Microbial decontamination of urea formaldehyde bonded medium density fiberboard

By

Roudi Bachar

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Approved: Dr. Zhongmin Dong
[Supervisor]

Approved: Dr. Gavin Kernaghan
[Supervisor]

Approved: Dr. Madine VanderPlaat
[Committee Member]

Approved: Dr. Lei Liu
[External Reader]

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Abstract

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Urea-formaldehyde (UF) is a resin commonly used as an adhesive for medium density fiberboard (MDF). Diverting MDF from Canadian landfills is important to decrease annual methane emissions. The use of anaerobic digestion and aerobic fungi were investigated as potential decontamination methods for the decontamination of toxic UF resins; specifically the formaldehyde (FA) component. For anaerobic digestion batch reactors with three inoculum types were used; diluted cow manure (DCM) inocula and decaying sea weed (DSW). FA concentration, gas production, and reactor conditions were measured. Reactors with 10 g L^{-1} MDF (1.1 – 1.4 g UF) were used with retention times of 38 days. Thermophilic (55 °C) reactors with fresh DCM as an inoculum had the greatest UF decontamination and gas production. Final FA concentration was 5.523 (SD = 1.516) ppm. GC-FID suggested partial UF resin un-hydrolyzed still in solution. The aerobic fungus *Paecilomyces variotii* was tested at various temperatures for its FA degrading potential. At 40 °C *P. variotii* could decontaminate autoclaved MDF with FA concentrations dropping from 400 ppm to 3.970 ppm (SD 3.832) ppm in 7 days, but pH rapidly increased and fungal death occurred.

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Acknowledgements

Abbreviations

2,4-DNPH	**2,4-Dinitrophenylhydrazine**
AB	**Anaerobic Bioreactor**
AD	**Anaerobic Digestion**
DCM	**Diluted Cow Manure**
DSW	**Decaying Sea Weed**
EWP	**Engineered Wood Products**
FA	**Formaldehyde**
FA-2,4-DNPH	**Formaldehyde-2,4-Dinitrophenylhydrazine**
FCM	**Frozen Cow Manure**
GC-FID	**Gas Chromatography – Flame Ionization Detector**
HPLC	**High Performance Liquid Chromatography**
HTPT	**High Temperature Pre-Treatment**
LTPT	**Low Temperature Pre-Treatment**
MDF	**Medium Density Fiberboard**
UF	**Urea-Formaldehyde**
VFA	**Volatile Fatty Acid**

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1.0 Introduction

1.1 Current waste disposal and recycling programs

Life cycle assessment studies have shown classic containment-style landfills are the worst waste management option for sustainability (Abduli et al., 2011; Hellweg, Hofstetter, & Hungerbuhler, 2005; Allen, 2001). With a global population increase unlike any in the past, the build up of municpal solid waste increases every year; pressuring waste management experts to find appropriate disposal methods. Significant environmental savings can be achieved via energy recycling and waste diversion programs (Cherubini et al., 2009).

Many countries in the EU have already adopted energy recycling waste management plans, with incineration of garbage and fermentation of organic products being the two main energy recycling programs. France annually treats roughly 12.6 Mt of non-hazardaous waste by incineration, creating 2,900,000 Mwh of electricity and 9,100,00 Mwh of heat. It was estimated that costs for sustaining these numbers were between € 580-730 million annually (Autret et al., 2007). However these figures do not take into account the revenue per tonne waste, from incinerators. Incinerators are far more economically sustainable than landfills, creating three times more revenue per tonne waste (Rabl et al., 2008). Our current models for archaic landfill style waste systems are quickly becoming out dated, with more research focusing on waste divertion and energy recovery. Sweden diverts over 95% of its municiple solid waste from landfills into

incinerators or anaerobic digesters (Swedish Government, 2015). Landfills have energy potentials of roughly 20 – 40 % that of coal burning. (Abu-Qudais & Abu-Qdais, 2000; Dolgen et al., 2005; Murphy & McKeogh, 2004). While these estimations make incinerators appear to be a promising form of energy production, they come with environmental payoffs.

Incineration produces emissions such as NO_x, heavy metals, poly acrylic hydrocarbons, N_2O and CO_2 (Harrad & Harrison, 1996). These green house gases are linked to global warming and climate change (Johnke, 2013; Panepinto & Genon, 2014; Athanasiou et al., 2015). Emission from incineration plants burning pure waste or using a form of co-combustion are three to nine times lower than traditional fossil fuel energy sources (Astrup, Moller, & Fruergaard, 2009). However, the energy from incineration is heavily dependant on the composition of the waste, with oil rich wastes such as plastics having the highest energy yeilds (Astrup, Moller, & Fruergaard, 2009; Cherubini, Bargigli, & Ulgiati, 2009).

Hazardous emissions have been linked to potential health effects as well. Researchers in London found residents living in communities near landfills had babies born with significantly lower weight than national averages (Vianna & Polan, 1984). Even worse, one study found an increase in miscarriages and birth defects in communities living near incinerators (Berry & Bove, 1997), resulting in new laws being formed in the UK and throughout much of the western world. Technology to reduce these emissions has been

created with much success, however many of these toxic compounds are still above acceptable health and environmental levels (Porteous, 2001).

Gasification is another form of energy production, but requires high capital cost and energy requirements to reach the temperatures necessary to induce energy production. This type of technology although considered green, is not renewable. As the few large scale gasification factories currently in operation primarily use coal as their fuel source.

1.2 Municipal solid waste (MSW) characterization

Analyzing municipal solid wastes (MSW) common properties may provide insight in creating appropriate disposal alternatives. MSWs are grouped based on their physical and chemical characteristics, as well as their use (residential, commercial, etc.). Each nation sets its own standards and guidelines for appropriate waste disposal. In Canada, a considerable portion of our MSW is in the form of engineered wood products (EWPs). These wastes are classified under section 241 – Lumber and other sawmill and millwork products (Government of Canada, 2011). Many of the wood products under classification 241 are used in industrial and commercial industries such as housing development, telecommunication landline poles and most types of construction. EWPs like fiberboard are also used in residential and office furniture products. EWPs come in a variety of wood and resin compositions, resulting in different product integrity and resistances (Tohmura, Inoue, & Sahari, 2001); including moisture resistance, biodegradation resistence, and

decreased formaldehyde (FA) emissions (Kim et al., 2006; Kartal & Ill, 2003; Tohmura, Inoue, & Sahari, 2001; Que et al., 2007).

1.3 Engineered wood products (EWP)

Fiberboard is an EWP created by bonding wood fibers or pulps with adhesive resins to produce durable building material with a diverse array of applications. Canada produces approximately 14,000,000 m^3 of EWPs annually (FAOSTAT, 2013), with 810,000 m^3 being specifically made into medium density fiberboard (MDF) (Statistics Canada, 2010). MDF is composed of individual wood fibers from an assortment of different types of woods bonded together with an adhesive, usually a formaldehyde-based polymer (Composite Panel Association, 2002). MDF is created with recycled materials but is not recyclable itself, resulting in a large quantity of these materials being sent to be landfills, throughout North America and the rest of the world (Grimm et al., 2008). Nearly 201,600 tonnes of MDF were found in Canadian landfills in 2010 (Statistics Canada, 2010) and MDF accounted for 5% of total industrial and commercial waste in landfills for all of North America (DECCW, 2009; WRAP, 2005). In Canada approximately 20% of methane emissions are from these landfills (Environment Canada, 2014). Under anaerobic conditions fiberboards can undergo rapid degradation, contributing to these large volumes of methane production. It has been estimated that landfills are one of the largest sources of anthropogenic-produced methane today (Thompson et al., 2009). Churubini et al., 2009, estimated that methane production from landfills constitutes to nearly 85% of

the total ecological footprint of these failing waste management systems; creating an immediate need to reduce the total waste input to landfills.

1.4 Urea-formaldehyde (UF)

MDF and the majority of fiberboards are bonded with urea-formaldehyde (UF) resins. UF is classified as an aldehyde condensation polymer, due to the release of a water molecule during formation (USA Patent No. US 5674971 A, 1995). Under warm and humid conditions UF resins hydrolyze into their constituent compounds; urea and formaldehyde (Park & Jeong, 2011). It is estimated that UF resin contain as much as 36% free (unbound) FA when the resin is first polymerized (Allan, Dutkiewics, & Gilmartin, 1980; Hun, et al., 2010). Levels of FA as high as 2000 ppm were found in wood dust from fiberboard factories in Quebec, Canada. (Gosselin, Brunet, & Carrier, 2002). This is more than 10x the 50% inhibition rate for the majority microbial communities in anaerobic waste environments (Pfleiderer Industrie, 1993, Lu & Hegemann, 1998). These FA concentrations are significantly lower than the amount used in MDF manufacturing, with industry standards for MDF production approximately 10-15% (w/w) UF resin.

There are also numerous effects to human health. FA has also been listed as a human carcinogen (World Health Organization, 2006) and may be the single largest cancer risk from the air in North America (Sax et al., 2006). It has been potentially linked to pneumonia, inflammation, organ damage, and acute death (Fischer, 1905). These negative health effects resulted in major regulation for FA as a controlled product, but it

is still considered safe while in its polymerized adhesive which has lower regulation standards.

Urea is another constituent released from UF degradation when the UF polymer is hydrolysed. This organic molecule containes two amine groups which can both be mineralized into ammonia/ammonium under anaerobic conditions. Ammonia is one of the most toxic components of landfill leachates (Bernard, Colin, & Anne, 1997). Ammonia is commonly used in the agricultural industry but its high solubility has led to a conerns over leachate runoff and containment . Flooding of highly fertilized areas can have impacts on water systems in the surrounding environment, where increases of ammonia levels can be seen for days in the water levels of rivers and lakes, and for months in sediment (Leidig & Mollenhauer, 1983). Ammonia toxicity in plants has been know for some time. Plant root growth inhibition as well as cell death have been directly linked to ammonia (Britto & Kronzucker, 2002). When ammonia is found in soil with large amounts of plant roots, there is also a significant pH drop, causing further imbalance and potential threat to the biodiversity of an ecosystem.

The health affects of ammonia in adult humans are minor in small doses but in large or chronic amounts there can be fatal health affects (Ryer-Podwer, 1991; Weisenburger, 1993; Verberk, 1977). The most common victims of ammonia poisoning are children, with much of the damage occuring to the respiratory and circulatory system, as well as a stunted growth (Schwartz & Neas, 2000; Chen & Kou, 1992; Drummond et al., 1980). The main source of ammonia poisioning is from leachate runoff. Ammonia from

landfill leachate is soluble in groundwater, poisoning potential drinking sources as well as any organisms native to those bodies of water (Randall & Tsui, 2002). EWP are a major source of the ammonia of landfills. It as been estimated that a 1% increase of fiberboard in landfills could raise landfill ammonia leachate levels by as much as 20% (Wang et al., 2011). Nitrification also causes the potential threat of loading nitrates (NO_3^-) into an ecological system. Nitrates have been linked to cancer and miscarriages in humans. They are also the limiting factor for ecological systems, fluxuations in nitrates are linked to changes in biodiversity (Spieles & Mitsch, 1999; Nilsson, 1988). A system which can contain anthropogenic-methane and reduce landfill leachate would be economical and better for human health.

1.5 MDF and UF environmental impact

Medium density fiberboard is one of the most commonly engineered wood products (EWPs) produced in Canada, with approximately 810,000 m^3 of MDF manufactured annually (Statistics Canada, 2010). MDF is composed of individual soft wood fibers combined with a formaldehyde-based polymer, most commonly UF (Composite Panel Association, 2002; Ye et al., 2007). Under wet and humid conditions UF resins hydrolyze into their constituent components, urea and formaldehyde. This characteristic is due to high susceptibility towards hydrolysis of the amino-methylene link within the UF polymer (Dunky, 1998). Waste disposal of MDF is an ongoing issue due to the environmental problems associated with their resins, limiting waste management strategies to primarily landfills.

As stated above, in landfills under anaerobic conditions, fiberboards undergo rapid degradation, producing large amounts of methane, a greenhouse gas. MDF accounts for 5 % of the total industrial and commercial waste in landfills for all of North America (DECCW, 2009; WRAP, 2005), contributing to the large volumes of methane produced from landfills. Landfills are one of the largest sources of anthropogenic-produced methane, it is estimated that 20 % of Canada's methane emissions are from landfills (Environment Canada, 2014). Leachate runoff is another concern with MDF waste. Effluents from MDF manufacturing plants contain high organic compound concentrations. This wastewater can disturb local communities by affecting biodiversity. Toxicity of FA has been known for years, being linked to numerous health disorders, including cancer. The World Health Organization listed FA as the single largest airborne carcinogen in the world. Ammonia and nitrates, the two most common compounds produced from urea metabolization, play pivotal roles in the nitrogen cycle. Nitrate is one of the major limiting factors in any ecosystem; large influxes of either of these compounds affect community biodiversity.

The wastewater generated by these MDF manufacturing plants has been found to contain high concentrations of carbon and nitrogen compounds suitable for anaerobic digestion (Portenkirchner et al., 2003, Galehdar et al., 2009, Barbu, Stassen, & Fruhwald, 2008). This waste recycling strategy has been in use since the early 1990's; converting the organic fraction of municipal solid waste into biogas and a nutrient rich digestate.

1.6 UF metabolic pathways

1.6.1 Anaerobic pathways

Understanding the mechanisms and metabolic pathways involved with these toxic compounds can help to create an effective means of disposal. FA can be metabolized into carbon dioxide (CO_2) and/or methane (CH_4) through multiple metabolic pathways, and is an intermediate in anaerobic fermentation pathways (Dalton, Stirling, & Quayle, 1982; Harder et al., 1987). Four different pathways for FA detoxification are known in bacteria (Hoog et al., 2001; Lee et al., 2002; Marx et al., 2004; Kato et al., 1983). Knowing that certain microbes can successfully metabolize these toxic compounds creates the potential use for bioremediatory methods, such as mixed acid fermentation, in the disposal of EWP's and UF based resins.

Two main products are formed when FA is oxidized into formate/formic acid. A new food source for microbes in the form of volatile fatty acids (VFA), and $NADH_2$, an electron carrier, is replenished for use in more metabolic processing. (Colby, Stirling, & Dalton, 1977; Adroer et al., 1990; Yurimoto, Kato, & Sakai, 2005).

VFAs are the primary digestive food source in anaerobic environments (Roediger, 1980). If left unaccounted for these VFAs impose a problem for farmers. Fresh manure is considered too acidic to use as fertilizer, causing problems such retardation of seedling germination and lower harvest yields (Schuman & McCalla, 1975). Once fresh manure is composted under anaerobic conditions then we see a falling off of VFAs; allowing the

organic matter is used as fertilizer (Dickinson et al., 2014; Patni & Jui, 1985). Even though

FA is generally a non-growth class material, meaning microbes cannot fully feed and grow

using it as a food source alone, it can be metabolized and its products are capable of

supporting cellular growth and proliferation (Slater & Somerville, 1979).

1.6.2 Aerobic pathways

In addition to anaerobic breakdown of formaldehyde (FA), cases have been found

of aerobic metabolization of FA from isolated fungi. *Paecilomyces variotti* is an

Ascomycetes with FA resistant properties. It can tolerate FA levels up to 0.5 %; with the

ability to fully metabolize FA as a sole carbon source (Kondo, Morikawa, & Hayashi, 2008).

Its tolerance towards FA and other C_1 compounds have been studied for some time

(Sakaguchi, Kurane, & Murata, 1975), making it a possible candidate as an aerobic

organism for the purpose of UF decontamination. Hydrolysis may not be possible

enzymatically however, as the documented cases of FA metabolization have been done

with free FA as a carbon sources (Achkor et al., 2003; Harms et al., 1996). Manipulating

chemical and physical conditions mechanically as a pre-treatment for hydrolysis of UF to

occur may help resolve this issue. Although *P. variotti* is capable of utilizing FA, it is

unknown how it will react to urea, with respects to environmental contamination. *P.

variotii* is capable of using urea as a nitrogen source metabolizing it into ammonia or

ammonium ion (NH_3 or NH_4^+) as well as CO_2 (Navarathna et al., 2010). However due to

the nature of batch bioremediation processing, these closed systems do not allow for a

continuous flow of effluent. Ammonia accumulation or bicarbonate ion production may result in a pH fluctuation, causing system failure. A possible solution to this is to inoculate batch reactors with a secondary organism, which feeds off of the products from primary inoculant organism. Ammonia fungi are fungi are capable of fruiting and metabolizing in ammonia rich environments (Sagara, 1975). They have been found throughout the world on forest floors including Canada (Raut et al., 2011). It may be possible to either use a known strain of ammonia fungi as a secondary inoculant or to find an entirely new one using soil samples collected from local forest floors.

1.7 Anaerobic Digestion

1.7.1 Anaerobic digestion background

Anaerobic digestion is a process in which microbes are able to break down organic materials in the absence of oxygen. This results in a mixture of gases such as methane (CH_4) and carbon dioxide (CO_2), as well as simple organic acids (e.g, lactic acid, acetic acid). Anaerobic digesters have been used in different industries to break down short chain fatty acids, volatile fatty acids, wastewater sludge, organic compost, and toxic biodegradable compounds such as FA (Kaszycki et al., 2001; Kraket et al., 2010; Sakar, Yetilmezsoy, & Appleseed, 2009). In the past researchers have tried to detoxify FA by anaerobic digestion and have found that 250-400 ppm of free FA is the threshold for microbial activity and growth (Lu & Hegemann, 1998). However, in most industrial anaerobic digesters, ammonia is the main chemical of concern for inhibition (Yenigun & Demirel, 2013).

