An Investigation into the Bioprocesses of DiCOM®:
A Technology Combining Composting and
Thermophilic Anaerobic Digestion for the
Treatment of Municipal Solid Waste

School of Biological Sciences and Biotechnology
Murdoch University
Western Australia

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In Biotechnology

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Lee R. Walker
It is difficult to understand that ...
High value fossil fuel and nuclear energy is invested to destroy the renewable solar energy which is fixed in the chemical compounds in biogenic waste.

(Edelmann et al., 2000)

God gave men and women a privileged place among all creatures and commanded them to exercise stewardship over the earth (Genesis 1:26-28). Stewardship implies caretaking, not abusing. We are to intelligently manage the resources God has given us, using all diligent care to preserve and protect them.

To My Darling Wife, Mandy ... I love you!
Declaration

I declare that this thesis is my own account of my research and contains, as its main content, work that has not previously been submitted for a degree at any university.

Lee Walker
The aim of this study was to investigate a novel municipal solid waste (MSW) treatment system called DiCOM®. This process diverts organic waste from landfill and consequently abates the adverse environmental impacts associated with the practice. The DiCOM® process exposes the mechanically sorted organic fraction of municipal solid waste (OFMSW) to sequential aerobic, thermophilic anaerobic and aerobic conditions in a sealed vessel during a twenty one day batch treatment cycle. The outputs of the technology are renewable energy, in the form of biogas, and compost.

The features that differentiate DiCOM® from other waste treatment systems include:

- Treatment is performed within a sequencing batch reactor.
- During the aerobic treatment the internal pressure is raised to enable hyperbaric composting in an attempt to maximise oxygen availability and consumption.
- Some of the bio-methanation potential of the OFMSW is sacrificed to enable aerobic microbial metabolism to raise the temperature of the OFMSW to that required for thermophilic anaerobic digestion (AD), minimising the need for external energy input.
- The residue of the thermophilic AD phase is composted within the vessel, minimising the plant footprint and the generation of off-site odours.
- The anaerobic liquid is reused thus minimising the production of wastewater.
- The small plant footprint can allow for processing of MSW close to its source in an attempt to minimise transport costs.

The DiCOM® process vessel is loaded with organics over a five day period during which the material is exposed to aerobic conditions. Aeration consists of cyclically pressurising the vessel with air to 25kPa, holding the pressure to maximise oxygen
consumption, followed by vessel depressurisation. During aeration microbial metabolism raises the temperature of the organics to that necessary for the thermophilic AD phase.

The 7 day thermophilic AD is initiated by flooding the reactor with anaerobic liquor from a previous batch thus inoculating the system with a viable and active methanogenic culture. The biogas produced can be captured and used to meet the energy requirements of the process. As the anaerobic liquor is reused, the production of a wastewater stream can be minimised.

Aerobic maturation of the compost occurs after the anaerobic liquor has been drained from the vessel and the organics mechanically dewatered. After 5 days of aerobic treatment the vessel is unloaded (2 days), completing the treatment cycle, with the vessel ready to receive the next charge of organics.

The study of the transition from aerobic to anaerobic conditions was of interest, as methanogens are known to be oxygen sensitive. A laboratory–scale DiCOM® reactor demonstrated a seamless transition from aerobic to anaerobic metabolism with methane being produced within 4 hours of the establishment of anaerobic conditions. The reuse of anaerobic liquor improved bio–methanation as noted by, a more rapid onset of sustained methane production, an increase in mean biogas production rate (from 18.2 to 28.7 L/kg of TVS/day) and an increase in total solid degradation (from 41 to 45% of TVS) during the anaerobic phase. An electron balance showed that the direct liquor transfer provided a 50% increase in electron flow during the digestion phase and provides a significant energetic advantage to the process. The compost was found to be
more stable (self–heating test) than the control, which is consistent with greater destruction of organics.

Inevitably the liquor reuse led to ammonia accumulation. While the higher levels of ammonia were shown to inhibit methanogenesis, the process of ammonia release had the benefit of providing alkalinity and hence decreased the risk of acidification. Modelling of ammonia and propionate accumulation suggested that the methanogens in the DiCOM® process were, to some degree, always inhibited, by either free–ammonia or free propionic acid, and that a “pH window” could be defined within which this inhibition was minimised.

A study into the presence and survival of methanogens indicated that endogenous thermophilic acetoclastic and hydrogenotrophic methanogens in OFMSW were found to survive the 5 day pressurised aeration phase of the process and resumed methanogenesis upon initiation of anaerobic conditions. Characterisation of the laboratory–scale anaerobic liquor identified the key methanogen present to be *Methanoculleus thermophilus*, a hydrogenotrophic methanogen. Maintenance of the anaerobic liquor is critical to efficient DiCOM® processing as the bulk of the methane produced (79%) was via hydrogenotrophic methanogenesis. Toward the end of digestion however, acetoclastic methanogenesis was found to be the dominant pathway for methane production. The dominant acetoclastic methanogen, *Methanosarcina thermophila*, was attached to the solid organics. Hence, the use of stabilised digested solid, as an inoculum, improved AD of OFMSW in DiCOM® trials resulting in a decrease in volatile fatty acid accumulation (44%) and the time required for solid stabilisation (3.5 days).
Laboratory trends could be observed at pilot–scale however, the performance of an optimised laboratory–scale DiCOM® reactor was not able to be reproduced at pilot–scale, as AD was incomplete within the DiCOM® timeframe. The high temperature predicted, from modelling of the initial aeration phase, was confirmed at pilot–scale (85°C) and may hold the key to understanding the observed variation in the micro–flora between laboratory and pilot anaerobic liquor. Solids degradation during the initial aerobic phase, measured as electron flow, was found to be significantly less (70%) than optimised laboratory trials, which resulted in a greater proportion of easily degradable organics introduced into the AD phase. Pilot–scale DiCOM® trials suggested that for sustainable operation, under test conditions, either a longer AD phase and/or an improved inoculum transfer may be necessary.

This study has contributed to the optimisation of the DiCOM® process by providing critical information to enable rapid waste processing and energy self sufficiency.
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<thead>
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<th>Symbol</th>
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<tr>
<td>ΔG°'</td>
<td>Change in Standard Gibbs Free Energy (Adjusted to pH 7.0)</td>
</tr>
<tr>
<td>AD</td>
<td>Anaerobic Digestion</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
</tr>
<tr>
<td>CSTR</td>
<td>Continuously Stirred Tank Reactor</td>
</tr>
<tr>
<td>COD</td>
<td>Chemical Oxygen Demand</td>
</tr>
<tr>
<td>DI H₂O</td>
<td>Deionised Water</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>GI</td>
<td>Germination Index</td>
</tr>
<tr>
<td>HRT</td>
<td>Hydraulic Retention Time</td>
</tr>
<tr>
<td>Kᵢ</td>
<td>Inhibition Constant</td>
</tr>
<tr>
<td>kₛ</td>
<td>Half Saturation Constant</td>
</tr>
<tr>
<td>LCFA</td>
<td>Long Chain Fatty Acid</td>
</tr>
<tr>
<td>mg/L</td>
<td>milli Grams per Litre</td>
</tr>
<tr>
<td>mM</td>
<td>milli Moles per Litre</td>
</tr>
<tr>
<td>MPN</td>
<td>Most Probable Number</td>
</tr>
<tr>
<td>MPR</td>
<td>Methane Production Rate</td>
</tr>
<tr>
<td>Ms</td>
<td>Endogenous Decay Coefficient</td>
</tr>
<tr>
<td>MSW</td>
<td>Municipal Solid Waste</td>
</tr>
<tr>
<td>mV</td>
<td>milli Volt</td>
</tr>
<tr>
<td>µₘₐₓ</td>
<td>Maximum Specific Growth Rate</td>
</tr>
<tr>
<td>NH₃ –N</td>
<td>Ammonia Nitrogen</td>
</tr>
<tr>
<td>NAD⁺</td>
<td>Nicotinamide Adenine Dinucleotide</td>
</tr>
<tr>
<td>NADH</td>
<td>Reduced NAD⁺</td>
</tr>
<tr>
<td>OFMSW</td>
<td>Organic Fraction of Municipal Solid Waste</td>
</tr>
<tr>
<td>OHPA</td>
<td>Obligate Hydrogen Producing Acetogen</td>
</tr>
<tr>
<td>OUR</td>
<td>Oxygen Uptake Rate</td>
</tr>
<tr>
<td>pKa</td>
<td>Acid Dissociation Constant</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>PAH</td>
<td>Polycyclic Aromatic Hydrocarbon</td>
</tr>
<tr>
<td>pressate</td>
<td>the liquid mechanically squeezed from the stabilised solid at the conclusion of the anaerobic phase of the DiCOM® process</td>
</tr>
<tr>
<td>recyclate</td>
<td>the anaerobic liquor which is recycled through the previous, mature reactor</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>SC</td>
<td>Spontaneous Combustion</td>
</tr>
<tr>
<td>SRB</td>
<td>Sulfate Reducing Bacteria</td>
</tr>
<tr>
<td>SRT</td>
<td>Solids Retention Time</td>
</tr>
<tr>
<td>TAN</td>
<td>Total Ammonia Nitrogen</td>
</tr>
<tr>
<td>TOC</td>
<td>Total Organic Carbon</td>
</tr>
<tr>
<td>TS</td>
<td>Total Solids</td>
</tr>
<tr>
<td>TVS</td>
<td>Total Volatile Solids</td>
</tr>
<tr>
<td>UASB</td>
<td>Up–flow Anaerobic Sludge Blanket</td>
</tr>
<tr>
<td>VFA</td>
<td>Volatile Fatty Acids</td>
</tr>
<tr>
<td>Y</td>
<td>Biomass Yield Coefficient</td>
</tr>
<tr>
<td>VS</td>
<td>Volatile Solids</td>
</tr>
</tbody>
</table>
1.1 Historical Approaches to Waste Disposal

In early human history, municipal solid waste (MSW) had little or no environmental impact due to the nomadic way of life of the people and the nature, and quantity, of the waste generated. The fate of such waste was either bacterial degradation or consumption by animals (Scrudato and Pagano, 1994). This approach continued even after the nomadic lifestyle was traded for permanent dwellings and people established townships and cities. Here people simply threw their waste into the streets. The sanitary conditions in medieval cities lead to rampant disease and death during the middle–ages, where diseases directly related to human waste killed many hundreds of thousands of people. Following the major plagues of the 12th century, changes began to take place and waste management became a priority.

Though there was still little understanding about disposal, by the 16th Century human wastes were collected in major cities, with sewage discharged directly into rivers and other human generated waste disposed of in waste dumps on the city limits. By the early 19th Century, despite the fact that sewage treatment had undergone significant advancement, treatment of municipal wastes had remained virtually unchanged with wastes dumped far enough from the town so that sight and smell were not an issue (Scott et al., 2005). At this time, no restrictions were placed on the nature of the waste or the location of disposal with all waste types disposed of together, and waste often used to reclaim land by disposal in swamps and wetlands (Scott et al., 2005).

During the 1920s and 30s health concerns regarding waste dumping led to the development of the “sanitary landfill”, where on a daily basis, the waste was covered by
a layer of clean soil to decrease surface decay and odour generation (Scott et al., 2005). However, by the 1960s and 70s, serious contamination of local environments by the uncontrolled dumping of industrial wastes (e.g. Love Canal: Beck, 1979; Cunningham and Cunningham, 2002) has lead to significant changes in solid waste management (Scott et al., 2005).

1.2 Waste Disposal – A Potential Methane Source

The resource-intensive societies of the developed world create enormous amounts of waste, to the point where today solid waste is considered to be a major problem of our society (Qasim and Chiang, 1994; Hartmann and Ahring, 2006). During the late 1990’s, globally and within Australia, up to 95% of refuse (consisting of MSW, construction, demolition, industrial and hazardous wastes) was disposed of in landfills (Scott et al., 2005; Beeton et al., 2006). However by 2006, within Australia (and mirrored around the world), the trend to reuse and recycle components of waste streams had decreased this figure to 48% (ABS, 2010). Even though this trend is encouraging, regrettably 60% of MSW is still being sent to landfill (ABS, 2010) with worldwide estimates approaching 1.5 billion tonnes per year (Themelis and Ulloa, 2007).

Historically, landfills have been a common, convenient and inexpensive option for waste disposal (Cunningham and Cunningham, 2002; Scott et al., 2005; Sabahi et al., 2009) however this situation is changing rapidly. Rising land and transport prices, lack of space, and demanding landfill construction and maintenance requirements (as a result of more stringent environmental protection regulations) have made it a more expensive disposal method (Cunningham and Cunningham, 2002). This situation is also compounded by public opposition to the development of new landfill sites (Qasim and Chiang, 1994). Landfilling of MSW has been implicated in a number of environmental
(Hartmann and Ahring, 2006) and health issues associated with the vermin attracted (Cunningham and Cunningham, 2002), the generation of unpleasant odours, contamination of ground water aquifers and surrounding surface water (Banar et al., 2006; Sabahi et al., 2009) and loss of valuable resources (Qasim and Chiang, 1994; El–Fadel et al., 1997). A life cycle assessment has found landfill to be the worst waste management option (Cherubini et al., 2009).

The recent rapid rise in global urban population has not only led to an increase in the quantity of waste produced but also a change in its nature, with a higher proportion of organic material being disposed. Historically landfill sites were often shallow and small in area, which allowed the biodegradable organic substances present in the waste to be aerobically degraded to carbon dioxide (CO$_2$), water and other by–products such as microbial biomass (Campbell, 1989). Modern landfills, however, are large and deep and the waste undergoes compaction as it is processed so waste decomposition is primarily anaerobic (Campbell, 1989). This anaerobic, or oxygen (O$_2$) free, microbial degradation results in the formation of biogas, a mixture of methane (CH$_4$) and CO$_2$ and is the basis for considerable environmental concern. Even though the CH$_4$ produced during the anaerobic degradation of organic waste provides the opportunity for energy recovery (Ham et al., 1979), it has often been considered as a liability because it (El–Fadel et al., 1997):

- Is flammable and can create an explosive mixture with the O$_2$ in air.
- Can migrate away from the landfill boundaries (e.g. Brookland Greens: Cooper, 2008).
- Can displace soil O$_2$ resulting in vegetation damage.
• Can escape into the atmosphere contributing to global warming. Current estimates by the Australian Greenhouse Office predict 900kg of CO₂ equivalents are emitted to the atmosphere per metric ton of MSW landfilled (Oakes, 2009).

On a molecular basis CH₄ is 20 times more efficient than CO₂ as a greenhouse gas (Bitton, 2005) as it has a greater capacity to absorb infrared radiation (Blake and Rowland, 1988) and tends to persist longer in the atmosphere (Gardner et al., 1993) with an estimated atmospheric life–expectancy of 12 years (World Meteorological Organisation, 2002). CH₄ is the second most important greenhouse gas emitted from human activity (CO₂ being the first) with total anthropogenic sources (355 TgCH₄/year in 2002) more than doubling that emitted from natural sources such as wetlands, termites and oceans (160 TgCH₄/year) (Wuebbles and Hayhoe, 2002). It has been estimated that CH₄ contributes approximately 18% towards total global warming (Church and Shepherd, 1989). However, this view is not unanimously supported within the scientific community, with a growing number of scientists opposing the concept of global warming claiming that there is “no detectable” warming in the lower troposphere (Gray, 2006).

1.3 Anaerobic Digestion

1.3.1 A Controlled Environment

Placing biodegradable organics into an uncontrolled landfill can lead to the release of odour and CH₄ into the atmosphere and contaminated leachates into groundwater courses. However, the production of CH₄ via biological degradation of these substances is not undesirable; it is the uncontrolled release of CH₄ (odour and leachate) into the environment that is objectionable. Consequently, significant environmental benefits can be achieved by confining this biological degradation process and harnessing the CH₄
produced. One process that exploits the capabilities of anaerobic communities by enclosing them in a controlled environment, so that polluting substances can be degraded and products captured at optimal rates (Hobson and Wheatley, 1993), is termed anaerobic digestion (AD).

AD is the process that converts organic matter into gaseous products, in particular CH\textsubscript{4} and CO\textsubscript{2}, by a mixed population of micro–organisms in the absence of light and O\textsubscript{2} (Hawkes and Hawkes, 1987; Schink, 1997; Mata–Alvarez, 2003). The overall process can be described as (Polprasert, 1989):

\[
\text{Organic matter} \rightarrow \text{CH}_4 + \text{CO}_2 + \text{H}_2 + \text{NH}_3 + \text{H}_2\text{S} \quad \text{(Eq. 1.1)}
\]

It is not a new process and has long been used for the stabilisation of wastes with the microbial nature of CH\textsubscript{4} production being discovered more than a century ago (Koster, 1988). AD represents an attractive waste treatment methodology due to its many advantages which include (Bitton, 2005):

- Inexpensive running costs (Bitton, 2005) as O\textsubscript{2} does not need to be provided. CO\textsubscript{2}, which is readily available, is the principle electron acceptor (Anderson et al., 2003).
- A small yield of biomass (McCarty, 1964a) (3–20 times less when compared to aerobic processes), decreasing the burden on down–stream processing.
- Waste reduction, with a large amount of organic matter converted to gaseous end–products.
- Energy production, with about 90\% of the energy available within the organic substrate being conserved in the CH\textsubscript{4} produced (Bryant, 1979; McInerney and Bryant, 1981) which can be used to offset energy consumption.
- The process is suitable for high–strength industrial wastes and there exists the possibility of accepting high loading rates.
• Anaerobic activity can be preserved during periods of extended starvation.
• Pollution reduction and odour control (Hawkes and Hawkes, 1987).

Disadvantages consist of slower degradation rates (compared to aerobic digestion), increased sensitivity to toxicants and long start–up periods (Bitton, 2005).

The degradation of organic matter to CH₄ and CO₂ is widespread in natural anoxic environments such as freshwater sediments, flooded soils, tundra, peat bogs and marshes (Bitton, 2005) when O₂, sulfur, sulfate, nitrate (Bryant, 1979; McInerney and Bryant, 1981) or oxidised iron or manganese species (Schink, 1997) are available in limited supply. During the formation of CH₄ from organic matter, electrons from within the waste are transferred to CO₂ resulting in the formation of CH₄. The presence of other electron acceptors, such as those listed above, initiates competition with CO₂ to be the final electron acceptor, decreasing CH₄ production.

AD involves four (4) major functional groups of micro–organisms that work synergistically to allow CH₄ formation from complex organic substances, with only one of these groups having the capacity to produce CH₄ (Mah et al., 1977; Bryant, 1979). These functional groups are hydrolytic, fermentative acidogenic bacteria, acetogenic bacteria and methanogens (Anderson et al., 2003; Bitton, 2005).

1.3.2 Stages of Anaerobic Digestion

Micro–organisms that produce CH₄ (methanogens) utilize a very limited number of substrates, chiefly acetate, and hydrogen (H₂) and CO₂ (Hawkes and Hawkes, 1987). Consequently the first stage of AD is to break down (hydrolyse) the large organic molecules (e.g. lipids, proteins, carbohydrates) present in the feedstock. Hydrolytic micro–organisms secrete exo–enzymes (e.g. cellulases, proteases and lipases), which
cleave large molecules into smaller ones that can be directly metabolised by microorganisms and include fatty acids, glycerol, peptides, amino acids, oligosaccharides and sugars (Miron et al., 2000; Anderson et al., 2003; Bitton, 2005) (Fig. 1.1). The hydrolysis of complex polysaccharides, contained within cellulolytic substrates, occurs slowly and is thus considered to be the rate limiting step (Hawkes and Hawkes, 1987) of AD processes. Even though cellulose, hemicellulose and pectin undergo hydrolysis anaerobically, lignin does not (Hawkes and Hawkes, 1987; Angelidaki and Sanders, 2004) and may protect the polysaccharides from enzymatic attack (Hawkes and Hawkes, 1987). It is also important to note that cellulose degradation is limited in media containing simple sugars as cellulase expression is down-regulated in the presence of glucose (De Vries and Visser, 2001).

The hydrolysed products are further degraded by fermentative acidogenic bacteria, into short chain fatty acids (e.g. acetic, propionic, butyric, formic, lactic and succinic acid) alcohols and ketones (e.g. ethanol, methanol, glycerol, acetone), CO$_2$ and H$_2$ (Fig. 1.1), with the products formed being dependent upon the bacterial type and environmental conditions (Bitton, 2005). The hydrolytic and fermentative bacteria contain both obligate and facultative anaerobes; the latter being responsible for removing small amounts of O$_2$ present in the system thus maintaining anaerobic conditions (Hawkes and Hawkes, 1987). Anaerobic conditions are essential for AD as methanogens require the redox potential to be maintained below about −300mV for biological growth (McInerney and Bryant, 1981).

The main function of the acetogenic bacteria in AD is the production of acetate, CO$_2$ and H$_2$, the primary substrates for CH$_4$ production. Two distinct groups of acetogens can be identified based on their metabolism: the obligate hydrogen producing acetogens
(OHPA) and the homoacetogens (Anderson *et al.*, 2003) (Fig. 1.1). The OHPA degrade short (propionate and butyrate) and longer chain fatty acids (via β-oxidation), alcohols, and aromatic compounds under conditions of low H₂ partial pressure (Anderson *et al.*, 2003; Bitton, 2005). In contrast, the homoacetogens produce acetate from CO₂ and H₂ but under certain conditions (temperature: 50–65°C and low H₂ partial pressure) can produce H₂ and CO₂ from acetate (Lee and Zinder, 1988b).

The methanogens, which produce CH₄ as the end-product of their metabolism, are distinct from bacteria and classified in a separate domain called *Archaea*. They use a
limited range of substrates with acetate, CO₂ and H₂ being the most important. Based on substrate specificity, the methanogens can be subdivided into two (2) categories, hydrogenotrophic and acetoclastic methanogens (Anderson et al., 2003). Hydrogenotrophic methanogens utilize H₂ to reduce CO₂ to CH₄ while acetoclastic methanogens cleave acetate to CH₄ and CO₂ with the methyl moiety of the acetate being reduced to CH₄ and the carboxyl moiety oxidised to CO₂ (Mah et al., 1978).

Almost all known species of methanogen utilize H₂ and CO₂ for growth (McInerney and Bryant, 1981) as shown by Equation 1.2 (ΔG°' derived from Thauer et al., 1977):

\[ 4 \text{H}_2 + \text{HCO}_3^- + \text{H}^+ \leftrightarrow \text{CH}_4 + 3 \text{H}_2\text{O} \quad \Delta G^{\circ'} = -135.6 \text{ kJ/mol HCO}_3^- \quad (\text{Eq. 1.2}) \]

The large negative value of ΔG°' indicates that the reaction is spontaneous, under standard conditions, and that the equilibrium favours the consumption of H₂. Due to the high affinity of methanogens for H₂ (the half saturation concentration (kₛ) for H₂ is between 2.5 and 13 μM: Robinson and Tiedje, 1984; van Bodegom and Scholten, 2001) the partial pressure of H₂ is kept low at around 10⁻⁴ atm in a well balanced reactor (Hawkes and Hawkes, 1987), which avoids OHPA inhibition (Anderson et al., 2003).

Acetate is an important substrate for methanogens and about 70% of CH₄ produced from organic matter in anaerobic digesters is produced via acetoclastic methanogenesis (Anderson et al., 2003) (the remaining 30% is derived from hydrogenotrophic methanogenesis). However, only two methanogenic species, Methanosarcina and Methanosaeta (Jetten et al., 1992; Zinder, 1990) metabolise acetate. Interestingly, the amount of free energy available from using this key substrate (Eq 1.3) (ΔG°' derived from Thauer et al., 1977) is barely sufficient, under standard conditions, to produce one mole of adenosine triphosphate (ATP) (ΔG°' = -30.0 kJ/mol ATP). This limited energy
yield could explain the relatively low maximum specific growth rate ($\mu_{\text{max}}$) of acetoclastic methanogens on this substrate (McInerney and Bryant, 1981) compared to hydrogenotrophic methanogens (Table 1.1).

$$
\text{CH}_3\text{COO}^- + \text{H}_2\text{O} \leftrightarrow \text{CH}_4 + \text{HCO}_3^- \quad \Delta G^\circ' = -31.0 \text{ kJ/mol CH}_3\text{COO}^- \quad (\text{Eq. 1.3})
$$

Comparing $\Delta G^\circ'$ from Equations 1.2 and 1.3, it can be inferred that a greater biomass yield would be possible when $\text{H}_2$ is used as substrate, compared to acetate, and therefore methanogens would preferentially consume $\text{H}_2$ if present in the reactor (Ferguson and Mah, 1983a).

**Table 1.1:** Maximum specific growth rate ($\mu_{\text{max}}$) of methanogens during the metabolism of acetate or $\text{H}_2/\text{CO}_2$ as the principle energy source.

<table>
<thead>
<tr>
<th>Species</th>
<th>Substrate</th>
<th>$\mu_{\text{max}}$ (h$^{-1}$)</th>
<th>Temperature (°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Methanosarcina</em> sp.</td>
<td>Acetate</td>
<td>0.030–0.064</td>
<td>50–55</td>
<td>Mladenovska and Ahring, 2000</td>
</tr>
<tr>
<td><em>Methanosarcina</em> barkeri</td>
<td>Acetate</td>
<td>0.028</td>
<td>30</td>
<td>Schöenheit <em>et al.</em>, 1982</td>
</tr>
<tr>
<td><em>Methanosarcina barkeri</em></td>
<td>Acetate</td>
<td>0.008</td>
<td>37–42</td>
<td>Maestrojuan and Boone, 1991</td>
</tr>
<tr>
<td><em>Methanosarcina</em> mazei</td>
<td>Acetate</td>
<td>0.013</td>
<td>37–42</td>
<td>Maestrojuan and Boone, 1991</td>
</tr>
<tr>
<td><em>Methanosarcina</em> barkeri</td>
<td>$\text{H}_2/\text{CO}_2$</td>
<td>0.087</td>
<td>37–42</td>
<td>Maestrojuan and Boone, 1991</td>
</tr>
<tr>
<td><em>Methanosarcina</em> mazei</td>
<td>$\text{H}_2/\text{CO}_2$</td>
<td>0.082</td>
<td>37–42</td>
<td>Maestrojuan and Boone, 1991</td>
</tr>
<tr>
<td><em>Methanoculleus</em> sp</td>
<td>$\text{H}_2/\text{CO}_2$</td>
<td>0.084</td>
<td>50–55</td>
<td>Cheng <em>et al.</em>, 2008</td>
</tr>
</tbody>
</table>

1.3.3 Anaerobic Digestion – a Hydrogen Balancing Act.

Wolin (1974) demonstrated that the concentration of $\text{H}_2$ in an ecosystem plays an important role in influencing the products formed by fermentative bacteria. Sugars generated from the hydrolysis of polysaccharides are primarily metabolised, by the Embden–Meyerhof pathway, to pyruvate. During this process, energy in the form of ATP is produced and nicotinamide adenine dinucleotide (NAD$^+$) is reduced to NADH (Madigan *et al.*, 2000). The ultimate fate of the pyruvate formed is dependent upon environmental factors and the need for the bacteria to regenerate their limited pool of NAD$^+$ (Wolin, 1974) to enable further energy generation from sugars (Eq. 1.4).
positive value of $\Delta G^{\circ}$ indicates that under standard conditions NADH oxidation will not occur, with the reaction proceeding only when $H_2$ levels are very low (McInerney and Bryant, 1981).

$$NADH + H^+ \leftrightarrow H_2 + NAD^+ \quad \Delta G^{\circ} = +18.0 \text{ kJ/mol NADH} \quad (Eq. 1.4)$$

The low $H_2$ partial pressure required for oxidation of the coenzyme, NADH, can be obtained in an anaerobic environment when the methanogens present, due to their high affinity for $H_2$ ($k_s \approx 4 \mu M$: Robinson and Tiedje, 1984), can remove the $H_2$ (Eq. 1.2) as it is formed. This co-operation between fermentative bacteria and hydrogenotrophic methanogens, or indeed any dependent partner micro-organisms, is termed syntrophy (Schink, 1997). That is, with respect to the Gibb’s free energy ($\Delta G$) of a biological process, neither partner can operate without the other, and together they exhibit a metabolic activity that neither one could accomplish on its own. However, in a stressed anaerobic system (overloading the system with substrate, incorrect pH, the presence of inhibitors, low numbers of methanogens, etc.), where the methanogens present are not capable of consuming the $H_2$ at the same rate it is produced, the partial pressure of $H_2$ will rise and NADH will no longer be able to be oxidised via $H_2$ production. As the re-oxidation of NADH is critical for bacterial energy production, at elevated $H_2$ partial pressures electrons carried by the NADH are most commonly transferred onto pyruvate (Wolin, 1974) and reduced “electron sink” fermentation products are formed such as lactate, propionate and longer chain fatty acids (Wolin, 1974; McInerney and Bryant, 1981). Therefore, in an anaerobic system where methanogens are efficiently removing $H_2$, the tendency will be for fermentative bacteria to produce more acetate, $H_2$ and $CO_2$ and less propionate, butyrate, lactate and ethanol.
The \( H_2 \) partial pressure is also a critical parameter in propionate and butyrate catabolism. The positive \( \Delta G^{\circ'} \) for the degradation of propionate (Eq. 1.5) and butyrate (Eq. 1.6) to acetate, \( CO_2 \) and \( H_2 \), indicates that neither reaction will proceed under standard conditions. However, due to the syntrophic relationship between OHPA and hydrogenotrophic methanogens present in methanogenic environments, the \( H_2 \) partial pressure is maintained at a very low level and consequently, the consumption of propionate and butyrate become thermodynamically feasible. Studies have indicated that butyrate and propionate degradation will only occur when \( H_2 \) partial pressures are below \( 2 \times 10^{-3} \) and \( 9 \times 10^{-5} \) atm, respectively (McInerney and Bryant, 1981). Therefore a small rise in \( H_2 \) partial pressure will first inhibit the degradation of propionate followed by butyrate. This sequential inhibition was reported by Borjesson and colleagues (1997), and Marchaim and Krause (1993), where propionate was found to be the first volatile fatty acid (VFA) to accumulate during the failure of a continuously–stirred–tank reactor (CSTR). It is therefore clear that \( H_2 \) not only influences the fermentation end–products but also their degradation, highlighting the important role that methanogens play in anaerobic ecosystems.

\[
\begin{align*}
CH_3CH_2COO^- + 3 H_2O & \leftrightarrow CH_3COO^- + HCO_3^- + H^+ + 3 H_2 \\
\Delta G^{\circ'} & = +76.1 \text{ kJ/mol propionate} \quad \text{(Eq. 1.5)}
\end{align*}
\]

\[
\begin{align*}
CH_3CH_2CH_2COO^- + 2 H_2O & \leftrightarrow 2 CH_3COO^- + H^+ + 2 H_2 \\
\Delta G^{\circ'} & = +48.1 \text{ kJ/mol butyrate} \quad \text{(Eq. 1.6)}
\end{align*}
\]

### 1.3.4 Environmental Factors Affecting Anaerobic Digestion

Effective anaerobic degradation of organic material requires viable populations of the relevant microbial groups described in Section 1.3.2 above. The micro–organisms reported to be the most delicate in anaerobic environments are the OHPA and acetoclastic methanogens (Rozzi, 1991; Kayhanian, 1994). As the bulk of \( CH_4 \) in AD
systems is typically generated via the acetoclastic pathway, factors which influence the efficient metabolism of the methanogens have a direct influence on the effectiveness of digestion. To elaborate: the culmination of hydrolytic, fermentative and acetogenic bacterial groups during AD, is the production of organic acids, and in particular, acetic acid. If, in an anaerobic system, the methanogens present were functioning sub-optimally, the likely outcome would be an accumulation of organic acids and, in a poorly buffered system, a decrease in pH. Since methanogens are most active in the pH range from 6.7 to 7.4 (Bryant, 1979; McInerney and Bryant, 1981; Anderson et al., 2003), continued pH depression would result in loss of methanogenic activity and the cessation of the breakdown of organic material. Consequently, the critical nature of the syntrophic relationships that exist between these micro–organisms requires that environmental conditions be stringently monitored and controlled if process failure is to be avoided (Anderson et al., 2003). These factors include nutrient availability, temperature, pH, hydraulic retention time, agitation and toxicity.

1.3.4.1 Nutrients

The nutritional requirements of anaerobic bacteria are simple in that they only require inorganic minerals and organic substrates that are utilized as energy sources. Other nutritional requirements are supplied from either cell lysis or the metabolites secreted by other micro–organisms (McInerney and Bryant, 1981). These nutrient requirements can be categorized as macro– or micronutrients. The function of these nutrients on the growth and metabolism of anaerobic micro–organisms is well documented in the literature, as summarised in Table 1.2. Trace elements (micronutrients), although needed in low concentrations, and macronutrients are essential for optimal microbial growth (Rajeshwari et al., 2000; Kim et al., 2002; Lettinga, 2005), efficient fermentation (Kim et al., 2002) and granulation (Tiwari et al., 2006). Nutritional
deficiencies can result in an incomplete, unstable bioconversion (Speece and Parkin, 1987) as reported by Speece and colleagues (2006) where the lack of calcium and nickel limited the rate of anaerobic treatment. It has been estimated that micronutrients should be present at twice the optimum concentration required (Rajeshwari et al., 2000), as the metabolic processes of anaerobic bacteria can be significantly decreased even with slight nutrient deficiencies (Anderson et al., 2003). Nevertheless, many essential nutrients can become toxic when present in excess (Lin and Chen, 1999; Mata–Alvarez, 2003; Anderson et al., 2003). Most crude substrates utilized for CH₄ fermentations generally contain sufficient minerals to satisfy the nutrient requirements for microbial growth (Mata–Alvarez, 2003). The biodegradable organic fraction of municipal solid waste (OFMSW) has however been reported to be deficient in most macro and micro–nutrients (Kayhanian and Rich, 1995) with many urban wastes requiring the addition of ammonia (NH₃), phosphate, sulphide or iron (McInerney and Bryant, 1981). Conversely, Mata–Alvarez (2003) reported OFMSW to contain macro– and micronutrients at quantities high enough for micro–organism growth not to be limiting. It is therefore likely that the nutrient content of OFMSW is region/site specific and appropriate analysis must be applied to each feedstock to ensure stable bioconversion.

1.3.4.2 Temperature

Temperature is one of the most influential environmental factors on the activity of micro–organisms. Generally, a rise in temperature gives a corresponding rise in the rate of biological reactions and hence, increased growth rates. For example, during acetate consumption by methanogens, an increase in temperature gives an increase in $\mu_{\text{max}}$ and a greater affinity for its substrate resulting from a decrease in $k_s$ (Lawrence and McCarty, 1969). However, above a species specific temperature, irreversible protein denaturation occurs resulting in disruption of cell membrane and cell death (Madigan et


**Table 1.2**  Concentrations of macro- and micronutrients that have been reported to improve process performance and the effect on some physiological and operational processes of anaerobic digestion (Adapted from Kayhanian and Rich, 1995).

**Legend:**  
CODH = the enzyme carbon monoxide dehydrogenase  
FDH = the enzyme formate dehydrogenase  
SODM = the enzyme superoxide dismutase  
MR = the enzyme methyl reductase

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Concentration (mg/L)</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Macronutrients</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbon, C</td>
<td></td>
<td>Basic building block of bacterial cell material and the primary source of energy 1.</td>
</tr>
<tr>
<td>Nitrogen, N</td>
<td>C:N = 30:1 6</td>
<td>Amino–nitrogen (R–NH₂) is essential for protein synthesis 1.</td>
</tr>
<tr>
<td>Potassium, K</td>
<td></td>
<td>Increases cell wall permeability by aiding transport of nutrients and cation balancing 1.</td>
</tr>
<tr>
<td>Sulfur, S</td>
<td>0.001–1.0 4</td>
<td>Enzyme synthesis including CODH a key enzyme in the acetyl–CoA pathway 2 and FDH 1.</td>
</tr>
<tr>
<td><strong>Micronutrients</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium, Ca</td>
<td>100–200 3</td>
<td>Granulation and increase activity 3.</td>
</tr>
<tr>
<td>Magnesium, Mg</td>
<td>75–150 3</td>
<td>Granulation and increase activity 3.</td>
</tr>
<tr>
<td>Sodium, Na</td>
<td>100–200 3</td>
<td>Increase activity 3.</td>
</tr>
<tr>
<td>Iron, Fe</td>
<td>10–110 7</td>
<td>Found in and increases activity of specific enzymes and sulphide precipitation 1,3 and promote the excretion of extra cellular enzymes 3.</td>
</tr>
<tr>
<td>K</td>
<td>200–400 3</td>
<td>Increase activity 3.</td>
</tr>
<tr>
<td>Ba</td>
<td>0.01–0.1 3</td>
<td>Biomass granulation 3.</td>
</tr>
<tr>
<td>Cobalt, Co</td>
<td>20 3</td>
<td>Present in specific enzymes such as CODH and corrinoids 1. Corrinoids contain a corrin ring as the core structure such as vitamin B12. CODH plays an important role in activity of acetogens and methanogens 2.</td>
</tr>
<tr>
<td>Se</td>
<td>0.8 3</td>
<td>Formate dehydrogenase, glycerine reductase, hydroxylase and dehydrogenase dependent.</td>
</tr>
<tr>
<td>Copper, Cu</td>
<td>0.08 8</td>
<td>Copper may be a component in SODH and hydrogenase 1.</td>
</tr>
<tr>
<td>Molybdenum, Mo</td>
<td>&lt;1–5 1</td>
<td>Present in FDH and may inhibit sulphate reducing bacteria 1.</td>
</tr>
<tr>
<td>Nickel, Ni</td>
<td>0.05–0.29 5</td>
<td>Present in coenzyme F430 5 which is used in the terminal step of methanogenesis as part of the MR complex 2 and CODH.</td>
</tr>
<tr>
<td>Selenium, Se</td>
<td>&lt;0.05 1</td>
<td>Present in anaerobic bacterial enzymes such as FDH and nucleic acids. Enzymes have a low redox potential and may assist in the metabolism of fatty acids 1.</td>
</tr>
<tr>
<td>Tungsten, W</td>
<td>&lt;1 1</td>
<td>Component of FDH and may assist in CO₂ and H₂ metabolism 1.</td>
</tr>
<tr>
<td>Zinc, Zn</td>
<td>0.41 8</td>
<td>May be part of FDH, SODM and hydrogenase 1.</td>
</tr>
</tbody>
</table>

**References:**  
2. Madigan *et al.*, 2000  
4. Speece, 1996  
5. Diekert *et al.*, 1981  
6. Anderson *et al.*, 2003  
7. Speece and Parkin, 1987  
8. Zoetemeyer *et al.*, 1982  
Consequently, even though AD can occur over a wide range of temperatures between 0 and 100°C, distinct groups of micro–organisms possess different temperature ranges for optimum growth and performance. These include psychrophilic species: 15–20°C (Anderson et al., 2003); mesophilic: 25 to 40°C; thermophilic: 50 to 75°C (Bitton, 2005) and some hyper–thermophilic species: 80–85°C (Prokofeva et al., 2009).

Operating AD at higher temperature provides significant advantages including:

- The kinetics are faster and biogas yields are greater at higher temperatures. Thermophilic AD has been reported to be more efficient than mesophilic AD (Kim et al., 2002) with CH₄ production being two to three times greater (Cecchi et al., 1991).
- The process can accept higher loading rates (Cecchi et al., 1991).

Operating AD processes at elevated temperature also has some disadvantages such as:

- Less stable (Anderson et al., 2003). AD is sensitive to small changes in temperature (Bitton, 2005) and imbalances may occur due to the complexity of the steps involved (Mata–Alvarez, 2003). Methanogens grow more slowly than acidogens (Bitton, 2005) thus, at higher temperature, acidogenesis may produce more acids than can be consumed via methanogenesis (Mata–Alvarez, 2003) with changes as small as 1–2°C having significant adverse effects (Anderson et al., 2003) particularly during the digestion of highly degradable substrates.
- Requires more energy to maintain an elevated temperature. In many cases the increased energy demand is the same as the excess energy production from the greater biogas yield (Mata–Alvarez, 2003).
- More sensitive to toxicants (Koster, 1988).
- Micro–nutrient addition appears to be more critical (Speece et al., 2006).
Even though biogas production and reaction kinetics are more favourable at thermophilic temperatures, optimal conditions depend upon the biodegradability and concentration of the substrate (Mata–Alvarez, 2003).

