Automated manometric method to assess anaerobic toxicity of chemicals

F. Fdz-Polanco, P. Nieto, S.I. Pérez-Elvira and M. Fdz-Polanco

Department of Chemical Engineering and Environmental Technology, University of Valladolid, P° Prado de la Magdalena, s/n. 47005 Valladolid, Spain

Abstract Industrial additives eventually used for different purposes (antifoaming, cleaning, bactericides, antiscale, etc) are discharged to the wastewater treatment plant. The anaerobic toxicity of these commercial products is not provided by suppliers. A new manometric method is developed and tested to evaluate anaerobic toxicity or inhibition using four different commercial products. Antifoaming Cleron 6 (50–200 ppm), bactericide Divosan-forte (0.05–1.0% v/v), bleach (0.1–1.0% v/v) and cleaning agent Topax 66 (0.10–1.0% v/v). According to the different methods proposed in the literature, from the methane production rate, it is possible to calculate both methanogenic activity evolution and final substrate removal and quantify the potential inhibitory effect of commercial additives. The experimental method is simple and reliable.

Keywords Anaerobic toxicity; anaerobic treatment; manometric method

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Introduction

A large number of additives are used in industry with different purposes (antifoaming, cleaning, bactericides, antiscale, etc) and then discharged to the wastewater treatment plant. In many cases their chemical formulation is not provided by the suppliers and little is known about their fate and effect on biological reactions and treatment plant performance. However, digester failures and imbalances have been detected after their release, giving some hints about their toxic behaviour.

Toxicity assays are necessary in order to assess the potential effects of chemicals on anaerobic microorganisms. Existing methods are founded on methanogenic activity and anaerobic biodegradability protocols. A great number of proposed methods can be found in the literature (Rozzi and Remigi, 2004) but direct methods based on gas production monitoring are the most frequently used. Toxic/inhibitory effect is evaluated as: (i) a decrease in biogas/methane production rate (% reduction of specific methanogenic activity) or (ii) a drop in biogas/methane production over a time period. There is a recent standard protocol for anaerobic toxicity evaluation (ISO 13641-1, 2003) although previous protocols are still in use. Table 1 presents some relevant characteristics of toxicity assays described in the literature.

The objective of this work is to develop and check an automated manometric device for anaerobic activity and toxicity assessment. In order to perform this study several industrial additives which were supposed to have a negative effect on the behaviour of full-scale anaerobic reactors were tested.

Methodology

Equipment derives from an early study (Fdz-Polanco *et al.*, 2005). The main difference is the 2-chamber flask (Figure 1) instead of the two separate bottles (digestion and gas-collection) initially proposed. This new configuration results in a more compact, easier to operate and less prone to leakages set-up.

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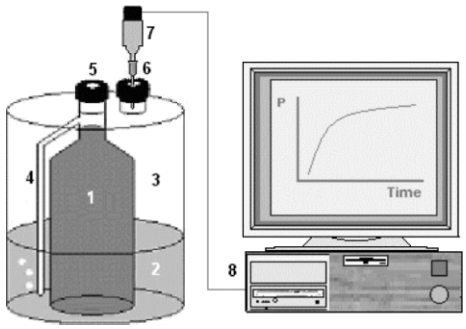
Table 1 Characteristics of anaerobic toxicity assays described in literature

