noted in Figure 5. In another experiment, a filter was fed 250 mg/l of copper for 3 consecutive days and the soluble copper in the effluent was only 12 mg/l.

Nickel

The unacclimated methanogens were slightly inhibited by 50 mg/l of nickel. For the first 4 days, the gas production with 100 mg/l of nickel was identical with that for 50 mg/l. However, total gas production ceased at about 80% of the control volume with 100 mg/l of nickel. Figure 6. At 250 and 500 mg/l of nickel, gas production rates and total volumes of gas produced were considerably more reduced than at 100 mg/l. Nickel was observed to characteristically take several days up to a week before the full inhibitory effect was noted.

Occasionally nickel has exhibited a slight stimulatory effect on gas production. As shown in Figure 7, the addition of a one day slug of 5 meq/l of nickel resulted in a slight increase in gas production. This was also noted sporadically in other cases but could not be consistently replicated. Even though nickel chloride is as soluble as sodium chloride, it was not determined to what extent the nickel was precipitated from solution or adsorbed onto the biomass.

Continuous additions of nickel chloride to the feed of a filter over protracted periods indicated that 200 mg/l could be tolerated with no adverse effect on gas production. An increase from 200 to 400 mg/l abruptly decreased gas production as shown in Figure 8. However, gas production abruptly resumed when

Fig. 1 Response of Unacclimated Methanogens to Cr^{+++}

Fig. 2 Response of Anaerobic Filter to Toxicant, Cr^{+++}

FIG. 3 RESPONSE OF ANAEROBIC FILTER TO TOXICANT

Fig. 4 Response of Unacclimated Methanogens to Cu^{++}

Fig. 5 Response of Anaerobic Filter to Toxicant
50 mg/l Cu^{++}

Fig. 6 Response of Unacclimated Methanogens to Ni^{++}

Figure 7 5 meq Ni^{++} in 500ml

Fig. 8 Response of Anaerobic Filter to Toxicant - Ni#

nickel addition ceased; but was followed by a secondary relapse with eventual resumption to full rate. At this point, 300 mg/l of nickel was added continuously with a rapid deterioration in gas production. In a parallel study shown in Figures 9 and 10, 250 mg/l of nickel caused reduction in gas production after 20 days continuous dose. The gas production rate dropped below 200 ml per day. Full gas production recovered in about 24 days. At this point, 250 mg/l of nickel was again added continuously with no adverse effect after 30 days. After a period of 7 days with no addition of nickel in the feed, 350 mg/l was added continuously and sharp decline in gas production resulted.

One day slug additions of nickel were added to the filter in increasing concentrations as shown in Figure 11. At slug nickel concentrations up to 500 mg/l, there was only about 25% decrease in gas production with full recovery in a day or two. However, at 1000 mg/l of nickel in the feed the gas production abruptly dropped and did not recover significantly after 5 days.

Ammonium Ion

Ammonium ion was added as the chloride salt and the pH in the liquor was below 7.5. Therefore, less than 1% of the ammonium ion was in the un-ionized form and even at the highest concentration never exceeded 100 mg/l as NH_3 . Acclimation of a filter to continuous additions of ammonium ion at step increases in concentration showed no decrease in gas production up to 6000 mg/l. Figures 12 and 13. (The increase in effluent COD is due to the excessive chlorides.) At 7000 mg/l of ammonium ion, progressive failure was noted.

One day slug additions of ammonium ion to an unacclimated filter showed a sharp decrease at 4000 mg/l, but it recovered to about normal in one day. Figure 14. A one day slug of 8000 mg/l resulted in an abrupt decrease in gas production without rapid recovery.

Phosphate

No decrease in gas production resulted from continuous addition of phosphate (as Na_2HPO_4) at step increases in concentration. Addition of 2000 mg/l (as P) was the highest concentration assayed. Figure 15.

Quarternary Amine Compound (QAC)

Quarternary amine compounds are used as bacteriacidal agents. QAC showed relatively little inhibition at 25 mg/l to unacclimated methanogens but essentially no gas production resulted at concentrations of 50 mg/l and above. Figure 16.

The first attempt to add 10 mg/l of QAC continuously to a filter for 30 consecutive days resulted in progressive failure of gas production. Figure 17. A second attempt resulted in no decrease in gas production. Figure 18. An increase

Fig. 9 Response of Anaerobic Filter to Toxicant Ni^{++}

Fig. 10 Response of Anaerobic Filter to Toxicant
 Ni^{++}

FIG. 11 RESPONSE OF ANAEROBIC FILTER TO TOXICANT

Fig. 12 Response of Anaerobic Filter to Toxicant - NH_4^+

Fig. 13 Response of Anaerobic Filter to Toxicant NH_4 (as N)

FIG. 14 RESPONSE OF ANAEROBIC FILTER TO TOXICANT

Fig. 15 Response of Anaerobic Filter to Toxicant
 Na_2HPO_4 (as P)

FIG. 16 KINETIC RESPONSE TO TOXICANT - QAC

Fig. 17 Response of Anaerobic Filter to Toxicant - QAC

Fig. 18 Response of Anaerobic Filter to Toxicant QAC

to 25 mg/l for 43 consecutive days resulted in a steady decline in gas production with indications of progressive failure over the last 10 days. Subsequently, 50 mg/l of QAc was added with reasonably normal and stable gas production for 20 days. Figure 19.

One day slug additions of QAc were made to an unacclimated filter. Figure 20. When 50 mg/l and 100 mg/l of QAc were added gas production abruptly decreased to 10 to 20% of normal. Gas production recovered rapidly to normal after the 50 mg/l dose but not after the 100 mg/l dose.

Sodium Ion

The nutrient salt solution in which the unacclimated methanogens were grown contained approximately 2200 mg/l of sodium which was added as sodium bicarbonate to provide alkalinity. To assay sodium ion toxicity, additional amounts of sodium ion in the form of sodium chloride were added to the system as shown in Figure 21. An additional 2500 mg/l (4700 mg/l total) showed no inhibition. An additional 4000 mg/l (6200 mg/l total) resulted in a lag with subsequent gas production rates comparable to the control. Additions of 7000 and 10,000 mg/l (9200 and 12,200 mg/l total) retarded the gas production rate to about 40 and 30% of the control respectively.

Long term acclimation of a filter to increasing levels of continuous sodium additions showed no adverse effect up to additions of 7500 mg/l as Na^+ (9700 mg/l total). Figures 22 and 23. Additions of 10,000 mg/l of Na^+ for 35 consecutive days resulted in 30 to 40% decrease in gas production. There was no indication of progressive failure.

One day slug additions of sodium as the chloride salt were added to an unacclimated filter. Figure 24. No significant decrease in gas production was noted at 10,000 mg/l as Na^+ (12,200 mg/l total) but 20,000 mg/l caused a rapid 40% decrease in gas production which recovered to normal in one day. At 40,000 mg/l of the Na^+ , gas production ceased abruptly.

