A Feasibility Study in eThekwini Municipality on Anaerobic Digestion for the Treatment of Toxic and High Strength Organic Wastes: A Study of the Business Case of Treating High Strength Industrial Wastes

Report to the
Water Research Commission

by

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EXECUTIVE SUMMARY

Waste minimisation and general environmental awareness results in the identification of concentrated toxic or high-strength organic wastes that should not be discharged directly to the environment, wastewater treatment works or to landfill sites. Most companies do not have the technical expertise for in-house treatment of these streams. Previous research (Sacks, 1998) has indicated that there is a significant volume of unused digester capacity in KwaZulu-Natal. eThekwini Municipality has unused digestion capacity at the Southern Wastewater Treatment Works. The Water and Waste Department have indicated that they would be prepared to motivate to Council to refurbish the facilities so as to be able to provide a service to industry by co-digesting toxic (recalcitrant) and high strength (labile) organic wastes. If the lead were to be taken by one municipality, and if it proves to be successful, many other municipalities would be expected to implement such a co-digestion facility. Currently the market for such a facility remains invisible, as companies prefer to remain ignorant as to the fate of their waste products, and the concept of waste minimisation and waste segregation is not widely practiced. However the increase in the number of ISO 14 001 registered companies will result in a proactive stance towards responsible waste management practices becoming more widespread. Landfill sites are often inappropriate for the disposal of liquid effluents and of compromised foodstuffs. Controlled liquid anaerobic digestion facilities would be a preferred technique for the responsible disposal of such wastes.

A previous project (*WRC K5/1074 Co-digestion of high strength/toxic organic effluents in anaerobic digesters*) investigated the scientific basis for operating a co-digestion service. When industrial effluents which are characterised by a high content of organic matter are fed to an anaerobic digester, an organic overload could seriously impair the performance of the process, particularly the methanogenic stage, and possibly lead to the failure of the digester. Industrial effluents may also contain compounds which exert a toxic effect on the anaerobic consortium. This effect may be temporary, i.e. providing that the micro-organisms are exposed to sufficiently small concentrations of the compounds for a sufficiently long period of time, they can eventually adapt to their presence in the feed and possibly even degrade them to a certain extent. But the toxic effect may be permanent and therefore result in suboptimal performance, or, in more severe cases, in the failure of the digester. The project therefore developed methods to assess whether a particular industrial effluent:

1. Can be safely treated in an anaerobic digester
2. Must be mixed with some proportion of another readily biodegradable effluent in order to be simultaneously digested (co-digestion)
3. Is amenable or not to anaerobic digestion.

Having established the scientific basis for operating a co-digestion scheme, it is necessary to evaluate the other aspects required to put it into practice.

AIMS OF THIS REPORT

- To assess the feasibility for a specialised municipal organic liquid waste treatment facility using the eThekwini Municipality's Southern Wastewater Treatment Works as a case study
- To estimate the market for such a service
- To determine the capacity of the existing facilities at the Southern Wastewater Treatment Plant
- To determine the potential for power generation from the methane gas production
- To determine the scientific tests and equipment needed to accept waste at the facility and to ensure safe operation
- To determine staffing levels required
- To estimate the running costs and tariffs necessary to make such a facility financially viable

EQUIPMENT CAPACITY

The anaerobic digesters at the Southern Wastewater Treatment Works, in Jacobs, have been off-line since 1985. There are two digesters, each 4 620 m^3 in volume. The installation had a 2 500 m^3 storage capacity for biogas, which was used to heat the digesters.

Table 1: Data for the Anaerobic Digesters at the Southern Wastewater Treatment Works

Digester volume	m^3	9 240 (2×4 620)
Operating temperature	°C	37
Residence time	D	30
Feed rate	m^3/d	308
Volatile solids loading	$kg/m^3/d$	1.6
	kg/d	14 784
Volatile solids destruction rate	%	55
	kg/d	8 131
Gas production rate	m^3/kg VS/d	1
	m^3/d	8 131
	kg COD/d	21 880
Proportion of methane in gas	%	60
Methane production rate	m^3/d	4 874

Typical operational parameters ranges given by Vesilind (2003) were used to estimate the treatment capacity of these digesters. The data are summarised in Table 1. Some of the assumptions on which these figures are based may be conservative in that they are derived from experience in the treatment of conventional sewage sludges, in which the biodegradable organics are largely particulate. The hydrolysis of particulate organics is usually the limiting rate process in anaerobic digestion, so if industrial effluents contain a greater fraction of soluble organics, the digestion rates may be higher. On the other hand, the anticipated presences of toxic components that tend to reduce the rates suggest that conservative assumptions should be used.

POWER GENERATION POTENTIAL

The heat of combustion of methane is 39 000 kJ/Nm^3. Thus burning the 4 874 m^3/d of methane will release 2 200 kW of thermal power. The requirement for heating the digesters is of the order of 220 kW, about 10% of the total thermal power released by the methane.

European experience with small-scale internal combustion engines with a rated capacity of less than 200 kW indicate an electrical conversion efficiency of up to 25 %. Larger internal combustion engines (up to 1.5 MW) have a much-higher electrical conversion efficiency, 30 to 35 %. For larger-scale systems, combined cycle power stations consist of gas turbines, steam turbines, and waste heat recovery boilers all working together to produce electricity. Units as small as 200 kW are available, but only at scales of greater than 800 kW does their electrical conversion efficiency equal or surpass an internal combustion engine-based system. However, the use of a gas turbine allows a greater fraction of the waste heat to be recovered as more valuable steam. In this fashion, overall gas turbine efficiency (i.e. both gas and steam turbines) can be greater than 70 %, although a more conservative figure of 60 % is probably appropriate.

However, eThekwini has experience of an electricity-from-biogas project in the Marianhill landfill project. It was estimated that the total cost of generating electricity will be US $0.0422 per kWh, whereas the cost of electricity purchased from Eskom over the 10 year lifetime of the project would be US$ 0.0225 per kWh. The project was therefore dependent on Cleaner Development Mechanism (CDM) funding from the World Bank to make it viable. CDM funding is a mechanism which could be explored for this co-digestion project in the future, but for the present purpose it was taken that electricity generation will not be intrinsically viable, and so was not considered as part of this business plan.

POTENTIAL MARKET FOR A CO-DIGESTION FACILITY

To estimate the extent of the market for such a facility in the Durban area, data were collected on liquid effluents currently disposed to marine pipeline and to low-hazard and high-hazard landfill sites.

During the 6 months from September 2005 to February 2006 an average of 135 m^3/d of industrial effluent was trucked to the Southern Wastewater Treatment Works for discharge via the marine pipeline. In addition, 2 570 kℓ/d of high strength effluent (58 g/ℓ COD) were sent to the works by pipeline (data supplied by eThekwini Water and Sanitation). These fall into the category of high strength effluents, as toxic effluents may not be discharged to the sea. This means that the high-strength component of the potential market for a co-digestion scheme consists of companies that already dispose of their effluent at the Southern Wastewater Treatment Works.

Considering the potential toxic liquid effluents, these can be divided into two categories: low-hazard effluents that are currently sent to local landfill sites, and high-hazard effluents that are currently trucked to the Holfontein landfill site. It is estimated that the Shongweni site receives an average of 800 kℓ/month (26 kL/d) of organic liquid wastes (mainly solvents) from 10 to 15 regular customers. An average of 75 kℓ/month (2.5 kℓ/d) is sent to Holfontein from Durban, originating from 2 to 3 regular customers. This amounts to a little less than 10% of the total volumetric loading of the digesters. The proportion of toxic liquids that the digesters will be able to handle can only be established by testing, however it seems unlikely to be more than 10%, so there appears to be a reasonable match between the volumes available and the capacity of the digesters.

The size of the market in financial terms can be estimated from the prices charged for disposal to landfill. These are roughly R6 000 per kℓ for disposal of high hazard liquids to Holfontein, and R350 per kℓ for low hazard liquids to Shongweni. Combining these rates with the volume estimates yields a figure of R0.73 M per month, or R8.76 M per annum.

Although the details of the hazardous effluents are confidential, so that their exact natures are not known, it appears that in Durban they probably arise as effluents as a result of washing out ships' tanks and harbour storage tanks. The hazardous components contribute R5.4 M to the total estimate of R8.76 M for the potential market. This means that the viability of the scheme will be critically dependent on the being able to successfully treat these effluents, which clearly means that they must be tested before any decision is made to go ahead with the co-digestion scheme.

SCIENTIFIC SUPPORT

The operation of the digester must be protected against failure due to an overload of substrate or due to the death or inhibition of the biomass. Scientific support will be required for the anaerobic digestion facility in order to determine (i) if a new effluent can be accepted by the facility; (ii) the conditions under which the new feed should be fed to the digester; (iii) the current state of the digester; and (iv) remedial action to be taken if the digester shows signs of failure.

Before a new effluent can be accepted there has to be available capacity (hydraulic and organic load) in the digester. Procedures have been devised to determine the excess capacity of the digester. If capacity is available a new effluent is assessed by undertaking the following steps:

- Obtain an understanding the nature of the source of the effluent and its variability to ascertain its consistency and information on its composition
- A quick screening procedure is then used to determine its organic load, biodegradability and methanogenic toxicity. The effluent is classified into one of three groups (acceptable, possibly acceptable and unacceptable). If an effluent passes the criteria (acceptable) then more extensive confirmatory tests are undertaken; if the effluent is classified as possibly acceptable more comprehensive tests can be undertaken.
- Confirmatory tests are used to provide quantitative data on the rates of degradation and degree of biodegradation. A comprehensive chemical analysis is undertaken
- If considered necessary, a laboratory-scale digestion test is undertaken using the actual biomass from the digester and the effluent
- An effluent which is classified as possibly acceptable can be evaluated to see if the biomass would adapt to the effluent over time and procedures for its gradual introduction would be determined
- The resulting effluent from the digester will be assessed for aerobic toxicity and acceptance for discharge in the marine outfall.

Procedures for the monitoring of the digester have been developed. A database would be used as a tool for monitoring the full-scale and laboratory-scale digesters. A computer based model of the digestion process would be developed and continuously improved so as to provide a deeper understanding of process and to provide guidance on its operation. An equipment inventory and staffing schedule has been prepared for this activity.

TARIFF DEVELOPMENT

The objectives of the tariff structure should be:

- To finance the scheme in a way that is transparent and equitable to all parties
- To encourage environmentally responsible behaviour among users of the scheme

The tariff should logically have 4 components:

- A fixed charge for all users of the scheme, which covers the overheads of having the scheme in operation
- An effluent volume based charge covering volume based costs
- A ‘per delivery’ charge covering costs associated with receiving a batch of effluent for treatment
- Charges for testing or investigating effluent from a factory.

These may not appear separately in the tariff structure, for instance it might be that Item 1 would be a hidden component in 2, or that 1 and 2 would be part of 3.

There are entries related to Items 3 to 4 in the current eThekwini Municipality Sewage Disposal Tariffs. The nature of the scheme will require that effluents have to be tested to determine at what rate they can be fed into the digesters. The testing requirements will vary from effluent to effluent depending on the risk it poses to the digester operation, its variability and the reliability of the factory from which it comes. It therefore makes sense that the cost of

testing should be explicitly born by the factories themselves, and should be administered on the basis of a set of charges for each test carried out. The components that are most in need of definition at this stage are 1 and 2, that is, the projected costs of setting up and operating the scheme.

Table 2: Estimated costs associated with setting up and operating the co-digestion scheme (Million Rands, 2006 value)

Item	Capital cost	Annual(ised) Cost
Refurbish digesters	23. 3	4.3
Laboratory	5.0	0.91
Storage site	0.5	0.09
Operation and		2.9
Effluent disposal		0.072
Salaries		1.5
R & D		0.5
Totals	28. 8	10.3

The current cost of disposal of low and high hazard liquids was estimated at R8.76 M per annum (see above, under Potential Market for a Co-digestion Facility). This means that the cost of providing the co-digestion service would be almost 25% higher than what is currently (2006) being paid to dispose of the wastes.

The market survey was far from exhaustive, so there are probably more wastes available, which could improve the picture. However, the biggest contributors to the revenue are the hazardous toxic wastes, and these carry with them the greatest risk of causing digester failure. The wastes already identified would constitute about 10% of the capacity of the digesters, and it may not be feasible to increase this proportion much further. From the operational risk perspective, additional business should come in the form of high-strength readily biodegradable wastes. However, these can currently be disposed of at very low cost via the marine pipeline, and it would be difficult to persuade customers to switch to a more expensive service.

However, a different perspective is provided by comparing the anaerobic digestion cost with that of aerobic treatment. Per kg of COD destroyed, the annualised cost, calculated above works out at R1.30/kg COD, which is comparable with the industrial tariff for aerobic treatment, for which the corresponding calculation yields R0.944/kg COD. The availability of the marine pipeline as an alternative strongly influences the viability of the co-digestion scheme. The cost calculated here is lower than it might be, because the digesters already exist, but would not make much difference to the comparison. In places where marine disposal is not an option, or if political pressures (which already exist) lead to marine disposal being discontinued, the situation would change significantly.

CONCLUSIONS AND RECOMMENDATIONS

- This study has shown that the co-digestion scheme has the potential to provide a service to industries that is environmentally attractive, but which appears to be not quite

financially viable since the total cost of operating it is estimated to be approximate 25 % higher than the current cost of disposal to landfill

- The realisation of this potential is critically dependent on the successful co-digestion of liquid effluents characterised as high hazard since these are the most expensive to dispose of to landfill. An experimental investigation is needed to confirm that these effluents can be successfully treated before deciding to implement the scheme.
- The extent of the potential market for the co-digestion service has not been established as adequate to sustain the scheme. However, it is difficult to obtain information on the nature and quantities of effluents, and it is probable that this investigation has not identified all of the potential market. It is likely that much more will come to light once the scheme goes into operation, providing which might make the economics more attractive
- Waste management involves a wide range of stake-holders, and ideally these should complement each other. The co-digestion scheme will create a new player, and might be seen as taking business away from, for instance, the landfill site operators. On the other hand, it could provide them with an additional resource with which to offer a waste management service to industries. A workshop involving key stake-holders could help to define how the co-digestion scheme could be introduced and managed to serve as wide a range of interests as possible, and thereby establish its financial viability.

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- Lisa McAllister and Kasthuri Govender of Enviroserv, for assistance in estimating the extent and cost of landfill disposal of hazardous liquid effluents.

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INTRODUCTION

Waste minimisation and general environmental awareness results in the identification of concentrated toxic or high-strength organic wastes that should not be discharged directly to the environment, wastewater treatment works or to landfill sites. Most companies do not have the technical expertise to treat these streams in-house. Previous research (Sacks, 1998) has indicated that there is a significant volume of unused digester capacity in KwaZulu-Natal. eThekwini Municipality has unused digestion capacity at the Southern Wastewater Treatment Works. The Water and Waste Department have indicated that they would be prepared to motivate to Council to refurbish the facilities so as to be able to provide a service to industry by co-digesting toxic (recalcitrant) and high strength (labile) organic wastes. If the lead were to be taken by one municipality and if it proves to be successful, many other municipalities could be expected to implement such a co-digestion facility. Currently the market for such a facility remains invisible as companies prefer to remain ignorant as to the fate of their waste products, and the concept of waste minimisation and waste segregation is not widely practiced. However the increase in the number of ISO 14 001 registered companies will result in a proactive stance towards responsible waste management practices becoming more widespread. Landfill sites are often inappropriate for the disposal of liquid effluents and of compromised foodstuffs. Controlled liquid anaerobic digestion facilities would be a preferred technique for the responsible disposal of such wastes.

A previous project (*WRC K5/1074 Co-digestion of high strength/toxic organic effluents in anaerobic digesters*) investigated the scientific basis for operating a co-digestion service. When industrial effluents that are characterised by a high content of organic matter are fed to an anaerobic digester, an organic overload could seriously impair the performance of the process, particularly the methanogenic stage, and possibly lead to the failure of the digester. Industrial effluents may also contain compounds which have a toxic effect on the anaerobic consortium. This effect may be temporary, i.e. providing that the micro-organisms are exposed to sufficiently small concentrations of the compounds for a sufficiently long period of time, they can eventually adapt to their presence in the feed and possibly even degrade them to a certain extent. But the toxic effect may be permanent and therefore result in compromised performance, or, in more severe cases, in the failure of the digester. The project therefore developed methods to assess whether a particular industrial effluent:

- Can be safely treated in an anaerobic digester
- Must be mixed with some proportion of another readily biodegradable effluent in order to be simultaneously digested (co-digestion)
- Is not amenable to be anaerobically digested at all.

Having established the scientific basis for operating a co-digestion scheme, it is necessary to evaluate the other aspects required to put it into practice.

Aims of this report

- To assess the feasibility for a specialised municipal organic liquid waste treatment facility using the eThekwini Municipality's Southern Wastewater Treatment Works as a case study
- To estimate the market for such a service
- To determine the capacity of the existing facilities at the Southern Wastewater Treatment Plant
- To determine the potential for power generation from the methane gas production
- To determine the scientific tests and equipment needed to accept waste at the facility and to ensure safe operation

- To determine staffing levels required
- To estimate the running costs and tariffs necessary to make such a facility financially viable.

Methodology

This is a desk study, based largely on scientific work previously presented in the MScEng thesis of Sacks (1997) and WRC project K5/1074 *Co-digestion of high strength/toxic organic effluents in anaerobic digesters*. This was supplemented by information provided by the eThekwini Water and Sanitation department on the anaerobic digesters and on industrial effluents received by the Southern Wastewater Treatment Works, and information on low hazard and high hazard liquid effluents disposed of to landfill provided by Enviroserv.

The most significant new contribution arising from this investigation is therefore the information related to the practical and financial viability of the scheme, i.e. the estimates of the physical and financial size of the potential market for a co-digestion service, and the estimates of the costs of setting up and operating the system. It should be noted that the approach taken has been to be conservative in these estimates: the potential market is likely to be larger than was estimated, because the investigation was terminated when it was found to be adequate to sustain the co-digestion scheme, and the costs might be less if certain subsystems prove to be dispensible. So this report should be seen as support for a decision on whether to go ahead with implementing the scheme, rather than a comprehensive plan on how to implement it, if it is decided to go ahead.

On the other hand, the scientific support component of the report is very comprehensive, because of the level of detail available from previous work.

POTENTIAL MARKET FOR A CO-DIGESTION FACILTY

Background information

Many industries produce high-strength organic effluents that are monitored by the local authorities to ensure compliance with the stipulated discharge standards. To meet the standards for discharge to sewer, the effluents are often diluted with excessive potable water. Another option for the disposal of effluents with a COD too high to be accepted at a sewage works, without either dilution or the imposition of high tariffs, is discharge to sea, e.g. CG Smith. Marine discharge standards are much lower than those for discharge to sewer since it is believed that the rapid turnover of water prevents adversity to marine ecosystems. The end result, with marine discharge, is a loss of water. Industries use fresh water from the rivers, however, when this is discharged to sea, the water can not be re-used. Co-disposal in municipal landfill sites or encapsulation in HH (High Hazard) landfill sites are the only options for very high-strength or toxic organic effluents. A potential problem with co-disposal is the generation of excess leachate which, if the landfill is not properly lined, may cause contamination of the groundwater. The co-disposal of liquid effluents has even resulted in landfill subsidence e.g., the BulBul Drive landfill site in Chatsworth, Durban, where a large mass of compacted waste collapsed on 8th September 1997.

Examples of industries that produce high-strength organic effluents are dairies, bakeries, chemical and pharmaceutical manufacturers and yeast processors. Many of these effluents have been found to be amenable to anaerobic degradation (van der Merwe-Botha and Britz, 1997). Pesticide effluent is an example of an effluent which contains toxic organic compounds. Effluents of this nature are usually co-disposed in municipal or hazardous landfill sites. They could, however, be treated by anaerobic digestion upon acclimation of the biomass. Also, implementation of waste-minimisation practices and identification and segregation of point sources would reduce the volumes of toxic effluent being produced. These lower volumes could then be treated in a digester without causing toxic shock.

Sources of industrial effluents in the Durban area.

Table 2.1 shows the industries identified (Sacks, 1997) as producing problematic effluents with CODs >2000 mg/ℓ that might be appropriately treated in a co-digestion facility located at the Southern sewage works.

An average of 135 m^3/d of industrial effluent was trucked to the Southern Wastewater Treatment Works during the 6 months from September 2005 to February 2006.. In addition, 2 570 kℓ/d of high strength effluent (58 g/ℓCOD) were received via a pipeline. (Data supplied by eThekwini Water and Sanitation). Since these were discharged to the sea, they were high-strength rather than toxic effluents. This is well over the nominal 308 m^3/d capacity of the digesters (see Chapter 3). This suggests that the majority of the potential high-strength market for a co-digestion scheme consists of companies that already dispose of their effluent at the Southern Wastewater Treatment Works.

More detailed evaluation of effluents will be necessary, since the biodegradability of their COD is not known at this stage. If there is a problem with a lack of biodegradability, it will always be possible to blend-in a proportion of primary sewage sludge.

A problem for the co-digestion scheme is that the current charge for disposing of these effluents via the marine pipeline is very low: R0.65/kℓ. This is almost negligible relative to the costs of operating the digesters, so a cost-saving cannot provide any incentives to those factories that use the pipeline. There is increasing pressure to ban marine disposal of effluents, and if this occurrs it would change this situation entirely, as the default alternative to anaerobic digestion would be aerobic treatment, which is many times more expensive..

Table 2.1: Industries with potentially suitable effluents for co-digestion at the Southern Wastewater Treatment Works in Durban (Sacks, 1997)

Albany Bakery	Engen Refinery	Paperkem
Beacon Sweets	Fine Foods	Plascon Paints
Beier Industries	Frame Textiles	Printpak
Bromor Foods	Golden Lay Farms	Revertex
Cargo Carriers	Huletts Refinery	S.A. Sugar Terminals
CG Smith	Ind. Oil Processors	Sapref Refinery
Chemical Specialities	Lever Bros./ Unifoods	Sorghum Breweries
Coates Brothers	Marachia Laundry	Tanker Services
Competition Motors	Mondi Paper	Unitrans Natal
Drum Services	NCP Yeast	Vision Creations
Durban Confectionery	NCP/Sentrachem	Waste Services
Elida Ponds	O.T.H. Beier	

Considering the potential toxic liquid effluents, these can be divided into two categories: low-hazard effluents that are currently sent to local landfill sites, and high-hazard effluents that are currently trucked to the Holfontein landfill site. It is estimated that the Shongweni site receives an average of 800 kℓ/month (26 kℓ/d) of organic liquid wastes (mainly solvents) from 10 to 15 regular customers. An average of 75 kℓ/month (2.5 kℓ/d) is sent to Holfontein from Durban, originating from 2 or 3 regular customers (estimates supplied by Enviroserv). This amounts to a little less than 10% of the total volumetric loading of the digesters. The proportion of toxic liquids that the digesters will be able to handle can only be established by testing, however it seems unlikely to be more than 10%, so there appears to be a reasonable match between the size of the potential market and the capacity of the digesters. Sacks (1997) found that the Darvill Wastewater Treatment Works near Pietermaritzburg operated efficiently with 10 % industrial effluent, however this fraction would not have been toxic.

Financial implications

The size of the market in financial terms can be estimated from the prices charged for disposal to landfill. These are roughly R6 000 per kℓ for disposal of high hazard liquids to Holfontein, and R350 per kℓ for low hazard liquids to Shongweni. Combining these rates with the volume estimates yields a figure of R0.73M per month, or R8.76M per annum.