Reference	Inhibitor/toxic	Substrate	Inoculum	Met. clas	Inh. calc.
Johnson and Young (1983)	Semivolatile org. comp. (1–100 mg/L)	Ethanol (1 mL/L)	Synthetic	M-GC (MN)	GP
Koster *et al.* (1986)	Sulphide (<1000 mg/L)	Acetate (0.5–2.0 g/L)	Granular sludge (0.08–0.40 g VSS/L)	GC	A
Campos and Chernicharo (1990)	Lithium chloride (0.25–2 g Li^+/L)	Acetate (0.6–1.9 g/L)	Synthetic granular sludge (2.5 g VSS/L)	M (PT)	A
Sierra-Álvarez *et al.* (1991)	Pulping wastewaters	VFA (4 g COD/L)	Granular (1.5 g VSS/L)	V (LD)	A
Young and Tabak (1993)	Toxic organic chemicals (25–800 mg/L)	Ethanol (20 g COD/L)	Adapted to ethanol	M (MN)	GP
Feijoo *et al.* (1995)	Sodium (0.8–27.7 g Na + /L)	VFA (3 g/L)	(a) Adapted (b) Not adapted	V (LD)	A
Madsen and Rasmussen (1996)	Surfactants, phenols, pesticides	Ethanol methanol	Municipal	M (PT)	GP (7 d)
Eismann *et al.* (1997)	Phosphine (10–1000 ppm)	Sodium acetate (0.8 g/L)	Enriched sediments 0.4 g TSS/L	V (SPD)	A
Schonberg *et al.* (1997)	Petrochemical waste	Acetic acid (1120 mg/L)	(a) Enriched (b) Acclimated	V (LD)	GP
Fang and Chan (1997)	Phenol (<3000 mg/L)	VFA ratio So/Xo = 2.5	Synthetic (500–2000 mg VSS/L)	V (SPD)	A
Vidal *et al.* (1997)	Chlorine bleaching effluents (0–100% v/v)	Mixture of VFA (4.3 g COD/L)	Granular form sugar factory (2 g VSS/L)	V (LD)	A
Lu and Hegemann (1998)	Formaldehyde (<3000 mg/L)	Glucose processing WW	(a) Adapted (2.5 g VSS/L) (b) Not adapted	V (LD)	GP (20 d)
García *et al.* (2000)	Surfactants (20–200 mg C/L)	Glucose (100 mg C/L)	Municipal (4 g TS/L)	M (PT)	GP (100 d)
González-Gil *et al.* (2002)	Formaldehyde (0.2–1 g/L)	Acetate, VFA (1–3 g/L)	Granular from brewery (2 g VSS/L)	V (LD)	A
Sponza (2003)	TCE (1–40 mg/L) CT (0.5–8 mg/L)	Glucose (3.5 g COD/L)	Yeast factory 7 g TSS/L	V (LD)	A
ISO 13641–1 (2003)	Substances	Glucose (2 g/L)	Sewage plant or other treating domestic sewage (20–40 g_TS/L)	M (PT)	GP (up to 3 d)
ISO 13641–2 (2003)	Substances	Degradable substrate	Sewage plant or other sources of anaerobes (0.20 g TS/L)	M (PT)	GP (aprox. 7 d)
This work	Industrial additives	Mixture of VFA 2–3 g COD/L	Granular from brewery and sugar factory (5 g VSS/L)	M (PT)	A

Met. clas: classification of the method, GC: gas-chromatographic, M: manometric, V: volumetric, LD: liquid displacement, MN: manometer, PT: pressure transducer, SPD: syringe piston displacement. Inh. calc.: inhibition calculation A: decrease in gas production rate; GP: drop in gas production over a period of (days)



1. Digestion chamber (200 mL)
2. Alkaline solution (15 g NaOH/L)
3. Headspace in gas collecting chamber
4. Connecting pipe
5. Liquid and gas sampling port
6. Syringe needle
7. Pressure transducer
8. PC with data acquisition card

Figure 1 Experimental set-up for methanogenic activity and toxicity assessment

The flask consists of an inner 150 mL digestion chamber and an outer 600 mL gas collecting chamber that can be partially filled with an alkaline solution or acidified water. Thus pressure increase is directly related to the production of methane or biogas. The volume of the alkaline solution in these assays was 250 mL, but it could be chosen depending on the expected pressure increase and the range of the pressure transducer. In these conditions, the final pressure was lower than 500 mbar. Each flask is connected by low-permeability tubing to a commercial pressure transducer (0–500 mbar). 12 flask-transducer units are placed inside a temperature controlled cabin (35°C), and pressure readings are directly plotted and registered in an Excel spreadsheet. Methane production is calculated based on the volume of the headspace and pressure readings. Activities and inhibition are easily reported by means of a macro.

