

**RENEWABLE ENERGY EXTRACTION FROM ORGANIC WINERY WASTES
THROUGH ANAEROBIC TREATMENT**

by

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Abstract

The wine industry produces large amounts of liquid and solid organic wastes in the form of lees and pomace. The high concentration of organics, measured as chemical oxygen demand (COD) contained in these wastes complicates disposal or reuse, however, this same characteristic makes them ideal for anaerobic conversion to methane gas, a cleaner energy source than fossil fuels. The main objective of this research was to determine whether these wastes could be treated anaerobically to produce methane, and to isolate ideal conditions for conversion. Research activities included 1) a waste audit of a medium-sized winery to determine the type and quantities of organic waste produced, 2) characterization of the waste, 3) enrichment of anaerobic cultures, 4) a set of biochemical methane potential (BMP) tests to assess the impact of different waste combinations on methane yields, and 5) a second set of BMP tests to assess the impact on methane yields by different waste types at different food to microorganism ratios (F/M) and at different waste combinations.

The research yielded the following general results. 1) Two anaerobic cultures were successfully enriched to degrade lees and tartaric acid. 2) BMP testing showed the enriched cultures could be overwhelmed by an excess of lees, and pomace could be degraded to some extent ($\sim 30\%$ COD) but over long periods of time (> 100 days). Both wastes were successfully converted to methane but at different rates. Post-experiment analysis showed an F/M ratio of 5 might be ideal for methane conversion. 3) Another set of BMP tests revealed lees was converted to methane more efficiently at an F/M ratio of 4, yielding 0.25 ± 0.01 L CH_4/g COD consumed. An ideal F/M ratio was not identified for pomace. Co-treatment of lees and pomace at different combinations did not reveal an ideal mix as all three combinations tested were statistically similar in yields, an average of 0.13 ± 0.02 L CH_4/g COD consumed. It was found that pomace had inhibited methanogenesis in the co-treatment tests; however, further research is needed to determine the cause and extent of this inhibition.

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List of Abbreviations

COD	Chemical Oxygen Demand
TCOD	Total COD
SCOD	Soluble COD
BOD5	Biological Oxygen Demand, 5 day assay
TS	Total Solids
TSS	Total Suspended Solids
VS	Volatile Solids
VSS	Volatile Suspended Solids
VFA	Volatile Fatty Acids
F/M	Food to microorganism ratio (mass of COD added per mass VSS of inoculum)
RO	Reverse Osmosis
STP	Standard temperature and pressure

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For my son Evan

“Look with your eyes.

Hear with your ears.

Taste with your mouth.

Smell with your nose.

Feel with your skin.

Then comes the thinking, afterward,

And in that way knowing the truth.”

Syrio Forel - George R.R. Martin’s “A Game of Thrones”

Chapter 1 Introduction

This chapter provides an introduction to the BC wine industry and environmental issues related to the disposal of organic waste generated in the wine making process. This is followed by the project objectives and an outline of the thesis document structure.

1.1 Background

British Columbia (BC) has seen significant growth in the production of wine-grapes and quality wines in the last two decades, and is positioning itself as a competitive industry locally and abroad. According to the BC Grapegrowers Association, the number of grapes produced between 1999 and 2010 tripled, as did the number of licensed wineries between 2000 and 2009 (McIntyre 2010). Despite the global economic downturn, BC wineries are still expected to continue to grow and mature as an industry. Environmental issues have taken prominence in the public eye in the last decade and this appears to be having some influence on consumer purchasing decisions. The wine industry is not immune to this and many regions have worked toward implementing or influencing their producers to move toward more sustainable practices. Producers have focused on a range of practices such as reducing waste through improvement of the wine making process, extracting valuable marketable products from the wine processing waste, and reusing effluent and biosolids from the fermentation process (Andreottola et al. 2009).

The vast majority of BC wineries are concentrated in the Okanagan Valley, where the semi-arid climate and sandy soils are ideal for the production of high-quality grapes. However, the same environment also poses challenges, such as lack of precipitation and poor soil retention of moisture and nutrients, which results in the application of large volumes of water and fertilizer. Additionally, the high strength wastewater released during vintage activities puts a tremendous load on municipal wastewater treatment plants. Regulatory bodies in the Okanagan region have expressed their desire to regulate or make regulations more stringent for winery wastewater discharges in the future. There is no known implementation timeline in the region but a move toward more stringent regulation of this waste has been observed in

other more mature wine producing markets such as California, where biological treatment is more common (e.g., aerated lagoons).

In general, wineries tend to compost the organic waste they produce, and liquid waste is usually discharged into dry wells, ponds, sanitary sewers or septic fields (Arvanitoyannis et al. 2006a). A small number of wineries in the Okanagan region have implemented on-site biological treatment methods; however, there is ample room in the industry for customized, cost-effective and sustainable solutions. Large amounts of money and other resources are invested yearly to produce quality grapes to turn them into quality wines. Getting more out of this investment in the form of energy recovery is a logical step toward making winery operations more sustainable. Recovered energy may be used for space heating, powering vehicles, selling it to a utility company, etc. More sustainable operations would benefit the wineries economically, the community and the natural environment. Wide-spread energy recovery from waste would also be beneficial to power utilities and local governments, and the cumulative effect would be of direct economic benefit to Canada.

The author obtained support from Tinhorn Creek Vineyards in the form of waste samples, wine production data and valuable communications for data gathering. Tinhorn is an all-estate winery that produces wines from grapes grown on their 130 acres of vineyards in the Oliver region of BC. 500 tonnes of grapes are harvested each year, from which 315,000 L of wine are produced (35,000 cases) and approximately 100 tonnes of solid organic and 110,000 L of liquid organic waste are produced.

1.3 Objectives

The main objective of this research was to determine whether the liquid and solid waste streams from a winery could be treated anaerobically to produce energy in the form of methane, and to begin to understand the conditions at which this conversion is optimal.

To this end, the following activities were carried out:

- 1) Conducted waste audit of a medium-sized winery to determine the type and quantities of organic wastes produced.
- 2) Obtained, prepared and characterized the main wastes identified in the waste audit in preparation for enrichment.
- 3) Obtained and enriched anaerobic cultures (inocula) to enhance their ability of degrading winery waste.
- 4) Conducted a set of biochemical methane potential tests to assess the impact on methane yields by different waste combinations in proportions determined in the waste audit.
- 5) Conducted a second set of biochemical methane potential tests to assess the impact on methane yields by different waste types at different food to microorganism ratios (F/M) and different combinations of the wastes at a constant F/M ratio.

1.4 Thesis Structure

The thesis is divided into five chapters: Chapter 1 Introduction, Chapter 2 Literature Review, Chapter 3 Methods, Chapter 4 Results and Discussion, and Chapter 5 Conclusions and Recommendations. Chapter 1 introduces the reader to the BC wine industry and environmental issues related to the organic waste produced by wineries. Chapter 2 delves into the general characteristics of the waste and chemicals of importance, and the treatment of winery wastewater through anaerobic methods, leading to the reasoning behind the technology chosen for this research. Chapter 3 covers research and analytical methods employed in the development of this project. Chapter 4 presents and discusses the results obtained. Chapter 5 outlines the main conclusions derived from the results, limitations encountered and recommendations for future development of this research. Chapter 6 contains the list of references cited throughout the document and Chapter 7 contains the appendices with standard curves related to analytical methods used.

Chapter 2 Literature Review

This chapter is divided into two main sections providing information on wine production wastes and winery wastewater treatment methods. It includes a discussion on the seasonality, general characteristics, qualities specific to each of the three wine production wastes, and important parameters relevant to the treatment of this waste. It provides information on winery wastewater treatment, focusing on anaerobic treatment and its intricacies, and concludes with a section on the treatment technology used for this research.

2.1 Winery Organic Waste

Wineries vary greatly in the manner in which they produce their wines. This includes everything from cleaning agents and equipment used, wine-making techniques, filtration materials used, and the variety of wines produced. Similarly, waste production can differ greatly between regions and within regions. For instance, the Spanish wine industry produces six times the amount of wastewater that the Italian or French wine industries produce (Bustamante et al. 2005). No studies have been conducted estimating the wastewater volumes produced by the BC wine industry.

2.1.1 Waste Production

Most wineries operate on a seasonal basis, and a year can be divided into vintage and non-vintage. Vintage is a 6 to 20 week period that includes the harvest of grapes and the extraction and fermentation of the juice into wine. Stabilization of the wine, along with maturation, blending and bottling continue into the non-vintage season. During vintage, winery wastewater is of high-strength due to the high organic content from suspended particles such as pulp and dead yeast, and from dissolved contents such as sugar, ethanol and organic acids. Cleaning activities can also impact the wastewater contents with soap, detergent or other cleaning products (Chapman et al. 1995). Non-vintage is the rest of the year when wine is usually maturing but no new production is in progress.

The vast volumes of water produced come from cleaning/washing activities. During vintage, the rinse water mixes with the high strength components from the crushing and pressing of grapes, washing and rinsing of tanks and barrels, etc. During non-vintage the effluent is composed of mostly water but can carry some organics and cleaning agents such as soap, detergents and sterilization agents (Mosse et al. 2011). Most wineries sterilize tanks using caustic agents such as sodium hydroxide, however, some use sodium percarbonate, a peroxide-based cleaner used in a variety of environmentally-friendly cleaning products. When dissolved in water it yields hydrogen peroxide and sodium carbonate (soda ash). The hydrogen peroxide acts as the cleaning oxidizing agent (pH 10-11), which breaks down into water and oxygen after approximately 28 days (Jones 1999). Table 2-1 provides a breakdown of a season along with typical activities that may impact wastewater quality and quantity.

Table 2-1 - Wastewater generation from wineries broken down by vintage phases.

Stage	Description
Pre-vintage	Bottling, caustic washing of tanks, non-caustic washing of equipment in readiness for vintage.
Early vintage	Wastewater production rapidly rising to peak vintage flows and has reached 40% of the maximum weekly flow; vintage operations dominated by white wine production.
Peak vintage	Wastewater generation is at its peak; vintage operations are at a maximum.
Late vintage	Wastewater production has decreased to 40% of maximum weekly flow; vintage operations dominated by production of red wines; distillation of ethanol spirit may coincide with this period.
Post vintage	Pre-fermentation operations have ceased; effect of caustic cleaning etc. is at its greatest and wastewater quality may be poor.
Non-vintage	Wastewater generation is at its lowest - generally less than 30% of maximum weekly flows during vintage; wastewater quality is highly dependent on day-to-day activities.

Adapted from (Chapman 1996).

From a wine making perspective, the most valuable components end up in the finished product, however, a large portion of the original organic compounds go to waste in the form of pomace, lees and wasted wine; estimates range from 20-30% of the total production (Arvanitoyannis et al. 2006a). The combined liquid effluent (predominantly water from rinsing and washing activities mixed with lees and wasted wine) can be as high as 0.7 to 1.2 times the volume of finished wine produced in a given year (Andreottola et al. 2009; Vlyssides et al. 2010). Given the significant resources invested in producing wine in the first place, it is logical to attempt to recover valuable components from the waste.

2.1.2 Winery waste characteristics

Figure 2-1 shows the processes that produce the organic wastes of interest.

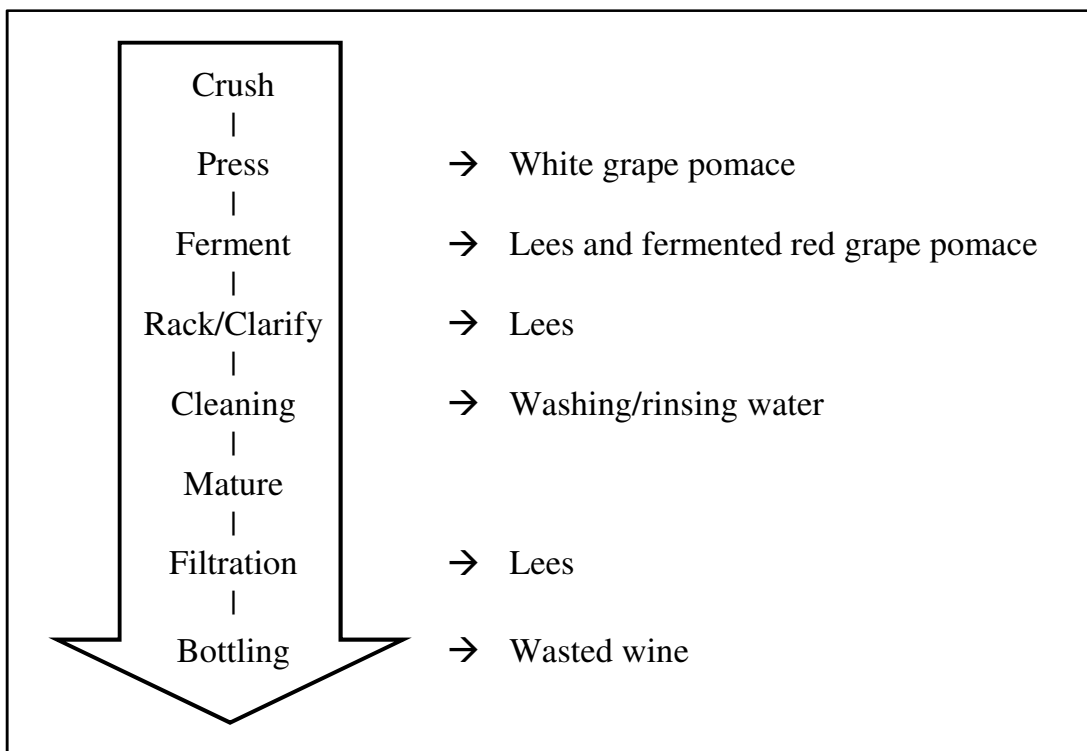


Figure 2-1 - Wine making processes and related wastes.

Table 2-2 lists the main parameters that make winery wastewater unfit for raw discharge or reuse.

Table 2-2 - General wine wastewater characteristics.

Parameter	Issue/ Concern
High Oxygen Demand	A measure of strength of waste and the oxygen requirement for microbial oxidation. High organic load from sugars, ethanol and organic acids. Depletes dissolved oxygen in aquatic environments due to degradation of organic compounds. Refer to section 2.1.3.1 for more information.
Settleable solids	Gross solids such as skins, stalks and seeds.
Suspended Solids (TSS)	High in suspended organic matter (dead yeast cells and dirt) can clog soil pores, and suffocate aquatic ecosystems.
Dissolved Solids (TDS)	High in dissolved organic matter (sugar, ethanol, organic acids).
Nitrogen and Phosphorus	High nutrient content, released through biodegradation. May pollute groundwater. Ammonia-N is toxic to fish. Nutrient overload may lead to eutrophication.
Sulphurous compounds	From disinfection and preservatives.
Salinity	Moderately saline due to cleaning agents (i.e., caustic soda)
Odours	Biodegradation of organics causes noxious odours.
Low pH	Organic acids found in pomace and lees contribute to the acidity. Harmful to most ecosystems that thrive at neutral levels (pH 7.0).

(Storm 1997;Malandra et al. 2003)

Lees and pomace were selected as waste substrates to test because of their significant contribution to the organic load. Wine was not deemed necessary to test separately as lees already contain a significant portion of wine. Lees are a liquid mixture of wine, dead yeast, bacteria, pulp, proteins, tannins and pectins; by-products of the fermentation process. A distinction must be emphasized between the terms *lees* and *wastewater*, which are not synonymous but related. The term ‘wastewater’ is used in the literature to refer to the mixture of lees and rinse waters; in some wineries, sewage is also part of this definition. In other

words, the high strength inherent to winery wastewaters is mainly due to the presence of lees. The bulk of the lees are discharged during the racking process, the process where the wine and lees mixture are allowed to settle and the clearer wine is decanted from the barrel. The remaining lees are washed away during barrel clean up. To a minimal extent they are also wasted after clarification, which is done to remove the turbidity produced by the remaining suspended lees, which is done mainly for aesthetic purposes (Chapman et al. 1995).

Pomace is mainly composed of the solid constituents of the grape: 50% stalks, 35% pulp, 15% seeds. The moisture content of pomace is highly variable depending on the pressing equipment used but has been reported in the literature as between 42% to 80% (wb) moisture (Mishra et al. 2008). Grape pomace also contains high concentrations of lignin and tannins, the latter of which are phenolic compounds and the main contributors to colour in the skin. During red wine production, the tannins diffuse from the skins into the wine during fermentation. There is a large variability in the concentration of phenolic compounds between cultivars and vintage, however, no significant differences were found between red and white grape varieties (Kammerer et al. 2004). Grape pomace is also a rich source of marketable products such as ethanol, tartrates, malates, citric acid, grape seed oil, fibre, and polyphenols (Arvanitoyannis et al. 2006a). During white wine production, the white grape pomace is discarded immediately after the press, while red grape pomace is fermented with the juice. White grape pomace therefore has a significantly higher concentration of sugars as it does not go through fermentation prior to disposal (Chapman et al. 1995).

At the start of this research in the spring of 2011, Tinhorn Creek opened a 65-seat Mediterranean style restaurant at its premises in Oliver, BC. The kitchen food waste generated is presently composted, however, being an organic waste produced by the winery, it was considered in the waste audit and in the first set of BMP tests.

2.1.3 Parameters of importance

The main parameters of importance for a biological treatment system are oxygen demand, solids, organic acids and any inhibiting compounds in the waste such as polyphenols in this case.

2.1.3.1 Oxygen demand

Wastewater strength is typically reported in units of biological oxygen demand (BOD) or chemical oxygen demand (COD), both of which are indicative of the amount of oxygen consumed by microorganisms through their growth, reproduction and decay. COD is a chemical oxidation test that uses chromic acid to oxidize all organic matter in the sample, biodegradable or not, which is why COD concentration is always higher than BOD concentration of the same sample. Inert inorganic substances present in a sample may also react with the acid, and may give elevated COD results. In short, BOD represents the readily aerobically biodegradable organic fraction in a sample while COD represents the total organic content, including biodegradable and inert non-biodegradable material. Therefore, COD is used where anaerobic systems are being studied. The range of maximum daily values of winery wastewater during vintage are 3,000 – 7,000 mg/L COD, 400-500 mg/L suspended solids and a pH of around 4 (Storm 1997). (Chapman et al. 2001) reports typical ranges in BOD concentration as 1,500 – 6,000 mg/L during vintage and 500 – 3,500 mg/L during non-vintage.

Discharge of high-strength wastewater into the environment can lead to the depletion of dissolved oxygen and to rapid growth of bacteria and algae, which can out-compete other organisms for oxygen, leading to a destabilization in the food chain and ultimately to eutrophication where vast volumes of algae suffocate other organisms and may turn a water body into an anoxic environment, killing aerobic organisms (Droste 1996;Atlas, R.,Bartha, R. 1998). Lees are the most problematic to dispose of, as the high levels of suspended organic material can clog soil pores, impairing hydraulic conductivity and limiting plant growth. The literature overwhelmingly agrees that lees and pomace are not suitable for untreated disposal, but are very suitable for biological treatment (Tofflemire 1972;Kammerer

et al. 2004;Andreottola et al. 2009;Bustamante et al. 2009;Mosse et al. 2011;Silva et al. 2011).

2.1.3.2 Solids

Solids are important parameters to monitor when assessing the quality of a wastewater. In treatment applications it is important to know the quality of the influent and effluent so removal efficiencies can be measured. The term “solids” is the overarching term referring to different types of solids within a sample. Comparing the concentrations of each provides valuable information that can help guide treatment process design and operation.

Total solids (TS) represent all matter in water, and are composed of volatile (VS) and fixed (FS) fractions of suspended (SS) and dissolved solids (DS) in an aqueous sample. VS are the fraction of the total solids that volatilize when incinerated at a temperature of 550 degrees Celsius. This measurement includes dissolved and suspended volatile solids. This measurement provides an approximation of total organic content of the sample. FS are the fraction of the total solids that do not volatilize and remain in the dish after incineration at 550 degrees Celsius. Total Suspended Solids (TSS) are composed of volatile (VSS) and fixed (FSS) fractions of suspended solids in an aqueous sample. VSS are the fraction of the suspended solids that volatilize when incinerated at a temperature of 550 degrees Celsius; it is a common estimate of biomass in a sample. FSS are the fraction of the suspended solids that do not volatilize and remain in the dish after incineration at 550 degrees Celsius, typically as left-over ash.

2.1.3.3 Organic acids

Organic acids are naturally found in high concentrations in the grape seeds and red grape skins, and tend to accumulate in the liquid fraction of the lees. The most significant organic acids are lactic, acetic, and malic and tartaric, the two most dominant species (Palma and Barroso 2002). Figure 2-2 presents the structural formula of the common acids.

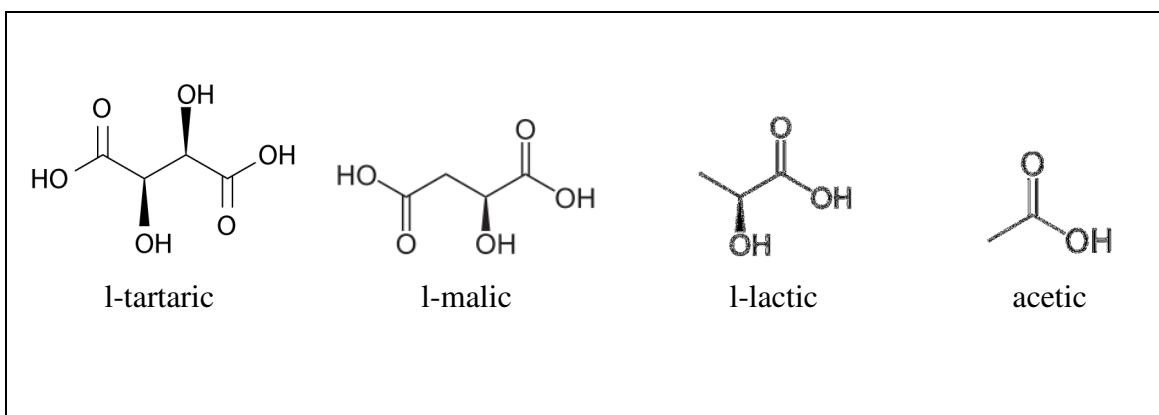


Figure 2-2 - Main organic acid components of winery wastewater.

Organic acids such as tartaric, malic, lactic and acetic are important as they affect the chemical and biological stability and consequently the flavour (tartness), colour and aroma of the wine derived from it. Determining the organic acid content is useful in determining the freshness of a grape juice, the maturation and acidity of a wine, and for establishing a juice “fingerprint” with which tampering can be detected (i.e., mixing of expensive juices with inferior product).

Tartrate species in lees have been reported at 100-150 kg per tonne of wine lees and 50-75 kg per tonne of pomace. These concentrations vary greatly with cultivation practices, climate, and production techniques (Yalcin et al. 2008). Tartaric acid is a valuable additive in the food industry and, from a food industry perspective, recovery from products such as pomace or lees is considered economical since its chemical production is costly (Dionex 2003). However, from a winery perspective this may be too costly of an endeavour as the transport costs may outweigh the financial benefit (Walsdorff et al. 2005).

Due to the importance of organic acids, many methods have been developed to determine their concentration in a variety of foods. These methods include spectrophotometric, enzymatic, and chromatographic methods (i.e., gas chromatography (GC), high performance liquid chromatography (HPLC) and ion chromatography (IC) (Masson 2000).

IC is useful in determining and separating organic acids in grape juice and wines. It works by separating ionic species in a sample using an ion-exchange process. Ionic species of different sizes and types interact with an ion-exchange resin at different rates. When the sample is moved through the resin, a staggered (or layered) separation is created. This is then passed through a column containing the solid phase that absorbs ions. An eluent is continuously passed through the column to move the ions, which are detected by the conductivity detector. IC with conductivity suppression is used in practice for routine analysis of organic acids in grape juice and wine and has been proven effective without the need of sample pre-treatment such as extraction and derivatization (Kupina et al. 1991; Dionex 2000).

2.1.3.4 Polyphenols

The phytotoxic and antibacterial properties in winery waste are principally contributed by polyphenols, which can impair biological treatment processes. Polyphenol concentrations in white and red wines have been reported at 280 and 1450 mg/L respectively. While not readily biodegradable, they can be degraded through anaerobic treatment processes (Mosse et al. 2011). Identification of polyphenols was not part of the scope of this research.

2.2 Winery Wastewater Treatment

Biological treatment processes have been utilized for the treatment of winery wastewater for decades. However, the application of these processes is a challenge given the variability in the timing, quantity and quality of the waste produced by a typical winery, which may lead to start-up failures or inefficiencies when going from periods of very low to very high wastewater production (Andreottola et al. 2005). The most common types of biological treatment can be divided into aerobic and anaerobic processes. Aerobic systems operate by encouraging the growth of aerobic microorganisms that degrade and consume organic compounds in the waste and convert it into biomass, carbon dioxide and water. Biomass can be separated from the effluent either by settling in complete mix suspended growth systems or immobilized in some form of media in attached-growth processes. Anaerobic systems work similarly to aerobic systems in that microorganisms consume organics and generate biomass, except in absence of oxygen. Without oxygen, anaerobic microorganisms resort to

anaerobic respiration, where carbonaceous material in the waste becomes the electron donor and carbon dioxide (CO_2) becomes the electron acceptor, and results in complete catabolism of the electron donors, many of which are fermentation products not previously utilized. In addition to carbon dioxide, methane, water and biomass are produced. Aerobes typically convert 50% of carbon in a substrate into biomass, while anaerobes only convert 5% of carbon in a substrate into biomass. Wasting of excess biomass adds to the cost of operating a treatment system; this is a key advantage that anaerobic systems have over aerobic (Droste 1996;Atlas, R.,Bartha, R. 1998).

Various processes for anaerobic treatment have been documented as feasible, albeit for larger wineries, but no instances were found during the literature review that combine the organic solid and liquid wastes with energy production as the main objective.

Table 2-3 - Summary of biological treatment systems reviewed.

Treatment System	References
Activated sludge	(Brucculeri et al. 2005;Fernandez et al. 2007)
Membrane bioreactor	(Artiga et al. 2007)
Sequencing batch reactor	(Torrijos and Moletta 1997)
Upflow anaerobic sludge blanket	(Keyser et al. 2003;Moletta 2005a;Akarsubasi et al. 2006;Andreottola et al. 2009;Diamantis and Aivasidis 2010)
Anaerobic digestion	(Daffonchio et al. 1998;Moletta 2005a)
Anaerobic sequencing batch reactor	(Edelmann et al. 1999;Ruiz et al. 2002;Angenent et al. 2004;Arvanitoyannis et al. 2006b;Donoso-Bravo et al. 2009)
Anaerobic filters	(Vijayaraghavan and Ramanujam 2000;Moletta 2005a)
Hybrid upflow sludge bed filter	(Molina et al. 2007)
Fluidized bed reactor	(Andreottola et al. 2005;Moletta 2005a)
Constructed wetland	(Mulidzi 2007)
Granular bed anaerobic baffled reactor	(Akunna and Clark 2000)
Hybrid anaerobic baffled reactor	(Boopathy and Tilche 1991)
Fixed bed biofilm	(Andreottola et al. 2005;Moletta 2005a)
Moving bed biofilm	(Andreottola et al. 2009)
Sequencing batch biofilm	(Andreottola et al. 2002)

The literature reports that winery wastewater is considered unbalanced for effective biological treatment, due to insufficient nitrogen and phosphorus content (Tofflemire 1972;Moletta 2005b;Silva et al. 2011). However, anaerobes require fewer nutrients than aerobes, and lees can provide sufficient nutrients given the right dilution.

2.2.1 Anaerobic Treatment

Anaerobic treatment has been in use for over a century, and has performed better than aerobic systems in treating high strength wastes as it requires less energy input, produces less biosolids for disposal, and allows for the recovery of energy through methanogenesis, however, this comes at a higher capital cost. Anaerobic treatment systems can be customized to meet desired outcomes such as producing higher quality solids, optimizing biogas capture and distribution, or reducing offensive odours. Methane production through anaerobic treatment of industrial and agricultural wastewaters is a mature technology being used in

large-scale facilities worldwide. However, anaerobic metabolism is a complex process involving many different microorganisms that interact in many ways that are still not fully understood. The inherent complexity of operating these systems often leads to inefficiencies and system failures that can lead to avoidance of such systems (Steyer et al. 2006). Therefore bioreactor design, configuration and operation need to be customized and optimized for the specific industry (Angenent et al. 2004).

Despite their complexity and intricacies in operation, anaerobic treatment systems are predicted to become more important in the future, due to the following advantages, as sustainable waste management becomes more mainstream (Edelmann et al. 1999):

- 80-90% of the biochemical energy can be recovered as biogas.
- Methane combustion produces less air pollution as it burns cleaner than fossil fuels.
- The methane generated during the process is most often recycled to heat the reactor or for electricity generation through a boiler, and has in some cases proven to be more than sufficient to run the reactor (Tofflemire 1972; Angenent et al. 2004). This can lower energy costs and reduce reliance on fossil fuels.
- Methane has a global warming potential (GWP) between 21 and 25 times that of carbon dioxide (DiStefano and Belenky 2009). However, when used as a fuel and all life cycle steps are considered, the shift from fossil fuels to bio-methane translates into reductions of carbon dioxide emissions as high as 65-85%. However, these figures are largely dependent on the original waste substrate used to produce the methane (Linné and Jonsson 2004).
- Methane tends to have better combustion properties than conventional fuels making it less noisy and energy efficient (i.e., better mileage) (Rutz and Janssen 2008).
- Proven effective in removing 90% or more COD and 80-90% removal of BOD from high-strength organic wastewaters.
- Less biomass wasting required as anaerobic systems produce less than comparable aerobic systems.
- Production of fertilizer from composting of biomass, reducing reliance on synthetic fertilizers.

- Anaerobic process consumes less energy compared to aerobic systems that require aeration.

Some of the disadvantages of anaerobic treatment systems include:

- Slow start-up phase if inoculum (microorganisms) are not acclimatized to the waste to be treated.
- Can be sensitive to fluctuations in environmental conditions (i.e., temperature, pH, etc.).
- The most efficient anaerobic systems operate at thermophilic temperatures (45-65 degrees Celsius), which require a significant input of energy to maintain adequate conditions. However, mesophilic bioreactors can be operated at lower temperatures (20-40 degrees Celsius) requiring less energy.
- Biogas may need to be scrubbed of trace sulphur compounds to avoid odours and to produce a cleaner burning fuel. This adds to the potential costs. (Pant and Adholeya 2007).
- Can be costly to build, construct and maintain. Monitoring and modelling may be needed for optimization.
- Post-treatment of the effluent is typical following anaerobic treatment, though this depends on actual effluent quality objectives (Poggi-Varaldo et al. 1999).

2.2.1.1 Methanogenesis

Methanogenesis is a metabolic process consisting of four main steps carried out by different groups of anaerobic microorganisms. The process begins with hydrolytic facultative bacteria breaking down insoluble organic molecules such as proteins, lipids and carbohydrates into their basic components: amino acids, fatty acids, glycerol and sugars. These sugars and amino acids are then fermented into hydrogen, ammonia, carbon dioxide and organic acids, including volatile fatty acids (VFA). Acetogenic bacteria then use carbon dioxide and organic acids to produce acetic acid, more hydrogen, ammonia and carbon dioxide. Lastly, methanogens convert the hydrogen, acetic acid, and a portion of the carbon dioxide into methane. The resulting products are methane, carbon dioxide, biomass from bacterial growth

and water. In short, carbon from the waste substrate is the electron donor and carbon dioxide is the electron acceptor. The total gas (or biogas) produced from methanogenesis is a mixture of methane, carbon dioxide and trace sulphuric compounds and hydrogen. While the ratio of gases produced varies depending on the waste consumed, the methane content typically ranges between 50% and 70% of the total gas (Converti et al. 1990).

The literature provides much information on empirical data often used to estimate operational parameters of biological treatment processes. While helpful, these data is often based on municipal sewage and large scale treatment plants. Given the heterogeneity of winery waste quality in a given year, and the variability in waste produced between wineries, it is clear that the data presented in the literature is useful only to an extent and therefore, biodegradation potential tests must be run to better understand the intricacies of anaerobic treatment of winery waste substrates for the purpose of producing energy in the form of methane.

Many anaerobic biodegradation potential tests were found in the literature, all involving closed-system incubation of an anaerobic culture and a substrate in a bottle or tube. All relied on the theory that the rate of COD removal is proportional to the rate of methane production. Substrate degradation is determined by either monitoring the concentration of substrate or the generation of an end-product such as methane gas. All these assays involved the use of control blanks, incubated and monitored with the rest of the bottles, but containing only inoculum. These help quantify biological activity contributed by endogenous respiration or presence of other substrates in the inoculum (Converti et al. 1990).

2.2.1.2 Factors affecting methanogenesis

A number of key parameters are typically monitored to better understand the health of an anaerobic treatment system; these are discussed in the following sub-sections.

2.2.1.2.1 Volatile Fatty Acids

VFAs are the most important intermediates in anaerobic treatment processes. The four principal VFAs monitored as indicators of anaerobic treatment performance are acetate,

propionate, butyrate and valerate; acetate and propionate being the two most dominant ones. A high concentration of VFAs in treated effluent is indicative of accumulation of these intermediate compounds, which can inhibit methanogenesis directly and indirectly. The direct method of inhibition is through toxicity exerted by components such as propionate, which are known to be toxic to some methanogen species at concentrations over 1,000 mg/L (Hobson and Shaw 1976). The indirect route is through the acidification of the environment as the VFA accumulation leads to the consumption of available alkalinity and an eventual drop in pH below the range preferred by methanogens, which decreases the rate of methane production (Pind et al. 2003).

2.2.1.2.2 Alkalinity

Excessive accumulation of carbon dioxide in the headspace (due to desired methanogenic activity) can lead to its solubilization and an increase in acidity; unbalanced conditions can also prevent methanogens from consuming volatile fatty acids produced by acetogens, leading to an accumulation of acids that can negatively impact the methanogenesis. The ideal alkalinity range for anaerobes is reported as being as between 2,000 and 18,000 mg CaCO_3/L (Alvarez et al. 2010).

2.2.1.2.3 Ammonia-N

Ammonia nitrogen is a vital component in an ideal environment for methanogens. Concentrations between 500 mg/L (very low) and 4000 mg/L (very high) can impact methanogens negatively, leading to low biomass and low methane yields. The negative effects at the low concentrations are mainly due to nutrient insufficiency or low buffering capacity and the high concentrations become toxic. The anaerobic metabolism of wastes containing high concentrations of solids has been shown to produce ammonia nitrogen in concentrations that can inhibit optimal reactor operation (Hadj et al. 2009).