1.3.4.3 pH
Anaerobic micro–organisms are sensitive to extremes in pH. The hydrogen ion concentration has a critical influence on the biochemistry of the micro–organisms, membrane integrity and permeability, system buffering and the availability of dissolved ions. Most methanogens function optimally in a pH range of 6.5 – 7.8 (Anderson et al., 2003), with process failure occurring if the pH is below 5.5 (Kim et al., 2002) and limited methanogenesis above a pH of 8.0 (McInerney and Bryant, 1981). Acidogenic bacteria produce acids which lower the pH but under normal conditions the pH is buffered by bicarbonate produced by methanogens (Pretorius, 1994). However, under adverse conditions, the buffering system can be overcome resulting in decreased pH. As acidity is a greater inhibitor of methanogens than acidogenic bacteria (Bitton, 2005), organic acids would continue to be produced resulting in further suppression of the pH, and ultimately, methane production would cease. Consequently ensuring adequate buffer in an AD system is a critical parameter in maintaining efficient bioconversion.

1.3.4.4 Toxicity
1.3.4.4.1 Oxygen
Methanogens are regarded as obligate anaerobic micro–organisms with little tolerance to O₂ (Thauer, 1998). The presence of molecular O₂ increases the redox potential of an anoxic environment. It is generally accepted that, for the production of CH₄, methanogens require a highly reduced (no oxygen) environment, having a redox potential less than –150mV (Wang et al., 1993; Yu and Patrick, 2003). Fetzer and
Conrad (1993) reported that the inhibitory effect of O₂ on methanogenesis may be explained by the rapid increase in redox potential (above the critical potential of +50mV) resulting from O₂ addition (>0.5%) but could not discount the possibility that CH₄ inhibition was via a toxic mechanism. However, methanogens are present, and metabolically active, in habitats that are not completely devoid of O₂ (Peters and Conrad, 1995; Conklin et al., 2007; Liu et al., 2008). It has been proposed that facultative and aero–tolerant micro–organisms in these environments can decrease molecular O₂ concentrations rapidly, thus maintaining an anoxic condition for more O₂ sensitive species (Kato et al., 1993). However, studies with some pure methanogenic strains have demonstrated not only methanogenic activity but growth in the presence of low levels of O₂ (Leadbetter and Breznak, 1996; Tholen et al., 2007). The superoxide radical (O₂⁻*), one of the products of incomplete oxidation of molecular O₂, is highly reactive (Fridovich, 1978). It damages cellular proteins and metabolic intermediates (Farr and Kogoma, 1991) and must be neutralised to maintain cell integrity. Superoxide dismutase and catalase, enzymes which aid in the neutralisation of O₂⁻*, have been found in some methanogenic strains (Brioukhanov et al., 2002) and are hypothesised to be induced by the presence of O₂ (Brioukhanov and Netrusov, 2004). It therefore seems reasonable to suppose that the ability to survive oxic conditions is partially associated with the activity of these detoxification enzymes (Brioukhanov et al., 2002; Liu et al., 2008).

1.3.4.4.2 Ammonia

Ammonia (NH₃) is released by the biological degradation of nitrogenous matter such as amino acids, proteins and urea (Anderson and Yang, 1992; Gallert and Winter, 1997; Kayhanian, 1999). The NH₃ in an AD system acts to buffer against the acidity generated during the digestion (Anderson and Yang, 1992). As nitrogen is an essential
nutrient, NH$_3$ is beneficial to methanogens at low concentrations (< 200mg/L (12mM): Liu and Sung, 2002) but at high concentrations it has been found to be inhibitory (Kayhanian, 1994; Poggi–Varaldo et al., 1997; Hansen et al., 1998; Bujoczek et al., 2000; Anderson et al., 2003), with the inhibitory effect being very acute (Poggi–Varaldo et al., 1997). Several mechanisms have been proposed for the inhibition, such as change in intracellular pH, increase in maintenance energy requirement (Sprott et al., 1984; Whittmann et al., 1995) and inhibition of specific enzymes (Whittmann et al., 1995). Depending on the pH, the total ammonia–nitrogen (TAN) can be in the form of an ammonium ion (NH$_4^+$) or molecular NH$_3$ (free NH$_3$) as described in equation 1.7:

$$\text{NH}_4^+ (\text{aq}) \leftrightarrow \text{NH}_3 (\text{aq}) + \text{H}^+ (\text{aq}) \quad \text{(Eq. 1.7)}$$

Both forms of TAN are toxic to some degree (Whittmann et al., 1995), however, free NH$_3$ is suggested to be the main cause of inhibition (McCarty and McKinney, 1961; de Baere et al., 1984; Sprott et al., 1984; Zeeman et al., 1985; Sprott & Patel, 1986; Weigant and Zeeman, 1986) as the molecule can diffuse passively through the cell membrane of methanogens (de Baere et al., 1984). The pK$_a$ for equation 1.7 (the pH where the concentrations of free NH$_3$ and NH$_4^+$ are equal) is 9.25 (Kayhanian, 1999) consequently, at pH 7 most of the ammonia nitrogen (NH$_3$–N) is present as the less toxic NH$_4^+$ (Anderson et al., 2003). An increase in pH increases the concentration of free NH$_3$ and therefore the degree of toxicity. Even though methanogens are considered to be the most sensitive of the AD micro–organisms, and are most likely to be inhibited by free NH$_3$ (Kayhanian, 1994), there exists conflicting evidence in the literature about the NH$_3$ sensitivity of acetoclastic or hydrogenotrophic methanogens. Some researchers (Koster and Lettinga, 1984; Zeeman et al., 1985; Sprott & Patel, 1986; Angelidaki and Ahring, 1993; Borja et al., 1996b; Hansen et al., 1998; Schnürer et al., 1999; Schnürer and Nordberg, 2007) found acetoclastic methanogens to be more sensitive to NH$_3$
toxicity than hydrogenotrophic methanogens, while others (Wiegent and Zeeman, 1986) observed NH₃ to have only a minor effect on acetoclastic methanogenesis.

The equilibrium described in equation 1.7 is endothermic therefore an increase in temperature will favour the formation of free NH₃. An elevated temperature will also decrease the solubility of CO₂ resulting in elevated pH. Consequently, the combined effect of raising the AD temperature will elevate the free NH₃ concentration and its associated toxicity (Gallert and Winter, 1997). Angelidaki and Ahring (1994) found poor digestion performance when elevated temperature (55–64°C) and high NH₃ (6.0gN/L) were combined (free NH₃ = 0.7gN/L). The observed poor performance could be alleviated by decreasing the temperature (<55°C). Conversely, Gallert and Winter (1997) found that thermophilic AD flora were able to tolerate at least twice as much free NH₃ than mesophilic flora (NH₃ KI = 0.69 and 0.22 g/L respectively).

The toxic effects of NH₃ have been reported to be, to some extent, reversible (Koster and Lettinga, 1984) and are able to be minimised during AD. Lowering the system pH will increase the concentration of the less toxic NH₄⁺. Process instability due to ammonia often results in the accumulation of VFA which decreases the pH and in turn lowers the free NH₃ concentration initiating an “inhibited steady state”, a condition where the process is operating stably but at a lower CH₄ production rate (Angelidaki et al., 1993b). Air stripping of NH₃ from process water, at high NH₃ concentrations, has been reported to be effective in diminishing NH₃ toxicity and increasing biogas yield (20%: Weiss, et al., 2009). The addition of zeolite (Borja et al., 1996a; Tada et al., 2005) and bentonite (Angelidaki and Ahring, 1992b) can lower the overall TAN concentration and partially counteract the NH₃–N inhibitory effects perhaps through the presence of cations such as Ca²⁺ and Na⁺ (Tada et al., 2005). Dilution of substrate
(Kayhanian, 1999; Bujoczek et al., 2000), increasing biomass retention times (Chen et al., 2008a) and acclimation of biomass (Koster and Lettinga, 1984; Angelidaki and Ahring, 1993; Bujoczek et al., 2000; Sung and Liu, 2003; Calli et al., 2004) have also been shown to decrease the effects of NH$_3$ toxicity. Acclimation at high TAN concentration was found to decrease the overall CH$_4$ activity, however, it increased the tolerance to TAN and pH variations (Sung and Liu, 2003). Chemical addition has also been investigated (powdered phosphorite ore: Krylova et al., 1997; Mg$^{2+}$ for struvite generation: Yoshino et al., 2003).

A wide range of inhibiting NH$_3$–N concentrations have been reported in the literature, with toxicant (TAN and free NH$_3$) concentrations that cause a 50% decrease in CH$_4$ production (K$_i$) summarised in Table 1.3. The significant difference in K$_i$ values can be attributed to differences in substrate, inocula, environmental conditions (temperature and pH) and acclimation (Chen et al., 2008a).

1.3.4.4.3 Fatty acids

VFA (acetate, propionate and butyrate) are toxic to acetoclastic methanogens (Aguilar et al., 1995; Ahring et al., 1995). Dogan et al., (2005) found that, under mesophilic conditions and pH 6.9–7.2, acetoclastic methanogenesis was completely inhibited by acetate, propionate and butyrate at 417, 68 and 284mM (25,000; 5,000 and 25,000 mg/L) respectively, while the K$_i$ was found to be 217, 47 and 170mM (13,000; 3,500 and 15,000 mg/L) respectively. Dhaked et al., (2003) found propionate not only to be inhibitory to methanogenesis but decreased the methanogenic count by a factor of 100. Acetate has been reported to inhibit propionate metabolism (Fukuzaki et al., 1990; van Lier et al., 1993; Pind et al., 2003).
Table 1.3  Concentrations of toxicant leading to 50% inhibition of methanogenic activity (K_I) reported in the literature.

<table>
<thead>
<tr>
<th>Toxicant</th>
<th>Temperature (°C)</th>
<th>Organism</th>
<th>Substrate</th>
<th>K_I</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH_3</td>
<td>35</td>
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<td>Acetate</td>
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</tr>
<tr>
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<td>Acetate</td>
<td>17</td>
<td>1.2</td>
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<tr>
<td>NH_3</td>
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<td>Anaerobic Sludge</td>
<td>Peptone</td>
<td>88</td>
<td>6.3</td>
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<td>Glucose</td>
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<td>16</td>
</tr>
<tr>
<td>NH_3</td>
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<td>6.8</td>
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<td>Acetate</td>
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<td>Peptone</td>
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<td>TAN</td>
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<td>214</td>
</tr>
<tr>
<td>TAN</td>
<td>39</td>
<td>Anaerobic Sludge</td>
<td>Simulated OFMSW</td>
<td>2300–2400</td>
<td>135–141</td>
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<tr>
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<td>Anaerobic Sludge</td>
<td>Peptone</td>
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<tr>
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<td>Cattle Manure</td>
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<td>107</td>
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<tr>
<td>Propionic Acid</td>
<td>35</td>
<td>Acetoclastic Methanogen</td>
<td>Acetate</td>
<td>3500</td>
<td>47</td>
</tr>
</tbody>
</table>

References:
2. Angelidaki et al., (1993a)
9. Borja et al. (1996b)
Lipids (fats) are an important substrate for AD due to their high CH₄ potential. The anaerobic hydrolysis of neutral fats proceeds easily to glycerol and long chain fatty acids (LCFA) (Hanaki et al., 1981) (Fig. 1.1), however LCFA (e.g. capric, lauric, myristic and oleic acids) have been found to inhibit the activity of acetoclastic methanogens (Hanaki et al., 1981, Koster and Cramer, 1987; Angelidaki and Ahring, 1992a) leading to failure of digestion. However, some research groups have reported on cultures capable of consuming LCFA (Angelidaki and Ahring, 1995; Grabowski et al., 2005; Shigematsu et al., 2006; Hatamoto et al., 2007; Sousa et al., 2007). Recent advances in this area of research have been reviewed by Sousa et al., (2009).

1.3.4.4.4 Heavy metals

Heavy metals (Cu²⁺, Pb²⁺, Cd²⁺, Ni²⁺, Zn²⁺, Cr⁶⁺) are inhibitory to anaerobic digesters (Lin, 1992; Mueller and Steiner, 1992; Lin, 1993; Yenigun et al., 1995; Lin and Chao, 1996; Sanchez et al., 1996; Yenigun et al., 1996; Leighton and Forster, 1998; Lin and Chen, 1999; Tiwari et al., 2006). The severity of the inhibition is dependent upon the concentration of the free metal ion (Mueller and Steiner, 1992), trophic group (Lin, 1992; Lin, 1993; Sanchez et al., 1996; Lin and Chen, 1999), pH, VFA concentration, reactor configuration and the type and form of the metal ion (Lin and Chen, 1999). Regarding heavy metal toxicity, acidogens are generally considered being less sensitive than methanogens (Tiwari et al., 2006) and propionate degrading acetogens more sensitive than hydrogenotrophic methanogens, for some metals (Cr & Zn: Lin, 1992). The sequence of toxicity demonstrated by mesophilic sewage sludge was found to be Cd>Cu>Cr=Zn>Pb>Ni (Lin, 1992), however acclimation has been found to change microbial sensitivities (Mueller and Steiner, 1992).
1.3.4.4.5 Sulfide

Sulfide (S^{2−}) is a potent inhibitor of AD (Li et al., 1996; O'Flaherty, 1999; Bitton, 2005). During AD sulfide can be produced by protein degradation and sulphate reduction (by sulphate–reducing bacteria (SRB)). Sulfide toxicity of acetoclastic methanogenesis is pH dependent with the hydrogen sulfide (H_{2}S) molecule (generally accepted as the toxicant) diffusing through the cell membrane more rapidly than ionised species (HS^{−} & S^{2−}: Koster et al., 1986). Using Eq. 1.8 (Kim et al., 2001):

\[
[H_{2}S]_{\text{free}} = [S^{2−}]_{\text{total}} \frac{[H^{+}]^{2}}{[H^{+}] \times K_1 + [H^{+}]^{2} + K_1 \times K_2}
\]

(Eq. 1.8)

where K_1 and K_2 are 9.1×10^{−8} and 1.1×10^{−12} (Weast et al., 1983) respectively, at neutral pH (6.8–7.2), where methanogens are most active, between 63 and 41% of the sulphide is present as H_{2}S. Methanogens have been reported to be more sensitive to H_{2}S than acidogens, with hydrogenotrophic methanogens more sensitive than acetoclastic methanogens (Rinzema and Lettinga, 1988). Koster et al., (1986) found a K_1 for methanogenic bacteria of 250mgS/L (7.8mM) at pH 6.4–7.2 while Isa et al., (1986) reported a K_1 of 1000mgS/L (31.3mM) and proposed that methanogens may be able to adapt to high levels of H_{2}S.

As metal sulfides readily precipitate (Mueller and Steiner, 1992), sulfide toxicity of methanogens may result from a lack of available essential micro–nutrients (Isa et al., 1986; Anderson et al., 2003). The insolubility of S^{2−} can also be used to mitigate H_{2}S toxicity by the addition of iron (Anderson et al., 2003).

An apparent inhibition of methanogenesis may also be a result of substrate limitation. If sulphate is present, methanogens and SRB compete for the same electron sources, acetate and H_{2} (Isa et al., 1986; Li et al., 1996; Bitton, 2005). A study of the growth
kinetics indicates that SRB have a higher affinity for acetate ($k_s = 9.5\text{mg/L} = 0.16\text{mM}$) than methanogens ($k_s = 32.8\text{mg/L} = 0.56\text{mM}$) (Yoda et al., 1987). The affinity of SRB for $\text{H}_2$ ($k_s$) has also been reported to be lower than for methanogens (Hawkes and Hawkes, 1987), therefore SRB out-compete methanogens when acetate and/or $\text{H}_2$ concentrations are low.

### 1.3.4.4.6 Agitation

The CH$_4$ produced during AD provides minimal mixing and is considered to be the rate limiting factor for efficient mass transfer (Anderson et al., 2003). Contact between organic matter and micro-organisms can be increased by agitation (mechanical stirring, pumping, gas recirculation). However, excessive mixing has been found to decrease reactor performance (Anderson et al., 2003) and suggests that stable digestion is dependent upon an optimal distance between syntrophic micro-organisms (McCarty and Smith, 1986; Kim et al., 2002; Speece et al., 2006).

Chlorinated hydrocarbons (Blum and Speece, 1992; Hickey et al., 1987), benzene compounds (Patel et al., 1991), cyanide (Gijen et al., 2000), tannins (Field and Lettinga, 1987) and salinity (Bitton, 2005) have all been shown to inhibit methanogens. Factors influencing the inhibition of AD have been recently reviewed by Chen et al., (2008a).

### 1.4 Municipal Solid Waste Treatment

#### 1.4.1 The Need for Action

Many people believe that humanity's greatest resource challenge is the imminent prospect of "peak oil" (Cawood, 2010) and that identifying alternative/renewable sources of energy is a matter of urgency (Greben et al., 2009). An equally important milestone in modern history will be the inevitable tightening of global supplies of
phosphorus with few nations possessing enough phosphorus to supply their own agricultural needs (Butler, 2009; Cawood, 2010). Consequently, it has been suggested that “peak phosphorus” will sound the end of chemical agriculture (Insidewaste, 2010). Appropriate waste management can address both of these issues. Western cultures must embrace the value of organic waste, in terms of its potential for energy generation, its nutrient value and its capacity to improve soil quality for food generation. Stabilised organic waste, returned to the soil, not only recycles valuable nutrients (decreasing the need for chemical fertiliser addition) but improves moisture retention in the soil and increases crop yield and soil carbon (Insidewaste, 2010).

1.4.2 MSW Treatment Regimes

In response to these issues, during recent decades, numerous waste management treatment strategies have been investigated for the treatment of MSW.

1.4.2.1 Incineration

Incineration, the simplest means of waste management (Neklyudov et al., 2006) has been reported as having the following advantages (McKay, 2002):

- The volume and mass of the waste is decreased and this decrease is immediate.
- Incineration facilities can be constructed close to the MSW sources or collection points, decreasing transportation costs.
- Using heat recovery technology, the cost of the operation can be decreased (Porteous, 2001).
- Air discharges can be controlled to meet environmental limits.
However, incineration does have its problems which include (McKay, 2002):

- Poor operating practices and the presence of chlorine in the MSW may lead to emissions. For example: particulates, unburned hydrocarbons (CH\textsubscript{x}), carbon monoxide (CO), nitrogen oxides (NO\textsubscript{x}), sulfur oxides (SO\textsubscript{x}), hydrochloric acid, fluorides, heavy metals, dioxin, furans and volatile organic compounds (VOCs) (Zhang, 1998; Porteous, 2001) such as polycyclic aromatic hydrocarbons (PAHs), phenol and benzene hydrocarbons (Chen et al., 2008b), that need to be captured and neutralized/treated. The expenses incurred during treatment of these emissions typically exceed the savings obtained from the low cost of incineration (Neklyudov et al., 2006).
- The control of metal emissions may be difficult for inorganic wastes containing heavy metals, such as arsenic, cadmium, chromium, copper, lead, mercury, nickel.
- Supplementary fuels are required to achieve the necessary high combustion temperatures.
- The bottom ash of incineration must be disposed of as a hazardous waste (Hartmann and Ahring, 2006).

Hartmann and Ahring (2006) in a recent review of MSW treatment reported that the content of OFMSW is inappropriate for incineration and concluded that incineration should only be used to treat the non–biodegradable component of MSW. Porteous (2001) reported that incineration was a natural companion to composting and recycling and should not be seen as a competing technology, reinforcing that incineration alone is an unsuitable solution for OFMSW management.

1.4.2.2 Composting

Composting is a well accepted method of processing organic waste with commercial–scale composting facilities (windrow) gaining credibility during the 1950’s and 60’s.
Since that time, significant advances have been made in understanding the interdependence of physical parameters and the biology of the process (Golueke and Diaz, 1996). Composting refers to the natural biological decomposition of organic matter accomplished by a diverse group of aerobic micro–organisms. The final product of such decomposition is a humus–like substance, which can be successfully used as an agent for soil improvement or organic fertilizer (Neklyudov et al., 2006). The main factors affecting the composting process have been determined to be temperature, moisture content, bulk density, porosity, O2 availability (Sharma et al., 1997; Mohee and Mudhoo, 2005) and carbon to nitrogen ratio (C:N) (Sharma et al., 1997).

**Temperature** undergoes considerable changes during composting with all composting processes known to go through three stages: (1) mesophilic (40–55°C), (2) thermophilic (5 to 25 days or possibly longer, with temperatures rising to 70°C or greater), and (3) cooling stage (or the final compost maturation) which may last for many weeks or months and ends with the compost cooling to ambient temperature. The duration of each stage depends on the composition of the organic waste and the degree of aeration. At elevated temperature the discharge of hazardous volatile compounds, which pollute the environment, intensifies (Neklyudov et al., 2006).

**Moisture content** in composted mixtures is an important parameter affecting the quality of a compost. Adequate composting efficiency is commonly observed if the moisture content of the material is within 50–70% (Stentiford and Dodds, 1992; Richard et al., 2002). Low moisture conditions decrease the microbial activities (Sharma et al., 1997) and restrict the movement of bacteria (Richard et al., 2002) while excess moisture will increase film thickness and fill the smaller pores between particles, limiting O2 transport (Richard et al., 2002).
Oxygen availability regulates the rate of biological reactions during composting, thus the mechanism of air delivery and/or airflow provides the primary means to control biological activity. Gas transfer is strongly affected by the physical properties of the substrate, which determine the geometry of a network of gas-filled pores (Sharma et al., 1997; Richard et al., 2004). Consequently, the bulk density and porosity of the material also become significant factors in the composting process. Three types of aeration of compost piles are known: (1) forced aeration, where air is pumped into the compost pile; (2) passive aeration, where air is drawn, via convection, through a series of pipes into the compost pile; and (3) natural aeration, where air is drawn naturally into the pile, which is typically coupled with compost turning to maintain porosity and ensure adequate aeration (Imbeah, 1998). Forced aeration has been reported to increase the composting rate and, in part, regulate the temperature of the process however, considerable amounts of volatile organic substances are lost (Liao et al., 1993) and maintaining appropriate moisture content becomes more complex (Neklyudov et al., 2006). Ruggieri and associates (2008) concluded that the added investment cost in forced–aeration systems is not necessary and recommended natural aeration and pile turning for OFMSW composting.

The optimum C:N ratio for composting has been reported to be 25:1, with higher values (more carbon) slowing down the rate of decomposition and lower ratios (more nitrogen) leading to nitrogen volatilisation (de Bertoldi et al., 1983; Sharma et al., 1997) resulting in elevated odour emission.

Trace elements, such as Ca and Mg, also affect the composting process. Such elements stimulate microbial activity and catalyse biochemical functions (Sharma et al., 1997). However, odorants of the dimethylsulfide type have been reported to increase
proportionally with sulfur–containing compounds in the raw material (Neklyudov et al., 2006).

The pH of the material during composting typically undergoes considerable change, varying within the range 4.5–8.1 (Neklyudov et al., 2006). During early composting, the formation of organic acids can result in pH values of approximately 5–6, however, as the process progresses, the pH value can rise to 8–8.5 (Sharma et al., 1997). Optimum activity of the micro–organisms have been reported at pH 7.0–8.0 for protein degradation, whereas carbohydrate degradation (taking place during the early stages of compost maturation) attained maximum rates at pH 6.0–9.0 (Nakassaki et al., 1993).

Recent studies in the composting of OFMSW have included the following:

• Weppen (2001) found that calorimetry could be used as a tool to predict microbial kinetics during composting and for full–scale composting plants the data could facilitate on–line calculation of bioactivity and mass balance within the system.

• Gea and associates (2004) investigated the suitability of different biological indices (oxygen uptake rate (OUR), respirometric index, and respiratory quotient) to monitor the biological activity of the composting process. They concluded that OUR provided the most reliable indicator of microbial activity in a compost environment. A static respirometric index, measured at 37°C, was found not be useful with samples obtained during the thermophilic phase, as it provided an underestimation of respiration.

• Norbu et al., (2005) showed that unsorted MSW, composted for 1 week, facilitated easier inorganic separation than untreated MSW and a 40% decrease in total mass. They concluded that a 1 week composting pre–treatment provided a significant advantage to developing countries utilising landfill.
• Barrena et al., (2006a) reported that the temperature of a large compost pile could be predicted by using a ‘static respiration index’, a measure of the biodegradable organic matter content of compost.

• Barrena and colleagues (2006b) found that the addition of a commercial inoculum (MicroGest 10X, Brookside Agra L.C.), at a concentration of \(10^6\) CFUg\(^{-1}\) of OFMSW, produced a significant acceleration of the composting process, with high levels of biological activity in the thermophilic phase, and a decrease (by approximately half) in total composting time, compared to the control. They also reported that respirometric index (determined at 55°C) and maturity grade appeared to be the most reliable tests to follow the biological activity of composting.

• Castaldi and associates (2008) reported that enzymatic activities and the chemical parameters of the water–soluble fraction, taken during composting, were suitable parameters for compost characterization. However, since the level of activity/concentration, at the end of composting, is dependent on the raw material used for composting, a single point determination was inadequate for accurate compost characterization.

• Ruggieri et al., (2008) investigated the performance of static and forced aeration composting systems and found that a static pile composting process out performed a static, and turned, pile with forced aeration. They concluded that the investment in providing forced aeration was not justified.

1.4.2.3 Anaerobic digestion

In light of the approaching, and inevitable, energy crisis resulting from dwindling world oil supplies, AD has attracted much attention in recent years primarily due to its positive energy balance (de Baere, 2006; Hartmann and Ahring, 2006). In a recent study, Kalyuzhnyi (2008) reported that the Russian agro–industrial sector could become
energy self–sufficient through proper utilisation of AD technologies for treating organic wastes. The challenge is therefore to improve process performance and stability so as to optimise biogas yield and consequently energy recovery. Accordingly, numerous process configurations have been applied to AD.

1.4.2.3.1 Wet verses dry

Depending on the moisture content of the AD, the process is termed ‘wet’ (low solids) if the total solids (TS) < 20%, ‘dry’ (high solids) if TS > 20% or ‘semi–dry’ if TS approximately 20% (Hartmann and Ahring, 2006). Six and De Baere, (1992) reported on the performance of a DRANCO (TS = 32%) installation treating 10,500t of OFMSW per annum in Belgium. de Laclos et al., (1997) reported on a Valorga (TS = 30%) full–scale plant treating 52,000t of OFMSW per annum in the Netherlands. Luning et al., (2003) compared the wet Vagron (TS = 12%) process with the dry Valorga process and found identical gas production between the two processes, though the dry process produced less wastewater. ten Brummeler (2000) reported on the BIOCEL process, a high solids (30–40%) AD treatment for solid organic wastes. In high solids AD, as complete mixing is not guaranteed, full contact between biomass and substrate is not guaranteed. Consequently, optimal co–operation between microbial groups may not be achieved leading to a decrease in biogas yield (Luning et al., 2003; Hartmann and Ahring, 2006).

1.4.2.3.2 Thermophilic verses mesophilic

Traditionally most AD was conducted at mesophilic temperatures as thermophilic processes were believed to be less stable, leading to process failure (Hartmann and Ahring, 2006). However, as understanding of the AD process has grown, many waste treatment plants now operate under thermophilic regimes (Ahring, 1994). Thermophilic
AD has also been reported to provide higher reaction rates, and therefore lower retention times and better pathogen removal but a greater sensitivity to toxicants (Hartmann and Ahring, 2006). Six and De Baere, (1992) found no faecal coliforms present in the end–product of the thermophilic DRANCO process whereas the feedstock contained 3000 CFU/g dry weight. Engeli et al., (1993) found 14 days thermophilic AD to be helpful in the elimination of weed (millet and tomato) seeds and decreasing the infectivity of plant pathogens. Schnürer and Schnürer (2006) found that AD decreased the diversity of fungal species but not total fungal colony forming units with thermotolerant species able to survive the thermophilic (55°C) anaerobic degradation process. Kim et al., (2002) found that thermophilic batch fed non–stirred reactors, treating synthetic primary sludge, outperformed its mesophilic equivalent and batch and continuously fed stirred reactors. The authors concluded that microbial consortia proximity was important for process stability, particularly with respect to propionate removal.

1.4.2.3.3 Two–phase verses single phase

In conventional single–stage AD processes, all phases (i.e. hydrolysis, acidification and methanogenesis) take place in one vessel which has been claimed to be suboptimal for all members of the AD consortia (Hawkes and Hawkes, 1987; Kim et al., 2002). Ongoing investigations attempt to optimise the process by separating the phases and, in doing so, provide optimum conditions for each microbial group.

1.4.2.3.4 Process configuration

The effect of process configuration has been found to be more important in anaerobic systems than aerobic systems (Speece et al., 2006). Azbar et al., (2001) explored the effect of process configuration on AD performance. Configurations included batch and
continuously fed single–stage CSTR, two stage CSTR and single–stage up–flow anaerobic sludge blanket (UASB) reactors. The authors reported that UASB provided the best performance, based on effluent quality, with the single–stage CSTR being the worst reactor configuration (severely limiting propionate metabolism: Speece et al., 2006), which is surprising as it is the most commonly used system in the field (Azbar et al., 2001).

New approaches have been applied to the treatment of OFMSW which include combinations of aerobic and anaerobic treatments and manipulation of AD process configurations.

• Six and De Baere, (1992) reported on the DRANCO process, a continuously fed, thermophilic (55°C), high solids (TS = 32%), AD treatment process that converts OFMSW to energy and a humus–like final product. OFMSW was treated in a single vessel (56m³) for between 14 to 21 days after which process liquid is removed from the product and used to adjust the moisture content of incoming waste. The dewatered end product is further stabilised via aerobic composting (10 days).

• A pilot–scale combined high–solids (TS = 25–30%) AD and aerobic composting process for the treatment of OFMSW was reported by Kayhanian and Tchobanoglous (1993). The complete–mix, semi–continuous (fed once per day) digestion of source sorted OFMSW occurred in the thermophilic range (55°C) and was most stable at a solids retention time (SRT) of 30 days. The end product was reported to be useful as a soil amendment or could be fired directly.

• Mata–Alvarez et al. (1993) described the performance of a pilot–scale (3m³) semi–dry (TS = 16–22%), complete–mix, thermophilic (55°C) anaerobic digester treating pre–composted and fresh OFMSW. The pre–composting step occurred during up to 1 week storage after sorting where the material aerobically self–heated. Pre–
composted OFMSW was found to generally provide lower biogas yields than fresh OFMSW.

- Wellinger et al., (1993) studied the KOMPOGAS system, a thermophilic (55°C), continuous (retention time: <40 days), horizontal, intermittently mixed (4 in 15min) anaerobic digester specifically designed for fruit, yard and vegetable waste (TS = 15–40%). The humus like product, a small amount of which is recycled as an inoculum, was found to be free of the introduced heat stable virus (Bacteriophage f2) demonstrating the sanitation potential of the process.

- Kübler and Schertler, (1994) investigated a three–phase AD process at pilot–scale called the BTA process. They concluded that: adequate anaerobic micro–organisms were present in the waste to provide acidification; process water recirculation may improve acidification; and that the separation of acidification, hydrolysis and methanogenesis overcame the diauxie effect of glucose on cellulase production and therefore enabled cellulose degradation, resulting in improved digestion.

- de Laclos et al., (1997) reported on a Valorga full–scale plant treating 52,000t of OFMSW per annum in the Netherlands. It is a semi–continuous, mesophilic (40°C), high–solids (TS = 30%), single–stage plug flow process having a retention time of 30–60 days. Removed solids are dewatered and stored for 4 weeks under aerobic conditions in enclosed windrows before being used as a soil conditioner.

- The BIOCEL process is a batch–wise, high solids (TS = 30–40%) AD process which converts OFMSW into compost and biogas. The 21 day digestion is performed under mesophilic conditions (35–40°C) with the temperature maintained by leachate recirculation that has been heated in a heat exchanger. ten Brummeler (2000) reported that a full–scale (50,000t/a) BIOCEL plant in the Netherlands was energy positive and the process was found to inactivate several types of pathogens resulting in improved compost quality.
• Bolzonella et al., (2003a) investigated the changes in digester parameters during transient conditions. They found that more highly degradable substrates provided greater variations in process stability parameters (VFA, alkalinity, biogas production) and that VFA concentration and alkalinity are the best parameters to monitor process stability. pH was found to be the least significant of the stability parameters.

• Bolzonella et al., (2003b) found that a short start–up for a pilot and full–scale thermophilic semi–dry (TS = 20%) CSTR AD of mechanically sorted OFMSW was possible. In this study, a mesophilic anaerobic culture was reported to be easily adapted to thermophilic conditions. This was achieved by stopping feeding of the mesophilic culture for 1 week (Cecchi et al., 1993), followed by an abrupt increase of temperature from 37 to 55°C, with thermophilic steady–state attained within a 40 day period.

• The SEBAC (SEquential Batch Anaerobic Composting) system (Chynoweth et al., 2003a; Chynoweth et al., 2003b; Forster–Carneiro et al., 2004; Teixeira et al., 2004) is a high–solids, thermophilic (50°C) leach–bed AD process comprising 3 sequential stages, occurring in 3 bioreactors, over a period of 15–40 days. Leachate is exchanged between ‘old’ and newly loaded bioreactors to enhance CH₄ production and process stability.

• Luning et al., (2003) compared the wet Vagron (TS = 12%) and dry Valorga processes and found no significant difference in gas production. Valorga was found to provide an environmental advantage in that it produced less wastewater. These authors also indicated that a problem with wet digestion was the formation of a ‘floating layer’ which, if not removed, could negatively affect reactor operations.

• Bolzonella et al., (2006) reported that source sorted OFMSW produced three times more CH₄/kgTVS than mechanically sorted OFMSW when treated in a dry AD process (Valorga) at full–scale.
Müller et al., (2006) reported on the 3A–Biogas process, a mesophilic high solids (TS=30–60%) AD treatment for OFMSW consisting of 3 sequential phases (aerobic/anaerobic/aerobic) occurring within the same vessel. These authors concluded that the 3A–Biogas process can reach the best synergies of composting and fermentation technologies.

A laboratory–scale dual digestion process was investigated under batch and semi–continuous conditions (Borowski and Szopa, 2007). Dual digestion with a 1d SRT aerobic thermophilic pre–treatment followed by a mesophilic anaerobic step with 20d of SRT provided 6 and 2% greater biogas and VS destruction respectively.

Nguyen et al., (2007) and Juanga et al., (2007) found that high rate percolation of water through OFMSW with micro–aeration had a beneficial effect on biogas production in a pilot–scale combined AD process.

Juanga et al., (2007) investigated the effect of leachate cross–recirculation between an anaerobic digester containing fresh waste and one containing mature waste (AD for 2 months) in a thermophilic sequential staging AD process. They reported improved CH₄ yield (65%), waste stabilisation and mass and volume decrease when compared to a combined AD process.

Abdullahi et al., (2008) investigated the influence of aerobic composting on the phytotoxicity of anaerobic digestate using seed germination tests. These authors observed that seed germination increased with dilution and incubation time and suggested that lower soil application rates and increased digestion and/or aerobic post–treatment periods would enhance the benefits of anaerobic digestate compost as a soil amendment.

Forster–Carneiro et al., (2008) examined the influence of different OFMSW wastes (food waste; OFMSW; shredded OFMSW) during dry (TS = 20%) thermophilic (55°C) anaerobic treatment. As experimental results showed important differences
in process performance, they concluded that the nature of organic substrate has an important influence on the biodegradation process and CH$_4$ yield and, for OFMSW, pre–treatment was not necessary.

- With the application of AD technologies growing worldwide due to its economic and environmental benefits, determination of the methanogenic potential of solid organic wastes has become a focus. With this in mind, Angelidaki et al., (2009) defined a protocol for the determination of the ultimate CH$_4$ potential for any given solid.

- Greben and co–workers (2009) suggested that South Africa could benefit from applying AD technology to OFMSW to generate renewable energy. Adding OFMSW to anaerobic digesters treating waste sludge at wastewater treatment plants would decrease the interruptions in electricity supply that are currently experienced (Greben and Oelofse, 2009).

- Nwabanne et al., (2009) investigated the biokinetics (maximum rate of substrate utilization, saturation constant ($k_s$), endogenous decay coefficient ($M_s$), biomass yield ($Y$) and maximum specific growth rate ($\mu_{max}$)) of AD of MSW and concluded that digesters treating MSW will require inoculation of the feed to increase the rate of digestion.

- Walker et al., (2009) investigated a rotating drum mesh filter bioreactor (RDMFBR) with a 100µm mesh in series with an anaerobic filter (AF) in a two stage configuration to treat OFMSW. Leachate from the RDMFBR was pumped to the upflow AF reactor before being returned to the RDMFBR. After an initial operating period (56 days) the RDMFBR produced the bulk (86–87%) of the total system CH$_4$, which suggests that separation of the system provided no significant advantage. These authors concluded that even though the system was robust, scale–up of the
process may be limited by the trans–membrane pressure flux, which would restrict the reactor working volume to a maximum of around 50 m$^3$.

1.5 The DiCOM® Process

The DiCOM® process is a novel biological treatment for OFMSW. It represents emerging Australian technology in combining thermophilic AD with in–vessel composting within a sequencing batch reactor. The end–products of the process are a renewable energy source, in the form of biogas, and a soil conditioner (compost). DiCOM® has been shown, at pilot scale, to be energy neutral, as biological heat production heats the solid waste to operating temperature, with the biogas produced typically providing an excess of energy. Nutrients in the waste are conserved in the stable compost that can be safely returned to the environment as soil conditioner. Little, or no, wastewater is produced during operation as process water is reused.

The process was designed and developed by AnaeCo Ltd. to address environmental concerns related to the disposal of OFMSW to landfill. Treatment consists of 21 days of sequential aerobic–anaerobic–aerobic treatment of mechanically sorted OFMSW. It occurs within a single sealed vessel enabling complete control over the internal environment, capture of produced biogas and treatment of odorous air. The timing of DiCOM® phases has been selected to allow for integration of the process into existing waste management infrastructure and routines and continuous processing of MSW. MSW in Western Australia is typically collected and delivered to Waste Transfer Stations during 5 consecutive days (Monday to Friday). Consequently, the initial 5 days of aeration allows the DiCOM® vessel to remain aerobic while the MSW is processed and OFMSW loaded into the reactor. During this time aerobic microbial metabolism raises the temperature of the OFMSW to that required for thermophilic treatment and
decreases the requirement for external energy input. 3 DiCOM® vessels, operating on a 3 week cycle, would provide continuous MSW processing. Accordingly, to allow for uninterrupted reuse of anaerobic liquid, each DiCOM® vessel can provide at most 7 days of AD. Finally, as the vessel must be empty to receive “fresh” OFMSW on a 3 weekly cycle, and 2 days are required to unload the end-product, the final aerobic polishing is performed over a 7 day period.

The centrepiece of the technology is the anaerobic thermophilic treatment of solid waste producing sustainable energy, in the form of biogas, as a by–product. At the commencement of digestion, the solid waste is completely submersed with anaerobic liquor from a previous DiCOM® trial. The high operating temperature of the process ensures maximum pathogen removal from the waste. It is anticipated that the rate of hydrolytic enzyme production during the pre–composting stage plays a significant role in the digestion process, as it is considered to be the rate–limiting step. To overcome the mass transfer problems typically encountered in hydrolysis reactions, and to maintain temperature, a liquid recycling loop is an integral component of reactor design. It is anticipated that the liquor recycle will enable uniform dispersion of micro–organisms and enzymes, the removal of undesired by–products, and improved process monitoring and control.

