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EFFECTS OF SOME INDUSTRIAL CHEMICALS ON ANAEROBIC ACTIVITY MEASURED BY SEQUENTIAL AUTOMATED METHANOMETRY (SAM)

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ABSTRACT

An experimental study was conducted into the effects of exposure of anaerobic bacteria to some commercial industrial chemicals. Anaerobic activity was tested using Sequential Automated Methanometry (SAM). SAM measures small pressure increases caused by gas production in vials containing anaerobic bacteria.

Tested were bleaching agents including hydrogen peroxide and sodium metabisulphite, mothproofing and insect repelling agents containing synthetic pyrethroids, a bacteriostatic agent and non-ionic detergents commonly used in the wool scouring industry. Actively digesting bacterial material was obtained from an experimental anaerobic system treating concentrated effluents from wool scouring industry.

None of the tested chemicals, with the exception of the bleaching agents, displayed any serious adverse effects on anaerobic activity. One of the tested detergents and one of the tested bacteriostatic agents mildly stimulated gas productivity, while strong increases in gas productivity were observed with one of the pyrethroid-containing chemicals. Sodium metabisulphite inhibited gas production but inhibition was reversible. Hydrogen peroxide was highly toxic and completely inhibited methane production even at the lowest added concentrations.

KEYWORDS

Anaerobic digestion, woolscouring, bacterial toxicity assay, detergent, bleaching agent, mothproofing agent, sanitizing agent

INTRODUCTION

Woolscouring involves a process in which raw wool is cleaned with detergents and other chemicals in order to yield a product which is suitable for further processing such as manufacturing of clothes and carpets. The disposal of woolscouring effluents represents a particular problem because no acceptable answer to the degradation problems of this particular waste has yet been given. The situation has been worsened in recent years leading to increasing official concern over pollution. This concern has shown itself in stringent anti-pollution measures or threats of such measures in countries like Japan, U.S.A., the Soviet Union, U.K. and the other EEC countries. It is therefore of vital importance that the scouring industry has an effective and economic means for effluent treatment processes available.

Woolscouring waste is obtained from washing dirty wool with detergent in a slightly alkaline environment (pH=8), in a solution containing about 0.5 - 2 g/l of non ionic detergent; COD ranges between 50,000 and 100,000 mg/l and may contain 5,000 - 15,000 mg/l woolgrease.

The objectives of the research were: (i) a demonstration of the use of a new analytical method (SAM) for measuring anaerobic activity in bacterial samples and; (ii) to study the effects of additions of slug doses of commonly used industrial chemicals on the activity of the methanogenic population in an anaerobic reactor treating woolscouring waste.

Several anaerobic toxicity assays based on the measurement of gas production have been described (Owen *et al.*,1979; Valcke and Verstraete, 1983). An automated pressure-based method for monitoring gas production by bacterial cultures was described by Concannon *et al.* (1988). The method used a number of solid-state pressure transducers (full scale approx. 100 kPa) fitted to an equal number of individual test vials containing bacterial samples. Data were collected on-line from the individual pressure transducers.

The SAM system uses only one pressure transducer which measures pressure build-up in a number of test vials by a mechanical multiplexing system, effectively connecting the pressure sensor to different test vials, one at a time. This approach has the following advantages. (i) Only one high-quality pressure transducer is used; observations obtained on different samples are directly comparable. (ii) Gas production is measured by a differential rather than a cumulative method by releasing gas pressure after each measurement interval. This results in increased sensitivity and virtually constant pressure conditions in the test vials. (iii) A low detection limit and very high sensitivity are achievable as the used pressure transducer operates on an adjustable full scale limit of 1.5 - 7.5 kPa.

This paper describes an initial demonstration of the SAM technique based on total gas production by individual test samples. In this study anaerobic activity was measured by monitoring of biogas production and manual analysis of the composition of the gas. The designation "Sequential Automated Methanometry" was choosen for the SAM system as the final purpose of the system is projected to achieve the following objectives: (i) measurement of methane production only by chemical trapping of carbon dioxide; (ii) on-line kinetic and stoichiometric analysis of produced methane. Further development of the SAM system is projected in the future.