It is reported that 200 mg/L of ammonia nitrogen as a safe concentration for methanogens. Additionally, levels of 1,100 mg/L caused partial inhibition of acetoclastic methanogenesis and concentrations of 8,000-12,000 mg/L caused total inhibition. The variability in the total

inhibition range was due to pH and type of biomass in question (Sung and Liu 2003). (Debaere et al. 1984) reported that levels of ammonia nitrogen over 4,000 mg/L were toxic and inhibited methanogenesis. One of the most sensitive known methanogens, *Methanosaeta concilii*, prefers an environment with an ammonia nitrogen concentration in the range of 250-1,100 mg NH₄-N /L (Steinhaus et al. 2007). Interestingly enough, high ammonia-N concentrations have been shown to prevent methanogenic inhibition where there is an accumulation of VFAs (> 160 mmol/L) (Procházka et al. 2012). The presence of nutrients in the effluent can be problematic if it is to be used for irrigation or discharged into a water body after treatment. For instance, excessive nitrogen and phosphorus content can lead to eutrophication of water bodies (if discharged) or soil nutrient imbalances (if used for irrigation).

2.2.1.2.4 Substrate co-treatment

Co-treatment is when two or more wastes are mixed to improve economic viability by treating both in one system, while hopefully enhancing the organic removal efficiency of the system. Wastes are typically chosen to complement each other to reduce limiting factors (i.e., nutrients). While the combination may prove beneficial, it could also introduce inhibiting substances; therefore empirical data is needed when treating two or more wastes in the same system (Mata-Alvarez et al. 2000). This is explored in the second set of BMP tests (BMP2) where co-treatment of lees and pomace is evaluated.

2.2.2 Anaerobic Sequencing Batch Reactor

The variable nature of winery wastewater means there is no single cost-effective option that would suit all wineries (Andreottola et al. 2009). Up-flow Anaerobic Sludge Blanket (UASB) technology is widely reported in the literature as effective in treating liquid winery waste but efficiency declines when treating substrates with high suspended solids, as would be expected when combining lees and pomace. As a solution to this problem, anaerobic sequencing batch reactors (ASBR) were developed to handle wastewaters high in suspended solids (Angenent et al. 2004). Additionally, ASBR is considered cost-effective when comparing its life cycle to those of other disposal options such as incineration, pyrolysis,

composting and even thermophilic anaerobic digestion, mainly because of the energy production potential (Edelmann et al. 1999; Arvanitoyannis et al. 2006b).

ASBRs are single or multiple vessel reactors that operate in cycles composed of four distinct steps: fill, react, settle and decant. During the filling stage, a reactor pre-seeded with biomass is filled with the influent to be treated at the desired F/M ratio. The reaction stage involves mixing of the reactor contents to improve mass transfer kinetics and allow the microorganisms in the biomass to interact with the substrate, thereby improving biodegradation efficiency. The goal is to sustain the reaction stage as long as necessary to decrease the concentration of pollutant in the effluent to acceptable levels; referred to as hydraulic retention time (HRT) in the wastewater industry. After the HRT has passed, the mixing is stopped and the contents of the reactor are allowed to settle. The microorganisms in the biomass forms flocs as they reproduce during the reaction stage, and these flocs tend to settle. A distinct biomass layer forms at the bottom of the reactor after enough settling time has passed, the clearer phase on top is the treated effluent (also called supernatant). The supernatant can then be decanted during the final stage and the cycle can be started again. Excess biomass generated as a result of the treatment process can be wasted during an additional idle stage when needed. The average amount of time the biomass stays in the reactor is the solids retention time (SRT).

ASBR was the main treatment technology studied in this project for the following reasons:

- The ability to treat wastewater in batches mitigates the variability in quality and quantity of winery wastewater over time, as cycle timing can be adjusted to deal with current fluctuations.
- The removal of soluble and non-soluble compounds in winery wastewater requires different solids and hydraulic retention times (SRT and HRT). SRT and HRT can be run independently of each other in an ASBR system.
- ASBRs have been shown to treat more volume of a soluble substrate per unit time than other more conventional treatment methods (Ndegwa et al. 2005).

- The operational flexibility of an ASBR has been credited in the effective treatment of phenolic wastewaters, and wine grape waste products are known to contain phenols (Donoso-Bravo et al. 2009).
- An ASBR operating at mesophilic temperatures (20-45 degrees Celsius) effectively reduced organic content of wastewater by as much as 98% (soluble COD). The lower end of the mesophilic range includes ambient temperature (~ 20 degrees Celsius), which requires less energy to run when compared to thermophilic processes (45-70 degrees Celsius) which require heating of the reactor.

ASBR is a proven technology but efficiency is dependent on design and operations customized for the specific application and desired outcome. Efficiency is largely affected by mixing during the reaction stage, food to microorganism ratio (F/M), geometric configuration, and feeding strategy. The effect of F/M ratio on performance of anaerobic treatment is one of the items explored in this research. Winery waste is not homogeneous in composition, therefore design criteria for treatment systems has to be based on empirical evidence with a measure of contingency based on experience. Full scale reactor design must be approached in stages, from laboratory testing to bench scale reactors, to pilot scale, and eventually to full scale. This research was the first step in obtaining empirical evidence about these particular wastes; the following chapter discusses the methods employed to this end.

Chapter 3 Methods

This chapter presents the methods used in various stages of this research, including the waste audit, inoculum culture enrichment, biochemical methane potential test setup and methane gas determination, as well as general analytical methods employed throughout.

3.1 Waste Audit

A waste audit of Tinhorn Creek's winery was conducted in April 2011 focusing on organic wastes created from the production of wine that could potentially be treated in an anaerobic treatment system. The audit consisted of data gathering about the wastes produced, which included a site visit in April 2011, a review of wine production logs and water metering records from 2009-2010, and restaurant serving projections for 2011.

3.2 Culture Enrichment

In order to conduct biochemical methane potential (BMP) tests, an acclimatized inoculum is required that can degrade the substrate to be tested. Enrichment minimizes the lag time during BMP tests, which is the time taken by bacteria to overcome toxicity and activate the relevant enzyme systems for the exploitation of the substrate in the environment. Constant feeding and maintaining optimal conditions for methanogens encourages the proliferation of the right mix of microorganisms.

Inoculum was provided by Tantalus Vineyards in Kelowna, BC, from their sequencing batch reactor that treats winery wastewater and domestic sewage. Spike feedings included waste substrate, nutrient medium and buffer. They were fed three times a week. The treated effluent was decanted every seven days and replaced with fresh waste substrate and nutrient medium at the time of feeding. Two cultures were enriched in separate bench scale ASBRs (W1 and W2). W1 was fed red wine lees (Cabernet Sauvignon in 2011 and Merlot in 2012) from Tinhorn Creek's barrel washing operations, at a concentration of 3100 mg COD/L for the first 214 days, and 2800 mg COD/L for the remainder of the enrichment. The strength of winery wastewater containing lees is highly dependent on the winery, the concentrations

used here were within the COD concentrations found in the literature. Reactor W2 was fed tartaric acid at a concentration of 600 mg TA/L (days 0 – 250) and 660 mg TA/L (days 250-300). The typical tartaric acid concentration of winery wastewater is between 350 mg/L (non-vintage) and 530 mg/L (vintage) (Chapman et al. 1995). Both reactors were enriched and monitored regularly over several months in the Environmental Engineering Laboratory at UBC Okanagan. Treated effluent samples were collected on a regular basis and tested for COD, TS, VS, TSS, VSS, pH and alkalinity, and ammonia-N and nitrate. Figure 3-1 presents the reactor and gas collection configuration.

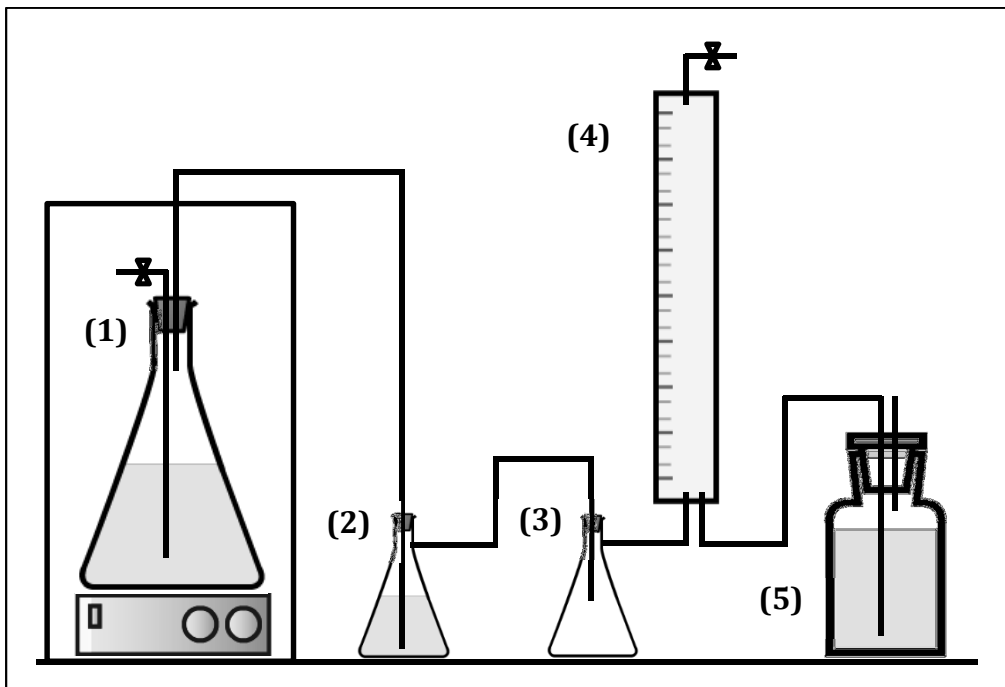


Figure 3-1 - Apparatus setup for each enrichment culture.

The components in Figure 3-1 are described below:

- 1) Each reactor consisted of a liquid fraction of 1.5 L. They were continuously mixed using a stirring plate set to 350 RPM to improve mass transfer during the reaction stage, and were kept at room temperature (at approximately 22 degrees Celsius).
- 2) A sodium hydroxide trap (1 N NaOH) was used to precipitate carbon dioxide as bicarbonate and allow only methane to pass through.

- 3) Another trap was placed to prevent any unexpected water from the manometer to back up into the sodium hydroxide trap.
- 4) A manometer filled with water was used to monitor the methane gas produced by the culture. As methane entered the manometer from below, water was displaced into the bottle (5).

Nutrient medium was supplied to both reactors during feedings to ensure the right balance of nutrients was present for the healthy development of the anaerobic methanogenic culture. The medium was prepared in accordance to the recipe proposed by (Herman and Roberts 2005).

The macronutrient solution consisted of the following:

- 50 g/L NaCl
- 50 g/L NH_4Cl
- 50 g/L $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$
- 50 g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$

The micronutrient solution consisted of:

- 10 g/L $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$
- 0.1 g/L $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$
- 0.3 g/L H_3BO_3
- 1.5 g/L $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$
- 10 g/L $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$
- 0.03 g/L $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$
- 0.03 g/L $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$
- 0.1 g/L $\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$

The medium consisted of 1 mL macronutrient solution, 0.1 mL micronutrient solution, 1 mL phosphate solution (50g/L KH_2PO_4), and 1 mL resazurin solution (0.01%) for every 100 mL of culture. Preparation involved boiling of all the ingredients at approximately 390 degrees Celsius for three minutes to expel dissolved oxygen from solution. After boiling, the solution

was cooled over ice and the headspace flushed with 99.999% nitrogen to prevent oxygen intrusion. Alkalinity was added in the form of 0.57 g sodium bicarbonate for every 100 mL of culture to prevent acidification of the culture due to the acidic substrate added or from potential VFA accumulation.

Resazurin was added in marginal volumes to serve as a redox indicator. It is blue in colour in its original form and turns to pink and then colourless as it is reduced. Chloride salts were used to prepare the media as opposed to sulfate salts, to prevent the use of sulfate as an electron acceptor and the subsequent production of hydrogen sulfide gas. This ensured anaerobes utilized carbonaceous material in the waste as the electron donor.

3.3 Biochemical methane potential tests

The method chosen for assessing the methane production potential of cultures was the biochemical methane potential test (BMP). BMP is an assay used to evaluate the biodegradation of a given substrate by an anaerobic inoculum culture. By monitoring the products of anaerobic metabolism (principally total gas and methane) it can be used to determine the methane production potential of the substrate by the anaerobic inoculum. Different batches representing different conditions can be set up, which can help identify ideal conditions for the conversion of the substrate to methane. BMP benefits include minimal cost of set-up, ease of repeatability, ease of monitoring, and reliability in comparing the capabilities of an inoculum in degrading various substrates (Owen et al. 1979;Moody et al. 2009) . As previously mentioned, BMP assays require an enriched inoculum that has been acclimatized to the actual or similar substrate to be tested. If the inoculum is not acclimatized, the BMP test tends to yield conservative results. The purpose of the assay is to obtain a measurable but not excessive rate of methane production during the experimental period.

Anaerobic technique was used during media preparation and inoculation of all BMP serum bottles to avoid the introduction of oxygen. Serum bottles (158.4mL actual volume) were sealed with blue butyl rubber stoppers secured with aluminum crimp seals. The liquid phase (inoculum, waste substrate and nutrient medium) took up 75 mL of the total volume in each

bottle. The control blanks consisted of inoculum in the same concentration as all the bottles with the balance being composed of nutrient media. The water used for all tests was Type 1 Pure Water, unless otherwise indicated. For the BMP tests the alkalinity was set to 2,000 mg/L for BMP1 and 2,670 mg/L for BMP2, both as CaCO_3 . These concentrations were at the lower end of the range reported in the literature but were proven to be more than adequate given observations from the culture enrichment process.

For both sets of BMP tests, pomace was homogenized as much as possible by mincing it in water in a commercial blender to create a workable suspension. This was done to minimize the variability in substrate composition used in the experiments. BMP tests are typically run for 20 to 30 days when soluble substrates are tested. Logically, substrates that are not as readily soluble take longer to degrade, and the test should be run for an extended period of time. In this case, pomace was not as readily soluble as lees; therefore BMP1 test was run for 100 days and BMP2 for 90 days. Regular monitoring of all BMP bottles was done throughout each set of experiments as per the methane determination method outlined in Section 3.3.3 .

3.3.1 BMP1

A composite inoculum was prepared consisting of 50% biomass from culture W1 and 50% biomass from culture W2 as VSS (mg/L). It was prepared by centrifuging well mixed samples from each enrichment culture and adding the corresponding mass of solids from each centrifuged sample to the composite. The balance of the volume was made up of nutrient media. The lees used for this set of BMP tests was from the production of 2010 Cabernet Franc wine. The assay was started in the spring of 2011, a time of year when fresh pomace was not available; therefore a synthetic pomace composite was prepared as per the professional recommendation of Winery Consultant Gary Strachan. The pomace composite consisted of 60% red Cabernet Sauvignon pomace and 40% synthetic white grape pomace, which consisted of 90% red Cabernet Sauvignon pomace and 10% white sultana grape raisins to approximate the higher sugar content found in white grape pomace. The 60/40 split was done to mimic Tinhorn Creek's production of 60% red pomace and 40% white pomace in a typical year.

As previously mentioned, the kitchen food waste generated at Tinhorn Creek's restaurant would be eligible for biodegradation in an anaerobic treatment system, following pre-treatment by way of food grinding system. The rate of kitchen food waste biodegradation is heavily dependent on its macronutrient components, which are proteins, lipids, carbohydrates and cellulose. Its heterogeneous nature leads to significant fluctuations in methane production rates, therefore similarly to pomace, it must be homogenized first. For this research study, dog food was used as a synthetic substitute as it contains all the macronutrients typically found in actual kitchen food waste. Advantages of using canned dog food included storage durability, ease of use, homogeneity (paste), and macronutrient composition. Its use as a synthetic alternative to kitchen food waste is heavily supported by literature on biogas production testing, and substrate biodegradability (VanderGheynst et al. 1997; Chang et al. 2006; Neves et al. 2008; Hadj et al. 2009) The Miradoro Restaurant had very recently opened at the time of this study and there were no historical records of waste production to review. Therefore, an estimate of food waste per person served was calculated using a figure of 0.48 kg of food waste per person served, which was derived from a restaurant food-waste survey conducted by the Sustainable Restaurant Association in London, England (SRA 2010). Similar reports for North American restaurants were not found during the literature review.

The waste audit results were broken down by month of a typical year and five months were selected as representative examples of how waste composition can sharply change in a given year. The representative months were May, July, September, November and December, times when the respective predominant wastes are lees, food, pomace, a combination of lees and pomace, and lees again. While some of the predominant wastes repeat, their concentrations are very different. Actual volumes and concentrations, including the composition of the BMP samples tested are shown Table 3-4.

Table 3-4 - Volume composition of BMP1 batch bottles.

Batch ID	Represents	Inoculum (mL)	Food (mL)	Lees (mL)	Pomace (mL)	Medium (mL)	Total (mL)
Food	July	10	1	0	0	64	75
Lees	December	10	0	22	0	43	75
Pomace	September	10	1	0	11	53	75
LP	November	10	0	7	6	52	75
FL	May	10	1	10	0	54	75

Each batch was composed of 5 replicate bottles for a total of 30 bottles including the control blanks. These were incubated in a dark rotary shaker incubator at 20 degrees Celsius and mixed constantly at 110 RPM for the duration of the test. Results are corrected for blanks.

3.3.2 BMP2

The second phase of BMP testing involved the feeding enriched inoculum lees at different F/M ratios, pomace at different F/M ratios, and three combinations of lees and pomace at a constant F/M ratio. Anaerobic technique was used to prepare all bottles for this test.

Sampling for testing of pH, alkalinity and organic acids (VFA and tartaric acid) required sampling of the liquid fraction from each bottle though it was important to avoid disturbing the cultures as much as possible. As a precaution the liquid fraction was not changed from 75 mL by more than 5% in total. For this reason, during each sampling, small volumes (0.5 mL) were extracted from each bottle and a composite was prepared of each batch of F/M ratios.

Table 3-5 - Composition of each bottle in batches fed lees.

Batch	F/M ratio	Lees (mg/L)	Inoculum (mg/L)
L2	2.2	1,405	650
L4	4.3	2,810	650
L6	6.5	4,215	650
L8	8.7	5,620	650

Twenty four grams (24 g) of fresh merlot pomace were blended with 650 mL water purified by reverse osmosis to make a suspension of 37 g/L of pomace.

Table 3-6 - Composition of each bottle in batches fed pomace.

Batch	F/M ratio	Pomace (mg/L)	Inoculum (mg/L)
P3	3.1	1,977	650
P4	4.1	2,636	650
P5	5.2	3,348	650
P6	6.2	4,007	650

For BMP2, the ranges of F/M ratios tested were derived from the results of BMP1. In theory, the F/M ratio should not affect the final volume of methane produced per unit of degradable COD, but it will change the rate at which it is produced, which is a very important factor in determining the feasibility of any biological treatment system. This assumption, however, does not take into account the presence of inhibitory substances that may have an effect at certain concentrations. Experimental testing of multiple F/M ratios helps shed light on inhibition or imbalances in the system.

Table 3-7 - Composition of each bottle in batches fed lees and pomace.

Batch	F/M ratio	Lees (mg/L)	Pomace (mg/L)	Inoculum (mg/L)	Composition of substrate (by COD)
L25P75	5.2	843	2,504	650	25% lees, 75% pomace
L50P50	5.2	1,686	1,661	650	50% lees, 50% pomace
L75P25	5.2	2,529	817	650	75% lees, 25% pomace

The inoculum composite prepared for this set of tests consisted of 75% of W1 biomass and 25% of W2 biomass as VSS (mg/L). The lees used for this set of tests were from the production of 2012 Merlot wine. Each batch in the BMP2 set of tests was composed of 4 replicate bottles for a total of 48 bottles including the control blanks. These were incubated in a dark rotatory shaker incubator at 22 degrees Celsius and mixed constantly at 110 RPM for the entire duration of the test. Results presented are corrected for control blanks.

3.3.3 Methane Determination

The main goal of the BMP tests was to determine the methane yield potential of cultures under different scenarios. This was accomplished through the following steps:

- A standard curve was prepared to allow correlation of the pressure to the volume of gas in the headspace of a bottle filled with the same liquid volume as the test bottles (75mL). Different volumes of air were injected into the standard bottle's headspace and pressure was immediately read using a pressure transducer and recorded.
- The atmospheric pressure was recorded and used to correct for STP.
- The pressure in the headspace of each test bottle was measured, and the standard curve for that day was used to calculate the total gas volume. Standard curves are presented in Figure 3-2 and Figure 3-3.
- Total gas volumes were corrected for STP.

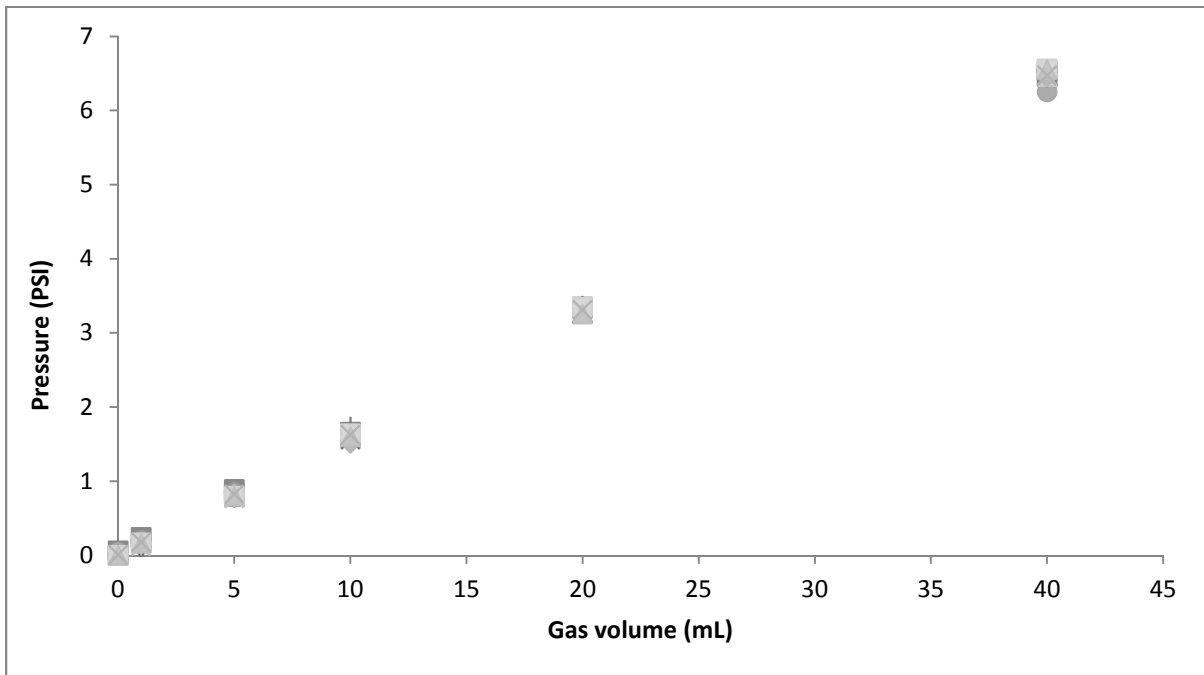


Figure 3-2 - Total gas standards (BMP1).

Different symbols represent different days, many of these overlap. The slopes ranged from 6.0776 to 6.40123, the intercepts ranged from -0.44520 to 0.15066, and the correlation coefficients ranged from 0.99949 to 0.99999.

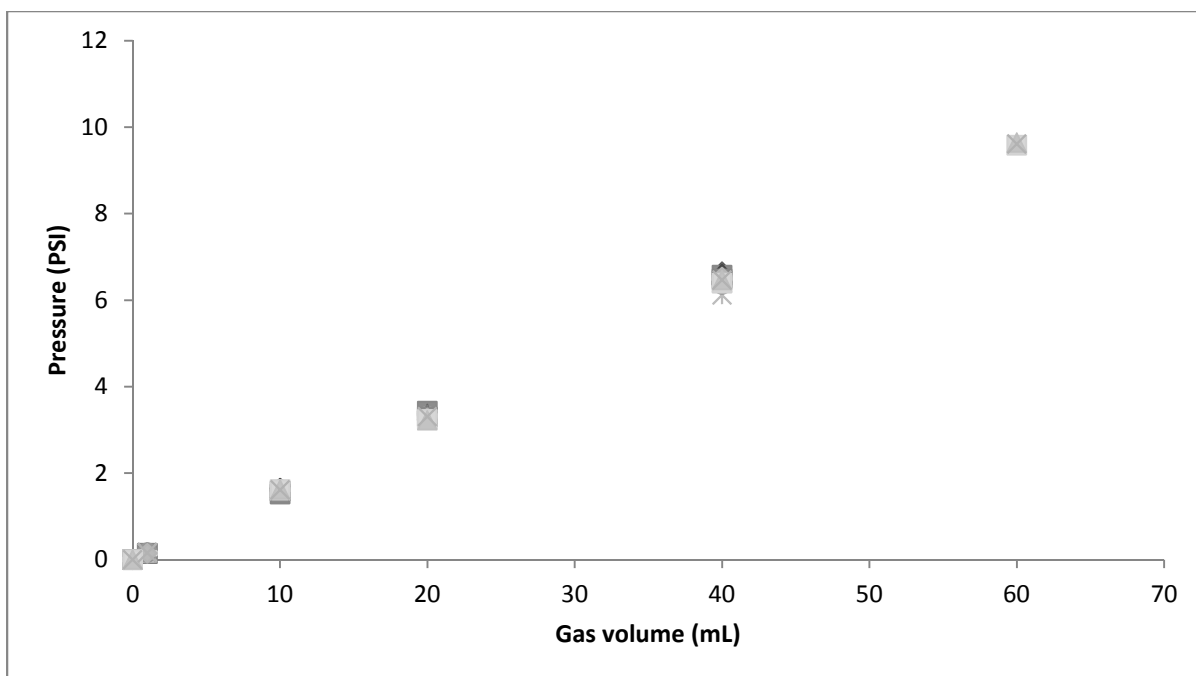


Figure 3-3 - Total gas standards (BMP2).

Different symbols represent different days, many of these overlap. The slopes ranged from 5.9928 to 6.4986, the intercepts ranged from -0.3178 to 0.1518, and the correlation coefficients ranged from 0.99936 to 1.00000.

A Shimadzu gas chromatograph (GC-2014) equipped with a carboxen-1006 column (operated at 50 degrees Celsius) and a flame ionization detector (FID) (operated at 230 degrees Celsius) was used for methane determination:

- Methane standards were prepared by injecting known volumes of “pure” (99.23%) methane gas into empty sealed serum bottles. After vigorous mixing, samples from each standard were injected into the GC in triplicate. The data was used to create a standard curve of peak area vs. percent methane. The standard curves for methane are presented in Figure 3-4 and Figure 3-5.
- Following standard injections, 0.5 mL of headspace gas from each bottle were injected into the GC. The resulting peak areas were converted to a percentage using the standard curve for that specific day.

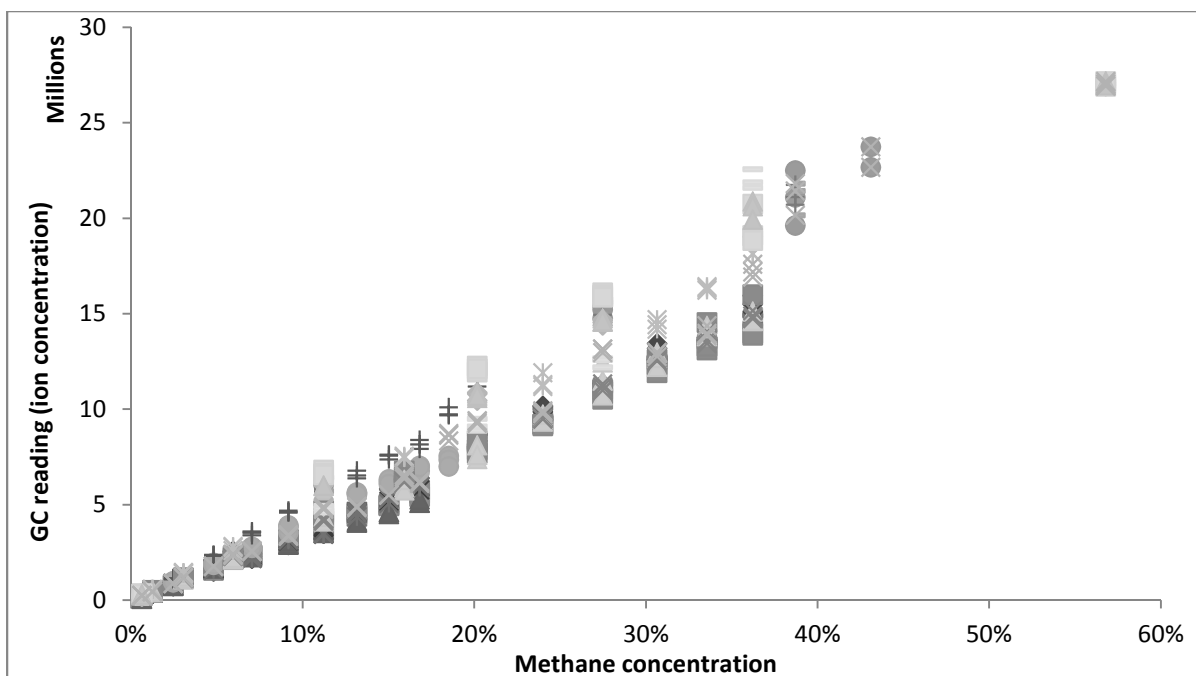


Figure 3-4 - Methane standards (BMP1).

Different symbols represent different days, many of these overlap. The slopes ranged from 1.81×10^{-8} to 3.22×10^{-8} , the intercepts ranged from -2.67×10^{-2} to 1.57×10^{-2} , and the correlation coefficients ranged from 0.98043 to 0.99956.

The differences in slope of the standards in Figure 3-4 and Figure 3-5 may be due to the small errors introduced during the standards preparation and transfer of sample to the GC injection port (i.e., unintended gas release during needle extraction through septum). Although the slopes of the lines were not consistent from day to day, the correlation coefficients for each day were very good (0.98043 to 0.99956). Data were analyzed using the slope for that particular day.

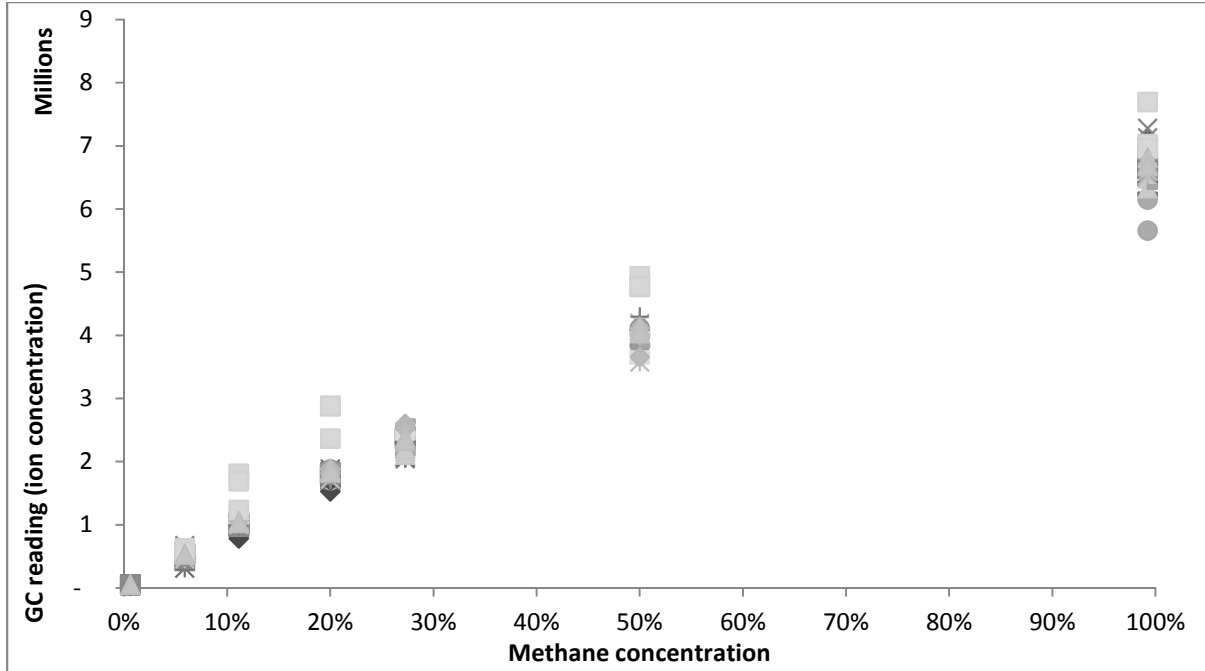


Figure 3-5 - Methane standards (BMP2).

Different symbols represent different days, many of these overlap. The slopes ranged from 1.18×10^{-7} to 1.63×10^{-7} , the intercepts ranged from -1.04×10^{-1} to -3.56×10^{-4} , and the correlation coefficients ranged from 0.97508 to 0.99919.

The resulting total gas volume and percent methane in the total gas were then used to calculate the volume of methane in each sample at any given day, using the following formulas.

$$\text{Corrected percent } CH_4 = \frac{\text{percent } CH_4}{1 - \left(\frac{D \times 22.4 \text{ L/mol}}{18 \text{ g/mol} \times 1000} \right)}$$

where D is the density of water vapour at saturation in grams per cubic meter

$$\text{Volume of } CH_4(\text{mL}) = (S + V) \times \left(\frac{P - P_w}{T + 273} \right) \times \left(\frac{\text{Corrected \%}CH_4}{100} \right) \times \left(\frac{273}{760} \right)$$

where

S is the excess gas production

V is the volume of headspace

P is the barometric pressure in mm Hg

T is the temperature in degrees Celsius

P_w is the vapour pressure of water at T

Corrected %CH₄ is the percent methane content of total gas

The results of the BMP test can be used to evaluate waste substrates for the optimization and design of an anaerobic wastewater treatment system (Moody et al. 2009). The methane yields from this assay can be used to assess inoculum performance in converting the waste to methane and to make comparisons between the conversions of different substrates.

3.4 General Analytical Methods

3.4.1 COD

Photometric Analysis was used to measure Chemical Oxygen Demand (COD) concentrations following CHEMetrics manufacturer instructions. The process is outlined below:

- A standard curve was calculated for each box of COD tubes used. A standard curve was created using six standards dilutions of potassium hydrogen phthalate (KHP) at known COD concentrations (5, 200, 400, 600, 800 and 1000 mg/L). Standard curves are presented in Figure 3-6.
- Samples were diluted tenfold or one hundred fold to obtain absorbance readings within the pre-established standard curve range to improve the reliability of results.
- As per the manufacturer's instructions, 2 mL of diluted sample were pipetted into each COD vial and each vial was inverted several times after securing the cap to mix the contents.
- A blank was prepared for every batch of samples, using the Type 1 water used for making the dilutions as the sample.
- Each vial was wiped clean and inserted into a digester block or oven, preheated to 150 degrees Celsius.
- The vials were left in the digester block for two hours before turning the block off and allowing the vials to cool.
- After 20 minutes of cooling, the vials were inverted several times and placed in a dark cabinet to allow cooling to room temperature.