1.6 Thesis Objectives

The bioprocesses which occur within DiCOM® are not well understood. DiCOM® provides periods of aerobic and anaerobic treatment during process operation. These conditions are mutually exclusive, with aerobic microorganisms unable to respire without O₂ and O₂ being toxic to anaerobic methanogens. Consequently, it is not well understood as to how DiCOM® will perform across aerobic/anaerobic transitions.
Additionally, aerobic processes are thought to be more rapid than anaerobic processes. During DiCOM® operation, the rates of aerobic and anaerobic processes have not yet been quantified.

At the commencement of AD, the solid waste is completely submerged with anaerobic liquor from a previous DiCOM® trial. AnaeCo Ltd. believes that the reuse of the anaerobic liquor not only avoids the production of a wastewater stream but improves process performance. However, as only a single pilot–scale reactor has been constructed, the immediate reuse of the anaerobic liquor has not yet been investigated. Moreover, to provide efficient DiCOM® operation, the maintenance of the anaerobic liquor is critical however no liquor management regime has been investigated.

The DiCOM® process demands that sufficient CH₄ is generated during the thermophilic AD to meet the energy requirements of the process. It is anticipated that the reuse of anaerobic liquor will provide sufficient methanogenic culture to meet the CH₄ demands of the process. However the types of methanogens present in the liquor and the sources of these methanogens are yet to be quantified.

Even though the DiCOM® process has been operated at pilot–scale, no attempt has been made to optimize the process, or to investigate the microbial consortium upon which the process depends. Additionally, no analysis has been performed on the accumulation of fermentation intermediates during the methanogenic phase of the process.
Consequently, the purpose of this thesis is to:

1. Design and construct a sophisticated computer controlled DiCOM® twin reactor system capable of responding to environmental changes and logging on-line data.

2. Develop a precise generic tool that enables precise comparison of the relative rates of biological solids degradation occurring in aerobic and anaerobic processes.

3. Test that the direct transfer of anaerobic liquid improves the performance of a laboratory-scale DiCOM® reactor.

4. Investigate whether the accumulation of intermediates, specifically NH₃ and propionate, during the continued reuse of anaerobic liquor, can be detrimental to methanogens and CH₄ yield during DiCOM® operation.

5. Identify the sources of the methanogens for the DiCOM® process and determine whether the key methanogenic species are present in these sources of inoculum.

6. Investigate whether the control of the heat generated during the initial aeration phase of the DiCOM® process is essential to initiate rapid methanogenesis during anaerobic treatment.

2.1 Introduction

Organic waste is a valuable resource but it has not always been looked upon in such a favourable light, with disposal being the main focus. Landfill and incineration are common methods of disposal that have recently lost public favour due to negative environmental impacts (Six and De Baere, 1992; Edelmann and Engeli, 1993) such as land degradation, pollution of ground water and the generation of potent greenhouse gases (CH$_4$, CO$_2$ and N$_2$O) (El–Fadel et al., 1997; Mathur, 1998). As more countries legislate against physical disposal of organic waste, alternatives must be sought. Biological treatments offer such an alternative.

Biological organic waste treatment processes for organic waste include composting and AD (Mathur, 1998). Composting, an aerobic degradation process, converts putrescible organic materials into a humus–rich, hygienic product which improves soils and nourishes plants. The organic material is aerated (for weeks or months) during which time microbial heat generates thermophilic temperatures, nutrients are assimilated and mineralised, and humification occurs (Mathur, 1998). However, wet and easily degradable materials can pose a problem for composting systems due to odour production (Edelmann and Engeli, 1993; Van Durme et al., 1992) and greenhouse gas emission (Edelmann and Engeli, 1993).

AD is a strictly O$_2$ free process, in which some of the waste is biologically converted to biogas and a more stable solid product. The biogas produced from this process can be used to generate electricity, making the process more economical and sustainable than composting, with energy being generated rather than consumed (as is the case with composting). However, the end products of AD are generally not suitable for direct
land application as they contain phytotoxins such as sulfides, organic acids or ammonia (Mata–Alvarez et al., 2000).

Composting and AD both provide their own unique advantages, with the composting process being simpler to operate and AD energetically more sustainable. A combination of these technologies, with aerated composting occurring before and/or after AD, could provide improved process performance. Combinations of composting and AD have been described in the literature (Chynoweth et al., 1990; Six and De Baere, 1992; Kayhanian and Tchobanoglous, 1993; Mata–Alvarez et al., 1993; Kübler and Schertler, 1994; McIntosh and Oleszkiewics, 1997; Smet et al., 1999; Edelmann et al., 2000; ten Brummeler, E., 2000) and have been reported as being cheaper to operate than pure composting plants as the energy produced during the process can be used to operate the plant (Edelmann et al., 2000).

DiCOM® is a combined composting and thermophilic AD process that occurs within a single closed vessel under batch conditions. The process, invented, developed and patented by AnaeCo Ltd. (formerly Organic Resource Technologies Ltd.), based in Perth Western Australia, has been proven at pilot–scale (140tpa). The process exposes the organic fraction of mechanically sorted MSW (OFMSW) to 5 days of aeration, followed by 7 days of thermophilic AD and 7 days of aerobic maturation to produce a composted end–product. The reactor is a single vessel operated as a sequencing batch reactor which minimizes process handling. During composting, pressurized air is introduced into the reactor using an AnaeCo patented aeration system.

The purpose of this chapter is to characterise the performance of a laboratory–scale DiCOM® reactor by quantifying the different process phases.
2.2 Materials and Methods

2.2.1 Reactor Design and Operation

A cylindrical, 7L high temperature PVC computer controlled laboratory–scale DiCOM® reactor, insulated with closed cell elastomeric foam (Aeroflex®) (Fig. 2.1), was operated in a 19 day cycle which consisted of three distinct phases.

![Diagram of the DiCOM® reactor design](image)

Figure 2.1: Laboratory–scale DiCOM® reactor design.

1. Initial Aeration: After the reactor was filled with organic feed material, pressurized air was introduced into the sealed reactor until the internal pressure was raised to 25kPa, at which time inflow air was stopped and the internal pressure maintained before being released. This aeration regime was repeated every 10 minutes for a period of 4.875 days. At this time aeration was discontinued, the reactor depressurized and sealed for 0.125 days to establish an anaerobic environment. The external reactor temperature throughout the initial aeration period was maintained, via a heat tape wrapped around the reactor, at a temperature equivalent to that of the reactor core, with a maximum of 60°C ± 2°C.
2. Thermophilic Anaerobic Digestion: The reactor was flooded with liquid (anaerobic inoculum) (4.1L) and re-circulated (maximum rate: 70mL/min) through an external heat exchange unit to maintain the temperature at 55°C ± 2°C. Liquid was withdrawn from the top of the reactor and reintroduced to the bottom of the reactor to minimize feed compaction and liquid channelling. After a period of 6.875 days, the anaerobic liquid was withdrawn. The solid was removed from the reactor and mechanically squeezed to remove excess moisture prior to being reintroduced into the reactor.

3. Secondary Aeration (Maturation): Once drained, the reactor was aerated for 7 days using the aeration regime outlined in the initial aeration phase though aeration cycles were repeated more rapidly (every 5 minutes). The reactor temperature was maintained via the heating tape which was set at 35 ± 2°C.

2.2.2 Organic Feed Material

Mixed MSW collected in the metropolitan area of Perth, Western Australia in March, 2005 was mechanically sorted using a screen aperture of 50mm. Larger inert objects (plastic, metal, glass) in the sorted OFMSW were removed by hand before the organics were further shredded (< 25mm). Portions of the well mixed batch of OFMSW were combined with shredded paper (Hygenex® 2187951) and Jarrah wood chips (trapped between 1 and 5mm screens) in the ratio of 1000:17:67 (w/w) (to replace that removed during the mechanical sorting process and provide a solid matrix) and frozen (−20°C) to provide an identical starting material for all trials and reproducible outcomes. Prior to use, samples (2.4kg; wet bulk density 578 kg.m⁻³; free air space 55%; C:N was 18:1; 55% moisture content; 56% total volatile solids content (VS); 8.3% protein; 4.3% fat and 45% carbohydrate) were thawed at room temperature and de-ionised water (≈ 400mL)
added to provide a positive ‘fist test’, as described in Australian Standard 4454 (AS 4454, 2003).

Anaerobic inoculum (liquid) was obtained from the DiCOM® pilot-plant and stored at ambient temperature under an N₂ atmosphere until required. The inoculum was comprised of “recyclate”, the anaerobic liquor which is recycled through the previous, mature reactor and “pressate”, the liquid mechanically squeezed from the solid of a mature reactor after the free-draining liquid (recyclate) was removed at the end of the anaerobic phase of the DiCOM® process. In preparation for use, the anaerobic liquid was heated in a water-bath from room temperature to 55°C over a 48 hour period and maintained at this temperature for 3 days prior to being introduced to the reactor.

2.2.3 Logged Data

O₂ concentration, airflow rate, internal pressure, core and outside reactor temperature, pH and biogas generation rate were logged by computer using a National Instruments data acquisition card (NI PCI-6224 M Series DAQ) and National Instruments LabView 7 control software. O₂ concentration in the exit gas during the aeration phases was measured using a polarographic (Mettler Toledo InPro6100) O₂ sensor. Biogas production was determined by the downward displacement of oil (Dow Corning 200 Fluid 50CS) with real-time O₂, CO₂ and CH₄ concentrations in the exit gas logged by a gas analyzer (Geotechnical Instruments GA 2000).

2.2.4 Chemical Analysis

Anaerobic liquor samples required for analysis were centrifuged (Hermle Z233M: 13,000rpm for 5min) and the supernatant stored at –20°C. A Varian Star 3400 gas chromatograph (GC) fitted with a Varian 8100 auto-sampler was used to analyze the
VFA concentration of liquid samples. Samples were acidified with formic acid (to 1% (v/v)) before 1μL samples were injected onto an Alltech ECONOCAP™ EC™ 1000 (15m x 0.53mm 1.2μm i.d.) column. The carrier gas (N₂) was set at a flow rate of 5mL/min. The oven temperature was programmed as follows: initial temperature 80°C; temperature ramp 40°C/min to 140°C, hold for 1 min; temperature ramp 50°C/min to 230°C hold for 2 min. Injector and detector temperatures were set at 200 and 250°C respectively. The peak area of the Flame Ionisation Detector (FID) output signal was computed via integration using STAR Chromatography Software (© 1987–1995).

The moisture content and VS of the OFMSW was obtained by heating at 105°C and 550°C respectively, until constant mass was achieved, as described by American Public Health Association (1992). Liquid extracts from compost samples were obtained and used to determine NH₄⁺ content, pH and conductivity as per Australian Standard (AS 4454 – 2003). NH₄⁺ concentration in compost extracts and anaerobic liquor samples were determined as described by American Public Health Association (1992). Total organic and inorganic carbon and Kjeldahl nitrogen were analyzed by Marine and Freshwater Research Laboratory, Murdoch, Western Australia. Protein, Lipid and Carbohydrate analysis was performed by ChemCentre (W.A.), Perth, Western Australia. Lactate concentrations were determined using a YSI Select 2700 Biochemistry Analyser using a lactate specific membrane (John Morris Scientific, Perth, W.A.).

2.2.5 Compost Stability Test

Compost stability was assessed using the self–heat test. The tests were carried out according to the Australian Standard (AS 4454 – 2003), except 1.9L stainless steel thermos flasks (Aladdin Australia) were substituted for Dewar flasks. An independent study (Cord–Ruwisch, R. and Longford, D. Work not yet published) demonstrated more
sensitive self–heating data due to the lower heat loss coefficient of the thermos flasks. Temperature data was computer logged using National Instruments LabView 7 control software and a thermocouple connected via a National Instruments data acquisition card (NI PCI–6224 M Series DAQ).

2.2.6 Compost Maturity Tests

Compost maturity was determined using a phytotoxicity assay (root elongation) adapted from Tiquia et al., (1996). Aqueous compost extracts were prepared by mechanically shaking a fresh compost sample with deionised water (DI) at 1:10 w/v for 10 min. The mixture was centrifuged (Thermo CENTRA CL3) at 1000rpm for 10 min before the supernatant was filtered under vacuum through a Whatman #41 ashless filter paper. 10 seeds of Long Scarlet Radish (Fothergills, South Windsor, N.S.W., Australia) (AS 4454 – 2003) were incubated in a 90mm Petri dish containing a Whatman Number #1 filter paper and 8mL of extract. After 5 days incubation at 22°C, in the dark, root length was determined and compared to a control grown in DI water. Tests and controls were performed in triplicate. In preparation, seeds were soaked overnight in DI water at ambient conditions.

Humic and fulvic acids were extracted from compost samples and analyzed using a method adapted from Domeizel et al., (2004). Alkaline extractions of compost (10g) were acidified to separate humic and fulvic fractions. The fulvic acid fraction was purified by elution on an XAD–8 (Swift, 1996) column before the humic/fulvic acid ratio (C HA/C FA) was determined using total organic carbon (TOC) according to the dichromate acid oxidation method (American Public Health Association, 1992).
2.3 Results and Discussion

2.3.1 Initial Aeration Phase

A laboratory–scale DiCOM® reactor was operated in which the mechanically sorted OFMSW was aerated for 5 days, followed by 7 days of thermophilic AD and a final 7 days of aerobic maturation of the final product. With the reactor sealed, compressed air was introduced into the reactor to obtain a predetermined operating pressure. It has been reported in the literature that forced aeration of static compost piles enhances degradation and thus decreases the active decomposition period by up to 50% (Finstein et al., 1980). Consequently, it is anticipated that the pressurization of the reactor increases the availability of O₂ (elevated O₂ partial pressure and increased penetration of O₂ into the substrate void spaces) for microbial activity, while minimizing the aeration inefficiencies resulting from channelling, and as a result speeds up the initial composting process. The airflow rate during the initial 5 days of aeration was 57L/kg/d, 55% less than that reported by Smet et al., (1999) (130 L/kg/d) during the first 5 days of a continuously aerated pilot–scale reactor treating source separated OFMSW. These data suggest that the patented aeration regime used, as compared to continuous aeration systems, decreases the volume of air required during initial composting.

During the initial aeration phase the temperature rose to a maximum of 61°C (Fig. 2.2) which correlated to O₂ consumption, as monitored in the exit gas. The temperature elevation provides a significant energy saving to the process, as the heat is generated by aerobic microbial activity rather than requiring heat from an external heat exchange system. The temperatures attained provide an increased rate of thermophilic microbial and chemical reactions (Mathur, 1998), enabling rapid initial composting, and are believed to provide destruction of most weed seeds and plant pathogens (McIntosh and Oleszkiewicz, 1997).
2.3.2 Thermophilic Anaerobic Phase

Anaerobic liquid, initially drained from the DiCOM® pilot plant, was introduced into the reactor to allow even distribution of exo–enzymes and hydrolyzed substrate throughout the reactor, providing the opportunity for enhanced solid degradation. No additional buffer was required to maintain the pH of the anaerobic liquid between 7 and 8 (Fig. 2.3), highlighting its significant buffer capacity. The acetate concentration in the re–circulated anaerobic liquid was found to rise to 90mM (5300mg/L) during the first 2 days of anaerobicity (Fig. 2.3) causing a drop in pH from 7.5 to 7.1. The drop in pH resulted in elevated biogas production (Fig. 2.3 & 2.4) during the first 12 hours of anaerobic operation due to a release of CO₂ from the HCO₃⁻ contained within the anaerobic liquid. The pH was restored after the rate of acetate consumption exceeded that of production (day 7).
Butyrate accumulated during the first two days of anaerobic operation (14mM; 1200mg/L), with significant degradation only occurring when acetate levels fell below 20mM (1000mg/L). No significant propionate degradation was observed during the anaerobic phase. Propionate has been found to inhibit methanogenesis from acetate at levels exceeding 20mM (1480mg/L) (Barredo and Evison 1991) particularly at low pH where the more toxic propionic acid form prevails (Anderson et al., 2003). In the current study, propionate accumulated to 25mM (1800mg/L) and, as a result, CH₄ production may have been decreased. The lactate concentration in the recirculated liquor was found to be always less than 2mM. As the amount of lactate was consistently low during digestion no further investigation into this intermediate was conducted.

The onset of methanogenesis was rapid and commenced within 4 hours of the anaerobic liquid being introduced into the reactor (Fig. 2.4). The maximum methane production
rate (MPR) occurred when acetate consumption was most rapid (day 7–9) (Fig. 2.3) with the biogas produced containing 65% CH₄. The mean MPR was determined to be 10.9L/day/kg VS (18.2Lbiogas/day/kg VS), a lower value than many continuous (Six and De Baere, 1992; Sinclair and Kelleher, 1995; Krzystek et al., 2001) and batch (Krzystek et al., 2001) AD systems reported in the literature. However, these systems expose the micro–organisms to non–composted feeds containing easily degradable substrates which would provide greater availability of substrate for biogas generation.

As the soluble COD during the initial aeration period typically decreases from about 70 to less than 20 g COD/kg dry solid, much of the easily degradable substrate is consumed while thermophilic conditions are generated. In a study of semi–dry thermophilic AD of MSW, Mata–Alvarez et al., (1993) demonstrated that not only is biogas production decreased by using composted MSW, but the effect is more pronounced with increased composting duration. The DiCOM® process offers the

Figure 2.4: The rate of production and composition (%) of the biogas produced during the anaerobic phase of a laboratory–scale DiCOM® reactor.

Legend: (▬) CH₄; (––) CO₂; (—) Biogas generation rate.
flexibility to optimize for biogas production by decreasing the duration and/or intensity of the initial aeration phase.

2.3.3 Maturation Phase

At the conclusion of the anaerobic phase, the anaerobic liquid was drained ready to serve as the inoculum for a twin reactor. Air was reintroduced to commence maturation of the solid which has been found to be beneficial in decreasing phytotoxicity, remove odour and stabilize the solids (Edelmann and Engeli, 1993). The core reactor temperature was maintained via a heating tape set at 35 °C (Fig. 2.2) as self heating did not occur in this trial. Towards the end of maturation no significant O₂ consumption (Fig. 2.2) occurred and it was inferred that the compost had matured, a conjecture supported by decreased CO₂ emission in the exit gas (Fig. 2.5). It could also be inferred that lack of self heating is due to the destruction of aerobic micro–organisms during anaerobic conditions. However, previous trials have demonstrated post–anaerobic aerobic microbial activity resulting in self heating during maturation.

![Figure 2.5: Typical CO₂ emission profile in the exit gas (%) during the second aerobic (maturation) phase of a laboratory–scale DiCOM® reactor.](image-url)
2.3.4 Degradation Occurring During Each Phase

As the reactor is a closed system, monitoring O\textsubscript{2} consumption and CH\textsubscript{4} production allows the determination of the rate of degradation by establishing an electron flow balance. Each mole of CH\textsubscript{4} produced is equivalent to 2 moles of O\textsubscript{2} used, in both cases representing 8 electrons. During the process, 41% of VS was degraded of which the aerobic and anaerobic phases accounted for 57 (44+13) and 43% of the electron flow, respectively (Fig. 2.6). Of the electron flow during the anaerobic phase, 9% can be attributed to degradation of the VFAs introduced into the reactor within the anaerobic liquid. Hence the actual percentage of anaerobic conversion of solids to gas was about 34% in this case. Therefore, a significant fraction of the reducing power in the solid waste was conserved as a fuel which can be used to operate the industrial plant. Significantly more conversion of solids to gas was obtained during process optimisation which is the subject of a further study.

![Graph showing electron equivalents during each phase of the DiCOM reactor](image)

**Figure 2.6:** Electron equivalents during each of the phases of the laboratory–scale DiCOM\textsuperscript{®} reactor showing the percentage degradation (mmol/kg) during each phase.
Considering that the aerobic conversion was deliberately O$_2$ limited, a faster aerobic conversion could be achieved by increasing the rate of air delivery. However the increased heat production could exceed the maximum acceptable temperature for composting (70°C: Mathur, 1998). As significant soluble COD had not accumulated in the solid after aeration (typically decreased from about 70 to less than 20g COD/kg dry solid), it can be assumed that, during microbial heat generation, hydrolysed substrates were completely oxidised to CO$_2$ and released from the reactor. Therefore, during aeration the minimum carbon–hydrolysis rate can be estimated to be:

\[
\text{C–Hydrolysis Rate} = \text{CO}_2 \text{ Release Rate} \quad \text{(Eq. 2.1)}
\]

When switching to the anaerobic phase the initial CH$_4$ production and electron flow were low (Fig. 2.4 & 2.6). However, by considering the accumulation of VFAs (Fig. 2.3) the hydrolysis rate can be reconstructed. During the first 2 days, gas generation was limited by methanogenic activity (VFA accumulation) followed by hydrolysis limitation (VFA levels decrease). Therefore, under anaerobic conditions the hydrolysis rate can be estimated to be (Eq. 2.2):

\[
\text{C–Hydrolysis Rate} = \text{C accumulation rate as VFA} + \text{CH}_4 \text{ Production Rate} + \text{CO}_2 \text{ Release Rate} \quad \text{(Eq. 2.2)}
\]

where:

\[
\text{VFA C–Accumulation Rate} = (2 \times \text{acetate}) + (3 \times \text{Propionate}) + (4 \times \text{Butyrate}) \text{ Accumulation Rates}
\]

(NOTE: a negative VFA accumulation rate results when VFA consumption exceeds production).

The hydrolysis rate did not change significantly across phase transitions which highlights the seamless transition between aerobic and anaerobic degradation (Fig. 2.7). The hydrolysis rate was highest during the initial aeration phase and continued at a
similar level for the initial 2 days of the anaerobic phase, after which time a decrease in rate suggests exhaustion of easily degradable substrate and the onset of hydrolysis limitation. These data support the finding that the aerobic phases account for a greater portion of the solid degradation in this trial.

![Graph showing hydrolysis rates during phases of a DiCOM® reactor](image)

**Figure 2.7**: A comparison of hydrolysis rates of OFMSW (measured as hydrolysed carbon) during each of the phases of a laboratory–scale DiCOM® reactor.

### 2.3.5 Compost Stability and Maturity

Compost stability is a measure of the amount of easily degradable substrate available for microbial degradation and can be determined via a self heating test. The compost self–heated to 39°C during the 10 day test therefore, according to the Australian Standard (< 40°C) (AS4454, 2003), the compost was considered to be stable.

Compost maturity is defined by the lack of phytotoxic compounds contained within the compost and can be determined via a root elongation test as the inhibition of plant growth is directly related to the presence of water soluble phytotoxic substances in the
compost (Inbar et al., 1990). The Australian Standard (Long Scarlet Radish) seed grown in aqueous compost extracts produced a 100% increase in average root length (126mm) when compared to the control (56mm) suggesting that the product is mature. However, the pH (8.8) and NH$_4^+$ (273 mgN/L of extract) content of an aqueous compost extract were found to exceed that required by the Australian composting standard (pH 7.5 and <200 mgN/L of extract) (AS4454). Tiquia et al., (1996) in a study on the phytotoxicity of spent pig–manure sawdust litter noted that root elongation was sensitive to NH$_4^+$–N concentration in the compost extract, however phytotoxicity was not noted in this study. As sensitivity of a plant is dependent upon the size of the seed and the food reserves contained within (Cheung et al., 1989), the lack of sensitivity could be attributed to the size of the radish seed (3mm spherical).

Other measures, which have been reported in the literature, to determine compost maturity include the germination index (GI) (Zucconi et al., 1981) and the humic/fulvic acid ratio (C$_{HA}$/C$_{FA}$) (Sugahara and Inoko, 1981). The GI has proven to be a very sensitive parameter in determining the toxicity of composts as it combines both root growth and seed germination. Tam and Tiquia, (1994) determined that a GI value greater than 80 to 85% coincided with the disappearance of phytotoxicity in the compost. Jiménez and Garcia (1992) found that the humic/fulvic acid ratio (C$_{HA}$/C$_{FA}$) was the best indicator of compost maturity and humification. In a study on the maturity of MSW compost they identified that the C$_{HA}$/C$_{FA}$ increased from 0.96 to 1.96 after 1 day and 165 days of composting, respectively. As the GI for the radish seed was determined to be 195% and the C$_{HA}$/C$_{FA}$ of the compost was determined to be 5.0, it was concluded that the compost was mature.
2.4 Conclusions

The DiCOM® process was found to operate stably under both aerobic and anaerobic conditions and provided a seamless transition, between these two opposing conditions, with no significant change in degradation rates occurring across transitions. Based on electron flow, anaerobic treatment accounted for only a minor quantity (34%) of the solid degradation, with the bulk of the reducing power within the OFMSW being wasted. As the world focuses more intently on renewable energy sources, the limited capture of reducing equivalents as CH₄ must raise concerns regarding the energy efficiency of DiCOM®. The challenge must therefore be to improve the degradation efficiency during the anaerobic phase of this process and, as a consequence, increase energy capture.
3.1 Introduction

Biological organic waste treatment offers an alternative to physical treatment, such as landfill or incineration, without the negative environmental impacts of land degradation, pollution of ground water and the generation of potent greenhouse gases (CH₄, CO₂ and N₂O) (El–Fadel et al., 1997; Mathur, 1998). Composting, an aerobic biodegradation process, converts putrescible organic materials into a humus–rich, hygienic product which improves soils and nourishes plants, whereas AD is a strictly O₂–free process, in which some of the waste is biologically converted to CH₄ and can be used to generate electricity. The DiCOM® process combines composting and thermophilic AD of solid waste in a single closed vessel operated as a sequencing batch reactor, the final products of which are composted organics and renewable energy in the form of biogas.

The success of the DiCOM® process is reliant on the ability of the system to produce sufficient CH₄ during the anaerobic phase for the process to be energy positive or, at worst, energy neutral. As the anaerobic phase of the process operates for only 7 days, the stability of the process and the early onset of methanogenesis during AD start–up are critical. Even though AD can be commenced with only the addition of water, improved start–up performance has been reported when using inocula (Lopes et al., 2004; Angelidaki et al., 2006; El–Mashad et al., 2006; Walker et al., 2006a), recirculation of leachate (El–Fadel, M., 1999; Ledakowicz and Kaczorek, 2004; El–Mashad et al., 2006) and cross–recirculation of leachate between immature and mature reactors (Adhikari et al., 2006; Juanga et al., 2007).
Chapter 3: Direct Transfer of Inoculum In a Laboratory–Scale DiCOM® Reactor

As the DiCOM® process consists of three separate phases, the process can be performed such that three separate reactors can operate simultaneously with each reactor completing one of the three phases but each being out of phase with the others, therefore providing continuous batch processing. Thus at a particular moment in time, one reactor would be completing the anaerobic phase while another was just commencing the anaerobic phase. The anaerobic inoculum could therefore be transferred directly from one reactor to another (direct transfer – Fig. 3.1) thus reusing the liquor, providing an active microbial inoculum (Chynoweth et al., 2003b) and avoiding the production of a wastewater stream.

![Figure 3.1: Timing of phases during operation of two sister laboratory–scale DiCOM® reactors allowing direct transfer (shown by ▶) of the anaerobic inoculum on day 12.](image)

Significant methanogenic activity did not occur immediately when using an inoculum which had been stored for eight weeks at ambient temperatures (Section 2.3.2 Fig. 2.4 & Section 2.3.4 Fig. 2.6). In order to avoid potential deterioration of the inoculum during storage, this chapter looks at the direct transfer of fresh, hot inocula from one reactor to another, to investigate its effect on the performance of a laboratory–scale DiCOM® reactor.

3.2 Materials and Methods

3.2.1 Reactor Design and Operation

Two cylindrical, 7L, high temperature PVC, computer controlled laboratory–scale DiCOM® reactors were setup, monitored and operated as described in Section 2.2.1 and 2.2.3.
3.2.2 Organic Feed Material

OFMSW feedstock was prepared as outlined in Section 2.2.2. Anaerobic inoculum (liquid) for the initial trial (reactor 1) was obtained from the DiCOM® pilot-scale reactor and had been stored for 8 weeks at ambient temperature under an N₂ atmosphere.

3.2.3 Chemical Analysis

VFA concentrations of liquid samples, moisture content of OFMSW, NH₄⁺ content of liquid extracts from compost samples, pH, conductivity, total organic and inorganic carbon and Kjeldahl nitrogen were analyzed as described in Section 2.2.4.

3.2.4 Compost Stability and Maturity Analysis

Compost stability and maturity were assessed using the self-heat test (AS 4454) and a phytotoxicity assay (root elongation) adapted from Tiquia et al. (1996) respectively as described in Section 2.2.5 and 2.2.6.

3.3 Results and Discussion

3.3.1 Direct and Non–Direct Inoculum Transfer

In the operation of the DiCOM® process, the solids in the reactor are flooded when moving from the initial aerobic phase to AD. In comparison to using clean water as the flooding liquid, stored anaerobic liquid from a previous trial showed improved performance (data not shown). Assuming that the enhanced reactor performance was due to the transfer of solutes, providing increased buffer capacity, and thermophilic bacterial consortia, it was tested to determine whether the direct reuse of hot inoculum could provide further improvement in performance over the previous method of storage at ambient temperature between trials (in the current case: 8 weeks). To this end, two
reactors were operated so that hot anaerobic inoculum could be transferred directly from a reactor completing its anaerobic phase to a sister reactor commencing its anaerobic phase (Fig. 3.1).

3.3.2 Thermophilic Anaerobic Phase

The direct transfer of anaerobic inoculum decreased VFA (acetate & butyrate) accumulation. Even though both reactors showed transient acidification during the first two days of anaerobicity (Fig. 3.2), accumulation of acetate and butyrate was decreased with the direct transfer of anaerobic inoculum. The acidification however did not significantly alter the pH of the liquor with pH profiles of both reactors showing similar trends.

Butyrate concentrations peaked between days 7 and 8, in both reactors, with direct transfer providing a 35% decrease in total accumulation (maximum 9 mM). No significant propionate degradation was observed during the anaerobic phase of either trial. In fact propionate accumulation was almost linear throughout the anaerobic phase and reached concentrations of 25mM (1850 mg/L) and 60 mM (4440 mg/L) for reactors 1 and 2 respectively. Propionate has been found to inhibit methanogenesis from acetate at levels exceeding 20 mM (1480 mg/L) (Barreto and Evison 1991) particularly at low pH where the more toxic propionic acid form prevails (Anderson et al., 2003). As propionate concentrations were in excess of 20 mM, particularly in reactor 2, it is likely that CH₄ production was decreased in this study. It is important to note however, that propionate degradation has been demonstrated in subsequent trials.

The VFA profiles of both reactors were remarkably similar, with the average maximum rate of net acetate accumulation, during day 5 to 7, (32 & 31 mM/d) being almost
identical (Fig. 3.2). It could therefore be argued that the effect of direct transfer of fresh, hot inoculum was insignificant. However, the considerable increase (80%) in maximum CH$_4$ generation rate and overall CH$_4$ production (50%), in reactor 2 (Fig. 3.3), shows that direct transfer of inoculum improves methanogenic performance.

The direct transfer of anaerobic inoculum increased the MPR from 11.2 to 17.0L/day/kg VS, a rate that is comparable with the literature as reviewed by Hartman and Ahring (2006). It should be noted that the CH$_4$ generation from MSW is highly dependent upon the composition of the waste, particle size and nature of any pre–treatment (i.e. aerobic pre–composting). It has been reported in the literature that not only is CH$_4$ production decreased by using composted MSW (Mata–Alvarez et al., 1993; Krzystek et al., 2001), but the effect is more pronounced with increased composting duration (Mata–Alvarez et al., 1993). A further increase in CH$_4$ generation has been shown to be feasible by decreasing the duration and/or intensity of the initial aeration phase (Refer to Section 6.3.3).
3.3.3 Degradation Occurring During Each Phase

As the reactor is a closed system, monitoring O₂ consumption and CH₄ production allows the determination of the rate of degradation by establishing an electron flow balance. Each molecule of CH₄ produced is equivalent to 2 molecules of O₂ used, in both cases representing 8 electron equivalents. Aerobic degradation during both trials (reactor 1 and 2) provided similar amounts of degradation as indicated by electron flow (33.8 and 36.6mol/kgVS respectively) (Fig. 3.4). The direct transfer of anaerobic inoculum however provided a 50% increase in electron flow during the anaerobic phase (22.9 and 34.6mol/kgVS) (Fig. 3.4), resulting in a notable increase in the amount of reducing power, from the solid waste, conserved as a fuel (i.e. 50% more CH₄; Fig. 3.3).

Figure 3.3: The CH₄ generation rate (thin lines) and total CH₄ volume (bold lines) produced in the biogas of reactors with direct transfer of inoculum (solid lines) and a control run (dashed lines) during the anaerobic phase of a laboratory–scale DiCOM® reactor.
The direct transfer of anaerobic liquid provided a significant increase in hydrolysis rate during the anaerobic phase (cumulative hydrolysis: 53.9 and 92.5gC/kgVS) (Fig. 3.5). The increased hydrolysis could be the result of:

- an active microbial community being introduced within the inoculum or
- residual exo–enzymes and/or biologically active species contained in the anaerobic liquid.

![Graph showing electron equivalents during each phase of a laboratory-scale DiCOM® reactor.](image1)

**Figure 3.4:** Electron equivalents during each of the phases of two sister laboratory-scale DiCOM® reactors. Solid lines indicate results from the direct transfer of anaerobic inoculum.

![Graph showing hydrolysis rate during each phase of a laboratory-scale DiCOM® reactor.](image2)

**Figure 3.5:** A comparison of hydrolysis rates of OFMSW (measured as hydrolysed carbon) during each of the phases of a laboratory-scale DiCOM® reactor. Solid lines indicate results from the direct transfer of anaerobic inoculum.
3.3.4 Inhibition of Methanogenesis

The results shown indicate that the immediate reuse of anaerobic inoculum improved the AD process in terms of process stability, overall CH₄ recovery and production rate and destruction of organics. Without proper management however the build–up of soluble components (such as salinity: Rolle et al., 1997 and NH₃: Angelidaki and Ahring, 1994; Koster and Lettinga, 1984; McCarty, 1964b; Sung and Liu, 2003) is likely to compromise the prolonged reuse of the anaerobic inoculum. In fact signs of methanogenic inhibition were evident as CH₄ generation slowed down (Fig. 3.3: day 7) while the key substrate, acetate (Fig. 3.2), was at saturable levels for acetoclastic methanogens (kₐ approx 3mM; Schoenheit et al., 1982) and that significant propionate degradation was not observed.

Ways to manage the NH₃–N concentration such that it provides useful buffer capacity without causing inhibition and propionate build–up will be reported later (Chapter 4). Example test trials, with controlled ammonium and salinity levels (here by dilution), showed complete VFA degradation and the exhaustion of easily degradable substrate (Fig. 3.6).

3.3.5 Compost Stability and Maturity

At the conclusion of the anaerobic phase of each trial the anaerobic liquid was drained ready to serve as the inoculum for a subsequent trial. Air was reintroduced to commence maturation of the solid which has been found to be beneficial in decreasing phytotoxicity, remove odour and stabilize the solids (Edelmann and Engeli, 1993). The core reactor temperature was maintained at 35°C as self–heating did not occur in either reactor. Towards the end of maturation no significant O₂ consumption, or CO₂ production, occurred suggesting that the compost had matured, which was confirmed by
the self–heating test (Table 3.1). Direct reuse of the inoculum provided greater
destruction of TVS (Table 3.1) and as a consequence a more stable product.

![Figure 3.6: Volatile fatty acid accumulation during the anaerobic phase of a laboratory-scale DiCOM®
reactor showing complete degradation of accumulated VFAs.](image)

Legend: (■) Acetate; (●) Propionate; (▲)Butyrate; (—) pH.

Compost maturity is defined by the lack of phytotoxic compounds contained within the
compost and can be determined via root elongation testing, as the inhibition of plant
growth is directly related to the presence of water soluble phytotoxic substances in the
compost (Inbar et al., 1990). For reactors 1 and 2, Long Scarlet Radish seeds grown in
aqueous compost extracts produced a 100 and 73% increase in average root length
respectively when compared to the control. The germination index (GI) (Zucconi et
al., 1981), an alternative measure of compost maturity, combines both root growth and
seed germination, and in both tests was found to exceed 85%, the level at which Tam
and Tiquia (1994) observed the disappearance of phytotoxicity in a compost. Both
measures suggest that the composts were mature (Table 3.1).

Even though total nitrogen present in the compost suggests a benefit to plant nutrition
(Table 3.1), the pH and NH₃ content of aqueous compost extracts were found to exceed
that required by the Australian composting standard (AS4454) (Table 3.1). Tiquia et al. (1996) noted that root elongation was sensitive to NH₃–N concentration in compost extracts. The compost extract obtained from reactor 2 produced shorter root lengths and a lower GI (Table 3.1) than that obtained for reactor 1 and it is likely that the higher NH₃ concentration of the extract (Table 3.1) was responsible. As there is a logical correlation between the NH₃ concentration of the anaerobic liquor and that in the final compost, for liquor reuse to continue to provide enhanced degradation, a greater understanding of process and control is essential to ensure end–product maturity.

Table 3.1: Stability and maturity parameters of compost produced in two sister laboratory–scale DiCOM® reactor.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reactor 1</th>
<th>Reactor 2 (Direct Transfer)</th>
<th>Australian Standard Requirement (AS 4454)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Volatile Solid (TVS) Destruction (%)</td>
<td>41</td>
<td>45</td>
<td>–</td>
</tr>
<tr>
<td>Maximum Temperature Attained During Heat Test (°C)</td>
<td>39</td>
<td>36</td>
<td>&lt; 40°C</td>
</tr>
<tr>
<td>pH of Compost Extract</td>
<td>8.8</td>
<td>8.8</td>
<td>5 – 7.5</td>
</tr>
<tr>
<td>NH₃–N (mgN/L Extract)</td>
<td>273</td>
<td>341</td>
<td>&lt; 200</td>
</tr>
<tr>
<td>Total Nitrogen (% dry matter)</td>
<td>1.1</td>
<td>1.4</td>
<td>≥ 0.6 If plant nutrition claimed</td>
</tr>
<tr>
<td>Root Elongation Test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (mm)</td>
<td>56</td>
<td>56</td>
<td>&gt; Control</td>
</tr>
<tr>
<td>Test (mm)</td>
<td>123</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>Germination Index (GI)</td>
<td>195</td>
<td>166</td>
<td>–</td>
</tr>
</tbody>
</table>

3.4 Conclusions

The direct transfer of fresh, hot inocula, from one DiCOM® reactor to another, was found to provide:

- A 58% increase in the mean CH₄ generation rate (from 18.2 to 28.7 L/kg of TVS/day) and
- A 50% increase in electron flow to CH₄ (22.9 and 34.6mol/kgVS).

As the success of DiCOM® is dependent upon the production of sufficient CH₄ for the process to be, at worst, energy neutral, and that the AD is performed over a very short (7 day) time period, the rapid start-up of methanogenesis during AD is critical. This
chapter demonstrates that the reuse of an active anaerobic inoculum can address both of these considerations. However, while the reuse of liquor can provide a methanogenic advantage to the process, it is also anticipated that it will provide a liability from the accumulated salts and microbial intermediates.
4.1 Introduction

The AD of easily degradable organic material is known to lead to digester acidification when organic material is available in excess. This is due to the fermentation of sugars into organic acids being a faster reaction than the degradation of organic acids into CH₄ gas. A similar trend has been found for the batch AD of solid waste. The start–up of a batch thermophilic AD process of OFMSW leads to VFA accumulation (ten Brummeler 2000; Walker et al., 2006a; Walker et al., 2006b). In the presence of a suitable buffer capacity the organic acid production may not cause permanent digester failure but a delay in the onset of CH₄ production. By definition the accumulation of organic acids is caused by the over–production of organic acids relative to their degradation. It can in principle be avoided by the presence of increased number of methanogenic, acid degrading consortia, hence the risk of digester acidification and the initial lack of CH₄ production (lag phase).