Ammonia has been shown to only inhibit methane producing archaea but other microbes can still produce gases such as H_2 and CO_2; these however are not as profitable as CH_4 (Valdez-Vazquez et al., 2005). To combat problems such as inhibitions a number of different abiotic conditions can be tweaked depending on the inhibitor of interested; the main environmental factor being temperature.

1.7.2 Four stages of anaerobic digestion

1.7.2.1 Hydrolysis

Hydrolysis is the rate limiting step in anaerobic digestion (Noike et al., 1985; Veeken & Hamerlers, 1999). During hydrolysis complex organic matter such as cellulosic materials, proteins, fats, and carbohydrates are all solubilized into soluble organic molecules (Gunaseelan, 1997). The main solid compound found in many anaerobic digesters is cellulose or cellulosic derivatives. The rate of depolymerisation of cellulosic material or possibly UF polymers depends mainly on pH and temperature (Weiland, 2010). These two factors play an important role in physical degradation of polymers as well as enzyme activity. A large number of bacteria are responsible for the breakdown of solids during hydrolysis (Pavlostathis & Giraldo-Gomez, 1991). There are numerous mathematical models to explain the order of the hydrolysis reaction, but a first-order kinetics model is the most widely accepted (Vavilin, Rytov, & Lokshina, 1996).

1.7.2.2 Acidogenesis and acetogenesis

During acidogenesis the newly hydrolyzed organic compounds are degraded even further. Sugars, amino acids, and long chained fatty acids are all degraded into simple short chain fatty acids or H_2 (Ghosh, Conrad, & Klass, 1975). Metabolic pathways for acidogenesis are unique to the microbe and substrate. Microbes may also use multiple pathways to break down the same aldehydes/alcohols into VFA (Marx et al., 2004; Amador-Noguez et al., 2011). It is important to note that acidogenesis, like hydrolysis, is performed by anaerobic bacteria. The next step, acetogenesis, is the conversion of some short chain fatty acids into primarily acetic acid. There is evidence to show that this is done by both anaerobic bacteria and anaerobic fungi (KeQiang, et al., 2004). Acidogenesis is required before acetogenesis can occur, but as soon as any substrate is available for acetogenesis to occur the microbes involved will usually start to metabolize their energy sources right away (Ragsdale & Pierce, 2008).The interweaving between substrates and products in both these steps is why some researchers group acetogenesis as a sub-step of acidogenesis.

1.7.2.3 Methanogenesis

The Archaea perform the last stage of fermentation, methanogenesis (Shin et al., 2010). The biochemical pathways and genes associated with this process are still under dispute (Galand et al., 2005; Deppenmeier, Muller, & Gottschalk, 1996), however the use of a CO_2 reduction pathway with formate and H_2 present is accepted as necessary. This is

the stage where CH_4 is produced and often the most focused stage in industry due to its profitability. Unfortunately this is where most problems occur with biogas production. The sensitivity of the archaeans to numerous factors including but not limited to ammonia, pH, and temperature has proven to be the main challenge to researchers with regards to biogas optimization versus cost effectiveness.

1.7.3 Anaerobic digestion reactor temperature conditions and resilience

Anaerobic digestion can occur in mesophilic (Bonmati & Flotats, 2003) and thermophilic (Ahring, 1994) temperature ranges depending on the requirements of the fermentation. Thermophilic (40 - 63 °C) digestion is found to be more resilient towards ammonia inhibition of methane production. Up to 4 g L^{-1} of ammonia can be tolerated in methane gas production, however if ammonia is slowly added into the system, a concentration of up to 6 g L^{-1} can be sustained before methane inhibition occurs (Angelidaki & Ahring, 1993). This increase in ammonia inhibition allows cheap inocula such as swine manure and nitrogen rich wastewater to be used in profitable renewable energy programs such as biogas production (Yenigun & Demirel, 2013). The main trade off for thermophilic reactors however, is the energy input requirements for heating and sustaining the high temperatures required for digestion (Labatut, Angenent, & Scott, 2014).

In mesophilic (20 - 39 °C) anaerobic digestion inhibition levels of ammonia have been found to be approximately 1.7 g L^{-1} (Albertson, 1961). Total biogas production was

found to be 88 mg L^{-1} and 297 mg L^{-1}, at 37 °C and 55 °C respectively within anaerobic

digesters with 6.5 g L^{-1} of ammonia (Gallert, Bauer, & Winter, 1998). Indicating a higher

tolerance for ammonia by thermophilic anaerobes than their mesophilic counterparts.

Mesophilic reactors have also required longer start up times when having high ammonia

concentrations (Blomgren, Hansen, & Svensson, 1990). Mesophilic digestion also is known

to be less resilient to VFA accumulation; this leads to an eventual over-acidification of the

system killing the microorganisms (Wilsonet al., 2008). Thermophilic digestion is more

resilient to VFA accumulation but is more sensitive to temperature fluctuations, requiring

constant energy input and operational maintenance (Kim, Ahn, & Speece, 2002; van Lier,

1996). Environmental conditions are not the only things that operators can control with

anaerobic digestion; the reactor type is also another variable one can consider for their

type of digestion. Operation of anaerobic digesters can be conducted with different types

of reaction vessels, each of these reactor types, has different economical and operational

payoffs.

1.7.4 Batch and continuous reactors

There are primarily two types of anaerobic digesters, batch and continuous

reactors. Batch culture digesters are created for a set period of time, with inoculation and

substrate addition occurring at the beginning of the fermentation process (Hamdi, 1992).

Inoculant can be transferred from previous digesters containing microbes that are

acclimatized to the conditions required, or inoculates can acclimatize while the

fermentation is occurring, depending on the stage of decomposition (hydrolysis, acidogenesis, methanogenesis). Batch cultures have a set fermentation time and are rotated periodically when the specific organic compounds have been degraded (Song, Kwon, & Woo, 2004). The second type of digester, continuous, uses chemostats and sometimes-fuzzy logic systems (electronic self-mediated) to constantly flush biodegraded organic compounds out and feed new waste in. They can also change abiotic conditions such as pH and temperature as needed, usually creating a more resilient microbe community, but not as efficient of an overall system (Dickinson et al., 2014; Polit, Estaben, & Labat, 2002). Operators can choose their abiotic and system conditions but ultimately the products of the fermentation are determined by the inoculant used; the group of bacteria/fungi involved directly determines the final products (Chojnacka, 2006).

1.7.5 Mixed-acid fermentation

In a mixed-acid fermentation a whole assortment of microbes would be used to produce different organic acids. This represents a distinct advantage, as there is little to no requirement for sterile conditions, as the inoculum sources are usually considered "dirty" (Domke et al., 2004). The first two reactions of a mixed acid fermentation are hydrolysis followed by acidogenesis.

A mixed-acid fermentation would be the first step in anaerobic digestion, followed by methanogenesis (Lovley & Klug, 1983). However, due to the high content of nitrogen in the form of dissolved ammonia from metabolized urea, it is likely to inhibit

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methanogens (Chen, Cheng, & Creamer, 2008). This causes a difficulty in methane production from UF bonded EWPs (Padgett, 2009). Most industrial uses of anaerobic digestion try to combat the effects of environmental and chemical conditions that inhibit methane production (Wilson et al., 2012), however some processes take advantage of these mechanisms. In MixedAlco® fermentation, most biodegradable materials can be broken down into various simple organic acids and eventually into alcohols and/or carbon dioxide. All of which can be used as energy or fertilizer sources, but methane production is deliberately inhibited (Hotzapple et al., 1999).

1.7.6 Hydrolysis rates

Enzymes responsible for hydrolysis of cellulosic material as well as sugars have been studied intensely due to their medical and industrial potentials (Hawksworth, Drasar, & Hill, 1971). Microbes encounter multiple complexes and shapes in the form cellulosic material, causing them to produce an array of polysaccharide hydrolysates; including xylanases, mannases, chitanses, and glucanases (Tomme, Warren, & Gilkes, 1995; Adams, 2004). For the biodegradation of UF resins, this allows for a third factor acting on hydrolysis rate. Enzyme activity, temperature, and pH all effect hydrolysis rates.

1.7.6.1 Pre-treatments for increased hydrolysis rates

The use of a pre-treatment prior to digestion can increase biogas production and lower the retention time required (Ariunbaatar et al., 2014). This is mainly due to speeding up the rate limiting step and allowing quicker and more complete digestion. Pre-

treatments are either thermal or thermochemical, both of which have their advantages. Thermal pre-treatments are less effective overall than thermochemical pre-treatments but have their advantages such as lower costs and no addition of chemicals into the system (Montgomery & Bochmann, 2014). Thermal pre-treatments are split into two main groups, either low temperature pre-treatments (LTPT) or high temperature pre-treatments (HTPT). LTPT are between 40-110 °C and HTPT are above 110 °C and under increased pressure. LTPT have shown small increases in biogas production but have failed to fully degrade more resistant cellulosic polymers (Prorot et al., 2011; Barjenbruch & Kopplow, 2003) but did show an increase in solubility of proteins (Neyens & Baeyens, 2003). LTPT showed an increase pathogen removal effectiveness versus digesters with no pre-treatment (Khalid et al., 2011). However, HTPTs were found to have greater pathogen destruction than LTPT, but had mixed results with biogas production (Ma et al., 2011). There have also been reports of unwanted bi-products occurring as a result of such intense thermal pre-treatments (Prorot et al., 2011; Rafique et al., 2010).

In the degradation of UF polymers it is necessary to allow the solid state polymer to be released into solution as its monomeric components. Using too intense of a pre-treatment may result in high levels of urea and FA in solution; which would cause microbial death and reactor failure. Using a gentler pre-treatment may be more appropriate for hydrolyzing a polymer with such a high solubility sensitivity. A LTPT may be used in this experiment due to a higher priority being put on FA degradation than cellulose digestion and biogas production.

1.8 Experimental design

1.8.1 Anaerobic digestion inoculum

The first inoculum choice for this experiment is cow manure. Cow manure is a carbon rich inoculum with a wide array of micro flora that has been a staple inoculum used for anaerobic digestion in the past (Macias-Corral et al., 2008; Hartmann & Ahring, 2005). The presence of a large amount of VFAs in cow manure suggests the post-digestion sludge may contain a lower concentration of smaller VFAs or organic acids. Due to VFAs being the main source of energy for the flora in this system (Patni & Jui, 1985). Due to the complex nature of the system a number of dependent and changing variables had to be tracked in order to confirm urea-formaldehyde (UF) degradation. These include initial present compound concentration levels, fermentation products concentration levels, and gas production levels. There have also been studies using algae as an active bioremedatory organism of sludge waste (Tedesco, Benyounis, & Olabi, 2013). This is another potential inoculum source for this experiment.

Gas must be monitored for two main reasons, fermentation confirmation and economic viability of inoculum. A system which utilized the changes in water levels through a graduated tubing was previously created by Saint Mary's University professor Dr. Zhongmin Dong, and B.Sc. (hons) candidate Damin Lee. Gas pressure created from the accumulation of CO_2/CH_4 in the reactor pushes fluid in a tube, raising the total fluid level. This allows a visual conformation that gas production is occurring, and that the reactors

are active. Gas production is a secondary form of control and analysis; it should not be used to confirm UF breakdown. Chemical identification through Purpald spectrophotometry and GC-FID were the main methods used to determine FA and VFA presence within post-fermentation reactors.

1.8.2 Fungal isolates

Previous work by Kondo et al. 2008, has shown *P. variotii* to have high potential as a UF degrading organism. Its natural resilience to high levels of FA in nature and industrial settings are characteristics that highlight potential use in UF degradation. In Dr. Gavin Kernaghans Atlantic Root Symbiosis Lab at MSVU there are fungal cultures of *P. variotii* available for potential use of FA decontamination.

In previous research carried out by Dr. Zhongmin Dong and Damin Lee at Saint Mary's University, fungi were found in reactors containing FA at levels above 200 ppm. During initial testing for this research, reactors containing just MDF were also found to contain FA tolerant fungi. These natural forming fungi could be a potential aerobic bioremediatory organism for FA decontamination. It is unknown what genus/species these fungi are, but fungal identification is possible after isolation.

1.8.3 Analysis Method Determination

Working with systems involving cow manure creates a unique set of challenges with regards to qualitative chemical analysis. In the paper and pulp industry, a Purpald colorimetric reaction is the standard method for FA determination in air and

solution (Quesenberry & Lee, 1996). This is a cheap, quick, and accurate method to determine FA in solution that could be used alternately to high-performance liquid chromatography (HPLC) or gas chromatography (GC) (Jiang, Adamec, & Weeks, 2013). As stated before, the overall "dirty" characteristics of anaerobic digestion reactors may present as a problem with Purpald analysis. Either physical or chemical inhibitors may be present in the samples, lowering accuracy of analyte detection. Cleaning up samples with centrifugation and supernatant extraction prior to analysis may solve this problem (Jiang, Adamec, & Weeks, 2013).

Purpald FA detection is precise on the order of ppm but it is not the most accurate method available. Other low weight aldehydes and alcohols may react with Purpald seemingly inflating total concentration of FA in solution (Anthon & Barret, 2004). This is why it may be necessary to use an alternative method to confirm our results. Using HPLC can be a costly and tedious process for FA monitoring due to run time and reagent cost. It may also be difficult to accurately identify and quantify FA concentration due to the dirtiness of the samples. Pre-treatment cleaning protocols for dirty HPLC samples are found throughout the literature to help deal with these issues (Heidorn et al., 2012; Nascimento et al., 2005). Most pre-treatments involve a use of centrifugation followed by a solid phase extraction of the sample. Using HPLC as an alternative method to confirm FA concentration data may be a possible if these sample pre-treatments are effective.

1.9 Objectives

Decontamination of UF polymers from MDF will help reduce MSW from Canadian landfills through waste diversion. It may also be possible to take MSW currently sitting in landfills and decontaminate EWPs, relieving stress on the waste management industry. We predict that UF resins in MDF can be metabolized into non-toxic compounds through anaerobic digestion and / or aerobic fungi. Once this is successful then it is necessary to optimize the conditions used for decontamination. The following objectives help support this hypothesis and reach our goal:

- **Selecting the most effective inoculum sources for anaerobic digestion and fungi for aerobic metabolization**

  o Anaerobic digestion uses a number of different inoculum sources. Finding one that is both effective and economically viable is key.

  o Finding and identifying fungi that are capable of UF breakdown. The conditions and methods around fungal FA degradation must be effective and economically viable.

- **Optimizing abiotic conditions for FA decontamination**

  o There are a number of different factors when it comes to optimizing abiotic conditions that have to be taken into account. These include optimal temperature for FA decontamination, retention period, substrate concentration, inoculum concentration, pH, and nutrients.

- **Identifying final products after successful decontamination**

  o Identifying what compounds are present in post-decontamination digestate / solution. This may include organic acids, alcohols, and nitrogen compounds such as ammonia / ammonium ion.

  o Using analytical methods such as GC-FID or HPLC it is possible to determine final FA concentrations and presents of other compounds (if appropriate standards are determined). These methods may also help confirm FA data in case any low weight carbon compounds remain in the post-digestion sludge; interfering with Purpald accuracy.

2.0 Material and Methods

2.1 Material and methods for anaerobic digestion

2.1.1 MDF pre-treatment

A pre-treatment of MDF (Flakeboard, New Brunswick, Canada) containing approximately 13-19 % UF resin content (Padgett, 2009, Taheri et al., 2016, Loxton et al., 2000) was used to accelerate hydrolysis of UF into its monomeric constituents. 1 L batch reactors containing 10 g MDF (1.3 – 1.9 g UF) cubes, in 450 mL of dH_2O. Samples were sealed and heated to 40 °C for 48 hours in a water bath. After the pre-treatment period samples were blended into a slurry using a handheld blender.

2.1.2 Inoculum Type

Three different inoculums were used to test anaerobic digestion (AD) viability of FA degradation. Fresh cow manure was obtained from a cattle farm located in Noel Shore, Maitland, Hants County, Nova Scotia, Canada and used within 72 hours. Some cow manure was frozen at -80 °C. This frozen cow manure (FCM), was thawed the morning of an experiment and used as the 2nd inoculum type. The 3rd inoculum source was decaying seaweed (DSW) collected from Point Pleasant Park Beach, Halifax, Nova Scotia. The DSW was rinsed with tap water and used within 24 hours of collecting. All reactor types including their inocula can be found in table 3.

2.1.3 Reactor hardware and environmental conditions

All treatment groups were done in triplicates. Manure was diluted in a 1:1 ratio with tap water then sifted through 2 mm and 1 mm sieves. 400 mL of fresh diluted cow manure (DCM) was added to each jar containing pretreated MDF. 400 g of washed DSW partially shredded. DSW was then added to 450 mL of dH_2O. Two sets of controls were used. A negative MDF control was used to monitor natural FA concentration in the inocula and gas production. The positive MDF control was used to confirm FA in solution and moniter UF hydrolysis. Jars containing 450 mL of dH_2O with 400 mL / g of inocula (MDF-), and jars containing 450 mL of pretreated MDF with 400mL of H_2O (MDF+). For MDF+ control groups, 4 g of Caplan fungicide was added to each jar as to limit possible contamination from formaldehyde/urea tolerant fungus found within the MDF blocks.

