Enhanced Biodegradation of Model Lignocellulosic Wastes in Laboratory-Scale Bioreactors & Landfills

by

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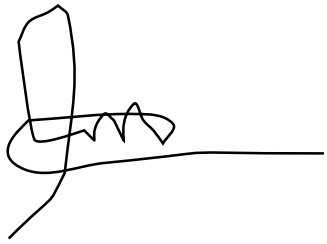
Plagiarism Statement

I, **Syed Muaaz-Us-Salam**, hereby declare that this doctoral thesis is a result of my own work, apart from where references have been made noticeably to the work of others.

Signed: Syed Muaaz-Us-Salam 

Abstract

In municipal solid waste (MSW) landfills, lignocellulosic wastes degrade slowly and cause the slow and prolonged release of biogas into the atmosphere. This release is adding to anthropogenic climate change, which is arguably the biggest challenge humankind faces today and requires immediate attention. As a solution to this problem, the overall aim of this study was to enhance biodelignification in landfills. This aim was supported by two research questions - To what extent can enzymatic & bacterial biodelignification systems breakdown lignocellulose in realistic lignin wastes, with the prospect of enhanced biogas recovery? What is the impact of flow & heterogeneity on bacterial biodelignification systems in model lignocellulose-containing bioreactor landfills? Two representative lignocellulosic wastes found largely undegraded in old landfills, i.e. newspaper and softwood, were used. Lignin peroxidase enzyme and a recently isolated lignin-degrading bacterial strain (*Agrobacterium sp*.) were used in tests conducted in stirred bioreactors with methanogens from sewage sludge. Lignin peroxidase resulted in ~20% enhancement in cumulative methane produced in newspaper reactors. It had a negative effect on wood (~10% decrease in total methane generated compared to controls, possibly due to simultaneous depolymerisation and repolymerisation of lignin on the surface of the wood preventing further depolymerisation). *Agrobacterium sp.* strain enhanced biodegradation of both wood (~20% higher release of soluble organic carbon and enhanced breakdown) and newspaper (~2-fold biogas yield). Furthermore, homogeneous and heterogeneous pore-structure configurations containing newspaper and sand were prepared to mimic old landfills. In the homogeneous case, 2-fold enhancement of biogas yield occurs, which is consistent with soluble organic carbon (sOC)/pH profiles. In the heterogeneous case, there is no significant enhancement. This is likely due to the much lower hydraulic conductivity of the newspaper/sand mixture compared to the outer sand zone, resulting in preferential flow paths through the sand. This paired with very low pH and very high sOC in the column impacts the microbial communities and their activity adversely. Overall, this thesis has surveyed the literature and identified the problem of slowly degrading newspaper and woody wastes in landfills. It has formulated research questions addressing this problem by studying accelerated degradation of these wastes, and the application of this technology to conditions close to real-life field-scale conditions (flow, heterogeneity). Enzymatic and bacterial biodelignification systems show promise under stirred-bioreactor conditions, as well as homogeneous lab-scale landfills. However, under heterogeneous conditions, the biodegradation process is more complicated.

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I would like to thank Heidi for her continued moral support throughout the course of my PhD. There were difficult times, and times that were very happy and you were there through it all by my side. Your companionship means the world to me. I also thank my parents and sister. I would also like to acknowledge the immense influence of Richard Feynman on myself as a person, and my approach to teaching and scientific work, especially when I had just started my PhD. His books helped me very much to realise that a good scientist comes in all shapes and sizes, and his love of finding things out, I have found almost contagious. To the reader, if they have not already, I highly suggest watching his 1964 Messenger Lectures.

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"What a fine day for science!" - Dexter McPherson

Contributions[1]

Muaaz-Us-Salam, S., Cleall, P.J., Harbottle, M.J.; (2017). "Enhanced Delignification and Methane Generation in Waste Repositories – Overcoming Heterogeneity." Proceedings Sardinia 2017, 16th International Waste Management and Landfill Symposium.

Muaaz-Us-Salam, S., Cleall, P.J., Harbottle, M.J.; (2019). "The Case for Examining Fluid Flow in Municipal Solid Waste at the Pore-Scale – A Review." Waste Management & Research, Vol. 37 Issue 4.

Muaaz-Us-Salam, S., Cleall, P.J., Harbottle, M.J.; (2020). "Application of Enzymatic & Bacterial Biodelignification Systems for Enhanced Breakdown of Model Lignin Wastes." Science of the Total Environment, Vol. 728, 138741.

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Muaaz-Us-Salam, S., Cleall, P.J., Harbottle, M.J.; (). "Impact of Flow & Pore Structure on the Activity of *Agrobacterium sp.* In Lab-Scale Landfills." (In preparation).

[1] Produced during the course of this project.

Awards & Honours[2]

- Chapter 4 Nominated for International Solid Waste Association Publication (ISWA) Award 2020 (2020)
- Best Speaker – Young Persons' Lecture Competition (Wales) (2018), IOM3 – Institute of Materials, Minerals & Mining
- Best Speaker - Judge's Choice Award (2017), Speaking of Science Conference
- Best Speaker (2017), 9th Gregynog PGR Conference
- Emerging Engineers Award (Wales) - (2017), Institution of Civil Engineers
- Summer School Scholarship (2017), Tsinghua & Cardiff Universities
- International Travel Bursaries (2017)
- David Douglas Award - Outstanding Paper (2017), South Wales Institute of Engineers Educational Trust
- Wastepack Awards (2016 and 2017), Chartered Institution of Wastes Management
- Environmental Research & Education Foundation (EREF) Scholar (2017)
- Postgraduate Research Scholarship - PhD (2017), School of Engineering, Cardiff University

[2] Awarded during the course of this project.

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III. Nomenclature

MSW	Municipal Solid Waste
MBT	Mechanical Biological Treatment
GHG	Green House Gas
MRF	Materials Recovery Facility
AD	Anaerobic Digestion
VFA	Volatile Fatty Acids
FSQ	Final Storage Quality
IPCC	Intergovernmental Panel on Climate Change
TC	Total Carbon (%)
OC	Organic Carbon (%)
sOC	Soluble Organic Carbon (mg/L)
sTC	Soluble Total Carbon (mg/L)
VS	Volatile Solids (%)
TS	Total Solids (%)
k	Hydraulic Conductivity (m/s)
BMP	Biomethane Potential (ml/g)
ABS	Absorbance at 660nm (OD-optical density)
Υ	Maximum Biogas Yield (ml/g)
μ	Specific Production Rate (day^{-1})
t	Time - modelling (day)

1 Introduction

By 2025, the global MSW generation by urban residents is expected to surpass 6 million tonnes per day (Hoornweg & Bhada-tata, 2012). The planet is struggling to cope with the adverse effects of existing waste that was generated (greenhouse gas emissions, contamination of the geoenvironment etc.), all the while current projections estimate that by the end of this century waste generation rates will exceed 11 million tonnes per day (greater than three times today's rate) (Figure 1-1) (Hoornweg et al., 2013). Today, landfilling is the most widely employed technique for waste disposal in the world, especially in developing countries (Manfredi et al., 2009; Renou et al., 2008). As such, it is estimated that landfills will be in existence for the foreseeable future.

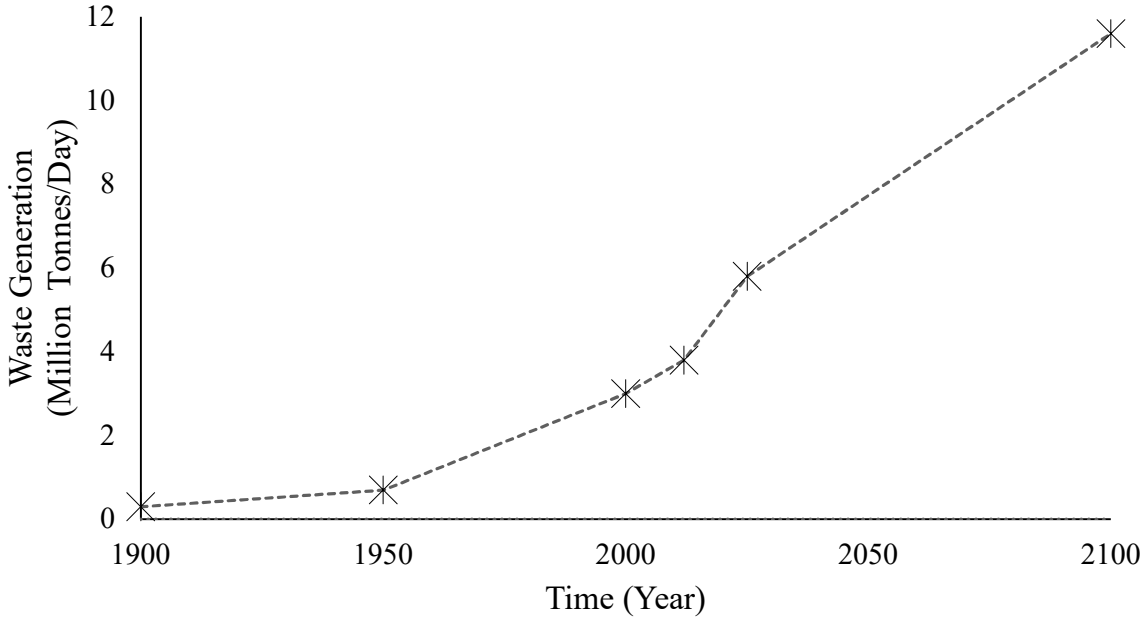

Figure 1-1. Past and projected global waste generation. Figure modified from Hoornweg et al., 2013:p.616.

Globally over 300 million tonnes of MSW are landfilled every year, over 50 million tonnes go to open dumps and only 135 million tonnes are recycled (Hoornweg & Bhada-tata, 2012). Recent research has shown that GHG emissions, mainly through slow release of methane throughout the life of a landfill, substantially contribute to the GHG accounting (up to 1000 kg of CO_2 -eq. per ton of waste for open dump landfills) and hence, the anthropogenic climate change of our world (Manfredi et al., 2009). Approximately 15% of the global methane emissions originate from landfills (West et al., 1998).

The methanogenic stage in landfills can last for centuries due to the presence of the slowly biodegradable fraction of MSW, i.e., lignin-rich wastes (Liu et al., 2014; Williams, 2005). Carbon dioxide and methane are predominant landfill gas components. While both are major contributors to climate change, in comparison with carbon dioxide, methane traps approximately 20 times more infrared energy and its global warming potential is 25 times higher over a 100-year period (De la Cruz, 2014; Eleazer et al., 1997; Jung et al., 2011). Therefore, it is widely accepted that landfills release GHG emissions and other contaminants (through leachate), e.g. dissolved heavy metals, organic matter etc., for at least several decades and stabilisation of the bulk of the waste may take hundreds of years (Fei & Zekkos, 2012; Reinhart & Townsend, 1997). Even for lined landfills, the trouble is that the time-scale over which these processes occur is often greater than the design life of the containment system (landfill cap, liner etc.). As such, the current practice of landfilling is not considered to be sustainable (Reinhart & Townsend, 1997; Reinhart et al., 2002; Townsend et al., 2015; Williams, 2005).

In addition to the geoenvironmental concerns highlighted above, a key issue for landfill operators is the slow methane release and its low concentrations accompanying the degradation of lignin wastes which cannot be utilised for energy production (Barlaz, 2006; Barlaz & Reinhart, 2004; Reinhart et al., 2002; Warith, 2002). This results in the operators having to carry out, by law, extensive monitoring (pre- and post- closure), which is costly and time-consuming (Benson et al., 2007; Townsend et al., 2015).

The above information demonstrates the significance of landfills in our world. It establishes the importance of understanding the physical processes occurring in landfills so that we may engineer them to be more sustainable.

1.1 Detailed Description of the Problem

In the UK approximately 57% of the total municipal waste generated was landfilled in 2015 (DEFRA, 2016). Likewise, around 52% of MSW in the US is currently landfilled (EPA, 2016). In the US, paper (26.6%), food (14.9%), yard trimmings (13.3%) and wood (6.2%) collectively amount to around 61% of the total MSW and 52% of the 136 million tons that are landfilled. Due to the presence of construction and demolition waste, landfills may contain up to 40% wood (Staley & Barlaz, 2009; Staley, 2009). In 2014, 11 million tons of wood and 19.5 million tons of paper and paperboard were disposed in US landfills (EPA, 2016). Likewise, a

significant proportion of waste landfilled in Europe is rich in lignocellulose (e.g. 460,000 tonnes of woody waste sent to landfill in 2014 alone) (Eurostat, 2017; O'Dwyer et al., 2017). Prior to 2008, 1.5 million tonnes of wood waste were deposited in Austrailian landfills (Ximenes et al., 2015). Similarly, a significant proportion of wastes generated and landfilled in Asia is rich in lignocellulose (Yadav & Samadder, 2018). As such, significant amounts of woody and paper wastes are present in landfills around the world, both types of fractions are derived from plant biomass, i.e. lignocellulose.

10-30% of plant lignocellulosic biomass is typically made up of lignin. It is an aromatic polymer comprised of phenylpropanoid aryl-C-3 units linked through different types of ether and C=C bonds (Bugg et al., 2011a). Due to its complex chemical structure, lignin is highly resistant to biodegradation. As paper, yard trimmings, wood and food waste are derived from plants and trees, they are the predominant source of lignin in landfills. In plant biomass, lignin forms a matrix where cellulose filaments and hemicelluloses are immersed. In turn, their bioavailability for degradation is severely reduced (Ahmad, 2010; Bugg et al., 2011a; Cragg et al., 2015; Martinez et al., 2009). The lignin content of MSW typically varies between 7% and 30% depending on the source and geographical location. Wood and paper products contain some of the highest lignin contents (e.g. ~28.3% and ~16.3% respectively) (Barlaz, 2006; Wang et al., 2011).

As such, these fractions are widely believed to contribute to the prolonged life of a landfill and give rise to the long tail characteristic of the classical landfill gas generation curve (Figure 1-2) (Wang, 2015). In MSW landfills, hemicellulose and cellulose degrade relatively quickly and contribute to biogas generation (Figure 1-2,sharp rise and peak). However, lignin is very hard to degrade for the micro-organisms and this drastically prolongs the life of a landfill (long ‘tail’ of emissions as shown in Figure 1-2) (Barlaz, 1999). The long methanogenic stage, which can last several decades is the result of lignin’s resistance to biodegradation and results in a gradual and slow release of biogas until the start of the aerobic stage, this could take over 100 years (Reinhart & Townsend, 1997; Townsend et al., 2015). As aforementioned, the time-scale for slow release of biogas into the atmosphere likely exceeds the design life and monitoring period of a typical landfill. This practice is unsustainable, from an environmental as well as management viewpoint.

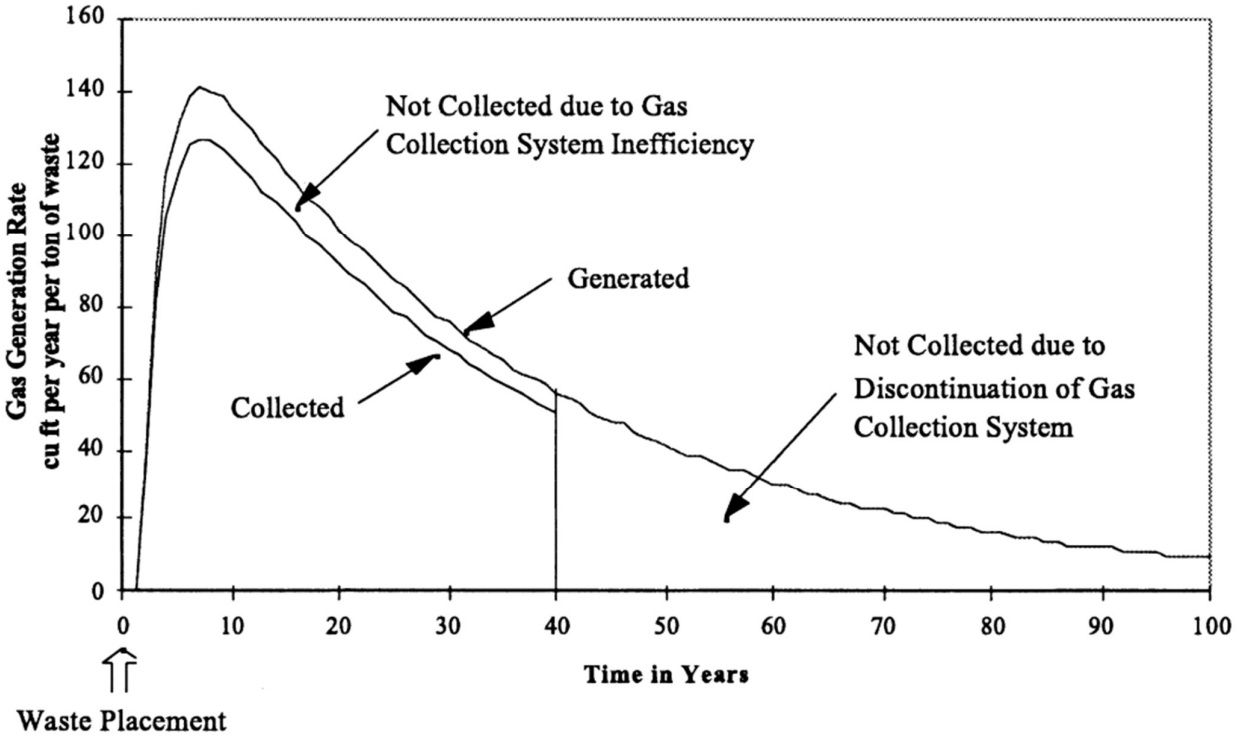

Figure 1-2 Profile for the rate of biogas generation in a landfill, notice the long 'tail' of emissions due to the slowly degrading lignocellulosic fraction mainly made up of woody and newspaper wastes. Figure reproduced from Camobrecco at al., 1999:p401.

Upon exhumation of 30-year old MSW from landfills, researchers found the lignin content of the waste to be approximately 81%, while the hemicellulose and cellulose had been degraded (Jayasinghe et al., 2011). This goes to show the extent of recalcitrance of lignin in a landfill. Woody and newspaper wastes are rich in lignin content, and have been found typically undegraded in landfills (in some reported cases, the newsprint preserved well enough to be read, and wood virtually identical to its counterpart prior to landfilling) upon exhumation. As such they are at the heart of this problem (Barlaz, 2006; Ximenes et al., 2015; Ximenes et al., 2017; Ximenes et al., 2008).

Accelerated MSW waste degradation has been of interest in the field, whereby controlling and optimising the various degradation processes can provide efficient and faster biodegradation of the waste mass. From some of the first laboratory-scale leachate recirculation tests for refuse decomposition, pioneered in the 1970s, to field-scale bioreactor demonstration projects in the US today, advances in MSW research in the last 30 years have provided potential avenues towards the prospect of sustainable landfilling (Pohland, 1980, Barlaz and Reinhart, 2004). Researchers have investigated the degradation of MSW with the addition of sewage sludge, inocula from different sources and leachate recirculation (Barlaz et al., 1987, Barlaz et

al., 1989a, Barlaz et al., 1989b, Barlaz et al., 1990, Borghi et al., 1999, Weaver, 2013). The cellulose (C) and hemicellulose (H) fractions of MSW degrade reasonably well. However, due to the recalcitrance of lignin (L) to degradation, its content in MSW does not change significantly (Barlaz, 2006, Bugg et al., 2011a, Bugg et al., 2011b).

Enzymatic and bacterial degradation of lignocellulosic fraction of MSW, initially with a focus on cellulose, has been studied, (Lagerkvist and Chen, 1993, Frank et al., 2016). More recently, lignin has been the focus of enzymatic and microbial degradation in MSW with promising results (Jayasinghe et al., 2011, Jayasinghe et al., 2013, Jayasinghe, 2013, Hettiaratchi et al., 2014, Jayasinghe et al., 2014, Hettiaratchi et al., 2015, Rashid et al., 2017). The benefit here is that accelerating lignocellulose degradation by these biotechnological methods could help to shorten the period over which the landfill produces biogas, thereby making its management easier (i.e., preventing escape and capturing for renewable energy). However, studies to date have some shortcomings. The experiments have mainly been carried out in ideal lignin-containing sample wastes (model molecules etc.) and information is lacking on the applicability of these biodelignification systems on 'real' waste material found in landfills. Likewise, they have also either homogenised the waste by shredding it to a very small size and/or carried out the experimental work at a scale that may not be representative of the bulk of the MSW size fraction (small-scale experiments aimed at achieving mechanistic insights mainly). This essentially removes the physical heterogeneity element from the study, as well as some other factors that apply to 'real-life' landfills (e.g. leachate flow). As such, it is difficult to evaluate the in-situ applicability of such treatments. The advantages of in-situ treatment include application to existing and prospective landfills.

Contributions studying leachate flow in MSW have been made and the existence of preferential flow has been well established (Bendz et al., 1998, Bendz and Singh, 1999, Rosqvist and Bendz, 1999, Powrie and Beaven, 1999, Al-Thani et al., 2004, White et al., 2011, Beaven et al., 2011, Woodman et al., 2013, Woodman et al., 2014, White et al., 2014, Woodman et al., 2015). However, the mechanisms governing transport in MSW (e.g. two phase-flow involving downward leachate flow and predominantly upwards landfill gas flow, governed by advection, mechanical dispersion and diffusion) are still not fully understood, especially at the pore- and meso-scale. To effectively accelerate MSW degradation in landfills, further understanding of biodelignification systems and their operation is required. Flow mechanisms that control transport in waste also determine the delivery of such biotechnological

systems to key areas where the lignocellulosic waste is present and will need to be understood well. This is particularly the case in a material where preferential channelling is very apparent, such as MSW. The treatment will not work well if the leachate (augmented with the biodelignification system) travels preferentially through the larger pores bypassing the key areas containing the 'target' fraction.

In addition to studying the applicability of enzymatic and bacterial systems on 'real' lignocellulose-containing wastes found typically undegraded in landfills, studies examining the impact of preferential flow on lignin degradation will be beneficial. An integration of the aforementioned research areas of accelerating lignocellulose degradation and studying this process with realistic factors at play, that would be present in a landfill (i.e. heterogeneity of waste, leachate flow) should provide some insights into the possibility of efficient and accelerated in-situ biodelignification of MSW, which could then lead to a future with more sustainable waste management.

1.1.1 Research Questions

In the preceding text the problem of slowly degrading lignocellulosic wastes in landfills has been established. Potential biotechnological ways to address this problem have been touched upon. This leads on to the overall aim and the research questions (RQ) investigated in this thesis.

Overall Aim: To assess the potential to enhance lignocellulose degradation of typical lignin-containing landfill materials via biodelignification systems & study its relationship with preferential flow.

- RQ1: To what extent can enzymatic & bacterial biodelignification systems breakdown lignocellulose in realistic lignin wastes, with the prospect of enhanced biogas recovery?
- RQ2: What is the impact of flow & heterogeneity on bacterial biodelignification systems in model lignocellulose-containing bioreactor landfills?

1.2 Thesis Outline

- *Chapter 1* highlights the problem that was investigated in this project, the overall aim and the research questions.

- *Chapter 2* reviews the state of the art surrounding slow waste degradation in bioreactor landfills, the main cause and prospective solutions offered by biotechnological intrervention. Moreover, the gaps in knowledge regarding biotechnological intervention, its application and delivery to key target areas are also explored. Based on these gaps and our understanding, it is further shown how the RQs were formulated.

- *Chapter 3* contains the materials and methods used in this project, including the experimental workflow (to answer the RQs), the model lignocellulosic wastes, analytical methods, statistical analyses etc.

- *Chapter 4* addresses RQ1. Specifically, application of enzymatic and bacterial biodelignification systems to model wastes in stirred bioreactors is carried out. The results and insights obtained are then discussed following by concluding remarks.

- *Chapter 5* addresses RQ2. Specifically, it looks at application of a bacterial biodelignification system to lab-scale homogeneous and heterogeneous bioreactor landfills containing a model lignocellulose waste with and without leachate recirculation. The results and insights obtained are then discussed following by concluding remarks.

- *Chapter 6* presents an overall discussion, focusing on insights obtained from the literature review, work carried out to answer RQ1 and RQ2 and the contribution to knowledge made by this thesis. It then summarises and concludes the entire body of work presented in this thesis and touches up on prospects and ideas for further work.

- *Chapter 7* detailes the reference list.

- Finally, the appendix contains supplementary data from preliminary and/or miscellaneous experiments, miscellaneous modelling work on biogas profiles etc. is given.

2 State of the Art[3]

This chapter presents a targeted review of the state of the art in i) bioreactor landfills, ii) enhanced lignocellulose degradation and iii) flow mechanisms in MSW landfills, specific gaps in knowledge are explored within these sections which in turn have helped formulate the RQs listed in section 1.1.1. Figure 2-1 provides a brief overview of the different processes that occur in the waste mass. It is around these processes that this targeted review resides. As such, Section 2.1 lays out a brief overview of the principle of a bioreactor landfill, fundamental degradation processes, and major factors affecting these processes. Section 2.2 leads into the literature on currently slow-degrading lignocellulosic wastes in landfills, prospect for their enhanced degradation, the biotechnological methods used for this purpose and implications for field-scale applicability. Section 2.3 specifically is related to literature on the understanding and modelling of the leachate flow aspect of MSW landfills, since (as also mentioned in RQ2) delivery of these biotechnological agents, determined by where the leachate will go, for enhanced degradation is of paramount importance when it comes to real-life field-scale applicability. Given the gaps in knowledge regarding understanding flow in landfills, a case is made for developing a deeper mechanistic understanding of these fundamental flow processes at the pore-scale to inform and correct models at larger scales. Section 2.4 briefly discusses and concludes the findings of this chapter, and highlights where the RQs sit in the gaps in knowledge pointed out earlier in the chapter.

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[3] This chapter is based, in part, on these previously published papers :

Muaaz-Us-Salam, S., Cleall, P.J., Harbottle, M.J.; (2017). "Enhanced Delignification and Methane Generation in Waste Repositories – Overcoming Heterogeneity." Proceedings Sardinia 2017, 16th International Waste Management and Landfill Symposium.

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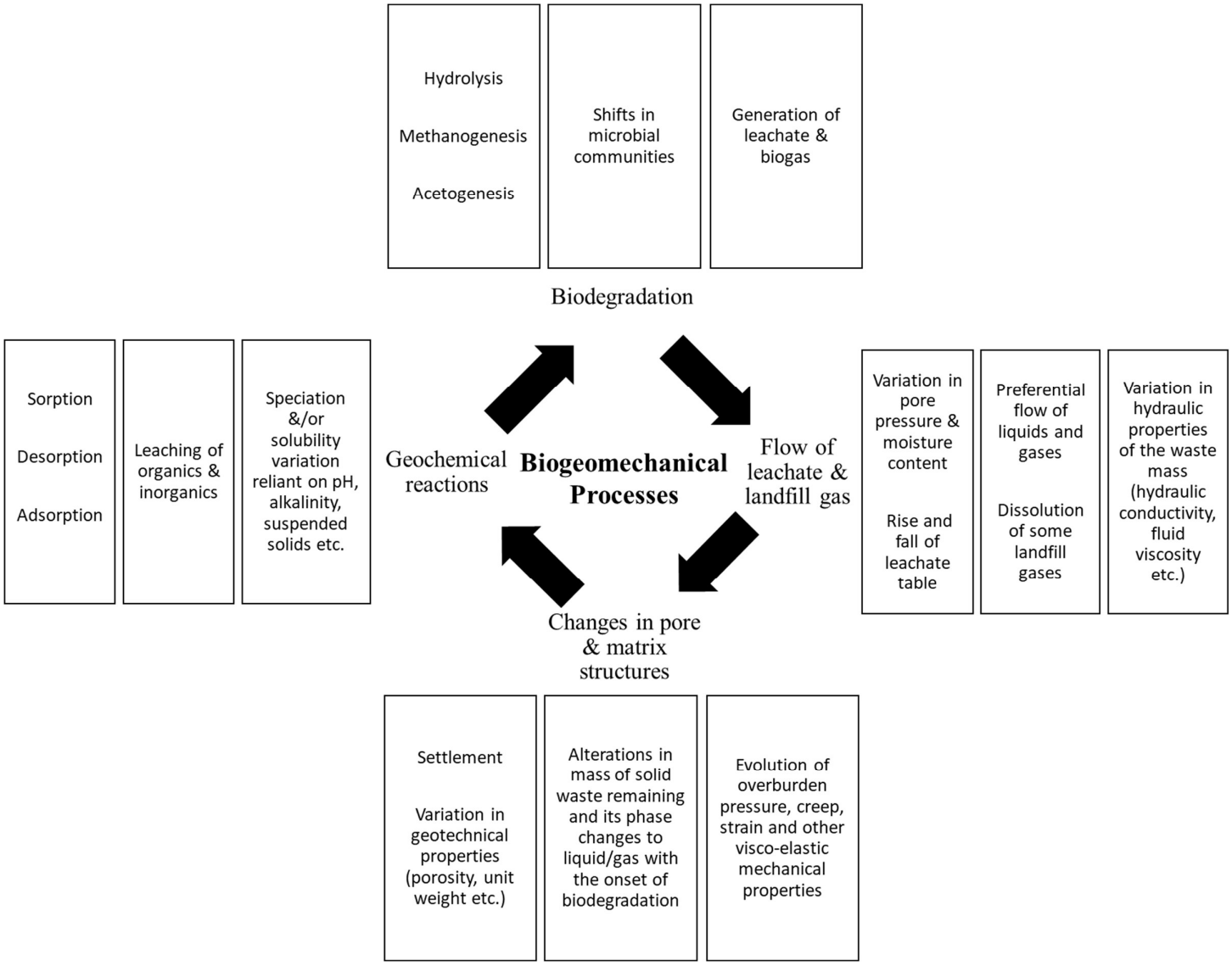

Figure 2-1 Different processes that take place in the MSW system. Conceptual model informed by the works of Datta et al., 2018, (Fei et al., 2014a).

2.1 Bioreactor Landfills

The modern landfill has been the result of an iterative process that has evolved from land disposal in open dumps to containment-only landfills and the bioreactor landfill. The idea behind the bioreactor landfill is to operate it as a contained system due to the health, safety, aesthetic and geoenvironmental problems associated with open-dump disposal (Reinhart & Townsend, 1997; Townsend et al., 2015). It uses microbial processes to stabilise the readily decomposable organic waste within 10 years of implementation (Jayasinghe, 2013). Figure 2-2 shows the layout of a modern bioreactor landfill. The waste in the landfill is placed in compacted cells. The waste is usually deposited every day, compacted (2-4 m in height) and covered with a daily cover. Once a layer of cells has been deposited, an intermediate soil cover is placed and the process is repeated until the landfill is full. Upon completion, the landfill surface is covered with a final cap (Tchobanoglous et al., 1993). At this point, the energy required to operate, construct and close a landfill may be less than the energy recovered, resulting in a renewable source of energy. As such, there is growing interest in landfill gas-to-energy projects worldwide (Barlaz, 2006).

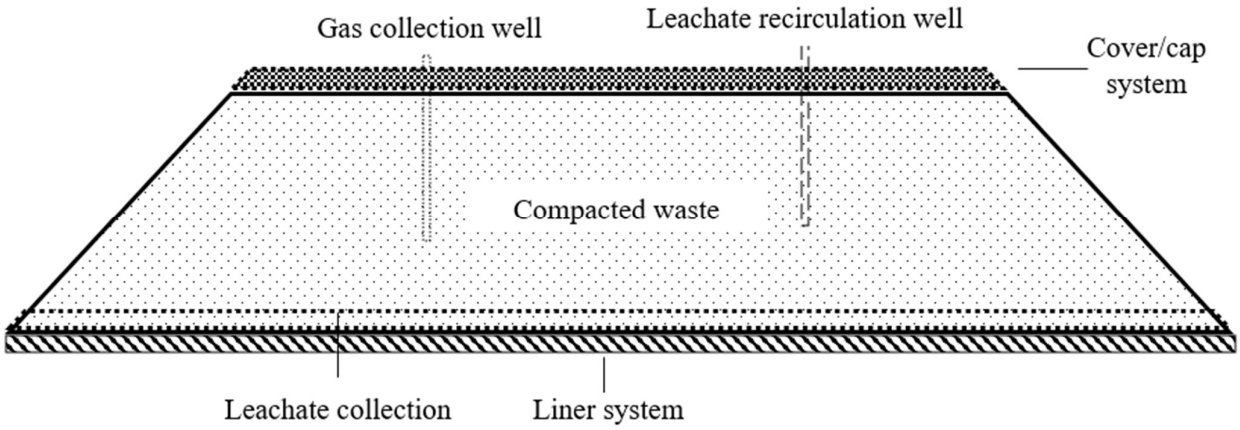

Figure 2-2. General cross-section of a present-day bioreactor landfill.

Leachate collection wells and recirculation systems are also installed throughout this emplacement phase in order to encourage microbiological degradation processes in the waste mass (Figure 2-2) (Barlaz & Reinhart, 2004; Reinhart et al., 2002). Leachate is generated due to the biodegradation of the waste, as well as the infiltration of water into the waste mass (rainfall, for e.g.). Leachate moves through the waste mass and may carry dissolved or suspended material arising from the degradation of the MSW. Leachate quality is highly dependent on the composition of the waste, its age and the biological activity within the waste

mass (Reinhart & Townsend, 1997). Relatively younger landfills produce leachate that has a high concentration of organic matter, as well as a high dissolved solids content. This may be attributed to the degradation of the 'easily' biodegradable matter present in the waste mass (the relatively steeper first half of the gas generation curve). Leachate could also contain trace amounts of hazardous material that may be present in the waste (Christensen et al., 2001; Kjeldsen et al., 2002; Kjeldsen & Beaven, 2011). To prevent the leachate from escaping the waste mass, site selection (low groundwater table, hydraulic conductivity of the nearby soil etc.) is an important design consideration. In addition to that, liner and collection systems (at the base of the waste mass) are also used to collect, monitor and recirculate the leachate as it has been shown to increase the rate of biodegradation (Pohland, 1980).

The collection of landfill gas, which is generated as a product of the biodegradation of MSW, is carried out with gas collection systems (Figure 2-2), and providing a cap on the surface of the landfill (prevents escape of gas). Gas recovery through gas wells in a bioreactor landfill may begin within 2 years of refuse burial. It is important to collect landfill gas as not only does it contain methane and carbon dioxide predominantly, which are GHG gases that are major contributors to warming the planet's climate, methane can also be used as a renewable energy source, and in doing so, its global warming potential is reduced (Mou et al., 2015; O'Dwyer et al., 2017). The design of the containment and collection systems must be robust, as it determines the efficiency of capture and minimises risk of escape into the atmosphere (Townsend et al., 2015).

In anaerobic bioreactor landfill operation, anaerobic bacteria degrade waste to generate methane and carbon dioxide (Jayasinghe, 2013). On the other hand, in only semi-aerobic operation, intrusion of air throughout the waste mass will predominantly produce carbon dioxide, which unfortunately cannot be used for energy generation. Bioreactors can also be operated under hybrid conditions (aerobic phase, followed by an anaerobic phase which then leads back to aerobic conditions successively) (Cossu et al., 2016; Morello et al., 2017; Raga & Cossu, 2013). Successful demonstration projects of the bioreactor landfill have been carried out with success and their in-depth analyses can be found in the literature (Mehta et al., 2002; Reinhart et al., 2002; Townsend et al., 2015). The inner workings of the landfill waste mass itself and the various processes it undergoes are presented in the following sub-sections, alongside the main factors that affect them.

2.1.1 Decomposition Processes

This section detailed the various biochemical processes that the landfilled waste mass is believed to undergo.

2.1.1.1 Stage 1: Hydrolysis

This stage occurs in the presence of trapped oxygen in the waste mass, during the waste emplacement phase (El-Fadel et al., 1997). Aerobic micro-organisms use oxygen and degrade a fraction of the organic waste into simpler hydrocarbons (Krause et al., 2016; Sang et al., 2012), water, carbon dioxide and heat (Figure 2-3). The temperature during this stage can rise to 70-90℃. This stage lasts for a few days and is highly dependent on the availability of oxygen in the trapped air within the waste mass and compaction. The main products of stage 1 are water and carbon dioxide, which may dissolve in water to form carbonic acid and acidify the leachate (Petrovic, 2016; Williams, 2005).

2.1.1.2 Stage 2: Acidogenesis

From the previous stage, a lack of oxygen results in an anaerobic environment within the waste mass (Figure 2-3) (El-Fadel et al., 1997). This triggers the activity of anaerobic/facultative micro-organisms. The proteins, lipids and carbohydrates are decomposed to sugars, which are further broken down into carbon dioxide, hydrogen, organic acids and ammonia (Krause et al., 2016; Petrovic, 2016; Sang et al., 2012). The concentration of ammoniacal nitrogen in the leachate is high at this stage. The temperature drops to 30-50℃. The composition of the landfill gas is usually around 20% hydrogen and 80% carbon dioxide. (Williams, 2005).

2.1.1.3 Stage 3: Acetogenesis

Acetogen micro-organisms convert the organic acids from the previous stage to acetic acid, its derivatives (Kjeldsen et al., 2002; Sang et al., 2012), hydrogen and carbon dioxide (Krause et al., 2016). Other microbes produce acetic acid directly from carbohydrates in the presence of carbon dioxide and hydrogen (Barlaz et al., 1990; Barlaz et al., 1989). The concentration of hydrogen and carbon dioxide starts to decrease in this stage. The solubility of the present metal ions is increased due to the acidic conditions of this stage which results in an increase in the concentration of metal ions in the leachate. Hydrogen sulphide gas may also be generated during the anaerobic stages of biodegradation. The pH during this stage can be around 4 or less due to the presence of organic acids in solution (Eleazer et al., 1997; Wang et al., 1997; Williams, 2005).

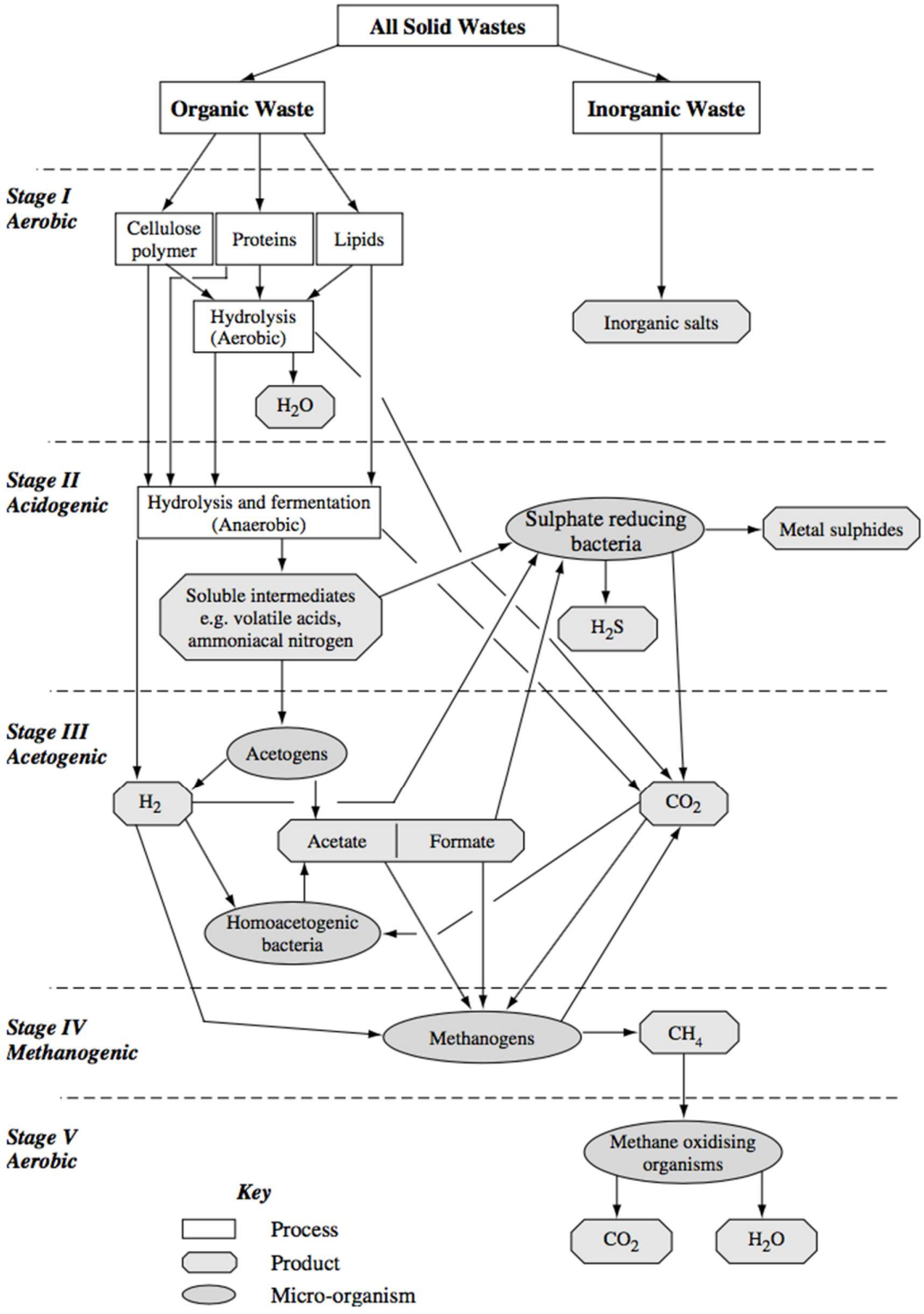

Figure 2-3. Waste degradation stages in anaerobic MSW landfills. Figure reproduced from Williams, 2005:p.200.

2.1.1.4 Stage 4: Methanogenesis

The redox potential of the waste mass decreases and encourages the growth of methanogens. This is the main gas production stage (Krause et al., 2016; Petrovic, 2016; Townsend et al., 2015) where the concentration of landfill gas is approximated at 60% methane and 40% carbon dioxide (Jung et al., 2011; Krause et al., 2016; Williams, 2005). Organic acids from the previous stage alongside the hydrogen from the previous stages are used by the methanogens to produce methane and carbon dioxide. It is this methanogenic stage that provides the best quality landfill gas, and once the easily degradable matter has been converted to biogas, the rate of gas production diminishes considerably as the waste starts to accumulate lignin-rich wastes (Townsend et al., 2015).

Methanogens used to be classified as bacteria by earlier studies, however, since then they have been classified as a distinct type of prokaryotes known as Archaea (Krause et al., 2016; Sang et al., 2012; Staley et al., 2012). The methanogens may be divided into two categories (Trzcinski, 2009):

- mesophilic (active between the 30-35℃ temperature range);
- thermophilic (active between the 45-65℃ temperature range);

Therefore, the landfill gas generation occurs between 30-65℃, with an optimum range of 30-45℃. Due to the degradation of the organic acids, the pH rises to 7-8 during this stage. Since methanogenesis is considered the rate-limiting stage, for optimal efficiency, pH should be maintained close to neutral (Khanal, 2008). This is the longest stage in the life of a landfill and may not start for several years, but however it does last several decades, up to 100 years (Barlaz et al., 1990; El-Fadel et al., 1997; White et al., 2004; Williams, 2005). The predominant cause of this slow and prolonged gas release towards the end of this stage, as mentioned previously in Chapter 1, is the presence of lignocellulose and lignin-rich wastes which are very difficult to degrade. It is precisely this problem that is at the heart of this thesis.

Stage 5: Oxidation

This is believed to be the last stage of waste degradation (El-Fadel et al., 1997; Owens & Chynoweth, 1993; Sang et al., 2012; Tchobanoglous et al., 1993). As the concentration of organic acids from the previous stage decreases due to their conversion to landfill gas, there is not a lot of substrate available for the methanogens to degrade (Williams, 2005). The conditions

within the waste mass return to aerobic conditions because the temperature cools down after methanogenesis and intrusion of air increases the conc. of oxygen in the waste (Figure 2-3) (Petrovic, 2016), perhaps with some hydrogen sulphide formation from sulphate-reducing micro-organisms. The waste mass is likely lignin-rich at this stage, due to the easily degradable fraction having been depleted.

2.1.2 Major Factors affecting Degradation

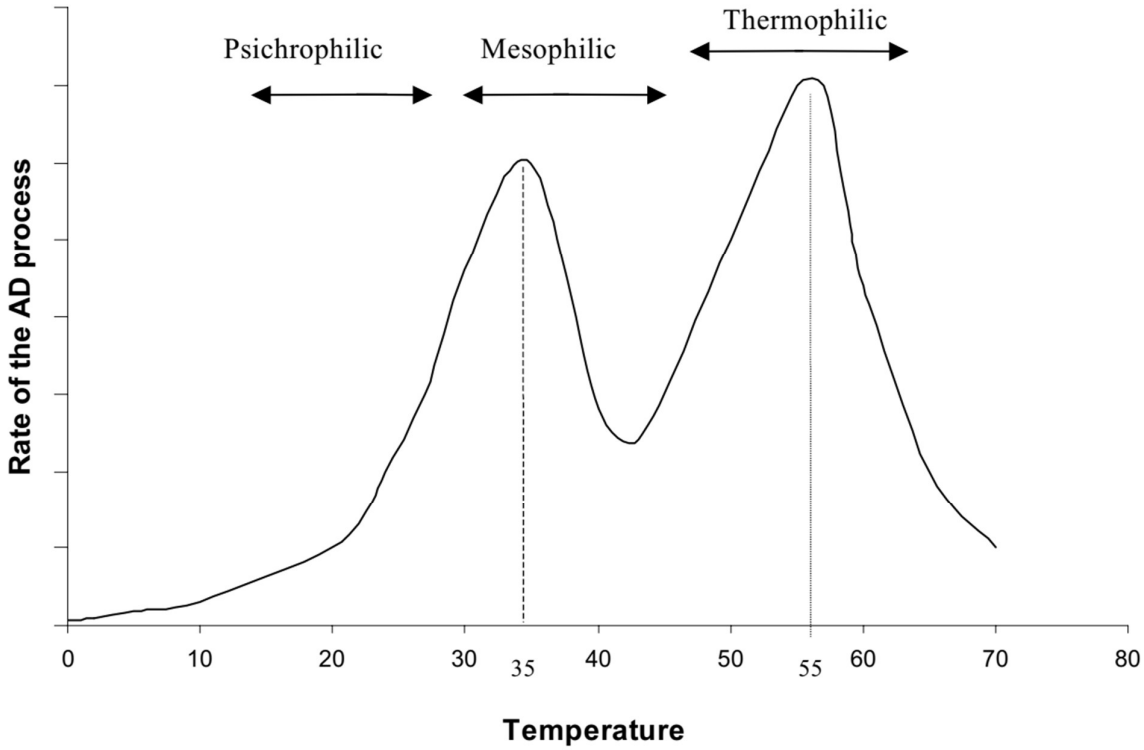

Figure 2-4. Temperature range for AD. Figure reproduced from Mata-Alvarez et al., 2003:p.14.

2.1.2.1 Temperature

Anaerobic digestion can take place over a large temperature range: from psychrophilic temperatures around 10℃, up to severe thermophilic temperatures over 70℃ (Figure 2-4) (Khalid et al., 2011; Mata-Alvarez, 2003). However, this will negatively impact the rate of biodegradation if the temperature is not in the optimal range. Too low or too high a temperature could force the activity to cease, either temporarily (until the temperature is back at a level where the micro-organisms can operate, provided everything else remains constant) or permanently. Methanogenesis is strongly influenced by the temperature parameter (Krause et al., 2016; Mata-Alvarez, 2003). Most landfill sites have temperatures between the 30-35℃ range during the methanogenic stage. Less gas is produced if the site is cold (Williams, 2005).

In the landfill environment, methanogenesis generally occurs during the mesophilic and thermophilic temperature ranges (Barlaz et al., 1990; Barlaz et al., 1987; Eleazer et al., 1997; Krause et al., 2016).

2.1.2.2 pH

The pH of a landfill site is generally neutral at first, stages 2 and 3 make the conditions acidic due to the production of the organic acids. These organic acids in stage 4 are then consumed by methanogenic bacteria, resulting in a rise in pH. The methanogenic microbes are the most sensitive to pH (Trzcinski, 2009) and are most effective in the 6.8-8.5 pH range (Figure 2-5) (Khalid et al., 2011; Khanal, 2008; Williams, 2005). If the pH is not in this range, the gas production is substantially reduced.

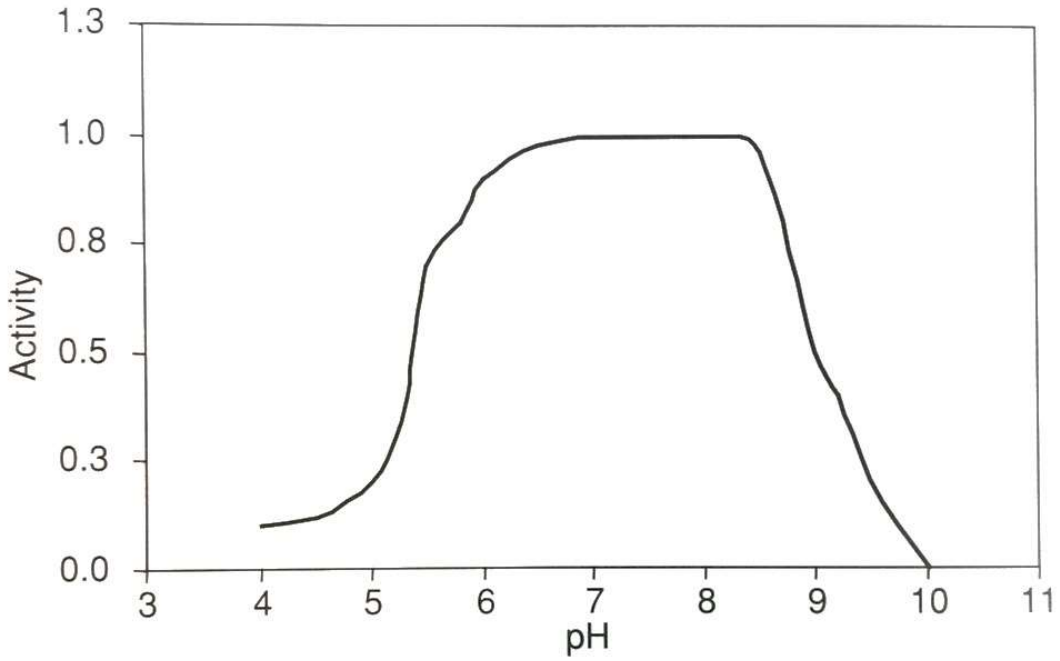

Figure 2-5- Methanogenic activity with changes in pH. Figure reproduced from Khanal,

2.1.2.3 Toxic compounds

Toxic substances are compounds that, at a certain concentration, negatively affect bacterial activity (Table 2-1). Methanogenic bacteria are especially sensitive to toxic substances. These compounds include high concentrations of volatile fatty acids (VFAs), hydrogen sulphide and free ammonia (Chen et al., 2016; Krause et al., 2016; Mata-Alvarez, 2003). Xenobiotics can also act as toxic substances and inhibit microbial activity. The presence of oxygen will inhibit the growth of micro-organisms that operate strictly under anaerobic conditions, such as methanogens (Jung et al., 2011). The presence of certain substances at

lower concentrations may be stimulating, and at higher concentrations have the opposite effect. This is particularly true for sodium which encourages microbial activity between 100-200 mg/L in batch reactor AD, whereas near 3000 mg/l it is inhibiting (Jayasinghe, 2013).

Table 2-1. Important parameters for waste biodegradation and their optimal ranges. Table reproduced from Vazquez, 2008:p.7.

Influencing factors	Optimal ranges/comments
Moisture	Generally above field capacity[1]: 55 -75% by wet weight
pH	6.5 - 7.5
Temperature	32°C - 38°C
Density	800 - 1000 kg m^{-3}
Nutrients	Generally adequate except local nutrient-deficient pockets due to heterogeneity
Inhibitors	Cation concentration producing moderate/severe inhibition (mg L^{-1}): Sodium 3500-5500 Potassium 2500-4500 Calcium 2500-4500 Magnesium 1500-3000 Ammonium (total) 1500-3000 Heavy metals: no significant influence Organic compounds: inhibitory effect only in significant amount

The toxicity of certain substances may be dependent on the chemical state in which they are present and could be influenced by other factors. For example, volatile fatty acids are intermediate compounds made as part of the biodegradation process, and undissociated species have been reported to cause more inhibition (very high concentrations) because they can easily diffuse into the inner parts of living cells. pH and alkalinity levels affect the toxicity of volatile fatty acids (Mata-Alvarez, 2003), so just the concentration of these acids would not be enough to describe their toxicity. Phenolics under high concentrations have also been found to be inhibitory to the activity of methanogenic bacteria and generally inhibit biogas production. Although, conditioning over a very long period of time (slowly increasing conc. and letting the microbes acclimatise) may help in 'restarting' the biogas production (Hernandez & Edyvean, 2004; Hernandez & Edyvean, 2008).

2.1.2.4 Heterogeneity of MSW

MSW is heterogeneous by nature. It arises in the waste mass due to the variety of components landfilled, as well as their particle size, accessibility/ease of degradation and biodegradation induced changes in certain waste regions (Bareither et al., 2012; Breitmeyer & Benson, 2014; Breitmeyer et al., 2020; Staley et al., 2011), while the inert areas are relatively unchanged (Dixon et al., 2008b; Grellier, 2007; Reddy et al., 2009a; Reddy et al., 2011). Waste streams are very different from one another, and waste composition in different parts of the world varies depending on the specific socio-economic factors of that region (Burnley, 2007; Dixon et al., 2008a; EPA, 2016; WRAP, 2010), which also in-turn adds to the heterogeneity found within landfilled waste. This heterogeneity varies spatially and temporally within a landfill. The state of degradation within a landfill also varies and adds to the heterogeneity (e.g. changes in particle size distribution) (Sormunen et al., 2008). The 'degree' of heterogeneity is a function of the composition of the waste streams, their physico-chemical structure, extent of biodegradation in the landfill etc. (Krause et al., 2016). Waste heterogeneity at the field-scale has been well-established and shown to have a significant impact on key biochemical variables (e.g. distribution of moisture content, biogenic carbon, temperature) (Audebert et al., 2016a; Hanson et al., 2010; Kulkarni & Reddy, 2012). As such, local variations in biodegradation stages and microbial communities have been recorded due to waste heterogeneity which then ultimately impact the overall degradation of the waste mass at the representative elementary volume-scale (Staley, 2009; Staley et al., 2012; Staley et al., 2011).

Following collection and prior to landfilling, some pre-treatment methods, such as shredding may be used to reduce particle size and allow for better compaction, so as to minimise heterogeneity (Vazquez, 2008). With shredding, the density of the waste will also increase (up to 29%) upon compaction, and result in a relatively more homogeneous structure, providing efficient use of space in comparison with the landfilling of 'as-discarded' waste (Ham et al., 1978). Decreasing the particle size increases the surface area to volume ratio, this provides the micro-organisms to secrete enzymes on a larger surface area for the same mass of MSW. This improves the efficiency of degradation and has shown to increase the rate of degradation (Zhu et al., 2008).

2.1.2.5 Leachate Flow & Recirculation

At the lab-scale, researchers have found that recirculation of leachate to operate the waste mass as a bioreactor tends to have a positive effect on biogas generation (Haydar &

Khire, 2005; Ko et al., 2016; Sanphoti et al., 2006). This has been attributed to advective and diffusive transport of nutrients as well as substrates in the leachate due to recirculation. Likewise, at the field-scale it has also been shown that degradation of waste in a landfill is accelerated in response to leachate recirculation (Barlaz & Reinhart, 2004; Benson et al., 2007; Hettiaratchi et al., 2015; Reinhart et al., 2002).

2.1.2.6 Solid:Liquid Ratio

Laboratory-scale experimental work has been carried out to study the impact of varying solid:liquid (S:L) ratio on anaerobic digestion efficiency of various wastes. Researchers have found that often very high S:L ratios may result in high organic loading of the bioreactor, hence overloading the microorganisms (Hettiaratchi et al., 2015; Pearse et al., 2018). This overloading often results in accumulation of organic acids and drops the pH to low and inhibitory levels for the microorganisms, which then has a negative impact on the overall biogas production/process efficiency (Li et al., 2018b; Saha et al., 2018). As such, there is a balance and optimum solid:liquid ratio for biodegradation which must be achieved in order to reach optimal biodegradation and biogas production (Begum et al., 2020; Liu et al., 2009; Neves et al., 2004).

Furthermore, miscellaneous factors impacting biodegradation (e.g. impact of bulk density, compaction, alkalinity, organic fraction of MSW, waste:inoculum ratio etc. on waste degradation) have been discussed and investigated elsewhere (Caicedo et al., 2017; Clarke, 2017; Guilford et al., 2019; Laner et al., 2012; Wang, 2016).

2.2 Enhanced Delignification & Methane Generation in Municipal Solid Waste

Certain micro-organisms (e.g. fungi such as *C. subvermispora*, bacteria such as *Agrobacterium sp*.) and enzymes (e.g. Peroxidase enzymes) have been proven to efficiently degrade lignin. The leachate can be augmented with these biodelignification systems and recirculated through the waste mass to enable lignin degradation. However, they have only recently received attention in MSW literature and have typically been studied under highly controlled conditions (e.g. model lignin molecules) with limited consideration of the heterogeneous physical nature of waste and the fractions that are found to be not degrading in landfills. Studies based on simple lignocellulose-like materials help with mechanistic insights,

however, it is difficult to gauge how 'real' as-discarded lignocellulosic wastes may respond to these biodelignification systems. Waste heterogeneity results in preferential flow paths and the leachate taking the path of least resistance and predominantly flowing through the larger pores. The lignin associated with the smaller pores with relatively lower hydraulic conductivities (micro- or nano- pores) may have very little interaction with this augmented leachate, with little to no lignin degradation in these pores. The landfill mass could potentially be a dual or multi porosity system where water may be retained in the micro-pores with comparatively lower hydraulic conductivities, leaving these areas untreated. Therefore, it is important to understand the flow around lignin-containing wastes in MSW, including the 'residence time' that this augmented leachate is in contact with these wastes for delignification. Such issues regarding flow will severely impact the effectiveness of any biodelignification treatment and currently present difficulties with upscaling laboratory-scale studies for potential in-situ application.