In an industrial context, both the risk of digester acidification and the “lag phase” of initial low gas formation rates need to be controlled for optimum process performance. In an effort to develop a very short batch thermophilic anaerobic treatment of OFMSW that stabilizes the waste and only recovers the biogas that originates from the degradation of rapidly degradable (putrescent) organics, we investigated the DiCOM® process, a hybrid aerobic/anaerobic process with 5 days of aerobic and 7 days of anaerobic treatment.

As the duration of the AD step was only 7 days, it was necessary to maximize methanogenic activity and keep the lag phase as short as possible which required:
• A rapid onset of methanogenic activity and a large number of methanogens present at start–up so as to avoid acidification,
• A strong buffering system to limit pH fluctuations,
• Maintaining a high rate of methanogenic activity throughout the 7 days by minimizing inhibition by any by–product such as NH₃.

In the batch AD of solid material a suitable inoculum is typically provided by transferring the liquor from a previous batch. This reuse of anaerobic recyclate (Walker et al., 2006b), the addition of inoculum (Meisgeier et al., 2003; Lopes et al., 2004; Angelidaki et al., 2006; El–Mashad et al., 2006; Walker et al., 2006(b); Maroun and El–Fadel, 2007; Nguyen et al., 2007) and the recirculation of leachate (Kubler and Schertler, 1994; Poggi–Varaldo et al., 1997; El–Fadel, 1999; Ledakowicz and Kaczorek, 2004; El–Mashad et al., 2006; Juanga et al., 2007; Nguyen et al., 2007) have been shown to be effective in attaining rapid methanogenesis and stabilizing the reactor against acidification.

The continued reuse of anaerobic recyclate (Walker et al., 2006b) however must raise concerns. Even though it assists reactor stability by providing increased buffer capacity (Georgakakis et al., 1974; Chynoweth et al., 2003b; Murto et al., 2004) and inoculum, soluble salts and microbial metabolites will accumulate. Of those it is in particular NH₃, which may have a detrimental effect on the methanogenic population and decrease the rate of methanogenic activity (Koster and Lettinga, 1984; Wiegant and Zeeman, 1986; Angelidaki and Ahring, 1993; Angelidaki and Ahring, 1994; Kayhanian, 1994; Gallert and Winter, 1997; Poggi–Varaldo et al., 1997; Gallert et al., 1998; Kayhanian 1999; Sung and Liu, 2003; Calli et al., 2004) leading to incomplete digestion and VFA accumulation.
As NH$_3$ and propionate accumulation, in the anaerobic liquor, has been observed, the purpose of this chapter is to quantify the effect of NH$_3$ and propionic acid accumulation, caused by repeated liquor transfer, on the thermophilic AD phase of the DiCOM® process and to devise process control measures that minimise the inhibition effect.

### 4.2 Materials and Methods

#### 4.2.1 Reactor Design and Operation

Two cylindrical, 7L, high temperature PVC, computer controlled laboratory–scale DiCOM® reactors were setup, monitored and operated as described in Section 2.2.1 and 2.2.3.

#### 4.2.2 Organic Feed Material

OFMSW feedstock was prepared as outlined in Section 2.2.2.

Synthetic OFMSW (SOFMSW) was produced by mixing 305g Marron Pellets (Specialty Feeds, Glen Forrest, Western Australia), 490g wheat chaff, 200g Jarrah wood chips (as above) and 5g paper (as above) having a C:N ratio of 23:1 and a moisture content of 53%.

Anaerobic inoculum (liquid) was obtained from a previous laboratory trial and stored at ambient temperature under an N$_2$ atmosphere until required. In preparation for use, the anaerobic liquid was heated in a water–bath from room temperature to 55°C, in 5°C increments, over a 24 hour period and maintained at this temperature for 3 days prior to being introduced to the reactor.
4.2.3 Inhibition Constant Determination

OFMSW was treated as described in Section 2.1 until the accumulated propionate was removed from the recyclate at which time the recyclate was drained and diluted 1 in 4 with deoxygenated de-ionized (DI) water. The digested solid was removed from the reactor and mechanically pressed to remove excess moisture.

To determine the free–NH$_3$ inhibition constant for CH$_4$ from acetate, digested solid, recyclate, 2M NH$_4$Cl (adjusted to pH 7.5 with 2M NaOH) and 1M CH$_3$COONa (adjusted to pH 7.5 with 2M HCl) were added to 100mL serum vials (Wheaton 223747) as described in Table 4.1. Deoxygenated DI water (degassed with N$_2$/CO$_2$ (80/20% (v/v)) and adjusted to pH 7.0 with 2M NaOH) was added to make a final liquid volume of 50mL. The vials were sealed with a butyl rubber stopper, crimped with an aluminium cap and the headspace flushed with N$_2$/CO$_2$ (80/20% (v/v)) before a 1mL liquid sample was removed from each vial for analysis. Vials were equilibrated in a shaking 55°C water bath for 30min before being depressurized, the pH adjusted to 7.5±0.1 with 2M NaOH and incubated for 24h at 55°C. After 24h, gas volume and composition were determined using an air–tight glass syringe (Eterna-Matic) and GC analysis. A 1mL liquid sample was removed for analysis. All vials were repeated in triplicate.

To determine the free–NH$_3$ inhibition constant for CH$_4$ production from hydrogen, digested solid, recyclate, 2M NH$_4$Cl (adjusted to pH 7.5 with 2M NaOH) and 1M CH$_3$COONa (adjusted to pH 7.5 with 2M HCl) were added to 100mL serum vials as described in Table 4.2. Deoxygenated DI water (degassed with N$_2$/CO$_2$ (80/20% (v/v)) and adjusted to pH 7.0 with 2M NaOH) was added to make a final liquid volume of 50mL. The vials were sealed with a butyl rubber stopper and aluminium crimp seal, the
Chapter 4: Accumulation of Inhibitors During Reuse of Anaerobic Recyclate

Headspace was flushed with H\(_2\) (except for the negative control which was flushed with N\(_2/CO_2\) (80/20\% \,(v/v))\)) before 10mL overpressure of CO\(_2\) was added which produced a 80/20\% \,(v/v) H\(_2/CO_2\) atmosphere. A 1mL liquid sample was removed from each vial for analysis. The pH of the vials were adjusted to 7.6±0.1 with 2M NaOH and incubated for 2h at 55°C, at which time gas volume and composition were determined using an air–tight glass syringe (Eterna-Matic) and GC analysis. A 1mL liquid sample was removed for analysis. Each vial was repeated in triplicate.

Table 4.1: Contents of 100mL serum vials for the determination of the constant for NH\(_3\) inhibition (\(K_{\text{NH}_3,\text{Ac}}\)) on CH\(_4\) production from acetate.

<table>
<thead>
<tr>
<th>Flask</th>
<th>Mass of Digested Solid (g)</th>
<th>Volume of Recyclate (mL)</th>
<th>TAN Concentration (mM)</th>
<th>CH(_3)COONa Concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ Control</td>
<td>1</td>
<td>10</td>
<td>12</td>
<td>51</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>12</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>12</td>
<td>127</td>
<td>80</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>12</td>
<td>187</td>
<td>80</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>12</td>
<td>217</td>
<td>80</td>
</tr>
<tr>
<td>– Control</td>
<td>6</td>
<td>10</td>
<td>12</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4.2: Contents of 100mL serum vials for the determination of the NH\(_3\) inhibition constant (\(K_{\text{NH}_3,\text{H}_2}\)) for CH\(_4\) production from hydrogen.

<table>
<thead>
<tr>
<th>Flask</th>
<th>Mass of Digested Solid (g)</th>
<th>Volume of Recyclate (mL)</th>
<th>TAN Concentration (mM)</th>
<th>CH(_3)COONa Concentration (mM)</th>
<th>Headspace Gas</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ Control</td>
<td>1</td>
<td>10</td>
<td>12</td>
<td>49</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>12</td>
<td>81</td>
<td>1</td>
<td>H(_2/CO_2)</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>12</td>
<td>118</td>
<td>1</td>
<td>H(_2/CO_2)</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>12</td>
<td>158</td>
<td>1</td>
<td>H(_2/CO_2)</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>12</td>
<td>188</td>
<td>1</td>
<td>H(_2/CO_2)</td>
</tr>
<tr>
<td>– Control</td>
<td>6</td>
<td>10</td>
<td>12</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Propionic acid inhibition constants for CH\(_4\) production from acetate (\(K_{\text{Prop,Ac}}\)) and hydrogen (\(K_{\text{Prop,H}_2}\)) were carried out as described above except TAN was replaced with 1, 21, 42, 70 and 105 mM sodium propionate. All solutions and final vial contents were adjusted to pH 6.5 with 1M HCl.
4.2.4 Chemical Analysis

VFA concentrations of liquid samples, moisture content of OFMSW, \(\text{NH}_4^+\) content of liquid extracts from compost samples, pH, conductivity, total organic and inorganic carbon and Kjeldahl nitrogen were analyzed as described in Section 2.2.4.

Gas composition of serum vial tests was determined using a Varian Star 3400 GC and peak area of the thermal conductivity detector (TCD) output signal was computed via integration using STAR Chromatography Software (© 1987–1995). 50μL gas samples were manually injected onto an Alltech 2m stainless steel Porapak Q (80/100) packed column (0.183m x 3.175mm) using a Hamilton 100μL gas tight syringe. The injector, oven, detector and filament temperatures were set at 120, 40, 120 and 170°C respectively. The \(\text{N}_2\) carrier and reference gas flows were set to 30mL/min. Peak area for \(\text{CO}_2\) and \(\text{CH}_4\) standards (0, 10, 20, 40, 60 and 80% (v/v)) were used to construct a standard curve which was used for concentration determination.

4.3 Results and Discussion

4.3.1 Predicted Build-up of Ammonia Inhibition During Reuse of Anaerobic Recyclate

4.3.1.1 Theoretical considerations on ammonia inhibition of methanogenesis

AD of high protein wastes, such as animal manures, some food wastes and municipal solid waste, may lead to accumulation of total \(\text{NH}_3\) in the leachate (Kayhanian, 1999; Hafner et al., 2006). In fact, Sommer et al. (2007) found, even during anaerobic storage of animal waste at 20°C, about 80% of organic nitrogen was mineralised. The accumulation of total ammonium nitrogen (TAN) in anaerobic leachate can lead to poor reactor performance or even reactor failure (Wiegant and Zeeman, 1986; Angelidaki and Ahring, 1994; Poggi–Varaldo et al., 1997; Gallert et al., 1998; Kayhanian, 1999; Sung and Liu, 2003; Calli et al., 2004; Eldem Nursen et al., 2004). It is also well
known that in aqueous solution, a pH dependent equilibrium exists between an ammonium ion and NH$_3$ molecule (often described as free–NH$_3$) and can be described by the following equation:

\[
\text{NH}_3 (\text{aq}) + \text{H}_2\text{O (l)} \leftrightarrow \text{NH}_4^+ (\text{aq}) + \text{OH}^- (\text{aq}). \quad (\text{Eq. 4.1})
\]

It has also been reported in literature that the NH$_3$ molecule is more toxic to methanogens than the ammonium ion (Sprott et al., 1984; Zeeman et al., 1985; Sprott & Patel, 1986; Weigant and Zeeman, 1986). Consequently, during the AD of high protein wastes, the concentration of free–NH$_3$ becomes a critical parameter and can be determined via an expression based on pH and the temperature dependent dissociation constant ($K_a$), as follows (Kayhanian, 1994; Kayhanian, 1999):

\[
[\text{NH}_3]_{\text{free}} = \frac{\text{TAN} \times \frac{K_a}{[\text{H}^+]}}{\frac{K_a}{[\text{H}^+]} + 1} \quad (\text{Eq. 4.2})
\]

or in the rearranged form (Østergard, 1985 as used by Hansen et al., 1998):

\[
[\text{NH}_3]_{\text{free}} = \frac{\text{TAN}}{1 + 10^{(pK_a - \text{pH})}} \quad (\text{Eq. 4.3})
\]

where [H$^+$], [NH$_3$]$_{\text{free}}$ and TAN are the hydrogen ion, free–NH$_3$ and total NH$_3$–N concentrations (mol/L) respectively and $pK_a = -\log_{10}(K_a)$.

As $pK_a$ is temperature dependent, the temperature effect, which must be considered for reactions occurring under thermophilic conditions, can be incorporated using the van’t Hoff equation. However, in this study, a novel approach, based on thermodynamic first principles (Gibb’s Free energy) was used to determine NH$_3$ speciation.
As $K_a = \frac{K_w}{K_b}$ and $\frac{1}{[H^+]} = 10^{pH}$, equation 4.2 can be rewritten to include the pH as follows:

$$[\text{NH}_3]_{\text{free}} = \frac{\text{TAN} \times 10^{pH}}{10^{pH} + \frac{K_b}{K_w}} \quad (\text{Eq. 4.4})$$

where $K_w$ and $K_b$ are the temperature dependent dissociation constants for the auto-ionization of water and Eq. 4.1 respectively. If the reaction in Eq. 4.1 is at equilibrium, then $\Delta G^a = -RT(K) \times \ln(K_a)$ and it therefore follows that $K_a = e^{\frac{\Delta G^a}{RT(K)}}$. $[\text{NH}_3]_{\text{free}}$ can now be written as:

$$[\text{NH}_3]_{\text{free}} = \frac{\text{TAN} \times 10^{pH}}{10^{pH} + e^{\frac{\Delta G^a - \Delta G^b}{RT(K)}}} \quad (\text{Eq. 4.5})$$

where $\Delta G_w^o$ and $\Delta G_b^o$ are the Gibb’s Free energy for water and Equation 4.1 (79.92 and 27.10 kJ/mol – calculated from Thauer et al., 1977), R is the universal gas constant $(8.314 \times 10^{-3} \text{ kJ/mol.K})$ and T(K) is the temperature in Kelvin and provides a single expression allowing $[\text{NH}_3]_{\text{free}}$ to be determined from the measurable parameters of TAN, pH and temperature (Refer to Appendix A for the complete derivation of Equation 4.5).

The major assumption made with this approach is that TAN is either in the form of $\text{NH}_4^+$ or $\text{NH}_3$ and the speciation is only dependent upon $K_a$ and pH. This assumption is approximately true for pure water, however in solutions having high concentrations of other solutes or dissolved or suspended organics (i.e. AD of MSW), $\text{NH}_4^+$ ions or $\text{NH}_3$ molecules may bind to these other species present, resulting in a decrease in their free
concentrations (Hafner et al., 2006). It is therefore appropriate to conclude that, in this study, calculations of free–NH₃ concentration will be over–estimated.

Values obtained from Equation 4.5 (Fig. 4.1) are the same as those generated by Kayhanian, (1999) (Eq. 4.2) and Hansen et al., (1998) (Eq. 4.3) and not only show an increase in [NH₃]free with increased pH but a significant increase in [NH₃]free at elevated temperatures. For example at pH 6.5, a temperature increase from 35 to 55°C (mesophilic to thermophilic) can provide a 3.5 fold increase in free–NH₃ concentration at the same level of TAN (compared to a 3.2 fold increase at pH 7.5). Accordingly, thermophilic anaerobic digesters would be expected to be 3.5 times more susceptible to NH₃ toxicity. However, in a study conducted by Gallert and associates (Gallert and Winter 1997; Gallert et al. 1998), thermophilic methanogens were found to tolerate at least twice as much free–NH₃ as

Figure 4.1: Predicted effect of pH and temperature on free–NH₃ concentration in a solution having a total NH₃ nitrogen concentration of 100mM.
mesophilic methanogens even though they were found to be more sensitive to TAN. In an attempt to determine the actual effect in the current study, inhibition constants ($K_i$) were determined for mixed cultures of the thermophilic acetoclastic and hydrogen utilizing methanogens used in the DiCOM® process.

### 4.3.1.2 Measured inhibition constants for free–ammonia

Inhibition constants for free–NH$_3$ on CH$_4$ production from acetate ($K_{1 \text{NH}_3, \text{Ac}}$) and H$_2$ ($K_{1 \text{NH}_3, \text{H}_2}$) were determined. The CH$_4$ generation rate of mixed cultures of acetoclastic and H$_2$–utilising methanogens were monitored during batch digestion of acetate and H$_2$/CO$_2$ at varying TAN concentration. A plot of reciprocal CH$_4$ generation rate against TAN gave $K_{1 \text{NH}_3, \text{Ac}}$ and $K_{1 \text{NH}_3, \text{H}_2}$ of 18 and 11mM respectively (Fig. 4.2) which are in the same order of magnitude as those presented in the literature (Refer to Table 1.3) (Experimental data is presented in Appendix B). The $K_i$ values obtained in the current study indicate that the organisms involved in the production of CH$_4$ from H$_2$ were more sensitive to free–NH$_3$ than those using acetate as substrate. This finding is supported by a study by Wiegant and Zeeman (1986) on thermophilic AD of cattle manure but is contradictory to other studies reported in the literature (Koster and Lettinga, 1984; Zeeman et al., 1985; Sprott & Patel, 1986; Borja et al., 1996b; Hansen et al., 1998; Schnürer et al., 1999). Acetoclastic methanogens were found to be twice as sensitive to free–NH$_3$ during thermophilic biomethanation of cattle manure (Angeladaki & Ahring, 1993). Schnürer and Nordberg, (2007) found elevated NH$_3$ concentration, under mesophilic conditions, caused a shift from acetoclastic methanogenesis toward syntrophic acetate oxidation, a process dependent upon CH$_4$ production from H$_2$. Schnürer and Nordberg’s observation suggests hydrogenotrophic methanogens are less sensitive to free–NH$_3$ toxicity however, no inhibition constant was provided.
Figure 4.2: Plot of reciprocal CH₄ generation rate at varying TAN concentrations to determine the NH₃ inhibition constants (K_{NH₃}) for CH₄ produced (A) from acetate and (B) hydrogen. Values of the inhibition constants were determined from the equations of the least square regression line for (A) y = 0.18x + 3.2; r = 0.87 and (B) y = 0.75x + 8.39; r = 0.83, where r is the linear correlation coefficient. Dashed lines indicate where the inhibition constants could be read from the graph and were found to be K_{NH₃,Ac} = 18mM and K_{NH₃,H₂} = 11mM.

Acetate–utilising methanogens in cattle manure, adapted to NH₃, have been shown to grow in the presence of free–NH₃ concentrations of up to 41mM (Angelidaki and Ahring, 1994) whereas methanogens present in swine manure were inhibited by 79mM
free–NH₃ (Hansen et al., 1998). It is likely that the variation in inhibiting free–NH₃ concentrations reported in the literature is likely due to differences in culturing temperature, pH and acclimation procedures (Gallert et al., 1998). In the Wiegant and Zeeman (1986) study mentioned above, even though the original inoculum was obtained from the same source, acetate and H₂ consuming methanogens were enriched under dissimilar conditions, including background ammonium concentrations. Wiegant and Zeeman concluded that acclimation of the culture to high TAN concentrations may play a significant role in their finding. It is unlikely that acclimation is a significant factor in the current study; as both acetoclastic and H₂ consuming methanogens are maintained within the same liquor. Conversely, dilution may play a role in the finding that H₂ consuming methanogens are more sensitive than acetoclastic methanogens to free–NH₃.

Given that the concentration of free–NH₃ is influenced by both pH and temperature, and that free–NH₃ is the inhibitor of methanogenesis, the degree of methanogenic inhibition can be predicted from the TAN, pH and temperature. The rate of acetoclastic methanogenesis for a given TAN, determined from the equation of the least square line of regression (Fig. 4.2A), can be compared to the uninhibited rate (TAN = 0) to estimate the percentage inhibition (Fig. 4.3). The inhibition is enhanced at elevated pH and is inline with the literature (Poggi–Varaldo et al., 1997). It is interesting to note that the point of inflection of the curve, that is, the point at which the inhibition is increasing most rapidly, occurs between pH 7.3 and 7.7 for 250 and 100mM TAN, respectively. This is of concern as it is under these conditions (pH and TAN concentration) that the DiCOM® process typically operates toward the conclusion of digestion.
Figure 4.3: Predicted percentage inhibition of methanogenesis as a function of pH and TAN for CH$_4$ produced from acetate (K$_{\text{NH}_3,\text{Ac}} = 18$ mM) assuming that no volatilization of NH$_3$ occurs.

4.3.1.3 Effect of ammonia build–up during liquor recycle/reuse

The biological degradation of nitrogen containing compounds within OFMSW generates NH$_3$ (Anderson and Yang, 1992; Gallert and Winter, 1997; Kayhanian, 1999), which may be in excess of that required for microbial growth. It is therefore anticipated that reuse of the anaerobic recylcate during repeated DiCOM® operation will lead to an accumulation of NH$_3$ in the absence of adequate NH$_3$ sinks. Apart from NH$_3$ volatilisation within the biogas (contributing to less than 0.05% loss and was therefore not included in predictions), the only significant NH$_3$ sink in the DiCOM® process is the solid itself. When the recylcate is drained from the reactor, the solid is wet with the recylcate. Even though the solid is mechanically squeezed, the moisture content is approximately 60%, which accounts for approximately 7% of the NH$_3$ contained within the recylcate. Using the formula:
\[ \text{TAN}_{\text{(end recycle)}} = \text{TAN}_{\text{(start recycle)}} + \text{NH}_3^{\text{(generated)}} - \text{TAN}_{\text{(removed in solid)}} \]  
(Eq. 4.6)

the accumulation of TAN in the anaerobic recycle can be predicted (Fig. 4.4). This prediction assumes that the amount of NH$_3$ produced per cycle is constant (33mM as per laboratory data) and its production is not influenced by the amount of NH$_3$ which is present in the recycle. Based on these assumptions, the TAN concentration would approach an equilibrium at a point where the amount of NH$_3$ produced during each trial is equal to that removed in the solid.

When combining the NH$_3$ inhibition values and the predicted NH$_3$ build–up, it can be concluded that any more than 6 reuses the accumulated NH$_3$ will inhibit methanogenesis by more than 50% (Fig. 4.4). If the aim is for rapid digestion, then an additional NH$_3$ sink must be developed.

![Figure 4.4: Predicted build–up in of NH$_3$ and toxicity as a function of number of reuses of anaerobic recycle (temperature = 55°C; pH = 7.5: the pH typically noted toward the completion of digestion in laboratory trials and NH$_3$ accumulation = 33mM/trial). Also indicated is an “NH$_3$ window” showing the number of reuses which provide moderate methanogenic inhibition.](image)

In laboratory DiCOM® trials, as is typical of batch AD of solid waste, acidification of the recycle was observed (Fig. 4.5) within the initial few days of digestion, which is in
line with what has been reported in the literature (ten Brummeler, 2000; Chynoweth et al., 2003a; Chynoweth et al., 2003b; Müller et al., 2006). During poorly performing trials, total VFA have been found to accumulate to 250mM. In a trial where only water was introduced into the reactor, rather than reusing the anaerobic liquor (data not shown), VFA were found to accumulate, pH fell to 5.5 and the digestion failed. However, in trials where NH\textsubscript{3} was present in the recylcate, even though VFA accumulation has been observed, pH has been stable (6.5 – 7.5) and digestion was successful. Therefore the NH\textsubscript{3} produced during digestion not only has a negative influence on the methanogens at high concentrations and pH but increases the buffer capacity of the recylcate (Georgakakis et al., 1974; Anderson and Yang, 1992) and stabilizes the pH of the digester under conditions of acidification (Angelidaki and Ahring, 1993). Hence an “NH\textsubscript{3} window” can be specified at which sufficient buffer capacity is provided without causing significant inhibition (Fig. 4.4).

4.3.2 Predicted Build–up of Propionate Inhibition During Reuse of the Anaerobic Recyclate

Propionate was found to accumulate in laboratory DiCOM\textsuperscript{®} trials. High concentrations of organic acids have also been found to be toxic to methanogens (Koster and Cramer, 1987; Barredo and Evison, 1991; Mösche and Jördening, 1999). In an attempt to determine whether organic acid toxicity could be a problem in the DiCOM\textsuperscript{®} process, laboratory trials were operated under non–ideal conditions and demonstrated that propionate and butyrate could accumulate through fermentative metabolism during the 7 day digestion period (Fig. 4.5). The implication is that the residual VFA (primarily propionate and butyrate) would be introduced into a subsequent trial, not only providing a greater organic input but the digestion would need to function under a higher VFA concentration. Since propionate is converted to acetate, only after the initial concentrations of acetate and butyrate are completely degraded (Duarte and Anderson,
1982; Öztürk, 1991), and propionate consumption has been proposed to be the rate limiting step due to slow consumption rate (Duarte and Anderson, 1982; Aguilar et al., 1995; Pind et al., 2003), propionate will continue to accumulate during subsequent sub–optimally performed trials (as noted in laboratory trials (data not shown)) possibly resulting in process failure.

Propionate inhibition has been well documented and has been implicated as a major cause of digester failure (Hobson and Shaw, 1976; Barredo and Evison, 1991) and the best indicator of process imbalance (Nielsen et al., 2007). Barredo (Barredo and Evison, 1991) showed that, under mesophilic conditions, propionate inhibited both biological growth and cumulative CH$_4$ production at concentrations as low as 20mM. Dhaked et al. (2003) observed propionate inhibition of methanogenesis during psychrophilic treatment of night soil and a 100 fold decrease in methanogenic count. Dogan et. al., (2005) found that mesophilic granular sludge from a UASB reactor treating alcohol distillery effluents was susceptible to propionate inhibition and that degree of inhibition was greater than for other VFA. Hajarnis and Ranade (1994) demonstrated that not only was CH$_4$ production decreased by elevated propionate levels but methanogenic inhibition was drastically enhanced as pH was reduced suggesting that methanogens are inhibited by the protonated form of this VFA (propionic acid) which is present in the reactor at low pH. In contrast however, Pullammanappallil et al., (2001) reported that CH$_4$ production from a mesophilic (35°C) digester was not effected by elevated propionate concentrations (38mM and pH 6.5). As acidification and propionate accumulation have been noted in laboratory trials (Fig. 4.5), it is essential to determine whether propionate is inhibitory in this system and, if so, the magnitude of such inhibition.
Figure 4.5: Laboratory–scale VFA profile showing incomplete propionate metabolism during 7 days of anaerobic treatment. pH data during day 12 was lost due to probe failure.

Legend: (■) Acetate; (●) Propionate; (---) pH.

The concentration of propionic acid can be determined from total propionate, temperature, pH and \( \Delta G^\circ \) from the following:

\[
\left[\text{CH}_3\text{CH}_2\text{COOH}\right] = \frac{[\text{Total Propionate}]}{1 + 10^{\text{pH}} \times e^{-\frac{-\Delta G^\circ_{\text{CH}_3\text{CH}_2\text{COOH}}}{\text{R} \times \text{T}}}} \tag{Eq. 4.6}
\]

where \( \Delta G^\circ_{\text{CH}_3\text{CH}_2\text{COOH}} = 27.80 \text{kJ/mol} \) (Speight, 2005), is the Gibb’s free energy of the reaction:

\[
\text{CH}_3\text{CH}_2\text{COOH (aq)} \leftrightarrow \text{CH}_3\text{CH}_2\text{COO}^- (\text{aq}) + \text{H}^+ (\text{aq}) \tag{Eq. 4.7}
\]

(Refer to Appendix C for the complete derivation of Equation 4.6). As expected, the propionic acid concentration increases as pH decreases (Fig. 4.6). However, for a given total propionate concentration and pH the concentration of propionic acid decreases with increased temperature (Fig. 4.6). A change from mesophilic (35°C) to thermophilic (55°C) conditions would result in a 2 fold decrease in propionic acid.
concentration. From this point of view, thermophilic anaerobic digesters could be expected to be more stable than mesophilic digesters under the same pH and propionate stress. However, this conclusion ignores the sensitivity of the organisms toward propionic acid, which needs investigation.

![Diagram](image)

**Figure 4.6:** Predicted concentration of propionic acid as a function of pH and temperature with a total propionate concentration of 20mM.

Propionate was found to be inhibitory to the methanogens in the DiCOM® process. Inhibition constants for propionate inhibition of acetoclastic and hydrogenotrophic methanogenesis were determined, however the data set was not well clustered, resulting in lower than desired correlation coefficients (r). Equations of the least square line of regression for inhibition of acetoclastic and hydrogenotrophic methanogenesis were $y = 51.6x + 10.2; r = 0.64$ and $y = 33.1x + 19.5; r = 0.55$, providing inhibition constants of $K_{I_{PropAc}} = 0.2$ and $K_{I_{PropH_2}} = 0.5$ mM respectively. These values are of the same order of magnitude as those used by Mosche and Jordening (1999) while modelling...
inhibition of CH$_4$ production from acetate ($K_{\text{Prop,Ac}}$ = 0.36mM; 27mg/L). The experimental results from the current study suggest that the micro–organisms involved in producing CH$_4$ from H$_2$, during the DiCOM$^\circledR$ process, were less susceptible to the toxic effects of propionic acid than acetoclastic methanogens.

In the absence of more reliable data, the inhibition constants determined from laboratory experiments were used to predict inhibition of methanogenic activity during laboratory–scale DiCOM$^\circledR$ trials (Fig. 4.7). Approximately 45% inhibition of methanogenic activity from acetate is predicted for pH and total propionate concentration of 6.5 and 20mM, respectively. As this pH and total propionate concentration are typically observed during laboratory experiments, where residual propionate is transferred, propionic acid inhibition could play an important role in process operation.

![Figure 4.7](image_url)

**Figure 4.7:** Predicted propionic acid inhibition on the production of CH$_4$ from acetate as a function of pH and total propionate concentration (where total propionate concentration = propionic acid + propionate concentrations and $K_{\text{Prop,Ac}}$ = 0.2mM).
4.3.3 Predicted Combined Inhibition by Ammonia and Propionate – A “pH Window” for Stable Digester Operation

During AD, methanogens are inhibited by free–NH$_3$ at high pH and by propionic acid at low pH. It is therefore appropriate to assume that there exists an optimum pH to operate a digester where the influence of these two inhibitors is minimised. Methanogenic activity, from acetate, can be expressed as:

\[
\text{Methanogenic Activity (\%) = } v_{\text{max}} \times \frac{K_{1NH_3Ac}}{K_{1NH_3Ac} + [NH_3]^3_{\text{free}}} \times \frac{K_{1PropAc}}{K_{1PropAc} + [\text{propionic acid}]}
\]  

(Eq. 4.8)

where $v_{\text{max}} = 100$ and $K_{1NH_3Ac}$ and $K_{1PropAc}$ are the inhibition constant for free–NH$_3$ and propionic acid respectively. Thus methanogenic activity, from acetate, can be represented diagrammatically (Fig. 4.8) as a function of pH and TAN. It is interesting to note, that even at the optimum pH for methanogens (6.5–7.8: Anderson et al., 2003) methanogenic activity from acetate was, to some degree, always inhibited during DiCOM$^\circledR$ operation. Within this optimum pH range, the methanogenesis from acetate was inhibited by propionic acid and free–NH$_3$ as the regions of inhibition overlap (compare Fig. 4.3 & Fig. 4.7).

The predicted optimum pH, to provide maximum methanogenic activity from acetate (at a total propionate concentration of 20mM) (Fig. 4.8), varies according to TAN concentration. At 75 and 250mM TAN the optimum pH is predicted to be 7.2 and 6.9, respectively, with maximum methanogenic activities being only 69 and 53% respectively. Methanogenic activity is predicted to improve with a decrease in TAN and at 25mM TAN the pH optimum is 7.4, allowing 80% methanogenic activity. However under these conditions, it is likely that a lack of buffer would be unable to maintain process stability under acidification. Therefore a compromise must be sought between the amount of NH$_3$ necessary to provide stability under acidification and the
degree to which the methanogens are inhibited. A higher TAN concentration (250mM) would provide greater buffer capacity, and stability against acidification, but the methanogens would be more inhibited. Furthermore, based on the modelled “pH window” (Fig. 4.8) methanogenic activity can only be maintained above 50% over a very small range of pH values (6.7 – 7.1). On the other hand, a decrease in TAN (75mM) decreases buffering capacity, and as a consequence process stability, but increases the rate of methanogenesis (for a given pH) and the pH range at which maximum methanogenic activity can be maintained (e.g. above 50%: pH 6.5 – 7.8).

Figure 4.8: Predicted activity of CH₄ from acetate during thermophilic anaerobic digestion exhibiting a “pH Window” (> 50% methanogenic activity) between VFA inhibition and NH₃ inhibition. Data were modelled using: acetate concentration = 100mM; K_ac = 0.399mM (Ahring and Westermann, 1987); K_I_NH₃ = 18mM; K_I_Prop = 0.2mM and typical propionate (20mM) as observed in laboratory (Fig. 4.5) and pilot–scale trials (Section 8.3.1).
4.3.4 Testing of Model Predications With Experimental Results

4.3.4.1 Set-up and results of experimental trials

To investigate whether the modelled “pH window” for maximum methanogenic activity can accurately predict laboratory-scale reactor performance three laboratory trials (Fig. 4.9) were set-up as described in Table 4.3. It was not the intention to compare the performances of these trials per se but to assess whether the model could predict methanogenic activity, and therefore reactor performance, under a variety of diverse conditions such as high TAN (Trial A), low buffer (Trial C) and optimised conditions (Trial B).

Table 4.3: Experimental constraints for laboratory digester trials.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Experimental Constraint</th>
<th>Substrate</th>
<th>TAN Concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>High TAN</td>
<td>OFMSW</td>
<td>220</td>
</tr>
<tr>
<td>B</td>
<td>Optimised</td>
<td>OFMSW</td>
<td>80</td>
</tr>
<tr>
<td>C</td>
<td>Low Buffer</td>
<td>Synthetic OFMSW</td>
<td>190</td>
</tr>
</tbody>
</table>

Trial A (Fig. 4.9 A), a standard DiCOM® trial, was performed using a recyclate high in TAN concentration. The pH of the recyclate fell from 7.4 to 7.0 under acidification and rose to 7.9 by the conclusion of the digestion period. Rapid acetate consumption was noted (day 7) followed by a period of decreased acetate uptake (days 9 – 12) while the acetate was well above the saturating concentration for the acetate degrading *Methanosarcina* and *Methanothrix* (*Methanosaeta*) (3mM and 0.2mM respectively: Min and Zinder, 1989). The rate of CH₄ production declined toward the end of digestion (day 8 onwards) even though the substrate for methanogenesis remained, suggesting the build–up of inhibitory effects. In this trial, digestion was incomplete as VFA were not completely degraded; free–NH₃ was found to rise to 72mM (day 12); and propionic acid was found to rise to a maximum of 0.03mM (day 6 and 7).
Trial C (Fig. 4.9 C) was performed under standard DiCOM® constraints using a synthetic OFMSW which had decreased buffer capacity (40.6 mg CaCO₃/g dry OFMSW compared to 6.9 mg CaCO₃/g dry synthetic OFMSW). VFA were found to rise swiftly (total VFA 650mM) resulting in a concomitant decrease in pH from 7.2 to 6.3, within the first 24h, and ultimately to 6.1 (day 12). CH₄ production was poor throughout the trial. Free–NH₃ was found to fall from 12mM (day 5) to 1mM (day 12) and propionic acid was found to rise to 1.5mM (day 12).

Trial B (Fig. 4.9 B), an optimised DiCOM® trial, experienced early acidification followed by rapid VFA consumption. CH₄ generation commenced immediately after the recyclate, containing moderate TAN, was introduced and peaked during maximum acetate consumption (day 6). The CH₄ generation rate by day 12 was low and corresponded to exhaustion of VFA in the recyclate. Free–NH₃ rose to 14mM (day 11) while propionic acid concentration peaked at 0.12mM (day 6).

4.3.4.2 Methanogenic inhibition can be predicted in experimental trials

When comparing the experimental outcomes of the three trials with the model predictions, it can be concluded that the predicted methanogenic inhibition by propionic acid and free–NH₃ was confirmed by the laboratory data (Fig. 4.9). Trial A and C show inadequate CH₄ production, particularly when the pH of the recyclate was outside the “pH window” (6.6–7.2 Fig. 4.8: based on 200mM TAN), which coincides with less than 50% methanogenic activity (day 7 – 12). As a consequence, this decreased activity could account for the incomplete VFA metabolism observed in these two trials.
Figure 4.9: Performance of laboratory–scale anaerobic digestion trials. Optimum performance (B) showed complete removal of propionate with methanogenic activity greater than 50%. Sub–optimal performance was demonstrated under high TAN loading (A) (TAN increased linearly from 220 to 275 mM) and acidification (C) with incomplete metabolism of VFA and significant methanogenic inhibition towards the conclusion of each trial.

Legend: (■) Acetate; (♦) Propionate; (▲) Butyrate; (●) Free–NH₃; (◊) Propionic Acid; (×) pH; (— —) Methanogenic Activity; (—) CH₄ Generation Rate.
However Trial B (Fig. 4.9 B), which operated within the “pH window” (6.5 – 7.8 Fig. 4.8: based on 80mM TAN), had greater than 50% activity and allowed complete degradation of the organic acids. Significant methanogenic inhibition was noted toward the end of the anaerobic period in trial A (Fig. 4.9 A: day 8 – 12), as the free–NH₃ concentration rose rapidly, due to the elevated pH of the recyclate. This decrease in methanogenic activity is also reflected in a decrease in acetate consumption during days 8 – 10.

The elevated concentration of free–NH₃ is likely to provide an explanation for the incomplete propionate degradation in trial A. Siegrist et al. (2002) also noted that propionate is not removed at elevated NH₃. This is in line with the observation that propionate degrading acetogenic bacteria have been found to be more sensitive than methanogens to free–NH₃ (Calli et al., 2004). Interestingly, poor CH₄ generation in this trial was noted during the initial days of anaerobic treatment (Fig. 4.9 A: day 5 – 7) when methanogenic activity was predicted to be at its highest. The low CH₄ production, coupled with the accumulation of VFA, particularly acetate (the primary substrate for methanogenesis) suggests a limited number of methanogens were present. It was anticipated that this poor performance was related to the use of an inactive (or inadequate) anaerobic inoculum which had been stored for an extended period (8 weeks).

Extreme acidification leading to reactor failure could also be predicted. In Trial C (Fig. 4.9 C), due to the lack of buffer provided by the solid, the pH rapidly fell below 6.5. Even though TAN levels were relatively high (220mM = 190mM plus NH₃ accumulation during the trial), methanogenic inhibition was not the result of NH₃ toxicity (as free–NH₃ concentration was low due to the low pH) but rather propionic
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acid toxicity as a consequence of the elevated propionate concentration and low pH of the recyclate.

The principal cause of inhibition is not explained by the absolute amount of the inhibiting species, for example propionate or TAN, but by a pH drift that enhances the inhibitory effect. As it is the free–NH$_3$ that is toxic to micro–organisms, the pH drop from accumulation of VFA will decrease the methanogenic inhibition. This was most clearly demonstrated in Trial A, where the predicted activity rose during the first 2 days of digestion (Fig. 4.9 A: day 5 – 7), when the digester experienced acidification. The lowering of pH to remove NH$_3$ toxicity of methanogens is not new and has been reported in a study conducted by Angelidaki et al. (1993a). However, in a batch system, if VFA accumulate, the less inhibited anaerobes may consume more of the VFA. If VFA consumption exceeds production, the pH will rise and the toxic effect of the free–NH$_3$ will again be evident.

4.4 Conclusions

Modelling the reuse of anaerobic liquor in the AD process has identified that:

• A narrow “pH window” exists whereby the concentrations of microbial metabolites, in this case NH$_3$ and propionate, influence the optimum pH for process operation.

• Without intervention, the number of reuses of the anaerobic liquor is limited before methanogenesis experiences toxicity from the accumulated salts and microbial metabolites.