The constitution of the hazardous effluents is confidential, so that their exact natures are not known. It appears that that in Durban they probably arise as effluents as a result of washing out ships' tanks and harbour storage tanks. These hazardous components contribute R5.4M to the total estimate of R8.76M for the potential market. This means that the viability of the scheme will be critically dependent on the being able to successfully treat these effluents, which clearly means that they must be tested before any decision is made to go ahead with the implementing the scheme.

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EQUIPMENT CAPACITY AND POWER GENERATION

Background information

Sacks' (1997) investigation of the potential for using existing anaerobic digestion capacity in the vicinity of Durban for co-digestion considered digesters at a number of treatment works besides the Southern Wastewater Treatment Works. Although the Southern Wastewater Treatment Works digesters have been out of operation since 1985, an estimate of their capacity can be made by considering some of the reactors that were operating at other sites.

The Darvill Wastewater Treatment Works, in Pietermaritzburg, was operating at 91.7 % (v/v) of its design capacity. The anaerobic digesters were run at a retention time of 20 d with a high organic load (2.03 kg VS/m^3.d). The two egg-shaped digesters were fed in alternating sequence, i.e., 3.5 d to the first then 3.5 d to the second. Thus, the digesters could accept a greater load in the form of high-strength industrial effluents, as both digesters could be loaded simultaneously. The digesters were heated to 35°C which is optimal for mesophilic digestion. Gas production was measured at ca. 10 000 m^3/d with a 60% (v/v) methane content, thus 6 000 m^3 CH_4 were produced per day. With a totally degradable solids reduction of 64.6 %, this was equivalent to 1.01 m^3 CH_4 per kg VS destroyed. These parameters indicated efficient performance of these digesters. The sludge parameters were ideal and the mixing was efficient. Only 10% (v/v) of the influent to the works was industrial effluent.

The Northern Wastewater Treatment Works, located in Springfield Park, Durban, is a large works, treating 43 Mℓ/d. It was, however, only operating at 61 % of its design hydraulic capacity. There were 3 anaerobic digesters on site, one was fed an extra 35 m^3/d, resulting in a lower HRT (24 d) than the other 2 digesters (36 d). These extended HRTs indicated the availability of hydraulic capacity that could be utilised for the treatment of effluents from the many industries in the Springfield Industrial Park. The performance efficiency of these digesters was good, with an average organic load of 1.6 kg VS/m^3.d, volatile solids reduction of 62% and methane production of 0.3 m^3 CH_4/kg VS destroyed. Biogas production was measured at ca. 3 550 m^3/d, with a CH_4 content of 64.7% (v/v). A portion of the methane was used to heat the digesters to 37 °C. Mixing was achieved by recirculation of the digester sludge.

The anaerobic digesters at the Southern Wastewater Treatment Works, in Jacobs, have been off-line since 1985. There are two digesters, each 4 620 m^3 in volume. The installation had a 2 500 m^3 storage capacity for CH_4, which was used to heat the digesters. Some recorded data were available from the time when the digesters were operational. During their operation, these digesters were efficient. Sacks (1997) report that they were operated at 37 °C, a 30 d retention-time and a volatile-solids loading of 58 kg VS per day per m^3 of digester volume. They achieved a 55% reduction in VS and a conversion efficiency of 1.2 m^3 CH_4/kg VS. However, these figures clearly must be wrong, because the VS loading is at least 20 times too high. Consequently is has been decided to use typical operational parameters in the ranges given by Vesilind (2003).

Table 3.1 : Assumed Data for the Anaerobic Digester at the Southern Wastewater Treatment Works

Digester volume	m^3	9 240 (2×4 620)
Operating temperature	°C	37
Residence time	D	30
Feed rate	m^3/d	308
Volatile solids loading	$kg/m^3.d$	1.6
	kg/d	14 784
	kg COD/d	21 000
Volatile solids destruction rate	%	55
	kg/d	8 131
	kg COD/d	21 880
Gas production rate	m^3/kg VS/d	1
	m^3/d	8 131
Proportion of methane in gas	%v/v	60
Methane production rate	m^3/d	4 874

Power Generation Potential

The heat of combustion of methane is 39 000 kJ/Nm^3 (Vesilind, 2003). Thus burning the 4 874 m^3/d of methane will release 2 200 kW of thermal power.

The City of Besançon, in France uses the biogas produced at its sewage plant to generate heat and electricity (Energie-Cités, 1999). That scheme uses 220 Nm^3/h of gas containing 65 % in an internal combustion engine to produce electricity at an efficiency of 37.1 % and heating at 46.3 %.

European experience (AEA Technology, 2001) with small-scale internal combustion engines with a rated capacity of less than 200 kW indicate an electrical conversion efficiency of up to 25 %. Larger internal combustion engines (up to 1.5 MW) have a much higher electrical conversion efficiency, 30 to 35 %. For larger-scale systems, combined-cycle power stations consist of gas turbines, steam turbines, and waste-heat recovery boilers all working together to produce electricity. Units as small as 200 kW are available, but only at a scale of greater than 800 kW does their electrical conversion efficiency equal or surpass an internal combustion engine-based system. However, the use of a gas turbine allows a greater fraction of the waste heat to be recovered as more valuable steam. In this fashion, overall gas turbine efficiency (i.e. both gas and steam turbines) can be greater than 70 %, although a more conservative figure of 60 % is probably appropriate.

Another factor which will influence the choice of engine is the level sulphate in the digester feed and consequently the level of H_2S in the biogas. Internal combustion engines should be supplied with gas containing <200 ppm H_2S to avoid excessive corrosion, whereas turbines can tolerate up to 10 000 ppm (Chambers and Potter, 2002). Industrial wastes frequently contain relatively high levels of sulphates, so a sulphide removal system will probably be required if an internal combustion system is chosen.

The heating requirement to maintain the digester temperature at 37 °C can be estimated. To heat 308 m^3/d feed from 25 °C to 37 °C requires 180 kW. Vesilind (2003) gives heat transfer coefficients through concrete walls exposed to air the range 0.7 to 1.2 kg-cal/m^2.h.deg C (8.12×10^{-4} to 1.4×10^{-4} kW/m^2.deg C). The surface area of the two digesters is estimated at 2 440 m^2 and the average outside temperature at 20°C. The estimated heat loss through the walls is therefore between 34 and 58 kW. The heating requirement is therefore of the order of 220 kW, about 10% of the total thermal power released by the methane.

To summarise, if internal combustion engines are used, the electrical efficiency will be between 25% and 35%, and this could go to60 % for a combined cycle turbine system (gas and steam turbines). The heating of the digesters themselves will take up less than the waste heat available from any of these options. Table 3.2 indicates the potential power output for these options.

Table 3.2: Estimated electrical power output for the different generation efficiency options

Electrical efficiency [%]	Electrical Power [kW]
25	550
35	770
60	1 320

eThekwini has experience of a electricity-from-biogas project in the Marianhill landfill project (Strachan and Bowers 2004). It was estimated that the total cost of generating electricity will be US$ 0.0422 per kWh, whereas the cost of electricity purchased from Eskom over the lifetime of the project would be US$ 0.0225 per kWh. (World Bank, 2006). The project was therefore dependent on Cleaner Development Mechanism (CDM) funding from the World Bank to make it viable. CDM funding is a mechanism which could be explored for this co-digestion project in the future, but for the present purpose it will be taken that electricity generation will not be intrinsically viable, and so not considered as part of this business plan.

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TARIFF DEVELOPMENT

Introduction

This chapter sets out the conceptual basis for setting up a system of tariffs that would be charged for accepting industrial effluents to be treated in an anaerobic co-digestion facility at Durban's Southern Wastewater Treatment Plant. Because efficient management of such a facility, dealing with intrinsically difficult effluents from a variety of different factories, is a much more complex matter than operating a conventional wastewater treatment plant, the basis for setting rational tariff structure is similarly complex. This need not necessarily lead to a complex set of tariffs: practical considerations might favour a simplified tariff structure which is easier to administer. However the levels at which charges are set need to be based on a proper assessment of the costs and benefits of the service to all the parties involved.

Identification of potential costs and benefits

The issue of disposal of difficult effluents affects a range of entities: the companies from which the effluents originate, the municipality, the local economy, the local environment and the national economy and environment. There are both direct and indirect costs and benefits for these parties. Some of the indirect costs and benefits are somewhat intangible, but others can be quantified in terms of alternatives which have to be used in the absence of the envisaged scheme.

Table 4.1: Benefits of the co-digestion service

Description	Affected Party	Comment
Toxic waste treatment	Company of origin	Alternative to Holfontein landfill site at ~R6 000 per m^3, while retaining all future environmental liabilities
Labile waste treatment	Company of origin Co-digestion scheme	Alternative to aerobic treatment Assists in digestion of toxic material
Attract industries	City	The presence of an effective and economic waste disposal system could be a factor in where an industry chooses to locate.
Strategic industries	National economy	The chemical storage facilities associated with the harbour result toxic effluents which must be dealt with.
Free existing capacity	Municipality	Reduce loads on WWTWs; allow capital expenditure to be delayed or reduced.
Replace existing treatment costs	Company of origin	Costs may be both in terms of finance and environmental risk.
Electricity generation	Co-digestion scheme	An environmental/economic decision

Table 4.2 Costs of the co-digestion service.

Description	Affected Party	Comment
Capital Costs	Co-digestion scheme	Refurbishing digesters Tanks (effluent storage, sludge reserve) Laboratory Gas storage Heat and power plant Monitoring and control instrumentation Pilot reactor Membrane plant (retain sludge)
Operational overheads	Co-digestion scheme	Staff (including training) Maintenance Safety, health and environment Regulatory compliance Ongoing R&D
Variable costs	Co-digestion scheme	Energy (pumping and heating) Sludge treatment/disposal
Testing Protocols	Company of origin	Initial screening Factory survey Rapid toxicity assay Full toxicity assay Biological methane potential Co-digestion test Laboratory digester trial Effluent OUR and toxicity

Conceptual basis for the tariff structure

The objectives of the tariff structure should be:

To finance the scheme in a way that is transparent and equitable to all parties.

To encourage environmentally responsible behaviour among users of the scheme.

The tariff should logically have 4 components:

1. A fixed charge for all users of the scheme, which covers the overheads of having the scheme in operation
2. An effluent volume based charge covering volume based costs
3. A 'per delivery' charge covering costs associated with receiving a batch of effluent for treatment
4. Charges for testing or investigating effluent from a factory.

These may not appear separately in the tariff structure, for instance it might be that Item 1 would be a hidden component in 2, or that 1 and 2 would be part of 3.

The nature of the scheme will require that effluents have to be tested to determine at what rate they can be fed into the digesters. The testing requirements will vary from effluent to effluent depending on the risk it poses to the digester operation, its variability and the reliability of the factory from which it comes. It therefore makes sense that the cost of testing should be explicitly born by the factories themselves, and should be administered on the basis of a set of charges for each test carried out.

It is expected that testing of effluents will arise in the following circumstances:

- When a factory applied to join the scheme, or when a significant change occurs in the nature of its effluent, a comprehensive evaluation will be carried out to establish the characteristics of the effluent and the conditions at the factory
- For a regular member of the scheme, periodical testing will monitor the effluent to detect any significant changes and to ensure responsible behaviour of the factory
- In the case of a process upset, testing of effluents will be undertaken to establish the probable cause of the problem.

The following scheme is proposed to charge for this testing:

- A fee will be charged for a new effluent (or a significantly altered effluent) joining the scheme, which will cover the initial investigative work
- Based on this investigation and effluent will be assigned to risk class which will determine the level of routine testing. The criteria for this will be based on the perceived risk that the effluent poses to the process, and will include factors such as its toxicity, variability and the reliability of the factory. Whenever a batch of effluent is received, a sample will be taken, but will not necessarily be tested. Samples will be selected randomly for testing at an average frequency depending on the risk class, and the tests that will be performed will depend on the nature of the effluent (e.g. whether it could contain toxic components or not). The fee that is charged will then depend directly on the nature and frequency of the tests.
- Samples which are not selected for routine testing will be retained for a suitable time (e.g. 30 days). In the case of a process upset, these can be tested to trace the problem. The costs of the investigative testing would be recovered from the contravening party.
- Statistical evidence from the monitoring programme, or evidence of irresponsible behaviour from an upset investigation could lead to a customer being demoted to a more expensive testing regime, or conversely promoted to a less expensive one.

Tariff Components

To develop the tariff from this basis involves assigning numerical values to the costs and benefits set out in Tables 4.1 and 4.2, and combining them in an appropriate calculation scheme.

New installations

The codigestion scheme will require two new items, not previously provided at the Southern Wastewater Treatment Plant. These are a set of holding tanks for storage of materials which has to be blended into the feed to the digesters gradually because of its toxic or inhibitory nature and a laboratory for testing such materials and monitoring the state of the digesters.

Providing tankage for an uncertain number of industrial customers potentially poses a difficult problem, however a simple solution would be to require each regular customer to provide their own storage tank or container. The scheme then only has to provide a suitable area for the tanks and a pumping system. A tank could also be provided to cater for occasional customers. The cost of setting up such a facility is estimated at R500 000.

The laboratory will be a relatively simple one, without any very sophisticated instrumentation, as can been judged from the testing protocols that have been developed. Its cost to set up and equip is estimated at R5 000 000.

Refurbishing of Digesters

The cost of refurbishing the two anaerobic digester at the Southern Wastewater Treatment Works was assessed by the design department of eThekwini Water and Sanitation. The calculations are presented in Table 4.3.

If it proves to be unnecessary to supplement the readily biodegradable proportion of the feed with primary sewage sludge, the refurbishment of the thickener and the thickener pump station can be omitted, thereby reducing the estimated total cost between R24M and R20 M. The available quantity of high strength industrial effluent is far greater than can be processed in the digesters, however the extent to which it is readily biodegradable has not been established, and it is possible that it would need to be blended with a proportion of primary sewage sludge. There may also be problems associated by the relatively high sulphate content of many industrial effluents, which are reduced to H_2S during anaerobic digestion. Too high a concentration of H_2S causes problems with inhibition of the micro-organisms and corrosion. Consequently, the higher figure will be accepted as a conservative basis for estimating the required level of tariffs.

Table 4.3: Cost estimate for the refurbishment of the SWWTW digesters.

			Nov-88	Scaleup	Jun-06	Labour
Primary Digesters (2)						
A	Equipment			1.8	6.93	25%
	Scum breaker pumps 4 x Gorman Rupp T6				R 240 000	
	Sludge Recirc pumps	4 x T3A7			R 300 000	
	Water Recirc pumps	4 x KSB			R 200 000	
	Roof extractor fans (3)				R 150 000	
	Water seal pressure relief valves (2)				R 100 000	
	800 liter s/s water tank				R 80 000	
	solenoid valves (200mm) (4)				R 100 000	
			108893	196007.4	R 1 358 331	R 339 583
B	Digester Pipework	coated carbon steel	11534	20761.2	R 180 000	R 45 000
C		stainless	9000	16200	R 112 266	R 28 067
D	Instruments		24615	44307	R 307 048	R 76 762
	thermocouples/gas flow/water flow					
E	Hamworthy Heaters		37200	66960	R 250 000	R 62 500
F	Heat Exchangers		30000	54000	R 374 220	R 93 555
G	Civils				R 250 000	R 62 500
H	new MCC /electrical cabling				R 550 000	R 100 000
					R 3 381 865	R 807 966
Raw Sludge Pump Station						
	pipework/valves/actuators				R 80 000	R 20 000
	refurbish 2x Gorman Rupp T6 pumps				R 40 000	R 10 000
	replace magflow & level control				R 40 000	R 10 000
	Civils				R 100 000	R 25 000
	New MCC/electrical				R 70 000	R 17 500
					R 330 000	R 82 500
Thickener						
	New inlet pipework & valves (Join to AC Pipe)				R 150 000	R 37 500
	New inlet stilling chamber				R 150 000	R 37 500
	Refurbish thickener Structure				R 200 000	R 50 000
	New weir plate				R 50 000	R 12 500
	New rotating bridge				R 350 000	R 87 500
	New Scum box				R 50 000	R 12 500
	New pipework, valves for effluent, scum and thickened sludge				R 300 000	R 75 000
					R 1 250 000	R 312 500
Thickener Pump Station						
	pipework/valves/actuator				R 100 000	R 25 000
	civils				R 150 000	R 37 500
	New 2x Gorman Rupp T6 Sludge pumps				R 120 000	R 30 000
	Magflow / level control				R 40 000	R 10 000
	New MCC				R 70 000	R 17 500
					R 480 000	R 120 000
Secondary Digester						
	pipework/valves				R 120 000	R 30 000
	new bridge				R 650 000	R 162 500
	Pumps				R 200 000	R 50 000
	Civils				R 200 000	R 50 000
	new MCC				R 120 000	R 30 000
	Instrumentation				R 100 000	R 25 000
					R 1 390 000	R 347 500

Continued on the next page ….

Table **4.3** continued

Gas Holder							
	Bell (Floating Roof)			R	4 100 000	R	1 025 000
	Water Traps/pipework/valves	35000	63000	R	436 590	R	109 148
	Instruments			R	300 000	R	75 000
	MCC/cabling			R	120 000	R	30 000
				R	4 956 590	R	1 239 148
Gas Flare		25000	25000	R	173 250	R	43 313
Tanker Discharge Bay				R	300 000		
Primary Sedimentation tanks 5 and 6							
Install Flow control penstock				R	100 000		
Refurbish PST bridges/pipework/electrics/launders				R	400 000		
			Totals	**R**	**10 871 705**	**R**	**2 605 426**
Tender Price	**R 13 477 131**	**P&G 20%**					**R 17 972 557**
Excavation &additional Pipework	**R 1 500 000**	**Contingency 10%**					**R 19 769 813**
		Design & Supervision 20%					**R 23 723 775**

Capital redemption

An annualisation factor of 0.18144 was used for the Durban Landfill project (World Bank, 2006) and is adopted here.

Operation and maintenance

Operation and maintenance is estimated at 10 % of the capital cost per annum.

Effluent disposal

It is assumed that digester effluent will be disposed via the marine pipeline at the prevailing tariff of R0.65 per kℓ

Staffing

The scheme will require relatively sophisticated staff to manage its technical complexities, 2 BSc graduates and 2 BTech graduates, and two employees with matric. The staffing cost is estimated at R1 500 000 per annum. (see Appendix H)

Research and Development

Ongoing research and development will ensure that the system is able to adapt to changing industrial, environmental and regulatory circumstances, and to make the service available to as wide a range of industrial customers as possible. The cost is estimated at R500 000 per annum.

Total annualised cost of the scheme (Million Rands, 2006)

Item	Capital cost	Annual(ised) Cost
Refurbish digesters	23.3	4.3
Laboratory	5.0	0.91
Storage site	0.5	0.09
Operation and maintenance		2.9
Effluent disposal		0.072
Salaries		1.5
R & D		0.5
Totals	28.8	10. 3

Conclusions

The estimated annualised cost for co-disposal of waste by anaerobic digestion is R10.3M/y. The current cost of disposal of low and high hazard liquids in the Durban area was estimated at R8.76M per annum (Section 2.3). This means that the co-digestion scheme will be, on average, 23% more expensive than the landfill options currently being used.

However this result is critically dependent on the treatability of the high hazard liquids that represent R5.4M of the estimated R8.76M per annum market size.

The market survey was far from exhaustive, so there are probably more wastes available, which could improve the picture. However, the biggest contributors to the revenue are the hazardous toxic wastes, and these carry with them the greatest risk of causing digester failure. The wastes already identified would constitute about 10% of the hydraulic capacity of the digesters, and it may not be feasible to increase this proportion much further. From the operational risk perspective, additional business should come in the form of high-strength readily biodegradable wastes.

Unless further hazardous effluents can be identified, the shortfall of R2.04M/y would have to be made up by treating high-strength non-toxic effluents. Given that the COD loading capacity of the digesters was estimated (Table 3.1) at 21 880 kg COD/d or 7 665 000 kg COD/y), this means about R0.3 /kg COD would have to be charged. For comparison, the 58 kg COD/m^3 effluent, referred in Section 2.2, is currently discharged to the marine pipeline at a cost of R0.65 /m^3, or R0.011 /kg COD. Persuading the factory to switch to a service which is nearly six times more expensive will not be easy.

However, a different perspective is provided by comparing the anaerobic digestion cost with that of aerobic treatment. Per kg of COD destroyed, the annualised cost calculated above works out at R1.30/kg COD, which may be compared with the industrial tariff for aerobic treatment, for which the corresponding calculation yields R0.944/kg COD. The availability of the marine pipeline as an alternative strongly influences the viability of the co-digestion scheme. The cost calculated here is lower than it might be, because the digesters already exist, but would not make much difference to the comparison. In places where marine disposal is not an option, or if political pressures (which already exist) lead to marine disposal being discontinued, the situation would change significantly.

Given that the market survey was unable to identify sufficient opportunities to cover the costs of the scheme, a detailed tariff scheme would be hypothetical, and, it does not seem useful to attempt to draw one up at this stage.

Reference

World Bank (2006), Durban Landfill Project Design Document, http://carbonfinance.org/Router.cfm?Page=DocLib&CatalogID=5574 Accessed 22 August 2006.

SCIENTIFIC SUPPORT

The operation of the digester must be protected against failure due to an overload of substrate or due to the death or inhibition of the biomass. Scientific support will be required for the anaerobic digestion facility in order to determine (i) if a new effluent can be accepted by the facility; (ii) the conditions under which the new feed should be fed to the digester; (iii) the current state of the digester; and (iv) remedial action to be taken if the digester shows signs of failure.

Before a new effluent can be accepted there has to be available capacity (hydraulic and organic load) in the digester. Procedures have been devised to determine the excess capacity of the digester. If capacity is available a new effluent is assessed by undertaking the following steps.

- Obtain an understanding the nature of the source of the effluent and its variability to ascertain its consistency and information on its composition
- A quick screening procedure is then used to determine its organic load, biodegradability and methanogenic toxicity. The effluent is classified into one of three groups (acceptable, possibly acceptable and unacceptable). If an effluent passes the criteria (acceptable) then more extensive confirmatory tests are undertaken; if the effluent is classified as possibly acceptable more comprehensive tests can be undertaken.
- Confirmatory tests are used to provide quantitative data on the rates of degradation and degree of biodegradation. A comprehensive chemical analysis is undertaken
- If considered necessary, a laboratory-scale digestion test is undertaken using the actual biomass from the digester and the effluent.
- An effluent which is classified as possibly acceptable can be evaluated to see if the biomass would adapt to the effluent over time and procedures for its gradual introduction would be determined
- The resulting effluent from the digester will be assessed for aerobic toxicity and acceptance for discharge in the marine outfall.

Procedures for the monitoring of the digester have been developed. A database would be used as a tool for monitoring the full-scale and laboratory-scale digesters. A computer-based model of the digestion process would be developed and continuously improved so as to provide a deeper understanding of process, and to provide guidance on its operation.

An equipment inventory and staffing schedule has been prepared for this activity.

Protocol for screening of industrial effluents

Before a new effluent can be accepted there has to be available capacity (hydraulic and organic load) in the digester.

Procedures have been devised to determine the excess capacity of the digester. If capacity is available, a new effluent is assessed by undertaking the following steps.

There is a need to obtain an understanding the nature of the source of the effluent and its variability to ascertain its consistency and information on its composition. The experimental approach will necessarily have to address the two key characteristics, which determine the behaviour of a test material in respect to an existing operational anaerobic digester: these are its toxicity and its biodegradability.

▪ Toxicity denotes a detrimental effect that the effluent exerts on the activity of a microbial population. It indicates the impact of the effluent on the baseline (i.e. prior to the co-digestion trial) performance of the digester. That is to say: if we feed this effluent (under certain

conditions) to a given, stable anaerobic digester, how will the overall performance of this system be affected as a consequence of this additional stream ?