In this section, the nature of the problem described above is examined and discussed in detail. A concise review of the current research carried out in the areas of (i) microbial and enzymatic lignin degradation and (ii) flow in MSW is discussed, with specific emphasis on the potential impacts of preferential flow on lignin degradation.

The aim of the following sections is to highlight opportunities and challenges regarding in-situ accelerated MSW degradation in landfills. Due to the multidisciplinary nature of the problem, this is accomplished by presenting a review and analysis of (i) microbial and enzymatic biodelignification systems, their delivery and applicability to MSW, (ii) flow-through studies on MSW, specifically the potential impacts of preferential flow on lignin degradation.

2.2.1 Degradation of Lignocellulosic Wastes in MSW

The following sub-sections focus on the degradation of these lignin-rich wastes under laboratory and field-scale operations.

2.2.1.1 Biodegradation Studies of Paper & Wood

A strong body of literature exists that has examined the decay of wood and newspaper under a variety of conditions (Barlaz, 2006; Blanchette et al., 1989; Kumar et al., 2020; Love et al., 2017; O'Dwyer et al., 2017; Palmqvist & Hahn-Hagerdal, 2000; Stinson & Ham, 1995; Wang et al., 2011; Ximenes et al., 2018). Researchers have found that wood and newspaper

under anaerobic conditions degrade very poorly, and have attributed this to the lignocellulosic structure of these wastes, i.e. the physical association between lignin (difficult to degrade) and cellulose/hemicellulose (relatively-easily degradable matter) (Figure 2-6). Not only is lignin recalcitrant to biodegradation, it also forms a 'glue'-like jacket around the aforementioned easily-degradable matter (cellulose and hemicellulose) decreasing its bioavailability to degradation as well (Brandt et al., 2013).

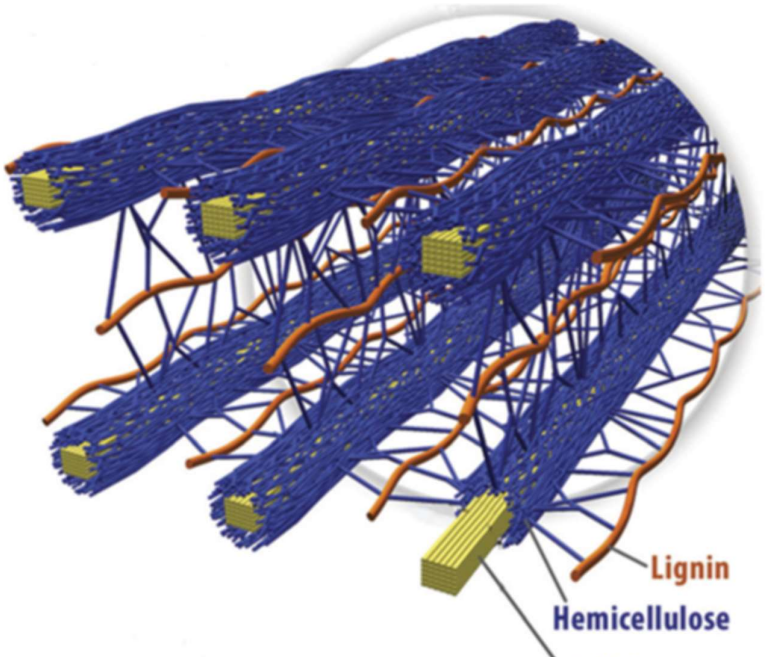

Figure 2-6- Structure of lignocellulose, lignin can be seen acting as a 'glue' between and around the easily degradable matter Figure reproduced from (Brandt et al.,2013).

Wang et al. (2011) evaluated the anaerobic biodegradability of major wood products in MSW. As reported in other studies, softwoods (SW) and engineered woods were generally found to contain more lignin than hardwoods (HW) (Wang et al., 2011) (De la Cruz et al., 2014). Woods with higher (Cellulose+Hemicellulose)/Lignin values (a measure of the degree of lignification - amount of bioavailable matter against bio-unavailable matter) generally exhibited greater methane yields and degrees of decomposition. This was attributed to the fact that cellulose and hemicellulose are relatively more easily degradable than lignin. It should be noted that this experiment lasted 1400 days (approximately 4 years) under ideal conditions (temperature, nutrients etc.) with only little decomposition. The highest methane yield obtained during this study (84.5 (8.2) mL of CH_4/g) in comparison with the typical yield of food waste (300-550mL of CH_4/g) was roughly at least 4 times lower (Krause et al., 2016). Likewise, the carbon conversion during the experiment ranged from 0% to 19.9%, suggesting that the bulk

of the carbon in the wood is recalcitrant, presumably due to lignin. This study highlighted the variability in the biodegradation of different woods, the very low methane yields and extent of decomposition obtained by wood under ideal conditions. It also shed light on the importance of lignin in wood products and how its presence negatively impacts wood biodegradation.

Wang et al. (2015) studied the decomposition and carbon storage of various paper products (newsprint, copy paper, magazine, diaper). The methane yields and carbon storage factors (portion of the amount of bioavailable carbon that was not transformed to CH_4 or CO_2) indicated that papers made from mechanical pulping processes (e.g. newsprint) are more resistant to degradation than those made via chemical pulping (e.g. copy paper). The authors suggested that this is most likely due to the chemical pulping processes removing most of the lignin, while the pulp obtained via mechanical processes contains significant amounts of lignin (Eleazer et al., 1997). As such, newspaper was found to contain more lignin than copy and magazine papers (Wang et al., 2015). The diaper samples, not made from chemical pulps, contained gel and plastic and exhibited limited degradation. The highest carbon storage factors (a measure of the amount of biogenic carbon not converted to methane and carbon dioxide) were obtained for newsprint (0.31), followed by magazine (0.13) and copy paper (0.02). These correlate directly with the lignin contents of the samples (24.5%, 11.5% and 0.96% respectively). This study demonstrated the adverse impact of lignin on paper biodegradation.

Studies focusing on the decomposition of different MSW components in actual landfills have been carried out (Baldwin et al., 1998; De la Cruz et al., 2013; Wang et al., 2013). Walsh and LaFleur (1995) excavated MSW from a New York landfill that had been buried for up to 100 years. Not only did they recover wood, it also composed 12.3% of the waste (Walsh & LaFleur, 1995). Likewise, Ximenes et al. (2008) studied the decomposition of wood products in excavated MSW samples from three Sydney landfill sites that had been closed for 19, 29 and 46 years respectively. The wood samples recovered (at least 46 years old) from one of the sites showed little or no visual signs of degradation. At least 19-year old paper samples showed very little signs of degradation as well. Identifiable food scraps were not found in either of them, indicating that these landfills were indeed biologically active and that the wood and paper products simply were degrading extremely slowly, if at all. In comparison with control samples, 46-year old refuse had a carbon loss of 8.7% for hardwoods, 9.1% for softwoods. The changes in the lignin content of the samples were not statistically significant. This study supports the previous results regarding slow decomposition of wood and paper products and

suggests that even with the passage of several decades in landfills, very little to no lignin degradation occurs (Cummings & Stewart, 1994; Krause et al., 2017).

Wang et al. (2013) conducted a study by burying different wood and paper products to assess their state of degradation 1.5 and 2.5 years later. The carbon storage factors decreased with an increase in the holocellulose (hemicellulose+cellulose) decomposition (Wang et al., 2013). This indicated that the degrading fractions were at least mainly hemicellulose and cellulose. The remaining carbon stored was largely attributed to lignin. The rates in the field were much slower than those observed in the optimal conditions in the laboratory (Wang, 2015; Wang & Barlaz, 2016; Wang et al., 2015; Wang et al., 2011). The carbon storage factors for the wood samples ranged from 0.39-0.45 over 2.5 years, indicating significant amounts of material left over. Relatively higher degradation was recorded in all paper products, with the storage factors ranging from 0.09 to 0.27. This study pointed out that wood and paper require many years to decompose in the landfill environment and the extent of decomposition is at least mainly attributed to holocellulose within the samples, with very little to no contribution from lignin.

This section has highlighted the evidence present in the literature regarding the slow degradation of lignocellulosic wastes in landfills. It has shown that these wastes are mainly comprised of woody and newspaper wastes. Further similar work can be found in-depth in the literature (Blanchette et al., 1989; Cummings & Stewart, 1994; De la Cruz et al., 2015; Ham et al., 1993; Hayes et al., 2017; Morello et al., 2018; Schilling & Norcutt, 2010; Ximenes et al., 2015; Ximenes et al., 2018).

2.2.2 Accelerated Newspaper & Wood Lignocellulose Degradation

The previous section has discussed the problem of slowly degrading lignin-rich wastes in MSW landfills. Since current waste degradation rates in the engineered and biogeochemically active environment of a landfill are not sustainable, is it possible to accelerate the degradation of these wastes artificially? Since the lifespan of a landfill can be over hundreds of years (Reinhart & Townsend, 1997), there are many old and existing landfills in the world with several still releasing significant amounts of greenhouse gases and contamination into the geoenvironment. Currently in the UK, with rising sea levels, over 1000 landfill sites near the coasts are at risk of being breached and releasing contaminants into the geoenvironment (Carrington, 2016). Accelerating the decomposition of these wastes (Figure 2-7) could help mitigate some of the issues associated with existing landfills, presently

containing lignin-rich wastes in significant amounts (Leonowicz et al., 2001; Leonowicz et al., 1999; Susmel & Stefanon, 1993; Toumela et al., 2000). For instance, accelerated decomposition would allow the landfill mass to stabilise relatively early, making it possible to remediate/restore the site and reduce the risk of leakage by repairing the containment-barrier system, where needed.

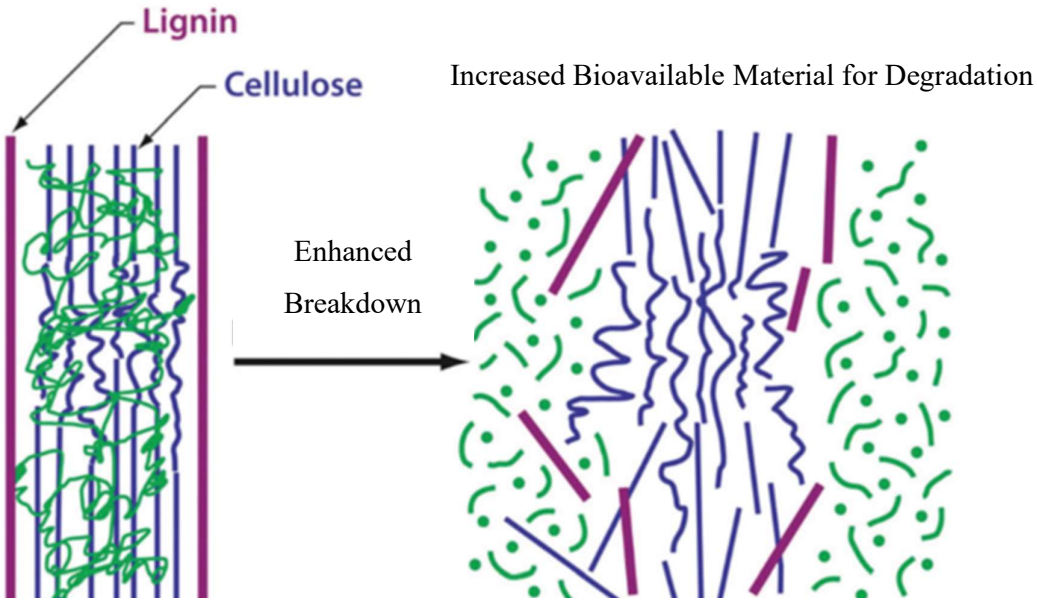

Figure 2-7 Conceptual model of increased bioavailability of lignocellulose in wood/paper due to the action of biodelignifying systems. Figure reproduced from (Brandt et al., 2013).

Some advantages of biotechnological treatment include early stabilisation of the waste mass (new and old sites), with relatively quicker methane recovery for energy generation and capture. This is particularly important as recent estimates indicate that currently less than 10% of the methane potential of landfills worldwide is utilised (Themelis & Ulloa, 2007). It is also suggested that biodelignification systems are not limited to in-situ application and could also be applied to anaerobic digestion plants to improve the methane yields and increase the bioavailability of the substrates (Copete-Pertuz et al., 2019; Geun Yoo et al., 2020; Gonzalez-Estrella et al., 2017; Li et al., 2018a). On the other hand, presently, the impact of accelerated waste degradation on the geotechnical properties of the landfill mass is unknown. Geotextiles, which are made of polymeric substances, may be subject to degradation by biodelignification systems due to the production of highly reactive free radicals. This may be a significant concern in landfills which are lined with high-density polyethylene (HDPE) geotextiles (Muller & Saathoff, 2015), as white-rot fungi and manganese peroxidase have been shown to degrade HDPE (Iiyoshi et al., 1998). In addition, at this moment in time, biodelignificating agents are

expensive. Therefore, their high cost will be a significant issue for potential field-scale application.

The acceleration of MSW stabilisation has traditionally focused on leachate recirculation (Campuzano & Gonzalez-Martinez, 2016; Gunaseelan, 1997; Li et al., 2011; Mata-Alvarez, 2003; Mata-Alvarez & Llabres, 2000). This technique has been proven to accelerate the overall decomposition of waste, and its hemicellulose and cellulose fractions with little to no effect on the lignin content (Barlaz et al., 1990). Other acceleration techniques with similar limitations include shredding, pH neutralisation, removal of releasable carbon via flushing etc. (Barlaz et al., 1990; Bolyard & Reinhart, 2016). However, these enhancement techniques do not affect the lignin degradation and therefore, there will still be significant amounts of biogenic carbon trapped in the landfill mass (De la Cruz, 2014; De la Cruz & Barlaz, 2010; De la Cruz et al., 2013; De la Cruz et al., 2015). To the best of the author's knowledge, lignin degradation has received very little attention with respect to MSW. Even though lignin is highly resistant to degradation, certain microorganisms have evolved for biodelignification. For nearly 50 years, there has been evidence of enzymatic and microbial delignification systems (Crawford & Crawford, 1980; Crawford & Crawford, 1976; Flaig, 1964; Hofrichter, 2002; Martinez et al., 2009; Martinez et al., 2005; Perez et al., 2002; Zimmermann, 1990). However, it was not until very recently that researchers applied them to MSW.

2.2.2.1 Fungal & Enzymatic Systems

Fungal lignin-degrading species include: Ascomycetes, Basidiomycetes (such as white-rot), and certain anaerobic species such as Orpinomyces sp. These species produce extracellular peroxidase enzymes and laccases for lignin degradation (Higuchi, 2006; Higuchi, 2004). Researchers have studied fungal delignification in wood chips pre-treated with Ceriporiopsis subvermispora followed by incubation with inoculum from anaerobic digesters. A 400% increase in methane yield has been reported (Ge et al., 2016). This is likely as white-rot fungi are capable of depolymerising all the components of wood, including lignin, cellulose and hemicellulose (Lundell et al., 2010). Unlike most microorganisms, white-rot fungi have been reported to have a unique ability for lignin degradation (Sanchez, 2009). Their extracellular lignolytic system produces peroxidase enzymes and targets lignin and open phenyl rings (Lundell et al., 2010; Makela et al., 2016). Up to 83.5% lignin degradation has been recorded in literature by *P. chrysogenum* (Rodriguez et al., 1994). However, a significant proportion of

the biotechnological literature in this area is focused on sustainable production of bioethanol and similar chemicals (Rabemanolontsoa & Saka, 2016; Ravindran & Jaiswal, 2016). Many delignificating fungal strains, such as white-rot require aerobic conditions to function and are sensitive to temperature, pH, moisture and UV radiation (Turpeinen, 2007). The landfill environment is hostile and anaerobic, therefore fungal application to delignificate MSW may be faced with some potential issues (fungal lignocellulose degradation is believed to be an aerobic process) (Cragg et al., 2015). However, as of the writing of this thesis, information regarding the application of white-rot fungi to delignification in landfills is somewhat limited, whilst one of the biggest problems with fungal biodelignification systems is that they require oxygen for breakdown, whereas the waste mass in a landfill is anaerobic for the majority of the landfill's life (Crestini, 2011; Lebuhn et al., 2014; Lopez et al., 2013; Ragauskas et al., 2014; Rollin et al., 2011).

Jayasinghe et al. (2011) evaluated the effect and optimisation of different peroxidase enzymes on 30-year old MSW. The waste was shredded down to an average particle size of 2 mm, thereby homogenising it. As this MSW was very old, its volatile solids (VS) content was low (18.6%), and the lignin content was high (81.9% of the total solids) (Jayasinghe et al., 2011). The authors studied the effect of three different types of enzymes: lignin peroxidase (LiP), manganese peroxidase (MnP) and soybean peroxidase (SbP). In comparison with the control reactors, all the enzyme-augmented reactors showed significantly more methane production and lignin degradation (6.2% lignin conversion in control, 68.4% with MnP). During the 40-day long experimental run, the control reactors produced less than 6 mL of CH_4/g VS whereas the methane yields exhibited by the enzymatic delignification systems ranged from 21-200 mL of CH_4/g VS.

Jayasinghe (2013) studied the action of peroxidases in flow-through columns (1 m tall and 14 cm wide) fitted with leachate recirculation. The columns were packed with 9 kg of the same MSW as used in the study above (Jayasinghe, 2013). The cumulative methane production in the enzyme augmented reactors was approximately 5 times the yield observed in the control reactors, i.e., around 2000 ml. The maximum methane concentration in the control and enzyme augmented reactors was around 32% and 51%. The transition from batch to flow-through studies exhibited similar methane generation trends, albeit with very different overall yields. The highest MnP yield from the flow-through tests was less than 100 times the value obtained from batch reactor operation. This study highlighted the issues with upscaling batch reactor

laboratory-scale studies. Jayasinghe (2013)'s MSW was shredded down to an average particle size of 2 mm, and thereby homogenised. It is hypothesized that incorporating the typical heterogeneity of actual MSW from a landfill should result in significantly different degradation behaviours and methane yields in flow-through studies. However, as of the writing of this thesis, this has not been investigated and is unknown.

Researchers have isolated and identified new facultative anaerobic delignifying bacteria from landfill soil and demonstrated the possibility of in-situ bacterial biodelignification to enhance gas production (Rashid et al., 2017). At first, the bacterial strains and bacterial enzymes were tested on pine lignocellulose. 20-25% delignification (Klason assay) was recorded for 4 strains after 7 days. Delignification for bacterial enzymes was found to be dose-dependent and the highest delignification (26-31% - Klason assay) was observed at the 1 mg/g of lignocellulose dose. The best performing bacteria or bacterial peroxidase enzymes were then added to a 10 mL plastic syringe containing isolated MSW soil mixed with 1% (w/w) lignocellulose from chopped pine. In comparison to the control, *Agrobacterium sp.* and *L. sphaericus* exhibited a 10-fold increase in gas production. A larger scale test with 0.5 kg of organic compost with *Agrobacterium sp.* was also employed, which resulted in a 3-5-fold increase in gas production. An alternative two-step strategy of aerobic pre-treatment with lignin-degrading strains followed by anaerobic digestion with MSW soil was also employed. This study highlighted the potential of facultative anaerobic bacteria to breakdown lignin in the absence of oxygen. As anaerobic conditions exist for the bulk of the life of a landfill, these types of biodelignification systems could potentially be used for in-situ application (Higuchi, 2004; Hofrichter, 2002; Martínez, 2002; Perez-Boada et al., 2005; Sun et al., 2005) .

2.2.2.2 Bacterial Systems

Fungal lignin degradation has been studied very well in literature. However, only in recent years has research regarding bacterial lignin degradation deepened, partly due to the limitations of applying fungal systems to anaerobic digestion systems and bioreactor landfills (i.e. requiring oxygen, slow rates of degradation). Even though bacteria may not be as efficient at degrading lignin as some fungal strains, evidence has emerged of bacteria also employing the use of extracellular oxidative enzymes (e.g. peroxidases and laccases) (Brown & Chang, 2014). Researchers have isolated lignolytic bacteria (*Citrobacter freundii-FJ581026, Citrobacter sp.-FJ581023*) from paper mill sludge and analysed their activity on kraft and synthetic lignin via HPLC (Chandra & Bharagava, 2013). Up to 58% was degraded to simpler

polymers that could be potentially taken into the cell for metabolism (Asina et al., 2016). In the landfill environment, the indigenous microorganisms could potentially use these intermediate simpler polymers for metabolism, resulting in enhanced overall degradation (Asina et al., 2016; Bugg & Rahmanpour, 2015; Cragg et al., 2015; de Gonzalo et al., 2016; Mathews et al., 2016; Mathews et al., 2015).

It is only in recent years that research regarding bacterial lignin degradation and the enzymes involved has intensified, whereas fungal lignin degradation and the respective enzymes have been studied extensively. The bacterial strains that are capable of lignin breakdown fall into the following categories (Bugg et al., 2011b):

- Actinomycetes
- $\propto$-proteobacteria
- γ-proteobacteria

The major bacterial lignolytic enzyme classes can be divided into the following (de Gonzalo et al., 2016):

- Lignin-modifying bacterial laccases
- DyP-type peroxidases

Laccases are a group of enzymes made of multi-copper proteins and are capable of catalysing the oxidation of aromatic compounds. They are widespread in fungi, bacteria, and have a broad substrate specificity, and thus have been investigated widely in literature (Alexandre & Zhulin, 2000; Asina et al., 2016; Bugg et al., 2011a; de Gonzalo et al., 2016; Higuchi, 2004; Pollegioni et al., 2015; Roth & Spiess, 2015).

DyP peroxidases are a superfamily comprising heme-containing peroxidases, and have been identified in bacteria, fungi and archaea (Colpa et al., 2014; Rahmanpour & Bugg, 2015). Recently, a substantial amount of bacterial DyPs have been described in literature. Compared to the well-known peroxidases mentioned earlier, which have limited stability and a difficult heterologous expression, DyP seem extraordinarily robust. Several types of DyP have been implicated in lignin degradation, and/or oxidation of phenolic parts of the lignin biopolymer (de Gonzalo et al., 2016).

Recently, evidence has arisen of other bacterial enzymes that also play a role in lignin degradation. Bacterial manganese-dependent superoxide dismutases have recently been discovered to be involved in lignin degradation (Rashid et al., 2015). Catalase-peroxidases have also been identified as a heme-containing enzyme secreted by *Amycolatopsis* upon incubation with lignocellulosic material. Bacteria do not produce the same peroxidases that are found in fungi. However, bacterial peroxidases and laccases have gained a lot of attention recently and their number has grown (de Gonzalo et al., 2016).

The use of bacterial strains has been carried out to obtain mechanistic insights into the degradation of lignin-like model molecules with promising results. Mathews et al. isolated bacterial strains from black liquor (pulping waste) and used the organism to see how well it grows on different lignocellulose-like model substances, by monitoring optical density of the reactors in a nutrient-rich stirred environment (Mathews et al., 2016; Mathews et al., 2015; Mathews et al., 2014). They found the bacterium to grow on a variety of lignin-rich model substrates as the sole carbon source under aerobic and anaerobic conditions, ading to the growing literature regarding bacterial lignolytic systems. Similar work involving model lignin-containing wastes has been carried out by other researchers with varying degrees of success regarding breakdown of the lignocellulosic matrix (Chong et al., 2018; Fang et al., 2018; Malayil & Chanakya, 2019; Yang et al., 2017; Zhu et al., 2017).

Similarly, lignolytic bacterial strains from termite guts have been isolated and used for enhancing biogas generation in model MSW reactors, with promising results (~2-3-fold increase in biogas) (Rahimi, 2019; Rahimi et al., 2020). Another study looked at enhancing biogas production in wheat straw with a lignolytic consortium (isolated from composting) and achieved enhancement (~1.2-1.4 fold) (Kong et al., 2018). Similar work has been carried out by other researchers with varying degrees of success (upto ~1.4 fold) (Ecem Öner et al., 2017). For application of bacterial biotechnological techniques at the field-scale, coupling of bacterial lignocellulose degradation with phenomena that occur in-situ is required.

2.2.3 Flow in MSW & Potential Impacts of Preferential Flow on Delignification

Heterogeneity of waste at multiple scales and its geographically and spatially varying composition makes it extremely difficult to study and characterise flow processes in this porous medium. Bendz et al. (1998) visualised the heterogeneity of MSW at three scales: the 1 m horizontal (refuse element) lens scale, the truck-load scale and the field-scale (entire landfill). Vertical flow should be permitted via the tears and gaps within and around the plastic bags and

other refuse elements (Bendz et al., 1998). As MSW is a heterogeneous medium, the permeability within the waste mass varies due to anisotropy, partial saturation, changes in waste density or effective stress. The total and drainable porosity of MSW determine the rate at which leachate levels build up in response to infiltration or miscellaneous water inputs, and the rate of decline when dewatering a landfill site. However, as of the writing of this thesis, there is very little experimental information regarding the pore-structure characterisation of MSW. Similarly, to the best of the authors' knowledge, understanding of flow through MSW at the pore- and meso-scale is extremely limited.

Han et al. (2011) carried out multi-step outflow experiments on the newspaper fraction of MSW to study the gas and leachate transport with two different particle sizes and compaction pressures. Single-porosity and dual-permeability models at the elementary-scale were tested against experimental data to assess conceptual models for the pore structure of MSW (Han et al., 2011). In the former, the waste is assumed to be a homogeneous porous medium consisting of impermeable particles separated by fractures, through which fluid flow takes place. In the latter, the porous medium is conceptualised to be made up of a fracture (larger pores in the waste mass) and matrix domain (micro-pores within the waste matrix). This model allows transfer of water and solutes within and between the two domains. Inverse parameter estimation in HYDRUS-1D was carried out to fit the equations of the respective models to the experimental data. The dual-permeability model was significantly better than the single-porosity model to describe the cumulative outflow with the passage of time. This indicated that the newspaper is potentially a dual porosity system, and possibly exhibits similar hydraulic properties within the landfill mass. However, the single-porosity model sufficiently described gas transport, suggesting that perhaps gas predominantly travels through the large pores. However, these types of elementary models do not offer mathematical descriptions of the transport processes occurring at the pore-scale. As of the writing of this thesis, this line of inquiry has received little to no attention in the literature.

Pore-scale understanding of transport phenomena in MSW requires detailed characterisation of the pore shapes, size distribution (from macro- to nano-pores), particle density etc. Researchers have studied the hydraulic characteristics of MSW, and there is a wealth of information available on the particle-size distribution of the various fractions (Dixon et al., 2008b; Kazimoglu et al., 2006; Reddy et al., 2009c; Stoltz et al., 2010). However, extremely limited information is available on the pores size characteristics of MSW. Stoltz et

al. (2012) applied the Young-Laplace equation to determine the pore-size distribution of MSW. However, in this approach, the pores are assumed to be a bundle of tubes which may not be accurate. To successfully understand multi-phase transport through waste (leachate and landfill gas), further understanding of these phenomena at the pore-scale is required. It is suggested that this is key to understanding enhanced biodelignification reactions, which take place at the pore scale and their coupling with transport phenomena to predict biodelignification and upscale from laboratory studies.

The particle size of MSW components varies between 0.1 mm to 100 cm (Reddy et al., 2015). Therefore, this heterogeneity results in tremendously unpredictable flow through MSW and the existence of preferential flow. Woodman et al. (2015) studied the transport of tracer breakthrough curves (i.e. plot of test duration against concentration of tracer) in saturated MSW (i.e. transport beneath the leachate table). In one of the test columns, the lithium breakthrough curve was spotted between days 1 and 2. This indicated preferential flow as it was estimated that a sharp-front trace would approximately take 38 days to arrive, if it were passing uniformly through the whole pore space. Similarly, dye-stained solutions have been used to study flow paths in MSW through visualisation (Caicedo, 2013). The dye-stained solution was observed to have channelled through the waste mass with a large proportion of the cross-section showing no visual signs of staining, hence indicating preferential flow.

Now let us conduct a thought experiment, suppose that this tracer instead was the active agent of a biodelignificating system (e.g. MnP), and was introduced in the waste mass via leachate recirculation. The leachate would take the path of least resistance, as has been confirmed by previous studies, and hypothesised to predominantly flow through the largest pores with little to no interaction with areas with lower hydraulic conductivities. This type of behaviour may result in some areas of waste experiencing significant degradation, whereas other areas (lower hydraulic conductivities, micro- or nano- pores) may be left untreated. Since lignin is found in large size fractions (blocks of wood), and smaller size fractions (paper, food waste), in order to achieve uniform delignification, the large and small pores must be treated. As such, it is noted that flow is likely to occur around the larger fractions present in the waste mass where the surface area to volume ratio is much lower, which is expected to significantly hinder the rate of accelerated lignin degradation.

Moreover, electrical resistivity tomography (ERT) has been used to study the infiltration and flow of leachate in landfill cells (Audebert et al., 2016a; Audebert et al., 2016b). The widths of the infiltration areas in the studied cells were significantly higher than the depths, highlighting the horizontal anisotropy of the waste mass due to compaction and stratification. Suppose that the leachate from this study was augmented with the same biodelignification system as mentioned in the preceding paragraph. The preferential growth of the infiltration zone in the horizontal direction would significantly discourage vertical penetration of the active agent resulting in non-uniform treatment. This uneven waste degradation, as a result of the combination of preferential flow and accelerated degradation may cause differential settlement, hence endagering the integrity of the containment system through strectching of the liner or cap.

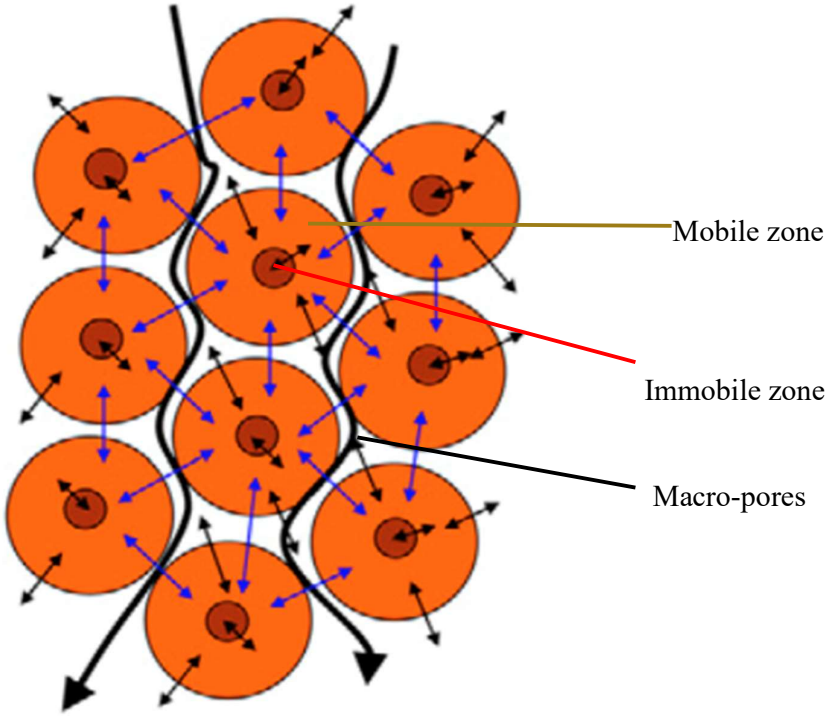

Figure 2-8 A conceptual dual-permeability model for flow with mobile and immobile (MIM) zones. Figure reproduced from Simmunek and van Genuchten, 2008:p.783.

As discussed in the previous sub-section, the landfill mass could be a dual-permeability system. Figure 2-8 shows a conceptual dual-permeability model with spherical particles and mobile-immobile (MIM) zones (Simunek & van Genuchten, 2008). These zones are hypothesised to occur due to the porosity of the particulate material itself. Here, it is assumed that two overlapping pore domains exist which allow fluid flow (inter-particular voids and

intra-particular mobile regions). These mobile regions within the particulate matrix permit flow at a slower rate than the voids.

Likewise, the concept is further refined by assuming that within the particles, there are immobile regions into which solute movement via molecular diffusion can occur. The solute may diffuse into the particles and be held in these immobile zones.

Landfills contain a broad spectrum of materials (plastics, wood, food waste etc.) which affect flow in different ways. Contributions regarding MSW classification have characterised waste components in terms of their effect on flow (Caicedo, 2013; Dixon et al., 2008b). Flow diverting particles are generally impermeable and will not allow flow through them. Whereas impeding particles may divert, as well as permit flow through them (Figure 2-9). With respect to enhanced delignification, the possible dual-permeability conceptualisation needs to consider the individual waste components. It may be the case that the intra-particular voids of different impeding particles would have MIM zones with distinct characteristics – some may hold the active agent indefinitely, or for longer periods of time than others. Indeed, the flow behaviour within the MIM zones, as well as around the MSW components at larger scales would be affected by the geometrical characteristics of the particles and the pore space morphology. This type of flow behaviour, particularly due to the different types of wastes present in the landfill with varying flow diverting or impeding properties, would most certainly hinder uniform biodelignification of the waste mass.

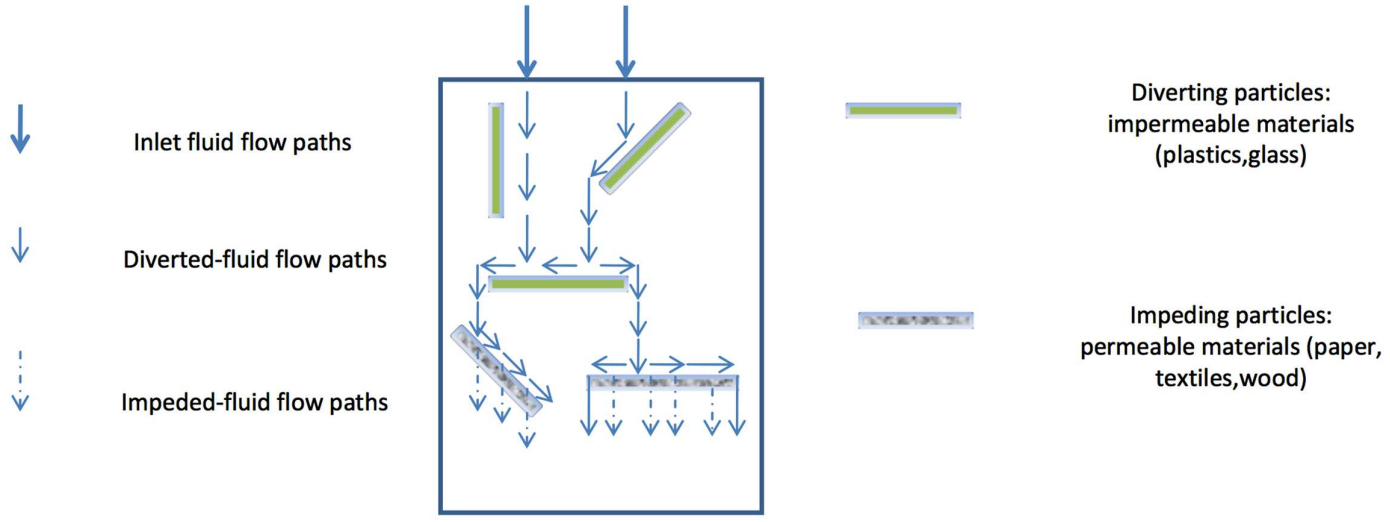

Figure 2-9 A conceptual model to illustrate the impact of different MSW components on flow. Figure reproduced from Caicedo, 2013:p.98.

2.3 Understanding Transport of Leachate in MSW

In this section, discussion of recent efforts from the last 20 years to describe transport in municipal solid waste (MSW) takes place. First emerging themes in the field to draw the reader's attention to a series of significant challenges are discussed. Then examination of contributions regarding the modelling of leachate flow to study transport via mechanistic and stochastic approaches, at a variety of scales is done. Since MSW is a multiphase, biogeochemically active porous medium, and with the aim of providing a picture of transport phenomena in a wider context, then a discussion of a selection of studies on leachate flow incorporating some of the complex landfill processes (e.g. biodegradation, settlement) takes place. It is clear from the literature survey that our understanding of transport phenomena exhibited by landfilled waste is far from complete. Attempts to model transport have largely consisted of applying representative elementary-scale models (the smallest volume which can be considered representative of the entire waste mass). Due to our limited understanding of fluid flow through landfilled waste, and the influence of simultaneously occurring biogeomechanical processes within the waste mass, elementary-scale models have been unable to fully describe the flow behaviour of MSW. Pore-scale modelling and experimental studies have proven to be a promising approach to study fluid flow through complex porous media. Here, it is suggested that pore-scale modelling and experimental work may provide valuable insights into transport phenomena exhibited by MSW, which could then be used to revise elementary-scale models for improved representation of field-scale problems. The objective of this section is to provide a case for examining fluid flow in municipal solid waste at the pore-scale. Following from El-Fadel et al. (1997), particular attention is paid to the development of the field within the last 20 years. Whilst attempting to make a case for moving past the REV this section is not intended to provide an exhaustive overview of the field. Instead, reflection on a focussed collection of recent key contributions is made with the aim of painting an accurate picture of the state of the art, highlighting the limitations of and gaps in our knowledge to identify emerging themes in the field and provide suggestions for future work. First emerging themes in the field are discussed to draw the reader's attention to a series of significant challenges. Contributions regarding the modelling of leachate flow and chemical transport via mechanistic and stochastic approaches, at a variety of scales are then examined. This is followed by consideration of contributions regarding leachate flow and chemical transport incorporating some of the complex landfill processes (e.g. biodegradation). The section is concluded with future needs and recommendations to improve our understanding of transport

phenomena within MSW. These recommendations are focused around obtaining pore-scale insights (Figure 2-10) into these processes with the ultimate aim of better field-scale prediction. The literature search methodology as part of supporting information for this article which is available online.

2.3.1 Emerging Themes In The Field

As shown in Table 2-2, in the last 20 years, work in the field has developed from consideration of homogeneous 1D models primarily focused on the liquid phase to representation of complex 3D transport processes (e.g. solute transport, biodegradation, settlement), and the interaction of these phenomena with the waste structure. However, from the literature discussed hereafter, it is evident that our understanding of the transport phenomena at play in MSW is far from complete.

As discussed in the following sections, elementary-scale models (e.g. the Richards equation) have been applied extensively in the last 20 years to study flow in MSW. While researchers have tried to consider the physical and biogeochemical processes taking place in the waste mass, current modelling approaches simplify these processes in comparison to the high level of complexity found in a typical MSW landfill system, likely adding to the discrepancies between experimental data and models; all the while there is growing evidence that these complex processes play a significant role in transport through MSW. Of course, models are always a simplification of reality, and even coupling of relatively simple processes produces models that can be difficult to validate against typical, easily available datasets. However, validation exercises are vital to identify the range of validity of a particular model and its weaknesses. For instance a relatively recent model comparison exercise, which is explored in detail later, found inconsistencies between available models and their ability to predict experimental data from a well-constrained lab-scale MSW landfill (Beaven, 2008; Beaven et al., 2008), suggesting that perhaps our understanding of underlying coupled processes within the waste matrix needs improvement before reliable short-term and long-term predictions can be made. It is also important to note that models are made for a specific objective and in many cases the models are performing at an acceptable level to achieve that objective. An example of this is the use of landfill gas models in practice (for example GasSim (GolderAssociates, 2012) is a widely used model in the UK) (Clewes et al., 2008). Such models are extreme simplifications of reality (e.g. based on zero or first-order decay functions) but match the measured trends well and are used to make operational decisions, even though our

understanding of the underlying processes in the waste body is still quite poor. The same applies to the stoichiometric equations used in recent geochemical speciation modelling work for landfills (van der Sloot et al., 2017; van der Sloot et al., 2007). While this may be the case for models with a certain objective, when it comes to flow/transport models incorporating biogeomechanical phenomena to describe the landfill system and predict its behaviour, they are difficult to validate due to lack of complete data sets, and/or they become highly parameterized requiring empirical data to infer model parameters, and even then, the high number of degrees of freedom make it difficult to parameterize the models. Within this body of work, it has also been found that there is a significant lack of full validation of a number of flow and transport models which attempt to incorporate biogeomechanical processes, against real experimental or field data. Where model comparison exercises have occurred, discrepancies between modelled and experimental data are suggestive of our lack of understanding of the MSW system. As such, it is difficult to say at this moment in time whether these complex models are applicable to real-life scenarios for the operational needs of the waste industry.

Recent studies (Woodman, 2007; Woodman et al., 2017; Woodman et al., 2014; Woodman et al., 2015; Woodman et al., 2013), contrary to previous work (Bendz et al., 1998; Bengtsson et al., 1994; Oman & Rosqvist, 1999; Rosqvist & Bendz, 1999; Rosqvist & Destouni, 2000; Rosqvist et al., 2005), have discovered that some tracers (e.g. lithium, deuterium) exhibit anomalous transport in MSW, with tracers previously thought to be geochemically inert (Oman & Rosqvist, 1999; Reinhart, 1989; Rosqvist & Destouni, 2000) in their passage through the pores of MSW being found to exhibit non-conservative transport (Woodman et al., 2014; Woodman et al., 2015). Current mechanistic REV-based approaches have not been able to predict this behaviour; thus, we do not fully know what happens to these tracers as they travel through the pore space. Upon studying the impact of mechanical compression of the waste matrix on diffusion of different tracers with varying diffusivities, researchers have also found that while compression decreases the hydraulic conductivity block diffusion times do not vary significantly, contrary to predictions by continuum-scale models (e.g. Richards' equation), suggesting that our understanding of diffusive transport through MSW may not be entirely representative of real-life behaviour (Woodman et al., 2014). From anomalous tracer transport, to conceptual models of the structure of MSW, it is clear that our understanding of the role of MSW structure and its fluid-structure interaction (leachate and

waste/soil interface) with leachate as it travels through the pore space is incomplete and requires further development.

It is also clear that the structure of the waste plays a significant role in the transport of leachate. Generally, attempts to describe the structure as a homogeneous matrix have been unsuccessful, with leachate exhibiting preferential channelling. Typically, in attempts to describe preferential channelling, the waste structure has been split into two domains representing slow- and fast-moving water. It is possible that these dual-porosity/permeability models are an oversimplification of the complex flow behaviour exhibited by MSW, where the flow regimes are instead more likely to be a continuous spectrum rather than just two categories of flow. However, it is important to acknowledge that the simplifications within these models are a direct consequence of the intended purpose of modelling. If the purpose is understanding, then simplification allows a focus on the interaction of the main governing principles, if the purpose is prediction then the focus is interpolation and extrapolation. However, it is important to note here that it is likely that this preferential channelling, at least in part, is a direct consequence of the structure of MSW and the fluid-structure interaction exhibited by the system as the leachate flows through the pore network.

To further understanding of these phenomena, conceptual models of the waste structure have been proposed, where the waste mass is assumed to contain low and high permeability objects in layers. Here, preferential pathways occur through the large gaps between these objects, and advection dominates, whereas within these layers, diffusion dominates and occurs mainly in the horizontal direction within the more permeable layers (Bendz et al., 1998; Woodman et al., 2014). However, such proposed conceptual models of the structure of MSW are likely to be, at best, difficult to validate via continuum modelling approaches that are prevalent in the literature. As such, when considering transport of leachate in landfills, it may be necessary to adopt more complex models offered by REV approaches to add an extra layer of detail and consider the composition and hydraulic properties of MSW components and the resulting pore network. As discussed below, it is likely that different waste components exhibit different permeability characteristics and may cause local variation of flow properties within the waste matrix (Muaaz-Us-Salam et al., 2017), and it might be important to take these into account, especially due to the ever-evolving pore-structure of MSW due to biodegradation, mechanical creep etc. (Fei, 2016; Fei & Zekkos, 2013; Fei et al., 2014; Fei et al., 2013).

Table 2-2 An overview of recent transport models.

Author(s)	Objectives	Features	Assumptions & Limitations
Bendz and Singh (1999); Bendz et al. (1998)	Modelling of unsteady water flow in landfilled MSW, later modified for solute transport.	DP under steady and transient conditions incorporating solute transport.	1D, NH, IC.
McCreanor and Reinhart (1999, 2000)	Better understanding of leachate movement mechanisms.	Stochastic modelling incorporating heterogeneity.	2D, IC.
Suk et al. (2000)	Develop a numerical model to compute leachate quality, gas composition, and gas pressure distribution over time in a landfill.	2-phase, multispecies solute transport incorporating biodegradation.	1D, NH and DP, ignores impact of biodegradation.
Rosqvist and Destouni (2000)	Study and quantify water and solute transport through preferential flow paths in biodegraded MSW by model interpretation of experimental BTCs.	DP, under steady and transient conditions.	IC, NH, unable to explain spreading of breakthrough curves.
Zacharof and Butler (2004a, b)	Mathematical modelling of the landfill environment.	Stochastic modelling incorporates biodegradation and waste heterogeneity.	Unable to simulate transient fluxes, limited testing against field data.
Rosqvist et al. (2005)	Study and quantify pollutant concentrations after long-term leaching at relatively low flow rates and residual concentrations after heavy flushing of an MSW sample.	Transfer function model able to simulate tracer BTCs.	IC, NH, overpredicting tracer concentrations.

(continued)

Statom et al. (2006)	Simulate the overall trend in chloride concentration from a closed landfill cell.	DP, able to predict long-term leachate concentrations.	IC, NH, steady-state conditions only, failed to predict high chloride concentrations.
Kindlein et al. (2006)	Numerical analysis of coupled transport and reaction processes inside landfills.	2-phase transport incorporating degradation, heat and heterogeneity.	2D, neglects relative biodegradability of different components and DP.
(Garcia de Cortazar & Tejero Monzon, 2007; Garciadecortazar & Monzon, 2007)	Simulation of the hydrological and biodegradation behavior of MSW landfills.	Able to calculate daily leachate flow, organic pollution and the generation and composition of biogas in landfills.	Impact of waste mechanics neglected, limited classification of biodegradable matter.
McDougall (2007)	Integrated analysis of the hydraulic, biodegradation and mechanical behavior of MSW.	Coupled biodegradation, hydraulics, mechanics.	No DP, NH, simplifies waste to cellulose, resulting in overestimation of biogas.(Datta et al., 2017)
Sanchez et al. (2010)	Generation and transport of the major gaseous components of landfill gas. Study flow of both the leachate and the gases.	Coupled 3D, 2-phase reactive-transport, incorporating biodegradation and heterogeneity.	Neglects DP, biodegradability and rates of degradation of different components.
Reddy et al. (2013, 2014, 2015)	Leachate distribution in a bioreactor landfill, evaluate the performance of drainage blankets as leachate recirculation systems.	2-phase flow.	Gas and leachate considered immiscible, IC, NH.
Woodman et al. (2013, 2014, 2015, 2017)	Quantification of the flow and transport of leachate in pilot- and field-scale MSW.	DP, also developed a DP-AD hybrid.	IC, DP, NH, failed to predict at the laboratory-scale.

(continued)

Slimani et al. (2016)	Describe the flow around a well during pumping and injection at the field scale.	Simulates response to pumping and injection, exponential relationship between hydraulic conductivity and depth used.	No DP, anomalous behaviour at the transition phases, IC.
Feng et al. (2013, 2014, 2015, 2016 2017a,c)	Investigate the hydrodynamic and biochemical behavior within a bioreactor landfill subjected to leachate recirculation.	3D 2-phase model with biogas generation.	Gas and leachate regarded as immiscible, ignores mechanical effects of biodegradation, NH.
De Donno and Cardarelli (2017)	Evaluate the benefit of a priori information for the characterisation of landfills.	Data-driven, utilizes resistivity and chargeability to limit variation of parameters.	IC, 2D snapshots of the landfill, dependent on ERT sensor-placement, NH.

Note: NH, Neglects heterogeneity; IC, Ignores coupled phenomena; AD, Advection-dispersion; DP, Dual-porosity; BTC, Breakthrough curve

2.3.2 Modelling Of Leachate Flow & Solute Transport

In this section development of mechanistic and stochastic modelling approaches to represent leachate flow in MSW and seek to critically assess how well these models have been able to capture experimentally observed behaviour is considered. Whilst a considerable number of studies have been reported e.g. (Abbaspour, 2005; Abbaspour et al., 2004; Al-Thani et al., 2004; Brun & Engesgaard, 2002; El-Fadel, 1999; Haydar & Khire, 2005; Islam et al., 2001; Olaosun, 2001; Oman & Rosqvist, 1999; Powrie & Beaven, 1999; Rosqvist & Bendz, 1999; Ünlü & Rowe, 2004), a selected few representative contributions are discussed in detail to highlight the significant issues.

2.3.2.1 Mechanistic techniques:

Earlier studies on flow were focused on simple REV-based approaches, predominantly involving models treating MSW as a homogeneous porous medium based on Darcy's law incorporating advection-dispersion phenomena to represent solute transport (Ahmed et al., 1992; Deeley et al., 1985; Demetracopoulos et al., 1986; El-Fadel et al., 1997; Khanbilvardi et al., 1995; Pohland, 1980; Reinhart, 1995; Reinhart, 1996; Sykes et al., 1982). For instance, the theory of unsaturated flow through homogeneous and isotropic porous media has been applied to study flow through MSW by (Korfiatis et al., 1984). Their model used a vertical 1D equation for downward flow through an unsaturated porous medium, considering the variation of moisture content with time, hydraulic conductivity with depth and a source-sink term. Overall, the agreement between the modelled and experimental data was poor. To the author's knowledge, this was one of the first recorded studies to demonstrate the existence of preferential flow and spatial variance of hydraulic properties of municipal solid waste. Much of this earlier work highlighted the unsuitability of assuming MSW to be a homogeneous, porous medium and the importance of including the heterogeneous nature of waste in the modelling framework.

In recent years, researchers have also used commercial codes and simulation software (e.g. HYDRUS, MODFLOW-SURFACT, COMSOL Multiphysics etc.) to model transport in MSW (Audebert et al., 2016b; Beaven et al., 2011; Fellner & Brunner, 2010; Haydar & Khire, 2005; Khire & Kaushik, 2012; Olivier et al., 2009; Saquing et al., 2012; Tinet et al., 2011a). Amongst commercial codes, HYDRUS has been a recurrent choice for modelling flow through MSW (Fellner & Brunner, 2010; Haydar & Khire, 2005; Haydar & Khire, 2007; Khire & Kaushik, 2012; Khire & Mukherjee, 2007; Reddy et al., 2013). HYDRUS is based on a

modified form of the Richards equation solved for saturated-unsaturated flow, and the advection-dispersion equation for solute and heat transport. The Richards equation may also be modified to include dual-porosity/permeability effects (Simunek & van Genuchten, 2008; Šimůnek et al., 2011). For instance, transport of phenol as a model contaminant in a laboratory-scale reactor containing simulated MSW has been studied and its transport modelled via HYRUS-1D (Saquing et al., 2012; Simunek et al., 2003; Simunek & van Genuchten, 2008). Solute transport in the liquid phase was described by the advection-dispersion equation. When the combined effects of sorption and biodegradation on phenol transport were studied, the model was in very poor agreement with the data, yielding an inversely derived biodegradation rate that was two orders of magnitude higher than the independently measured rate, suggesting that transport through the MSW medium is complex and the fluid-structure interaction exhibited through the medium is of relevance for hydrological prediction.

In recent years, as demonstrated below, the scale of interest for modelling leachate transport has shifted from bench scale towards pilot- and field-scales, partly due to developments in computational capacity but also because engineers, waste managers and regulatory authorities are ultimately interested in the field-scale. For instance, researchers have adopted a kinetic wave model, first proposed by Beven and Germann (1981) for describing water flows in soils with macropores, to determine the channel flow in landfills (Bendz et al., 1998). A source/sink term was used to account for flow from and into the channel from the matrix (Beven & Germann, 1981). Upon moisture intrusion into the landfill due to precipitation or leachate recirculation, water would filtrate from the channel into the matrix domain, whereas during dry periods it would be released to the channel domain. They tested their approach against a pilot-scale MSW sample and found that the model could describe the arrival of the wetting front and the drainage front during unsteady flow, whereas it was not able to describe the observed dispersion through the MSW sample. Their work highlighted the unsuitability of assuming the flux laws through MSW to be strictly convective in nature, and the importance of considering the spatial variability of this porous medium for hydrological modelling.

A very popular mechanistic approach to modelling of flow has been to apply different formulations of the Richards equation, dividing the domain into two homogeneous and isotropic overlapping continua (e.g. mobile and immobile regions of liquid) in an attempt to capture the complex pore space of MSW (Beaven et al., 2011; Di Bella et al., 2012; Di Bella et al., 2015; Fellner & Brunner, 2010; Han et al., 2011; Kjeldsen & Beaven, 2011; McDougall,

2011; Slimani et al., 2016; Statom et al., 2006; Tinet et al., 2011b). For instance, vertical flow in MSW samples at the pilot-scale has been investigated by (Woodman et al., 2015) interestingly in their study lithium did not behave conservatively as a tracer. The positively skewed tracer breakthrough curves exhibited tailing, as observed in previous studies (Bendz et al., 1998; Oman & Rosqvist, 1999; Rosqvist & Bendz, 1999; Rosqvist & Destouni, 2000; Rosqvist et al., 2005). They compared advection-dispersion, dual-porosity and hybrid advection-dispersion/dual-porosity models. In the advection-dispersion approach, different processes responsible for non-uniform flow are essentially lumped together into the dispersivity parameter. The dual-porosity model consistently offered a better fit. The hybrid advection-dispersion/dual-porosity model only performed well when either advection-dispersion or dual-porosity behaviour dominated. This research shed light on the previously mentioned anomalous transport within MSW, in terms of REV domain-based modelling approaches, indicating that multi-porosity mechanisms may be significant, and considering the variety of components present in MSW (wood, paper, card, food waste etc.), this is entirely plausible (Beaven et al., 2011; Gotze et al., 2016; Grellier, 2007; Hossain, 2002; Reddy et al., 2009c; Zekkos, 2005; Zekkos et al., 2010). For instance, researchers have studied the hydraulic properties of different MSW components (e.g. paper and wood (Ghane et al., 2014; Ghane et al., 2016; Han et al., 2011; Subroy et al., 2014) where both these components' hydraulic properties could be described by dual-permeability Richards equations, but their intrinsic permeability varied by 1-2 orders of magnitude, suggesting that if they were both present in a waste matrix, due to their varying hydraulic characteristics, it may not be possible to model the dual-porosity characteristics of the entire waste body by assigning them a single set of properties.

It is important to note that any model be it analytical or numerical is an approximation of real behaviour. Whilst analytical models cannot really handle heterogeneity, and therefore have lumped parameters, numerical models do allow us to include heterogeneity however, the number of parameters required make it very difficult to parameterize the models. All assumptions, the manner in which the models are implemented (reaction pathways/solution algorithms/numerical schemes) and how the boundary conditions are integrated into the model also have a significant impact on the model outcome. This could help explain why models based on the same governing equations, initial and boundary conditions can yield varying predictions (Beaven, 2008; Beaven et al., 2008).

The increasingly popular dual-porosity approach was recently tested against field-scale data by (Woodman et al., 2017). Solute transport and horizontal fluid flow between well pairs in a saturated MSW landfill via the use of lithium and bromide tracers along with a fluorescent dye were investigated. Poor fits were obtained with the advection-dispersion model, while the dual-porosity model considered offered a better fit to the breakthrough curves. However, simply because dual-porosity models tend to fit the data better than others is not sufficient to conclude that this is absolutely and the only manner in which fluid flows through MSW, it is more of an indication that REV-based dual-porosity approaches are relatively better at describing the behaviour than other simpler REV-based approaches. This research also added to the growing body of evidence regarding the anomalous behaviour of lithium as a tracer in MSW (Woodman et al., 2014; Woodman et al., 2015; Woodman et al., 2013). More importantly, this anomalous behaviour highlights the significance of the fluid-structure interaction of the MSW with the mobile liquid, tracers and transport phenomena in general. Fitting parameters in a model to match data is an approach that is adequate if interpolation and limited extrapolation is the objective of model. Nevertheless, models developed to increase our mechanistic understanding should be based on independently determined material parameters. However, this is only practical for relatively small simple waste samples and upscaling to full-scale landfills will require some form of fitting (determination of parameters of a probability distribution), thereby moving the model away from its mechanistic basis.

Similar to the above, the Richards equation has also been applied to model leachate pumping and injection data at the field-scale by Slimani et al. (2016). They tested the Richards equation under homogeneous conditions, as well as stratified conditions by decreasing the permeability with depth in order to represent 'real-life' conditions, drawing support from the conceptual model of the layered structure of MSW first presented by Bendz and Singh (1999). They found the homogeneous assumption to be inappropriate to describe the flow behaviour, and that consideration of stratification yielded better fits to the data. It should be noted that REV-based modelling approaches, where the domain is essentially homogenized, albeit segmented in some approaches, as discussed above, may not be entirely suitable to carry out a deeper investigation into the role of the structure of MSW, or that of its different components and their dual-/multi-porosity characteristics. This is because the transport processes at play take place inside the pores of this porous medium and it is likely that it is their multi-scale behaviour that governs transport at the field-scale. In another study, researchers developed a dual-porosity flow model to study the flow of leachate to vertical wells (Ke et al., 2018). As is

typical for this type of model, the waste mass was divided into matrix and fracture domains, whereby flow could occur horizontally and vertically towards the vertical well with the possibility of mass exchange between the two continua. Sensitivity analysis indicated that the hydraulic properties of the fracture domain influence leachate drawdown more so than those of the matrix domain. Interestingly, the degree of anisotropy (horizontal hydraulic conductivity ÷ vertical hydraulic conductivity) was found to have a negative impact on leachate drawdown as it gets sequentially harder for leachate to flow vertically. Furthermore, the authors also tested their model against field-scale drawdown test data. Whereby upon fitting the data to the proposed model to obtain parameters such as hydraulic conductivities of the fracture and matrix continua, the authors were able to obtain a reasonably good fit. their study shed light on the need for field-scale data which is required to inform current elementary-scale models, without which the predictive capabilities of current elementary-scale models are very limited.

