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IMPROVING THE RATE OF DEGRADATION OF SOLIDS FROM VIP LATRINES

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List of Acronyms:

BNMS:	Basal Nutrient Mineral Solution
COD:	Chemical Oxygen Demand
DWAF:	Department of Water Affairs and Forestry
EMMA:	eThekwini Municipality Metropolitan Area
FAS:	Ferrous Ammonium Sulphate
JMP:	Joint Monitoring Program
MDG:	Millennium Declaration Goals
RDCOD:	Readily Biodegradable COD
STP:	Standard Temperature Pressure
TS:	Total Solids
UD:	Urine Diversion
UN:	United Nation
UNICEF:	United Nations International Children's Emergency Fund
UKZN:	University of KwaZulu-Natal
VIP:	Ventilated Improved Pit
VS:	Volatile Solids
WHO:	World Health Organisation

List of Symbols:

A_T	Total alkalinity	(mg $CaCO_3.l^{-1}$)
α	Milliéquivalent of oxygène	8000
m	Mass of empty crucible	(g)
M	Morality if FAS	(M)
$M_{25°C}$	Mass of sample at 25°C (g)	(g)
$M_{105°C}$	mass of sample and crucible after evaporation at 105°C overnight (g);	(g)
$M_{550°C}$	Mass of sample and crucible after drying in a furnace at 550°C for 2 h (g).	(g)
μ	Mean (true)	
n	Number of sample	
N_a	Normality of H_2SO_4	(N)
s	Standard deviation	
S_A	Fraction of volatil acids	(%)
S_F	Fraction readily fermentable	(%)
S_i	Fraction non biodégradable soluble	(%)
t_a	Student parameter	
X_H	Fraction of biomass	(%)
X_i	Fraction non biodégradable particulate	(%)
X_S	Fraction slowly biodégradable	(%)
V	Volume of sample	(ml)
V_a	Volume of H_2SO_4	(ml)
V_B	Volume of FAS used to titrate blanks	(ml)
V_S	Volume of FAS used to titrate sample	(ml)
$\bar{x}$	Estimated mean	

Chapter 1: Introduction

This chapter assesses the global water and sanitation situation and challenges and then focuses on the eThekwini area in order to understand the setting of this project.

1.1. Context

Safe drinking water and sanitation are not only the basic necessities for human life but are also fundamental to reduce diseases, mortality and inequalities. However, according to the report *Meeting the MDG Drinking Water and Sanitation Target: The Urban and Rural Challenge of the Decade* produced by the World Health Organisation (WHO) and United Nations International Children's Emergency Fund (UNICEF) Joint Monitoring Program for Water Supply and Sanitation (JMP) in 2006, over 1.1 billion people (18 % of the world population) did not have access to an improved source of water and in 2004 some 2.6 billion (more than 40 % of the world population) lacked basic sanitation. The coupled effect of unsafe water with a lack of basic sanitation kills at least 1.6 million children under the age of 5 years.

At the United Nation Millennium Summit in September 2000, 189 countries adopted the UN Millennium Declaration in order to solve the world's main development challenges by 2015. The Declaration consists of 8 Millennium Development Goals (MDGs) which are to fight poverty and hunger (**Goal 1**), achieved universal primary education (**Goal 2**), promote gender equality and women empowerment (**Goal 3**), reduce child mortality (**Goal 4**), improve maternal health (**Goal 5**), eradicate HIV/AIDS and Malaria (**Goal 6**), ensure environmental sustainability (**Goal 7**) and develop global partnerships (**Goal 8**). The MDG drinking water and sanitation target (**Goal 7** target 10) aims to reduce by half the proportion of people who have not access to safe drinking water and basic sanitation in 2015 that is to say, provide safe water to 1.1 billion of people and sanitation to 1.6 billion of people.

There are huge disparities between rural and urban areas. In 2004, 84 % of the rural population did not have access to an improved source of drinking water whereas more than 95 % of the population in urban areas are covered; more than three out of every five rural people did not have access to a basic sanitation whereas 90 % of urban areas are covered. This implies that a greater effort must be made in rural areas while maintaining the same level of services in urban areas which continue to experience a migration input. The overall population is increasing which means that in 2015 an increased number of people will need

access to drinking water and sanitation and it is estimated that the unserved (after meeting the Millennium Goals) will number over 900 million for safe water and 1.7 billion for sanitation.

In South Africa, water is recognized as human right. After the Apartheid regime, when the first non-racial democratic government took power in April 1994, 15.2 million people had no access to safe water and 20.5 million people lacked basic sanitation out of a total population of 40 million people. Before 1994, some initiatives had been taken to standardise the water supply and sanitation policies of local governments in the four provinces. The national policies are managed by the Department of Water Affairs and Forestry (DWAF) which is responsible for providing equitable access to water supply and sanitation. The ... *access to sufficient water*... and ... *an environment not harmful to human health*... are constitutional rights. These rights are expanded through the Water Services Act 108 of 1997. To ensure these rights and to avoid the affordability problem, a stepped block tariff is recommended for each category of user and free basic water is provided (6 000 l/household/month). The government, helped by development partners, has supplied, through the national water and sanitation programme, safe drinking water to some 16 million people and sanitation to more than 9 million people between 1994 and 2006 (Water & Sanitation Africa January/February 2007). This progress is the fastest in Africa but it depends of the national economy being sufficiently robust to afford the necessary subsidies. The initial emphasis was on water supply but an outbreak of cholera in 2000 emphasised the need for sanitation services. According to the DWAF data for 2006 (April 2006), 11.8 million South Africans still do not have access to free basic water services and more than 15 million people lacked sanitation services out of a population of 49 million people. The country is currently working to reach the government's goals which are to provide basic water to all by 2008 and basic sanitation to all by 2010.

The eThekwini Municipality Metropolitan Area (EMMA) is located on the eastern seaboard of South Africa within the province of KwaZulu-Natal and covers an area of 2 297 square kilometres with an estimated population (2004) of just over 3 million people. In July 2004, according to the eThekwini Water and Sanitation unit, there were still 74 680 households without a water supply and 187 419 households without basic sanitation. These households are mainly located in peri-urban and existing informal settlements areas. The free basic sanitation strategy is being developed to complement the free basic water strategy for the poorest category of people (indigent). eThekwini Municipality also plans to provide around 16 000 houses with water and sanitation services per annum. Based on the prevention principle, maintenance is a key factor to avoid that infrastructures will become a future

financial burden. The routine operation and maintenance costs of a basic level of sanitation service are low, and households themselves are best placed to address these (Draft White Paper on Water Services, 2002). The intermittent costs of pit emptying may be significant. Municipalities should develop clear policies on what assistance, if any, they will provide. In general the municipality chose to install Urine Diversion (UD) toilets instead of Ventilated Improved Pit (VIP) toilets due to the cost of emptying pits.

There are a total of about 100 000 VIP and pit latrines in the eThekwini Municipal area. The majority of these were built in areas before their consolidation into the expanded municipal area. The design filling time of a pit latrine is 7 years. Many of the pits have reached the end of their service life. The design of the government approved VIP has a permanent top structure which means it can not be relocated when the pit is full. Provision has to be made in the design of the structure for emptying. A further problem is that in some settlements (e.g. Besters Camp) the houses have been extended so that the area is so dense that there is no place to relocate the pits.

This cost is all the more important that pits are located in inaccessible areas. According to Gounden (eThekwini Municipality project manager), the municipality will have to spend R 70 million to empty 30 000 pits that need emptying over the next 3 years.

A way to reduce the emptying frequency is thus a major stake for the municipality. It is in this context that the Pollution Research Group of the University of KwaZulu-Natal (UKZN) has been commissioned to undertake this study.

1.2. What is a basic water and sanitation service?

The Millennium Joint Monitoring Programme (JMP) defines safe drinking water as water with microbial, chemical and physical characteristics that meet WHO guidelines or national standards. The source should be less than 1 km from its place of use and provide at least 20 litres per person per day. eThekwini Water and Sanitation unit uses more restrictive criteria to define the basic level. The criteria are different if households are situated in rural, non rural formal or non rural informal area but basically, the water supply must be less than 200 metres from households. The restrictive criteria results in an increased number of people who do not receive an adequate level of service.

The JMP defines the basic sanitation as the lowest-cost technology ensuring hygienic excreta and a clean and healthful living environment both at home and in the neighbourhood of users. The definition implies that there is not a unique sanitation model but sanitations in function of

the country, area, etc. In eThekwini Municipality Metropolitan Area the specific sanitation system depends on the locality of the households .In rural areas, households must have a toilet which is at least VIP standard. In non rural formal areas, households must either have sewage reticulation within 100 metres of the property boundary or have adequate sanitation in the form of a septic tank, conservancy tank, package plant or similar. Finally, for non rural informal areas, it is define as households which can easily access a communal toilet block.

Dry toilets are the more appropriate solution to face the lack sanitation in peri-urban and rural areas with low density (Mara, 1996) where water supply is also deficient. They do not waste potable water as with traditional waterborne sewage systems, they are environmentally friendly and inexpensive compared to traditional flush toilets. Peri-urban and rural areas constitute the main challenge for the eThekwini Municipality. A basic package of sanitation and water in the form of dry toilets (UD toilets) and 200 litre yard tanks are providing by the authorities to these communities.

1.3. Dry toilets

There are several dry on-site sanitation options available but the basic one is the ventilated improved pit latrine the concept can be modified into a urine diversion toilet.

1.3.1. Ventilated improved pit latrines

Ventilated improved pit (VIP) latrines are improvements to a pit latrine to minimize odours and flies. They are recognized as a minimum level of acceptable sanitation in South Africa. VIPs are composed of a pit into which excreta and anal cleansing material are deposited. The pit is sealed with a slab which is provided of a hole or a seat. A superstructure is built (mainly in bricks) to offer privacy to users and to prevent flies. A ventilation pipe with fly screen is then added from the pit to at least 500 mm above the superstructure to allow the gas circulation. The pipe is painted in black to increase the temperature thus promoting the convection of air from the pit. The gas circulation is facilitated by the presence of a gap (3 times the cross-section of the pipe) usually above the door. The overall design is shown in the Figure 1.

