Microbial Bioremediation and Physiochemical treatment of Landfill Leachate

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Declaration

I certify that this thesis which I now submit for examination for the award of PhD is entirely my own work and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

This thesis was prepared according to the regulations for postgraduate study by research of the Institute of Technology Carlow and has not been submitted in whole or in part for an award in any other Institute or University.

The work reported on in this thesis conforms to the principles and requirements of the Institute's guidelines for ethics in research.

Signature ____________________________

Date ________________

Dedication

To my dad, Joseph Sexton.

Thank you for all the wonderful memories, fun, love and laughter. Thank you for always believing in me. You are truly missed.

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Abstract

Landfill leachate (LFL) is an environmentally hazardous waste characterised by elevated levels of organic and inorganic compounds. LFL is produced when water percolates through a landfill picking up the by-products of waste degradation. This process occurs throughout the working life of the landfill, often continuing for up to a century after the landfill is decommissioned. The discharge of untreated LFL, rich in ammonia, phosphate and nitrate, can cause the contamination of ground and surface water and the subsequent pollution of rivers, lakes and soil. As such, LFL must be collected from the bottom of the landfill and stored in tanks or lagoons before treatment. In Ireland, the treatment of LFL normally occurs off-site, as a combined treatment with domestic sewage in wastewater treatment plants (WWTPs). Although commonly practised, this process is unsuitable due to the stringent discharge limits imposed on these facilities.

In light of this, the main objective of the current research was to investigate alternative options for the treatment of LFL, that were both cost-effective and had the ability to treat important LFL constituents, such as biological oxygen demand (BOD), chemical oxygen demand (COD), ammonia, nitrate and phosphate to national discharge standards. An active local landfill, Powerstown landfill, Co. Carlow, was chosen as the source of LFL for this study. Initially, microbes were isolated and characterise from LFL generated in Powerstown. Microbial strains were screened based on their ability to remediate LFL. Secondly, a variety of adsorbents, including pumice stone and oyster shells, were also screened for their ability to adsorb ammonia, phosphate and nitrate, common LFL constituents.

After optimisation, both treatment options, bioremediation and adsorption, were combined into a novel fixed bed column system which was employed for the treatment of LFL on-site at Powerstown landfill. In the first instacne, a pilot study was conducted to access its ability to treat LFL. This study achieved high percentage removal rates, alonside achivign the national dichrage standard for reciving bodies. After succefully optimisation, this fixed bed system was employed on-site to LFL. This novel, cost-effctive treatment was employed for 40 day,and achieved high percentage removals of ≥81 % for BOD, COD, ammonia, phosphate and nitrate, resulting in their respective discharge limits being met. Thus, this research has proven the feasibility of a combined biological and physiochemical fixed bed system for the treatment of LFL. This system offers an exciting alternative to current treatment practices for LFL. However, additional studies are needed in order to access the ability of this treatment to successful treat LFL over longer period of time, while also taking into account the ever changing concentration of LFL.

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Safety Protocol

LFL is a toxic wastewater (WW) produced when water percolates though the landfill picking up the by-products of waste degradation. This WW can contain a wide range of toxic compounds, so caution needs to be taken when sampling and working with LFL. Due to sampling and on-site treatment occurring at an active landfill site, which contains vermin and other environmental hazards, due care must be taken.

Key safety requirement;

(1) Any personnel working with LFL samples should obtain Hepatitis C vaccinations.
(2) Stored LFL should be clearly labelled with parafilm around the lid to prevent leakage.
(3) PPE should be worn when working with LFL.
(4) Individuals should not sample at the landfill unaccompanied.
(5) Individuals should inform the landfill staff and personnel of their intention to visit the landfill for the purpose of sampling and system maintenance.
(6) All visitors to the landfill should adhere to the signing in and out procedures.
(7) All visitors must wear the necessary safety equipment (Hi-vis jackets, hard shoes and hard hats) at all times when on-site.

List of Abbreviations / Symbols

AC	Activated Carbon
AD	Anaerobic digestion
AOP	Advanced Oxidation Process
AOXs	Absorbable Organic Halogens
ARDA	Amplified rDNA Restriction Digest
As	Arsenic
ASBR	Anaerobic Sequence Batch Reactor
AsO_4^{3-}	Arsenate
A_w	Available Water
Ba	Barium
BAF	Biological Aerated Filter
BO_3^{3-}	Borate
BOD	Biological Oxygen Demand
BTC	Breakthrough Curve
c.	Capacity
C1	Column 1
C11	Cell 11
C2	Column 2
C3	Column 3
C4	Column 4
C5	Column 5
Ca^{2+}	Calcium
Cd	Cadmium
Cd_2^+	Cadmium
CH_4	Methane
Cl^-	Chloride

Co	Cobalt
CO_2	Carbon Dioxide
COD	Chemical Oxygen Demand
Cr_3^+	Chromium
Cr_6^+	Chromium 6
Cu_2^+	Copper
CW	Constructed Wetlands
$d.H_2O$	Deionised water
DL	Discharge Limit
DLA	Discharge Limit Achieved
EB	Electron Beam
EBPR	Enhanced Biological Phosphorus Removal
EPA	Environmental Protection Agency
EU	European Union
Fe^{2+}	Iron
GAC	Granular Activated Carbon
H_2	Hydrogen
H_2O	Water
H_2O_2	Hydrogen Peroxide
H_2S	Hydrogen Sulphide
H_2SO_4`	Sulfuric Acid
H_3PO_4	Phosphoric Acid
HCl	Hydrochloric Acid
HCO_3^-	Hydrogen Carbonate
Hg	Mercury
hrs	Hours
HRT	Hydraulic Retention Time
HYBRID	Hybrid Error Model

IC	Internal Capacity
IPPC	Integrated Pollution Prevention and Control
K^+	Potassium
L	Litres
LFL	Landfill Leachate
Li	Lithium
LT	Leachate Tank
MAPs	Magnesium Ammonium Phosphate
MBBR	Moving Bed Biofilm Reactor
MBR	Membrane Bioreactor
Mg^{2+}	Magnesium
MgO	Magnesium Oxide
Mins	Minutes
MM	Minimal Media
Mn^{2+}	Manganese
MPE	Mean Percentage Removal
MSW	Municipal Solid waste
N_2	Nitrogen
N_2O	Nitrogen Oxide
NA	Nutrient Agar
Na^{2+}	Sodium
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
NH_3	Ammonia
NH_4	Ammonium
Ni	Nickel
Ni_2^+	Nickel
NM	Not Measured

NO^{3-}	Nitrate
O_2	Oxygen
O_3	Ozonation
OD	Optical Density
OLR	Organic Loading Rate
OP1	Option 1
OP2	Option 2
OS	Oyster Shells
OTUs	Operational Taxonomic Units
P1	Phase 1
P2	Phase 2
P3	Phase 3
PAC	Powdered Activated Carbon
PAHs	Polycyclic Aromatic Hydrocarbons
PAOs	Polyphosphate Accumulating Organisms
Pb_2^+	Lead
PCBs	Polychlorinated Biphenyls
PCR	Polymerase Chain Reaction
pH	Potential of Hydrogen
PO_4^{3-}	Phosphate
PRE	Percentage Removal Rate
PS	Pumice Stone
PVC	Polyvinyl Chloride
RBC	Rotating Biological Contractors
RL	Regeneration Liquid
RMP	Revolutions Per Minute
RT	Retention Time
S_2	Sulphide

SBR	Sequence Batch Reactor
SO_4^{2-}	Sulphate
SP	Sample Point
SRB	Sulphur Reducing acteria
TKN	Total Kjeldahl Nitrogen
TN	Total Nitrogen
TOC	Total Organic Carbon
UASB	Up-flow Anaerobic Sludge Bed
UV	Ultra Violet
VFA	Volatile Fatty Acids
VOAs	Volatile Organic Acids
VOCs	Volatile organic compounds
w/v	Weight Per Volume
WW	Wastewater
WWTPs	Wastewater Treatment Plants
XOC’s	Xenobiotic Organic Compounds
Zn_2^+	Zinc
A	Area
C	Influent ($mg.L^{-1}$)
C_{ad}	Adsorbed concertation
c_e	Influent concentration ($mg.L^{-1}$)
C_o	Effluent ($mg.L^{-1}$)
K_{AB}	Adam-Bohart kinetics constant ($mg.min^{-1}$)
K_F	Indicator of adsorption capacity ($mg^3.g^{-1}$)
K_L	Langmuir adsorption equilibrium constant ($mg^3\ g^{-1}$)
K_{TH}	Thomas kinetic coefficient ($mg.min^{-1}$)
m	Mass of adsorbent used (g)
m_{total}	Total amount of ions delivered to the system (mg)

n	The strength of adsorption in the adsorption process
N_o	Exhaustion Point
N_O	Saturation concentration ($mg.L^{-1}$);
p	P-value
Q	Flow rate ($ml.min^{-1}$)
q_e	Mass of contaminate adsorbed per gram of adsorbent ($mg.g^{-1}$)
q_e.cal	Calculated adsorption capacity using an isotherm ($mg.g^{-1}$)
q_e.exp	Adsorption capacity obtained from experiments ($mg.g^{-1}$)
q_e.th	Mass of contaminate adsorbed per gram of adsorbent, theoretical value ($mg.g^{-1}$)
q_{eq}	Adsorption capacity ($mg.g^{-1}$)
q_{max}	Maximum adsorption at monolayer coverage ($mg.g^{-1}$)
q_o	Adsorption capacity ($mg.g^{-1}$)
q_{total}	Total amount of pollutant adsorbed by the column (mg)
R^2	Correlation co-efficient
R_L	Dimensionless constant sepration ration factor
T	Time (mins)
t_{total}	Total flow time (mins)
U_o	represent the linear velocity
v	Volume of liquid used (L)
Z	Bed depth (cm)
η^2	Eta Squared
Λ	Wilk’s lambda
χ^2	Non-linear Chi-square

Chapter 1 Introduction

1.1 Aim and objectives

Landfilling is the most commonly used method of MSW disposal which results in the production of large volumes of landfill leachate (LFL), the product of water that has percolated through waste picking up the products of degradation. In general, LFL contains dissolved organic matter, inorganic macro components, heavy metals, and xenobiotic organic compounds, which if not correctly managed, can infiltrate soil causing the pollution of receiving waters (Aziz *et al.*, 2010; Connolly, 2010). In addition, the generation of LFL is greatly affected by the infiltration of groundwater, precipitation, and rainwater through uncapped landfill (McCarthy *et al.*, 2010; Kamaruddin, 2015; Couto *et al.*, 2017). As such, the management of landfill leachate is essential for the protection of the surrounding environment, specifically ground and surface water.