Inoculum

Depending on the chemical assessed sludge from different anaerobic industrial plants (sugar, brewery) were used. In order to readapt the inoculum and minimize endogenous biogas production during the assay, the sludge was stored for 3 days at 35°C.

Reactives

A VFA mixture 100 g COD/L (acetic, propionic and butyric, 4:1:1) pre-neutralized stock solution was used as substrate. 100 mg $Na_2S{\cdot}9H_2O$ and 1.0 g $NaHCO_3$ per litre of medium were added as reducing and buffer, respectively. A mixture of nutrients and minerals (Field *et al.*, 1988) was introduced to avoid limitations by other factors different from the toxicant.

Step by step procedure

The procedure was: (a) addition of calculated amount of dilution water, (b) addition of reducing solution and buffer, (c) addition of sludge, nutrients and minerals, (d) addition of substrate and inhibitory compound, (e) pH adjustment (7.2 ± 0.1), (f) introduction of the flasks into the thermostated cabin and (g) when the operational temperature is reached (30 min), close the gas-collecting chamber, connect the pressure transducers and run the registering program.

The digestion chamber has a negligible headspace so it is no longer necessary to flush the system with oxygen-free gas. When anaerobic sludge has been stored for long periods a previous feeding without toxicant should be added to facilitate the reactivation and to avoid long lag phases. Analysis of every inhibitory concentration was performed in duplicate.

Table 2 Summary of anaerobic toxicity assays and range of concentrations

Product name	Use	Composition	Inh. concentration	Inoculum
Cleron 6	Antifoaming	Silicone emulsion	50–200 mg/L	Granular from sugar factory
Divosan-forte	Bactericide	Peracetic acid solution	0.05–1.0% (v/v)	Granular from brewery
Commercial bleach	Disinfectant	Hypochlorite solution	0.10–1.0% (v/v)	Granular from brewery
Topax 66	Chloro-alkaline cleaning	n.a.	0.10–1% (v/v)	Flocculent from brewery

n.a. not available

Inhibition calculation

Inhibition (% INH) is calculated by means of equation 1 that compares specific methanogenic activity of a control assay (free of toxicant) and an assay prepared with the desired concentration of the chemical under study.

$$\%\mathrm{INH} = \frac{(\mathrm{ACT_k} - \mathrm{ACT_a})}{\mathrm{ACT_k}} * 100 \quad (1)$$

where: $\mathrm{ACT_k}$ maximum specific methanogenic activity in the assay without toxicant (control); and $\mathrm{ACT_a}$ maximum specific methanogenic activity in the assay with predetermined concentration of toxicant.

Results and discussion

Different toxicity assays were performed to check industrial additives (Table 2). The influence of the additives on the rate of methane production was determined by adding