Researchers have also applied electrical resistivity tomography (ERT) subsurface modelling to understand the flow in two landfill cells and subsequently model the flow of leachate within them (Audebert et al., 2016a; Audebert et al., 2016b). Despite the inherent heterogeneity of landfilled waste, similarities between the leachate injection experiments were reported. They proposed a hydrodynamic model (based on the dual permeability model in HYDRUS-2D) with one parameter set to predict leachate flow for the waste deposit cells. Similar to recent studies, they found the dual continuum approach better described the flow of leachate in comparison with the single-continuum assumption (Han et al., 2011; Woodman, 2007; Woodman et al., 2017; Woodman et al., 2014; Woodman et al., 2015; Woodman et al., 2013).

2.3.2.2 Stochastic and probabilistic modelling:

Instead of mechanistic approaches, some researchers albeit comparatively few in number, have adopted stochastic and/or probabilistic modelling approaches (McCreanor & Reinhart, 1999; McCreanor & Reinhart, 2000; Reinhart et al., 2002; Rosqvist & Destouni, 2000; Rosqvist et al., 2005; Zacharof & Butler, 2004a; Zacharof & Butler, 2004b). For instance, the U.S. Geological Survey's saturated-unsaturated transport model (SUTRA) has been applied to model flow in MSW in homogeneous anisotropic and heterogeneous waste masses (McCreanor & Reinhart, 1999; McCreanor & Reinhart, 2000) using a stochastic approach to model the heterogeneous nature of MSW. They used normal, exponentially

increasing and exponentially decreasing probability density functions to model the frequency-hydraulic conductivity relationships for anisotropy and heterogeneity. The flow in the model itself was described by a general form of Darcy's law (Voss, 1984). They compared results for the homogeneous, isotropic case, due to low computation times, against field data for cumulative leachate volumes generated and found errors ranging from 27 to 160%, indicating the unsuitability of modelling the waste mass isotropically. They discussed that the discrepancies were likely due to preferential flow. Overall, their study was one of the first to highlight the possibility of applying stochastic approaches to tackle the problem of waste heterogeneity. Similarly, a probabilistic Lagrangian modelling approach was adopted to interpret tracer breakthrough curves by (Rosqvist & Destouni, 2000; Rosqvist et al., 2005). To account for preferential flow, they divided the domain into mobile and immobile water (Hopmans et al., 2002; Kohne et al., 2009; Simunek et al., 2003; Simunek & van Genuchten, 2008; Vereecken et al., 2016). Likewise, another approach divided the waste into regions of fast and slow flow paths, where the solute advection variability between these fast and slow flow paths was described by a bimodal probability density function (BIM). The tracer breakthrough curves had a long tail, and the early peaks were indicative of rapid solute transport in preferential flow paths, while the prolonged tails were possibly due to transport in the slow regions. Overall, the experimental work indicated the existence of nonuniform transport. Interestingly, the authors claimed that the MIM model was able to fit to the data adequately, however, the dispersivity values were unreasonably high suggesting that the spreading of the breakthrough curves could not be explained by local dispersion alone. The BIM model achieved good agreement with the tracer tests. Interestingly, the model interpreted that 90% of total water flow occurred through 47% of the water content of the waste sample, suggesting that preferential flow dominated the flow regime. This study showed that the landfill system cannot be described by models based on homogeneous isotropic media and indicated that two-domain models are better at describing transport through MSW. Interestingly, recent work (Caicedo-Concha, 2016; Caicedo-Concha et al., 2011; Caicedo, 2013; de Vries et al., 2017; Kohne et al., 2009; Simunek et al., 2003; Simunek & van Genuchten, 2008; Vereecken et al., 2016) has suggested that different MSW fractions affect flow in different ways and as such, the validity of assigning the same immobile region characteristics to the entire waste matrix is debatable. Of course, the suitability of adopting such assumption depends significantly on the objectives of the model, as accuracy is operationally defined and not an absolute term.

2.3.3 Modelling Of Leachate & Gas Transport Incorporating Degradation & Deformation

Here, the development of modelling approaches to represent leachate flow in MSW coupled with biogeomechanical processes occurring within the waste mass are considered. Within this body of work, it has been demonstrated that there is a significant lack of validation against real experimental or field data. Where validation has occurred, the differences between modelled and experimental data suggest a lack in our understanding of the MSW system as a whole. For instance, as noted earlier a recent model comparison exercise was conducted where modellers were provided with set-up and operational data for two experimental lab-scale landfills and invited to submit predictions for variables such as waste settlement, gas generation, changes in leachate chemistry etc. (Beaven, 2008; Beaven et al., 2008; Clewes et al., 2008; Ivanova et al., 2008; Lobo et al., 2008; McDougall, 2008; Reichel & Haarstrick, 2008; White, 2008; White & Beaven, 2013). The majority of the models underpredicted the cumulative biogas production, with one of the models overpredicting the yield (for one of the experiments, by almost twofold). Most of the models predicted the trends in data such as settlement and volatile fatty acid concentrations with varying degrees of accuracy. Detailed descriptions of some of these models and their underlying frameworks are discussed later.

To describe two-phase flow (gas, liquid) in landfills, REV-based models obeying Darcy's law overall, and in some instances explicitly modelled via variants of the Richards equation, with van Genuchten functions to describe relative permeabilities of leachate and gas, have been applied widely (Feng & Zhang, 2013; Feng et al., 2017; Feng et al., 2015; Feng & Zheng, 2014; Feng et al., 2016; Kindlein et al., 2006; Sanchez et al., 2006; Sanchez et al., 2007; Sanchez et al., 2010; White & Beaven, 2013; White et al., 2011; White et al., 2014). As a typical example, Reddy et al. (2014) applied the finite-difference based Fast Lagrangian Analysis of Continua (FLAC) model to simulate two-phase flow in bioreactor landfills. They assumed leachate and biogas to be immiscible fluids whose flow was governed by leachate saturation and capillary pressure (pressure difference between pore water and pore gas). The flow of these two fluids was described via Darcy's law, and the relative permeabilities were related to the saturation of the waste via van Genuchten functions (van Genuchten, 1980). Upon validation against data obtained from the literature (-laboratory & field-scale) & similar single-phase modelling work, the authors claimed that FLAC was on par with currently available/used models.

2.3.3.1 Implicit and explicit modelling of biogeomechanics.

Likewise, variants of the two-phase approach have been coupled with models of other biogeomechanical processes in landfills in attempts to describe the whole system. For instance, a 2D multiphase flow and transport model incorporating degradation was presented by (Kindlein et al., 2006). They modelled the landfill system as a homogeneous domain arguing that the landfill heterogeneity at the field-scale can be neglected, which, as previously discussed, may not be a suitable assumption. The hydraulic model for multiphase flow was based on the work of Bear and Helmig by applying Darcy's law for fluid flow incorporating diffusion and dispersion (Bear, 1972; Helmig, 1997). The relative permeability of waste and gas was based on the Brooks and Corey functions (Brooks & Corey, 1964). Monod kinetics was employed to model biodegradation and the evolution of organic compounds with time. Biodegradation was coupled with multiphase flow implicitly by including sinks and sources in the multiphase equations for leachate and biogas. Although they did not validate their model against field-scale or laboratory-scale data, their model suggested that leachate tends to move preferentially around regions of waste exhibiting gas production. Overall, their study showed the possibility of modelling flow of leachate and gas exclusively, while considering biodegradation as sources and sinks instead of explicitly modelling individual degradation stages (i.e. specific reaction pathways starting with a substrate). However, their study lacked the inclusion of the inherent heterogeneity of the waste, which might have impacted their results.

In many instances, many of the two-phase flow models in the literature have not been fully tested against experimental or field-data, and where reported, the agreement between these types of models and measured data has been poor. For instance, a hydro-bio-mechanical model to represent the behaviour of landfilled waste has been developed (Datta et al., 2017; Kazimoglu et al., 2006; McDougall, 2007; McDougall, 2011). The hydraulic model was based on the 2D formulation of Richards' equation, and the van Genuchten parameters were used to express the relationship between suction and moisture content in order to solve unsaturated flow scenarios. The biodegradation model was based on modelling individual anaerobic degradation reactions explicitly (hydrolysis, acetogenesis, methanogenesis). However, the biodegradation model assumed a perfectly-mixed two-stage anaerobic digester, while all the degradable waste was classified as cellulose in the modelling of these reactions. Recently, Datta et al. applied this model to a laboratory-scale experiment studying coupled processes in MSW (Datta et al., 2017). The overall predicted methane generation volume was more than double

the experimental value, suggesting that approximating all the degradable content of MSW as cellulose for modelling purposes is likely an unsuitable assumption, particularly due to the presence of hemicellulose and the more recalcitrant, lignin components of the biodegradable matter within MSW. Similarly, researchers have developed a 3D two-phase flow model for leachate and gas flow in landfills (Feng et al., 2018; Feng et al., 2017). As with Kindlein et al. (2006) they modelled the leachate and gas flow via Darcy's law, with source-sink terms for gas and leachate resulting from biodegradation from the landfill, ignoring intermediate degradation products (Feng & Zhang, 2013; Feng et al., 2017; Feng & Zheng, 2014; Feng et al., 2016; Kindlein et al., 2006). The relative permeabilities were modelled by adopting the van Genuchten and Mualem model and assuming that gas and leachate are immiscible, the porosity of the waste remains constant and isothermal conditions prevail (Mualem 1976; van Genuchten 1980). Comparison against field data for spatial variation of pore water pressure showed poor fits, and the authors discussed the possibility of heterogeneity of the waste hydraulic properties causing disagreements between measured and predicted data. This study highlighted the importance of considering the flow of leachate and gas as coupled phenomena, and the unpredictability that arises in modelling these phenomena when the waste structure and its heterogeneity are not considered, as is typical of REV-based modelling strategies.

In addition to modelling two-phase flow with biogeomechanical processes, some researchers have opted for a compromise between modelling biodegradation explicitly, reaction-by-reaction (McDougall, 2007; Kindlein et al., 2007) and simply including it as a source-sink term by modelling bulk biogas generation as a first-order process. For instance, a 2D coupled hydro-bio-mechanical model was recently developed (Reddy et al., 2017a; Reddy et al., 2017b). The two-phase hydraulic model was based on Richards' equation, where the biogas and leachate were considered immiscible and the relative permeabilities of the leachate and gas were modelled via the van Genuchten model. A Mohr-Coulomb based plane-strain plasticity model was adopted to model the settlement of the waste. USEPA's LandGEM model was used to model first-order biodegradation of the waste mass (USEPA, 2005). It should be pointed out that whilst this model has not been verified against field data as of yet, the authors have performed parametric case studies to identify the importance of certain parameters to inform bioreactor landfill design. Their modelling framework does not consider heterogeneity of the hydraulic, biochemical and geotechnical properties of the waste mass, which would likely impact their model's predictions at the field-scale. Their framework also assumes a first order bulk gas generation and degradation behaviour from the waste mass. Recent evidence

has shown that different MSW components which are biodegradable exhibit variable degradation behaviour and that lignin-rich components of MSW generally do not undergo biodegradation in the landfill environment (Jayasinghe et al., 2014; Muaaz-Us-Salam et al., 2017; Wang & Barlaz, 2016; Wang et al., 2015; Wang et al., 2011; Warwick et al., 2018; Ximenes et al., 2015; Ximenes et al., 2008). Overall, their studies have provided valuable insights into the importance of coupled processes in designing bioreactor landfills for leachate recirculation and early stabilization of the waste mass.

2.3.3.2 Consideration of heterogeneity.

In addition to coupling biogeochemical processes with the aforementioned two-phase REV-based approaches, some researchers have also attempted to capture the heterogeneity of the waste mass (McCreanor & Reinhart, 1999; McCreanor & Reinhart, 2000; Sanchez et al., 2006; Sanchez et al., 2007; Sanchez et al., 2010; Zacharof & Butler, 2004a; Zacharof & Butler, 2004b). For example, flow has been modelled stochastically through MSW incorporating waste heterogeneity and biogas production (McCreanor & Reinhart, 1999; McCreanor & Reinhart, 2000; Zacharof & Butler, 2004a; Zacharof & Butler, 2004b). In the latter model, biochemical pathways (hydrolysis, acetogenesis and methanogenesis) were modelled individually for the various components of the organic fraction represented by carbohydrates, fats and proteins. Model molecules for each of these components were chosen and growth/decay functions were used to model the rates of change in the molar mass of these components during hydrolysis, acetogenesis and methanogenesis. Flow was modelled stochastically to include the effects of waste heterogeneity by taking the overall flow through the landfill to be time invariant. It was also assumed that the flows through the waste were log-normally distributed against the average vertical water velocities. The statistical velocity model was then used to calculate the travel times of the leachate particles by using the random function given by the ratio of the distance travelled to the average velocity experienced. Since time was the key variable in the hydrological and biochemical modules, it was used as the basis to produce the integrated model with the overall aim of predicting leachate and biogas compositions. However, similar to other field-scale models discussed in this section, testing against actual field data was not reported. It should also be noted that whilst stochastic modelling may be suitable to fit experimental data and gain some insight into the flow regime of the porous medium, unlike mechanistic approaches, it is not an ideal way to gain in-depth understanding of the physics and biogeochemistry of these phenomena. In another attempt to consider the impact of the inherent heterogeneity of MSW on flow and biogeochemical phenomena, a 3D model for

biodegradation, and flow of landfill gas and leachate has been developed (Sanchez et al., 2006; Sanchez et al., 2007; Sanchez et al., 2010). They modelled individual aerobic and anaerobic degradation reactions by employing Suk et al. and Lee et al.'s models for the dissolved carbon, its conversion to organic acids and the rate of growth of microorganisms.(Lee et al., 2001; Suk et al., 2000) They then employed El-Fadel et al.'s strategy to model the bulk biodegradation of the waste by including relative biodegradability of certain fractions (El-Fadel et al., 1996; El-Fadel, 1996). The biodegradation module linked with the standard convection-diffusion-reaction equation to model the concentration of landfill gas. Their hydraulic model was based on Richard's equations, while the relative permeabilities of gas and leachate were modelled via van Genuchten functions. They considered heterogeneity of the waste mass by introducing spatial variation of permeabilities and porosities in 3D by employing the sequential Gaussian simulation technique. Although they did not test their model against actual field data, they simulated a variety of scenarios for homogeneous and heterogeneous landfills. In summary, their study demonstrated the impact of waste heterogeneity on flow of leachate and gas, and the significance of including two-phase flow to realistic modelling of landfill processes, since the inter-phase interactions impact the gauge pressure within the waste mass and influence the stability of landfills.

2.3.4 Future Needs & Recommendations

In light of the state of the art reviewed above, the following challenges are identified and on this basis, provide recommendations for future research needs and potential multidisciplinary approaches to address them.

(1) *The 'black box' of waste* – As perhaps suggested by other researchers, it is recommended that gaining a better understanding of the aforementioned challenges requires penetrating the 'black box' of waste. To achieve this an extra layer of detail is required in our current continuum-scale understanding of transport. For instance, geophysics and petroleum engineering literature is rich with contributions successfully exploring transport in permeable geological media at the pore-scale (Bijeljic et al., 2011; Blunt, 2017; Blunt et al., 2013; Mostaghimi, 2012; Mostaghimi et al., 2010; Mostaghimi et al., 2012; Mostaghimi et al., 2016; White & Beaven, 2008). Currently, a popular approach to modelling transport at the pore-scale involves using micro-CT X-ray scanning techniques to provide image data which are compiled to produce a digital 3D representation of pore-structure which is then used to define the modelling domain for

flow simulations via various methods (e.g. Lattice-Boltzmann, Navier-Stokes, Figure 2-10) (Bijeljic et al., 2013; Davit et al., 2013; de Vries et al., 2017; Liu et al., 2017; Roman et al., 2016; Seetha et al., 2017; Soulaine et al., 2011). It is suggested that studying flow and transport at the pore-scale in MSW would help us understand the complex mechanisms involved and generate vital information which can then be used to inform and/or modify our existing models for better prediction. For instance, micro-CT (depending on the resolution and sample size) might also be able to identify the pores within different MSW components (wood, food waste etc.) and with the help of pore-scale computational fluid dynamics (CFD) simulations, could shed light on the dual-porosity/permeability characteristics of MSW at the component-level and their role in impacting flow through the pore space.

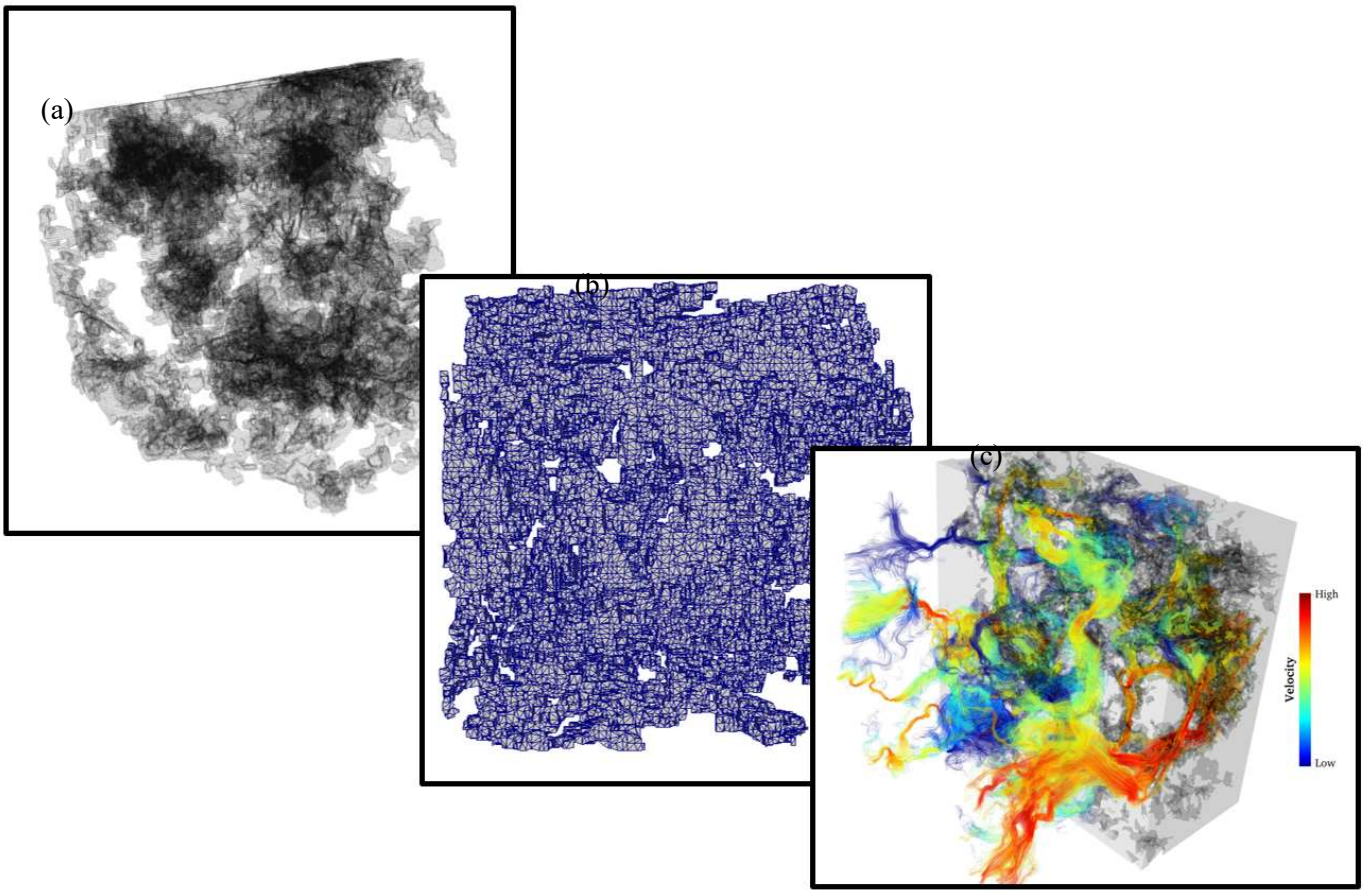

Figure 2-10 Schematic of a pore-scale simulation. (a) Scanning, (b) pre-processing, (c) CFD simulation.

(2) *Fluid-structure interaction* – The variable nature of MSW composition, and the resulting fluid-structure interaction resulting from various biogeochemical processes needs to be better understood, with particular attention to biodegradation, mechanical creep and

other processes that result in a transient system with an evolving pore space (Caicedo, 2013). In order to understand the fluid-structure interactions and similar processes well, it may be beneficial to study the structure at the component-scale, including the packing between different components, as well as flow through individual material types, for instance, the fluid-structure interactions resulting from wood might be different to those resulting from food waste (Caicedo-Concha, 2016; Caicedo-Concha et al., 2011).

(3) *Heterogeneity* – Throughout this literature review, it has been evident that researchers have found the multiscale heterogeneity of MSW, paired with other factors discussed above, has resulted in preferential flow and added to the complexity of modelling flow through this porous medium. This has been, and likely will continue to be, a significant challenge. Measuring and quantifying the variability of waste components in the matrix, their arrangement and the resulting multiscale heterogeneities is challenging, and has not been, to date, fully investigated. However, this review has highlighted how understanding the impact of heterogeneity on flow through MSW is an integral part of improving our predictions. Here some suggestions are offered to study heterogeneity. As discussed above, pore-scale experimental and modelling investigations of flow will likely improve our understanding of transport mechanisms. Fractal theory has been widely investigated in the sciences to study the inherent irregularity in nature and natural phenomena (Dekking et al., 1999; Hutchinson, 1981; Mandelbrot, 1982; West et al., 1997). Recently, fractals have also been applied to porous media to study the properties of pore structures including multifractal analyses and their ability to quantitatively describe multi-scale pore-structure heterogeneities (Bird et al., 2006; Bird et al., 2000; Caplan et al., 2017; Gibson et al., 2006; Jaya et al., 2013; Liu & Ostadhassan, 2017; Lopes & Betrouni, 2009; Morató et al., 2017; San José Martínez et al., 2010; Wang et al., 2016a; Wang et al., 2016b; Xie et al., 2010; Xu, 2015; Xu et al., 2015; Zhang et al., 2014). It is suggested that this line of inquiry combined with pore-scale modelling of flow might help us quantitatively relate heterogeneity of MSW to the spatial distribution of its components and their individual dual permeability characteristics. It might also help us quantitatively determine the changes in heterogeneity due to the evolution of the pore space due to biogeochemical processes at a variety of scales to study their impact on transport phenomena.

(4) *Data and sources of error* – Thus far, this section on flow has discussed modelling efforts to describe fluid flow in MSW, as well as fluid flow and transport incorporating

biogeomechanical phenomena. In making the case for pore-scale modelling in MSW, a crucial focal point is the data that is required to study flow, transport and biogeomechanical processes and validate pore-scale modelling efforts. Since to the best of the author's knowledge such experimental data is sparse, within the context of MSW, it is important to explore possible experimental techniques and ideas at the pore-scale which could help in generation of data to gain a better understanding of the above to study and model these processes at the pore-scale. Following this, since the ultimate scale of interest is the field-scale, exploring the integration of insights obtained from pore-scale experiments and models into existing elementary/field-scale models could perhaps form the next step. As highlighted at the start of the chapter, Figure 2-1 shows some of the processes that take place in the MSW system. Studying these processes at the pore-scale could be addressed with micro-model experiments combined with modern imaging techniques currently widely used in digital rock physics work (Blunt et al., 2013; Xiong et al., 2016). Micromodel experiments in pore-scale literature have been vital in understanding fluid properties and fluid flow in permeable media. For instance, researchers have successfully used these techniques for investigating the evolution of the pore space in certain rocks due to typical geochemical dissolution reactions whereby the pore space is modified (Al-Khulaifi et al., 2017; Blunt, 2017; Menke et al., 2016; Menke et al., 2017). However, detailed examination and review of these experimental techniques and possible lines of inquiry are beyond the scope of this thesis.

2.4 Discussion & Concluding Remarks

This literature review has highlighted the problem of slowly degrading lignocellulosic wastes in landfills, which have been shown to be mainly woody and newspaper-based in nature. Likewise, this slow degradation gives rise to a 'long'-tail of green-house gas emissions over a long period of time, adding to anthropogenic climate change as we know it. As a result, a potential solution to the problem has been discussed, accelerating the degradation of these wastes so as to increase the amount of overall biogas produced in the same amount of time, which can then be quickly captured and used for bioenergy generation. This potential solution has been discussed in the form of biotechnological systems (enzymatic and bacterial) which could specifically target the lignocellulosic fraction to provide more bioavailable and intermediate degradation products to methanogens for conversion to biogas. These systems could hold the potential to provide significant lignin degradation in MSW landfills. As with

any engineering problem, there will be limitations and advantages to many of these systems. E.g. most fungi require aerobic conditions to function, which simply do not exist for the bulk of the life of a landfill. Analogously, DyP have been found to be operational only under acidic conditions. It would be desirable to develop this enzyme, and the others discussed here, to work for a variety of conditions under which it could delignificate. It may even be the case that a combination of enzymatic, bacterial and fungal delignification systems is required. Perhaps bacteria may be more flexible to changes in pH and thrive in certain pores or only at certain stages of the degradation process, whereas enzymatic delignification behaviour may be different in the biogeochemically active landfill environment. However, as pointed out earlier, these biotechnological systems have either not been extensively studied in MSW or 'real' lignocellulosic wastes found undegraded in landfills, or have been studied with the elimination of the heterogeneous physical nature of MSW and other processes that occur at the field-scale (e.g. leachate flow). Due to the multidisciplinary nature of this problem, delivery of these systems to key lignocellulose-rich areas, in a heterogeneous waste matrix is paramount. Moreover, heterogeneity results in preferential flow, which is a major cause of the lack of biodegradation at the field-scale in the first place. This leads on to the flow processes in the waste mass at play, as discussed below.

Presently, dual-porosity and dual-permeability models have been applied to identify flow mechanisms at the elementary scale. As discussed previously, in-situ delivery and operation of these biodelignification systems will be significantly affected by the flow of leachate. Hence a deeper understanding at the pore-scale is required, which would need to potentially be coupled with biodelignification reactions occurring within the pores. We may then be able to upscale laboratory column delignification studies and be able to predict the extent of biodelignification in relation to the lignin content, leachate recirculation flowrates etc. Only once sufficient understanding of these phenomena has been achieved can they potentially be applied at the field-scale with the prospect of sustainable landfilling. A better understanding of biodelignification systems and the flow mechanisms within heterogeneous MSW, for delivery and operation, could potentially help us decrease the time to stabilisation for a landfill, resulting in significant environmental benefits.

While pore-scale investigation of fluid flow and biogeochemical processes might lead to new insights, as is the case with any experimental/modelling technique, sources of error and challenges will arise. One of the key points addressed in this literature survey has been the

heterogeneity of the waste. This inherent heterogeneity makes representative sampling of a waste body very difficult and casts doubt on extrapolating the conclusions from one particular study to another. Pore-scale studies with micro-model experiments/models will likely encounter these doubts and difficulties. However, the purpose of this line of work would be to further understanding of the fundamental processes of the MSW system and the interaction between the different processes from Figure 2-1. What does this mean for currently existing models? Hopefully, experimental and numerical pore-scale studies as described previously, when put against currently existing data at the lab and field scales will help in establishing a relationship between scaling of flow/transport and biogeochemical/physical processes. This relationship between the scales should then help in understanding how averaging will work to upscale from the pore-scale, to the centimetre scale, up to the metre scale, leading all the way up to the field scale. In conclusion, modelling transport phenomena in MSW is challenging due to its inherent multi-scale heterogeneity and ever-evolving pore space due to various biogeochemical/physical processes. Continuum-scale models have not been able to sufficiently describe transport due to the impact of the aforementioned processes. Studying transport is suggested at the pore-scale to further our understanding of transport within the pores of the waste, since it is these pore-scale processes that ultimately govern transport at the field-scale. The insights obtained could then be used to modify existing continuum-scale models for better prediction.

To summarise, this literature review has painted a picture of the current state of the art regarding degradation of lignocellulosic wastes in landfills, and potential ways of accelerating that degradation via biotechnological methods. Furthermore, gaps in knowledge and potential challenges that exist in applying the enzymatic and bacterial lignocellulose-degrading systems to accelerate breakdown/biogas production have been discussed. Namely, heterogeneity of the waste mass, understanding of transport phenomena within the waste mass and further information about the workings of these biotechnological systems with ‘real’ wastes and experiments mimicking the environmental conditions that may be found in a landfill (e.g. leachate recirculation, presence of methanogens/other microbes, heterogeneous configurations of waste and older landfilled waste with a mainly ‘inert matrix’ with zones containing lignocellulosic material). Lastly, the formulated RQs and overall aim for this project sit well within the aforementioned gaps, as discussed at length within this chapter.

3 Materials & Methods

This chapter details the materials used in the experimental work in this project, detailed experimental design, as well as the analytical methods, statistical analyses and the overall workflow.

3.1 Materials

Model lignin wastes consisted of newspaper (52 g/m^2standard recycled paper) and softwood (kiln-dried Nordic redwood pine timber, high heartwood proportion). For particle-size reduction, prior to starting an experimental run, approx. 200 g of waste were fed to a Fritsch 55743 rotary knife mill (approx. feed size: 2 cm long squares for newspaper and cubes for wood) with a 2mm screen; for experiments requiring powdered wood (<0.15mm), the knife-milled material that passed through the 2mm screen was collected and passed through a 0.15mm sieve. These wastes were chosen to represent the range of lignocellulosic wastes found in MSW landfills, with newspaper containing a smaller amount of lignin in comparison to softwood which is very lignin-rich. Sewage sludge from an anaerobic digester at a wastewater facility in Cardiff, was used as a source of methanogens (5.52% ± 0.002 dry solids of which 59.46% ± 0.023 volatile solids). Sewage sludge was used due to its relatively more homogeneous behaviour as an inoculum across the literature for anaerobic biodegradation in relation to landfill leachate, which varies much more widely around the world (Pearse et al., 2018). The sludge was sampled from the digester with a hose-pipe attached to the tank in three 5-litre high-density polyethylene jerrycans, immediately transferred to the lab and used to start the bioreactor experiments. For experiments where acclimatisation at 30℃ was required, the sludge was transferred to an incubator maintained at 30℃ for three days and shaken manually twice a day for homogenisation. For every experimental run, fresh sludge was sampled from the exact same site and the exact same sampling point. Commercially available Lignin peroxidase (Merck product code: 42603-10MG-F) and *Agrobacterium sp.* (GenBank accession JX872342, bacterial phylum α-Proteobacteria) from a previous study (from T. Bugg, Warwick University, UK) (Rashid et al., 2017) were used. For experiments requiring construction of porous lab-scale bioreactor landfills (heterogenbeous and homogeneous), washed fine silica sand (particle size distribution in Figure A-0-27) was used as the 'inert' background matrix to represent inert material left after the degradation of the readily degradable material.

3.2 Experimental Layout & Workflow

Experimental work was planned and carried out in response to the RQs that were formed for this project. As a reminder to the reader and to provide an overview of the entire project, the RQs and the experimental structure for each RQ are presented below:

- RQ1: To what extent can enzymatic & bacterial biodelignification systems breakdown lignocellulose in realistic lignin wastes, with the prospect of enhanced biogas recovery? For RQ1, as detailed in Table 3-1 and 3-2, a fungal peroxidase (lignin peroxidase) enzyme and a recently isolated lignin-degrading bacterial strain (*Agrobacterium sp*.) were used. In the first instance, small-scale bacterial biodelignification tests were conducted to identify optimal conditions for later bioreactor experiments on wood, as it is the more recalcitrant of the two model wastes. This was followed by tests conducted in stirred bioreactors with methanogens from sewage sludge added to produce biogas from breakdown products. These bioreactor experiments tested the application of the enzyme and *Agrobacterium sp*. (Table 3-1 and 3-2). Furthermore, the impact of varying the solid:liquid ratio on the activity of *Agrobacterium sp*. was also evaluated, as the literature suggests that this is an important variable for to consider for anaerobic biodegradation applications.

- RQ2: What is the impact of flow & heterogeneity on bacterial biodelignification systems in model lignocellulose-containing bioreactor landfills? For RQ2, as detailed in Table 3-3, *Agrobacterium sp.* was added to homogeneous and heterogeneous pore-structure configurations containing methanogens, newspaper and sand to mimic old lab-scale landfills. A peristaltic pump was used to provide recirculation in flow reactors. The above allowed to study the impact of landfill waste heterogeneity and leachate flow (field-scale, 'real-life' variables) on the ability of *Agrobacterium sp.* to enhance biodegradation/biogas production.

Table 3-1-Experimental work-flow and summary of the small-scale experiments, highlighting the context and relationship to RQ1.

Condition	**Variable**							
-Wood + M9 -*Agrobacterium* sp.+ Wood + M9	<0.15mm size only	<0.15mm size + Autoclaved	<0.15mm size + Intermediate acclimatisation only	<2mm size+Intermediate acclimatisation only	<0.15mm size + Autoclaved + Intermediate acclimatisation	<2mm size only	<2mm size + Autoclaved	<2mm size + Autoclaved+ Intermediate acclimatisation

Note: Abbreviations: Sludge, S; lignin peroxidase enzyme, E; Newspaper, N; Wood, W Agrobacterium, A; Methanogens, M; M9 solution, M9; 4 grams mass of waste, 4g; 12 grams mass of waste, 12g, Autoclaved, AT. These abbreviations are used in Ch. 4 (in combination, as well as stand-alone) and also briefly defined within it.

Table 3-2-Experimental workflow and summary of the bioreactor-scale experiments in RQ1.

Condition	**Variable**			
-*Agrobacterium sp.*+M9 +Sludge -M9+Sludge	(no waste)	Newspaper	Wood	-
-*Agrobacterium sp.*+M9 +Sludge	Newspaper (low solid:liquid)	Wood (low solid:liquid)	Newspaper (high solid:liquid)	Wood (high solid:liquid)
-LiP+Sludge -Sludge	(no waste)	Newspaper	Wood	-

In RQ1 and RQ2 experiments, biogas, pH and soluble organic carbon were the main variables monitored. The specific details of each individual experiments (thorough justification, type, controls, replicates etc.) are provided in the following sub-headings dedicated to each RQ. Moreover, miscellaneous work was carried out which was related to, but did not directly inform the RQs. This included: Preliminary biogas potential tests. Preliminary lignin peroxidase tests with landfill leachate. Impact of size of model lignocellulosic wastes (wood and newspaper) on the biogas potential. Impact of size of wood on the wellbeing of bacteria contained within sewage sludge. Growth curve for *Agrobacterium sp.* (optical density & staining living cells). Hydraulic conductivity tests for varying sand and newspaper mixtures. Modelling of biogas generation and flow data from the experimental work. Pore-scale flow modelling on real-pore structure images from CT-scanning & Elementary-scale modelling work.

Table 3-3- Experimental work-flow and summary of the project, highlighting the context and relationship to RQ2.

Type of Experiment	Condition	Variable
Bioreactor-scale	-*Agrobacterium sp.*+M9+Sludge+Sand (Homogeneous) -M9+Sludge+Sand (Homogeneous)	-Flow+Newspaper -No-flow+Newspaper
	-*Agrobacterium sp.*+M9 +Sludge+Sand (Heterogeneous) -M9+Sludge+Sand (Heterogeneous)	-Flow+Newspaper -No-flow+Newspaper
	-M9 +Sludge (Stirred)	Newspaper

Note: Abbreviations: Homogeneous, Ho; Heterogeneous, Ht; Flow, F; No-flow, N; Agrobacterium, A; Control (lacking Agrobacterium), C. These abbreviations are used in Ch. 5 (in combination, as well as stand-alone) and also briefly defined within it.

3.2.1 Experimental Design RQ1

3.2.1.1 Preliminary Small-scale Bacterial Biodelignification:

To identify optimal conditions for later bioreactor experiments on wood a series of preliminary experiments were undertaken. This is particularly critical for wood due to the typically lengthy degradation time-scales (experiments lasting years in some studies) (Wang et al., 2013) and the lignin content of wood and its various sizes found undegraded in landfills (small cm-scale chips to m-scale blocks) (De la Cruz et al., 2013; Wang & Barlaz, 2016; Ximenes et al., 2017; Ximenes et al., 2018). Since wood contains roughly 1.5 times more lignin than newspaper and is unprocessed lignocellulose, where the structure is intact, it represents some of the most difficult to degrade wastes in landfills and should prove to be a more challenging substrate for biodegradation. To get an understanding of the impact of the physical state of the wood on biodegradation by *Agrobacterium sp.*, small-scale tests in 50 ml sterile tubes were conducted.

Four wood sample types were tested in these experiments exploring the impact of waste form/size and accessibility of biodegradable materials. Each consisted of one particle size range (<2 mm or <0.15 mm) and was either tested as-is or autoclaved prior to the experiment. In the latter case wood was autoclaved at 120℃ for 15 mins to test its effect on deconstructing the lignocellulosic matrix for easier microbial access to key polymers, i.e. lignin, cellulose and hemicellulose (Pecorini et al., 2016). Each type was then tested with and without

Agrobacterium sp. in duplicate specimens. Each specimen comprised 1 g of wood added to 20 ml of M9 mineral medium in 50 ml tubes with either 1 ml of *Agrobacterium sp.* starter culture or 1 ml sterile M9 medium (control) (Rashid et al., 2017).

Flasks were placed in a shaking incubator at 200 rpm and 30°C for seven days. For these small-scale tests, sludge was absent since the purpose was to test the ability of this strain to breakdown lignocellulose, whilst the presence of other microbial communities from the sludge could interfere with the monitoring of the activity of only this strain. Total carbon released into the liquid phase during the test and the organic carbon content of the solid residue after bacterial treatment were analysed. Biogas production, pH and chemical oxygen demand were not monitored in these preliminary small-scale tests.

3.2.1.2 Bioreactor System:

The experimental apparatus (supplied by Anaero Technology UK) consisted of 15 one-litre reactors submerged in a water bath maintained at the required temperature. Gas flow meters based on the water displacement method (Wickham et al., 2016) combined with an Arduino (for data logging) were used to monitor biogas production. Biogas was collected in 5 litre Tedlar® bags attached to the outlets of the gas flow meter for each reactor, and all biogas data are reported at STP. The reactors were continuously stirred at 45 rpm during the experimental work and the duration of the experiments was between 30-35 days.

3.2.1.3 Bioreactor-scale Bacterial Biodelignification (Experiment 1):

Mechanistic insights from the preliminary small-scale tests were used to inform the design of larger-scale 1 L (total volume) tests. The rationale behind larger-scale experiments was to be able to study whether enhanced biodegradation could be carried out using the *Agrobacterium sp.* whilst monitoring for key variables such as biogas production, release of organic carbon etc, all of this done in the presence of methanogenic microbial communities from sewage sludge. These bacterial experiments contained 4g of waste, and employed a mixture of sludge and M9 minimal media (for the *Agrobacterium sp.* (Rashid et al., 2017).

Each bioreactor comprised 600 ml M9 medium (650 ml in controls), 50 ml suspension of *Agrobacterium sp*. (0 ml in controls), 50 ml methanogen-rich sewage sludge (acclimatised at 30 ºC for three days prior to introduction) and 4g of lignocellulosic material (wood or newspaper), together with 300 ml of headspace. The headspace comprised of air due to *Agrobacterium sp.* being a facultative anaerobe, i.e. able to work under anaerobic conditions

but preferring aerobic conditions (Rashid et al., 2017; Taylor et al., 2012). This reflects typical conditions in bioreactor landfills, where the waste mass is initially aerobic and the system slowly goes anaerobic with the passage of time (Benson et al., 2007). Five cases were tested (*Table 3-4*), each in triplicate, with *Agrobacterium sp.* applied to vessels containing wood (<0.15 mm, non-autoclaved), newspaper and no waste, whilst control vessels with only methanogens (no *Agrobacterium sp*.) were supplied with wood or newspaper. All bioreactors were incubated in the water bath apparatus at 30 ºC for 35 days. Liquid samples (5 ml) were taken periodically for analysis (section 3.3) from the sampling port using sterile pipettes and transferred to 15 ml sterile containers. Solid residue was obtained from all bioreactors by collection on Whatman No. 42 ® filter paper (2.5 μm) via vacuum filtration then drying at 105 ºC.

Progressively diminishing rates of biogas and organic carbon release for newspaper reactors prompted a 'restarting' of the experiment, where the contents of the bioreactors were centrifuged at 3394 rcf for 10 min under aseptic conditions to remove the supernatant and fresh autoclaved M9 was added in the same amounts as the beginning of the test (see above). This allowed for the hypothesis that towards the end of the experiment (whilst there is still carbon present in the liquid phase but gas production has almost stopped), accumulation of recalcitrant or toxic substances in the liquid phase inhibits further conversion/breakdown to biogas to be tested. 'Restarting'/removing the liquid and introducing a fresh medium could be beneficial and allow for biogas production to continue again from the left-over solid residue. 'Restarted' bioreactors were incubated under the same conditions for 20 additional days, with liquid and gas sampling.

3.2.1.4 Impact of Solid:Liquid Ratio on Bacterial Biodelignification (Experiment 2):

Previous work has shown that microbial activity is affected by the waste to inoculum ratio (with particularly low ratios shown to inhibit methanogenesis) (Moset et al., 2015). As mentioned previously, to test the hypothesis that the solid:liquid ratio might also play an important role in the ability of the *Agrobacterium sp*. to breakdown lignocellulose, the impact of the Solid:Liquid (S:L) ratio on bacterial biodelignification of these wastes was also studied by increasing the mass of waste added to 12 g. The experiments (*Table 3-4*) are otherwise the same as the earlier bioreactor-scale tests, apart from there not being a 'waste-free' control triplicate. A 4 g test (for wood and newspaper respectively) was also carried out as a repeat of

that in the previous experiment to allow comparability and to serve as controls to test the above hypothesis.

3.2.1.5 Bioreactor-scale Enzymatic Biodelignification (Experiment 3):

1 L (total volume) tests to study whether enhanced biodegradation could be achieved using lignin peroxidase. Treatments are summarised in *Table 3-4*. 4 g of waste ≤2 mm, i.e. newspaper or wood (non-autoclaved), and 2.22 mg of lignin peroxidase (LiP) were added to 600 ml of sludge (source of methanogens) for a volatile solids ratio of sludge to waste of 4:1 (Labatut et al., 2011). The reason for a much larger sludge volume here pertains to the fact that inoculum to substrate ratio is a major factor in the determination of the biomethane potential of a substrate (Raposo et al., 2006), hence the enzyme experiments were conducted at the ideal volatile solids ratio of the sludge to the waste based on previous studies (Moset et al., 2015; Peña Contreras et al., 2018). The chosen waste:LiP mass ratio was based on previous work (Hettiaratchi et al., 2014; Hettiaratchi et al., 2015; Jayasinghe et al., 2011; Jayasinghe et al., 2014; Jayasinghe et al., 2013) where the volatile solids (VS):LiP ratio was optimised for maximum biogas production. LiP was activated with H_2O_2 prior to addition at the optimal LiP: H_2O_2 ratio (Hettiaratchi et al., 2014; Jayasinghe et al., 2011; Jayasinghe et al., 2013). The controls comprised of exactly the same experimental setup but lacked the peroxidase enzyme in 'waste-containing' controls. 'Waste-free' controls were also carried out with 0 and 2.22 mg of LiP. All bioreactors were incubated at 38 ºC, as this is the optimal operational temperature employed by the anaerobic digester used as the source of methanogens. Biogas collected during the experiment in 5 litre Tedlar gas bags was analysed for methane content.

Table 3-4 Conditions tested in the three main experiments. Numbers refer to number of replicates for each treatment.

	Experiment 1		**Experiment 2**		**Experiment 3**	
	With *Agrobacterium sp.*	**Without *Agrobacterium sp.***	**With *Agrobacterium sp.***	**Without *Agrobacterium sp.***	**With enzyme***	**Without enzyme***
No waste	3				3	2
Newspaper (4 g)	3	3	3		3	2
Newspaper (12 g)			3			
Wood (4 g)	3	3	3		3	2
Wood (12 g)			3			

*Note: Wood sample size < 0.15 mm, apart from samples denoted with * which had size < 2mm. Other experimental conditions are as described in the text.*

3.2.2 Experimental Design RQ2

Table 3-5 details the experimental plan. Two scenarios investigated comprised of homogeneous and heterogeneous landfill bioreactor configurations. These configurations were selected based on the general consensus in the literature on the typical pore structure of MSW landfills. Field-scale tracer tests, as well as pilot- and lab-scale flow experiments suggest that MSW is typically present in the landfill as a collection of zones of varying permeability, with a possible network of spaces running through and/or between them which may allow for preferential channelling of leachate (Bendz & Singh, 1999; Caicedo, 2013; Woodman, 2007; Woodman et al., 2014). Hence why, an idealised heterogeneous structure was chosen to represent a repeatable heterogeneous landfill bioreactor. In bioreactor landfill operation, research suggests that waste homogenisation techniques (i.e. shredding, uniform mixing) help to achieve optimal biodegradation of the waste mass (Barlaz et al., 1990; Barlaz et al., 1987; Pellera et al., 2016). As such, the homogeneous case represented a repeatable best-case scenario where the waste is distributed uniformly for optimal biodegradation and biogas production. Moreover, stirred bioreactor controls were also included. Stirred controls served as a baseline response for the biodegradation of newspaper prior to the newspaper being fixed in a solid

matrix, this allows for valuable biogas kinetics information to be studied which is free of physico-hydro-mechanical effects (leachate flow, permeability, physical mixing, advection an diffusion) which would otherwise occur in the solid matrices of homogeneous and heterogeneous landfill bioreactors. Moreover, they also allowed us to ensure that the response of the sludge used for all the experiments conducted was similar (accounting for inter-experimental variability), since homogeneous and heterogeneous experiments were conducted at different times due to the limitation of having a maximum of 15 reactors at any given time. Figure 3-1 shows a schematic of the experimental setup for the 1-L (total volume) homogeneous and heterogeneous reactors.

3.2.2.1 Homogeneous pore space

In the homogeneous reactors, a 3D-printed diffuser (see Figure A-0-29 for detailed design) was placed at the base of the reactors for even distribution and mixing of the recirculated leachate. There were 3 ports from which samples were taken, i.e. 3cm from the base (BOTTOM port), 6cm from the base (TOP port) and approximately 8.3 cm from the base (RECIRCULATION port-surface of the solid matrix). The reactor lid contained the connections to the ports below in the solid matrix, the port connecting the headspace to the gas flow meter and housed the 6mm acrylic tube going into the diffuser at the base of the reactor. In the homogeneous case, a newspaper and sand (hand-compacted with mallet & 3-D printed tool-see Figure A-0-28 for detailed design, bulk density: 1492.7 kg/m^3) mixture containing 12g of newspaper and 600g (1.96% newspaper by mass content) of sand was prepared and 50 ml of sewage sludge was uniformly mixed and hand-compacted.

3.2.2.2 Heterogeneus pore space

In the heterogeneous reactors, the diffuser was placed in the exact same manner as described above. The reactor head housed the 3 ports from which samples were taken, which here were 6cm from the base inside the newspaper/sand zone (INSIDE port) and 6cm from the base inside the outer sand only section (OUTSIDE port), and approximately 8.3cm from the base (RECIRCULATION port-surface of the solid matrix). Here, the same mass of newspaper was present, albeit not distributed homogeneously (same overall bulk density as noted for the homogeneous case). Here, 12g of newspaper was homogeneously mixed with 250g of sand (4.58% by mass content of newspaper) and placed as a column in the middle of the reactor (Figure 3-1) this was then hand-compacted exactly as noted previously (same overall bulk density as the homogeneous case). This was surrounded by 350g of sand , and 50ml of sewage

sludge was mixed uniformly in the newspaper/sand zone as well as the outside sand based on volume ratio (approximately 1:3, respectively).

Within the pore structure configurations described above, bacterial enhancement reactors contained 50ml of *Agrobacterium sp*. culture and sterile 600ml of M9 mineral media. The respective solid-phase controls contained sterile 650ml of M9 only. The reactors with leachate recirculation were recirculated using a Watson-Marlow 205U peristaltic pump at 0.5 rpm (approximately 6ml/hour). The tubing used for sampling and recirculation was Saint Gobain Tygon® S3 (6.4mm external diameter). It is noted that the newspaper percentage of the newspaper/sand mixtures used in the homogeneous and heterogeneous cases was 1.96% and 4.58% respectively. The effective bulk density for these bioreactors (using only the sand mass) was 1463.4 kg/m^3, which is within 2% of the total bulk density (as stated previously, which includes the newspaper mass as well). As such, the structure of the sand likely remained predominantly intact while the newspaper resided largely in the pores. This is further supported by calculating the bulk density of the bioreactor columns by taking into account the sand-only mass, which amounts to 1463.4 (within 2% of the bulk density calculation with newspaper).

3.2.2.3 Stirred controls

As described previously, the ideally mixed liquid-phase stirred controls containing 650ml of M9 and 50ml of sewage sludge with 12g of newspaper stirred at 45rpm continuously. It is noted that the homogeneous experiments (HO**) were carried out first, and these experiments including their controls (HoSC) ran for a duration informed by our previous stirred bioreactor experiments and the as-generated data (35 days) (Muaaz-Us-Salam et al., 2020). The heterogeneous experiments (Ht**) and their controls (HtSC) ran for longer (47.5 days) since the as-generated data indicated continued activity beyond the originally planned duration of 35 days.

3.2.2.4 Sampling

Sampling of leachate was carried out by sealing off the headspace with a pinch clamp, then connecting the sampling tubing to the respective port and using a peristaltic pump to retrieve a 5ml sample. For sampling of the RECIRCULATION port, a T-connector was incorporated into the apparatus (i.e. recirculation circuit) at the start of the experiments with a pinch clamp. Upon taking samples of the leachate (5ml) in all the experiments and all the sampling ports, the same volume of sterile M9 (5ml) was added to the specific port to replace the lost liquid. Post-sampling, all analyses were performed after filtration through a 0.2µm

filter to remove suspended solids and bacterial cells. As highlighted earlier, gas flow meters based on the water displacement method (Wickham et al., 2016) combined with an Arduino (for data logging) were used to monitor biogas production.

Table 3-5- Experimental plan. Note: "R#" refers to number of replicates.

Experiment description		**Flow condition**		
	Materials	**Flow (6 ml/hr)**	**No Flow**	**Liquid-phase\ stirred\ control**
Homogeneous	Newspaper+Sand+Sludge +M9	*HoFC (R3)*	*HoNC (R3)*	*HoSC1 (R3)*
	Newspaper+Sand+Sludge +M9+Agro	*HoFA (R3)*	*HoNA (R3)*	*(-)*
Heterogeneous	Newspaper+Sand+Sludge +M9	*HtFC (R3)*	*HtNC (R3)*	*HtSC2 (R3)*
	Newspaper+Sand+Sludge +M9+Agro	*HtFA (R3)*	*HtNA (R3)*	*(-)*

3.3 Analytical Methods

Lignin content analysis on the solid residue was adapted from Rashid et al., 2017. The samples were dried at 105℃ until constant weight, then 0.25 g of dry mass was added to 3.75 ml of 95% H_2SO_4 with stirring for 2 h at RTP. Then 140 ml of deionised water was added to the resulting solution followed by reflux for 4 h in round bottom flasks. The residual content was collected via Whatman No. 42 ® filter paper (2.5 µm) and washed with deionised water. Then the residue was dried at 105℃ until constant weight. The residue was then volatilised at 550℃ to correct for ash. The lignin content was determined by subtracting the mass of ash from that of the dry residue and then calculating the ratio of this to the original sample dry mass.

The Folin-Ciocalteau method was used for measuring phenol release (Meda et al., 2005; Rashid et al., 2017). 0.8 ml of deionized water and 0.5 ml Folin-Ciocalteau's reagent (Merck F9252) were added to 0.2 ml filtered liquid sample (0.2 µm pore size). 2.5 ml of 20% Na_2CO_3 was then added and the samples incubated in the dark for 30 min. Then the absorbance was measured using a spectrophotometer at 760 nm and p-hydroxybenzoic acid was used to calibrate the absorbance as a standard.

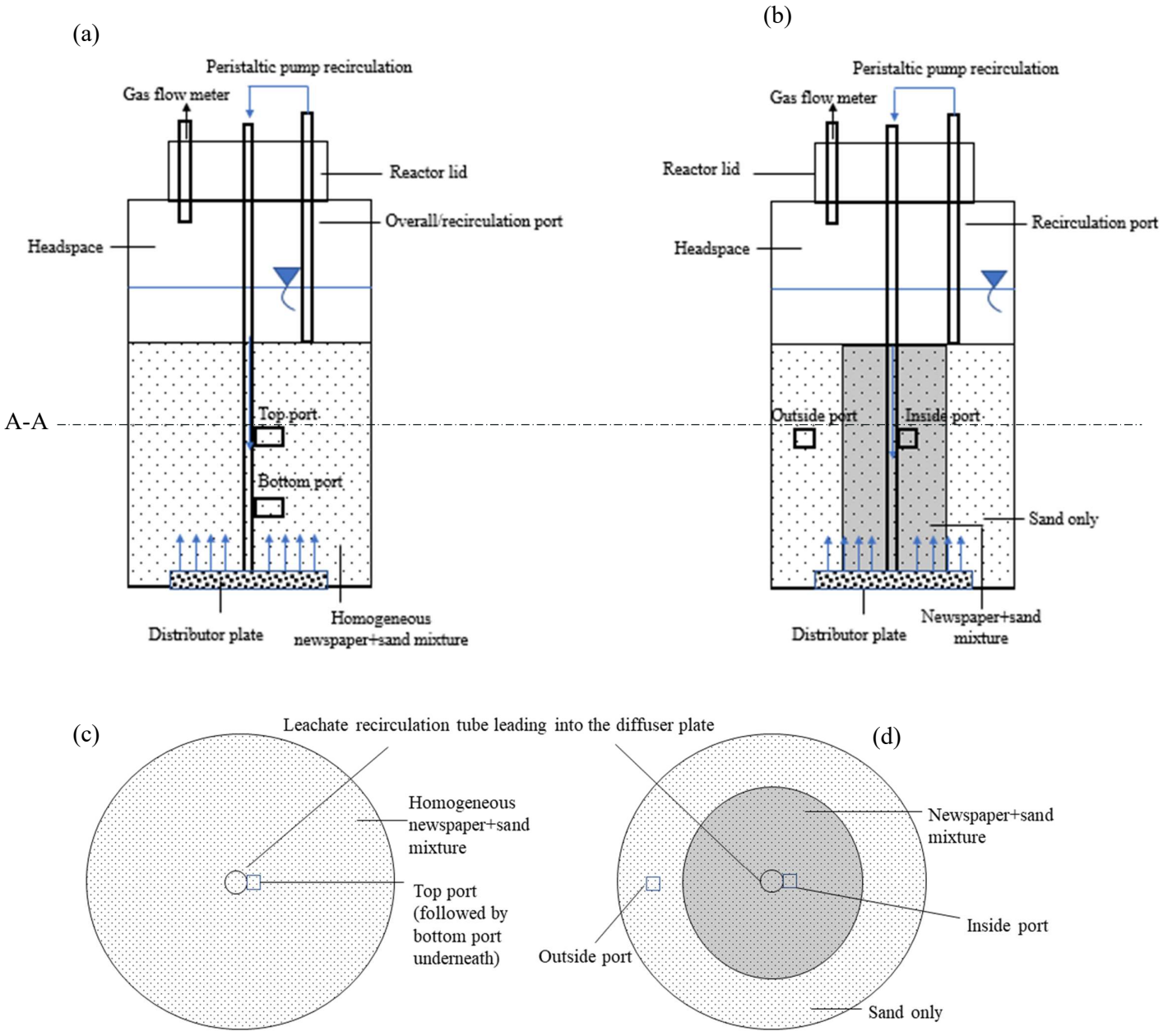

Figure 3-1-Laboratory-scale bioreactor landfill configurations. (a) Homogeneous configuration. (b) Heterogeneous configuration (c) Plan cross-section (A-A) homogeneous configuration. (d) Plan cross-section A-A heterogeneous configuration Note: Blue arrows denote leachate flow direction.

The pH, total/soluble chemical oxygen demand (TCOD/sCOD), volatile/total solids (VS/TS were measured according to standard methods (analyses for variables beginning with the letter 's' done on 0.2 µm filtered liquid samples) (APHA, 2012). The liquid and solid

total/organic carbon (OC/TC) were measured via a Shimadzu TOC-VCPH following the manufacturer's instructions.

Biogas volume measurements were based on the water displacement method (Wickham et al., 2016). The methane concentration of the biogas was determined via gas chromatography (GC) analysis on a Varian 450 GC equipped with a flame ionisation detector and a methaniser using a CP SiL5 CB column (50m, 0.33mm diameter, He carrier gas).

3.4 Statistical Analyses

Two-way ANOVA and Student's T-test were the statistical analyses carried out on the data in Microsoft Excel (at a significance level of 0.05). Statistical significance was attributed to $P<0.05$ (i.e. ANOVA F-value >F-critical). Correlations between data (specifically for the sCOD sOC and pH values) were identified via Pearson correlation initially and plotted against each other to further study the correlations.

3.5 Bacterial Culture

The *Agrobacterium sp.* cultures were maintained on Luria-Bertani (LB) agar. The bacterium was cultured for biodelignification tests in LB broth and the cultures were harvested (centrifugation at 3394 rcf for 10 min) in the exponential phase according to its growth curve. The cultures were then washed with M9 to remove any carbon from the LB broth that could go into the bioreactors, followed by centrifugation again and resuspended in M9 for addition to the bioreactors.