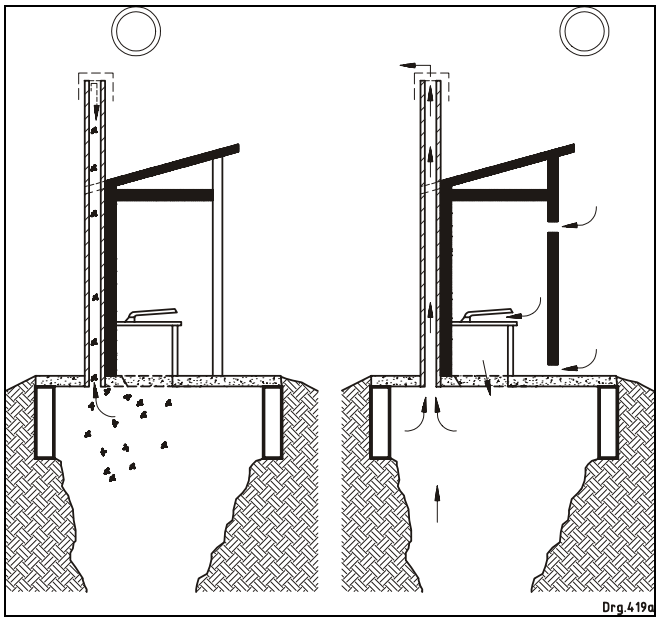

Figure 1: Fly control and air (source: SANS 10365-1:2004)

The liquid fraction (urine, water) infiltrates into the surrounding soil. The solid fraction (human excreta) is mainly decomposed under anaerobic conditions by producing biogas (see section 2.1). The decomposition rate is lower than the inflow of human excreta which leads to an excreta accumulation. Increase the decomposition velocity is a key factor to balance the system and avoid an accumulation. The higher accumulation rate the more frequent the need to empty the pits. Increase the decomposition rate is the aim of this study in order to decrease the accumulation and thus the emptying cost for the existing pits.

1.3.2. Urine diversion toilets

Urine diversion (UD) toilets are just a particular design of VIP toilets where urine and faeces are separated. It has been shown at the UKZN experimental site that faecal material can be used as a soil additive to improve plant growth and to produce specific food crops which are safe for human consumption (WIN-SA 2).

Chapter 2: Literature Survey

It is widely understood that the anaerobic digestion is the main degradation process which occurs in pit latrines (Chaggu, 2004). The aerobic digestion which takes place at the interface air/faeces will not be described in this report.

In this section, the anaerobic digestion theory will be explained (section 2.1) followed by the serum bottle tests principle (section 2.2) and the parameters which characterize the raw material (section 2.3).

2.1. Anaerobic digestion

The biodegradable fraction of the waste is principally composed of polymeric substances such as carbohydrates, proteins and lipids. These compounds are converted to methane, carbon dioxide and water by anaerobic or facultative anaerobic bacteria in an oxygen depleted or oxygen free environment, (Anderson et al., 2003). The overall process is explained in the next section and a short description of parameters influencing the biogas production rate will given.

2.1.1. General model for anaerobic digestion

The anaerobic digestion is composed of four major steps which are hydrolysis, acidogenesis, acetogenesis and methanogenesis (Lubberding, 1998). During the *hydrolysis* (first step);the hydrolytic bacteria hydrolyze polymeric substances to mono-saccharides, amino acids and long-chain fatty acids by action of extracellular enzymes after the disintegration of the complex particulate into smaller units. Next, the hydrolysis products (mono-saccharides and amino acids) are fermented during the *acidogenesis* (second step) by producing simple organic acids such as acetic, butyric, propionic, valeric, lactic acids but also hydrogen gas. These volatile fatty acids lower the pH of the medium since they dissociate, releasing H^+ ions, (Anderson et al., 2003). Long-chain fatty acids are oxidised which leads to the production of acetate and hydrogen gas (anaerobic oxidation). Short-chain fatty acids longer than acetic acid are oxidized by the hydrogen-producing acetogenic bacteria, resulting in the formation of hydrogen, acetate and carbon dioxide. The end products (acetate, hydrogen, carbon dioxide) from *acetogenesis* (third step) are transformed into methane by the methane-producing micro - organisms (*methanogenesis,* fourth step*).* Methane can be either converted from acetate by acetoclastic methanogenic micro-organisms with carbon dioxide production or from hydrogen and carbon dioxide by hydrogenotrophic methanogenic micro-organisms.

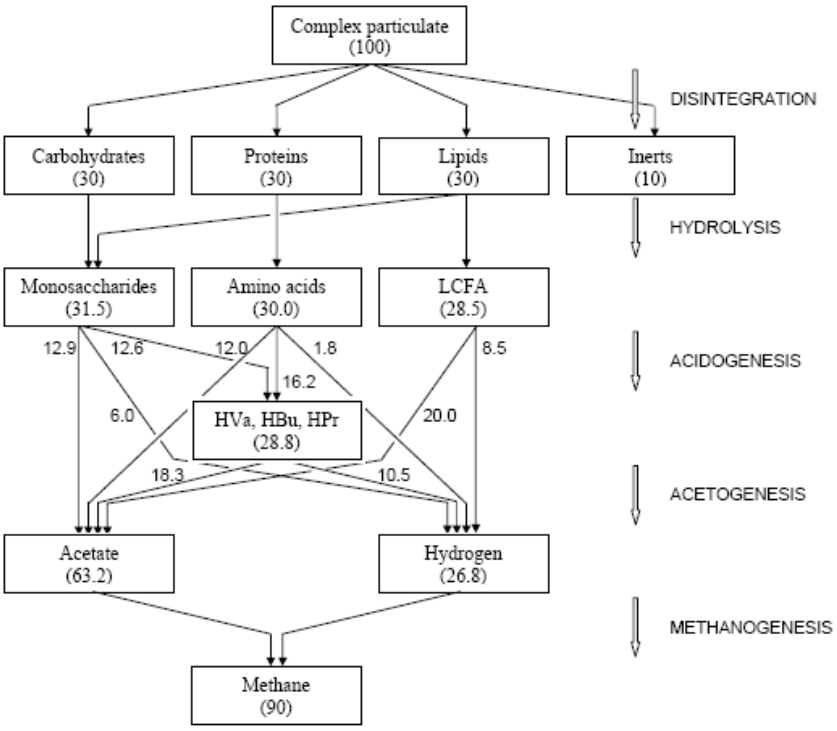

Figure 2: Flow-diagram for the anaerobic degradation of a composite particulate material. Valerate (HVa), Butyrate (HBu) and Propionate (HPr) are grouped for simplicity. Figures indicate COD fractions (Batstone et al 2002)

The Batstone model (Figure 2) gives an overview of the anaerobic transformation of organic material but to have a more adequate model, other groups of micro-organisms must be considered. Indeed, homoacetogenic micro-organisms degrade hydrogen and carbon dioxide producing acetate as the only fermentation end-product. Acetate oxidizing bacteria perform the opposite reaction by oxidizing acetate to hydrogen and carbon dioxide. And, acid synthesizing bacteria can reverse the transformation of short chain fatty acids when the concentration of hydrogen and acetate or ethanol is high.

2.1.2. Influent parameters

Temperature: Most experiments with anaerobic digestion have been done in the mesophilic (30 to 40°C) and in the thermophilic (50 to 60°C) temperature range. The rate of microbial processes increases with temperature (within limits). In addition at higher temperatures the substrate has a lower viscosity and the diffusion of dissolved material greater.

pH: Methane production is governed by the pH value as it affects the growth of the micro-organisms. The anaerobic process works best near neutrality, as methanogens have an optimum pH range from 6.5 to 7.6

(Rittmann and McCarty, 2001). A lower pH value (pH 5.0 to 6.0) is optimal for acid-forming bacteria. The pH value influences the degree of dissociation of weak acids and weak bases. The decrease in pH by the accumulation of hydrogen and/or volatile fatty acids causes the inhibition of methanogenesis and stops the degradation process at the acidogenesis stage (Anderson et al., 2003).

Nutrients: The main elements in organic compounds are hydrogen, nitrogen (provided by ammonia), oxygen and carbon. Other elements, such as sulphur (provided by sulphide), phosphorus, calcium, magnesium and iron are also necessary but are required in small concentrations to avoid inhibitory effects.

2.2. Serum bottle tests principle

Serum bottles (125 ml) are small-scale batch tests widely used to study the anaerobic biodegradability. These inexpensive and simple tests provide a good reproducibility of the results. However, some parameters must be determined in order to make sense (mass balance calculation) and avoid any misunderstanding during the results interpretation. The serum bottle tests principle will be described in this part followed by the key parameters [chemical oxygen demand (COD), pH, total solid (TS), volatile solids (VS), ash content, osmotic pressure, Alkalinity] used to characterise the substrate (section 2.3).

An inoculum (sludge), a substrate and a Basal Nutrient Mineral Solution (BNMS) are introduced into serum bottles at the working temperature before flushing with CO_2/N_2 gas mixture (50/50) and sealing them with a rubber septum and an aluminium crimp cap. The bottles are then stored in a thermostatically controlled room at 37°C in the dark. The pressure in the headspace is re-equilibrated after 2 h.

The gas volume is measured with a graduated glass syringe lubricated with distilled water by holding it horizontally to minimize the effect of the weight of the plunger. The gas composition is then analysed with a gas chromatography when the gas production is sufficient (100 µl). Otherwise, the gas is re-injected into the bottle. The gas sample volume is recorded and taken into account in subsequent calculations as well as temperature and atmospheric pressure. The frequency of these measures is obviously a function of the reaction progress.

Controls must be included to quantify the baseline gas production from the inoculum as shown below in Table 1 and all the bottles are replicated in triplicate.

Table 1: Set-up of the serum bottles assays (WRC Report No 1074/1/06)

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	To asses the biodegradability of the substrate and possible influence of substrate concentration

2.3. Characterisation of the substrate

2.3.1. Chemical oxygen demand

The total organic matter content is measured as chemical oxygen demand (COD). The total COD can be divided into several fractions to provide a more complete of picture of the effluent. The wastewater fractionation of Wentzel et al, (1995) and Orhon and Cokgör, (1997) (see Figure 3) permits a better understanding and interpretation of the gas production results.