- The spectrophotometer was warmed up and set up following the manufacturer's instructions. Wavelength was set to 620 nm and the instrument was zeroed using the blank.
- The absorbance of each vial was measured and recorded.
- Test results were then converted to a concentration (mg COD/L) using an Excel spreadsheet, using the standard curve data. Standard curves can be found in the appendices.

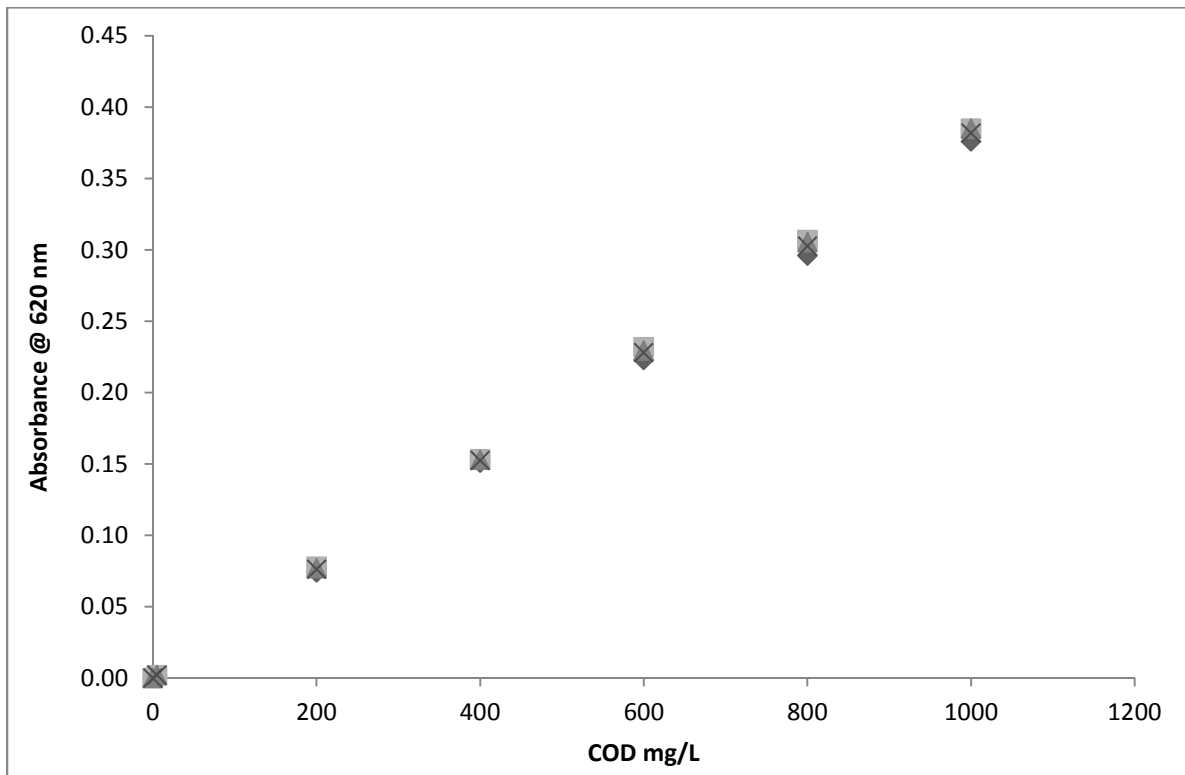


Figure 3-6 - COD standards.

Different symbols represent different sets of standards, many of these overlap. The slopes ranged from 2600.7 to 2675.0, the intercepts ranged from -0.75182 to 0.52823, and the correlation coefficients ranged from 0.9999 to 1.0000.

3.4.2 Solids

The materials and methods used for solids determination were as per Standard Methods for the Examination of Water and Wastewater (Franson et al. 2002). The solid tests are

gravimetric measurements of different types of solids contained within a liquid sample. They involve drying and incineration steps to isolate or remove different types of solids.

The total solids (TS) and volatile solids (VS) procedure consisted of the following steps:

- Evaporating dishes (ceramic crucibles) were cleaned in an acid bath and later dried in an oven to remove any moisture. These were then weighed.
- Samples were homogenized by mixing with a vortex mixer before testing.
- 10 mL of liquid sample were placed in the dishes and allowed to dry overnight at 104 degrees Celsius. This left only solids in each dish, which include organic, inorganic, dissolved and suspended solids.
- Samples were then removed from the oven and were allowed to cool in a desiccator before weighing.
- Samples were transferred to a furnace pre-heated to 550 degrees Celsius and allowed to incinerate for two hours to remove volatile (organic) material in the sample.
- Samples were allowed to cool and transferred to a desiccator before weighing again.
- The masses recorded were used to calculate TS and VS.
- All samples were analyzed in triplicate.

To determine the total suspended solids (TSS) and volatile suspended solids (VSS) in each sample, the following steps were followed:

- Tin dishes and new glass fibre filters were weighed and their mass recorded.
- Samples were homogenized by mixing with a vortex mixer before testing.
- A vacuum apparatus was used to filter 5 mL or 2 mL (if high in solids) through the glass filter. This allowed the liquid portion and the solids dissolved therein to pass through the filter, leaving only the suspended fraction.
- The dishes and filters were allowed to dry overnight at 104 degrees Celsius. This left only solids (organic and inorganic) in each dish.
- Samples were removed from the oven and allowed to cool in a desiccator before weighing.
- Samples were transferred to a furnace pre-heated to 550 degrees Celsius and allowed to incinerate for two hours to remove volatile (organic) material in the sample.

- Samples were then allowed to cool and transferred to a desiccator before weighing again.
- The masses recorded were used to calculate TSS and VSS.
- All samples were run in triplicate.

The samples processed using these techniques included enrichment culture effluent samples, waste substrate samples, and BMP samples.

3.4.3 Alkalinity and pH

Alkalinity was measured following the titration method outlined in Standard Methods for the Examination of Water and Wastewater (APHA et al. 2005). The pH of undiluted five millilitre effluent samples was measured and recorded before titrating the sample with 0.1N sulphuric acid (H_2SO_4) to a pH of 4.5. The volume of H_2SO_4 required to lower the pH to 4.5 was used to determine the sample's alkalinity in mg CaCO_3/L .

3.4.4 Ammonia-N and nitrate

Ammonia-N was measured using a Thermo Scientific Orion ISE meter equipped with an ammonia electrode. "Technical Bulletin: Nitrate Measurements in Environmental Samples" was followed; the method allows for the determination of ammonia-N and nitrate concentrations in aqueous samples.

- A set of ammonia-N standards were prepared using a standard stock solution. These were used to prepare a standard curve of millivolts (mV) versus known concentrations of ammonia-N.
- Through experience it was determined that a 100 fold dilution of samples was adequate to ensure operation within the method's range of detection.
- 2 mL of diluted sample were added to a glass test tube containing a small magnetic bar. The procedure was carried out over a magnetic stirring plate to allow adequate mixing of the reagents used with the sample.

- 200 μ L of alkaline reagent were added to the sample to drive the pH to 11 and volatilize ammonia in the sample. A reading was taken immediately using the ammonia electrode.
- 40 μ L of reducing agent were then added to the sample to convert any nitrate to ammonia-N, which could then be read using the ammonia electrode.
- The resulting mV readings were used with the standard curve to determine the concentration at each reading.
- The first reading was used to determine the ammonia-N concentration, while the nitrate concentration was determined by subtracting the first concentration from the second one and converting a positive result to a nitrate equivalent.

3.4.5 Organic Acids

Organic acids (VFA and tartrates) were measured in filtered effluent samples from the second set of batch tests (BMP2) using a Dionex (Thermo Scientific) Ion Chromatograph (IC). The IC was not available during most of the enrichment process or during the first set of BMP tests. The method followed was “Dionex Application Note 143 - Determination of Organic Acids in Fruit Juices” (Dionex 2003):

- Standard curves were created using Supelco Volatile Acid Standard containing the following acids in different concentrations: acetate, propionate, isobutyrate, isovalerate, valerate, hexanoate, isocaproate, heptanoate, and formate/butyrate; the standards were also spiked with tartaric acid at different concentrations. The standard curves are presented in Figure 3-7.
- Samples were thoroughly mixed and 5 mL were extracted using a syringe and needle.
- The sample was then passed through a syringe filter (0.2 μ m filter) into a test tube.
- 1 mL of filtered sample was used to create 10 fold dilutions of each sample.
- 5 mL of filtered, diluted sample were transferred to Dionex IC plastic vials, which were labelled and placed in IC sample carousel.
- IC was programmed using Chromeleon 6 software and program AN143 was run and left overnight to process samples.

- The chromatogram peak areas were used to determine the corresponding concentrations.

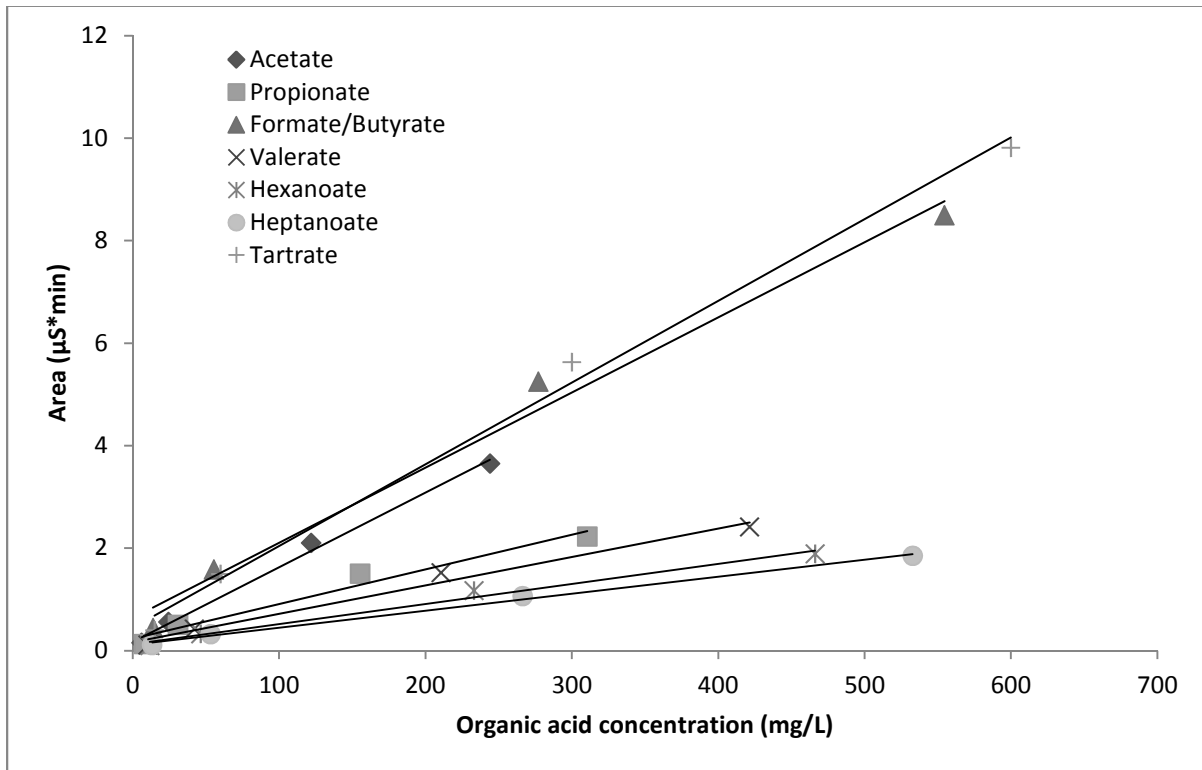


Figure 3-7 - VFA standards.

A single standard curve was prepared for each acid. Different symbols represent different sets of standards, many of these overlap. The slopes and intercepts for each acid varied, however, the correlation coefficients ranged from 0.98381 to 0.99768.

Chapter 4 Results and Discussion

This chapter discusses the result of the waste audit, ongoing monitoring of the culture enrichment, and both sets of BMP tests.

4.1 Waste Audit

The following section presents the results of the waste audit conducted. These include actual data from the 2009-2010 season and projections for the 2011. Figure 4-1 shows the contribution of wash-water and wastes as calculated from the waste audit and estimations of similar activities from the literature review.

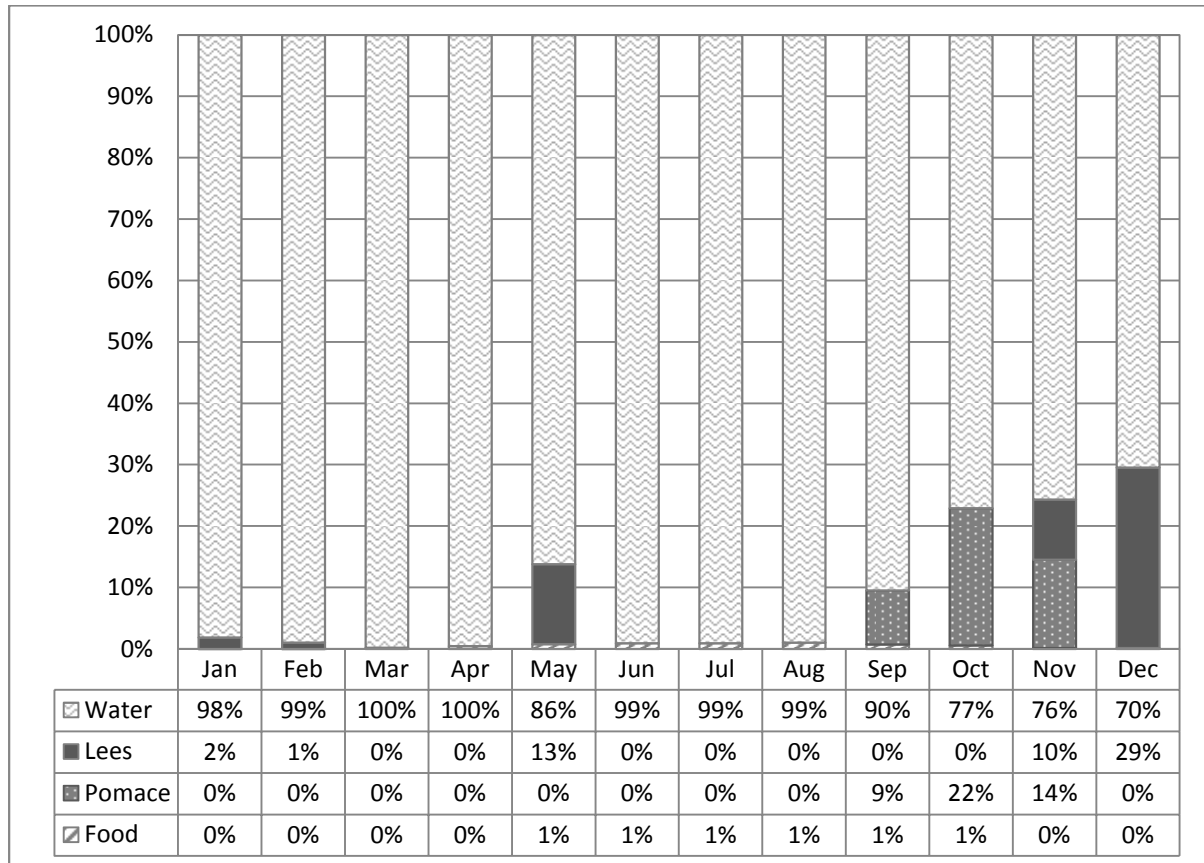


Figure 4-1 - Estimated contribution of four main waste outputs from the winery.

Water from rinsing and washing operations is the most dominant output from the winery in terms of volume. At the time of this project this water is directed to a septic system that is separate from lees or pomace disposal, however, it was important to consider this discharge as it could be used in a future treatment system to dilute the other waste. Lees are produced in the most significant quantities in May, November and December, while pomace is mainly produced in the fall during crush and shortly after in winter when fermented red grape pomace is separated from the wine. Food waste projections appear to be insignificant in volume compared to the other outputs, peaking at 1% of the total outputs produced in the summer months, when tourist season is expected to attract the most patrons to the restaurant. The percentages presented in Figure 4-1 were used as guides in the design of the BMP1 test. Volume considerations are important for the sizing of storage infrastructure for an eventual treatment system. The volume contribution from lees, pomace and food waste was considered and the results are presented graphically in Figure 4-2.

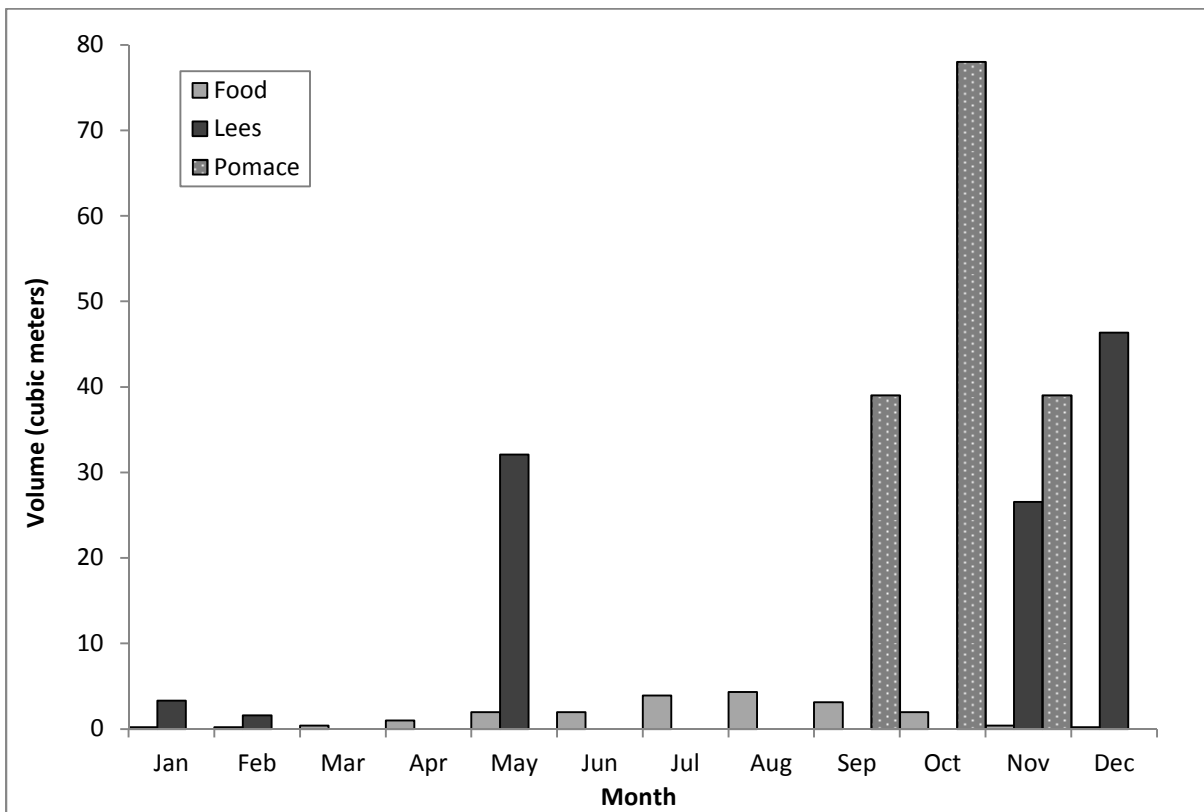


Figure 4-2 - Estimated monthly contribution from three main organic wastes.

Lees volumes were estimated from cellar records detailing the volumes of wine transferred from one stage to the next. The logs showed volumes of product progressively decreasing with each stage. This was expected as a fraction of the product is lost as wasted wine between stages, most prominently during the racking stage where wine is clarified through settling of lees, with the lees being discarded and the wine progressing further through the other production stages. Pomace and food waste are solid wastes and as such, their densities had to be considered in order to arrive at an estimate of their volumetric contribution. Densities of 514 g/L and 640 g/L were used for food and pomace respectively (Mishra et al. 2008;SRA 2010). Following volume considerations, the strength as total COD of each waste was considered. The COD concentrations of waste samples were determined to be 52,300 mg/L in the pomace suspension, 233,300 mg/L in pure lees, and 105,900 mg/L in the synthetic food suspension. These COD concentrations and the estimated volumes of waste produced per month were used to produce Figure 4-3, which shows the mass of total COD contributed on a monthly basis by each of the three wastes.

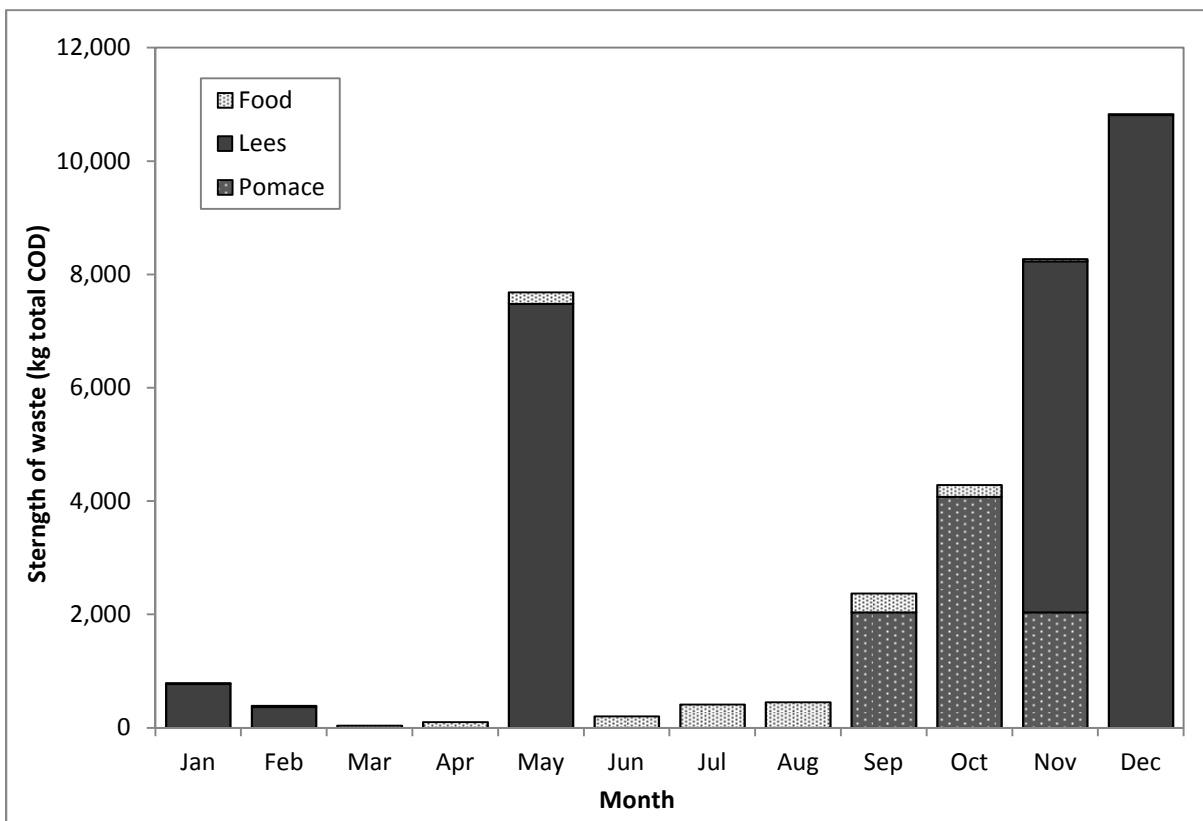


Figure 4-3 - Estimated mass of total COD contributed per waste by month.

The vast majority of the high strength waste is produced in the fall and winter during vintage and later on in the spring in the form of lees and wasted wine during racking and bottling of red wines from the previous year. The contribution of food waste was predicted to be small but significant enough to consider in the first BMP test. Lees was the most significant waste in terms of COD contribution to the total output. This supported the reasoning behind enriching the first inoculum culture with lees, as it is the strongest waste the inoculum would have to degrade. Culture enrichment is discussed in the following section.

4.2 Culture Enrichment

Two cultures were seeded from the same inoculum; one was enriched to degrade lees (W1) and the other one to degrade tartaric acid (W2), two significant components of winery waste.

4.2.1 Culture W1

Culture W1 was enriched on lees for 325 days. The following sections discuss methane production and parameters of importance monitored throughout the enrichment process.

4.2.1.1 Methane produced

Figure 4-4 shows the cumulative gas production by culture W1 over time for the totality of the enrichment period. Two distinct stable phases were identified and are labelled in the graph. These are defined as healthy stages of methanogenic culture growth that produced volumes of methane per COD mass of feed at steady rates.

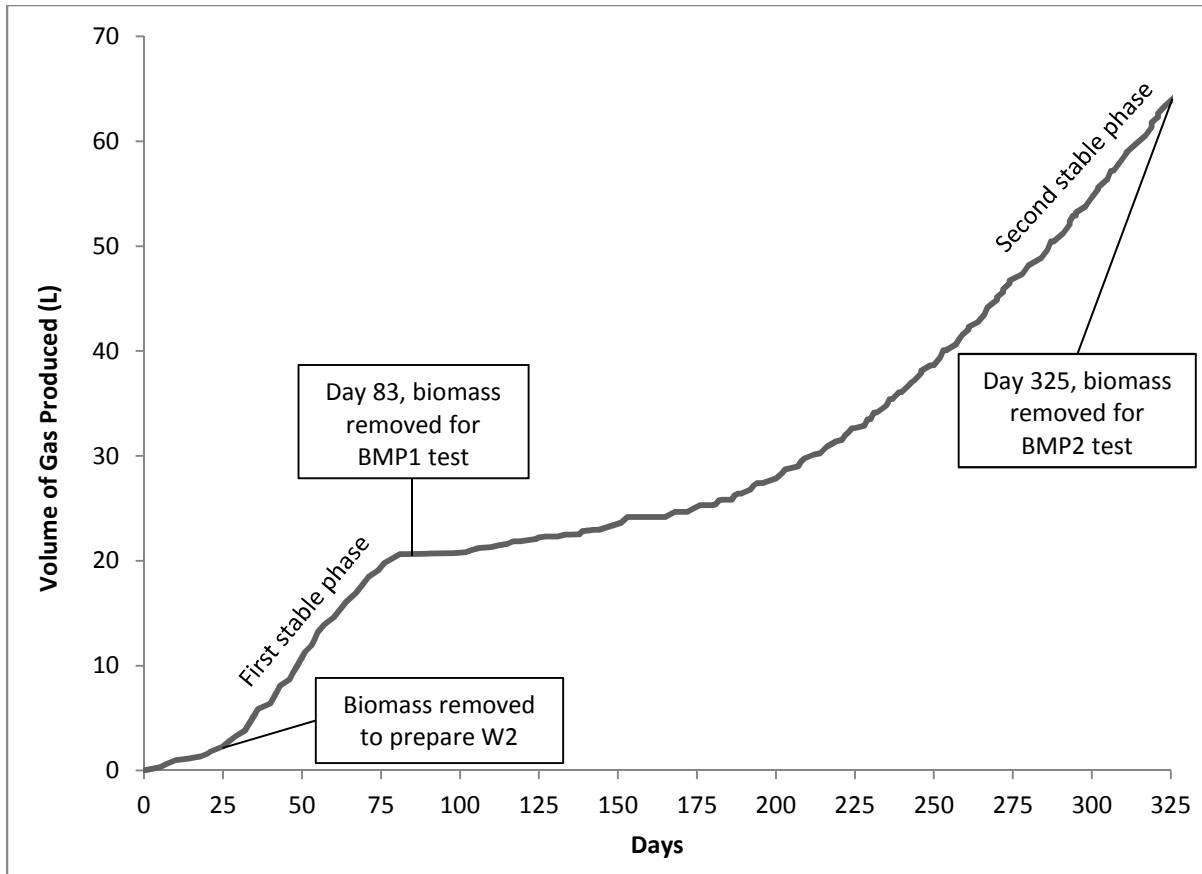


Figure 4-4 - Cumulative methane gas production by culture W1.

Culture W1 was fed red wine lees at a concentration of 1550 mg COD/L for the first 25 days, after which biomass was removed to create culture W2. Following day 25, W1 was fed lees at a concentration of 3100 mg COD/L (4650 mg COD in 1.5 L) every 2-3 days. At day 274 new red wine lees was introduced and fed at a concentration of 2810 mg COD/L (4220 mg/1.5 L) every 2-3 days. Nutrient medium was changed on a weekly basis. Biomass was removed from the culture on days 83 and 325 for BMP tests.

The first stable phase of methane gas production was achieved after approximately 40 days. Given the volatile fraction in lees (VS/TS = 0.75) it was assumed 3490 mg COD was biodegradable ($4650 \times 0.75 = 3490$). At an average temperature of 22 degrees Celsius, the theoretical maximum methane yield would be 0.378 mL/mg of COD consumed; therefore it would have been possible to produce 1320 mL of methane from the lees it was fed

$(3490 \times 0.378 = 1320 \text{ mL})$. Actual methane gas production during the first stable phase held at an average of 660 mL methane per feed, or 50% of theoretical maximum.

At day 83, biomass was extracted from the reactor to prepare the inoculum composite for the first set of BMP tests. After this occurrence there was a decrease in methane production, observed as a plateau, until around day 100. The shock to the culture was significant enough that it had to be 'jump-started' by adding glucose as an additional substrate to enhance biomass growth. Methane production continued to climb through day 228 and to converge on a steady rate of methane production reached at around day 250. It was at the end of the enrichment period (day 325) that biomass was extracted again to seed the second set of BMP tests. If monitoring had been continued, it is logical to assume that a decrease or cease in methane production would have been observed again.

During the latter part of the second stable phase, W1 was being fed Merlot lees at 2810 mg COD/L (4220 mg/1.5 L) every 2-3 days. Given the volatile fraction in lees ($VS/TS = 0.75$) it was assumed 2110 mg COD were biodegradable ($2810 \times 0.75 = 2110$). At an average temperature of 22 degrees Celsius, the theoretical methane yield is 0.378 mL / mg of COD consumed; therefore the theoretical yield is 800 mL of methane ($2110 \times 0.378 = 800 \text{ mL}$). Actual methane gas production held at an average of 670 mL methane per feed, or 84% of the theoretical maximum.

The relatively low methane conversion rates observed in the first stable production phase could be due to several reasons. However, the reason was not evident from the parameters that were monitored throughout the enrichment process (i.e., total COD, TS, VSS, pH, alkalinity, ammonia-N and nitrate). It is possible that polyphenol content was high enough to inhibit methanogenesis; however, polyphenols were not in the scope of this research and this is an assumption. The methane conversion rate observed in the second stable production phase is considered reasonable.

4.2.1.2 Total COD

Total COD was monitored in the effluent throughout the enrichment process. The estimated concentration of degraded COD is also presented here. While the degraded COD is an estimate, the results for the COD found in the effluent are actual analytical results and thus discussed in more detail. However, it can be said both parameters agree, that is when more COD is degraded a lower concentration is found in the effluent (Figure 4-5).

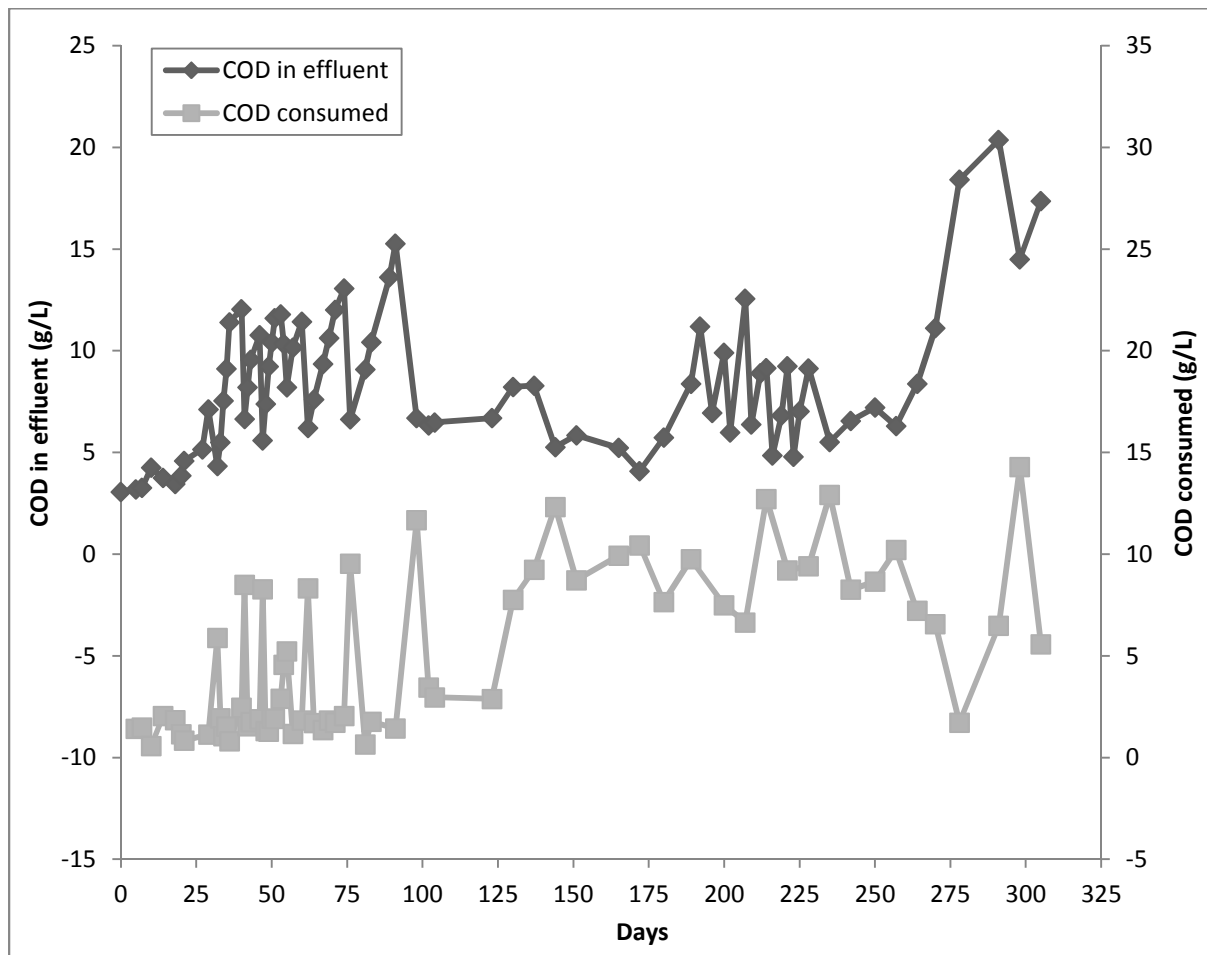


Figure 4-5 - Concentration of total COD consumed and in effluent from reactor W1.