Sulfide

With unacclimated cultures, there was no inhibition of gas production up to 100 mg/l of sulfide. However, at 250 mg/l, there was no methane production for the first 9 days, only the carbon dioxide evolved from neutralization of the glacial acetic acid feed. Gradually methane production resumed. Figure 25. The lag in methane production was longer and the rate of resumption of methane production was slower at sulfide concentrations of 500 and 1000 mg/l. It is to be noted, however, that even at 1000 mg/l of sulfide, eventual acclimation and methane production resulted.

Fig. 13 Response of Anaerobic Filter to Toxicant QAC

Fig. 20 Response of Anaerobic Filter to Toxicant QAC

Fig. 21 Response of Unacclimated Methanogens to Na⁺

Fig. 22 Response of Anaerobic Filter to Toxicant NaCl (as Na^+)

Fig. 23 Response of Anaerobic Filter to Toxicant Na⁺

FIG. 24 RESPONSE OF ANAEROBIC FILTER TO TOXICANT

Fig. 25 Batch Kinetics for Toxicant - $S^=$

Continuous addition of sulfide at step increases in concentration were fed to a filter. Figures 26, 27 and 28. With acclimation, it is noted that no adverse effect on gas production resulted at concentrations up to about 600 mg/l, even though this concentration was strongly inhibitory to unacclimated methanogens. At 800 mg/l of sulfide there was about a 30% decrease in gas production over the 40 consecutive days of sulfide addition with a slight upward trend indicating acclimation. Addition of 1000 mg/l for 30 consecutive days resulted in about a 40% decrease in gas production with the gas rate being somewhat erratic. After sulfide addition ceased the gas production quickly resumed at the normal rate. Subsequently, 2000 mg/l of sulfide was added continuously for 5 days and gas production almost ceased.

One day slug additions of sulfide were administered in the daily feed to an unacclimated filter. No effect was noted at 500 mg/l. Figure 29. At 1000 mg/l there was an unexplainable increase in gas production. At 2000 mg/l gas production plummeted but quickly returned to normal. A 4000 mg/l slug of sulfide caused gas production to almost cease and recovery to normal was slow.

Sulfite

All concentrations of sulfite exhibited toxicity immediately. At 25 and 50 mg/l, there was an increase in gas production rate after Day 2 which was nearly the same as the control. Figure 30. However, 75 and 100 mg/l inhibited the gas production rates until about Day 12 when acclimation was indicated by an increase in rate of gas production.

Acclimation to relatively high concentrations of sulfite was possible with only normal variations in gas production. Figures 31 and 32. At 3500 mg/l a gradual reduction in gas production was noted. However, even at 5000 mg/l gas production was sporadic but reached 50% of normal.

ORGANICS

Acetaldehyde

The aldehyde group on this compound causes its toxicity. At a concentration 300 mg/l, acetaldehyde inhibited methane production to 60% of the control. However, acetaldehyde at a concentration of 250 mg/l in the feed to an anaerobic filter for durations of 1, 2, 4 and 7 days showed no inhibition of gas production except for a temporary decrease at the end of the 4 day duration feeding. Figure 33.

The acetaldehyde concentration in the feed was increased to 500, 1000, 2000 and 3000 mg/l for 3 consecutive days in the feed to anaerobic filters. The filters received only nutrient salts and acetate for 3 to 5 days between the successively increasing acetaldehyde doses. As shown in Figure 34, there was evidence

Fig. 26 Response of Anaerobic Filter to Toxicant Sulfide

Fig. 27 Response of Anaerobic Filter to Toxicant Sulfide

Fig. 28 Response of Anaerobic Filter to Toxicant -
S⁼

FIG. 29 RESPONSE OF ANAEROBIC FILTER TO TOXICANT

Fig. 30 Response of Unacclimated Methanogens to Sulfite

Fig. 31 Response of Anaerobic Filter to Toxicant SO_3^{2-}

Fig. 32 Response of Anaerobic Filter to Toxicant SO_3^-

FIG. 33 RESPONSE OF ANAEROBIC FILTER TO TOXICANT

Fig. 34 Response of Anaerobic Filter to Toxicant Acetaldehyde

of acetaldehyde being metabolized in the filter when the concentration was 2000 mg/l. Gas production rose and the effluent COD gradually increased. At 3000 mg/l, gas production ceased and COD rose rapidly. However, gas production was back to normal within three days after acetaldehyde was eliminated from the feed.

Acetone

Acetone is a ketone and ketones did not demonstrate toxicity to unacclimated methanogens. Figure 35 confirms acetone was not toxic to the filter. The gas production was slightly increased by the acetone supplement in the feed. There was an increase in effluent COD during the 30 day continuous addition of 1000 mg/l of acetone to the filter. The effluent COD subsequently decreased to background levels, however, before acetone supplementation was discontinued.

Acrolein

Acrolein has two components which exhibit toxicity to unacclimated methanogens. One is the double bond and the other is the aldehyde group. At 25 mg/l, acrolein exhibited no inhibition to unacclimated methanogens. Figure 36. However, at 50 mg/l, the rate of gas production for Day 1 was only half of the control and total cessation of gas production was noted when only 15% of the acetate was exhausted.

Figure 37 shows that when 2 filter volumes of feed containing 10 mg/l were administered to a filter in an 8 hour period, gas production almost ceased. It took about 10 days for gas production to return to the background level after the acrolein was added. Subsequently, 100 mg/l of acrolein was included in the feed for a 2 day period and it caused total gas cessation for several days with very slow rate of recovery as shown in Figure 38.

Continuous addition of 10 mg/l of acrolein to the filter feed caused a drop in gas production from 1400 to 900 ml/day. Acclimation to this concentration of acrolein resulted in gradual increase in gas production to background levels in about 20 days as shown in Figure 39. The level of acrolein in the feed was subsequently increased to 40, 75 and 150 mg/l with complete resumption of gas production equal to the background rate as shown in Figure 40.

Additions of acrolein to the daily feed to the filters were made over prolonged periods. Acrolein additions were stopped for a number of days. Then continuous additions of acrolein at an increased level were resumed. Figures 41 and 42 indicate that 100 mg/l of acrolein could be tolerated in the feed with no decrease in gas production. Subsequent addition of 200 mg/l resulted in total cessation of gas production.

Fig. 35 Response of Anaerobic Filter to Toxicant Acetone

Fig. 36 Response of Unacclimated Methanogens to Acrolein

Figure 37 10mg/l Acrolein 21 in 8hrs

Figure 38

100mg/1 Acrolein in 2 liters

Figure 39 Acrolein - continuous feed

Figure 40 Acrolein - continuous feed

Fig. 41 Response of Anaerobic Filter to Toxicant - Acrolein

Fig. 42 Response of Anaerobic Filter to Toxicant-Acrolein

One day slug additions of acrolein to a filter were made at increasing concentrations. Relatively little decrease in gas production resulted with 250 and 500 mg/l of acrolein in the feed. However, a one day slug addition of 1000 mg/l of acrolein markedly decreased gas production which recovered only slowly as shown in Figure 43.