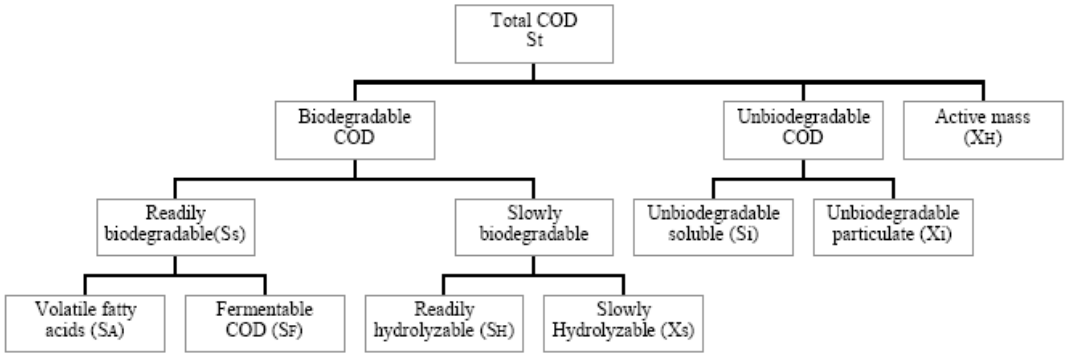

Figure 3: Division of influent COD in its component fractions (Wentzel et al, 1995; Orhon et Cokör, 1997)

The total COD is fractioned into total biodegradable COD and total inert COD which leads to an overestimation of the gas production if the total COD were to be taken into account. The total biodegradable COD is then sub-divided into three fractions: readily biodegradable, rapidly hydrolysable and slowly hydrolysable. The use of the slowly hydrolysable fractions lead to an over estimation of gas production during tests under laboratory time scales because the serum bottle test is for a short period of time (up to 3 months) whilst the solids in VIP may be present for up to 7 years between an eThekwini emptying cycle. Typical ranges for the domestic wastewater fractions are given by Henze et al., (1995) (see Table 2).

Table 2: Typical ranges for wastewater fraction (from Henze et al, 1995)

Symbol	Fraction	% of total COD
S_F	Readily biodegradable fermentable fraction	10 to 20
S_A	Volatile acids (acetate)	2 to 10
Si	Inert, non-biodegradable soluble	5 to 10
Xi	Inert, non-biodegradable particulate	10 to 15
X_S	Slowly biodegradable fraction	30 to 60
X_H	Heterotrophic biomass	5 to 15

From Table 2, if it is considered that the slowly biodegradable fraction is not transformed into biogas and biomass, then only a range from 12 to 30 % of total COD (fermentable and volatile acid fraction which compose the readily biodegradable fraction) is used in the anaerobic digestion process. This soluble fraction consists of small biodegradable particles whereas rapidly and slowly hydrolysable fractions consist of larger particles (Wentzel et al, 1999). The size of particles is then a key factor in the response of biomass and consequently in the production of gas. Since the readily biodegradable COD (RBCOD) is modelled as simple, soluble compounds, the samples are filtered through 0.45 µm filters in order to obtain the RBCOD fraction. This method leads to an overestimation of the RBCOD because a small fraction of the slowly biodegradable fraction passes through the filter (Dold et al., 1980). However, this method will be used in following experiments to assess to the RBCOD which should approximately represent from 12 to 30 % of the total COD (WRC Report No. 820/1/00).

2.3.2. pH

The anaerobic digestion process is limited to a pH range from 6.5 to 7.8 (see section 2.1.2). Outside this range, the process is inhibited. The initial pH value of the substrate provides an estimate of its buffer capacity and of its possible degradation. If the pH value is close to or within this range, an acclimatization period will be necessary for micro-organisms but if the pH value is too far from this range then the microbial growth is not possible. An initial alkaline pH value will balance the pH drop during the initial acidogenesis step of the anaerobic digestion.

2.3.3. Total solid

The total solids (TS) are the total amount of organic and inorganic material in a sample which remains after being dried at 105° C. By difference the amount of water in a sample can be estimated.

2.3.4. Volatile solids and ash

The volatile solid (VS) content of a sample describes the content of organic material in the sample and is also often used as a measurement of the biomass concentration. It is determined by loss of weight after ignition of the total solids sample in a furnace at 550° C.

Ash content describes the content of inorganic material in the sample. It is determined by the residual mass after ignition in a furnace at 550° C

2.3.5. Osmotic pressure or water activity

The osmotic pressure of a solution is a direct measure of the activity of water in the solution. A low water activity corresponds to a high osmotic pressure.

The osmotic pressure is the pressure difference between two cells containing liquid mixtures with different concentrations, separated by a membrane that is permeable to some of the species and impermeable to others, needed to maintain thermodynamic equilibrium (Sandley, 1999). Osmosis is the movement of water across a semi-permeable membrane (cell membrane for bacteria) due to the difference in solute concentrations on either side of the membrane. Most bacteria are capable of growing in a wide range of salinity by maintaining a relatively constant internal salt concentration. If the salt concentration outside the cell is too high, then water is lost from the cell and growth is inhibited. Sugars and other substances also influence osmotic relationships between cells and their environment. A micro-organism has to expend energy in order to transfer chemicals (substrates or products) up an osmotic gradient.

2.3.6. Alkalinity

The alkalinity is a simple parameter to characterize the capacity of a solution to accept acid inputs without becoming “too acidic” (Benjamin, 2002). Alkalinity [A_T] is a measure of the ability of a solution to neutralize acids to the equivalence points of the carbonate or bicarbonate ion.

$$A_T = [HCO_3^-]_T + 2[CO_3^{-2}]_T + [B(OH)_4^-]_T + [OH^-]_T + 2[PO_4^{-3}]_T + [HPO_4^{-2}]_T + [SiO(OH)_3^-]_T - [H^+] - [HSO_4^-] \quad \textbf{(eq 2.1)}$$

(Subscript T indicates the total concentration of the species in the solution as measured)

Alkalinity can be measured by titrating a sample with a strong acid until a pH value of 4.5. At this point, all the bases of interest have been protonated to the zero level species; hence they no longer cause alkalinity.

Chapter 3: Preliminary Considerations

In order to build a coherent and reasonable experimental plan some additional considerations have to be taken into account. This chapter provides an over view of the alkalinity effect on anaerobic digestion of faeces (section 3.1). It includes preliminary calculations of the amount of $NaHCO_3$ required to improve the biodegradation rate by providing buffer capacity (section 3.2). The theoretical gas production is calculated based on the Buswell model (section 3.3).

3.1. The alkalinity effect on faeces anaerobic digestion

An earlier investigation (Naidoo, 2005) revealed that the addition of alkalinity enhances the degradation process and that without additional alkalinity samples of faecal material alone, and samples containing faeces and a small amount of anaerobic inoculum were unable to convert most organic material in the sample to methane gas within the time frame of the experiment (30 d). A further conclusion from these tests was that the addition of micro-organisms (sludge) does not significantly improve the rate or extent of degradation.

3.2. Preliminary calculations of $NaHCO_3$ amounts needed

According to the Figure 2, for every 100 moles of COD from complex particulate, 44.9 moles of COD are transformed into acetate. Knowing that 1 mole of acetate is equal to 2 moles of COD, 100 moles of COD produce 22.5 (44.9/2) moles of acetate.

In the same way, 28.8 moles of COD are transformed into simple acids (HVa, HBu, HPr) from the 100 moles of COD of the complex particulate. Thus, as 1 mole of propionate is equal to 3.5 moles of COD, 8.2 (28.8/3.5) moles of propionate are formed. Finally, 30.7 (22.5 + 8.2) moles of acids are produced from 100 moles of COD of substrate thus according to the stoichiometry a maximum of 30.7 moles of $NaHCO_3$ are required per 100 moles of COD of substrate.

In the bottles, ca 3 g of COD were introduced, which is equivalent to 0.09 (3/32) mole of COD thus the maximum amount of $NaHCO_3$ that needed to be added was 0.029 (30/100*3/32) mole per bottle or also 2.4 ($0.029*M_{NaHCO3}$) g of $NaHCO_3$ per bottle.

However the quantities of $NaHCO_3$ must comply with the pH constraint. It is important for methanogenesis for the pH to be below a value of 8. A concentration value of 6.10^{-3} M or 0.5 g/l was chosen to comply with this constraint.

The experiments were based on these considerations it was realised that this is an overestimation because the calculations have been based on the total COD. According to the WRC Report No. 820/1/00, the RDCOD represents around from 12 to 30 % of the total COD.

3.3. Biogas production

If the composition of the organic material is known and all the material is converted to biogas, the theoretical methane yield potential can be calculated with the Buswell equation.

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right)H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right)CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right)CO_2 \qquad \textbf{(eq 3.1)}$$

And the theoretical methane yield is given by the following relation:

$$B_{o,th} = \frac{\left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right)22.4}{\left(n + \frac{a}{4} - \frac{b}{2}\right)32} \quad \left(STP\frac{l\ CH_4}{g\ COD}\right) \qquad \textbf{(eq 3.2)}$$

The Table 3 provides the methane production from different substrates.

Table 3: Characteristic of a number of typical organic materials suitable for anaerobic digestion (from Angelidaki lecture)

Substrate Type	Composition	COD/VS g COD/g VS	CH_4 yield STD l/g VS	CH_4 yield STD l/g COD	CH_4 %
Carbohydrate	$(C_6H_{10}O_5)n$	1.19	0.415	0.35	50
Proteins	$C_5H_7NO_2$	1.42	0.496	0.35	50
Lipids	$C_{57}H_{104}O_6$	2.90	1.014	0.35	70
Ethanol	C_2H_6O	2.09	0.730	0.35	75
Acetate	$C_2H_4O_2$	1.07	0.373	0.35	50
Propionate	$C_3H_6O_2$	1.51	0.530	0.35	58

By using Table 3 and assuming that the main compounds in VIP latrines are carbohydrates, proteins and lipids, we obtain a CH_4 yield of 0.35 STPl/g COD. The methane represents ca 50 % of the gas production thus the total volume of gas produced is ca 0.70 STPl/g COD. By considering only the RDCOD, thus a gas production range comprise between 0.084 and 0.21 STPl/g COD can be expected.

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Chapter 4: Materials and Methods

This section describes the protocol for studying the combined effect of moisture and alkalinity. The first part describes the experimental plan for the modified serum bottle test (sections 4.1 & 4.2) followed by characterisation of the substrate (section 4.3). A summary of the tests is provided in the last section (section 4.5).

Caution, the general serum bottle tests was modified to study the moisture effect.

4.1. Experimental plan

A factorial matrix with two factors (moisture and alkalinity) and six levels (from 0 to 25 ml of water for the moisture and from 0 to 2.5 mg $NaHCO_3$ for the alkalinity were added per bottle) was built. Thus, $6^2 = 36$ experiments were required and were conducted in triplicate. The experimental plan relative to the experimental matrix was describe in section 4.5.

Note 1: the water quantities were estimated.

Note 2: additional experiments werre added for controls (see part 4.5).

Note 3: additional bottles were included so that they could be sacrificed part way through the experiment in order to determine parameters such as COD, pH, alkalinity, gas composition (see part 4.5)

4.2. Modified serum bottle tests

In order to study the combined effect of moisture and alkalinity, some modifications to the serum bottle were necessary. If basal nutrient medium solution is introduced into the serum bottles and water is then added, the first levels of the test (0 ml of water) for the moisture cannot be performed. It was consequentially assumed that the substrate (VIP content) is rich enough in nutrients for the growth of micro-organisms and thus basal nutrient medium solution is not necessary (**Assumption 1**). In an analogous manner it was assumed that the VIP contents contain a sufficient concentration of micro-organisms thus the addition of an inoculum is not necessary for the (**Assumption 2**).