Currently, many different methods are employed for the treatment of LFL, most of which are adapted from WW treatment methods (Raghab *et al.*, 2013). In general, a combination of both biological and physiochemical methods are effective for the treatment of LFL, as it can be difficult to obtain satisfactory results with just one method (Figure 2.9; Kargi and Pamukoglu, 2004). In addition, the LFL generated in different facilities often vary greatly in composition and require different types of treatments.

The objectives of the proposed research include; (1) Assess the ability of a range of microbial species to bioremediate LFL, (2) Design and optimise a physiochemical treatment based on the adsorption of xenobiotic compounds and

(3) Develop a treatment option combining both biological and physiochemical treatments, that has the potential to treat LFL on-site within an Irish landfill.

The results of the proposed project will determine some important fundamental insights into leachate bioremediation. Briefly they including determining the following; (1) discovery of novel isolates capable of leachate bioremediation; (2) bioremediation rates of typical leachates; (3) optimum conditions required for leachate bioremediation. Furthermore it will determine the potential for adsorption to be used in leachate treatment. This will focus on development and optimisation of a physiochemical adsorption step, with the aims of (1) determining the most effective low cost adsorbent, (2) optimising bed hed and flow rates for a fixed bed, (3) treating leachate to national discharge standard, and (5) determining eh longevity of adsorption columns.

Finally the potential to combined both treatment onsite will be accessed to determine (1) the potential to combine both treatment into a single treatment units, (2) to treat LFL on-site within and Irish landfill site and finally (3) to access that ability to regenerant adsorption materials.

1.2 Structure of the thesis

Chapters 3-7 in this thesis are written and formatted as journal manuscripts, i.e. each chapter has an abstract, introduction to the theme discussed in that specific chapter, the methodologies employed, the results and respective discussion and conclusions. Chapter 8 contains a brief conclusion and future recommendation. Lastly, Chapter 9 contains all references used throughout this thesis. Below are several highlights from Chapters 1-7.

Chapter 2; This chapter contains an overview of the current literature, with emphasis on current LFL treatment options, including both biological and physicochemical processes.

Chapter 3; This chapter focuses on the isolation and identification of bacteria from LFL samples, with emphasis on isolating strains that are capable of LFL bioremediation. This chapter also contains a bioremediation trial which was carried out to determine if the isolates enhanced the bioremediation of LFL within a fixed bed column system.

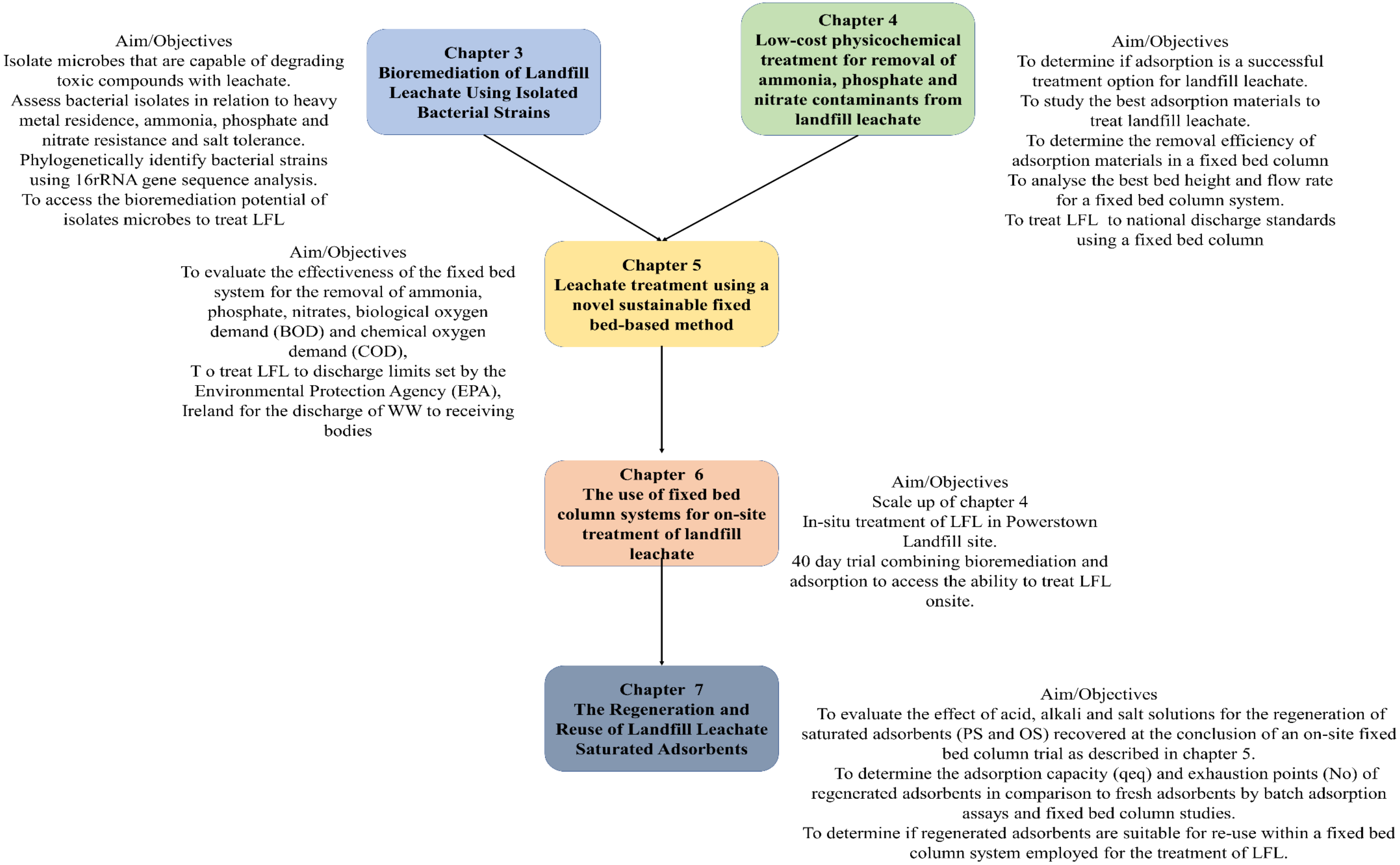
Chapter 4; The adsorption capacity of oyster shells, pumice stone, zeolite and sand was investigated using batch and column studies. These experiments specifically identified the most effective adsorbents for the removal of priority constituents, ammonia, phosphate and nitrate, from LFL.

Chapter 5; This chapter focused on the optimisation of a combined bioremediation and adsorption fixed bed column system. The lab-based system was trialled over a 16 hrs period for the continuous treatment of LFL, focusing on the reduction of BOD, COD, ammonia, phosphate and nitrate to below their national discharge limits.

Chapter 6; the main objective of this chapter was to employ the optimised system for the treatment of LFL on-site at Powerstown Landfill, Co. Carlow. The system was operated over a 40-d trial period for the continuous treatment of LFL.

Chapter 7; in this final experimental chapter, the regeneration of the adsorbents utilised in chapter 5 was investigated. The ability of regenerated adsorbents to be reused within the system for the treatment of LFL was also investigated

The flow diagram below describes the experimental outline for this study, indicating the object of each chapter.



Chapter 3
Bioremediation of Landfill Leachate Using Isolated Bacterial Strains

Aim/Objectives
Isolate microbes that are capable of degrading toxic compounds with leachate.
Assess bacterial isolates in relation to heavy metal residence, ammonia, phosphate and nitrate resistance and salt tolerance.
Phylogenetically identify bacterial strains using 16rRNA gene sequence analysis.
To access the bioremediation potential of isolates microbes to treat LFL

Chapter 4
Low-cost physicochemical treatment for removal of ammonia, phosphate and nitrate contaminants from landfill leachate

Aim/Objectives
To determine if adsorption is a successful treatment option for landfill leachate.
To study the best adsorption materials to treat landfill leachate.
To determine the removal efficiency of adsorption materials in a fixed bed column
To analyse the best bed height and flow rate for a fixed bed column system.
To treat LFL to national discharge standards using a fixed bed column

Chapter 5
Leachate treatment using a novel sustainable fixed bed-based method

Aim/Objectives
To evaluate the effectiveness of the fixed bed system for the removal of ammonia, phosphate, nitrates, biological oxygen demand (BOD) and chemical oxygen demand (COD),
To treat LFL to discharge limits set by the Environmental Protection Agency (EPA), Ireland for the discharge of WW to receiving bodies

Chapter 6
The use of fixed bed column systems for on-site treatment of landfill leachate

Aim/Objectives
Scale up of chapter 4
In-situ treatment of LFL in Powerstown Landfill site.
40 day trial combining bioremediation and adsorption to access the ability to treat LFL onsite.

Chapter 7
The Regeneration and Reuse of Landfill Leachate Saturated Adsorbents

Aim/Objectives
To evaluate the effect of acid, alkali and salt solutions for the regeneration of saturated adsorbents (PS and OS) recovered at the conclusion of an on-site fixed bed column trial as described in chapter 5.
To determine the adsorption capacity (qeq) and exhaustion points (No) of regenerated adsorbents in comparison to fresh adsorbents by batch adsorption assays and fixed bed column studies.
To determine if regenerated adsorbents are suitable for re-use within a fixed bed column system employed for the treatment of LFL.

Figure 1.1 Flow diagram showing the aim/objective of each chapter .