3.6 Hydraulic Conductivity

It was important to understand the hydraulic behaviour of the model waste material prior to the homogeneous and heterogeneous experiments. Hence why, hydraulic conductivity was measured via a constant head permeameter in hand-compacted samples of newspaper and sand mixtures. The test was done in accordance with BS ISO 1713:2004 (BSI, 2004).

4 Application of Enzymatic & Bacterial Biodelignification Systems for Enhanced Breakdown of Model Lignin Wastes[4]

4.1 INTRODUCTION

This chapter explores the extent to which enzymatic and bacterial biodelignification systems can breakdown lignocellulose in model wastes to potentially enhance biogas generation. The objective of this chapter is to test the hypothesis that certain enzymes and bacteria can break down lignocellulose in complex and realistic waste materials, found to be largely undegraded in landfills (Ximenes et al., 2015; Ximenes et al., 2017; Ximenes et al., 2008), and so enhance rates of biogas production (RQ1). Conversion of breakdown products to biogas through methanogenic activity (provided through addition of methanogen-rich sewage sludge) is recorded as volume of gas produced, alongside key parameters of the system (chemical oxygen demand, organic carbon, pH etc.). As part of the bacterial biodelignification experiments, the hypothesis that the solid:liquid ratio might also play an important role in the ability of the *Agrobacterium sp*. strain to enhance lignocellulosic breakdown and subsequent methanogenesis is also tested. As aforementioned in Chapter 3 the representative lignocellulosic wastes commonly found largely undegraded in old landfills comprised of newspaper and softwood. In the first instance, preliminary small-scale bacterial biodelignification tests were done on wood. This was followed by lab-scale bioreactor tests utilising the *Agrobacterium sp.* and lignin peroxidase enzyme. The results are then discussed and also placed within the context of the state of the art.

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[4]Chapter published in part as **Muaaz-Us-Salam, S**., Cleall, P.J., Harbottle, M.J.; (2020). "Application of Enzymatic & Bacterial Biodelignification Systems for Enhanced Breakdown of Model Lignin Wastes." Science of the Total Environment, Vol. 728, 138741.

4.2 RESULTS & DISCUSSION

4.2.1 Waste Characterisation

The composition of the model wastes is shown in Table 4-1 . Volatile solids content of a substrate indicates the fraction that has the potential to be converted to biogas (Barlaz et al., 1989; Eleazer et al., 1997; Wang et al., 1994). The wood was almost entirely composed of volatile matter, whereas newspaper had some ash content. This is comparable to recent work studying the chemical composition of different wastes (Chickering et al., 2018; Krause et al., 2017; Krause et al., 2016). As the aforementioned studies also suggested, ash content likely comes from fillers and ink constituents within the newspaper. The wood was very rich in lignin and also has a higher organic carbon content compared to the newspaper. Bearing in mind the variability in waste composition that arises around the world, in relation to recent contributions, these values fall within the typical ranges reported (De la Cruz et al., 2014; Wang & Barlaz, 2016).

Table 4-1 Waste Characterisation.

Sample	VS (%)	OC (%)	Lignin (%)
Wood	99.62 ± 0.00	50.6 ± 0.40	38.45 ± 0.13
Newspaper	84.26 ± 0.00	36.72 ± 8.09	25.73 ± 0.89

Note: Data presented as percentage of dry mass

4.2.2 Bacterial Enhancement

4.2.2.1 Small-scale:

Total carbon release profiles from the preliminary small-scale experiments conducted on the non-autoclaved and autoclaved ≤0.15 mm and ≤2mm wood are shown in Figure 4-1a,b. In the presence of the *Agrobacterium sp*., a steady and significant increase in the carbon present in the liquid phase occurred for the non-autoclaved ≤0.15 mm samples (black circle, Figure 4-1a)) (~20% relative to the control, $P<0.05$, ANOVA F-values>F-critical) during the 7-day experiment. Since the only source of carbon in these experiments was lignocellulose from wood, and the soluble carbon content was determined post-filtration through 0.2 μm pore-size filters (even bacterial cells should not be passing through into the supernatant), it is highly

likely that the release of carbon corresponds to biodegradation due to the bacterium. The profiles for the non-autoclaved <0.15 mm wood controls (grey circle, Figure 4-1a) remain steady and virtually unchanged, suggestive of no activity in the absence of the bacterium. No significant impact on the sTC release profiles due to the *Agrobacterium sp.* on 2 mm particles (Figure 4-1b, autoclaved and non-autoclaved) was recorded ($P>0.05$, ANOVA F-values<F-critical). The sTC profile in the presence of the *Agrobacterium sp.* is consistently lower for 2 mm samples (non-autoclaved and autoclaved, dotted lines denote bacterium augmentation), which could be explained by the bacterium metabolising leached carbon from the wood.

It is noted that the <0.15 mm autoclaved wood containing the *Agrobacterium sp.* (Figure 4-1a,b) starts at a lower sTC on day 0 (black dotted line, Figure 4-1a), however, the increase in total sTC released in solution is ~30% higher in comparison to the increase found in the control, hence indicating lignocellulosic breakdown of the solid matrix due to bacterial enhancement.

To confirm the liquid phase carbon release results, the organic carbon analysis of the solid residue post-treatment with the *Agrobacterium sp.* for wood under different conditions is shown in Figure 4-1c. The organic carbon in ≤0.15 mm samples is significantly lower (two-way ANOVA, $P<0.05$, F-values>F-critical) in the treated samples for both autoclaved and non-autoclaved wood samples (by 8.6 and 7.5% respectively), indicating higher levels of breakdown of the lignocellulosic structure due to *Agrobacterium sp.* treatment. With 2 mm wood there is no significant difference due to bacterial enhancement. It is expected that the much higher surface area to volume ratio, and accessibility of the <0.15 mm samples is the key cause of the much better solid OC breakdown due to *Agrobacterium sp.* treatment in this set of samples in comparison to the 2mm size.

Overall, the preliminary small-scale data suggest that *Agrobacterium sp.* can break down woody lignocellulose, and that the particle size is an important factor in the rate of reaction when applying this *Agrobacterium sp.* strain for biodegradation. Treating wood by autoclaving does not significantly impact the biodelignification ability of this bacterium.

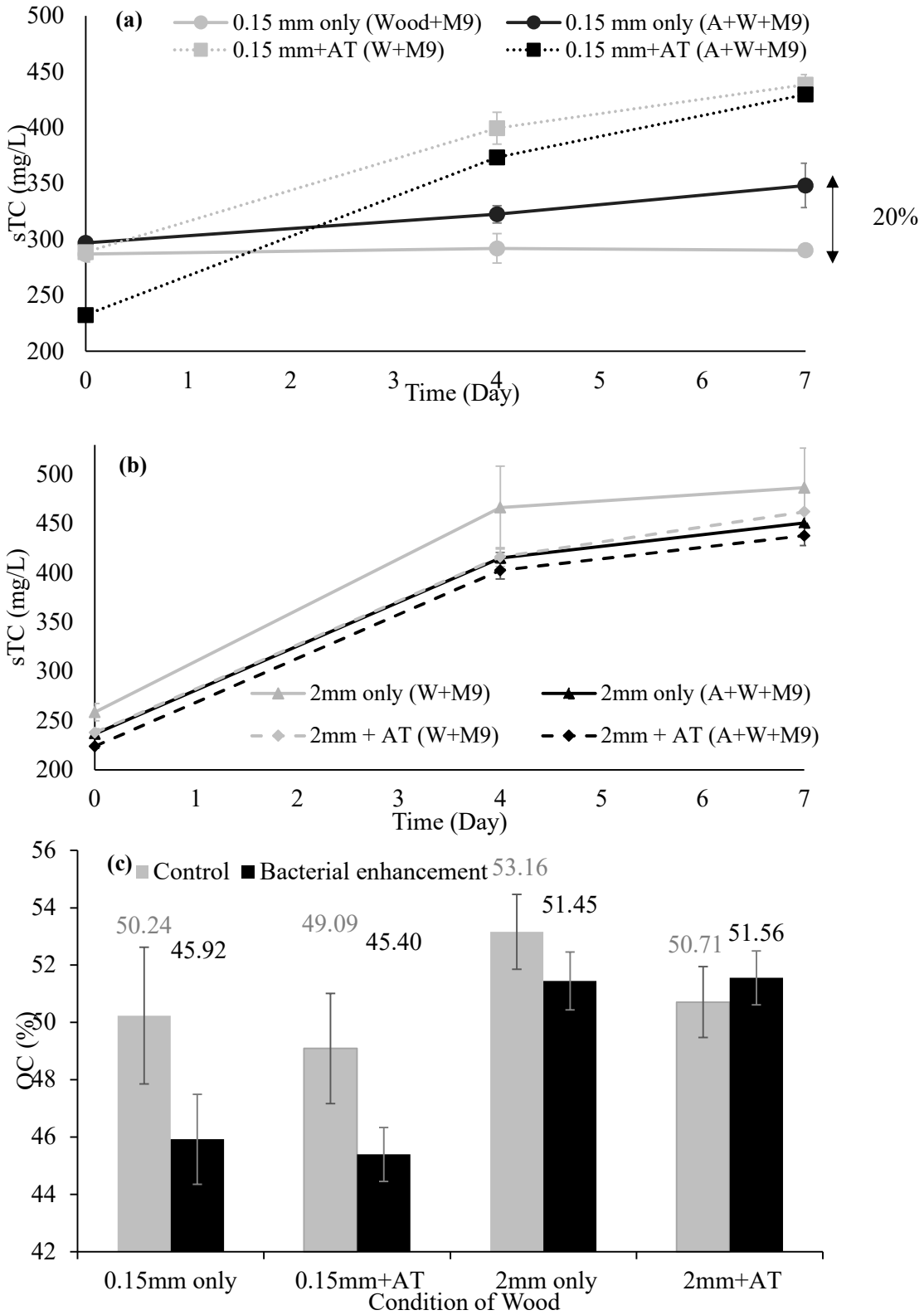


Figure 4-1 Assessing the impact of Agrobacterium sp. treatment on wood lignocellulose under different conditions. (a) Soluble liquid total carbon release results for the small-scale 0.15 mm tests. (b) Soluble liquid total carbon release results for the small-scale 2mm tests. (c) Solid phase organic carbon results for the small-scale experiments. All error bars represent +/- 1 standard deviation. Abbreviations: Agrobacterium, A; Autoclaved, AT; Wood, W; M9 solution, M9.

4.2.2.2 Bioreactor-scale (Experiment 1):

The cumulative biogas profiles released from newspaper in Experiment 1 are shown in Figure 4-2a. After a lag-phase of ~7-8 days, *Agrobacterium sp.* nearly doubled the biogas production in newspaper reactors compared to controls without Agrobacterium ~92% enhancement, $P<0.05$) whilst no response was observed without waste materials. At the same time, the sCOD and sOC profiles (Figure 4-2c,d) exhibit a consistently lower amount of organic carbon in solution with newspaper and *Agrobacterium sp.* than in newspaper controls. These data suggest that the *Agrobacterium sp.* enhance the release of carbon from the solid phase and increase the conversion of dissolved carbon to biogas. In controls with newspaper, the total amount of OC released into solution is ~0.32 g whilst the OC released in total as biogas is 0.04 g, giving a total of ~0.36 g. In specimens with *Agrobacterium sp.* and newspaper, ~0.31 g of OC was released into solution by the end of the experiment, but the amount of biogas was 0.08 g, giving a total of ~0.39 g. These data indicate greater release of solid organic carbon into solution and greater conversion of that soluble OC to biogas in the presence of *Agrobacterium sp*., leaving less in solution. With 1.4 g of solid OC present initially, in the presence of the Agrobacterium sp. there is an ~8.2% increase in the total release of OC in relation to the control. The observed effects most likely result from the *Agrobacterium sp.* breaking down either solid phase or leached organic materials into a form utilisable by the methanogens.

For the newspaper reactors, the sOC and sCOD profiles increase gradually with increasing biogas production and result in overall accumulation towards the end of the experiment of organic carbon in the liquid phase. It is hypothesised that the cause was accumulation of recalcitrant organics and other degradation products which are not converted to the gas phase and which hinder the activity of the microorganisms. Therefore, an attempt was made to 'restart' the experiments by replacing the liquid fraction with fresh autoclaved M9 on Day 35. Following this, however, little to no additional activity was observed (Figure 4-2). As such, the flat-lining of the biogas and sCOD/sOC curves is likely to be due to a different limiting factor.

No significant effect was observed on biogas production due to the addition of the *Agrobacterium sp.* strain to wood (Figure 4-2b). Similarly, no significant increase in sCOD or sOC was observed in this experiment. A possible explanation for this, and an important difference between the preliminary small-scale and bioreactor experiment 1 is the concentration of wood in the overall volume. For the small-scale wood biodegradation tests,

the S:L ratio was 0.05 g/ml, and a significant impact of bacterial treatment on the woody lignocellulose was observed, whereas here it was 0.006 g/ml (~8.3 times more dilute). It is possible that the wood was not sufficiently concentrated in the bioreactors to either observe a measurable response or to stimulate microbial activity. This is supported by work from other researchers where the S:L ratio has had an impact on biogas production in bioreactors (Costa et al., 2016; Raposo et al., 2006; Sawatdeenarunat et al., 2015).

4.2.2.3 Impact of increasing solid:liquid (S:L) ratio (Experiment 2):

The lack of a response from wood exposed to *Agrobacterium sp.* in the first bioreactor experiment was hypothesised to be due to a combination of a slow biodegradation rate and a low S:L ratio. Increasing the S:L ratio from 0.006 g/ml to 0.017 g/ml had a significant impact on the cumulative biogas volume for both lignocellulosic wastes (Figure 4-3a, Figure 4-4a).

In the case of wood, the biogas for the higher S:L ratio was increased by ~205%. Changing the S:L ratio has been shown to have a significant impact on the biogas kinetics and cumulative volume of the organic fraction of MSW (Krause et al., 2017; Krause et al., 2016; Raposo et al., 2006). Although, to the best of the author's knowledge, this has not been investigated in the application of bacterial biodelignification systems on wood lignocellulose. The wood sCOD profile (Figure 4-3c) increases and reaches a peak around day 14 then decreases gradually. The sOC profile (Figure 4-3b) does not seem to strictly follow this exact trend of reaching a peak followed by gradual decrease. sCOD is indicative of the amount of oxygen required to oxidise the carbon present, and the more oxygen required, the more carbon is present in solution or roughly the same amount is present, but in a more complex structure which is more difficult to oxidise. The general trend in sCOD is an increase then a gradual decrease. The sOC increases and then stays reasonably steady with a higher value measured in the wood cases. A possible explanation of these observations might have to do with the form of the carbon released. The fact that sCOD lowers but the sOC does not perhaps indicates that the form of the soluble compounds might have gone from more complex structures, to more simple structures that may be relatively easier to convert to biogas, i.e. end-product of anaerobic digestion.

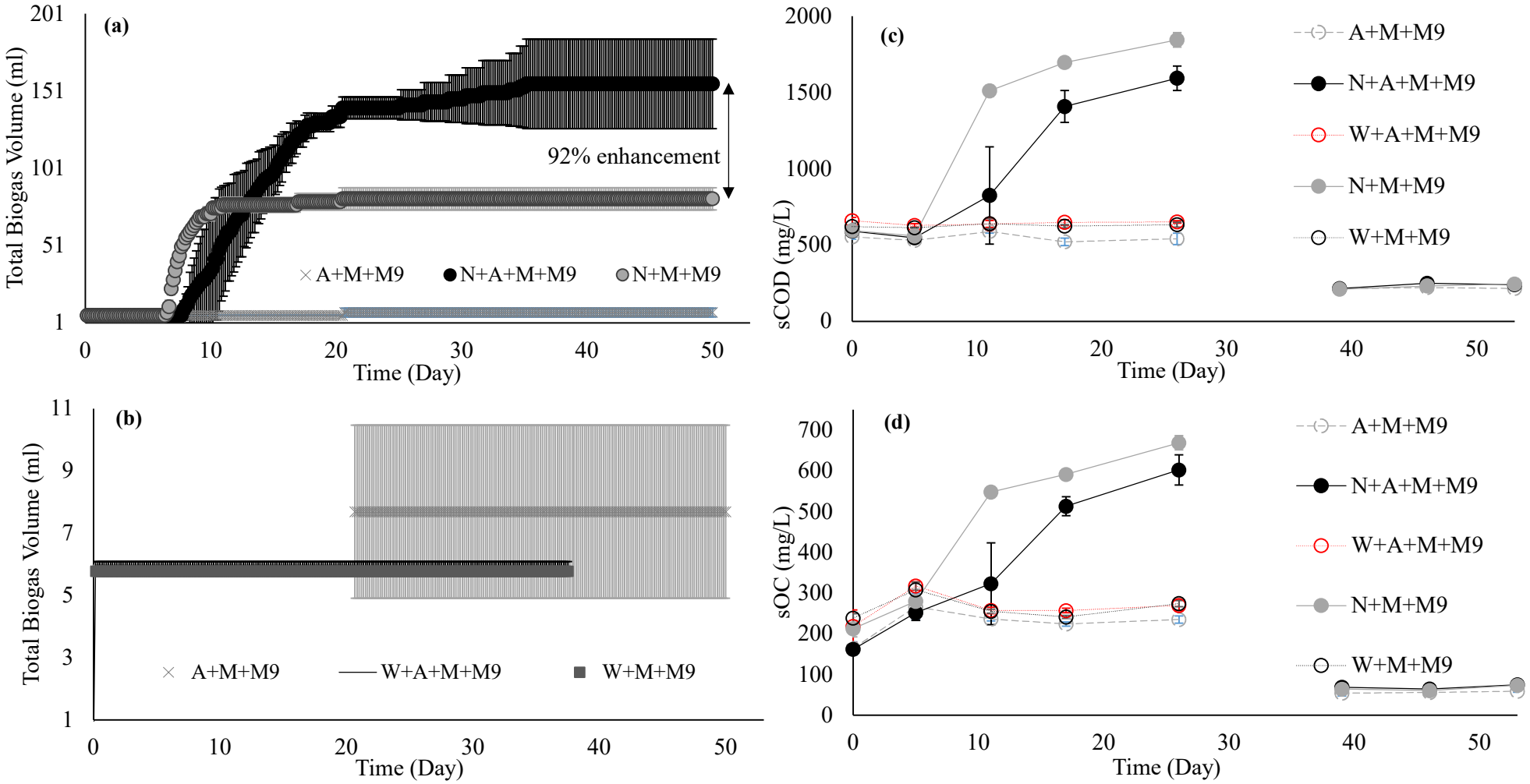


Figure 4-2 Assessing the impact of Agrobacterium sp. treatment on newspaper and wood lignocellulose in the presence of sludge. (a) Cumulative biogas volume results for newspaper. (b) Cumulative biogas volume results for wood. (c) Soluble chemical oxygen demand profiles. (d) Soluble organic carbon profiles. Note: system 'restarted' on Day 35, last liquid sampling point was at day 26 before the 'restart'. All error bars represent +/- 1 standard deviation. Abbreviations: Agrobacterium, A; Methanogens, M; Newspaper, N; Wood, W; M9 solution, M9.

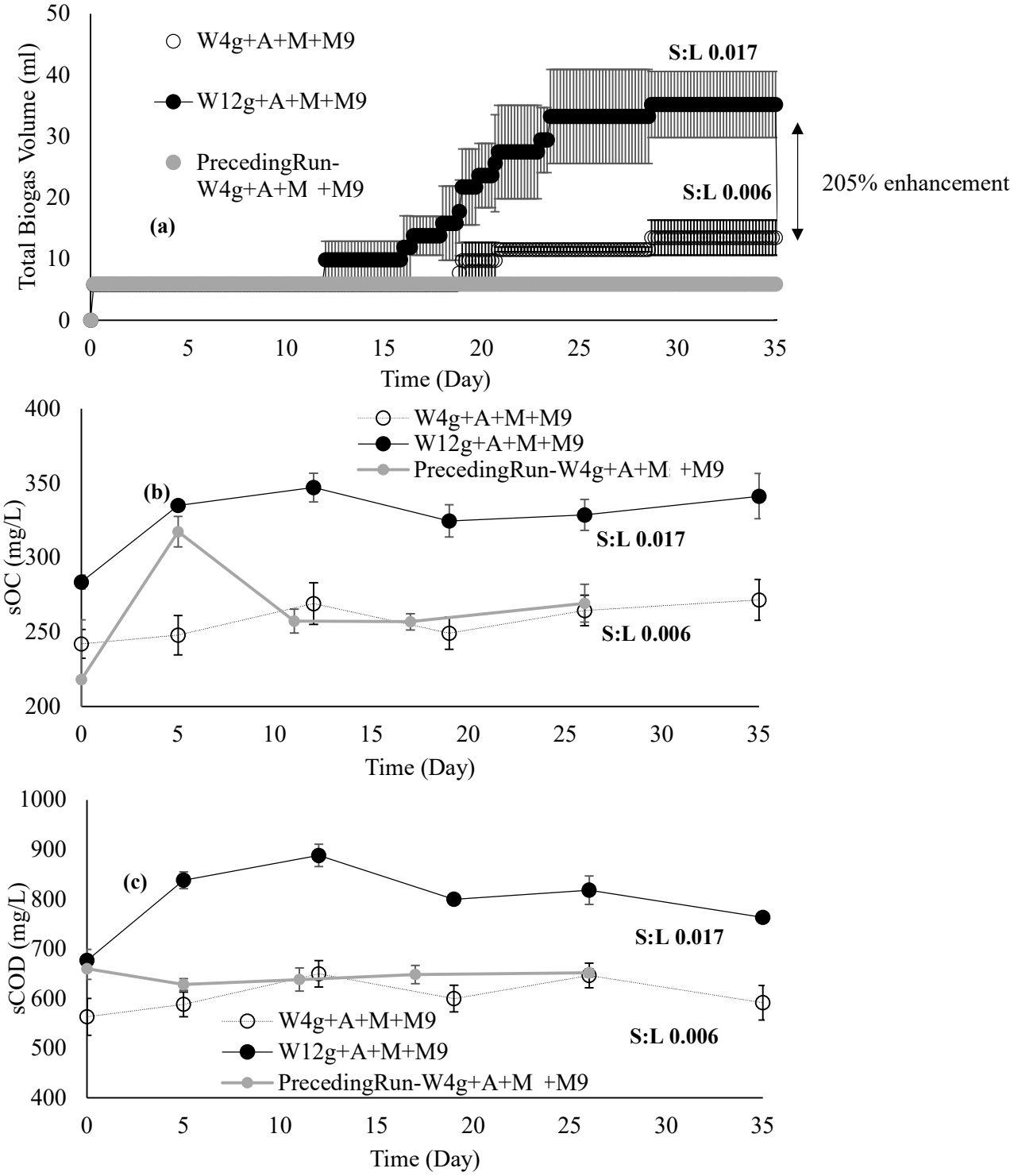


Figure 4-3 Assessing the impact of increasing S:L ratio on Agrobacterium sp. treatment of wood lignocellulose with sludge. (a) Cumulative biogas volume for wood. (b) Soluble organic carbon profiles for wood. (c) Soluble chemical oxygen demand profiles for wood. All error bars represent +/- 1 standard deviation. Abbreviations: Agrobacterium, A; Methanogens, M; Newspaper, N; Wood, W; M9 solution, M9; 4 grams mass of waste, 4g; 12 grams mass of waste, 12g.

It is important to note that at the very high S:L of 0.05 g/ml in the small-scale tests, a very flat line for the total carbon profile for the ≤0.15 mm wood controls was recorded. Whereas here, the sCOD profile due to the addition of the bacterium is transient and follows a logical pattern of lignocellulosic breakdown (i.e. ~31% increase in the first two weeks) and methanogenesis to convert that carbon to the gas phase (i.e. evidence from biogas production profile, and decrease of sCOD by ~16% relative to the maximum peak-value reached in the first two weeks). As such, it is suggested that the liquid analyses results discussed here are most likely due to the *Agrobacterium sp.* acting in synergy with the microbes from the sludge for depolymerisation of wood.

In Figure 4-4b,c the newspaper sOC and sCOD profiles are shown respectively. In the case of newspaper, the biogas volume was 280% greater than that at the lower S:L ratio. The cumulative overall volume for newspaper at the lower S:L ratio was significantly different ($P < 0.05$; approximately double) to that obtained previously with the same conditions (Figure 4-4a, Figure 4-1b,c). This may have arisen due to variability in the newspaper composition (a different sub-sample was milled for this test) or variability in the microbial community from the sludge, since this was sampled 4 months after the previous test, albeit from the same site/digester/sampling point. However, it is clear that with all else equal the higher S:L ratio gives a significantly greater degree of biodegradation. For newspaper, the sOC and sCOD profiles follow a similar trend to the previously reported (Figure 4-2).

To study the impact of S:L ratio on the activity of the *Agrobacterium sp.* and overall biogas generation as a continuum, this ratio could be further increased or decreased under similar experimental conditions. This would perhaps allow for optimisation of the microbial processes studied. However, since the aim of this RQ has been to gain mechanistic insights, optimisation has been beyond the scope of this study.

From the above bioreactor experiments, it is obvious that under all conditions, newspaper clearly produces much higher biogas yields than wood and shows a greater degree of enhancement. This is likely due to a combination of two main factors. Firstly, due to the much higher lignin content of softwood (~1.5 times more than newspaper), which results in more recalcitrant biomass in comparison to wood. Secondly, newspaper, is a mechanical pulp which in relation to softwood is more processed, this likely also results in better accessibility to key polymers for degradation (Baldwin et al., 1998; Barlaz, 2006; Eleazer et al., 1997; Stinson & Ham, 1995; Wang et al., 1994).

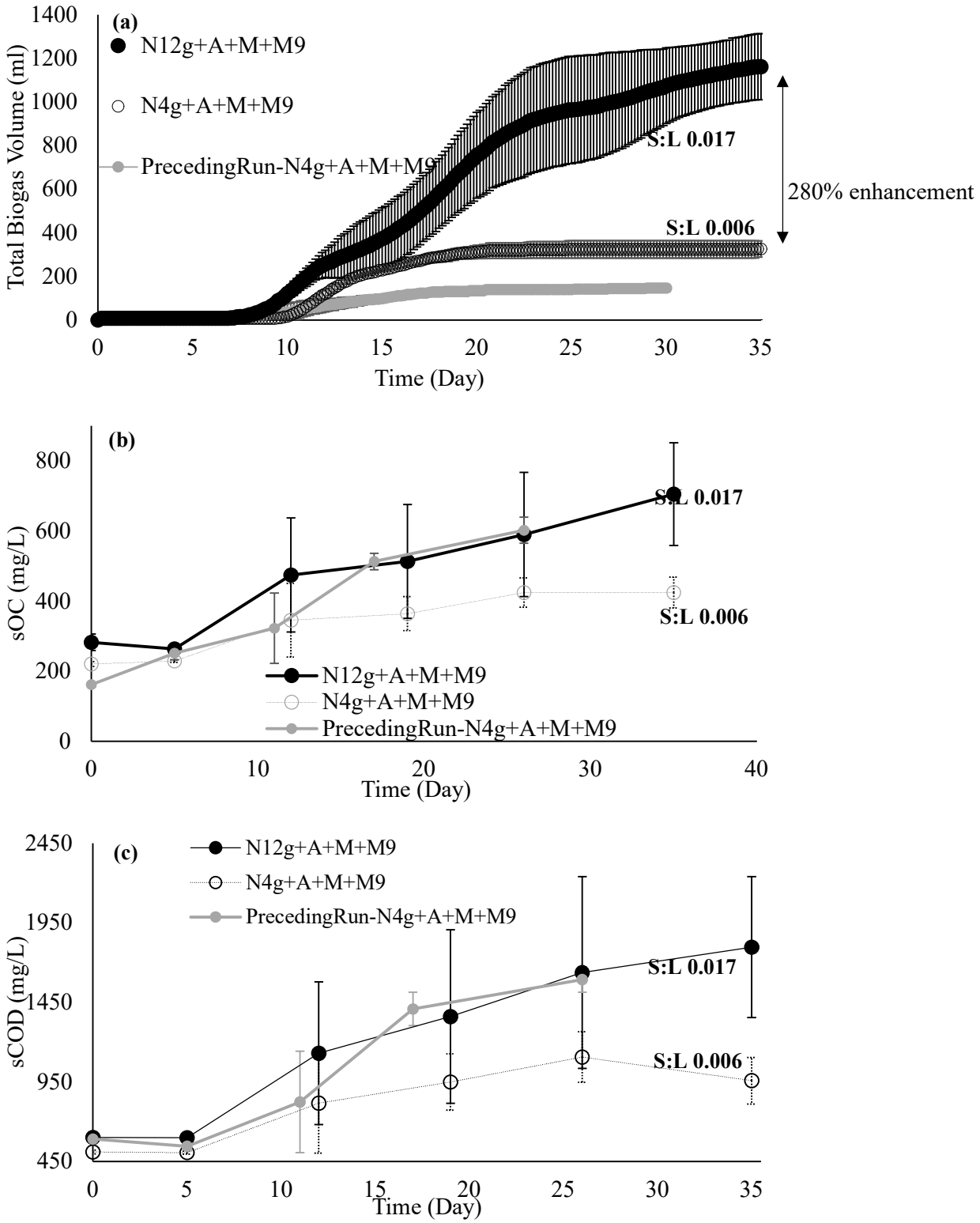


Figure 4-4 Assessing the impact of increasing S:L ratio on Agrobacterium sp. treatment of newspaper lignocellulose with sludge. (a) Cumulative biogas volume for newspaper. (b) Soluble organic carbon profiles for wood. (c) Soluble chemical oxygen demand profiles for newspaper. All error bars represent +/- 1 standard deviation. Abbreviations: Agrobacterium, A; Methanogens, M; Newspaper, N; Wood, W; M9 solution, M9; 4 grams mass of waste, 4g; 12 grams mass of waste, 12g.

4.2.3 Enzymatic Enhancement

In Figure 4-5 and Figure 4-6 biogas profiles/values and net biomethane yields per gram of VS due to the action of LiP are shown. A small positive effect on biogas/biomethane yields due to the enzyme for just the bioreactors containing sludge and no waste took place ($P<0.05$) (Figure 4-5 and Figure 4-6). This is likely a result of the enzyme breaking down suspended and/or dissolved recalcitrant organics present in the sludge which would otherwise not be broken down by the microbial communities present, which can then be utilised for metabolism and eventually be converted to biogas.

A significant ($P<0.05$) enhancement in biomethane potential from newspaper of ~41% was achieved with LiP present. LiP is thought to form reactive free radicals that attack the non-phenolic parts of the lignocellulosic structure (Bugg et al., 2011a; Bugg et al., 2011b; Cragg et al., 2015). The enhancement is possibly a result of the enzyme attacking the lignocellulosic structure of newspaper, thereby providing additional substrate to the microorganisms from the sludge which can then be converted to biogas.

In the enzyme-containing bioreactor cases discussed above, in addition to the overall biogas volume being enhanced, the proportion of methane also seems to be higher in the presence of the enzyme. This observation suggests that enzymatic enhancement in these reactors not only increased the overall biogas yield, but also the specific methane production, thereby increasing its concentration in the biogas mixture.

In bioreactors containing wood, there was a significantly lower amount of biomethane production ($P<0.05$, Figure 4-5 and Figure 4-6) in the enzyme-augmented reactors compared to the control. This effect was also found by Schroyen et al. (Schroyen et al., 2017; Schroyen et al., 2014; Schroyen et al., 2015), where not all lignocellulosic substances responded positively to pre-treatment through peroxidase enzymes prior to anaerobic digestion, in some instances the treatment negatively impacted methane production (e.g. with corn stover, wheat straw, maize). No significant changes in phenolics (which at high concentrations can inhibit methanogenesis (Hernandez & Edyvean, 2004)), TCOD or any transient changes in pH were observed within the first two weeks in comparison to the controls (data not shown).

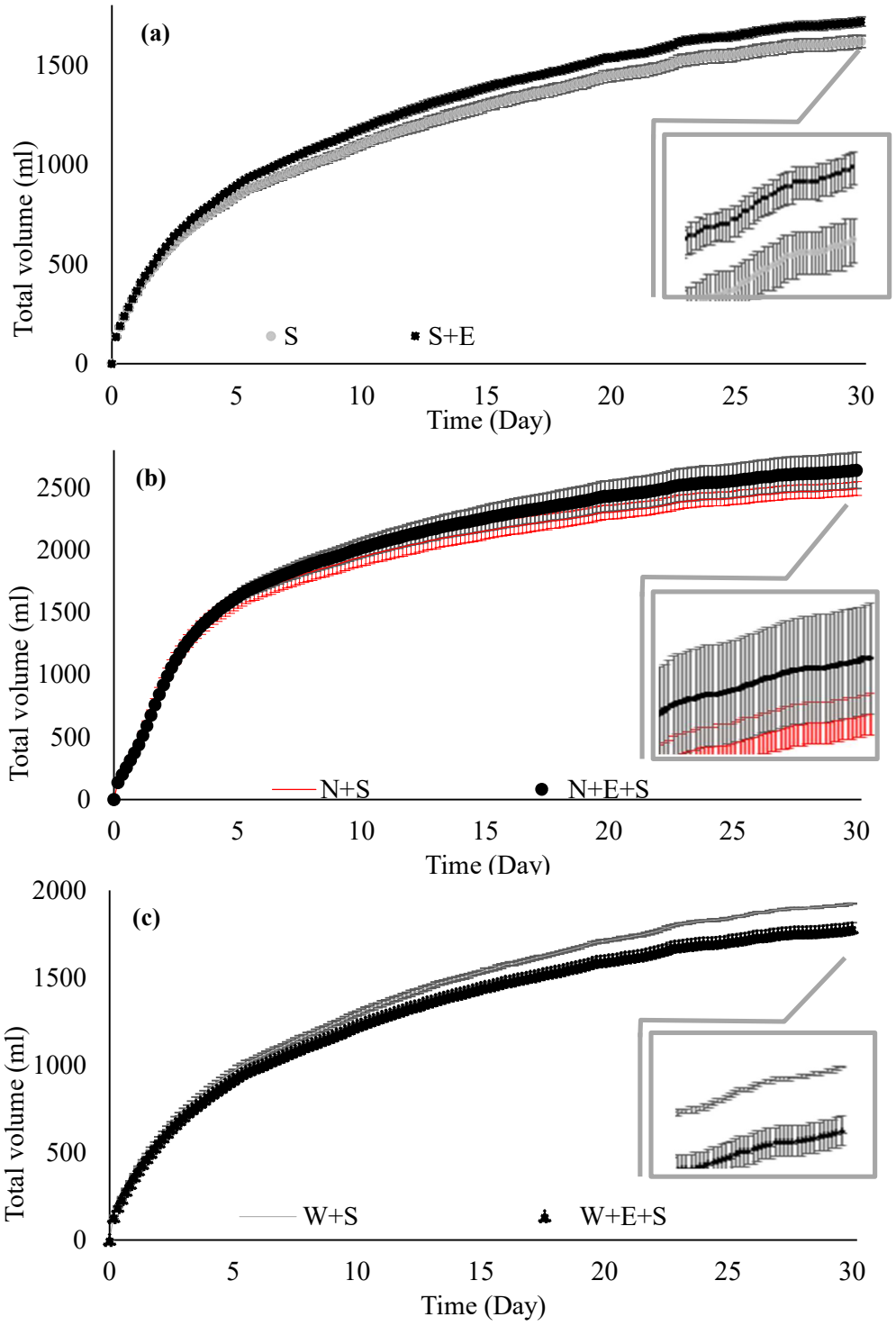


Figure 4-5 Cumulative biogas generation profile results. Assessing the impact of lignin peroxidase application on newspaper and wood. (a) Sludge only. (b) Newspaper. (c) wood. All error bars represent +/- 1 standard deviation. Abbreviations: Sludge, S; lignin peroxidase enzyme, E; Newspaper, N; Wood, W. Inset figures included for closer examination of the gas generation profiles towards the end of the test.

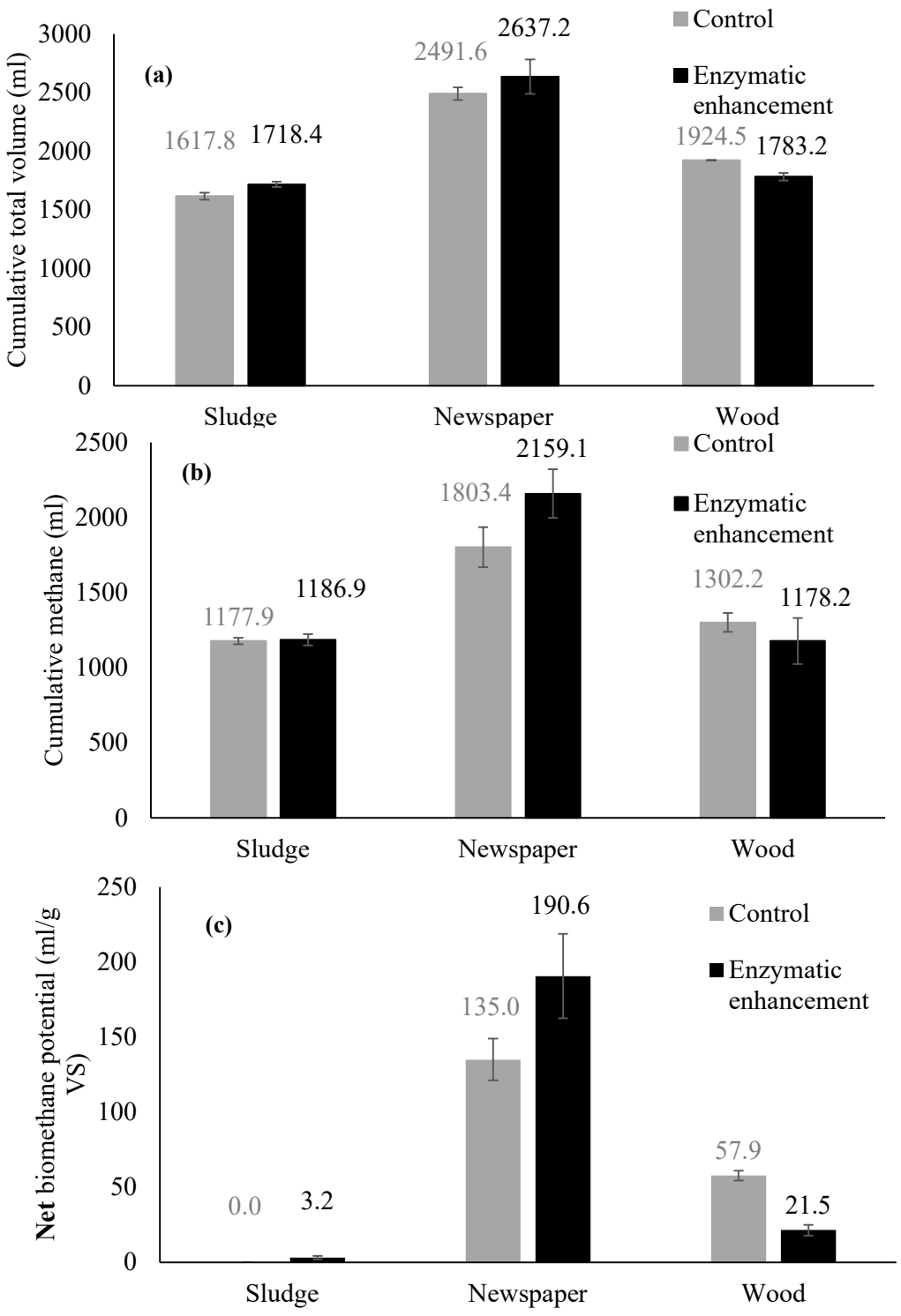


Figure 4-6 Biogas and methane yield results. Assessing the impact of lignin peroxidase application on newspaper and wood. (a) Cumulative biogas volume comparison for bioreactors. (b) Cumulative methane generated in bioreactors over the 30-day experimental run. (c) Net methane yield per gram of VS. All error bars represent +/- 1 standard deviation.

At low concentrations of LiP and similar fungal peroxidases in relation to the amount of lignin, the peroxidase enzyme has been said to catalyse repolymerisation of lignin alongside depolymerisation (Cragg et al., 2015; Rahmanpour et al., 2017; Rahmanpour et al., 2016; Rashid et al., 2017). Repolymerisation of lignin means that lignin would precipitate back onto the surface and recent work has shown that this has a negative impact on the accessibility of the lignocellulosic structure to biodegradation (Li et al., 2007; MacAskill et al., 2018; Oliva-Taravilla et al., 2016; Wiman et al., 2012). Due to the very high lignin content of softwood (nearly double that of newspaper), it is possible that the concentration of LiP was low for these reactors, and that depolymerisation and simultaneous repolymerisation led to the lower methane yield in the enzyme-amended reactors. The lignin content of the wood residue is slightly higher (38.17±0.42) in the enzyme reactors in relation to the controls (37.22±0.02), one possible reason for which could be re-polymerisation, although this difference is not statistically significant (two-way ANOVA $P>0.05$, F-values<F-critical). Although not confirmed in this study, but has been found in the literature, it is possible that lignin droplets (post-depolymerisation) coalesced onto the surface of the wood (re-polymerisation) and resulted in slightly higher lignin contents in relation to the controls and lesser access for the microbes to the cellulose fibres resulting in lower methane yields as shown in Figure 4-5 and Figure 4-6 (Rahmanpour et al., 2017).

4.2.4 General Discussion of Bacterial and Enzyme Experiments

Agrobacterium sp., isolated from MSW soil, was tested (Rashid et al., 2017). for its ability to break down softwood bark chips in compost under aerobic conditions, showing an approximately 2.3-3.5-fold enhancement in biogas production The ~2-fold enhancement reported here is comparable to this, despite the methanogenic microbial communities from organic compost used by (Rashid et al., 2017) and the anaerobic sewage sludge (this study) being very different. In addition, compost certainly contains aerobic strains which could result in much better initial hydrolysis of the lignocellulose making it easier for the various strains tested to then attack the structure. Much of the work carried out on bacterial breakdown of lignocellulosic wastes in the literature (Chandra et al., 2007; Mathews et al., 2016; Mathews et al., 2015; Mathews et al., 2014; Mnich et al., 2017; Tsapekos et al., 2017) has not specifically paired this process with simultaneous methanogenesis as has been done in the current present study and that of (Rashid et al., 2017) as such this study has helped to shed more light into this gap in understanding.

Schroyen et al. applied peroxidase enzymes to lignocellulosic materials (Schroyen et al., 2017; Schroyen et al., 2014; Schroyen et al., 2015). Interestingly, the case where they were able to obtain maximum enhancement of biogas from corn stover (4.5% lignin) was with a laccase treatment, not peroxidase or their mixture, and it resulted in a 17% increase in methane yield. In comparison in this study a ~41% enhancement was obtained in biomethane potential (25.7% lignin in this newspaper, Table 4-1). In Schroyen et al.'s mixture of laccase and peroxidase pre-treatments, they studied 7 substrates and obtained biogas enhancement only in 4 (hemp: 4.8%, miscanthus: 9.5%, flax: 14%, willow: 40%), whilst the pre-treatment had a negative impact on the biogas production of the rest of the substrates (in ensilaged maize, the pre-treatment instead of enhancing biogas production, nearly halved it). Their work suggested that enzymatic biodelignification is a treatment that depends highly on the nature of the substrate, it may actually have a negative impact in some lignocellulosic wastes, the reasons for which are not clear as of yet. Interestingly, the substrates studied in their study and ours suggest that it is not entirely clear as to why the peroxidase enzyme treatment works with some wastes, and not with others. Looking at the lignin contents, there does not seem to be a relationship between high lignin contents and proportionally negative impact on peroxidase enzyme action. However, lignin is predominantly made up of guaiacyl and syringyl monomers, where the former is less cross-linked than the latter and in comparison, is an easier lignin-monomer to breakdown. Syringyl-rich lignin has also been shown to negatively impact the growth of some lignin-degrading fungi (Hooker et al., 2018). It might be the case that the monomeric composition of lignin plays an important role in the success of peroxidase treatment, whereby wastes with high syringyl monomeric units (e.g. softwood) may be more difficult to attack than say wastes made up predominantly of guaiacyl units (e.g. hardwood).

Jayasinghe et al. applied LiP and similar fungal peroxidases to partly degraded 30-year old excavated MSW (Hettiaratchi et al., 2014; Hettiaratchi et al., 2015; Jayasinghe et al., 2011; Jayasinghe et al., 2014; Jayasinghe et al., 2013). They also observed a positive impact of LiP on biomethane generation. However the increase in biogas yields recorded by them was nearly an order of magnitude larger than the control compared to the 41% increase in biomethane potential in the case of newspaper in this study. This may be due to the age of their waste, since for very old landfilled waste, significant degradation of the biomass likely occurred making the structure of the waste more accessible to enzymatic attack. On the other hand, this study used virgin materials and since newspaper is made from a mechanical pulp, much of the lignocellulosic structure is still intact (Eleazer et al., 1997; Wang et al., 2015; Wang et al.,

1994). From the experimental work carried out in this chapter, it has been obvious that both the lignocellulosic wastes, wood and newspaper, responded to treatment by the *Agrobacterium sp.*, whilst only newspaper responded positively to the peroxidase enzyme treatment. Possible reasons for these results and biogas yields obtained have been discussed. It has been highlighted that one of the major factors resulting in the different response exhibited by wood and newspaper has been due to the lignocellulosic structure and particularly, lignin content of the individual wastes. Finally, in comparison with enzymatic biodelignification, delignifying bacteria are present in landfills (the strain used here was isolated from landfill soil) and so there may be the potential to enhance their activity to encourage enhanced biogas recovery.

4.3 CONCLUSIONS

Agrobacterium sp. enhances the biodegradation of lignin-containing wastes, specifically newspaper and softwood, under idealised small-scale conditions, containing numerous other microbial communities. The solid:liquid ratio is a potentially important variable for the application of *Agrobacterium sp.* and should be considered for pilot-/field-scale trials (e.g. by adjusting leachate table). Lignin peroxidase enhances the biodegradation of newspaper (not wood), in conjunction with methanogenic bacteria. These results suggest that enhanced breakdown of real wastes in MSW landfills and processes such as anaerobic digestion is feasible using either technique but that the waste form is an important factor in the rate and extent of breakdown. This chapter has directly provided answers to RQ1 (i.e. To what extent can enzymatic & bacterial biodelignification systems breakdown lignocellulose in realistic lignin wastes, with the prospect of enhanced biogas recovery?) and helped in informing the overall aim of the thesis. Hence, developing on these liquid-phase experiments to inform RQ2 will be the purpose of the next chapter.

5 Impact of Flow & Heterogeneity on Bacterial Biodelignification Systems in Model Lignocellulose-containing Bioreactor Landfills [5]

5.1 INTRODUCTION

In this chapter the hypothesis that the impact of waste heterogeneity and its control on fluid flow is a major restriction on enhancing biogas generation via a lignocellulose-degrading bacterium is explored. This has been carried out in direct response to RQ2: What is the impact of flow & heterogeneity on bacterial biodelignification systems in model lignocellulose containing bioreactor landfills? As aforementioned in Chapter 3, homogeneous (uniform lignocellulose distribution) and heterogeneous (non-uniform) pore-structure configurations were prepared from newspaper and sand to mimic extremes of waste distribution in landfills. This allowed the study of the impact of these key variables (leachate flow and pore space heterogeneity), at the laboratory-scale in bench-scale bioreactor landfills, on the activity of a lignocellulose-degrading bacterium with the prospect of enhanced biogas generation from lignocellulose-containing landfill materials. As detailed experimental design and justification was provided in chapter 3, here the results obtained and the discussion of those results within the state of the art takes place.

[5] Chapter in preparation as **Muaaz-Us-Salam, S**., Cleall, P.J., Harbottle, M.J.; (). "Impact of Flow & Pore Structure on the Activity of *Agrobacterium sp.* In Lab-Scale Landfills." (In preparation).

5.2 RESULTS & DISCUSSION

5.2.1 Hydraulic Conductivity of Newspaper/Sand Mixtures

100% washed fine silica sand had a hydraulic conductivity of approximately 2.14 $\times 10^{-3}$ m/s, which falls within the range found in the literature for fine sands (Şen, 2015). As shown in Figure 5-1 the relationship between newspaper content shows two distinct regions. Between 0% and 4.2%, there is a steep exponential drop by 2 orders of magnitude, after that until 50% the decrease continues but at a much lower rate. The values obtained for newspaper/sand mixtures lie within the range of typical hydraulic conductivities for MSW reported in the literature (approximately 2×10^{-8}m/s - 2 x10^{-3}m/s) (Dixon et al., 2011; Dixon et al., 2008b; Powrie & Beaven, 1999; Reddy et al., 2009b). The mixtures prepared and used in this study (0, 1.98 and 4.58% newspaper in sand) therefore offer regions of relatively high (~2.14 $\times10^{-3}$m/s), intermediate (~1.55 $\times10^{-4}$m/s) and low (~2.46$\times10^{-5}$ m/s) hydraulic conductivity in experiments and allow construction of idealised models of heterogeneous flow conditions representative of real landfilled waste.

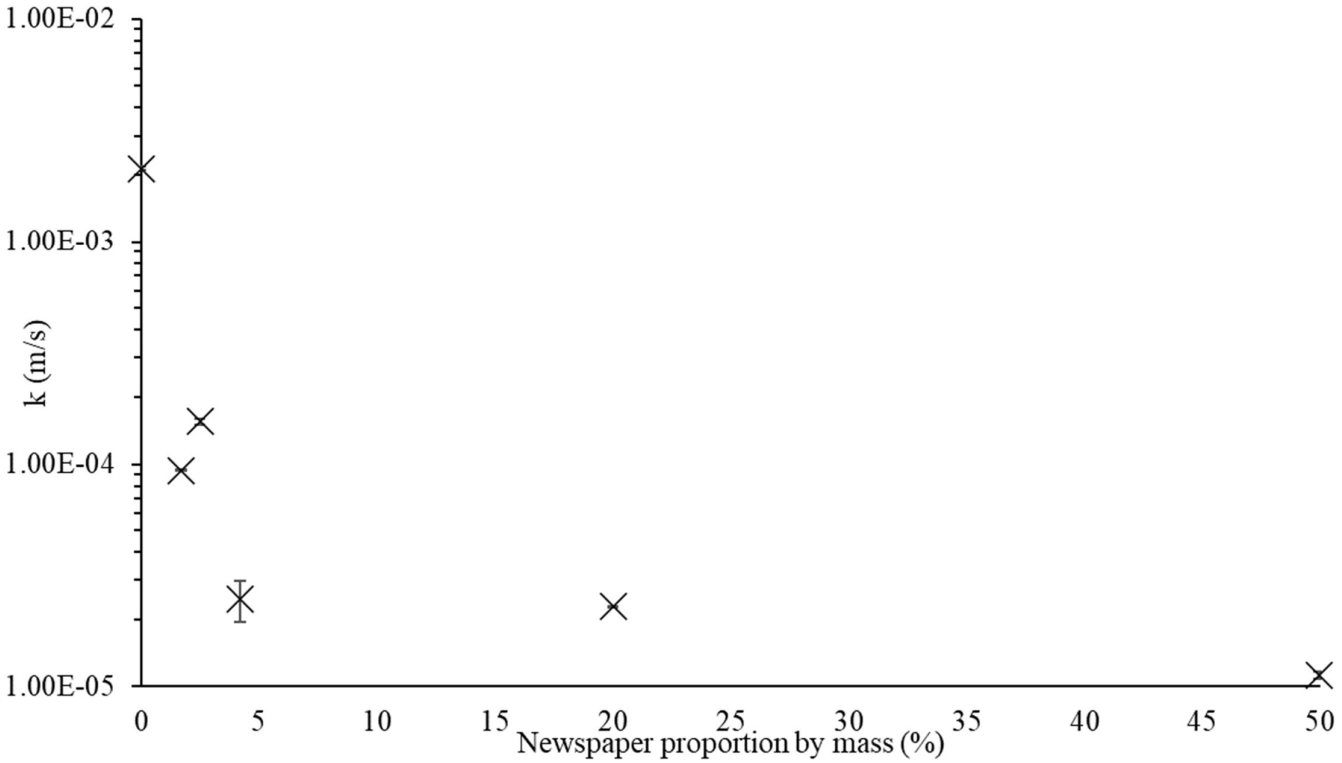


Figure 5-1- Hydraulic conductivity of newspaper and sand mixtures. All error bars represent +/-1 standard deviation.

5.2.2 Stirred Controls

Figure 5-2 shows the biogas, pH and sOC profiles for HoSC1 and HtSC2. An approximate lag phase of approximately 5 days existed in nearly all the reactors before measurable biogas production was observed. With the onset of biogas production, an increase in sOC and a proportional decrease in pH was recorded. The proportional decrease in pH was most likely due to the organic acids in the liquid phase from acetogenesis, hence the inverse proportionality between sOC and pH (Barlaz et al., 1989; Wang et al., 2018).

If the increase in the sOC due to acetogenesis is very rapid, the accompanying drop in pH is just as swift (e.g. HOSC2.2, Figure 5-2) and potentially acts as a shock for the methanogenic bacteria (ideal pH range: 6.8-7.4) within the sludge, and the total biogas volume remains constant, i.e. gas production stops (Cabrera et al., 2019). There may be potential for the recovery and adaptation of the bacteria and the biogas may start to be produced by the methanogens again (e.g. HOSC2.3, Figure 5-2), however, that may not always be the case (e.g. dark-grey line, Figure 5-2). The ideal scenario, i.e. for optimal biogas yields and efficiency (e.g. HOSC1.1, Figure 5-2) seems to be a very gradual and sustained release of sOC over a long period of time, this helps in keeping the pH very close to 7 (well within the ideal pH range for methanogens) and still provides a continuous stream of organic acids for conversion to biogas.

A single factor ANOVA suggests that the two sets of data for the two experimental runs for the HoSC1 and HtSC2 bioreactors do not significantly differ from each other ($P>0.05$, F-value<F-Critical). As such we can be confident that the sampling of the inocula at different times for the two experimental runs for the homogeneous and heterogeneous reactors did not significantly impact the results, and that interexperimental variability was insignificant.

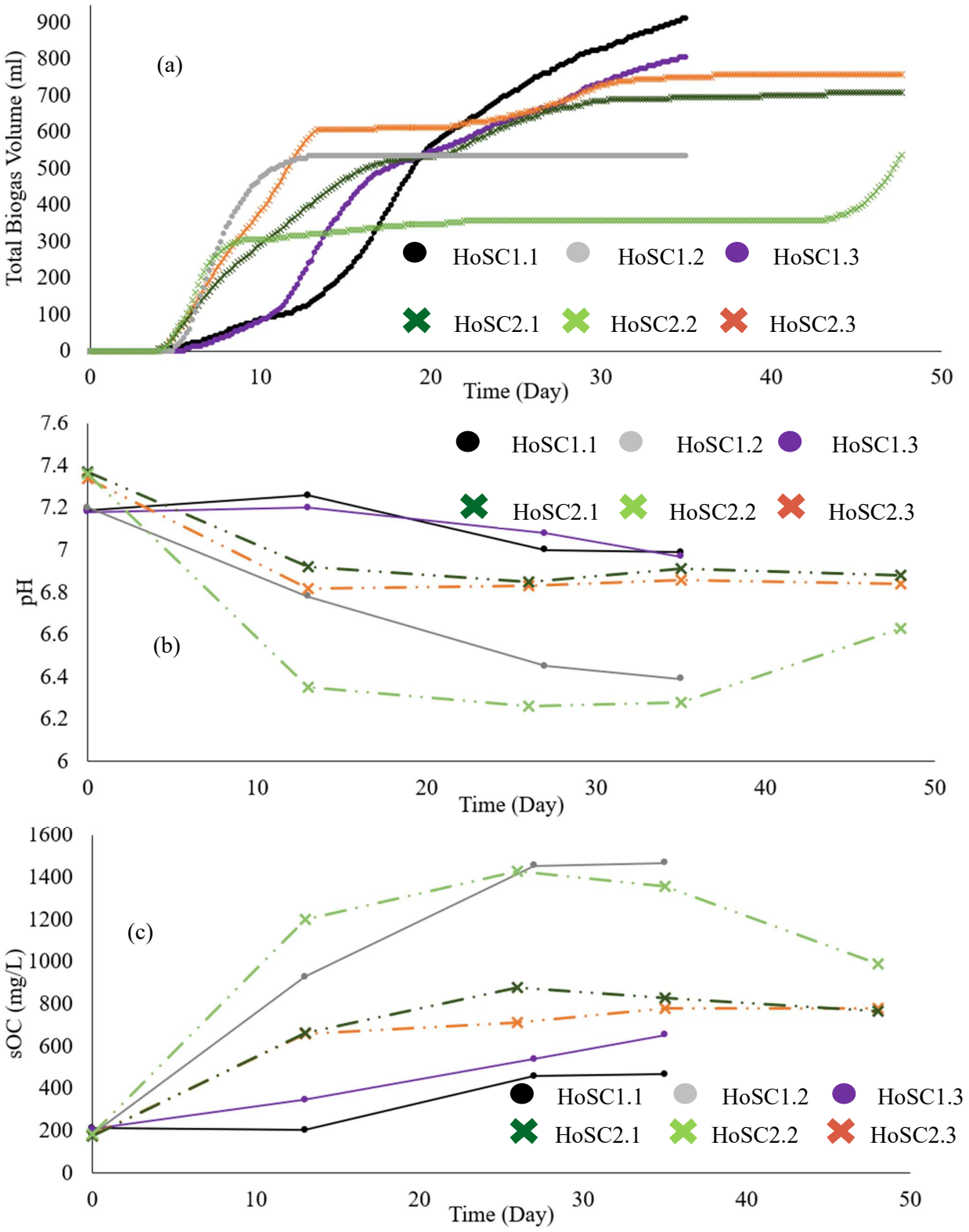


Figure 5-2- Stirred cross-experimental control data, biogas (a), pH (b) & sOC (c). Each colour represents one and the same reactor across all the graphs. Note: black, dark and light grey -HoSC1, Dark, medium-dark and light green – HtSC2.