• Methanogens in a batch thermophilic AD process of OFMSW may be, to some degree, always inhibited (as is the case with DiCOM®), even in the optimal pH range for methanogens (6.5 – 7.5), with the degree of inhibition able to be predicted under a given set of conditions.
5.1 Introduction

The reuse of anaerobic liquor (Walker et al., 2006b) offers a significant environmental advantage in that there is a decrease in volume of the liquid waste stream that requires further processing and accordingly, a decrease in energy consumption and demand for fresh water. In fact, the DiCOM® process specifies that no liquid waste stream will be produced from the process which makes this technology attractive within the current climate where water is considered to be a limited and valuable resource. In addition to the benefit of decreased water consumption and the advantages/disadvantages associated with accumulation of salts and intermediates (as described in Chapter 4), liquor reuse is a potential source of microbial inoculum.

The successful start–up of an AD system requires a balance between the hydrolytic and fermentative micro–organisms, on the one hand, and the methanogenic micro–organisms, on the other. Typical batch AD of solid waste goes through a phase of acidification, compromising the time required for bio–stabilisation. However, the addition of inoculum has been shown to decrease the duration of reactor start–up (Meisgeier et al., 2003) and the time required for stabilisation of the solid (Lopes et al., 2004). Generally the inocula originate from sewage sludge (Krzystek et al., 2001; Meisgeier et al., 2003; Maroun and El–Fadel, 2007), animal products (Lopes et al., 2004), animal manure (Juanga et al., 2007; Maroun and El–Fadel, 2007; Nguyen et al., 2007; Sommer et al., 2007) and sludge, leachate or digested/stabilized solid from anaerobic digesters (Chynoweth et al., 2003b; Angelidaki et al., 2006; El–Mashad et al., 2006; Climent et al., 2007; Juanga et al., 2007; Nguyen et al., 2007). Thermophilic systems are often started using a mesophilic culture that is gradually acclimated to
thermophilic conditions (Bolzonella et al., 2003b; van Lier et al., 1993; Fang and Lau, 1996).

With rapid processing becoming a priority in waste management systems, inocula, their source and the inoculum/waste ratio have become the focus of much investigation. Lopes et al. (2004), while studying the influence of bovine rumen fluid inoculation on batch AD of OFMSW, found that rumen fluid (15% (w/w)) doubled the CH$_4$ production rate and accordingly, halved the time required for solid stabilisation, when compared with no added inoculum. The improved reactor performance noted may be the result of an increase in the number of micro–organisms contributing to the degradation of organic matter present in the reactor.

El–Mashad et al. (2006) found that addition of inoculum improved reactor performance when investigating AD of cattle waste (faeces, urine and bedding material) under thermophilic conditions (50°C) in a laboratory–scale (30L) non–mixed accumulation (fed–batch) reactor. Inoculum (10% (v/v)), effluent from a mature sister reactor during leachate recirculation, was equally divided and added in doses with feed (0.1L/L/week) during a 60 day period. Results indicated that semi–continuous inoculation allowed a higher MPR resulting in a 25% increase in MPR compared to a once only inoculum addition.

Juanga et al. (2007) observed improved pilot–scale (375L; working volume 260L) reactor performance when fresh MSW (mainly food waste) was inoculated with leachate via a thermophilic (55°C) sequential stage batch system. Mature leachate, obtained from a second reactor containing stabilised waste, was cross–recirculated between fresh and mature waste. As a consequence of this cross–circulation not only
did the leachate from the mature reactor provide inoculum to the fresh waste but the solid matrix of the mature reactor provided an established microbial consortium (inoculum) for treatment of acidified leachate from the fresh waste. During operation of the sequential staging system, the onset of methanogenesis was earlier and more rapid providing a 3–fold increase in MPR in the first 24h when compared to a system without transfer of mature leachate but inoculated with a mixture of cow manure, digested waste and anaerobic sludge.

Sommer et al. (2007) reported that, an inoculum (7.6% (w/w)) of digested piggery and cattle slurry (faeces and urine) provided an immediate 5–fold improvement in MPR during storage (10 days at 20°C) of the fresh slurry. Forster–Carneiro et al. (2007) explored the effect of a variety of inoculum sources on the performance of dry (30% TS) thermophilic (55°C) AD of OFMSW. They found that mesophilically digested wastewater treatment sludge, used as an inoculum, provided a 2.5–fold increase in average MPR when compared to thermophiliacally digested separately–collected OFMSW.

From the above discussion, it is clear that the addition of an appropriate inoculum provides the opportunity for improved process performance and shorter start–up and waste processing times (Lopes et al., 2004). It would seem logical that greater inoculum levels would enable higher MPR. However, results from Fernández et al. (2001) and Angelidaki et al. (2006) indicated that maximum CH₄ yields and best degradation rates of organic waste were achieved at relatively low inoculum levels. Both studies implicated high TAN concentrations, which were introduced by larger inoculum volumes, as the source of methanogenic inhibition.
The DiCOM® process relies on 2 primary sources of methanogenic inoculum, the “recyclate” and the “pressate”. The effectiveness of these inocula has not yet been examined. The purpose of this chapter is to investigate the sources of methanogenic inocula in the DiCOM® process and the roles they play in process performance.

5.2 Materials and Methods

5.2.1 Locating The Organisms That Produce Biogas In The DiCOM® Process

Samples of recyclate, pressate and exhausted digested OFMSW solid were removed from the laboratory–scale reactor at the completion of the anaerobic phase of a standard DiCOM® trial. Each component (recyclate, pressate, digested OFMSW solid, sterile de–oxygenated DI H₂O and a combination of all three components) was introduced into one of four sterile 100mL serum vials (Wheaton) to a total volume of 40mL. To a fifth vial, solid that had been aerated using a standard DiCOM® protocol for 5 days, was added to sterile de–oxygenated DI H₂O (total volume 40mL). An excess of acetate (30mM) was added to all vials after which they were sealed with 1cm thick butyl rubber stoppers and capped with aluminium crimp seals. The head space of each vial was then flushed with nitrogen gas before vials were incubated in a 55°C shaking water bath (Lab–Line Instruments: 3540–1 at 200rpm). After the medium had equilibrated to 55°C (20min), the bottles were vented via a syringe needle to atmospheric pressure. Biogas produced during a 24h incubation period was measured using the downward displacement of water. The rate of biogas production from each flask was used to predict the number of active biogas producing micro–organisms.

5.2.2 Methanogen Survival During Aeration In The DiCOM® Process

1g samples of OFMSW solid were removed daily from a laboratory–scale DiCOM® reactor during the initial aeration phase of a standard trial. The solid was immediately
mixed with 50mL basal media and then homogenised for 30sec in a blender, which had been sterilised using 70% ethanol. The mixed liquor (0.5mL) was then serially diluted in tubes (16mL Hungate) containing 4.5mL of anaerobic medium. Dilutions were performed by transferring 0.5mL between Hungate tubes. The headspace of Hungate tubes were flushed with N$_2$/CO$_2$ (80:20) (for acetoclastic methanogens) and H$_2$/CO$_2$ (80:20) (for H$_2$ utilising methanogens) before being incubated at 55°C. After incubation, positive growth was recorded if CH$_4$ was present in the headspace.

The counts of methanogen populations present in the solids during the initial aerobic phase of the DiCOM® reactor were estimated by 3–tube most probable number (MPN) technique according to standard method described by de Man (1975). Anaerobic media consisted of basal media and 80mM acetate (for acetoclastic methanogens) and 10mM acetate (for H$_2$ utilising methanogens).

Basal media, based on DSM 334 (DSMZ, 1983), was prepared by dissolving 0.3g KH$_2$PO$_4$; 0.6g NaCl; 0.1g MgCl$_2$.6H$_2$O; 0.08g CaCl$_2$.2H$_2$O; 1.0g NH$_4$Cl; 1.0mg Resazurin; 2.0g KHCO$_3$; 10.0mL vitamin solution and 10.0mL trace element solution in 1L of DI H$_2$O. The substrate (acetate) was added, as its sodium salt, to the required concentration. Medium was dispensed into screw–cap tubes (16mL Hungate) fitted with butyl rubber septa (Bellco Glass Inc.) before headspaces were flushed with N$_2$/CO$_2$ (80/20 v/v) and autoclaved at 126°C for 20 min. Prior to inoculation, the medium was reduced with sterile stock solutions of Na$_2$S.9H$_2$O and cysteine–HCl.H$_2$O each to a final concentration of 0.3g/L.

Trace element solution was prepared by dissolving 12.8g Nitrilotriacetic acid; 1.35g FeCl$_3$.6H$_2$O; 0.1g MnCl$_2$.4H$_2$O; 0.024g CoCl$_2$.6H$_2$O; 0.1g CaCl$_2$.2H$_2$O; 0.1g ZnCl$_2$;
0.025g CuCl$_2$.2H$_2$O; 0.01g H$_3$BO$_3$; 0.024g Na$_2$MoO$_4$.2H$_2$O; 1.0g NaCl; 0.12g NiCl$_2$.6H$_2$O and 0.026g Na$_2$SeO$_3$.5H$_2$O in 1L of DI H$_2$O. The vitamin solution was prepared by dissolving 2.0mg biotin; 2.0mg folic acid; 10.0mg pyridoxine hydrochloride; 5.0mg thiamin hydrochloride; 5.0mg riboflavin; 5.0mg nicotinic acid; 5.0mg DL–calcium pantothenate; 0.1mg vitamin B$_{12}$; 5.0mg p–aminobenzoate; and 5.0mg lipoic acid in 1L of DI H$_2$O.

5.2.3 The Role Of The Methanogen Population From Pre–Aerated Solids During The Start–Up Of The Anaerobic Phase Of The DiCOM® Process

Batch experiments were set–up in 50ml serum vials (Wheaton). Each vial contained 5g of solid, aerated for 5 days in a laboratory–scale DiCOM® reactor according to standard DiCOM® constraints (as per Section 2.2.1), and 25mL of either DiCOM® recyclate, the filtrate of DiCOM® recyclate (anaerobically filtered through 0.45µm filter paper) or sterile DI H$_2$O. After the contents were introduced, the serum vials were sealed, headspaces flushed, incubated, equilibrated and depressurised as described above (Section 5.2.1). The volume of biogas produced during the 24h incubation period was measured using the downward displacement of water, while biogas composition was measured by gas chromatography as described in Section 4.2.4. All trials were conducted in duplicate.

5.2.4 The Effect Of DiCOM® Recyclate Inoculation On Early Methane Production

DiCOM® recyclate, freshly drained from a laboratory–scale reactor, was divided into 2 equal portions. One portion was used without treatment while the other was centrifuged (Hermle Z233M: 13,000rpm for 5min) and the supernatant filter sterilised using a 0.2µm syringe filter. Trials were set up in 100ml serum vials (Wheaton) as shown in Table 5.1.
**Table 5.1:** Contents of 100 mL serum vials used to investigate the effect of varying concentrations of DiCOM® recyclate micro–organisms has on CH₄ production.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Fresh MSW (gm)</th>
<th>% inoculum</th>
<th>fresh recyclate ml</th>
<th>filtrated recyclate ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5gm</td>
<td>100</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>5gm</td>
<td>10</td>
<td>4</td>
<td>36</td>
</tr>
<tr>
<td>3</td>
<td>5gm</td>
<td>1</td>
<td>0.4</td>
<td>39.6</td>
</tr>
<tr>
<td>4</td>
<td>5gm</td>
<td>0</td>
<td>0</td>
<td>40</td>
</tr>
</tbody>
</table>

After the contents were introduced, the serum vials were sealed, headspaces flushed, incubated, equilibrated and depressurised as described above (Section 5.2.1). Biogas generation was monitored routinely over a 13 day period, by the downward displacement of water, and biogas composition determined by gas chromatography. All experiments were prepared in duplicate.

### 5.2.5 Chemical Analysis

Biogas composition was determined as per Section 4.2.4

### 5.3 Results and Discussion

#### 5.3.1 In the DiCOM® Process, Where Are the Organisms That Produce Biogas?

It has been established (Chapter 3) that the introduction of anaerobic liquid and “pressate” has a positive effect on the onset of methanogenesis. Based on this observation it can be concluded that the transferred material contains methanogens which can be used as an inoculum for subsequent trails. As the duration of the digestion phase of the DiCOM® process is only seven days, methanogenesis must commence rapidly, without the typically reported delay, and as a consequence, the size of the inoculum could play an important role in shortening the digestion phase.
In an initial attempt to determine the capacity of each component of the inoculum/solid system to produce biogas, serum vial experiments were conducted (Raw data is displayed in Appendix D). Results demonstrated that, while most of the biogas activity at the beginning of the anaerobic phase (an estimated 72%) was attached to the OFMSW that had been aerated (Fig. 5.1), at the onset of the anaerobic phase an estimated 28% was transferred from the previous trial (Fig. 5.1 A). Consequently, the activity of the inoculum could have a significant impact on the rate of biogas, and as a result CH₄ produced early in the anaerobic phase and therefore on overall process performance. The gas production by the micro–organisms contained within the OFMSW was surprisingly high but as the composition of the biogas produced was not analysed, no conclusion can be drawn as to the survival of methanogens during aeration.

![Diagram of total biogas production](image)

**Figure 5.1:** Percentage of total biogas (an unspecified mixture of CH₄, CO₂ and H₂) produced from each component of the DiCOM® process at (A) the beginning of the anaerobic phase and (B) the end of the anaerobic phase. Percentages were obtained from data in Table D.1 (Appendix D).

### 5.3.2 Methanogens Survive the Initial Aerobic Phase of the DiCOM® Process.

The large amount of CH₄ produced from landfills (Themelis & Ulloa, 2007) indicates that a significant number of methanogens exist within MSW. As methanogens are obligate anaerobes, the effect of aerating the MSW prior to AD on the endogenous methanogens is not well known. To investigate the survival of methanogens contained within the OFMSW feedstock during the initial aerobic phase of the DiCOM® process,
the growth of acetoclastic and hydrogenotrophic methanogens was monitored using a MPN technique (Dr Wipa Charles).

Although the OFMSW used in this experiment had been frozen for 2 years the number of active methanogens were found to be approximately $10^5$ and $10^3$ cells/gm OFMSW for H$_2$ utilising and acetoclastic methanogens, respectively (Fig. 5.2). The population of acetoclastic methanogens was found to be quite stable during the period of aeration. Conversely the number of H$_2$ consuming methanogens was found to rise during the first day of aeration, while aerobic microbial activity was at its peak and the O$_2$ level in the reactor was at the lowest (<1 ppm) (Fig. 5.3). The number of these methanogens then decreased close to the initial level, possibly due to increasing O$_2$ concentration within the reactor. This suggests that *Archaea* are present on the OFMSW starting material and survive the aerobic period, hence some methanogenic inoculum is provided by the starting material (Charles *et al.*, 2009).

![Figure 5.2: Methanogen numbers present in the OFMSW solid during the initial aerobic phase of the DiCOM® process. Legend: (●) Hydrogenotrophic methanogens; (▲) Acetoclastic methanogens.](image-url)
5.3.3 The role of the methanogen population from pre-aerated solids during the start up of the anaerobic phase of the DiCOM® Process

As large numbers of methanogens were found to be present in aerated OFMSW prior to the inoculation with the anaerobic recylcate and pressate, an experiment was designed to determine the contribution of these microbes during the initial 24h of the anaerobic phase of the DiCOM® process.

During the first 24h of anaerobic treatment, the type of liquid added had no significant effect on the volume of biogas produced (Fig. 5.4) but did influence the composition of the biogas. No CH₄ formation was observed when only water was added (Fig. 5.4 C), which was possibly due to the accumulation of VFA leading to acidification (pH = 5.6). The buffer capacity of the anaerobic liquor added to the remaining tests provided stability against this acidification (pH > 6.0) and enabled methanogenesis to occur (Fig. 5.4 B & A). Significantly higher CH₄ production (26%) was obtained when using full
anaerobic recyclate demonstrating that the recyclate provided an inoculum essential for the rapid start-up of methanogenesis during the anaerobic phase.

![Biogas Production Chart](image)

**Figure 5.4**: Biogas and CH₄ production during the 24h incubation period at 55°C of 100mL serum vials containing 5-day aerated OFMSW and recyclate, filtered recyclate (filtered through a 0.45μm paper filter) or sterile (autoclaved) de-ionised water.

### 5.3.4 Increasing DiCOM® Recyclate Inoculation Size Enhances Early Methane Production

To investigate the impact of inoculum size on methanogenesis during the anaerobic phase of the DiCOM® process, an experiment was conducted whereby varying amounts of DiCOM® recyclate inoculum (40, 4, 0.4 and 0mL) were added to OFMSW samples (5g), while buffer capacity was maintained at a constant level by adding a complimentary volume of filter sterilised recyclate (0, 36, 39.6, 40mL). CH₄ production indicated that the onset of methanogenic activity was enhanced by larger inoculum sizes (Fig. 5.5) which is consistent with theoretical biokinetics and observations by El–Mashad *et al.* (2006). It is interesting to note that the final quantity of CH₄ gas formed was not significantly affected by the size of the inoculum. This observation suggests that, given adequate buffering, the endogenous methanogens, contained within the solid,
form an adequate inoculum to consume the fermentation intermediates and stabilise the waste. However the time required to stabilise the solid, when using only the innate methanogens, was extended beyond the 7–day time constraint for the anaerobic phase of the DiCOM® process. Therefore it can be concluded that the transfer of active microorganisms, presumably methanogens and fatty acid degrading bacteria, from one batch to the next, is important to enable the rapid on–set of methanogenesis during the start–up of the AD of OFMSW using the DiCOM® process.

The CH₄ production from the introduced inocula can be obtained by subtracting that produced by the innate methanogens and showed that after 6 days the 1 and 10% inocula provided 22 and 54% of that produced by the 100% inoculum, respectively (Fig. 5.6). These data allow an estimation of the average doubling time of the methanogens present in the inoculum during maximum CH₄ production (below).

![Cumulative CH₄ Production](image)

**Figure 5.5:** Cumulative CH₄ production during the 13 day incubation period from OFMSW samples combined with 100%, 10%, 1% and no microbial inoculum from DiCOM® recylcate in 100mL serum vials.
**Legend:** (■) 100%; (●) 10%; (▲) 1%; (×) 0%.
Microbial doubling time and microbial growth can be described as (Pirt, 1975):

$$\text{doubling time} = \frac{\ln(2)}{\mu}$$  \hspace{1cm} (Eq. 5.1)

and (Pirt, 1975):

$$X = X_0 \cdot e^{\mu t}$$  \hspace{1cm} (Eq. 5.2)

respectively, where $X$ is biomass growth, $X_0$ is the biomass at $t=0$, $t$ is the elapsed time and $\mu$ is the specific growth rate. Based on the assumption that CH$_4$ generation can be equated to methanogen growth, the average doubling time for the methanogens present in the DiCOM$^\text{®}$ recyclate was estimated to be 1.6 days (Table 5.2).

<table>
<thead>
<tr>
<th>Inoculum Size (%)</th>
<th>Starting Gas Production ($X_0$) (L/kg)</th>
<th>Final Gas Production (X) (L/kg)</th>
<th>Time Period For Maximum Activity (days)</th>
<th>Estimated Doubling Time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.89</td>
<td>6.89</td>
<td>4 – 6</td>
<td>1.60</td>
</tr>
<tr>
<td>10</td>
<td>7.04</td>
<td>17.10</td>
<td>4 – 6</td>
<td>1.56</td>
</tr>
<tr>
<td>100</td>
<td>9.93</td>
<td>23.03</td>
<td>2 – 4</td>
<td>1.65</td>
</tr>
</tbody>
</table>
DNA sequencing (Dr Lucy Skillman – data not shown) of enriched and pure cultures (Dr Wipa Charles – data not shown) of methanogens isolated from the DiCOM® recyclate has identified the predominant H₂ utilising and acetoclastic methanogens as *Methanoculleus thermophilus* and *Methanosarcina thermophila*, respectively. The minimum doubling times for thermophilic *Methanoculleus spp.* have been reported in the literature to be between 1.8 and 3.8h (Ferguson & Mah, 1983b; Rivard and Smith, 1983; Zabel *et al.*, 1985) and between 12 and 14h (Zinder and Mah, 1979; Zinder *et al.*, 1984; Min and Zinder, 1989; Thauer *et al.*, 1989) for *Methanosarcina thermophila*, times which are significantly shorter than the estimated doubling time (1.6 days) for the methanogens in this study. However, the methanogens in the current study were cultivated in less than ideal conditions, resulting from the changing conditions during batch operation and high NH₃ concentrations of the liquor (100 – 150mM); factors which may account for the longer estimated doubling time.

Using the estimated average doubling time (1.6 days) and Eq. 5.1 and 5.2, the time delay obtained when providing inoculum of different sizes can be modelled (Fig. 5.7). The modelled data predicts time delays of up to 10 and 5 days for a 1% and 10% inoculum. However, data represented in Figure 5.5 indicates the delay to be about 7 days for both of these inocula. The very short anaerobic phase of the DiCOM® process (7 days) requires that the delay in methanogenic activity be minimized and as a consequence maintenance of, and maximum transfer of, inoculum is critical as evidenced by a loss of only 35% of the inoculum (i.e. Inoculum Size = 65%) providing a 24 hour delay in methanogenic activity (Fig. 5.7).
5.3.5 Hydrogen Utilising Methanogenesis Is Significant During The Commencement Of Anaerobic Digestion

Two mechanisms for CH$_4$ formation from acetate have been described in the literature. Firstly, acetate can be metabolised to CH$_4$ by the acetoclastic reaction where the methyl group of acetate is decarboxylated (Anderson et al., 2003). A second pathway exists where acetate is initially oxidized to H$_2$ and CO$_2$ and subsequently converted to CH$_4$ via a syntrophic partnership between two organisms (Zinder and Koch, 1984). Acetoclastic methanogenesis has been considered to be the dominant pathway for the metabolism of acetate to CH$_4$ (Pavlostathis and Giraldo–Gomez, 1991a, Siegrist et al., 1993). However, syntrophic acetate oxidation has been found to be more robust toward variations in operational conditions, where it becomes the main mechanism for acetate metabolism in the presence of inhibitors, particularly ammonium and VFAs or at high temperature (Karakashev et al., 2006, Schnuerer et al., 1999).

In a separate study of the DiCOM® process (Charles et al., 2007), the mechanism by which CH$_4$ was formed from acetate, the key methanogenic substrate, was investigated.
In the study, samples of recyclate and OFMSW were withdrawn from a laboratory-scale DiCOM® reactor and exposed to C–1 (carboxyl group) and C–2 (methyl group) labelled acetate, and the percentage of $^{13}\text{CH}_4$ produced during a 5–hour incubation period was monitored. Results from this study showed that 60% of the \(\text{CH}_4\), formed toward the conclusion of the anaerobic period, originated from acetoclastic methanogenesis (Fig. 5.8), while during start–up of the anaerobic period, \(\text{CH}_4\) was derived from hydrogenotrophic methanogenesis. Roy et al., (2006) while studying the initial phase of anoxia in rice soil slurries also noted that the majority of \(\text{CH}_4\) production, at the beginning of the incubation, was the result of hydrogenotrophic methanogens. They reported that, after 3 days of incubation, there was a shift from hydrogenotrophic to acetoclastic methanogenesis with the latter then found to be the dominant source of \(\text{CH}_4\). Findings in the current study emphasize the important role that both methanogenic groups play in the DiCOM® process.

**Figure 5.8:** Proportion of \(\text{CH}_4\) formed from acetate (after 5 hrs incubation) by samples taken from the OFMSW digesting reactor between day 0 and 7, as calculated from $^{13}\text{CH}_4$ formed from C–2 labelled acetate (Used with permission from Charles et al., 2007).

Legend: (■) Acetate; (▲) \(\text{CH}_4\).
As previously discussed (Section 5.3.4), the predominant H₂ utilising and acetoclastic methanogens have been identified as *Methanoculleus thermophilus* and *Methanosarcina thermophila*, respectively (15 – 50% and <10%, respectively: Dr Lucy Skillman – data not shown). This is in line with the literature where Weiss *et al.*, (2009) reported that in thermophilic AD of solid waste *Methanoculleus* spp. was found to be the dominant methanogen. Furthermore, in the current study, the *Methanoculleus* and *Methanosarcina* species were found to predominate in the DiCOM® recyclate and OFMSW, respectively (approximately 90% and 50%, respectively: Dr Lucy Skillman – data not shown). The fact that the liquid recyclate contained relatively little acetoclastic methanogens has been suggested to be due to the tendency of *Methanosarcina*, the major acetoclastic methanogen in the process, to undergo clump and biofilm formation (Chen and Lun, 1993; Liu *et al.*, 2003).

The above observations suggest that the introduction of recyclate provides mainly an inoculum of hydrogenotrophic methanogens. While the presence of these methanogens alone may not be adequate, it provides a process advantage at the beginning of the anaerobic phase. Due to rapid hydrolysis and fermentation the H₂ partial pressure was typically high during the startup of AD (Fig. 5.9). As a result, the hydrogenotrophic methanogens, introduced in the recyclate, can initiate rapid CH₄ production, avoiding the delay in CH₄ formation typically noted during the start–up of batch AD. The ensuing decrease in H₂ partial pressure is essential for acetate degradation by both syntrophic (Lee and Zinder, 1988a) and acetoclastic metabolism (Charles *et al.*, 2007).
Figure 5.9: Typical hydrogen and volatile fatty acid (VFA) concentration in the anaerobic liquid during batch anaerobic digestion of OFMSW using a laboratory–scale DiCOM® reactor.

Legend: (■) Acetate; (♦) Propionate; (▲) Butyrate; (▬) H₂.

Results indicated that the bulk (79%: Table E.1: Appendix E) of the CH₄ generated during laboratory–scale DiCOM® processing was produced via hydrogen metabolism. This is in contrast to the stoichiometry of the reaction describing the metabolism of glucose to CH₄ (Eq. 5.3–5.4: Mata–Alvarez, 2003 & Eq. 5.5: McInerney and Bryant, 1981), where 33% (1 CH₄ molecule: Eq. 5.5) is generated from hydrogen metabolism while the remaining 66% (2 CH₄ molecules: Eq. 5.4) is formed via acetate cleavage.

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 2 \text{H}_2\text{O} \rightarrow 2 \text{CH}_3\text{COO}^- + 2 \text{HCO}_3^- + 4 \text{H}^+ + 4 \text{H}_2 \quad \text{(Eq. 5.3)}
\]

\[
2 \text{CH}_3\text{COO}^- + 2 \text{H}_2\text{O} \rightarrow 2 \text{HCO}_3^- + 2 \text{CH}_4 \quad \text{(Eq. 5.4)}
\]

\[
\text{CO}_2 + 4 \text{H}_2 \rightarrow \text{CH}_4 + 2 \text{H}_2\text{O} \quad \text{(Eq. 5.5)}
\]

During mesophilic AD of sewage sludge, Siegrist et al., (1993) reported that the fluxes were closer to 30 and 70% for CH₄ production from acetate and H₂, respectively. However, under thermophilic conditions acetate conversion to H₂, via a syntrophic microbial relationship, becomes more thermodynamically favourable (Lee and Zinder, 1988a; Ahring, 2003). Consequently, methanogenesis could proceed via H₂ in
preference to direct acetoclastic metabolism and account for the higher percentage of CH$_4$ produced via H$_2$ in the current study.

The discovery that the methanogens transferred in the DiCOM$^\text{®}$ recyclate are predominantly *Methanoculleus spp.* raises a question as to the effectiveness of this inoculum. It is clear that the success of the DiCOM$^\text{®}$ process hinges on the efficient operation of the anaerobic phase. As the larger portion (79%: Table E.1: Appendix E) of the CH$_4$ produced during DiCOM$^\text{®}$ trials was generated from H$_2$ (Fig. 5.8), it is evident that the transfer of *Methanoculleus spp.* is important for the process. However, the anaerobic phase of some DiCOM$^\text{®}$ trials have operated in a non–sustainable manner in that propionate accumulation has been observed (Fig. 5.9; Section 3.3.2 Fig. 3.2). As propionate is the last of the VFA to be degraded, and can only be metabolised when the acetate concentration is low (Duarte and Anderson, 1982; Ozturk, 1991), acetate degradation becomes a critical factor.

If the recyclate contained significant amounts of both hydrogenotrophic and acetoclastic methanogens, metabolism of their respective substrates could occur more rapidly enabling propionate removal, thus facilitating long–term sustainable processing. As *Methanosarcina thermophila*, the only acetate degrading methanogen detected, was found to predominate within the solid, it is reasonable to hypothesise that DiCOM$^\text{®}$ process performance will be improved if solid material was transferred between trials.

### 5.3.6 Using Solid Inoculum Improves Anaerobic Digestion

In an attempt to investigate whether the transfer of a solid inoculum improves AD, 20% (v/v) of digested solid from the end of the anaerobic phase of one trial was introduced immediately to non–aerated OFMSW (80% v/v) prior to the DiCOM$^\text{®}$ recyclate being
introduced. AD was performed at 55°C until VFA were exhausted. A control experiment was operated under the same conditions without solid transfer.

The extra solid inoculum added avoided the typical VFA accumulation in the DiCOM® recyclate (Fig. 5.10A and 5.10B) and the VFA profile indicated that the AD was more stable than under normal operation. During the initial 7 days of anaerobic treatment, propionate was not degraded in the control (Fig. 5.10B) while the trial containing solid inoculum (Fig. 5.10A) had complete degradation of all residual VFA. Acetate accumulation reached its peak after 24h at 17mM and 56mM for the test and control, respectively. In much the same way the solid addition suppressed the accumulation of propionate from 40mM to 9mM.

No significant difference in total CH₄ production was observed between the two trials, with both producing approximately 170 L CH₄/kg TVS (165 (solid inoculum) and 173 (control). NOTE: the CH₄ production for the test case (solid inoculum) was adjusted to account for the 20% decrease in OFMSW loaded). It can be concluded that the addition of solid inoculum did not provide extra solid degradation capacity but decreased the time required for VFA degradation. This finding is inline with those reported by Lopes et al. (2004) where they found that inoculum addition (15% bovine rumen fluid) decreased the time (50%) required to stabilise OFMSW.

5.3.7 DiCOM® Pilot–Scale Recyclate Contains Less Methanogens Than Laboratory–Scale Recyclate

Anecdotal evidence indicated that the anaerobic liquor used in pilot–scale trials was “cleaner” than that used in the laboratory where the liquor remained more gritty and opaque. In an attempt to determine whether this “cleanliness” could also be reflected in
the number of methanogens present in the recylcate, real–time PCR (Dr Lucy Skillman) was used to enumerate the bacteria and methanogens.

Figure 5.10: VFA and pH profiles during anaerobic digestion of non–aerated OFMSW (A) with a 20% (v/v) solids inoculums and (B) without inoculum.

Legend: (■) Acetate; (♦) Propionate; (▲) Butyrate; (—) pH.

The average number of methanogens in the DiCOM® recylcate (days 6 – 12) was approximately 10–fold lower in the pilot–scale than in the laboratory–scale reactor (3×10⁴ and 5×10⁵ cells/mL, respectively: Fig. 5.11 A). This difference was also reflected in total cell density (bacteria and methanogens) showing 2.4×10⁸ and 8×10⁹
cells/mL of recyclate, respectively (Fig. 5.11 B). It is possible that this is a consequence of the greater consolidation of the material due to the increased mass of solid loaded into the reactor (pilot: 8000kg compared with laboratory: 2.4kg). As the recyclate passes up through the material the fine particulate matter and microbial cells may be filtered out, remaining in the solid.

**Figure 5.11:** Total (A) methanogen populations and (B) microbial populations in the anaerobic liquid from lab–scale reactor compared to the pilot–plant (P), measured by real–time PCR. The “P” denotes days of pilot plant operation.
The lower number of methanogens contained within the pilot plant recylcate (Fig. 5.11 A: P6–P12) makes the recylcate less effective in its capacity to inoculate subsequent trials compared to the trends in laboratory trials (Fig. 5.11 A: 6–12). As a consequence the transfer of solid inoculum in pilot trials may need to be considered to provide satisfactory process performance.

5.4 Conclusions

Results from laboratory DiCOM® trials indicated that:

- Endogenous thermophilic acetoclastic and hydrogenotrophic methanogens in OFMSW survived pressurised aeration for a period of 5 days and resumed methanogenesis upon initiation of anaerobic conditions. The survival of methanogens during the aerobic phase may be significant for the efficient operation of the DiCOM® process and accordingly may need to be optimised.

- Reliance upon the low numbers of endogenous methanogens in OFMSW to initiate methanogenesis can lead to a significant lag phase. The adequate transfer of inoculum via transfer of recylcate may be critical.

- Acetoclastic methanogenesis was significant towards the conclusion of AD of OFMSW during DiCOM® trials however hydrogenotrophic methanogens were responsible for the major portion (79%) of the CH₄ produced.

- The reuse of anaerobic recylcate was found to provide an inoculum dominated by hydrogenotrophic methanogens, while acetoclastic methanogens were found to adhere to the digesting OFMSW.

- The use of stabilised digested solid, as an inoculum, improved AD of OFMSW in DiCOM® trials resulting in a decrease in VFA accumulation and the time required for solid stabilisation.
6.1 Introduction

Historically, strategies for the management of MSW included burial, burning and ocean dumping; practices which are now known to lead to contamination of land, air and sea (Earle et al., 1995). Until recently landfill was the main waste treatment method utilised. Waste management has become one of the largest environmental concerns in recent decades, with the problems in disposal compounded by the ever-increasing quantity of refuse to be managed. The scarcity of land and the uncontrolled contamination with gas and leachate emissions have made landfill, particularly of organics, no longer a sustainable option (Hartmann and Ahring, 2006). It is now accepted that no single solution exists for the management of MSW, with an integrated approach most likely to succeed (Earle et al., 1995).

MSW, a heterogeneous waste, consists of a biogenic organic fraction, which is readily degradable (i.e. kitchen wastes, grass cuttings, etc.); a combustible fraction (i.e. slowly degradable and non-degradable organic matter i.e. wood, paper, cardboard and plastics); and an inert fraction (i.e. stones, sand, glass, metals, etc.). In general, approximately 50% of MSW consists of organic matter, with the composition of the OFMSW being an important parameter in determining the most appropriate method for its treatment. Typically, food waste, which is too wet and lacks the structure for composting, is treated via AD whereas green waste (plant material) is composted (Edelmann and Engeli, 1993; Braber, 1995).
Both composting and AD have their own specific advantages and disadvantages (Table 6.1), with composting generally accepted as being a more rapid process than anaerobic treatment (Lopes et al., 2004; Mohaibes and Heinonen–Tanski, 2004; Bitton, 2005). However, based on an energy balance, AD has an advantage over composting, incineration, a combination of composting and digestion (Edelmann et al., 2005) or land–filling (Haight, 2005). Composting has been reported as having an electricity demand between 30 and 70kWh/ton of input waste. AD, on the other hand, is capable of providing an energy surplus between 40 and 170kWh/ton of input waste (Braber, 1995; Mata–Alvarez et al., 2000; Fricke et al., 2005), a gross heat yield between 210 and 390kWh(heat)/ton (Fricke et al., 2005) and is capable of being energy sufficient if only one quarter of the biogenic waste is converted to biogas (Edelmann et al., 2000).

<table>
<thead>
<tr>
<th>Composting Advantage</th>
<th>Composting Disadvantage</th>
<th>Anaerobic Digestion Advantage</th>
<th>Anaerobic Digestion Disadvantage</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple</td>
<td>Inexpensive</td>
<td>More Complex</td>
<td>More Expensive</td>
<td>a, b, f, h</td>
</tr>
<tr>
<td>Larger Area</td>
<td>Smaller Area</td>
<td></td>
<td></td>
<td>a, b</td>
</tr>
<tr>
<td>Odour Pollution</td>
<td>Less Odour Via</td>
<td></td>
<td></td>
<td>a, b, c, e</td>
</tr>
<tr>
<td>Uncontrolled</td>
<td>Biogas Combustion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leachate Emission</td>
<td>High Strength</td>
<td></td>
<td></td>
<td>j</td>
</tr>
<tr>
<td>Uncontrolled</td>
<td>Wastewater Formed</td>
<td></td>
<td></td>
<td>b, c, g, i</td>
</tr>
<tr>
<td>CH₄ Production</td>
<td>Net Energy</td>
<td></td>
<td></td>
<td>d</td>
</tr>
<tr>
<td>Net Energy Consumer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

References:  

- a Edelmann and Engeli, (1993)  
- c De Baere, (1999)  
- d Edelmann et al., (1999)  
- e Smet et al., (1999)  
- f De Baere, (2000)  
- g Edelmann et al., (2000)  
- h Mata–Alvarez et al., (2000)  
- i Edelmann et al., (2005)  
- j Hartmann and Ahring, (2006)

One recognised disadvantage of AD is the fact that the solids produced are not typically suitable for direct land application as they tend to be odorous, too wet and too high in VFA concentration, which are phytotoxic. In addition, if the digestion is not performed under thermophilic conditions, the solids are not sanitised (Mata–Alvarez et al., 2000).
Consequently, a post treatment of these solids is required (Poggi–Varaldo et al., 1999) with composting providing an appropriate management solution (Fricke et al., 2005; Meissl and Smidt, 2007).

Providing aerobic maturation for the solid digestate, obtained from anaerobic treatment of solid waste, has been found to improve the quality of the end–product (Edelmann and Engeli, 1993) and decrease odour emission. De Baere (1999) found a 90% decrease in volatile compound emission when biowaste was matured aerobically, following AD, compared to composting alone. Smet et al. (1999) found that combined anaerobic/aerobic treatment provided a decrease in emission of volatile compounds (17 fold), and air requirement (10 fold), when compared to aerobic treatment alone.

Recent years have seen the development of numerous integrated waste treatment systems utilising composting (external to the digester) of anaerobic digestate, with the costs of these combined systems anticipated to be lower than isolated solutions (Edelmann and Engeli, 1993). The DRANCO process (Six and De Baere, 1992) exposes OFMSW to 2–3 weeks of thermophilic (55°C) high solids (32%) AD followed by 10 days of aerobic treatment. The BIOCEL process (ten Brummeler, 2000), combines 21 days of mesophilic (35–40°C) high solids (30–40% (w/w)) AD of bio–waste with a short (1–3 weeks) post–digestion composting period for drying and “ripening” the compost. The BTA–process (Kübler and Schertler, 1994) is a three–phase (acidification, fermentation and methanisation) mesophilic low solids (10%), multiple reactor, anaerobic treatment for OFMSW which is followed by aerobic conditioning of the non–degraded solids. Kayhanian and Tchobanoglous (1993) investigated a pilot–scale 2–phase system for treatment of OFMSW that combined high
solids (25–30%) AD and aerobic post–treatment for the production of recoverable energy and compost. After 30 days of AD, the 3 day composting period, eliminated biodegradable VS from the solid indicating a stable product. These are just a few of a growing number of processes.

Heat generated biologically, through aerobic metabolism of readily degradable substrates, can offer energy savings to AD processes in that the material can be raised to digestion temperature without the addition of external heat. Depending on the degradability of the organic substrate, the O₂ supply and heat loss, the temperature of the material can rise to 70°C (or more) which contributes to the elimination of pathogens from the material (Neklyudov et al., 2006). To investigate the effect of aerobic pre–treatment, Krzystek et al. (2001) compared aerobic/anaerobic and anaerobic/aerobic treatments of OFMSW. From a slurry of waste (2–4% TS), under mesophilic temperatures (aerobic: 30°C; anaerobic: 36°C) it was found that VS destruction was similar for both process configurations. However the amount of biogas produced was four times lower when the first phase was aerobic. It should be noted that the duration of the aeration was not provided for this configuration.