Acrylic Acid

The double bond in acrylic acid is the toxic component. It demonstrated a very uniform inhibition pattern to unacclimated methanogens as shown in Figure 44. Progressive inhibition of the gas production rate is noted at increasing acrylic acid concentrations as the acetate is utilized. At 500 and 750 mg/l, only 60 and 40% of the control volume of gas was produced after 22 days.

Continuous addition of 200 mg/l of acrylic acid in the feed of an unacclimated filter depressed gas production about 50% as shown in Figure 45. When 2 liters of feed were pumped through a filter in a 1 hour period, gas production almost ceased, but rapidly recovered as shown in Figure 46. An 8 hour exposure to 1500 mg/l of acrylic acid had a much more drastic long term effect in an unacclimated filter as shown in Figure 47.

Gradual acclimation of a filter to increasing acrylic acid concentrations is shown in Figure 48. After 90 days, 600 mg/l of acrylic acid in the feed displayed no inhibition of gas production. At 800 mg/l, gas production decreased to about 70% of the background rate as shown in Figure 49. Gas production remained reasonably constant, however. An increase to 1000 mg/l further depressed gas production to 35% of the background rate. At this point, the acetic acid in the background feed was eliminated to observe the metabolism of acrylic acid. Partial metabolism of acrylic acid was indicated by the gas production and COD reduction in the effluent. Acrylic acid concentrations of 500, 1000 and 1500 mg/l were assayed with partial metabolism indicated. Figure 50.

Finally, one day slug additions of acrylic acid were made to the feed, followed by a period of unadulterated feeding as shown in Figure 51. A direct inhibition of gas production resulted, followed by a rapid recovery of gas production when the unadulterated feed was added. Even at 2500 mg/l acrylic acid rapid recovery was noted, but 5000 mg/l caused total inhibition of gas production with relatively slow recovery.

Acrylonitrile

The nitrile group on this compound is the toxic component. Toxicity to unacclimated methanogens is exhibited in two modes. The rate of methane production is retarded as the concentration of acrylonitrile is increased from 10 to 25 mg/l.

FIG. 43 RESPONSE OF ANAEROBIC FILTER TO TOXICANT

Fig. 44 Response of Unacclimated Methanogens to Acrylic Acid

Figure 45

2000mg/l HAC + 200mg/l
Acrylic Acid (continuous feed)

Figure 46 1500mg/l Acrylic Acid - 2 liters / 1 hr

Figure 47 1500 mg/l Acrylic Acid - 8hr Exposure

Fig. 48 Response of Anaerobic Filter to Toxicant Acrylic Acid

Fig. 49 Response of Anaerobic Filter to Toxicant Acrylic Acid

Fig. 50 Response of Anaerobic Filter to Toxicant
Acrylic Acid

Fig. 51 Response of Anaerobic Filter to Toxicant,
Acrylic Acid

Then the total methane production is retarded as the concentration is increased to 50 and 100 mg/l. Figure 52. In this batch assay, the gas production rate stopped before all of the acetate was exhausted with acrylonitrile concentrations of 50 and 100 mg/l. Whereas, at 10 and 25 mg/l of acrylonitrile, gas production did not cease before the acetate was all converted to methane.

Continuous addition of 100 mg/l acrylonitrile to a filter for 5 days resulted in an increase in effluent COD which continued to rise for 2 days after acrylonitrile additions were stopped. Figure 53 indicates effluent COD returned to the background level 9 days after cessation of toxicant.

A one-day slug addition of 500 mg/l acrylonitrile to a filter abruptly decreased gas production. Figure 54 indicates 90% recovery of gas production occurred in 11 days.

An attempt to acclimate a filter to 50 mg/l of acrylonitrile is shown in Figure 55. For 17 days, 50 mg/l of acrylonitrile was added with no noticeable affect for about 6 days. Subsequently, gas production dropped to near zero. Recovery of gas production was slow and had not returned to background level after 30 days.

A second attempt was made to acclimate a filter to 50 mg/l of acrylonitrile. The filter had not returned to normal gas production, but the addition of 50 mg/l of acrylonitrile for 22 days did not retard gas production as shown in Figure 56. However, 100 mg/l of acrylonitrile for 7 days resulted in complete cessation of gas production.

Anionic Detergent

An anionic detergent with the trade name WXN and produced by American Cyanamid Corporation was assayed. The British have reported on the toxicity of anionic detergents to anaerobic digestion. Concentrations of 25 to 250 mg/l of anionic detergent were assayed. Figure 57. The mixed liquor volatile suspended solids of the unacclimated methanogenic assay cultures was about 600 mg/l. Therefore, the ratio of anionic detergent to dry solids was 4 to 42%. At 25 mg/l (4%), the gas production rate was approximately 80% of the control. At 50 mg/l, the gas production rate gradually declined for the first 10 days then subsequently increased. At 100 mg/l, the initial gas production rate was about 20% of the control. Subsequently, it decreased and later showed acclimation after 20 days. Gas production ceased after 10 days at 12% of the control volume in the system with 250 mg/l of anionic detergent. Continuous additions of 15 and 30 mg/l showed no significant inhibition. However, 60 mg/l for 28 days showed a gradual decrease as indicated in Figure 58. However, the gas production did not significantly improve for 30 days after the WXN additions were stopped. After gas pro-

Fig. 52 Response of Unacclimated Methanogens to Acrylonitrile

FIG. 53 RESPONSE OF ANAEROBIC FILTER TO TOXICANT

Figure 54. 500mg/l Acrylonitrile in 500ml

Fig. 55 Response of Anaerobic Filter to Toxicant Acrylonitrile

Fig. 56 Response of Anaerobic Filter to Toxicant - Acrylonitrile

Fig. 57 Response of Unacclimated Methanogens to Anionic Detergent

Fig. 58 Response of Anaerobic Filter to Toxicant
Anionic Detergent

duction finally was restored to normal, the addition of 200 mg/l was observed to cause rapid, progressive failure. Figure 59.

Carbon Disulfide

A filter receiving 2000 mg/l of acetate in the feed was subjected to progressively increasing doses of carbon disulfide. Figure 60. Inhibition of gas production was not observed until 1500 mg/l was added. Gas production dropped from 1000 to about 500 ml/day. No increase in gas production was observed during a 7 day period in which the toxicant was not administered. Resumption of carbon disulfide at 2000 mg/l in the feed did not inhibit gas production further. Figure 61.