EXPERIMENTAL PROCEDURE

Sequential Automated Methanometry (SAM). The principle of SAM is schematically represented in Fig. 1. The instrument consists of a valve with a static part (1) and a rotating part (2). Multiple outlet ports are connected to the static part of the valve (3). Each of the outlets are connected with leak-proof test vials containing bacterial samples (4, only one vial shown in Fig.1). Test vials are fitted with a septum holder on the top (5) for introduction of reagents. The rotating part of the valve is driven by an electromotor (6) and rotation is monitored by a phase encoder (7). Pressure in the rotating part of the valve is measured by a Rosemount 1151DP Alphaline Differential Pressure Transmitter (8) against atmospheric pressure. Temperature of the test vials is measured by a Pt100-type temperature sensor (9, ±0.1°C). Electric signals to electromotor and from sensors are controlled and monitored by a microcomputer running a control program (11) via an interface module (10). Valve, sensors and test vials are located inside a constant temperature cabinet (35±0.5°C) and contents of test vials are mixed by an intermittently operating slow-moving rotary shaker (not shown in Fig.1). The SAM system was capable of simultaneous monitoring of 34 test vials.

Pressure increments in the test vials were measured at 30 minute intervals and recorded together with temperature data. After each pressure measurement the gas pressure inside the test vials was equilibrated to atmospheric pressure by release of the formed gas through connecting hose (3) for a few seconds at the point of the rotating valve.

Pressure increment readings were calibrated into volumetric changes under the prevailing conditions of the experiments as follows. A calibrated 1 ml gas sampling syringe was used to inject measured quantities of air into the test vials filled with 50 ml water. The entire system was then equilibrated to 35°C including test vials and gas sampling syringe. The resulting pressure changes correlated well with injected volumina of air (r=0.998). Detection limit of the SAM system under the used experimental conditions was approx 10-20 µl of gas. Gas pressure increments measured during the toxicity trials were corrected to standard temperature and pressure (STP).

Toxicity assays and analytical procedures. A two-litre sample of bacterial sludge was taken from a 250 l anaerobic reactor operating on raw woolscour effluent and thoroughly mixed by a variable speed mechanical stirrer. Sub-samples were taken from the mixed sludge for determination of TS and VS concentrations. 50 ml subsamples were transferred into individual test vials, each vial containing 1.3 g VS (first series of experiments) or 1.9 g VS (second series). Headspaces of the vials were flushed with dry nitrogen and stored at 35°C for 6 hours to achieve a low steady level of gas production before recording was started. Different quantities of the tested chemicals were dissolved in 1 ml distilled water and injected into the test vials to start the toxicity assay. Parallel blank tests were performed in 5 vials with 1 ml distilled water added and the results averaged. All vials were shaken twice hourly for one minute by a rotary shaker during recording.