The following conditions have to be ensured: the gas flow rate must not be dramatically reduced, as methane represents COD reduction; the stabilisation of the waste (e.g. the extent of the COD removal) needs to be maintained, as this is a prerequisite that the legislation imposes for the final discharge (and disposal of residues); and the overall stability of the digester must not be compromised, as rescuing a failing digester or starting-up a new digester can be very expensive and time-consuming.

However, a reasonable reduction in the process performance might be acceptable, as the combined treatment will counterbalance the specific losses.

The detailed protocol is presented in **Appendix A.**

Anaerobic activity tests using the serum bottle method

A serum bottle is a closed vessel, in which the **sample** (i.e. the compound to be tested), a **defined medium** and a **seed inoculum** (the anaerobic sludge) are incubated at the desired temperature. The gas which is generated inside the vial is periodically released and quantified using a syringe. There are two main types of serum bottle assays to evaluate biodegradability and toxicity. These are termed Biological Methane Potential (BMP) assay and Anaerobic Toxicity Assay (ATA) respectively. Both tests comprise *i*) a control set, which enables to quantify the *baseline* conditions and *ii*) a number of test units, in which the micro-organisms are exposed to a variety of conditions.

The key difference between the two assays is the targeted variable . A biodegradability test is aimed at evaluating the susceptibility of a compound to undergo a microbial degradation; therefore, the test compound acts as the substrate for the anaerobic micro-organisms. A toxicity test is aimed at evaluating the negative effect of a compound on the microbial activity compared to 'normal' conditions, i.e. in the presence of a labile substrate.

Detailed descriptions of the serum bottle tests are provided in **Appendix B.**

pH-stat titrimetric activity tests

- A **titrimetric method** relates the microbial activity to the rate of titrant addition
- A **pH-stat method** maintains the pH of the solution constant, within a limited range
- A combined **pH-stat** and **titrimetric method** detects the change in concentration of protons [H^+] and compensates this change by adding an equivalent amount of the appropriate titrating solution.

A pH-stat titrimetric sensor can be applied to monitoring the methanogenic step of the anaerobic digestion process, as the degradation of acetate to methane influences the pH of the solution by generating alkalinity.

Two operational modes can be chosen, i.e. a simple pH-stat mode, whereby an inorganic acid is used as titrant to compensate the increase in pH; and a combined pH- and substrate-stat mode, in which acetic acid simultaneously compensates the change in pH and the acetate consumption by the micro-organisms. The latter mode is particularly suited for conducting toxicity tests in which the concentration of the base substrate is maintained nearly constant throughout the test and the test material is spiked at intervals. This application can be proposed as being as reliable as established serum bottle assays, offering the advantages of:

i) a shorter duration of the test: the activity must be monitored for only few hours after each spike

ii) potentially isolating the true effect of the test material on the methanogenic activity from other effects that could be due to a decrease in pH or substrate concentration.

However, the quality of the outcome is highly dependent on the correct start-up of the test: great care must be taken in ensuring that all other pH-affecting processes are neutralised, with particular regard to the bicarbonate/carbon dioxide system that must be equilibrated prior to starting the test, using a 1:1 N_2:CO_2 gas mixture.

Detailed descriptions of the pH-stat titrimetric tests are provided in **Appendix C.**

Operation of a laboratory-scale anaerobic digester

It will be advisable for the laboratory to maintain a small scale digestion in parallel with the full-scale system in order to provide a facility for testing operating issues where it is necessary to use conditions that are as close as possible to those in the full-scale digester. **Appendix D** summarises the experience that has been accumulated by the research team in the operation of laboratory-scale completely stirred digesters.

Appendix D describes following main subjects:

i) how to **operate** an anaerobic digester

ii) how to **monitor** the performance of an anaerobic digester

iii) how to **assess** the performance of an anaerobic digester.

A database for monitoring laboratory-scale anaerobic digestion experiments

Unless an anaerobic digestion unit is highly automated, e.g. with online data acquisition of gas production and composition, pH, automated feeding and sampling procedures, etc., the accurate monitoring of the process can be very time-consuming.

Appendix E describes a specifically designed database that contributes significantly to improving the data logging and management tasks.

General laboratory requirements

Appendix F describes the materials and methods for the range of general test procedures that will be used in the laboratory, **Appendix G** lists the required analytical equipment, and **Appendix H** lists the staffing requirements.

CONCLUSIONS AND RECOMMENDATIONS

- This study has shown that the co-digestion scheme has the potential to provide a service to industries that is environmentally attractive, but which appears to be not quite financially viable since the total cost of operating it is estimated to be approximate 25% higher than the current cost of disposal to landfill.
- The realisation of this potential is critically dependent on the successful co-digestion of liquid effluents characterised as high hazard since these are the most expensive to dispose of to landfill. An experimental investigation is needed to confirm that these effluents can be successfully treated before deciding to implement the scheme.
- The extent of the potential market for the co-digestion service has not been established as adequate to sustain the scheme. However, it is difficult to obtain information on the nature and quantities of effluents, and it is probable that this investigation has not identified all of the potential market. It is likely that much more will come to light once the scheme goes into operation, providing which might make the economics more attractive.
- Waste management involves a wide range of stake-holders, and ideally these should complement each other. The co-digestion scheme will create a new player, and might be seen as taking business away from, for instance, the landfill site operators. On the other hand, it could provide them with an additional resource with which to offer a waste management service to industries. A workshop involving key stake-holders could help to define how the co-digestion scheme could be introduced and managed to serve as wide a range of interests as possible, and thereby establish its financial viability.

Appendix A

Protocol for screening effluents

The experimental approach will necessarily have to address the two key characteristics, which determine the behaviour of a test material in respect to an existing operational anaerobic digester: these are its toxicity and its biodegradability.

▪ Toxicity denotes a detrimental effect that the effluent exerts on the activity of a microbial population. It indicates the impact of the effluent on the baseline (i.e. prior to the co-digestion trial) performance of the digester. That is to say:

If we feed this effluent (under certain conditions) to a given, stable anaerobic digester, how will the overall performance of this system be affected, as a consequence of this additional stream?

The following conditions have to be ensured: the gas flow rate must not be dramatically reduced, as methane is the primary economical output of the process and represents COD reduction; the stabilisation of the waste (e.g. the extent of the COD removal) needs to be maintained, as this is a prerequisite that the legislation imposes for the final discharge (and disposal of residues); and the overall stability of the digester must not be compromised, as rescuing a failing digester or starting-up a new digester can be very expensive and time-consuming.

However, a reasonable reduction in the process performance might be acceptable, as the combined treatment will counterbalance the specific losses.

Methanogenesis is generally the most sensitive stage of the entire process and certainly this is the case for high strength organic effluents that may be relatively easily converted to acidic intermediates but thereafter cause a stress overload for methanogens. Further, methanogenesis is immediately related to the production of methane. It is therefore obvious that the general practice is to address methanogenic toxicity.

▪ Biodegradability indicates the inherent property of a test material, which depends primarily on its molecular structure and refers to its susceptibility of undergoing a biologically-mediated degradation. It is related to the actual conversion of the test material to degradation products (or intermediates) which will likely pose a lesser concern in terms of final disposal. That is to say:

If we feed this effluent (under certain conditions) to a given, well operating anaerobic digester, to what extent will the biomass succeed in degrading the organic constituents to intermediates and ultimately to methane?

Since the research focused on organic effluents, we adopted the approach whereby if the organic content of the test material is reduced during the anaerobic digestion process and the extent of this reduction is reflected in a stoichiometrically equivalent output of methane, this is sufficient to conclude that the test material had undergone a certain degree of biodegradation.

Limits of the protocol, This protocol is by no means intended to be a comprehensive and a rigorous instrument which enables to assess the fate of the test material, simply because other forms of toxicity may in fact be induced by particular chemical species contained in the test material that are not necessarily limited to inhibiting the methanogenic biomass. Similarly it may happen that the degradation intermediates and/or the non-degradable fractions of the test material are in fact equally or even more environmentally dangerous than the original effluent and although it may be seen that the test material is degraded to some extent, the disposal issue persists.

Other more specific analytical tools exist and generally require a more in-depth study of the degradative pathway of the test material.

However these circumstances may be anticipated by a preliminary knowledge of the general composition of the effluent to be treated and possibly of the industrial process that generates it. This step is also indispensable.

Nevertheless, it is believed that the protocol illustrated in this section can be a reliable decision support tool for high organic strength effluents: purposely, a relatively simple and inexpensive method such as the serum bottle method has been adopted, since it is believed that it is sufficiently simple and accurate to enable the characterisation of a test material in a relatively short period of time and without a great amount of laboratory work. Table A.1 contains a list of the material needed to perform the experimental assessment described hereafter.

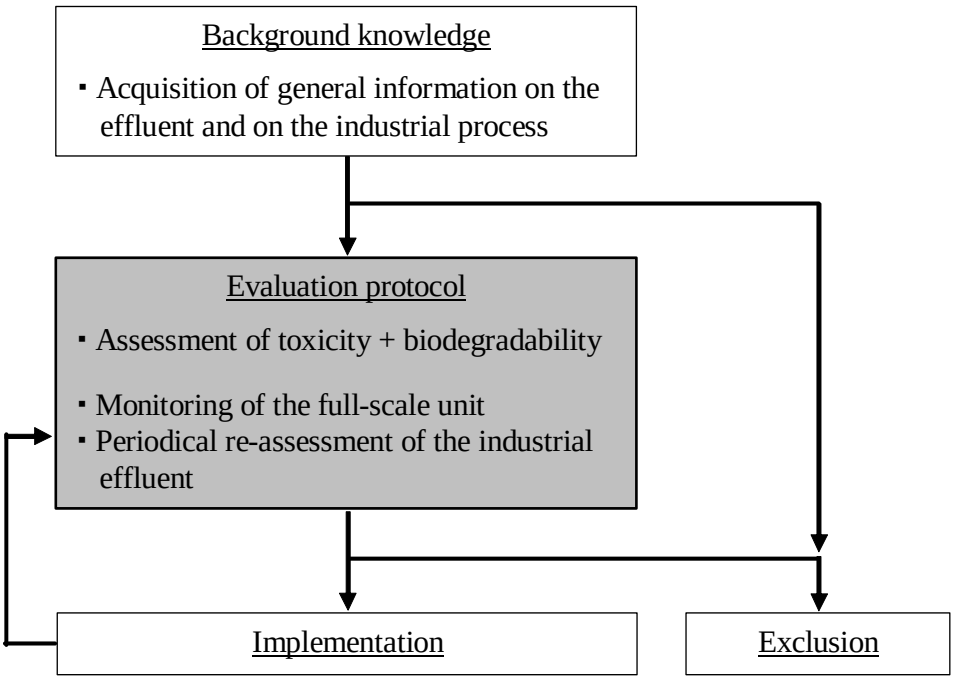

Figure A.1 – Flow diagram of the Protocol for the screening of high strength industrial effluents.

This discussion of concepts can be visualised as in Figure A.1, which summarises the general approach of this research.

Prior to undertaking the experimental assessment, the operator of the co-digestion facility should acquire sufficient information regarding the general characteristics of the effluent under study and of the process that generates it. This preliminary step should enable the selection of high strength effluents, which do not pose other more specific and possibly dangerous threats to health and environment, than their high content in organic matter, salts, colour, detergents, etc. The evaluation protocol serves the double function of initial assessment of the industrial effluent to investigate the feasibility of the co-digestion option for its disposal, as well as continuous monitoring tool of both the actual performance of the full-scale application and the periodic reassessment of the effluent, should its make-up and characteristics vary in time, due to changes in the production process.

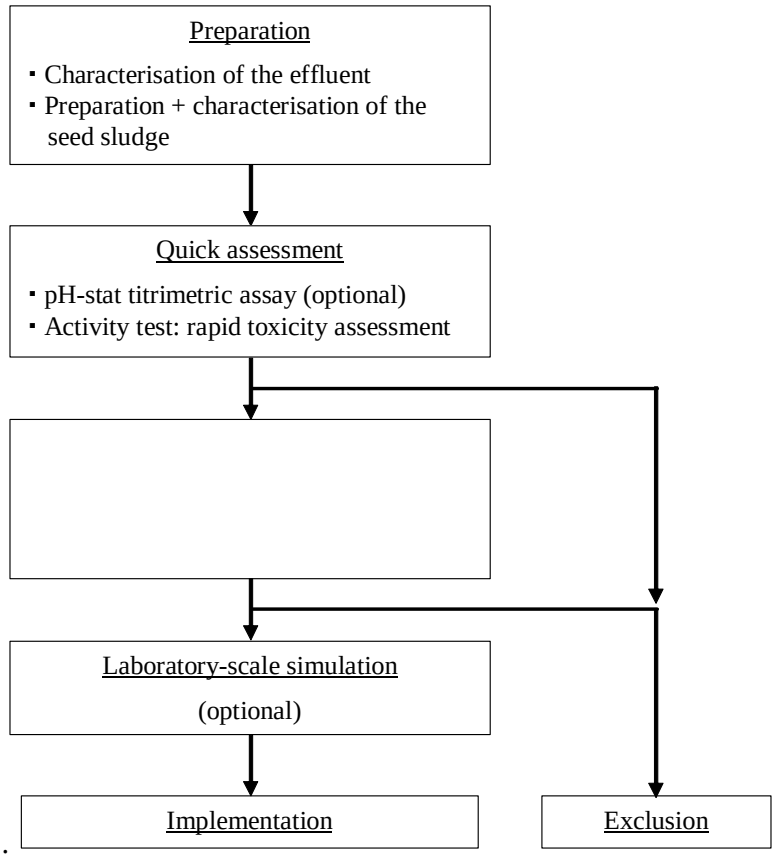

Figure A.2 – Experimental stage of the Protocol for the screening of high strength industrial effluents.

A The evaluation protocol

Young and Tabak (1993) proposed a comprehensive 3-level protocol which provided a means of determining the fate and the effect of toxic organic chemicals in anaerobic wastewater treatment processes. Level one consisted in a 3-step screening protocol to assess toxicity. Level two was a kinetic protocol to assess the kinetics of acetogenesis and methanogenesis under the effect of the toxicant. Ultimately, level three was a protocol to assess the kinetics of the degradation of the toxic compound.

The protocol proposed herein is aimed at assessing the methanogenic toxicity and the biodegradability of the effluent under study (the test material). Insight in the kinetics of the degradation process can be obtained, although it is not a primary objective. It therefore corresponds to the level-one stage of the former/earlier protocol, as depicted in Figure A.2.

A.1. Characterisation of the test material and preparation of the seed sludge.

The test material will be characterised in terms of its organic content (as COD), pH and alkalinity and solids concentration (total and volatile solids).

For specific applications additional parameters, that may be relevant to the anaerobic degradation process and yet relatively simple and inexpensive to obtain, can be determined: e.g. it might be of interest to assess to what extent the colour can be removed through the anaerobic (co)digestion of the test material.

The characterisation of the effluent must be carried out within the shortest possible time after collection, in order to minimise alterations that may occur due to the incorrect handling of the effluent

or to (bio) chemical or physical degradation processes that may intervene. Once the characterisation is completed, the test material should be stored at low temperature (4 °C), in the dark, in a sealed container.

A generic, i.e. unadapted, sludge must be used for the small-scale assessments (which include the Quick and the Confirmatory assessment stages; Table A.2), in order to *objectively* assess the toxicity and the biodegradability of the test material in a manner which will be independent from the specific application and therefore comparable to other similar assessments as well as to evaluate a possible adaptation of the biomass to the test material upon long-term exposure. The unadapted sludge could be sampled at a wastewater works and maintained for a certain time on a readily biodegradable substrate, e.g. acetate.

For the laboratory-scale simulation, a well operating digester will be used in order to accelerate the start-up phase. The sludge used must be characterised in terms of its solids content and have a low residual organic content. This can be done by subjecting it to a period of starvation prior to starting the assessment.

A.2 Quick toxicity assessment

The objective of this first stage is to characterise the test material as potentially inhibitory or readily biodegradable and to identify a concentration range to be extensively investigated in the following step.

A first activity test (AAT) will be conducted by using three values of concentration of the test material. The indications which can be given here are inevitably qualitative, as the actual concentrations will vary from case to case taking into account primarily the strength of the test material and also the technical viability of a particular dilution.

- A very high strength effluent will have to be significantly diluted, so that the highest concentration level does not exceed the value reported in Table B.2; a higher COD concentration is likely to negatively affect the performance of the biomass and should therefore be avoided *at this stage*

- An excessive dilution of the test material may not be of practical interest at the real scale, because it would not meet the rate of production of the test material itself and/or would require storage capacity that might be not available. This is clearly a less precise limit that the former one, but it should similarly be avoided, *at this stage*.

Tentatively, we could take as the *high*, *intermediate* and *low* concentrations the values of 100, 50 and 10 % v/v respectively (these volumetric ratios obviously refer to the aliquot of liquid volume available for the test material). It is important to stress that discretion should be applied in ensuring the primary objective which must always be that the outcome of the AAT is meaningful.

This AAT will comprise **15 units** (6 controls, three containing the seed inoculum only, and three supplemented with the reference substrate; and 3 replicates per concentration level: Table A.2) and should be monitored for not less than 7 d. Based on the outcome of this test, the next step will be set-up, according to the following reasoning.

a) If no significant inhibition is detectable, even at the high concentration level: the test material is likely to be readily biodegradable with no or negligible harm for the digestion process. In the following step, higher concentrations may be investigated or the current test extended for a longer time.

b) If the low concentration level shows a significant inhibition, the test material is likely to result in a strong impairment of the digestion process, unless the biomass would be able to adapt upon exposure. In the following step, if lower concentrations are still technically of interest/viable, lower concentration ranges could be investigated in order to identify a concentration range of low toxicity.

A.3 Confirmatory assessment

This experimental step is aimed at characterising the biodegradability and/or the toxicity of the test material: the former will be expressed as the fraction of the organic matter content of the test material that is converted to methane. The latter, will be given as inhibition curve and, if possible, as 50% inhibition concentration (IC_{50}). Three or four data points are required in order to define a meaningful inhibition curve (or segment),.

Further, if the test material shows to be inhibitory in the early stage of the incubation, the test period can be extended until signs of biomass adaptation are seen. In our experience, we believe that if after 60 to 90 d, no gas production has occurred, we can conclude that biomass adaptation is not feasible.

Should the test material be amenable to digestion, two further objectives could be achieved at this stage: *i*) the simulation of the co-digestion treatment and *ii*) the identification of a set of 'safe' operational parameters to start-up the laboratory-scale experiment or to directly implement the same treatment option on a full scale.

This AAT will comprise **15 units** (3 controls, with the seed inoculum and the readily biodegradable substrate; 3 controls, with the seed inoculum and the test material; and 3 replicates per concentration level) and should be monitored *until the methanogenic activity decreases to zero*.

A second level of AAT could be started specifically aimed at testing different volumetric ratios between the test material and the designated readily biodegradable effluent. It is assumed that such effluent that will possibly be used in the co-digestion treatment has been identified, as for instance the targeted digester unit on site is currently treating that effluent. It is beyond the scope of this protocol to actually screen various options of readily biodegradable effluents, unless specifically required.

A.3.1 Data interpretation

An anaerobic activity test will provide the Specific Methanogenic Activity (SMA) of the seed inoculum, which indicates the (maximum) activity attainable, i.e. the rate of substrate uptake (g COD/g VSS/d), and the Biodegradability (B) of the test material, which indicates the COD fraction which is actually converted to methane (g COD/g COD).

A tentative value for the Organic Loading Rate (OLR, g COD/ℓ/d) to apply to a laboratory-scale digester can be estimated as:

$$\mathrm{OLR} = \frac{\mathrm{SMA}}{\mathrm{B}} \cdot \mathrm{X} \quad \text{(A.1)}$$

where X is the concentration of solids in the reactor (g VSS/ℓ).

A.4 Laboratory-scale simulation

This phase is aimed at verifying the findings of the screening phase, i.e. the extent of biodegradation, the inhibitory effect to the methanogenic biomass, the potential for adaptation and the optimal volumetric ratio effluent-to-substrate, and at simulating the full-scale co-digestion process. It is crucial that the operation of the laboratory-scale unit is closely monitored, to enable the optimisation of the co-digestion performance. The composition of the biogas is relatively easy to determine and is a very sensitive parameter that could serve as main indicator of the process performance.

A.5 Monitoring

Once the co-digestion process has been implemented at the full-scale, the same methodology underlying the *screening* protocol can be used to off-line monitor the performance of the process and to periodically re-assess the characteristics of the industrial effluent.

A serum bottle AAT can be carried out at regular intervals (e.g. monthly) on a sludge sample withdrawn from the digested and incubated in the vials with no addition of other components. During

a first period, the activity of the sludge is monitored until the residual organic substrate is depleted. Thereafter a dose of reference substrate is spiked into the vials daily (e.g. 30 µℓ of acetic acid) and the activity monitored until it levels off. The determination of the maximum (or potential) methanogenic activity on acetate, as opposed to the *actual* methanogenic activity on the mixed substrate inside the digester, enables to semi-quantitatively estimate the active methanogenic biomass which is a most direct indication of the health of the digester.

The periodical re-assessment of the industrial effluent is a necessity to ensure that its characteristics do not significantly deviate from those initially assessed, as the result of changes in the production process. The set-up described for the AAT of the Confirmatory Assessment of the protocol (Table A.2) will be used; conditions close to the actual conditions in the co-digestion facility (e.g. effluent concentration, substrate-to-biomass ratio, etc.) will also be used as reference. Should the response be significantly different to the initial one, the option of diverting the effluent until an extensive assessment has been completed must be considered.

Conclusions

An experimental protocol based on a very simple methodology and requiring little additional laboratory work has been developed to screen high strength effluents prior to being co-digested. The same concept is applied to monitor the performance of the co-digestion process, once implemented at the full-scale.

Table A.1: List of material required to execute the Protocol for screening industrial effluents.

▪ Temperature controlled room (or equivalent device, e.g. thermostated cabinet).

The assays are ideally to be conducted in a mesophilic temperature range, i.e. at 35 °C, and the temperature must be maintained constant throughout the test.

▪ Material to conduct anaerobic activity tests using the serum bottle method (**Annexure B**).

Glass vials (125 mℓ), butyl rubber septa and aluminium crimps (Separation Pty Ltd.), glass syringes (various sizes, e.g. 2, 10 and 50 mℓ), glass syringe (100 µℓ) for gas sampling.

▪ Gas-chromatograph, equipped with a column capable of detecting carbon dioxide and methane (**Annexure F**).

▪ Nutrients and mineral salts stock solution (**Annexure F**).

▪ Apparatus for the determination of COD, solids, alkalinity and VFA (**Annexure F**).

Table A.2: Protocol guidelines, detailing the number of tests and the time needed to complete the experiment.

▪ Characterisation of the test material (Overall time: 2 days)

Perform the following analytical determinations.

- COD: 1 to 3 determinations, with 4 replicates each (time: 2 days)

- Alkalinity and VFA: 1 determination, with 3 replicates (time: negligible)

- Solids: (total and volatile, unless otherwise needed) 1 determination, with 4 replicates (time: 1 day)

▪ Preparation of the seed sludge (Overall time: 1 day)

The following steps are to be undertaken.

- Collect a sample of the sludge from the plant (or from a laboratory-scale reactor).

- Characterise the sample. As per 'Characterisation of the test material', except for the item *Solids*, where total and volatile suspended solids should be undertaken.

- Our experience indicates that the sludge sample should not exceed the 30 to 50 g TS/ℓ, to ensure that a representative sample can be obtained.

▪ **Stage 1** Quick toxicity assessment (Overall duration: 8 days)

Perform 1 test, using 15 vials.