5.2.3 Homogeneous Experiments

5.2.3.1 Flow:

The lag phase in all flow reactors was longer than the SC systems (approximately 8-12 days), presumably due to ideal mixing and contact in the SC systems as this has been identified to be an important factor in MSW degradation (Barlaz et al., 1990; Krause et al., 2016), whereas in the porous media setting dispersion and advection complicate and impact the transport of nutrients (Figure 5-3, Figure 5-4, Figure 5-5).

In the flow reactors, at the start of the test, the sOC values along the length of the reactor for HoFA and the HoFC were not significantly different (Figure 5-3, Figure 5-4, Figure 5-5). At approximately 14 days, the variability in the sOC along the reactor length was much lower in the flow cases in comparison to no-flow cases, suggesting better movement/distribution of sOC and miscellaneous degradation products due to advection and dispersion by flow. Moreover, from this point onwards, the sOC for HoFC remained comparatively more elevated than the HoFA. This was accompanied by more rapid and higher biogas production in the HoFA reactors, suggesting lesser accumulation of degradation products in solution, and much more efficient conversion to biogas, supported by the sOC values in the HoFA reactors (Figure 5-3, Figure 5-4, Figure 5-5). This is further supported by data obtained in the HoFC (accumulation of sOC with time in the BOTTOM port at very high values, decreasing the pH sharply and stunting biogas generation). This accumulation does not occur in the enhanced case (HoFA) due to the activity of the *Agrobacterium sp.* because it helps in achieving higher biogas production by increasing the efficiency of degradation and allowing faster conversion of the sOC into biogas. This keeps the pH in the optimal range for the methanogens (6.8-7.4, see previous section), which are the main organisms from sludge involved in producing biogas.

As such, by the end of the test, HoFA had produced approximately twice the average amount of biogas in relation to HoFC, suggestive of enhancement due to the addition of this bacterium to the reactors ($p<0.05$). This observation is comparable to our previous work in liquid phase only, where approximately 2-fold biogas enhancement was obtained in stirred-ideal conditions due to the presence of the *Agrobacterium sp.* (Muaaz-Us-Salam et al., 2020). Likewise, the sOC/pH trends were consistent with what was found in these tests as well, i.e. *Agrobacterium sp*. causing biogas enhancement, in synergy with lesser sOC/higher pH in solution due to more gas production when compared to identical controls lacking *Agrobacterium sp.*

It should be noted that the behaviour of SCs in comparison to the homogeneous experiments is similar mechanistically, but some key differences are present. For instance, accumulation of sOC/accompanying low pH in SCs (Figure 5-2) does not reach the same values as those seen in the homogeneous experiments (Figure 5-3, Figure 5-4, Figure 5-5). The relationships between sOC/pH and biogas are the same, but in the porous media setting, sOC accumulation and low pH reach more 'extreme' values than SCs when biogas production is inhibited. This is likely at least in part due to preferential channelling where some pores due to little-to-no advective flow could reach high and inhibitory levels of intermediate organic acids resulting in extreme conditions where the biogas production is ultimately affected. This can be seen in the HoFC2,3 reactors (Figure 5-3, Figure 5-4, Figure 5-5) where the 'inhibitory' zone of high sOC is dominant at the bottom in the BOTTOM port and less pronounced in the TOP port, but present nonetheless. This suggests there is greater potential for recovery in stirred systems. However, flow of leachate has clearly had a positive impact on biogas production generally amongst the lab-scale landfills. Particularly, the flow has had a similar effect in 'stirring' in SCs and distributing nutrients etc. to enhance biodegradation. Although there is larger variability in the columns without *Agrobacterium sp.* under flow conditions.

5.2.3.2 No-Flow:

Within all the no-flow reactors, the lag phase here compared to SCs is not only longer (approximately 8-10 days), but there is more variability in how long the lag phase lasts. However, the lag phase is similar to the homogeneous flow experiments. The heterogeneity in the initiation of biogas production in no-flow and flow reactors can be partially explained by zones within the waste mass where initiation of methanogenesis occurs first. Previous work has found similar behaviour in reactors containing MSW with periodic recirculation (Staley et al., 2011). In their reactors, initiation of methanogenesis was shown to occur in zones, where the pH profiles within the reactors themselves varied and higher pH zones were correlated to methanogenesis initiation. A similar pattern is observed here. The TOP port which lies in the middle of the reactor remains in acetogenesis conditions the longest (lower pH/higher sOC than the other two ports,Figure 5-3, Figure 5-4, Figure 5-5). This may be due to key nutrients being used up nearer the injection point (closer to the BOTTOM port) and it takes time for them to move to the TOP port, since it is in the centre of the waste mass (furthest from both entry and exit areas of recirculated leachate). The pH in the other two ports remains close to neutral and in the optimal range for methanogenesis, whilst biogas production is taking place. Here, the presence of heterogeneous degradation phases simultaneously (methanogenesis, acetogenesis)

has not been recorded in no-flow conditions and under flow-conditions with a biodelignificating bacterium. For example, after the lag phase the TOP port within all no-flow reactors shows elevated sOC in comparison to the port below and above (Figure 5-3, Figure 5-4, Figure 5-5). This suggests accumulation of sOC in this zone (predominantly acetogenic phase, supported by low pH in this zone outside the ideal methanogenic range), whilst the upper and lower halves are likely the major contributors to the production of biogas at this point in time (predominantly methanogenic phase). Towards the end, accumulation of sOC starts to occur not only in the TOP port, but also the BOTTOM port and this pushed the pH out of the optimal range, to very low values as the biogas curves flat-line.

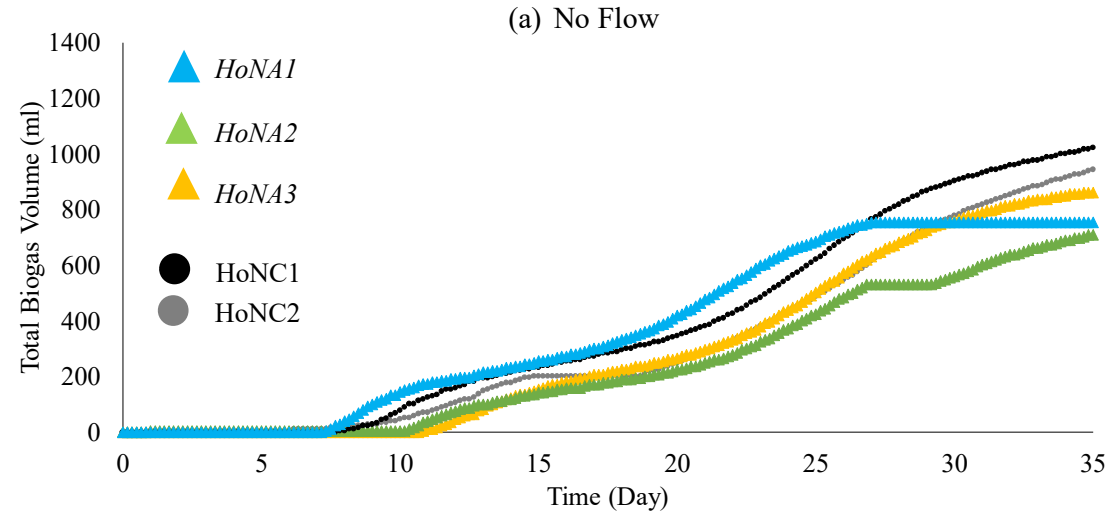
The presence of the *Agrobacterium sp*. in HoNA does not seem to have an enhancing effect on biogas production ($p>0.05$). This is possibly a result of localised nutrient limitation due to ineffective distribution of M9 nutrients, as well as degradation products, since there is no flow/recirculation.

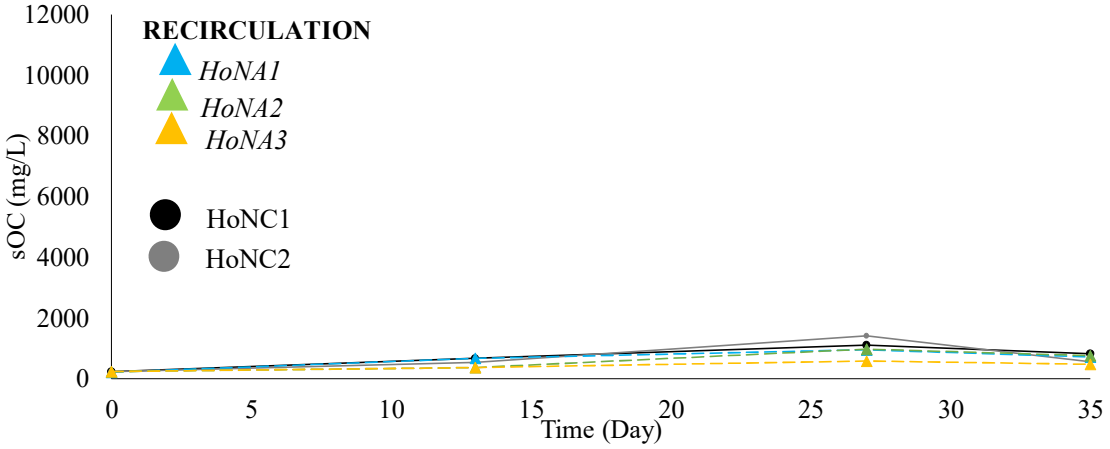
5.2.4 Heterogeneous Experiments

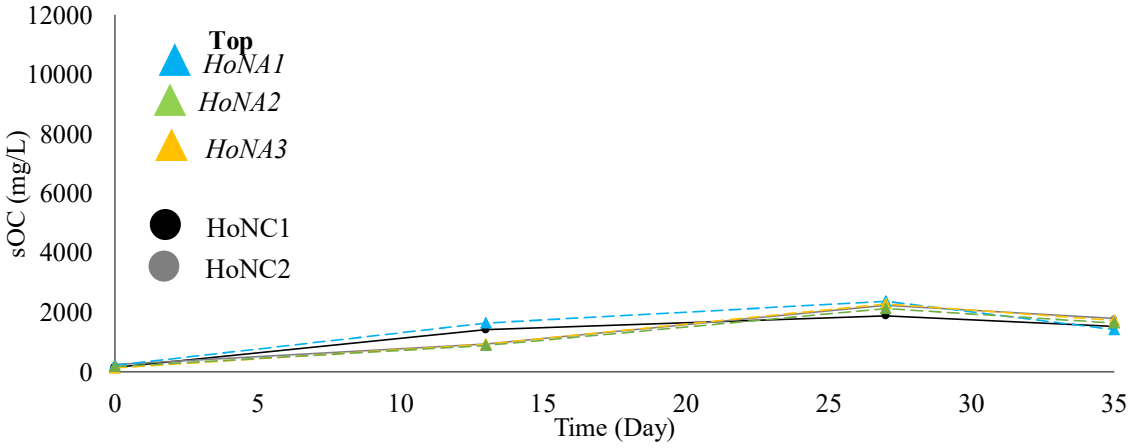
5.2.4.1 Flow:

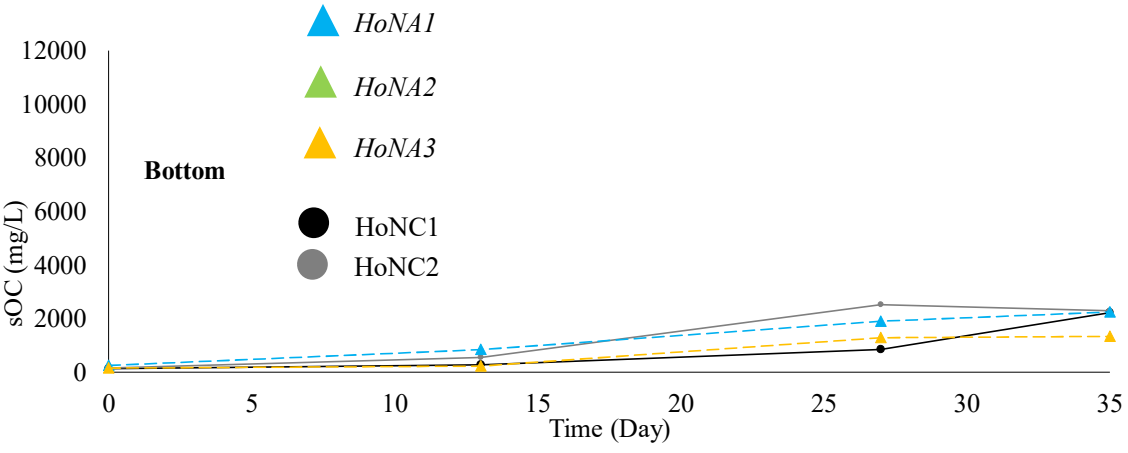
In the flow reactors, the lag phase was longer than that present in the SC systems (Figure 5-2). In addition, variability in the lag phase was also far greater that the SC systems. The variability and average lag phase duration was not significantly different to that of the homogeneous reactors, although one outlier existed (one reactor with a lag phase of approximately 15 days whereas all the homogeneous reactor lag phases were ≤ approximately 10 days). This variability extended all the way to biogas kinetics, as well as overall volume.

In general, the flow reactors produced a higher amount of biogas, presumably, due to better distribution of nutrients and sOC degradation products compared to no-flow systems. However, the heterogeneous physical nature of waste here complicates matters. There does not appear to be an absolutely clear difference between all flow and all no-flow reactors for biogas profiles, the variability due to heterogeneity has resulted in some overlap of biogas profiles and this must be clearly noted.


(a) No Flow
Total Biogas Volume (ml)
1400
1200
1000
800
600
400
200
0
HoNA1
HoNA2
HoNA3
HoNC1
HoNC2
0
5
10
15
20
25
30
35
Time (Day)


RECIRCULATION
sOC (mg/L)
12000
10000
8000
6000
4000
2000
0
HoNA1
HoNA2
HoNA3
HoNC1
HoNC2
0
5
10
15
20
25
30
35
Time (Day)


Top
sOC (mg/L)
12000
10000
8000
6000
4000
2000
0
HoNA1
HoNA2
HoNA3
HoNC1
HoNC2
0
5
10
15
20
25
30
35
Time (Day)


Bottom
sOC (mg/L)
12000
10000
8000
6000
4000
2000
0
HoNA1
HoNA2
HoNA3
HoNC1
HoNC2
0
5
10
15
20
25
30
35
Time (Day)

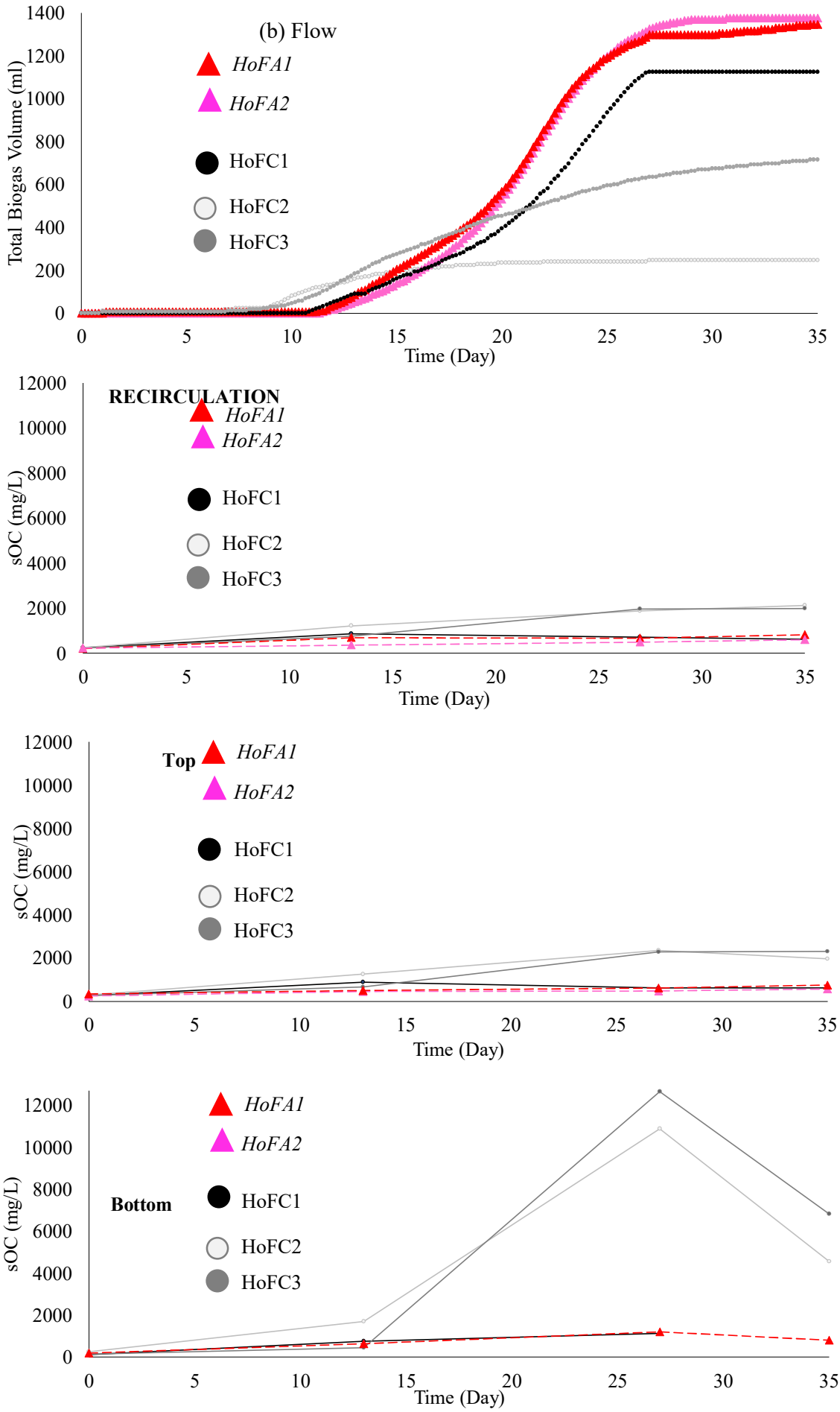


Figure 5-3- Biogas and sOC data for the homogeneous experiments. The two columns (a) and (b) represent the experimental conditions and their corresponding data. Each colour represents one and the same reactor across all the graphs vertically.

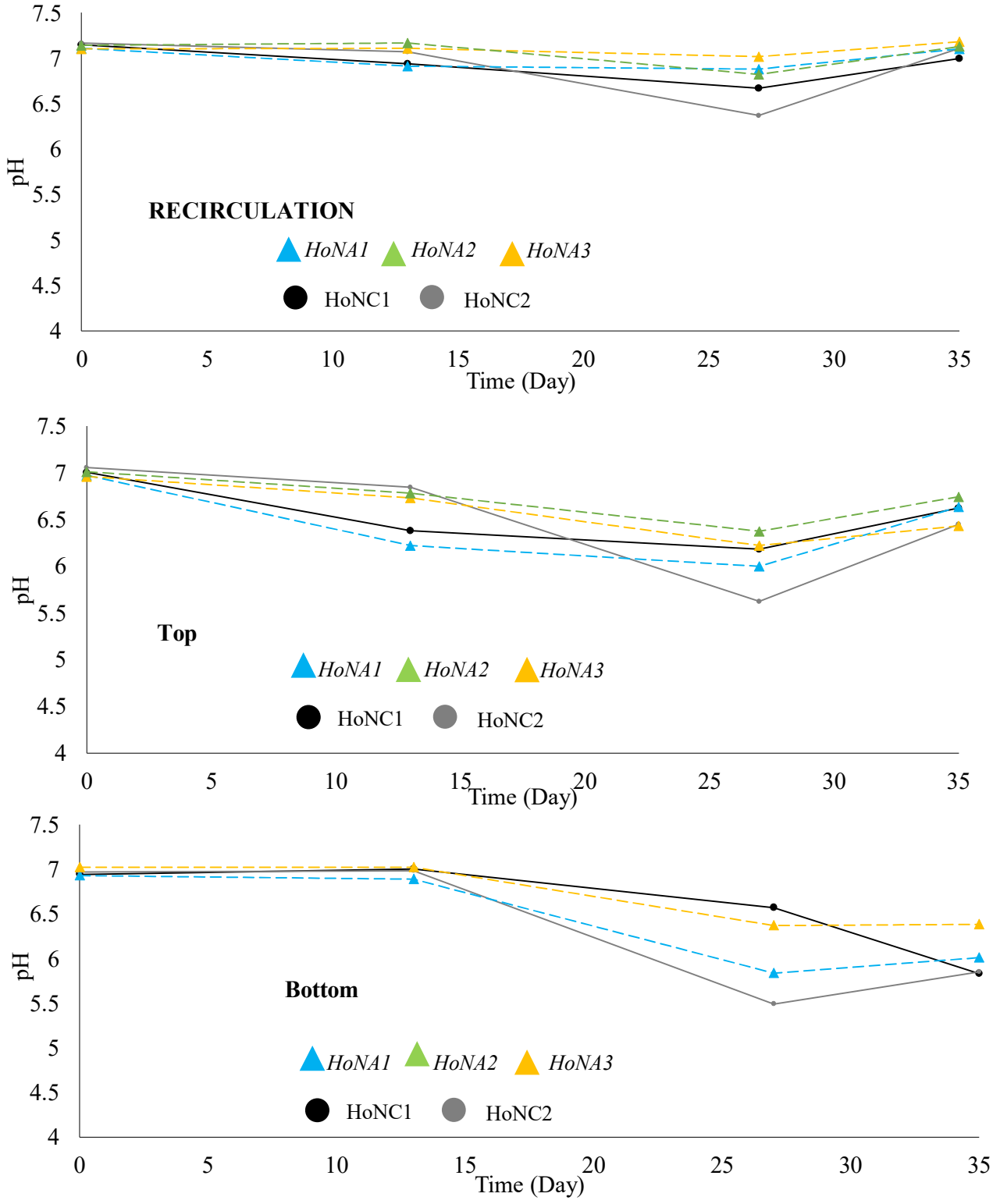


Figure 5-4- pH data for the homogeneous experiments with no flow. Each colour represents one and the same reactor across all the graphs vertically.

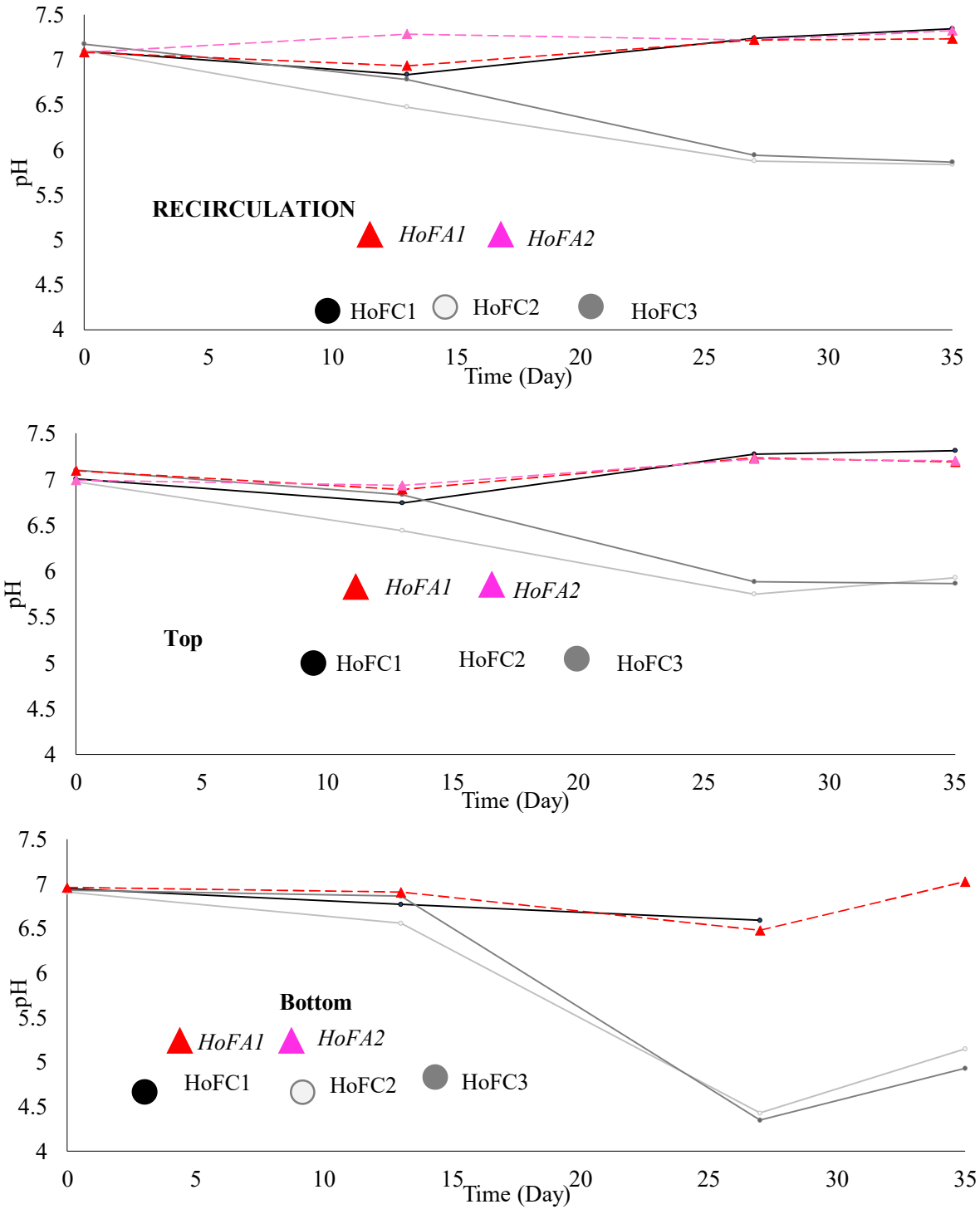


Figure 5-5- pH data for homogeneous experiments with flow. Each colour represents one and the same reactor across all the graphs vertically.

There seems to be a clear relationship between the sOC release and the impact that has on the system over the course of the experiment (Figure 5-6, Figure 5-7, Figure 5-8). If the release in sOC is rapid during a short period of time, this is accompanied by a sharp decrease in pH as well (e.g. HtFC3) and pushes the pH well out of the ideal range for methanogens. This has a clear impact on biogas production which slows down (e.g. HtFC3,4). Recovery from this is possible (HtFA2), which requires adaptation by the microbial communities. However, it does not always occur (HtFC3) and the system can remain in this phase, this has been shown to occur in landfill bioreactor systems where the organic load is very high (Han et al., 2020; He et al., 2006; Kim & Kim, 2015).

It can be seen that in the flow-reactors, there is relatively less accumulation of sOC in the OUTSIDE (sand) and the RECIRCULATION ports, compared to the INSIDE port, which is likely a consequence of channelling of the fluid and nutrients recirculating/flowing predominantly through the sand rather than the newspaper/sand mixture, which has a hydraulic conductivity two orders of magnitude lower (Figure 5-1). This is further supported by the much higher sOC/lower pH within the newspaper/sand zone (INSIDE port) compared with the relatively lower sOc/higher pH in the sand only medium (OUTSIDE port) (Figure 5-6, Figure 5-7, Figure 5-8). The sOC remains elevated in the central region containing the waste throughout the experiment, this is also paired by very low pH values throughout the experiment. These extreme pH/sOC values have been shown to negatively impact the biogas generation profiles, in this chapter and previous work (Muaaz-Us-Salam et al., 2020). This can again be related back to channelling of the fluid through the higher hydraulic conductivity zone (outer ring of sand). It should be noted that there likely was an interface between the inner newspaper-containing region and the outer sand ring with ‘good’ access to both carbon source and other nutrients, but this was probably much smaller than the ‘good access’ regions in the homogenous reactors.

The presence of the *Agrobacterium sp*. (HtFA) had no significant impact on the biogas yield in relation to the flow controls (HtFC) ($p>0.05$). It is likely that the very low pH values, particularly in the inner region where newspaper is present, inhibited the activity of the *Agrobacterium sp*., as observed for other lignocellulose-degrading bacterial strains (Mathews et al., 2016; Mathews et al., 2015; Mathews et al., 2014), and methanogens (ideal pH-range between 6.8-7.4 (Barlaz et al., 1990)). Moreover, researchers have shown that low pH conditions are detrimental to the health and activity of methanogens in comparison to pH closer

to neutral. Researchers found that at pH 5.1, the rate of decay of living methanogen cells is approximately 10 times faster than that at pH 7 (Sun et al., 2020), which can explain why recovery from this low pH is possible in some reactors and perhaps difficult in others.

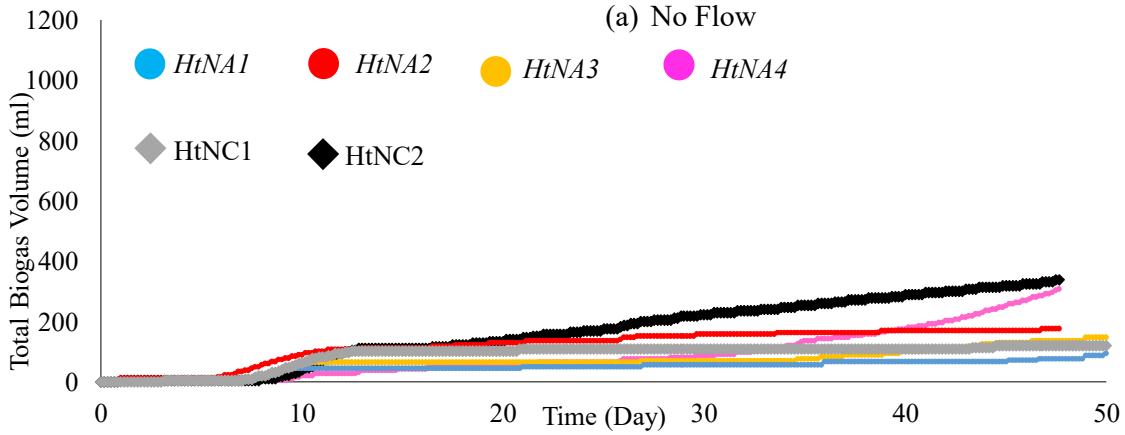
5.2.4.2 No-Flow:

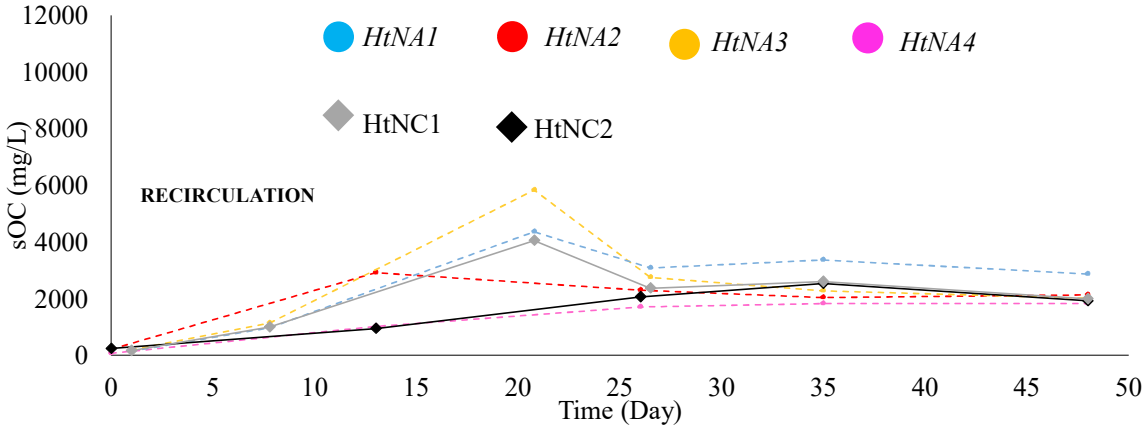
Within the no-flow reactors, the lag phase was similar to that obtained in the flow reactors. However, the volume of biogas generation varied a lot less within replicates and was generally lower compared to that obtained within flow-reactors (Figure 5-6, Figure 5-7, Figure 5-8). Amongst the no-flow reactors, it seems that better dispersion amongst the no-flow reactors generally results in higher biogas generation in the reactors (e.g. HtNC2, HtNA4). For instance, the sOC in these reactors in the OUTSIDE port gradually increases and remains elevated, rather than just being concentrated in one zone (INSIDE-port), which allows for better distribution of nutrients and degradation products. Likewise, the opposite is true, suggesting that stunted biogas generation profiles due to less dispersion across the cross-section of the waste mass, i.e. high concentration of sOC in the newspaper/sand region.

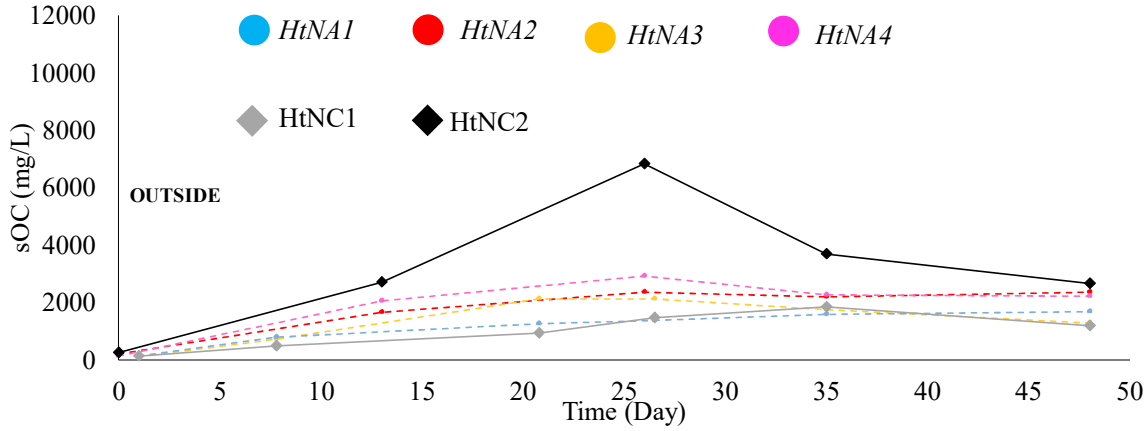
The variability in the sOC values due to the heterogeneity of the medium is present in the same pattern as that from flow experiments. Higher concentration of lignocellulosic material in the central region (newspaper/sand mixture) resulted in very high sOC values in this section, and relatively lower values in the sand throughout.

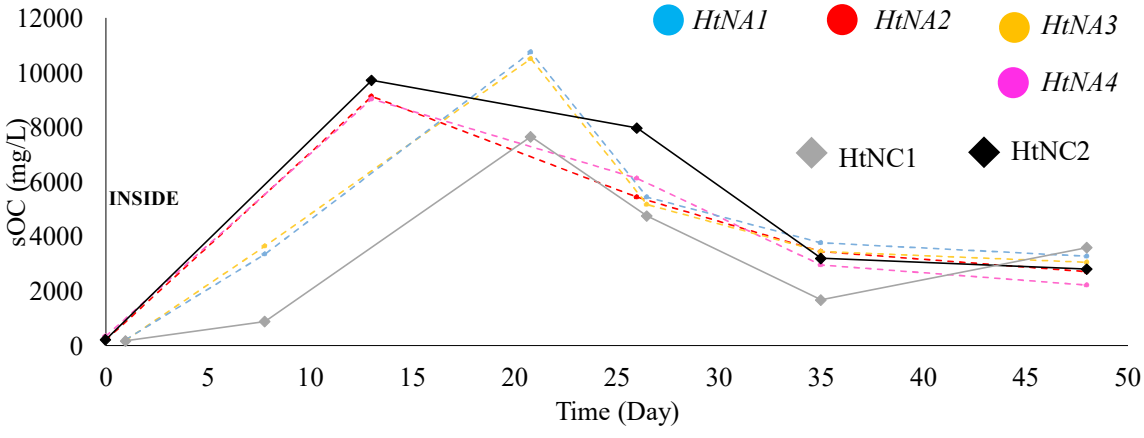
Just like in the flow reactors, the presence of the *Agrobacterium sp*. (HtNA) did not have an impact on the biogas yield compared to HtNC, likely due to similar reasons aforementioned for the flow-reactors (e.g. very low pH values, toxicity) ($p>0.05$) with the addition of localised nutrient limitation as a result of no recirculation here.

Moreover, as with the flow bioreactors, there will be an interface between the inner newspaper-containing region and the outer sand ring where access to nutrients and degradation products is optimal. However, it will likely not have lasted this way for long, since lack of fluid flow would have resulted in no advective flow, and hence the flushing and transport of nutrients and carbon will have been affected. Likely contributing to the very low overall biogas yield obtained in the HtNC and HtNA reactors.


(a) No Flow
HtNA1
HtNA2
HtNA3
HtNA4
HtNC1
HtNC2
Total Biogas Volume (ml)
Time (Day)


HtNA1
HtNA2
HtNA3
HtNA4
HtNC1
HtNC2
RECIRCULATION
sOC (mg/L)
Time (Day)


HtNA1
HtNA2
HtNA3
HtNA4
HtNC1
HtNC2
OUTSIDE
sOC (mg/L)
Time (Day)


HtNA1
HtNA2
HtNA3
HtNA4
HtNC1
HtNC2
INSIDE
sOC (mg/L)
Time (Day)

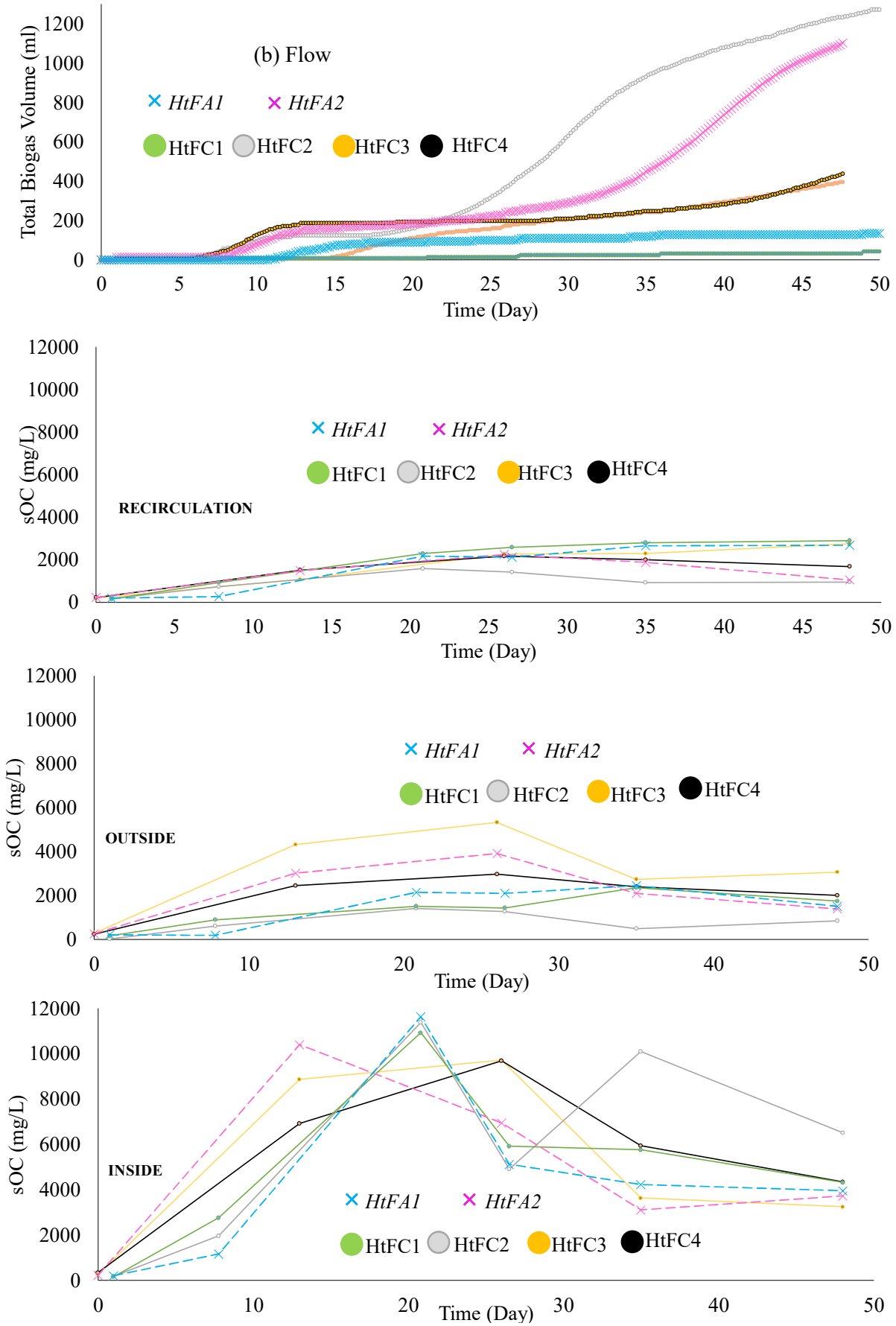


Figure 5-6- Biogas and sOC data for the heterogeneous experiments. The two columns (a) and (b) represent the experimental conditions and their corresponding data. Each colour represents one and the same reactor across all the graphs vertically.

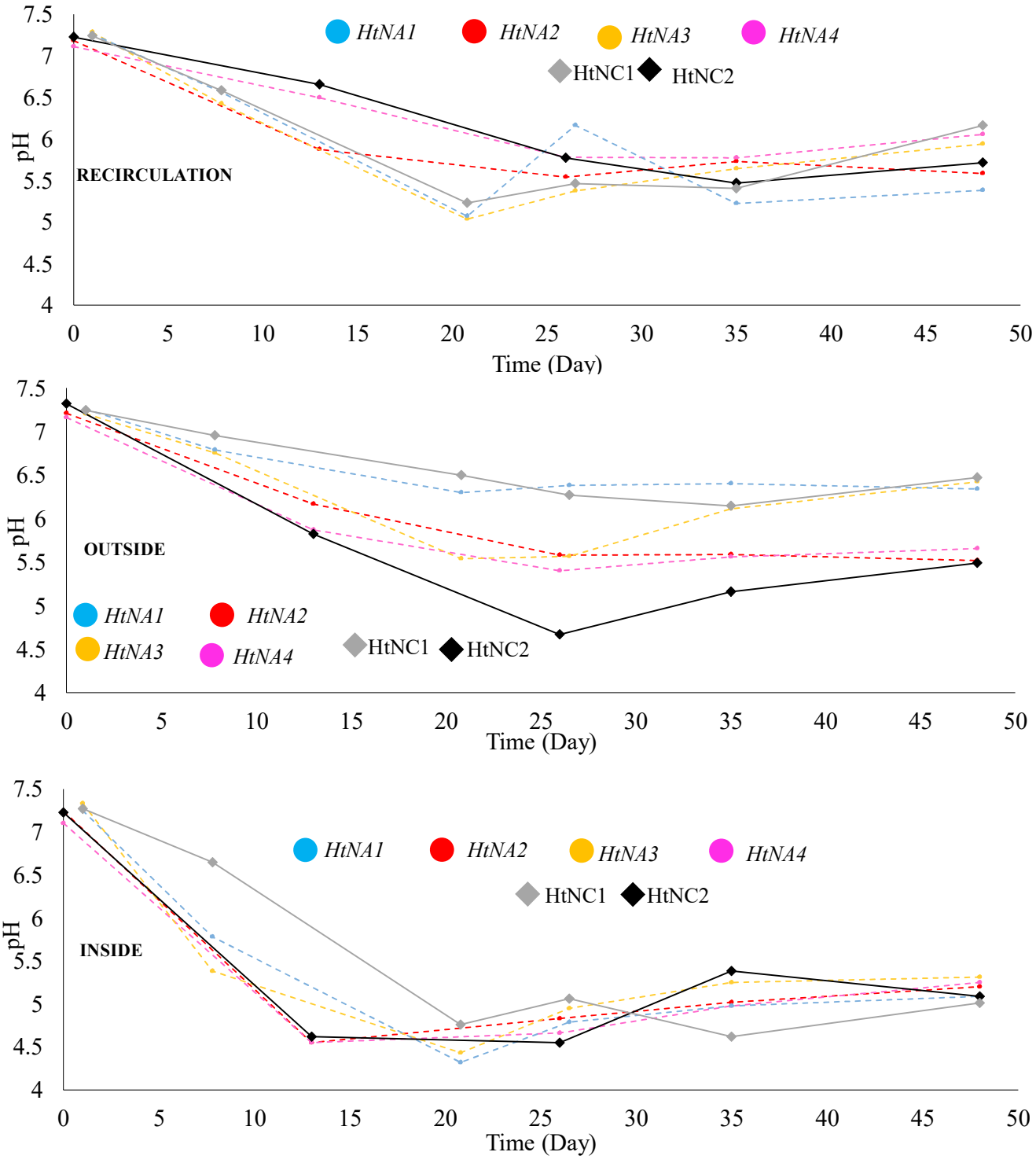


Figure 5-7- pH and sOC data for the heterogeneous experiments with no flow. Each colour represents one and the same reactor across all the graphs vertically.

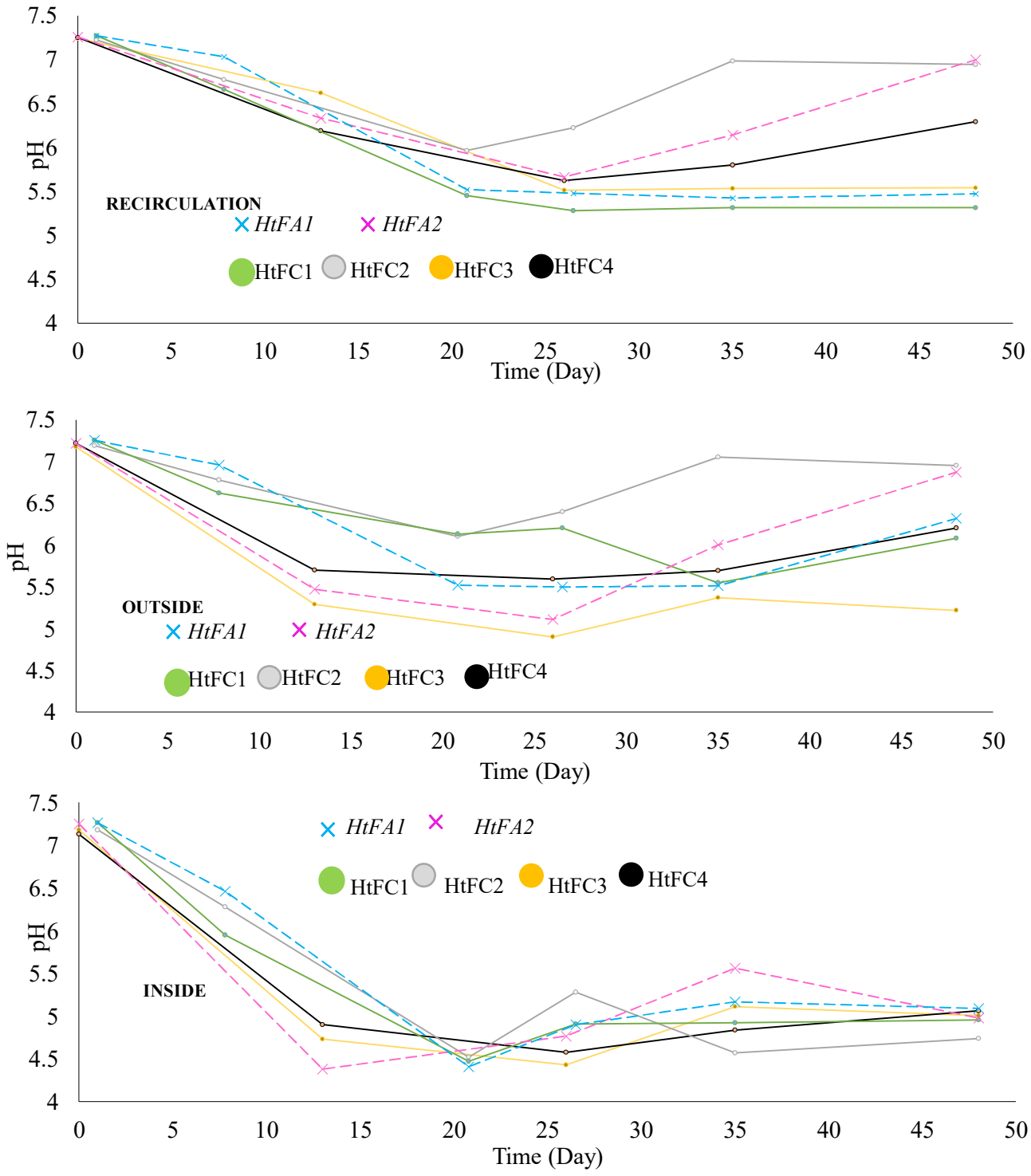


Figure 5-8- pH data for the heterogeneous experiments with flow. Each colour represents one and the same reactor across all the graphs vertically.

5.3 Overall Discussion & Implications for Field-Scale Applicability

The results discussed above have shown the importance of the physical and hydraulic properties of lignocellulosic wastes when it comes to the application of biodelignificating bacteria to landfills for enhanced biogas production. Under ideal-stirred conditions in liquid phase reactors, enhancement of biogas production (approximately 2 fold) was observed with the same newspaper used in this chapter (Muaaz-Us-Salam et al., 2020). In this chapter, the same newspaper when homogeneously distributed in an inert sand matrix, shows enhancement in biogas as well (approximately 2-fold), comparable to our work under stirred conditions. Researchers have used a bacterium from termite gut in homogeneous conditions and found enhancement of biogas production in lignocellulose-containing waste (approximately 1.2 – 2.5 fold over 200 days vs ¼ of the time in this study) (Rahimi et al., 2020). Similarly, researchers have used bacterial isolates very similar to the one used in our study and improved gas production in 1% pine powder and composed supplemented with 10% softwood chips approximately 3-fold (Rashid et al., 2017). This shows that the bacterium used in this study is comparable to upcoming bacterial strategies to tackle the problem of lignocellulose-containing wastes in landfills.

However, once that newspaper is heterogeneously present in a particular zone in the waste mass, the system becomes more complex. A two-factor ANOVA suggested that all the homogeneous biogas yields were significantly different to all the heterogeneous biogas yields ($p<0.05$, F-value>F-critical). In the experiments presented here, change in pore structure from homogeneous to heterogeneous results in decreased hydraulic conductivity in the key target area and likely results in preferential channelling which prevents ideal contact of the circulating nutrients and *Agrobacterium sp.* with the waste, as well as the contact of the nutrients and degradation products in the form of sOC with the bacteria from the sludge in the target zone. Locally, the acetogenic bacteria break down the waste in this zone into organic acids, however, due to reduced flow through this zone very high sOC concentrations are achieved, which result in a steep drop in pH. This is harmful for the methanogenic bacteria and *Agrobacterium sp.* and negatively impacts their activity, stunting biogas generation.

Figure 5-9 shows a plot of all the sOC values plotted against their pH for the entirety of the samples taken for this study. It can be seen that the higher the sOC, the lower the pH, which can be described by as a bi-linear line with a distinct change around pH 5. This further suggests that the very large drops in pH have been due to accumulation of intermediate organic acids and would explain the inhibition observed in the heterogeneous cases (central region vs outer ring). This is also true of accumulation of sOC/low pH in certain ports in the homogeneous controls (without *Agrobacterium sp*.) towards the end of the experiments, as discussed previously in detail. Consequently, lower sOC values relate very well with higher pH values, which is generally obtained in the enhanced cases with the *Agrobacterium sp.* reactors in homogeneous conditions (HoFA) since the bacterium is enhancing the breakdown of the newspaper lignocellulose and producing a higher amount of gas, thus much less accumulation of sOC in solution and pH stays in the optimal range for methanogens and *Agrobacterium sp.*

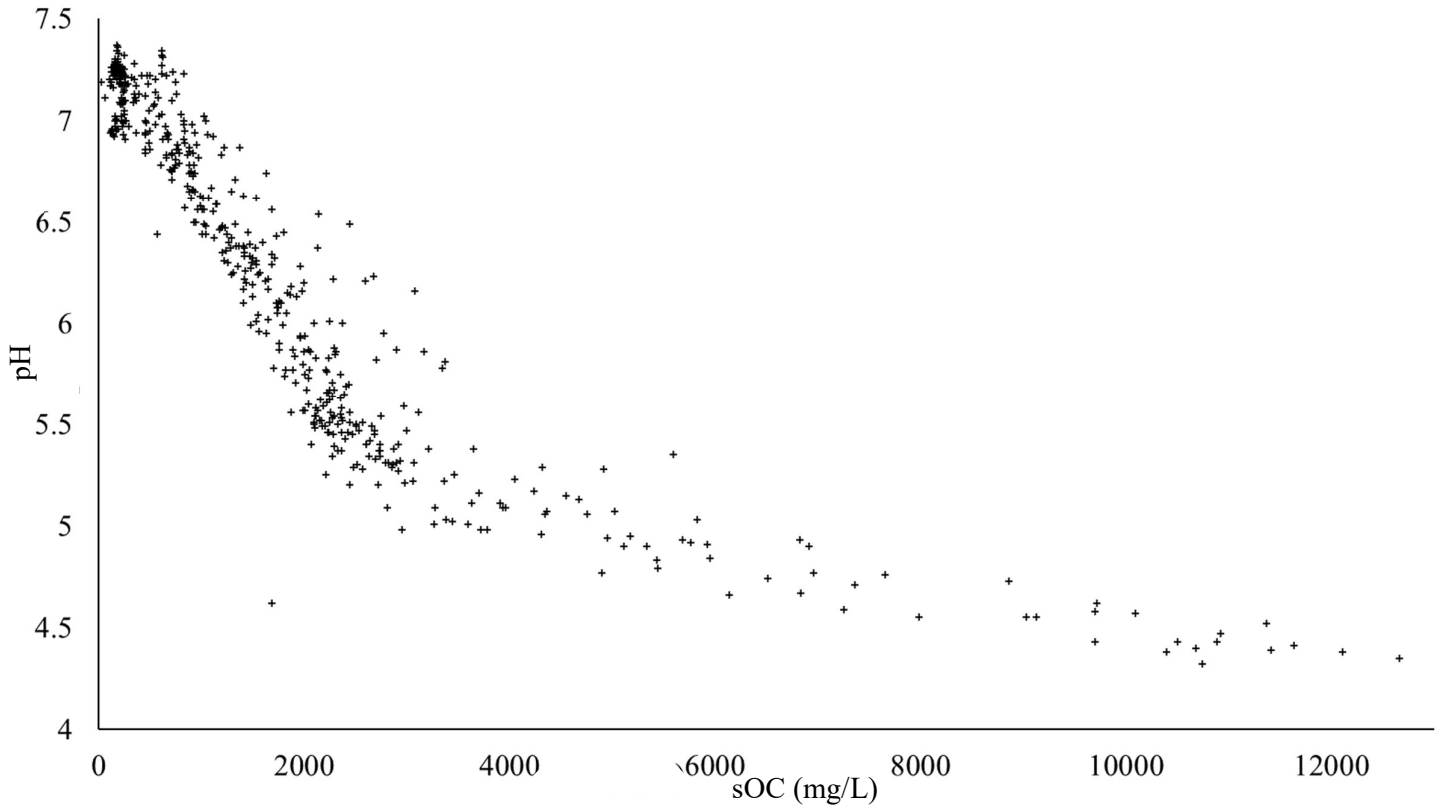


Figure 5-9- Correlation between pH and sOC for stirred, homogeneous and heterogeneous systems. At high concentrations sOC does not cause a directly proportional degrease in pH.

For lignocellulose-containing waste present relatively homogeneously within a landfill waste-cell could potentially benefit from this technology and enhance biodegradation/gas release. However, in very heterogeneous cells, the delivery of the bacterium to the area of interest (low hydraulic conductivity) and local pH and sOC effects might become a significant

hurdle in applying this technology. As such, for application of this technique at the field-scale, pilot-scale studies with real excavated MSW, homogenised by mixing could be explored as the next step to build on this work and extend it all the way to the field- scale. For existing heterogeneous waste cells in the landfill where excavation and homogenisation are not an option, potential flow-diversion techniques could be explored in order to manipulate the flow regime under highly heterogeneous soil/waste configurations. For future waste cells/projects wanting to utilise such biotechnological methods to accelerate biogas generation, homogenisation of waste (shredding, mixing etc.) should be carried out prior to landfilling for this type of technology to work well. Furthermore, it is hypothesised that it may not be the presence or absence of particular microorganisms that causes the long tail of emissions and slow decay (these may well be present anyway), but the heterogeneity of the matrix that significantly limits bioavailability of the lignin-rich materials. Therefore, if techniques could be developed to increase bioavailability, we might have a more significant impact than bioaugmentation alone.

5.4 CONCLUSIONS

This chapter contributes towards bridging the gap between small-scale, stirred-ideal enhanced biogas production experiments (typically found in the literature) and potential field-scale application of this technology to enhance biogas production by investigating the impact of variables such as leachate flow and heterogeneity on bacterial biodelignification. Furthermore, the results have shown that waste pore structure and flow through it are important factors to consider before bacterial biodelignification can be applied at the field-scale in landfills. When the lignocellulose is homogeneously distributed in an inert sand matrix, 2-fold enhancement of biogas yield occurs, which is consistent with sOC/pH profiles. Once the lignocellulose is concentrated in one zone, there is no significant enhancement due to bacterial biodelignification. This is likely due to the much lower hydraulic conductivity of the newspaper/sand mixture compared to the outer sand zone, resulting in preferential flow paths through the sand. This paired with very low pH and very high sOC (toxic to the activity of the methanogens and Agrobacterium sp.) due to the newspaper/sand mixture in the column impacts the microbial communities and their activity adversely.This chapter has directly provided answers to RQ2 (i.e. What is the impact of flow & heterogeneity on bacterial biodelignification systems in model lignocellulose-containing bioreactor landfills?) and helped in informing the overall aim of the thesis.