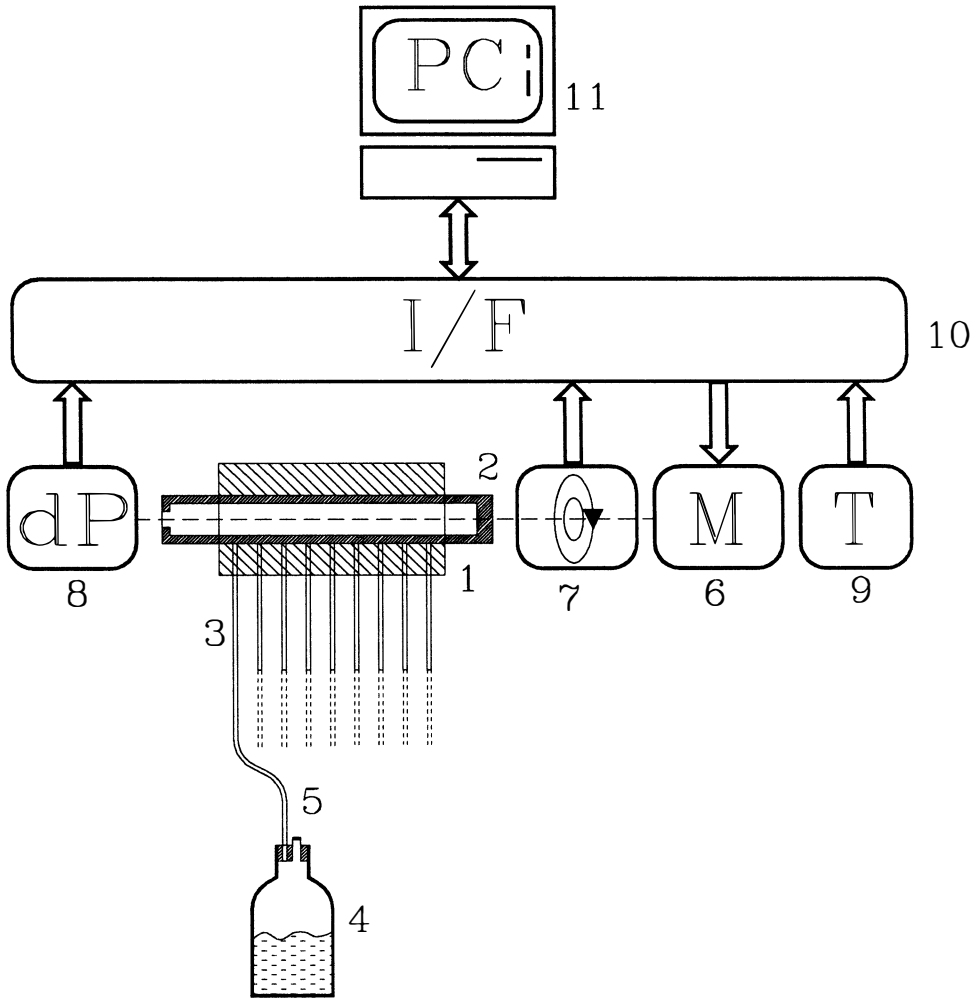

Fig.1. Schematic representation of Sequential Automated Methanometry. 1: gas multiplexing valve; static part, 2: gas multiplexing valve; rotating part, 3: multiple valve outlet ports, 4: test vial with bacterial sludge, 5: reagent injection port, 6: electromotor, 7: phase encoder, 8: differential pressure transducer, 9: temperature sensor, 10: interface module, 11: microcomputer

At the end of the trials 20 ml gas samples were collected from the headspaces of the test vials and analyzed for methane and carbon dioxide. Final pH was measured in all sludge samples. Total solids (TS) were determined by drying of the sample overnight at a temperature of 105°C. Volatile solids were determined by heating of the dried sample to 600°C overnight; the weight loss by the ignition of the sample was defined as VS. Biogas samples were analyzed for methane content with gas chromatography. 500 µl samples were injected on a 6 ft x 1/8"

ss Porapak T column. Oven temperature was 60°C, injector 180°C, thermal conductivity detector 210°C. Quantification was done by normalization.

TABLE 1. Tested Industrial Chemicals

Product[1]	Description/use	Max. Conc. (mg/l)
ICI Teric GN9™	Non-ionic detergent; nonyl phenol/8-10 mols ethylene oxide condensate	5000
Shell Dobanol 91-6™	Non-ionic detergent; fatty alcohol/6 mols ethylene oxide condensate	5000
Wellcome Perigen™	Insect-resist agent; synthetic pyrethroid	5000
Mitin AL™	Insect-resist agent; synthetic pyrethroid plus hexahydropyrimidinmethroine derivate	5000
Sanitized SN™	Bacteriostatic agent	5000
Hydrogen peroxide	Bleaching agent	180
Sodium metabisulphite	Bleaching agent	5000

[1]These products were used for scientific purposes only and no endorsement nor discouragement for their use is intended.

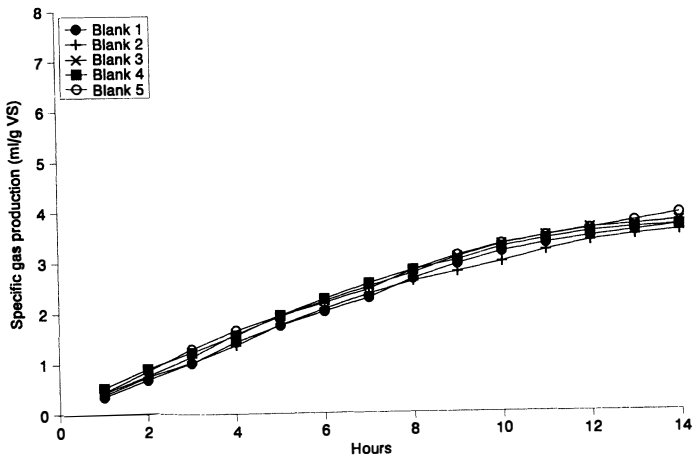

Fig. 2. Cumulative specific gas production curves in five blank samples (no chemical added).