$NaHCO_3$ was used in preference to Na_2CO_3 as it produces solutions with a pH value closer to the optimum pH range for most methanogens. $NaHCO_3$ was be added as a liquid due to the small quantities required. However the first level (0 ml of water) could not be achieved because of this technical restriction.

4.3. Protocol for substrate characterisation

4.3.1. Chemical oxygen demand

The total COD was determined according to the Standard Method 5220 (APHA, AWWA and WEF, 1998). A 10 g sample of VIP solids was first diluted with distilled water in a 1 l volumetric flask and homogenised well. The VIP solids were further diluted by transferring a 100 ml aliquot to a 500 ml volumetric flask and diluting to the mark with distilled water. A 10 ml aliquot of the diluted VIP waste was then transferred into an Erlenmeyer flask with the aid of a pipette. Eight glass beads, 0.04 g of mercuric sulphate, 5 ml of potassium dichromate (0.25 M) and 15 ml of 98 % reagent grade sulphuric acid were then added to the Erlenmeyer flask. The same ingredients were added to an Erlenmeyer flask containing 10 ml of distilled water (blank). All the Erlenmeyer flasks were replicated at least three times, placed in a reflux apparatus and heated on a hot plate for 2 h. After cooling, 80 ml of distilled water and 3 drops of Ferroin indicator were added to each flask. The solution was titrated with a solution of standardized ferrous ammonium sulphate (ca 0.25 M).

The RBCOD was determined as described above by using the filtrate of a 10 g of sample (diluted with distilled water in a 1 l volumetric flask) which had been filtered through a 0.45 μm membrane filter.

The concentration of ferrous ammonium sulphate (FAS) was determined by titration prior to every batch of analyses as it is not stable. A 5 ml aliquot of potassium dichromate (0.25 M) was diluted with distilled water in a 50 ml volumetric flask. A 50 ml aliquot was then placed in an Erlenmeyer flask to which 15 ml of concentrated sulphuric acid was added. After cooling, two drops of Ferroin indicator were added and the solution was titrated with the FAS solution.

The COD is calculated equation (4.1):

$$COD = \frac{(V_B - V_S)M}{V}\alpha \quad (mg\ O_2 / l) \qquad \textbf{(eq 4.1)}$$

Where:

V_B and V_S are the volumes of FAS (ml) used to titrate the blank and samples respectively;

M = molarity of FAS (M);

V = volume of sample (ml);

α = 8 000 (milliequivalent weight of oxygen x 1 000 ml/l).

4.3.2. pH

The pH was measured with a pH electrode previously calibrated against two buffer solutions.

4.3.3. Total solids

The total solids (TS) were determined according to the Standard Method 2540 (APHA, AWWA and WEF, 1998). A 25 to 30 g sample was weighed and placed into a cool dry crucible (previously weighed). The crucible containing the sample was then placed overnight in an oven at a temperature of 105°C. After cooling in a dessicator, the crucible was weighed. The total solids represent the difference of the weights before and after drying at 105°C. The calculation to determine the TS is given in equation 4.2:

$$\%TS = \frac{M_{105°C} - m}{M_{25°C}} \qquad \textbf{(eq 4.2)}$$

$$\%moisturecontent = \frac{M_{25°C} - M_{105°C} + m}{M_{25°C}} \qquad \textbf{(eq 4.3)}$$

Where:

m = mass of empty crucible (g);

$M_{25°C}$ = mass of sample at 25°C (g);

$M_{105°C}$ = mass of sample and crucible after evaporation at 105°C overnight (g);

4.3.4. Volatile solids and ash

The volatile solids (VS) and ash were determined according to the Standard Method 2540 (APHA, AWWA and WEF, 1998). The previous sample was ignited in a muffle furnace pre-heated to 550°C for 2 h. The crucible was partially cooled in air and then transferred to a dessicator for final cooling. The dish was weighted once completely cool. The loss of weight represents the fraction of volatile solids (organic fraction) in the sample and the ash (mineral fraction of the sample) is the counter part of the VS.

Equations 4.4 and 4.5 permit the calculation of VS and Ash respectively:

$$\%VS = \frac{M_{105°C} - M_{550°C}}{M_{105°C} - m} \qquad \textbf{(eq 4.4)}$$

$$\%Ash = \frac{M_{550°C} - m}{M_{105°C} - m} \qquad \textbf{(eq 4.5)}$$

Where:

m = mass of empty crucible (g);

$M_{105°C}$ = mass of sample and crucible after evaporation at 105°C overnight (g);

$M_{550°C}$ = mass of sample and crucible after drying in a furnace at 550°C for 2 h (g).

4.3.5. Osmotic pressure

The osmotic pressure was determined using an osmometer (OSMOMAT 030). The instrument was calibrated with distilled water and a calibration solution of known osmotic pressure. The osmotic pressure of the sample was determined under identical conditions. A 50 µl sample volume was used for calibration. It was carefully transferred with a pipette to avoid trapping air bubbles into a clear measuring vessel which was then placed into the OSMOMAT. The automatic initiation of crystallization takes place on reaching the freezing point temperature.

4.3.6. Alkalinity

Standard Method 2320 (APHA, AWWA and WEF, 1998) was used. A 15g sample of VIP solids was diluted with distilled water in a 100 ml vial and mixed. A 20 ml aliquot was transferred in a flask and was titrated with 0.1 N solution of sulphuric acid (H_2SO_4) to a pH value of 4.5 (the end-point) without recording intermediate pH values and without undue delay. A pH meter was used in preference to an indicator due to the dark colour of the sample. The alkalinity is calculated using equation 4.6.

$$A = \frac{V_a \times N_a \times 50\,000}{V} \quad \left(mg\ CaCO3/l\right) \qquad \textbf{(eq 4.6)}$$

Where:

V_a = volume of H_2SO_4 used (ml);

N_a = normality of H_2SO_4 (N);

V = volume of sample titrated (ml);

4.4. Gas analysis

The gas composition in the serum bottle was analysed by gas chromatography (GOW-MAC 350, Thermal Conductivity Detector) to quantify nitrogen, methane, and carbon dioxide production.

A 20 µl of biogas was drawn using a 100 µl precision syringe and injected into a gas chromatograph equipped with a thermal conductivity detector. The analysis parameters are given in Table 4:

Table 4: The gas chromatography analyse parameters to quantify nitrogen, methane and carbon dioxide

Parameters	Values
Carrier gas	Helium
Gas flow rate	40 ml/min
Gas pressure	400 kPa
Column temperature	80°C
Detector temperature	95°C
Injector temperature	95°C
Current bridge	100 mA

The retention times under these conditions are 0.98, 1.31 and 1.84 min respectively for nitrogen, methane and carbon dioxide. The chromatographs were treated with the Clarity Lite® software.

Calibration curves were prepared by injecting volumes (20 µl to 100 µl) of calibration gas into the GC. Using the fact that the peak area is proportional to the quantity of individual gasses injected, the peak areas can be plotted against the volumes injected.

4.5. Summary of the tests

The quantities of substrate were based on the results from the investigation by Naidoo, 2005.Table 5 shows the control to quantify the baseline gas production and the Table 6 summarize the experimentation plan.

Table 5: Summary of blank

Activity Assay	Substrate	NaHCO3 (mg/btle)	Water (ml/btle)	Objective
I = T1	VIP content (15 g)	No	No	Quantify the "background" gas production

Table 6: Summary of experimentation plan to assess the biodegradability of the substrate. (15 g VIP solids). S1: 100 mg/l, S2: 200 mg/l, S3: 300 mg/l, S4: 400 mg/l, S5: 500 mg/l of NaHCO3.

$NaHCO_3$ (mg/bottle) \ H_2O (ml/bottle)	**0**	**5**	**10**	**15**	**20**	**25**
0	0 mL H_20 0 mL S_1 (T_1)	0 mL H_20 5 mL S_1 (T_2)	5 mL H_20 5 mL S_1 (T_3)	10 mL H_20 5 mL S_1 (T_4)	15 mL H_20 5 mL S_1 (T_5)	20 mL H_20 5 mL S_1 (T_6)
0.5	0 mL H_20 0 mL S_2 (T_7)	0 mL H_20 5 mL S_2 (T_8)	5 mL H_20 5 mL S_2 (T_9)	10 mL H_20 5 mL S_2 (T_{10})	15 mL H_20 5 mL S_2 (T_{11})	20 mL H_20 5 mL S_2 (T_{12})
1	0 mL H_20 0 mL S_3 (T_{13})	0 mL H_20 5 mL S_3 (T_{14})	5 mL H_20 5 mL S_3 (T_{15})	10 mL H_20 5 mL S_3 (T_{16})	15 mL H_20 5 mL S_3 (T_{17})	20 mL H_20 5 mL S_3 (T_{18})
1.5	0 mL H_20 0 mL S_4 (T_{19})	0 mL H_20 5 mL S_4 (T_{20})	5 mL H_20 5 mL S_4 (T_{21})	10 mL H_20 5 mL S_4 (T_{22})	15 mL H_20 5 mL S_4 (T_{23})	20 mL H_20 5 mL S_4 (T_{24})
2	0 mL H_20 0 mL S_5 (T_{25})	0 mL H_20 5 mL S_5 (T_{26})	5 mL H_20 5 mL S_5 (T_{27})	10 mL H_20 5 mL S_5 (T_{28})	15 mL H_20 5 mL S_5 (T_{29})	20 mL H_20 5 mL S_5 (T_{30})
2.5	0 mL H_20 0 mL S_6 (T_{31})	0 mL H_20 5 mL S_6 (T_{32})	5 mL H_20 5 mL S_6 (T_{33})	10 mL H_20 5 mL S_6 (T_{34})	15 mL H_20 5 mL S_6 (T_{35})	20 mL H_20 5 mL S_6 (T_{36})

Grey squares represent the bottle which cannot be performed.

In order to test the first assumption (Basal Nutrient Mineral Solution is not necessary), additional bottles were prepared with the Basal Nutrient Mineral Solution. For this aim, the test T_{15} was replicated because it should perform good results at the middle of the ranges (see Table 6). The BNMS added must comply with the water quantity required for the test T_{15}. Three others bottles with BNMS and VIP only were also prepared.

The second assumption (inoculum is not necessary) was tested and three additional bottles were prepared with anaerobic sludge and VIP.