2.1.4 Batch reactor setup and digestion period

Each batch reactor (1 L Mason jars) was sealed with Vaseline and a rubber stopper. The tops of the reactors contained two tubes. One tube was used to collect samples from the reactor; running from the effluent of the digester towards a 3-point valve and into a collection syringe. The other tube located in the gaseous phase of the reactor, was used to release gas pressure and measure total gas produced. With a 3-point control valve running towards a manometer used to measure gas volume produced, while another tube running underwater allowed for gas release without compromising anaerobic conditions within the reactor (Figure 1). Digestion took place over 38 days in both mesophilic (40 °C)

and thermophilic (55 °C) temperatures. Samples were collected after 2, 10, 22, and 38 days.

2.1.5 Sample collection and non-specific preparation

Samples were collected in 2 mL aliquots. Centrifugation at 13900 rpm for 3 minutes was used limit possible inhibitors and remove solid residue from samples. Supernatant was collected and analyzed for FA and pH.

2.1.6 Purpald formaldehyde analysis

The Pulp and Paper industry standard method for FA detection is a spectrophotometry reaction involving Purpald (4-Amino-3-hydrazino-5-mercapto-1,2,4-triazole,4-Amino-5-hydrazino-1,2,4-triazole-3-thiol) **(**162892, Sigma-Aldrich, Canada)**.** This method was adopted to be used for FA detection. All DCM samples were collected and stored at 4 °C for a maximum of 48 hours prior to analysis. Standard curves prior to MDF testing were used to determine FA sensitivity. Due to the sensitivity of reactions new standard curves were prepared every time a reading was taken. This was to minimize user error and allow the same Purpald solution to be used for both the standard curve and the treated samples. All solutions were created fresh every day, approximately 2 hours prior to testing. For FA concentrations between 1 - 10 ppm a solution of 34 mM Purpald in 0.25 M NaOH solution was used. Purpald solutions were mixed in 1:1 volume ratio with FA samples; 1 mL of each solution. Samples were allowed to oxidize for 60 minutes in petri dishes open to air, followed by spectrophotometric (Genesys 20, Thermo Fisher Scientific,

Canada) absorption readings taken at λ = 549 nm. Samples greater than 10 ppm FA were diluted until an absorption reading could be measured. Dilution ratios were used to calculate their concentrations.

DCM with no MDF was mixed with known FA concentration standards. Solutions were centrifuged under 13,000 rpm for 5 minutes. Supernatant was filtered through a 2 µM filter and analyzed with Purpald. This was to determine if any interference occurred during Purpald spectrophotometry of FA concentration levels due to DCM.

2.1.7 VFA analysis

Total VFA's concentrations at start and end of digestion periods were analyzed. Column chromatography was used to separate the eluent solutions. C-18 silica gel column with methanol solvent was used to extract VFAs into 10 mL flasks. All fractionized samples were immediately sealed and placed into 4 °C storage overnight. GC-FID analysis was performed within 24 hours. Samples VFA content was found through the use of GC-FID, using the Standard Methods Committee organic and volatile acids method, 5560 D gas chromatographic SOP (Standard Methods Committee, 2001). Samples were cleaned of sludge and microbial components before GC-FID analysis. 10 mL of liquid sample was centrifuged at 13,000 rpm at 4 °C. The supernatant was removed and centrifuged under the same conditions. The supernatant was removed again and filtered through 0.2 µM pore-size filters (SLGP033NB EMD Millipore. USA). Samples were acidified to pH 2 with 85 % phosphoric acid.

An external calibration curve was prepared using diluted 10 mM standard volatile free acid mix (CRM46975 Sigma-Aldrich, USA). Concentration ranged from 0 to 10 mM. Dilutions were prepared by mixing the VFA standard with 1 % phosphoric acid aqueous solution to 0.0, 0.1, 0.5, 1.0, 5.0 and 10.0 mM concentrations. Instrument type was a Varian 3800 GC (Agilent Technologies, USA) with an FID detector (39175000 Agilent Technologies, USA) run at 240 °C. The column used was a Supelcowax-10, fused silica capillary size of 30 m x 0.33 mm and 0.25 μm film thickness (24080U Sigma-Aldrich, Canada). Helium was used for the gas phase with a rate of 1.0 mL / min. Injection volume was 0.2 μL at 150 °C.

2.1.8 HPLC Analysis

A series of standards were made for organic products which were expected within the system. This included: formaldehyde, acetic acid, formic acid, lactic acid, and succinic acid. The organic acids are known products from fermentation within natural anaerobic systems; all of which are now are created on an industrial scale for commercial and private use. The hardware used for HPLC is listed below.

- CBM-20A System Controller (Shimadzu, Canada)
- LC-20A Solvent Delivery Unit (Shimadzu, Canada)
- SIL-20A Auto Sampler (Shimadzu, Canada)
- CTO-20A Column Oven (Shimadzu, Canada)
- SPD-20A UV-VIS detector (Shimadzu, Canada)
- Zorbax Eclipse Plus C18 4.6 x 250 mm, 5μm (Agilent Technologies, USA)

Solutions consisting of 100 ppm concentrations of each standard were used to determine control peak rates. A low pressure gradient was used with a 60 % acetonitrile mixture used for the liquid phase and a flow rate of 1.2 mL / min. Peak wavelength absorbance varies for the organic acids (Table 1). It was determined that the best wavelength to use for organic acid analysis was 210 nm. A variety of mixtures containing different standards were used to simulate the conditions and trends found within a real fermentation system. The standard methods given in Thermo Scientific's Application Note 97 (Thermo Fisher Scientific, 2001) option 1 were used as a standard operating procedure for FA and organic acid analysis.

2.1.8.2 HPLC analysis pre-treatment

FA had to be cleaned up with a pre-treatment and undergo derivatization before HPLC analysis could take place. Roughly 50 mL of sample was centrifuged at 13,900 g for 10 minutes. After centrifugation the supernatant was removed and filtered through a 0.2 μm pore syringe filter (SLGP033NB EMD Millipore. USA) into a 50 mL Erlenmeyer flask.

The FA in solution was derivatized using a 2,4-dinitrophenylhydrazone (2,4-DNPH) derivatizing agent. 2 mL of 1 M citrate buffer (80 mL 1 M citric acid in 20 mL of 1 M sodium citrate) was added. The pH was adjusted to 6 with HCl or NaOH. A volume of 3 mL of 2,4-DNPH was added to the sample. The sample was sealed and placed in an incubator (Thermo Fisher Scientific, Canada) at 40 °C shaking at 90 rpm for 1 hour.

A liquid-solid extraction was used to separate the derivatized formaldehyde (F-2,4-DNPH) from solution. A vacuum manifold was created by attaching 3 valves in a parallel line with a small electric vacuum at the end. Each valve had a Hypersep C18 2 g / 15 mL column (Thermo Fisher Scientific, Canada) attached to the end, acting as the solid state of the extraction. 10 mL of 40 mM citrate buffer was passed through the column for conditioning. Once the buffer was conditioned 5 mL of saturated NaCl solution was added to the original sample. The sample was mixed and poured into the column at a rate of 3 to 5 mL / min. Each column was then eluted with roughly 4 mL of acetonitrile. The solution was then diluted to 5 mL total volume with acetonitrile. Samples were then stored for a maximum of 48 hours in 4 °C before being analyzed by HPLC.

2.1.8.3 HPLC analysis reactor design

A series of anaerobic bioreactors (AB) were set up at Saint Mary's University. Each AB was created by the same methods stated in 2.1.3 / 2.1.4. 1 L Mason jars were filled with a 1:1 mixture of DCM and deionized water. Reactors were kept at a temperature of 37 °C in water baths. Anaerobic conditions were constantly maintained via a system of valves and tubes; this ensured if a sample had to be collected or air pressure lowered that no oxygen would enter the system. A total of 10 reactors were created (Table 2). One reactor contained 10g of pinewood sawdust as a control (Table 2). This was to create a control consisting of just wood fibers and no UF. Samples were collected, once a week for 8 weeks. Samples were stored at 4 °C until analysis could be undertaken; this storage

period could range from 24 – 48 hours. Samples were syringed through 2 μM filters before

being placed into the SIL-20A auto sampler loading dock.
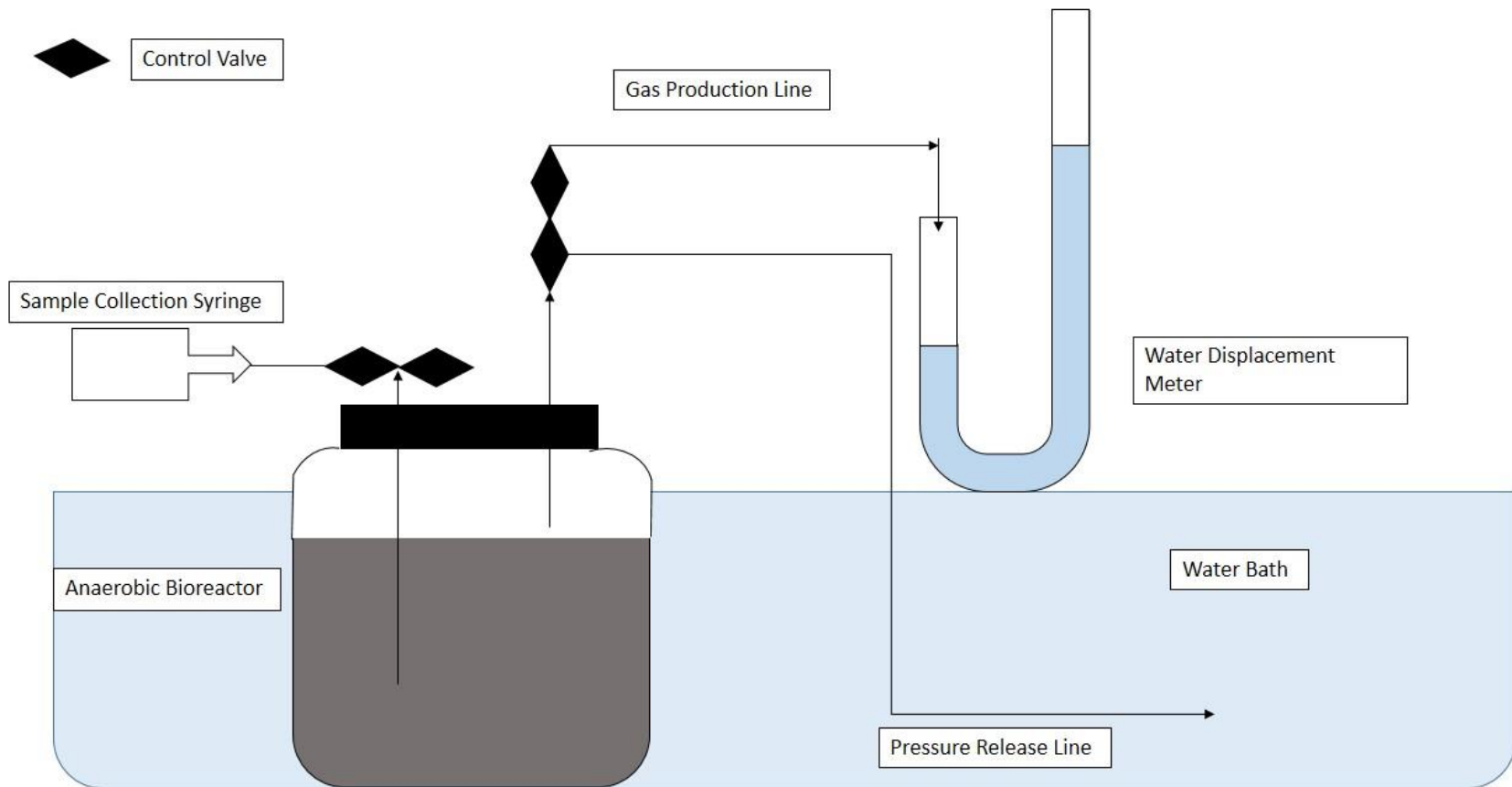
Figure 1 – Anaerobic digester setup including water bath and gas production water ladder. Two sets of 3-way control valves were used to collect samples and monitor gas production while keeping the anaerobic atmosphere from compromising. A manometer was used to measure total gas production.

Table 1 – Organic acids typically found as products of anaerobic fermentation with peak wavelengths.

Compound Name	Peak wavelength (nm)	Reference
Succinic Acid	215	(Technologies, 2011)
Acetic Acid	200	(Bielejewska & Bronislaw, 2005)
Lactic Acid	215	(Shui & Leong, 2002)
Formic Acid	226-250	(Ramsperger & Porter, 1926)

Table 2 – Bioreactor set up for HPLC analysis optimization trials. DCM composition can be found in table 3.

Reactor Conditions	Number of Reactors	Sample ID
10g MDF + 900mL DCM	4	JS1-4
10g Pinewood sawdust + 900mL DCM	2	JS5 JS6
910mL DCM	2	JS7 JS8
900mL dH$_2$O + 10g MDF	2	JS11 JS12

Table 3 – Bioreactor conditions used through anaerobic digestion experiments.

Reactor ID (temperature)	Composition	Experiment	Purpose
DCM (40 & 55 °C)	Diluted cow manure (200 mL fresh cow manure + 200 mL H_2O) + 450 mL dH_2O + 10 g MDF	3.1.1 & 3.2	Treatment type
FCM (40 & 55 °C)	Frozen cow manure (200 mL frozen cow manure + 200 mL H_2O) + 450 mL dH_2O + 10 g MDF	3.1.1	Treatment type
DSW (40 °C)	Decaying Seaweed (400 g) + 450 mL dH_2O + 10 g MDF	3.1.1	Treatment type
MDF- (40 & 55 °C)	Medium density fiberboard negative control. 400 mL DCM + 450 m L dH_2O	3.2	Control
MDF+ (40 & 55 °C)	Medium density fiberboard positive control. 10 g MDF + 4g Caplan fungicide + 950 mL dH_2O	3.2	Control

2.2 Material and methods for aerobic fungal degradation of UF

2.2.1 Fungal isolation and cultivation

Three different fungal isolates were tested for their FA degradation potentials. Two of the isolates used were strands of *Paecilomyces variotii.* These fungi were obtained from the Atlantic Root Symbiosis Lab. The two *P. variotti* isolates were grown at Mount Saint Vincent University, Halifax, Nova Scotia, Canada. Fungi were obtained from jars containing 20 g MDF in 500 mL H_2O (Sample ID P20) and 30 g MDF in 500 mL H_2O (Sample ID P30). The third fungi used was isolated from bioreactors at Saint Mary's University. The MDF+ control in the anaerobic digestion experiment showed fungal growth within the reactors. This fungus was collected and transported to the lab at MSVU for testing (Sample ID ClPa). The third fungus was identified as containing both *P. variotti* and a *Cladosporium spp*. through microscopic examination.

2.2.2 FA degradation and optimal temperature test

The three fungi were tested for their FA degrading abilities at three different temperatures. These temperatures were room temperature (RT), 32 °C, and 40 °C. Room temperature was determined to be roughly 23 °C at the time.

10 mL 5 % w/v MDF solution were inoculated with each fungus. Prior to inoculation MDF was soaked for 48 hours in 2 L of dH_2O. After soaking, the MDF solution was autoclaved then pressed with a potato ricer to obtain a sterile UF liquid. Glass jars with lids left slightly unsealed to allow partial air diffusion were used as reactors. Cultivation were done in two separate incubators and a laminar flow hood. A period of 7 days was given to test how effective the fungi were at FA degradation.

It is important to note that the fungi were obtained from liquid media with a significantly higher amount of FA than all the treatment groups. If 5% MDF was used in each sample then a quick calculation can be done to find theoretical maximum yield.

MDF = 5% w/v (solution)   V (solution) = 500 ml   MDF = 25 g

FA = 7% w/w of MDF (roughly)   FA = 25 g * 0.07 = 1.75 g   1.75/500 * 100 = 0.35%

Therefore, the available FA in solution = 0.35% = 3500 ppm.

However, there was likely was FA loss due evaporation from autoclaving. This changes the final FA in solution. This greatly lowered the maximum FA concentration of ppm.

2.2.3 Formaldehyde and pH analysis

Purpald analysis was used to determine FA concentration. The same methods were used as in section 2.1.6 for FA concentration determination. FA concentration were

analyzed on day 0 and 7. An initial pH reading was taken using a pH probe and a final pH

reading was taken after the 7 day period. Visual observations were also recorded.

2.2.4 Mathematical analysis

2.2.4.1 Statistical analysis

Paired t-tests were used during data analysis for single variable changes. FA

concentration, gas production, and VFA concentration were all analyzed as treatment

versus control. In experiments where there were multiple treatment groups and controls,

such as 2.1.4.2 a one-way ANOVA test was used using Prism 6's Graph Pad software.