Mata–Alvarez et al. (1993) investigated the performance of a pilot–scale thermophilic (55°C) semi–dry (16–22%) semi–continuous AD process treating fresh and pre–composted OFMSW. The pre–composted (defined as self–heating of the OFMSW during the less than 1 week after sorting) material showed a decrease in biogas yield and production rate. However a higher specific biogas rate from pre–composted OFMSW (at 12 day HRT) was attributed to composting making complex organic matter available for anaerobic metabolism. This claim was refuted by Hartmann and Ahring (2006), in a
recent review of AD of OFMSW, as the organic loading rate of the pre–composted trial, conducted by Mata–Alvarez et al. (1993), was higher (30%) than for the fresh OFMSW. In contrast, a recent study by Borowski and Szopa, (2007) has shown that aerobic thermophilic pre–treatment (ATPT) of sewage sludge enhanced mesophilic AD efficiency when compared to conventional single stage mesophilic AD. They found that an aerobic reactor operated under O₂ limiting conditions, at thermophilic temperature, in conjunction with the short hydraulic retention time (HRT: 1 day), resulted in the accumulation of soluble products, providing a shorter SRT (30% less), higher VS destruction (2%) and greater pH stability. However, from an energy balance perspective, Hartmann and Ahring (2006) propose that there is no significant improvement from combining aerobic treatment as a precursor for AD of OFMSW due to the resulting decrease in energy yield.

The DiCOM® (Walker et al., 2006a) and 3A–biogas (Müller et al., 2003; Müller et al., 2006) processes attempt to utilise biological heat generation during pre–composting in an attempt to minimise the reliance on an external heat source to heat solid wastes to AD temperature. Both expose organic solid waste to sequential aerobic, anaerobic and aerobic conditions within a single reactor. A comparison of process parameters for these two solid waste stabilisation systems (Table 6.2) highlights the significant decrease in processing time offered by DiCOM®.

The aim of this chapter is to compare rates of degradation of OFMSW under static, in–vessel aerobic composting and thermophilic AD conditions and a combination of the two processes. Here, the DiCOM® process is used as an example of a full–scale process that combines aerobic composting and thermophilic AD. Data produced are expected to
be helpful in designing the most efficient combination of the two methods for the degradation of OFMSW.

### Table 6.2: Comparison of the DiCOM® and 3A–Biogas process parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DiCOM® Process</th>
<th>3A–Biogas Process a b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding Regime</td>
<td>Batch</td>
<td>Batch</td>
</tr>
<tr>
<td>Type of Composting</td>
<td>Static In–Vessel</td>
<td>Static In–Vessel</td>
</tr>
<tr>
<td>Duration of Initial Aerobic Treatment (days)</td>
<td>5</td>
<td>&lt; 6</td>
</tr>
<tr>
<td>Transition From Aerobic to Anaerobic Conditions</td>
<td>Reactor Is Sealed</td>
<td>Reactor Is Sealed</td>
</tr>
<tr>
<td></td>
<td>Aerobic Microbial Metabolism</td>
<td>Aerobic Microbial Metabolism</td>
</tr>
<tr>
<td></td>
<td>Removes Remaining O₂</td>
<td>Removes Remaining O₂</td>
</tr>
<tr>
<td>Anaerobic Digestion Temperature</td>
<td>Thermophilic (55°C)</td>
<td>Mesophilic (35–45°C)</td>
</tr>
<tr>
<td>Duration of Anaerobic Treatment (days)</td>
<td>7</td>
<td>25–40</td>
</tr>
<tr>
<td>Solids Content During Digestion</td>
<td>Dry ≈ 30% (TS)</td>
<td>Dry (30–60% TS)</td>
</tr>
<tr>
<td>Inoculum/Metabolite Distribution</td>
<td>Liquor Recirculation</td>
<td>Leachate Percolation</td>
</tr>
<tr>
<td>Methanogenic Inoculum Source</td>
<td>Previous Liquor Use +</td>
<td>Endogenous Methanogens</td>
</tr>
<tr>
<td></td>
<td>Endogenous Methanogens</td>
<td></td>
</tr>
<tr>
<td>Duration of Post–Anaerobic Aerobic Treatment (days)</td>
<td>7</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>End Products</td>
<td>Biogas and Compost</td>
<td>Biogas and Compost</td>
</tr>
</tbody>
</table>


b Müller et al., (2006)

### 6.2 Materials and Methods

#### 6.2.1 Experimental Design

OFMSW for all trials was prepared as described in Section 2.2.2.

The OFMSW was treated in an insulated cylindrical, 7L high temperature PVC computer controlled laboratory–scale reactor as described in Section 2.2.1. The reactor was operated as a sequencing batch reactor capable of providing in–vessel composting, AD or combinations of both.
Trials consisted of at most 12 days of treatment (aerobic, anaerobic or a combination of both) followed by 7 days of aerobic maturation (Table 6.3). During aerobic operation, pressurized air was introduced into the reactor until the internal pressure was raised to 25kPa. The internal pressure was maintained (10 min) before being released and the aeration regime repeated. This aeration regime was used to prevent channelling of air through the essentially unmixed material. Small scale composting trials typically underperform due to limited heat build-up (high surface to volume ratio causing increased heat loss). To prevent the heat loss typical for small scale composting experiments, a highly insulated vessel (heat loss coefficient 0.0912 h⁻¹) was used and the external reactor temperature controlled, by means of a heat tape, to that of the reactor core, but not beyond 60°C.

<table>
<thead>
<tr>
<th>Trial Description</th>
<th>Length of Initial Aeration (days)</th>
<th>Length of Anaerobic Treatment (days)</th>
<th>Length of Final Aeration (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermophilic Anaerobic Digestion (B)</td>
<td>0</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>Combined Aerobic and Thermophilic Anaerobic Digestion (C)</td>
<td>1</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>DiCOM® (A)</td>
<td>5</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Static In–Vessel Composting (D)</td>
<td>12</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Thermophilic Static Composting (E)</td>
<td>12</td>
<td>0</td>
<td>7</td>
</tr>
</tbody>
</table>

For those trials where a thermophilic anaerobic phase was present (Trial A, B and C; Table 6.3), digestion was initiated by sealing the reactor and allowing aerobic microbial metabolism to consume residual $O_2$ and establish an anaerobic environment. Once anaerobic conditions had been established, the reactor was flooded with 4.1L liquid (anaerobic inoculum obtained from a laboratory-scale DiCOM® reactor) ($NH_4^+–N = 1400mg/L$). Then the liquor was re–circulated (70mL/min (max)) from the reactor top to its base and maintained at $55^\circ C ± 2^\circ C$. At the conclusion of digestion, the anaerobic
liquid was drained and the solids mechanically squeezed (to remove excess moisture and provide a positive “fist test” (AS 4454, 2003)).

Aerobic post–digestion maturation consisted of 7 days of aeration as described above. Again, to limit heat loss from this small scale reactor, external heating was used to ensure the core reactor temperature did not fall below 35± 2°C.

6.2.2 Specific Trial Information

**Trial A:** The DiCOM® trial was conducted as described in Section 2.2.1 and 2.2.2.

**Trial B:** For the anaerobic trial, the OFMSW was heated to 55°C in an incubator (3h) prior to being introduced to the reactor.

**Trial C:** The combined aerobic/anaerobic trial was conducted as per Trial A except pre–aeration was decreased to 1 day while AD was operated for 10 days.

**Trial D:** For the static in–vessel composting trial the introduced air was heated and moistened by sparging through water (VanderGheynst et al., 1997a) at 55°C ± 2°C to avoid evaporative cooling and drying of the OFMSW (Mata–Alvarez et al., 1993; Mason and Milke, 2005). During days 5 to 12 the O₂ was added at a frequency (pressurising and releasing) such that the O₂ concentration in the exit gas was between approximately 1 and 3%.

**Trial E:** Thermophilic composting was conducted as per Trial D except during day 5–12 where the core temperature was forced to remain above 55°C ± 2°C. This was to allow a fair comparison of composting at the same temperature as the thermophilic AD.
6.2.3 Data Collection and Chemical Analysis

O₂, CO₂ and CH₄ concentration, airflow rate, internal pressure, core and outside reactor temperature, pH and biogas generation rate were logged as described in Section 2.2.4. pH and VFA and NH₄⁺ concentration of liquid samples and percent moisture, VS, total organic and inorganic carbon, Kjeldahl nitrogen, protein, lipid and carbohydrate content of OFMSW samples were conducted as described previously in Section 2.2.4. Compost stability was assessed using the self–heat test (AS 4454 – 2003) as described in Section 2.2.5 while compost maturity was determined using a phytotoxicity assay (root elongation) adapted from Tiquia et al., (1996) as described in Section 2.2.6.

6.3 Results and Discussion

6.3.1 Operation as a Sequential Aerobic–Anaerobic–Aerobic System

As an example of a full–scale hybrid aerobic/anaerobic OFMSW treatment process the aerobic–anaerobic–aerobic regime, as used in the DiCOM® process, was carried out and monitored (Trial A). Significant biological oxidation commenced during the initial 5 days of aerobic treatment where O₂ was consumed and easily degradable organics, present within the waste, were mineralised (Fig. 6.1). The heat generated brought the reactor to thermophilic conditions for the ensuing thermophilic AD.
Figure 6.1: Oxygen uptake rate (OUR) during static, in–vessel aeration phases and temperature profile of a laboratory–scale DiCOM® reactor (Trial A) during OFMSW processing. Reactor temperature was maintained through aerobic microbial activity during aeration and via an external heat exchanger during anaerobic processing. Legend: (▬) Temperature; (—) OUR.

Anaerobic conditions initiated CH$_4$ production with the onset of methanogenesis facilitated by the transfer of anaerobic inoculum in the liquor obtained from a laboratory–scale DiCOM® reactor. CH$_4$ generation was noted within 4 hours of liquor addition, with peak generation (32L/day/kgVS) attained within 36h and a total CH$_4$ production of 103L/kgVS (270L$_{\text{biogas}}$/kgVS). These values are lower than many semi/continuous (450L$_{\text{biogas}}$/kgVS (DRANCO): Six and De Baere, 1992; 280L$_{\text{biogas}}$/kgVS (BTA): Kübler and Schertler, 1994; 630–710L$_{\text{biogas}}$/kgVS: Hartmann and Ahring, 2005a; 640–790L$_{\text{biogas}}$/kgVS: Hartman and Ahring 2005b) and batch (550L$_{\text{biogas}}$/kgVS: Hartman and Ahring, 2005b) AD systems reported in the literature but higher than other continuous (230L$_{\text{biogas}}$/kgVS: Bolzonella et al., 2003b) and batch (50L/kgVS: Forster–Carneiro et al., 2008) systems. These data highlight that the nature of the organic substrate and the treatment methodology applied have an important influence on the biodegradation process and CH$_4$ yield (Hartmann and Ahring, 2006; Forster–Carneiro et al., 2008).
VFA accumulated (total VFA = 75mM) during the initial 2 days of anaerobic treatment however minimal pH variation occurred during this time (7.3 to 6.6). Process acidification did not occur, presumably due to the high buffer capacity of the anaerobic liquor and the release of ammonia. The strong correlation between the decrease in CH$_4$ generation rate (4.5L/day/kgVS) and residual VFA (Fig. 6.2A), during the final 24h of digestion, indicated the exhaustion of easily degradable organics within the OFMSW. After draining of the liquor, the subsequent aerobic decomposition did not cause a significant increase in temperature, which also indicated exhaustion of easily degradable organics (Fig. 6.1).

6.3.2 Operation as Static, In–Vessel Composting of OFMSW

In general, composting is considered to be a faster but less energy efficient degradation system. To verify whether a purely aerobic treatment provides benefits in terms of degradation rate or product stability, static in–vessel composting was also carried out. Fig. 6.3 displays temperature and OUR profiles for a completely aerobic in–vessel composting trial (Trial D). A second composting trial was carried out under the temperature constraints of the DiCOM$^\circledast$ process (Trial E) to investigate whether the efficiencies of microbial degradation are significantly influenced by the different temperature regimes.
Chapter 6: Comparison of Aerobic and Anaerobic Microbial Treatment

![Graph showing the effect of aerobic pre-treatment on volatile fatty acid build-up.](image)

**Figure 6.2:** Effect of (A) 5, (B) 0 and (C) 1 days of static in-vessel aerobic pre-treatment on volatile fatty acid build-up during the subsequent anaerobic phase of thermophilic OFMWS treatment.

Legend: (■) Acetate; (♦) Propionate; (▲) Butyrate; (▬) pH.

In order to directly compare the degradation rate under aerobic and anaerobic conditions the aerobic OUR and the anaerobic CH$_4$ formation rate were converted to a molar electron flux defined as the rate at which electrons are removed from the OFMSW. Considering that an O$_2$ molecule, can accept 4 electrons and that a CH$_4$ molecule represents 8 electron equivalents, the oxidation of organic molecules into the final
gaseous end–products, CO₂ and CH₄, can be directly compared via the flow of electrons from the solid (Fig. 6.4).

**Figure 6.3:** Temperature and OUR profiles of static in–vessel composting of OFMSW (Trial D).

**Legend:** (▬) Temperature; (— ) OUR.

**Figure 6.4:** Molar electron flow of OFMSW stabilised using the DiCOM® process (Trial A) and two static in–vessel composting trials (Trial D and E).

**Legend:** ( — ) Trial A; (▬) Trial D; (▬ —) Trial E.
In terms of overall mineralisation rates the hybrid system (DiCOM®) showed a greater overall degradation than static aerobic composting, even though the switch to anaerobic conditions during the DiCOM® trial temporarily (for 1 day) slowed the degradation rate as measured by electron flux. Interestingly, despite the decreased electron flow during days 6 to 12 of the fully composted trial, the product (based on electron flow) appeared to be as stable as the product from the hybrid process.

6.3.3 Effect of Duration of the Anaerobic Treatment

Considering the above result, that during anaerobic treatment the rate of mineralisation of OFMSW did not slow down but was enhanced, the effect of longer durations of anaerobic treatment was investigated by extending the anaerobic phase from 7 to 10 (Trial C) and 12 days (Trial B) at the expense of the initial aerobic phase (Table 6.3).

During the anaerobic phases of all trials (A, B & C) acetate accumulated initially, accompanied by H₂ production (data not shown) and followed by propionate accumulation, which degraded by day 11 (Fig. 6.2). Butyrate accumulation, only observed in the trial where prior aerobic treatment was absent (Trial B), indicated availability of easily fermentable material. Without adequate buffer capacity, and VFA degrading microbial communities, such a build–up of butyrate is known to cause acidification and digester failure.

When comparing the overall microbial conversion as electron flow to either O₂ or CH₄, it can be shown that by decreasing the duration of the initial aerobic phase a greater amount of electrons flow towards CH₄, both in terms of total amount and maximum rates produced (Fig. 6.5). The low electron flow rate in the final aerobic phase of all
trials is in line with completed AD of VFA (Fig. 6.2). The low level of VFA and electron flow towards the end of the purely anaerobic trial (Trial B) indicated methanogenic substrates were being depleted and that the treatment may be further shortened.

![Graph of molar electron flows and reactor run time](image)

**Figure 6.5:** Aerobic and anaerobic molar electron flows, as measured by OUR (4 electrons per mol of O₂ used) and CH₄ production rate (8 electrons per mole of CH₄ formed), for Trials B, C, A and D having 0, 1, 5 and 12 days of static in–vessel initial aerobic treatment. 
**Legend:** (▬▬) 0 days; (▬) 1 day; (▬▬▬) 5 days; (▬▬▬) 12 days.

The duration of the initial aerobic phase had a clear effect on overall CH₄ recovery, showing that the shorter it was the more CH₄ gas could be recovered (Table 6.4) which is in line with the literature (Mata–Alvarez *et al.*, 1993).

### 6.3.4 Compared to Composting, Anaerobic Treatment Enhanced Hydrolysis Rates

Hydrolysis has been reported in the literature as being the limiting factor in some AD processes (Pavlostathis and Giraldo–Gomez, 1991b). In an effort to determine whether hydrolysis is also a limiting factor encountered during static in–vessel composting, the likely carbon hydrolysis rates of both aerobic and anaerobic processes were estimated.
As no significant soluble COD had accumulated in the solid after aeration (data not shown), it can be assumed that, during microbial heat generation, hydrolysed substrates were completely oxidised to CO₂ and released from the reactor. Therefore, under the assumption that any CO₂ produced from non–soluble material originated from the oxidation of monomers that were hydrolysed from non–soluble macromolecules, during aeration the minimum hydrolysis rate is directly indicated by the CO₂ production rate and can be estimated as described in Section 2.3.4 (Eq. 2.1).

**Table 6.4:** The effect of the length of aeration on the proportion of electron flow conserved as CH₄ during anaerobic digestion of OFMSW. Electron flows represent treatment during the initial 12 days only.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Aeration (days)</th>
<th>Total Electron Flow (mmol/kg VS)</th>
<th>Electron Flow to CH₄ (mmol/kg VS)</th>
<th>Electron Flow to CH₄ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermophilic Anaerobic Digestion (B)</td>
<td>0</td>
<td>56400</td>
<td>56400</td>
<td>100</td>
</tr>
<tr>
<td>Combined Aerobic and Thermophilic Anaerobic Digestion (C)</td>
<td>1</td>
<td>49000</td>
<td>42700</td>
<td>87</td>
</tr>
<tr>
<td>DiCOM® (A)</td>
<td>5</td>
<td>58700</td>
<td>33500</td>
<td>57</td>
</tr>
<tr>
<td>Static In–vessel Composting (D)</td>
<td>12</td>
<td>42800</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Thermophilic Static Composting (E)</td>
<td>12</td>
<td>42000</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Under anaerobic conditions the hydrolysis rate can be reconstructed considering the CH₄ generation and VFA accumulation rates and estimated as described in Section 2.3.4 (Eq. 2.2).

The dropping respiration activity after 5 days of aerobic treatment indicates that easily degradable substrates were exhausted and aerobic composting became hydrolysis limited (Fig. 6.5 & 6.6 Trial D). The decrease in hydrolytic rate was unlikely to be due to the available moisture as the moisture content of the OFMSW (at the conclusion of the fully aerobic trials) was found to be 60 and 62% (w/w) which is in the optimal range.
for composting (50–70%: Stentiford and Dodds, 1992; Richard et al., 2002). The continued decrease in C–hydrolysis rate over the following days of treatment indicated that the solid was approaching stabilisation.

![Graph: Hydrolysis Rate vs Reactor Run Time](image)

**Figure 6.6:** Aerobic and anaerobic hydrolysis rates, estimated from CO₂ release, CH₄ production and intermittent VFA accumulation, for trials with 0, 1, 5 and 12 days of static in–vessel initial aerobic treatment corresponding to Trials B, C, A and D.

Legend: (▬▬) 0 days; ( —— ) 1 day; ( — — — ) 5 days; (▬▬▬) 12 days.

The described, early drop in carbon hydrolysis rate under aerobic conditions could be overcome by switching the conditions to anaerobic and flooding the reactor with anaerobic liquor containing an active inoculum (Fig. 6.6 Trials A & D). The improved hydrolysis observed may be due to one or more of the following:

- The anaerobic liquid contained residual exo–enzymes from previous uses and increased the concentrations of active enzymes.
- The enzymes generated during the composting phase were water limited. In an aerobic composting system, the water available for enzyme activity is found as a film on the surface of the solid. It is also within this liquid film that water–soluble
metabolites accumulate, decreasing the free–water available for enzymatic activity.
Flooding the reactor may provide the free–water necessary for optimal activity.

- The enzymes had completely degraded the substrate in their immediate vicinity and the addition of liquid enabled contact with fresh substrate (mass transfer improvement).
- Bacteria producing hydrolytic enzymes may have been introduced with the anaerobic inoculum.

A comparison of total electron flow (62,000; 51,300; 64,400 and 46,400 mmol/kg VS for Trials B, C, A and D, respectively; Fig. 6.5) and C–hydrolysis (11,300; 10,000; 10,500 and 8000 mmol C/kg VS, respectively; Fig. 6.6) suggests that treatment containing an anaerobic period, during which the solid waste was completely submerged, increased the degree of solid degradation in the waste. The waste in trials A, B and C appeared to reach stability, indicated by low molar electron flows (Fig. 6.5) and C–hydrolysis rates (Fig. 6.6), between days 10 and 12; yet each of these trials was exposed to a different length of aerobic treatment. It can therefore be deduced that it is not the duration of the aerobic treatment that is critical in rapid waste stabilisation in hybrid systems but the presence of an effective anaerobic component.

While both processes could potentially be further improved by controlling environmental conditions, there is an additional limitation on aerobic static in–vessel degradation when up–scaling the process. This limitation can be derived theoretically and has been tested practically (See Chapter 7). Assuming that the heat generated by respiration is 18MJ/m$^3$ O$_2$ (Kaiser, 1996); negligible heat loss from a large, full–scale reactor; a cooling effect of dry air at 20°C of 36MJ/m$^3$ of air (leaving the reactor fully
saturated with moisture and at 60°C; it can be estimated that, to avoid overheating (>60°C) of the reactor, a maximum of 28L O₂/m³ reactor/h can be used for degradation. This would result in a maximum hydrolysis rate of 19mmolC/h/kgVS which is 3 and 2 times less than the average anaerobic and aerobic degradation rates observed in our laboratory trials (Fig. 6.6 Trials B and D: average hydrolysis rates during the initial 5 days for Anaerobic Trial B: 62mmol C/h/kg VS and Aerobic Trial D: 40mmol C/h/kg VS). While in industrial applications the use of cooling systems can overcome this problem, it would add additional expense to the process which is avoided by switching to anaerobic conditions.

6.3.5 Effect of Treatment Regimes on Compost Stability and Maturity

Apparent product stability can be inferred from the final aerobic phase showing little electron flow for all treatments, indicating that adequate product stability had been obtained (Fig. 6.5). However this assumption is not supported by data obtained from self–heating tests (Fig. 6.7) which showed that static in–vessel composting did not adequately stabilise the product, with the trial failing the Australian Standard (AS 4454 – 2003) self–heat test. To meet the requirements of this Standard, the compost must not self–heat to temperatures higher than 40°C. The failure to meet this requirement indicated that readily available substrate was still present within the solid and the end–product was not yet stable, which is inline with static aerobic composting providing the lowest total amount of degradation as measured by cumulative electron flow and total C–hydrolysis. This lack of stability is inline with the literature where OFMSW treated (3 months) in a static pile with forced aeration, when compared to a turned pile, and turned pile with forced aeration, showed the lowest level of product stability (Ruggieri et al., 2008). Szanto et al., (2007) found a decrease (17%) in organic matter
degradation when straw–rich pig manure was composted (4 months) in a static pile as compared to piles that were turned monthly. The decrease in product stability, and organic matter degradation, in both of these studies was attributed to compaction of the material resulting in a decrease in air permeability within the aggregates formed; a deduction that may also be applicable in the current study.

![Graph showing the effect of initial aeration phase duration on compost maturity indicators, self heating test and germination index (GI) with Radish Long Scarlet and Chinese Flowering Cabbage for trials B, C, A and D.](image)

*Figure 6.7:* Effect of initial aeration phase duration on compost maturity indicators, self heating test and germination index (GI) with Radish Long Scarlet and Chinese Flowering Cabbage for trials B, C, A and D. *A GI > 85 indicates the disappearance of phytotoxicity (Tiquia *et al.*, 1996). In the above, a GI > 100 resulted from enhanced root growth in the test when compared to the control (deionised water).

It could be suggested that the lack of heat generation during self–heating tests, for material treated anaerobically, is the result of aerobic micro–organisms having been destroyed during anaerobic treatment periods. However, an increase in electron flow (Fig. 6.5 Trial A, B & C) and hydrolysis rate (Fig. 6.6 Trial A, B & C) at the onset of post–digestion aeration indicated that aerobic micro–organisms had survived anaerobic treatment and were active when exposed to O₂.
Root elongation and germination index (Tiquia et al., 1996) have been found to be sensitive indicators of the phytotoxicity and maturity of compost (Tiquia et al., 1996). Shortening the initial aerobic component of the first 12 days of processing had no apparent adverse effects on the germination index of the seed species selected (Fig. 6.7; Table 6.5), suggesting that a longer anaerobic phase could provide a greater biogas yield without compromising the maturity of the final end–product.

Table 6.5: Mathematical parameters (mean ($\bar{x}$) and standard deviation ($\sigma$)) for results of root elongation tests for extracts from composts obtained with varying aeration duration.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Chinese Flowering Cabbage Test</th>
<th>Radish Long Scarlet Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$</td>
<td>$\sigma$</td>
</tr>
<tr>
<td>A</td>
<td>24</td>
<td>7</td>
</tr>
<tr>
<td>B</td>
<td>27</td>
<td>10</td>
</tr>
<tr>
<td>C</td>
<td>25</td>
<td>9</td>
</tr>
<tr>
<td>D</td>
<td>32</td>
<td>11</td>
</tr>
</tbody>
</table>

6.4 Conclusions

From a comparison of aerobic and anaerobic processes, for the treatment of OFMSW, it has now become clear that:

- Contra to the popular belief that aerobic processes are faster than anaerobic, when measured as electron flow, the maximum rate of solids degradation, during AD (550mmol e⁻/kgVS/h) can be more rapid than for composting (350mmol e⁻/kgVS/h).
- The hydrolysis limitation that can be experienced by aerobic composting systems can be overcome by changing to anaerobic conditions and flooding the system with an appropriate anaerobic inoculum.
- The inclusion of an anaerobic step in waste stabilisation improves end–product stability, as measured by self–heating, when compared to static composting alone.
In light of these results and, in conjunction with the world’s increased needs for renewable fuels, the common practice of composting easily degradable feedstock, such as those containing food wastes, must be bought into question. Not only does composting use energy to destroy the reducing power contained within the waste, but the methane emission observed from composting operations adds to the greenhouse effect.

Additionally, it is evident that:

- The DiCOM\textsuperscript{®} process can be optimised by extending the anaerobic phase, at the expense of aerobic treatment, to provide a greater CH\textsubscript{4} yield (up to 75\% increase).
- AnaeCo Ltd. should investigate the possibility of loading OFMSW into the DiCOM\textsuperscript{®} process under anaerobic conditions to maximise CH\textsubscript{4} yield and product stability. However, an energy balance needs to be performed to ascertain whether the additional heat required to raise the temperature of the OFMSW to 55\(^\circ\)C is offset by the increased energy yield resulting from greater CH\textsubscript{4} production.
Chapter 7

*Prediction of Core Reactor Temperature During the Initial Aerobic Phase and the Effect of High Temperature During this Phase on the Methanogenesis Phase of the DiCOM® Process*

7.1 Introduction

The initial aeration phase of the DiCOM® process is a static in-vessel composting process. Composting is described as a controlled bio-oxidative process of heterogeneous organic solids, involving a thermophilic phase, leading to the production of CO₂, water (H₂O), minerals and stabilised organic matter (Zucconi and de Bertoldi, 1987). The heat generation that occurs during composting of OFMSW is said to be critical to the composting process as it quickly removes putrescible substances present in the waste (Finstein and Morris, 1975) and sanitises the end-product. Assuming adequate moisture (40–70%) (Stentiford and Dodds, 1992), OFMSW typically contains sufficient micro-organisms to initiate self-heating (Finstein and Morris, 1975; Stentiford and Dodds, 1992), as microbial growth during composting occurs in surface-bound water (Finstein and Morris, 1975) described as “free-water” (Buggeln and Rynk, 2002).

During composting, heat is initially generated by aerobic respiration of micro-organisms (biotic) and the direct oxidation of plant chemicals that react with the O₂ present within the solid matrix (abiotic). There is uncertainty as to the fraction of heat generation due to the respiration of still-living plant cells (Kubler, 1990) however, non-microbial heat output must be considered to be very slight as, in MSW, plant cells are sparse compared to agricultural products (Finstein and Morris, 1975). Abiotic reactions typically do not play a large role until biological agents raise the temperature to elevated levels (>80°C: Kubler, 1987) (Buggeln and Rynk, 2002).
In a recent review of the current compost modelling literature, Mason (2006) stated that biological heat production and latent heat of evaporation of water have been shown to be the most significant terms in heat balance. The evaporation of water is the principal mechanism for losing heat from a composting mass. Failure to remove excess heat from composting material will cause the temperature to rise to a point where microbial mediated degradation ceases (Stentiford and Dodds, 1992). In most ecosystems however the heat generated is dissipated too quickly to cause a temperature increase large enough to result in the cessation of microbial degradation. In contrast, the self–heating that occurs in large masses of decomposing organic materials can lead to spontaneous combustion (SC) as the mass provides insulation against heat loss to the surroundings (Finstein and Morris, 1975). As a consequence, the possibility of SC occurring during DiCOM operation must be considered.

SC is an infrequent but real phenomenon that occurs during composting of mixed vegetable and yard waste and is defined as the combustion of material in the absence of an externally applied spark or flame. For SC to occur, heat must be generated to raise the temperature of the waste to that greater than the ignition temperature of the woody materials contained within (120–145°C). The accumulation of heat depends upon the balance between the rate of internal heat production and heat loss to the external environment. When the former rate is higher, a “critical temperature” may be reached at which point “run–away” temperature is unavoidable (Buggeln and Rynk, 2002).

Water plays an important role in controlling the self–heating of a composting system. If the moisture level is outside the optimum for composting (40–65% (w/w): Guanzon and Holmer, 2003), microbial activity, and heat generation, will be restricted by either water
limitation (moisture <40%) or O₂ limitation (moisture >65%), due to the poor solubility of O₂ in the liquid boundary layer, limiting self-heating. If optimum moisture is provided and the temperature of the waste rises (due to biotic aerobic respiration), heat is lost to the external environment through evaporation of water (Buggeln and Rynk, 2002), an action which safeguards against over-heating of the waste. In fact, while heat in the form of water vapour is being lost from a self-heating composting system, the temperature of the waste will not rise above 70–90°C until all of the free-water has been removed.

The availability of O₂ is an important factor in controlling the progression of SC (Buggeln and Rynk, 2002). The activation energy (Eₐ) for the biological oxidation of organic substrates is less than for the direct action of O₂ in air. As a consequence, initial oxidation of organic waste occurs via biotic processes, which raises the temperature of the waste. The elevated temperature not only increases the rate of biological reactions but allows chemical degradation to occur more easily (Buggeln and Rynk, 2002). As the material heats to 70–90°C, two important changes occur: heat kills the micro-organisms and free-water is eliminated from the system. However, air will still be drawn into the pile via convection and O₂ will react abiotically with plant chemicals to produce more heat (Buggeln and Rynk, 2002). Heat generation from abiotic auto-oxidation begins to be detectable at about 80°C (Kubler, 1987) with the process being self-catalytic, as higher temperatures cause faster reaction rates, which further raises both temperature and demand for O₂ (Buggeln and Rynk, 2002). Consequently, O₂ availability is a key component in the development of SC.
As the temperature of the compost continues to rise, pyrolysis is initiated, which is defined as the decomposition of a substance by the action of heat under O₂ starved conditions. The delivery of O₂ to the system at this time becomes a critical factor as the pyrolysis products can still react with O₂ to produce heat (Buggeln and Rynk, 2002).

Care must also be taken when composting dry cellulosic material as moist air added to dry material can raise its temperature. The latent heat of condensation is released when moisture is absorbed by a dry substrate (this is the reverse of evaporation where latent heat is absorbed and the material cooled) generating enough heat to result in SC (Gray et al., 2002).

An industrial scale DiCOM® plant will treat a large mass (about 700 tonnes/trial) of mixed vegetable and yard waste (OFMSW) in a single insulated reactor with forced aeration. It is expected that the forced aeration provided will increase the composting rate, compared to passive aeration, and as a consequence decrease the duration of the composting process (Neklyudov et al., 2006). However, the increased rate of aerobic metabolism will provide an increase in heat generation, which when combined with the insulation provided by the mass of material and the reactor insulation, may result in over–heating of the waste or even SC. This chapter develops simple mathematical models, based on laboratory data, to predict heat accumulation during aerobic DiCOM® processing and the possibility of over–heating. Model predictions are tested in a pilot–scale DiCOM® reactor. This chapter also investigates the effect of elevated temperature during the initial aeration phase on the methanogenic inoculum provided by the OFMSW.
7.2 Materials and Methods

7.2.1 Pilot Scale Trial

7.2.1.1 Reactor design and operation

An insulated (185mm thick fibreglass batt R3.5), cylindrical (1.5m I.D.), stainless steel, fully automated (PLC control system) DiCOM® reactor (8m³ capacity), designed and built in 2000 by AnaeCo Ltd. at Jandakott, in the metropolitan area of Perth, Western Australia, was operated in a 19 day cycle which consisted of three distinct phases.

1. Initial Aeration: Pressurized air was introduced into the sealed reactor until the internal pressure was raised, via a patented aeration system, to 25kPa. At this time the inflow air was stopped and the internal pressure maintained before depressurisation. This aeration regime was repeated every 10 minutes for 5 days. During sorting/loading of waste, this aeration regime was discontinued and the reactor opened to the external atmosphere (Fig. 7.1). On the morning of the sixth day, aeration was discontinued, and the reactor was depressurized and sealed (for approximately 3h) to establish an anaerobic environment. During aeration the temperature of the peripheral edge of the waste was monitored.

![Aeration protocol during the initial aeration phase of a pilot–scale DiCOM® reactor, indicating periods of passive aeration while OFMSW was loaded into the reactor.](image-url)

**Figure 7.1:** Aeration protocol during the initial aeration phase of a pilot–scale DiCOM® reactor, indicating periods of passive aeration while OFMSW was loaded into the reactor.
2. Thermophilic Anaerobic Digestion: The reactor was flooded with liquid (recyclate/anaerobic inoculum) (5 m$^3$) from a previous DiCOM® trial and re-circulated (maximum 1.7 m$^3$/h) through an external heat exchange unit to maintain the temperature at 55°C. Liquid that had drained freely through vertical internal screens along the sides of the reactor was withdrawn and, after heating, reintroduced to the bottom of the reactor. After a period of 7 days, the free-draining anaerobic liquid was withdrawn.

3. Secondary Aeration (Maturation): Once drained, air was introduced into the reactor, to inhibit CH$_4$ production while avoiding the production of an explosive mixture. CH$_4$ at concentrations between 5–14% in air (O$_2$>12%) are explosive (Coward and Jones, 1952). To avoid this flammable mixture, equations were generated to define a “WAIT” region whereby the CH$_4$/O$_2$ mixture produced was not explosive (Appendix F: Fig. F.1 Eq. F.1–F.4 and Table F.1). The reactor was pressurised with air to 25 kPa and the internal gas composition monitored. If the percentage composition was outside the “WAIT” region (Appendix F: Fig. F.1), the reactor was depressurised immediately and re-pressurised with fresh air. If however, the gas composition fell within the “WAIT” region (i.e. CH$_4$ ≤ 3.72×$O_2$ – 17.6), the reactor remained pressurised and sealed until aerobic microbial activity consumed the O$_2$ and the gas composition moved out of the “WAIT” region (i.e. CH$_4$ > 3.72×$O_2$ – 17.6). At this time the reactor was depressurised and re-pressurised with air. When the CH$_4$ content within the reactor had been diluted such that CH$_4$ < −0.333×$O_2$ + 7 (Appendix F: Fig. F.1) the reactor was pressurised and depressurised rapidly, serially diluting the contained CH$_4$ with air. Once the CH$_4$ content within the reactor was less than 0.5%, aeration continued for 7 days using the aeration regime outlined in
Point 1 above however, aeration cycles were repeated more rapidly (every 6 minutes). After 24 and 72h of aeration, pressurised aeration was interrupted (for approximately 4h) and the reactor opened to the external atmosphere. At this time solids were re-circulated in the reactor and mechanically squeezed to remove excess moisture.

After 19 days of treatment, the reactor was unloaded.

7.2.1.2 Organic feed material

Mixed MSW, collected from Mosman Park (a suburb of Perth, Western Australia) on 23rd January 2006, was placed on the sorting floor of the AnaeCo pilot–plant facility where it remained until sorted. Sorting was conducted over 3 consecutive days commencing on 24th January, 2006. Large items in the waste were removed by hand before the mixed MSW was introduced into a 1.5×2m rotating, bag–opening trommel having a square screen aperture of 50mm. Sorted OFMSW (3110kg), having an average moisture content of 43% (w/w), was introduced into a DiCOM® pilot–scale reactor.

Anaerobic inoculum (recyclate) was stored (3 weeks) at ambient temperature under anaerobic conditions until required. In preparation for use, the anaerobic liquid was heated to 55°C over a 48 hour period and maintained at this temperature for 24h prior to being introduced to the reactor.

7.2.1.3 Data collection

Internal pressure, peripheral reactor temperature, recyclate pH and temperature and biogas generation rate were logged by computer using DCS software. CO₂, CH₄ and O₂
concentrations in the exit gas were logged by a gas analyzer (Geotechnical Instruments GA 2000).

Pilot–Scale Core Temperature Profile was obtained at the completion of the initial aeration phase, prior to transitioning to anaerobic conditions. Temperature readings were obtained, at 20cm depth intervals, via a temperature probe (1m: INS digital thermometer), inserted through sampling ports in the side of the reactor.

### 7.2.2 Effect of Elevated Temperature During Aeration on Methanogenic Activity

MSW was prepared as described in Section 2.2.2 and exposed to the initial aeration phase of a standard DiCOM® laboratory trial as described in Section 2.2.1. At the conclusion of the initial aeration phase, the solid was removed, subdivided and stored at –21°C. 10g samples of the 5–day aerated OFMSW, thawed at ambient conditions, were introduced into 100mL serum vials (Weaton) and incubated at 55°C, 65°C, 75°C and 85°C. After 24h incubation, 45mL of sterile filtered anaerobic liquor was introduced into each vial. All vials were then sealed with butyl rubber bungs and aluminium crimp seals and the headspaces flushed with N₂/CO₂ (80/20 (v/v)). All vials were incubated for 7 days in a 55°C shaking water bath (Lab–Line Instruments Model No. 3540–1) at 200rpm. The volume of gas in the headspace of the vials was measured daily using a 50mL glass syringe fitted with 23G × 30mm needles. The composition of gas samples were determined as described in Section 4.2.4 from which cumulative CH₄ production and production rate were calculated.
7.2.3 Determination of the Heat Loss Coefficient for the Laboratory–Scale DiCOM® Reactor

A laboratory–scale DiCOM® reactor was filled (to working capacity) with hot (approximately 80°C) water (6L) in a temperature controlled room (21±2°C). The contents of the reactor were stirred constantly via a magnetic stirrer to avoid temperature striations. Core reactor temperature was logged, by computer, over an 18h period.

7.3 Model Development

7.3.1 Part 1: Model To Predict Core Temperature During The Initial Aerobic Phase In A DiCOM® Reactor Without Considering Microbial Growth and Death

7.3.1.1 Assumptions

The initial objective was to model the temperature of an aerobic bioreactor without considering microbial growth or death. To simplify this task the following assumptions were made:

1. Cellulose is the primary feedstock which is hydrolysed to glucose, ethanol and lactate only. These intermediates are the substrates consumed during aerobic microbial metabolism.

2. The above substrates are consumed in a ratio of 1:1:1 and are never limiting.

3. The average ΔH_{molO2} is −460kJ/mol of O₂ used (see Section 7.3.1.2).

4. The primary feedstock and as a consequence, substrate, is always saturating.

5. O₂ is never limiting.

6. The moisture content of the feedstock (cellulose) is constant (58%: as per experimental data).
7. Air exiting the reactor is saturated with water vapour (Finstein and Morris, 1975; VanderGheynst et al., 1997b).