Chloroform

Chloroform was injected to yield concentrations of 0.5 to 10 mg/l in serum bottles containing enriched acetate cultures. As shown in Figure 62, the chloroform resulted in a lag in gas production and a reduced rate of gas production in proportion to the concentration. Since 0.1 ml of glacial acetic acid was injected, approximately 40 ml of carbon dioxide was produced by neutralization. Therefore, methanogenic activity is indicated by gas production above 40 ml. A series of serum bottles containing 50 ml of acetate enrichment cultures was operated at 35°C and 50 day SRT. The acetate concentration was restored to 2000 mg/l daily. On day 3, chloroform was injected to yield 2.5 to 40 mg/l within the bottle as shown in Figure 63. Gas production was again retarded in proportion to the concentration of chloroform injected. Injection of 5 mg/l chloroform reduced the gas production to 50 percent of the control with complete recovery 12 days after injection. With 40 mg/l of chloroform, gas production ceased after 5 days for a total period of 48 days. Then gas production rapidly resumed to 100 percent of the control in an 8 day period. The rapid resumption of gas production indicated the culture was dormant, yet viable. About 64% of the chloroform and biomass would have been lost in one hydraulic detention time. Very little loss of chloroform would have been due to volatilization with no gas production.

Figures 62 and 63 demonstrate the crucial role of SRT in facilitating acclimation of anaerobic digestion to slug and/or chronic exposure to toxicants in the feed stream with only temporary process inhibition. It follows from kinetic considerations that low synthesis ratios and low specific utilization rates result in long wash out times. The synthesis ratio for anaerobic systems (2 to 5%) is generally only 1/10 of that for aerobic systems. In addition, toxicity directly reduces the specific utilization rate. Therefore, anaerobic systems exposed to

Fig. 59 Response of Anaerobic Filter to Toxicant
Anionic Detergent.

Fig. 60 Response of Anaerobic Filter to Toxicant CS_2

Fig. 61 Response of Anaerobic Filter to Toxicant CS_2

Fig. 82 Kinetic Response to Toxicant - Chloroform

Fig. 63 S.R.T. Response to Toxicant – Chloroform
50 days S.R.T., 35°C

toxicants are greatly susceptible to wash out. Figure 64 shows an example how formaldehyde toxicity extends wash out time. The relatively long SRT of 50 days for the systems in Figure 63 prevented solids wash out prior to acclimation.

Acclimation was studied in detail using two methods. In the first, repeated injections of 2.5 mg/l of chloroform (based in the serum bottle volume) were made into methanogenic suspended growth cultures and the gas was passed through an alkali CO_2 absorber to record only methane production round-the-clock. These suspended growth cultures were serum bottles operated at an SRT of 50 days. As shown in Figure 65, the first injection of 2.5 mg/l chloroform retarded methane production severely. Then, when methane production had returned to normal, a second injection of 2.5 mg/l chloroform was made. Subsequently, a third, fourth and fifth injection were made. There was no noticeable effect on methane production rate from the subsequent injections of 2.5 mg/l chloroform, indicating that acclimation had occurred.

The second general method of studying acclimation was long term studies using the submerged anaerobic filter. Acclimation was observed by gradually increasing the chloroform concentration in the feed. Initially the feed to the filter contained 1 mg/l chloroform. Before increasing the chloroform concentration in the feed each time, a period of 7 days with no chloroform addition was provided to observe the filter response. Then a higher chloroform concentration was administered. After a period of 6 months, the feed to the filter was receiving 40 mg/l of chloroform with no significant loss in acetate utilization ($3.3 \text{ kg/m}^3\text{-day}$). Increasing the chloroform to 50 mg/l however caused gas production to nearly cease. Subsequently it took 45 days for full gas production to resume after chloroform addition ceased. This is shown in Figure 66.

One-day slug additions of chloroform in increasing concentration were administered to another submerged anaerobic filter receiving acetate in a nutrient salt solution, as shown in Figure 67. One-day feed slug additions were made at feed concentrations from 5 to 200 mg/l of chloroform. The filter exhibited excellent acclimation potential. Gas production was severely retarded for about three days following the chloroform slugs, but subsequently recovered rapidly to full gas production. However, at 300 mg/l, recovery took about 30 days. Since the filters had a nominal 1 day hydraulic retention time, the exposure to the chloroform was nominally 1 day also.

Fig. 64 Steady State Characteristics for Toxicity
Kinetic Parameters - Formaldehyde

Fig 65 Effect of Repeated Injections of 2.5 mg/l Chloroform on Methane Production

Fig. 66 Response of Anaerobic Filter to Toxicant Chloroform

Fig. 67. Response of Anaerobic Filter to Toxicant Chloroform

The effect of chloroform exposure time on gas production inhibition was studied. Various concentrations of chloroform were injected into suspended growth serum bottles. After the prescribed exposure time, the bottles were centrifuged, the adulterated supernatant discarded and replaced with centrifuged unadulterated supernatant from an inoculum digester, as described in the Experimental Procedures. Results are shown in Figure 68. There was a residual and accumulative effect of exposure to chloroform and the effect was partially reversible, as indicated by a shorter period of zero gas production (compare with data in Figure 63) followed by a rapid return to full gas production. The shorter period of zero gas production was most likely due to removal of the centrifugate containing some of the chloroform and replacement with unadulterated supernatant from the enrichment culture. The residual effect could be due to chloroform associated with the biomass, since only chloroform present in the centrifugate was discarded. As mentioned previously, these experiments only studied the effect of time of exposure to toxicant in the liquid phase.

2 Chloropropandiol

The two hydroxyls on this compound render it less toxic than chloropropionic acid. In the first attempt, there was no inhibition of gas production until the 2 chloropropandiol concentration reached 2000 mg/l. At that concentration, gas production dropped off rapidly. Figures 69 and 70. A second attempt was made to assay 2000 mg/l of 2 chloropropandiol in the filter feed as shown in Figure 71. Gas production decreased from about 1500 to 1100 ml/day and held steady during the 40 day period of addition.

Chloropropionate

The chloro substitution on propionic acid renders it quite toxic to unacclimated methanogens. Even 5 mg/l inhibited the gas production rate. A pattern of delayed inhibition, followed by acclimation was noted at 10 and 20 mg/l. The rate of gas production for the first day for all concentrations was only slightly less than the control. Figure 72. However, the rate progressively decreased until Day 7 for chloropropionate concentrations of 10 and 20 mg/l. After Day 7, the rate of gas production progressively increased until the acetate was exhausted, indicating progressive acclimation.

Addition of 400 mg/l for 4 days to a filter stopped gas production. Figure 73. Recovery, after chloropropionate addition was stopped, was very gradual indicating regrowth. Continuous addition of 50 mg/l to a filter caused progressive decrease in gas production over a 25 day period. Figure 74.

FIG. 68 EFFECT OF TIME OF EXPOSURE TO CHLOROFORM ON GAS PRODUCTION

Fig. 69 Response of Anaerobic Filter to Toxicant -
2 Cl Propandiol

Fig. 70 Response of Anaerobic Filter to Toxicant -
3 Cl 1, 2, Propandiol

Fig. 71 Response of Anaerobic Filter to Toxicant
3 Cl 12 Propandiol

Fig. 72 Response of Unacclimated Methanogens to Chloro Propionate

Figure 73

400mg/l 2-Chloropropionic 21 Acid
Added over 110 hrs

Figure 74 50mg/l 2-Chloropropionic Acid +
2000mg/l HAC (continuous feed)

Cyanide

Batch serum bottle studies with 0.5 to 10 mg/l of KCN (as CN^-) resulted in the gas production patterns shown in Figure 75. Figure 75 is a plot of cumulative gas production; methane production is indicated by gas production in excess of 40 ml. Methane production began almost immediately in the presence of 1 mg/l CN^- , but at a rate only about 50 percent of the control. Lag periods of 9, 18 and 24 days took place before methane production commenced in the presence of 2.5, 5 and 10 mg/l CN^- . However, the rate of methane production was almost equal to the control, once it started.