The culture was fed by spiking, leading to cumulative increases in COD (observed as peaks) and weekly media changes led to a decrease in COD (observed as valleys). COD concentrations in the effluent were the lowest in the first 25 days, when lees were being fed at a concentration of 1550 mg COD/L, after this time lees COD concentration was increased

to 3100 mg COD/L and consequently the total COD increased in the effluent. As time progressed past day 30, the total COD in the effluent became more predictable, a cyclical spiking effect was seen where there was a gradual rise in COD and then a drop before repeating itself again. The rise in COD was due to the cumulative effect of feeding three to four times a week. The drops in COD were due to changes of nutrient medium done weekly, this reset things on a weekly basis as any suspended organics were discarded with the treated effluent. At around day 90, feeding of the culture was scaled back to once weekly. This was done to see if a reduction in total feed and a prolonged starvation period would lead to catabolism of the more recalcitrant compounds and the removal of excess COD in the effluent. The COD in the effluent did decrease in concentration, however, methane continued to be produced at an increasing, though irregular rate.

After day 175, feeding frequency was resumed to three times a week and COD appears to have remained at around $7940 \pm 2,240$ mg/L during this time period. After day 228, monitoring was scaled back due to a diminishing supply of resources. On day 274 new lees was introduced as the main feed for the culture. A significant increase in COD concentration was observed in the effluent, going from $7,180 \pm 1,360$ mg/L in the previous period to $17,660 \pm 2450$ mg/L in this one, even though the new lees were of a lower total COD concentration than the previous stock. Perhaps there were more complex organic compounds in the new lees as it had not gone through as many fermentation steps as the previous lees stock. Interestingly enough, methane production did not vary much after this introduction, indicating that a comparable concentration of COD was degraded at before.

4.2.1.3 Total solids and volatile suspended solids

TS and VSS were monitored for the duration of the experiment. The results are presented graphically in Figure 4-6. TS represent dissolved and suspended solids while VSS only represents suspended or particulate organic compounds.

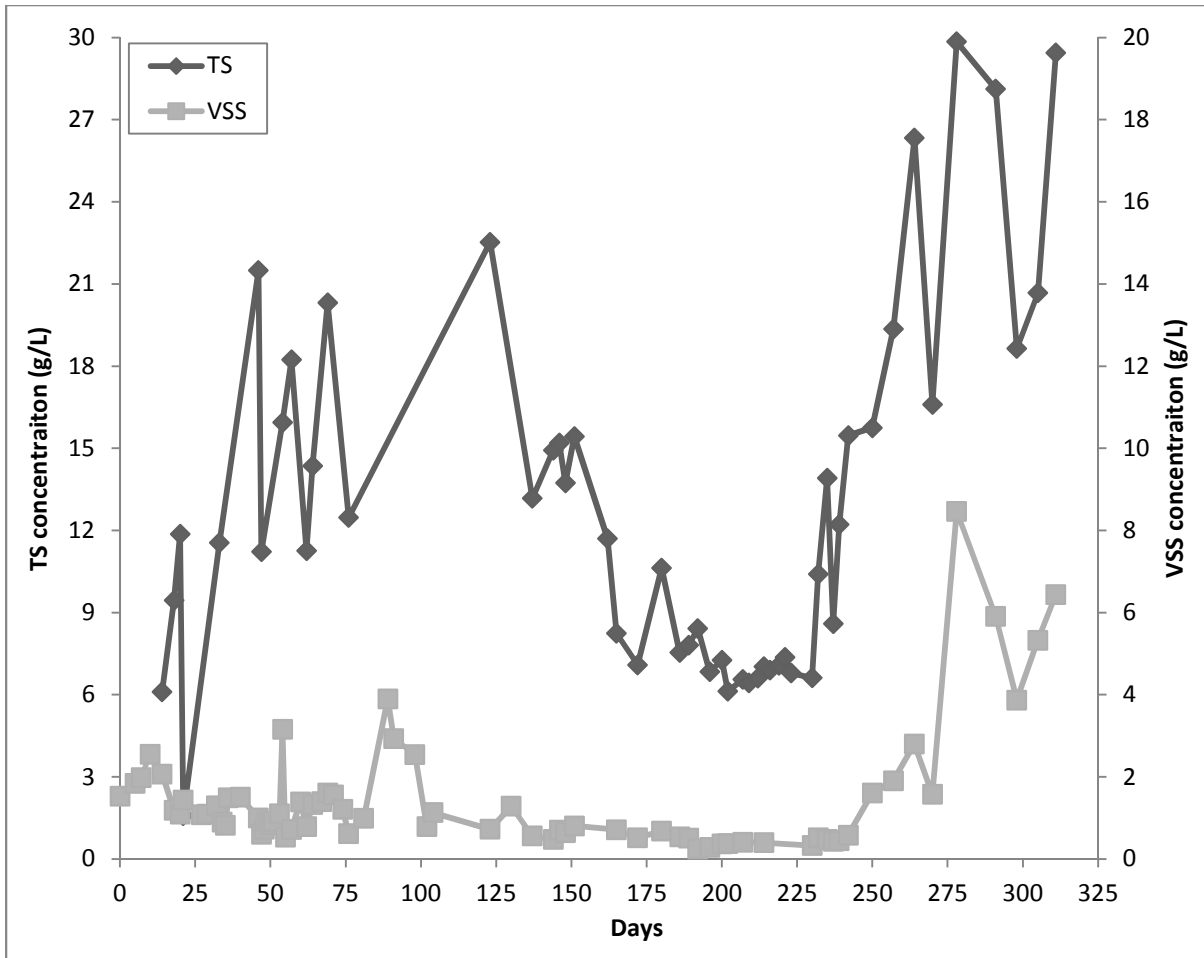


Figure 4-6 - Concentration of TS and VSS in effluent from reactor W1.

The peaks observed are the result of cumulative spike feedings, and the valleys are due to weekly media changes that removed suspended solids periodically. TS monitoring was started on day 14; it was very variable in the early stages (days 14-75) hovering between 1,500 and 21,000 mg/L. Although the culture experienced a decline in methanogenic effectiveness as seen in the plateau in methane production during the same time period, the total solids appear to have remained within the concentration range previously mentioned. By day 123, TS registered at 22,530 mg/L but gradually decreased in concentration, settling at around 6100 mg/L by day 230, after which a sudden increase was observed, increasing to 16,600 mg/L by day 270. TS concentrations from day 200 to day 228 hovered at around 6820 ± 390 mg/L. On day 274, a new lees stock feed was introduced, and TS again increased significantly to $28,980 \pm 1230$ mg/L in the last stage of the test.

VSS is used in the wastewater treatment field to estimate biomass concentration in aqueous samples. The relationship between VSS and TS is useful in determining how much of the total solids are due to biomass and how much is due to other types of solids such as dissolved solids and inert particulate. VSS were monitored through the entirety of the test. While variable in the early phase (days 0-75), VSS was found to hover around 1,000 mg/L before experiencing a rise in concentration at around day 90. This was most likely due to the inactive period following biomass extraction for BMP1 testing. What this means is more biomass was washed out with the effluent at the time of testing; meaning the settling efficiency of the culture had also been impacted. This could be due to the loss of floc mass. Perhaps at this time a longer settling period was required. Past day 83, VSS in the effluent had increased to around 4000 mg/L; however as the weeks progressed it decreased to 700 mg/L. VSS stabilized after day 200 through day 228 at 390 ± 20 mg/L and increased to $1,970 \pm 570$ mg/L after day 250, which coincides with the increase in TS. On day 274, a new lees stock feed was introduced, and VSS increased to 5390 ± 1110 mg/L through the end of the test. Most of the TS is dissolved organics represented by COD and dissolved salts.

Decreases in concentration of TS and VSS were significant between days 123 and 235, making it more probable that as feeds were reduced in frequency, the culture consumed more of one or two things: 1) soluble compounds that accumulated in the settled fraction or 2) itself through catabolism, resorting to endogenous respiration due to starvation. If accumulated soluble material was the sole source of food then VSS in the effluent should have remained relatively steady or increased, however, a reduction was observed indicating that perhaps a fraction of biomass had remained suspended after the settling phase and a reduction in overall biomass due to endogenous respiration was observed through this parameter in the effluent. In other words, biomass was consumed and this was reflected in better settling and in lower concentrations of suspended biomass.

4.2.1.4 pH and alkalinity

pH and alkalinity were monitored regularly and the results are presented in Figure 4-7.

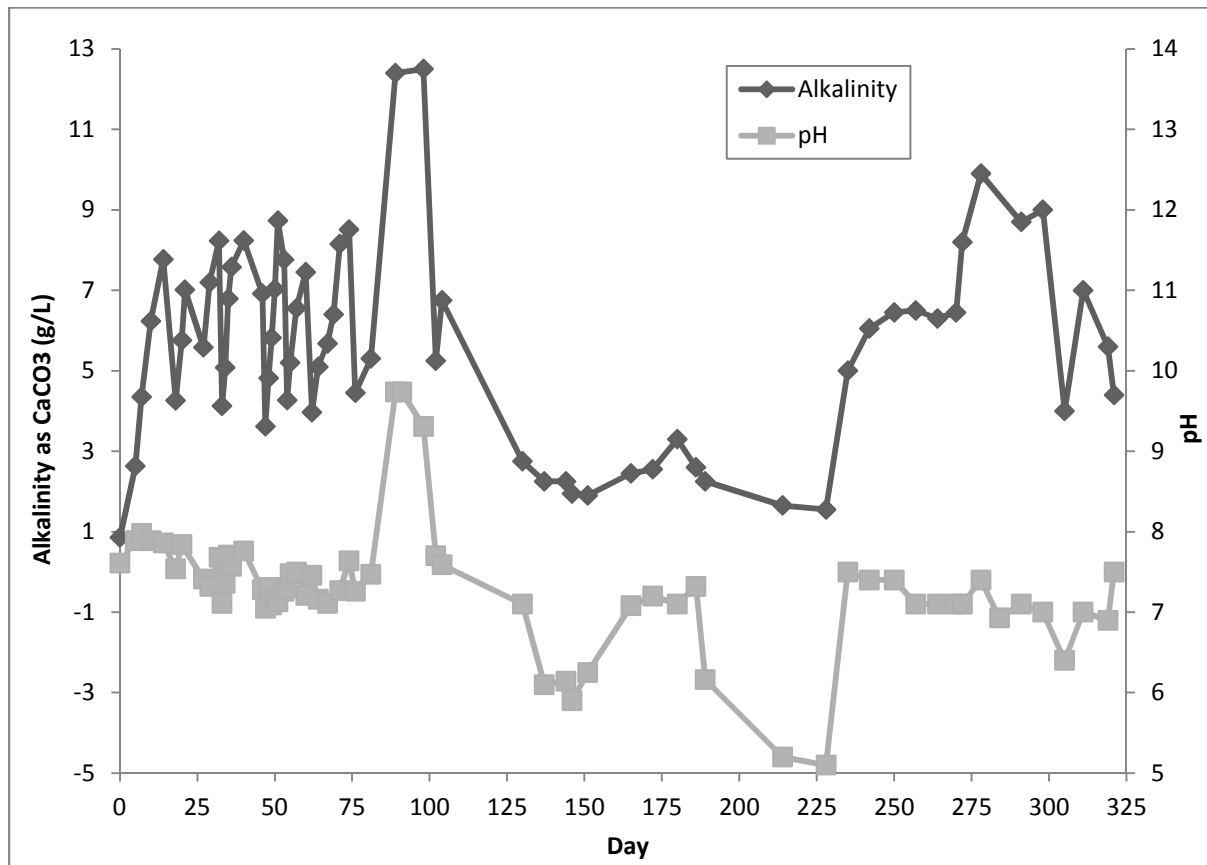


Figure 4-7 - Concentration of pH and alkalinity in effluent over time from reactor W1.

The peaks observed are the result of cumulative spike feedings that replaced alkalinity periodically. Reactor acidity hovered at around pH 7.7 and did not fluctuate much in the first 30 days. Alkalinity gradually accumulated to 7,770 mg/L as CaCO₃ until day 14, after which it fluctuated between 6,000 \pm 2,000 mg/L. It is assumed the rise in metabolic activity eventually produced enough acidic intermediate compounds by day 14 to start consuming alkalinity in significant quantities. After day 30, acidity and alkalinity both remained relatively steady at pH 7.35 \pm 0.21 and 6,230 \pm 1,590 mg/L as CaCO₃ due to weekly medium changes and the alkalinity provided in the medium was deemed sufficient for the application. It was during this stable time that the culture achieved stable and predictable production of methane. Following day 83 after removal of biomass from the culture for BMP testing, the pH was seen to increase, peaking at around day 90 at 9.74. Similarly, alkalinity peaked on

day 98 at 12,500 mg/L as CaCO₃. Alkalinity and pH increased due to the lack of metabolic activity, which typically produces enough acidic intermediate compounds (VFAs) to consume the alkalinity and keep the pH at a lower range. Spike feedings were scaled back to once per week between days 100 and 225; the less frequent addition of alkalinity in the nutrient medium during feedings led to a gradual decrease in reactor alkalinity and pH.

As metabolic activity resumed, both parameters decreased to 5.9 and 1,900 mg/L as CaCO₃ by day 150 before rebounding back to neutral range for pH at 7.2 and 2,500 mg/L as CaCO₃ for alkalinity. This decrease was expected as the frequency of feedings and input of supplemental alkalinity had been scaled back during that same period. With the reintroduction of more frequent feedings and media changes, pH climbed to a neutral range of 6.97 ± 0.8 after day 228 and remained relatively steady. Alkalinity rose in a more gradual manner to a range of 5810 ± 1930 mg/L as CaCO₃. On day 274, new lees were introduced, however, pH remained relatively steady following this change (though slightly more variable) at 7.0 ± 3.4 , while alkalinity decreased temporarily before stabilizing in the range of 5250 ± 1350 mg/L as CaCO₃.

4.2.1.5 Ammonia-N and nitrate

The monitoring of ammonia-N and nitrate in the effluent was started on day 71 and continued through the rest of the experiment. The concentrations observed for both parameters are presented in Figure 4-8.

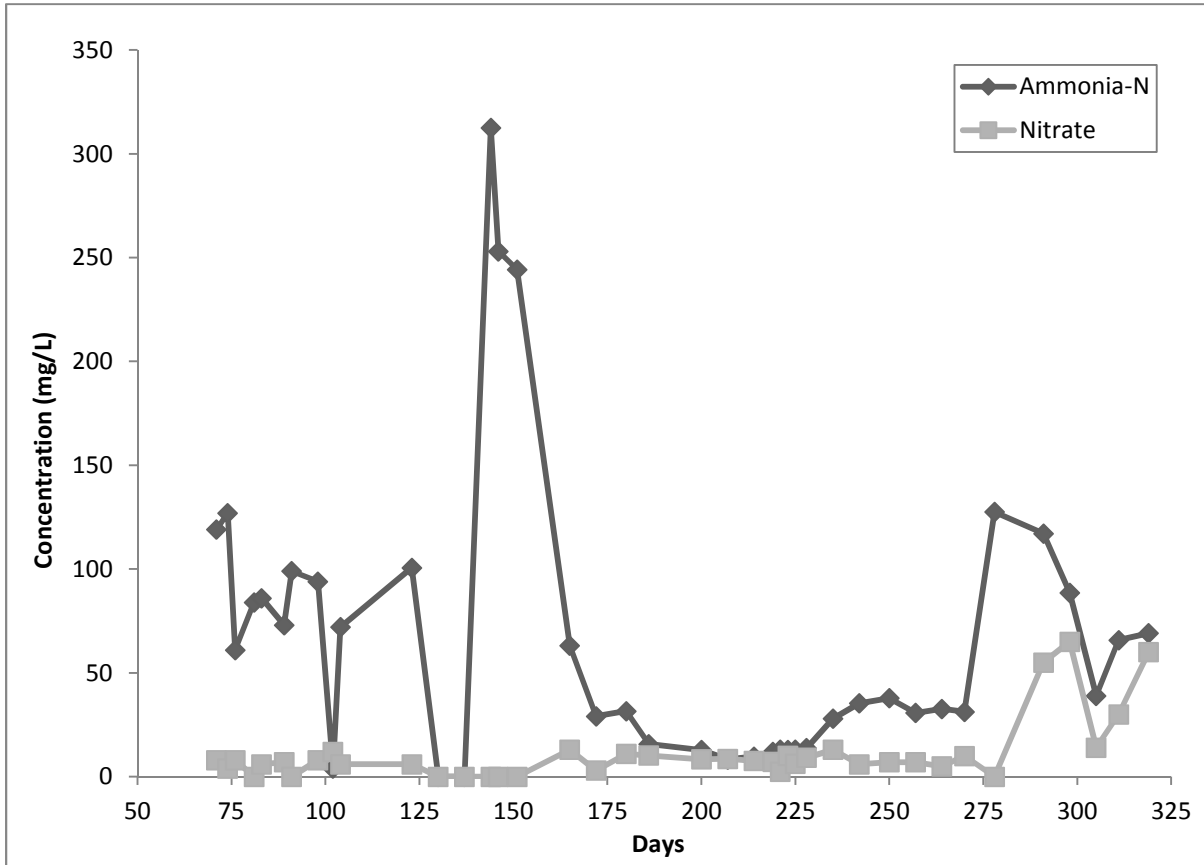


Figure 4-8 - Concentration of ammonia-N and nitrate in effluent from reactor W1.

The ammonia-N profile does not appear to be directly affected by cumulative spike feedings; however, weekly media changes removed ammonia-N from the effluent (valleys). Nitrate was found in marginal concentrations for most of the enrichment process until new red wine lees (Merlot) was introduced on day 274, when a relatively significant increase was observed. Ammonia-N concentrations varied greatly at 95 ± 27 mg/L, with nitrate at much lower concentrations, 5 ± 3 mg/L very close to the lowest detectable limit of 1 mg/L. Marginal nitrate concentrations were to be expected as effective anaerobic treatment systems should consume any soluble nitrate available. Ammonia-N fluctuated greatly and the

maximum concentration observed was an uncharacteristic spike on day 144 when it reached 310 mg/L. By day 172, ammonia-N concentrations had dropped dramatically to 29 mg/L. Nitrate concentrations remained relatively low at 4 ± 5 mg/L. Ammonia-N concentrations remained low through days 172 to 228, stabilizing at 14 ± 6 mg/L. Nitrate concentrations remained low at 8 ± 3 mg/L. After day 228, the ammonia-N concentration increased and stabilized at around 30 mg/L and nitrate remained low at 7 ± 2 mg/L. Ammonia-N decreased after the introduction of new lees on day 274, but later stabilized in the range of 66 ± 20 mg/L through the end of the experiment. For the first time in 274 days, nitrate increased above 15 mg/L before stabilizing in the range of 42 ± 24 mg/L. It is most likely that nitrates were introduced in a greater concentration in the new lees. At no point did ammonia-N accumulate to inhibiting concentrations above 1100 mg/L.

4.2.2 Culture W2

It was considered important to adapt a second culture to high concentrations of tartaric acid. On day 25 of culture W1 enrichment, biomass was removed to create a second culture to be enriched with tartaric acid (W2). Day 58 for culture W2 is the same calendar day as day 83 for culture W1 as culture W2 was started 25 days later.

4.2.2.1 Methane produced

Figure 4-9 shows the cumulative gas production by culture W2 over time for the totality of the enrichment period.

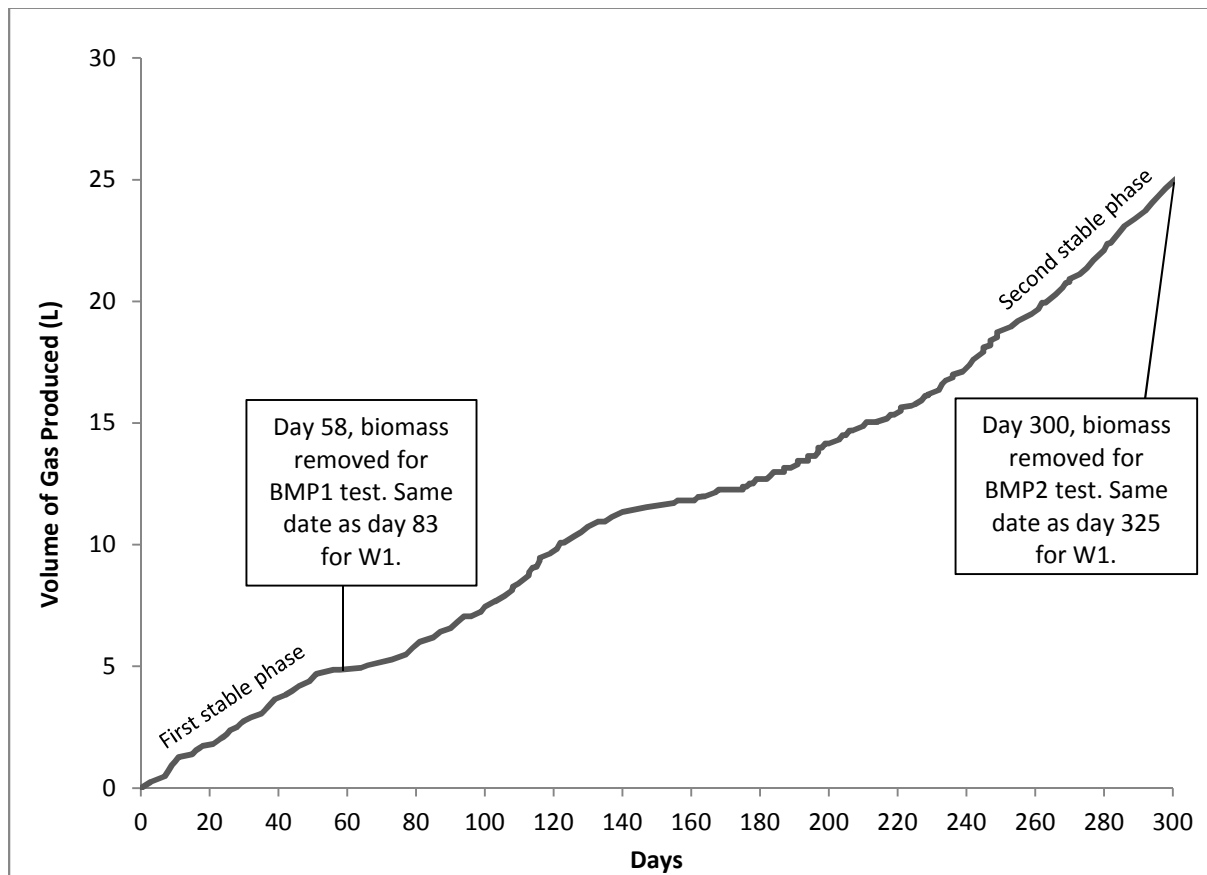


Figure 4-9 - Cumulative methane gas production by reactor W2.

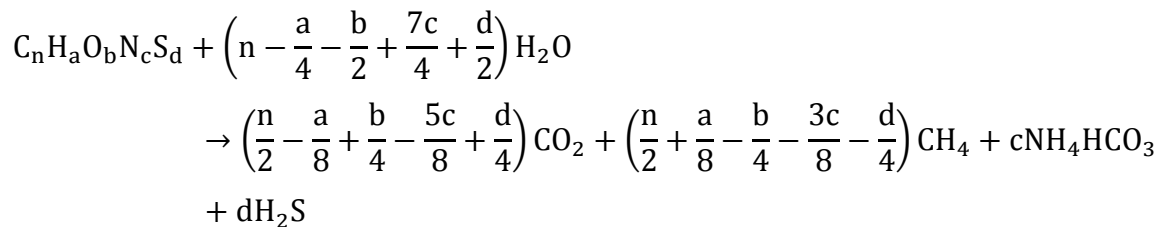
Culture W2 was fed tartaric acid at a concentration of 600 mg/L in spike feeds every 2-3 days for most of the enrichment period. Nutrient medium was changed on a weekly basis.

Biomass was removed from the culture on days 58 and 300 for BMP tests. The first stable phase was achieved between days 20 and 58, when W2 was being fed a total of 900 mg of tartaric acid in 1.5 L (600 mg/L). This concentration of tartaric acid (600 mg/L) would yield a maximum of 168 mL of methane according to the Buswell stoichiometric formula. The actual methane production in this phase held at an average of 160 mL methane per feed, or 95% of theoretical, which is an excellent yield. There was a brief stage of low activity after biomass was extracted from the culture (day 58) to seed the first set of BMP tests, though it did not come to a halt as W1 did.

The second stable growth phase was defined as days 260 to 300. The concentration of tartaric acid in the feed during the second stage had increased to 990 mg per 1.5 L (660 mg/L), which according to the Buswell stoichiometric calculation would yield a maximum of 185 mL of methane. Actual methane gas production in this phase held at an average of 288 mL methane per feed, or 155% of theoretical maximum. There could be many reasons for this apparent overproduction of methane. The most likely being that the organisms began to die off due to low concentrations of substrate to feed on, thus releasing organic material for other methanogens to feed on. It was not surprising that W2 outperformed W1 in percent of theoretical maximum as it was fed a purely soluble substance with no inhibitory compounds. The following sub-sections summarize the results of the parameters monitored throughout the enrichment process.

The Buswell formula (Herman and Roberts 2005) provides a stoichiometric estimate of the maximum number of moles of carbon dioxide and methane that can be produced from the anaerobic decomposition of organic substrate (see Equation A).

Equation A - Buswell formula



Where n, a, b, c and d represent number of atoms of each element. Once moles were calculated, the volume of one mole of methane was used to calculate the corresponding volume:

$$22.4 \text{ L} \times 1000 \text{ mL/L} = 22,400 \text{ mL at STP for 1 mol CH}_4$$

4.2.2.2 Total COD

Figure 4-10 shows how the culture's efficiency in converting tartaric acid improved, which is reflected as decreasing concentrations of COD detected in the effluent over time, albeit with the inherent variability of biological systems.

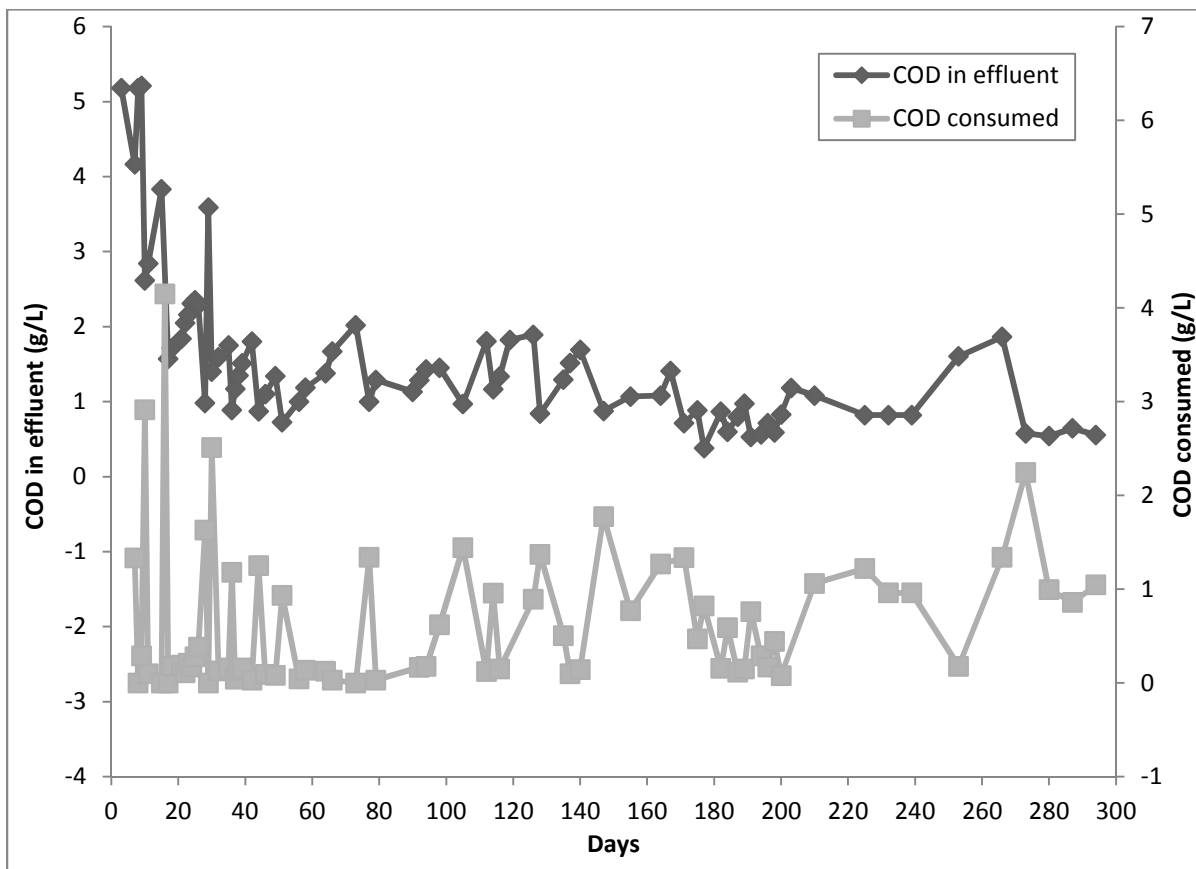


Figure 4-10 - Concentration of total COD consumed and in effluent from reactor W2.

Spike feedings led to cumulative increases in COD, though this was consumed quickly. The COD peaks observed here are believed to be related to biomass growth. Periodic media

changes led to a decrease in COD, seen as valleys. COD concentrations in the effluent at first were found in excess of 5000 mg/L but this quickly decreased as the culture became more efficient at degrading the tartaric acid. During the first stable phase (days 20-58), the COD in the effluent was around 1600 ± 670 mg/L. Between days 58 and 260, the COD remained in the range of 8120 ± 2660 mg/L. During the second stable phase (days 260-300), the COD went from a concentration of 1860 mg/L to a stable 580 ± 45 mg/L. The COD consumed coincides inversely with the COD found in the effluent; as more COD was consumed within the reactor the less of it was observed in the treated effluent.

4.2.2.3 Total solids and volatile suspended solids

Solids were monitored for the entirety of culture W2's enrichment. The results of this monitoring are presented graphically in Figure 4-11 below.

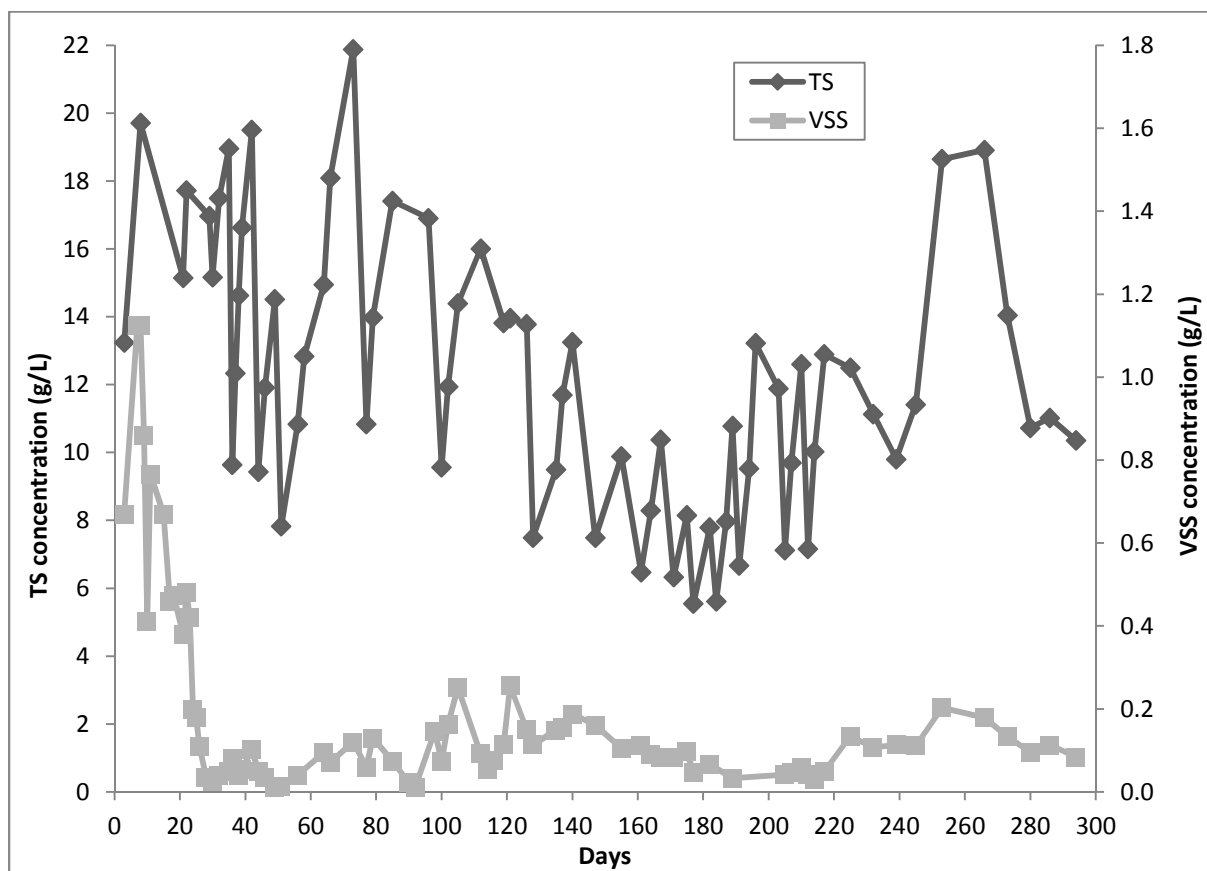


Figure 4-11 - TS and VSS concentrations in treated effluent from reactor W2.

Spike feedings led to biomass growth and an observable cumulative increase in solids (peaks) while periodic media changes led to the removal of solids in the effluent (valleys). TS in the effluent were on a slowly decreasing trend during the first stable phase of gas production, indicative of the culture's improving efficiency at the time, also reflected in the increase in methane production observed over the same time period. A cumulative increase was observed after day 58, when biomass was removed for the first BMP test. This was a time of recovery for the culture and after day 73 it began to consume organics in the solids again, which was reflected in lower concentrations in the effluent. Over time, TS in the effluent fluctuated but decreased gradually until day 180, after which the concentration began to gradually increase again. An unexpected sharp increase to 18,780 mg/L was observed at around day 260 as the culture entered the second stable growth phase, however, this decreased sharply by day 280 and remained at around $10,700 \pm 330$ mg/L into the end of the enrichment until biomass was extracted for the second BMP test.

As observed in the COD graph (Figure 4-10), the organic content in the effluent (VSS) diminished with time as the culture's efficiency improved, starting from concentrations as high as 1,100 mg/L and decreasing by the first stable phase to a range of 45 ± 25 mg/L. VSS fluctuated but remained in the range of 102 ± 57 mg/L during days 58 to 260. During the second stable phase (days 260 to 300) VSS was on a decreasing trend. After the initial stabilization period, from approximately day 30 onwards, VSS remained relatively constant under 300 mg/L, indicating that biomass settled well but marginal amounts were washed out in the effluent.