Samples were taken from each reactor a total of 5 times (day 0, 2, 10, 22, and 38) giving

5 total ANOVA tests. A Tukey's test ($\alpha = 0.05$) was used post-analysis to determine what

treatment groups, if any, were different from each other. Treatment types were grouped

together based on statistically significant similarity. Results were graphed with each

reactor types grouped and standard deviations. A confidence level of 95% ($\alpha = 0.05$) was

used to determine differences or similarities.

2.2.4.2 Gas production calculation

A manometer was used to determine total gas produced. The actual volume of gas created however had to be calculated. The amount of gas pressure needed to move water up through the water column was greater than the 1 atm pressure. This includes the presence of gas in the headspace of the reactor. The equations below were used to calculate actual gas production.

Boyle's law was used to determine the actual volume (V_a) using the equation $P_1 V_a = P_2 V_2$

Where: P_1 = 1 atm V_2 = Volume of gas in ladder and headspace

$$P_2 = \frac{(Change\ in\ ladder\ height\ (cm) * VHR\ )}{1000\frac{cm^3}{atm}} + 1$$

Equation 1 - Reactor and water column gas pressure equation. P_2 is given in units of atm.

$$V_a = \left(\frac{P_2 V_2}{P_1}\right) - RHS*$$

Equation 2 – Actual volume of gas produced in each digester. **RHS** = Reactor Head Space cm^3. A volume of 150 cm^3 (RHS) is subtracted from the final volume because we are just calculating the total volume of gas produced in the column, not including the headspace in the digester. This gives an accurate reading of the gas produced in total and is assuming that the gas in the headspace was always present in the system (this does not mean that it is always the same composition).

3.0 Results

3.1 Results for anaerobic digestion experiment

3.1.1 Identification of optimal inoculum source

All DCM samples mixed with FA standards showed no interference due to sample properties. Accurate FA concentrations were found in all tested DCM with little to no difference from FA standards. Small colour differences from purified DCM samples and pure FA standards could be visually observed but this did not interfere with readings at λ = 549 nm. Purpald was found to be an acceptable and method for FA determination in DCM samples (Figure 2).

3.1.2 Optimal inoculum sources FA concentrations

DCM was determined to be the most suitable inoculum to use for UF decontamination (Table 4). No significant difference in [FA] was found between FCM and DCM levels after day 38 (P = 0.2052, α = 0.05) (Figure 3). A significant difference was found between DSW and the other two inoculum sources. A decrease in FA levels in DSW occurred between days 10-22, this may be due to Captan's ineffectiveness after 14 days. Gas production data (Figure 4) shows evidence to support this. FCM and DCM levels are

shown to be constantly low with FCM taking slightly longer to reach near 0 levels (Figure 3).

The same methods as stated in 2.2.1 and 2.2.4 were used for this part of the experiment. Temperature as well as pH are the main two concerns with regards to UF degradation. This is due to the hydrolysis phase in anaerobic digestion being the rate limiting step kinetically. This step was sped up through the 48 hour pre-treatment stated in 2.2.1. Results presented show DCM as an inoculum digested at 40 °C and 55 °C as the only differences between treatment groups.

3.1.3 Optimal inoculum sources gas production

Gas production was monitored to determine bioreactor activity. Using a mamometer, gas activity could be visually monitored and recorded. Only gas production was monitored, no gas analysis was conducted. Total gas production in DCM reached approximately 857 ml (SD = 58.1). This was greater than FCM and DSW total gas production values of 423 mL (SD = 39.4) and 0.5 mL (SD = 9.3) respectively (Figure 4). DCM showed a quick increase in gas production and a total gas produced than the other two inoculum sources. DSW showed little to no gas production. DSW also produced negative gas production after 13-14 days. Indicating aerobic respiration.
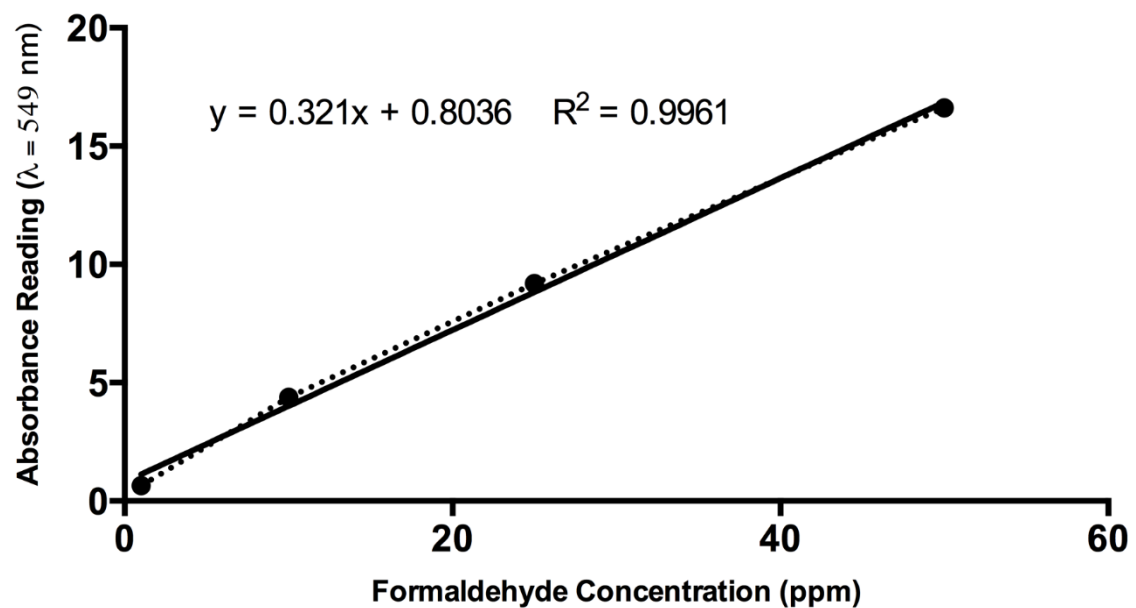
Figure 2 – Standard curve of Purpald solution used in FA concentration determination. 4-point concentration curves were used while determining FA concentration through Purpald colorimetric analysis. A solid line of best fit shows slope of curve. Curve standards were set to $r^2 > 0.99$.

Table 4 – FA degradation for three inocula at 40 °C. No significant difference was found between FCM and DCM levels after day 38 (P = 0.2052, α = 0.05).

SAMPLE	0 DAYS [FA] PPM	2 DAYS [FA] PPM	10 DAYS [FA] PPM	22 DAYS [FA] PPM	38 DAYS [FA] PPM
FCM	5.331 +/- 0.500	16.169 +/- 1.856	5.983 +/- 0.647	1.864 +/- 0.606	2.653 +/- 0.914
DCM	5.341 +/- 0.642	7.905 +/- 0.455	1.668 +/- 0.605	1.569 +/- 0.187	1.783 +/- 0.395
DSW	5.725 +/- 0.213	26.249 +/- 2.816	142.615 +/- 7.430	128.630 +/- 15.941	105.045 +/- 8.499
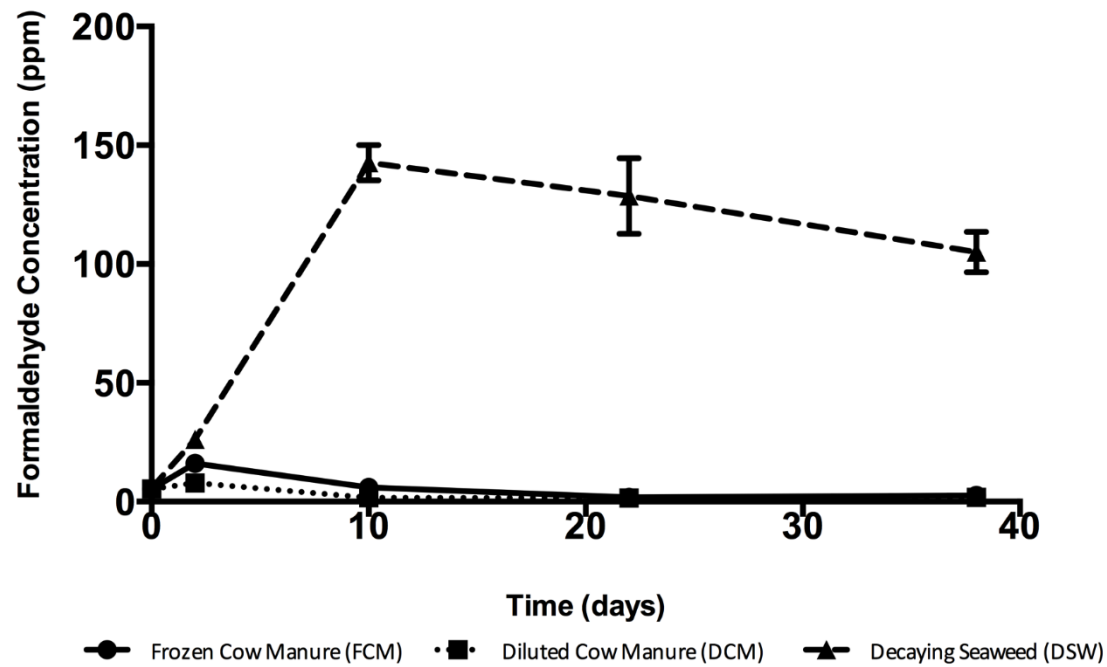
Figure 3 – FA degradation for three inocula at 40 °C. FCM and DCM levels are constantly low ([FA] < 17 ppm) with FCM taking slightly longer to reach near zero levels. DSW showed an increase in FA concentration until day 10 when a slight drop occurred until day 38. This may be due to DSW degrading FA or the presence of FA tolerant fungi taking over the reaction.
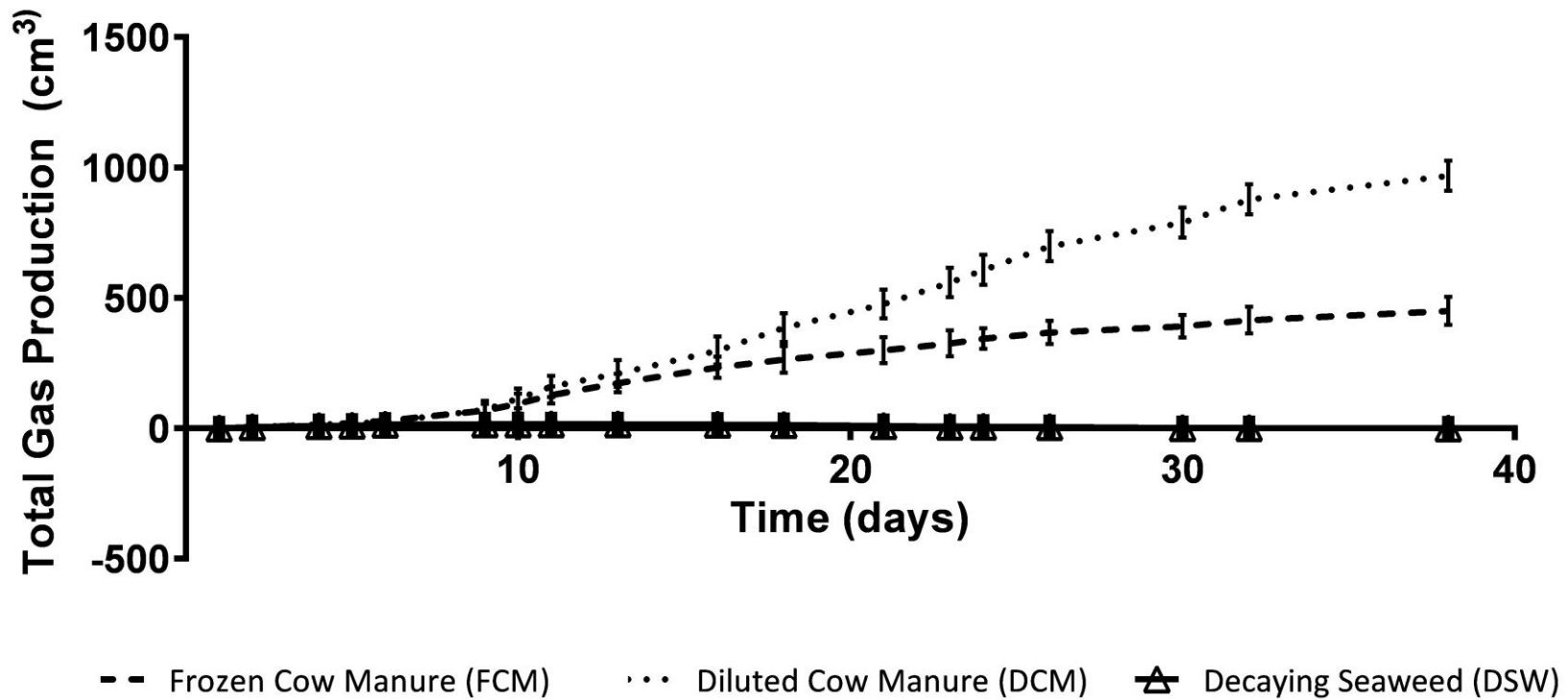
Figure 4 – Gas production data for optimal inoculum trials. DCM showed a quick increase in gas rate and a total gas produced than the other two inoculum sources. DSW showed little to no gas production. DSW also started to consume gas (negative gas production) production after 13-14 days indicating aerobic respiration.

3.2 Determining optimal abiotic conditions for anaerobic digestion results

FA concentrations in all treatments groups had significantly lower concentrations than positive control groups (Figure 5 & 6). FA concentrations after 38 days in mesophilic and thermophilic reactors were 3.189 ppm (SD = 1.472) and 5.523 ppm (SD = 1.516) respectively (Figure 5 & 6).

3.2.1 Mesophilic and thermophilic reactors FA degradation ability

Mesophilic and thermophilic temperatures showed similar FA concentrations post-digestion (Figure 5 & 6). No significant difference (P = 0.0970, α = 0.05) in FA concentrations were found between mesophilic and thermophilic reactors. Very low levels of FA were found in FA- controls. Naturally forming formaldehyde/low chain aldehydes were found in the 1 - 4 ppm range throughout the entire reactor cycles in negative controls. There was no significant difference (P = 0.0839, α = 0.05) found between mesophilic digestion reactors and 40 °C FA- control reactors FA levels (Figure 5). Thermophilic reactors and 55 °C FA- controls did however have a significant difference (P = 0.0563, α = 0.05) in FA levels (Table 2). Thermophilic digestion reactors were found to have higher final FA levels than their control counterparts.

3.2.2 Fungal growth in MDF+ / FA+ controls

Positive controls consisting of MDF slurry contained large concentrations of FA in solution. After 22 days a drop in FA concentration was observed (Figure 6). Upon visual

inspection it was found that a fungus formed a thin layer at the top of the reactor. In preliminary trials a large film fungal biomass grew on each FA+ control within 5-8 days after trials started. These fungi all appeared to be aerobic. This was observed in both 40 °C and 55 °C FA+ controls. Captan was originally used to prevent this fungal growth, however, it seemed to only be effective for up to 22 days (Figure 6). After this period fungus growth occurred again and FA concentration started to drop. Gas production levels in FA+ controls were negative, suggesting aerobic respiration from the fungus. Even with lowered FA concentrations a significant differences between all FA+ controls and treatment groups (Figure 6) were found, with a significantly smaller final FA concentration found in all treatment groups than in FA+ controls.

As stated earlier the presences of a natural forming UF tolerant forming fungi skewed earlier data with regards to FA- controls. Reactors in both 40 °C and 55 °C showed increasing levels of UF in solution (Figure 6). This was until day 22 when FA levels dropped and white, cloudy fungi was observed in solution. Further experimentation was conducted on this fungus and other FA degrading fungi (2.2).

3.2.3 UF hydrolysis rates

UF hydrolysis rates differed based on temperature. Total FA in solution after 22 days was significantly greater in the 55 °C samples versus 40 °C (P = 0.0137, α = 0.05) Final FA concentrations in 40 °C and 55 °C were lower than FA at 22 days (Figure 6). The average between FA levels was found not to be significantly different (P = 0.3907, α = 0.05)

between 40 °C and 55 °C groups with concentrations being 107.985 +/- 1.474 and 112.884 +/- 6.202 ppm respectively.

DCM treatment groups all have statistically significant different FA concentrations than their FA+ control counterparts. FA concentration for both temperatures in DCM groups are statistically similar with levels 100x below their FA+ control counterparts. A decrease in FA concentration can be observed around 22 days in both 40 °C and 55 °C FA+ controls. This may be due to UF tolerant fungi. The final FA concentration however is still lower in the DCM groups. Constant FA concentration increase can be observed until approximately 22 days. The FA level decrease coincides with fungi observation in solution. Final FA concentrations are not found to be significantly different between the two temperatures. Total FA released into solution was found to be greater in 55 °C conditions than in 40 °C. Using the 22 day point as a reference for FA release, it seems that the 55 °C DCM digester degraded a larger total of FA than the 40 °C DCM digester. The 55 °C reactor degrading 199.95 ppm of FA and the 40 °C DCM degrading 145.69. However it is important to note that the final concentrations of FA are equal after the 38 day digestion period.