Daily feed serum bottles operated at 50 day HRT and SRT incubated at 35°C responded to slug cyanide concentrations as shown in Figure 76. Daily wasting of the mixed contents and restoration of the acetate concentration to 2000 mg/l was practiced. In all cases, gas production nearly ceased for varying periods with recovery at nearly comparable rates for all cyanide concentrations. The period of no gas production was proportional to the cyanide concentration. As with chloroform, prolonged periods of zero gas production were observed. This would tend to lead one to the conclusion that the systems were "dead". However, the rapid recovery rate of gas production would suggest the system biomass remained viable during these prolonged periods of zero gas production, with metabolism blocked until detoxification or acclimation to the cyanide occurred.

Long term acclimation of cyanide in an anaerobic filter is shown in Figure 77. Initially, when the concentration reached 15 mg/l, gas production dropped sharply. The filter was fed for 7 days with no cyanide in the feed. Then 15 mg/l was again added continuously; the result was generally normal gas production. The cyanide concentration was then increased to 20 mg/l and normal gas production resulted. However, when 30 mg/l of cyanide was continuously in the feed, gas production fell sharply.

One-day slug additions of cyanide were then administered in the daily feed to an anaerobic filter. Figure 78. When gas production returned to normal, a higher concentration of cyanide was administered as a one-day slug. Initially 10 mg/l CN^- caused a temporary reduction of gas production to about 50 percent of normal followed by rapid recovery. Eventually a 200 mg/l CN^- slug had less effect on gas production than the initial 10 mg/l slug even though there was slight downward trend in gas production. A 500 mg/l CN^- slug caused a rapid decrease in gas production, but recovery was observed within 10 days.

Fig. 75 Batch Kinetics for Toxicant - CN^-

Fig. 76 S.R.T. Response to Toxicant - Cyanide
50 days S.R.T., 35°C

Fig. 77 Response of Anaerobic Filter to Toxicant KCN (as CN^-)

Fig. 78 Response of Anaerobic Filter to Toxicant CN^-

The remedial effect of various agents on cyanide toxicity was observed in batch serum bottles. Cyanide (5mg/l) was introduced into replicate, actively metabolizing acetate enrichment cultures in serum bottles. In replicate bottles containing the cyanide, chemicals suspected of being able to reduce cyanide toxicity were added. A control was maintained in which only cyanide was introduced. These chemicals were hydroxylamine, cysteine, thiosulfate and dithionite. Only the remedial effect of hydroxylamine is shown in Figure 79. However, all of the chemicals exhibited similar qualitative effects in causing gas production to resume sooner than in control.

The effect of cyanide exposure time to the biomass was observed. Acetate enrichment cultures were anaerobically transferred to serum bottles. Cyanide was introduced at various concentrations for various exposure times. Then the serum bottles were centrifuged, the adulterated supernatant discarded and replaced with unadulterated, centrifuged supernatant from an inoculum digester. Acetate concentrations were restored to 1000 mg/l daily and the daily gas production recorded. As noted in Figure 80, the effect of cyanide was quite reversible at concentrations of 10 mg/l or less as indicated by the near-normal gas production observed after removal of the CN^- in the liquid phase by centrifugation. At concentrations above 10 mg/l, and at prolonged exposure times, gas production decreased significantly. However, the systems recovered much faster than those systems described in Figure 76. This faster recovery was probably due to removal of the CN^- in the liquid phase by centrifugation.

Dinitrophenol

Dinitrophenol is an "uncoupling agent" of oxidative phosphorylation in aerobic cells. As such, metabolism continues, but synthesis of cells ceases. One day addition of 250 mg/l to a filter was followed by a sharp drop in gas production with abrupt recovery to normal. However, a one day addition of 500 mg/l resulted in about 80% inhibition with little recovery observed after 12 days. Figure 81.

Four day addition of 250 mg/l to a filter was followed by an increase in effluent COD. Eight days after dinitrophenol addition was stopped, effluent COD was back to normal. Figure 82.

Formaldehyde

Formaldehyde is an effective bacteriocide at high concentrations. The aldehyde group is the toxic component. Progressive inhibition of batch gas production assays were noted in the range of 100 to 500 mg/l. Figure 83. Both a lag in onset of gas production and a decrease in rate of gas production were observed as the formaldehyde concentration was increased.

Fig. 79 Remedial Effect of Hydroxylamine
on Cyanide Toxicity

FIG. 80 EFFECT OF TIME OF EXPOSURE TO CN^- ON GAS PRODUCTION

FIG. 81 RESPONSE OF ANAEROBIC FILTER TO TOXICANT

FIG. 82 RESPONSE OF ANAEROBIC FILTER TO TOXICANT

FIG. 83 KINETIC RESPONSE TO TOXICANT -
FORMALDEHYDE

When a background nutrient solution containing 200 mg/l of formaldehyde was passed through the filter in 1 hour, there was a subsequent increase in gas production. Figure 84. However, under the same exposure conditions with 500 mg/l, gas production decreased to 40% of normal but fully recovered in about 10 days. Figure 85.

Continuous addition of 100 mg/l had no significant effect on gas production. However, after 7 days with 400 mg/l gas production decreased to about 30% of normal. Figure 86. At this time, the formaldehyde concentration was increased to 1000 mg/l and the gas production continued on a steady decline. A filter was exposed to 500 mg/l of formaldehyde in the feed for progressively longer periods. Figure 87. It maintained normal gas production with 4 days continuous feed but failed completely with 7 days exposure.

Progressively increasing levels of formaldehyde in the feed initially resulted in increased gas production, indicating metabolism of the formaldehyde. Eventually, the filter accommodated 400 mg/l with no inhibition of gas production. Figure 88. However, 20 days continuous feed of 600 mg/l lead to progressive decrease in gas production, even when formaldehyde addition was stopped. Figure 89.

Gasoline

A direct injection of 500 ml of no-lead gasoline was injected into a filter over a one hour period. Since gasoline is lighter than water, it would not have accumulated in the filter, but would rise directly up through the filter and overflow. However, gasoline has a slight solubility in water at 35°C and this would remain in contact with the microbes until the bulk of the water was displaced, i.e., one day hydraulic detention time. Gas production decreased to 30% of normal, rapidly increased to 150% of normal temporarily after 5 days then abruptly dropped to normal. Figure 90.

Hydroquinone

Relatively high concentrations of hydroquinone were required to cause inhibition. At 6000 mg/l about 50% inhibition of the methane production rate was observed with unacclimated methanogens. Figure 91. At 10,000 mg/l gas production ceased after the first day.