Table 7: Summary of the assumptions tests (SET 4)

Activity assay	Bottle composition	Objective
A	10 ml BNMS + 15 g VIP	Test assumption 1
B	10 ml Sludge + 15 g VIP	Test assumption 2
C	10 ml BNMS + 10 ml Sludge + 15 g VIP	Test interaction 1/2
D	10 ml Sludge	Baseline sludge
16 +	Bottle T_{16} + 10 ml BNMS	Test assumption 1
E	1 mg $NaHCO_3$ + 15 g VIP + 10 ml BNMS	Test assumption 1 (cf bottle T_{15})

Sacrificed bottles were also added to follow and measure the evolution of pH, alkalinity and COD. The bottles to be sacrificed were replicated three fold and have been chosen with the aid of Table 5. It was hypothesised that the gas production would increase as the moisture content was increased because of improved mass transfer. And in the same way, we could hypothesise that when the alkalinity increased (the process was buffered) the gas production would also increase as the rise in pH value due to acidogenisis was counteracted by the increased alkalinity. The implication of these hypotheses was that a higher gas production rate was expected to be achieved under the conditions on the right bottom section of the Table 6. The tests replicated were T_6, T_{16}, T_{18}, T_{33}, and T_{36} to covert a large range and to analyse the evolution of biological activity.

The sacrificed bottles were opened when the total gas production reached roughly 20%, 40% and 60% of the theoretical production. A relatively low gas production was selected because the theoretical calculation over estimates the gas production.

Chapter 5: Results and Discussion

This chapter contains a critical analysis of the results obtained and recommendations are provided for future work. Due to time constraints, the experiments were undertaken in several stages. It was not possible to fill all the bottles and analyse the flushing gas composition in one day. In addition, the gas chromatograph broke down which delayed the filling of the second set of bottles. This incident provided an opportunity to observe the first set of serum bottles closely, thus a better understanding of the system was achieved.

The experiments were split into three sets: the first set was to screen specific bottles which were expected to have higher gas production (T_6, T_8, T_{16}, T_{18}, T_{31}, T_{33}, and T_{36}), and the bottles which contained no sodium bicarbonate (T_1, T_2, T_3, T_4, and T_5). The second set targeted the remaining bottles where high gas production was expected (T_{15}, T_{16}, T_{17}, T_{21}, T_{22}, T_{23}, T_{24}, T_{27}, T_{28}, T_{29}, T_{30}, T_{34}, T_{35} and T_{36}). The third completed the experimental plan (T_9, T_{10}, T_{11}, T_{12}, T_{14}, T_{20}, T_{26}, and T_{32}). Nineteen and fifteen days respectively separated the first from the second set and the second from the third set of experiments.

5.1. Characteristics of the VIP contents

The raw material was sample in Tongaat at the North of Durban during an emptying campaign. The material collected came from the lower part of one pit. The pits were around fifteen years old thus the material used was quite degraded and stabilised.

Table 8: Summary of the VIP contents characteristics

Parameter	Value	Units
TS	0.195 +/- 0.005	g/g
VS	0.64 +/- 0.02	g/g
Ash	0.36 +/- 0.02	g/g
COD_T	0.19 +/- 0.07	g O_2/g sample
$COD_{0.45}$	0.04 +/- 0.01	g O_2/g sample
Alkalinity	13.75 +/- 0.72	mg $CaCO_3$ /g sample

Note: the $COD_{0.45}$ represented 21% of the COD_T which corresponds to the theoretical range comprise between 12 and 30 % mentioned by Henze et al, (1995).

The gas production expected with this raw material was around 0.21 STPl of methane per bottle. Knowing that methane represents roughly 50 % of the gas produced, thus a gas production of 0.42 STPl per bottle was expected.

5.2. Set 1

5.2.1. Results

All the gas production results were expressed in Nml per g COD to provide an accurate view and avoid any misinterpretation.

Figure 4 shows the cumulative gas production during the first set of experiments.

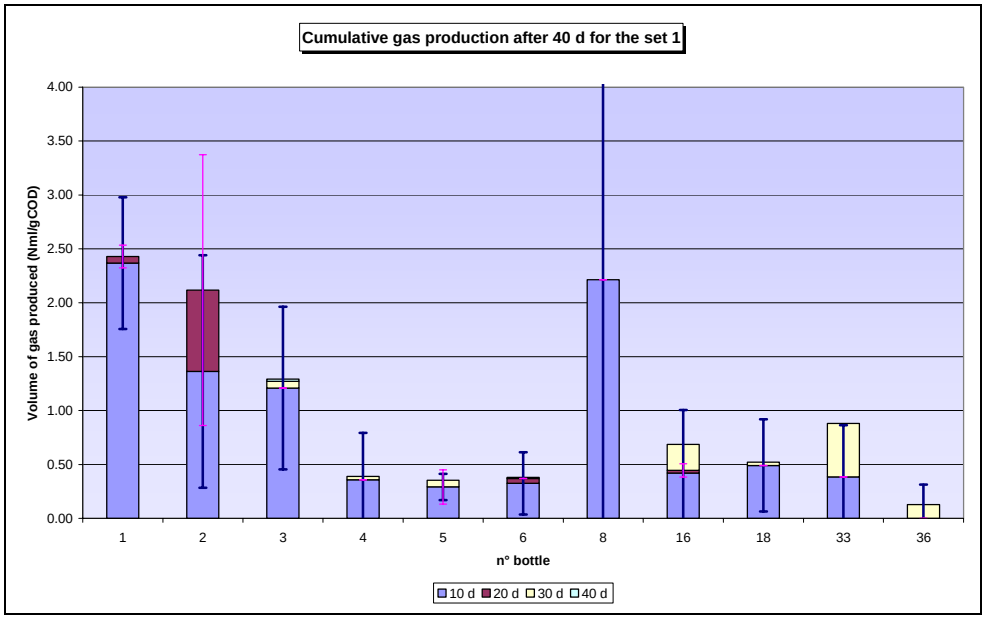


Figure 4: Cumulated gas production of the first set

5.2.2. Interpretation

After 10 days, it appeared that the serum bottles with lower amounts of water added, produced greater quantities of gas. Indeed, the bottles T_1, T_2 and T_3, containing 0, 5 and 10 ml of water respectively, produced gas volumes between 1.21 and 2.37 Nml/g COD whereas bottles T_4, T_5 and T_6 containing 15, 20 and 25 ml of water respectively, produced gas volumes between 0.29 and 0.49 Nml/g COD. The same behaviour was followed by the bottles T_8, T_{16}, T_{18}, T_{33} and T_{36}.

The addition of small quantities of sodium bicarbonate seems improve the gas production rate. A comparison of the gas production in bottles T_2 and T_8 which both had the same volume of water added but different quantities of $NaHCO_3$, shows that the gas production in bottle T_8 is 1.6 times higher than for the bottle T_2 (after 10 d). However, it appears that the addition of large quantities of $NaHCO_3$ does not produce a significant improvement in the rate of gas production (the bottles T_{16}, T_{33}, and T_{18}, T_{36} compared to bottles T_4, T_3 and T_6).

After 20 days, no or only a low gas production was measured with the exception of bottle T_2. A high gas production was recorded after 10 days whereas later, no or only a low gas production was recorded. Several hypotheses can be proposed such as:

- not sufficient nutrients,
- a gas leak,
- change of conditions,
- increase of temperature which leads to an increase of volume, etc.

If the Ideal Gas Law holds then the necessary increase of the temperature to produce the recorded gas volume is presented in Figure 5:

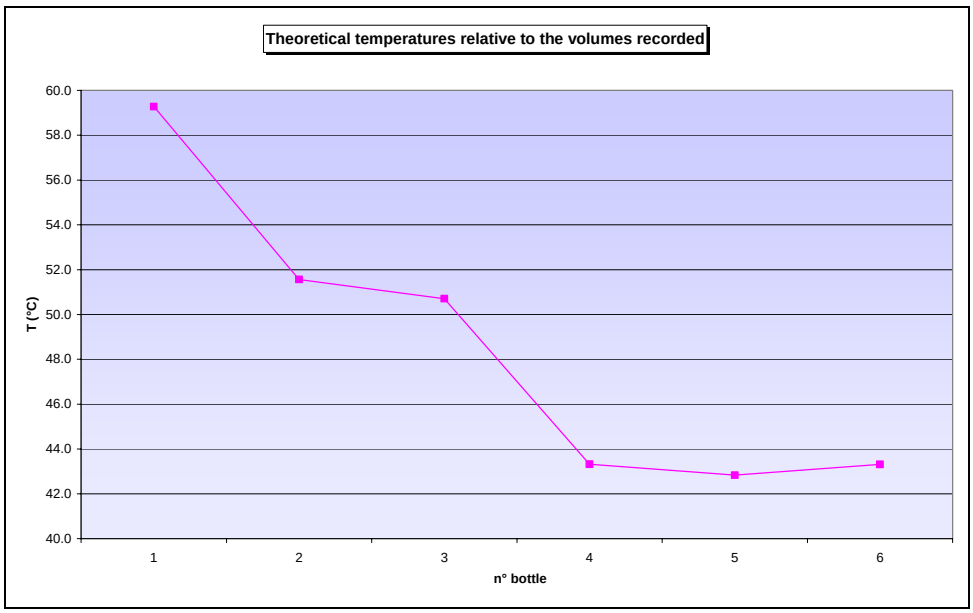


Figure 5: Theoretical temperatures relative to the volumes recorded

According to the Figure 5, a temperature of 59.3°C is needed to explain such an increase of gas for the bottle T_1 and more than 50°C for the bottles T_2 and T_3. These temperatures were not observed in the storage room so these volumes cannot be attributed to this phenomenon.

The other hypothesis were tested (SET 4) in order to understand this phenomenon.

After 30 days, some bottles (T_3, T_4, T_5, T_{16}, T_{18}, T_{33} and T_{36}) restarted to produce gas which leads to conclude that the change of conditions might be the explanation of the period of inactivity.

The anaerobic digestion was over after 40 days and no real gas production was recorded even later (followed during 2 months and half).

The standard deviations are quite high which makes it difficult to draw firm conclusions. Since the standard deviation is of the same order as the average gas production, (see bottles T_2 and T_8) this means that the results are not reproducible and the behaviour can be more attributed to a particular case than to a general trend.

5.3. Set 2

5.3.1. Results

Figure 6 shows the cumulative gas production during the second set of experiments.