6 Overall Discussion & Concluding Remarks[6]

Chapters 1-2, in this thesis have established the problem of slowly degrading lignocellulosic wastes within MSW landfills and the fact that these lignocellulosic wastes are comprised mainly of newspaper and woody fractions of the waste mass. These chapters have also surveyed the literature surrounding this problem, potential avenues for solutions (bacterial and enzymatic systems to accelerate lignocellulose degradation) and the challenges involved (e.g. waste heterogeneity, preferential flow).

The above in turn gave rise to RQ1 & RQ2 investigated within this project. Namely, RQ1: To what extent can enzymatic & bacterial biodelignification systems breakdown lignocellulose in realistic lignin wastes, with the prospect of enhanced biogas recovery? RQ2: What is the impact of flow & heterogeneity on bacterial biodelignification systems in artificial heterogeneous lignin-containing wastes? Chapters 3-5 have explored RQ1 & RQ2.

In Chapter 4, the small-scale tests containing *Agrobacterium sp.* have shown enhanced biodegradation of lignocellulose contained within wood. Likewise, at the larger 1L bioreactor-scale, this has also been true with enhanced biogas generation. In the enzyme systems, enhancement in all systems except for those containing wood was observed, for which some possible explanations have been explored. Specifically, *Agrobacterium sp.* enhances the biodegradation of lignin-containing wastes, particularly newspaper and softwood, under idealised small-scale conditions, containing numerous other microbial communities. The solid:liquid ratio is a potentially important variable for the application of *Agrobacterium sp.* and should be considered for pilot-/field-scale trials (e.g. by adjusting leachate table). Lignin

[6] Contents of this chapter and preceding chapters have partially informed and will be disseminated in the following article (reviewed by 2 experts and editor): **Muaaz-Us-Salam, S**., Cleall, P.J., Harbottle, M.J.; (). "Understanding & Improving Biodegradation of Wastes in Landfills." (Waste360 ONLINE:).

peroxidase enhances the biodegradation of newspaper (not wood), in conjunction with methanogenic bacteria. These results suggest that enhanced breakdown of real wastes in MSW landfills and processes such as anaerobic digestion is feasible using either technique but that the waste form is an important factor in the rate and extent of breakdown.

Similarly, in Chapter 5, the *Agrobacterium sp.* system was then applied to newspaper lignocellulose in homogeneous and heterogeneous bioreactor landfill experiments for RQ2. This chapter builds upon the insights obtaioned in Chapter 4. The contents of this chapter have contributed towards bridging the gap between small-scale, stirred-ideal enhanced biogas production experiments (typically found in the literature) and potential field-scale application of this technology to enhance biogas production by investigating the impact of variables such as leachate flow and heterogeneity on bacterial biodelignification. This, to the best of the authors' knowledge, was lacking from the literature. Furthermore, this chapter has shown that waste pore structure and flow through it are important factors to consider before bacterial biodelignification can be applied at the field-scale in landfills. This particularly links well with leachate flow, pore structure and coupled modelling insights obtained in Chapter 2 (Section 2.3). Briefly, better understanding of pairing flow and lignolytic activity with the delivery of the bacterium to the target region in heterogeneous zones would help with developing field-scale application.

In light of the findings of this thesis, the following can be said about potential field-scale implications. For lignocellulose-containing waste present relatively homogeneously within a landfill waste-cell could potentially benefit from this technology and enhance biodegradation/gas release. However, in very heterogeneous cells, the delivery of the bacterium to the area of interest (low hydraulic conductivity) and local pH and sOC effects might become a significant hurdle in applying this technology. As such, for application of this technique at the field-scale, pilot-scale studies with real excavated MSW, homogenised by mixing could be explored as the next step to build on this work and extend it all the way to the field- scale. For existing heterogeneous waste cells in the landfill where excavation and homogenisation are not an option, potential flow-diversion techniques could be explored in order to manipulate the flow regime under highly heterogeneous soil/waste configurations. For future waste cells/projects wanting to utilise such biotechnological methods to accelerate biogas generation, homogenisation of waste (shredding, mixing etc.) should be carried out prior to landfilling for this type of technology to work well. Furthermore, it is hypothesised that it may not be the

presence or absence of particular microorganisms that causes the long tail of emissions and slow decay (these may well be present anyway), but the heterogeneity of the matrix that significantly limits bioavailability of the lignin-rich materials. Therefore, if techniques could be developed to increase bioavailability, we might have a more significant impact than bioaugmentation alone.

6.1 Contribution to Knowledge

The literature survey in chapter 2 has contributed to current understanding of biodelignifying systems and flow phenomena in MSW (published in literature now) which had not been previously been surveyed and critiques as has been in this thesis (Muaaz-Us-Salam et al., 2019; Muaaz-Us-Salam et al., 2017). Investigation of RQ1 & RQ2 has provided mechanistic insights into the application of bacterial biodelignification systems and helped bridge the gap in knowledge that existed in taking this technology from the ideal experiments conducted thus far (Abraham et al., 2020; Ahmad et al., 2010; Bugg et al., 2011a; Crawford & Crawford, 1976; Huang et al., 2013; Martinez et al., 2009; Mathews et al., 2016; Mathews et al., 2015; Perez et al., 2002) to field-scale applicability (taking into account some of the main artefacts of real-life field conditions such as heterogeneity, and flow) (Muaaz-Us-Salam et al., 2020).

This thesis has surveyed the literature and identified the problem of slowly degrading newspaper and woody wastes in landfills. It has specifically formulated research questions addressing this problem by studying accelerated degradation of these wastes, and the application of this technology to conditions close to real-life field-scale conditions (flow, heterogeneity). Enzymatic and bacterial biodelignification systems show promise under stirred-bioreactor conditions, as well as homogeneous lab-scale landfills. However, under heterogeneous conditions, the biodegradation process is more complicated. The findings of this thesis have important implications for enhanced breakdown in old MSW landfills that are rich in these wastes, and AD operations utilising lignocellulosic wastes. In 6.2, possible avenues for further work at a variety of scales are considered.

6.2 Suggestions for Future Work

6.2.1 Small-scale

- Further research into the impact of waste heterogeneity on the activity of *Agrobacterium sp.* would be beneficial in understanding this biotechnology further, so as to assess its field-scale applicability even more. This may require monitoring the changes in the microbial communities in the areas where the pH drops drastically, and identifying transient trends in population changes, so as to understand and manipulate these processes better under heterogeneous pore structure configurations.

- Research into utilising direct interspecies electron transfer (DIET) within microbial communities to accelerate biogas production has been becoming popular (Baek et al., 2018; Lovley, 2017a; Lovley, 2017b; Martins et al., 2018; Pan et al., 2020; Rotaru et al., 2014; Stams & Plugge, 2009; Wu et al., 2020; Zhang et al., 2018). DIET uses extracellular modes of transfer to send and receive electrons within the methanogenic and acidogenic communities to accelerate biogas production, the work referenced previously shows promise. It is suggested that perhaps utilisation of simultaneous DIET & *Agrobacterium sp*.-like systems to accelerate and enhance biogas production further could be a very fruitful effort.

- Developing on the flow models (pore- and Darcy-scale) used/developed in this thesis by validation against real waste samples, extracting pore-structures via CT-scanning. Coupling the model with mechanistic insights obtained by experimental work from above, specifically activity of the biodelignifying bacteria & its relationship to enhanced biogas generation.

- Since anaerobic digestion plants are also taking lignocellulosic wastes for biogas production, there is potential to apply the systems studied in this thesis in anaerobic digestion tanks (either batch or continuously stirred systems with inflow and outflow segments). Augmentation with *Agrobacterium sp.*-like bacteria could potentially enhance biogas recovery/efficiency in this area. This would of course mean starting with preliminary work on the activity of such bacteria on lignocellulosic wastes most commonly going to digesters in the UK.

6.2.2 Up-scaling

- Up-scaling the flow-experiments at the pilot-scale perhaps incorporating real, exhumed waste containing inert waste mass (represented in this thesis as sand) and lignocellulosic waste.

- The pilot-scale studies would then need to be further up-scaled to the field-scale incorporating single and/or multiple landfill cells with appropriate controls.

6.2.3 Wider issues

- In this thesis and within the literature, the focus of employing/studying lignocellulose-degraqding bacteria is mainly on obtaining mechanistic insights and application at the lab-scale incorporating a variety of variables. An important consideration is that if a bacterium has shown promise at the lab-scale and the pilot-scale, and perhaps even within a landfill cell, how likely is it to be just as effective in other landfills around the world? This is an important question to answer as field-scale deployment would require one to know how well the biotechnological solution might work under different climates, waste environments and variable site-conditions (moisture content, soil organic carbon etc.).

- Lastly, an emphasis should be placed on more fundamentally revising our waste management systems such that lignocellulosic waste products stay within the circular economy. This would perhaps require intervention within developing countries such as Pakistan to establish waste collection/transfer station, anaerobic digesters and so on, so as to avoid exacerbating this problem of slowly degrading lignocellulosic wastes in landfills.

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Appendix[7]

This appendix contains supplementary data that was obtained through experiments that did not directly inform the RQS. Figure A-0-1 relates to suitability of the sludge for use in bioreactor experiments in chapters 4 and 5. Figure A-0-2 shows the growth curve of the *Agrobacterium sp* bacterium also used in chapters 4 and 5. Figure A-0-3 was some tangential testing of impact of particle size on biogas production. Figures A-0-4 and A-0-5 relate to phenolics data for small-scale wood/*Agrobacterium sp*. tests in chapter 4. Figures A-0-6, A-0-7, A-0-8 refer to some testing done on impact of addition of alternate easily biodegradable carbon source on bacterial activity and lignocellulose breakdown. Figures A-0-9 to A-0-13 relate to phenolics data and some correlations for stirred- bioractor tests in chapter 4. Table A-0-1 shows biogas modelling via the Gompertz equation of the stirred bioreactor tests in chapter 4. Figures A-0-14 to A-0-20 relate to phenolics data for the bioreactor landfill tests in chapter 5. Figures A-0-21 to A-0-24 relate to some retrospective fluorescent dye tracing for some of the experiments in chapter 5. Figures A-0-25 and A-0-26 are some examples of miscellaneous elementary and pore-scale modelling work conducted. Figures A-0-27 to A-0-29 relate to chapter 5 (sand particle size distribution, and bespoke 3-D printed parts).

[7] Contains supplementary data and modelling examples. Full raw data and developed codes during this project can be obtained by emailing the author.

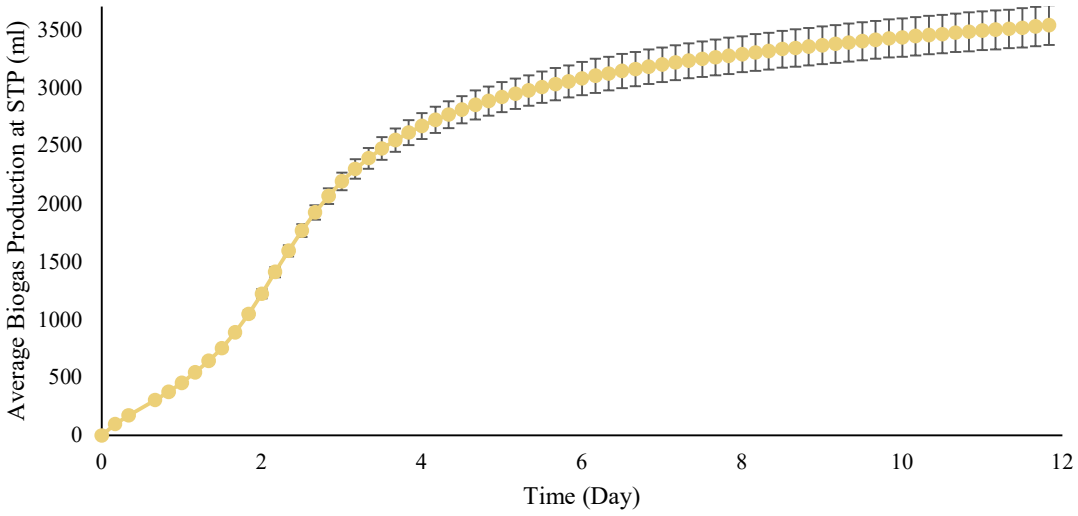


Figure A-0-2 Biogas production for cellulose (test done to see suitability of the sludge used in this study for biodegradation/biogas production).

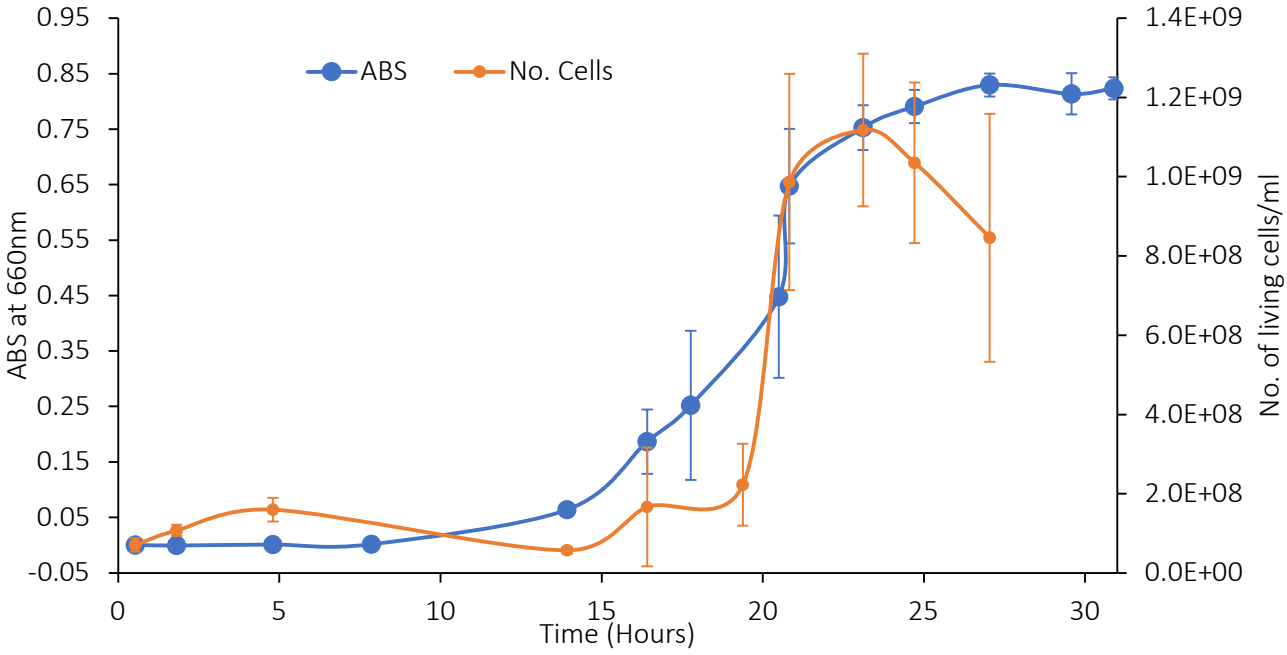


Figure A-0-1 Growth curve for the pure Agrobacterium sp. culture used in this project. Note: ABS- Absorbance at 660nm, No. Cells- Number of living cells stained and counted with 5-Cyano-Tetrazolium Chloride & fluorescence microscopy.

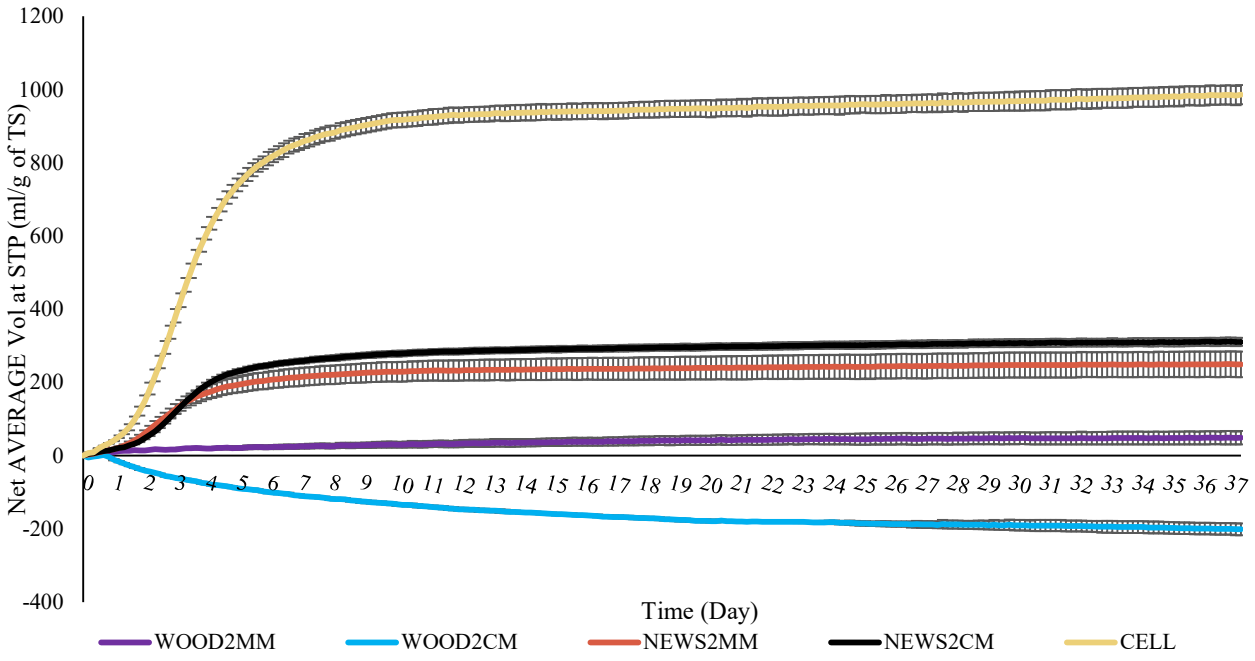
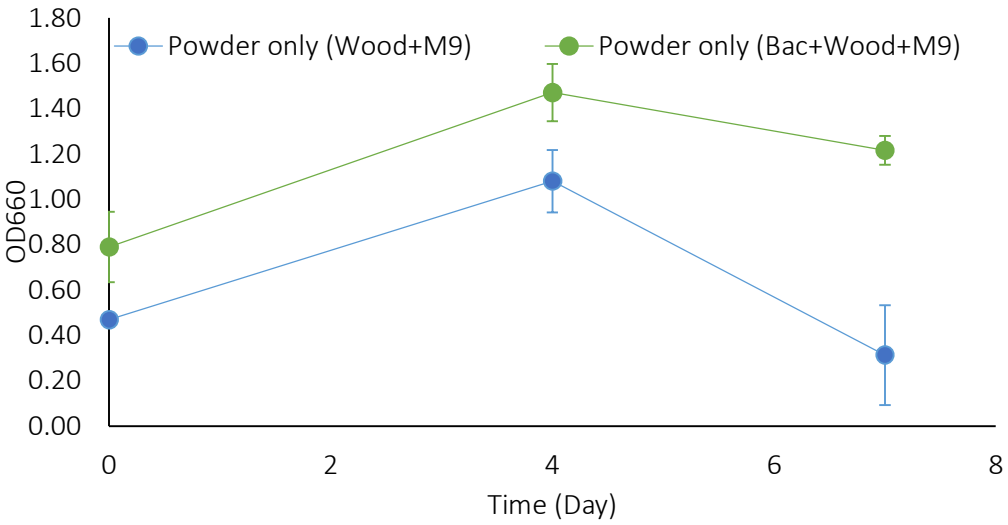
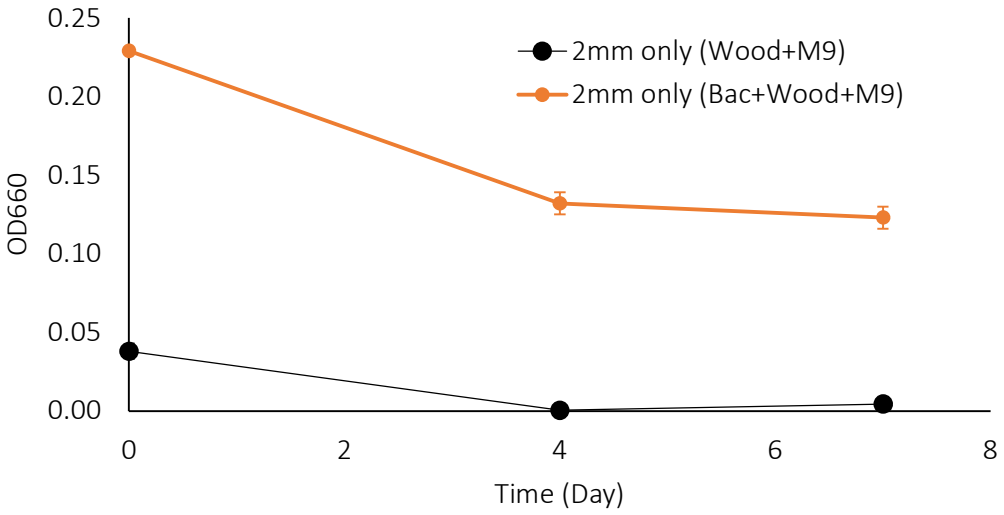
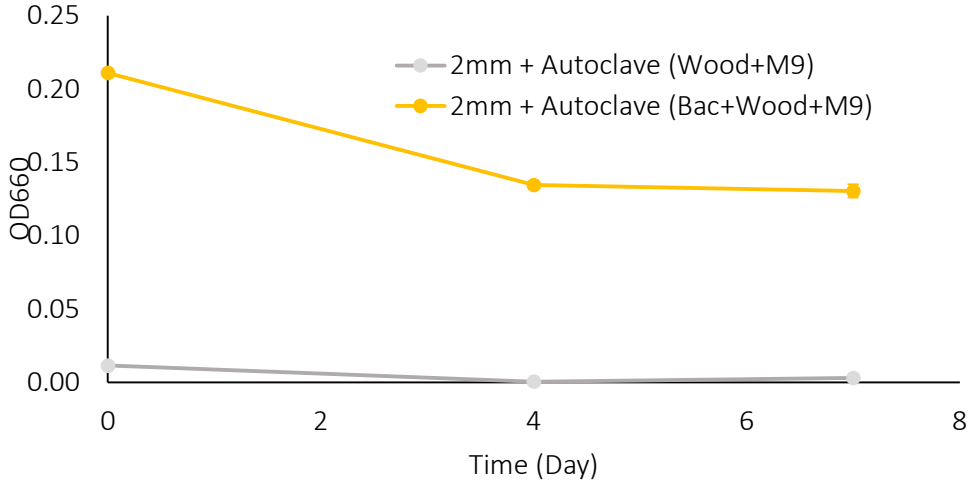


Figure A-0-3 Biogas results analysing the impact of particle size on biogas production. Note: CELL-cellulose, 2mm-2millimetre, 2cm-2cwntimetre.







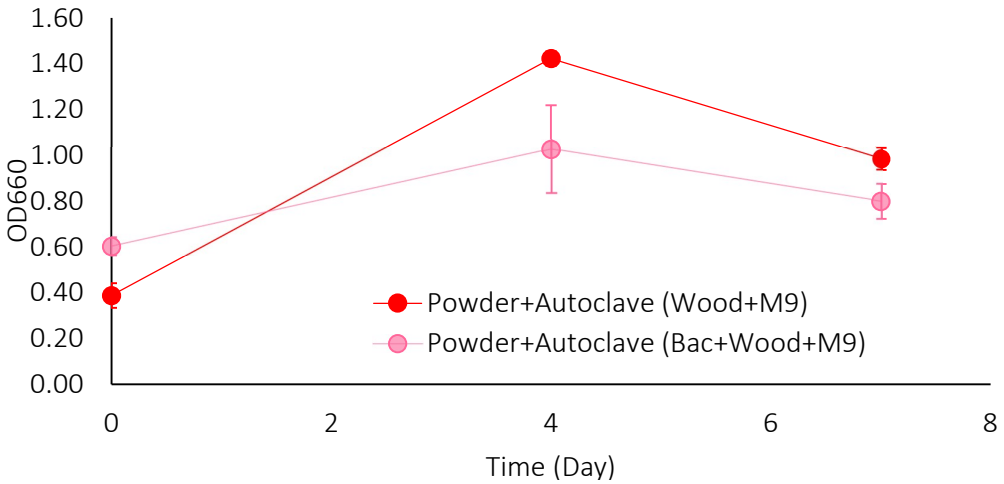
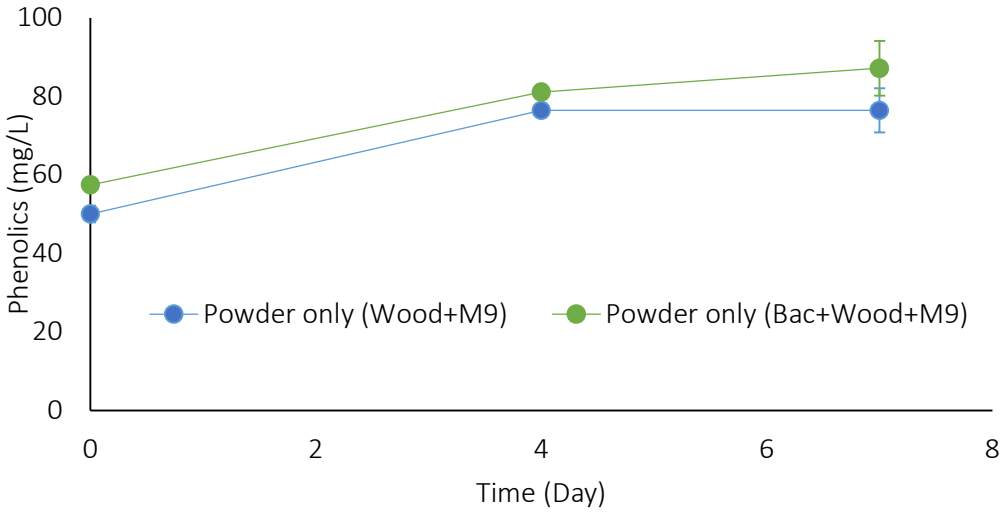
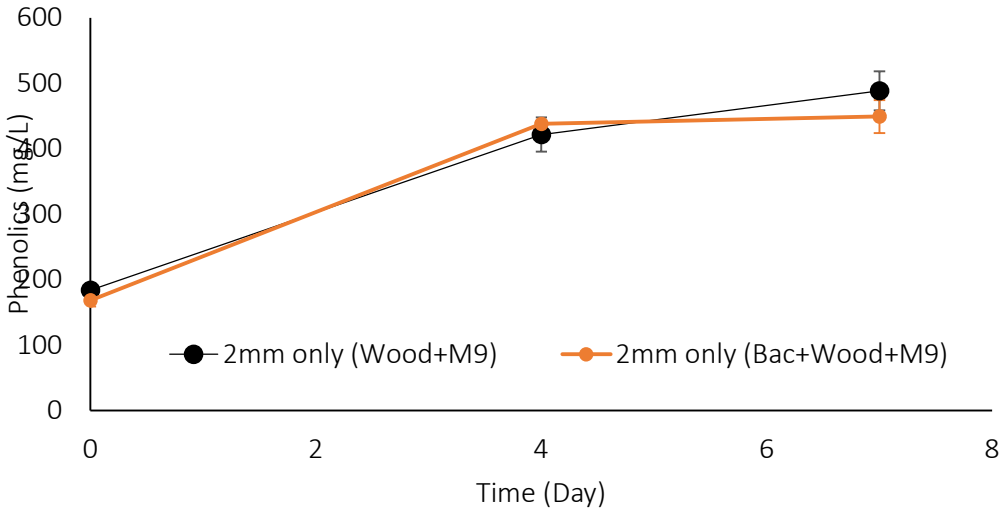
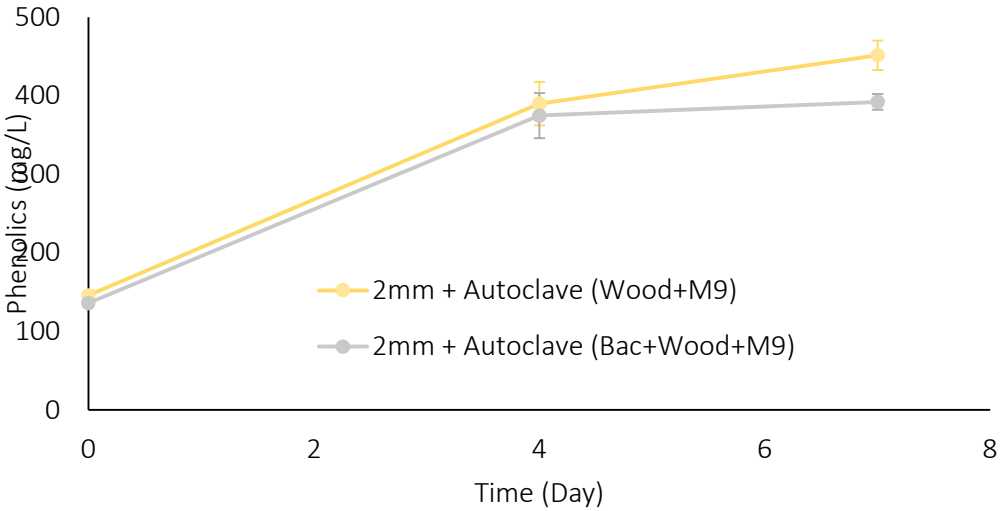


Figure A-0-4 Optical density measurements for week-long small-scale experiments containing Agrobacterium sp., M9 and wood in different formats.







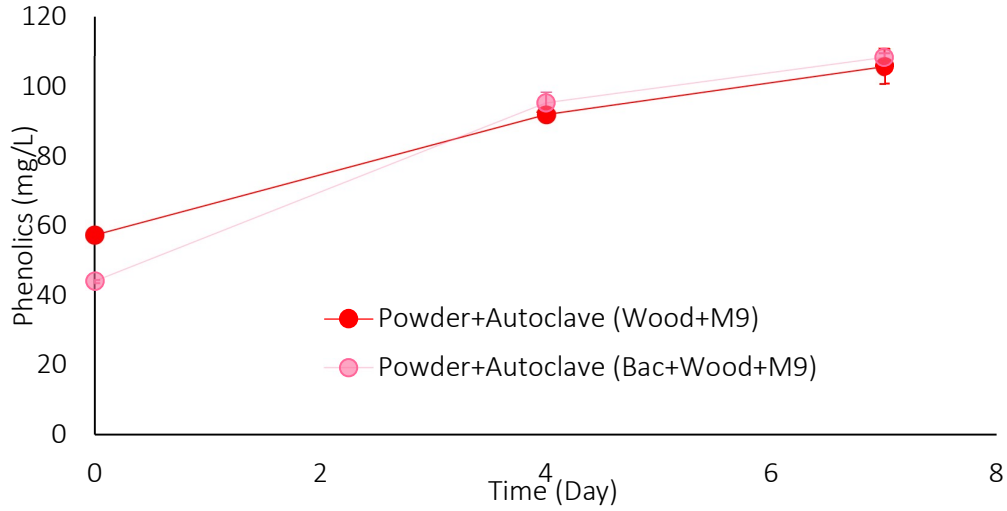
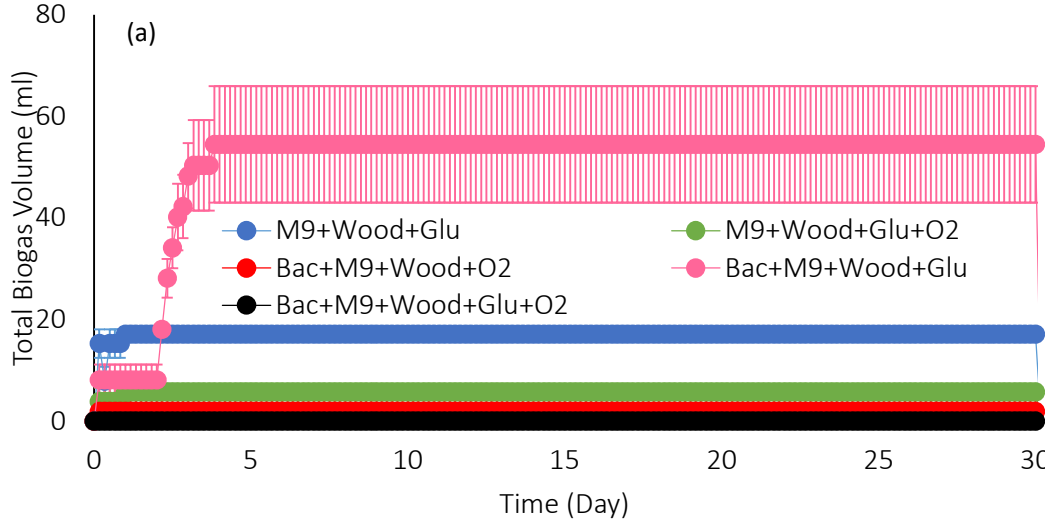
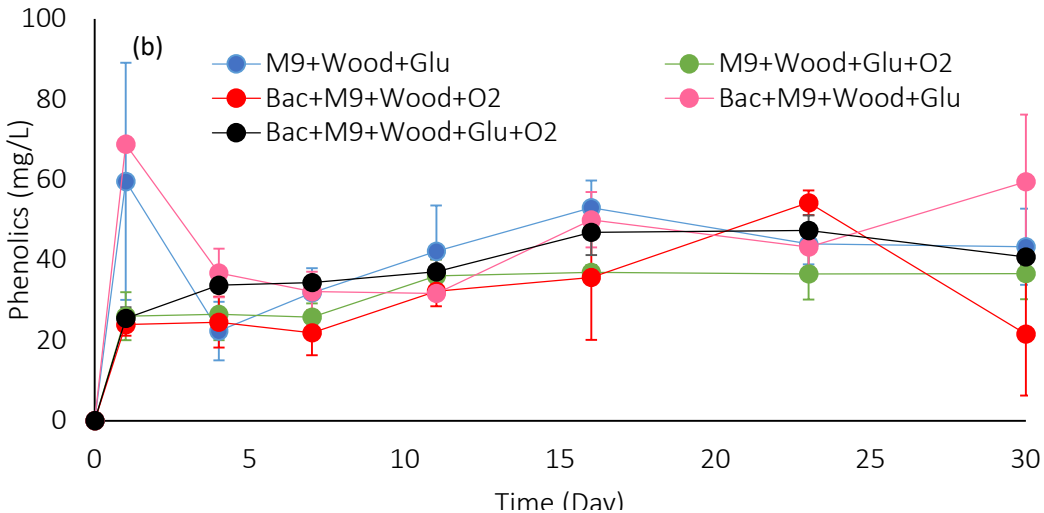
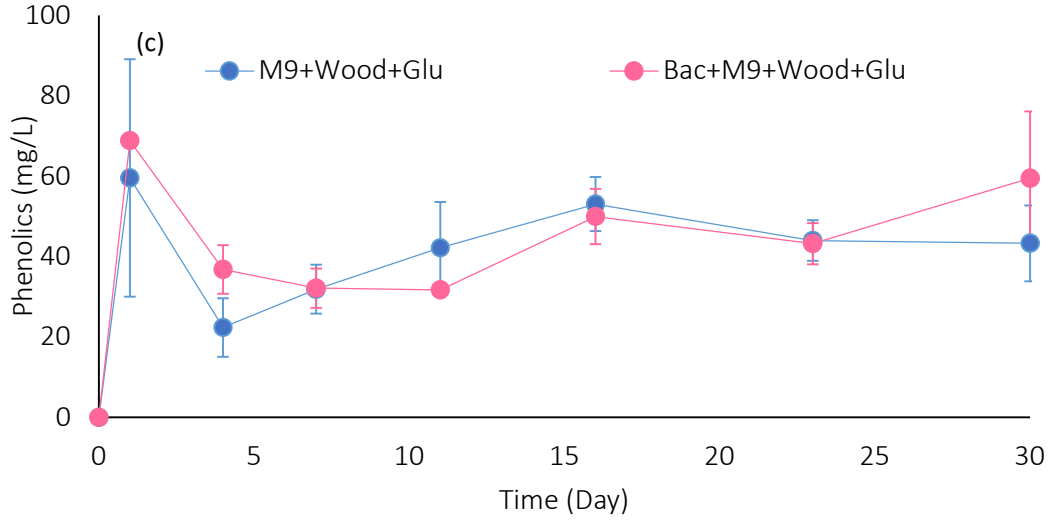


Figure A-0-5 Phenolics measurements for week-long small-scale experiments containing Agrobacterium sp, M9 and wood in different formats.







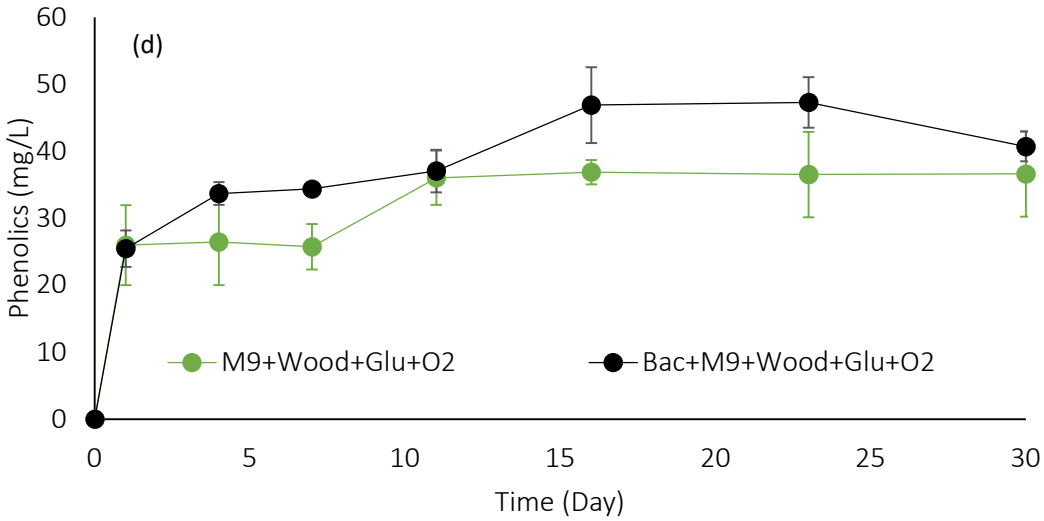


Figure A-0-6 1L-scale pure Agrobacterium sp. culture experiments with glucose and oxygen supplementation. (a) Biogas data, (b,c,d)

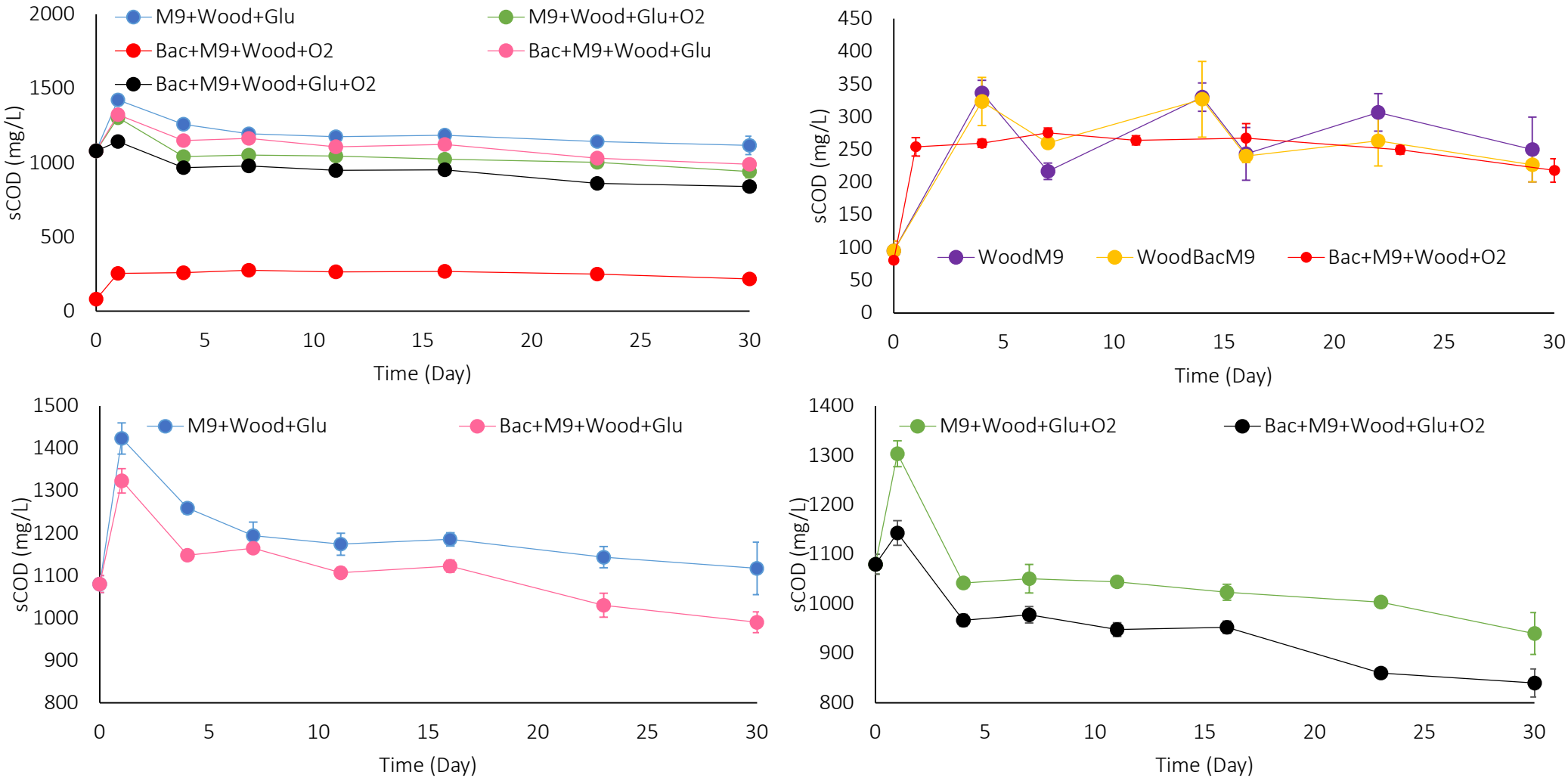


Figure A-0-7 1L-scale pure Agrobacterium sp. culture experiments with glucose and oxygen supplementation, sCOD data.

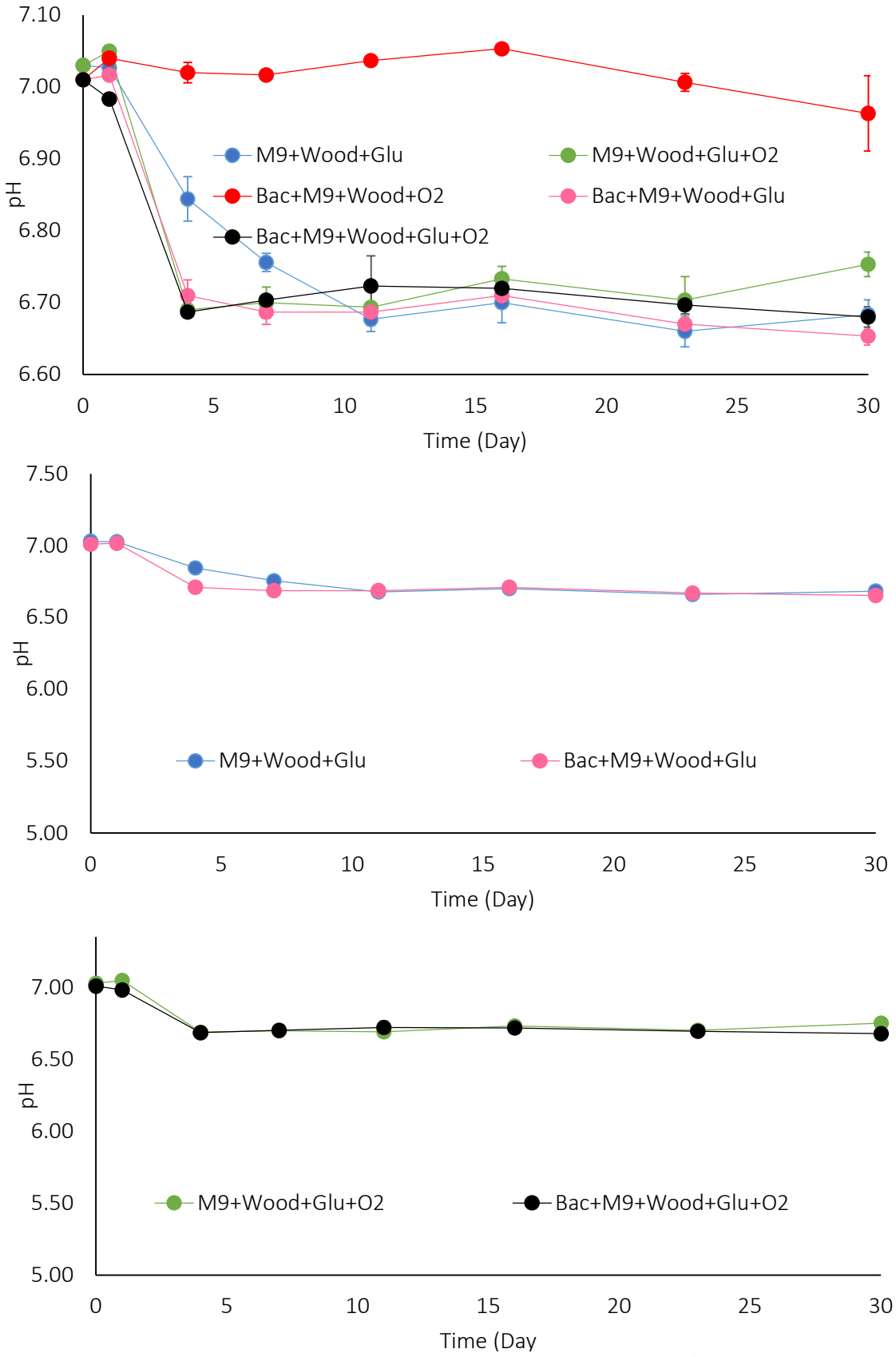


Figure A-0-8 1L-scale pure Agrobacterium sp. culture experiments with glucose and oxygen supplementation. pH data.

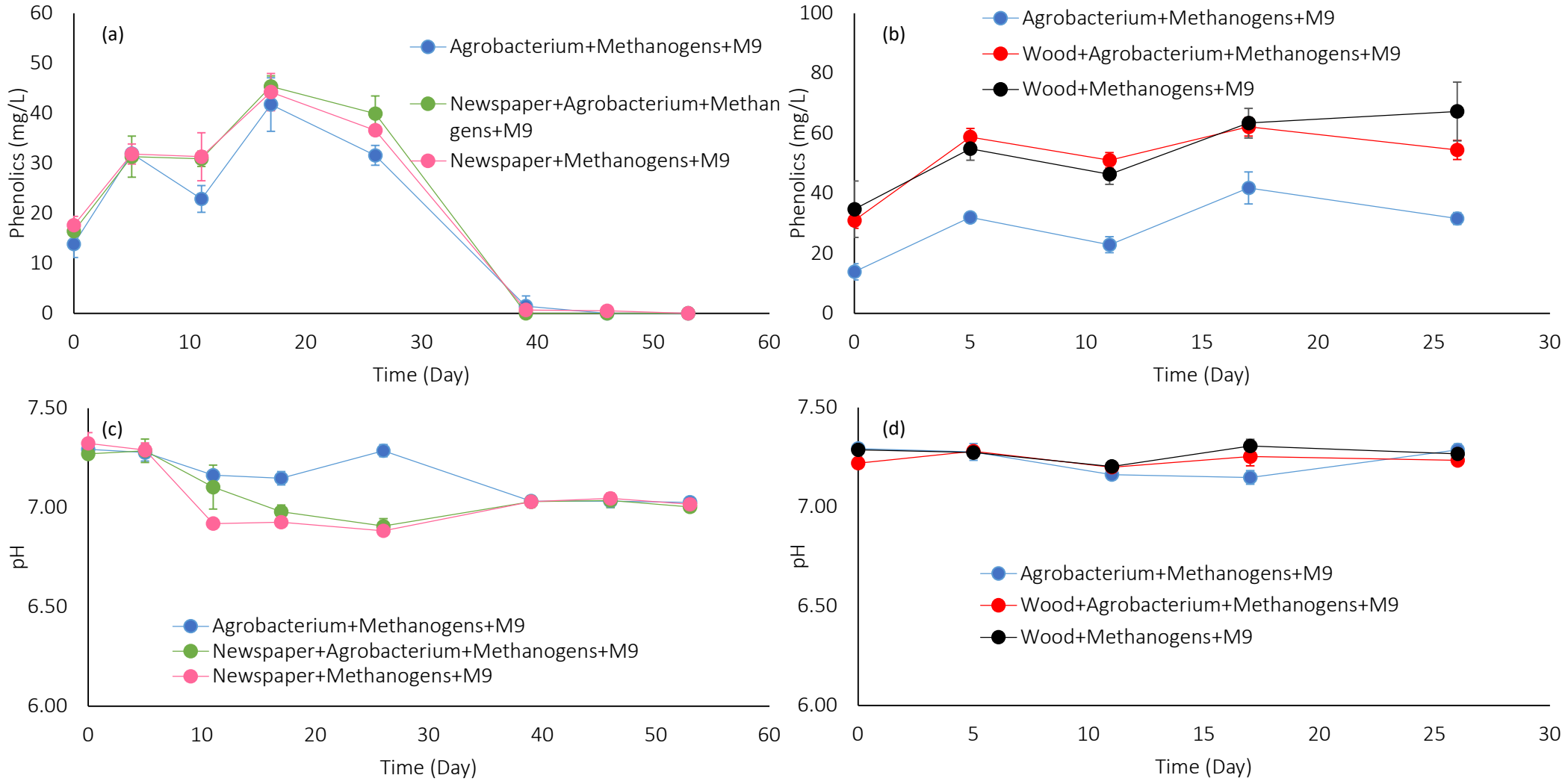


Figure A-0-9 Phenolics (a,b) & pH (c,d) data for the 1-L-scale experiments containing Agrobacterium sp. & methanogens.

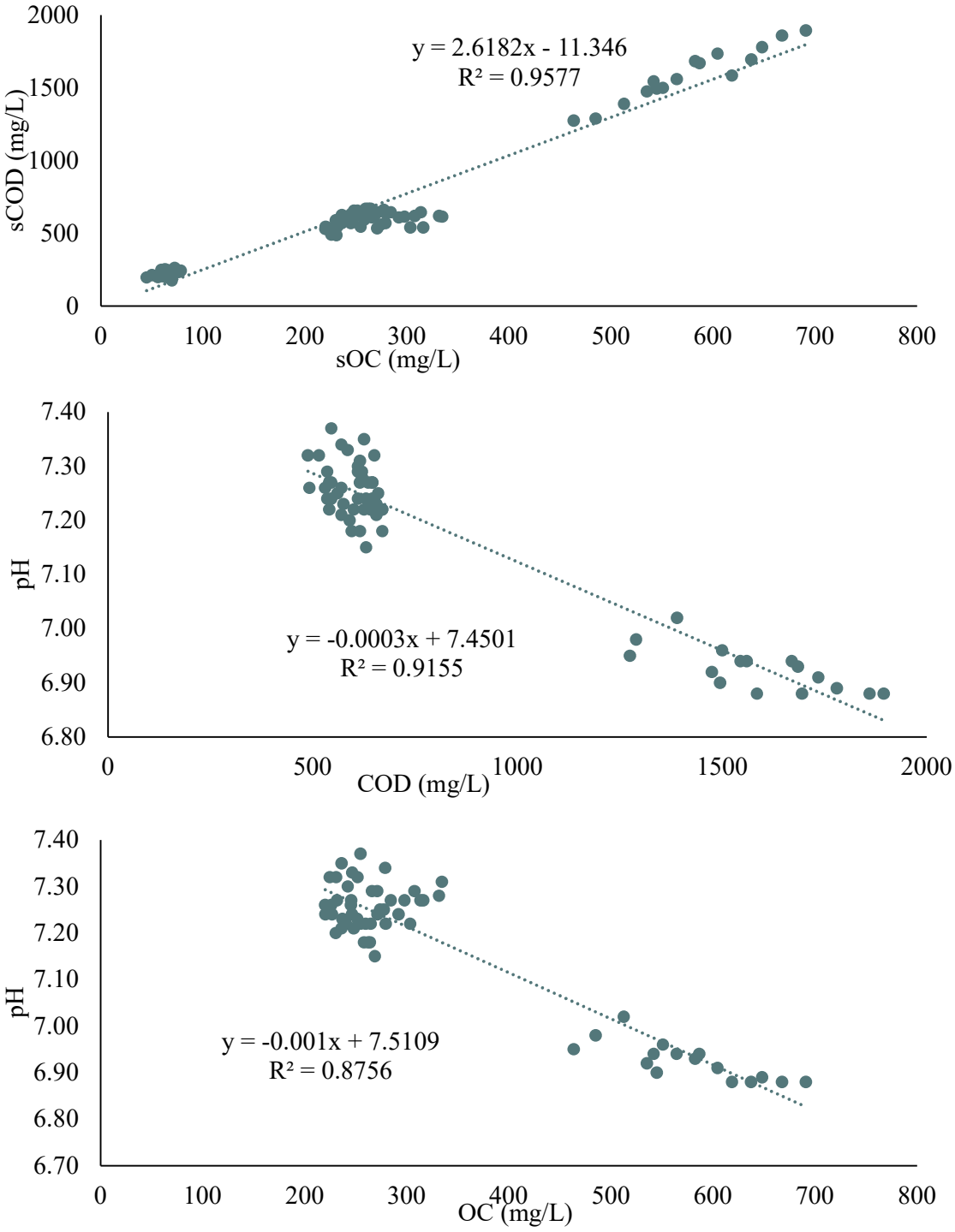
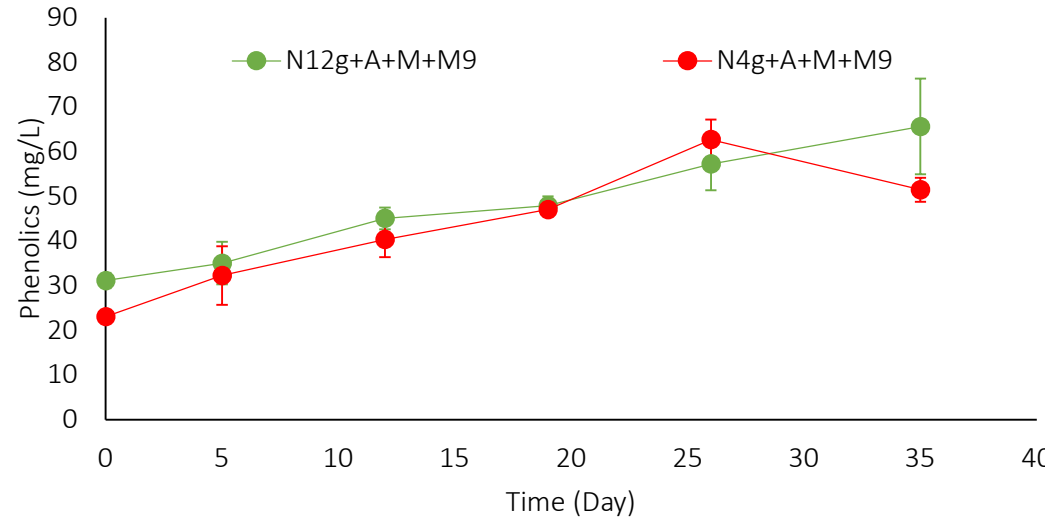


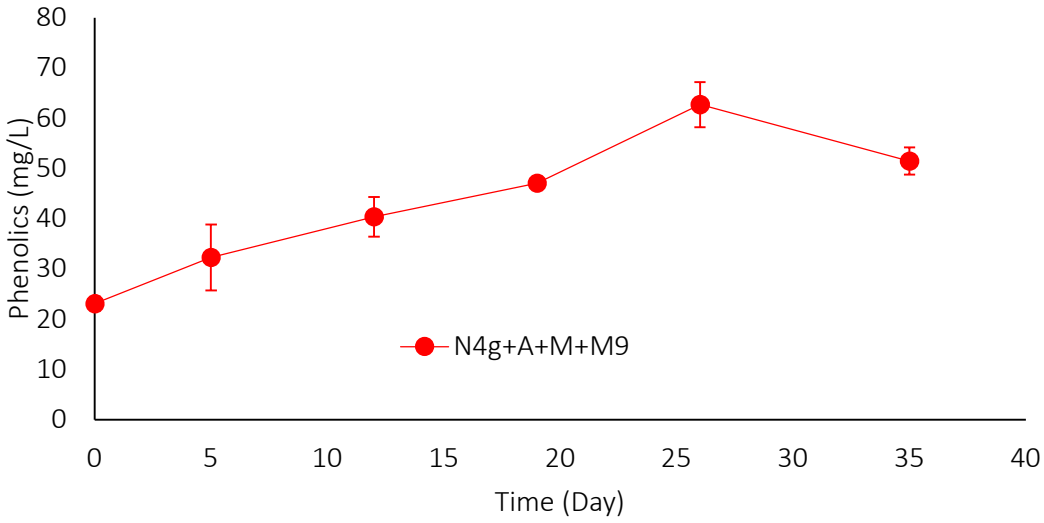
Figure A-0-10 Correlations & R^2 values for different variables for the 1-L-scale experiments containing Agrobacterium sp. & methanogens.