3.2.4 Mesophilic and thermophilic reactors gas production

DCM digesters at both temperatures 40 °C and 55 °C produced higher amounts of gas than all controls. Thermophilic DCM digesters produced a significantly higher amount of gas than mesophilic digesters (Figure 8). This may be due to an increase in hydrolysis

at higher temperatures and more VFA being metabolized to CO_2 + CH_4 as well as other gases. Thermophilic DCM digesters showed greater than 400 mL of gas production while mesophilic digesters were in the range of 100 - 200 mL. No control group ever produced more than 100 mL of gas.

3.2.5 VFA post digestion data

3.2.5.1 Quantitative data of identified VFAs

A total of 7 different VFAs were identified from pre- and post-digestion samples with GC-FID. Pre-digestion cow manure contained propionic acid, iso-valeric acid, iso-caprionic acid, iso-hexanoic acid, and n-heptanoic acid. Post-digestion cow manure contained acetic acid, butyric acid, iso-valeric acid, iso-caprionic acid, hexanoic acid, and n-heptanoic acid. The difference in identified compounds present in both samples can be found in Figure 9.

Four of the same compounds were found in both pre- and post-digestion cow manure. They were iso-valeric acid, iso-caprionic acid, hexanoic acid, and n-heptanoic acid.

Acetic acid and butyric acid (were found in the post-digestion cow manure but not in the pre-digestion cow manure. The presence of acetic acid supports the notation that digestion occurred and that acetogenesis is when it was produced. Identified VFAs total

concentrations were summed and t-test was used to find any difference in total concentration.

3.2.5.2 Presence of unidentified VFAs and un-hydrolyzed UF

Pre-digestion cow manure shows multiple unidentified peaks with retention times 5 – 6 minutes and 7 – 9 minutes (Figure 10). These peaks show a large number of unknown compounds in the pre-digestion cow manure that are not present in the post-digestion cow manure (Figure 11). There are three unidentified peaks in the post-digestion cow manure compared to the 30+ in the pre-digestion cow manure. This suggests in a larger amount of total organic compounds in pre-digestion cow manure than in post-digestion.

There is a large peak at 10.789 min in the post-digestion cow manure. This peak is very sharp and immediate followed by a curved fall off. These are the characteristics found in an incomplete hydrolysis of a polymer (Tan et al., 2014). Suggesting that un-hydrolyzed UF is present in the post-digestion cow manure. These oligopolymers may be various sized chains of UF that were partially hydrolyzed. No FA was present in the post-digestion cow manure.

3.2.6 HPLC analysis of mesophilic DCM reactors

Artificial mixtures of compounds present during digestion showed small peaks with varying levels of noise throughout each peak. Data was gathered on retention times and peak strength for each standard / mixture of standards (Table 5).

DCM were found to be too "dirty" to purify for detection of target analyte through HPLC (Figure 12). Sample cleanup and filtration had a minor effect on reducing background noise. This noise was still present and did not allow for confident identification of F-2,4-DNPH. Quantification analysis was not possible due to noise.

Peeks are not sharp within the LCsolution analysis reports; they have large bases that range for approximately 1 - 1.5 minutes. Low resolution is due to poor separation of these products. 2,4-DNPH purification on control samples showed a positive reading with identifiable peaks for each compound present (Figure 13).

Figure 5 & 6 – Formaldehyde concentration levels in co-digested DCM with MDF compared with controls. Figure 5 - DCM Treatment group and FA- Controls. DCM treatment groups have FA significantly similar to FA- Controls. All final FA concentrations are below 5 ppm. Figure 6 - DCM Treatment groups and FA+ Control. DCM treatment groups all have statistically significant difference FA concentrations than their FA+ control counterparts. FA concentration for both temperatures in DCM groups are statistically similar with levels 100x below their FA+ control counterparts. A decrease in FA concentration can be observed around 22 days in both 40 °C and 55 °C FA+ controls. This may be due to UF tolerant fungi. The final FA concentration however is still extremely lower in the DCM groups.
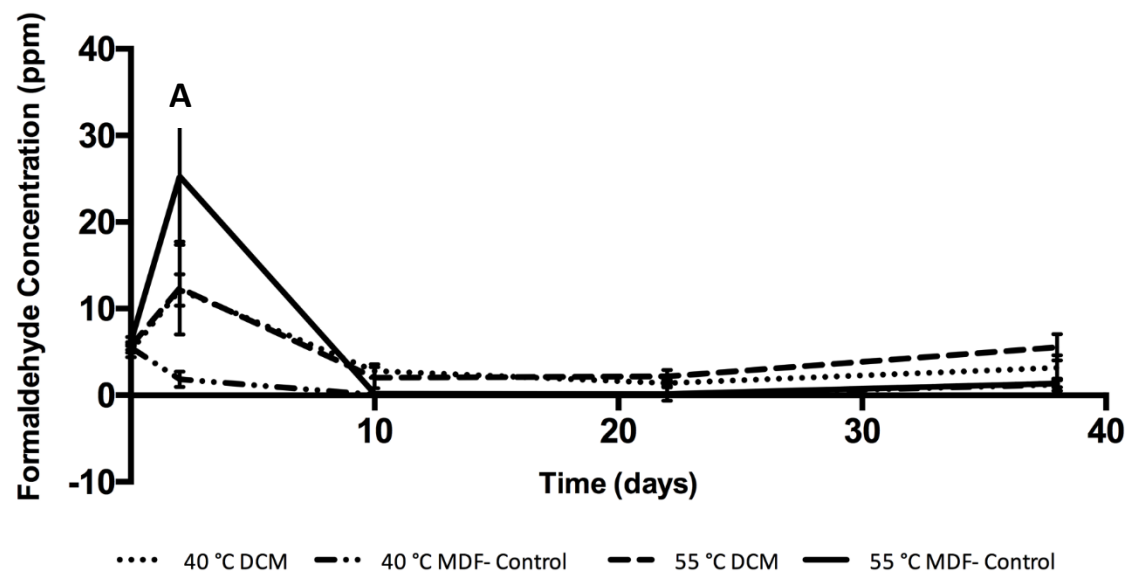
Figure 5 – FA concentration levels in DCM at 40 and 55 °C and their negative controls. One-way ANOVA test showed no significant differences between any of the groups except for one time (A). The 55 °C MDF- control after 2 days of digestion showed a FA spike.
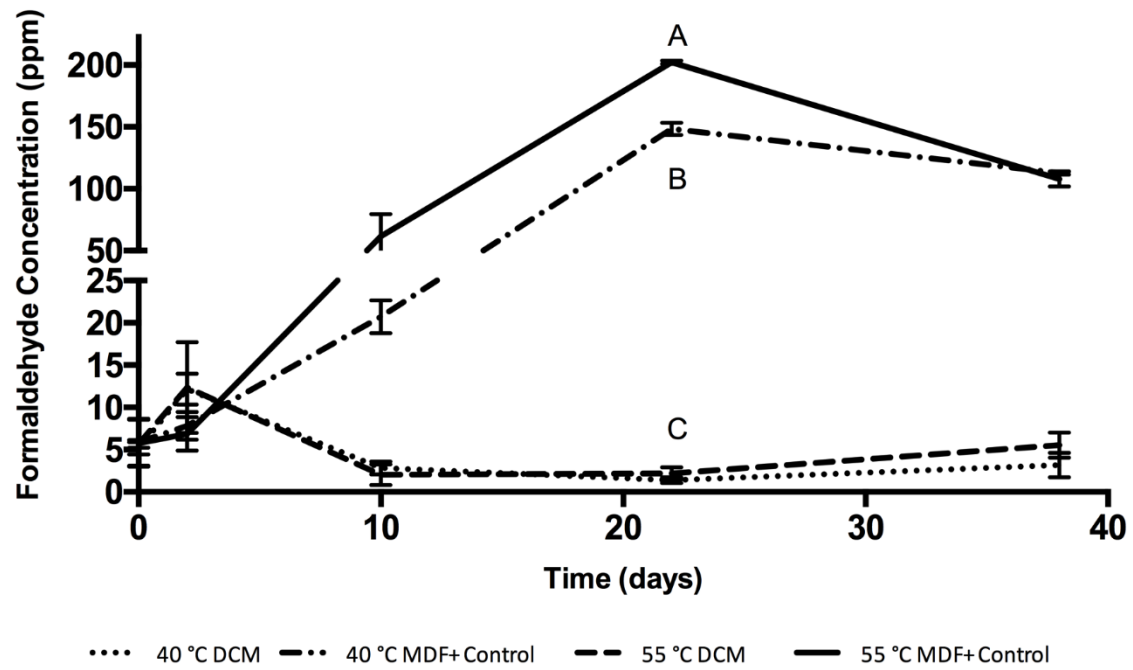
Figure 6 – FA concentration levels in DCM at 40 and 55 °C and their positive controls. One way ANOVA was used to determine any differences in the treatment groups. Data points within the same time period with different letters were significantly different.
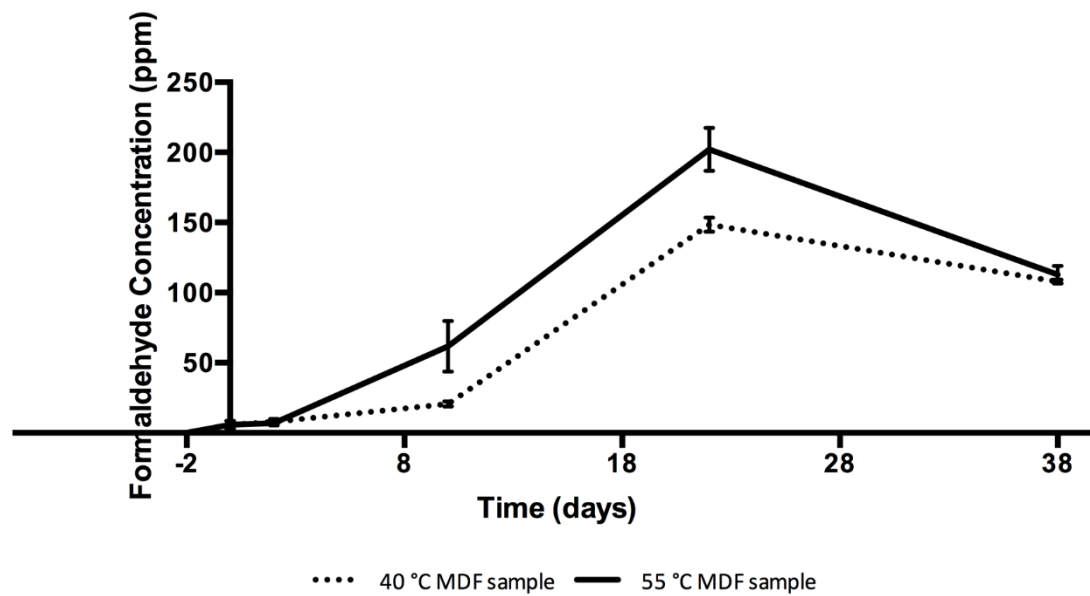
Figure 7 – Hydrolysis rates of UF through FA concentration monitoring of 40 and 55 °C MDF+ controls. Constant FA concentration increase can be observed until approximately 22 days. The FA level decrease coincides with fungi observation in solution. Final FA concentrations are not found to be significantly different between the two temperatures. Total FA released into was greater in 55 °C conditions than in 40 °C. Final FA concentrations are not found to be significantly different between the two temperatures.

Figure 8 – Gas production averages from the four treatment types. Each treatment type curve is created through combining data from each of the bioreactors based on their treatment. Samples with potential aerobic fungi continuously produced negative gas until the end of the retention period. Water displacement tubes were reset daily allowing gas to escape and resetting the water into its original position, this resetting may have involuntarily allowed oxygen back into the system in minute amounts daily, allowing the fungus to survive.

Total Gas Production (cm³) vs **Time (days)**

Legend:
- ···· 40 °C DCM
- ◇ 40 °C MDF -
- △ 40 °C MDF +
- —— 55 °C DCM
- ◆ 55 °C MDF -
- ▲· 55 °C MDF+

56
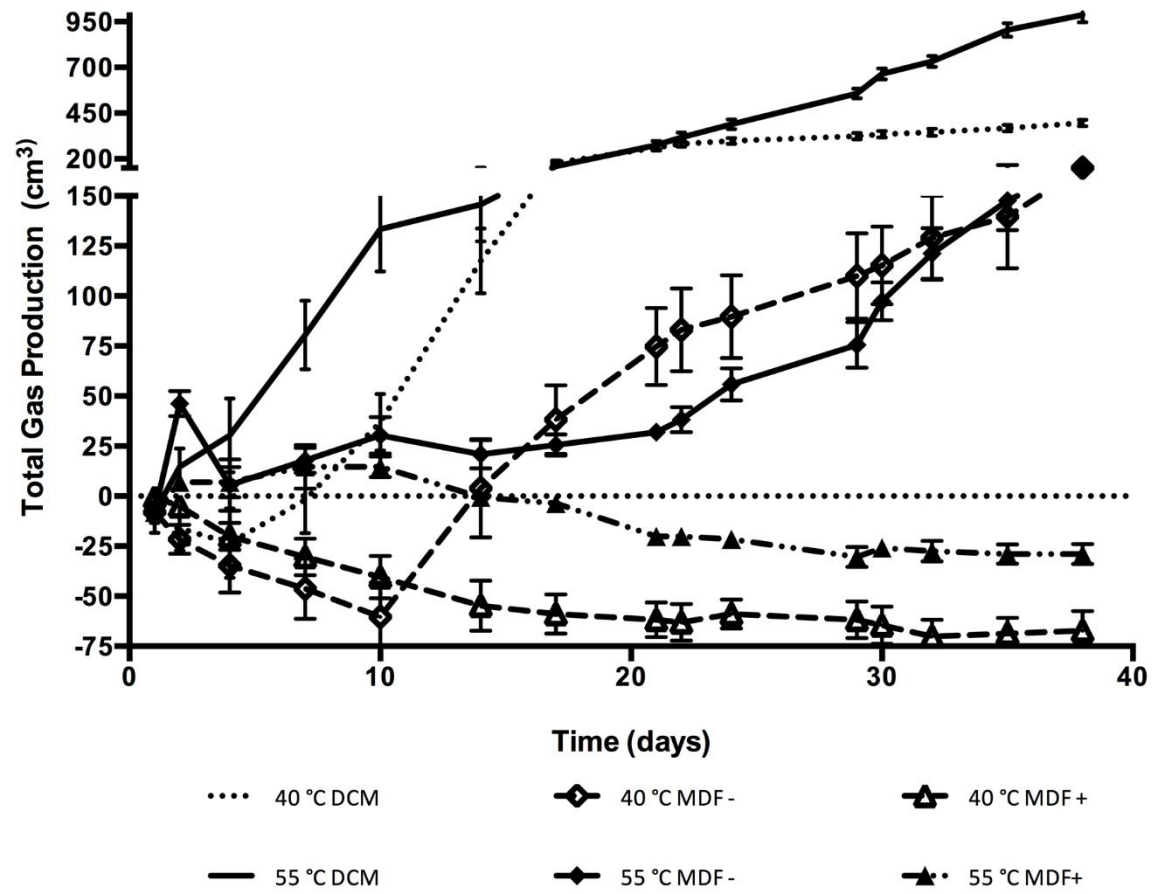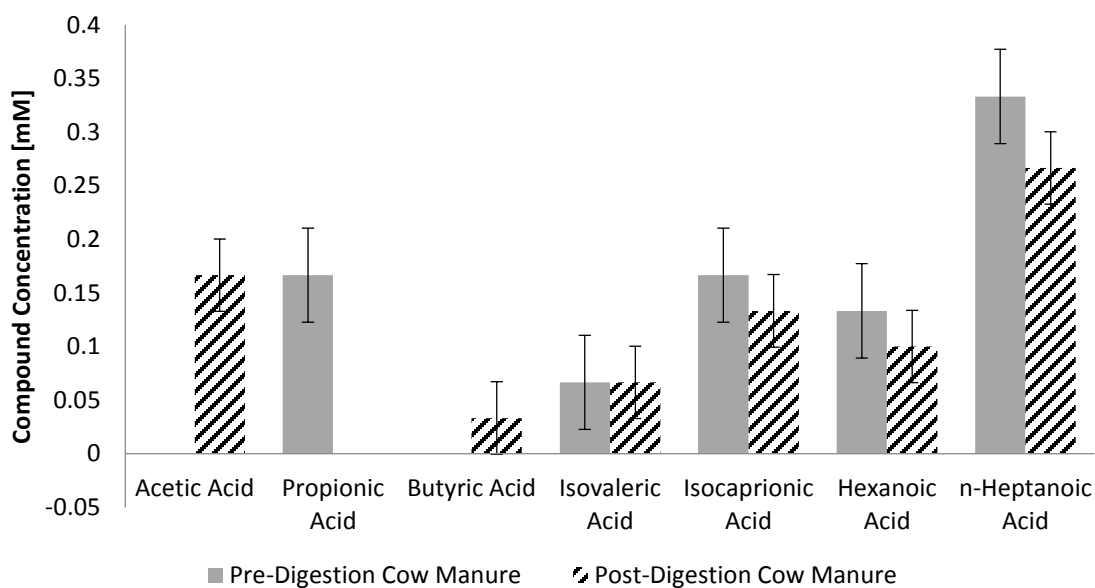
Figure 9 – Pre and post digestion levels of VFA in cow manure with standard error bars. In the pre-digestion manure there are higher amounts of propionic (5.464 min), isovaleric (7.370 min), and hexanoic (7.972 min) acid. In the post-digestion manure there are higher levels of acetic (4.581 min), butyric (6.503 min) and n-heptanoic (8.497 min) acid. There is an equal amount of isocaprionic acid (7.756 min) in both pre and post digestion manure.