1.3 Dissemination of research

1.3.1 Publication

Morris Sinéad, Ryan David, Enright Deirdre, Garcia-Cabellos Guiomar, Enright Ann-Marie. 2018. *Bioremediation of Landfill Leachate Using Isolated Bacterial Strains.* International Journal of Environmental Bioremediation & Biodegradation. DOI; 10.12691/ijebb-6-1-4

Morris Sinéad, Ryan David, Enright Deirdre, Garcia-Cabellos Guiomar, Enright Ann-Marie. 2018. *Leachate treatment using a novel sustainable fixed bed-based method.* Desalination and Water Treatment. DOI; 10.5004/dwt.2018.22406

1.3.2 Conference proceeding

Morris Sinéad, Ryan David, Enright Deirdre, Garcia-Cabellos Guiomar, Enright Ann-Marie (2019): *Low-cost physicochemical treatment for removal of ammonia, phosphate and nitrate contaminants from landfill leachate.* Environ 2019 – Institute of Technology Carlow . April 2019

Morris Sinéad, Ryan David, Enright Deirdre, Garcia-Cabellos Guiomar, Enright Ann-Marie *The in-situ treatment of landfill leachate through bioremediation and chemical adsorption in a fixed bed column*. European Waste Water Management. Manchester, United Kingdom. July 2018

Morris Sinéad, Ryan David, Enright Deirdre, Garcia-Cabellos Guiomar, Enright Ann-Marie (2018): *The Microbial and Physicochemical Treatment of Irish Landfill Leachate.* Environ 2018 –Cork Institute of Technology. March 2018

Morris Sinéad, Ryan David, Enright Deirdre, Garcia-Cabellos Guiomar, Enright Ann-Marie (2017). *Leachate treatment using a novel sustainable fixed bed based method*. CEST 2017. Rhodes, Greece. 31st August - 2nd September 2017

Morris Sinéad, Ryan David, Enright Deirdre, Garcia-Cabellos Guiomar, Enright Ann-Marie (2016): *Microbial Bioremediation and Physicochemical Treatment of Landfill Leachate.* Environ 2016 - Ecosystem, Services for a Sustainable Future, University of Limerick; 03/2016

Chapter 2

Literature Review

2.1 Overview

The main objective of this chapter is to look into the current literature in relation to landfills, with an emphasis on landfill leachate treatment. It will begin by looking at waste management practices, within EU and Ireland. Following this, it will look at leachate production and current treatment options. The focus is on identifying treatment option that may be suitable to treat LFL.

2.2 Introduction

Waste generation and management have occurred to some extent throughout human history. When humans lived nomadic lifestyles, waste management was not an issue as waste was simply dumped and individuals moved on. Around 10,000 BC nomadic existence was largely abandoned, and communities began to develop in single areas which led to the dumping of waste in relative proximity (National Solid Wastes Management Association, 2008). This type of primitive waste disposal continued in the towns and cities of the 19th century, where it polluted these urban centres and contributed to the spread of devastating diseases including the bubonic plague, cholera, and typhoid fever (Barbalace, 1999; Martin, 2008). It was also during this time that the connection between microorganisms and disease, "the germ theory", was postulated by Louis Pasteur (Feinstein, 2008). Spurred on to create better-living conditions for their inhabitants, city and town councils began waste collection and disposal in open dumps or the marine environment. Although this was an advancement in terms of disease control, little regard was given to the resulting environmental pollution. Nevertheless, at this time the majority of waste disposed of was biodegradable in nature and contained relatively low levels of hazardous materials.

At the turn of the 20th century, waste management practices had become more developed with waste collection and disposal occurring routinely in urban areas (Herbert, 2007). It was also during this time the practice of landfilling waste became commonplace. These original landfills consisted of specifically excavated holes or trenches into which waste was placed. Finally, when the trenches were filled, the waste was covered with topsoil. In most instances, the waste was placed directly on the soil at the base of the landfill without the implementation of a barrier to prevent water percolating through the waste. This practice resulted in the contamination of surrounding soil and groundwater by landfill leachate (LFL). However, at the time limited knowledge existed on the processes occurring within landfills such as leachate or landfill gas production, and the potential damage these sites had on the environment.

By the 21st-century, the dangers and environmental impact associated with landfilling became apparent and was noted by the scientific and technology communities (Herbert, 2007). A considerable shift in waste production also occurred during this time from waste that largely consisted of bio-degradable materials to waste categorised into three further groupings including: non-biodegradable waste, man-made plastic and toxic waste, each of which contributes to environmental pollution (European Commission, 1999; Ward, 2011). In addition, many developed nations began introducing legislation to regulate landfills in an effort to prevent and minimise pollution (Herbert, 2007). The main strategy introduced was to reduce, reuse and recycle waste wherever possible to prevent waste from being landfilled in the first instance (McCarthy *et al.*, 2010; EPA, 2011).

Currently, waste management in landfill sites continues to cause concern both nationally and internationally. Within the European Union (EU) the waste sector is undergoing dramatic changes due to the implementation of waste management acts and directives. Despite these changes, waste production in the EU is increasing, with a current average of 2.5 billion tonnes being produced annually (European Commission, 2016). Of this waste, 36% is recycled with the bulk being landfilled or incinerated (European Commission, 2016). Unfortunately, 600 million tonnes of this landfilled waste was suitable for recycling which highlights an inherent problem with current practices.

Fundamentally, a change is now required in the interpretation of waste in its current context as a problem that requires elimination. Instead, waste needs to be viewed as a resource from which valuable constituent need to be recovered as a priority of waste processing. As such, the use of landfilling in terms of the waste hierarchy (Figure 2.1) should be used as an option of the last resource. Landfilling waste has its own associated problems and environmental concerns. Specifically, landfills are known for the production of off-gases such as methane (CH_4) and hydrogen sulphide (H_2S) and thus can impact air quality as well as being as an eyesore in local communities. By improving waste management practices at these facilities, the emission of these harmful greenhouse gases can be reduced which in turn will result in the reduction of environmental and health problems, and landscape deterioration (European Commission, 2016; Wall *et al.*, 2016; Giersc *et al.*, 2018).

One of the major issues of landfilling waste is the production of LFL a toxic wastewater (WW) with the potential to cause the environmental contamination of groundwater (EPA, 2000; McCarthy *et al.*, 2010). LFL is characterised by high levels of biological oxygen demand (BOD), chemical oxygen demand (COD), ammonia, nitrogen, heavy metals, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs).

This literature review will focus on the production and composition of LFL and the current treatment options available for this environmentally damaging waste stream.

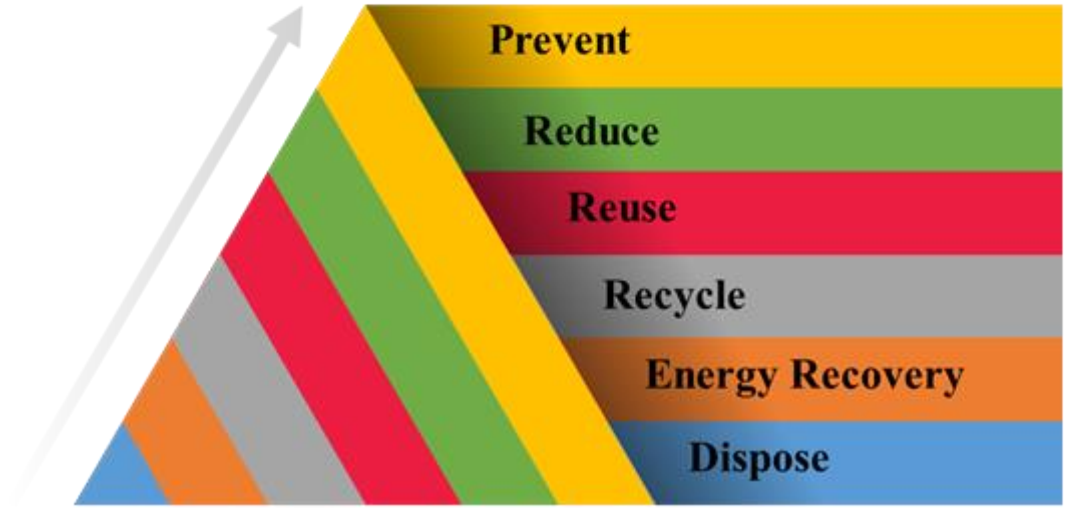


Figure 2.1 Waste Hierarchy (modified from Wall *et al* . 2016)

2.3 Municipal solid waste

Municipal solid waste (MSW) is defined as a combination of household and commercial waste (EPA, 2016; EPA, 2018). The generation of MSW represents an environmental burden worldwide, which continues to rise exponentially, due to population growth, lifestyle change and increased industrial activities (Renou *et al.*, 2008; Kamaruddin, 2015). In general terms, MSW generation is often used as an indicator of the economic activity and consumption behaviours rates within society (Torretta *et al.,* 2017). An Environmental Protection Agency (EPA) report entitled “Ireland’s Environment 2016; An Assessment” (Wall *et al.,* 2016) has estimated that 2.76 million tonnes of MWS was generated in Ireland during 2016, representing an increase of 6% from 2012, of which 41% was recycled and 26% was disposed of in registered landfills, with the additional waste being incinerated or recovered (EPA, 2016; Wall *et al.,* 2016b). The data also estimated that during 2016 the Irish population generated a staggering 331 kg of MSW per person (EPA, 2016; Wall *et al.*, 2016) which is 5 kg below the national EU average of 336 kg per person (Wall *et al.*, 2016).

In summary, the report highlights the following information in relation to MSW generation and disposal in Ireland during 2016;

- 2.71 million tonnes of MSW was managed.
- 0.44 million tonnes of MSW went unmanaged, i.e. waste was not collected or brought to a registered waste facility.
- Almost three quarters, 74%, of MSW was recovered (decrease from 79% in 2014).
- The rate of recycling was 41%, comparable to 2014.
- Over a quarter, 26%, was landfilled within six operational landfills (compared to 18 in 2012).

2.4 Waste Management in Europe

EU policy concerning the protection of the environment and natural resources has steadily grown in importance since the 1980s (European Commission, 1999, 2016). Unsurprisingly, waste production is at a record high, with each of the 500 million people living in the EU generating an approximate half a tonne of household rubbish annually (European Parliament, 2018; Eurostat, 2018). Further to this, vast amounts of MSW is also generated from manufacturing and construction activities. As such, the EU currently produces up to 2.5 billion tonnes of waste annually (European Parliament, 2018).