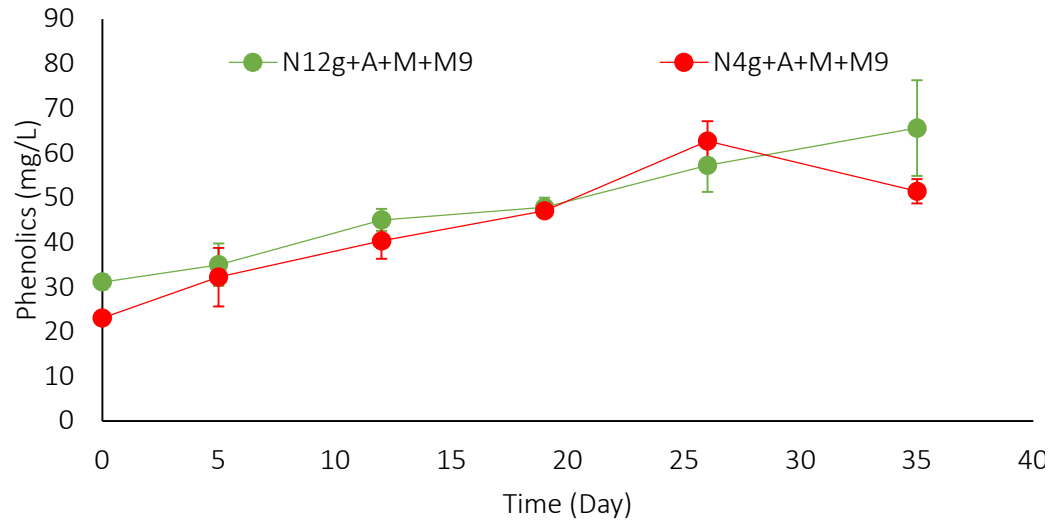
Table A-0-1 Biogas modelling using the Gompertz equation for the 1-L-scale enzymatic experiments & 1-L-scale experiments containing Agrobacterium sp.+methanogens.

Sample	Measured biogas volume (ml or ml/g VS)	Modelled biogas volume (ml or ml/g VS)	R^2	R_{max} (ml/day or ml/g VS day)	λ (day)
NewsSludge*	270.90 ± 9.70	270.96	0.990	89.15	0.72
NewsSludge-E*	318.6 ± 49.7	318.36	0.955	107.35	0.95
WoodSludge*	85.48 ± 0.80	85.36	0.959	8.44	1.54
WoodSludgeE*	41.12 ± 9.50	47.10	0.977	6.26	1.52
Agrobacterium+Methanogens+M9”	81.26 ± 7.16	81.26	0.995	35.97	6.33
Newspaper+Agrobacterium+Methanogens+M9”	153.63 ± 26.28	153.29	0.998	14.68	7.62
Newspaper(12g) +Agrobacterium+Methanogens+M9”	1162.11 ± 151.39	1120.67	0.996	73.14	9.78
Wood(12g) +Agrobacterium+Methanogens+M9”	35.18 ± 5.38	33.74	0.948	2.12	9.52

Note: *Data presented on the basis of ml/g of VS. “Data presented as ml.







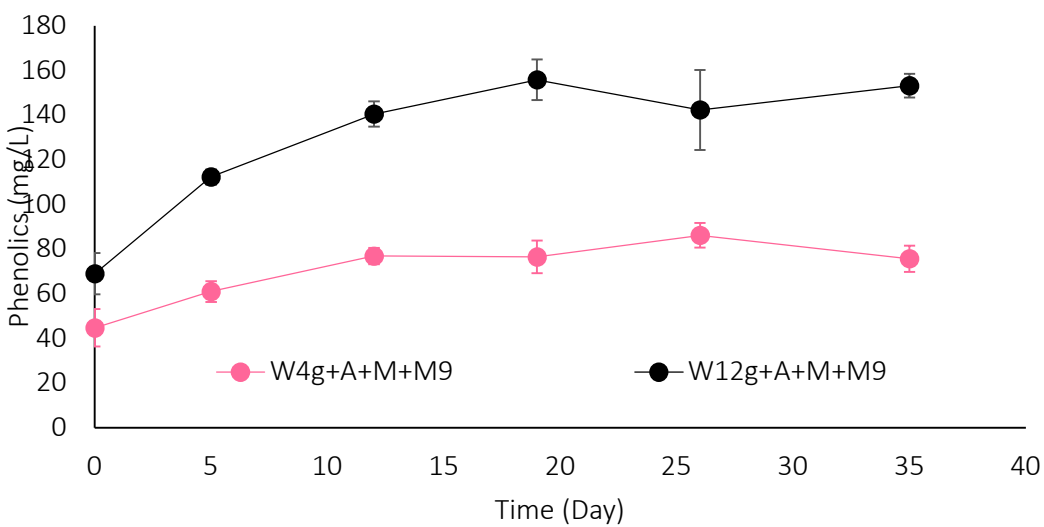
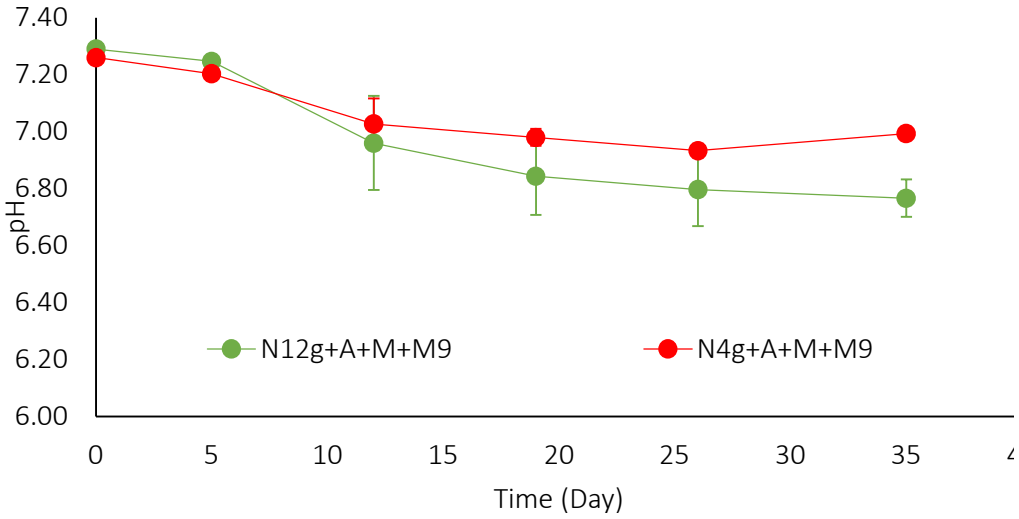
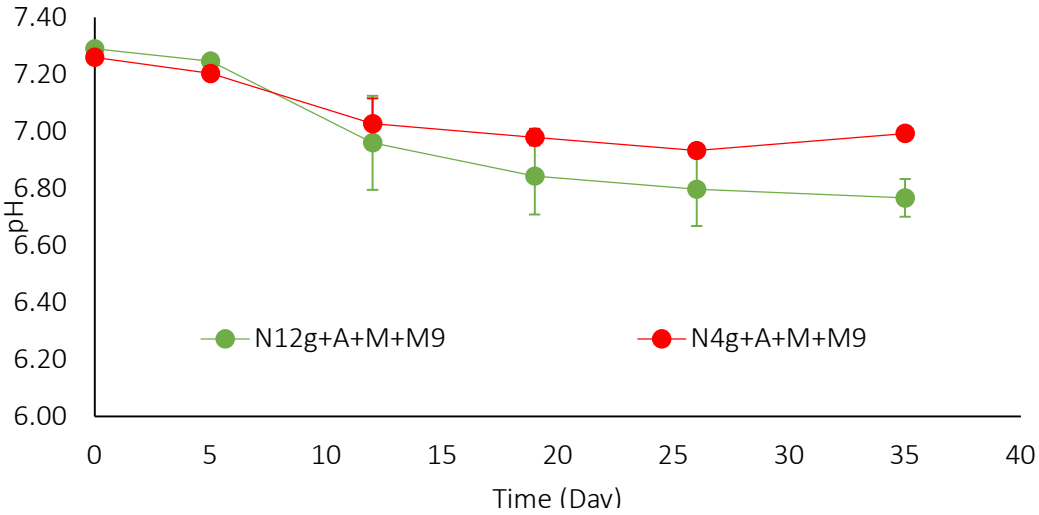
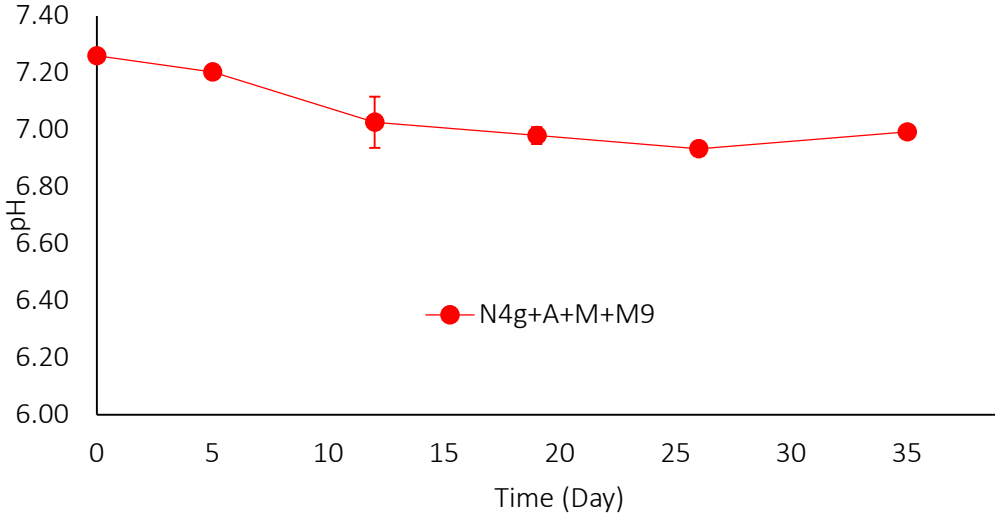


Figure A-0-11 Phenolics data for the 1-L-scale experiments containing Agrobacterium sp. and methanogens, studying the impact of increasing the Solid:Liquid







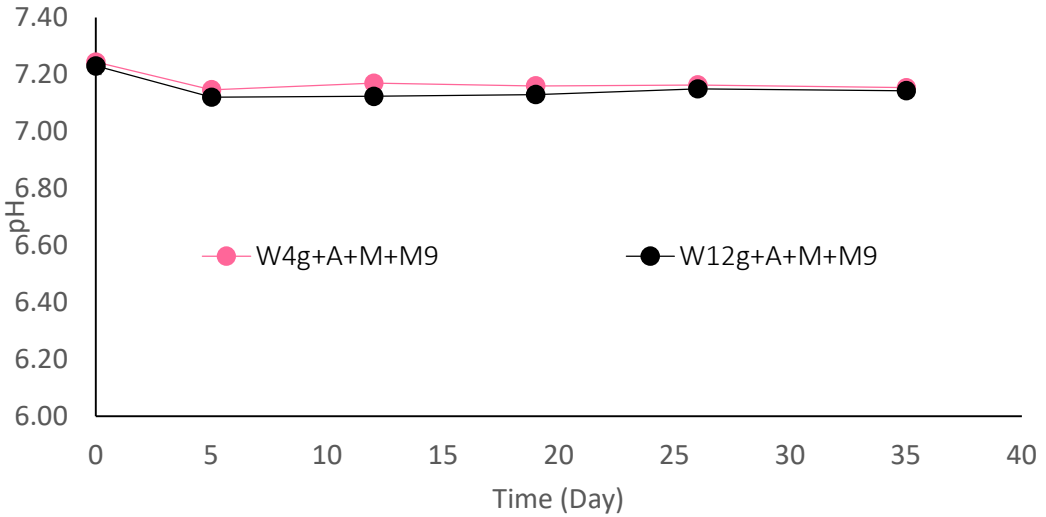


Figure A-0-12 pH data for the 1-L-scale experiments containing Agrobacterium sp. and methanogens, studying the impact of increasing the Solid:Liquid ratio.

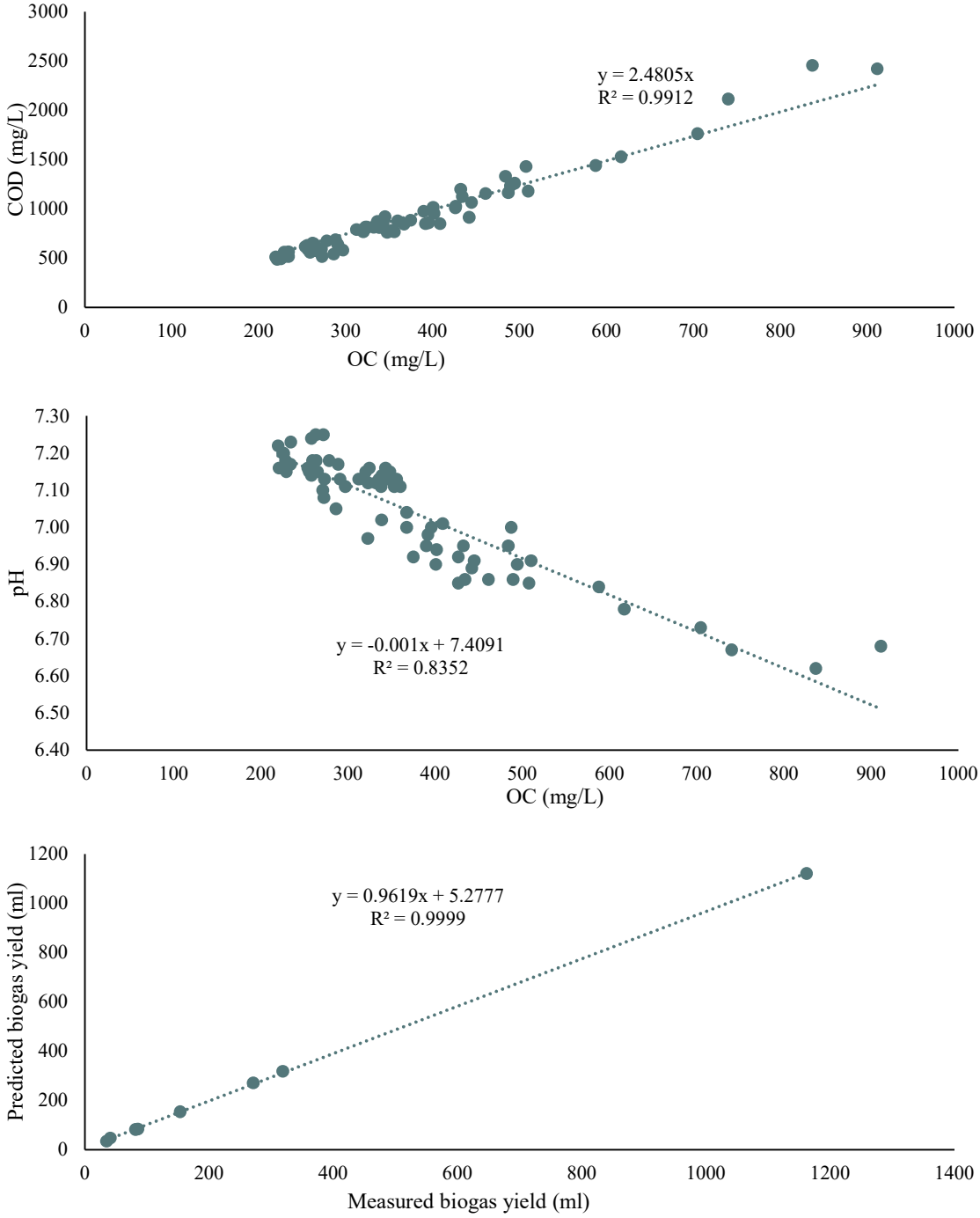
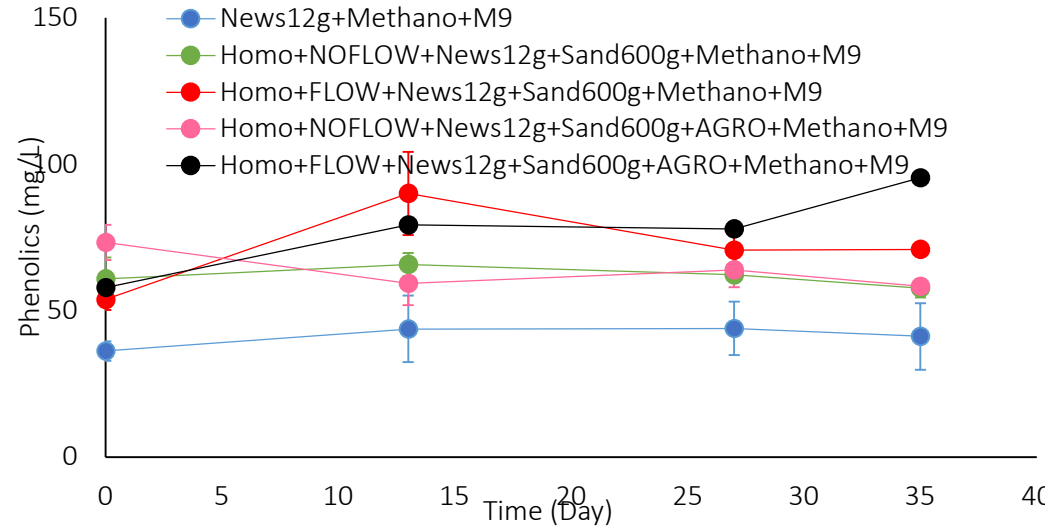
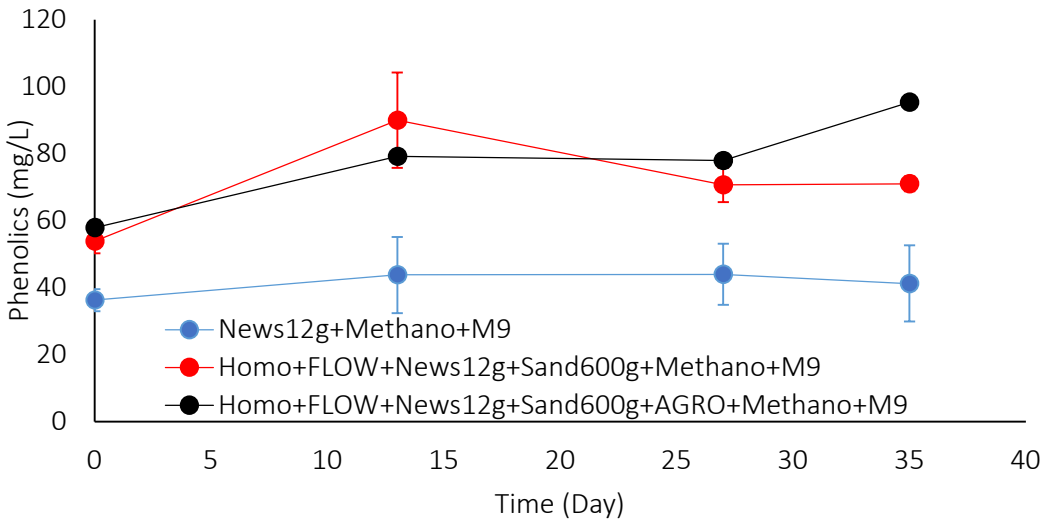
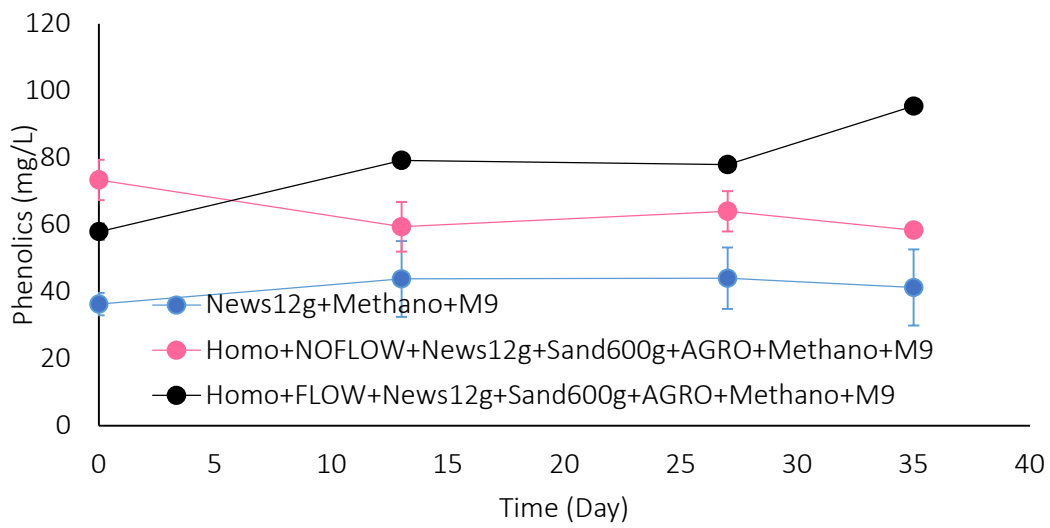


Figure A-0-13 Correlations for various variables in the 1-L-scale experiments containing Agrobacterium sp. and methanogens, studying the impact of increasing the Solid:Liquid ratio. (c) Modelled and measured biogas yields in this experiment with Gompertz equation.







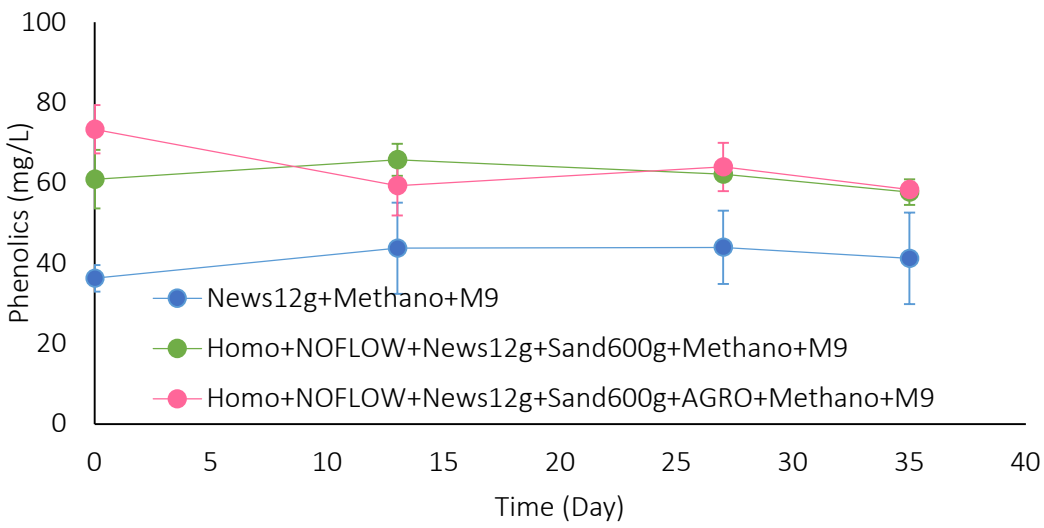


Figure A-0-14 Phenolics data for the homogeneous pore structure flow tests with Agrobacterium sp. and methanogens. OVERALL PORT.

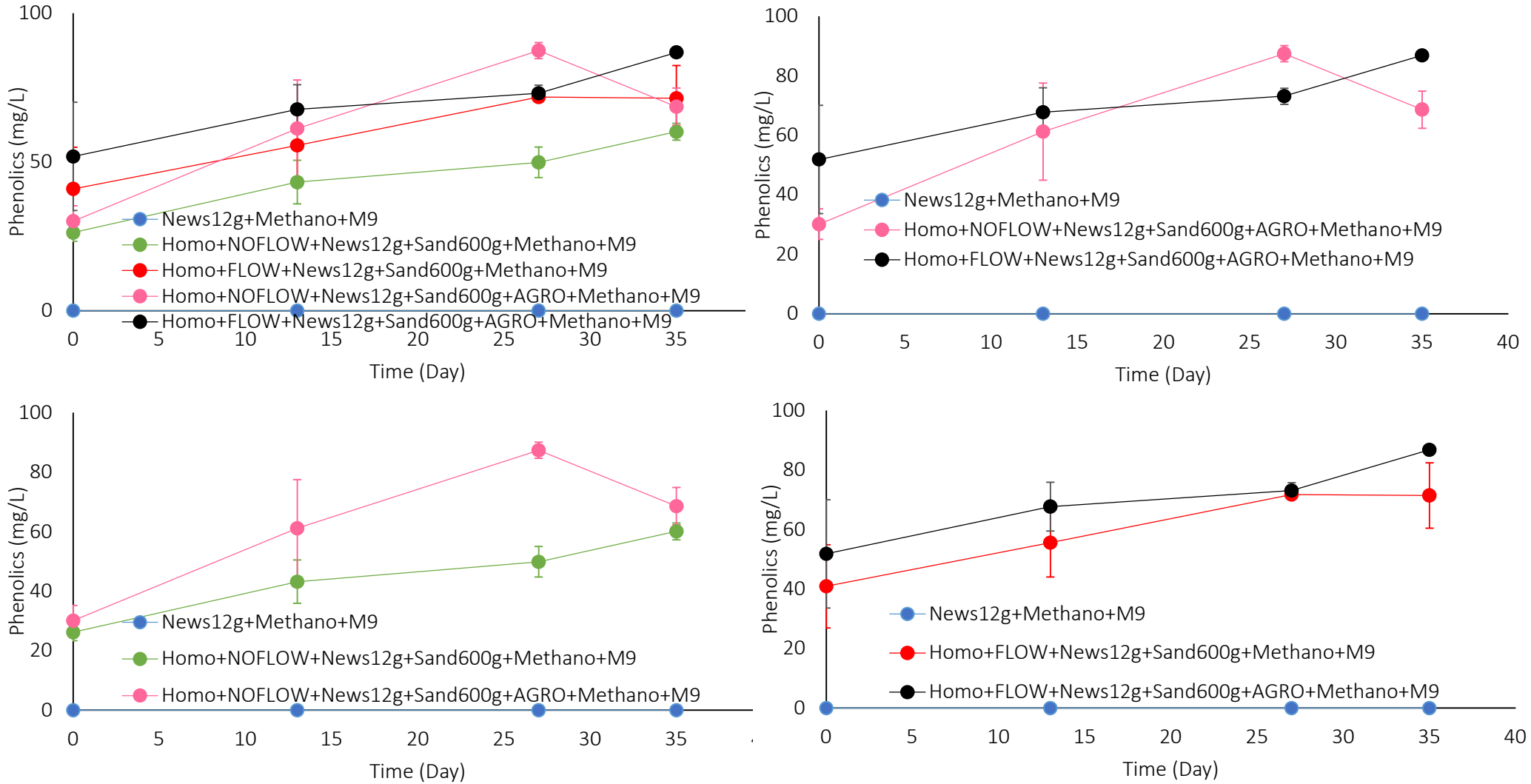
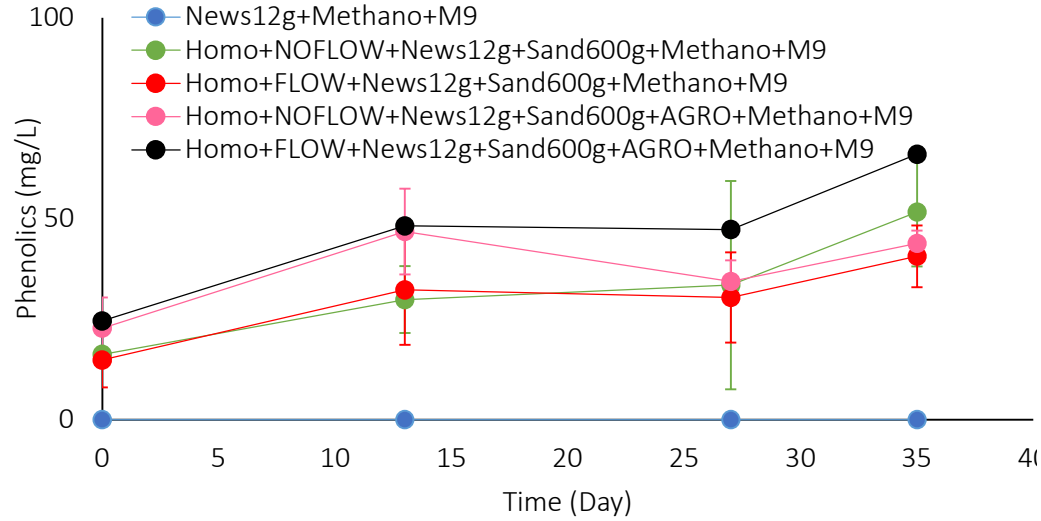
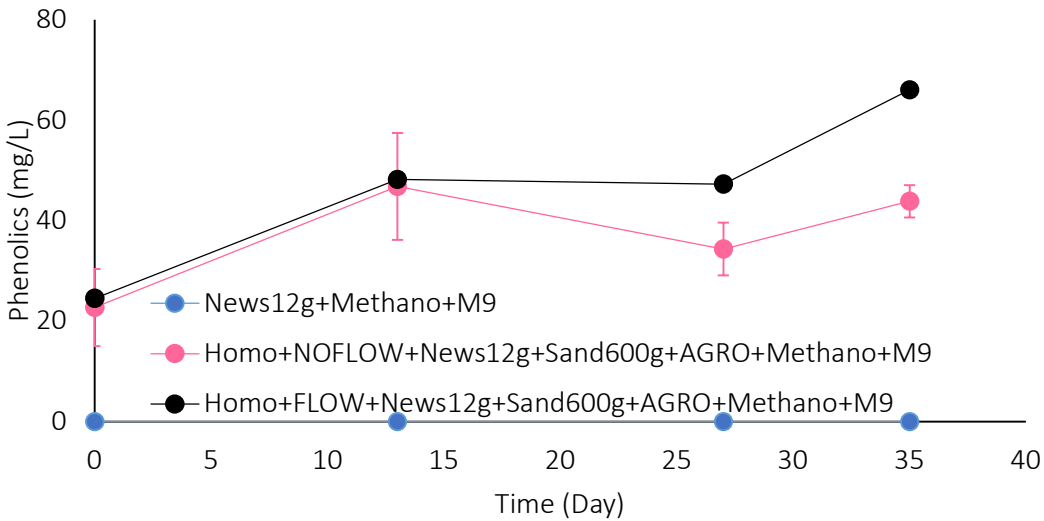
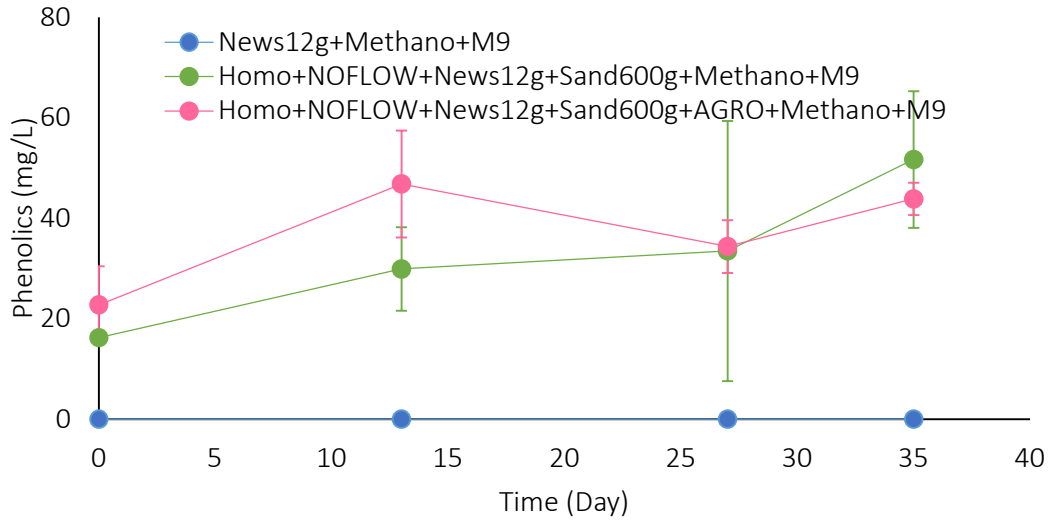


Figure A-0-15 Phenolics data for the homogeneous pore structure flow tests with Agrobacterium sp. and methanogens. TOP PORT.







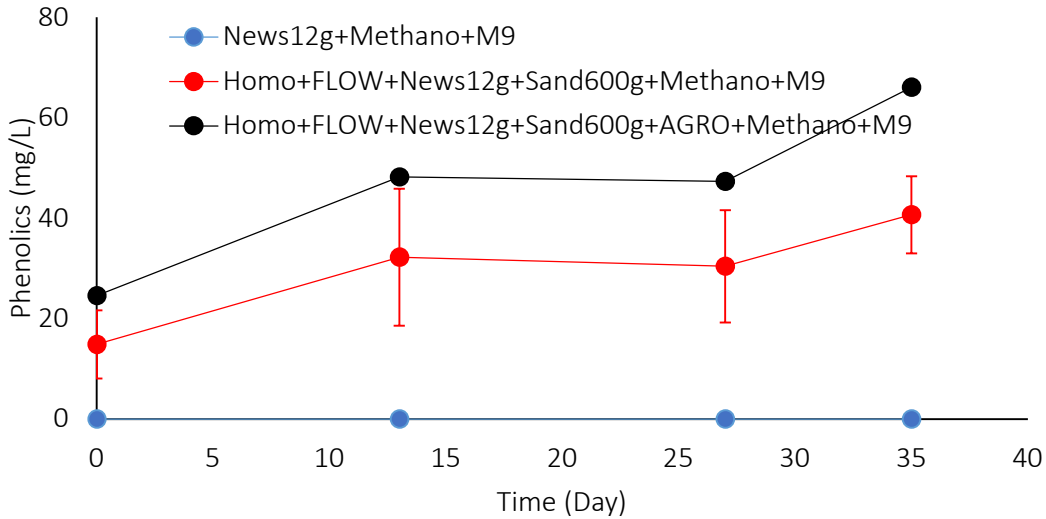
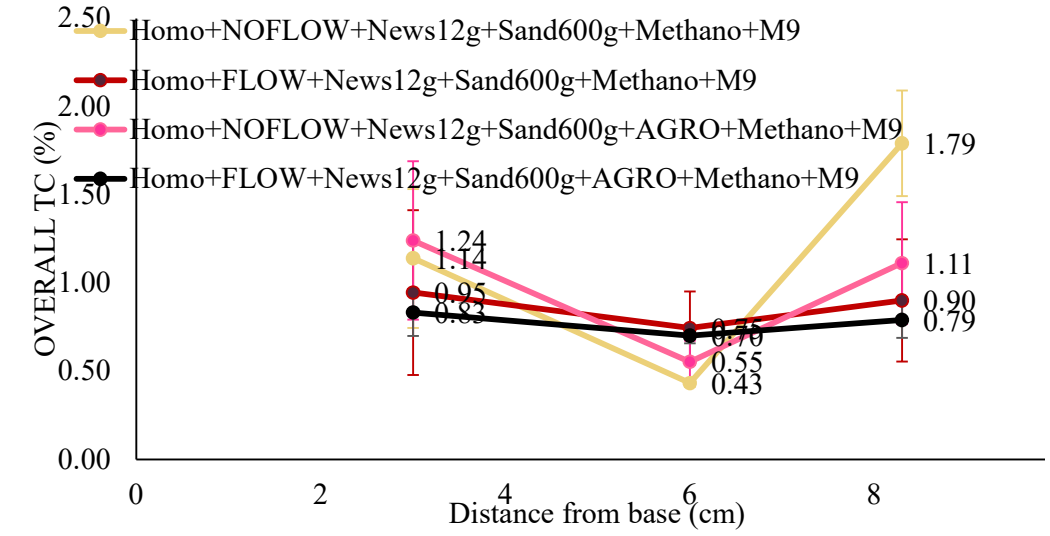
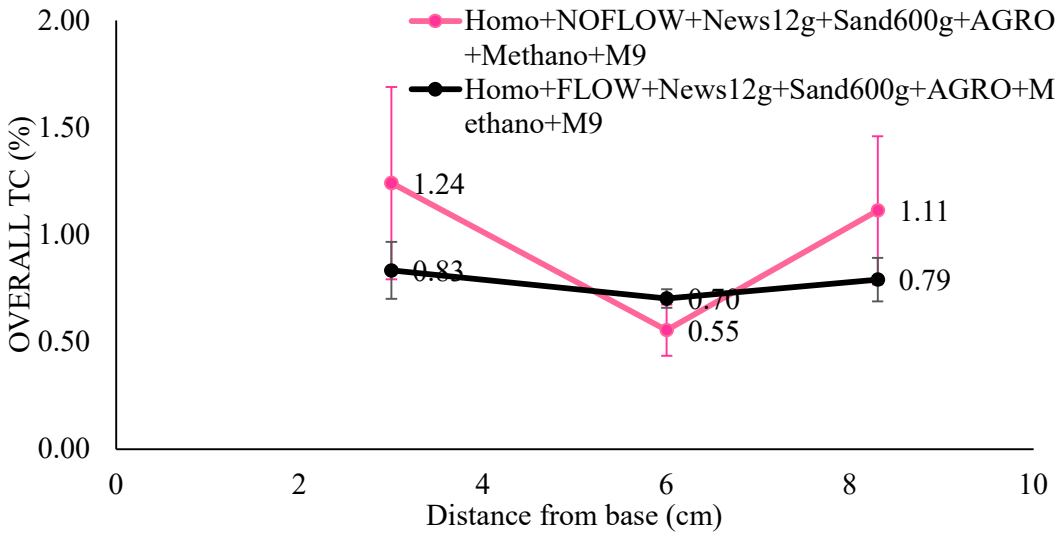
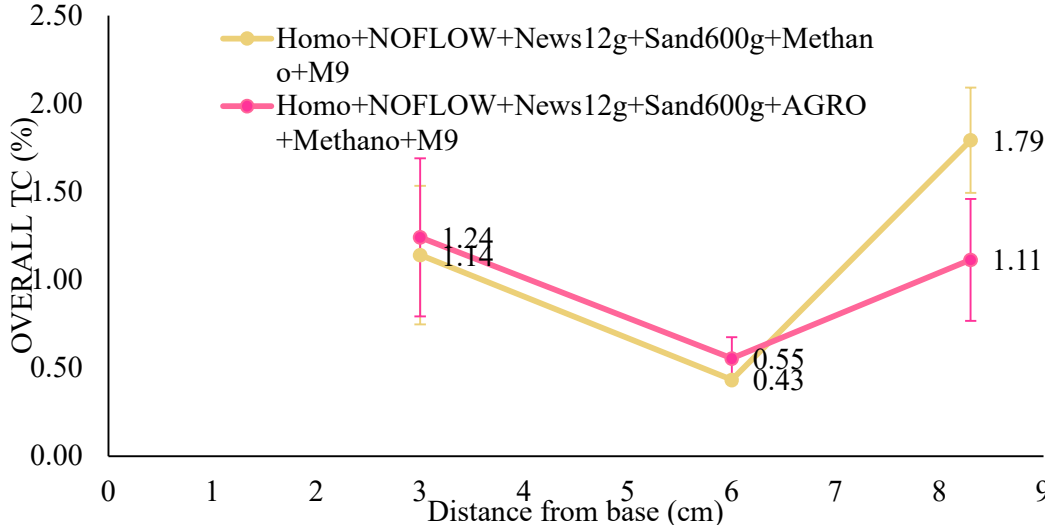


Figure A-0-16 Phenolics data for the homogeneous pore structure flow tests with Agrobacterium sp. and methanogens. BOTTOM PORT.







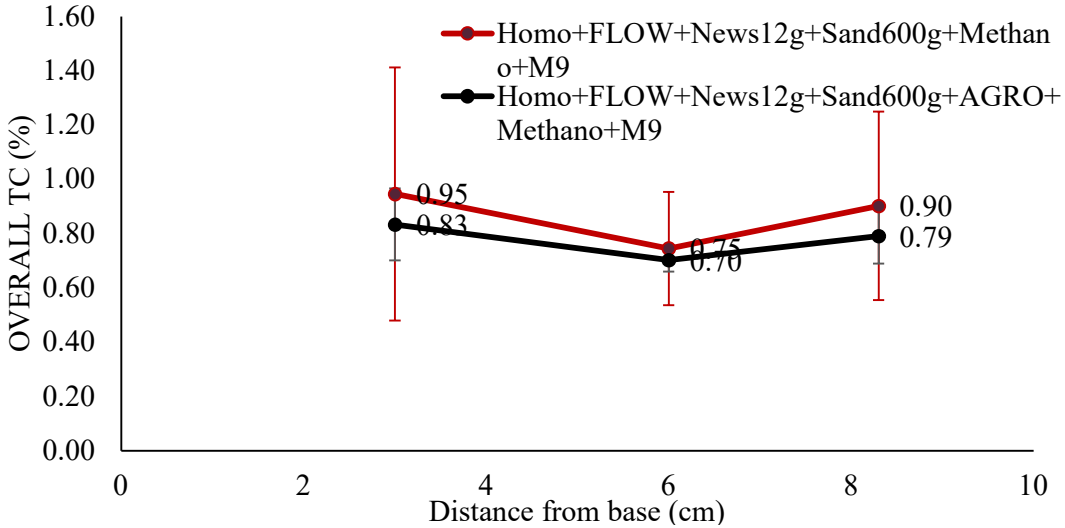
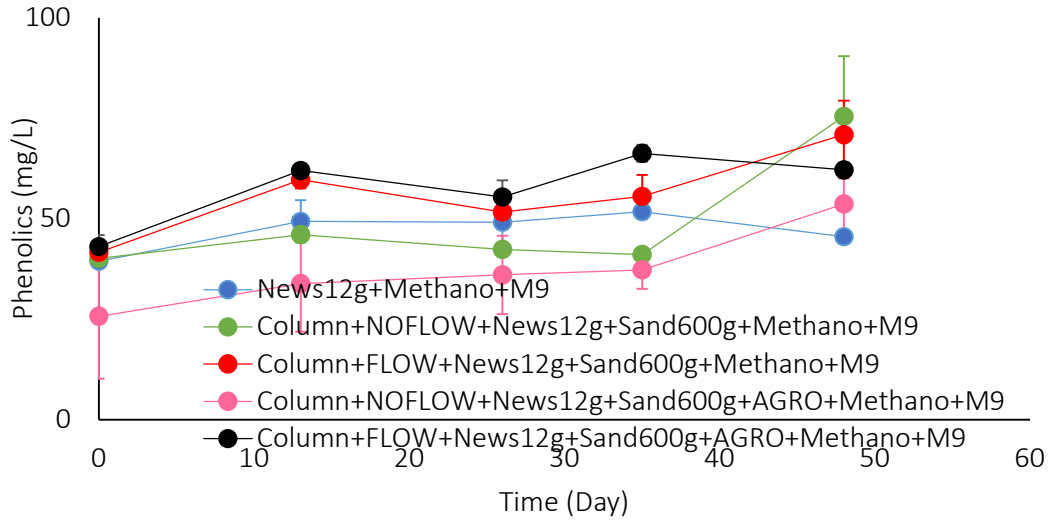
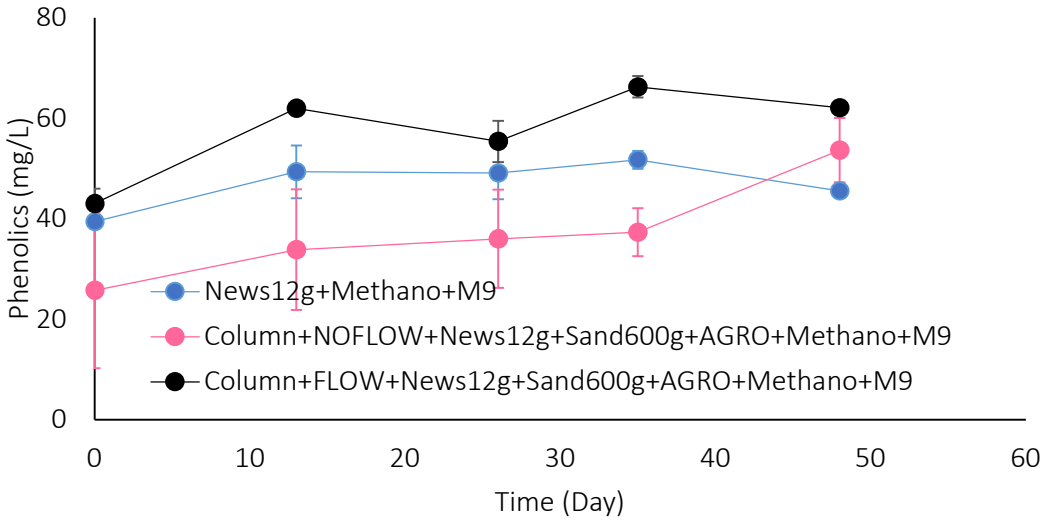
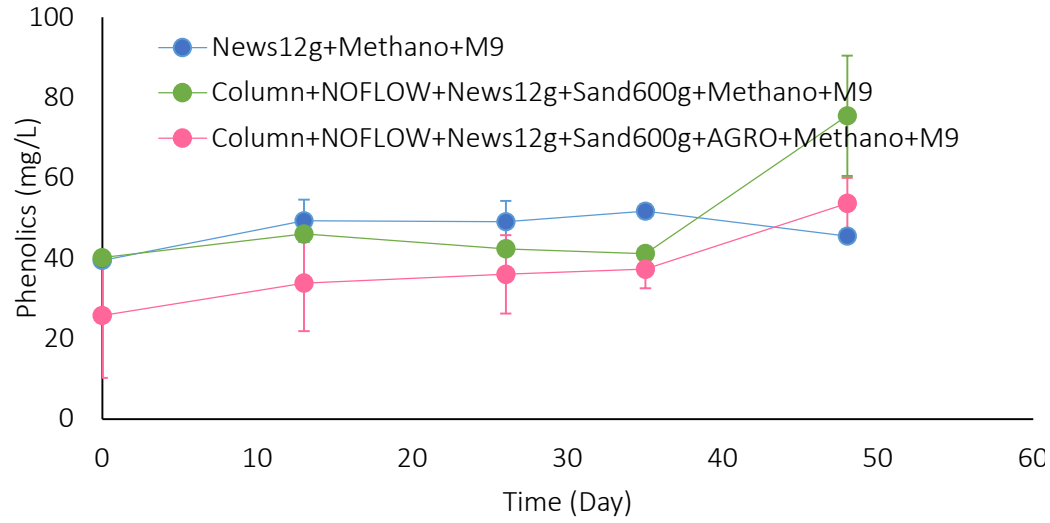


Figure A-0-17 TC data of the solid fraction containing sand and newspaper following the homogeneous pore structure flow tests with Agrobacterium sp. and methanogens.







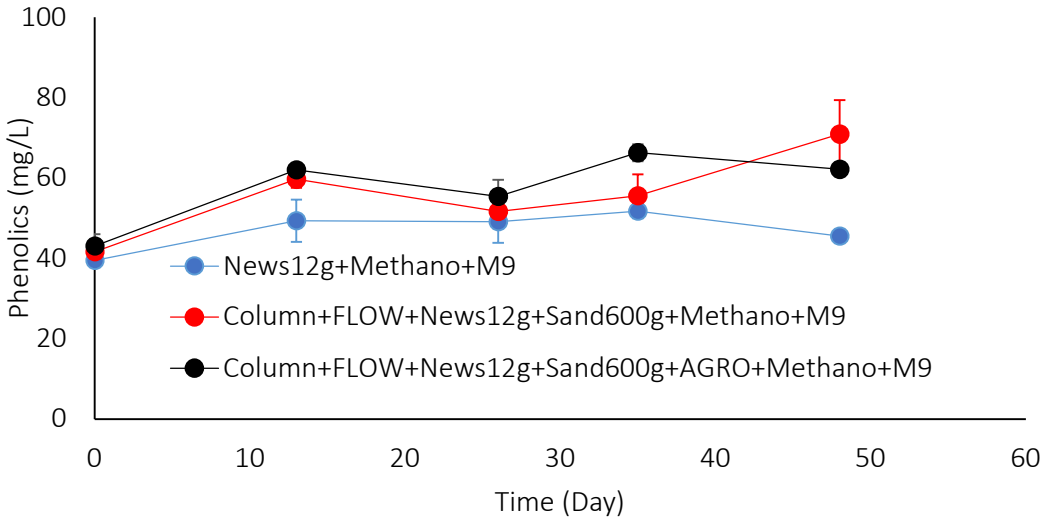
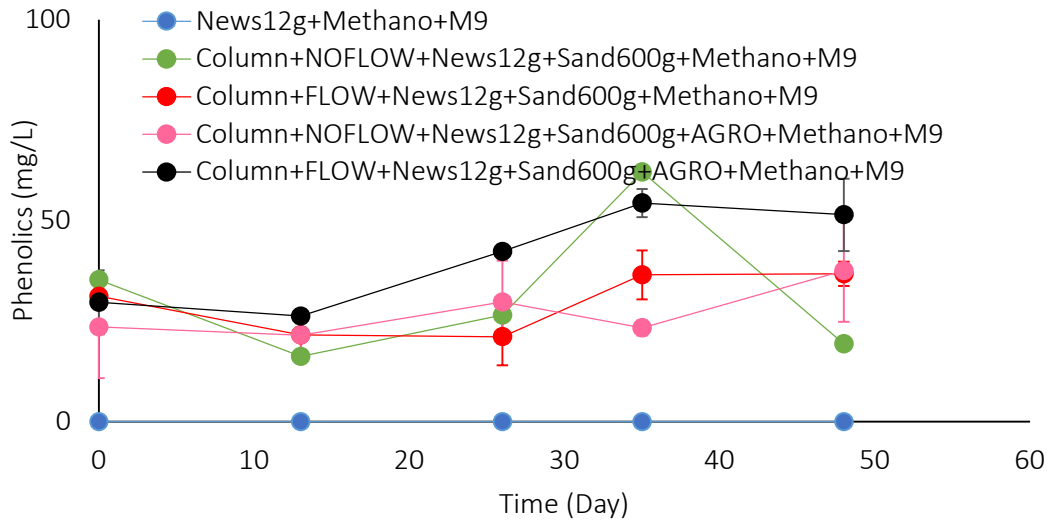
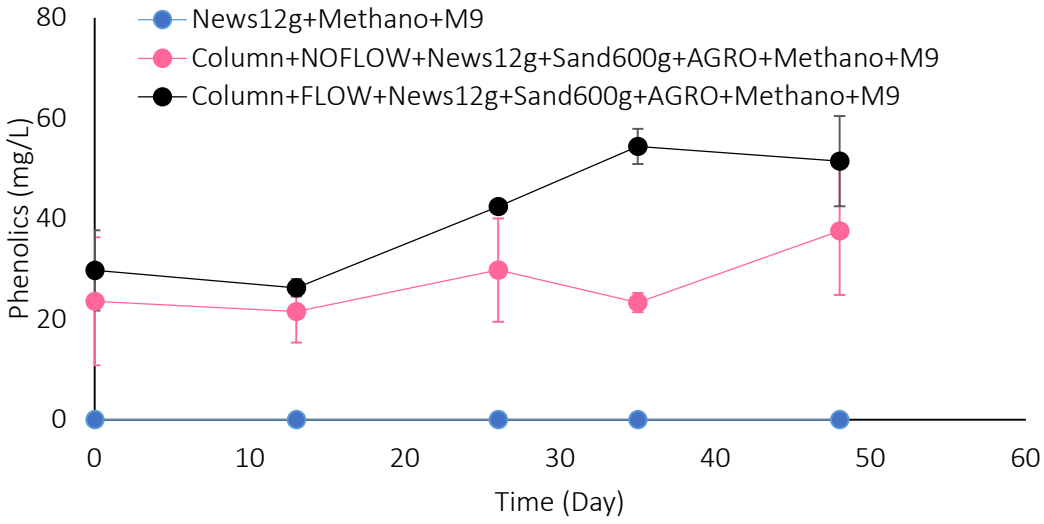
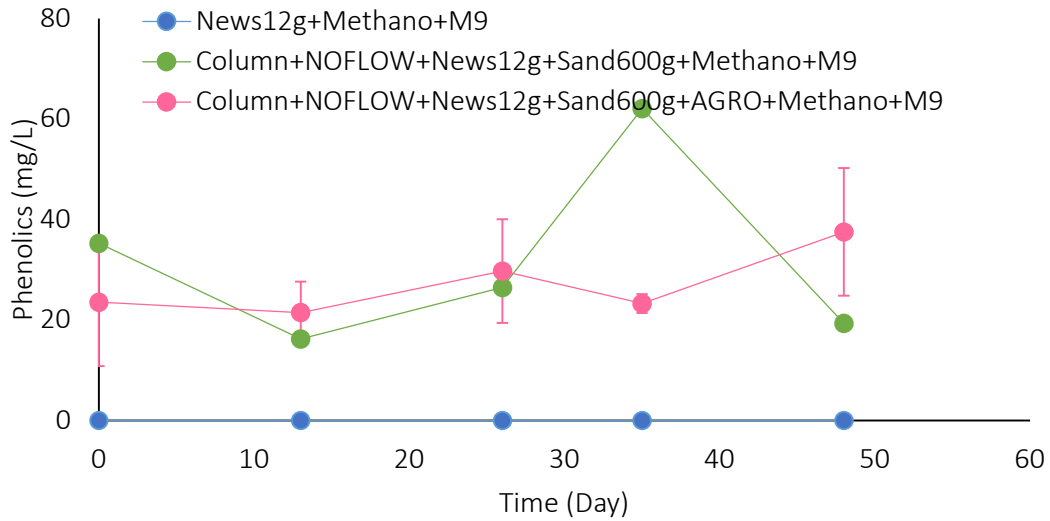


Figure A-0-18 Phenolics data for the 1st set of heterogeneous pore structure flow tests with Agrobacterium sp. and methanogens. OVERALL PORT.







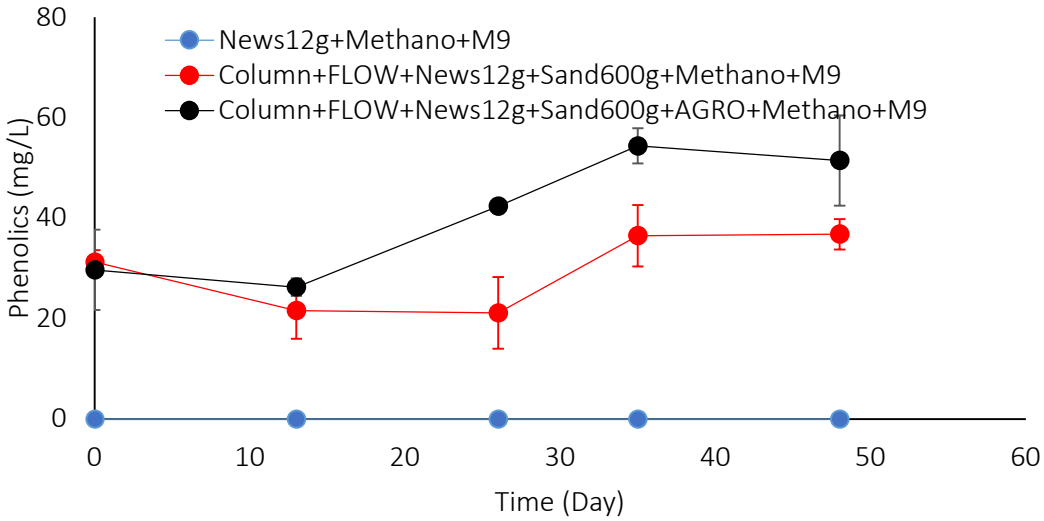
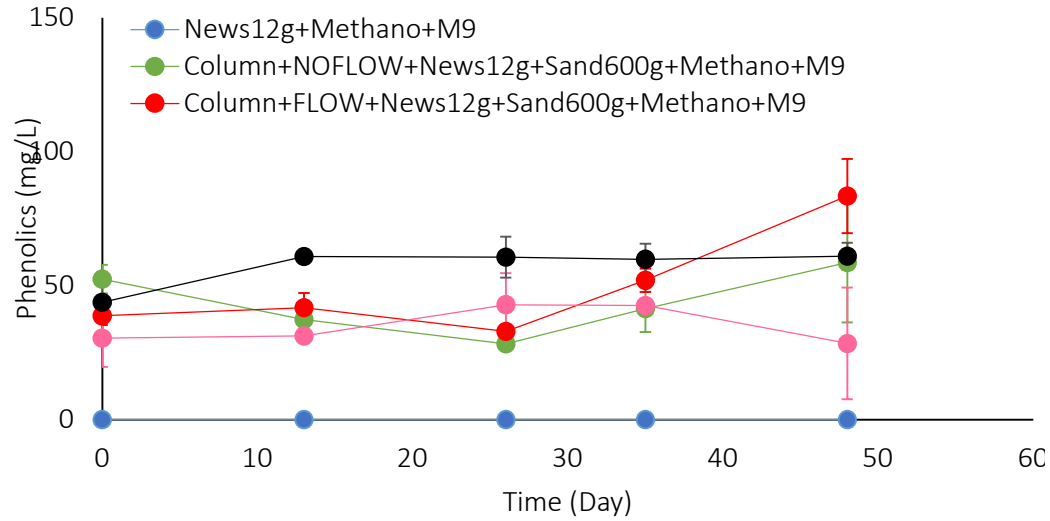
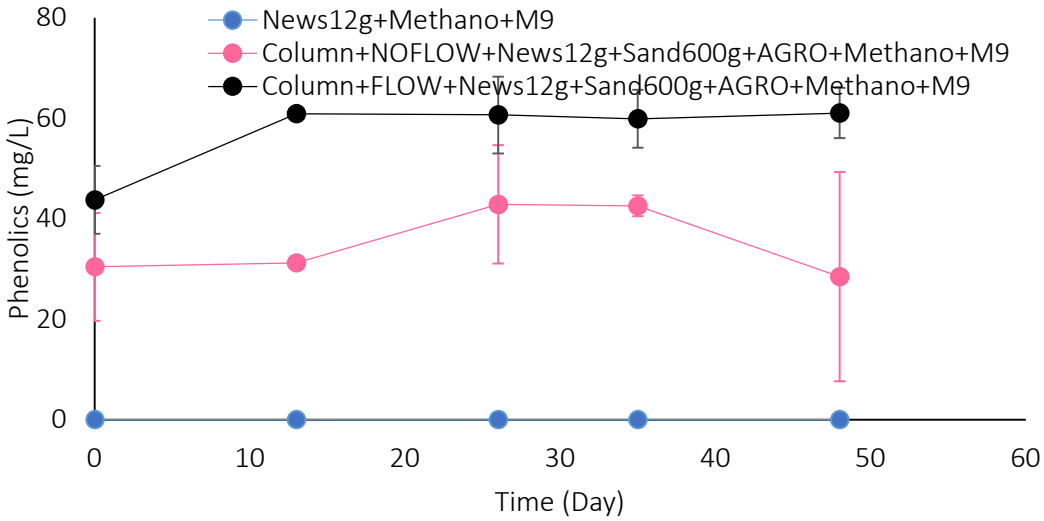
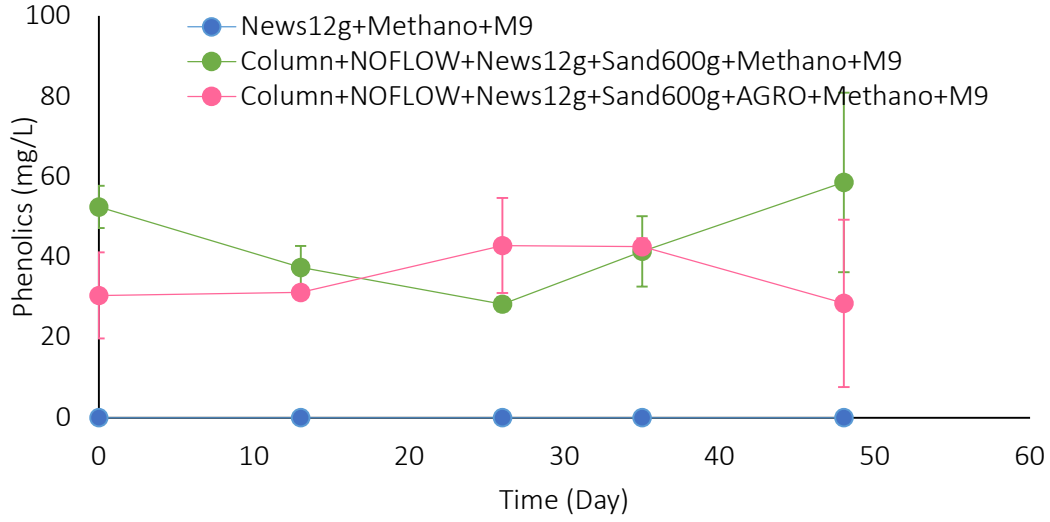


Figure A-0-19 Phenolics data for the 1st set of heterogeneous pore structure flow tests with Agrobacterium sp. and methanogens. INSIDE PORT.