RESULTS

Experiments were carried out with various bleaching agents, sanitizing agents, mothproofing agents and detergents (Table 1).

Fig. 2 shows cumulative gas production curves after incubation of five blank samples (incubated with 1 ml distilled water). The specific gas production curves were very similar demonstrating the reproducibility of the techniques used.

Table 2 summarizes the results, indicating accumulated gas production values and methane concentrations in the headspace gas of the test vials.

TABLE 2. Anaerobic Toxicity Screening of Woolscouring Chemicals

	Conc (mg/l)	Gas (ml/g VS)[1]	CH_4 (%)[2]	CO_2 (%)[3]	pH[4]
Blank (avg)	0	3.7	23.9	5.3	8.1
ICI Teric	2500	4.6	19.6	6.2	8.1
	5000	5.6	31.6	4.1	8.1
Shell Dobanol	2500	4.0	23.0	5.3	8.0
	5000	2.9	15.5	6.6	8.0
Mitin AL	2500	7.5	ND	ND	8.0
	5000	6.7	ND	ND	7.9
Wellcome Perigen	2500	3.6	12.5	4.7	8.1
	5000	4.1	22.8	4.8	8.0
Sanitized SN	2500	5.0	25.1	5.3	8.0
	5000	5.6	13.6	5.1	8.0
Hydrogen peroxide	90	0.3	ND	ND	8.1
	180	1.1	ND	ND	8.0
Sodium metabisulphite	2500	2.1	ND	ND	8.0
	5000	1.2	ND	ND	7.9

[1]total amount of gas produced after 14 hours per g VS; average values of two observations; [2]percentage methane in headspace gas measured after 16 hrs; [3]percentage carbon dioxide in headspace gas measured after 16 hrs; [4]final pH in samples. ND = Not determined

The results presented in Table 2 show a strongly stimulating effect by Mitin AL on gas production, whereas the addition of hydrogen peroxide and sodium metabisulphite appears to have inhibited gas production. Toxicity assays with these chemicals were repeated and studied in more detail. In order to investigate whether any methane was produced after addition of the chemicals, headspaces of the test vials were flushed a second time with nitrogen gas immediately after the addition of the bleaching agents hydrogen peroxide and sodium metabisulphite.

A summary of the results of this series of experiments is presented in Table 3.

TABLE 3. Anaerobic Toxicity Screening of Strongly Inhibiting and Stimulating Chemicals.

	Conc (mg/l)	Gas (ml/g VS)[1]	CH_4 (%)[2]	CO_2 (%)[3]	pH[4]
Blank (avg)	0	6.2	14.0	4.3	8.0
Mitin AL	500	6.2	14.9	4.7	8.0
	1250	7.1	15.5	4.3	7.9
	2500	8.6	20.5	4.8	8.0
	5000	10.1	22.0	5.2	8.0
Hydrogen peroxide	18	0.6	0.0	4.4	7.8
	45	2.8	0.0	5.5	7.7
	90	1.3	0.1	3.5	7.8
	180	4.3	0.0	8.2	7.6
Sodium metabisulphite	500	5.8	11.3	3.3	7.9
	1250	5.3	10.3	3.2	8.0
	2500	2.7	11.4	3.4	8.1
	5000	2.1	2.1	3.3	8.2

[1]total amount of gas produced after 27 hours per g VS; [2]percentage methane in headspace gas measured after 63 hrs; [3]percentage carbon dioxide in headspace gas measured after 63 hrs; [4]final pH in samples.

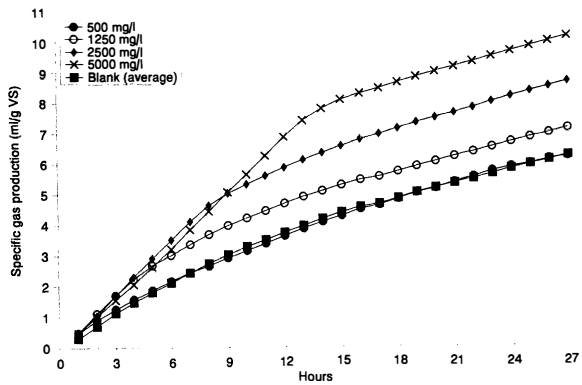

Fig. 3. Cumulative gas production curves after addition of Mitin AL.