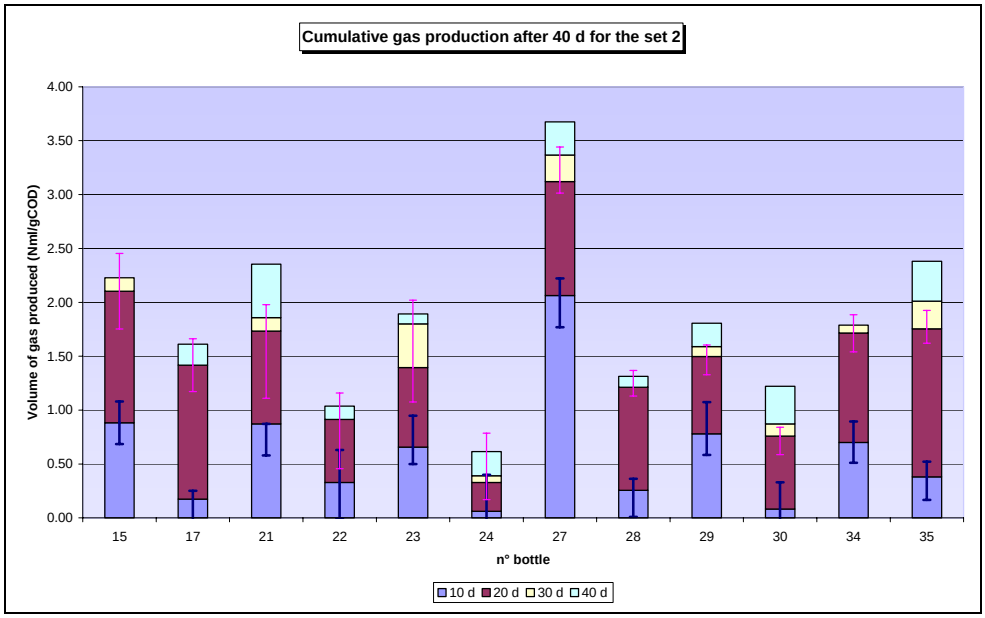


Figure 6: Cumulative gas production of the second set

5.3.2. Interpretation

After 10 days all the bottles produced gas but at different levels (from 0.06 Nml/g COD for the bottle T_{24} to 2.06 Nml/g COD for the bottle T_{27}). The highest gas productions were observed for a volume of water added of 10 ml with the bottles T_{15}, T_{21} and T_{27}. By symmetry the lowest gas volumes were produced at the highest water volume added (bottles T_{24} and T_{30}). Between these two extreme behaviours, no real trend can be observed. No conclusion can also be made regarding the quantities of $NaHCO_3$ introduced.

The gas production rate between the days 10 and 20 was the highest. It can be easily explained by the fact that after an acclimatising phase, the anaerobic digestion occurs with an optimum after 14 days. Next the production decreased due to probably the lack of nutrients,

the production of inhibitors etc. Note that this behaviour was completely the opposite from the SET 1. All the sets were compared together in the section 5.6.2.

After 40 days, gas production was still observed, and it can be noted that between the days 30 and 40 the production was all the more important that it was low during the 10 previous days. It might have been due to the fact that the compounds produced during the 20 first days unbalanced the system and thus a new equilibrium had to be reached before restarting any activity.

The standard deviations were similar for all the bottles in this set, thus the results are reproducible probably due to the fact that the raw material is homogeneous for this set.

5.4. Set 3

5.4.1. Results

Figure 7 shows the cumulative gas production during the third set of experiments.

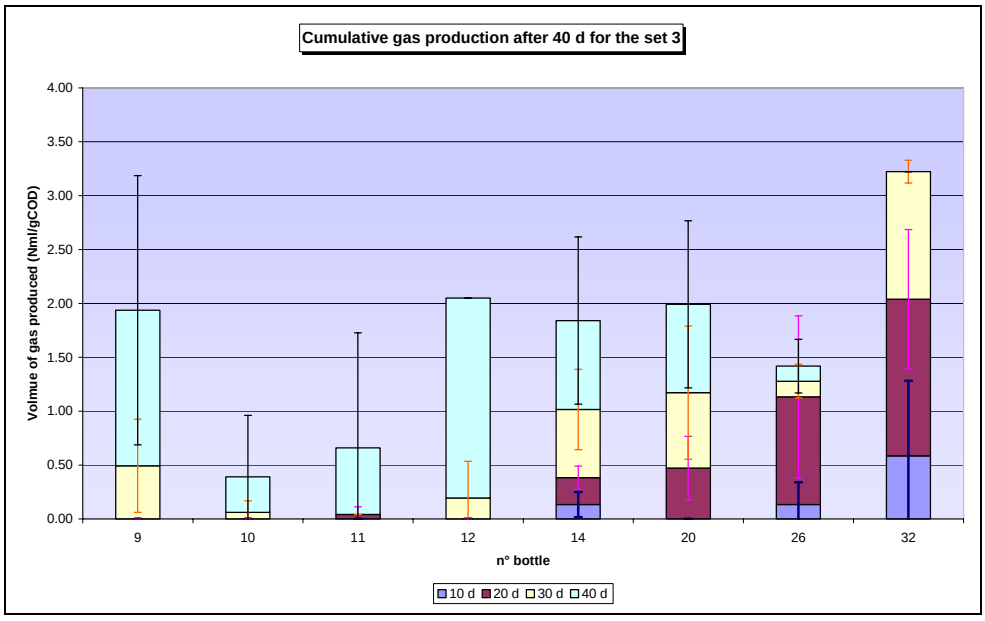


Figure 7: Cumulated gas production of the third set

5.4.2. Interpretation

After 10 days, almost no gas was produced. Only the bottles T_{14}, T_{26} and T_{32} produced but not in large amounts (from 0.13 to 0.59 Nml/g COD). These bottles had a low volume of water added (5 ml of water) and this behaviour was the same as the one of the SET 1 and 2. However contrary to the previous sets, it seems that a high quantity of $NaHCO_3$ improves the

biodegradability (bottle T_{32} has a production 4.5 times higher than the bottles T_{14} and T_{26}). These two trends (positive effect of low volume of water added and high amount of $NaHCO_3$ introduced) were observed throughout this set.

The highest gas production rate occurred after 20 days for the bottles T_{14}, T_{20}, T_{26} and T_{32} while the other bottles (T_9, T_{10}, T_{11} and T_{12}) started their production of gas only after 20 to 30 days and in small amounts (from 0.04 to 0.49 Nml/g DCO). This delay is quite large compared to the other sets.

The standard deviations are high which imply that one should be really careful before generalising the conclusions.

5.5. Set 4

This set was chosen to test the assumptions made at the beginning of the experiments and during the SET 1. The summary of these tests is given in Table 7.

5.5.1. Results

Figure 8 shows the cumulative gas production during the fourth set of experiments.

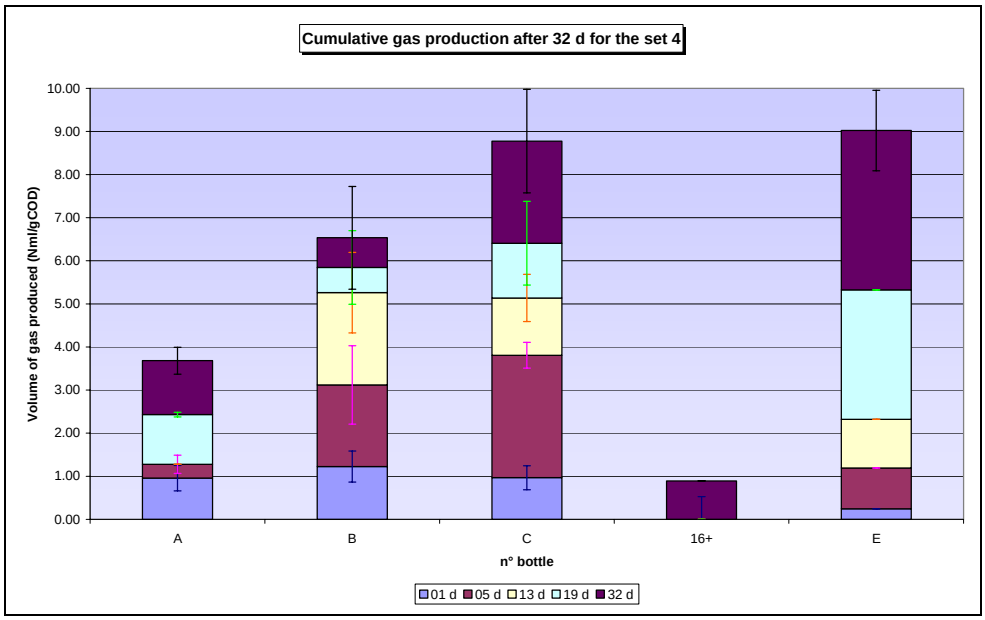


Figure 8: Cumulated gas production of the forth set

5.5.2. Interpretations

After 1 day, the bottles A, B and C which did not contain $NaHCO_3$ had a higher gas production than the bottle E which contained a small amount of $NaHCO_3$ (1 mg). The bottle with only 10 ml of sludge (no represented on the Figure 8) produced a large amount of gas on the first day, and then nothing. It might be explained by the fact that micro-organisms were stressed by the lack of nutrients. This conclusion is supported by the fact that this behaviour did not appear into the bottles B and C which contain VIP and/or BNMS.

The gas productions of the bottles B and C were almost the same, whereas the one of bottle A was lower. It leads to conclude that the sludge drives the gas production and that the addition of BNMS does not make a real difference. However, if bottle E was compared to its counterpart (bottle T_{15}), its gas production was 2.5 times higher. The result gives us to understand that the addition of BNMS has a positive effect on the degradation rate. Note that in the bottle C, 20 ml of liquid was introduced and that it did not decrease the gas production as was observed in the previous sets.

The main gas production occurred during the first days (from day 1 to day 13) for the bottles B and C whereas it occurred later (from day 13 to 19) for the bottle E. This means that the addition of $NaHCO_3$ leads to an acclimatising phase before the gas production.

The overall gas production was much higher in this set than in the previous ones. Indeed, the gas production of the SET 1, 2 and 3 was below 2.5 Nml/g COD, whereas in this set the bottles B, C and E the volumes recorded were above 5 Nml/g COD, thus more than twice higher. The gas production of the bottle containing only sludge was the baseline of the inoculum. This quantity must be subtracted to the volume of the bottles also containing inoculum to reach the net gas production. If it is done, then the gas production of the bottles B and C becomes nil. The inoculum has its own COD which leads to a potential gas production higher. Even if the experiments show a benefit, it must not be forgotten that the inoculum has increased the COD and this must be taken into account.

5.6. Global view

5.6.1. Results

Figure 9 shows the cumulative gas production for all the experiments.