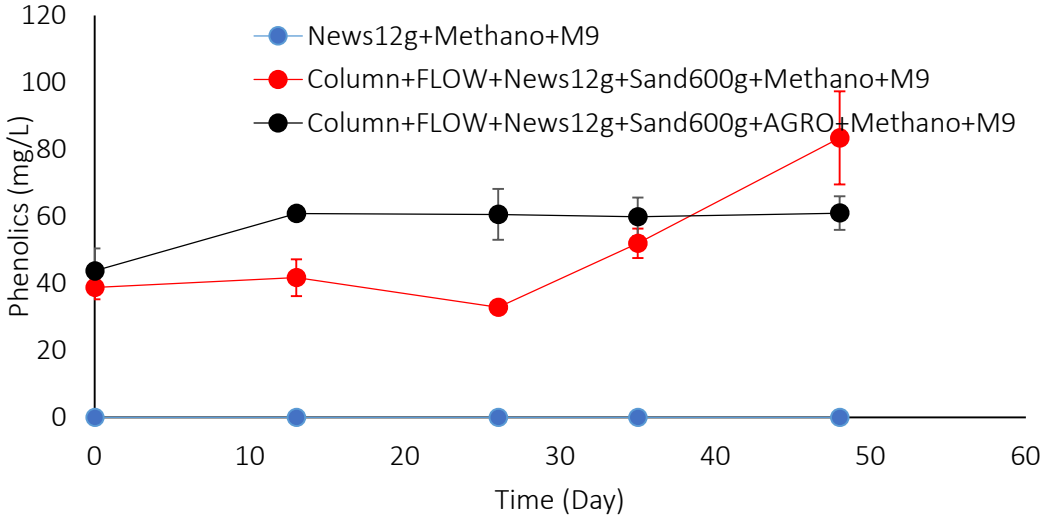


Figure A-0-20 Phenolics data for the 1st set of heterogeneous pore structure flow tests with Agrobacterium sp. and methanogens. OUTSIDE PORT.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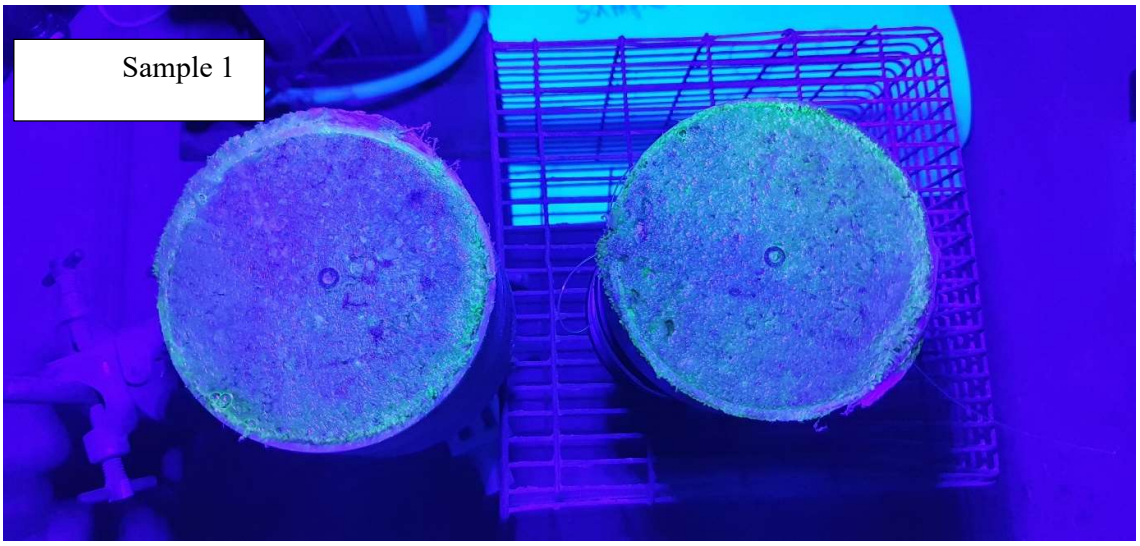

Sample 1


Sample 2
sample: 2

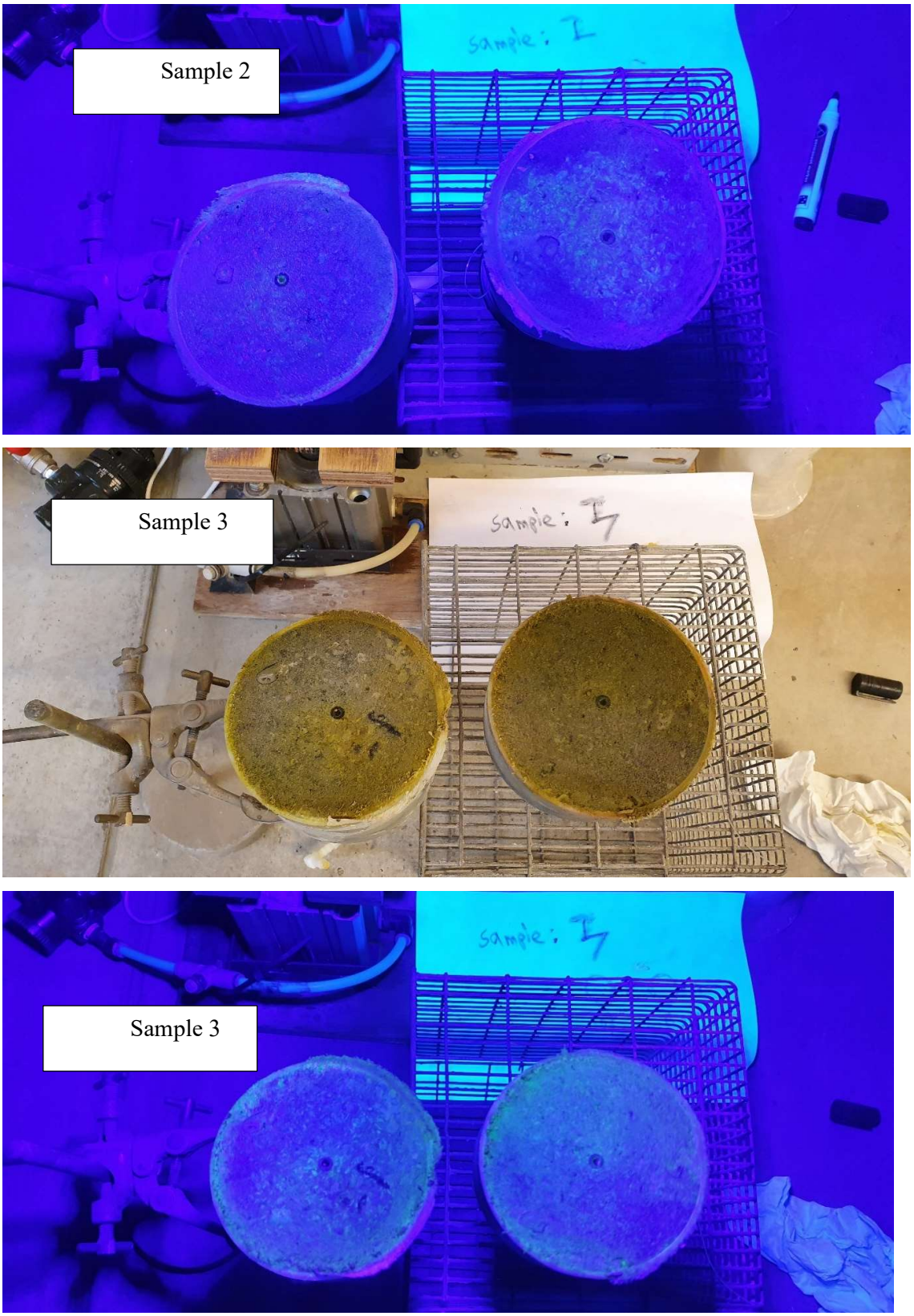
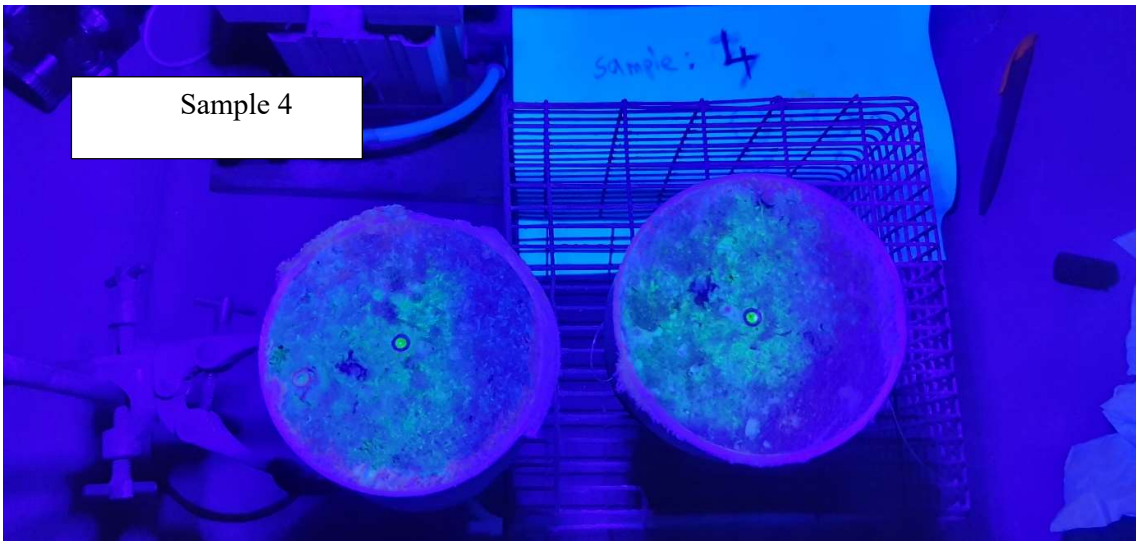
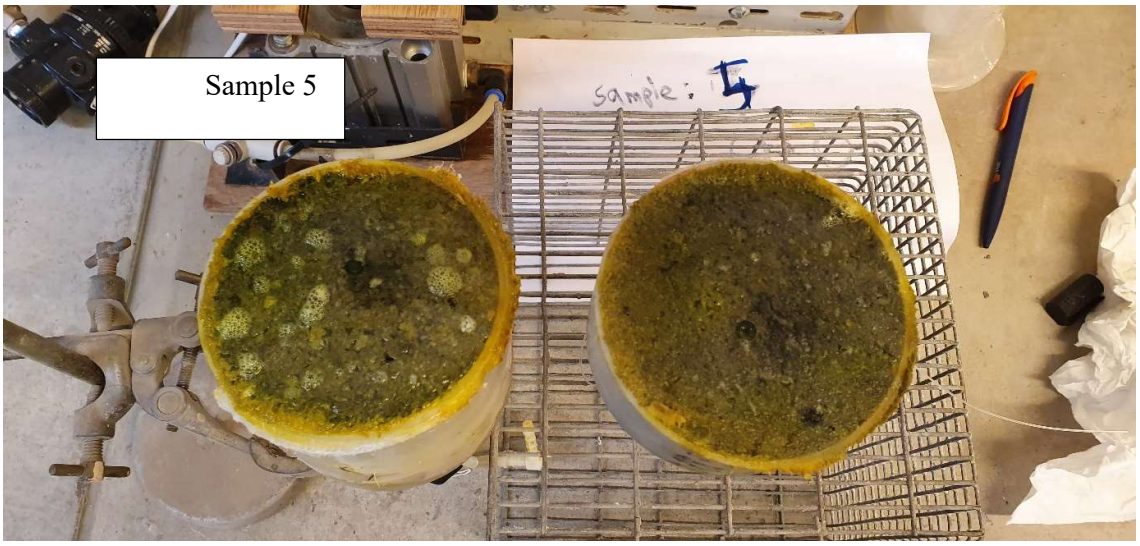
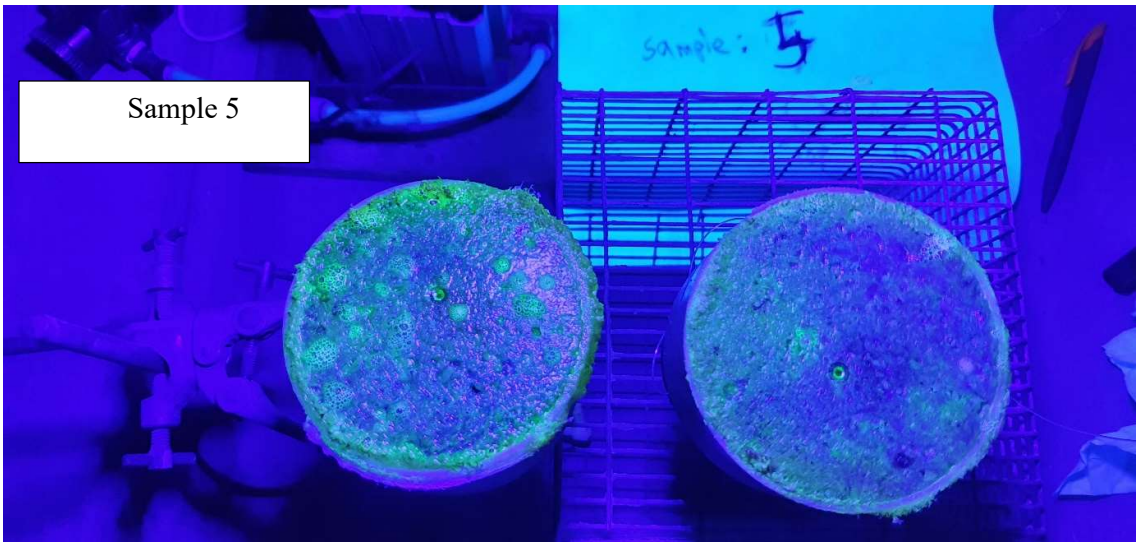


Figure A-0-21 Heterogeneous lab-scale landfills. Experimental conditions: NOFLOW+NEWS12g+SAND600g+METHANO+M9.

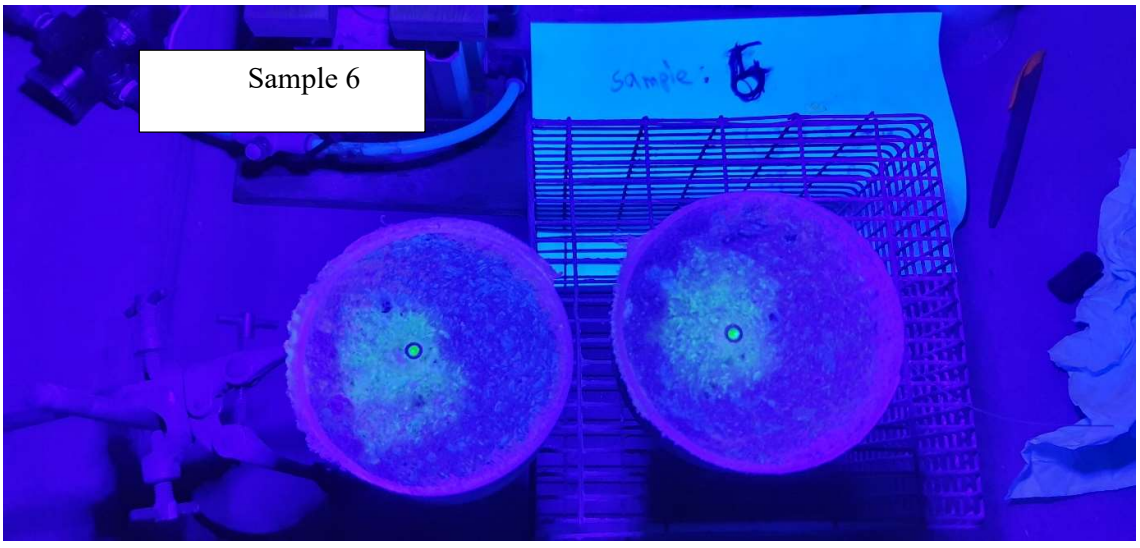

Sample 4
sample: 4


Sample 4
sample: 4


Sample 5
sample: 5


Sample 5
sample: 5


Sample 6
sample: 6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sample: 6

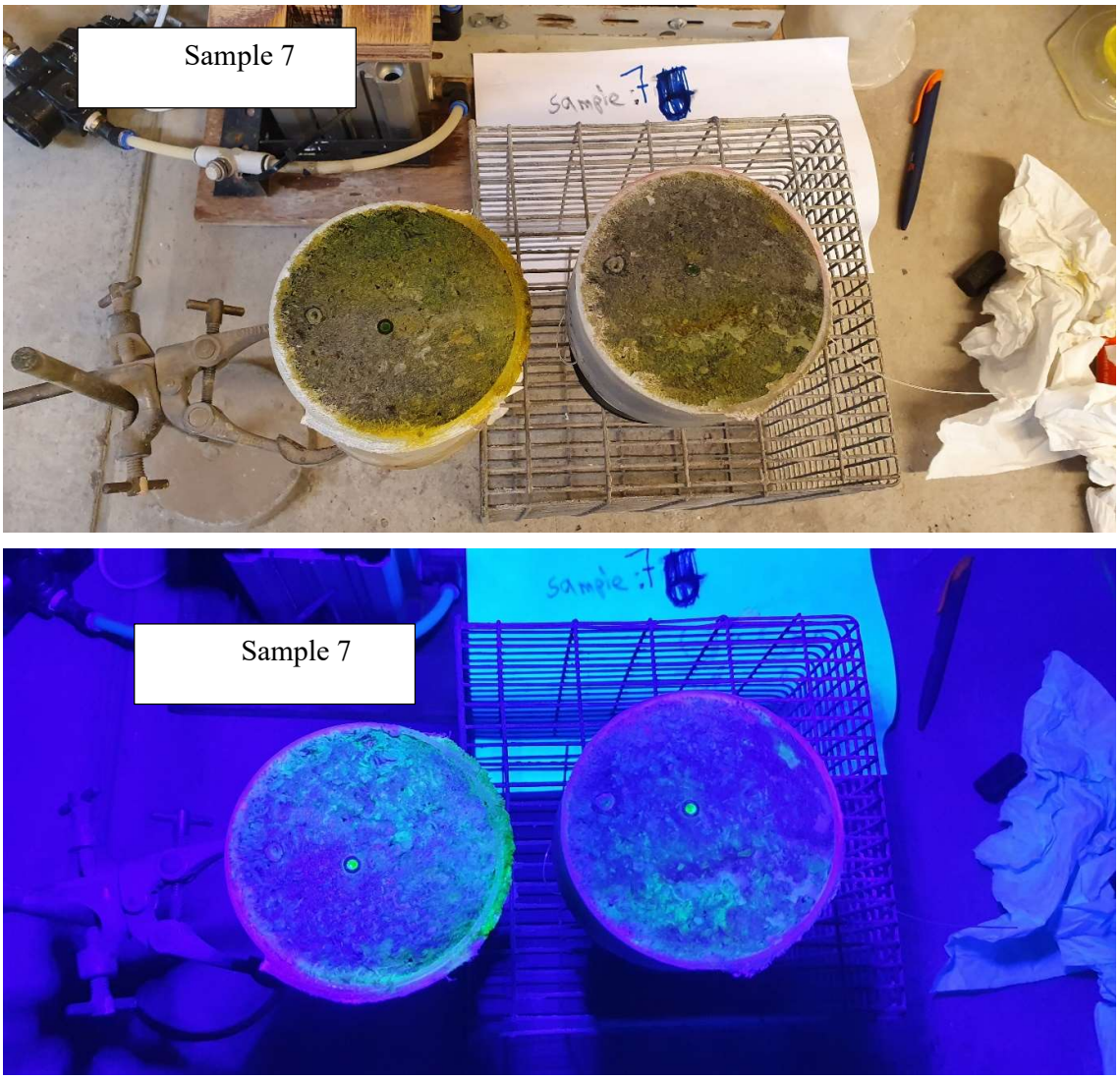
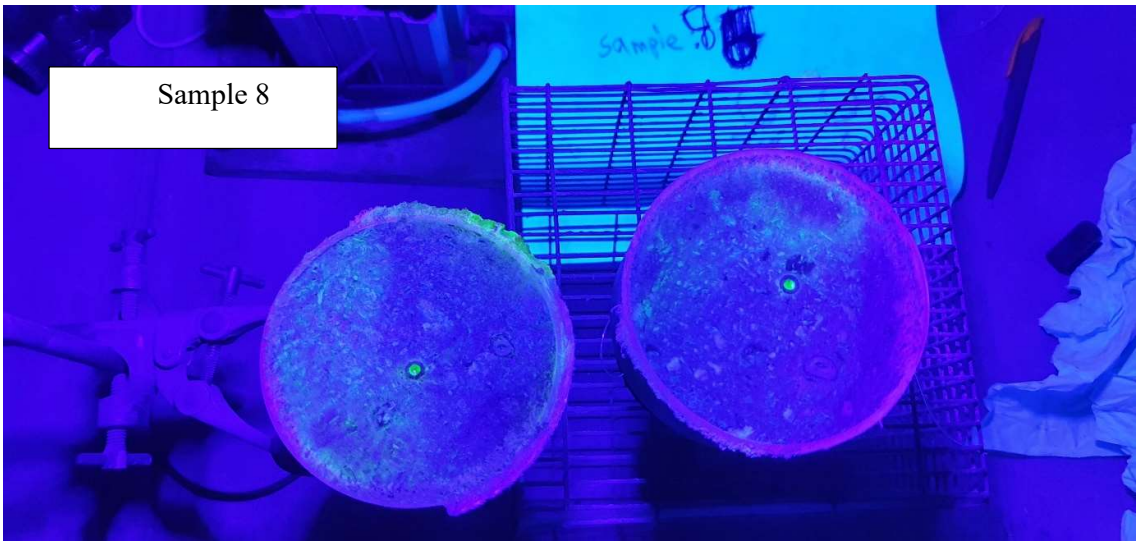


Figure A-0-22 Heterogeneous lab-scale landfills. Experimental conditions: FLOW+NEWS12g+SAND600g+METHANO+M9.


Sample 8


Sample 8


Sample 9

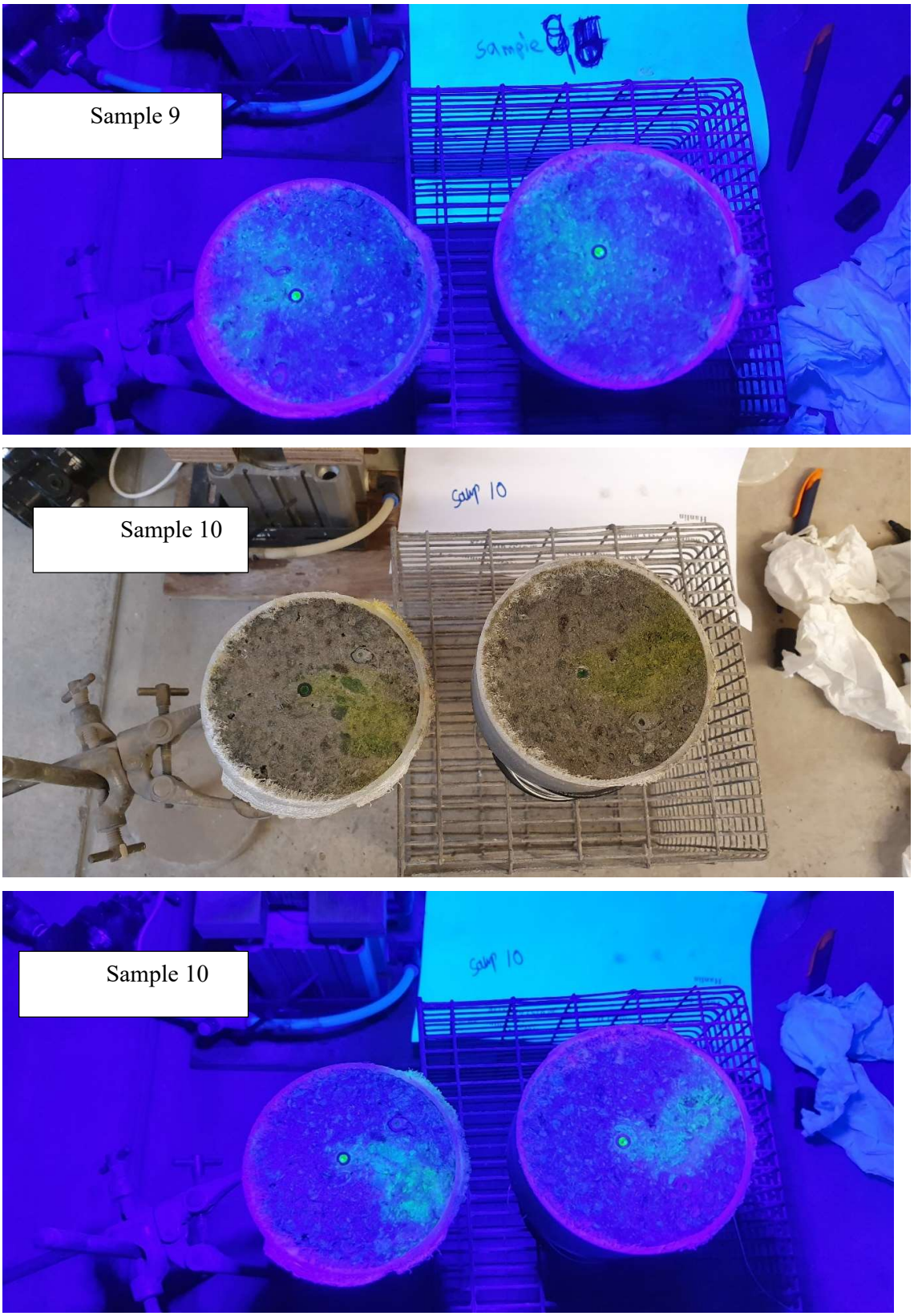
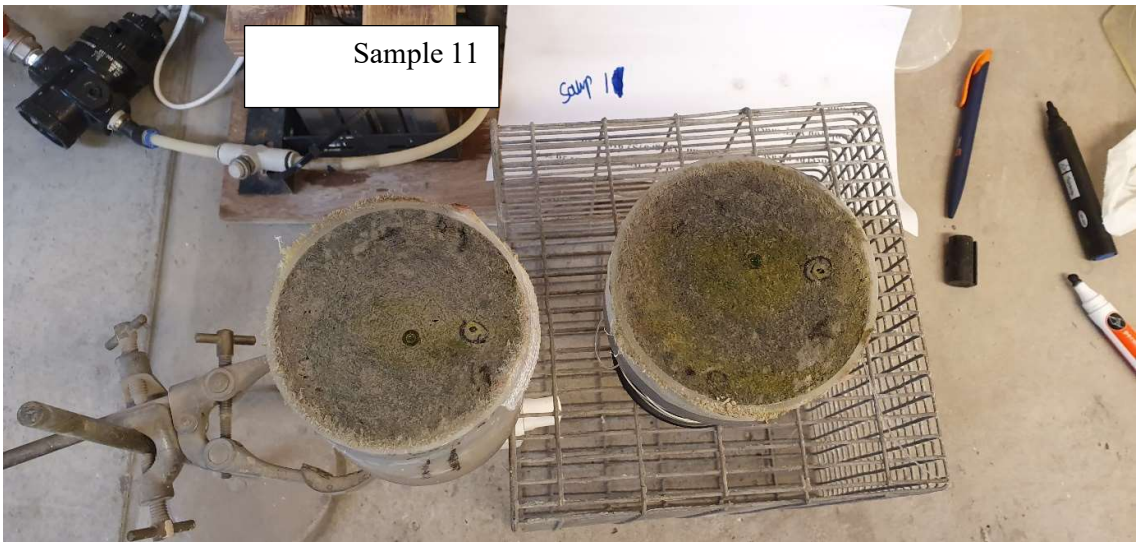


Figure A-0-23 Heterogeneous lab-scale landfills. Experimental conditions: NOFLOW+NEWS12g+SAND600g+AGRO+METHANO+M9.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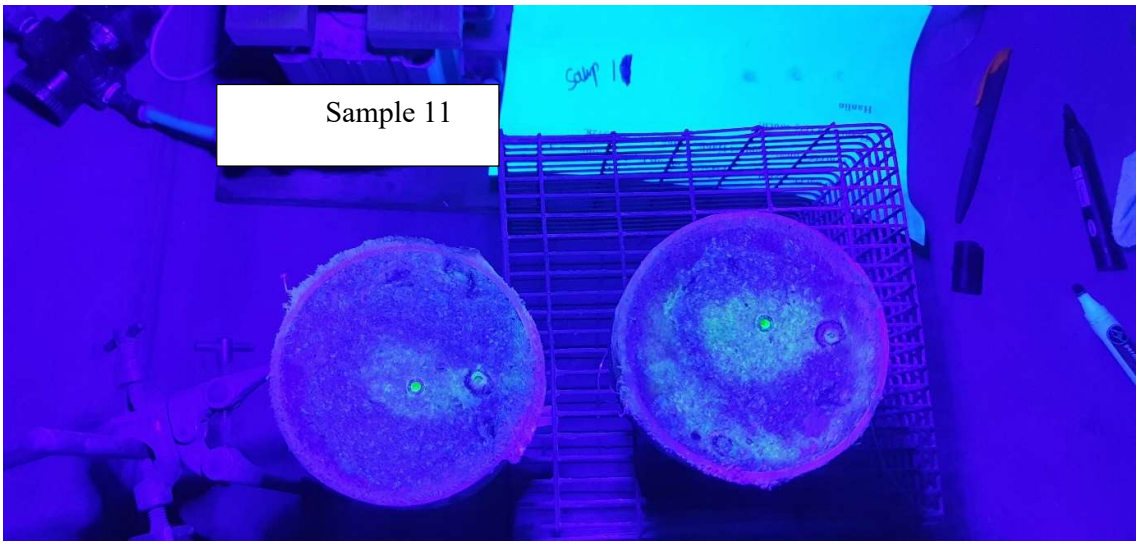
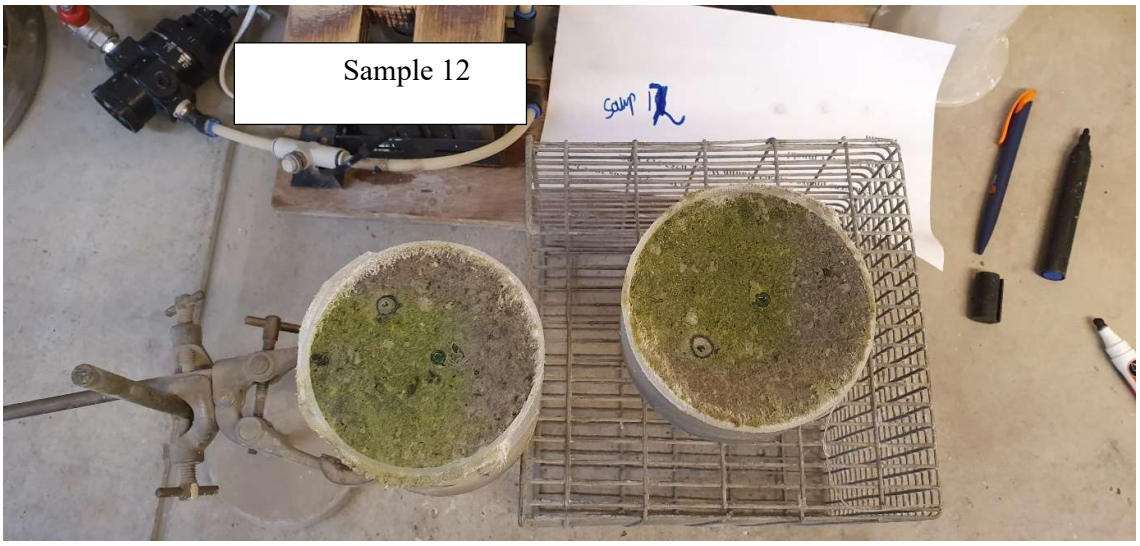
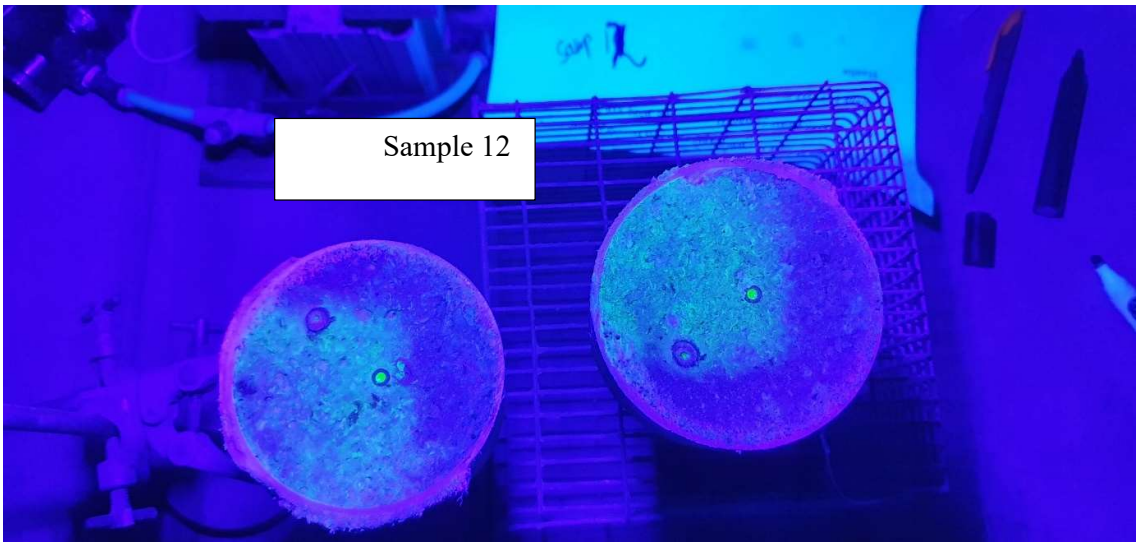

Sample 11


Sample 12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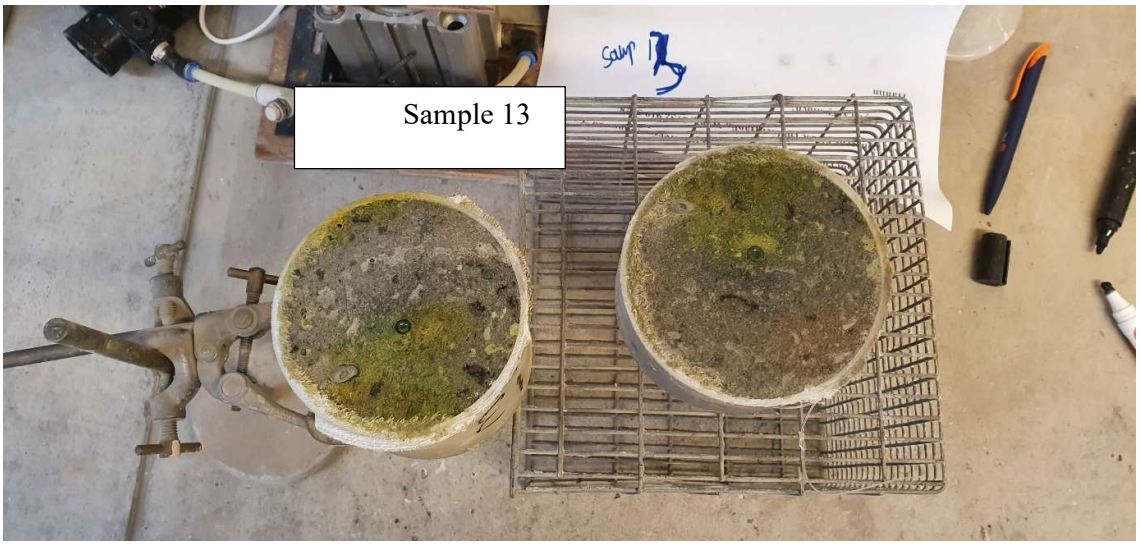
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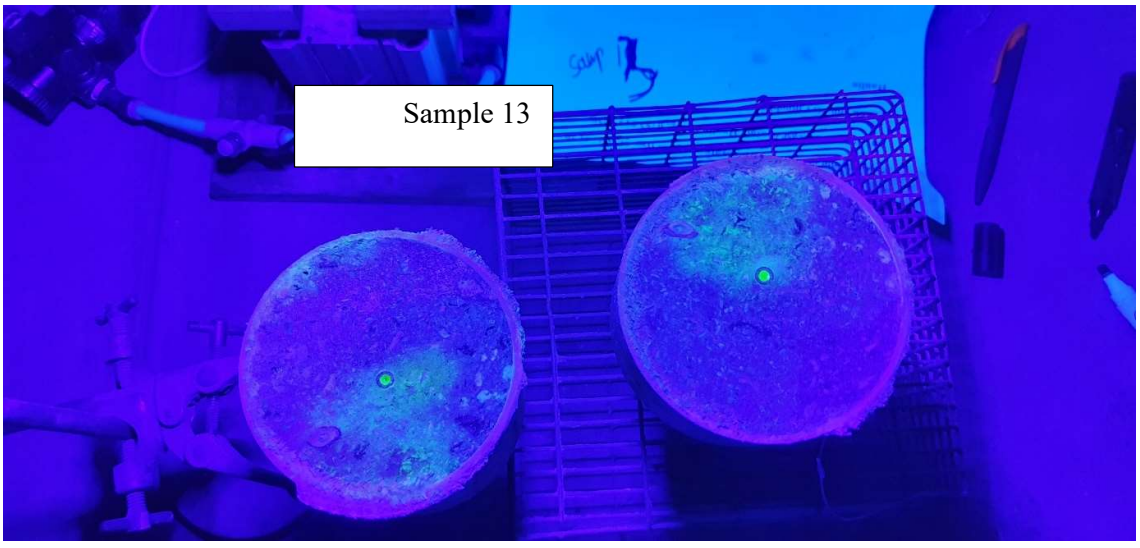
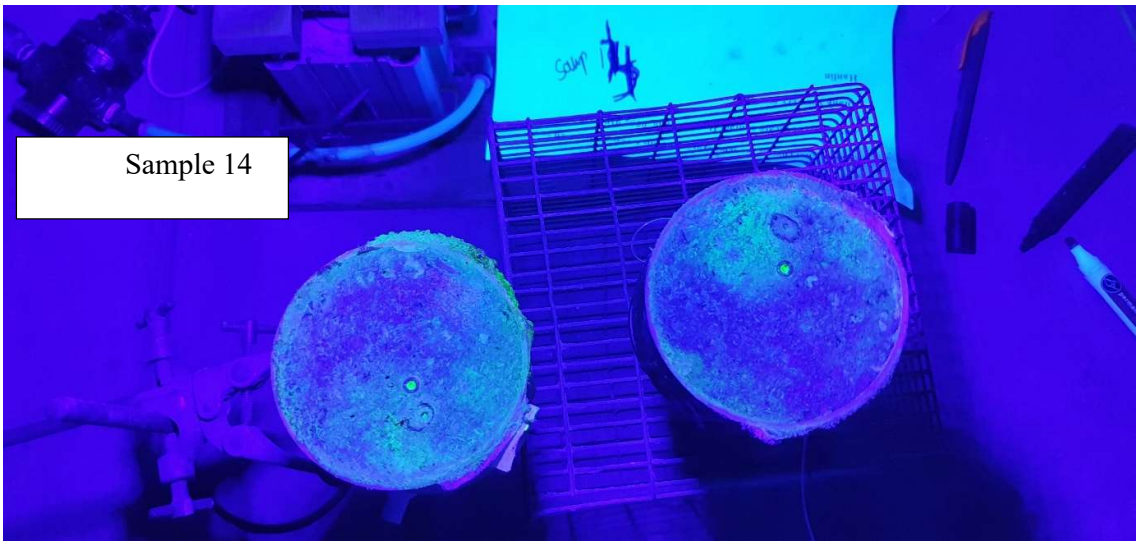

Sample 13


Sample 14


Sample 14

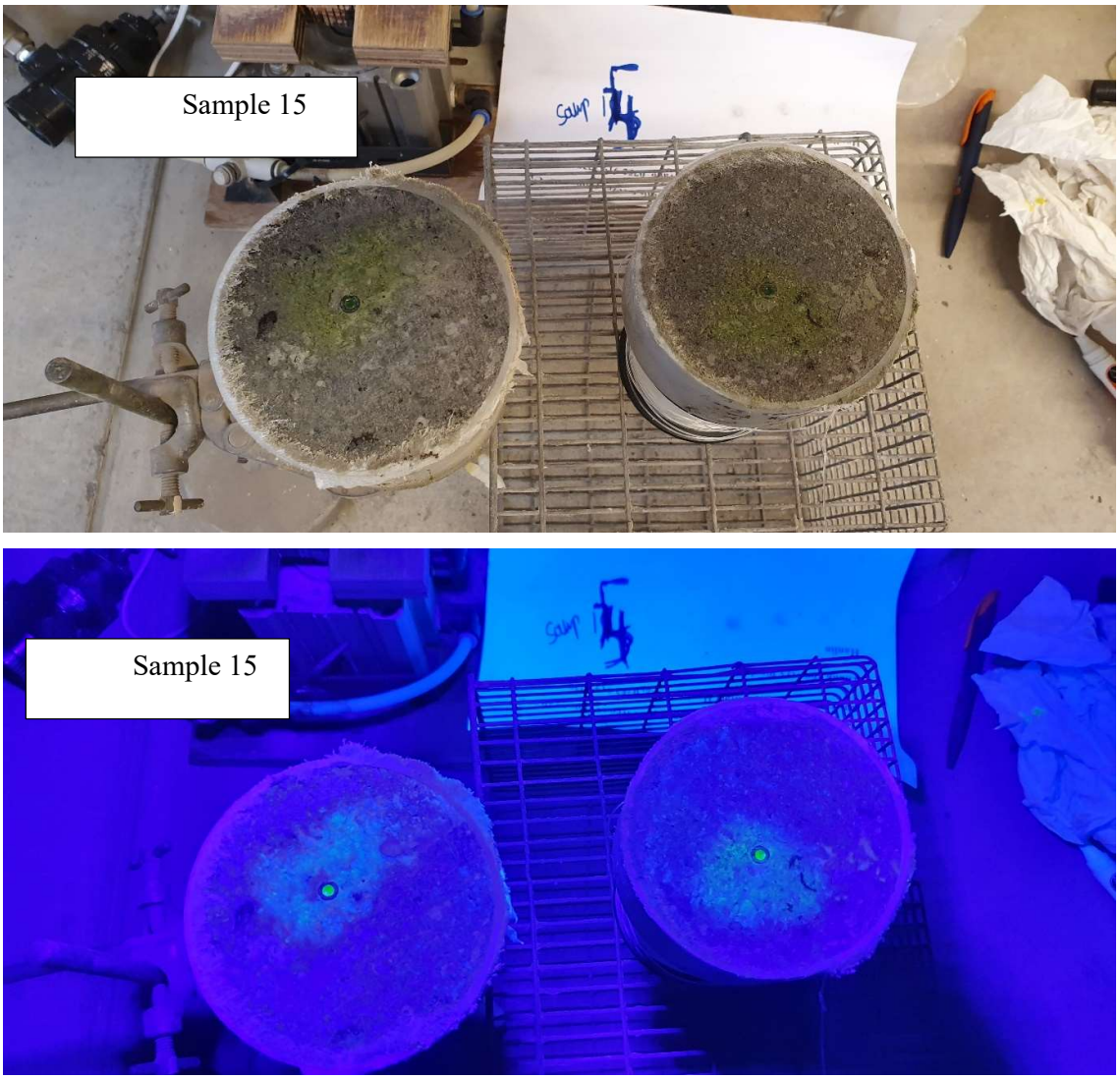


Figure A-0-24 Heterogeneous lab-scale landfills. Experimental conditions: FLOW+NEWS12g+SAND600g+AGRO+METHANO+M9.

Figure A-0-25 Heterogeneous Darcy-scale simulation example with two low hydraulic conductivity zones in the waste mass (velocity field of leachate shown, flowing in from the left).

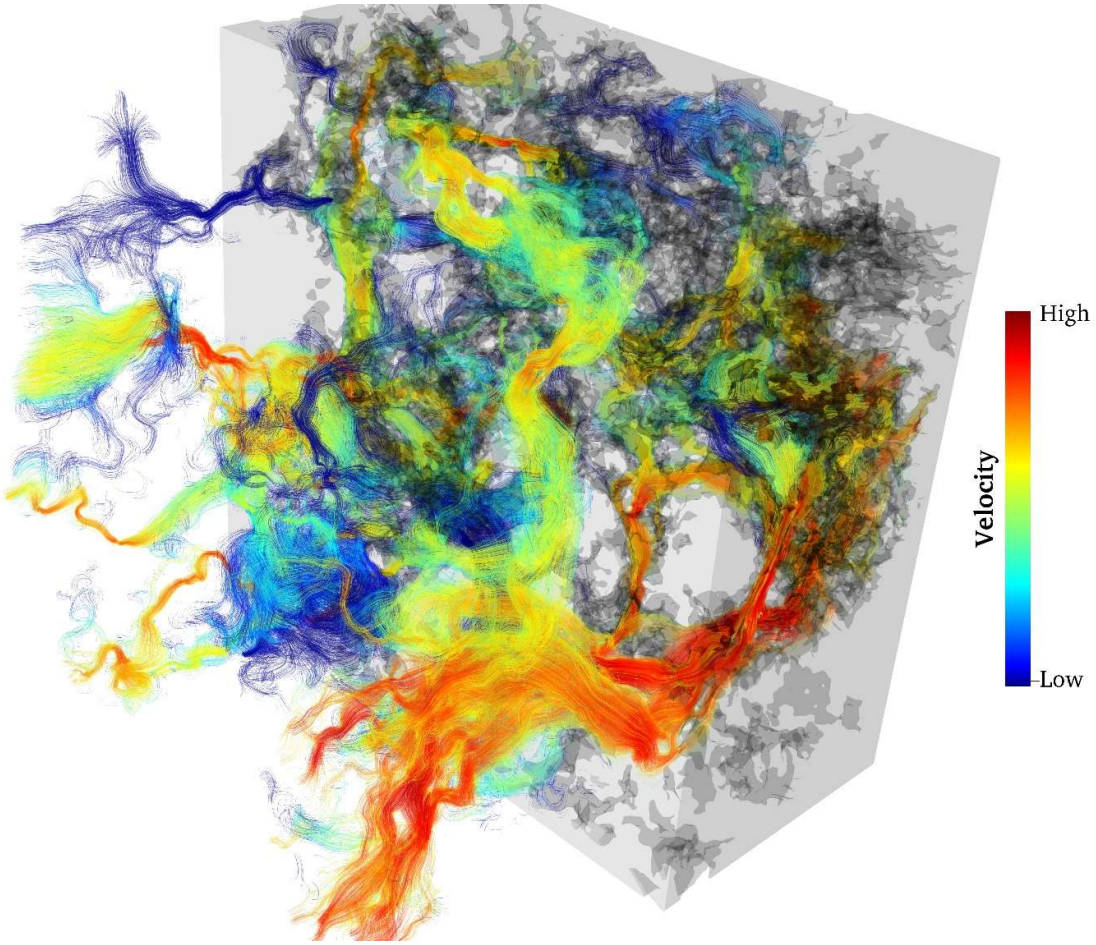


Figure A-0-26 Pore-scale simulation example using Navier-Stokes equation on a real porous space extracted via CT-scannings, and assuming Stokes flow (velocity field shown).

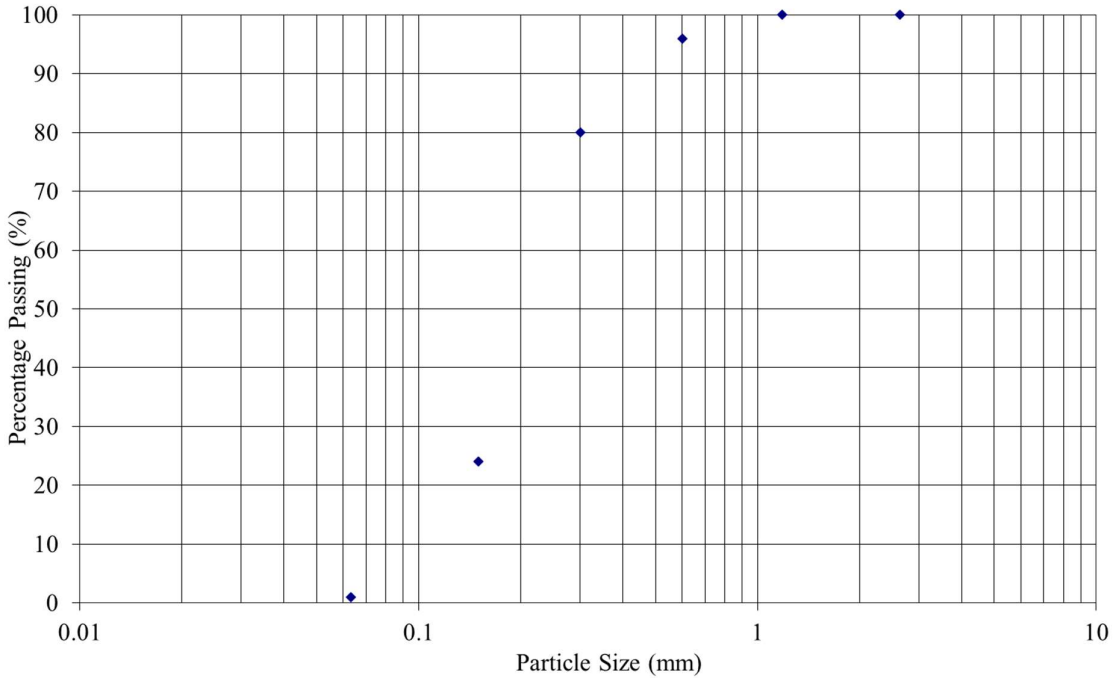


Figure A-0-27- Particle size distribution of washed fine silica sand used in the experiments.

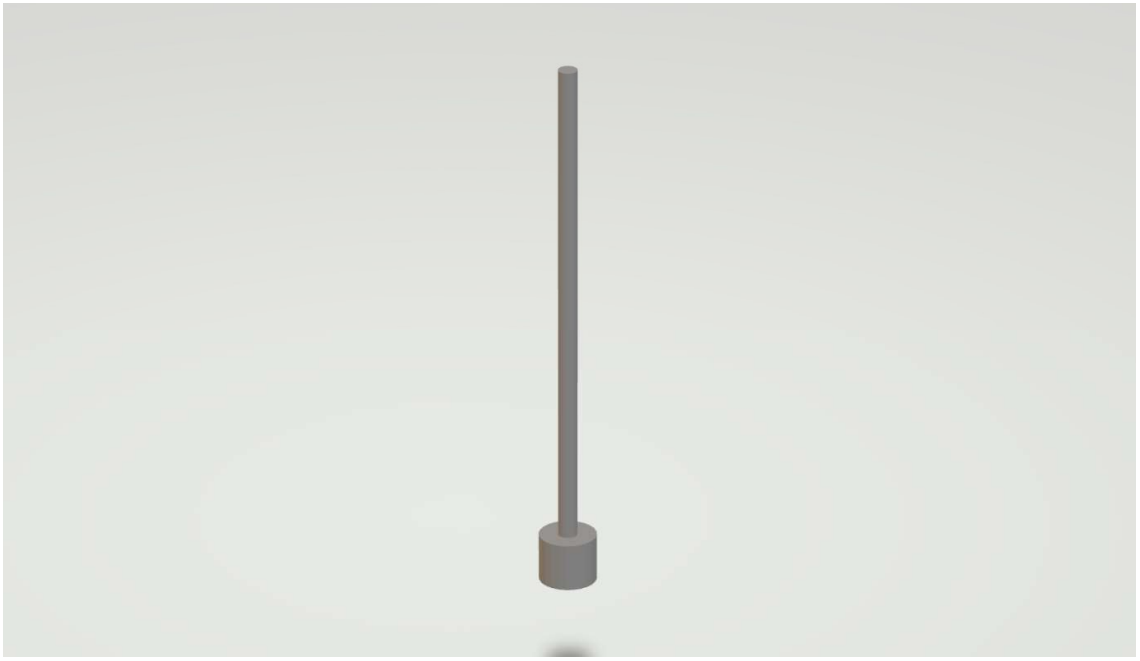


Figure A-0-28-3D Printed hand compaction tool designed and printed at Cardiff University's School of Engineering (Stereolithography).

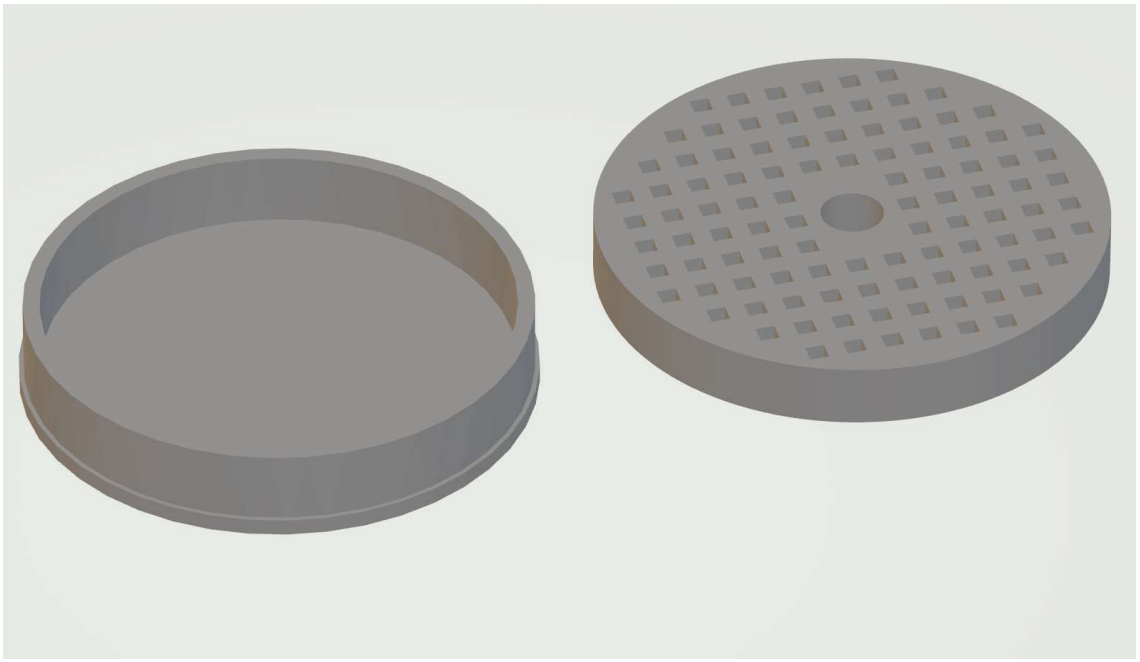


Figure A-0-29-3D Printed distributor tool designed and printed at Cardiff University's School of Engineering (Selective laser sintering). The inlet fitting in the centre (A) was threaded post-printing and fitted with a threaded-to-tube adapter (6mm, RS Stock No. 176-1404) for intrusion of leachate. The side length of each square (B) in the mesh was 0.18cm.