Mothproofing agent. Cumulative gas production curves of the test using Mitin AL are shown in Fig.3. It is apparent that increases of gas production occurred with increased added amounts of Mitin AL up to a maximum of about twice the amount of gas produced by the blank for the highest added concentration. Also initial gas production rates exceed those of the blank (most significant for the 2500 mg/l and 5000 mg/l Mitin concentrations), but level off to a gas production rate equal to the gas production rate of the blanks. It also appears from Table 3 that the concentrations of methane measured in the headspace of the vials increase upon increasing concentrations of added Mitin AL.

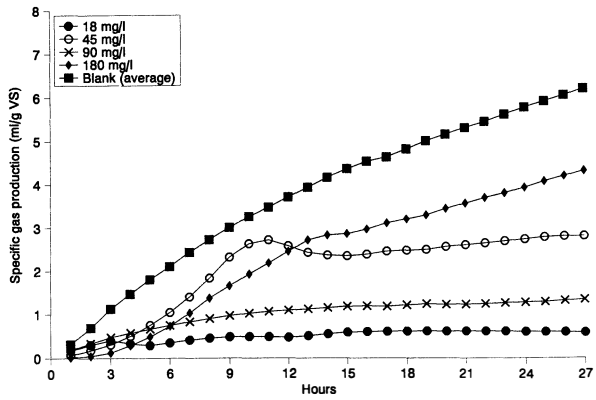

Fig. 4. Cumulative gas production curves after addition of hydrogen peroxide.

Hydrogen peroxide. Fig. 4 shows cumulative gas production curves after addition of hydrogen peroxide. Hydrogen peroxide appears to cause inhibition of gas production. Figures presented in Table 3 indicate that in none of the cases were any appreciable amounts of methane produced. Hydrogen peroxide was very toxic and terminated methane production even at the lowest added concentration. Addition of peroxide caused an immediate strong production of gas leading to excessive foaming in the vials with highest added concentrations, presumably by production of oxygen through a catalase mediated cleavage of peroxide (Gottschalk, 1988). SAM recording started after this initial burst of gas production. Accumulative gas production curves obtained during the first series of experiments (not shown) indicated initial negative gas production followed by a positive gas production for the highest added concentration (180 mg/l). Table 3 shows that no significant methane production had occurred even after more than 2 days although production of carbon dioxide exceeded the blank. Final pH values measured in the vials with added peroxide are slightly lower than in any of the other tested cases in Table 3.

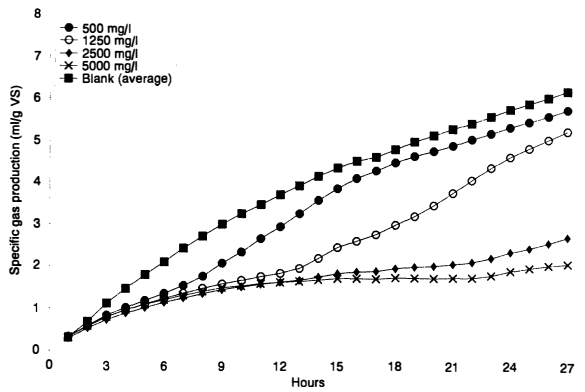

Fig. 5. Cumulative gas production curves after addition of sodium metabisulphite.

Sodium metabisulphite. Cumulative gas production curves after addition of sodium metabisulphite (Figure 5) show increasing inhibition of the gas production with increasing concentrations added. Complete inhibition was not achieved as in the case of hydrogen peroxide. The inhibition was temporary for the two lowest concentrations; the gas production resumed the rate of the blank after 12-15 hours. No smell of hydrogen sulphide was evident in the test vials after completion of the experiment.

DISCUSSION

Detergents. Non-ionic detergents slightly stimulated or inhibited gas production at 2500-5000 mg/l concentrations of the added chemical products. Khalil *et al.* (1989) reported inhibition of growth of *Methanosarcina barkeri* by sodium decylbenzene sulphonate at between 15 and 20 mg/l. As concentration of detergents in the products Shell Dobanol and ICI Teric was not determined, it is not possible to assess the actual concentration of the detergents during the toxicity assays.