Figures 10 & 11 – Figure 10 - GC-FID graph of compounds present in pre-digestion cow manure. Many peaks are grouped with retention times between 5 - 6 minutes and again between 7 – 9 minutes. An array of different light carbon compounds are found in the pre-digestate with more overall total peaks as well than post-digestate manure. Figure 11 - GC-FID graph of compounds present in post-digestation DCM. Some minor peaks are found between 7 – 9 minutes. N-Heptanoic Acid (8.495) is the only compound with a distinguishable peak that is identified. The large peak at 10.789 curving down to the 11.5 minute is unknown, but is most likely un-hydrolyzed UF. The variable in peak size may be due to different lengths of UF polymer in solution (eg. 4 carbon chain, 5 carbon chain etc.) There does not seem to be any formaldehyde present in the system.

File: c:\gc ms analyses\data\cear research\2015\09dec2015\tcmc1001.run
Channel: Front = FID Results
Last recalc: 12/15/2015 11:41 AM

c:\gc ms analyses\data\cear research\2015\09dec2015\tcmc1001.run

4

3

2
5.382

1
5.136
5.118
5.061
5.021
5.395
5.414
5.643
Propionic Acid (5.464)

5.047
5.108
5.257
5.512

Isocaproic Acid (7.753)

Hexanoic Acid (7.970)
8.022
8.056
8.072
8.237
8.358
8.396
8.439
Heptanoic Acid (8.495)
8.452
8.534

6.951
7.448

0

-0

5   6   7   8   9   10   11   12

Minutes
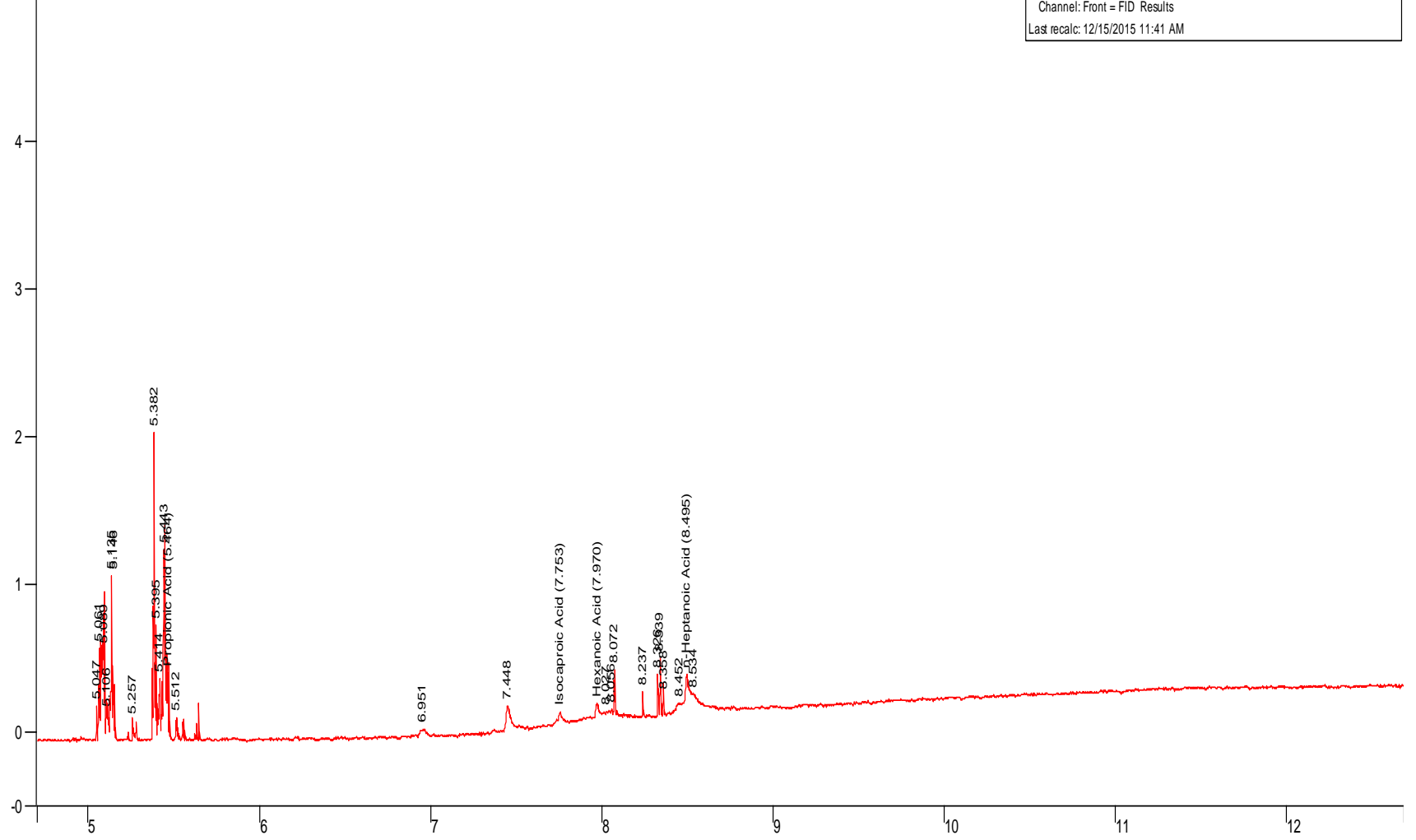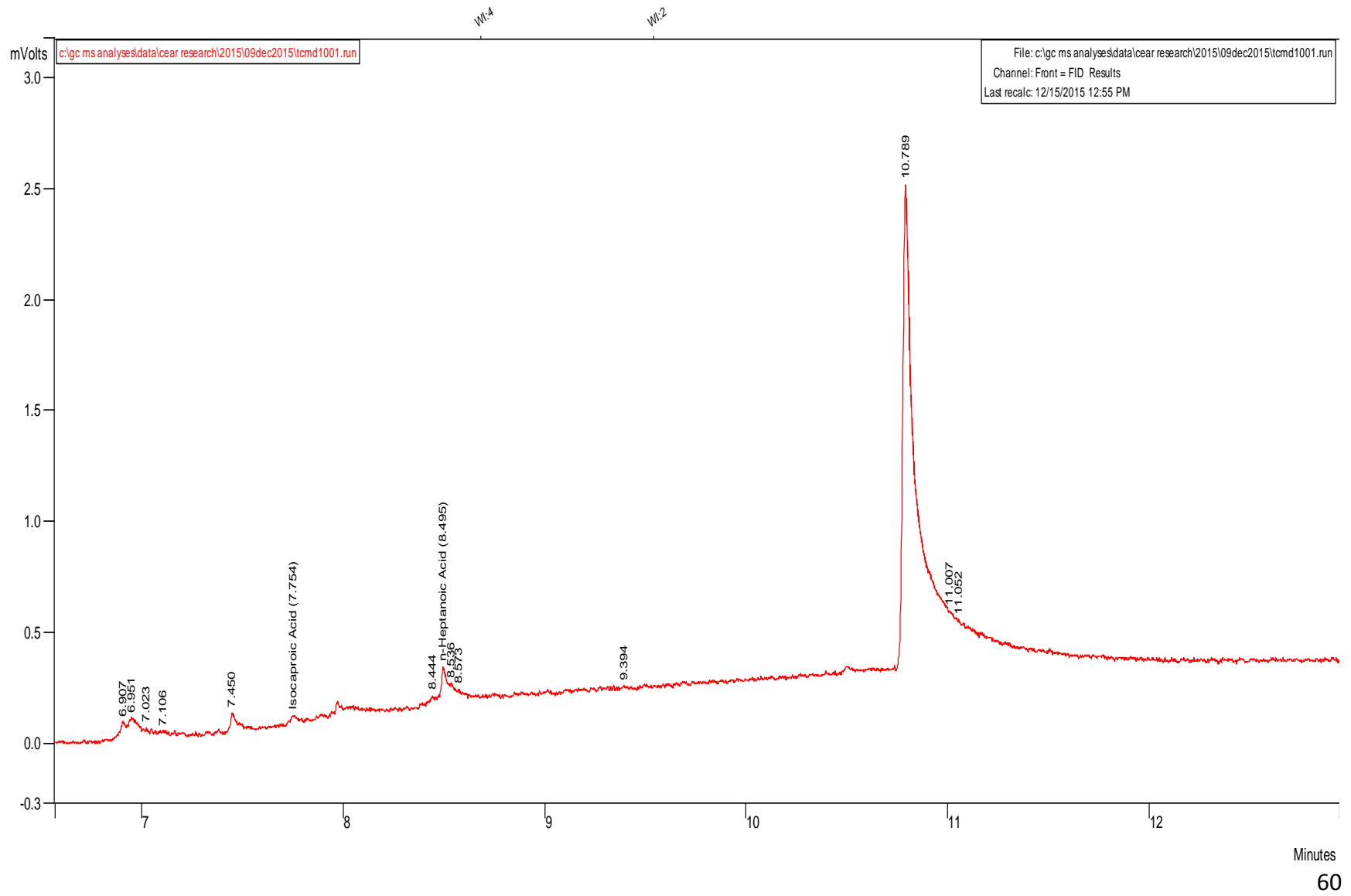
Table 5 – HPLC analysis of standards and stock solutions including retention times and peak strengths.

Sample ID	Retention time (min)	Notes
Deionized H_2O	N/A	Some peaks aprox. 1.5mV in size, most likely noise (λ = 360nm and λ = 210nm)
37% Formaldehyde Solution (Formalin)	0.553	Peak is at 2.5mV (small) (λ = 360nm)
1:1:1 mixture of Formaldehyde-2,4-DNPH (F-2,4-DNPH), H_2O, Formalin	0.642, 0.794, 1.074	1.074 peak is largest with mV = 250. 0.642 peak = 140mV (λ = 360nm) [1.074 peak < 5mV]
1:1 mixture of F-2,4-DNPH + H_2O	0.794, 1.086	(λ = 360nm)
2,4-DNPH + H_2O	N/A	(λ = 360nm)
1:1 2,4-DNPH + Formalin	0.628, 0.781, 1.062	(λ = 360nm)
1:1 mixture of 2,4-DNPH + Acetonitrile	0.759 1.086	0.759 = 440mV 1.086< 5mV (λ = 360nm)
Acetic Acid (pH 4.5 – 5)	1.214, 2.184	(Shimadzu, 2016)
Formic Acid (pH 5)	0.899, 1.762	0.899 peak = 55mV (λ = 210nm)
Lactic Acid (pH 4 - 5)	0.942, 1.797	0.942 peak = 120-130mV(λ = 210nm)
1:1:1:1 Acid Mixture	0.426, 0.770, 1.236, 1.858	Peaks 0.426 and 0.770 were less than 1mV 1.236 peak = 85mV 1.858 peak = 100mV (λ = 210nm)
Fresh Cow Manure (FCM)	1.511, 1.806, 2.192	Fresh Cow Manure, day zero of fermentation (collected on morning of analysis). (λ = 210nm) 1.511 peak = 1250mV 1.806 peak = 550mV 2.192 peak = 250mV *Many smaller peaks were also present, but due to lack of peak strength were not included.
2:1 DCM + Acid Mixture	1.290, 2.949	1.290 peak = 255mV 2.949 peak = 5mV (λ = 210nm) *Large volume on bottom of peak from 0.5-4.0min. This could be due to lack of separation of products.
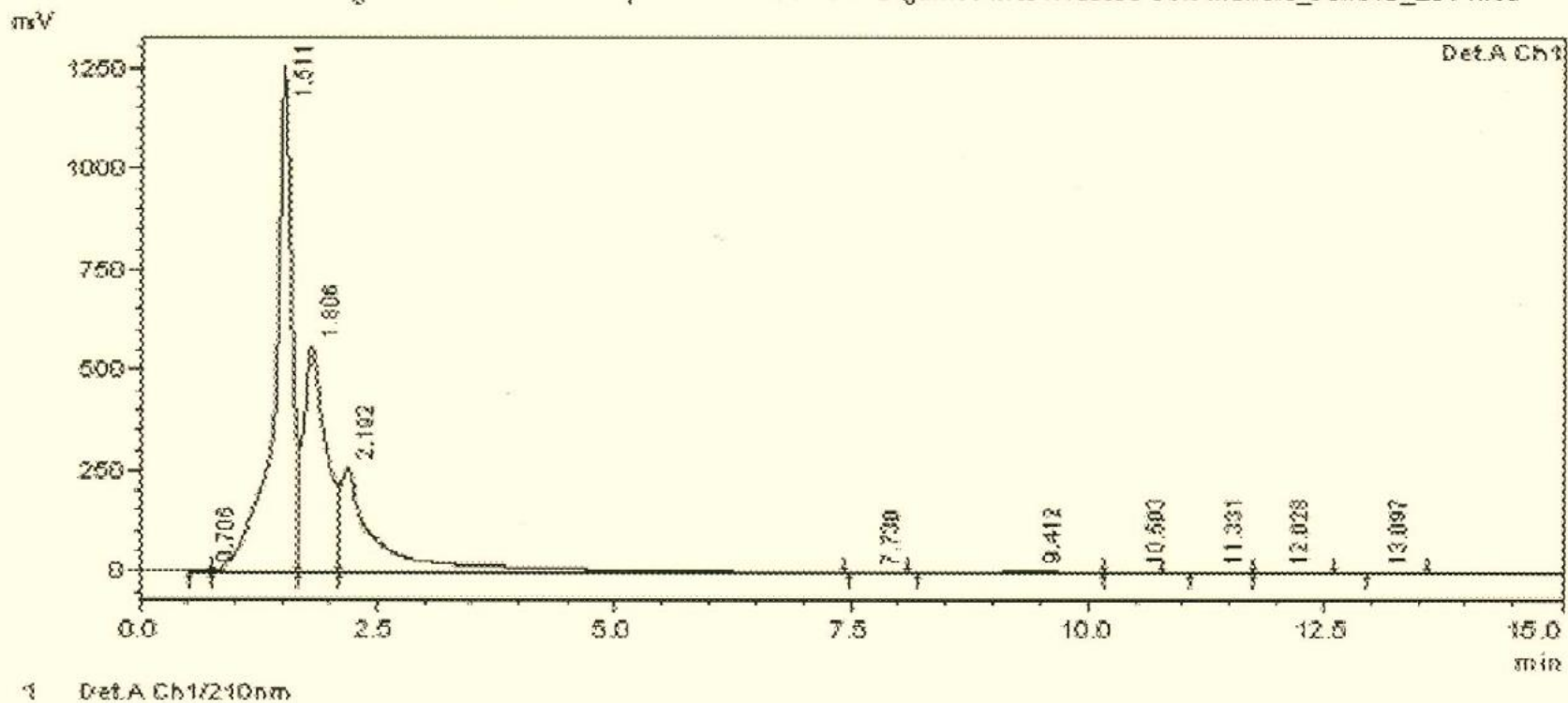
Figure 12 – F-2,4-DNPH in DCM sample. The 4 peaks found >2.5min have considerable amount of background noise in between their peaks. This interference allowed for no quantitative analysis. Identification of fermentation products or FA concentration levels were unsuccessful with this method.
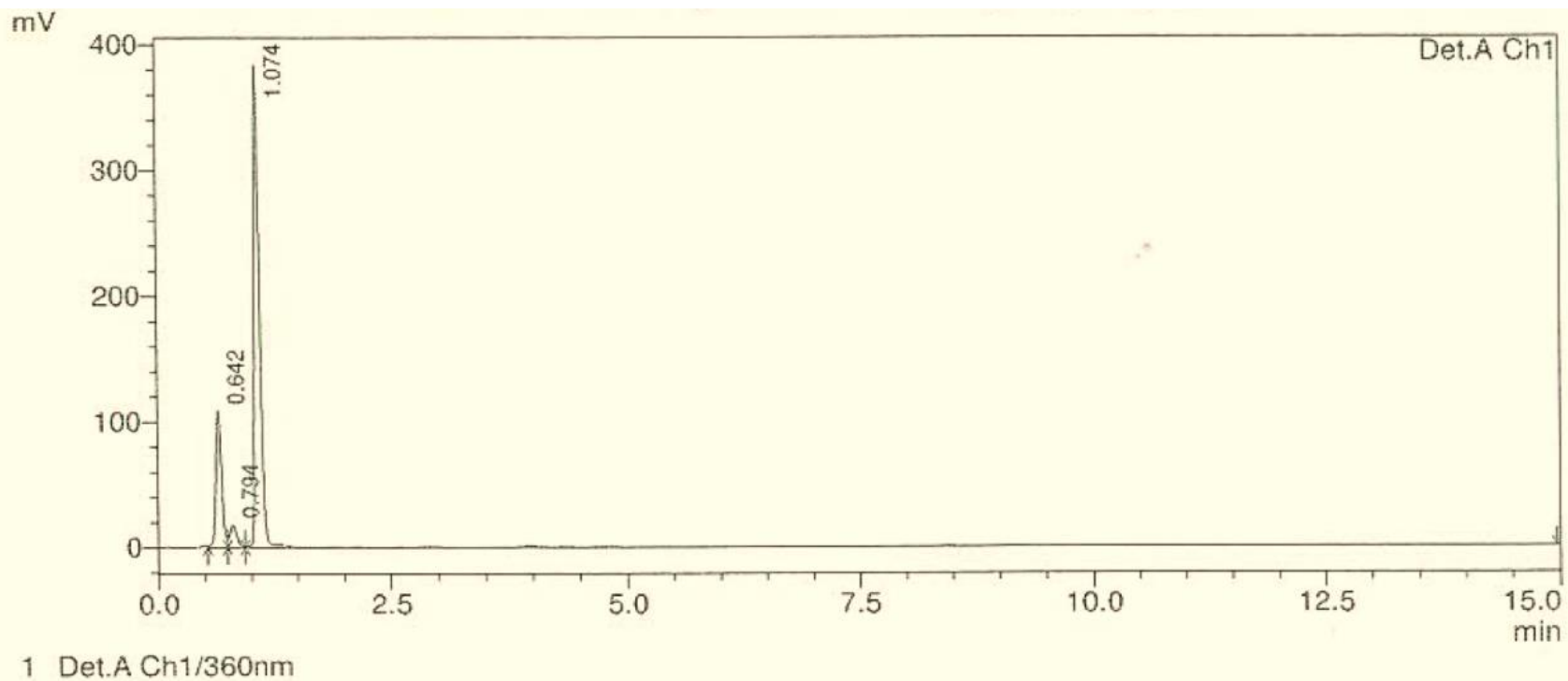
Figure 13 – 2,4-DNPH in water solution. Non-derived formaldehyde is shown at peak 1.074. F-2,4-DNPH is present at peak 0.642. A large amount of unreacted FA is still present in the system. This may be due to concentration differences, formalin (formaldehyde 40% (v/v)) concentration being significantly higher than 2,4-DNPH concentration levels.