- preparation of the test, e.g. preparation of the Nutrient and Mineral salts Solution (1 day).

- set-up of the test: add all components to the vials; purge with inert gas for 1 to 2 min; seal the vials; re-equilibrate after 1 h and GC determination of the gas composition at time zero (1 day).

- monitoring the test: every day, determine the gas volume and composition (recommended at least 2 replicates per gas sample; 0.75 day).

- analytical determinations at the end of the test: pH, COD, Alkalinity and VFA; as per 'Characterisation of the test material', to be conducted on one replicate per group of vials (2 days)

▪ **Stage 2** Confirmatory assessment - AAT (Overall duration: 30 to 90 days)

Perform 1 test, using 15 vials.

Follow the same indications of the 'Quick toxicity assessment': monitor the test every day, until the conditions reach equilibrium; thereafter, once every 3 days or every week.

▪ Confirmatory assessment - co-digestion (Overall duration: min 8 days)

Perform 1 test, using 15 vials.

Follow the same indications of the 'Quick toxicity assessment'. When the methanogenic activity decreases to zero, the operational mode can be switched to fed-batch by spiking one dose of the substrates, for a follow-up experiment. The duration is unknown, because it is function of the biodegradability of the test material (combined with the readily biodegradable substrate), but unlikely less than a week.

▪ **Stage 3** (optional) Laboratory-scale simulation (Overall duration: variable)

The set-up should resemble the actual configuration of the co-digestion facility. Otherwise, use a basic completely stirred vessel (e.g. 3 to 5 litre-volume). Feeding and effluent collection can be done manually. A variable volume headspace (e.g. a gas sampling bag) can prevent gas losses and air contamination during these operations. It is imperative to determine the biogas flow rate (a basic liquid displacement device can be used).

Perform the following determinations:

- daily: pH, alkalinity and VFA, COD, gas volume and composition;
- weekly: solids concentration.

Maintain the same operating conditions for approximately 1 HRT.

A.6 REFERENCE

Young JC and Tabak HH (1993). Multilevel protocol for assessing the fate and effect of toxic organic chemicals in anaerobic treatment processes. *Wat. Environ. Res.*, **65**(1): 34-35.

The serum bottle technique for the cultivation of anaerobic micro-organisms has been described by Miller & Wolin (1974).

A serum bottle is a closed vessel, in which the **sample** (i.e. the compound to be tested), a **defined medium** and a **seed inoculum** (the anaerobic sludge) are incubated at the desired temperature. The gas which is generated inside the vial is periodically released and quantified using a syringe. Owen and co-workers (1979) developed two serum bottles assays to evaluate biodegradability and toxicity, which they termed Biological Methane Potential (BMP) assay and Anaerobic Toxicity Assay (ATA) respectively. Both tests comprise *i*) a control set, which enables to quantify the *baseline* conditions and *ii*) a number of test units, in which the micro-organisms are exposed to a variety of conditions. To ensure the reproducibility of the determination, each unit is done in replicates (generally, three). The composition of each set varies depending on the type of test to be conducted and is summarised in Table B.1.

The key difference between the two assays is the variable which is targeted. A biodegradability test is aimed at evaluating the susceptibility of a compound to undergo a microbial degradation; therefore, the test compound acts as the substrate for the anaerobic micro-organisms. A toxicity test is aimed at evaluating the negative effect of a compound on the microbial activity under 'normal' conditions, i.e. in the presence of a labile substrate, which therefore must be supplemented in all the bottles: in a toxicity test, the substrate and the test compound are *antagonistic*.

Table B.1: Set-up of the serum bottles assays.

Type of assay		Sludge	Medium	Sample	Objective of the test
BMP	- sterile control	No	Yes	No	(optional) to verify the existence of non-biological degradation potential.
	- control units	Yes	Yes	No	To quantify the baseline gas production, due to e.g. residual organic matter in the sludge.
	- test units	Yes	Yes	Substrate [1]	To assess the biodegradability of the substrate and possible influence of substrate concentration.
ATA	- control units	Yes	Yes	Substrate [2]	To quantify the 'reference' activity, in the absence of the inhibitory test substance.
	- test units	Yes	Yes	Substrate [2] + Test compound	To assess the inhibitory effect of the test compound at different concentrations.

Underlined, the parameter which is varied in each test.

[1] If the test substance is recalcitrant and requires a co-substrate to be biodegraded, both species must be present.

[2] Reference, readily degradable substrate: e.g. acetate, propionate, etc.

B.1 Test preparation

The procedure outlined in this annexure was established in the course of this project, by progressively refining the methodology of Owen and co-workers (1979). Although the principle underlying a serum bottle test is very simple, several factors of seemingly little importance may in fact influence the outcomes.

We present a generalised procedure which applies to both a biodegradability and a toxicity test, since we believe that *i*) the key variable which is measured in both cases is the microbial activity and that *ii*) similar information can be obtained from either test. Therefore, the term Anaerobic Activity Test (AAT) could be used, to unify the two concepts.

A serum bottle AAT (Table B.1) comprises two control sets (which allow for the quantification of the baseline e.g. endogenous activity of the seed inoculum and of the reference activity, respectively) and a number of test units, in replicates, in which the anaerobic sludge is exposed to a variety of conditions. The activity is assessed at varying concentrations of the test material.

Table B.1: Set-up of the serum bottle activity test.

Set	Composition	Objective
Control units	▪ Seed inoculum (only)	To quantify the baseline gas production, due to e.g. residual organic matter in the sludge.
Reference units	▪ Seed inoculum ▪ Reference substrate	To quantify the reference activity, in the absence of the inhibitory test substance.
Test units	▪ Seed inoculum ▪ Reference substrate ▪ Test material	To assess the inhibitory effect of the test material at different concentrations and its biodegradability.

As mentioned, several aspects must be taken into account *prior* to performing a serum bottle test (Figure B.1).

▪ Test duration

The common practice is to say that a biodegradability test should last at least 30 d, or until the biogas production becomes negligible, whereas a toxicity assay should last 7 d.

We propose that both (therefore an AAT) are continued until the microbial activity becomes negligible: this enables to observe slow biodegradation processes as well as to discriminate between acute and permanent inhibitory effects.

It is therefore proposed that the main quantity to be monitored is microbial activity, i.e. the rate of biogas production.

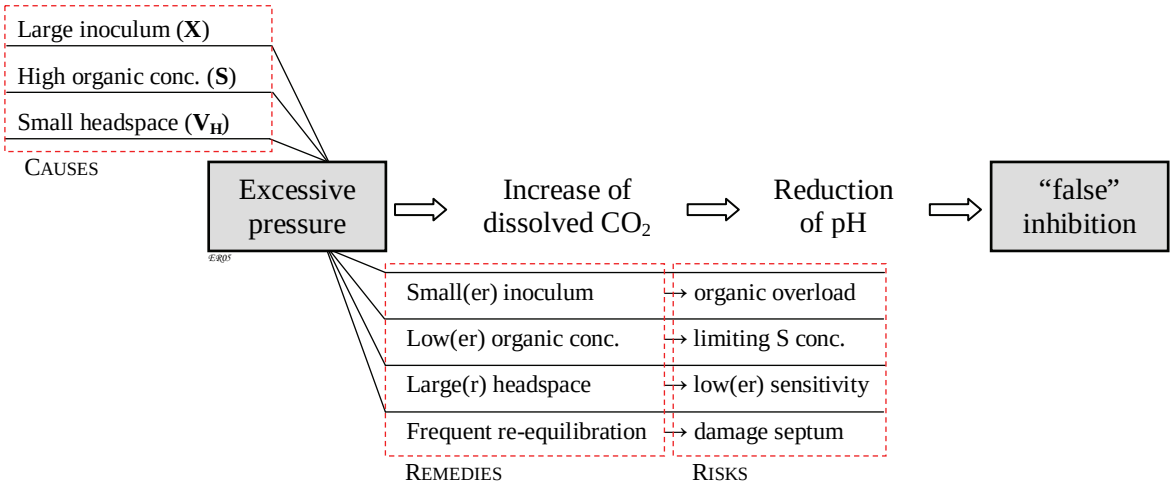

Figure B.1 – Schematic of the cause-effect interaction between the relevant aspects in the design of a serum bottle AAT (source: Remigi & Foxon, 2005).

▪ Total liquid volume in a vial (or: **headspace-to-liquid volume ratio**)

When the test materials are diluted, relatively large liquid volumes may be necessary to obtain a sufficiently dilute concentration of the organic matter. At the other extreme, if the test materials have a high strength, large liquid volumes are also necessary to lower the concentration of organic matter.

Low gas-to-liquid volume ratios enhance the sensitivity of the determination, as a higher overpressure is generated for a given amount of biogas produced. However, large liquid volumes leave little space for the biogas produced to expand. This requires that the manual release of the gas is sufficiently frequent to prevent the pressure from building up to an extent that the increased solubilisation of carbon dioxide does not cause a drop in pH which can be detrimental to the activity of methanogens.

▪ Frequency of pressure re-equilibration

The frequency of pressure re-equilibration primarily influences the physico-chemical equilibrium of the liquid and the gas phases; and also the long-term resistance of the seal. If the rate of gas production between two subsequent re-equilibrations is too high, the pressure might exceed the resistance of the butyl rubber septum causing it to burst, or even just swollen to an extent that gas may leak through previous punctures. Since the rate of gas production is a function of the inherent activity of the biomass, of the amount of biomass present and of the amount of organic substrate supplemented, these three variables should be carefully chosen in order to ensure a gas production which can be handled.

Clearly this issue is less important when an automated device (e.g. a pressure transducer) is used, as it is possible to set a threshold of pressure that triggers a vent which re-equilibrates the pressure.

▪ Inoculum type and concentration

The volume of sludge used to seed a serum bottle and its inherent specific activity will affect the rate of gas production and in turn the frequency of pressure re-equilibration, as discussed above.

Speece (1996) states that the cardinal rule in conducting a biodegradability assay is that the seed sludge has been acclimated to the test materials. However, in the first attempt of assessing biodegradability, the inoculum will inevitably be unacclimated to that particular test material; thereafter, the same sludge could be re-exposed to the test material: to verify if acclimation has occurred or not.

However, in a batch process, the use of a large inoculum or of a highly active inoculum, can prevent the accumulation of volatile fatty acids that would negatively affect the activity of methanogens.

▪ Substrate (or, test material) concentration

Excessively low or high substrate concentrations are equally detrimental to the accuracy of a AAT: in the former case, the activity displayed by the biomass will be low and the inherent inaccuracy of the manual gas volume determination may be of the same order of magnitude of the gas production; in the latter case, the consequence of a high rate of biogas production have been discussed above. In addition, at high substrate concentrations, the availability of nutrients and other 'growth factors' may become limiting. If the concentration of substrate is too high, it may even become inhibitory to the biomass (e.g. resulting in a fast production of volatile acids that inhibits methanogenesis). The **substrate-to-biomass ratio** has proven to be a crucial factor in batch conditions, which influences the kinetics of the process (Moreno et al., 1999).

▪ Number of units

To verify that the outcome of an AAT is reliable, it is recommended that three replicates per unit are prepared. If more accurate insight in the progression of the reaction is desired, a larger number of replicates must be prepared, so that some vials can be sacrificed at given intervals and their content analysed, e.g. to measure the residual COD. It is recommended that all determinations are conducted at least in triplicate, to calculate a meaningful average and standard deviation of the measurements: therefore, three additional replicates should be considered for each unit to sacrifice.

Table B.2: Tentative guidelines for the set-up of an AAT.

Parameter	Units	Value or interval
Headspace-to-total volume ratio (V_H/V_{TOT})	-	0.2 – 0.6
Substrate (preferably, acetate)	g COD/ℓ	3 – 6
Organic matter (i.e. substrate + test material)	g COD/ℓ	< 30
Solids (i.e. seed inoculum)	g VS/ℓ	10 – 20
Substrate-to-biomass ratio (S/X)	-	0.2 – 0.3
Re-equilibration period	D	1 – 2
Volume of gas per re-equilibration	mℓ	5 – 30

B.2 Execution of the activity test

A step-by-step guide for the execution of an AAT is illustrated hereafter.

Step 1: Prepare the following components:

▪ Nutrients and minerals stock solution (NMS): refer to Section **F.1**.

▪ Healthy, active and homogenised sludge (the seed inoculum). This must be characterised in terms of its solids content (as volatile or volatile suspended solids) and organic content (as COD).

▪ Test material: this is to be characterised in terms of its pH, alkalinity and organic content (as COD).

▪ Organic substrate (for toxicity assays, only), either as concentrated stock solution or pure reagents. Acetate, propionate or a mixture thereof are commonly used. The type of test can be slightly modified, i.e. from a strictly batch mode to a semi-continuous mode; and pure acetic acid or ethanol can also be used.

Step 2: Equilibrate all components to the working temperature.

Step 3: Pour the components into the vials.

▪ Ideally, the components should be anaerobically transferred into the vials. However, it has been demonstrated that by manipulating them with care, i.e. trying to minimise the exposure to air and thoroughly flushing the vials with oxygen free gas for 1 to 2 min prior to sealing, is sufficient to leave the anaerobic biomass almost unaffected.

▪ If the substrate (for toxicity tests) or the test material is in solid form (e.g. powder), it might be easier to add this first. In all other cases, they should be added immediately before sealing the vials.

Step 4: Flush with an oxygen-free inert gas.

▪ A nitrogen and carbon dioxide mixture would be ideal in that it removes residues of oxygen while establishing a CO_2 partial pressure above the liquid. If such a mixture is not available, nitrogen can be used to overgas the headspace, but must not be passed through the liquid as it would strip carbon dioxide, thus altering the equilibrium of inorganic carbon.

▪ Overgas for 5 min at low flow rate; then, immediately seal with butyl rubber septa (20 mm x 3 mm, Separations Pty Ltd.) and aluminium crimp (20 mm, Separations Pty Ltd.).

Step 5: Store at the working temperature, in the dark and motionless.

Step 6: Re-equilibrate the headspace, after 1 to 2 h after preparation.

As the pressure in the serum bottles might have changed as a consequence of the temperature change and of gas/liquid equilibria adjustment, it should be zeroed to atmospheric pressure.

▪ Puncture each septum with a needle and vent the excess gas that might have accumulated.

▪ Although the value does not have to be recorded as part of the AAT, quantifying the volume of excess gas could provide useful indications of biological activity. Clearly, all units containing the same volume of liquid are expected to exhibit the same volume of excess gas. Differences from this behaviour could be due to:

i) a prompt start-up of the microbial degradation process, resulting in the immediate production of gas: wasting the excess gas in this circumstances would neglect a portion of microbial activity; or

ii) an onset of physico-chemical reactions, in the presence of a test compound which contains volatile fractions; or

iii) non-homogeneous conditions across and/or within each unit, and therefore indicate an inaccurate set up of the test: the measurement of the excess gas might help in the identification of the faulty units, which should be discarded.

Step 7: Determine the gas composition, after re-equilibration.

▪ Although one can assume the initial composition of the headspace, based on the composition of the mixture used to sparge the vials, a measured value is certainly preferable. Moreover, knowing the actual gas composition, could help in understanding the circumstances discussed in Step 6.

Step 8: Record all information, including the starting time on a log sheet.

▪ An example is provided in Tables B.3 and B.4.

Step 9: Constantly monitor the progress of the test.

▪ As mentioned earlier, this consists in the periodical determination of both the volume of gas produced and the composition of the headspace.

▪ The gas produced should always be wasted to prevent an excessive increase of pressure; unless it is too small to be accurately quantified: in this latter case, it can be re-injected into the vials, so that the pressure can gradually build up to a detectable level. A value lower-than-zero (not zero) should be recorded on the log sheet, so that it can be taken into account during the data analysis stage.

▪ Due to the temporary increase in pressure between two subsequent determinations, a fraction of the gas produced remains dissolved in the liquid. The release of the excess gas will bring the pressure back to the atmospheric pressure. However, this will happen too rapidly for the gas/liquid equilibrium to re-establish and the excess dissolved fraction will likely remain undetected. Manually shaking the vials will favour gas/liquid re-equilibration. It is suggested that two measurements of the gas volume be performed, initially on the undisturbed vial the after shaking. In this way the conditions closed to equilibration will be established, as the 'driving force' will be *from* the liquid *to* the gas phase. Shaking the vial before releasing any excess gas would enhance the internal overpressure to an extent that could make it *practically* impossible to accurately measure the volume of biogas produced, as the excess pressure could force the gas to escape past the plunger. Under particular conditions, this excessive overpressure could also cause a surge of foam that would result in sludge entering the needle and possibly obstructing it.

▪ A syringe of suitable volume should be used to ensure that the measurement is sufficiently accurate: clearly, if the production is large, a small-volume syringe which is potentially very sensitive, would result in either a loss of gas (due to the leakage past the plunger) or in the necessity of performing numerous repeated insertions of the needle, that would damage the resistance of the butyl rubber. Conversely, a large-volume syringe would not be sufficiently accurate to quantify a small gas production, due to its low sensitivity.

These circumstances can be anticipated, and avoided to some extent, when preparing the serum bottles and the dilutions.

▪ The syringe should be lubricated with deionised water, prior to the measurement. The reading of the gas volume (or the last one, in case of multiple punctures) should be done by holding the syringe horizontally, in order to minimise the effect of the weight of the plunger, and by gently twirling it, thus allowing a free movement. Readings are verified, by drawing the plunger past the equilibrium point and released, to verify that it returns to the original equilibrium volume.

▪ Gas composition should be determined immediately after withdrawal from the headspace. The volume should be small enough to leave the headspace pressure unaffected. With a headspace volume of 50 to 100 mℓ, a sample of 40 µℓ would alter the pressure by 4 to 8 mbar. For accurate mass balances, this volume should be recorded and accounted for.

▪ The frequency of the re-equilibration (and determination of gas volume and composition) should be adjusted to the progress of the reaction. Typically, in the early stages of the process, when the gas production is stronger (and the gas composition changes rapidly), frequent determinations would enable the accurate monitoring of the degradation.

Step 10: Stop the test when the gas production has levelled off and the gas composition is also constant.

▪ If long-term information is required (e.g. when the potential adaptability of the micro-organisms to the test compound is investigated), the test can be continued further. Clearly, the frequency of the manual re-equilibration may be reduced, e.g. once per week, until signs of resumed activity are noted.

▪ If no follow-up is needed and the bottles can be opened, the following determinations should be done, to provide quantitative data for mass balances:

- pH, alkalinity and volatile acids concentration;

- COD concentration; and

- solids concentration.

A conceptually different way of performing a serum bottle AAT consists in batch-feeding the test units with the reference substrate, until a steady-state in methane production is reached (control); thereafter, both the reference substrate and the test material are spiked into the serum bottle (test unit). The drawback of such technique is that the length of the two phases is inevitably limited by volume that can be added into the vial. And more importantly, it seems to defeat the object of a serum bottle assay, since a conventional batch-fed reactor would be definitely more suitable for such regime.

However, the following discussion on data interpretation is equally applicable to an AAT that had been performed in this way.

Table B.3: Front page for a log-book, for serum bottle tests.

Type of test:

☐ Biodegradability

☐ Toxicity

☐ Other (please specify) _____

Unit nr	Type [1]	Composition				Comments [3]
		Inoculum	NMS	Test C. [2]	Substrate [2]	

[1] Type of unit, e.g. control, test unit *low* concentration, etc.

[2] Test C., test compound. Type and volume (or concentration) should be recorded.

[3] Record any other information that could be of help in interpreting the results.

Table B.4: Log-sheet for data capturing, for serum bottle tests.

Test ID		Date/Time		Day nr	

Conditions (i.e. T, P_{atm}, etc.)	

Unit nr	Volume (mℓ)	Gas composition (peaks area)				Comments
		N_2	CH_4	CO_2	Total	

B.3 Data interpretation

The objective of a biodegradability test is to answer the question: … to what extent is the test material biodegradable?

This translates into quantifying the portion of the organic matter contained in the test material that undergoes microbial degradation, i.e. that is converted into methane.

This is done by comparing the initial COD added in the serum bottle (COD_0) to the amount of methane produced in the course of the test ($V_{CH,\infty}$), as:

$$B \approx \frac{V_{CH,\infty}}{COD_0} \qquad \text{(B.1)}$$

where B denotes the extent of biodegradation.

Suitable units must be used, by converting COD into methane (or vice versa), using the equivalence factor:

1 g COD = 0.350 ℓ CH_4 at STP (B.2)

Note that the extent of biodegradation B can be affected by the concentration of the test compound (or, by the COD-to-biomass ratio): typically, at relatively high substrate concentrations, the activity of the micro-organisms might be inhibited due to excess substrate, whereas at low concentrations, the substrate might be not sufficient to promote the maximum activity.

For this reason (and the lack of an authoritative reference on this matter; Müller & Frommert 2004), it is recommended that more than one level of concentration be assessed.

The objective of a toxicity test is to answer the question, How does the test material affect the methanogenic activity of the biomass ?

This translates into comparing the methanogenic activity of the sludge exposed to the test material to the activity in the absence of the test compound.

The reason why acetate or other *direct* precursors of methane, e.g. propionate, are preferred to e.g. glucose as substrates is that the drop in pH which follows the initial break down of the substrate could result in a transient reduction in methanogenic activity, that would hide the actual effect of the test material, i.e. it would be interpreted as inhibitory effect, although the test material was not inhibitory or would worsen the true inhibitory effect of the test material and even make it irreversible, although it was in fact only temporary.

The effect of the test material (E) is quantified as:

$$E \approx \frac{A_{t.m.}}{A_{ref}} \qquad (B.3)$$

where:

$A_{t.m.}$ is the methanogenic activity in presence of the test material; and

A_{ref} is the methanogenic activity on the reference substrate.

Clearly, E is a function of the concentration of the test material, so that generally the effect of a compound ranges from *no-effect*, at low concentrations, to *full inhibition*, at high concentrations.

This effect/concentration relationship is formally described by an inhibition curve. For technical applications, the quantity EC_{50}, defined as the concentration of the test material which results in a 50 % reduction in the methanogenic activity as compared to the reference activity, is of more value than the entire curve.

B.3.1: Interpreting biodegradability test

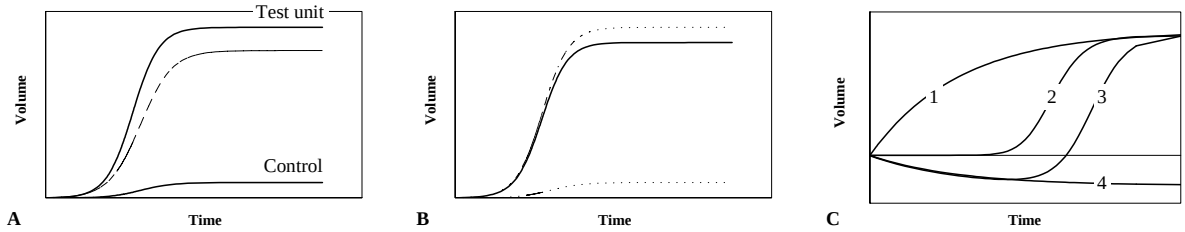

*Figure B.2: Anaerobic activity test for the assessment of biodegradability. (**A**) Average cumulative volume of biogas, in the test and control units and in the reference units (dashed line). (**B**) Net volume of methane (solid line), in the test units. (**C**) Possible patterns of net methane production (adapted from: Battersby & Wilson, 1988).*

A guide for interpreting the outcome of an AAT aimed at assessing the biodegradability of the test material is illustrated hereafter.

i) Plot the cumulative gas volume for the control and for the test units (Figure B.2, A).

ii) Calculate the range of uncertainty of the above curves by calculating the average and standard deviations of each data point. Ideally, the variation coefficient should not exceed 5 to 10 %. If one of the replicates is clearly out of range, it must be disregarded in the further calculations.

iii) Calculate the volume of methane, through a step-by-step mass balance, as follows:

$$\Delta v_{CH,t} = f_{CH,t} \cdot (\Delta v_t + V_{t-\Delta t}) - f_{CH,t-\Delta t} \cdot V_{t-\Delta t} \qquad (B.4)$$

where:

f_{CH} indicates the molar fraction of methane (at time t and $t-\Delta t$ respectively);

$V_{t-\Delta t}$ is the headspace volume at time $t-\Delta t$ (for simplicity, it can be considered constant throughout the test, unless a fed –batch regime is adopted); and

Δv is the volume of gas produced in the interval $(t-\Delta t, t)$.

iv) Calculate the *net* volume of methane produced in the test unit, i.e. the methane generated by the conversion of the biodegradable fraction of the test material, by subtracting the volume of methane measured in the control units to the one measured in the test units (Figure B.2, B). Ideally, the gas production in the control should be small compared to the one of the test material to make the determination reliable.