Injection of 2 liters of feed containing 3000 mg/l of hydroquinone over a 103 hour period resulted in total cessation of gas production. However, recovery was quite rapid after the hydroquinone was displaced. Gas production leveled out at 70% of background indicating some residual damage to the methanogens. Figure 92.

Figure 84

200mg/1 Formaldehyde 500ml - 1hr

Figure 85

500mg/l Formaldehyde 500ml - 1hr

Figure 86 Formaldehyde - Continuous Feed
Increasing Concentrations

FIG. 87 RESPONSE OF ANAEROBIC FILTER TO TOXICANT

Fig. 88 Response of Anaerobic Filter to Toxicant Formaldehyde

Fig. 89 Response of Anaerobic Filter to Toxicant, Formaldehyde

Figure 90 500ml No Lead Gasoline

Fig. 91 Response of Unacclimated Methanogens to Hydroquinone

Figure 92 3000mg/l Hydroquinone 103 hrs
2 liters

Hydroxylamine

Hydroxylamine is considered as an "aldehyde trapping agent" and has demonstrated remedial benefit in countering cyanide toxicity. It is a reducing agent. At 25 mg/l, a lag in gas production resulted with subsequent recovery of the gas production rate comparable to the control. Figure 93. No explanation is given for the anomalous behavior wherein gas production ceased before reaching the control volume. An increased lag in gas production was noted at 50 mg/l with rapid acclimation, approximating the control rate and volume. At 100 mg/l a small amount of gas was produced initially, but eventually ceased after producing only 6% of the control volume.

Lauric Acid

The nutrient salt solution contains some divalent cations which would precipitate lauric acid from solution. Gas production was inhibited to 70% of the control when 750 mg/l of lauric acid was added to the filter feed. Gas production recovered to normal in 6 days. Figure 94.

When 2500 mg/l of lauric acid was added to the feed, gas production almost ceased and after 26 days gas production was still only about 30% of background. Figure 95.

Nitrobenzene

Batch assay of nitrobenzene with unacclimated methanogens appeared to affect only the lag time before onset of methane production. At 25 mg/l, there was only a slight lag of about 0.1 day. At 50 mg/l, the lag time was about 1 day. At nitrobenzene concentrations of 100 and 200 mg/l, the lag time was approximately 2 and 3.5 days respectively. Figure 96. The rate of gas production after the lag period was essentially constant for all concentrations of nitrobenzene.

Continuous additions of nitrobenzene were made to the filter feed. No inhibitory effect was noted on gas production until the nitrobenzene concentration reached 600 mg/l. Figure 97. The gas production initially declined after the 600 mg/l additions commenced. There was a temporary recovery followed by a rapid decline as shown in Figure 98. Gas production was then erratic. Effluent COD did not rise in proportion to the decrease in gas production which is inexplicable.

Phenol

Phenol exhibited only slight toxicity to unacclimated methanogens. At 1000 mg/l, the gas production rate was about 85% of the control. Full gas volume was produced in the presence of 1000 mg/l of phenol. However, at 2000 and 3000 mg/l, the gas production rate was severely inhibited and gas production ceased at 20 and 9% of the control volume. Figure 99.

Fig. 93 Response of Unacclimated Methanogens to Hydroxylamine

Figure 94 750mg/l Lauric Acid 750ml - 1hr

Figure 95

2500mg/l Lauric Acid in 700ml

FIG. 96

KINETIC RESPONSE TO TOXICANT -
NITROBENZENE

Fig. 97 Response of Anaerobic Filter to Toxicant Nitrobenzene

Fig. 98 Response of Anaerobic Filter to Toxicant Nitrobenzene

FIG. 99

KINETIC RESPONSE TO TOXICANT-PHENOL

Phenol at a concentration of 2000 mg/l was added to a filter for increasing periods after a 5 day rest as shown in Figure 100. A one day slug increased the effluent COD but did not inhibit gas production. Effluent COD rapidly decreased as the phenol was displaced from the filter during the 5 day rest period during which the filter only received unadulterated feed. Subsequently, 2000 mg/l was fed for 2, 4 and 7 consecutive days. Gas production decreased to about 70% of background without further decrease.

An anaerobic filter was then fed phenol as the only organic carbon source for a period of a year. It was first fed phenol at a concentration of 1000 mg/l for over a month. The phenol concentration was then increased to 2000 mg/l. When the feed concentration was increased to 4000 mg/l gas production dropped abruptly.

Propanal

Propanal has an aldehyde component which exhibits toxicity to unacclimated methanogens. At a concentration of 1000 mg/l, there was an inhibition of the gas production rate. Total gas production volume was slightly above the control volume, indicating partial metabolism of the propanal. Figure 101. Progressive inhibition of the gas production rate was noted at propanal concentrations of 2000, 4000 and 8000 mg/l.

Extended periods of feeding propanal at step increases in concentration are shown in Figures 102 and 103. The drop in gas production during the 7 day resting period after one propanal concentration is ended and before a higher propanal concentration is begun indicates metabolism of the propanal. Eventually, 5000 mg/l of propanal was added to the feed for a 30 day period. Gas production increased to about 250% of background but showed deterioration at the end.

Thiocyanate

There was no significant inhibition by 1 mg/l of thiocyanate to unacclimated methanogens. Figure 104. However, at 5 mg/l, there was a slight lag in gas production and gas production rate. In addition, there was an abrupt cessation of gas production before all of the acetate was exhausted.

KCNS was assayed by continuous addition to a filter. No inhibition of gas production was observed until the thiocyanate concentration reached 100 mg/l. Even so, at this concentration, no decrease in gas production was observed for 18 days. Gas production precipitously declined to zero thereafter within 3 days. Approximately 30 days after thiocyanate addition ceased, gas production had recovered to normal. Figure 105.

FIG. 100 RESPONSE OF ANAEROBIC FILTER TO TOXICANT

Fig. 101 Batch Response to Propanal

Fig. 102 Response of Anaerobic Filter to Toxicant - Propanal

Fig. 103 Response of Anaerobic Filter to Toxicant Propanal

Fig. 104 Response of Unacclimated Methanogens to CNS⁻

Fig. 105 Response of Anaerobic Filter to Toxicant KCNS (as CN^-)

Vinyl Acetate

Vinyl acetate caused a lag in the gas production rate of unacclimated methanogens. However, at 1000 mg/l, the gas production rate was comparable to the control after 7 days and full gas production was noted. At 2000 and 3000 mg/l, the gas production rate progressively decreased so that after 21 days, the total volume of gas was only 50 and 25% of the control respectively. Figure 106.