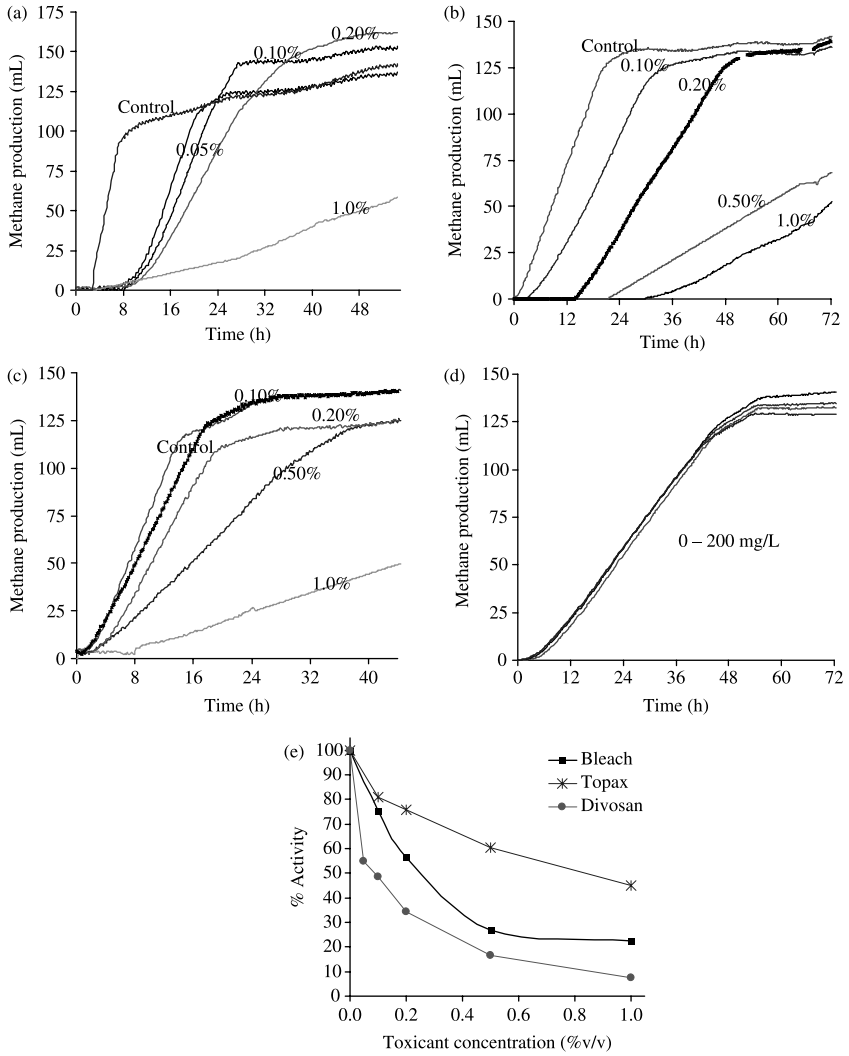


Figure 2 Methane production curves for different toxicant concentrations: (a) bactericide Divosan-forte; (b) bleach; (c) cleaning Topax 66; and (d) antifoaming Cleron 6; (e) activity reduction for Divosan-forte; Topax 66 and bleach

various amounts of the chemicals and measuring methanogenic activity. Figure 2 shows the effect of four additives tested on activity measurements.

According to Figure 2a, the bactericide (Divosan-forte) temporally inhibits methane productions as shown by the decrease of the slope of the methane production curve. After this inhibitory effect the bactericide is partially biodegraded as indicated by the lower values of total methane produced in the control flask compared to the assays in the flasks with different concentrations of bactericide. The plot shows similar lag-times for all the assays, except for the control, which started before the others.

The commercial bleach assays (Figure 2b) showed longer lag phases while toxicant concentration increases, however the same methane production as the control was reached after 3 days at 0.20 v/v%.

The chloro-alkaline cleaning tested (Figure 2c) presented a different pattern: control and toxicity assays showed similar lag phases in the range 0–0.5% v/v but the slope of the curve decreased as toxicant concentration increased. At higher concentrations (1%), a longer lag-time was observed.

The antifoaming agent (Figure 2d) did not show any toxic effect in the range tested. The maximum antifoam concentration tested was four times the value recommended by the suppliers, and it can be asserted that when using recommended concentrations of the chemical no problem is expected.

Figure 2e illustrates the effect of the concentration of the additives on the methanogenic activity of the sludge. Divosan-forte showed the highest toxic effect, with a sharply activity decrease even at low concentrations (0.05% v/v), while Topax-66 caused a slight decrease in the activity at the recommended concentration (0.20% v/v). The IC50 values reported were Divosan-forte 0.06% v/v, bleach 0.24% v/v and Topax 0.83% v/v.

Conclusions

A new experimental set-up has been developed to assess biological parameters of anaerobic sludge by means of an automated manometric method. It is easy-to-operate, fast and allows direct calculation of activities and inhibitions.

This method has been used to assess the methanogenic activity and anaerobic toxicity of several industrial additives. Short-term batch bioassays are adequate in order to evaluate the effect of certain toxicants on methane production but longer assays are required to assess the recovery and adaptation of sludge exposed to toxicants.

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References

Campos, C.M.M. and Chernicaro, C.A.L. (1991). The use of the SMA-TEST for measuring toxicity in anaerobic sludges. *Wat. Sci. Tech.*, **24**(12), 103–111.