Four cases are possible, which are schematically represented in Figure B.2, C:

- the test material is readily biodegradable (**1**);

- the test material is biodegradable after a lag-phase (**2**);

- the test material is inhibitory in the initial phase of incubation (**3**); or

- the test material is inhibitory throughout the entire period of incubation (**4**).

v) Calculate the ultimate biodegradation (or: biodegradability) of the test material, as:

$$B = \frac{V_{CH,\infty}}{V_{CH,theory}} \tag{B.5}$$

where:

$V_{CH,theory} = f_T \cdot 0.350 \cdot COD_0$; and

f_T is the temperature correction factor, from STP to the working conditions.

vi)To complement the interpretation of the results, one can calculate (and plot) the Specific Methanogenic Activity (SMA) in g COD/g VS/d (or: g COD/g VSS/d)

$$SMA = \frac{R_{CH}}{X_0} \tag{B.6}$$

where:

R_{CH} is the *net* rate of methane production; and

X_0 is the biomass initially present in the vial.

R_{CH} is calculated as the first derivative of the net volume of methane (Step 4)

The growth rate of anaerobic micro-organisms is low, so that in many applications it can be assumed negligible. However, over long periods such those of a biodegradability assay, it can actually result in a measurable increase in biomass concentration. Therefore, the use of the *initial* biomass concentration in equation B.6 would overestimate the SMA of the later stages of the process. If the solids concentration is measured at the end of the test (preferably, as volatile suspended solids), one can assume a linear increase between the initial and the final values, to obtain a more accurate estimate through equation B.6.

B.3.2 Interpreting toxicity test

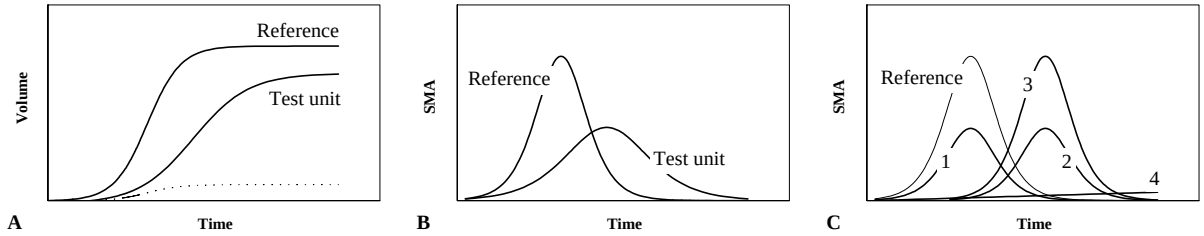

*Figure B.3: Anaerobic activity test for the assessment of toxicity. (**A**) Average cumulative volume of biogas, in the reference and test units and in the control units (dashed lines). (**B**) Specific Methanogenic Activity, in the reference and in the test units. (**C**) Possible patterns of activity curves.*

A guide for interpreting the outcome of an AAT aimed at assessing the toxicity of the test material is illustrated hereafter.

i) Plot the cumulative gas volume for the control and for the test units (Figure B.3, A).

ii) Calculate the range of uncertainty of the above curves by calculating the average and standard deviations of each data point. Ideally, the variation coefficient should never exceed 5 to 10 %. If one of the replicated is clearly out of range, it must be disregarded in the calculations.

iii) Calculate the volume of methane, according to equation B.4.

iv) Calculate the Sludge Methanogenic Activity, according to equation B.6.

Note that the SMA calculated for a toxicity assay is the rate of methane production in the test units and is conceptually different from the one calculated for a biodegradability assay, which was based on the *net* methane production. The reason is that in a toxicity assay we are interested in the microbial activity *in presence of* the test material, whereas in a biodegradability assay we want to *isolate* the effect of the test material from a baseline.

Four cases of inhibition are possible, which are schematically represented in Figure B.3, C:

- The test material is *partially* inhibitory; however, it does not delay the uptake of the reference substrate (**1**)

- The test material is *partially* inhibitory and requires a period of adaptation before the micro-organisms are able to display a detectable methanogenic activity (**2**)

- The test material is *temporarily* inhibitory: after a lag-phase, the micro-organisms display a methanogenic activity similar to that on the reference substrate (**3**)

- The test material is *permanently* inhibitory: the biomass is not able to adapt to the presence of the test material (**4**).

v) Calculate the inhibition effect as:

$$E = \frac{SMA_{t.m.}}{SMA_{ref}} \qquad (B.7)$$

where:

$SMA_{t.m.}$ is the activity in the test unit; and

SMA_{ref} is the baseline activity, in the control unit.

As mentioned above, this 'effect' is concentration-dependent and can be conveniently described through an inhibition curve which plots the relationship between concentration of the test material and inhibition. This latter is defines as:

$$I = 1 - E \qquad \text{(B.8b)}$$

An example of inhibition curve is presented in Figure B.4: the value EC_{50} is found on the horizontal axis in correspondence to $I = 0.5$.

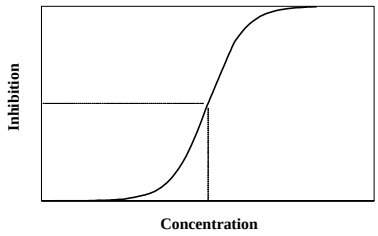

Figure B.4: Example of inhibition curve.

B.3.3 How does the frequency of re-equilibration affect the calculation of activity ?

The specific methanogenic activity for a serum bottle activity test is calculated as the rate of methane production per unit of biomass (equation B.6):

The accuracy of this estimation/calculation is inherently/inevitably dependent on the frequency of pressure re-equilibration.

Figure B.5 compares the Specific Methanogenic Activity that one can estimate using a *high* (**A**) or a *low* (**B**) frequency of re-equilibration. Ideally, however the operator should tune the frequency according to the actual gas production rate, i.e. the differential gas production, rather than maintaining a constant predetermined frequency. In fact, the period of interest for the determination of methanogenic activity (hence inhibition) is the from the beginning of the test up until the activity reaches its maximum and a little beyond that point; whereas for the estimation of biodegradability, one is mostly interested in the asymptote, therefore the frequency of re-equilibration after the activity has peaked may be reduced.

Since the manual re-equilibration is a discontinuous process, the curve of the calculated SMA will also be discontinuous. This makes it virtually (and statistically) impossible to precisely assess the *maximum* specific methanogenic activity, which ultimately is the parameter that is taken as representative of the performance of the seed inoculum under the given operating conditions. For instance, when the toxicity effect of a test material at a given concentration is to be assessed, the *maximum* SMA, calculated when the sludge is exposed to the substance, is compared to the *maximum* SMA, when the sludge is only exposed to the reference substrate.

We propose a way of presenting the estimates of *maximum* SMA which consists in calculating the average and standard deviation of the experimental maximum and the two immediately adjacent values (Figure B.5 C). Clearly, this standard deviation provides a purely qualitative indication of how accurate is the average value calculated.

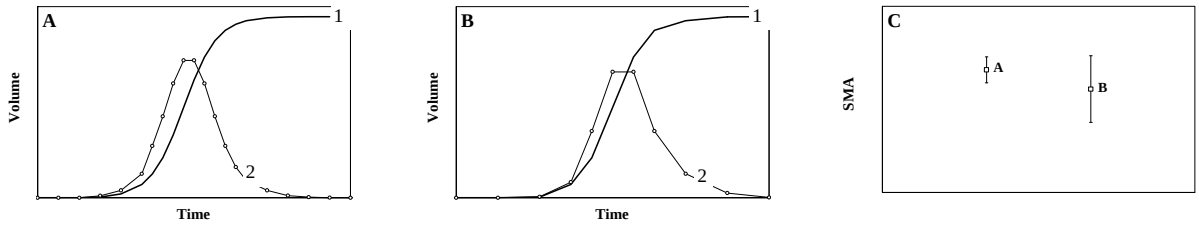

*Figure B.5 – Effect of the frequency of re-equilibration on the accuracy of the calculation of the SMA: high (**A**) vs. low (**B**) frequency; comparison of the two estimates of the maximum SMA (**C**).*

A high standard deviation should be taken as a warning that either of the following has occurred:

▪ the experimental determination of the methanogenic activity, in correspondence to the actual maximum, was not sufficiently accurate, i.e. the manual determination of the gas volume and/or the analytical determination of the gas composition had been inaccurate this leading to an outlier value of SMA;

▪ the frequency of re-equilibration, in the vicinity of the actual maximum was too low, thus leading to an apparent steep change of the SMA.

The advantage of this approach is two-fold:

- It provides an experimental ground to the evaluation of the inherent accuracy of a toxicity assessment

- It enables to visually compare the activity calculated for different conditions, e.g. at different concentrations of the test material, irrespective to the inherent accuracy of the determination.

Conclusions

A serum bottle AAT is a convenient, relatively inexpensive and a very accurate batch-test which enables to assess *i*) the potential negative effect of the test material on an anaerobic sludge as well as *ii*) its biodegradability under anaerobic conditions. This method only requires the manual determination of gas volumes and the analysis of the gas composition, as well as the quantification of the organic matter (as COD) and of the solids content (as VS or VSS).

The detailed protocol for conducting the test and for interpreting the data has been validated in the numerous tests conducted for this research.

The information obtained through an AAT should ideally form the ground for the next experimental stage.

B.4 REFERENCES

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The principles of pH-stat titration has been discussed in a previous project report in which some results obtained for the application to the methanogenic process have been presented.

Briefly, the concepts to bear in mind are:

- a **titrimetric method** relates the microbial activity to the rate of titrant addition with time
- a **pH-stat method** maintains the pH of the solution constant, within a limited range
- a combined **pH-stat** and **titrimetric method** detects the change in concentration of protons [H^+] and compensates this change by adding an equivalent amount of the appropriate titrating solution
- incidentally, the titrating solution might not only provide the protons to maintain the pH value constant, but also the substrate which is consumed by the micro-organisms. This additional feature of a pH-stat titrimetric method has been termed RePro-stat (whereby the concentration of a *Re*agent or a *Pro*duct is maintained constant) and offers some very interesting prospects for the development of these instruments (Rozzi & Ficara, 2001).

The above considerations provide the obvious limits to the applicability of such method:

i) the targeted process must result in the production or consumption of protons (or equivalent species);

ii) the targeted process must be the only one (or the prevalent one) which acts on the pH of the solution.

To limit the discussion to biological processes, there are several processes which comply with condition (*i*). Condition (*ii*) requires that the contribution to the pH change of all other process can be quantified separately or is zero. The case of monitoring methanogenic activity (i.e. a one-side control) falls into case (*ii*), as the interference generated by the chemistry of inorganic carbon can be effectively controlled by carefully setting the initial conditions of the activity test. In fact, this application has proven very accurate in measuring aceticlastic activity and inhibition (Rozzi et al, 2001; 2002).

The application to the monitoring of the anaerobic degradation of an organic material (i.e. a two-side control) is clearly more complicated and falls into the first case, i.e. unless we are able to separately assess the contribution of the acidification and methanisation phases or to suppress or control one at a time, the pH-stat titration will inevitably provide inaccurate information. This application is currently under investigation and the operating details have not been finalised yet.

The determination of methanogenic activity and inhibition is suitable for the option of maintaining the substrate concentration constant. Two species of acids can be used, i.e. acetic and hydrochloric acids. The description of both follows:

$$\text{Microbial uptake of acetate: } CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- \quad \text{(C.1)}$$

$$\text{Titration with acetic acid: } CH_3COOH + HCO_3^- \rightarrow CH_3COO^- + CO_2 + H_2O \quad \text{(C.2)}$$

$$\text{Titration with hydrochloric acid: } HCl + HCO_3^- \rightarrow Cl^- + CO_2 + H_2O \quad \text{(C.3)}$$

The experimental procedure to conduct a methanogenic activity test (termed MAIA, Methanogenic Activity and Inhibition Analyser) has been optimised in the course of this project, to be integrated in the procedure for screening the suitability of an effluent to be co-digested (Annexure A).

This procedure is illustrated hereafter.

C.1 Preparation of the components

▪ As common practice, the **sludge** can be stored for relatively long periods of time at temperature below 4 °C. During storage, it is not supplemented with substrate and the activity does not deteriorate significantly.

However, in order to shorten the lag-phase which would be displayed by a sludge that is recuperating its activity after a period of starvation, it is convenient to maintain an anaerobic culture (in a laboratory-scale digester) which can be sampled to provide a well-active seed sludge for the activity test. Should this not be possible, the sludge should be *at least* conditioned to the operating temperature overnight. This not only allows the gradual resumption of activity; but more importantly, avoids that changes in temperature affect the physico-chemical equilibria particularly that of bicarbonate/carbon dioxide: this would generate an initial transient that would hinder an accurate determination of activity through titration (Figure C.1 A).

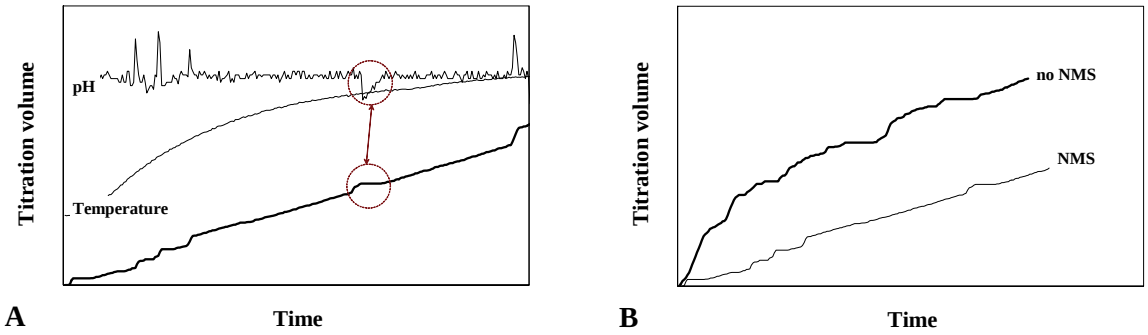


*Figure C.1: Effects of temperature variations (**A**) and of the use of a NMS (**B**) on the titration curve.*

The step-increase in the volume titrated are reflected in the local drops in the pH of the suspension (circles) and are an independent phenomenon: they are caused by non-homogenous mixing conditions (Rozzi et al., 2001).

In any circumstance, the initial period of the test (until a clear steady-operation is achieved, e.g. the titration process is regular) should be closely monitored by the operator, in that the determination of other parameters besides pH could be of help in obtaining a clear understanding of the start-up phase. Particularly, a frequent determination of the gas composition would allow detecting changes which can severely affect the titration rate.

▪ The use of a **nutrient and minerals solution (NMS)** has proven to have a positive effect on the titration process, in that it seems to reduce the background fluctuations (Figure C.1 B). More importantly, it provides the necessary supplements to the seed inoculum and a buffering capacity to the system. As most of these factors are generally present in a well-active sludge, the addition of a NMS might not be necessary. However, the following steps should be undertaken.

- Measure the solids concentration.

Our experience indicates that a concentration of approximately 10 g VS/ℓ is ideal to conduct the activity test. However, the "strength of the signal" is clearly a combination of the solids concentration and the inherent activity of the seed inoculum: therefore, this value should be regarded as indicative and should be changed according to the characteristics of the sludge utilised.

Should it be necessary to dilute the seed inoculum, this should be done using the NMS: deionised water should be avoided as it alters the osmotic pressure in the solution and might severely affect the micro-organisms.

Should it be necessary to concentrate the inoculum, the sludge sample can be centrifuged and resuspended in the NMS.

The contribution to alkalinity of the NMS must be taken into account, prior to correcting alkalinity (as explained later in this Section).

- Prepare the titrating solutions, using either acetic or hydrochloric acid.

The strength of the solution is immediately related to the sensitivity of the method (Rozzi et al., 2002).

Based on our experience, a concentration of 0.5 mol/ℓ is the ideal compromise between dilution effect and sensitivity.

C.2 Instrument preparation

A few advances in the manufacturing of the instrument have been made since the early set-up. This applies both to the hardware and to the software.

It is beyond the scope of this report to discuss the details of such modifications, but since future users may operate on instruments which do not resemble the one described hereafter, it is necessary to mention the main changes:

i) The use of a printed board instead of the Input/Output modules

ii) The DOS-based interface has been substituted with a Windows-based interface

iii) The gravity-delivery of the titrant through a Mariotte bottle has been substituted by a volumetric system of control, consisting of a peristaltic metering pump

iv) The ability of controlling two independent reaction vessels, on two process parameters simultaneously, i.e. pH and dissolved oxygen (however the DO probe could quite simply be substituted by a redox potential electrode, for instance).

These changes have resulted in user-friendlier control panels, on which the user presses buttons rather than typing commands on a blank screen. In particular, the change in the titration system is a great improvement as the calibration and the maintenance of the system became easier. Particularly, the Mariotte bottle system requires that any air bubble which might 'grow' in the line is carefully removed prior to calibrating (this can make the procedure very time consuming); also, air can relatively easily seep through, accumulate in the line during the test and make the delivery of the titrant very inaccurate. In this respect, the peristaltic pump ensures more stable performances throughout the test and a quicker calibration. The only drawback is that the tube can deteriorate due to the continuous rotation of the pump.

The following discussion applies to the original DOS-based version, equipped with Mariotte bottles for the delivery of titrants; but the translation to the new instrument should not present any problem.

Step 1: Calibrate the temperature sensors.

The pH-stat titrator is equipped with two sensors (PT100), to detect the temperature inside the reaction vessel and that of the titrant solution respectively.

The temperature of the reaction vessel affects the pH value generated by the signal of the pH electrode: a temperature dependency for the pH is implemented in the source code.

Since a constant head above the solenoid-valve is ensured by the use of the Mariotte bottle, the temperature of the titrating solution is the only variable that affects the flow rate, by altering the viscosity of the liquid. The flow rate is internally calculated from the number of openings of the solenoid-valve. It is therefore imperative that the temperature sensors are calibrated before the pH electrode and the electro-valve. The calibration of the temperature sensors can be performed periodically, as the sensors are more robust and the signal more stable.

Proceed as follows:

- Immerse the first electrode in a container with cold water, e.g. water and ice blocks, and wait until the signal displayed (in millivolts) is stable. Enter the temperature of the bath (a laboratory thermometer can be used) and confirm.

- Repeat the same operation with the hot bath: it is recommended to use temperature of 60 °C or above, to ensure a better accuracy of the calibration process.

- Repeat both steps, for the second sensor.

Note that even if the activity test is carried out in a temperature-controlled room, where both the reaction vessel and the titrant reservoirare exposed to the same temperature, both sensors have to be calibrated and connected to the Input/Output module.

Step 2: Calibrate the pH electrode.

It is recommended to perform this step at the beginning of each activity test, as the nature of the anaerobic sludge and the long duration of a test expose the electrode to a relatively fast deterioration of the response. A good practice is to log the results of the calibration in order to be able to promptly identify when it is time to *i*) regenerate the electrolyte or *ii*) substitute the electrode.

Proceed as follows:

- Immerse the electrode in the first pH buffer (e.g.pH 4) and wait until the signal (in millivolts) is sufficiently stable; then confirm and enter the corresponding pH value, i.e. 4.00.

- Repeat the operation for the second pH buffer (e.g. pH 10).

It is clearly preferable to use pH 4 and 10 as buffers being the test conducted at a set-point pH of around 7, which falls well between the two limits.

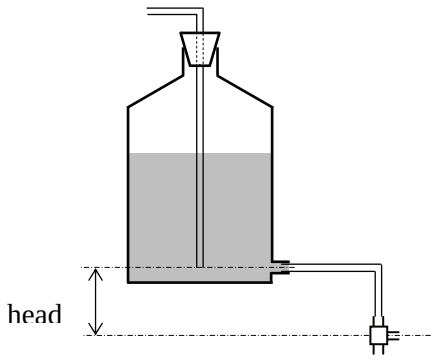


Figure C.2: Set-up of a Mariotte flask to maintain a constant head above a solenoid electro valve.

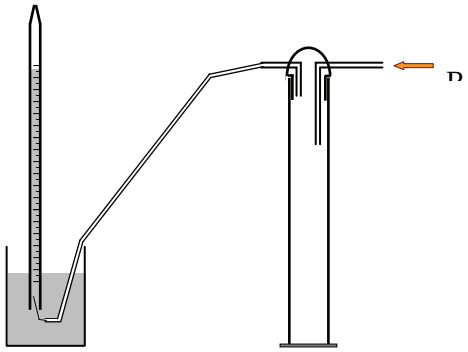


Figure C.3: Volumetric set-up consisting of a reversed burette, for measuring the biogas production.

Step 3: Set up the solenoid valve.

This step must also be performed at the beginning of each test and is aimed at removing air bubbles from the line connecting the Mariotte bottle to the solenoid valve; to correctly set the head above the solenoid valve (Figure C.2); and to adjust the settings of the solenoid valve to obtain the desired flow rate.

Based on our experience, a flow rate of 10-50 µℓ/pulse ensures a good sensitivity of the control system.

Proceed as follows:

- Set up the opening and closing times of the solenoid valve. These parameters primarily influence the flow rate of titrant and the sensitivity of the response of the controller, respectively. They are not totally independent however, because the longer the opening time, the smaller is the maximum number of openings in the unit time, hence lower is the overall sensitivity. On the other hand, the shorter the closing time, the higher the sensitivity but also greater the fatigue of the membrane inside the valve, which will lead to the overheating and eventually the break down of the solenoid valve itself.

The default value of 0.2 and 0.5 s respectively are generally good.

- Set up the data-logging interval.

This step does not strictly relate to the solenoid valve set up, but as mentioned above it indirectly affects the sensitivity of the determination by setting an upper boundary to the number of openings of the valve.

The data logging interval does not indicate the frequency of acquisition of the various signals (i.e. pH, temperature, etc.) which are continuously received by the I/O modules, but the frequency on which the signals are averaged and the average value logged as output datum. Clearly, the higher the frequency, the more sensitive is the control action, but the larger the amount of data the larger will be the size of the output file.

A frequency of 30 seconds is generally a good compromise for long-term tests such anaerobic activity ones.

- Ensure that the titrant line is bubble-free; set the head above the solenoid valve and the height of the solenoid valve above the titrant outlet. This set-up must not be altered after the calibration procedure has been completed, otherwise the flow rate will change, and the calibration value will be incorrect.

- Place a measuring cylinder underneath the titrant outlet and start the calibration. At any point, stop the valve, enter the exact volume cumulated to the point and confirm. Repeat this step not less than three times and do not exceed the 10 mℓ cumulative volume.

- It is advisable to log the data pairs on a separate spreadsheet and to recalculate the flow rate, using a linear regression. In this way, the titration volumes logged by the software can be validated at the end of the test. Moreover, one can keep a history of the performance of the solenoid valve (similarly to the suggestion for the pH electrode) and diagnose when the valve becomes unreliable.