4.2.2.4 pH and alkalinity

pH and alkalinity were monitored throughout the enrichment process. Both are presented together in Figure 4-12 to illustrate their relationship, the expected and observed trend being that as alkalinity was consumed, pH decreased, and when alkalinity accumulated sharply, pH increased.

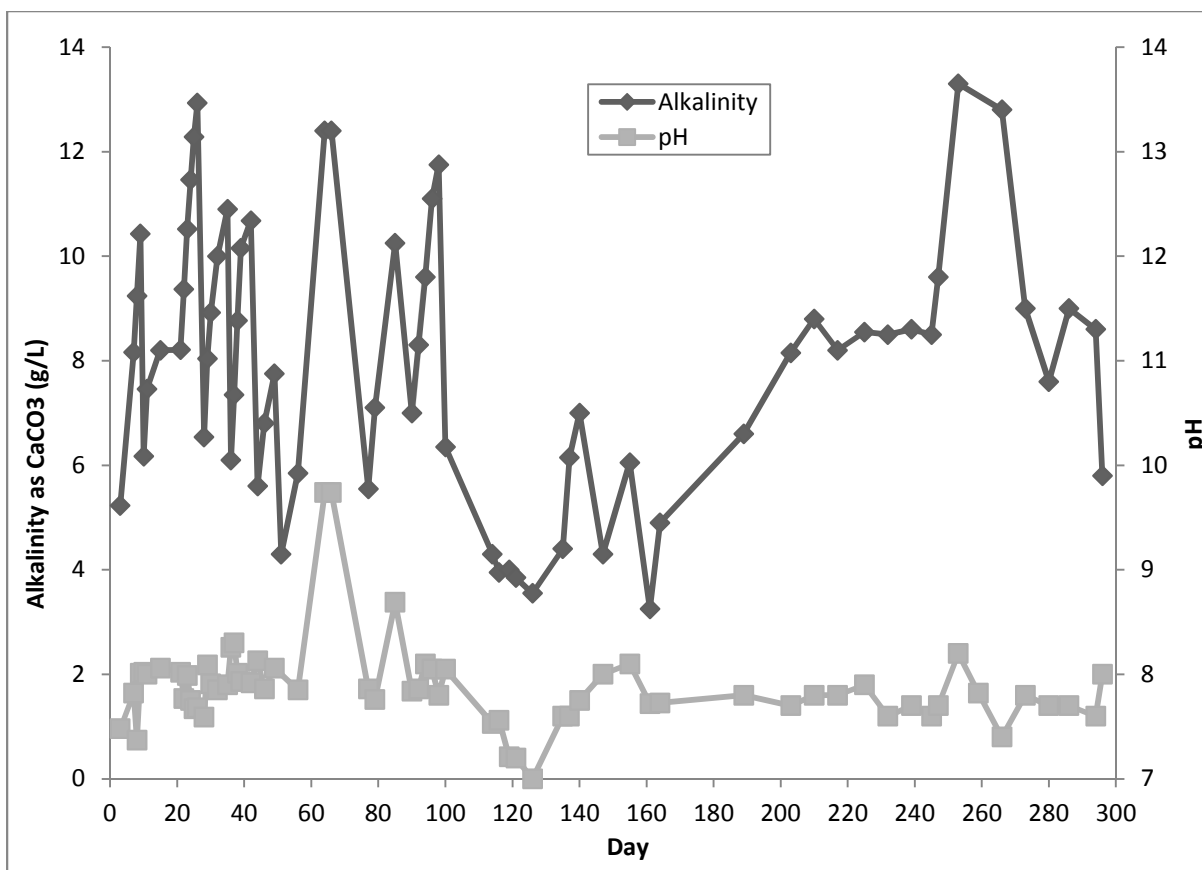


Figure 4-12 - pH readings and alkalinity concentration in effluent from reactor W2.

The cumulative effect of alkalinity addition is observed as peaks. Periodic media changes and metabolic activity removed excess alkalinity from solution (observed as valleys). W2 remained in the pH range of 7.9 ± 0.4 for the entire duration of the enrichment process. During the first and second stable phases, pH readings were 7.9 ± 0.2 and 7.7 ± 0.2 respectively. Alkalinity provided in the medium was deemed to be more than sufficient throughout the enrichment process. Even at the lowest alkalinity of around 3,300 mg/L as CaCO_3 , the pH remained at or slightly above the neutral range. Although exceeded twice, the pH remained well within the range preferred by methanogens of 6.5-8.5 for the majority of the process. The alkalinity fluctuated greatly during the enrichment process but remained at all times within the ideal range of 2,000 to 18,000 mg CaCO_3/L (Alvarez et al. 2010).

4.2.2.5 Ammonia-N and nitrate

Monitoring of ammonia-N and nitrate in the treated effluent started on day 45 of enrichment.

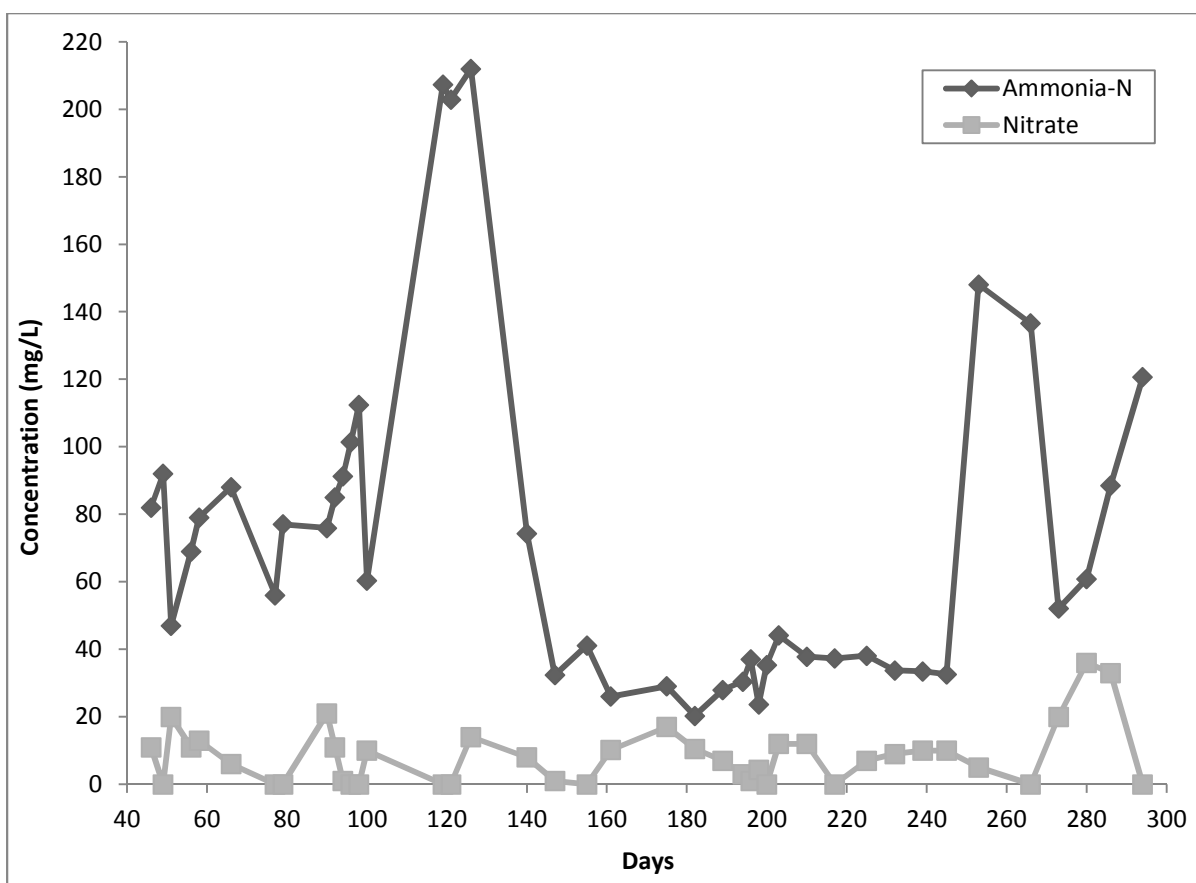


Figure 4-13 - Ammonia-N and nitrate concentrations in effluent from reactor W2.

The tartaric acid was consumed quickly and therefore there were periods of starvation in which the culture resorted to endogenous degradation. This resulted in an increase in cell protein breakdown that released ammonia-N and nitrate from the decaying biomass (peaks). Weekly media changes reduced the concentrations of ammonia-N and nitrate in the effluent (valleys). During the latter part of the first stable phase (Day 45-58), ammonia-N was detected in the range of 74 ± 17 mg/L and nitrate in the range of 11 ± 7 mg/L. A sharp increase in ammonia-N was observed between days 100 and 145, the same period in which a slight increase in methane production was also observed. It is possible that ammonia-N was accumulating as a result of increased metabolic activity. This is also supported by a decrease in residual alkalinity observed over the same time period; an increase in metabolic activity could lead to the accumulation of intermediate acidic compounds such as VFAs, which would consume alkalinity. The ammonia-N concentration dropped after around day 145 and

stabilized in the range of 35 ± 3 mg/L until day 245, after which a sharp increase was observed. Nitrate on the other hand, remained relatively steady in the range of 6 ± 6 mg/L between days 58 and 260. There was no nitrate in the tartaric acid or the nutrient medium added in the feed therefore the source of ammonia must be from cell protein breakdown.

During the second stable phase, ammonia-N appeared to fluctuate sharply to around 140 mg/L around day 260 before decreasing to 52 mg/L on day 273 and rising again toward the end of the enrichment process, ending at 121 mg/L on day 294. During the same time period, a significant rise in nitrate was observed to 36 mg/L on day 280 before dropping to below detectable limits at the end.

4.3 BMP1

The purpose of the first set of BMP tests was to learn the laboratory test methodology, and to explore the methane producing capabilities of the enriched inocula when fed the different organic wastes identified in the waste audit. The results of this test are discussed in the following sub-sections. Table 4-8 shows the mass of COD added to each batch and the resulting volumes of total gas and methane.

Table 4-8 - COD load and volumes of total gas and methane produced in BMP1.

Batch ID	Represents	COD load (mg)	Total gas produced (mL)	Total methane produced (mL)
Food	July	150	46 ± 3	9 ± 2
Lees	December	5170	149 ± 2	40 ± 3
Pomace	September	720	152 ± 4	54 ± 3
LP	November	1990	89 ± 2	15 ± 1
FL	May	2480	93 ± 2	14 ± 1

4.3.1 Total gas

The total gas production was monitored and Figure 4-14 presents the average cumulative volumes of total gas produced by the different batches.

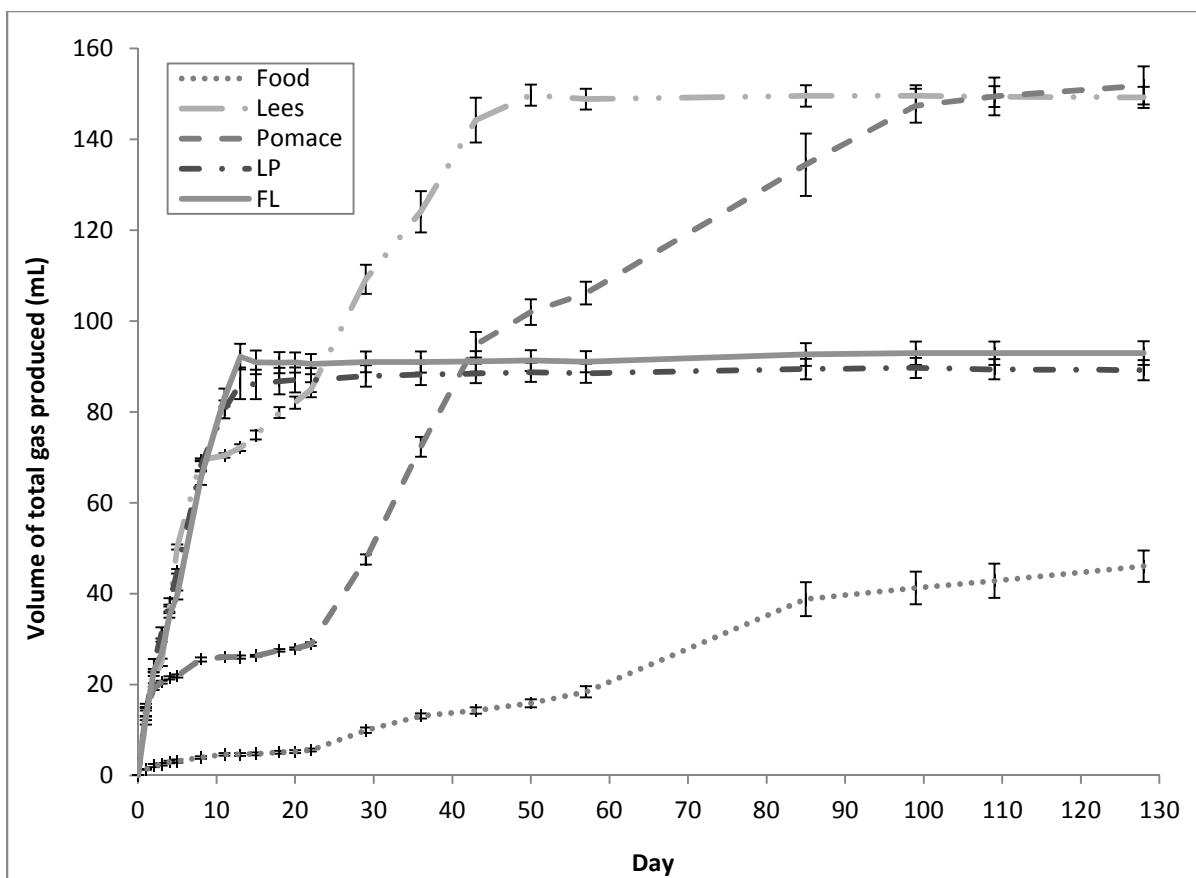


Figure 4-14 - Cumulative volumes of total gas produced over time in BMP1.

Each data point is the cumulative mean \pm 1 SD of 5 replicates.

The batch of cultures fed lees went through two stages of growth exhibited in the graph as two distinct plateaus and came to stop in activity by day 50. The first production stage was from day 0 to 8, in which 70 mL of total gas were produced, or 47% of the total. This was followed immediately by a slower but productive stage between days 8 and 50, accounting for an additional 80 mL of gas, adding up to 150 mL in total. Lees contained the highest COD load and as such was expected to produce the most gas.

The batch of cultures fed pomace also exhibited two distinct stages of growth, producing a total of 150 mL of total gas as well. The first productive stage was from days 0 to 8 where it produced 25 mL of gas or 17% of the total. This was followed by a period of inactivity, presumably a lag phase when bacteria were acclimatizing to inhibiting compounds and/or breaking down more complex compounds in the pomace. Activity picked up between days

20 and 50 during which an additional 100 mL of gas were produced; by the end of this period, 83% of the total gas had been produced. The gas production rate increased after day 50, producing an additional 25 mL of gas by day 110. The pomace COD load was approximately 1/7th the lees COD load, and yet that batch produced as much total gas as the one fed lees, though much more gradually.

The batch of cultures fed synthetic food had the lowest COD load and as expected produced much less total gas than the other batches; however, it did produce gas consistently throughout the experiment as complex compounds were broken down slowly. Three production stages can be observed in the graph, the first one occurring between days 0 and 22, at the end of which 6 mL or 13% of the total gas had been produced. The second productive stage occurred between days 22 and 50, at the end of which 16 mL of gas or 35% of the total gas had been produced. The third production stage went from day 50 to the end of the experiment at day 128, and the total gas produced reached 46 mL.

The batches of cultures fed food and lees (FL), and lees and pomace (LP) had very similar COD loads at 1990 and 2480 mg COD respectively and as could be expected exhibited very similar gas production patterns starting with rapid but short-lived periods of gas production ceasing by day 15 and producing a total of 90 mL and 88 mL of total gas respectively. No other stages of activity were observed from either batch from day 15 until the end of the experiment.

4.3.2 Methane

The methane production monitored and Figure 4-15 shows the cumulative volumes of methane produced over the course of the experiment. The methane production stages coincide with the total gas production stages as would be expected.

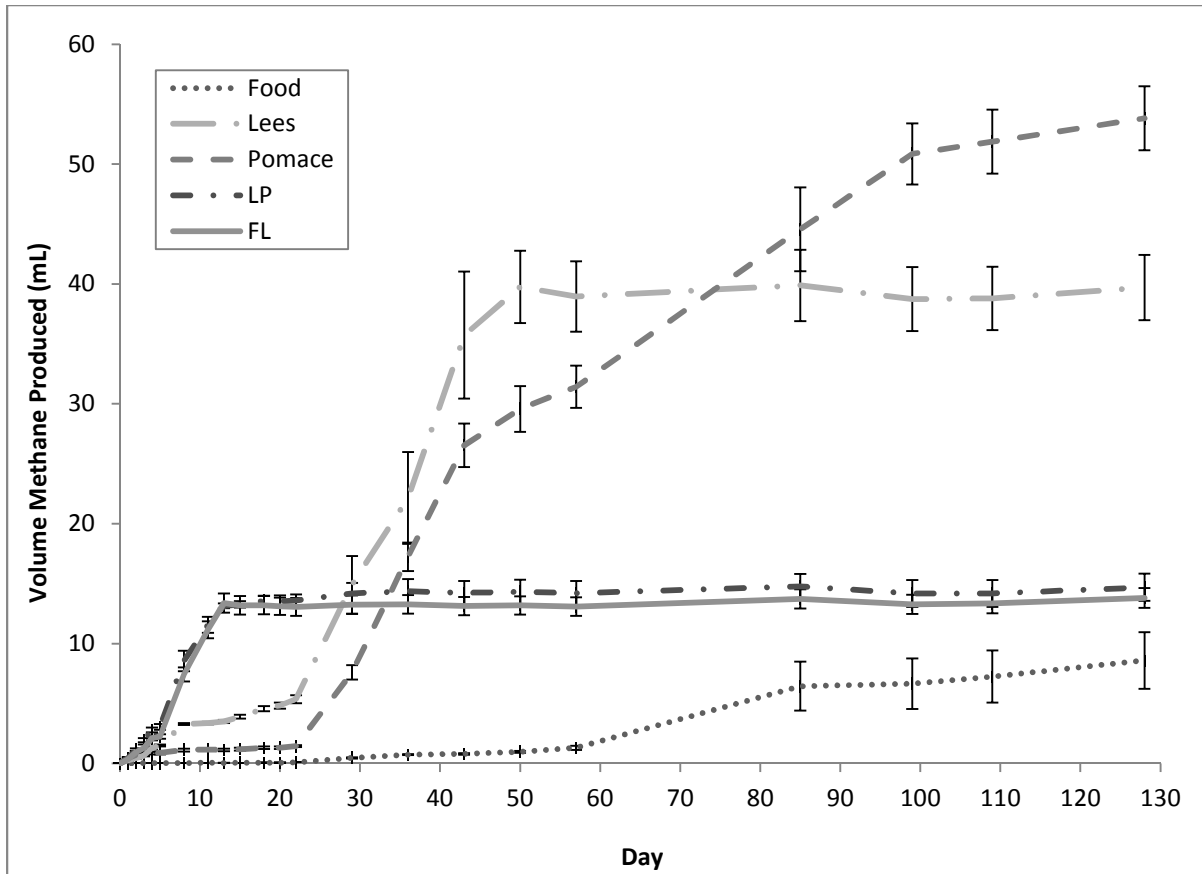


Figure 4-15 - Cumulative volumes of methane produced over time in BMP1.

Each data point is the cumulative mean ± 1 SD of 5 replicates.

Since each batch was fed different COD loads, the results were normalized by calculating the methane yields, which are defined here as the volume of methane produced per total mass of COD added and consumed. The resulting yields are presented in Table 4-9 below.

Table 4-9 - Summary of calculated performance parameters of BMP1 batches.

Batch ID	COD added (mg)	COD consumed (mg)	COD consumed (%)	Methane produced (mL CH ₄)	Methane yield (L CH ₄ /g COD added)	Yield efficiency (L CH ₄ /g COD consumed)
Food	150	10 \pm 0	7 \pm 2	9 \pm 2	0.06 \pm 0.01	0.90 \pm 0.20
Lees	5170	850 \pm 10	16 \pm 0	40 \pm 3	0.01 \pm 0.00	0.05 \pm 0.00
Pomace	720	230 \pm 60	32 \pm 8	54 \pm 3	0.08 \pm 0.00	0.23 \pm 0.01
LP	1990	280 \pm 30	14 \pm 1	15 \pm 1	0.01 \pm 0.00	0.05 \pm 0.00
FL	2480	340 \pm 0	14 \pm 0	14 \pm 1	0.01 \pm 0.00	0.04 \pm 0.00

All three batches that were fed lees in some form (Lees, LP and FL) performed quite poorly when methane yields are considered. The batches fed pomace produced the most gas and logically consumed the most mass of COD; however, it was outperformed in terms of yield efficiency by the batch fed synthetic food waste. The batch fed synthetic food performed poorly considering the COD added but performed exceptionally well, above the theoretical maximum of 0.35 L CH₄/g COD. This is most likely because the food contains more protein than the other substrates, which may bias the COD/CH₄ ratio. The nitrogenous compounds in the protein led to a greater CO₂ yield (see Equation A - Buswell formula).

To learn more about the inoculum itself, the F/M ratios of the BMP1 batches were considered along with the resulting methane yields and the time required achieving these.

Table 4-10 - F/M ratios, resulting methane yields and duration of productive stages.

Batch ID	F/M ratio	Methane production duration (days)	Methane produced (mL CH ₄)
Food	0.5	128	9
Pomace	3.3	110	54
LP	9.4	15	15
FL	11.7	15	14
Lees	24.6	50	40

Figure 4-16 shows the F/M ratios of batches fed Pomace, LP and FL plotted against their respective methane yields. The batch fed synthetic food was not considered in this analysis because it produced a very low volume of gas over an extended period of time, putting into question its repeatability given the variability inherent to biological processes, the effects of atmospheric pressure changes on total gas measurements (despite STP correction), and in the methane determination method using the GC-FID. The batch fed lees was ignored because while it produced significant volumes of gas it performed extremely poorly when methane yields are considered. It is believed the culture was overwhelmed by the volume of lees added. While the pH was not monitored throughout the experiment, it was measured at the end, registering an average of pH 4.5, well outside the range preferred by methanogens. It is believed this was due to the acidic nature of lees and possibly insufficient alkalinity added in

the medium at the beginning. Looking at the remaining data points, the data appears to suggest that an F/M ratio of 5 would provide measurable yields in a reasonable time frame (approximately 40 mL methane in roughly 80 days).

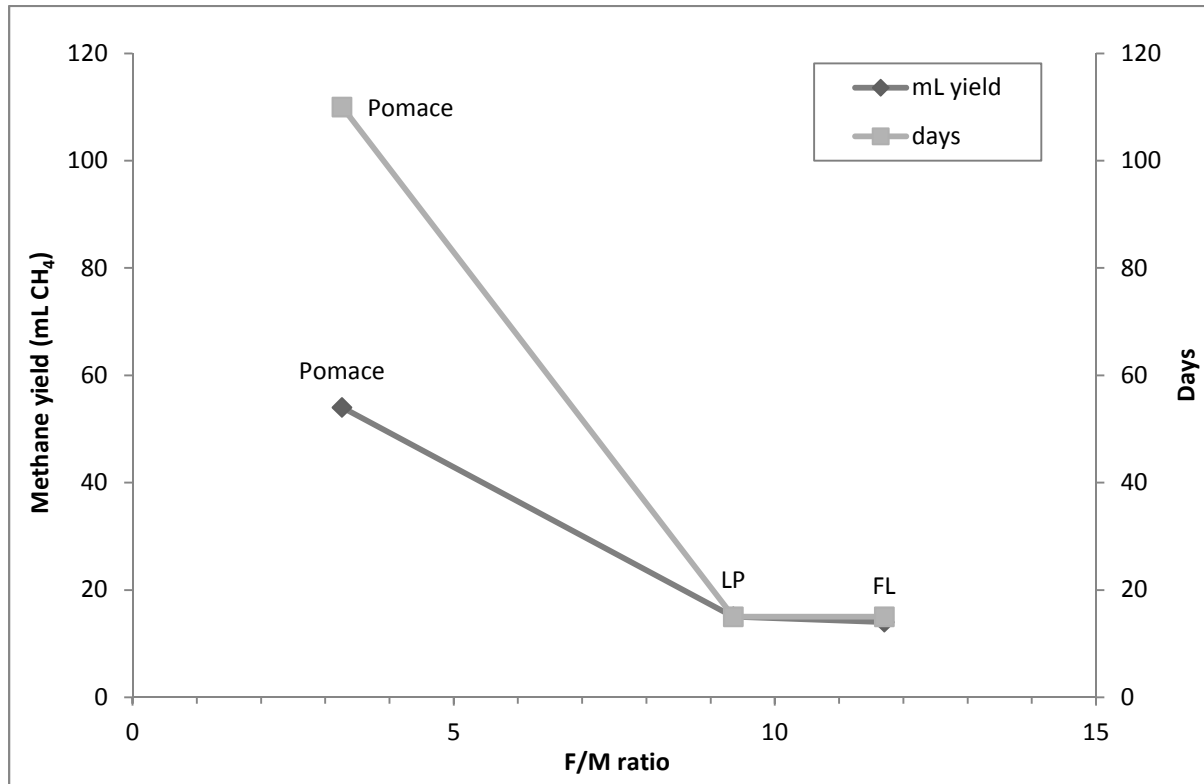


Figure 4-16 - Relationship between F/M ratios, methane yields and test durations.

These data was used as a rough indicator of the inoculum's capabilities and to design the BMP2 tests. Eighty days were considered reasonable as it was desirable to keep the test duration at less than 100 days. The actual yields and test duration of the BMP2 tests were not expected to be exactly as predicted; these data was merely used as a guide.

4.3.3 pH

The pH of each bottle was tested at the end of the experiment and the average for each batch calculated to determine whether or not the batches had moved outside the range preferred by methanogens (pH 6.5-8.5). All batches that contained lees were found to have a pH lower than 5.1, well outside the range preferred by methanogens. In the case of the batch fed only

lees, it is likely that the inoculum was overwhelmed with the concentration of COD added, thereby leading to an accumulation of VFAs, the consumption of the available alkalinity and the inhibition of methanogenesis. In batches LP and FL it is possible that lees consumed significant amounts of alkalinity and not enough was available to handle possible VFA accumulations later on. Batches fed pomace and synthetic food were found to have a pH of 7.7 ± 0.1 and 8.0 ± 0.1 respectively, slightly higher than ideal but well within the methanogen's preferred range. These two batches performed fairly well when considering the methane yield, although they also took the most time to produce it. The batches that went acidic did not contain enough alkalinity to support the degradation of the substrate added. To correct this (given the volume limitations of the bottles) a lower F/M ratio is required.

4.4 BMP2

The second set of BMP tests (BMP2) were conducted to assess impacts on methane yields of the culture when fed at different F/M ratios and by the co-treatment of lees and pomace in different combinations. Lees and pomace are the two largest waste streams of a winery and as such it was deemed important to study these two substrates. The tests consisted of the following batches, which were monitored for total gas and methane production for 90 days:

- Lees at four different F/M ratios: 2.2, 4.3, 6.5 and 8.7, referred to as L2, L4, L6 and L8 respectively.
- Pomace at four different F/M ratios: 3.1, 4.1, 5.2 and 6.2, referred to as P3, P4, P5 and P6 respectively.
- Lees and pomace in three different combinations: 25/75, 50/50 and 75/25 percent lees/pomace respectively, all at the same F/M ratio of 5.2.

4.4.1 Lees

4.4.1.1 Total gas

The total gas produced by the cultures was monitored throughout the experiment as explained in the methods section. Statistical analysis showed there had been no significant change in total gas produced since day 42 in L2 ($P = 0.798$, $N = 32$), day 70 in L4 ($P = 0.689$, $N = 12$), day 75 in L6 ($P = 0.443$, $N = 8$) and day 70 in L8 ($P = 0.103$, $N = 12$) meaning

biological degradation of the lees had stopped by day 75 at the latest. Table 4-11 summarizes the active stage duration and total gas produced for each batch. Figure 4-17 shows the cumulative volume of total gas calculated throughout the experiment. These data has been corrected for the control blank.

Table 4-11 - Summary of total gas produced by cultures fed lees.

Batch	Active stage (days)	Total gas produced (mL)	% of total gas produced in days 0-5
L2	42	65 ± 5	62
L4	70	118 ± 4	59
L6	75	145 ± 6	57
L8	70	162 ± 7	60

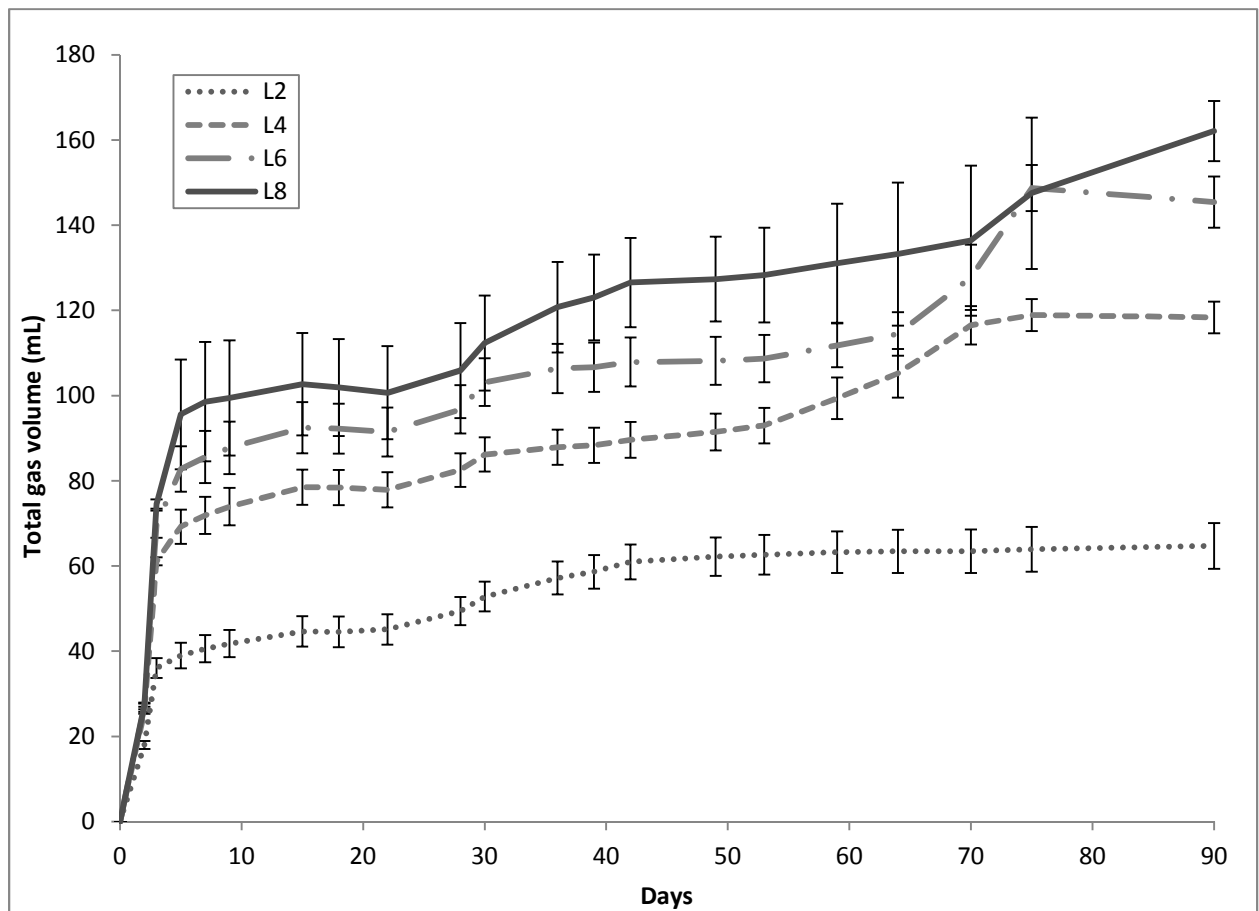


Figure 4-17 - Cumulative volumes of total gas produced by cultures fed lees.

Each data point is the cumulative mean ± 1 SD of 4 replicates.

As can be observed in Figure 4-17, all four batches had a very similar gas production pattern, starting with a sharp gas production rate at the beginning, yielding most of the total gas produced in the first few days and gradually producing the rest over several weeks. Batch L2 did not experience a late active phase from day 70 onward as the other three batches experienced. Statistical analysis showed L2 and L4 were significantly different than the rest ($P < 0.001$, $N = 48$), and there was no statistically significant difference between L6 and L8 ($P = 0.196$, $N = 24$).

4.4.1.2 Methane

The methane production was monitored and Figure 4-18 shows the cumulative volumes of methane produced over the extent of the BMP2 experiment. The periods of methane production roughly coincide with the total gas production stages as would be expected.