Very good acclimation was observed with vinyl acetate. When 1000 mg/l was added for a month to a filter, the effluent COD did not increase noticeably and gas production increased from a background level of 1600 to about 2100 mg/day. Figure 107. Likewise, gas production dropped back to about 1600 when the vinyl acetate additions were stopped for 7 days. Next, 2000 mg/l of vinyl acetate was added continuously. Gas production rose to 2500 ml/day and effluent COD remained constant for 14 days. Then there was an abrupt decline in gas production mirrored by an increase in effluent COD. Vinyl acetate additions were withheld for 7 days and the gas production and effluent COD quickly returned to normal. Next, 4000 mg/l of vinyl acetate was added to the feed. Gas production abruptly soared but within a few days, it dropped rapidly. The filter recovered rapidly when vinyl acetate additions were stopped. The filter was allowed to operate for about a month with only background feed. Again, 4000 mg/l of vinyl acetate was added to the filter. Figure 108. Gas production increased and the effluent COD went from 300 to 1400 mg/l in the first 10 days, followed by a return to 300 for the remainder of the 40 day period. After a 7 day rest, the filter operated quite efficiently with 6000 mg/l of vinyl acetate in the feed for 14 days.

ANTIBIOTICS

Bacitracin

This antibiotic showed no significant inhibition to unacclimated methanogens at concentrations of 100 to 1000 mg/l. At 2000 mg/l, the gas production rate was inhibited to approximately 60% of the control. Figure 109. Bacitracin at 500 mg/l inhibited gas production to about 50% of the background level. Figure 110. When Bacitracin was withheld from the feed, the gas and effluent COD returned to normal in about 7 days. At 1000 mg/l of Bacitracin in the feed, gas production abruptly decreased from 1600 to 700 ml/day and held steady. Figure 111.

Monensin

Monensin is a cattle feed additive. At very low concentrations, it is inhibitory to unacclimated methanogens. Figure 112. Even 0.5 mg/l inhibited the gas production rate to 50% of that of the control, but gas production continued to eventually reach the volume produced by the control. However, at 1.0, 2.5 and 5 mg/l, not only was the gas production rate initially inhibited to about 20%

Fig. 106 Response of Unacclimated Methanogens to Vinyl Acetate

Fig. 107 Response of Anaerobic Filter to Toxicant - Vinyl Acetate.

Fig. 108 Response of Anaerobic Filter to Toxicant Vinyl Acetate

Fig. 109 Response of Unacclimated Methanogens to Bacitracin

Fig. 110 Response of Anaerobic Filter to Toxicant Bacitracin

Fig. 111 Response of Anaerobic Filter to Toxicant-Bacitracin

Fig. 112 Response of Unacclimated Methanogens to Monensin

of the control rate, but gas production ceased at 20, 12 and 9% respectively of the volume produced by the control.

Even though monensin exhibited about 50% inhibition in the gas production rate at 0.5 mg/l, it acclimated very well. Initially, the filter was acclimated to 10 mg/l but the gas production declined to about 50% of background. Figure 113. The filter recovered with background feeding only. Again, 10 mg/l of monensin was added to the feed with no inhibition of gas production. Effluent COD increased somewhat. Eventually, the concentration of monensin was increased to 25, 50, 75 and 100 mg/l with no inhibition of gas production and low effluent COD. Figures 114 and 115. This represents an acclimation to 200 times the concentration which initially caused 50% inhibition of unacclimated methanogens.

Oxytetracycline

There was not significant inhibition of gas production with unacclimated methanogens until the oxytetracycline concentration reached 250 mg/l. Figure 116. This resulted in no lag period but only a decrease in the rate of gas production. Full gas production was achieved.

Oxytetracycline showed no inhibition until the concentration reached 250 mg/l. Figure 117. At this level, gas production did not drop but effluent COD tended to increase. Effluent COD did not decrease during the 7 day rest period when no oxytetracycline was added. Gas production steadily decreased after 500 mg/l of oxytetracycline was added to the feed. Figure 118.

Penicillin

A one-day slug of penicillin to a filter at a concentration of 837,500 units/l resulted in a 40% increase in gas production as shown in Figure 119. No inhibition of gas production was observed at penicillin doses of 400, 500, 750 and 1000 mg/l (1675 units/mg). Figures 120 and 121.

Fig. 113 Response of Anaerobic Filter to Toxicant - Monensin

Fig. 114 Response of Anaerobic Filter to Toxicant - Monensin

Fig. 115 Response of Anaerobic Filter to Toxicant Monensin

Fig. 116 Response of Unnaclimated Methanogens to Oxytetracycline

Fig. 117 Response of Anaerobic Filter to Toxicant Oxytetracycline

Fig. 1: Response of Anaerobic Filter to Toxicant - Oxytetracycline

Figure 119 500mg/l Penicillin (500ml) 1675 units/mg

Fig. 120 Response of Anaerobic Filter to Toxicant - Penicillin

Fig. 121 Response of Anaerobic Filter to Toxicant - Penicillin

SUMMARY AND CONCLUSIONS

The response of methane fermentation after exposure to selected toxicants has been semi-quantitatively evaluated. The toxicants and the corresponding concentrations which resulted in 50% inhibition are listed in Table III for the unacclimated batch methanogenic cultures as well as for the long-term acclimation studies with anaerobic filters. Tables IV and V group the toxicants into relative toxicity categories. These concentrations are approximate because only occasionally did a given toxicant concentration result in precisely 50% inhibition. Therefore, in most cases an interpolation was required to estimate the 50% inhibition concentration.

An attempt was made to estimate the acclimation potential of the methanogens to a toxicant. This acclimation potential was taken to be the ratio of the toxicant concentration which resulted in 50% inhibition to an acclimated filter to the toxicant concentration which resulted in 50% inhibition to unacclimated batch culture methanogens. Table VI groups the toxicants into relative acclimation potential categories.

The compounds have been grouped according to their relative toxicity in concentration ranges. As shown in Table IV, chloroform, cyanide, thiocyanate and Monensin demonstrated 50% inhibition at concentrations below 5 mg/l.

Copper, acrylonitrile and chloropropionate were in the second lowest concentration category of toxicity of 5 to 25 mg/l. It is significant to note the toxicity of the nitrile and chloro compounds.

QAC, sulfite, acrolein, anionic detergent, formaldehyde, hydroxylamine and nitrobenzene exhibited 50% inhibition at concentrations of 25 to 100 mg/l to unacclimated methanogens in batch culture. The toxicants which exhibited 50% inhibition to unacclimated methanogens in batch culture at concentrations of 100 to 500 mg/l were trivalent chromium, nickel, sulfide, acetaldehyde, acrylic acid and oxytetracycline.

In concentrations in excess of 1500 mg/l NH_4^+ , Na^+ , hydroquinone, phenol, propanal, vinyl acetate and Bacitracin exhibited 50% inhibition to unacclimated methanogens in batch culture.

The molecular structural characteristics reported by Chou, et al (1978) to be toxic to methane fermentation were generally shown to be the same in this study. The short, chlorinated organics were most toxic. Compounds containing nitriles were next in line of toxicity. Compounds containing both a double bond and aldehyde group were more toxic than those with only a double bond or aldehyde group. Hydroxyl substitution reduced toxicity. Propanal and vinyl acetate were relatively low in toxicity.