Eismann, F., Glindemann, D., Bergmann, A. and Kuschk, P. (1997). Effect of free phosphine on anaerobic digestion. *Wat. Res.*, **31**(11), 2771–2774.

Fang, H.H.P. and Chan, O. (1997). Toxicity of phenol towards anaerobic biogranules. *Wat. Res.*, **31**(9), 2229–2242.

Fdz-Polanco, F., Nieto, P., Pérez, S.E., Van der Zee, F.P., Fdz-Polanco, M. and García, P.A. (2005). Automated equipment for anaerobic sludge parameters determination. *Wat. Sci. Tech.*, **52**(1–2), 479–485.

Feijoo, G., Soto, M., Méndez, R. and Lema, J.M. (1995). Sodium inhibition in the anaerobic digestion process: Antagonism and adaptation phenomena. *Enz. Microb. Tech.*, **17**(2), 180–188.

Field, J., Sierra-Alvarez, R. and Lettinga, G. (1988). Ensayos anaerobios. 4° Seminario DAAR. Valladolid, Spain. pp. 52-81

García, M.T., Campos, E., Sánchez-Leal, J. and Ribosa, I. (2000). Anaerobic degradation and toxicity of commercial cationic surfactants in anaerobic screening tests. *Chemosphere*, **41**(5), 705–710.

González-Gil, G., Kleerebezem, R. and Lettinga, G. (2002). Assessment of metabolic properties and kinetic parameters of methanogenic sludge by on-line methane production rate measurements. *Appl. Microbiol. Biotechnol.*, **58**, 248–254.

ISO 13641-1 (2003). Water quality – Determination of inhibition of gas production of anaerobic bacteria – Part 1: General test, ISO, Geneva, Switzerland.

ISO 13641-2 (2003). Water quality – Determination of inhibition of gas production of anaerobic bacteria – Part 2: Test for low biomass concentrations, ISO, Geneva, Switzerland.

Johnson, D.L. and Young, J.C. (1983). Inhibition of anaerobic digestion by organic priority pollutants. *J. WPCF*, **55**(12), 1441–1450.

Koster, I.W., Rinzema, V., de Vegt, A.L. and Lettinga, G. (1986). Sulfide inhibition of the methanogenic activity of granular sludge at various pH-levels. *Wat. Res.*, **20**(12), 1561–1567.

Lu, Z. and Hegemann, W. (1998). Anaerobic toxicity and biodegradation of formaldehyde in batch cultures. *Wat. Res.*, **32**(1), 209–215.

Madsen, T. and Rasmussen, H.B. (1996). A method for screening the potential toxicity of organic chemicals to methanogenic gas production. *Wat. Sci. Tech.*, **33**(6), 213–220.

Rozzi, A. and Remigi, E. (2004). Methods of assessing microbial activity and inhibition under anaerobic conditions: a literature review. *Rev. Env. Sci. Biotech.*, **3**(2), 93–115.

Schonberg, J.C., Bhattacharya, S.K., Madura, R.L., Mason, S.H. and Conway, R.A. (1997). Evaluation of anaerobic treatment of selected petrochemical wastes. *J. Haz. Mater.*, **54**(1/2), 47–63.

Sierra-Alvarez, R., Kortekaas, S., Van Eekert, M. and Lettinga, G. (1991). The anaerobic biodegradability and methanogenic toxicity of pulping wastewaters. *Wat. Sci. Tech.*, **24**(3/4), 113–125.

Sponza, D.T. (2003). Toxicity and treatability of carbontetrachloride and tetrachloroethylene in anaerobic batch cultures. *Int. Biodet. Biodegrad.*, **51**(2), 119–127.

Vidal, C., Soto, M., Field, J., Mendez-Pampin, R. and Lema, J.M. (1997). Anaerobic biodegradability and toxicity of wastewaters from chlorine and total-free bleaching of eucalyptus Kraft pulp. *Wat. Res.*, **31**(10), 2487–2494.

Young, J.C. and Tabak, H.H. (1993). Multilevel protocol for assessing the fate and effect of toxic organic chemicals in anaerobic treatment processes. *Wat. Env. Res.*, **65**(1), 34–35.