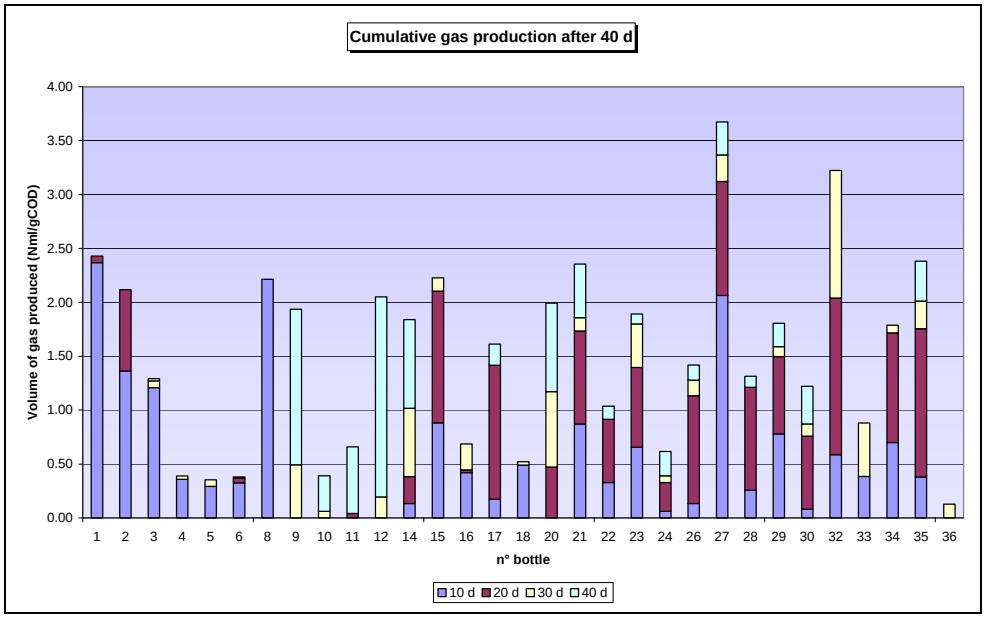


Figure 9: Cumulative gas production

Figure 10 shows the cumulative gas production after 40 days in 3D.

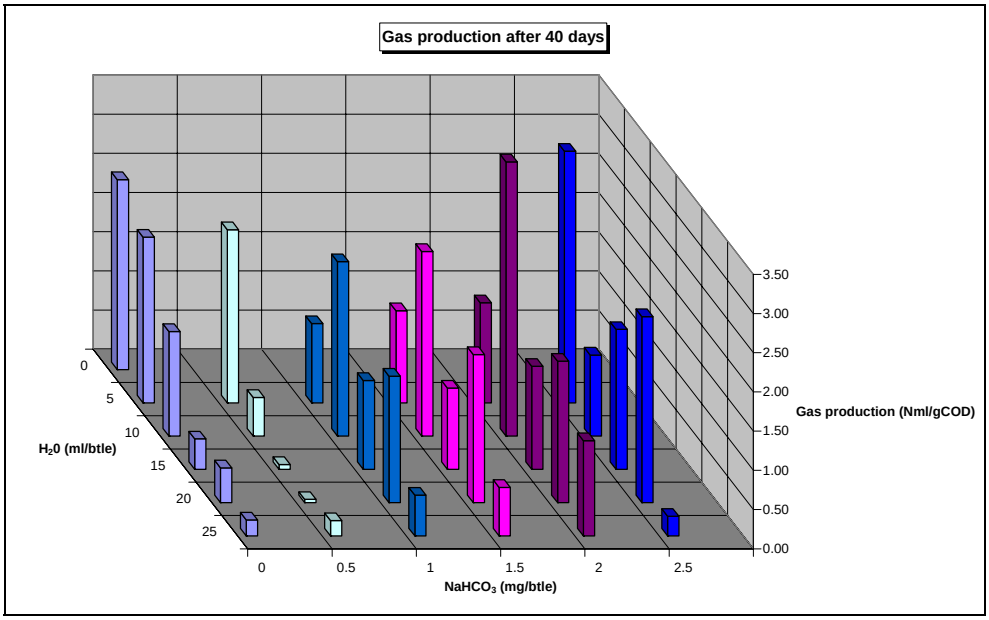


Figure 10: Gas production after 40 days (3D view)

5.6.2. Interpretation

Figure 9: the main gas production was observed during the 20 first days for most of the bottles. However, it might seem that the gas production is more correlated to the set than to the treatment applied. Indeed, the bottles of the SET 1 had a gas production during the first 10 days whereas the SET 2 recorded a small gas production during the first 10 days, but mainly during the following 10 days. Finally, the SET 3 had two kinds of trend; one with only a low gas production after 30 days and another one with a higher gas production which started mainly after 10 days. Bottle T_{16} was made the same in both SET 1 and 2 and it appears that the gas production was completely different from SET 1 to SET 2, and it followed the set trend instead of providing a specific answer relative to the conditions (see **Figure 11**).

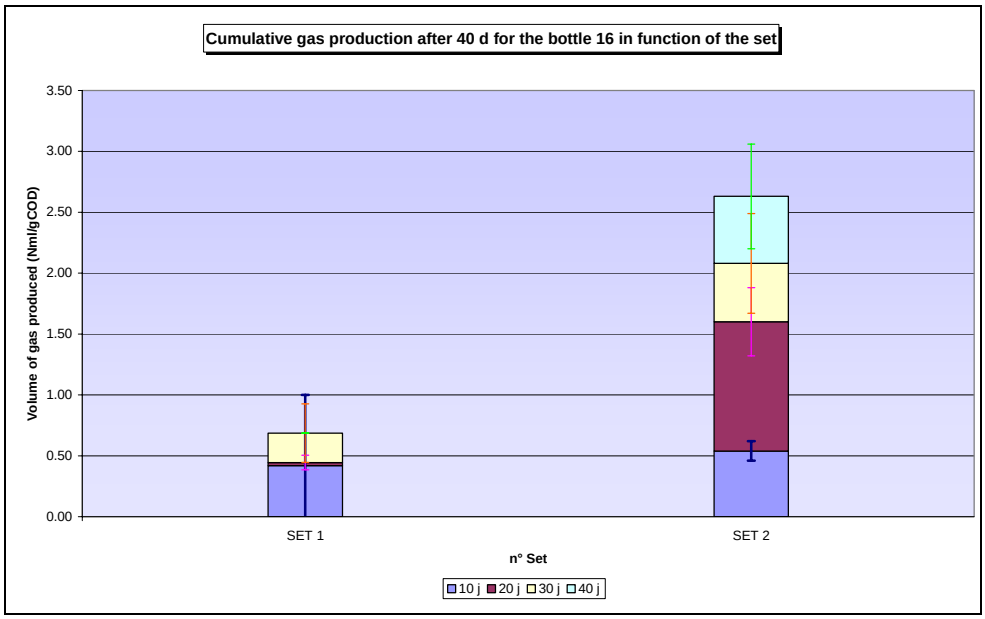


Figure 11: Gas production of the bottle T_{16}

Figure 10: the addition of water seems have a negative effect on the gas production. The best amount of water added was between 5 and 10 ml. Concerning the amounts of $NaHCO_3$, no real conclusion can be made. The results depend mostly on the set, and thus on the raw material used. Even though the VIP content came from the same sample, it is obvious that the material was not homogenous. Around 2 kg of faeces were necessary for these experiments and it cannot be expected to have exactly the same composition.

Knowing that the volumes of water into the bottles seem have an effect on the gas production and that CO_2 was present into the gas phase, it is possible that there is a correlation between these two parameters. The hypothesis was tested that the CO_2 was absorbed by the liquid phase using PHREEQ. PHREEQ is a programme written by D.L. Parkhurst and C.A.J. Appelo and distributed by the *US geological survey* to simulate particular chemical reactions and transfers into natural and polluted water. This programme also permits simulation of a batch system which is our case. The solution composition was defined by the $NaHCO_3$ quantities added into the water. The gas phase was specified by the flushing gas (N_2/CO_2, 50/50). Simulations were done by using these specifications at constant volume (closed system) and at a temperature of 37°C (experimental temperature). Other sets of simulations modelled the effect of the increase of pressure, and in particular the increase of CO_2 linked to the biogas production during the biodegradation. From all these trials, it appears that the greater the volumes of water, the more gas is absorbed. However, the total gas composed of CO_2, N_2 and H_2O for the first simulations, and with CH_4 for the next one, shows that for small volumes of water, the variation of gas volume is positive up to 10 ml of water, and then negative. This phenomenon explains the trends observed in the serum bottle experiments. When the CO_2 fraction in the gas phase increases, its absorption increases. In this way, when the degradation is taking place, there is production of CO_2 which is absorbed by water in the bottles, and all the more when the volume is high. The gas volumes measured are then lower than the theoretical volumes. The more degradation occurs, the more CO_2 is produced and the greater the discrepancy between the gas volume recorded and the gas produced by the degradation reactions. Consequently, the gas production measured is mainly composed of methane. It has been observed experimentally that even when no gas was recorded; it was possible to detect methane by analysing the gas from the headspace with the GC. It is important to note that the pH change due to the absorption has no effect on the alkalinity. This result is correct because the alkalinity is conservative and H_2CO_3 (from CO_2 dissolved) has no alkalinity itself. It only modifies the pH value which is independent of the alkalinity. The pH of the solution is around 4.7 which is a problem for the growth of micro-organisms and the objectives of our experiments.

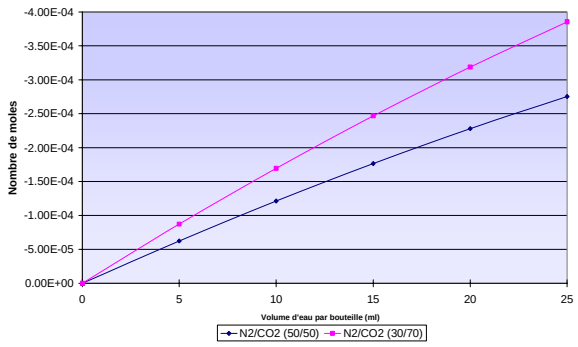
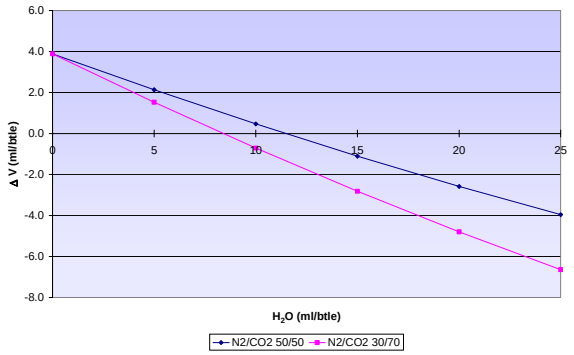
$N_{CO2\ absorbées} = f(V_{H2O})$	$\Delta V_{g\ total} = f(V_{H2O})$
	

Figure 12: CO2 absorption and gas variation due to the absorption.