EU waste policy has evolved over the last 30 years through a series of environmental action plans and a framework of legislation that aims to reduce negative environmental and health impacts and create an energy and resource-efficient economy (European Parliament, 2018; Eurostat, 2018). As such, and despite increases in MSW generation, a decrease in the volume of MSW being landfilled has been observed. For example, a decrease of 60 million tonnes (118 kg per capita) of MSW being landfilled has occurred during the period 1995-2016 (European Parliament, 2018; Eurostat, 2018). Specifically, the latest waste figures for the EU show that of the 2.5 billion tons of MSW generated in 2016 within the EU 47% was recycled or composted (European Parliament, 2018). Alongside this, countries such as Belgium, Sweden and German have reduced their landfilling requirements to <1% and instead rely on both incineration and recycling for the disposal/treatment of MSW. In contrast, several eastern countries such as Romania and Greece send c.80% of all MSW to landfills, with Spain and Portugal landfilling c.60% of MSW produced there.

In addition, the move away from landfilling can also be attributed to the implementation of various directives including, the Landfill Directive 1999/31/EC (EU, 1999), which aims to "prevent or reduce as far as possible negative effects on the environment, in particular, the pollution of surface water, groundwater, soil and air, and on the global environment, including the greenhouse effect, as well as any resulting risk to human health, from the landfilling of waste, during the whole life-cycle of the landfill” (EU, 1999). In addition, the Waste Framework Directive (2008/98/EC) (EU, 2008b) also aims to prevent or reduce as far as possible negative effects on the environment, in particular on surface water, groundwater, soil, air, and on human health from the landfilling of waste by introducing stringent technical requirements for MSW and landfills.

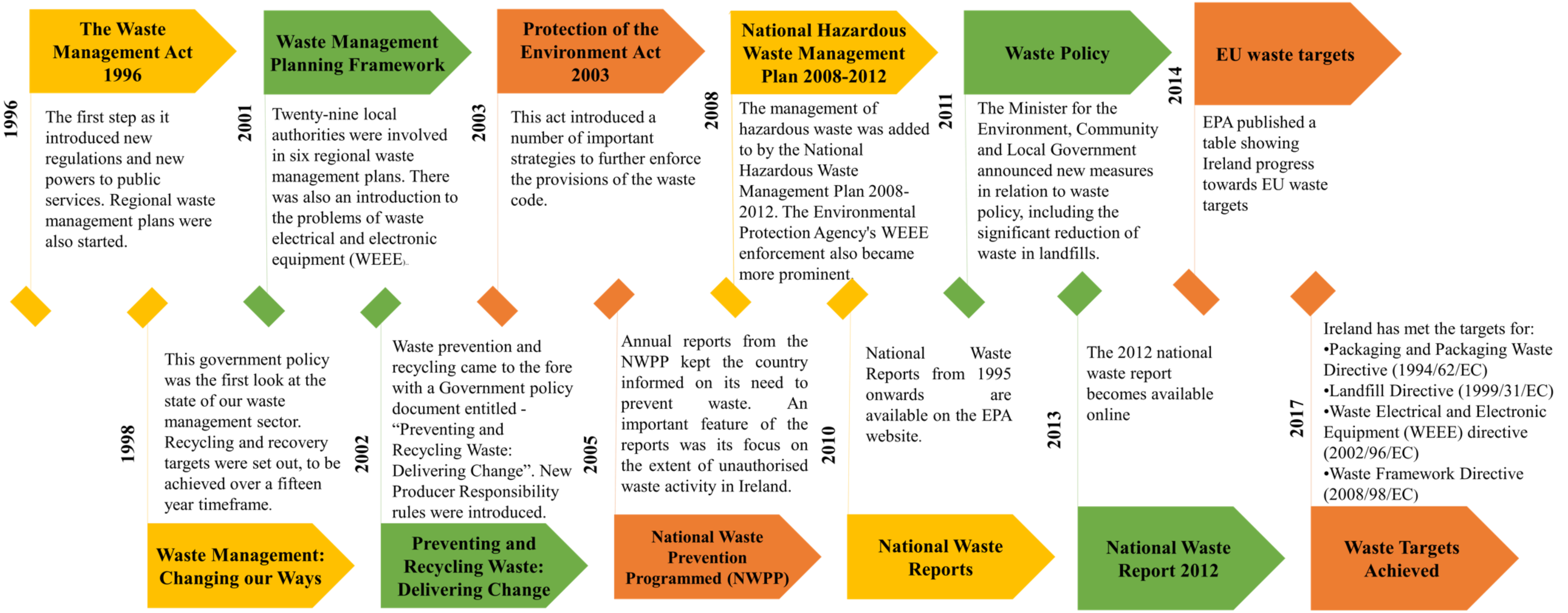
2.5 Landfilling in Ireland

Landfilling in Ireland had undergone a dramatic change over the past decade, due to the implementation of various directive set by the EPA (Brennan *et al.*, 2016). A timeline of these legislative changes is outlined in Figure 2.2. In particular, the Landfill Directive 1999/31/EC (EU, 2001) has implemented dramatic and much-needed changes to landfilling in Ireland. The Landfill directive was issued in 1999 and implemented in Ireland by July 2000. The main objectives are to prevent and reduce any negative effects on the environment and human health that are associated with landfilling.

As a direct consequence of the Landfill Directive, a reduction in the number of active landfill sites within Ireland has occurred. The timeline of these closures is described below (Burke, 2016; EPA, 2018);

- Mid-1980s → 200 landfills operational, consisting of MSW landfills, inert landfills and integrated pollution prevention and control (IPPC) landfills (McCarthy *et al.*, 2010).
- 1995-97 → 95 landfill sites operational.

- 2009 → 30 landfills with an operational capacity of 24 million tonnes of MSW, one-third of which was for the privatised waste sector and were expected to remain operational for twelve years (McCarthy *et al.*, 2010).
- 2012 → 18 landfill sites operational with a capacity of 910,000 tonnes until 2014.
- 2016 → 6 landfill sites operational.



1996

The Waste Management Act 1996

The first step as it introduced new regulations and new powers to public services. Regional waste management plans were also started.

1998

Waste Management: Changing our Ways

This government policy was the first look at the state of our waste management sector. Recycling and recovery targets were set out, to be achieved over a fifteen year timeframe.

2001

Waste Management Planning Framework

Twenty-nine local authorities were involved in six regional waste management plans. There was also an introduction to the problems of waste electrical and electronic equipment (WEEE).

2002

Preventing and Recycling Waste: Delivering Change

Waste prevention and recycling came to the fore with a Government policy document entitled - "Preventing and Recycling Waste: Delivering Change". New Producer Responsibility rules were introduced.

2003

Protection of the Environment Act 2003

This act introduced a number of important strategies to further enforce the provisions of the waste code.

2005

National Waste Prevention Programmed (NWPP)

Annual reports from the NWPP kept the country informed on its need to prevent waste. An important feature of the reports was its focus on the extent of unauthorised waste activity in Ireland.

2008

National Hazardous Waste Management Plan 2008-2012

The management of hazardous waste was added to by the National Hazardous Waste Management Plan 2008-2012. The Environmental Protection Agency's WEEE enforcement also became more prominent.

2010

National Waste Reports

National Waste Reports from 1995 onwards are available on the EPA website.

2011

Waste Policy

The Minister for the Environment, Community and Local Government announced new measures in relation to waste policy, including the significant reduction of waste in landfills.

2013

National Waste Report 2012

The 2012 national waste report becomes available online

2014

EU waste targets

EPA published a table showing Ireland progress towards EU waste targets

2017

Waste Targets Achieved

Ireland has met the targets for:
- Packaging and Packaging Waste Directive (1994/62/EC)
- Landfill Directive (1999/31/EC)
- Waste Electrical and Electronic Equipment (WEEE) directive (2002/96/EC)
- Waste Framework Directive (2008/98/EC)

Figure 2.2 Timeline of waste management events within Ireland from 1996-2017

2.6 Landfill leachate

LFL is a chemical cocktail, that represents a potential hazard to both human health and the environment if left untreated (Renou *et al.*, 2008; Bove *et al.*, 2015). LFL is the result of waste undergoing physiochemical and biological changes and is defined as "a liquid, which has percolated through waste, picking up suspended and soluble materials that originate from or are products of the degradation of the waste" (McCarthy *et al.*, 2010). In general, LFL contains dissolved organic matter, inorganic macro components, heavy metals, and xenobiotic organic compounds, which if not correctly managed, can infiltrate soil causing the pollution of receiving waters (Aziz *et al.*, 2010; Connolly, 2010). In addition, the generation of LFL is greatly affected by the infiltration of groundwater, precipitation, and rainwater through uncapped landfill (McCarthy *et al.*, 2010; Kamaruddin, 2015; Couto *et al.*, 2017). Typical water movement within a landfill is highlighted in Figure 2.3.

The guidelines and regulations for the control and containment of LFL at landfill sites are set out in the Landfill Directive. Specifically, the directive requires the implementation of a leachate management system prior to the commencement of a new landfill. A leachate management system should ensure that LFL produced within the landfill is contained within this site, with measures in place to reduce seepage out of the landfill through the side or the base, to prevent liquid levels rising to the extent that leachate spills over causing the uncontrolled release to the surrounding environment, and to minimise the interaction between the leachate and the landfill liner (EPA, 2000a; McCarthy *et al.*, 2010). As such, knowledge of the potential for leachate generation is a pre-requisite of both the conceptual design of the landfill and the subsequent leachate management strategies. Therefore, the design of all MSW landfills should include comprehensive leachate collection and removal systems. Importantly the leachate collection system must function over the landfill's design lifetime irrespective of the liquids management strategy being used. This should include; (1) a drainage layer constructed of either natural granular material or synthetic drainage material; (2) perforated leachate collection pipes; and (3) leachate collection sumps or header pipe systems where leachate can be removed, as outlined in Figure 2.4 (EPA, 2000a; Timoney, 2009; McCarthy *et al.*, 2010).