8. The mass of feed is 2.4 kg (as per experimental protocols).

9. The airflow rate is 10 L/h (similar to experimental data).

10. Mass transfer and heat diffusion within the feed are not considered.

7.3.1.2 Respiration heating rate

The only heat source is the aerobic metabolism of substrates which will be referred to as: Respiration Heating Rate (Resp HR). Three key intermediates of organic hydrolysis are glucose, ethanol and lactate and as such will be considered the substrate for microbial degradation. The equations for their aerobic metabolism are as follows:

\[
\begin{align*}
C_6H_{12}O_6 + 6 O_2 \rightarrow 6 CO_2 + 6 H_2O & \quad \Delta H_{\text{glucose}}^* = -2803 \text{kJ/mol glucose} = -467 \text{kJ/mol O}_2 \quad (\text{Eq. 7.1}) \\
C_2H_6O + 3 O_2 \rightarrow 2 CO_2 + 3 H_2O & \quad \Delta H_{\text{ethanol}}^* = -1367 \text{kJ/mol ethanol} = -456 \text{kJ/mol O}_2 \quad (\text{Eq. 7.2}) \\
C_3H_6O_3 + 3 O_2 \rightarrow 3 CO_2 + 3 H_2O & \quad \Delta H_{\text{lactate}}^* = -1364 \text{kJ/mol lactate} = -455 \text{kJ/mol O}_2 \quad (\text{Eq. 7.3})
\end{align*}
\]

(* \Delta H values were derived from Heats of Formation (\Delta H_f) values from Speight, 2005).

As can be seen from the \Delta H (change in enthalpy) values in equations 7.1 to 7.3, it is not the substrate type but the amount of O\textsubscript{2} used that determines the heat production. The \Delta H for the modelled substrate will be the average \Delta H of the above three reactions (–460 kJ/mol of O\textsubscript{2}) and will be referred to as: \(\Delta H_{\text{molO}_2}\). This value compares well with that used by Kaiser (1996) where the energy available from organic substrates was 448 kJ/mol O\textsubscript{2}.

Consequently, the energy generation rate can be defined as:

\[
\text{Resp HR} = \text{OUR} \times \Delta H_{\text{molO}_2} \quad (\text{kJ/h}) \quad (\text{Eq. 7.4})
\]
where OUR is the oxygen uptake rate (molO₂/h).

### 7.3.1.3 Heat losses from the reactor

Energy can be lost from the reactor in three different ways.

1. Through the walls of the reactor, referred to as: **Conductive Heat Loss Rate** (Conductive HLR).
2. Removal of warm air from the reactor after heating the incoming air to the internal reactor temperature, referred to as: **Sensible Heat Loss Rate** (Air HLR).
3. Evaporation, referred to as: **Evaporative Heat Loss Rate** (Evap HLR).

#### 7.3.1.3.1 Conductive heat loss rate

The feed is assumed to be 58% H₂O and 42% cellulose, thus the specific heat of the feed is 3.0 kJ/kg °C (0.58×4.186 kJ/kg H₂O °C + 0.42×1.423 kJ/kg cellulose °C).

The heat capacity of the feed is the amount of heat required to raise the temperature of one mole of the feed by one degree Celsius without change of phase and can be defined as:

\[
\text{heat capacity}_{\text{feed}} = \text{specific heat}_{\text{feed}} \times \text{mass}_{\text{feed}} \quad \text{(kJ/°C)} \quad \text{(Eq. 7.5)}
\]

Conductive heat loss from the reactor can be determined from the difference between internal and external temperatures as follows:

\[
\text{Conductive HLR} = \text{reactor heat loss coefficient} \times (\text{core temp} - \text{ambient temp}) \times \text{heat capacity}_{\text{feed}} \quad \text{(kJ/h)} \quad \text{(Eq 7.6)}
\]
7.3.1.3.2 Sensible heat loss rate

Similarly, the amount of heat that can be removed from the reactor by the airflow is a consequence of the amount of air passing through the reactor (airflow rate), the capacity of that air to take up heat (specific heat of the air) and difference between the temperature of the inflow and exhaust air and can be expressed as:

\[
\text{Air HLR} = \frac{\text{air flow rate}}{24.5} \times \text{specific heat}_{\text{air}} \times (\text{core temperature} - \text{ambient temperature})
\]

(kJ/h) \hspace{1cm} (Eq. 7.7)

7.3.1.3.3 Evaporative heat loss rate

Heat loss via evaporation is the result of water being removed from the reactor as vapour by a gas flow. The capacity of the air to remove water vapour is related to how dry it was when it entered the reactor. At any given temperature and humidity the water vapour, contained within the air, will exert a specific partial pressure (p(H_2O)) and, for the inlet air and the air contained inside the reactor, the partial pressures can be referred to as p(H_2O_{inlet}) and p(H_2O_{core}), respectively. As the p(H_2O) is temperature dependent, the saturating partial pressure of water vapour in air contained within the reactor can be estimated by Eq. 7.8 (equation derived from data in Weast et al., 1983 having a correlation coefficient of 0.9999):

\[
p(H_2O_{core}) = e^{\left(\frac{-5201.5}{(\text{core temp} + 273)} + 18.595\right)} \hspace{1cm} \text{(kPa)} \hspace{1cm} (\text{Eq. 7.8})
\]

Consequently, given the degree of saturation of the inflow air, the partial pressure of water vapour in the inflow air can be determined as:

\[
p(H_2O_{inlet}) = \left(\frac{\% \text{ humidity}_{\text{inlet air}}}{100}\right) \times e^{\left(\frac{-5201.5}{(\text{inlet air temp} + 273)} + 18.595\right)} \hspace{1cm} \text{(kPa)} \hspace{1cm} (\text{Eq. 7.9})
\]
Using the Ideal Gas Law \( n = \frac{PV}{RT} \) (Weast et al., 1983) and the fact that the number of moles of a gas are proportional to the partial pressure (Gay–Lussac’s Law) the rate at which water enters the reactor in the entry air is given by:

\[
\text{H}_2\text{O In Rate} = \frac{p(\text{H}_2\text{O}_{\text{inlet}})}{(\text{inlet air temp} + 273) \times R} \times \text{air flow rate} \quad \text{(mol/h)} \quad (\text{Eq. 7.10})
\]

where \( R \) is the Universal Gas Constant = 8.314 L.kPa/K.mol.

While the rate at which water leaves the reactor is given by:

\[
\text{H}_2\text{O Out Rate} = \frac{p(\text{H}_2\text{O}_{\text{core}})}{\text{(core temp} + 273) \times R} \times \text{air flow rate} \quad \text{(mol/h)} \quad (\text{Eq. 7.11})
\]

Therefore, the overall rate at which water is lost from the reactor (\( \text{H}_2\text{O LR} \)) can be determined by:

\[
\text{H}_2\text{O LR} = \text{H}_2\text{O Out Rate} - \text{H}_2\text{O In Rate} \quad \text{(mol/h)} \quad (\text{Eq. 7.12})
\]

Accordingly, the amount of energy being lost due to the removal of water vapour (Evap HLR) can be described by:

\[
\text{Evap HLR} = \text{H}_2\text{O LR} \times \Delta H_{\text{H}_2\text{O}} \quad \text{(kJ/h)} \quad (\text{Eq. 7.13})
\]

where \( \Delta H_{\text{H}_2\text{O}} \) (standard enthalpy change for \( \text{H}_2\text{O(l)} \rightarrow \text{H}_2\text{O(g)} \)) = 44.01kJ/mol (Weast et al., 1983).
7.3.1.3.4 Total heat loss rate (Total HLR)

The overall rate at which heat is lost from the reactor can be determined from the difference between the rates of heat generation and heat loss and can be defined as Total HLR, as follows:

Total HLR = Resp HR – conductive HLR – Air HLR – Evaporative HLR (kJ/h)  (Eq. 7.14)

7.3.1.3.5 Rate Of temperature change (Temp Change Rate)

Finally, the rate at which the contents of the reactor change temperature is dependent upon the rate at which heat is being generated within the reactor (Total HLR) and how much heat is required to increase the temperature of the solid (heat capacity_{feed}) and can be described as:

$$\text{Temp Change Rate} = \frac{\text{Total HLR}}{\text{Heat Capacity}_{\text{feed}}} \quad (^\circ\text{C}/\text{h}) \quad (\text{Eq. 7.15})$$

7.3.2 Part 2: Model Of Core Reactor Temperature During The Initial Aerobic Phase In A DiCOM® Reactor Considering Viable Microbial Cell Numbers

7.3.2.1 Assumptions

To adequately model reactor temperature, microbial growth and death must be considered. O₂ consumption by respiration, and consequently reactor temperature, is proportional to the number of viable cells. As the reactor temperature influences the rate of microbial growth, and death, the possibility of low cell numbers (e.g. early in the composting period or caused by overheating) needs to be considered.

To include microbial growth and death as part of the model to predict the temperature of an aerobic bioreactor the following assumptions were made:
1. All of the energy available in the substrate is available for heat generation.

2. There are only two microbial species present in the reactor, a mesophile and a thermophile.

3. Model parameters are for a generic mesophile and thermophile.

4. OUR is assumed to be $1 \times 10^{-13}$ mg O$_2$/h/cell for both organisms (estimated from laboratory data OUR).

5. The inoculum of mesophilic and thermophilic cells are both $4 \times 10^{10}$ cells/kg feed (similar to that found in spread plate laboratory experiments: data not shown).

**7.3.2.2 Maximum specific growth rate**

The maximum specific growth rate ($\mu_{max}$) is temperature dependent and it is possible to predict its value as follows (Zwietering et al., 1991):

$$
\mu_{max} = b^2 \times (temp - temp_{min})^2 \times (1 - e^{c(temp - temp_{max})}) \quad (h^{-1}) \quad (Eq. 7.16)
$$

Where:  

- $b$ and $c$ are Ratkowski numbers  
- $temp$ is reactor temperature in °C  
- $temp_{min}$ is the minimum temperature (°C) at which the organism can grow  
- $temp_{max}$ is the maximum temperature (°C) at which the organism can survive

The equation for $\mu_{max}$ above will generate positive and negative values at temperatures below $Temp_{min}$ and above $Temp_{max}$, respectively. Consequently, if the reactor temperature is below $Temp_{min}$, or above $Temp_{max}$, $\mu_{max}$ is set to 0.
7.3.2.3 Microbial death rate

It is common to model high temperature microbial inactivation with an Arrhenius–type equation which was proposed by Bigelow (1921) as follows (Van Impe et al., 1992):

\[
\text{Death rate} = 60 \times \left( \frac{2.303}{D_{\text{ref}}} \right) \times e^{\left( \frac{2.303}{z} \right) \times (\text{temp} - T_{\text{ref}})} \quad (\text{h}^{-1}) \quad \text{(Eq. 7.17)}
\]

Where: \( D_{\text{ref}} \) is the decimal reduction time that is, the time (h) required to decrease the population by 90% at a particular temperature (\( T_{\text{ref}} \))

\( T_{\text{ref}} \) is the reference temperature (°C) for the decimal reduction time

\( z \) is the number of degrees (°C) required to alter the decimal reduction time by a factor of 10.

7.3.2.4 Specific growth rate

The specific growth rate can include the death rate (\( \text{h}^{-1} \)) and be described as (derived from Pirt, 1975):

\[
\mu = \left( \frac{\mu_{\text{max}} \times S}{k_s + S} \right) - (\text{M}\_s \times Y) - \text{Death rate} \quad (\text{h}^{-1}) \quad \text{(Eq. 7.18)}
\]

Where: \( \mu \) is the specific growth rate

\( S \) is the substrate concentration (mol.L\(^{-1}\))

\( k_s \) is the Michaelis–Menten half saturation constant (mol.L\(^{-1}\))

\( M_s \) is the maintenance coefficient for the particular organism (gS.(gX.h)\(^{-1}\))

\( Y \) is the yield coefficient for the particular organism (gX.gS\(^{-1}\))
7.3.2.5 Biomass Generation

Finally biomass generation can be described according to (Pirt, 1975):

\[ X = e^{(\ln(x_o) + \mu \times t)} \]  

(cells)  (Eq. 7.19)

Where \( X_o \) is the initial biomass at \( t = 0 \).

7.4 Results and Discussion

7.4.1 Heat Loss Coefficient Of The Laboratory–Scale DiCOM® Reactor

The heat loss coefficient (0.091h\(^{-1}\)) was determined by plotting the driving force (the difference between core reactor and room temperatures) against the rate of temperature change, with the magnitude of the slope providing the heat loss coefficient (Fig. 7.2).

![Graph showing heat loss coefficient](image)

**Figure 7.2:** Determination of heat loss coefficient (slope of the least–squares line of linear regression) of a laboratory–scale DiCOM® reactor.

**Equation of Least–Square Regression:** Rate of Temperature Change = –0.0912*Driving Force

**Correlation Coefficient** = 0.9813.
7.4.2 Model Predictions

7.4.2.1 Modelling temperature predictions excluding viable cell numbers

Equations 7.4 to 7.15 formed the basis of a computer simulation, developed using National Instruments Labview 7.0 software, and was utilised to predict core reactor temperatures of DiCOM® reactors independent of microbial constraints.

The model predicted laboratory and large–scale DiCOM® reactor temperatures to reach 77 and 135°C respectively (Fig. 7.3) suggesting that overheating in a large–scale plant is possible (Refer to List 7.1 for model parameters). The model predicts that temperature will rise to a maximum where an equilibrium exists between the heat lost from the reactor and the heat gain. This prediction assumes that heat generation is constant and limited by O₂ consumption and is based on the maximum OUR observed in the laboratory–scale reactor laboratory. The predicted temperatures are greater than the optimum for composting (60°C: Strom, 1985) and for growth of Methanoculleus spp. (60°C: Spring et al., 2005) and Methanosarcina thermophila (55°C: Boone and Mah, 2001), which have been identified as the key methanogens in the DiCOM® process (Section 5.3.4). The predicted maximum temperature of 135°C for the full–scale reactor is a clear over–estimate as aerobic microbial activity would be expected to cease well before this temperature was attained and equations for SC are not included in the model. However, the predicted overheating indicates that excess heat generation during the initial aeration period of the DiCOM® process in large–scale plants is possible. The application of this model is obviously limited and needs to be expanded by considering the effect of temperature on cell growth and death.
List 7.1: Assumed model parameters for the prediction of reactor temperature without considering viable cell numbers.

<table>
<thead>
<tr>
<th>Model Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>OUR</td>
<td>0.1 mol O(_2)/h.reactor</td>
</tr>
<tr>
<td>Mass of Feed</td>
<td>2.5 kg</td>
</tr>
<tr>
<td>Specific Heat of Feed</td>
<td>3.0 kJ/kg.°C</td>
</tr>
<tr>
<td>Air Flow Rate</td>
<td>10 L/h</td>
</tr>
<tr>
<td>Specific Heat of Input Air</td>
<td>0.03 kJ/kg.°C</td>
</tr>
<tr>
<td>Percentage Moisture in Air</td>
<td>50 %</td>
</tr>
<tr>
<td>Input Air Temperature</td>
<td>20°C</td>
</tr>
<tr>
<td>Room Temperature</td>
<td>20°C</td>
</tr>
<tr>
<td>Reactor Heat Loss Coefficients</td>
<td></td>
</tr>
<tr>
<td>Laboratory–Scale Reactor</td>
<td>0.09 h(^{-1})</td>
</tr>
<tr>
<td>Full–Scale Reactor</td>
<td>0.0 h(^{-1})</td>
</tr>
</tbody>
</table>

Figure 7.3: Predicted maximum reactor temperature for laboratory (75°C) and large–scale (135°C) reactors ignoring the presence of micro–organisms while maintaining maximum OUR and that substrate is always in excess. Model parameters: mass of feed = 2.5kg; Specific heat of feed = 3.026 kJ/kg°C; Air flow rate = 10 L/h; Specific heat of input air = 0.0289 kJ/kg°C; Percentage moisture in Air = 50%; Input air temp = 20°C; Room temperature = 20°C.

Legend: (▬) Laboratory–scale reactor; (―) Pilot–scale reactor.

7.4.2.2 Modelling temperature predictions when viable microbial numbers are considered

By considering cell growth and death, the predicted maximum temperature (refer to Table 7.1 for biological model parameters) attained in laboratory (Fig. 7.4 A) and large–scale (Fig. 7.4 B) reactors, is equal to the temp\(_{\text{max}}\) (the maximum temperature at which
the organism can survive) of the thermophile present in the model. The model predicts that mesophilic biomass decreases as the temperature rises past the $T_{\text{max}}$ for the mesophiles (Fig. 7.4 A). Heat gain, now only driven by thermophilic metabolism, continues to raise the temperature past the $T_{\text{max}}$ for the thermophiles, causing death. This growth and death cycle is repeated until a stable temperature is attained. The important point here is, not whether a microbe can raise the temperature of its environment to that higher than the $T_{\text{max}}$ of the organism, but that the temperature becomes stable at that approximately equal to the $T_{\text{max}}$ of the most thermo–stable micro–organism. The above trend was also noted when a larger reactor (heat loss coefficient = 0.0h–1) was modelled (Fig 7.4B) however, more time was required for the temperature to stabilize (15 days) as heat is lost more slowly from the reactor.

**Table 7.1:** Assumed parameters for modelling of microbial growth. Parameters were estimated for (b, c, $T_{\text{max}}$, $T_{\text{min}}$, $T_{\text{ref}}$, $D_{\text{ref}}$ and $z$) based on data provided in Ratkowsky *et al.* (1983); Zwietering *et al.* (1991); Van Impe *et al.* (1992); Cunha *et al.* (1997) and van Asselt and Zwietering (2006).

<table>
<thead>
<tr>
<th>Model Parameter</th>
<th>Units</th>
<th>Mesophilic Organism</th>
<th>Thermophilic Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>b</td>
<td>–</td>
<td>0.025</td>
<td>0.0297</td>
</tr>
<tr>
<td>c</td>
<td>–</td>
<td>0.215</td>
<td>0.215</td>
</tr>
<tr>
<td>$T_{\text{max}}$</td>
<td>ºC</td>
<td>50</td>
<td>75</td>
</tr>
<tr>
<td>$T_{\text{min}}$</td>
<td>ºC</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>$T_{\text{ref}}$</td>
<td>ºC</td>
<td>50</td>
<td>121</td>
</tr>
<tr>
<td>$D_{\text{ref}}$</td>
<td>h</td>
<td>0.23</td>
<td>0.11</td>
</tr>
<tr>
<td>$z$</td>
<td>ºC</td>
<td>4.3</td>
<td>13</td>
</tr>
<tr>
<td>$k_s$</td>
<td>M</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Y</td>
<td>gX.gS$^{-1}$</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>$M_i$</td>
<td>gS.(gX.h)$^{-1}$</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Even though model values were based on laboratory data, at no time during laboratory–scale DiCOM® trials did the temperature of the reactor reach the predicted 75ºC (64ºC was the maximum during 22 trials). This could be due to an over–estimation of high temperature tolerance for the modelled thermophiles. Although it can be deduced that
thermophilic micro–organisms capable of raising the temperature beyond 64°C are not active in the laboratory reactor.

Figure 7.4: Predicted microbial growth and reactor temperature with heat loss coefficient = 0.0912 h⁻¹ (A): (laboratory–scale reactor) and 0.0 h⁻¹ (B): (full–scale reactor) assuming that substrate is always in excess. Model parameters: mass of feed = 2.5kg; Specific heat of feed = 3.026 kJ/kg°C; Air flow rate = 10 L/h; Specific heat of input air = 0.0289 kJ/kg°C; Percentage moisture in Air = 50%; Input air temp = 20°C; Room temperature = 20°C; Size of Mesophilic and Thermophilic Biomass Inocula = 1 x 10¹¹ cells; T_max for Mesophilic and Thermophilic Biomass = 50 and 75°C, respectively.

Legend: (▬) Core reactor temperature; (– –) Biomass cell number (× 10⁶).
7.4.3 DiCOM® Pilot–Scale Demonstrates Over–Heating

In an attempt to confirm the predicted over–heating in a larger–scale plant during aeration, the temperature profile of the DiCOM® pilot–scale reactor was obtained.

Overheating during initial aeration of the pilot–scale reactor was evident where a maximum temperature of 85°C was attained (Fig. 7.5) and consequently it can be predicted that hyper–thermophilic aerobic micro–organisms were present. The highest temperatures reached occurred toward the middle of the reactor, where the insulation provided by the waste is greatest, and within the top layer (approximately 50cm) of the OFMSW (i.e. close to the waste/reactor–headspace boundary). This observation suggests that even though O₂ is supplied under pressure, the availability of O₂ is greater toward the surface of the material, where it is more porous (or less consolidated), than at lower regions of the reactor.

Figure 7.5: Trans–sectional temperature profile of the OFMSW in a pilot–scale DiCOM® reactor at the completion of the loading and aeration phase (day 5 of waste processing).

Legend: (♦) 120cm from reactor base; (■) 210cm from reactor base.
SC during DiCOM® operation is possible since heat generation from abiotic oxidation of plant chemicals is detectible above approximately 80°C (Kubler, 1987). However the design of the DiCOM® process provides significant safeguards against SC becoming a reality. Abiotic oxidation is an essential precursor to establish the elevated temperature required for the initiation of SC and this process requires O₂ to be present. As DiCOM® operates within a sealed vessel the supply of O₂ can be controlled. If the reactor temperature was to exceed a temperature set–point (e.g. 90°C) the O₂ supply could be stopped therefore eliminating abiotic oxidation and consequently avoiding the onset of SC. If on the other hand SC had already been initiated, the DiCOM® reactor can be completely flooded with liquid to suppress the smouldering initiated via pyrolysis.

7.4.4 Effect of Elevated Temperature During Initial Aeration on the Onset of Methane Production

During the initial aerobic phase of the DiCOM® Process, microbial respiration generated a significant amount of heat. The core temperature in the laboratory–scale reactor typically reached 60°C within 24h of the commencement of aeration and was maintained at this temperature for the duration of the phase. Temperatures greater than 60°C were rarely reached in the laboratory–scale reactor due to a high heat loss coefficient. However, the heat loss coefficient of the pilot–scale reactor (−0.0002h⁻¹; personal communication AnaeCo Ltd.) was small (450 times less than the laboratory reactor) due to the significantly lower surface area to volume ratio (and the greater mass of feed material) which results in less heat lost to the environment. Consequently, during the initial aerobic phase, the core temperature of pilot–scale trials was found to be as high as 85°C.
Thermophilic methanogens present within the solid were previously found to play a significant role in methanogenesis during the anaerobic phase of the DiCOM® process (Section 5.3.5 and 5.3.6). Those found in the laboratory–scale reactor have an optimum temperature of approximately 55°C (*Methanosarcina thermophila*: Boone and Mah, 2001; *Methanoculleus spp.*: Spring et al., 2005) and may be inhibited by temperatures above 60°C. Hence, the high core temperatures achieved during the aerobic phase in the pilot plant may decrease the methanogen population, resulting in a decrease in CH₄ production and, as a consequence, VFA accumulation. To investigate the effect of high temperature during the initial aeration phase, on CH₄ production from OFMSW during the anaerobic phase of the DiCOM® process, samples of OFMSW were incubated for 24h at elevated temperatures (65°C, 75°C and 85°C).

Heat treatment of OFMSW at temperatures up to 85°C had no effect on total CH₄ production during the 7–day thermophilic (55°C) AD (14.9 to 16 L/kg wet OFMSW) (Fig. 7.6). The onset of CH₄ production was delayed (one day) for OFMSW pre–incubated at temperatures greater than 55°C. However, the rapid increase in CH₄ production on day 2 resulted in approximately equal cumulative CH₄ production from all trials by the end of day 3. CH₄ production peaked in all trials between days 3 and 4 (Fig. 7.7).

The delay before the onset of methanogenesis from heat–treated OFMSW, at temperatures between 65 and 85°C, indicated that exposure to high temperature may have been detrimental to some, or all, of the microbial communities (including methanogens) within the OFMSW. Even though there was no long lasting negative effect on CH₄ production, in a reactor stressed by VFA accumulation or ammonia...
inhibition, the lack of methanogenic activity during the initial 24h of AD may increase the risk of reactor failure.

**Figure 7.6:** Cumulative CH\(_4\) production from the thermophilic AD of MSW. 10g samples of pre–aerated MSW in 100mL serum vials were incubated at 55°C, 65°C, 75°C and 85°C for 24h. 45mL of filter sterilised anaerobic liquor (water for the control) were added, the vials sealed and flushed with N\(_2\)/CO\(_2\) and incubated at 55°C for 7 days. **Legend:** (♦) water; (○) 55°C; (Δ) 65°C; (◊) 75°C; ( ⟨) 85°C.

**Figure 7.7:** CH\(_4\) production rate (L/kg/day) of the thermophilic anaerobic digestion of MSW pre–incubated at 55°C, 65°C, 75°C and 85°C. **Legend:** (♦) water; (○) 55°C; (Δ) 65°C; (◊) 75°C; ( ⟨) 85°C.
DiCOM® pilot–plant operation could be adversely affected by exposing the feedstock to high temperatures (>60°C) during the initial aerobic (loading) phase. During these five days, approximately equal quantities of OFMSW are loaded into the reactor daily. By the end of the fifth day of loading, approximately 60% of the MSW could have potentially been exposed to high temperatures (>60°C) for more than 24 hours. This longer exposure (>24 hours) of the OFMSW to temperatures greater than 60°C is likely to have a proportionally stronger effect on methanogenic suppression during the start–up of the anaerobic phase; but further investigation is needed to quantify this conjecture. This aside, commercially the process demands a relatively constant combustible biogas production for use in meeting the energy requirements of the plant. Thus any delay in biogas generation must be avoided.

7.4.5 Temperature: A Selective Agent For The Bacterial And Methanogenic Species Present In DiCOM® Pilot–Plant And Laboratory Reactors

The above model, incorporating microbial growth and death (Section 7.4.2.2) indicated that, when no heat was lost from the reactor, the core reactor temperature would rise to the maximum temperature at which the most heat tolerant organism could survive. As the heat loss coefficient of the pilot–scale reactor is small, it was hypothesised that the most heat stable micro–organism in the pilot–scale system had a temp_{max} of approximately 85°C. To test this hypothesis, terminal restriction fragment length polymorphism (T–RFLP) profiles (Dr. Lucy Skillman) were obtained from pilot–scale reactor samples.

Due to the difficulty of reliable DNA extractions from solid samples, T–RFLP analysis was performed on anaerobic liquor samples taken daily during the anaerobic phase of the DiCOM® pilot–scale reactor. DNA extracted from the liquor samples was amplified
via a polymerase chain reaction (PCR) of the 16S ribosomal ribonucleic acid (rRNA) gene. Restriction digestion of the PRC products provided fragment length profiles which were used to determine the abundance of each species present.

Eubacterial communities present in pilot–plant OFMSW and recycle samples were dominated by *Thermotoga/Thermocrinis/Hydrogenobacter* and *Geotoga spp.* (up to 70%: data not shown). *Thermocrinis* and *Hydrogenobacter* species are hyper–thermophilic aerobic micro–organisms having an upper growth limit between 80 and 90°C (Huber et al., 2001; Ishii et al., 2001) (List 7.2). These data support the hypothesis that reactor temperature will be dominated by the \( \text{temp}_{\text{max}} \) of the most heat stable micro–organism. The dominant eubacterial groups present in the laboratory–scale reactor were found to be different from those from the pilot reactor in that, *Prevotella* spp. and *Thermodesulfobacterium* (up to 16%) were the dominant species, but both were absent from the pilot–scale samples. The physiological role of the above mentioned species is yet to be investigated.

As a consequence of the pilot–scale DiCOM® reactor self–heating to 85°C, it was anticipated that the types of methanogens detected in pilot–plant reactor samples would be different to those at laboratory–scale. Analysis of the anaerobic population of pilot reactor samples (Anaerobic Day 3) showed large numbers of *Methanosarcina thermophila* (up to 70%) unlike laboratory results where they accounted for less than 10% of the methanogenic population. This was unexpected as *M. thermophila* has an optimum growth temperature (50–55°C) well below the temperature to which the pilot reactor self–heated (85°C). Their presence in high numbers suggests that, in pilot trials, acetoclastic methanogenesis would account for a greater portion of CH₄ contra to trends
Chapter 7: Temperature and the DiCOM® Process

List 7.2: Characteristics of eubacteria and methanogens found in laboratory and pilot plant recylcate samples.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Prevotella spp.</em></td>
<td>A mesophilic, chemo–organotrophic, obligate anaerobic fermentative bacteria (Berger, 1994) found in the rumen and human oral and urogenital regions, having an optimum growth temperature of 37°C (Ueki et al., 2007).</td>
</tr>
<tr>
<td><em>Thermodesulfobacterium spp.</em></td>
<td>A thermophilic, chemo–organotrophic, strictly anaerobic sulfate–reducing bacteria with an optimal growth temperature between 60 and 70°C (Hatchikian and Ollivier, 2001).</td>
</tr>
<tr>
<td><em>Thermotoga spp.</em></td>
<td>Obligate anaerobic, heterotrophic, hyper–thermophilic organisms with an optimum growth temperature between 66 and 80°C and an upper growth limit of 90°C. (Huber and Stetter, 2001)</td>
</tr>
<tr>
<td><em>Geotoga spp.</em></td>
<td>A strictly anaerobic, fermentative bacteria which is moderately thermophilic having an upper growth limit of 60°C (optimum 50°C) (Davey et al., 2001).</td>
</tr>
<tr>
<td><em>Methanobacterium spp.</em></td>
<td>A H2–consuming strictly anaerobic, chemolithotrophic, mesophilic and thermophilic organisms with optimum growth temperatures &lt;70°C (<em>M. thermoautotrophicum</em>) (Boone and Mah, 1989).</td>
</tr>
<tr>
<td><em>Methanoculleus spp.</em></td>
<td>Mesophilic and thermophilic, chemolithotrophic, strictly anaerobic H2/CO2 utilizing CH4 producer (Chong and Boone, 2001) having an optimum growth temperature between 25–60°C (Spring et al., 2005).</td>
</tr>
<tr>
<td><em>Methanosarcina thermophila</em></td>
<td>Chemolithotrophic, obligate anaerobic, thermophilic (50–55°C optimum) organisms (Boone and Mah, 2001).</td>
</tr>
</tbody>
</table>

Definitions: (Prescott et al., 1999).

**Autotroph:** An organism that uses CO2 as the source of carbon for biosynthesis.

**Heterotroph:** An organism that uses reduced organic molecules from another organism as the source of carbon for biosynthesis.

**Chemotroph:** An organism that oxidises organic or inorganic compounds to obtain energy.

**Lithotroph:** An organism that uses reduced inorganic molecules as an electron source.

**Organotroph:** An organism that uses organic molecules as its electron source.
noted in laboratory experiments where the bulk of CH$_4$ was produced via hydrogenotrophic methanogenesis (Section 5.3.5). *Methanoculleus* spp., the dominant methanogen at laboratory–scale (15–50%), was not detected in pilot–scale samples but the more heat tolerant hydrogenotrophic methanogens assigned to the *Methanothermobacter* and *Methanobacterium* groups were identified. An understanding of these differences in microbial profiles may assist in optimizing process performance and stability at pilot and full–scale facilities.

### 7.5 Conclusions

- The developed mathematical model predicted that elevated temperature during the initial aeration phase of the DiCOM$^\text{®}$ process was possible, due to rapid aerobic degradation of the OFMSW organics. The predicted elevated temperature was confirmed in pilot–scale DiCOM$^\text{®}$ trials. As the DiCOM$^\text{®}$ process relies on the OFMSW to provide a source of methanogenic inoculum and, that methanogens are heat sensitive, control of temperature during aeration may be critical.

- Laboratory results demonstrated that exposing OFMSW to temperatures greater than 55°C provided a short delay in the onset of methanogenesis which may contribute to VFA accumulation, acidification, suboptimal reactor performance and an increased risk of reactor failure.

- T–RFLP analysis indicated that the microbial profiles of laboratory and pilot–scale reactors were different, with temperature variation a possible cause for the disparity: the implications of which are yet to be quantified.
Chapter 8

Pilot–Scale Trials of the DiCOM® Process

8.1 Introduction

Laboratory reactors are not typically reproduced in the dimensions of pilot and/or full-scale plants nor do they reflect materials compression (inter-particle bridging which can result in incomplete transfer of material weight to the base of the waste column), airflow wall effects (greater air flow in small-scale systems) and airflow channelling through the matrix (Mason and Milke, 2005). Additionally, particle size is not scalable and heat losses from laboratory-scale reactors can be significant. Consequently, laboratory data is not directly (1:1) transferable to pilot and/or full-scale systems, but the literature reports that trends observed at laboratory-scale can be recognised at larger scale (Körner et al., 2003).

The purpose of this chapter is to determine whether trends observed at laboratory-scale can be reproduced in a pilot-scale DiCOM® reactor.

8.2 Materials and Methods

8.2.1 Pilot Scale Trials

Three (3) consecutive pilot-scale DiCOM® trials were conducted at the AnaeCo facility with reactor design and operation, organic feed material, data collection and recyclate preparation conducted as described in Section 7.2.1.

8.2.2 Recyclate Storage

The DiCOM recyclate was stored for all trials at ambient temperature under anaerobic conditions. The liquor was stored at ambient temperature under anaerobic conditions
for an extended period (6 months) prior to use in Trial A, however for Trials B and C the duration of liquor storage was minimised (2 and 3 weeks, respectively).

8.3 Results and Discussion

8.3.1 Can Performance Trends of a Laboratory–Scale DiCOM® Reactor be Reproduced at Pilot–Scale?

The DiCOM® process has been successfully operated in a sustainable manner at laboratory–scale. To test whether these operational trends were reproducible at pilot–scale, in co–operation with AnaeCo Ltd., a number of consecutive pilot–scale trials were conducted. Specifically it was studied as to whether process improvements could be obtained by using a well maintained anaerobic inoculum. The initial trial (Trial A) demonstrated poor performance (Fig. 8.1 A) in that it provided incomplete VFA removal and required pH adjustment (NaOH addition) to maintain process stability. As the recyclate for Trial A had been stored for an extended period of time (approximately 6 months), and as laboratory experience has shown that the rapid reuse of recyclate improved process performance, it is likely that the observed poor performance could have resulted from an inactive inoculum.

The use of a fresh inoculum has been shown to improve overall performance and stability during laboratory–scale DiCOM® operation (Chapter 3). To test whether this observed process improvement could be reproduced during a pilot–scale trial, a second trial was performed (Trial B). The use of fresh liquor improved trial performance through decreased VFA accumulation (compare Fig. 8.1 B and A) and elevated CH₄ generation (Fig. 8.2) which is inline with results obtained during laboratory experiments (Section 3.3.2 & 3.3.3). Nevertheless, during Trial B, complete VFA removal was still not obtained within the timeframe of the DiCOM® process.
To further investigate the effect of a fresh and active inoculum that was free of accumulated VFA (intended to demonstrate optimum performance) on the performance of the DiCOM® process a third trial (Trial C) was performed. The accumulated VFA (here acetate and propionate) from the previous trials were degraded, to levels lower than the starting values in the recyclate, by allowing extended anaerobic incubation in trial B. The use of a fresh active inoculum low in VFA resulted in improved biogas production and a decrease in VFA accumulation during the anaerobic period (Fig. 8.2; Fig 8.1 C).

While improvement in AD performance was noted during successive operation, the pilot-scale DiCOM® process did not completely perform to specification. Repeated reuse of anaerobic liquor provided a decrease in VFA accumulation by day 12 (140, 85 and 55mM for Trials A, B and C, respectively), an improvement in CH₄ production (57, 100 and 110L/kgVS), maximum CH₄ production rate (0.60, 0.92 and 0.99 L/h/kgVS) and rapidity of the onset of methanogenesis (Fig. 8.2). This trend could be extrapolated to further trials but was beyond the scope of this study. However, unlike optimised laboratory trials (Fig. 8.3) the anaerobic phase of pilot-scale trials did not show successful biomethanation as the key VFA (acetate, butyrate and propionate) were not completely degraded. This is a significant limitation as almost complete degradation of VFA is needed to allow for continued re-use of the liquor and sustainable operation.
Figure 8.1: Incomplete VFA degradation during a time-course from the DiCOM® pilot-scale reactor trials treating approximately 3200kg of mechanically sorted OFMSW (VS: (A) 51%; (B) 56%; (C) 60%). Recyclate used for Trial A (A) had been stored at ambient temperature under anaerobic conditions for an extended period of time (approximately 6 months) while for Trials B (B) and C (C) the recyclate was stored for the minimum feasible time period (2 and 3 weeks, respectively). Arrows indicate time of pH adjustment.

Legend: (■) Acetate; (◆) Propionate; (▲) Butyrate; (O) Free-NH$_3$; (—) pH.
Figure 8.2: Specific CH₄ production rates during DiCOM® pilot–scale reactor trials treating approximately 3200kg of mechanically sorted OFMSW. The inoculum used for Trial A had been stored for approximately 6 months under anaerobic conditions at ambient temperature while for Trials B and C the inoculum was stored for the minimum practicable time period (2 and 3 weeks, respectively) before being reused.

Legend: ( — ) Trial A; ( — ) Trial B; ( — — ) Trial C.

Figure 8.3: Volatile fatty acid accumulation during the anaerobic phase of a laboratory–scale DiCOM® reactor showing complete degradation of accumulated VFAs.

Legend: ( □ ) Acetate; ( ♦ ) Propionate; ( ▲ ) Butyrate; ( O ) Free–NH₃; ( — ) pH.

8.3.2 Why Does a Laboratory–Scale DiCOM® Reactor Perform Better Than Pilot–Scale?

It is anticipated that the difference in scale has caused variations in environmental conditions and performance which may include;
1. Lower heat loss from the reactor leading to a greater propensity for overheating during aeration phases (Section 7.4.3).

2. Less methanogens in the anaerobic liquor (Section 5.3.7) leading to lower initial CH\textsubscript{4} production and VFA accumulation.

3. Higher solid to liquid ratio (dry solid to liquid: 0.4:1 compared to 0.3:1 for pilot and laboratory reactor, respectively) expediting the build-up of soluble substances in the liquor (Section 4.3.1.3 (NH\textsubscript{3})) and increasing the risk of digester failure.

4. Less exposure of the organic material to the initial aeration phase (as the material is loaded over a 3 day period and cannot be aerated during loading) resulting in a greater organic burden for anaerobic and aerobic maturation phases.

5. “Freshly” received OFMSW (compared to frozen OFMSW used in laboratory reactors) possibly providing differences in microbiological diversity, material structure and substrate availability.

6. Less subdivision of the solids (compared to shredded material used in laboratory trials) would provide slower reaction rates with a longer stabilisation period required.

7. Consolidation of the material may limit penetration of O\textsubscript{2} and recylcate into the solids resulting in decreased degradation.

In addition the physical embodiment of the process was not optimal in that:

- liquor heating was performed using contact heating elements rather than a heat exchanger, exposing micro–organisms to extremes of temperature;
- anaerobic dewatering was not possible; consequently, pressate was exposed to O\textsubscript{2}.

The incomplete VFA degradation observed during an optimised pilot–scale trial could be the result of deficient CH\textsubscript{4} production. Modelling, as outlined in Section 4.3.3, predicts that at pilot–scale acetoclastic methanogenesis experienced a similar degree of inhibition to optimised laboratory trials (Fig. 8.4). Accordingly, inhibition from free–
NH$_3$ and/or propionate was unlikely to account for the limited methanogenic activity observed. It has already been established that the concentration of methanogens in the pilot–scale liquor is lower than in laboratory liquor (Section 5.3.7 Fig. 5.11 A) which may result in lower methanogenic activity resulting in VFA accumulation. When comparing the best pilot–scale run (Trial C) with an optimised laboratory–scale trial a delay in maximum methanogenic activity was evident (Fig. 8.5). In the laboratory–scale reactor CH$_4$ production commenced more rapidly, limiting the initial build–up of VFA, and decreased towards the end of the digestion phase, where readily available substrate became limiting (Fig. 8.3). In contrast, the pilot CH$_4$ generation rate and VFA concentration were still high when the liquor was drained from the reactor. However the total amount of CH$_4$ formed during the 7 day digestion phase was approximately equal (110 & 103L/kgVS: Fig. 8.5). It can therefore be deduced that in the pilot trial:

- Significant methanogenic growth occurred during the digestion period.
- A greater fraction of readily degradable organic material was present in the material at the beginning of the anaerobic phase.

Figure 8.4: Predicted acetoclastic methanogenic activity based on the model presented in Section 4.3.3 for pilot–scale DiCOM$^\text{®}$ trials.