Mothproofing agents. Gas production was stimulated by the addition of Mitin AL, a pyrethroid containing product. Some pyrethroids have been shown to be biodegradable by aerobic bacteria (Maloney *et al.*, 1988). In case the pyrethroid ingredient of Mitin AL would be amenable to anaerobic degradation it seems very unlikely that the pyrethroid would have given rise to immediate increase of the rate of gas production by an unadapted bacterial culture. Observed increased gas production may have been caused by anaerobic fermentation of other organic constituents contained in the product Mitin AL.

Hydrogen peroxide. Re-adsorption of oxygen collected in the headspace of the vials after addition of hydrogen peroxide may occur by facultative anaerobes leading to an initial net decrease of the headspace pressure in the vials. It is likely that the increases of gas production recorded for the 180 mg/l added concentrations in Fig.4 at the end of the test period are caused by either the production of carbon dioxide formed by oxidation, or by a decrease of dissolved bicarbonate in solution caused by a lower pH. Addition of as low as 18 mg/l H_2O_2 completely terminated methane production and no recovery was evident. Hydrogen peroxide forms a serious threat to the anaerobic digestion process. Cocci *et al.*(1985) recommend a reduction of the peroxide concentration to less than 8 mg/l for safe operation of an anaerobic system. However in the conducted experiments the methanogens were directly exposed to peroxide, which is less likely to occur in practice. Peroxide in concentrated woolscour effluent caused by haphazard spillage in a practical situation is likely to rapidly disappear through reaction with reducing substances in the woolscour effluent before it would enter an anaerobic reactor. Cocci *et al.*(1985) recommend an eight-hour holding period to reduce the toxicity of peroxide containing waste.

Sodium metabisulphite. Sodium metabisulphite caused disruption of the anaerobic processes although in all cases recovery of the gas production seemed to occur, with the possible exception of the highest added concentration. This indicated an adaptation of the indigenous microorganisms to the sodium metabisulphite under the assay conditions. It is possible that inhibition was caused by the presence of Na^+ ions (Rinzema *et al.*, 1988).

CONCLUSIONS

- Sequential Automated Methanometry is a useful tool for studying effects of chemicals on gas productivity under anaerobic conditions, with good reproducibility and sensitivity.

- A number of chemicals, commonly used in the woolscouring industry, including mothproofing agents, bacteriostatic agents, sanitizers and detergents, were found to be slightly inhibitory, non-toxic or stimulatory to anaerobic activity and are not considered harmful to anaerobic treatment processes at the tested concentrations.

- Hydrogen peroxide has a highly toxic effect on anaerobic processes even at the lowest added concentration of 18 mg/l. Sodium metabisulphite causes partial inhibition of the methane production which is reversible without any remaining adverse effects on gas production.

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REFERENCES

Cocci, A., Landine, R.C., Brown, G.J. and Tennier, A. (1985). Pilot-scale treatment of peroxide bleachery waste, paper machine effluent and waste activated sludge. *Proc. 40th Indust. Waste Conf., Purdue Univ.*, 335-341.

Concannon, F., Reynolds, P.J., Hennigan, A. and Colleran, E. (1988). Development of a computerized continuous assay for specific methanogenic activity measurement. In: *Poster papers, proc. 5th Int. Symp. Anaer. Digest., Bologna, Italy, 22-26 May 1988*, pp. 177-181.

Gottschalk, G. (1988). In: *Bacterial Metabolism.* 2nd Ed. Springer Verlag, p35.

Khalil, E.F., Whitmore, T.N., Gamel-El-Din, H., El-Bassel, A. and Lloyd, D. (1989). The effect of detergent on methanogenesis by *Methanosarcina barkeri. FEMS Microbiol. Lett., 57,* 313-316.

Maloney, S.E., Maule, A. and Smith, A.R.W. (1988). Microbial transformation of the pyrethroid insecticides: Permethrin, Deltamethrin, Fenvalerate, and Fluvalinate. *Appl. Environ. Microbiol., 54,* 2874-2876.

Owen, W.F., Stuckey, D.C., Healy, J.B. Jr., Young, L.Y. and McCarty, P.L. (1979). Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Water Res., 13,* 485-492.

Rinzema A., van Lier, J. and Lettinga, G. (1988). Sodium inhibition of acetoclastic methanogens in granular sludge from a UASB reactor. *Enzyme Microb. Technol., 10,* 24-32.

Valcke, D. and Verstraete, W. (1983). A practical method to estimate the acetoclastic methanogenic biomass in anaerobic sludges. *J. Wat. Pollut. Control Fed., 55,* 1191-1195.