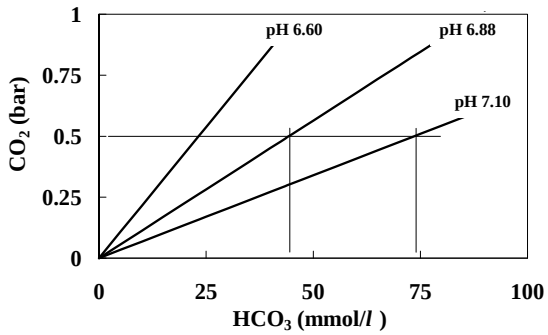
Step 4: Set up the reaction flask.

Proceed as follows:

- Pour the seed inoculum and the NMS (if needed); insert the temperature sensors and the pH electrode in the respective ports, and seal; insert the top cap with the titrant and substrate addition ports.

- Connect the liquid sample line to the gas bottle with the nitrogen/carbon dioxide mixture (see: Step 6).

- Set the biogas outlet in a way that gas can be vented while air is prevented from contaminating the headspace in the reaction vessel (water trap). It is sufficient to let the plastic tube bubble into a beaker with water.



Association/dissociation of carbon dioxide:

$$H_2O + CO_2 \leftrightarrow H^+ + HCO_3^-$$

$$K_1 = \frac{[H^+]\cdot[HCO_3^-]}{[CO_2]}$$

$$\frac{[HCO_3^-]}{[CO_2]} = 10^{-pK_1+pH}$$

Figure C.4: Graphical representation of the pH-stat condition (T 35 °C).

Step 5: Selection of the appropriate set-point pH.

A crucial aspect to take into account in the sensitivity of the control system: clearly, this depends on the intensity of the *signal* detected, i.e. of the variation in the pH value of the suspension resulting from a *unit* change in the concentration of reactants (or products). This in turn is dependent on the buffering capacity of the suspension, which can be interpreted as its *ability to resist* to changes in pH. Therefore, the lower the buffering capacity, the higher will be the sensitivity of the titration process, and hence the accuracy of the activity determination (Figure C.5).

The selection of the set-point value of pH must be a compromise between maintaining a sufficiently low buffering capacity in the system (i.e. a high sensitivity) and ensuring optimal conditions for the methanogens to operate. This latter objective requires the pH to be in the range 6.5 to 7.5. Under such conditions, the buffering capacity of a solution depends almost exclusively on the concentration of bicarbonate ions: the association/dissociation equilibrium of carbon dioxide (equation C.4) shows that the condition of constant pH (pH-stat) represents a straight line (C.4).

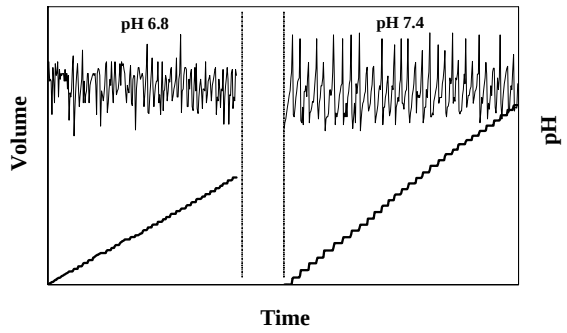


Figure C.5: Comparison of the sensitivity of the titration unit at different set-point pH.

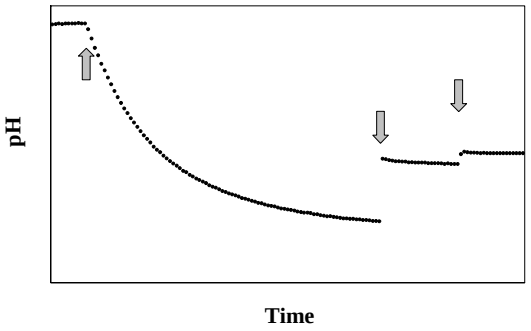


Figure C.6: pH adjustment prior to starting a pH-stat activity test, by flushing a nitrogen/carbon dioxide mixture and manually correcting alkalinity (arrows).

Step 6: Adjustment of pH and carbon dioxide molar fraction.

Proceed as follows:

- Start the program which detects the pH but disables the control system: time and pH data will be displayed on the screen, at the preset data logging frequency.

- Let the program run for a couple of minutes, until the reading is stable, then start flushing with the 1:1 N_2:CO_2 gas mixture.

- Open the bottle and regulate to a low gas flow. The efficiency of gas transfer through the liquid and into the headspace is inevitably limited, because of the line for sampling the liquor that is used, which necessarily has to have an outlet sufficiently large to ensure a homogeneous sludge sample: a needle would certainly make the gas bubbles much smaller, hence increase the transfer efficiency, but would also be rapidly clogged with biomass, when sampling. A gas recycle would improve the transfer efficiency but it is also complicated to make.

- As the carbon dioxide diffuses into the liquid and in the headspace, the pH should progressively decrease (Figure C.6) until reaching a second equilibrium point, which corresponds to the conditions of alkalinity of the sludge and to a CO_2 partial pressure of 0.5 bar. If the alkalinity has been accurately measured and corrected (Section C.1) and the temperature kept constant, this point should be sufficiently close to the desired set-point.

- If the desired set-point is not reached by simply sparging with gas, manually correct by dosing concentrated acid or alkaline solutions, through the needle (injection port) on the top rubber cap.

- Once the set-point value is reached (and maintained), turn off the gas, close the liquid sampling line and close the pH-monitoring mode.

Step 7: Set up the gas measuring device.

This step is not actually part of the pH-stat titrator, as the gas production is not logged by the software. However, it has been demonstrated that the information on gas production (thus on COD degraded) can be an ideal complement to the information on the titration rate, to calculate mass balances.

Proceed as follows:

- Connect the gas outlet to the volumetric measuring device. Simple devices can be a Mariotte bottle or a reversed gas-burette (Figure C.3), filled with alkaline solution, e.g 1 N.sodium hydroxide.

An alkaline solution is preferable to an acidic one, as it absorbs the carbon dioxide fraction of the biogas and the volume displaced will be directly equivalent to the volume of methane produced, which is the ultimate sink of the degraded COD.

- It is very important to hydraulically disconnect the measuring unit from the reaction vessel, to prevent accidental transfer of sodium hydroxide into the reaction vessel. This can be done, as shown in Figure C.3.

Step 8: Start the activity test.

Proceed as follows:

- Start up the control software and monitor the process for some time (e.g. 30 min). If the chemistry of the system has been properly adjusted in the preparatory phase, no titration or gas-production should occur. The addition of the titrant indicates that either the system was not at equilibrium (Rozzi et al., 2001) or that the sludge is endogenously active. The first situation should last few minutes, until the titration system has compensated for the imbalance. The second situation will indicate a continual baseline activity that would have to be quantified at the end of the test and would to some extent lower the titration efficiency of the response.

- When the above situation has been clarified, add the substrate i.e. sodium acetate (or acetic acid), by injecting it through the top port.

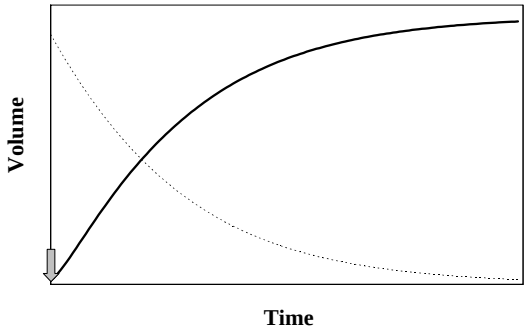


Figure C.7: Simulated titration curve, for a single dose of acetate (titrant: hydrochloric acid).

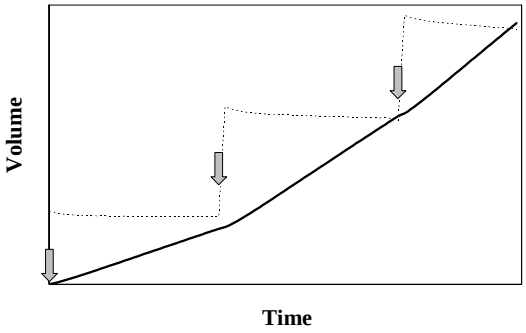


Figure C.8: Simulated titration curve, for three sequential additions of acetate (titrant: acetic acid).

Step 9: Follow-up

- For a pure activity test, aimed at measuring SMA at a non-limiting concentration of acetate, the use of hydrochloric acid is preferable. This mode (termed decreasing substrate concentration) allows replicate injections of acetate to be done and therefore a more reliable estimate to be obtained. A typical titration curve is simulated in Figure C.7, for a single addition of substrate.

- For an activity test aimed at assessing the kinetics of the process, either acetic or hydrochloric acid can be used. In the first mode (termed, at constant substrate concentration), multiple additions of acetate at progressively increasing concentration must be done (Figure C.8) and activity monitored for 2 to 3 h after each injection.

- For a toxicity test, acetic acid must be used as titrant to maintain a relatively constant acetate concentration throughout the test: this ensures that the different segments of the test only by the concentration of the test material, the other *relevant* conditions (i.e. pH, substrate concentration and carbon dioxide molar fraction in the gas phase) remain constant.

Step 10: Conducting the test.

- The key parameter of the test, i.e. the activity of the biomass, is estimated as the rate of titration i.e. the slope of the titration curve. To ensure an accurate estimation of the slope, it is recommended that there is a gap of 2 to 3 h between subsequent additions of substrate or of the test material. An extensive discussion of the aspects related to the execution of a test is reported in Rozzi et al (2001).

- Regularly monitor the gas production.

- In order to have an accurate and comprehensive overview of the situation, it is recommended to regularly download the data into a spreadsheet and to process the results off-line. To do so, stop the control software shortly, copy the log file (*.txt) to a disc and import the data on a spreadsheet. Immediately restart the control software.

C.3 Data interpretation

The interpretation of a pH-stat titrimetric activity test is very simple.

The objective parameter to estimate is the *specific methanogenic activity* of the sludge, which is defined as the rate of acetate uptake per unit of biomass. Since one mole of acetate yields one mole of bicarbonate (equation C.1) and the titrant solution (either acetic of hydrochloric acid) is a monoprotic acid, a 1:1 relationship exists between the moles of titrant dosed and the moles of acetate consumed:

rate of acetate uptake (mol/d) = titrant concentration (mol/ℓ) · rate of titration (ℓ/d)

COD units are generally preferred to moles, to indicate the concentration of organic substrates, therefore:

rate of acetate uptake (g COD/d) = $\boldsymbol{\alpha}$ (g COD/mol) · rate of acetate uptake (mol/d)

where $\boldsymbol{\alpha}$ = 64 is the COD equivalent of one mole of acetate.

Making the assumption that no biomass growth occurs in the vessel during the test, the activity A can be calculated as:

$$A = \alpha \cdot \frac{C \cdot Q}{X} \text{ (g COD/g VS/d)} \qquad \text{(C.5)}$$

where:

X is the amount biomass in the seed inoculum (g VS);

C and Q are the concentration and flow rate of the titrant respectively.

Although the assumption of absence of significant growth is reasonable on a short test, it can be verified by plotting the titration flow rate on a semi logarithmic plot (C.9), according to the following procedure.

The dynamics of the biomass concentration is generally described by a first order equation:

$$\frac{dX}{dt} = (\mu - b) \cdot X \qquad \text{(C.6)}$$

where μ (d^{-1}) and b (d^{-1}) are the growth and the decay rates respectively and X the biomass concentration (g VS/ℓ).

The dynamics of the substrate is proportional to the net growth of the biomass:

$$-\frac{dS}{dt} = \frac{1}{Y} \cdot \frac{dX}{dt} \qquad \text{(C.7)}$$

where Y is the biomass yield (g VS/g COD) and S the substrate concentration (g COD/ℓ).

By integrating equation C.6 and substituting into equation C.7, neglecting the biomass decay, one obtains:

$$-\frac{dS}{dt} = \frac{1}{Y} \cdot \mu \cdot X = \frac{1}{Y} \cdot \mu \cdot X_0 \cdot e^{\mu \cdot t} \qquad \text{(C.8)}$$

where X_0 is the initial concentration of biomass.

This can be rewritten as:

$$-\frac{dS}{dt} = \left(\frac{dS}{dt}\right)_0 \cdot e^{\mu \cdot t} \qquad \text{(C.9)}$$

where $\left(\frac{dS}{dt}\right)_0$ is the initial rate of substrate degradation.

As discussed above, the rate of substrate degradation is proportional to the rate of titration; therefore equation C.9 can be rewritten as:

$$\ln Q = \ln Q_o + \mu \cdot t \qquad (C.10)$$

The latter can be plotted as a straight line on a semi logarithmic plot, in which the growth rate is estimated as the slope.

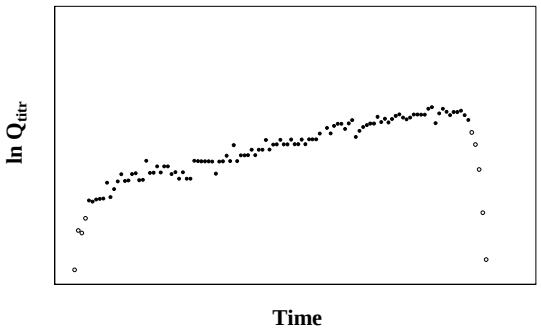


Figure C.9: The titration data plotted on a semi-logarithmic scale illustrate the growth of the biomass.

Conclusions

A pH-stat titrimetric sensor can be applied to monitoring the methanogenic step of the anaerobic digestion process, as the degradation of acetate to methane influences the pH of the solution by generating alkalinity.

Two operational modes can be chosen, i.e. a simple pH-stat mode, whereby an inorganic acid is used as titrant to compensate the increase in pH; and a combined pH- and substrate-stat mode, in which acetic acid simultaneously compensates the change in pH and the acetate consumption by the micro-organisms. The latter mode is particularly suited for conducting toxicity tests in which the concentration of the base substrate is maintained nearly constant throughout the test and the test material is spiked at intervals. This application can be proposed as being as reliable as established serum bottle assays, offering the advantages of:

i) A shorter duration of the test: the activity must be monitored for only few hours after each spike

ii) Potentially isolating the true effect of the test material on the methanogenic activity from other effects that could be due to a decrease in pH or substrate concentration.

However, the quality of the outcome is highly dependent on the correct start-up of the test: great care must be taken in ensuring that all other pH-affecting processes are neutralised, with particular regard to the bicarbonate/carbon dioxide system that must be equilibrated prior to starting the test, using a 1:1v/v N_2:CO_2 gas mixture.

The application of a two-unit pH-stat titration sensor, which could monitor simultaneously the acidogenic and methanogenic stages, is currently being studied. This new set-up will enable AAT equivalent to serum bottle ones, to be undertaken with the advantages of a shorter duration and automated data acquisition.

Table C.1: Summary of the recommended parameters to conduct a pH-stat titrimetric activity test.

Parameter	Units	Interval or value
Biomass concentration	g VS/ℓ	10
Alkalinity	mmol/ℓ	29-46 [(a)]
CO_2 partial pressure	bar	0.5 [(a)]
Set-point pH	-	6.7-6.9 [(a)]
Titrant concentration	mol/ℓ	0.5
Temperature	°C	30-35
Titrant flow rate	µℓ/opening	10-50

[(a)] The partial pressure of carbon dioxide is defined by the application, as aceticlastic methanogenesis generates an equimolar gas mixture; the set-point value of pH can be selected in the range indicated; the resulting value of alkalinity which ensures that the physico-chemical system is at equilibrium, hence does not interfere with the titration process, is calculated using equation C.4 (or graphically, on C.4). The interval reported in the Table refers to a temperature of 35 °C.

C.4 REFERENCES

Rozzi A and Ficara E (2001). Titration biosensors for risk assessment of contaminated sites and groundwater. *Proc. 1st Workshop SENSPOL: Sensing technologies for contaminated sites on groundwater*, Alcalà (Spain), 9-11 May.

Rozzi A, Remigi E and Buckley CA (2001). Methanogenic activity measurements by the MAIA biosensor: instruction guide. *Wat. Sci. Technol.*, **44**(4): 287-294.

Rozzi A, Castellazzi L and Speece RE (2002). Acetoclastic methanogenic activity measurement by a titration bioassay. *Biotechnol. Bioeng.*, **77**(1): 20-26.

The objective of this appendix is to summarise the experience that has been accumulated by the research team in the operation of laboratory-scale and, to a minor extent, of pilot-scale completely-stirred digesters.

This Annexure is structured as a list of topics that we encountered in the course of this project.

The reporting of such factors and the measures that were put in place to manage them may be of help for the researcher in anticipating similar circumstances in the future.

This Section is subdivided in the following main subjects:

i) How to **operate** an anaerobic digester

ii) How to **monitor** the performance of an anaerobic digester

iii) How to **assess** the performance of an anaerobic digester.

D.1 Operation of an anaerobic (CSTR) digester

The set-up for a generic laboratory-scale reactor is described below.

The essential components are:

- The reaction vessel

- The stirring device

- The temperature-controlling system

- The inlet and outlet ports

- The gas-measuring system.

The size and shape of the reaction vessel have an influence on other characteristics of the set-up and to some extent on the performance of the process itself. However, for general purposes, and certainly in the early stages of an investigation, a conventional cylindrical or conical flask would be adequate.

The use of a magnetic stirring device is obviously limited to small reaction vessel, where the turbulence generated by the rotation of the bar can be effectively transferred to the entire liquid volume. Because the rotation of the bar becomes highly unstable as the rotation speed increases, magnetic stirring is generally insufficient for reaction volumes larger than 5 ℓ and a directly-driven stirring device is preferable; this may hold two or three paddles to improve the mixing throughout the entire depth of the reactor.

Maintaining a relatively constant temperature is crucial in anaerobic applications because the temperature is a major factor controlling the reaction rates of the system (specifically regulating the solubility of carbon dioxide which in turn has an impact on the pH of the suspension) and biological reactions rates. Furthermore, generic applications are performed in the mesophilic range, around 30 to 35 °C: a drop below or an increase above this range would be detrimental for the process, resulting in the microbial activity slowing down and ultimately ceasing.

Three systems to control the temperature have been adopted in this research:

- A water bath in which water is heated and pumped into the hollow jacket that constitutes the external wall of the reactor

- A temperature controlled-room- A water bath, similar in principle to the first one, in which the sludge flowing into the recirculation circuit is heated prior to being pumped back into the reactor. This system was used for a larger pilot-scale set-up.

The reactor must be equipped with the following ports:

- An inlet port for pumping the feed in the digester

- Two outlet ports for the extraction of liquid samples and for venting the biogas respectively.

It is convenient for the inlet port to reach deep into the mixed liquor so that the feed is thoroughly homogenised as it is pumped into the digester: If the inlet hangs high in the headspace and there was either foam or a thick layer on the surface of the mixed liquor, the feed might not be homogeneously distributed.

To maintain the set-up as simple as possible, the feeding line can be used for the extraction of samples of the mixed liquor for analytical determinations, e.g. COD, solids, etc.

Great care should be taken in both feeding and sampling the digester: when feeding, the entire dose of feed should actually enter the digester and no residual should remain inside the pipe. This can be done by rinsing the tube with some sludge or by reversing the flow a number of times immediately after the feed has been injected. Similarly, when sampling, one should make sure that the sample is representative of the conditions inside the reactor at that time.

The outlet port for gas venting must hang as high as possible above the liquid surface, to prevent the possibility of it becoming clogged with foam.

If the feeding is done volumetrically, e.g. using a peristaltic pump, great care should be placed in ensuring that *i*) the feed was well mixed/homogenised when added to the digester; that *ii*) no (or very little) oxygen was dissolved in the medium, that would affect the activity of the anaerobic consortium; and that *iii*) the characteristics of the feed stock solution are sufficiently consistent: since the feed contains organic matter, microbial activity would easily establish and alter the characteristics of the feed, if this was not correctly handled and preserved. An option could be to maintain a feed stock solution in a cold bath.

The ideal set-up would be as follows:

- Immediately before starting the feed operation, the feed stock solution was thoroughly stirred for a period of time (few minutes are sufficient)
- The reservoir in which the feed stock solution is stored was sealed and equipped with a variable volume headspace, similar to that described for the actual digester
- Immediately after sealing the container, the headspace was flushed with nitrogen: this would ensure that no air was solubilised when the feed stock solution was stirred, nor was sucked in from outside when the feed was pumped into the digester.

The key factors that should be decided for operating a digester are the **sludge retention time** (SRT) and the **hydraulic retention time** (HRT) and the **organic loading rate** (OLR).

The sludge and the hydraulic retention times coincide if no sludge separation is performed prior to liquid extraction. The simple set-up does not allow sludge separation, even if stirring is stopped before extraction for a period of time that would allow the sludge to settle, because the pipe used for feeding and for extracting is deep into the mixed liquor.

In general, the SRT should be decided in a way that guarantees the minimum retention time needed for the most vulnerable microbial population.

The HRT will define the feed flow rate, according to the formula:

$$Q = \frac{V}{HRT}$$

where V indicates the volume of the contents of the reactor.

The OLR, being the hydraulic retention time fixed, will define the concentration of the feed (C) according to the formula:

$$C = \frac{OLR}{Q} \cdot V \qquad ( )$$

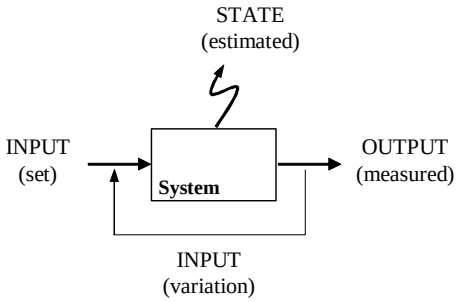


Figure D.10: The concept of monitoring an anaerobic process: the input variables are set by the operator; the output variables are analytically or instrumentally measured; the state of the system is estimated, based on the observation of output and the knowledge of the input. This ultimately enables to manipulate the input, in order to achieve better performance.

D.2 Monitoring a digestion process

Monitoring the performance of a laboratory-scale or pilot-scale digester requires the estimation of the *state* of the system in response to the *input* and operating conditions.

The question therefore becomes: which variables should be measured in order to reliably assess the behaviour of the digester?

It is beyond the scope of this report to discuss the topic comprehensively. Our experience however suggests that the following variables are sufficient to obtain an accurate picture of the prevailing state of an anaerobic process.

D.2.1 Gas production

This is the most direct/measureable output of an anaerobic process, in fact the majority of the existing methods to assess biodegradability of a substance and the activity of a sludge target this variable (Rozzi & Remigi, 2004).

Qualitatively one can say that a high biogas flow rate is indicative of an effective digestion process. However, the knowledge of this variable alone is not sufficient, and may even be misleading, if not accompanied with the knowledge of the composition of the gas: typically, a high gas flow rate with a low methane fraction may be indicative of a predominantly acidogenic process in which the methanogenic biomass may be inhibited. If these conditions are not detected in time, they may ultimately lead to the failure of the digester.

A simple device for measuring the gas production is by liquid displacement, whereby the gas produced in the digester displaces an equivalent volume of a liquid contained in a separate reservoir: the volume of gas is indirectly quantified by measuring the volume of liquid displaced. Numerous methods/techniques exiss: we used a U-tube filled with a barrier solution and equipped with a solenoid valve to release the excess pressure.

D.2.1.1 Set-up of the gas line

Great care should be taken into setting up the gas line in order to ensure that the measurement of the volume of gas is accurate and consistent throughout the experimental period..