The quality of LFL produced in an MSW landfill changes considerably over time, as the degradation of waste progresses inside this natural internal bioreactor (EPA Ireland, 2000a). Consequently, the classification of LFL is essential to determine the potential effects accidental release would have on the surrounding environment (EU, 2000; Renou *et al.*, 2008). LFL production and management are considered a major problem by landfill operators, due to the ever-changing nature of waste degradation and leachate compositions (EPA, 2000a; Wang, 2013).

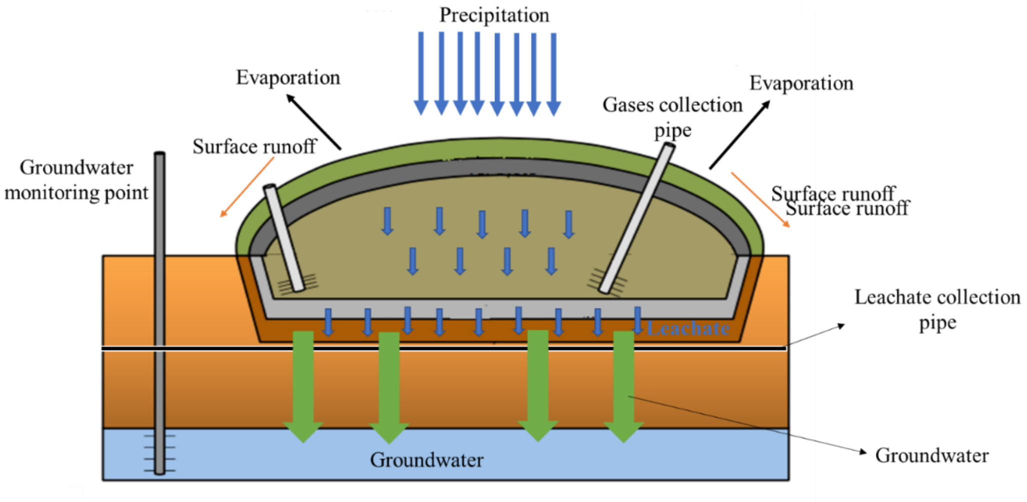


Figure 2.3. Water movement and leachate production within a landfill, adapted from Aziz *et al* ., (2012).

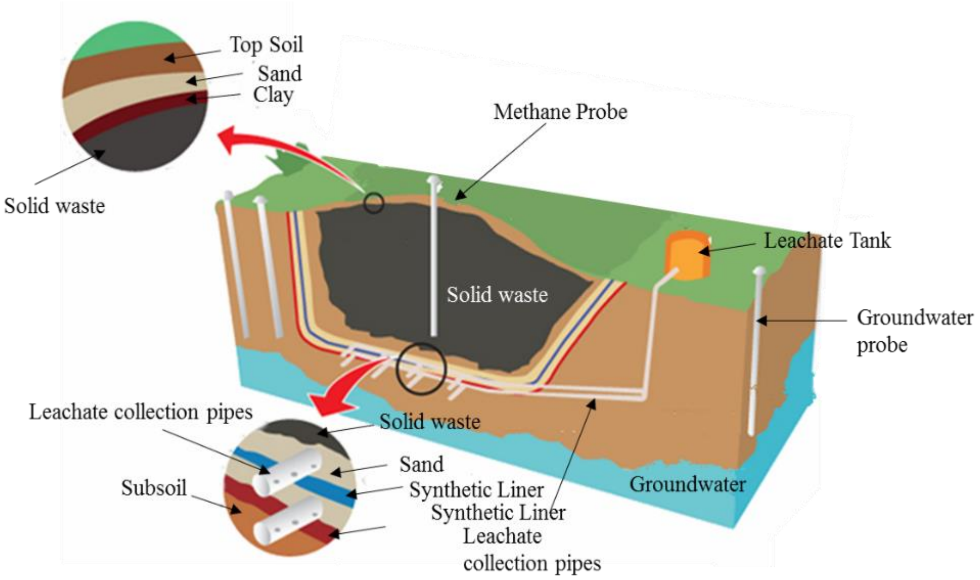


Figure 2.4. A cross-section of an MSW landfill, adapted from Moore (2018).

2.7 Legislation

The European Waste Management Act 1996 (Irish Statute Book) and the Landfill Directive 1999/31/EC (EU, 2001) have driven major changes in waste management within the EU over the past 20 years. The Waste Framework Directive 2008/98/EC (EU, 2008b), the Urban Wastewater Treatment Regulations Council Directive 99/31/EC (EU, 1991), and the Water Framework Directive 2000/60/EC (EU, 2000) also govern landfill operation and LFL disposal within the EU member states. The Landfill Directive and the Waste Framework Directive directly influence waste management practices and, in turn, LFL collection and disposal. The landfill directive also sets clear guidance that leachate management and collection systems need to be in place in landfills. Annex 1 and 2 of the Landfill Directive, states that a geological barrier must be in place to prevent leachate contamination of ground and surface waters and that leachate must be collected and treated where possible. This directive regulates not only the types of waste a landfill can receive but also landfill aftercare. A recent study by Wang (2013) highlighted that aftercare of landfill sites may be necessary for up to 200 years after the landfill has been decommissioned/closed. The directive also states that leachate volume and composition must be measured quarterly at active sites and bi-annual when aftercare is in place (EU, 1999; Brennan *et al.,* 2017a; Brennan *et al.*, 2017b).

Recently this legislation has been revised in light of the EU adoption of an ambitious Circular Economy Package, which includes proposals on waste management aimed to stimulate Europe's transition towards a circular economy (European Commission, 2018). This package sets clear targets for the reduction of waste and establishes a long-term path for waste management and recycling within the EU. Some of the key elements and targets are (EU, 2008a);

- A common EU target for recycling 65% of MSW by 2030;
- A common EU target for recycling 75% of packaging waste by 2030;
- A binding landfill target to reduce landfilling to a maximum of 10% of MSW by 2030.

The implementation of these directives has affected a positive change in the waste management sector in Ireland by decreasing the number of landfills and enforcing the collection and treatment of LFL. However, as a direct consequence of the closure of smaller landfill site, the remaining high-volume landfill sites have the potential to produce more concentrated LFL (Brennan *et al.,* 2016; Brennan *et al.,* 2017b). As such it is anticipated that the changes in leachate concentration combined with the stringent discharge limits will require a multi-action approach to manage leachate generation and treatment in the near future (Brennan *et al.,* 2016; Brennan *et al.,* 2017a).

2.8 Decomposition of waste within MSW landfill and LFL production

The decomposition of MSW can be divided into three categories i.e. physical, chemical and biological decomposition (Townsend *et al.,* 2015). These processes are largely dependent on the type of waste present in the landfill. Physical decomposition occurs during the separation of waste, alongside mechanical reduction. Chemical decomposition involves process such as gasification, oxidation and volatilisation, while biological degradation occurs as soon as waste is placed in the landfill (EPA, 2000a; Townsend *et al.,* 2015).

Biological decomposition of organic matter, which occurs as soon as the waste is transferred to the landfill, results in the production of LFL and landfill gas, CH_4

and CO_2 (Matsufuji, 2007; Reddy *et al.*, 2015). Biodegradation, the process by which organic substances are broken down into smaller compounds, is driven by aerobic and anaerobic microorganisms, already present in the landfill (Rajput *et al.*, 2016; Kamaruddin *et al.*, 2017). The rate of this degradation is dependent on numerous factors including the physical, chemical and biological environments of the landfill. As such, the degradation processes and rates differ between different landfill sites. In general, there are five unique phases involved in the process (Figure 2.5 and 2.6).

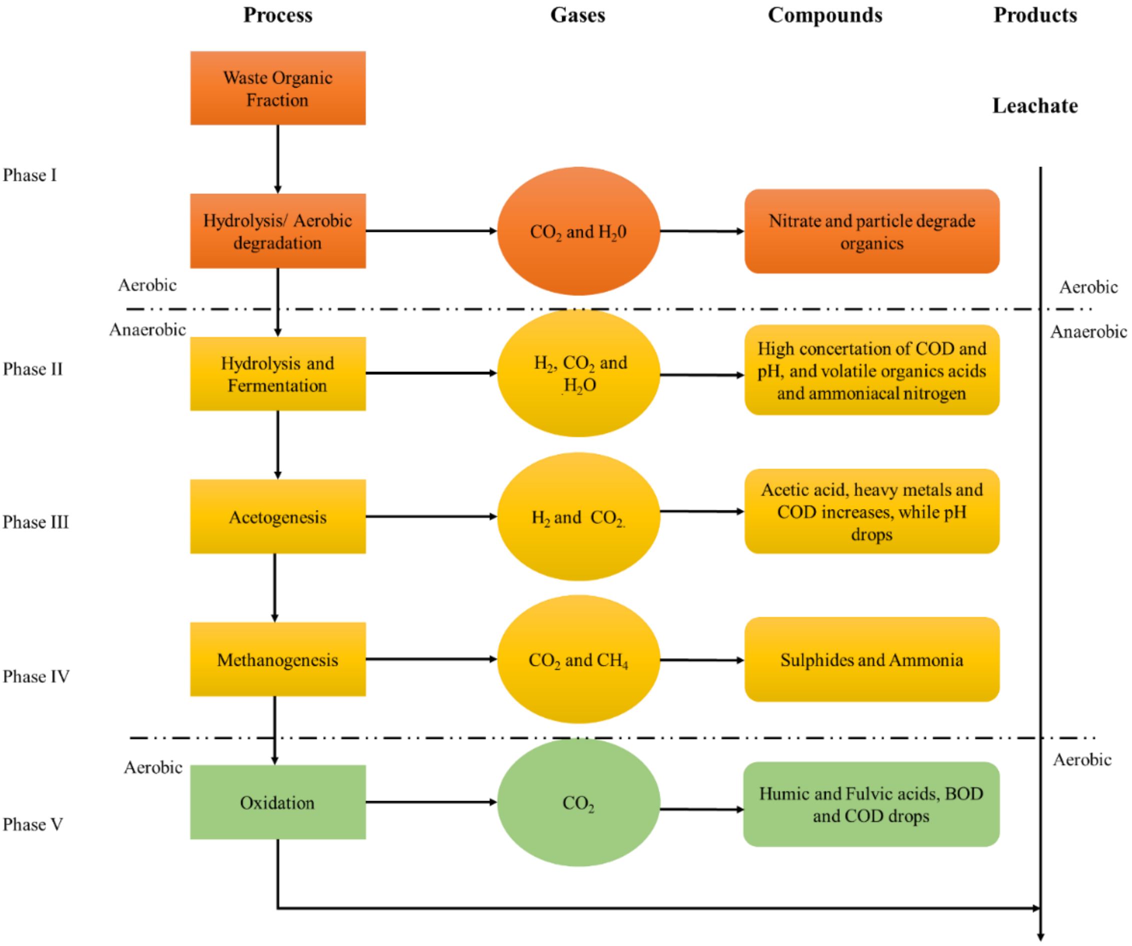


Figure 2.5. Stages of waste degradation and their associated by-products.