The relative toxicity to acclimated filters indicated that chloroform, chloropropionate and cyanide exhibited 50% inhibition at concentrations of 50 mg/l or less as shown in Table V. Copper, QAC, acrolein, acrylonitrile, anionic detergent, thiocyanate and Monensin were 50% inhibitory in the 50 to 250 mg/l range. Cr^{+++} , Ni^{++} , S^- , acrylic acid, formaldehyde, nitrobenzene, Bacitracin and oxytetracycline made up the 250 to 1000 mg/l category. In the 1000 to 5000 mg/l category were SO_3^- , acetaldehyde, acetone, CS_2 , chloropropandiol, lauric acid and penicillin. Finally in the concentration above 5000 mg/l for 50% inhibition were NH_4^+ , Na^+ , propanal and vinyl acetate.

The toxicants which exhibited to lowest acclimation potential as categorized in Table VI were: Cr^{+++} , Ni^{++} , NH_4^+ , QAC, Na^+ , anionic detergent, propanal, Bacitracin and oxytetracycline. All had acclimation potentials of 2 or less. This category is generally inorganic toxicants and antibiotics. Since propanal was not assayed in the acclimation filter above 5000 mg/l which was not only not toxic but metabolized, it may be misleading to include propanal because its 50% inhibition limit was never reached. It is significant to note the low acclimation potential of the anionic detergent.

The compounds with an acclimation potential of 2 to 5 were Cu^{++} , S^- , acrolein, acrylic acid, acrylonitrile, formaldehyde and vinyl acetate. It is probably misleading to include Cu^{++} and S^- because these are inorganics which were not measured in solution. In the acclimated filter studies, considerable S^- would have been stripped by the gas production.

It is significant that the toxicants which exhibited 50% inhibition in the lowest concentration category demonstrated the greatest acclimation potential. Chloroform and Monensin exhibited acclimation potentials of 90 and 200 respectively. CN^- and CNS^- exhibited acclimation potentials of 25 and 17 respectively.

Table III
Summation of Toxicants and Their Concentrations
Resulting in 50% Inhibition

Compound	Unacclimated Batch - R_{50} (mg/l)	Acclimated Filter - R_{50} (mg/l)	Acclimation Potential	Slug Dose to Filter R_{50} (mg/l)
Cr ⁺⁺⁺	350	> 300	1	< 250
Cu ⁺⁺	15	> 50	4	> 50
Ni ⁺⁺	200	300	1.5	> 500
NH ₄	> 7000	7000	1	4000
QAC	30	> 50	2	50
Na ⁺	7000	10000	1.5	> 20000
PO ₄ ⁼	--	> 2000	--	--
S ⁼⁴	250	1000	4	2000
SO ₃ ⁼	50	> 3500	70	--
Acetaldehyde	300	2500	8	2500
Acetone	--	> 1000	--	--
Acrolein	35	150	4	750
Acrylic Acid	400	1000	2.5	1000
Acrylonitrile	25	75	3	--
Anionic Detergent	50	60	1.2	--
CS ₂	--	2000	--	--
Chloroform	0.5	45	90	250
Chloropropandiol	--	2000	--	--
Chloropropionate	7.5	50	6.7	--
CN ⁻	1.0	25	25	200
2,4 Dinitrophenol	--	--	--	250
Formaldehyde	100	400	4	--
Gasoline	--	--	--	--
Hydroquinone	6000	--	--	--
Hydroxylamine	50	--	--	--
Lauric Acid	--	< 2500	--	--
Nitrobenzene	50	600	12	--
Phenol	3000	--	--	--
Propanal	3000	> 5000	1.7	--
CNS	5	85	17	--
Vinyl Acetate	1500	> 6000	4	--
Bacitracin	2000	1000	.5	--
Monensin	0.5	> 100	200	--
Oxytetracycline	250	500	2	--
Penicillin	--	> 1000	--	--

Table IV
Relative Toxicity to Unacclimated
Batch Methanogens

I.	<u>50% Inhibition at 5 mg/l or Less</u>
	Chloroform
	CN ⁻
	CNS ⁻
	Monensin
II.	<u>50% Inhibition at 5 to 25 mg/l</u>
	Cu ⁺⁺
	Acrylonitrile
	Chloropropionate
III.	<u>50% Inhibition at 25 to 100 mg/l</u>
	QAC
	SO ₃ ⁼
	Acrolein
	Anionic Detergent
	Formaldehyde
	Hydroxylamine
	Nitrobenzene
IV.	<u>50% Inhibition at 100 to 500 mg/l</u>
	Cr ⁺⁺⁺
	Ni ⁺⁺
	S ⁼
	Acetaldehyde
	Acrylic Acid
	Oxytetracycline
V.	<u>50% Inhibition at 1500 mg/l and Above</u>
	NH ₄ ⁺
	Na ⁺
	Hydroquinone
	Phenol
	Propanal
	Vinyl Acetate
	Bacitracin

Table V
Relative Toxicity to Acclimated Filters

I.	<u>50% Inhibition at 50 mg/l or Less</u>
	Chloroform Chloropropionate CN ⁻
II.	<u>50% Inhibition at 50 to 250 mg/l</u>
	Cu ⁺⁺ QAC Acrolein Acrylonitrile Anionic Detergent CNS ⁻ Monensin
III.	<u>50% Inhibition at 250 to 1000 mg/l</u>
	Cr ⁺⁺⁺ Ni ⁺⁺ S ⁼ Acrylic Acid Formaldehyde Nitrobenzene Bacitracin Oxytetracycline
IV.	<u>50% Inhibition at 1000 to 5000 mg/l</u>
	SO ₃ ⁼ Acetaldehyde Acetone CS ₂ Chloropropandiol Lauric Acid Penicillin
V.	<u>50% Inhibition at 5000 mg/l and Above</u>
	NH ₄ ⁺ Na ⁺ Propanal Vinyl Acetate

Table VI
Relative Acclimation Potential of Toxicants

Acclimated Filter
 Unacclimated Batch

Acclimation Potential of 2 or Less

Cr⁺⁺⁺
 Ni⁺⁺
 NH₄⁺
 QAC
 Na⁺
 Anionic Detergent
 Propanal
 Bacitracin
 Oxytetracycline

Acclimation Potential of 2 to 5

Cu⁺⁺
 S⁼
 Acrolein
 Acrylic Acid
 Acrylonitrile
 Formaldehyde
 Vinyl Acetate

Acclimation Potential of 5 to 25

Acetaldehyde
 Chloropropionate
 Cyanide
 Nitrobenzene
 CNS⁻

Acclimation Potential Above 70

SN₃⁼
 Chloroform
 Monensin

When proper attention was paid to SRT and acclimation procedure, methane fermentation of acetate was demonstrated to be stable in the presence of relatively high concentrations of selected toxicants. Slug doses of a wide variety of toxicants to plug flow anaerobic filters demonstrated that most of the toxicants assayed were relatively reversible in that gas production resumed promptly after the toxicants passed through the system. This points up the need to carefully specify the hydraulic flow pattern (CSTR vs plug flow) to minimize the impact of toxicity in industrial wastewater treatment by methane fermentation.

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