The biological activity might also be affected by water amounts due to the osmotic pressure modified by the addition of distilled water.

It seems, but it should be taken carefully, that there are two trends of gas production. The higher productions are observed at the extreme points of the both ranges of $NaHCO_3$ and H_2O added. At the middle, the gas production is the lower.

5.6.3. Statistical interpretation

Comparing treatments with a control: C.W. Dunnett has developed a test for determining if the difference between each treatment mean and a control is significant at a significance level α. In the present study, bottle T_1 was the control, and 30 different treatments replicated 3 times have to be tested. It was decided to use a two-tailed test because even if a significant larger gas production than that of the control is expected; it is also interesting to see if the treatment does not produce the opposite effect. The details of the calculations and the hypothesis are shown in the annexure I.

The conclusion from this test is that the treatments do not significantly affect (at a level $\alpha = 0.05$) the gas production relative to the control. However, it can be noted that the effect of the treatments is mainly negative compared to the control.

The assumptions were also tested with this statistic tool to confirm or refute their validity. It appears that there is no significant difference, but the addition of sludge or BNMS has a positive effect on the gas production.

Two-factor analysis: two factors (humidity and alkalinity) have been simultaneous studied to improve the biodegradation. Now, it is of interest to analyse their effects. The details of the calculations and hypothesis are given in the annexure II.

The conclusion of this test is that there is no interaction between the two factors but they both affect the biodegradation rate.

If the conclusions of the tests are combined, it leads to think that even if the treatments have not a significant effect, they affect the biodegradation rate. Unfortunately, it seems that this effect be negative.

The tests presented here are based on the gas production recorded by the bottles T_1, however it has been shown that a bottle without water could have a positive gas variation (see Figure 12) due to absorption effects only. This phenomenon biases the tests. In add the absorption of CO_2 leads to conclude that the effects of $NaHCO_3$ and water are negative on the degradation.

5.6.4. Mass balance:

Bottles are close systems thus the following equation can be written:

$$\Phi_{in} = \Phi_{out} \qquad \textbf{(eq 5.1)}$$

In terms of COD:

Φ_{in} = mean of COD introduced into the bottles +/- standard deviation

Φ_{out} = {mean of COD into the bottle at the end + COD from methane produced[1]} +/- pooled standard deviation[2].

[1] Note : $DCO(CO_2) = 0$ g O_2/l

[2] The pooled standard deviation of a sum is the square root of the sum of the square of the standard deviations. If y is a sum of experimental variables i with standard deviations s_i then the standard deviation of y is: $s_y = \sqrt{\sum_{i=1}^{n} s_i^2}$ (Skoog et al, 2003).

The bottle 15 is used as example. Its gas production is at the middle of the range.

$$\Phi_{in} = \Phi_{out}$$

$$2{,}79 \text{ +/- } 0{,}204 = \{2{,}14 + 0{,}01\} \text{ +/- } \sqrt{0{,}192^2 + 0{,}003^2}$$

$$2{,}79 \text{ +/- } 0{,}204 = 2{,}15 \text{ +/- } 0{,}192$$

In order to appreciate the difference between the initial COD and the final in the mass balance, the Student T test was applied. If the difference between the both averages $\bar{x}$ and μ is not significant at a level α then the relation $-t_\alpha < t = (\bar{x} - \mu)/(s/\sqrt{n}) < +t_\alpha$ is verified for n-1 degree of freedom where n is the number of replications and *s* the standard deviation. In our case a level $\alpha = 0.01$ is enough and $t_{0,01}$ for 2 degree of freedom is equal to 6.965. This test leads to the conclusion that the difference of COD is due to random effects.

Other bottles have been tested, and the system is almost all the time balanced for $\alpha = 0.01$. But if the interval is reduced, it leads to conclude that the system is unbalanced.

It has been assumed that methane represents 50% of the gas production because the GC broke down and the overall gas production has not been recorded. However, according to the previous part about the CO_2 absorption, the CO_2 is absorbed and the gas measured is mainly methane. An underestimation of the methane quantities and consequently of the COD from methane has be done.

5.6.5. Conclusion:

The gas production is really low, probably due to the fact that the raw material was too well degraded. Indeed, it was old VIP contents and it seems that they were well stabilized.

In order to test this first conclusion a new set was done with a younger sample of faeces.

5.7. New set

The new sample has been collected from a school in Pietermariztburg. This new material comes from the top layer of a pit thus it was not degraded. The colour of this new material was brown whereas the old one was black.

5.7.1. Results

The Figure 13 shows the gas production recorded for the new set.

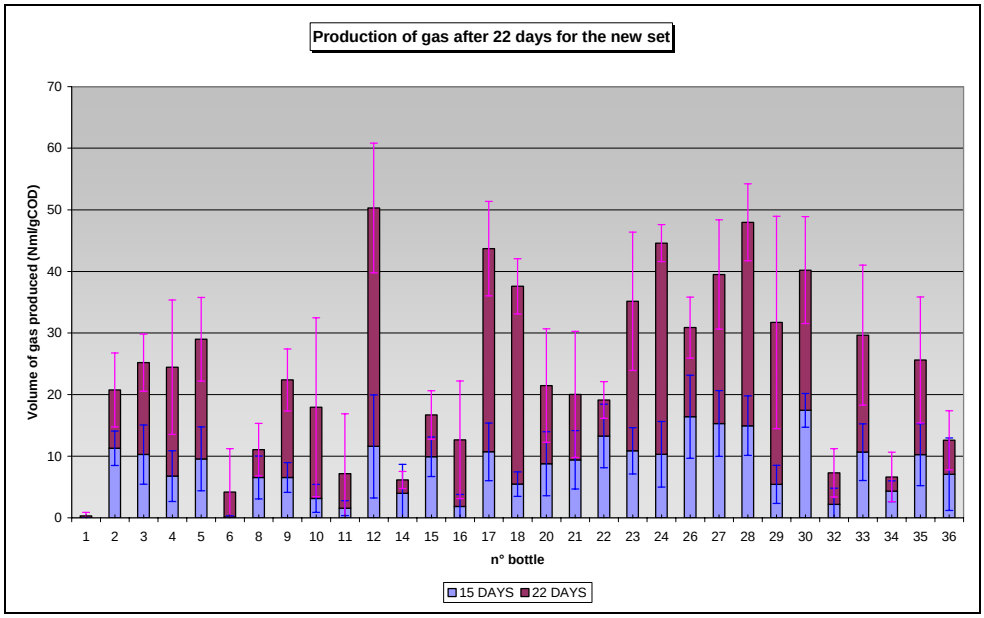


Figure 13: Gas production after 22 days for the new set.

5.7.2. Discussion

The first observation is that the gas production is much higher than for the previous sets. The average of the first matrix is around 2 Nml/g COD, whereas here the average is around 10 Nml/g COD and only after 15 d, thus 5 time more.

The global shapes are similar with a decrease of gas volumes with the water increase and a high production in second part of the graph.

The control produces almost no gas thus any addition of whatever increases the gas production. Compared to the first experiments, the gas production of the control leads to completely different conclusions.

The standard deviations for all replicate bottles are similar, which means that the results are reproducible.

Chapter 6: Conclusions and recommendations

This section provides the conclusions of this work, followed by recommendations for a future work.

Based on the first set of experiments, the CO_2 absorption increases with H_2O. This phenomenon has been explained with the PHREEQ simulations. The consequence of the CO_2 absorption is double: first, the absorption leads to a decrease of pH, potentially as low as 4.7, which is harmful for anaerobic digestion's micro-organisms and causes the opposite effect wanted. However, the pH value at the end of the experiments was above 7.9 which is in contradiction with the simulations results. Next, the absorption leads to an over estimation of the gas production when the volumes of water added to the bottles are between 0 and 10 ml, and to an under estimation of the gas production from 15 ml of water added. The control contains no water thus on the one hand, the reference is over estimated, and on the other hand the treatments with the higher conditions are under estimated. All these considerations minimize the benefit effect of treatments, and lead to a confused interpretation.

According to the first set again, the starting day was significant. The earliest set has high gas production on day 10 measurements.

If day 10 measurement is eliminated, low alkalinity takes a long time to produce gas and high alkalinity leads to gas production which begins sooner but does not appear change much with moisture or alkalinity.

The raw material used has an important effect on the gas production and makes the conclusion difficult due to its variability.

Based on the second set, increasing alkalinity above any (bottle T_1) shows an increase in activity. In the same way, increasing moisture from none to some shows a significant increase in activity.

Based on both sets, the best conditions to increase the biodegradability are an addition of $NaHCO_3$ of around 2 mg/bottle. It is difficult to conclude about the water due to the CO_2 absorption.

For future work, it can be recommended to flush the bottles with N_2 (free of CO_2) to avoid a decrease of pH. A low pH is harmful for micro-organisms and is what we would avoid by adding $NaHCO_3$. The CO_2 absorption makes the interpretations difficult.

Water with salts added to equalise osmotic pressure can be investigated instead of using distilled water.

Make more controls would be interesting because the interpretations and statistic tests are only based on these three bottles.

Homogenise the raw material to increase the reproducibility.

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Annexure

Annexure I: Statistical tests

Abstract

In South Africa, Ventilated improved pit (VIP) latrines which are improvements to a pit latrine to minimize odours and flies, are recognized as a minimum level of acceptable sanitation. The lack of space (density of settlements) and the design of the VIP chosen by the eThekwini Municipality (robust superstructure) does not allow any relocation hence full pits must be emptied. Thus in order to reduce the emptying costs supported by the municipality an increase in the decomposition rate to limit the sludge accumulation is investigated.

Into the pit, the solid fraction (human excreta) is mainly decomposed under anaerobic conditions by producing biogas and soluble products which may leach into the soil. Acidity, alkalinity and solubilisation are keys factors for the degradation process and the objective of this study is to evaluate the combined effect of moisture and alkalinity on the anaerobic biodegradation of VIP content. A factorial matrix with two factors (alkalinity and moisture) and six levels is built for an adapted serum bottle test. The bottles are filled with 15 g of VIP content, an alkaline solution (from 0 to 2.5 mg of $NaHCO_3$ per bottle) and water (0 to 25 ml of H_2O per bottle).

It was found that additional alkalinity could enhance the rate of anaerobic digestion with the first results but unfortunately there is an absorption of CO_2 phenomenon into the bottles which leads to an under estimation of the benefit of treatments. A new raw material (less stabilised) is tested in a new set of experiments. The degradation is in process but with the first results, it seems that the gas production is really higher than the previous one and that a quite large amount of $NaHCO_3$ (2 mg/bottle) increases the gas production.

Keywords: anaerobic degradation, serum bottle, biogas, alkalinity, buffering capacity.