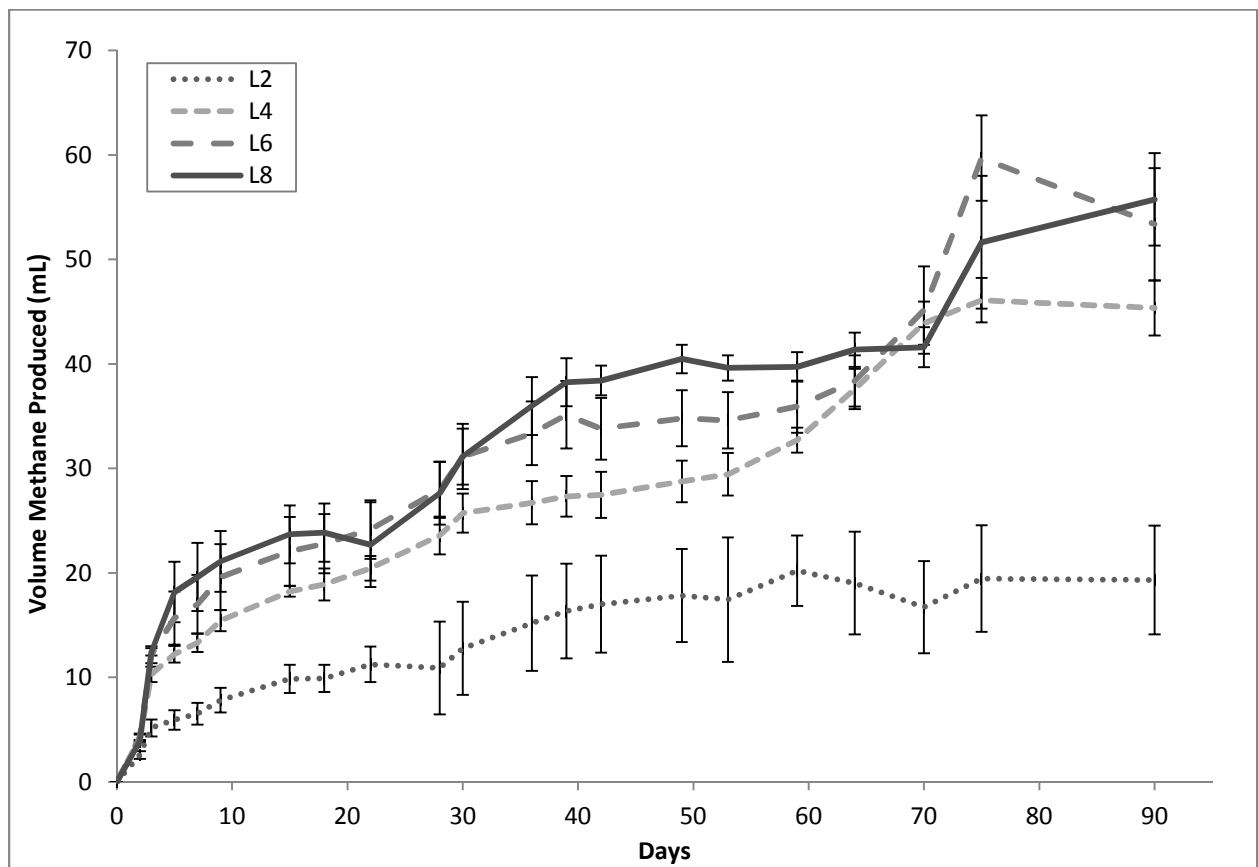


Figure 4-18 - Cumulative volume of methane produced over time.

Each data point is the cumulative mean \pm 1 SD of 4 replicates.

There was a statistically significant difference between the batch means ($P = <0.001$, $N = 44$). L2, L4 and L6 were found to be statistically different from each other; however, there was no statistically significant difference between L6 and L8 ($P = 0.363$, $N = 23$). The similarity between L6 and L8 was also determined in the total gas results.

A summary table of results is presented below containing the duration of the active stage, COD consumed, methane percentage, methane produced, methane yield and yield efficiency for each condition. In order to properly compare the different batches, methane yields were normalized and are presented as methane yielded per mass of COD added and COD consumed. The error bars for batch L2 are larger and reflect the issues associated with low gas producing samples, such as greater impacts on variability from atmospheric pressure and slight losses during sampling.

Table 4-12 - Results of COD consumption and methane production batches fed lees.

Batch ID	COD added (mg)	COD consumed (mg)	COD consumed (%)	Methane produced (mL CH ₄)	Methane yield (L CH ₄ /g COD added)	Yield efficiency (L CH ₄ /g COD consumed)
L2	400	130 ± 10	32	19 ± 5	0.05 ± 0.01	0.15 ± 0.04
L4	500	180 ± 10	36	45 ± 3	0.09 ± 0.01	0.25 ± 0.01
L6	610	240 ± 10	39	53 ± 5	0.09 ± 0.01	0.23 ± 0.02
L8	720	280 ± 50	40	56 ± 4	0.08 ± 0.01	0.20 ± 0.02

When looking at the methane yields per mass of COD added, all four batches appear to underperform as they are all lower than 0.35 L/g COD. However, when the actual COD consumed is taken into consideration, all four batches perform much better with L4 and L6 performing fairly similarly. Figure 4-19 was prepared to show the relationship between the F/M ratio and efficiency of each batch in converting COD to methane.

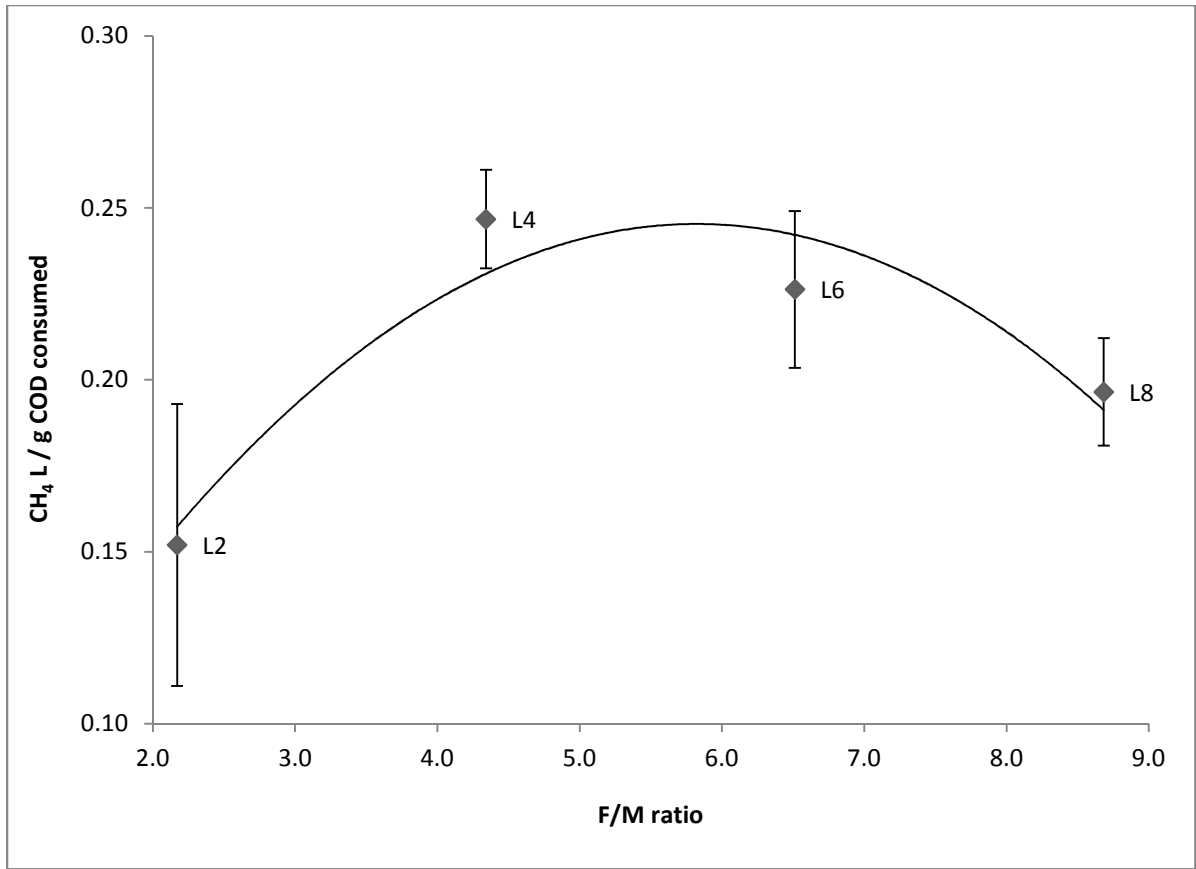


Figure 4-19 - Efficiency of cultures fed lees in converting COD to methane.

Statistical analysis of the data presented in Figure 4-19 showed there was a significant difference between methane yield efficiencies of the batches ($P = 0.003$, $N = 15$). Of the ratios tested, an F/M ratio of 4.3 (L4) yielded the most methane per gram of COD consumed. These data appears to support the hypothesis that an F/M ratio of around 5 is optimal in terms of methane production.

One litre of pure methane gas can produce 39.76 kJ of energy.

$$\frac{\text{mol CH}_4}{22.4 \text{ L CH}_4} \times \frac{890.8 \text{ kJ}}{\text{mol CH}_4} = 39.76 \text{ kJ/L CH}_4$$

The best average yield observed was from L4 at 0.25 L CH₄/g COD consumed, which translates to 9.94 kJ/g COD consumed. Pure lees were in the range of 211 g COD/L, and L4

consumed 36% of the COD available, therefore it could be said that 76 g COD from every litre of pure lees could be converted to methane, which translates to 755 kJ/L of pure lees, assuming the conditions in L4 could be replicated at a larger scale. The energy yields presented here are only applicable to the anaerobic cultures used in this research and are not mean to represent typical energy yields from these types of wastes. Winery waste production is highly variable and as such the reactor would experience periods of feast and famine, the effect of these conditions over the long term health of the reactor need to be assessed at a larger scale before making further inferences on yields.

4.4.1.3 Ammonia-N and nitrate

Filtered effluent samples were tested for ammonia-N and nitrate at the end of the experiment. L2, L4, L6 and L8 were found to have ammonia-N in concentrations of 19 ± 1 mg/L, 16 ± 1 mg/L, 18 ± 2 mg/L, and 16 ± 1 mg/L respectively.

All four batches had low concentrations of ammonia-N by the end of the experiment, as concentrations over 500 mg/L are considered ideal for methanogens. All four batches had ceased in total gas production, indicating conditions had either become inhospitable due to the lack of food or the accumulation of inhibiting substances. In this case, a lack of ammonia-N could be one of the factors affecting methanogenesis, though most likely not the main reason why gas production stopped. It is interesting to note that although each batch contained radically different loads of COD, they ended up in the same range (~ 17 mg/L). Nitrate in all batches was below detectable limits.

4.4.1.4 pH

The pH of the cultures was monitored every 15-20 days. The lees batches remained within the pH range preferred by methanogens throughout the experiment (6.5-8.5). As was expected, the pH observed at the beginning was inversely related to the concentration of lees added. As the experiment progressed the batches moved towards a pH range between 7.5 and 8.0 as organic acids were consumed.

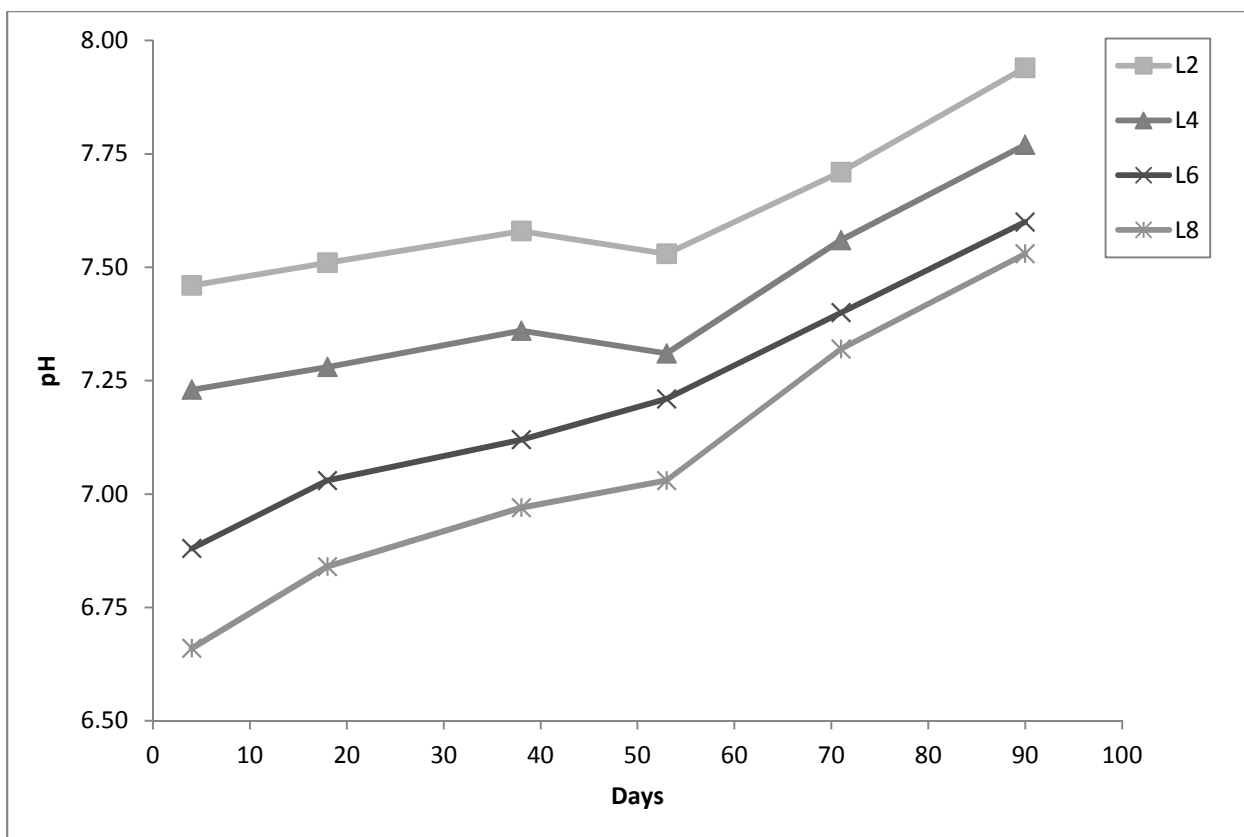


Figure 4-20 - pH of batches fed lees over the course of the BMP2 test.

pH of composite effluent samples from each batch on days 4, 18, 38, 53, 71 and 90.

After day 53, the pH in all four batches increased. This may be due to the lack of significant organic acid production and accumulation of basic components that increased pH. The pH meter was calibrated before each set of readings, therefore instrument error is not suspected for the drop observed in L2 and L4 on day 53.

4.4.1.5 VFA and tartaric acid

The inoculum was found to have significant concentrations of acetate and propionate, most likely left over from the enrichment process. Given its lower efficiency at converting substrate to methane, culture W1 was most likely responsible for the residual acetate and propionate found in the inoculum composite.

The inoculum and the waste substrates (lees and pomace) were analyzed for VFA and tartaric acid content and the results are summarized in Table 4-13 below. The pomace suspension did not contain any detectable soluble volatile organic acids, however, tartrate was found in high concentrations (700 mg/L). Lees was found to contain significant concentrations of acetate, propionate and tartaric acid, with very low but detectable levels of hexanoate.

Table 4-13 - VFA and tartrate content of inoculum and waste substrates for BMP2.

Organic acid	Inoculum (mg/L)	Lees (mg/L)	Pomace (mg/L)
Acetate	3050	1740	BDL
Propionate	1170	710	BDL
Tartrate	10	620	700

BDL – Below detection limit

VFAs and tartrate concentrations served as indicators of metabolic activity in the bottles. A healthy methanogenic system produces organic acids through fermentation that are then used by acetogenic bacteria to produce acetate, which methanogens then use to produce methane. Inhibiting conditions may occur when these intermediate products accumulate at a faster rate than methanogens can consume them. The lack of acetate in all batches at the end of the experiment indicates degradation had most likely stopped as acetic acid had been consumed and converted to methane and carbon dioxide. As expected, an increase the concentration of organic acids through a higher F/M ratio resulted in increased methane production.

Figure 4-21 below shows the results of analyses of samples corresponding to L2. Two main observations can be made about the batch fed lees:

- VFAs: formate/butyrate, valerate, hexanoate and heptanoate were not detected in any of the samples collected.
- Tartaric acid was consumed in all samples before day 18.

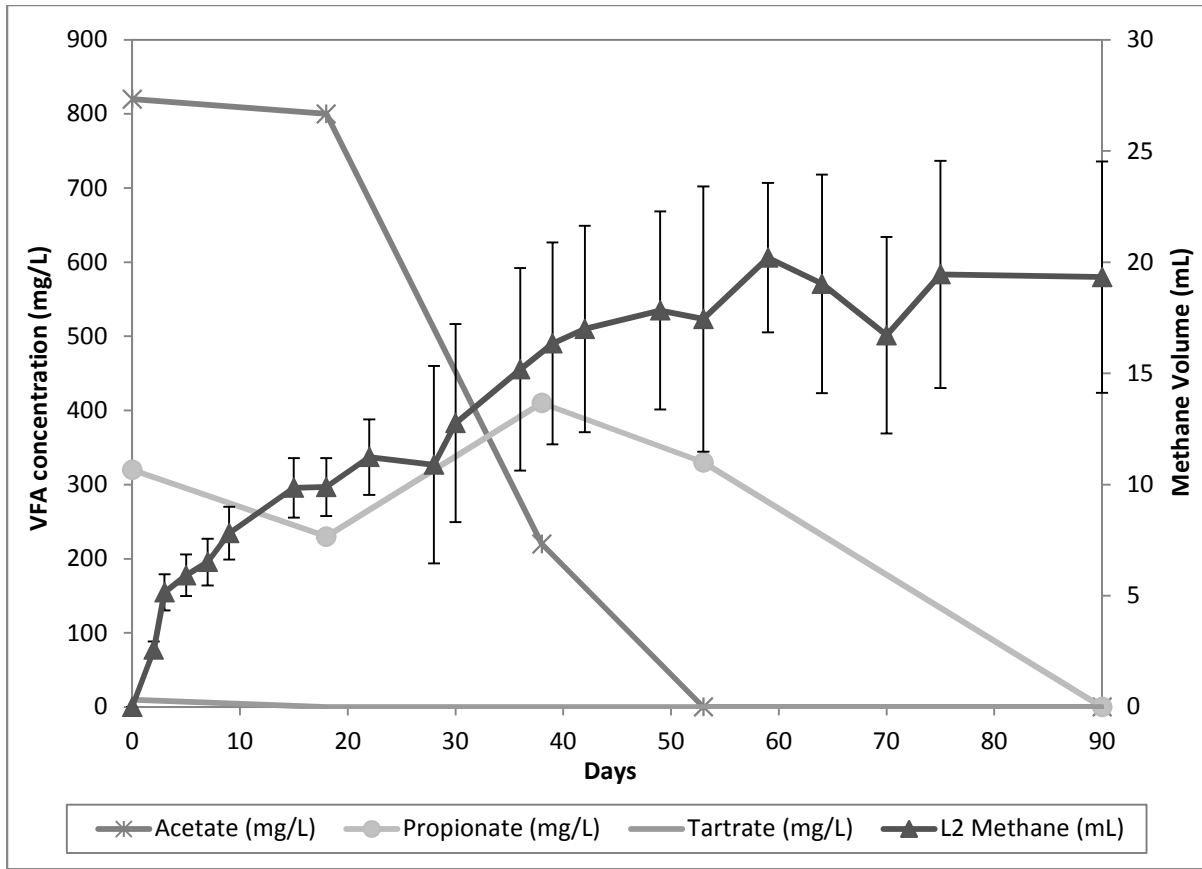


Figure 4-21 - VFA and tartaric acid concentrations vs. methane production in L2.

Acetate and propionate were the only two VFAs found in significant concentrations in L2. Methane production ceased after acetate had been consumed. Propionate was present in significant concentrations, increasing in concentration the first 40 days before declining by day 53 and not detected by day 90. This pattern was expected as it was produced first by fermentation processes and ultimately converted to acetate and converted to methane. L2 was the only batch that exhibited the depletion of propionate. At no point did it reach toxic concentrations of over 1,000 mg/L. The nuisance odour threshold of 230 mg/L was not exceeded by propionate by the end of the experiment, meaning disposal in open air of this batch would not present a problem.

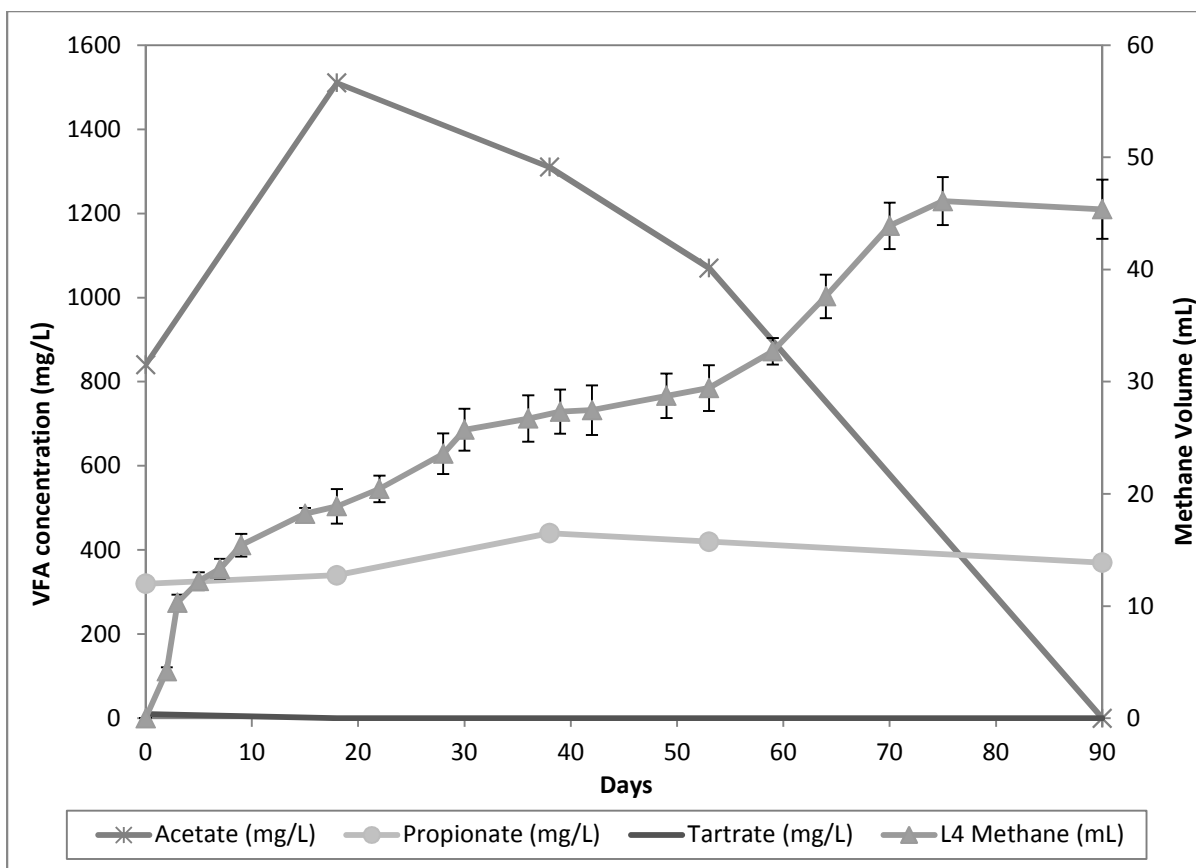


Figure 4-22 - VFA and tartaric acid concentrations vs. methane production in L4.

Acetate, as expected, decreased in concentration in L4 as the experiment progressed and the substrate was consumed. Propionate appeared to remain relatively stable throughout the experiment at around 400 mg/L. This was unexpected as it should have been consumed. It is quite possible that a surplus of acetate was present and aceticlastic bacteria preferred it over other organic acids present. The nuisance odour threshold of 230 mg/L was exceeded by propionate at the end of the experiment, meaning disposal in open air of this batch would present a problem. Methane production continued until at least day 75, and while VFA concentration was not determined on that specific day, it is safe to assume acetate was exhausted at the same time.

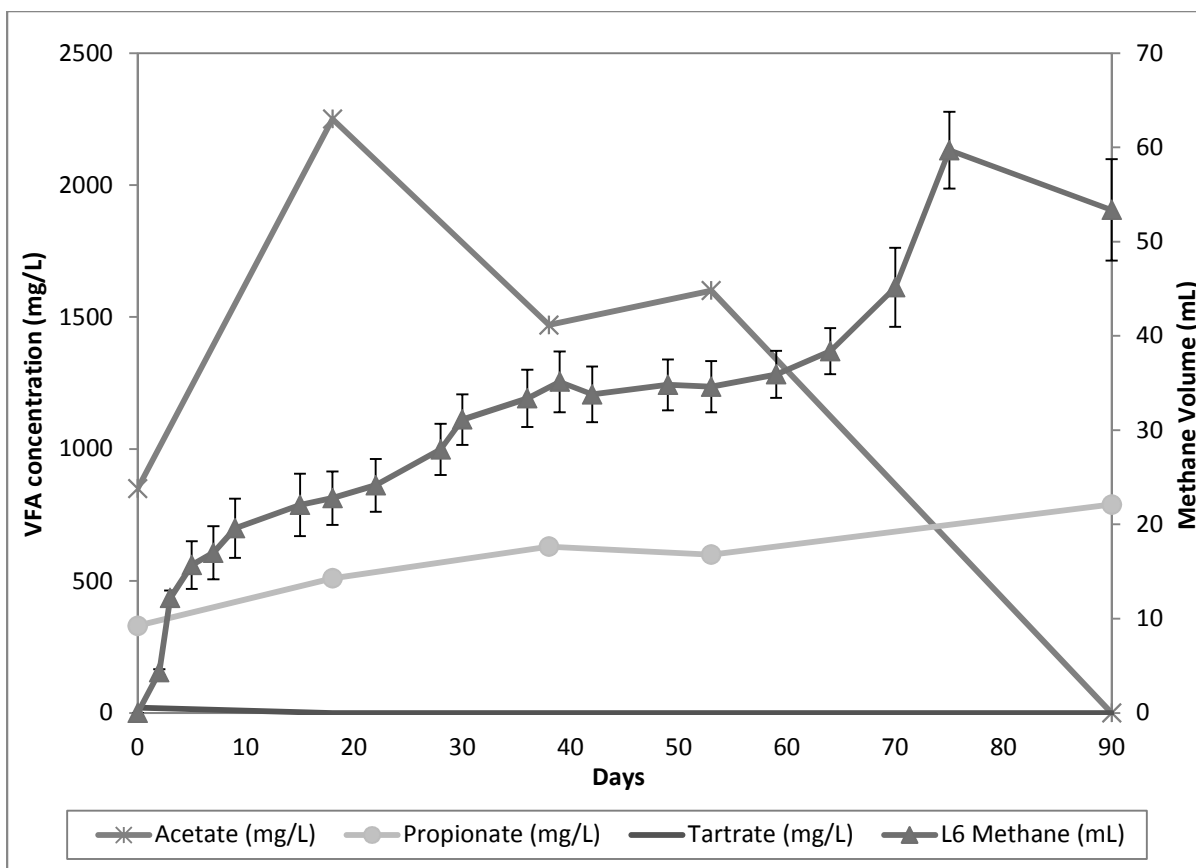


Figure 4-23 - VFA and tartaric acid concentrations vs. methane production in L6.

Acetate, as expected, decreased in concentration in L6 as the experiment progressed and the substrate was consumed. Propionate appeared to gradually accumulate throughout the experiment. Propionate can be toxic at concentrations over 1,000 mg/L and although this concentration was not exceeded, it was significant reaching ~ 800 mg/L. It is reasonable to assume that this had some negative effect on the performance of the culture. The nuisance odour threshold of 230 mg/L was exceeded by propionate at the end of the experiment, meaning disposal in open air of this batch would present a problem in this regard. Methane production continued until at least day 75, and while VFA concentration was not determined on that specific day, it is safe to assume acetate was exhausted at the same time.

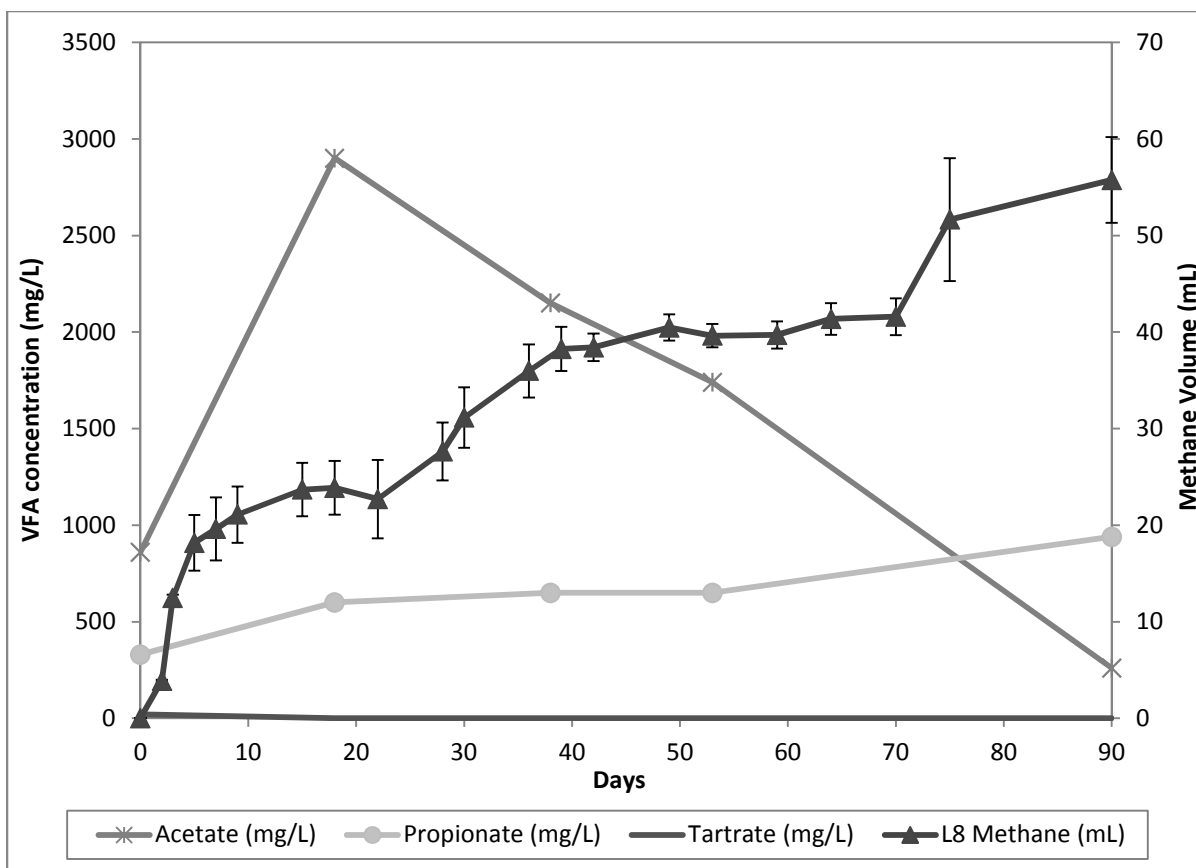


Figure 4-24 - VFA and tartaric acid concentrations vs. methane production in L8.

Acetate, as expected, decreased in concentration in L8 as the experiment progressed and the substrate was consumed. Propionate appeared to gradually accumulate throughout the experiment. It can be toxic at concentrations over 1,000 mg/L, a concentration that was almost reached and it is reasonable to assume that this had some negative effect on the performance of the culture. The nuisance odour threshold of 230 mg/L was exceeded by acetate and propionate at the end of the experiment, meaning disposal in open air of this batch would present a problem. Towards the end of the experiment, methane production was on an increasing trend despite the high variability observed. If the experiment had been extended it is quite possible methane production would have continued as some acetate remained in the bottles by day 90.

4.4.2 Pomace

4.4.2.1 Total gas

The total gas produced by the cultures was monitored throughout the experiment as explained in the methods section. Statistical analysis showed there had been no significant change in total gas produced since day 42 in P3 ($P = 0.700$, $N = 32$), day 50 in P4 ($P = 0.613$, $N = 12$), day 64 in P5 ($P = 0.496$, $N = 16$) and day 64 in P6 ($P = 0.478$, $N = 13$) meaning biological degradation of the lees had stopped by day 64 at the latest. Table 4-14 summarizes the active stage duration and total gas produced for each batch.

Table 4-14 - Summary of total gas produced by cultures fed pomace.

Batch	Active stages (days)	Total gas produced (mL)	% of total gas produced in days 0-5
P3	42	31 ± 3	35
P4	50	40 ± 1	38
P5	55	49 ± 2	43
P6	64	59 ± 2	39

Figure 4-25 below shows the cumulative volume of total gas calculated throughout the experiment. These data has been corrected for the control blank.

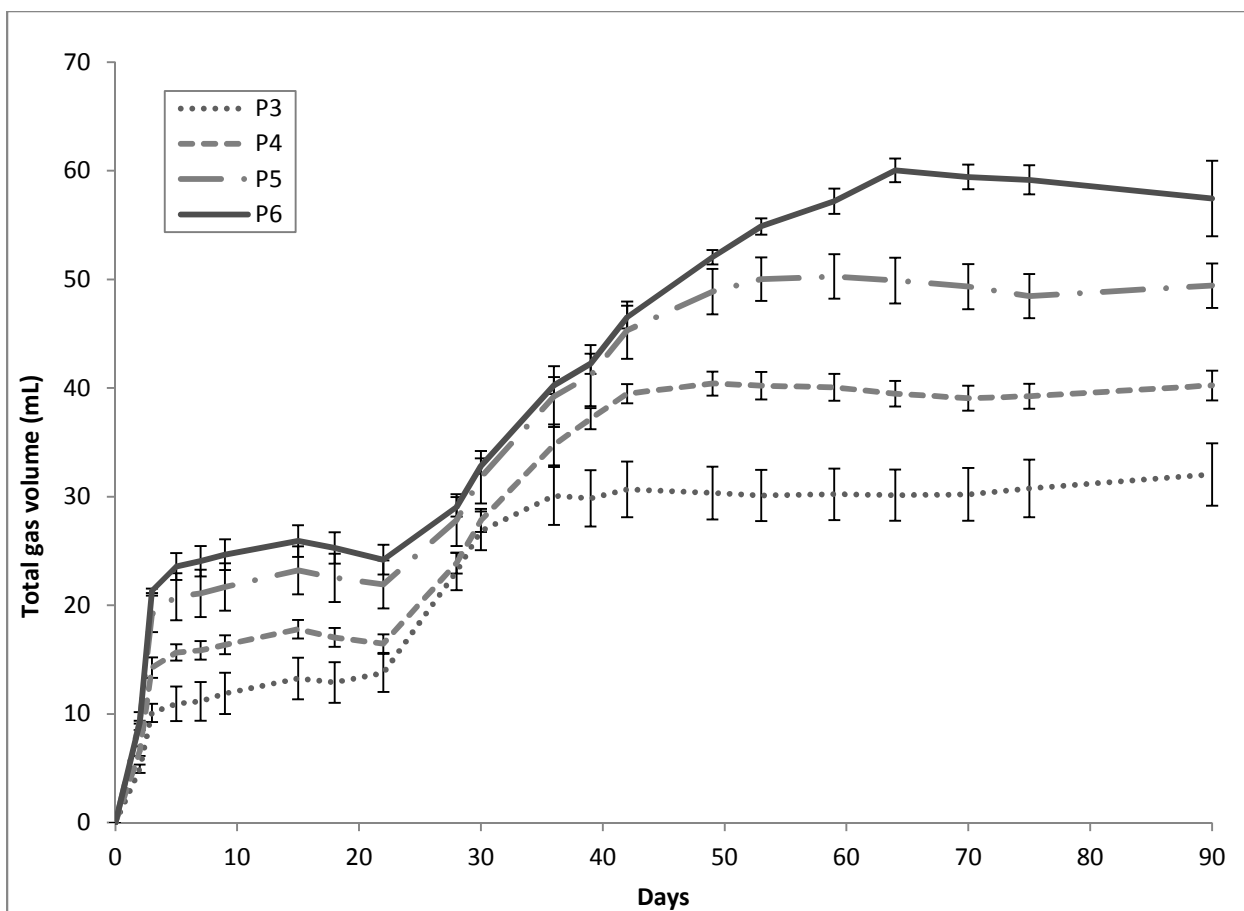


Figure 4-25 - Cumulative volumes of total gas produced by cultures fed pomace.