3.3 Fungal growth temperature test results

3.3.1 Effect of temperature on fungal FA degradation ability

Paecilomyces variotii strain P20 was the most effective at FA degradation at 32 °C and 40 °C with final FA concentrations of 6.79 and 3.76 ppm, respectively (Figure 14). It was however the least effective at FA degradation at room temperature (RT). P30 metabolized FA the most effectively at RT with a final FA concentration of 14.51 ppm. An inverse relationship was observed between final FA concentrations and temperature for all 3 fungi (Figure 15).

The mixed fungi treatment group ClPa was never the most effective for FA removal. The *Cladosporium sp.* showed little to no FA degradation or complementation with the ability of *P. variotii* to metabolize FA (Figure 14).

One-way ANOVA was used to test for any significant differences between FA levels after 7 days in all treatment types. Room temperature (RT) tests showed significant differences among means (P = 0.0131, α = 0.05). Tukey's multiple comparisons test showed significant differences between P20 and P30 at a 95 % confidence level (α = 0.05). For tests conducted at 32 °C there were no statistically significant differences among means found (P = 0.0503, α = 0.05). The 40 °C tests showed significant differences among the means (P = 0.0226, α = 0.05) and Tukey's multiple comparisons test showed significant differences between P20 and P30.

3.3.2 Fungal growth FA degradation pH and visual observation results

All samples started with the same pH levels close to neutral. A drastic increase of pH was observed over the seven day treatment period. Final pH levels close to 9.0 were observed in all treatment groups (Table 6). The only exception was P30 at room temperature, but a pH of 8.0 was observed, still deemed to be high. Visual inspection of the reactors showed increased darkening of all treatment solutions. This darkening increased over time until the fungi died.
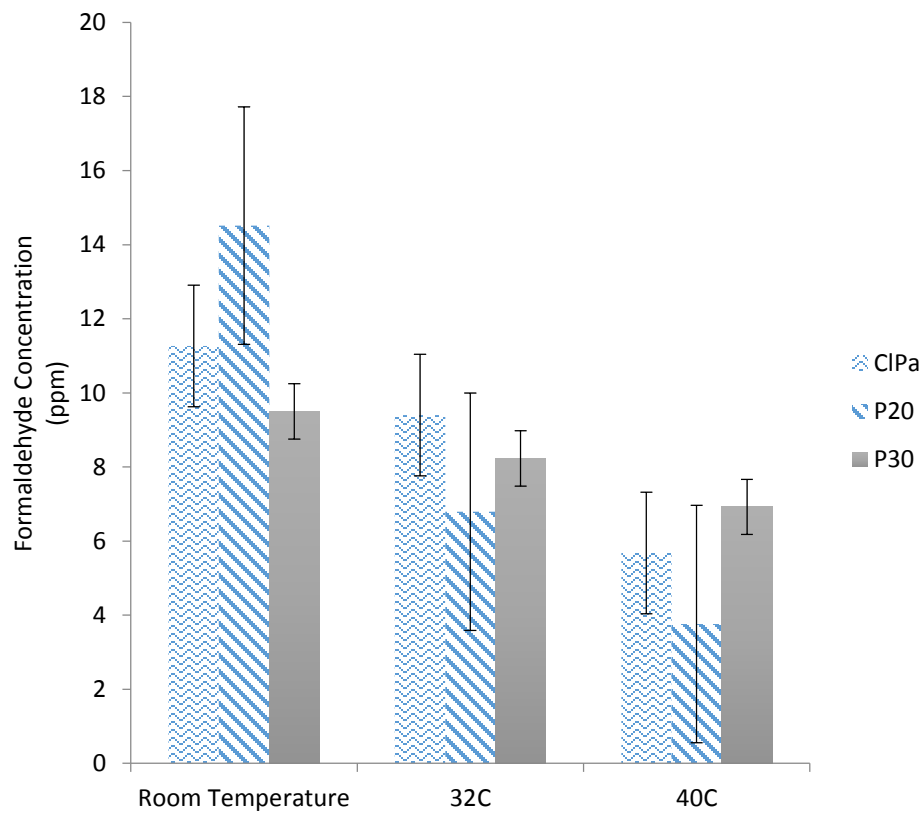
Figure 14 – Final FA concentration after seven day metabolization period for three fungi. An inverse relationship is seen between temperature and final FA concentration. 40 °C is the most effective temperature. Within temperature sets there were significant differences in final FA concentration at RT and 40 °C. At RT there was a difference between P20 and P30 strains. At 40 °C there was a difference between P20 and P30 again.

Table 6 – pH of fungi after seven day period of UF metabolization. Alkaline conditions were found in all treatment groups, ranging from pH 8-9.

SAMPLE ID		ROOM TEMPERATURE	32 C	40 C
P20	pH	**8.65**	**8.99**	**9.06**
	SD	0.25	0.09	0.08
CLPA	pH	**8.87**	**8.97**	**8.99**
	SD	0.06	0.04	0.04
P30	pH	**7.95**	**8.67**	**8.90**
	SD	0.47	0.06	0.15
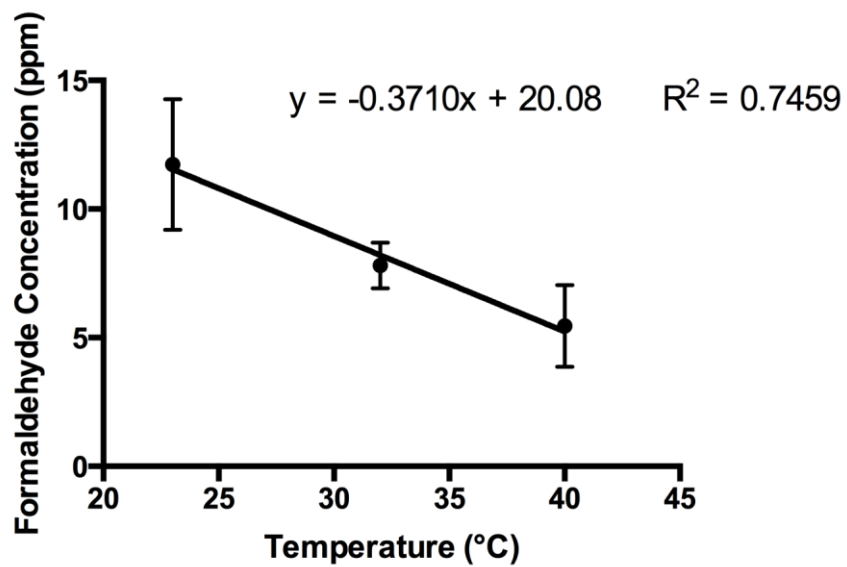
Figure 15 – Relationship between FA degradation ability and temperature for three fungi. An inverse linear relationship is found between the temperature and total FA levels in all treatment groups; data from all three treatment groups was pooled.

3.4 Differences between final FA concentration in anaerobic and aerobic treatments

One-way ANOVA was used to analyze the difference between final FA concentrations in both anaerobic treatment groups (DCM 40 °C and 55 °C) and the 40 °C fungal trials. There were significant differences found among the means of treatment groups ($p = 0.0223$, $\alpha = 0.05$) (Figure 16). Tukey's multiple comparison tests revealed a difference between the 40 °C DCM treatment and P30 treatment ($\alpha = 0.05$).
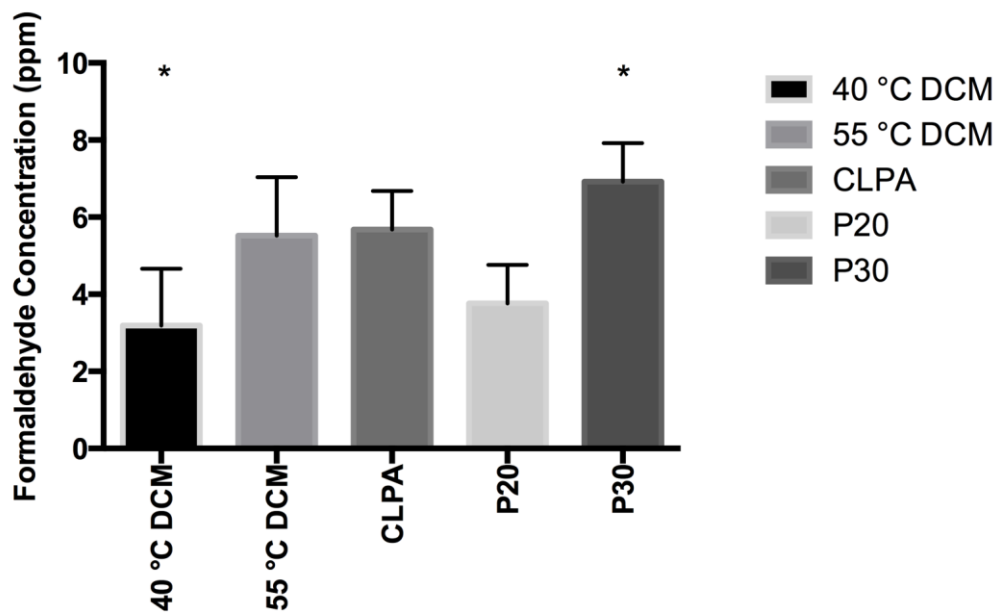
Figure 16 – One-way ANOVA test of most effective FA degradation treatment groups. A one-way ANOVA ($\alpha = 0.05$) analysis of both DCM treatment groups (40 & 55 °C) and the 40 °C fungal metabolization treatment groups. Final FA concentration after treatment was compared. ANOVA determined there was significant differences between the means, with a Tukey's multiple mean comparison analysis revealed the difference to be between the 40 °C DCM and P30 treatment group (*).

4.0 Discussion

4.1 Anaerobic digestion

4.1.1 Selecting the most efficient and economically viable inoculum for anaerobic digestion

Anaerobic digestion is shown to be a viable technique for decontaminating the formaldehyde (FA) out of urea-formaldehyde (UF) bonded medium density fiberboard (MDF). Both diluted cow manure (DCM) and frozen cow manure (FCM) reactors brought FA levels down to similar levels (Figure 3). Unfortunately the decaying sea weed (DSW) inoculum did not bring FA levels down to acceptable levels. There was a fall in FA levels at the ten day mark in DSW reactors, the final FA concentrations were still around 120 ppm. The use of DSW may be a viable inoculum if the retention time was increased or some abiotic conditions were changed, but with little gas production and a poor initial performance it was deemed to be the weakest inoculum of the three.

With regard to DCM versus FCM there were two main differences that were noticed in the data. The first being the total gas production data. In order for anaerobic digestion to be an economically viable waste diversion option the products must have some capital value. Both DCM and FCM post-digestion sludge could be used as a fertilizer. DCM however produced more gas, with >1500 cm^3 as opposed to roughly 500 cm^3 respectively (Figure 4). DCM also had a faster start up rate (Figure 4). Although both DCM and FCM have the same final FA concentrations the lag period in FCM is greater, taking

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longer for degradation to begin (Figure 3). This may be due to the microbes in the FCM taking longer to acclimatize to the temperature of the anaerobic bioreactors (AB). Heating the FCM before digestion was deemed a waste of energy.

Overall using DCM would be more economically viable and possibly more time efficient to use as an inoculum source. These are some of the factors that were taken into account when deciding to use DCM for the next series of experiments in this research.

4.1.2 Optimal abiotic conditions for anaerobic digestion

Once it was determined that DCM would be used as an inoculum source the next step was to determine what temperature would give the best results with FA degradation. A few factors were taken into account, such as final FA concentration, total amount of FA degraded, and gas production. These were the factors used to differentiate quality and efficiency of degradation, however resilience of reactors was also taken into account. If an AB at a certain temperature seemed to have more failures than normal or sudden pH drops (section 1.7.3) then this was seen to lower the overall efficiency of digestion at that temperature.

During the course of this research over 60 AB were created. Of those 60 only three failed. The three AB that failed did so during preliminary testing of AB set up. This led to little data being collected on pH and AB resilience.

4.1.2.1 Optimal temperature for FA degradation

Final FA concentration was statistically equal in both mesophilic (40 °C) and thermophilic (55 °C) treatments (Figures 5 & 6). A one-way ANOVA test was used during each of the sample periods to detect any differences between the 6 reactor groups. It was found that there were no significant differences between any of the negative controls and the treatment types (Figure 5). This may lead to the conclusion that the two temperatures are equally effective, however we must look at the hydrolysis rates to determine the total FA degraded.

It was found in the hydrolysis rate trials (Figure 7) UF hydrolyzed to a greater degree at 55 °C than 40 °C. This indicates that the total amount of FA degraded was greater in the thermophilic AB than their mesophilic counterparts. Taking this into consideration it seems as though the thermophilic conditions are better than mesophilic for total FA degradation. The resilience of these digesters with respect to FA degradation capacity does not seemed to have been reached as little to no reactor failure was observed. It is import to note here that increasing the amount of substrate (MDF) concentration in these reactors could be possible. However, it is likely that urea's metabolic products, such ammonia / ammonium, will be the likely cause of inhibition instead FA the ABs (Yenigun & Demirel, 2013). Potential inhibition factors are discussed in depth in section 4.1.2.2. Thermophilic conditions are better for increasing AB resilience to inhibitors compared to their mesophilic counterparts. Microbes in thermophilic reactors have shown almost twice as much resilience to ammonia / ammonium ion during

digestion; with mesophilic inhibition occurring roughly at 2.8 g L^1 total ammonia nitrogen (TAN) and thermophilic at roughly 5 g L^1 TAN (Poggi-Varaldo et al., 1996, Gallert & Winter, 1997, Sung & Liu, 2003). Allowing higher total FA degradation potential in the thermophilic reactors than mesophilic.

The gas data also agrees with this conclusion. The total amount of gas produced in the thermophilic digesters was greater than in mesophilic digesters (Figure 8). This could be due to greater bioactivity in the form of increased metabolization of the microbes, decreased chemical inhibition, and increase in viable substrate. This increase in gas production supports the use of anaerobic digestion as viable waste management option for EWPs.

Total organic acids in the systems seems to have decreased after digestion (Figure 9). This agrees with what was found in the literature, most of the organic acids being metabolized into carbon dioxide, methane, or other simple organic acids. It is possible that with a longer retention time that the longer chained organic acids may have been metabolized into smaller compounds.

4.1.2.2 Pre-treatment usage and remaining UF resin

The use of a pre-treatment has allowed the FA to slowly seep out of its polymer state into solution at a rate that is manageable by the microbial community in the digesters. This allows FA concentrations of 200 - 250 ppm (Figure 7) to be degraded down to 1 - 3 ppm without killing the microbes. The total amount of FA in the UF polymer is

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roughly 50 % (Padgett, 2009). These levels of FA would be too high for any microbial activity if the resin was all hydrolyzed at once. In the past researchers have tried to treat FA contaminated wastewater with microbial activity ceasing around 250 - 400 ppm (Lu & Hegemann, 1998). Due to the pre-treatment and nature of this technique it cannot be applied to decontaminate wastewater. This pre-treatment helps with hydrolyzing UF polymers quickly allowing a lower retention time for digestion. The GC-FID data however shows an unknown right-tailing peak at 10.789 min (Figure 11). This is the profile of partially hydrolyzed resin still left a solution (Burton et al., 2012, Nimtz, et al., 1997). Increasing the intensity of the pre-treatment through thermochemical means may be a possible solution. Lowering the pH through an organic acid and increasing the pre-treatment temperature to 90 - 140 °C are possible options.