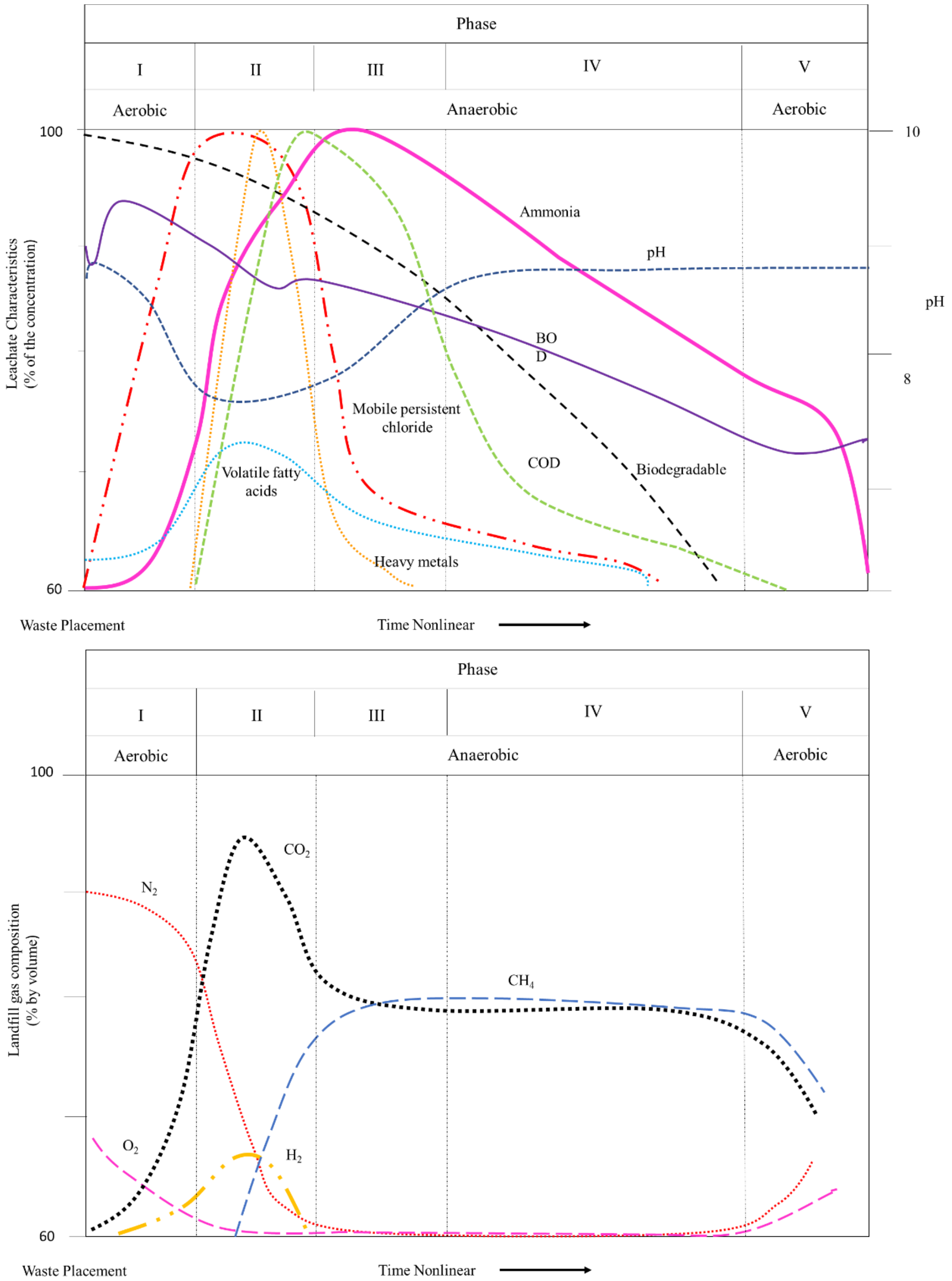


Figure 2.6. Landfill gases and leachate concentration over the different phases of waste degradation adapted from Kreith and Tchobanoglous (2002), Matsufuji (2007) and Ghosh and Hasan (2010).

2.8.1 Phase I – Initial adjustment

Initial adjustment begins when solid waste is placed in the landfill and covered. Microbial decomposition commences in this moisture-rich environment under aerobic conditions and lasts for several months at the surface layer of the landfill. In this phase, O_2 which is present in the waste is used up, while oxidation processes facilitate the corrosion of metals and acidification as a result of organic decomposition. LFL generated within this phase is characterised by low biological oxygen demand (BOD) and an elevated pH (5-6). The main products generated are CO_2, water, nitrate (NO^{3-}) and partially degraded organics. (Butkovskyi, 2009; Ghosh and Hasan, 2010; Schiopu and Gavrilescu, 2010; Last, 2015).

2.8.2 Phase II- Transition

During this phase, anaerobic conditions develop as O_2 has been depleted and CO_2 production is observed. NO_3^- and sulfate (SO_4^{2-}) are reduced to nitrogen gas (N_2) and hydrogen sulfide (H_2S). The LFL produced during this phase has increasing concentrations of chemical oxygen demand (COD) and pH values of 6-7, as well as volatile organic acids (VOAs) and CO_2 (Edward *et al.,* 1995; Kamaruddin, 2015).

2.8.3 Phase III- Acid formation

Acid formation, also termed the acidogenic phase, is carried out by anaerobic and facultative microorganisms, including acidogenic and acetogenic bacteria. These bacteria hydrolyse high molecular weight compounds such as lipids, organic polymers and proteins into less complex compounds such as short chains organic acids, acetic and formic acid and alcohols. Among the products of the acidogenesis phase are ammonia (NH_3) and H_2S, which result in an intense unpleasant smell (Ali Shah *et al.,* 2014).

This is quickly followed by acetogenesis, where acetogenic bacteria, such as *Syntrophomonas* and *Methanobacterium* (Ali Shah *et al.,* 2014), continue the sequential degradation to low molecular weight intermediate compounds, such as fulic acid and humic acids. During this phase, CO_2 continues to be generated, alongside CH_4 and hydrogen (H_2). LFL generated during this phase has an acidic pH (pH 5), while BOD and COD concentrations increase rapidly alongside heavy metal concentrations. The LFL produced during this stage is also characterised by high level of NH_3, total nitrogen (TN) and phosphate (PO_4^{3-}) (Butkovskyi, 2009; Schiopu and Gavrilescu, 2010; Last, 2015). The combined acidogenic and acetogenic phase and can last for up to five years (Christensen, 2011; Townsend *et al.,* 2015).

2.8.4 Phase IV- Methane fermentation

During the methanogenic stage, organic acids are converted to CH_4 and CO_2 by methanogenic archaea. Also, during this phase SO_4^{2-} and TN compounds are also reduced to sulfides (S^{2-}) and NH_3 via nitrification and sulphur reducing bacteria (SRB). Often methanogenesis and acidogenesis occur simultaneously (Kjeldsen *et al.,* 2002; Christensen, 2011). LFL generated during this phase has typical pH values of between 6-8 due to the production of landfill gases, CH_4 and CO_2. In addition, the marked reduction in the concentration of BOD and COD observed in this phase is often concomitant with an increase in the concentration of NH_3. Alongside this TN, PO_4^{3-} and heavy metals are found at lower concentrations

(Christensen *et al.*, 2001; Schiopu and Gavrilescu, 2010; Christensen, 2011). The transition from the acidic phase to the CH_4 fermentation phase takes between 4 to 10 years after waste placement within a landfill and may continue over a period of several years (Schiopu and Gavrilescu, 2010; Kamaruddin *et al.*, 2017).

2.8.5 Phase 5 – Maturation

Biological decomposition becomes less active during this stage, landfill gases start depleting and leachate generation becomes more stable. LFL generated at this stage contains humic- and fulic- acid, which can be difficult to degrade biological (Kreith and Tchobanoglous 2002).

2.9 Classification of leachate

The classification of LFL is largely dependent on landfill age, however, certain factors including, the composition of waste, the extent of rainfall infiltration and temperature can also affect LFL characteristics (Christensen *et al.*, 2001; Kjeldsen *et al.*, 2002; Renou *et al.*, 2008). Age-based classification, which refers to the age of the landfill in which the LFL was generated, includes, recent, young and mature. Tables 1-1 outline the typical age-based characteristic of LFL.

2.9.1 Fresh

Recent LFL is generated in landfills that have just begun receiving waste, within which waste decomposition is occurring at a fast rate. The pH of these LFL is close to netural, as O_2 which is present in the waste is being consumed by the indigenous microorganisms during the decomposition of organic material. Recent LFL contains elevated levels of BOD and COD and are generally characterised by

BOD/COD ratios of > 0.3 (Christensen *et al.,* 2001; Renou *et al.,* 2008; Kamaruddin, 2015).

2.9.2 Young

A young landfill (<10 years old) normally contains large amounts of biodegradable organic matter. This facilitates rapid anaerobic fermentation resulting in the production of large volumes of VFAs. Young LFL is characterised by high COD, TOC and BOD (Renou *et al.,* 2008; Kamaruddin, 2015). This young LFL produced during the hydrolysis- acetogenesis phases is also characterised by pH values of ≤7.5 and BOD_5/COD ratios of < 0.7. (Christensen *et al.*, 2001; Halim *et al.*, 2010; Lee and Nikraz, 2014; Contrera *et al.*, 2015).