Each data point is the cumulative mean ± 1 SD of 4 replicates.

All four batches fed pomace had a very similar gas production pattern, starting with a sharp gas production rate at the beginning, but producing only about 40% of the total gas before entering a lag phase after day 5, where marginal volumes of total gas were produced. Activity picked up after day 22 but at a slower rate than the initial stage. The low yields of total gas and methane observed were expected. Pomace is a complex substrate with large insoluble polymers that would yield less total gas and methane per mass of COD compared to lees. Additionally, inoculum culture W1 was enriched on lees and not pomace, requiring the inoculum to adapt to the pomace before acquiring the ability to degrade the more complex components effectively. The purpose of this degradation was to convert the readily biodegradable components in pomace to methane and the remaining COD would hypothetically be composted.

It should be noted that pomace was not used as an enrichment substrate due to the complications this would have presented. The apparatus components such as hoses and tubes could have been plugged by the solids in the pomace suspension, and rectifying such an issue would have required interruption of enrichment. Statistical analysis showed there was a significant statistical difference between the mean volumes of total gas produced by the four batches ($P < 0.001$, $N = 48$). A pairwise comparison confirmed this (Tukey test).

4.4.2.2 Methane

The methane production was monitored and Figure 4-26 shows the cumulative volumes of methane produced over the course of the experiment. The periods of methane production did not coincide with first major stage of total gas production as would be expected. While total gas was produced in significant volumes between days 0-5, methane production was marginal. This could be due to the presence of inhibiting compounds that affect methanogens in particular. The intermediate fermentation carried out by acidogens produces carbon dioxide, and this gas was the main contributor to the total gas given that methane concentrations were so low during this time period. As the inoculum acclimatized itself to pomace and its components, it improved in terms of methane production. This is seen from day 22 onward, when methane started to be produced in more significant quantities, contributing increasingly to the total gas produced.

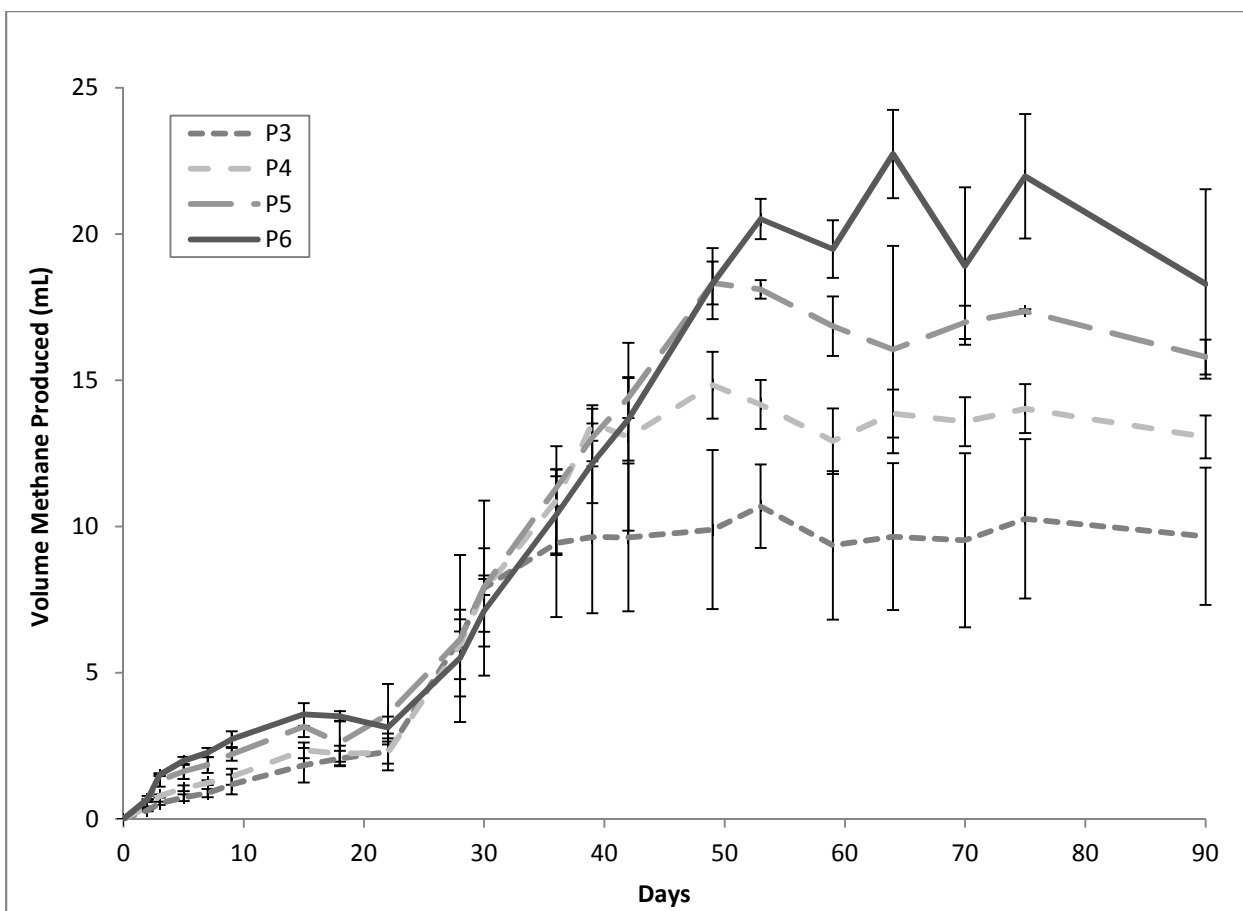


Figure 4-26 - Cumulative volume of methane produced over time.

Each data point is the cumulative mean \pm 1 SD of 4 replicates.

In terms of methane produced, there was a statistically significant difference between all four batches ($P = <0.001$, $N = 42$), however, pairwise comparison (t-test) showed there was no statistically significant difference between P5 and P6 ($P = 0.069$, $N = 23$). This is different than what was found in the total gas results where all four batches were significantly different than one another. It is quite possible that the high variability observed in P6 after around day 55 was due to a loss of gas from the bottle during pressure tests and/or headspace gas sample collection; this could have increased the error enough to make it statistically similar to P5, after all, the P value was 0.069, fairly close to the 0.050 threshold. The methane content (as percent of the total gas) was suspected as a possible explanation for this but statistical analysis showed there was no significant difference between the two batches in this regard either ($P = 0.995$, $N = 24$).

As was the case in the cumulative methane graph for the cultures fed lees, some dips are visible, especially at the later stages. As mentioned before, these were at times due to the control blanks outperforming a certain batch on a certain day, or highly variable methane percentage rates were calculated in the GC measurement procedure, perhaps due to human or equipment error. To minimize this error, standards were run through the GC every time methane percentages were measured, however, the variability could not be minimized more than what is presented here. Though odd, the general increasing trend can be observed in all batches.

A summary table of results is presented below containing the duration of the active stage, COD consumed, methane percentage, methane produced, methane yield and yield efficiency for each batch. In order to properly compare the different batches, methane yields were normalized and are presented as methane yielded per mass of COD added and consumed.

Table 4-15 - COD consumption and methane production of batches fed pomace.

Batch ID	COD added (mg)	COD consumed (mg)	COD consumed (%)	Methane produced (mL CH ₄)	Methane yield (L CH ₄ /g COD added)	Yield efficiency (L CH ₄ /g COD consumed)
P3	440	119 ± 16	27	10 ± 2	0.02 ± 0.01	0.08 ± 0.02
P4	490	142 ± 23	29	13 ± 1	0.03 ± 0.00	0.09 ± 0.01
P5	550	158 ± 22	29	16 ± 1	0.03 ± 0.00	0.10 ± 0.00
P6	600	200 ± 13	34	18 ± 3	0.03 ± 0.01	0.09 ± 0.02

At first glance, methane yields of all four batches appear to underperform as they were all much lower than the theoretical maximum of 0.35 L/g COD, however, low yields from pomace were expected because as mentioned before, pomace contains a larger insoluble fraction than lees. Figure 4-27 was prepared to show the relationship between the F/M ratio and efficiency of each batch in converting COD to methane.

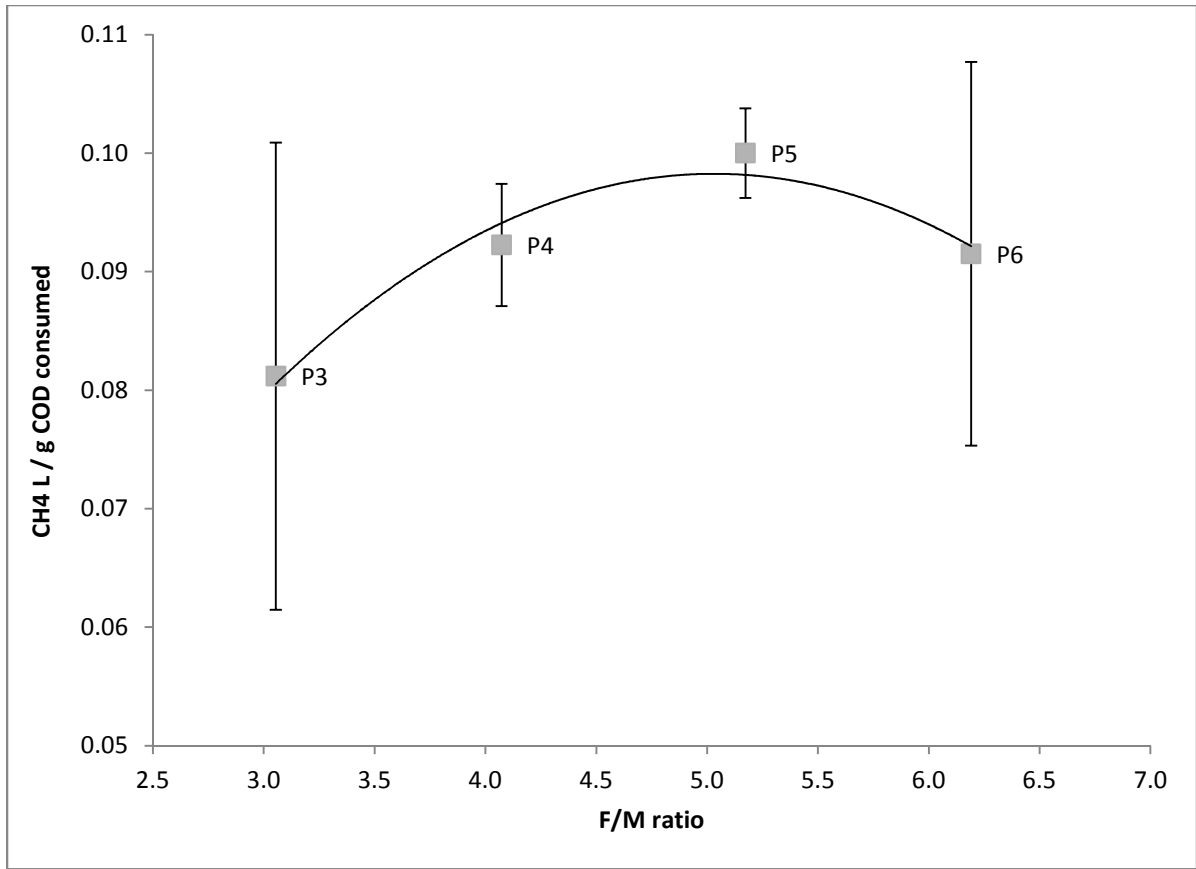


Figure 4-27 - Efficiency of cultures fed pomace in converting COD to methane.

At first glance, an F/M ratio of 5.2 (P5) appeared to yield the most methane per gram of COD consumed, however, statistical analysis showed that there was no significant difference between the four batches in terms of methane yielded per gram of COD consumed ($P = 0.433$, $N = 15$). Pairwise comparisons confirmed this (Tukey test).

As stated earlier, one litre of pure methane gas can produce 39.76 kJ of energy. The best average yield observed in the pomace batches was from P5 at 0.10 L CH₄/g COD consumed, which translates to 3.98 kJ/g COD consumed. Pomace suspension for these batches was in the range of 20 g COD/L, and P5 consumed 29% of the COD available, therefore it could be said that 24 g COD from every litre of pure lees could be converted to methane, which translates to 96 kJ/L of pomace suspension, assuming the conditions in P5 could be replicated at a larger scale. The pomace suspension prepared for this batch consisted of 24 g of fresh red grape pomace per litre of suspension; therefore the energy yield could also be

inferred to be 4 kJ/g of pomace. The energy yields presented here are only applicable to the anaerobic cultures used in this research and are not mean to represent typical energy yields from these types of wastes. Winery waste production is highly variable and as such the reactor would experience periods of feast and famine, the effect of these conditions need to be assessed at a larger scale before making further inferences on yields.

4.4.2.3 Ammonia-N and nitrate

Filtered effluent samples were tested for ammonia-N and nitrate at the end of the experiment. P3, P4, P6 and P6 were found to have ammonia-N concentrations of 17 ± 1 mg/L, 16 ± 1 mg/L, 17 ± 2 mg/L, and 17 ± 2 mg/L respectively. All four batches had low concentrations of ammonia-N by the end of the test considering 500 mg/L is an ideal concentration for methanogens. By day 65 all batches had stopped producing gas. The low concentrations observed here may be indicative of low concentrations throughout the experiment. Although each batch contained radically different loads of waste substrate, they ended up in the same range (~17 mg/L). Nitrate levels in all four batches were below detection. The effluent was not tested for phosphorus but it is reasonable to expect significantly lower concentrations than nitrate, which in this case were negligible.

4.4.2.4 pH

Composite samples from each batch were monitored every 15-20 days and results are presented in Figure 4-28. The pomace batches remained within the pH range preferred by methanogens throughout the experiment (6.5-8.5). The more pomace added the more acidic the final culture. For the first 40 days it appeared as if acids were being produced, driving the pH down, however, after day 53 all four batches increased in pH. The change in trend may be due to an increase in the rate of methanogenesis as more soluble substrates were made available by hydrolysis and acetogenesis of organic compounds in the pomace.

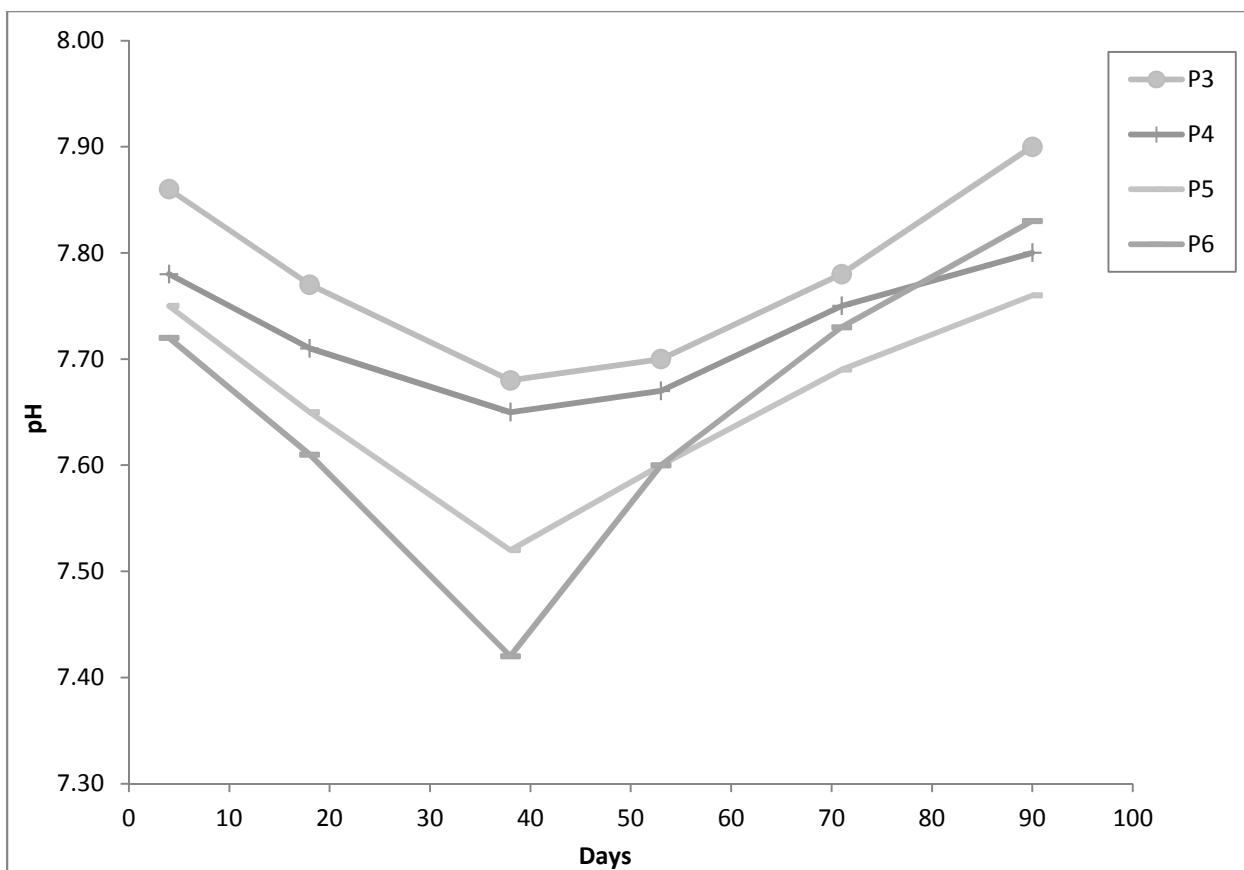


Figure 4-28 - Graph showing pH of batches fed pomace.

pH of composite effluent samples from each batch on days 4, 18, 38, 53, 71 and 90.

4.4.2.5 VFA and tartaric acid

Tracking of VFA and tartaric acid served as an indication of metabolic activity. The lack of acetate in all batches at the end of the experiment indicates degradation had most likely stopped as acetic acid had been consumed and converted to methane and carbon dioxide. As expected, an increase in the addition of organic acids (i.e., more lees) resulted in increased methane production. Acetate and propionate were the only two VFAs found in significant concentrations. As expected, acetate concentration appears to deplete at the same time gas production ceased though more data is needed to make a more conclusive statement on this. The blanks did not show significant concentrations of acetate at any point in the experiment meaning it was introduced in the waste. Tartrate is a significant component of pomace (700 mg/L) but was not found in significant concentrations. It is most likely that it was consumed before the first samples were collected on day 18.

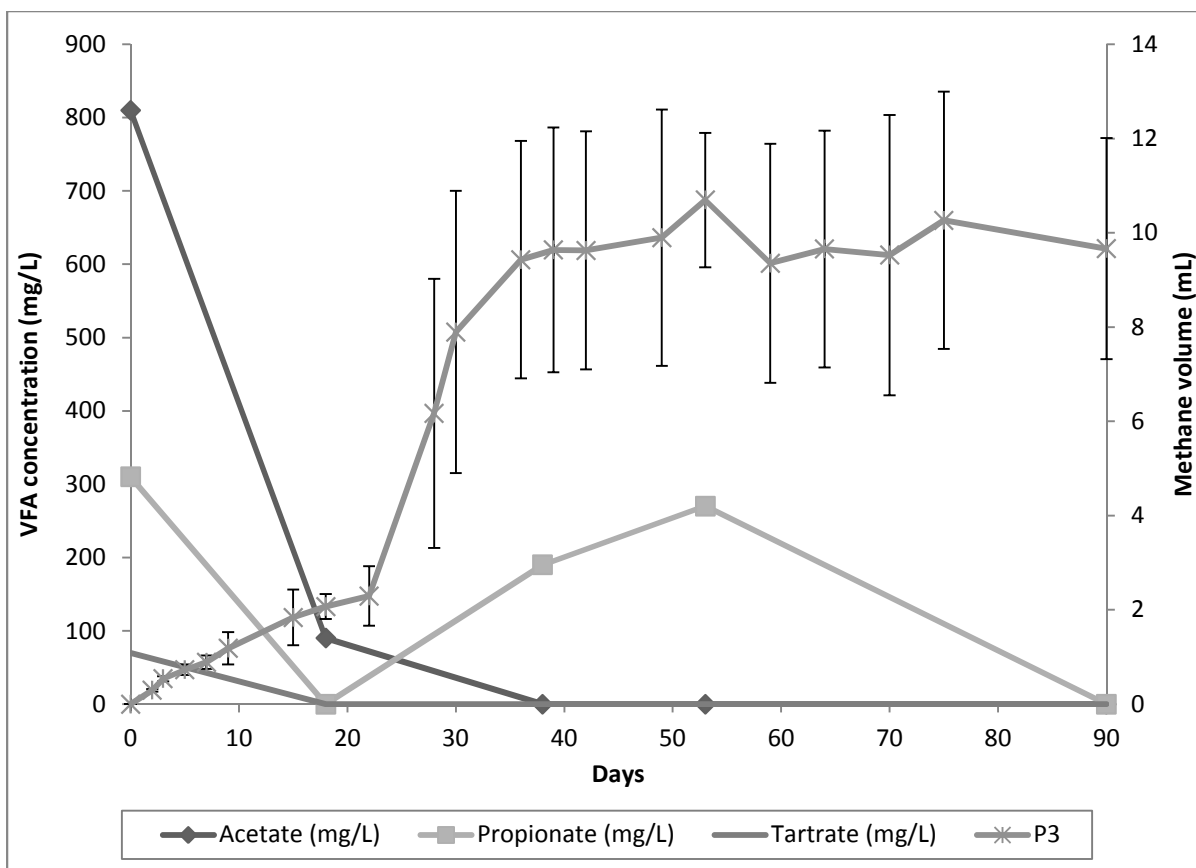


Figure 4-29 - VFA and tartaric acid concentrations vs. methane production in P3.

Acetate had been consumed before day 40 in P3 and was not detected in the latter samples. Propionate, however, was not detected in the day 18 sample but was found in increasing concentrations in the day 38 and 53 samples. It was not detected in the day 90 samples. At no point did propionate reach toxic concentrations of over 1,000 mg/L. The nuisance odour threshold of 230 mg/L was not exceeded by the end of the experiment, meaning disposal in open air of this batch would not present a problem in this regard.

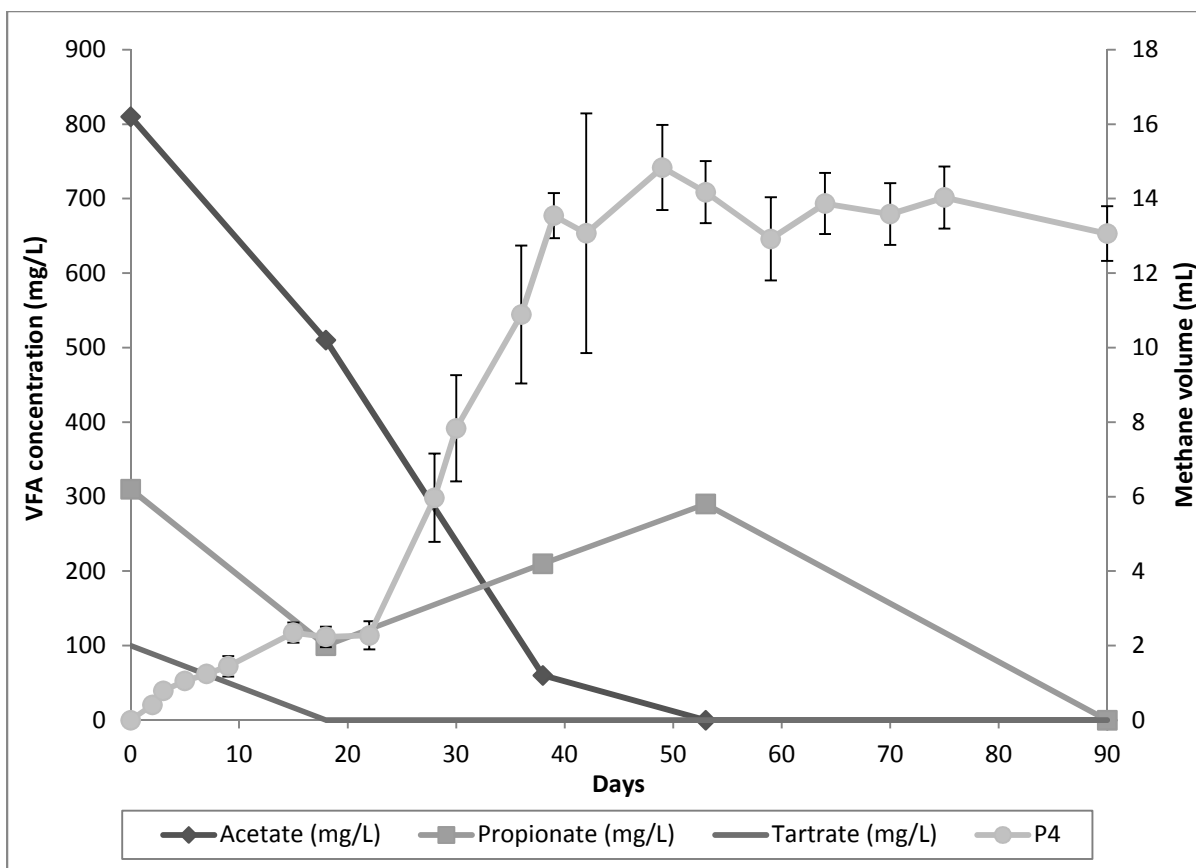


Figure 4-30 - VFA and tartaric acid concentrations vs. methane production in P4.

Acetate was consumed completely by day 53 in P4 and was not detected after that point. Day 53 is also around the time methane production stopped. Propionate increased in concentration but was not detected on day 90, it was presumably consumed. At no point did propionate reach toxic concentrations of over 1,000 mg/L. The nuisance odour threshold of 230 mg/L was not exceeded by the end of the experiment.

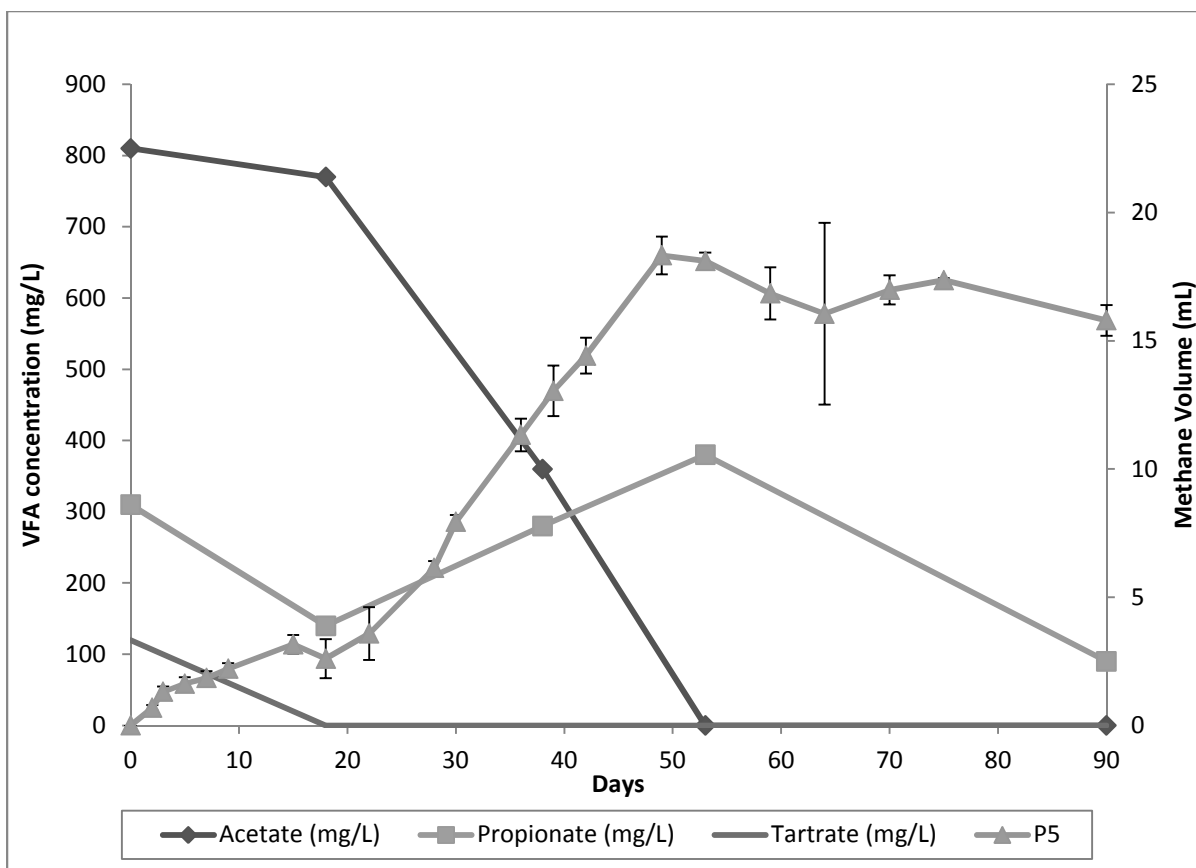


Figure 4-31 - VFA and tartaric acid concentrations vs. methane production in P5.

Acetate was depleted by day 53 in P5 and was not detected in the final effluent. Day 53 is also around the time methane production stopped. Propionate increased in concentration but did not reach toxic concentrations of over 1,000 mg/L; it was found at a concentration of 90 mg/L in the effluent. The nuisance odour threshold of 230 mg/L was not exceeded by the end of the experiment.

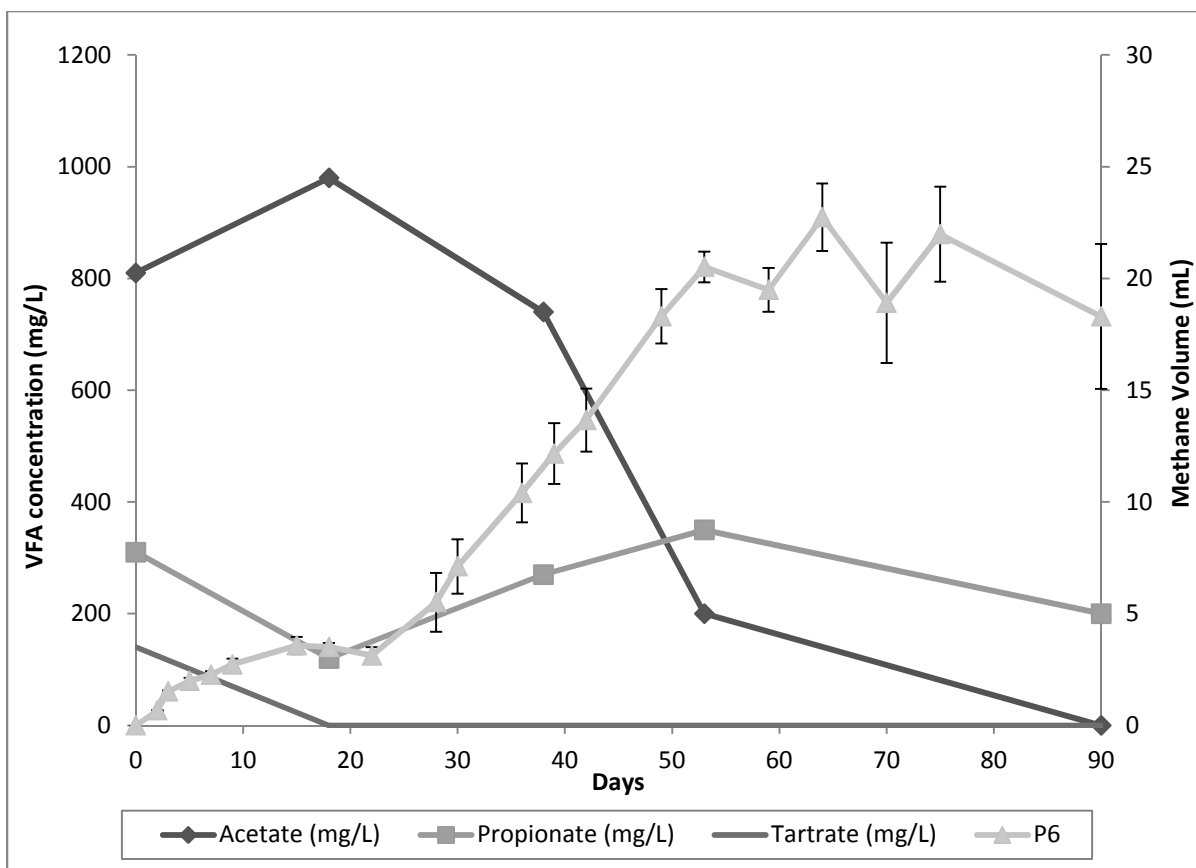


Figure 4-32 - VFA and tartaric acid concentrations vs. methane production in P6.

Acetate was gradually consumed in P6 as the experiment progressed, with no detectable concentration found in the final effluent. Methane production levelled off at around day 60; perhaps this was also the time when acetate was exhausted. Propionate increased slightly in concentration but was later depleted somewhat and found at a concentration of 200 mg/L in the effluent, however, at no point did it reach toxic concentrations of over 1,000 mg/L. Formate/butyrate, valerate, hexanoate and heptanoate were not detected in any of the samples collected. Tartaric acid was not detected in any of the samples even though it is known to be present in pomace; it is assumed it was consumed in the first 18 days. Although propionate came close, the nuisance odour threshold of 230 mg/L was not exceeded by the end of the experiment.

4.4.3 Co-treatment of lees and pomace

4.4.3.1 Total gas

The total gas produced by the cultures was monitored throughout the experiment as explained in the methods section. Statistical analysis showed there had been no significant change in total gas produced since day 64 in L25P75 ($P = 0.631$, $N = 12$), day 65 in L50P50 ($P = 0.917$, $N = 12$), and day 75 in L75P25 ($P = 0.944$, $N = 12$), meaning biological degradation of the lees and pomace had stopped day 75 at the latest. Table 4-16 below summarizes the active stage duration and total gas produced for each batch.

Table 4-16 - Summary of total gas produced by cultures fed pomace.

Batch	Active stages (days)	Total gas produced (mL)	% of total gas produced in days 0-5
L25P75	65	71 ± 2	49
L50P50	65	76 ± 1	53
L75P25	75	82 ± 3	61

Figure 4-33 below shows the cumulative volume of total gas calculated throughout the experiment. These data has been corrected for the control blank.