Besides ensuring that the system is gas-tight, one should set up the line so that the gas-measuring device can be disconnected for maintenance and repair without compromising the gas tightness of the reactor. The most frequent incidents that can happen are that the barrier solution evaporates, the solenoid valve fails, or the gas line becomes blocked (e.g. by biological foam) or leaks.

By installing the manual valve indicated in Figure D.10, the operator can isolate the gas line for inspection, without exposing the reactor to air. The accuracy of the measurement relies entirely on the accuracy of the calibration of the liquid-displacement device. *Theoretically*, the unit volume

corresponding to one pulse/opening of the valve can be calculated geometrically. However, in practice, it might be difficult to accurately measure the distance between the two levels. It is therefore advisable to actually calibrate the device, e.g. by flushing the headspace of the reaction vessel with inert gas at a constant flow rate. This can even be done on a regular basis, during the experiments without risk of disturbing the process.

The use of a variable volume gas reservoir can be used for a quick check on the gas tightness of the system and on the operability of the device: the operator can manually compress the bag and verify that the level in the U-tube moves accordingly, and if necessary, that the solenoid valve correctly opens and closes. This is a very simple check that can be routinely performed.

D.2.2 Gas composition

In our experience, this is possibly the most sensitive and easy to perform measurement that the operator can undertake. The gas-chromatographic method does not need to be very sophisticated (the detection of carbon dioxide and methane is sufficient) and the analytical apparatus is not too expensive. The maintenance and calibration procedure for the GC is illustrated in Annexure F. The most common incident is that the syringe gets blocked. This is almost inevitable as the needle is inserted through the septum into a fitting which very likely tends to accumulate biological material.

Nevertheless, we verified that a very high accuracy in determining the composition of the gas can be achieved: we generally take five to eight replicate samples and the variation coefficient has consistently been lower than 2 % (for carbon dioxide and methane).

We cannot recommend an ideal interval for gas composition, as this depends on the composition of the feed (**Figure F.4**). The operator should consider the variations in the gas composition, particularly increasing fractions of carbon dioxide.

D.2.3 pH, alkalinity and VFAs concentration

Unlike the gas flow-rate and composition which can be determined almost continuously, these variables are measured on a sample of mixed liquor hence at most once a day. In fact, a lower frequency is sufficient, and the operator can measure these variables alternatively to determine COD.

The measurement of pH, alkalinity and VFAs concentration only requires very simple equipment, i.e. a pH-meter, a burette and magnetic stirrer. The two-point titration procedure is illustrated in Section **F.3**.

All these variables are strictly dependent on the dissolved carbon dioxide, therefore great care must be taken in carrying out the determination immediately after having collected the sample, and in handling the sample correctly, i.e. minimising the gas exchange with atmosphere.

This is inherently contradictory, because all determinations require that the sample is homogeneous hence well mixed; however, stirring the sample also greatly enhances the gas exchange between the liquid and the atmosphere, thus enabling carbon dioxide to escape (due to the large difference in partial pressure between the conditions outside and inside the digester). In order to minimise this inconvenience, the following rules should be applied:

i. Collect the sample in a container which minimises the liquid surface exposed to air, e.g. a cylinder

ii. Measure pH immediately after sampling, directly in the container: stirring is not necessary and pouring the liquid into another container would favour the stripping of carbon dioxide gas

iii. Pour the sample for alkalinity and VFAs determination into a beaker and stir at a very low speed, only sufficient to thoroughly distribute the titrating solution in the entire volume

iv. Use a concentrated rather that diluted titrating solution in order to decrease the titration time. In our experience, a 0.5 mol/ℓ solution is ideal.

It is generally acknowledged that ideal ranges for pH, alkalinity and VFAs concentration are as follows (Speece ,1996):

D.2.4 COD concentration

The determination of COD, combined with that of the gas flow rate and composition, is necessary to conduct a mass balance on the organic matter and ultimately calculate the performance of the process. The determination is relatively quick and generally accurate; it is recommended to use four replicates to ensure that the average and standard deviation are meaningful.

D.2.5 Solids concentration

The determination of solids in the digester assists in ensuring that the biomass is not washed out of the system, but rather grows on the organic substrate. The determination is not immediate as it requires the sample to be diluted (if necessary), filtered, dried in the oven and ignited in the furnace and the outcome can only be available one or two days after the sample has been withdrawn.

For this reason and because the growth rates of anaerobic micro-organisms are relatively low, a frequency of one determination per week is generally sufficient. It is recommended to undertake the measurement of VSS in four replicates. Ideally, one should verify a constant or increasing trend in the concentration of VSS.

The estimation of the methanogenic fraction or activity of the biomass could effectively complement the determination of solids concentration: the method indicated is not immediate but is likely to be sufficiently accurate if a minimum of three doses of acetic acids are added. If the digester is not overloaded, the fed-batch phase of the test can start immediately after the sludge sample has been withdrawn, with no need for a preliminary (partial) consumption of the residual substrate. Therefore, the estimation of the methanogenic fraction of the biomass will take *at least* 3 to 4 days.

More importantly, it only provides a *relative* estimate, but is generally sufficient to monitor the process.

D.2.6 Mass balance

A closed mass balance calculation is essential to ensure that a data set is accurate.

D.2.7 Evaluation of the stability of an anaerobic digester

Alkalinity and VFA concentrations are commonly used as indicators of the stability of a digestion process. In the common practice, their ratio, i.e. VFA-to-Total Alkalinity ratio, is also used.

However, Speece (1996) warns that the *reserve bicarbonate alkalinity* should be regarded as a more accurate monitoring parameter, as it indicates the concentration of bicarbonate alkalinity which is available to neutralise *additional* free volatile acids, before the pH drops below a safe threshold value of 6.2 to 6.5.

A numerical example may help in understanding this concept.

If we consider the situation of the laboratory-scale digester with the conditions described below, it *apparently* seems sufficiently safe, as one can argue from the following parameters:

Temperature	pH	Alkalinity	VFA	pCO_2 (bar)
32 °C	7.93	300.4 mmol/ℓ	99.8 mmol/ℓ	0.28

The reserve bicarbonate alkalinity (RA) is calculated as:

$$RA = BA - BA_{CO_2} = (TA - TA_{VFA}) - BA_{CO_2} \quad \text{(D.11)}$$

where:

- BA and TA indicate the *bicarbonate* and *total* alkalinity respectively (mol/ℓ);

- the lower case CO_2 and VFA indicate the alkalinity required to buffer the carbon dioxide and the VFA respectively.

The quantities BA_{CO_2} and TA_{VFA} are calculated as follows:

$$BA_{CO_2} = \frac{K_{CO_2} \cdot K_H \cdot p_{CO_2}}{10^{-pH}} \quad (D.12)$$

$$BA_{VFA} = 0.83 \cdot VFA \quad (D.13)$$

where:

- K_{CO_2} is the dissociation constant for HCO_3^-/CO_2 (mol/ℓ);
- K_H is the Henry's law constant for carbon dioxide (mol/ℓ.bar);

Equation D.12 translates the equilibrium of carbon dioxide in water and between the liquid and the gas phase (Henry's law).

Values for the constants K_{CO_2} and K_H can be calculated as follows (T, temperature in K):

$$pK_{CO_2} = 17052 \cdot T^{-1} + 215.21 \cdot \log T - 0.12675 \cdot T - 545.56$$

$$K_H = 10^{2.6747 - 0.0139 \cdot T}$$

For the conditions summarised above, $K_{CO_2} = 4.6 \cdot 10^{-7}$ mol/ℓ and $K_H = 0.0272$ mol/ℓ.atm.

$$BA = Alk \cdot MW\left(HCO_3^-\right) = 18323 \text{ mg/}\ell$$

$$BA_{CO_2} = 0.299 \text{ mol/}\ell = 18255 \text{ mg/}\ell$$

This clearly leaves a very small safety margin of 68 mg/ℓ only to neutralise additional VFA, which corresponds to 68/0.83 = 82 mg/ℓ of VFA.

D.2.8 Calculating the performance of the process

The ultimate objective of an anaerobic digestion process is to effectively convert the organic carbon of the feed into methane. The efficiency of this process is generally measured in terms of COD removal efficiency as follows:

$$\eta_I = 1 - \frac{COD_{out}}{COD_{in}} \quad (D.14)$$

where COD_{in} and COD_{out} denote the COD concentrations in the feed and in the effluent respectively. However, this approach is correct when the system has reached a steady-state and not before one (or more) retention times have passed, as the efficiency defined according to equation D.14 does not account for the dilution effect (function of HRT) that occurs in a completely stirred digester. A mass balance on the COD is actually a better approach in that it compares the *total* amount of organic matter fed, to the *total* amount which residues, as:

$$\eta_{II} = \frac{COD_{removed}}{COD_{fed}} = \frac{\sum C_{in,t} \cdot V_{in,t} - \left[C_{out,t} \cdot V_R + \sum \left(C_{out,t} \cdot V_{out,t}\right)\right]}{\sum \left(C_{in,t} \cdot V_{in,t}\right)} \quad (D.15)$$

where:

$C_{in,t}$ and $C_{out,t}$ denote the COD concentration in the feed and in the effluent respectively, at time *t*;

$V_{in,t}$ and $V_{out,t}$ denote the volume of feed and of mixed liquor respectively, at time *t*; and

V_R is the liquid volume.

Ideally, the fraction of COD degraded should be stoichiometrically converted into methane and therefore the efficiency evaluated according to equation D.15 should equal the following alternative definition:

$$\eta_{III} \frac{COD_{toCH_4}}{COD_{fed}} = \frac{\sum M_{ch,t}}{\sum (C_{in,t} \cdot V_{in,t})} \quad (D.16)$$

where $M_{ch,t}$ is the amount of methane (in g COD) produced at time *t*.

Possible reasons for the efficiency (equations D.15 vs. D.16) not been equal are that:

i) The COD measured analytically is not accurate or not representative of the actual organic matter in the mixed liquor; this is actually possible since the COD is often measured on the filtered mixed liquor, i.e. the portion of COD that has possibly been stored into the biomass is not accounted for.

ii) The quantification of the methane produced is not accurate. In fact, the volume of methane produced is calculated through a mass balance which is based on a number of parameter that must be determined with sufficient accuracy for the overall mass balance to be accurate.

$$\Delta V_{CH} = \Delta V_{CH,out} + \Delta V_{CH,headspace}$$

where:

- $\Delta V_{CH,out}$ denotes the volume of methane that have left the reactor during the interval $(t-1, t)$ with the biogas; and

- $\Delta V_{CH,headspace}$ indicates variation of the methane volume in the headspace in the same interval.

The two terms can be calculated as:

$$\Delta V_{CH,out} = \int f_{CH}(t) \cdot Q_{gas}(t) \cdot dt \quad (D.17.a)$$

$$\Delta V_{CH,headspace} = V_H \cdot (f_{CH,t} - f_{CH,t-1}) \quad (D.17.b)$$

where:

- $f_{CH}(t)$ is the methane fraction in the off-gas; it is generally a function of time;

- $Q_{gas}(t)$ is the biogas flow rate (ℓ/d) and is also a function of time;

- $f_{CH,t}$ and $f_{CH,t-1}$ denote the methane fraction, at time *t* and *t-1* respectively; and

- V_H is the volume of the headspace (it is generally considered constant).

In the set-up described in, the determination of the gas composition was done discontinuously (i.e. once a day) and only the cumulative volume of gas produced was known (V_{gas}). This translates in the practical assumption that *i*) the biogas flow rate is constant and *ii*) the methane fraction varies linearly in the time interval. Therefore, equation (D.4.a) can be written as:

$$\Delta V_{CH} = \frac{f_{CH,t} + f_{CH,t-1}}{2} \cdot V_{gas} \quad (D.18)$$

Table D.1: Variables that should be measured to monitor a laboratory-scale digester.

Variable	Frequency of determination	Optimal range
Gas flow rate	Continuous (*at least* daily)	▪ Function of the sludge activity and organic loading rate
Gas composition	At least daily	▪ Function of the feed composition and microbial dynamics
pH	Whenever a liquid sample is collected	6 to 7.5
Alkalinity	As for pH, or every two pH determinations	(…)
VFAs concentration	As for alkalinity	(…)
COD	Daily or every two days	▪ Should not display a clear *increasing* trend
Solids	Weekly or every two weeks	▪ VSS should not display a clear *decreasing* trend
SMA (*)	As for solids	> 60 mg COD/g VSS/d

(*) The variable measured is the methanogenic activity of the sludge, combined with the previous knowledge of the solids concentration: the methanogenic fraction is a derived quantity.

Note. These guidelines are meant for the monitoring of the start-up phase and for an experimental digester, on which specific tests are performed, i.e. conditions vary. Steady-state and routinely operated digesters require a much lower frequency of determinations.

D.3 REFERENCE

Speece RE (1996). *Anaerobic biotechnology for industrial wastewaters*. Archae Press, Nashville (TN, USA).

Based on the day-to-day experience of laboratory work, we realise that unless an anaerobic digestion unit is highly automated, e.g. with online data acquisition of gas production and composition, pH, automated feeding and sampling procedures, etc., the accurate monitoring of the process can be very time-consuming. Particularly when the number of units to maintain is large (at one stage, due to the continuous lack of success in starting up the laboratory-scale reactors, we were simultaneously manually monitoring four units).

Nowadays, the implementation of *ad hoc* control software using commercial packages is relatively simple and inexpensive. However, it still requires time to write the code and then debug it. More importantly, this option would still not be feasible on the instrumentation side, as the various measuring devices should be interfaced with the software, and not all the devices in the laboratory are actually designed for that purpose.

For this reason, we focused our efforts in improving the data-logging and management step.

A spreadsheet is often not the ideal tool to be used when a large and diverse amount of data, coming from different sources, and which need a different type of analysis, are to be manipulated.

Typically, COD measurements are carried out daily and consist in the titration of a certain number of replicates; sometimes, it might be necessary to use two dilutions, to obtain meaningful average values. Therefore, the single datum that is obtained daily is in fact the result of a series of manual determinations.

Similarly, for the determination of alkalinity and VFA concentration using the two-point titration method, a preliminary level of manipulation of the raw data is required before the actual single measurement can be logged on the spreadsheet.

The determination of the gas composition is different. During the course of this project, it has been found to be the most sensitive indicator of process performance. Therefore, frequent gas sampling and analyses were conducted on various occasions and immediately after feeding the reactor. Since the parameter of interest is the gas composition, i.e. the molar fractions of nitrogen, carbon dioxide and methane in the gas phase, the raw output of the GC, i.e. the peak areas, had to be converted into molar fraction (as explained in Section F.2); the same had to be repeated for each replicate of the same sample; then the molar fraction, averaged and sometimes manually corrected (i.e. in some cases, replicates had to be disregarded) before the single datum for gas composition could be inputted on the spreadsheet, in correspondence to the time of sampling. On some occasions, multiple samples (up to 10 to 12) were taken in a day.

It is therefore clear that different levels of pre-treatment of the raw data are necessary prior to logging and that different time scales apply to the different pieces of information. It goes without saying that each one of the data inputting and manipulating steps is inherently exposed to accidental errors, particularly when the same operation is repeated several times. This and the fact that ideally it is good practice to provide an audit trail of each and every step from the actual raw data to the synthetic data at the end of the data-manipulating line, we oriented our interest to the use of a **database application**.

Clearly MS-Access was the obligate choice, as it is widely available and as it is ideally suited to interact with MS-Excel, which is still the preferential tool to generate plots.

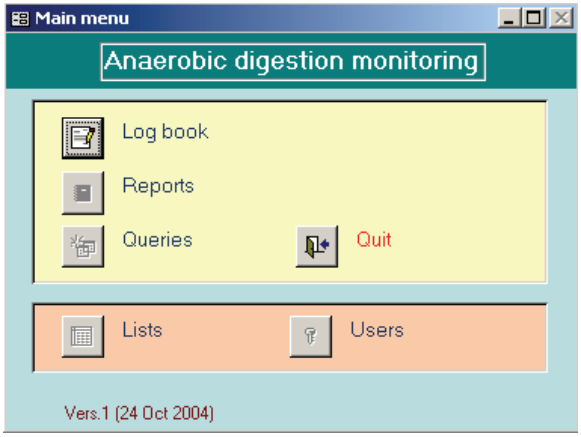


Figure E.1: Main menu of the application for monitoring anaerobic digestion processes.

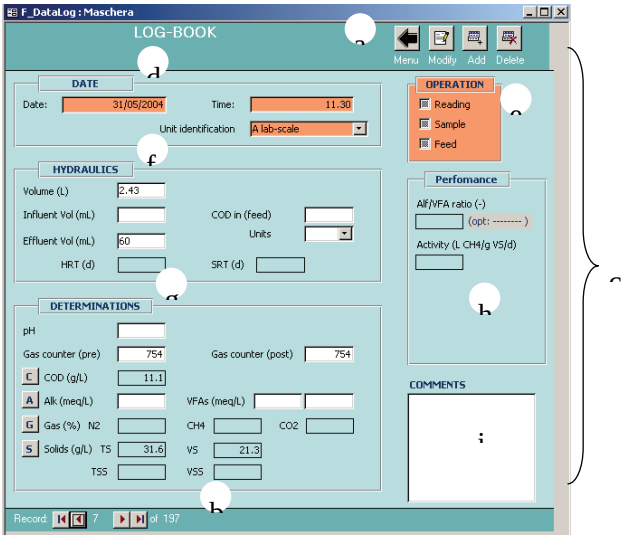


Figure E.2: Main page of the log-book for data entries.

The application that has been implemented consists of the user-interface and of the underlying database.

The main menu (Figure E.1) is displayed at start-up. It contains six buttons:

- Log-book: it opens the main sheet of the log-book, through which data will be inputted
- Reports: it allows the user to make an 'open' selection within a list of predefined sets of data (e.g. the OLR and the gas production rate and composition), that will be displayed, printed or saved as an ASCII file (e.g. to be imported in a spreadsheet for further analysis). This button is not active in the current release, as further debugging is needed.
- Quit: it shuts down the application
- Lists: this button is not active, but it will allow the user to manage data that can simplify routine calculations, such as molecular weights or COD-equivalents data of the test materials, etc.
- Users: since the application has been used for internal purposes only, the need has not arisen for creating different levels of users with different privileges. This however is generally a good practice when such an application is accessed by many users. Typically, to ensure that data are not accidentally manipulated, only some of the users will be authorised to write data (including modifications and deletions); some others will be authorised to query the database and generate reports, etc.

The main page of the **log-book** is showed in Figure E.2 and consists of the following areas:

- Commands bar (**a**): it contains four buttons, i.e. Menu (to return to the main menu), Modify (to modify the record which is currently active), Add (to display a blank form to enter a new record) and Delete (to erase the current record)

▪ nNavigation bar (**b**), which shows the position of the current record within the database and the total number of data stored; four small buttons with red arrows, i.e. First, Previous, Next and Last, enable to move to the corresponding record of the database

▪ The main body (**c**) contains the sub-areas Date, Operations, Hydraulics, Determinations, Performances and Comments:

- The sub-area Date (**d**) contains the fields Date, Time and Unit ID. As the application is designed to serve the control of multiple reactors, each record can be associated to the corresponding unit, e.g. laboratory-scale A. All these three fields are mandatory, to avoid the risk of inputting unrelated data.

- The sub-area Operations (**e**), also mandatory, allows to select the type of data that is going to be entered: a simple reading (i.e. gas volume and/or gas composition), a liquid sample (which supposedly will provide a value of pH, COD, alkalinity, VFAs and periodically of solids) and feed (which is associated with a volume or flow-rate fed to the reactor, as well as the organic load).

- The sub-area Hydraulics (**f**) manages the aspects related to the feed and sampling procedures. It also displays in *read-only* mode the current hydraulic and sludge retention times, which are internally calculated: it is assumed that the reactor is a completely stirred vessel with no sludge separation, as all the units used in this project, but independent calculations of the two could be implemented in a future version.

- Determinations (**g**) is the sub-area in which the actual analytical and instrumental data are recorded: pH, gas counter (i.e. gas volume), COD, etc. As discussed earlier, most of the parameters are 'manipulated' quantities which result from the elaboration of the raw information, e.g. replicated measurements which contribute to a single averaged value. This is used to be done on a separate spreadsheet, whereas here a secondary input form is displayed by clicking on the respective buttons.

For example, the Solids button opens the Solids calculation sheet reported in Figure E.3. This form will receive the raw data logged by the user, elaborate the output (click on Results) and transfer the end result onto the main log-book. The elaboration of the data is not rigid in that it is always possible for the user to control to some extent how the end result is obtained, e.g. an outlier from the set of data can be deactivated, in order to obtain a better estimate (i.e. with a lower VC). However, all the raw data will be saved in the database, should it ever become necessary to review the past data.

- The sub-area Performances (**h**) displays a range of summary information which is generally used as process performance indicators, besides the actual values of the measured parameters. At present, only two of such indicators are included, i.e. the Alkalinity-to-VFA ratio and the methanogenic activity.

Other possible indicators could be the gas flow-rate, the methane-to-carbon dioxide ratio. Clearly more than the current values of these indicators, it is of greater interest their trend over a certain predefined period of time. A more efficient way of displaying these indicators would be to generate a secondary form (or even a report as the data are to be *read only*) in which a series of data can be viewed over a selected period of time.

- The sub-area Comments (**i**) (present in all the forms) allows the user to input all information that may be considered of interest for a further analysis.

*Figure E.3: Calculation sheets for Solids (**a**) and for COD (**b**).*

Conclusions

The database application is far from a final tool for the monitoring of anaerobic digesters, not only because many of the features which have been planned have not been implemented yet, but also because thorough debugging has not yet been performed. However, it is believed that such tool, when perfected, could be of great help in managing large experimental trials as it would be a full-scale co-digestion trial. In this respect, i.e. when a large amount of data coming from various sources is to be managed (e.g. clinical trials), databases and not necessarily on Access platforms have proven very robust and efficient.

This Section contains further information on technical aspects encountered during the experimental work: a detailed description of the mineral salts and nutrients medium, which is used to supplement the anaerobic micro-organisms with all elements needed for their metabolism, is reported in Section F.1; the characteristics and the calibration procedure (and results) of the gas-chromatograph that has been employed for the determination of the gas composition are discussed in Section F.2; the two-point titration method to measure alkalinity and volatile fatty acids concentration is outlined in Section F.3; the organic carbon content of a test compound and the composition of the biogas resulting from its anaerobic biodegradation can be theoretically calculated, according to the procedures outlined in Section F.4.

F.1 Nutrients and Mineral Salts medium

Micro-organisms need **nutrients** and **trace elements** (minerals and vitamins) for their metabolism.

Nitrogen, phosphorous, sulphur, calcium, magnesium, iron, cobalt and nickel have proven to be essential to sustain high acetate utilisation rates: e.g. Takashima and Speece (1989) indicate that to prevent a reduction in activity of the biomass, the NH_4-N concentration in the reactor must be maintained in excess of 40 to 70 mg/ℓ. Several nickel or cobalt ion-containing enzymes, such as coenzyme F_{420}, are involved in methanogenesis and therefore it is not surprising that trace metals such as nickel, cobalt and iron are found to be stimulatory to anaerobic processes. Thus an obligate requirement of iron (0.020 mg), cobalt (0.004 mg) and nickel (0.003 mg) per gram of acetate is reported by Speece (1996). The empirical cell formula $C_5H_7O_2NP_{0.06}S_{0.1}$ indicates that there is an obligate requirement for sulphide. Zehnder and co-workers (1980) reported an optimal growth to occur at 0.001 to 1 mg S/ℓ (as sulphide).

There is no general consensus on whether these *growth factors* are readily available and at a sufficient concentration in a sludge; when low concentration of biomass (e.g. 1 g TS/ℓ) are used, it may be necessary to supplement the culture with nutrient and trace elements. Besides, the bioavailability of trace metals may be hindered in the presence of sulphide, carbonate and phosphate ions due to the low solubility of their precipitates (Speece, 1996).