2.9.3 Mature

As landfill matures, it enters the methanogenic phase of waste degradation as described in Section 1.7.7. During this phase, methanogenic archaea begin converting VFAs to biogas comprised of CH_4 (50-60 %), CO_2 (30-40%) and H_2S (< 1%) (Buchroithner, 2015). The LFLs generated in mature landfills (>10 years) are characterised by a basic pH (>7.5), low COD (<4000 $mg.L^{-1}$ O_2) and high levels of ammonia- nitrogen (>400 $mg.L^{-1}$) (Christensen *et al.,* 2001; Abbas *et al.,* 2009; Kamaruddin, 2015; Torretta *et al.,* 2017). Low BOD/COD ratios in the range of 0.05 to 0.2 are also indicative of this stage, as large amounts of humic and fulvic acids, as well as non-biodegradable recalcitrant organic compound, are present (Christensen *et al.,* 2001; Halim *et al.,* 2010; Lee and Nikraz, 2014; Contrera *et al.,* 2015).

Table 2.1. Landfill leachate classification based on age. This table was developed based on studies carried out by Renou *et al.* (2008), Christensen *et al.*,(2001), Kjeldsen *et al* ,(2002) and Kamaruddin *et al. (*2017).

	Recent	**Young**	**Mature**
Age (years)	<5	5-10	>10
pH	4.5-7.5	7.5-7.5	>7.5
COD (mg L^{-1})	>10,000	4,000-10,000	<4,000
BOD$_5$/COD	>0.5	0.2-0.5	<0.2
TOC	≤20000		80-160
COD/TOC	≤0.3	0.3-0.5	≥0.5
Ammonia	10-800	≤1800	20-90
Organic compounds	80% VFA	5-30% VFA + humic and fulvic acids	Humic and fulvic acids
Heavy metals	Low-medium		Low
Biodegradability	Important	Medium	Low

2.10 Composition of leachate

The composition of a specific LFL is a good indicator of the stage of the biological processes occurring at a given time (Jokela *et al.,* 2002; Kjeldsen *et al.,* 2002). The parameters of LFL are represented by dissolved organic matter, inorganic macro components, heavy metals and xenobiotic organic compounds (XOCs). Various compounds have been detected in LFL, including, for example, borate (BO_3^{3-}), arsenate (AsO_4^{3-}), barium (Ba), lithium (Li), mercury (Hg), and cobalt (Co) (Christensen, 2011; Kamaruddin, 2015). However, these compounds are usually found at low concentrations and are therefore of secondary importance (Christensen *et al.,* 2001; Kjeldsen *et al.,* 2002; Kamaruddin, 2015).

2.10.1 Dissolved Organic Matter

The parameters for measuring dissolved organic matter are COD, BOD and total organic carbon (TOC). This bulk parameter covers a variety of organic matter from volatile fatty acid (VFA), as well as fulvic- and humic-like compounds. Dissolved organic matter effects leachate composition in relation to other constituents through complex properties of the high molecular weight components of the dissolving organic matter. Unfortunately, there is very little information on the composition of the dissolved organic matter in LFL. At the most, a low BOD/COD ratio suggests leachate with low concentrations of VFA and relatively higher amounts of humic- and fulvic-like compounds (Christensen *et al.,* 2001; Kjeldsen *et al.,* 2002). However, this is all dependent on the stability and stage/age of the landfill (Christensen *et al.,* 2001; Kjeldsen *et al.,* 2002; Kamaruddin, 2015).

2.10.2 Inorganic macro compounds

This grouping contains compounds such as calcium (Ca_2^+), magnesium (Mg_2^+), sodium (Na_2^+), potassium (K^+), ammonium (NH_4^+), iron (Fe_2^+), manganese (Mn_2^+), chloride (Cl^-), SO_4^{2-} and hydrogen carbonate (HCO_3^-). Similar to dissolved organic matter the presence of inorganic macro-compounds also depends on the age of the landfill. For example, in the methanogenic phase of LFL production concentrations of Ca^{2+}, Fe^{2+}, Mn^{2+}, and Mg^{2+} are all low, due to the high pH and the lower dissolved organic matter. The concentration of SO_4^{-2} is also lower in this phase due to the microbial reduction of sulphate to sulphide by SRB communities (Christensen *et al.,* 2001; Kjeldsen *et al.,* 2002; Kamaruddin, 2015).

2.10.3 Ammonia

Ammonia (NH_3), together with organic nitrogen, are collectively termed total Kjeldahl nitrogen (TKN) and are found in high concentration in LFL. In particular, high concentrations of ammonia are a common feature of LFL produced throughout the working life of a landfill, with levels considerably above the national discharge standards of 4 mg.L^{-1} for WW to receiving bodies being recorded (EPA, 2001; McCarthy *et al.,* 2010). Ammonia is extremely toxic to the aquatic environment and therefore can only be released at low concentrations (EPA, 2000b). Ammonia is present in the initial stages of waste degradation and increases in the transition and acid formation phase, while in the metrogenic stages, as the landfill mature, theammonia levels begin to slowly decrease (Schiopu and Gavrilescu, 2010; Last, 2015). In general, the ammonia concentration of LFL ranges between 500 to 2000 mg.L^{-1} (Kjeldsen *et al.,* 2002).

This is of particular concern for its subsequent treatment in WWTPs as ammonia concentrations > 600 $mg.L^{-1}$ can negatively affect microbial growth within these mainly aerobic systems thus reducing treatment efficiencies (Li *et al.,* 1999). Consequently, refusal to accept this waste stream at WWTPs as a direct result of high NH_3 levels is not uncommon, resulting in increased cost to the landfill operator (Renou *et al.,* 2008; Brennan *et al.*, 2016; Brennan *et al.,* 2017b).

Finally, due to the persistent anaerobic conditions with a mature landfill, nitrates and nitrites are usually found at low concentrations, however high levels of nitrate occur during the methanogenesis phases of waste degradation (Figure 2.7) (Othman *et al.,* 2010). Environmental nitrate pollution is of concern for two reasons. Firstly, high nitrate coupled with high phosphate levels are responsible for accelerated eutrophication causing damage to aquatic ecosystems (Xu *et al.,* 2010a; European Environment Agency, 2015). Secondly, because of their solubility in water, nitrates can move easily through soil into drinking water supplies where they represent a public health risk (World Health Organization, 2011; European Environment Agency, 2015). Similar to ammonia, nitrates are often found in high concentrations in MSW LFL ($\geq$100 $mg.L^{-1}$) and are greatly affected by the presence of oxidising conditions, which can result in volatilisation and subsequent nitrification reactions. Volatilisation within these systems results in the production of free ammonia which is then converted to nitrate via nitrification (Hassan and Ramadan, 2005). However, when reducing (anaerobic) conditions persist the reduction of nitrate to ammonia or N_2 via dissimilatory nitrate reduction may result, thus decreasing nitrate concentrations and increasing ammonia concentrations (Hassan and Ramadan, 2005).

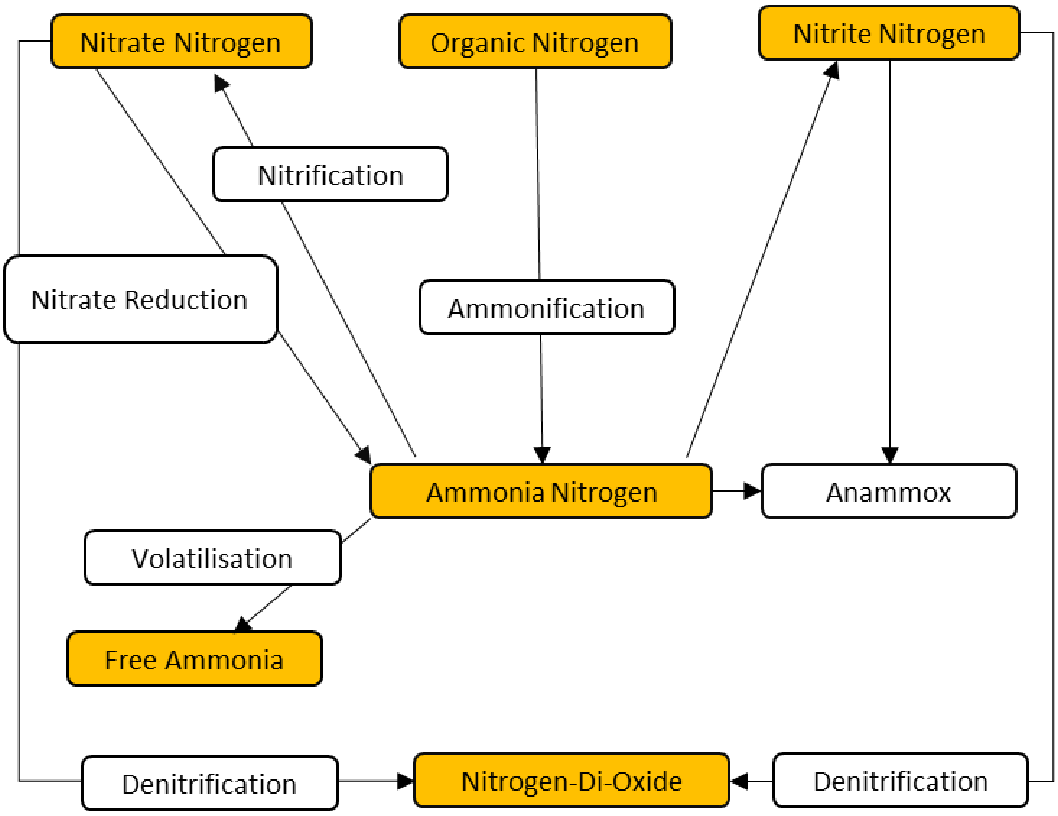


Figure 2.7. Breakdown of ammonia/total nitrogen within a landfill site. Adapted from Koda *et al* ., (2015)

2.10.4 Xenobiotic organic compounds

Xenobiotic organic compounds (XOC's) generally originate from industrial and household chemicals and are usually present at a low concentration within LFL (c.1 mg.L^{-1}). XOC's are water soluble and can negatively affect ground and surface water should contamination occur (Kjeldsen *et al.*, 2002). The most frequent compounds are halogenated hydrocarbons, such as tetrachloroethylene which enter landfill through household products such as aerosol cleaners and nappies (Leahy and Shreve, 2000; Shin *et al.*, 2002). The presence of hazardous waste within landfill has also contributed to the presence of XOC's in LFL and although this practice is now prohibited legacy effects are still being observed. Other XOC's present include phenols (cresols and 4, chlorophenol), aromatic hydrocarbons (toluene, xylenes and benzene), pesticides (bentazon and hexazinone) and halogenated hydrocarbons (chlorobenzene and tetrachloroethylene).