A sudden drop in pH is a problem that is faced sometimes in batch reactors but it was seen rarely in our trials. This may be due to such low substrate concentrations. The FA was tested and found to be mostly degraded, however it is unknown what happened to the TAN in the system. This TAN may be a limiting factor in raising the amount of substrate before inhibition occurs (Sung & Liu, 2003). The amount of MDF added to each digester was relatively low in the industrial sense. It may be possible to decontaminate more MDF through the use of anaerobic digestion by adding more than the standard ten g MDF that was used in this experiment. Due to the time constraint of this experiment we were unable to test the effects of adjusting substrate and inoculum concentrations. In theory the MDF used was roughly 11-13 % resin (Padgett, 2009). Past research shows

complete digestor inhibiton from TANs at 8 – 13 g L^{-1} (Sung & Liu, 2003). This allows the potential limitations of these reactors of MDF levels of 150 – 200 g MDF L^{-1}. However substantial methane production occurs at TAN concentrations roughly 5 g L^{-1} (Chen, Cheng, & Creamer, 2008). This brings the real limit of these reactors to roughly 75 - 100 g MDF L^{-1}. This could be a project for future research.

4.1.2.3 Method discrimination for detected formaldehyde (HPLC, Purpald, GC-FID)

The levels of FA in the post-digestion sludge is the same as the levels of FA found in manure (Figure 5). Upon further inspection with our VFA analysis, it was found that there were no detectable levels of FA. This discrepancy between our colorimetric analyses with Purpald versus GC-FID may come from Purpalds innate characteristic to react with other C_1 compounds such as methanol and other alcohols (Anthon & Barret, 2004). It highly likely that these C_1 compounds are present in the post-digestion sludge and may be reacting with Purpald. The complex and dirty nature of our system means that other C_1 carbons apart from FA will all be present. It is important to note however that the concentration of these C_1 carbons in the MDF treatment groups are the same as in just manure in our 55 °C treatment (MDF- control). Purpald is known to react with aldehydes including aldehyde resins (Harkin, Obst, & Lehmann, 1974). Possibly resulting in Purpald reacting with total FA in each reactor, including the un-hydrolyzed UF. If this is the case then Purpald would be a good indicator for future studies for total UF resin in solution. This claim would require further experimentation however.

The use of GC-FID revealed the un-hydrolyzed UF left in the AB; which would not have been possible with Purpald spectrophotometry. This gives GC-FID analysis certain advantages over Purpald analysis. These advantages do come at a cost however, including time and capital. GC-FID cannot qualitatively give total FA in solution including un-hydrolyzed UF due to too different lengths of the UF polymer possibly present. Again, as stated above, if Purpald can react with all FA in solution, including UF this could provide quantitative data of the amount of UF/FA left in solution. This is speculation and would need further work to determine. Depending on what the experimenter is trying to determine, both these methods are valuable with their own advantages and disadvantages.

The use of HPLC as an analysis tool for FA and organic acids in AB was unsuccessful. When analyzing standards and controlled acid mixtures there was strong resolution (Table 5). However when trying to isolate FA mixed in with DCM there was poor resolution (Figure 12). Too much noise was found in the base of peaks as a result of poor separation. Putting more effort towards fixing this issue and trying to increase peak resolution was deemed too diverting from the purpose of this research.

4.2 *P. variotii* metabolization of UF resin

4.2.1 *P. variotii* total breakdown for FA

The presence of *Cladosporium sp.* seemed to have little to no effect on *P. variotti's* degradation ability (Figure 14). Total FA levels in all of the temperatures were below 10

ppm and *P. variotti* was effective at breaking down free FA from UF resin. Both *P. variotti* isolates strands seemed to have statistically different effectiveness at room temperature and 40 °C. However with such a small difference and small data set, more trials would be required to confirm that statement.

The final FA concentrations in P30 and P20 at 40 °C showed similar levels with the mesophilic and thermophilic AB (Figures 5 & 14). The final FA concentrations found in the 40 °C trials were statistically similar to the FA concentrations in the anaerobic digestion treatment groups (Figure 14). This shows that the degradation of FA in these systems could be occurring much quicker and with the same effectiveness as their anaerobic digestion counterparts. Limitations to this and other issues with this hypothesis can be found in section 4.2.3.

4.2.2 *P. variotii* temperature and pH variation

A clear trend is seen with regards to temperature and total FA degradation (Figure 15). The main enzyme used in the FA degradation pathway, S-hydroxymethylglutathione dehydrogenase, an alcohol oxidase, has been studied in the past (Kondo, Morikawa, & Hayashi, 2008, Fukuda et al., 2012). This enzyme still retained activity of temperatures up to 50 °C (Kondo, Morikawa, & Hayashi, 2008) with optimal activity temperatures of 40 °C (Fukuda et al., 2012). The data found in this experiment are in line with data from the literature. There have also been studies into *P. variotti*'s ability to degrade FA with various pH levels. Stable pH levels for enzyme activity were found to be between 5 – 10 pH

(Kondo, Morikawa, & Hayashi, 2008; Fukuda et al., 2012; Estevez, Veiga, & Kennes, 2005).

However optimal pH has been found to be around a pH of 8. It is important to note that this is strictly for isolated enzyme activity. In our studies we used the fungal organism instead of just isolated enzyme. We found that the fungi most likely died from reaching such high pH levels in the batch reactors. These pH levels could be from a number of different sources. Metabolic bi-products resulting from FA degradation, urea seeping into solution with no microbe for degradation, or precursor compounds released from *P. variotii* prior to metabolization of UF.

4.2.3 Limitations of *P. variotii* degradation of UF resins

Just like the in the anaerobic digestion treatments it is important to look at the FA in the reactors. In this case however un-hydrolyzed FA may not be the issue with *P. variotii's* use for FA degradation. The main issue stems with the aseptic techniques used during reactor set up as well as its low potential for economies of scale. Autoclaving was used before inoculation to prevent any contamination. However hydrolysis during steam autoclaving of polymers is known to occur (Scheirs, 2000). UF polymers are less resistant to hydrolysis than melamine resins or other polymers with additional compounds for moisture induced hydrolysis resistance (Scheirs, 2000). Improper storage conditions such as high temperature and high humidity are known to increase the rate of hydrolysis of UF into free FA (Vargha, 1998). If relatively minor levels of humidity and temperature change can cause an increase of FA in solution, then the condition MDF is under during

autoclaving will definitely release free FA into solution and air. This creates the first issue with this method, the release of FA into the air. An incomplete understanding of the actual amount of FA degraded in solution, and the release of a toxic pollutant into the atmosphere are both scenarios we would like to avoid.

The second issue with regards to this technique is economies of scale. Unlike anaerobic digestion which can be scaled to large industrial settings this method cannot. Autoclaving such large amounts of MDF would be an expensive pre-treatment but a necessary one. Final products after fungal metabolization would also have very little capital potential. It is unknown what these compounds are as stated above but it seems unlikely that they could have the capital potential to offset the cost of autoclaving MDF.

Overall the use of *P. variotii* to decontaminate UF polymers can be an effective one in micro lab scale environments. However due to the aseptic techniques required for achieving successful UF decontamination this method has very high capital cost and may have more negative environmental effects than benefits.

4.3 Conclusion

It was found that formaldehyde (FA) can be degraded both aerobically and anaerobically. Each method used has its own set of advantages and disadvantages.

The use of *P. variotii* as a bioremediatory organism for FA degradation can be a viable option. Final FA levels after 7 days were found to be roughly 4 – 7 ppm. This FA-resistant organism has shown once again that it can be used to degrade FA however it may have a problem with prolonged periods of exposure to urea in solution. The pre-treatment involved in sanitizing MDF and the environment for degradation to take place may have environmental consequences as well. Autoclaving urea-formaldehyde (UF) resins is not an effective way to sterilize MDF for *P. variotii* degradation. This may cause release of free FA to the atmosphere and negates the effects of removing FA from UF resins in the first place. There is also the problem of scaling this method to any reasonable size that would be needed to create an impact on Canadian waste diversion. This method may be useful in the future if innovation occurs with regards to the pre-treatment and chemostat style reactors are used.

The use of anaerobic digestion may be the most effective, environmentally and economically for removal of MDF and its UF resins from Canadian landfills. FA concentration was found to be in the same range as the negative FA controls (MDF-). The effectiveness and resilience of a thermophilic anaerobic digester has been shown in this research. The next step would be to increase the intensity of the pre-treatment, through thermochemical means.

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APPENDICES

Table A1 – Formaldehyde concentration after 38 day retention period. Concentrations with superscript are all found to have no significant differences with other concentrations with the same superscript. 40 °C treatment groups showed FA concentration levels statistically equivalent to negative control digesters with no added MDF.

| SAMPLE	[FA] AT DAY 0 (PPM)	[FA] AFTER 2 DAYS (PPM)	[FA] AFTER 10 DAYS (PPM)	[FA] AFTER 22 DAYS (PPM)	[FA] AFTER 38 DAYS (PPM)
40 °C DCM	5.146 +/ 0.745	12.163 +/- 1.831	2.818 +/- 0.778	1.402 +/- 0.348	3.189 +/- 1.472[ab]
55 °C DCM	5.695 +/ 0.445	12.362 +/- 5.376	2.032 +/- 1.234	2.174 +/- 0.733	5.523 +/- 1.516[a]
40 °C MDF+	5.865 +/- 2.787	7.829 +/- 1.657	20.717 +/ 1.947	148.512 +/- 4.950	112.884 +/- 1.474
55 °C MDF+	5.751 +/- 2.787	6.871 +/- 1.996	61.598 +/- 18.03	202.130 +/- 1.352	107.985 +/- 6.202
40 °C MDF-	5.493 +/- 0.613	1.836 +/- 0.879	0.088 +/- 0.057	0.082 +/- 0.063	1.208 +/- 0.718[b]
55 °C MDF-	6.219 +/- 0.522	25.288 +/- 7.928	0.192 +/- 0.089	0.160 +/- 0.783	1.357 +/- 0.461[b]

Figure A1 – FA concentration levels in each reactor group after 2 days of digestion. All six-treatment groups were found to be statistically equal (P = 0.9800, α = 0.050).

Figure A2 – FA concentration levels in each reactor group after 2 days of digestion. Each digester had FA levels with means below 30 ppm with the exception of the 55 °C MDF – control treatments. This treatment group were the only reactors statistically different FA than the others (P = 0.0003, α = 0.050).

Figure A3 – FA concentration levels in each reactor group after 10 days of digestion. Both mesophilic and thermophilic DCM digesters and the negative controls had the same FA levels (group A). The mesophilic and thermophilic positive control had greater FA levels than the treatment groups and negative controls. The thermophilic positive control had greater FA levels than the mesophilic positive control (groups C and B respectively) (P < 0.0001, α = 0.050).

Figure A4 – FA concentration levels in each reactor group after 22 days of digestion. Following the same trends seen after 10 days of digestion, the two treatment groups and negative controls have statistically equal FA concentrations. Both mesophilic and thermophilic controls have FA levels greater than 100 ppm. Thermophilic positive control reactors have the greatest FA concentrations (P < 0.0001, α = 0.050).

Figure A5 – FA concentrations levels in each reactor group after 38 days of digestion. Both treatment groups and the negative controls all have statistically equal FA concentrations (group A). FA levels have dropped in the two positive controls from the previous sampling time. White hyphae like strands were visually seen in solution, and this drop in FA concentration may be due to the presence of a FA resistant fungus. Both the control groups had statistically equal FA concentration levels (P < 0.0001, α = 0.050).
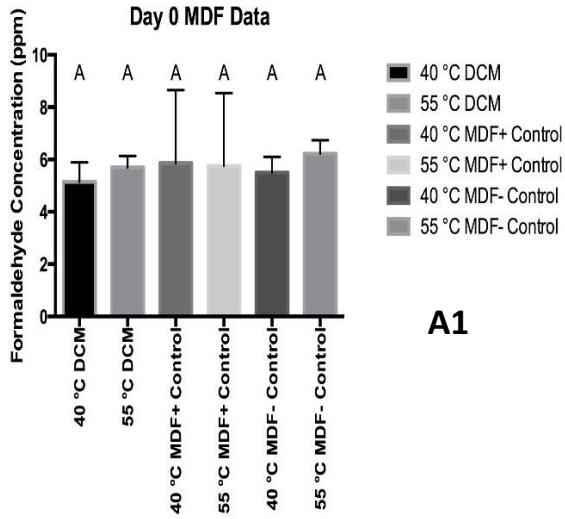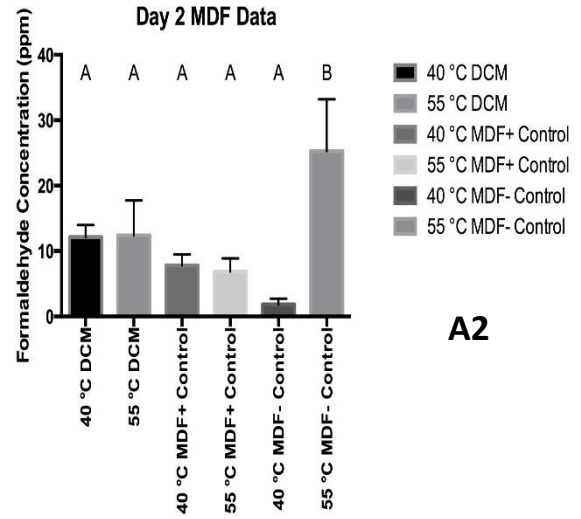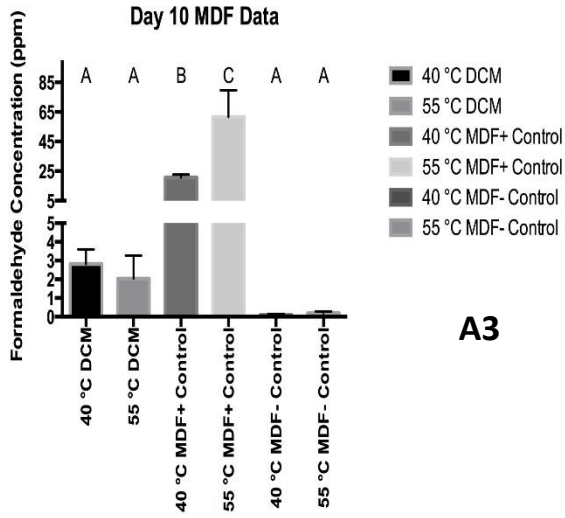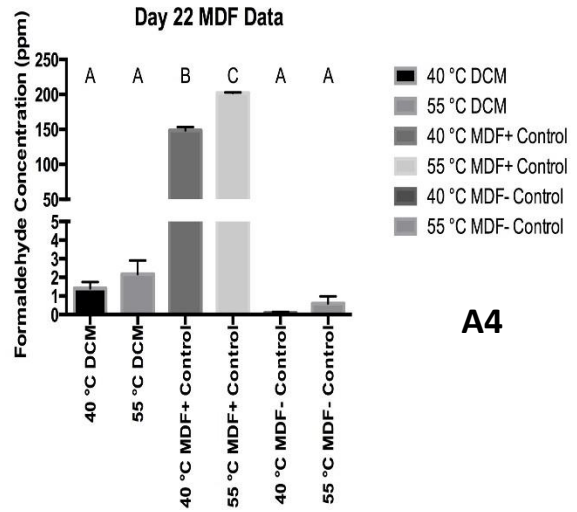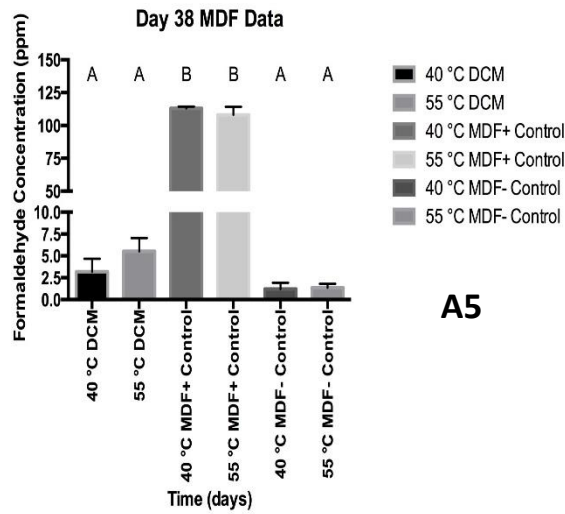
Table 2A – Rention times and presences of organic acids found in pre- and / or post-digestion cow manure through the use of GC-FID.

COMPOUND	RETENTION TIME (MIN)	PRESENCE IN PRE OR POST DIGESTION
ACETIC / FORMIC ACID	4.581	Post-digestion
PROPIONIC ACID	5.464	Pre-Digestion
BUTYRIC ACID	6.503	Post-digestion
VALERIC ACID	7.380	Post-digestion & Post-digestion
ISOCAPRIONIC ACID	7.754	Post-digestion * Post-digestion
HEXANOIC ACID	7.972	Post-digestion & Post-digestion
N-HEPTANOIC ACID	8.497	Post-digestion & Post-digestion