The theoretical minimum COD:N:P ratio is 350:7:1 for highly loaded systems and 1 000:7:1 for lightly loaded systems (Speece, 1996).

The presence of an additional carbon source can also enhance degradation, in co-metabolic processes.

The nutrients and minerals solution (NMS) that was used for the experiments presented in this report, contained trace elements, minerals and vitamins, according to Owen et al. (1979) with some modifications. The stock solutions for the preparation of the medium are presented in Table F.1. Each one is to be prepared in deionised water and stored in a cold room (at 4°C). We have not rigorously quantified their *shelf-life*: however. A safe approach is to prepare relatively small volumes that can be used up within 2 to 3 months after preparation.

Table F.1:Stock solutions for the preparation of the minerals and nutrients medium.

Stock solution	Composition	Concentration (g/ℓ)
S2	Resazurin	5755
S3	$(NH_4)_2HPO_4$	26.7
S4	$CaCl_2 \cdot 2H_2O$	16.7
	NH_4Cl	26.6
	$MgCl_2 \cdot 6H_2O$	120
	KCl	86.7

	$MnCl_2 \cdot 4H_2O$	1	.33
	$CoCl_2 \cdot 6H_2O$	2	
	H_3BO_3	0	.38
	$CuCl_2 \cdot 2H_2O$	0	.18
	$Na_2MoO_4 \cdot 2H_2O$	0	.17
	$ZnCl_2$	0	.14
S5	$FeCl_2 \cdot 4H_2O$	370	
S6	$Na_2S \cdot 9H_2O$	500	
S7	Biotin	0	.002
	Folic acid	0	.002
	Pyridoxine hydrochloride	0	.01
	Riboflavin	0	.005
	Thiamin	0	.005
	Nicotinic acid	0	.005
	Panthotenic acid	0	.005
	p-aminobenzoic acid	0	.005
	Thioctic acid	0	.005

The vitamins *p*-aminobenzoic acid and thioacetic acid were unavailable, therefore were omitted from stock solution S

The method for the preparation of the medium is illustrated hereunder.

Add 1 ℓ of deionised water to a 2 ℓ Pyrex beaker.

Add the following stock solutions: S2 (1.8 mℓ), S3 (5.4 mℓ) and S4 (27.0 mℓ).

Add deionised water up to a volume of 1.8 ℓ.

Boil for 15 min whilst flushing with oxygen-free nitrogen (1 ℓ/min).

Cool to room temperature.

Add the following stock solutions: S7 (18.0 mℓ), S5 (1.8 mℓ) and S6 (1.8 mℓ).

Add sodium bicarbonate ($NaHCO_3$: 8.4 g) in powder form.

Flush with oxygen-free nitrogen until pH stabilises around 7.1.

Autoclave at 121°C, for 30 min.

Store at 4°C until use.

F.2 GAS-CHROMATOGRAPHIC DETERMINATION OF GAS COMPOSITION

The biogas composition was analysed with a GOW MAC 350 gas chromatograph, equipped with a thermal conductivity detector (TCD) which was used to detect nitrogen, methane and carbon dioxide. A Haysep D stainless steel column (L 4 m, ID 2.2 mm, OD 3.2 mm) was used for the separation, with the following conditions:

▪ Conditions for the GOW MAC 350 and TCD:

Column oven: 25 °C

Detector: 25 °C, 100 mA

Filaments: 40 °C

Injection port: 40 °C

The carrier gas was helium at a flow rate of 24.5 mℓ/min The retention times of nitrogen, methane and carbon dioxide were approximately 1.05, 1.39 and 2.30 min respectively. Gas samples were withdrawn from the serum bottles or laboratory-scale reactors by inserting the needle of a gas-lock syringe (100 µℓ) through the butyl rubber septum (GR-2 9.5 mm, Supelco) and withdrawing 40 to 60 µℓ of headspace gas. The peak area was recorded on a Varian 4270 integrator with the attenuation set at 1.

The settings for the integrator were as follows.

Switch power on.

Press the DIALOG key.

Enter a FILE NAME if desired, and then press ENTER.

The batch program is ready to acquire the set of parameters which are needed to interpret the chromatogram and automatically integrate the peak areas. A matrix of (5 x 3) parameters is to be entered. The three parameters indicate the times (TT and TV) and the codes (TF) which identify one of five distinct events. E.g. Should the detector be receiving a signal stronger than the background after 5 min (TT: 5), this would be treated as an error (TF: ER) and the detection period would be terminated (Line 5 in the sequence corresponds to the 'safety' termination).

Press ENTER after each entry, to prompt the next parameter in the sequence.

	TT	TF	TV
Line 1	0.01	AZ	1
Line 2	0.01	CS	0.5
Line 3	0.01	PM	1
Line 4	0.01	AT	1
Line 5	5	ER	5

At the next prompt, press ENTER to exit.

Press ENTER, to END DIALOG.

Press PRINT FILE to display program code

The calibration of the gas chromatograph was performed by using the pure gases (UHP, Afrox Scientific). Gas samples of given volumes were withdrawn by inserting the gas-lock syringe into a

butyl rubber septum positioned on the outlet of the gas bottle. The flow rate on the regulator was set to 16 ℓ/min and 0.35 ℓ/min for carbon dioxide and methane respectively.

Sets of five to six volumes were injected (ranging from 10 to 60 $\mu\ell$), in 3 to 5 replicates.

The detailed calibration procedure was as follows:

▪ Calibration procedure:

Set up the sampling line. Make sure that the integrator and GC settings are correct.

Check the septum seal on the sampling line: as a precaution, the septum should be changed every 30 to 35 injections, to prevent gas leaks through the injection port.

Look at the reading on the pressure gauge and ensure that there is no fluctuation.

Record the ambient temperature and the gauge pressure.

Withdraw a sample of the gas *i* (*i* = N_2, CH_4 and CO_2) of defined volume ($v_{i,j}$, *j* = 10, …, 60 $\mu\ell$): lock the syringe until the point of injection.

Inject the gas sample into the GC and wait for integrator analysis.

Record the retention time (RT) and Area ($A_{i,j}$) of the gas with the highest area.

Repeat Steps 5 to 7, for all the *j* volumes of the first gas, from the lowest to the highest volume.

Replicate Steps 5 to 7, a number of times sufficient to generate a statistically significant set of data for the first gas (five replicates should suffice).

Repeat steps 5 to 9, for the other gases.

The raw data for the GC calibration on methane and on carbon dioxide are reported in Tables F.2 and F.3. The respective calibration curve are plotted in Figures F.1 and F.2: the data points (diamonds) are the average values of each set of replicates (column *Ave*, in the Tables); the solid lines are the linear regressions on the average values (the respective slopes and intercepts are indicated under the Figures, as *calibration factor* and *detection limit*, respectively); the dotted lines represent a sort of confidence intervals for the calibration curve and are calculated for each data point as $\mathrm{Ave} \pm 2 \cdot \mathrm{StDev}$.

As one can see, the determination coefficient of the regression is very high ($R^2 \geq 99.9\%$) and the detection limits very low ($v_0 \leq 5$ $\mu\ell$).

Table F.2: Raw data for the calibration curve of METHANE.

Volume (µℓ)	Area 1st	Area 2nd	Area 3rd	Area 4th	Area 5th	Ave	StDev	VC (%)
10	4183	4150	4495	4269	4343	4288	138	3
20	10557	11244	11047	11380	11320	11110	333	3
30	15299	16226	15939	17284	17316	16413	877	5
40	22471	22565	22609	23659	23800	23021	651	3
50	29938	29481	29579	30229	30209	29887	347	1
60	36069	35810	36004	36624	36724	36246	404	1

Table F.3: Raw data for the calibration curve of CARBON DIOXIDE.

Volume (µℓ)	Area 1st	Area 2nd	Area 3rd	Area 4th	Area 5th	Ave	StDev	VC (%)
10	6196	5755	5763	5269	5421	5681	359	6
20	15015	14192	13920	13866	14088	14216	465	3
30	21677	23511	22056	21035	21687	21993	925	4
40	29753	31234	31949	29617	29354	30381	1142	4

50	41226	40265	38024	37575	37632	38944	1 688	4
60	47783	45063	n.p.	n.p.	n.p.	46424	1 925	4

n.p., not performed; Ave, average; StDev, standard deviation; VC, variation coefficient: $VC = StDev/Ave$

For mass balances purposes, the number of moles of each component (n_i), in a biogas sample, can be calculated from the gas law:

$$P_i \cdot V = n_i \cdot R \cdot T \quad (F.1)$$

where:

P_i is the partial pressure (bar) of each constituent,

V is the total volume (ℓ),

R is the gas coefficient: 0.0821 ℓ·bar/(mol·K),

T is the temperature (K).

The partial pressure of a component is calculated as:

$$P_i = (P_{atm} - P_w) \cdot \chi_i \quad (F.2)$$

where:

P_{atm} is the atmospheric pressure: 1.013 bar,

P_w is the water vapour pressure (bar),

χ_i is the molar fraction of each constituent (mol/mol).

The water vapour pressure was calculated as (Batstone et al., 2002):

$$P_w = 3.225 \cdot 10^9 \cdot e^{0.05393 \cdot T} \quad (F.3)$$

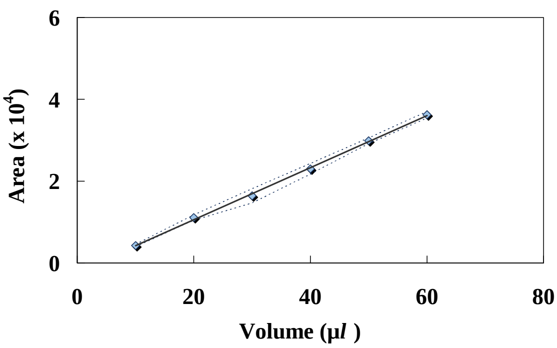


Figure F.1: Calibration curve for methane.

Calibration factor: **636** Area/μℓ
(R^2 99.90 %)

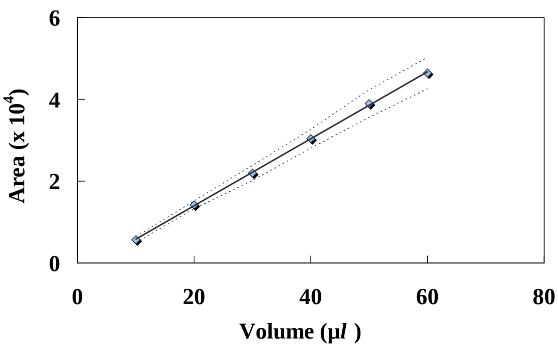


Figure F.2:Calibration curve for carbon dioxide.

Calibration factor: **818** Area/μℓ
(R^2 99.97 %)

Detection limit: **3** µℓ Detection limit: **3** µℓ

F.3 TWO-POINT TITRATION METHOD

A spreadsheet based on the two-point titration method developed by Anderson & Yang (1992) to determine the alkalinity and VFA concentration was implemented to process titration data and is described hereafter.

Input data	Symbol	Units
▪ Volume of the sample of mixed liquor	V	mℓ
▪ Concentration of the titrating solution	c	mol/ℓ
▪ Initial pH	pH_0	-
▪ Initial volume of titrating solution (*)	v_0	mℓ
▪ Volume of titrating solution dosed to reach pH 5.1	v_1	mℓ
▪ Volume of titrating solution dosed to reach pH 3.5	v_2	mℓ

(*) It denotes the volume of acid added to the sample prior to starting the titration, e.g. to inactivate the biological activity.

The following constants will be used:

$H_0 = 10^{-pH_0}$; $H_1 = 10^{-5.1}$; $H_2 = 10^{-3.5}$

$K_1 = 6.6 \cdot 10^{-7}$ $K_2 = 2.4 \cdot 10^{-5}$

Calculate the following:

$$A_i = \frac{H_i - H_0}{K_2 + H_i}; \quad B_i = \frac{H_i - H_0}{K_1 + H_i}; \quad C_i = \frac{v_i \cdot c}{V} \cdot 1000 \qquad (i = 1, 2)$$

Output	Formula	Symbol	Units
▪ Bicarbonate alkalinity	$\frac{C_1 \cdot A_2 - C_2 \cdot A_1}{B_1 \cdot A_2 - B_2 \cdot A_1}$	BAlk	mol/ℓ
▪ Dissociated VFA	$\frac{C_2 \cdot B_1 - C_1 \cdot B_2}{B_1 \cdot A_2 - B_2 \cdot A_1}$	dVFA	mol/ℓ
▪ Total VFA	$dVFA \cdot \frac{H_0 + K_2}{K_2}$	tVFA	mol/ℓ

F.3.1 The accuracy of alkalinity and VFA determination through titration

The equilibrium of the inorganic carbon is the key factor that one must take into consideration when measuring pH; since we used a titration method to determine alkalinity and volatile acids concentration, these estimates too can be affected, if the concentration of carbon dioxide in the liquid varies significantly.

Te problem obviously arises when a sludge sample is withdrawn from the digester and taken to the laboratory to be analysed. Factors that influence the equilibrium are primarily the carbon dioxide partial pressure, the temperature and the mixing conditions and, to a smaller degree, the microbial activity the turbidity of the suspension and the surface area exposed to air.

▪ The sudden drop in the partial pressure of carbon dioxide from the reactor (typically, 0.2 to 0.8 bar) to the environment (0.03 bar) favours the stripping of CO_2 hence the pH raises

▪ The drop in temperature from the operating temperature of 35 °C to ambient temperature opposes/counteracts the stripping of CO_2 hence the pH decreases

▪ Turbulent conditions favour the stripping of CO_2

▪ Microbial activity generates CO_2 within the suspension but this effect is partially counterbalanced by the simultaneous alkalinity generation (aceticlastic methanogenesis)

▪ A high turbidity, i.e. a high solid content of the mixed liquor may hinder the stripping of CO_2

▪ A wide surface area at the liquid/atmosphere interface favours the stripping of CO_2.

The first two factors could be quantified with precision, using the laws of aquatic chemistry, whilst the other may be more difficult to estimate. One would rather try to minimise these potential interferences by:

- minimising the exposure of the sludge sample to the air, e.g. by capping the container until the measurement is completed (if possible)

- performing all determinations which involve pH, at the operating temperature

- limiting or avoiding mixing the sample (if possible)

- using a narrow and deep container, e.g. a long cylinder.

It is beyond the scope of this discussion to analyse this topic in detail; we rather approached this issue from a heuristic/pragmatic point of view.

On a few occasions, we took a sludge sample, split it into two sub-samples (F, NF), immediately filtered one, and then titrated both. Mixing is required for an accurate titration, so we distinguished between a ‘quick’ and a ‘slow’ titration: in the former case, as soon as the sample had reached the titration points (i.e. 5.1 and 3.5), the volume titrated was recorded; in the latter case, after the titration points had been reached, the sample was left and the carbon dioxide allowed to re-equilibrate (the pH response is much faster than the kinetics of gas/liquid exchange) and additional titrant was carefully added until the titration points were passed.

The change in temperature was unavoidable, as the titration apparatus could not be housed inside the temperature controlled room; so temperature was recorded during the procedure.

The results (Figure F.3) seem to indicate that the factors mentioned above do not significantly affect the determination, at least when the entire procedure is completed within 1 h from sampling, the sludge is contained in a cylinder and magnetically stirred at low speed.

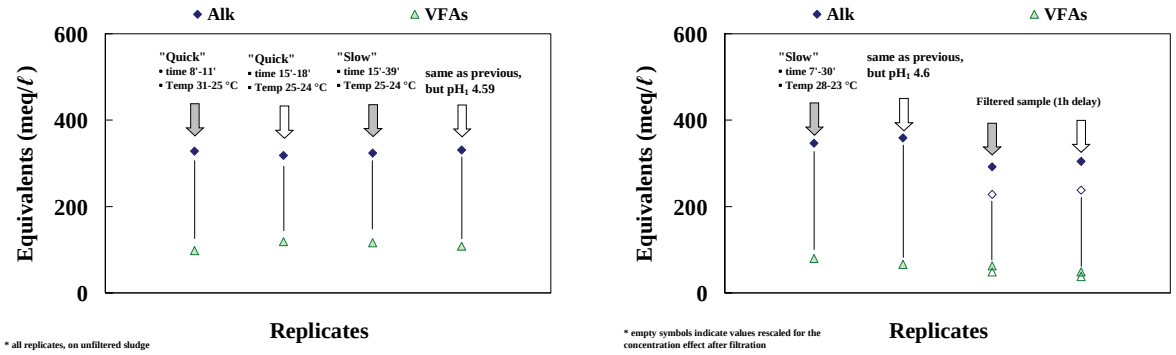

Alk
VFAs
600
400
200
0
Equivalents (meq/ℓ)
"Quick"
▪ time 8'-11'
▪ Temp 31-25 °C
"Quick"
▪ time 15'-18'
▪ Temp 25-24 °C
"Slow"
▪ time 15'-39'
▪ Temp 25-24 °C
same as previous, but pH1 4.59
Replicates
* all replicates, on unfiltered sludge
Alk
VFAs
600
400
200
0
Equivalents (meq/ℓ)
"Slow"
▪ time 7'-30'
▪ Temp 28-23 °C
same as previous, but pH1 4.6
Filtered sample (1h delay)
Replicates
* empty symbols indicate values rescaled for the concentration effect after filtration

Figure F.3 .

F.4 STOICHIOMETRIC CALCULATIONS

F.4.1 Organic carbon content

The theoretical COD of a compound of known chemical composition can be calculated as follows.

$$Cr_2O_7^{2-} + 8H^+ \rightarrow 2Cr^{3+} + 4H_2O + 3O \quad \text{(F.4)}$$

$$C_cH_hO_oN_n + \alpha \cdot Cr_2O_7^{2-} + (8\alpha + n) \cdot H^+ \rightarrow c \cdot CO_2 + \frac{h + 8\alpha - 3n}{2} \cdot H_2O + n \cdot NH_4^+ + 2\alpha \cdot Cr^{3+} \quad \text{(F.5)}$$

where:

$$\alpha = \frac{2}{3}c + \frac{1}{6}h - \frac{1}{3}o - \frac{1}{2}n \quad \text{(F.5a)}$$

$$COD = \frac{3}{2}\alpha \text{ mole } O_2 \quad \text{(F.5b)}$$

This calculation can be used to determine the theoretical COD of the mass balance components.

An example of the calculation is presented and few additional examples summarised in (Table F.4).

An empirical formula for **biomass** is: $C_5H_7O_2N$.

Substituting the values $c = 5$; $h = 7$; $o = 2$; $n = 1$ in equation F.5a, we obtain:

$\alpha = \frac{10}{3}$ mole O_2

And equation F.5b gives the COD equivalent of one mole of biomass:

1 mole $C_5H_7O_2N$ = $\frac{3}{2}\alpha$ = 5 moles of O_2

Considering the formula weight of the biomass (113 g/mol), this translates into 1.416 g COD/g of biomass.

Table F.4 – Stoichiometric calculation of the chemical oxygen demand.

Compound	Formula	Molecular weight (g/mol)	COD equivalent (g COD/g)
Biomass	$C_5H_7O_2N$	113	1.416
Nutrient stock solution	$C_{12}H_{14}O_3N_2$	234	1.709
Acetic acid	CH_3COOH	60	1.067
Ethanol	C_2H_5OH	46	2.087

F.4.2 Biogas composition

For monitoring anaerobic digestion, the methane-to-carbon dioxide ratio represents a rapid and sensitive parameter: any sudden marked change in this ratio is indicative of unbalanced conditions.

The volume of biogas produced per unit mass of volatile solids destroyed is an important and sensitive process control indicator to assess the activity of the micro-organisms and the progress of digestion.

The earliest definition of the stoichiometry of anaerobic digestion was that of Buswell and Müller (1952), which provides a good approximation of the biogas composition from a waste of known chemical composition:

$$C_cH_hO_o + \left(c - \frac{h}{4} - \frac{o}{2}\right) \cdot H_2O \rightarrow \left(\frac{c}{2} - \frac{h}{8} + \frac{o}{4}\right) \cdot CO_2 + \left(\frac{c}{2} + \frac{h}{8} - \frac{o}{4}\right) \cdot CH_4 \qquad (F.6)$$

The equation reflects algebraically that the higher the oxidation state of the carbon in the organic substrate, the lower the proportion of methane in the biogas (Figure F.4).

An example of the calculation is presented hereafter.

The formula for **starch (saccharide) subunits** is $C_6H_{10}O_5$, which gives the following values of the constants in equation F.6:

$c = 6;\ h = 10;\ o = 5$

which in turns results in the theoretical gas composition of:

$2 \cdot CO_2 : 2 \cdot CH_4$

This implies that the biogas produced from the anaerobic degradation of starch will be a 1:1 mixture of methane and carbon dioxide.

McCarty (1964) advanced this equation more comprehensively, as follows:

$$C_cH_hO_oN_n + \left(2c + n - o - \frac{9sd}{20} - \frac{ed}{4}\right) \cdot H_2O \rightarrow$$

$$\left(c - n - \frac{sd}{5} - \frac{de}{8}\right) \cdot CO_2 + \frac{de}{8} \cdot CH_4 + \frac{sd}{20} \cdot C_5H_7O_2N + \left(n - \frac{sd}{20}\right) \cdot \left(NH_4^+ + HCO_3^-\right) \qquad (F.7)$$

where:

$d = 4c + h - 2on - 3n$

s and e indicate the fraction of COD synthesised and converted to methane respectively.

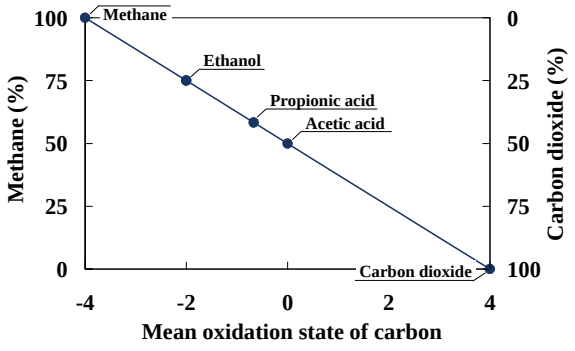


Figure F.4: The theoretical composition of the biogas is a function of the mean oxidation state of carbon in the substrate (adapted from: Gujer & Zehnder, 1983)

F.5 REFERENCES

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Class	Number	Item
Instruments		
	1	pH meter
	1	gas chromatograph and integrator
	1	pH titrator and computer
	1	pH-stat titrator (biosensor)
	1	mass balance (up to 5 kg)
	1	mass balance (up to 1 000 g)
Laboratory equipment		
	1	cold room
	1	temperature-controlled room
	1	incubator
	1	autoclave
	1	COD bench
	3	automated laboratory-scale anaerobic digesters
	1	oven
	1	Muffle furnace
	1	Centrifuge (what RCF range?)
	1	water bath
Small equipment		
	5	gas-tight syringes
	200	150 ml serum bottles
	1	crimping tool
	1 000	aluminium / butyl crimps
	3	gas bags
	filters	100 mm diameter glass (sinter, fibre-mat?)

APPENDIX H Staffing

This Annexure describes the proposed staffing requirements for a co-digestion system monitoring and management facility.

The proposed staffing is outlined in Table H.1

Table H.1: Staffing requirements

Number	Proportion[1]	Qualifications	Experience
1	0.5	MSc or MScEng	anaerobic digestion processes
1	1.0	BSc or BTech	analytical chemistry / microbiology
1	1.0	matric / HNC / in-service trainee	general laboratory procedures
1	1.0	matric	sampler and cleaner

[1] Proportion of working hours dedicated to anaerobic co-digestion unit