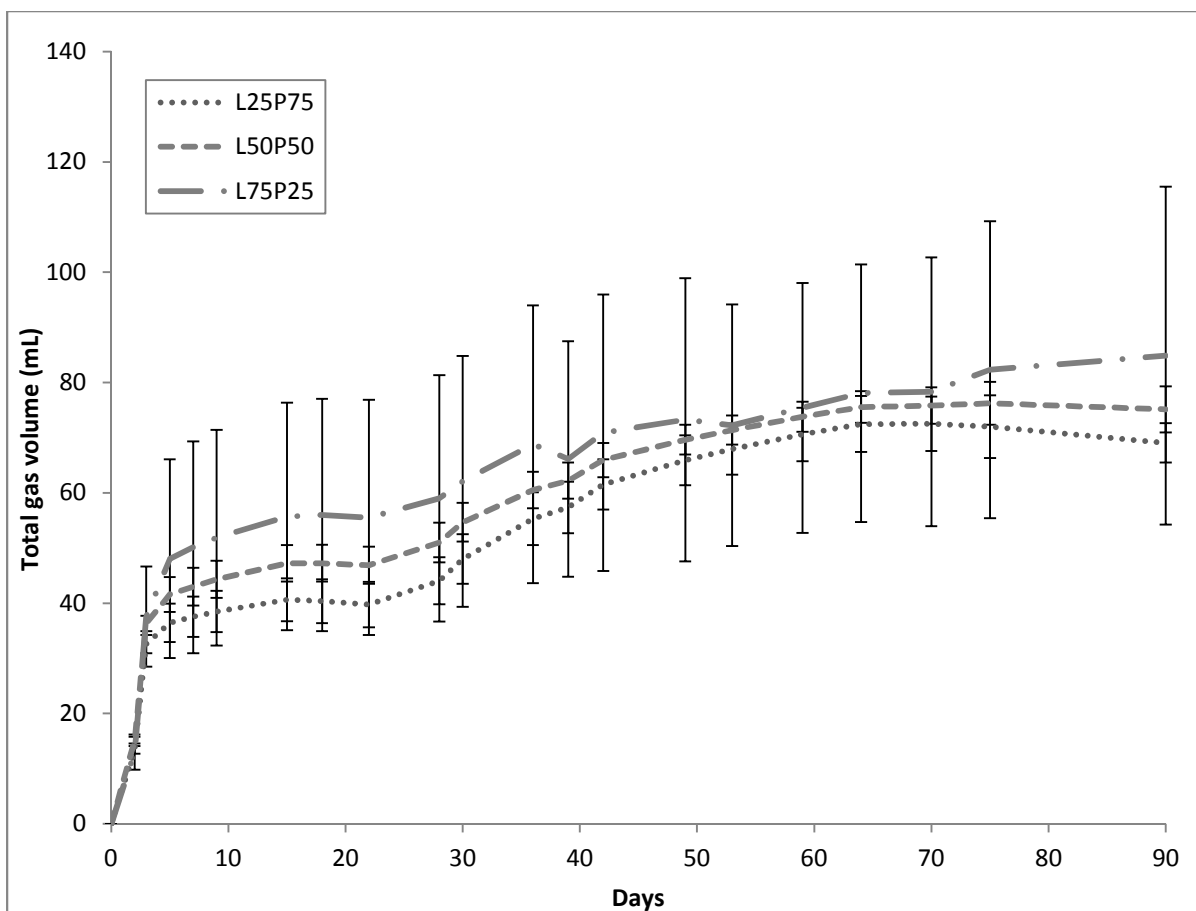


Figure 4-33 - Cumulative volumes of total gas from cultures fed lees and pomace.

Each data point is the cumulative mean ± 1 SD of 4 replicates.

All three batches had a very similar gas production pattern, starting with a high rate of gas production and levelling off by around day 15. Activity resumed after day 20 at a much slower rate, culminating at day 65 for L25P75 and L50P50, and day 75 for L75P25. Two of the replicates in batch L75P25 performed poorly and this increased the variability considerably. Statistical analysis showed there was no significant difference between the total gas volumes produced by the three batches ($P = 0.202$, $N = 36$).

4.4.3.2 Methane

The methane volume as a fraction of the total gas was monitored as described in the methods chapter and these data is presented below as cumulative volume of methane produced over time (Figure 4-34).

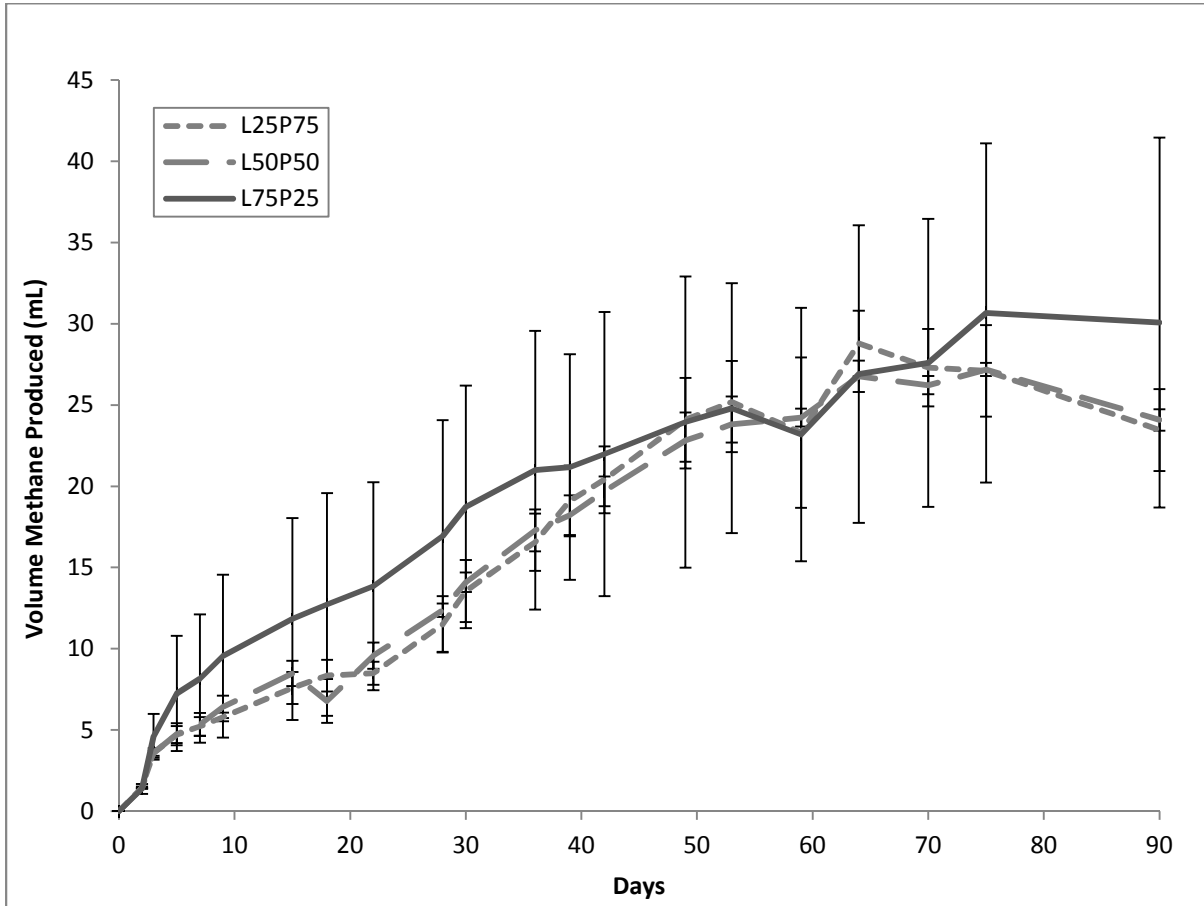


Figure 4-34 - Cumulative volume of methane produced over time.

Each data point is the cumulative mean \pm 1 SD of 4 replicates.

Statistical analysis showed there was no significant difference between the total gas volumes produced by the three batches ($P = 0.628$, $N = 30$). A summary table of results is presented below containing the duration of the active stage, COD consumed, methane percentage, methane produced, methane yield and yield efficiency for each batch. In order to properly

compare the different batches, methane yields were normalized and are presented as methane yielded per mass of COD added and per COD consumed.

Table 4-17 – Summary of results of batches fed lees and pomace.

Batch ID	COD added (mg)	COD consumed (mg)	COD consumed (%)	Methane produced (mL CH ₄)	Methane in total gas (%)	Methane yield (L CH ₄ /g COD added)	Yield efficiency (L CH ₄ /g COD consumed)
L25P75	550	186 ± 3	34	24 ± 3	56 ± 4	0.04 ± 0.00	0.13 ± 0.01
L50P50	550	200 ± 6	37	24 ± 1	55 ± 5	0.04 ± 0.00	0.12 ± 0.00
L75P25	550	217 ± 33	40	30 ± 11	56 ± 4	0.06 ± 0.02	0.14 ± 0.05

All batches had much lower methane yields than the theoretical maximum of 0.35 L CH₄/g COD. Figure 4-35 shows the relationship between the lees and pomace combinations (at an F/M ratio of 5.2) and the resulting efficiency of each batch at converting COD to methane.

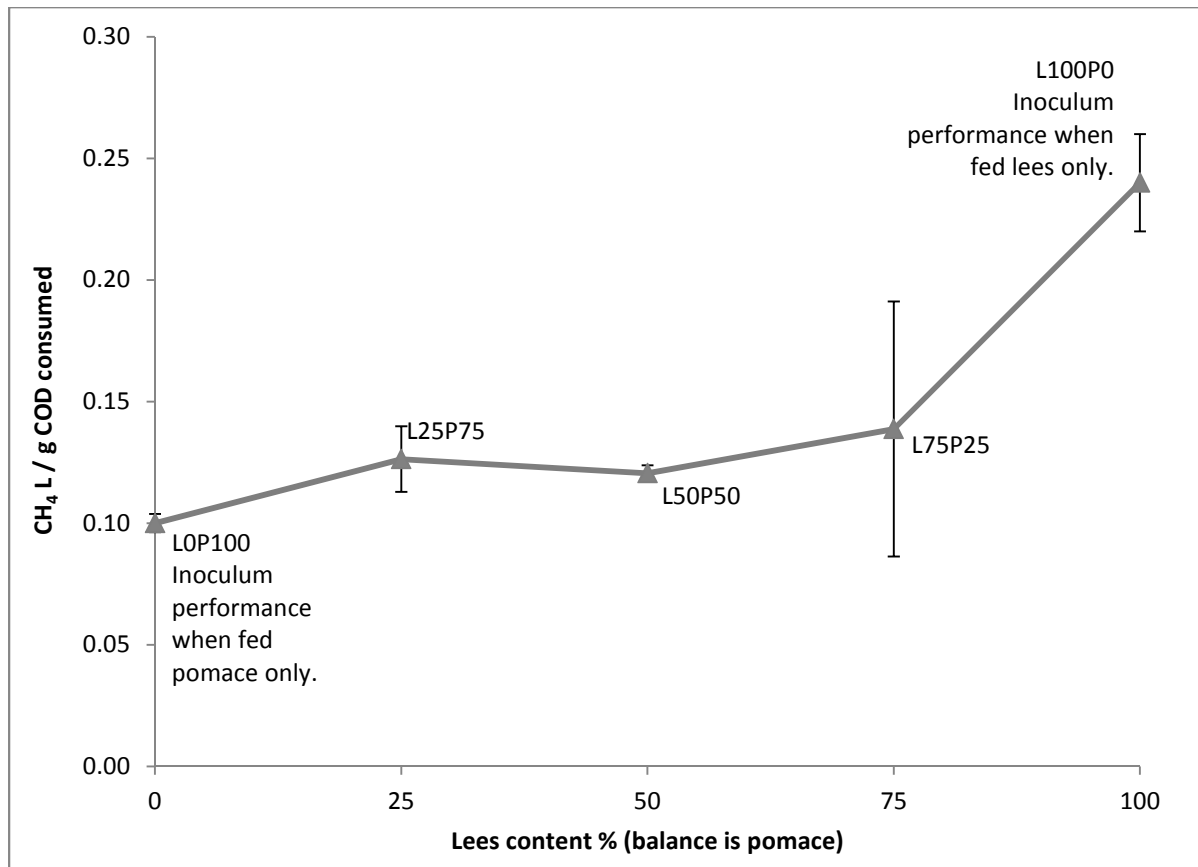


Figure 4-35 - Methane yield efficiency of batches fed lees and pomace combinations.

The data points labelled L0P100 and L100P0 respectively correspond to batch P5 and a hypothetical L5 batch interpolated from the lees batch data. Statistical analysis of the three batches in which lees and pomace were co-treated showed there was no statistically significant difference between the three ($P = 1.000$, $N = 9$). This can be interpreted to mean that the pomace added produced the same inhibitory effect in all three batches, regardless of the mass. It is likely that a threshold of an inhibiting compound in pomace was exceeded in all three batches; perhaps 25% pomace as COD (L75P25) border for inhibition expression. From this preliminary testing, co-treatment of lees and pomace does not appear to be desirable as the pomace seems to greatly reduce methane production efficiency.

4.4.3.3 Ammonia-N and nitrate

Filtered effluent samples were tested for ammonia-N and nitrate at the end of the experiment and concentrations were found to be 16 ± 0 mg/L, 19 ± 3 mg/L and 17 ± 2 mg/L for L25P75, L50P50 and L75P25 respectively.

All three batches had very low concentrations of ammonia-N by the end of the experiment, as levels over 500 mg/L are considered ideal for methanogens. All four batches had stopped producing gas for some time and it is possible that these low concentrations are not reflective of conditions during the active methane producing phases. In this case, a lack of ammonia-N could be one of the factors affecting methanogenesis, though most likely not the main reason why gas production was not optimal. It is interesting to note that although each batch contained radically different loads of COD, they ended up in the same range (~ 17 mg/L). Nitrate levels in all four batches were below detection. The effluent was not tested for phosphorus but it is reasonable to expect significantly lower concentrations than nitrate, which in this case were negligible.

4.4.3.4 pH

The pH in samples from each batch was monitored six times at different stages of the test and the results are presented in Figure 4-36.

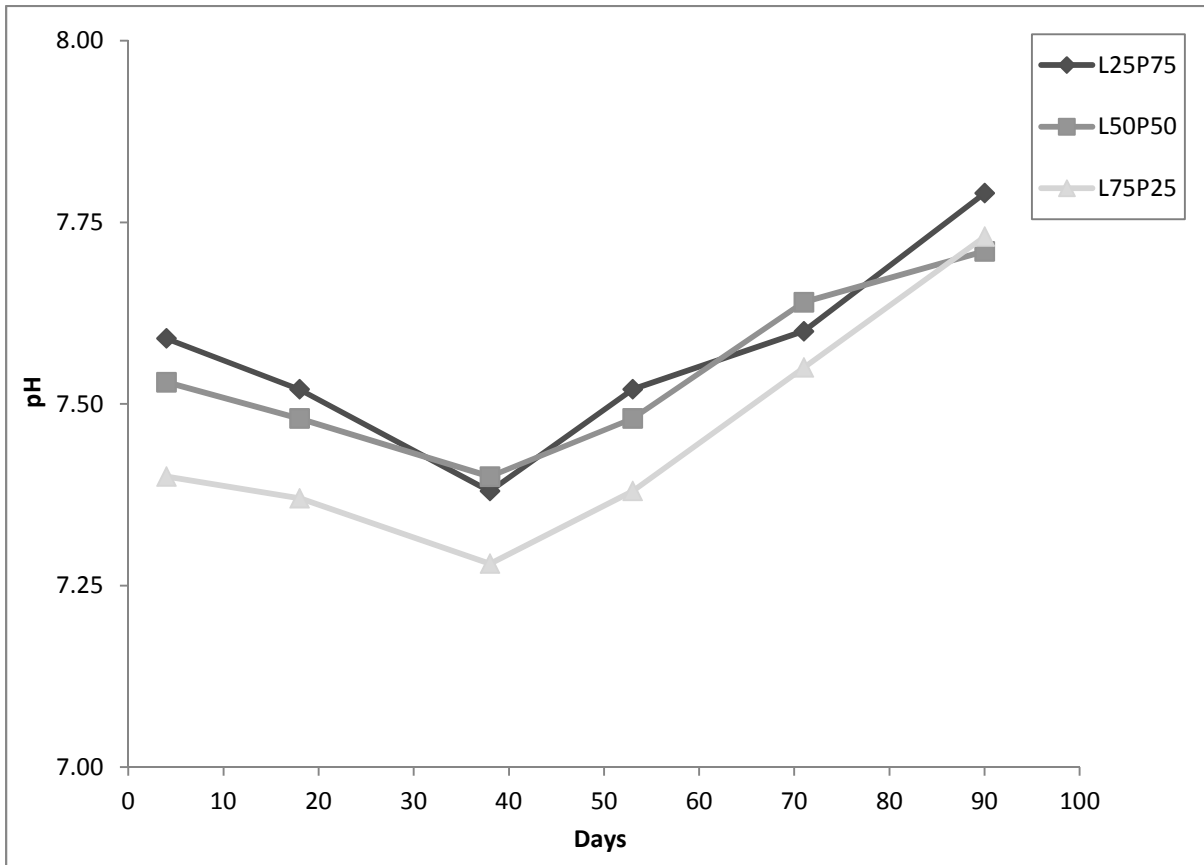


Figure 4-36 - Graph showing pH of batches fed combinations of lees and pomace.

pH of composite effluent samples from each batch on days 4, 18, 38, 53, 71 and 90.

All three batches remained within the pH range preferred by methanogens (6.5 - 8.5). The pH observed at the beginning appears to be related to the amount of lees in the combination – the more lees added the more acidic the culture became. For the first 40 days it appeared as if acids were being produced, driving the pH down, however, after day 38 all three batches increased in pH and converged around pH 7.75 by the end of the experiment.

4.4.3.5 VFA and tartaric acid

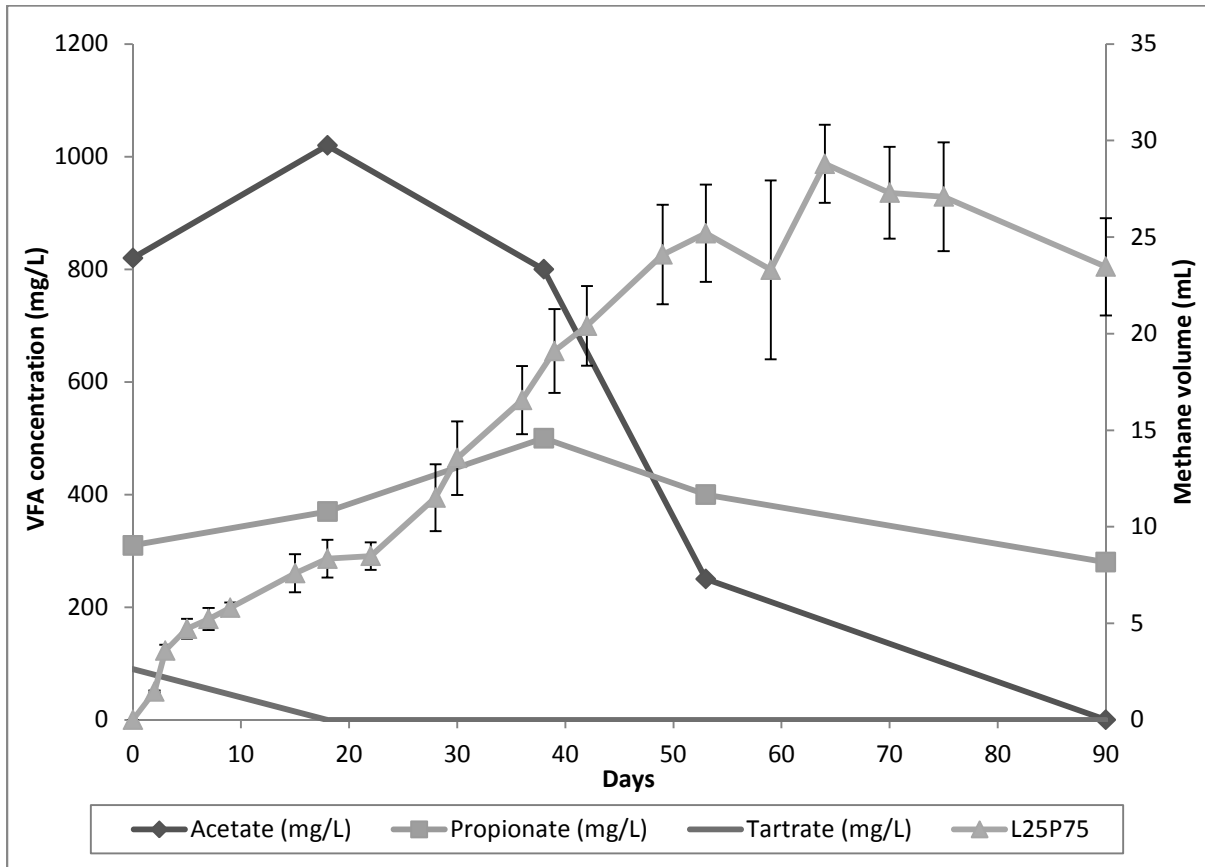


Figure 4-37 - VFA and tartaric acid concentrations vs. methane production in L25P75.

Acetate increased in concentration in L25P75 beyond 1,000 mg/L by day 20 before decreasing to below detection by day 90. As with the rest of the samples tested, the acetate concentration appeared to be inversely related to the methane gas produced. Propionate started at a concentration of 310 mg/L and slightly increased, peaking by day 40; however, levels decreased to 280 mg/L by day 90, close to the starting concentration. Propionate never exceeded toxic concentrations of over 1,000 mg/L. The nuisance odour threshold of 230 mg/L was exceeded by propionate at end of the experiment.

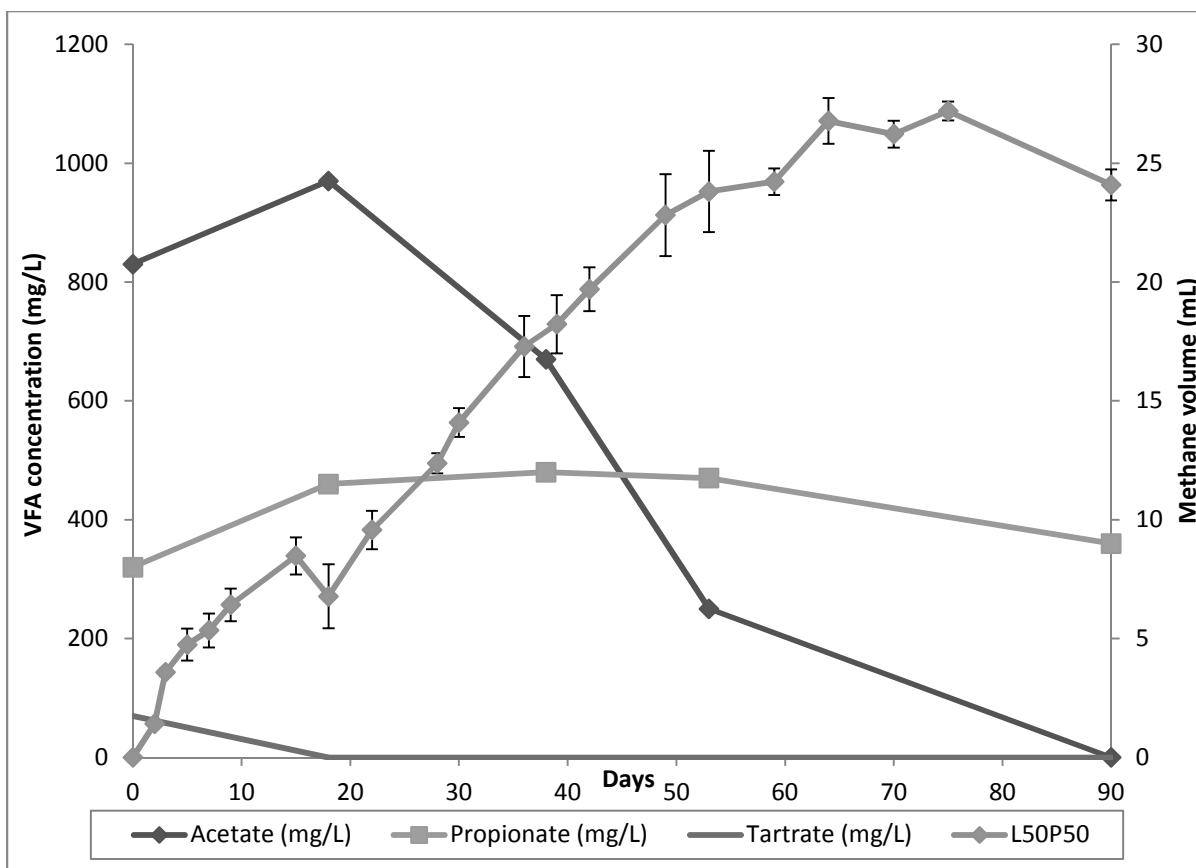


Figure 4-38 - VFA and tartaric acid concentrations vs. methane production in L50P50.

Acetate increased in concentration in L50P50 during the first 18 days, but declining thereafter to undetectable levels by day 90. As with the other cultures, acetate consumption appears to be related to methane gas production. Propionate increased slightly in concentration the first 18 days but remained relatively steady during the test and returned close to the starting concentration by day 90. Propionate did not reach toxic concentrations of over 1,000 mg/L. The nuisance odour threshold of 230 mg/L was exceeded throughout the test, including at the end of the experiment.

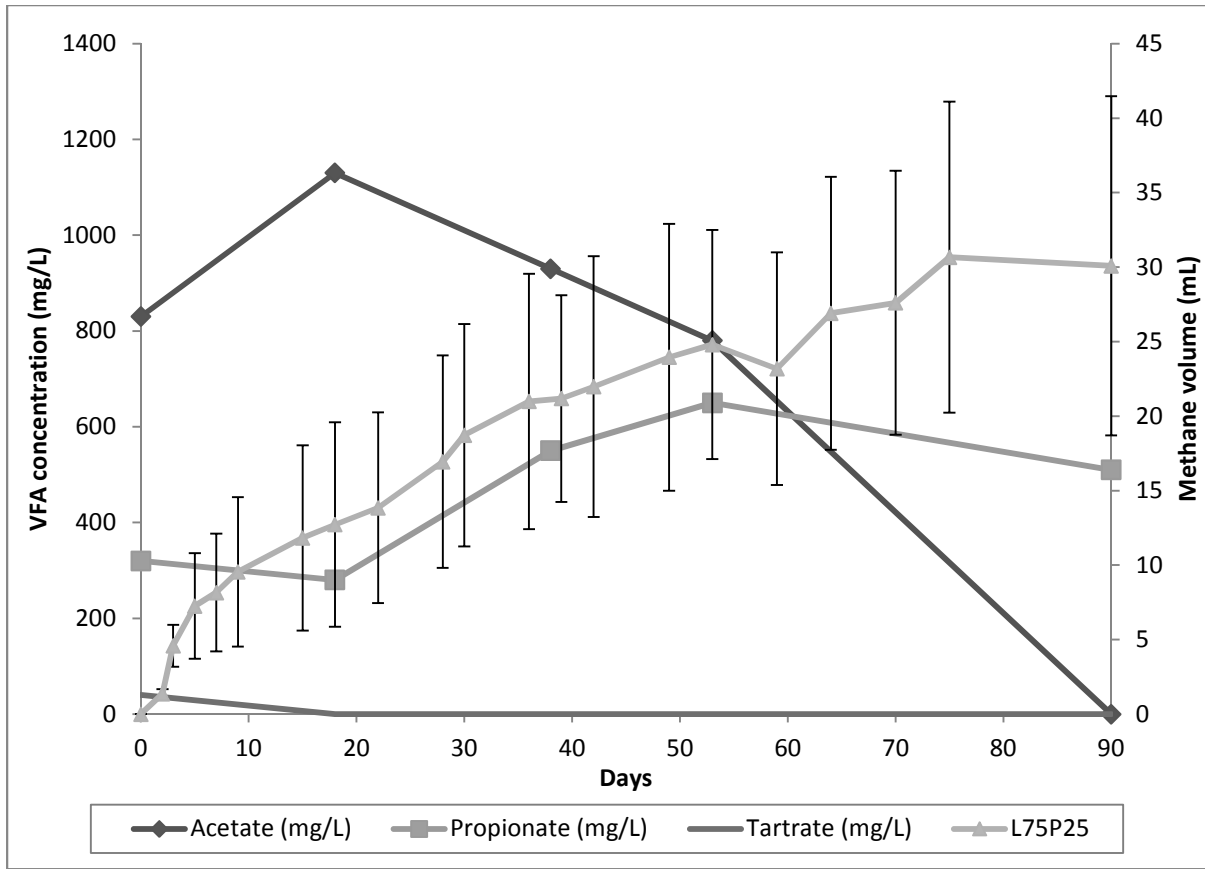


Figure 4-39 - VFA and tartaric acid concentrations vs. methane production in L75P25.

As in the other batches, acetate concentration in L75P25 was inversely related to the rate of methane production. It had increased by day 18 but gradually decreased thereafter. It was below detection in the final sample at the end of the experiment. Propionate gradually increased in concentration to around 600 mg/L by day 53 and decreased slightly by the end. Inhibiting concentrations exceeded 1,000 mg/L were not observed. Propionate exceeded the nuisance odour threshold of 230 mg/L through most of the process, including the final sample tested at the end of the experiment.

Chapter 5 Conclusions and Recommendations

The main objective of this research was to determine whether the liquid and solid waste streams from a winery could be converted anaerobically to methane, and to begin to understand the conditions at which this conversion is optimal.

5.1 General Conclusions

The results of the activities undertaken during this research have led to the following conclusions:

Waste audit

- The majority of the organic waste is produced in the fall and winter during vintage.
- Lees are the main contributor to winery wastewater organic strength.

Culture enrichment

- Two anaerobic cultures were successfully enriched with lees and tartaric acid over the period of 300-325 days. Methane production is affected by bacterial culture diversity which in turn is affected by the quantity and quality of the enrichment food substrate. Because of this, it is possible that the capabilities of the inoculum presented in this thesis can be improved by tweaking the nutrients added in the medium or through co-treatment with a complementary substrate; however, a benchmark had to first be set to improve upon.
- By the end of enrichment, culture W1 was converting lees into methane at a rate of 84% of the theoretical maximum.
- By the end of enrichment, culture W2 was converting tartaric acid into methane at an exceptional rate of 155% of the theoretical maximum (Buswell formula), however this unlikely yield was probably due to the consumption of dead cellular material accumulated over time. Analysis of treated effluent supports this as VSS concentrations were on a decreasing trend during the time these yields were observed.

Biochemical Methane Potential Test (BMP1)

- The batch of cultures fed synthetic food waste degraded approximately 7% of the total COD added. The COD consumed was converted to methane at an exceptional yield efficiency of 0.90 ± 0.20 L CH₄/g COD consumed, but at an extremely slow rate, over 128 days.
- The batch of cultures fed lees degraded approximately 17% of the total COD added. The COD consumed was converted to methane at low yield efficiency of 0.05 ± 0.00 L CH₄/g COD consumed, over a decent time period of 50 days.
- The batch of cultures fed pomace degraded approximately 32% of the total COD added. The COD consumed was converted to methane at decent yield efficiency of 0.23 ± 0.01 L CH₄/g COD consumed, but at a very slow rate, over 110 days.
- The batch of cultures fed lees and pomace (LP) degraded approximately 14% of the total COD added. The COD consumed was converted to methane at low yield efficiency of 0.05 ± 0.00 L CH₄/g COD consumed, at a very fast rate, over 15 days.
- The batch of cultures fed synthetic food waste and lees (FL) also degraded approximately 14% of the total COD added. The COD consumed was converted to methane at low yield efficiency of 0.04 ± 0.00 L CH₄/g COD consumed, at a very fast rate, over 15 days.
- Post-experiment analysis of the F/M ratios tested led to the hypothesis that an F/M ratio of 5 may be a good ratio for a BMP test, as it would yield measurable volumes of methane in approximately 80 days. This information was used in the design of the second set of BMP tests.

Biochemical Methane Potential Test (BMP2)

- Lees was fed to inoculum at F/M ratios of 2.2, 4.3, 6.5 and 8.7. Of these, a ratio of 4.4 was found to be the statistically more efficient at converting COD to methane, at a rate of 0.25 ± 0.01 L CH₄/g COD consumed, though it should be noted a ratio of 6.5 yielded very similar volumes at 0.23 ± 0.02 L CH₄/g COD consumed.

- Pomace was fed to inoculum at F/M ratios of 3.1, 4.1, 5.2 and 6.2. Statistical analysis of results failed to find a significant difference between the yields observed. All four F/M ratios tested yielded an average of 0.09 ± 0.01 L CH₄/g COD consumed.
- Lees and pomace were fed to the inoculum at a constant ratio of 5.2 co-treated in different combinations of 25/75, 50/50 and 75/25 percent lees/pomace respectively. Statistical analysis of the data failed to find a significant difference between the yields observed. All three combinations tested yielded an average of 0.13 ± 0.02 L CH₄/g COD consumed. This means that pomace at any of the three proportions tested inhibited methanogenesis to the same extent. This is interesting because it was believed that any inhibition would be proportional to the mass of pomace added, but it was not. This could hint at an inhibition from a certain compound in the pomace, perhaps polyphenols, though their quantification and testing their extent of inhibition was not within the scope of this research. The variability in methane yield efficiency of the L75P25 batch was much higher than the others. It is possible that a pomace contribution of 25% as COD is close to a threshold of inhibition.

5.2 Future Research

The conclusions drawn from the results of this research have led to a number of recommendations for future research in an attempt to move toward the stated general objective.

- Further BMP testing of a wider range of pomace F/M ratios to confirm the results observed here. It is possible that the pomace ratios tested were too similar in composition and thereby yielded similar results.
- A larger bench scale reactor would be the next logical step in verifying and refining the data, before moving to pilot scale testing if the endeavour could yield valuable data. The ultimate objective would be to isolate ideal design parameters such as the amount of waste to degrade (F/M ratio), volume capacity of the reactor, and hydraulic and solid retention times.
- A microbiological study could be conducted on the enriched inocula to determine the health and the identity of the bacterial consortium present.
- More cultures could be enriched with red and white wine lees, and red and white grape pomace. Inoculum enriched on pomace could then be tested to determine if enrichment mitigates the inhibition from pomace observed here.
- Pre-treatment of pomace could also be tested to assess how it impacts biodegradability, inhibition and resulting methane yields.
- Methane yields from the co-treatment of lees and pomace should be tested again at a constant F/M ratio of 5 but with pomace contributions of less than 25% total COD added. The goal being to determine the inhibitory effects of pomace and its components (i.e., polyphenols).
- Related research but on a different vein, could include research into sizing of energy storage and boiler components, and calculations of potential carbon credit offsets and return on investment.

The data obtained from this research and any future projects will hopefully lead to the determination of important design parameters that could improve the stability and reliability of future anaerobic reactors treating liquid and solid winery wastes.

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