Legend: (■) Laboratory Trial; (▲) Optimised Pilot Trial (Trial C).
Figure 8.5: The CH₄ production rate (solid lines) and cumulative CH₄ production (dashed lines) during the anaerobic phase of an optimised pilot–scale (Trial C) (bold lines) and laboratory–scale (thin lines) DiCOM® trials.

The approximately equal CH₄ production (/kgVS) during the anaerobic phase of optimised laboratory and pilot trials would initially appear to negate the concerns that overheating during the initial aeration phase may decrease the viable endogenous methanogenic population within the OFMSW (Section 7.4.4) and that the pilot–scale recyclate contained a smaller inoculum of methanogens (Section 5.3.7). It is evident that there existed adequate time, during the anaerobic phase, for the development of a methanogenic culture from a combination of methanogens endogenous to the solid and those transferred within the recyclate. However, a significant portion (59%) of the CH₄ production occurred during the final 3 days of anaerobic treatment (Fig. 8.5: days 9–12) with the production rate still significant (70% of maximum) at the time of recyclate removal. Importantly, it was during this time (days 9–12) in laboratory trials that VFA (acetate and butyrate) limitation resulted in a decreased CH₄ production rate (Fig. 8.5) and facilitated propionate removal (Fig. 8.3). As standard pilot trials have not shown acetate exhaustion leading to propionate removal, the above mentioned concerns must still be considered for sustainable long–term operation of the pilot–scale DiCOM® process.
It can be predicted, during pilot–scale processing, that a larger methanogenic inoculum could provide a more rapid onset of methanogenesis and a decrease in accumulated VFA, allowing for acetate exhaustion and propionate removal, as observed in laboratory experiments.

The quantity of volatile solids introduced to laboratory and pilot trials were approximately equal (58 and 60% VS/kg, respectively) however, measured as electron flow, the initial aeration phase of an optimised laboratory reactor degraded approximately three times more organics (13% compared to 38%) than at pilot scale (Fig 8.6). This decrease in aerobic activity accordingly provided a greater quantity of organics to the anaerobic phase of the pilot trial as previously suggested. With the anaerobic phases of both processes providing approximately equivalent electron flow it is therefore necessary for the final aerobic maturation phase to contend with the reducing power not processed during initial aeration. Consequently, elevated temperatures in the compost were achieved (63°C compared to 35°C). As electron flow was elevated at the conclusion of processing, it was not surprising that the compost produced at pilot–scale was not stable even though satisfying many maturity criteria (Table 8.1). The lack of pilot–scale end–product stability is in contrast to that found in optimised laboratory DiCOM® operation (Table 8.1).

When comparing laboratory and pilot performance, overall there was less total degradation of the solids at pilot scale (Fig. 8.6) but this was not due to decreased anaerobic activity but rather aerobic activity. Accordingly, it can be deduced that the underlying cause of the VFA accumulation, during the early part of the digestion phase, is the lack of degradation occurring during the initial aeration phase. Therefore, for improved process performance and product stability, the amount of degradation during
the initial aeration phase must be increased. This however creates a dilemma for pilot–
scale operation. It is necessary to increase aerobic degradation without raising the
temperature of the waste too much to endanger the endogenous methanogen population.
As current pilot–scale aerobic degradation rates generate temperatures in excess of that
which can be tolerated by the key endogenous methanogens (Section 7.4.5), an increase
in aerobic degradation is unlikely without the introduction of a cooling system internal
to the reactor.

![Figure 8.6](image)

**Figure 8.6:** Comparison of a time course of the flow of electron equivalents for an optimised pilot and
laboratory–scale DiCOM® trial. Electron flows for each phase are indicated (bold = pilot),
with total electron flows being 59536 and 66034 (mmol/kgVS) for pilot and laboratory–
scale trials, respectively.

*Legend:* (▬) Pilot; (―) Laboratory.

Increased aerobic degradation is likely to be biologically problematic, expensive or
mechanically restrictive. However the rate of degradation is greater under anaerobic
conditions (Fig. 8.6). As anaerobic metabolism will not induce run–away temperature,
the solution to improved solid degradation is likely found within anaerobic phase
operational parameters.
Table 8.1: Comparison of compost stability and maturity parameters for the end–product of laboratory and pilot–scale DiCOM® trials.

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<th>Pilot Trial C</th>
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</tr>
</tbody>
</table>

References: ¹ Tam and Tiquia, (1994)

8.3.3 Can a Pilot–Scale DiCOM® Reactor Be Operated Sustainably Such That Accumulated VFA are Exhausted?

As the DiCOM® recyclate provides significant buffer, it is anticipated that the VFA generated as a result of limited initial aerobic degradation should not cause acidification in pilot–scale operation. However pilot trials have shown that the removal of these VFA may be nontrivial with a stable end–product and sustainable operation attained only if VFA exhaustion is achieved. To investigate whether VFA exhaustion could be achieved within a pilot–scale DiCOM® reactor, the anaerobic phase of Trial B was extended.

Propionate was found to be degraded within the reactor, however, more slowly (4mM/d) than in laboratory experiments (24mM/d) (compare Fig. 8.3: days 9–10 & Fig. 8.7: days 20–26), suggesting that a significant propionate degrading culture was not present in the pilot–scale reactor. As expected, due to the long anaerobic incubation, lower degradation activity in the aerobic maturation phase (compare Fig. 8.8 & 8.6) and enhanced product stability (Table 8.1) were obtained.
The VFA removal required for sustainable operation of the pilot–scale DiCOM\textsuperscript{®} facility can be achieved. However to realize this outcome, one or both of the following is required.

- An extension of the anaerobic phase. Seven (7) days of AD is not sufficient to provide VFA exhaustion. If the liquor were to be drained according to the typical DiCOM\textsuperscript{®} timing schedule, approximately 50\% of the electron equivalent flux (38389 mmol/kgVS; shaded region in Fig. 8.8) which occurred during AD would remain either within the solid or the drained liquor. Reducing power remaining in the solid would become a liability to the aerobic maturation phase and, if not sufficiently oxidised, would result in an immature end–product. VFA remaining within the liquor would be transferred to another trial, resulting in a greater organic burden for the anaerobic phase and an increased risk of reactor failure.

![Figure 8.7: A time-course showing VFA degradation during the extended anaerobic phase (21 days compared to the standard 7 days) of a modified DiCOM\textsuperscript{®} pilot–scale reactor (Trial B) treating approximately 3200kg of mechanically sorted OFMSW. Legend: (■) Acetate; (●) Propionate; (▲)Butyrate; (O) Free–NH\textsubscript{3}; (----) pH.](image)
Figure 8.8: A time-course showing electron flow during the extended anaerobic phase of a modified DiCOM® pilot–scale reactor (Trial B) showing seamless transitions between phases. The electron flow for each phase is indicated, with the total electron flow being 84357 mmol/kgVS. If the anaerobic liquor were withdrawn on day 12 (7 days of anaerobic digestion) as per the typical DiCOM regime, the shaded region (38389 mmol/kgVS) indicates the electron equivalents which would either become a liability to the aerobic maturation phase or be transferred to another batch.

- An increase in CH₄ production especially at the beginning of anaerobic phase. The lag in methanogenic activity observed during pilot trials must be minimised. Consequently, the transfer of a viable active inoculum is critical.

8.4 Conclusions

Results from operation of a pilot–scale DiCOM® reactor indicated:

- Performance of an optimised laboratory–scale DiCOM® set–up was not able to be reproduced at pilot–scale.

- Limited aerobic degradation occurred during the initial aeration phase (70% less than that reported at laboratory–scale) increasing the organic demand on the AD phase.

- Laboratory trends could be recognised in pilot–scale data. That is, the reuse of anaerobic liquor provided improved process performance at pilot–scale, which could
be seen in a decrease in VFA accumulation by day 12, an improvement in CH$_4$
production and maximum production rate, and a more rapid onset of methanogenesis.

- The observed propionate consumption in laboratory trials was achieved within the
  pilot–scale reactor. However, metabolism of the propionate that accumulated within
  the trial was slower than in laboratory experiments and as a consequence did not
  occur inside the strict time frame of the DiCOM® process.

- Draining the anaerobic liquor at the normal time (day 12 i.e. 7 days AD) does not
  appear to be sustainable.

- For sustainable pilot–scale operation, under test conditions, the DiCOM® process
  would require either:

  - A mechanism for VFA removal from the recylcate,
  - A longer AD phase and/or
  - Enhanced methanogenic activity during AD, possibly by an improved inoculum
    transfer.
9.1 Conclusions

In this study a model 2-vessel computer controlled DiCOM® reactor was constructed. This enabled, for the first time, to study the effect of the direct liquor transfer between two sister–reactors on a prolonged basis. During the course of this study, the specific electron flow rate was developed as an effective tool for comparing the rates of aerobic and anaerobic solids degradation. Popular belief suggests that aerobic processes are faster than anaerobic processes. However, analysis of electron flow rate demonstrated that, contra to this belief, anaerobic solids degradation rates can, in fact, be faster than those experienced during aerobic degradation.

The delivery of air for aerobic treatment of solid waste is a significant energy expense. Further, composting systems release CH₄, adding to the global warming effect. Hence, the common practice of composting, rather than digesting, solid waste containing easily degradable substrates, such as food waste, must be questioned.

The key sources of methanogenic inocula in a laboratory–scale DiCOM® system were identified as being the recyclate and the OFMSW itself. The predominant methanogens in this system were found to be *Methanosarcina thermophila*, an acetoclastic methanogen, and *Methanoculleus thermophilus*, a hydrogenotrophic methanogen. *M. thermophila* was mostly found attached to the solids while *M. thermophilus* proliferated in the anaerobic liquor. As a consequence, this study suggests that for maximum inoculum effect, not only liquor but also solids need to be transferred from one reactor to another. This can be accomplished by transferring anaerobic liquor and “pressate”
(solids) therefore providing an inoculum of both acetoclastic and hydrogenotrophic methanogens and highlights the importance of liquor transfer in DiCOM® operation.

Thermophilic methanogens, present within the waste, not only survived the initial aerobic treatment, but some species were found to grow. This means that concern over the methanogenic inoculum being eradicated by the aerobic treatment is not founded. At the same time, the possibility of dangerous co-existence of CH₄ and O₂ during the aerobic phase needs to be addressed. As methanogens are known to be O₂ sensitive further investigation is required to determine whether these methanogens are O₂ tolerant or that the overpressure provided during aeration does not provide efficient O₂ penetration into the solid matrix. It would also be of value to investigate whether the growth of methanogens during the initial aerobic phase of the DiCOM® is critical for optimal AD performance.

The current study has demonstrated that the direct reuse of anaerobic liquor improves the efficiency of the DiCOM® process. This is anticipated to be due to the transfer of active methanogens and/or microbially active species. However, the continual reuse of the anaerobic liquor was shown to be a liability to the system in that microbial metabolites, such as NH₃ and, in the case of a suboptimally operated system, propionate, can accumulate. It is anticipated that prolonged reuse will continue to improve process performance however it will also result in greater salts accumulation (e.g. Na⁺, K⁺, Zn²⁺ ...) which may adversely affect process performance. It is anticipated that salts will accumulate to reach an equilibrium between those entering on the organics and those exiting on the compost. It has not been within the scope of this thesis to investigate the effect of salts accumulation on process performance however this is seen to be an important topic for further research.
A particular salt of concern is sulphate. The accumulation of sulfates is anticipated and accordingly the production of H$_2$S from anaerobic microbial sulphate reduction is predictable. Since methanogens are known to be sensitive to H$_2$S further investigation into the impact of H$_2$S on the operation of DiCOM® is essential.

This study revealed, from modelling and experimental findings, that aerobic composting can be heat limited. For composting, the heat production, from rapid oxygen consumption during solids degradation, can lead to excessive heat production however AD, at similar or greater solid degradation rates, is not. The control of heat generated during initial composting was confirmed to be important for the rapid onset of methanogenesis. Overheating (>55°C) of OFMSW samples was found to provide a delay (≈ 1 day) in the onset of CH$_4$ production. As the AD phase of the DiCOM® process is short (7 days) any delay in the onset of methanogenesis will adversely affect sustainable operation.

Results from laboratory–scale were unable to be reproduced at pilot–scale. Limited aerobic activity was observed during the initial aeration phase, placing a greater organic input demand on the anaerobic phase leading to unsustainable VFA build-up in the liquor. It was concluded that for stable DiCOM® pilot–scale operation, a longer AD phase, enhanced methanogenic activity or a mechanism to remove VFA from the liquor would be required.

9.2 Further Research Needs

9.2.1 Anticipated Challenges for Full Scale Implementation

Excessive self–heating was predicted, and confirmed, during initial aerobic treatment at pilot–scale. It is anticipated that this elevated temperature was responsible for the
noticed disparity in the profiles of key methanogen present in pilot and laboratory–scale reactors, with methanogens having higher optimal temperatures being present (*Methanothermobacter* and *Methanobacterium* rather than *Methanoculleus spp.*) at pilot–scale. The fact that overheating can occur during the initial aeration phase, must raise concern regarding operation at commercial–scale. As the solid is a significant source of methanogenic inoculum, the preservation of the endogenous methanogens may be critical for stable and rapid AD. Consequently, further investigation into the effect of exposing material to high temperature and its effect on methanogenesis during DiCOM® processing is necessary. Furthermore, the question of whether the temperature of the organics can be controlled by decreased air flow without anaerobic conditions being established should also be addressed.

The incomplete AD observed at pilot–scale (Section 8.3.1), compared to laboratory-scale (Section 8.3.1), was likely due to the limited degradation observed during the initial aerobic phase. This creates a dilemma. Increased aerobic degradation would decrease the load of readily degradable organic matter in the AD phase (at the expense of CH₄ production) and would produce more heat during the aerobic phase, which may limit the survival of endogenous methanogens. The effect of excessive heat generation during the aerobic phase may be even more pronounced at commercial–scale where the reactor would have less heat loss. The solution may reside with the quality of the introduced anaerobic inoculum (Section 5.3.3).

At commercial scale the ratio of solids to liquid will increase from 0.6 to 1.6. Consequently, the rate of VFA accumulation and the buffer capacity required to stabilise the digestion, at commercial–scale, must be quantified. In addition, the increased solid to liquid ratio will augment the rate of salts accumulation, further
highlighting the need to investigate salt toxicity on process stability. Furthermore, the formulation of a synthetic liquor/recyclate may be critical for commercial DiCOM® operation, allowing for the replacement of anaerobic liquor should poisoning of the liquor occur.

A commercial DiCOM® facility will process 700 tonnes of OFMSW in a single vertical vessel. Material consolidation is likely to influence O₂ penetration into the solid matrix and the rate of aerobic degradation and associated microbial heat generation. There exists the possibility that, due to limited O₂ penetration, anaerobic regions could exist within the matrix resulting in the accumulation of fermentation intermediates and acidification of the biomass. During AD consolidation may influence liquid flow paths and the ability to maintain appropriate buffering throughout the reactor. Therefore, the effect of material consolidation on O₂ penetration and anaerobic liquor flow–paths, at commercial–scale, needs to be studied.

9.2.2 Possible Process Modifications

The quality of the pressate (suspended solid material obtained from mechanically squeezing the stabilised organics at the conclusion of the anaerobic phase of the DiCOM® process) may be a critical factor for sustainable DiCOM processing (Section 5.3.3 and 5.3.4) at commercial–scale. The anaerobic liquor at pilot-scale was found to contain less methanogens than laboratory samples. It is postulated that this is due to the greater consolidation of the organics providing a matrix to filter the micro–organisms from the liquor and trap them in the solids, a conjecture likely to be exacerbated at commercial–scale. The transfer of stabilised digested solids was found to decrease the time required for solids stabilisation. Consequently, in a commercial setting, inoculation of the anaerobic phase with viable methanogens could be improved by transferring solids from
a vessel completing AD to one commencing AD. Whether this could be achieved anaerobically is unknown and dependent upon reactor design and configuration. Alternatively, the transfer of pressate is less problematic, as it is a silty slurry of high solids content and could be more readily transferred (e.g. pumping). Transferring this slurry may enable sufficient solids transfer to provide an adequate inoculum.

Extending the length of AD phase during DiCOM® processing would provide a greater biogas yield and greater energy production. Pilot-scale results indicated that VFA exhaustion could be achieved within the reactor with extended digestion. In a commercial situation, an extension of the digestion period would require a minimum of two anaerobic liquors and a corresponding increase in the plant footprint. The advantage however would be that two vessels would produce biogas concurrently (Fig. 9.1: shaded region). Energy generation requires a minimum of 30% CH₄ in the biogas (personal communication AnaeCo Ltd.). Consequently, the biogas produced during the initial few days of batch AD may not sustain energy generation due to its low CH₄ content (Section 2.3.2 Fig. 2.4). When using two liquors, the overlap in biogas production occurs during the last few days of one digestion (Fig. 9.1 Reactor 1), where the biogas has a high CH₄ content, and the initial few days of another digestion (Fig. 9.1 Reactor 2), where the biogas is CH₄ deficient. Combining of these gas flows could provide a biogas rich enough in CH₄ to allow energy generation from a wasted biogas stream (from Reactor 2: days 12–15).

Omitting the initial aeration phase of the DiCOM® process may allow the process to be more sustainable. Pilot trials, when compared to an optimised laboratory trial, have demonstrated decreased microbial activity during the initial aerobic phase (Section 8.3.2 Fig. 8.6). To achieve high rates of degradation aerobically, biological heat generation
could, without significant heat loss, result in high temperatures appreciably limiting microbial activity. However, high rates of anaerobic degradation, equivalent to those experienced under aerobic conditions, have been shown to be possible (Chapter 6). If no initial aeration was applied, OFMSW, upon being introduced into the bioconversion vessel, could be submerged under anaerobic liquor. This would increase the time available for the exhaustion of fermentation intermediates and solids stabilisation, as suggested for sustainable pilot–scale operation (Section 8.3.1). This approach, however, requires OFMSW to be anaerobically introduced into the process vessel, which would increase mechanical design complexity.

![Figure 9.1: Timing of phases during operation of two hypothetical DiCOM® reactors using two (2) anaerobic liquors and an anaerobic phase length of 10 days (optimum for anaerobic laboratory trials: Refer to Section 6.3.2 Fig. 6.2). The shaded region (between days 12 and 15) indicates the period when both reactor would be producing biogas.](image)

Draining the anaerobic liquor from the reactor after 2 days of digestion may allow the processing time of DiCOM® to be decreased, and the process more energy self–sufficient. Laboratory results indicated that VFA accumulation within the anaerobic liquor reached a maximum after 2 days of anaerobic treatment. At this point VFA consumption exceeded production and VFA concentrations in the anaerobic liquor fell. It could be suggested that the bulk of the easily degradable organics have been solubilised during this 2 day period, and that by draining the liquor at this stage, the majority of the methanogenic potential of the solids has been captured within the liquor. The VFA laden liquor could be fed to a dedicated methanogenic reactor (acclimated to
high salt and NH₃) and the organic solids exposed to post–digestion aerobic maturation, providing a 14 day treatment cycle (5 days loading, 2 days AD followed by 7 days aerobic maturation).

The inclusion of a fourth bioconversion vessel and a 28 day batch treatment cycle may improve DiCOM® process performance and sustainable operation at larger–scale. Due to the strict timing regime of DiCOM®, the time available for AD is limited. Consequently, any delay in the onset of methanogenic activity must be avoided. It has been suggested in this study that for sustainable DiCOM® operation, at pilot–scale, the length of digestion be increased. By providing a fourth bioconversion vessel the processing cycle can be increased to 28 days (i.e. 5 days loading, 14 days AD followed by 7 days aerobic maturation and unloading). Under this configuration, considering the time interval between days 14 and 21 (Fig. 9.2: shaded region), Reactor 3 would be aerobic while being loaded; Reactors 1 and 2 would be anaerobic and Reactor 4 would be performing aerobic maturation of the solids contained within. During this same time period, Reactor 1, having been anaerobic for 7 days, would contain an established methanogenic culture, while Reactor 2 would contain an immature culture. Exchange of anaerobic liquor between these 2 reactors would decrease the acidification experienced by Reactor 2 and eliminate the delay in the onset of methanogenesis experienced in pilot trials.
Figure 9.2: Timing of phases during operation of four hypothetical DiCOM® reactors having an anaerobic phase length of 14 days providing a 28 day treatment cycle. The shaded region (between days 14 and 21) indicates the period when a mature reactor (Reactor 1) could exchange anaerobic liquor with an immature reactor (Reactor 2).

9.3 Possible Further Applications of the DiCOM Process

The embodiment of the DiCOM® process may be useful in providing raw materials for other industrial biological processes. The literature has indicated that organics treated under thermophilic conditions and low O_2 partial pressures can result in an accumulation of VFA within the solid matrix (McIntosh and Oleszkiewicz, 1997; Borowski and Szopa, 2007). If the initial aeration phase of the DiCOM® process was operated under these conditions, such that complete oxidation of substrate could not occur, the VFA produced may be able to be used as feedstock for other biological processes. These could include the production of polyhydroxybutyric acid (PHB) a biopolymer useful in the manufacture of bioplastics or the production of electricity via microbial fuel cells.

It is anticipated that the biogas produced during DiCOM® processing is derived primarily from easily degradable substrates (such as sugars). As cellulase production is suppressed in the presence of sugars, it can also be presumed that cellulase activity
during the DiCOM® processing is limited. Accordingly, compost produced via the DiCOM® process may have a high cellulose content. With cellulose being an important substrate for ethanol production, DiCOM® compost may be a viable substrate for the production of ethanol.

ABS – see Australian Bureau of Statistics.


AS – see Standards Australia

AS/NZS – see Standards Australia/Standards New Zealand


*Biocycle*, 31(10):50–51.


References


References


Appendix A

Temperature and pH Dependent Speciation of TAN

For the equilibrium between NH$_3$ and NH$_4^+$ ...

\[
NH_3 + H_2O \leftrightarrow NH_4^+ + OH^- \\

K_b = \left[\frac{[NH_4^+][OH^-]}{[NH_3]}\right]
\]

\[
[NH_3] = \left[\frac{[NH_4^+][OH^-]}{K_b}\right]
\]

but \([OH^-] = \frac{K_w}{[H^+]}\)

\[
\therefore [NH_3] = \frac{[NH_4^+] \cdot K_w}{[H^+] \cdot K_b}
\]

(Eq. A.1)

let \(f(NH_3) = \) Fraction of NH$_3$ nitrogen present as free NH$_3$

\[
f(NH_3) = \frac{n(NH_3)}{n(NH_3) + n(NH_4^+)}
\]

\[
f(NH_3) = \frac{n(NH_3)}{n(NH_3) + \frac{n(NH_4^+)}{n(NH_3)}}
\]

\[
f(NH_3) = \frac{1}{1 + \frac{n(NH_4^+)}{n(NH_3)}}
\]

\[
f(NH_3) = \frac{1}{1 + \frac{V}{n(NH_3)}}
\]

\[
f(NH_3) = \frac{1}{1 + \frac{[NH_4^+]}{[NH_3]}}
\]

(Eq. A.2)

Substitution Equation A.2 into Equation A.1 gives ...

\[
f(NH_3) = \frac{1}{1 + \left[\frac{[NH_4^+] \cdot K_w}{[H^+] \cdot K_b}\right]}
\]
\[
f(NH_3) = \frac{1}{1 + \frac{[NH_4^+]}{1} \left( \frac{[NH_4^+]}{[H^+] \cdot K_w} \right)}
\]
\[
f(NH_3) = \frac{1}{1 + \frac{[H^+] \cdot K_b}{1 \cdot [NH_4^+] \cdot K_w}}
\]
\[
f(NH_3) = \frac{1}{1 + \frac{[H^+] \cdot K_b}{1 \cdot K_w}}
\]
\[
f(NH_3) = \frac{1}{1 + \frac{[H^+] \cdot K_b}{[H^+] \cdot K_w}}
\]
\[
\text{now } [H^+] = 10^{-pH}
\]
\[
f(NH_3) = \frac{1}{10^{-pH} + \frac{K_b}{K_w}}
\]
\[
f(NH_3) = \frac{10^{pH}}{10^{pH} + \frac{K_b}{K_w}}
\]

Therefore …

\[
[NH_3]_{free} = \frac{[\text{Total } NH_3 \times \text{Nitrogen}] \times 10^{pH}}{10^{pH} + \frac{K_b}{K_w}} \quad \text{(Eq. A.3)}
\]

By definition \( \Delta G_w^o = -R.T.\ln(K_w) \)

\[
\therefore \ln(K_w) = \frac{-\Delta G_w^o}{R.T}
\]

\[
K_w = e^{\frac{-\Delta G_w^o}{R.T}}
\]

It therefore follows that …

\[
K_b = e^{\frac{-\Delta G_b^o}{R.T}}
\]
Converting temperature from degrees Kelvin to degrees Celsius gives...

\[
\frac{K_h}{K_w} = e^{\frac{-\Delta G_w^o}{R . T}}
\]

\[
\frac{K_h}{K_w} = e^{\frac{-\Delta G_w^o - \Delta G_b^o}{R . T}}
\]

\[
\frac{K_h}{K_w} = e^{\frac{\Delta G_b^o - \Delta G_w^o}{R . T}}
\]

Converting temperature from degrees Kelvin to degrees Celsius gives ...

\[
\frac{K_h}{K_w} = e^{\frac{\Delta G_w^o - \Delta G_b^o}{R . T(273 + T)}}
\]  \hspace{1cm} (Eq. A.4)

Substituting Equation A.4 into Equation A.3 gives ...

\[
f(NH_3) = \frac{10^{pH}}{10^{pH} + e^{\frac{\Delta G_w^o - \Delta G_b^o}{R . T(273 + T)}}}
\]

Therefore ...

\[
[NH_3]_{free} = \frac{[\text{Total NH}_3 \text{ Nitrogen}] \times 10^{pH}}{10^{pH} + e^{\frac{\Delta G_w^o - \Delta G_b^o}{R . T(273 + T)}}}
\]

Where \( \Delta G_w^o = 79.92 \text{ kJ/mol} \)

i.e. \( \Delta G_w^o = \Delta G_{f\text{OH}^o} + \Delta G_{f\text{H}^o} - \Delta G_{f\text{H}_2\text{O}^o} \)

\( \Delta G_w^o = -157.37 + 0 - (-237.29) * \)

\( = 79.92 \text{ kJ/mol} \)

\( \Delta G_b^o = \Delta G_{NH_3}^o = 27.10 \text{ kJ/mol} \)

i.e. \( \Delta G_{NH_3}^o = \Delta G_{f\text{NH}_3^o} + \Delta G_{f\text{OH}^o} - (\Delta G_{f\text{NH}_3^o} + \Delta G_{f\text{H}_2\text{O}^o}) \)

\( \Delta G_w^o = -79.41 + 157.37 - (-237.29 + 237.29) * \)

\( = 27.10 \text{ kJ/mol} \)

\( R = 8.315 \times 10^{-3} \text{ kJ/mol.K} \) (Nelson and Cox, 2000)

* Values taken from Thauer et al., (1977)
Table B.1: Raw data for the calculation of inhibition constant ($K_i$) for inhibition of acetoclastic methanogenesis by NH$_3$.

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<th>TAN Conc. (mM)</th>
<th>pH</th>
<th>Free-NH$_3$ Conc. (mM)</th>
<th>Elapsed Time (h)</th>
<th>Headspace Volume (mL)</th>
<th>Volume Over Pressure (mL)</th>
<th>Total Gas Volume (mL)</th>
<th>CH$_4$ (%)</th>
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<th>CH$_4$ Production Rate (mL/g/h)</th>
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Table B.2: Raw data for the calculation of inhibition constant (K_i) for inhibition of hydrogenotrophic methanogenesis by NH3.

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Appendix C

Temperature and pH Dependent Speciation of Propionate

For the equilibrium between CH₃CH₂COOH and CH₃CH₂COO⁻ …

\[
\text{CH}_3\text{CH}_2\text{COOH} \leftrightarrow \text{CH}_3\text{CH}_2\text{COO}^- + \text{H}^+ \\
K_a = \frac{[\text{CH}_3\text{CH}_2\text{COO}^-][\text{H}^+]}{[\text{CH}_3\text{CH}_2\text{COOH}]} 
\]

\[
[\text{CH}_3\text{CH}_2\text{COOH}] = \frac{[\text{CH}_3\text{CH}_2\text{COO}^-][\text{H}^+]}{K_a} \quad \text{(Eq. C.1)}
\]

let \(f(\text{CH}_3\text{CH}_2\text{COOH})\) = Fraction of \(\text{CH}_3\text{CH}_2\text{COOH}\) present

\[
f(\text{CH}_3\text{CH}_2\text{COOH}) = \frac{n(\text{CH}_3\text{CH}_2\text{COOH})}{n(\text{CH}_3\text{CH}_2\text{COOH}) + n(\text{CH}_3\text{CH}_2\text{COO}^-)}
\]

\[
f(\text{CH}_3\text{CH}_2\text{COOH}) = \frac{n(\text{CH}_3\text{CH}_2\text{COOH})}{n(\text{CH}_3\text{CH}_2\text{COOH}) + n(\text{CH}_3\text{CH}_2\text{COO}^-) + n(\text{CH}_3\text{CH}_2\text{COOH})}
\]

\[
f(\text{CH}_3\text{CH}_2\text{COOH}) = \frac{1}{1 + \frac{n(\text{CH}_3\text{CH}_2\text{COO}^-)}{n(\text{CH}_3\text{CH}_2\text{COOH})}} \quad \text{(Eq. C.2)}
\]

Substitution Equation C.2 into Equation C.1 gives …

\[
f(\text{CH}_3\text{CH}_2\text{COOH}) = \frac{1}{1 + \frac{[\text{CH}_3\text{CH}_2\text{COO}^-]}{[\text{CH}_3\text{CH}_2\text{COOH}][\text{H}^+][K_a]}}
\]
\[
f(\text{CH}_3\text{CH}_2\text{COOH}) = \frac{1}{1 + \frac{[\text{CH}_3\text{CH}_2\text{COO}^-]}{[\text{CH}_3\text{CH}_2\text{COO}^-][\text{H}^+]} \times \frac{K_a}{[\text{H}^+]}}
\]

\[
f(\text{CH}_3\text{CH}_2\text{COOH}) = \frac{1}{1 + \frac{K_a}{[\text{H}^+]}}
\]

now \([\text{H}^+] = 10^{-\text{pH}}\)

\[
f(\text{CH}_3\text{CH}_2\text{COOH}) = \frac{1}{1 + 10^{-\text{pH}}}
\]

\[
f(\text{CH}_3\text{CH}_2\text{COOH}) = \frac{1}{1 + 10^{\text{pH}} \times K_a}
\]  
(Eq. C.3)

Therefore …

\[
[\text{CH}_3\text{CH}_2\text{COOH}] = \frac{[\text{Total Propionate Species}]}{1 + 10^{\text{pH}} \times K_a}
\]

By definition \(\Delta G_{\text{CH}_3\text{CH}_2\text{COOH}}^\circ = -R.T.\ln(K_a)\)

\[
\therefore \ln(K_a) = \frac{-\Delta G_{\text{CH}_3\text{CH}_2\text{COOH}}^\circ}{R.T}
\]

\[
K_a = e^{-\frac{-\Delta G_{\text{CH}_3\text{CH}_2\text{COOH}}^\circ}{R.T}}
\]

Converting temperature From degrees Kelvin to degrees Celsius gives …

\[
K_a = e^{\frac{-\Delta G_{\text{CH}_3\text{CH}_2\text{COOH}}^\circ}{R(273+T)}}
\]  
(Eq. C.4)
Substituting Equation C.4 into Equation C.3 gives …

\[
f(CH_3CH_2COOH) = \frac{1}{1 + 10^{\text{pH}} \times e^{-\Delta G_{CH_3CH_2COOH}^o/R(273+T)}}
\]

Therefore …

\[
[CH_3CH_2COOH] = \frac{[\text{Total Propionate Species}]}{1 + 10^{\text{pH}} \times e^{-\Delta G_{CH_3CH_2COOH}^o/R(273+T)}}
\]

Where \( \Delta G_{CH_3CH_2COOH}^o = 27.80 \text{ kJ/mol} \) (Speight, 2005)

\( R = 8.315 \times 10^{-3} \text{ kJ/mol.K} \) (Nelson and Cox, 2000)
## Appendix D

### Location of Biogas Producing Organisms in DiCOM® Process

Table D.1: Biogas (an unspecified mixture of CH₄, CO₂ and H₂) generation from acetate (30mM) in serum vial tests during 24h incubation at 55°C of DiCOM® process components (recyclate, squeezeate, aerated solid and digested solid). The biogas generation rates (mL/h/Reactor) are based on typical laboratory data: Weight of OFMSW (2670g), Volume of Recyclate (4000mL) and Volume of Squeezeate (650mL).

<table>
<thead>
<tr>
<th>Vial Contents</th>
<th>Liquid Volume (mL)</th>
<th>Mass of Solid (g)</th>
<th>Gas Volume (mL)</th>
<th>Biogas Generation Rate (mL/h/Reactor)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressate</td>
<td>40</td>
<td>50</td>
<td>0.046</td>
<td>L/h/L</td>
</tr>
<tr>
<td>Recyclate</td>
<td>40</td>
<td>9</td>
<td>0.012</td>
<td>L/h/L</td>
</tr>
<tr>
<td>Digested OFMSW + DI H₂O</td>
<td>5</td>
<td>10</td>
<td>39</td>
<td>L/h/kg OFMSW</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.18</td>
<td>490</td>
</tr>
<tr>
<td>Digested OFMSW + Recyclate</td>
<td>5</td>
<td>10</td>
<td>58</td>
<td>L/h/kg OFMSW</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.24</td>
<td>630</td>
</tr>
<tr>
<td>Aerated OFMSW + DI H₂O</td>
<td>5</td>
<td>10</td>
<td>29</td>
<td>L/h/kg OFMSW</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.074</td>
<td>200</td>
</tr>
</tbody>
</table>
Table E.1: CH$_4$ (°CH$_4$ and ¹⁷CH$_4$) production from a DiCOM® reactor and during 5h incubation at 55°C of OFMSW and recycle samples, removed from a DiCOM® reactor during the anaerobic treatment phase. Samples, 5g OFMSW and 25mL anaerobic recycle, were removed daily during the 7 days of DiCOM® anaerobic treatment and transferred into 50mL serum vials, sealed, degassed with N$_2$/CO$_2$ (80/20%) and spiked with 10mM C–2 labelled acetate. Test vials (C2) were spiked with ¹³C–2 labelled acetate while the controls (C0) (to determine the background concentration of ¹³CH$_4$) were spiked with unlabelled acetate (Data used with permission – Dr. Wipa Charles).

<table>
<thead>
<tr>
<th>Day</th>
<th>Sample</th>
<th>CH$_4$ Abundance</th>
<th>CH$_4$ Peak Count</th>
<th>°CH$_4$/¹⁷CH$_4$ (%)</th>
<th>Adjusted °CH$_4$/¹⁷CH$_4$ (%)</th>
<th>Acetate Present (mM)</th>
<th>Acetate Added (mM)</th>
<th>Total Acetate (mM)</th>
<th>Fraction Labelled Acetate</th>
<th>Acetate Dilution Factor</th>
<th>CH$_4$ Produced (L/day)</th>
<th>°CH$_4$ Produced (L/day)</th>
<th>¹⁷CH$_4$ Produced (L/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>C0</td>
<td>230398</td>
<td>1954</td>
<td>0.85</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>C2</td>
<td>103499</td>
<td>1015</td>
<td>0.98</td>
<td>0.13</td>
<td>48.4</td>
<td>10</td>
<td>58.4</td>
<td>0.17</td>
<td>5.84</td>
<td>0.77</td>
<td>0.03</td>
<td>5.57</td>
</tr>
<tr>
<td>1</td>
<td>C0</td>
<td>453920</td>
<td>9023</td>
<td>1.11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>C2</td>
<td>169142</td>
<td>1920</td>
<td>1.14</td>
<td>0.03</td>
<td>80.5</td>
<td>10</td>
<td>90.5</td>
<td>0.11</td>
<td>9.05</td>
<td>0.26</td>
<td>16.8</td>
<td>1.50</td>
</tr>
<tr>
<td>2</td>
<td>C0</td>
<td>841496</td>
<td>9448</td>
<td>1.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>2</td>
<td>C2</td>
<td>553858</td>
<td>17006</td>
<td>3.07</td>
<td>1.9</td>
<td>89.9</td>
<td>10</td>
<td>99.9</td>
<td>0.10</td>
<td>9.99</td>
<td>19.45</td>
<td>13.0</td>
<td>2.72</td>
</tr>
<tr>
<td>3</td>
<td>C0</td>
<td>877368</td>
<td>9919</td>
<td>1.13</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>C2</td>
<td>1054453</td>
<td>95119</td>
<td>9.02</td>
<td>7.9</td>
<td>32.1</td>
<td>10</td>
<td>42.1</td>
<td>0.24</td>
<td>4.21</td>
<td>33.24</td>
<td>11.0</td>
<td>3.16</td>
</tr>
<tr>
<td>4</td>
<td>C0</td>
<td>85939</td>
<td>683</td>
<td>0.79</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>4</td>
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<td>93789</td>
<td>21422</td>
<td>22.84</td>
<td>22.1</td>
<td>11.6</td>
<td>10</td>
<td>21.6</td>
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<td>2.16</td>
<td>47.53</td>
<td>7.6</td>
<td>2.71</td>
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<td>716759</td>
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</tr>
<tr>
<td>5</td>
<td>C2</td>
<td>1020931</td>
<td>345024</td>
<td>33.80</td>
<td>32.6</td>
<td>9.4</td>
<td>10</td>
<td>19.4</td>
<td>0.52</td>
<td>1.94</td>
<td>63.21</td>
<td>4.5</td>
<td>1.68</td>
</tr>
<tr>
<td>6</td>
<td>C0</td>
<td>251479</td>
<td>5732</td>
<td>2.28</td>
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</tr>
<tr>
<td>6</td>
<td>C2</td>
<td>397345</td>
<td>141559</td>
<td>35.63</td>
<td>33.4</td>
<td>6.8</td>
<td>10</td>
<td>16.8</td>
<td>0.59</td>
<td>1.68</td>
<td>56.12</td>
<td>1.9</td>
<td>0.72</td>
</tr>
<tr>
<td>7</td>
<td>C0</td>
<td>642094</td>
<td>9779</td>
<td>1.52</td>
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<td></td>
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</tr>
<tr>
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<td>440872</td>
<td>17787</td>
<td>40.33</td>
<td>38.8</td>
<td>6.8</td>
<td>10</td>
<td>16.8</td>
<td>0.59</td>
<td>1.68</td>
<td>65.31</td>
<td>Total (L) 12.5</td>
<td>47.9</td>
</tr>
</tbody>
</table>
Appendix F

Equations Defining Safe Gas Mixtures
Around The Flammable Region For CH₄ in Air

Figure F.1: Equations defining a safe region (defined as “WAIT”) containing a non explosive gas mixture around the CH₄/O₂ flammable region. Solid lines and dashed lines indicate non-explosive compositions of CH₄/O₂ mixtures with a safety factor of 2 and 1.25, respectively.

Table F.1: Oxygen and methane concentrations of the extremities of the flammable region for CH₄ in O₂. (*AS/NZS 60079.20:2000; # interpolated data from AS/NZS 60079.20:2000)

<table>
<thead>
<tr>
<th>Oxygen Concentration (%)</th>
<th>Methane Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.1</td>
<td>17*</td>
</tr>
<tr>
<td>17.4</td>
<td>4.4*</td>
</tr>
<tr>
<td>11.2</td>
<td>4.4*</td>
</tr>
</tbody>
</table>
Publications


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I would like to take this opportunity to thank Dr. Ralf Cord–Ruwisch for his continued support, encouragement and creative ideas, which I have found of more assistance than words can describe. I would also like to thank him for his continued “nagging” to finish this thesis, it has been greatly appreciated.

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