2.10.5 Heavy metals

Heavy m0etals, which are not biodegradable, can have a serious effect on both the environment and human health (Vaverková *et al.*, 2018). The bioaccumulation of heavy metals can result in the toxicity of living organisms and pose a risk to both animal and human health, if contamination of ground and surface water occurs (Sulaimon *et al.*, 2014; Vaverková *et al.*, 2018b). Heavy metals, including cadmium (Cd_2^+), chromium (Cr_3^+), copper (Cu_2^+), lead (Pb_2^+), nickel (Ni_2^+) and zinc (Zn_2^+), are commonly present in LFL (Baun and Christensen, 2004; Christensen 2011). In general, their concentrations remain low, as the pH increases associated with the methanogenic stages of waste degradation also increase the sorption capacity of soil rendering the metals immobile (Christensen *et al.*, 2001). As such, serval studies have described the presence of heavy metals in LFL as not of major concern (Christensen *et al.*, 2001). However, in some instances where the heavy metal concentration of LFL is above the national discharge standards a requirement for their removal/treatment is necessary.

2.10.6 Landfill Microbiology

Microorganism, including bacteria, archaea and fungi are found in abundance within landfill sites. Due to the presence of rich organic matter and substrate complexity within these regions, landfills are considered "microbial pools" (Song *et al.,* 2015a; Wang *et al.,* 2017). As discussed previously (Section 1.7) landfill decomposition is generally characterized by changes in the physicochemical parameters with little or no reference to the changing microbial ecology throughout its working life (Song *et al.,* 2015a; Song *et al.,* 2015b). Limited research, using both culture and culture-independent techniques, has been carried out in an effort to elucidate these communities within different landfill sites (Sawamura *et al.,* 2010; Gomez *et al.,* 2011). In general, research has indicated that the microbial community structure within landfill is strongly influenced by the environmental conditions present and their substrate-specificity (Song *et al.,* 2015b; Song *et al.,* 2015a). A general schematic of the anaerobic and aerobic bioconversions of complex organic wastes present in a landfill is presented in Figure 2.8 (Song *et al.,* 2015b; Song *et al.,* 2015a).

During the aerobic stage, bacteria and fungi convert organic waste to low molecular weight compounds, mainly CO_2, H_2O and humic-like substances. The resulting high energy yields enable a fast rate of microbial growth (Sang *et al.,* 2012). During the nitrification process, ammonia is converted to nitrite by ammonia-oxidizing bacteria which is further reduced to nitrate by nitrite-oxidizing bacteria. In anoxic conditions, denitrifying bacteria convert nitrogen into N_2 via N_2O. Without the combination of both these aerobic and anoxic reactions, nitrogen cannot be removed efficiently from landfills (Sang *et al.,* 2012). During the anaerobic stage complex, organic wastes such as carbohydrates, proteins, and lipids are hydrolysed to monosaccharides, amino acids, and fatty acids by acidogens. These bacteria reduce these products to H_2, CO_2, and organic acids such as lactate and acetate. These products can then be converted anaerobically to CH_4, via acetoclastic and hydrogenotrophic methanogenesis (Sang *et al.,* 2012). To date, a wide range of microorganism have been isolated from LFL. Wang *et al .,* (2017) examining the microbial community structure of an MSW landfill in Yangzhou City, East China, found Firmicutes, Proteobacteria, *and* Bacteroidetes to be dominant phyla. Similarly, a range of both gram-positive and negative bacteria were isolated and identified from LFL via plate count techniques and the Minitek microbial identification system (Hale Boothe *et al.,* 2001). This study found a range of Gram-positive spp. including *Bacillus megaterium*, *Bacillus pasteurii*, *Staphylococcus lentue, Staphylococcus delphini* and Gram-negative spp. that mainly belonged to the genera Acinetobacter, Pseudomonas, Yersinia and Enterobacter. Furthermore, Krishnamurthi and Chakrabarti (2013) isolated a diverse grouping of bacteria including *Bacillus spp., Staphylococcus ssp., Lysinibacillus spp., Brevibacillus spp.* and *Clostridium spp.* from LFL produced in Chandigarh, Northern India.

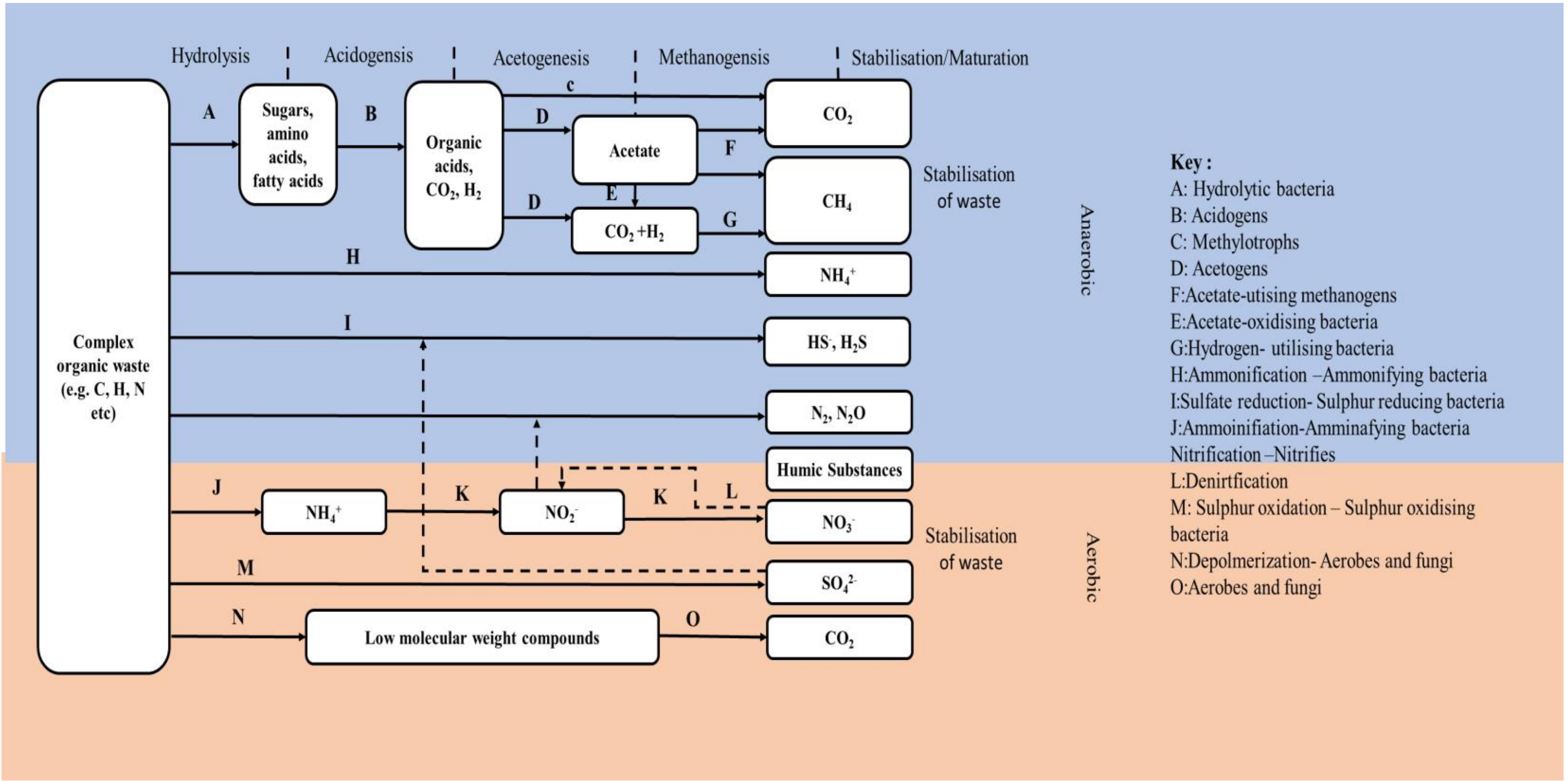


Figure 2.8. Anaerobic and aerobic bioconversions of complex organic wastes present in landfill. Adapted from Sawamura *et al* ., (2010).

2.11 Factors affecting leachate production

Leachate generation and composition are affected by various other factors such as climatic conditions and the composition of waste which will be briefly discussed below. Other factors that influence leachate generation, which are not extensively discussed in the literature, are the presence/absence of O_2 and H_2, the MSW retention time within the landfill, type of material used in the final cover of the landfill and leachate recycling (Fielding *et al.*, 1988; Chung and Poon, 2001; Borglin *et al.,* 2004; Sanphoti *et al.*, 2006; Francois *et al.*, 2007; Hossain and Haque, 2009).

2.11.1 Climatic variation

Changes in ambient temperature and landfill water balance strongly influence LFL characteristics, as water availability (A_w) and temperature impact microbial activity thus affecting waste degradation rates (Renou *et al.,* 2008; Christensen, 2011). For example, landfills situated in hot, arid regions tend to generate small amounts of concentrated LFL, whereas those located in tropical regions generate larger volumes of dilute LFL, because of the precipitation amounts in these respective areas (Renou *et al.,* 2008; Kamaruddin, 2015). Tränkler *et al .,* (2005) highlights that degradation rates in landfills are slower in areas with dry climates but improve significantly during rainy seasons. The study also calculates that >60% of LFL is composed of precipitation, which infiltrates landfill sites, a factor that cannot be avoided when a landfill cell is actively receiving waste. In addition, it should be noted that the volume of moisture-rich wastes (organic matter) and liquids that are accepted into a landfill also increase the production of LFL (Hartz and Ham, 1983; González *et al.,* 2011). Biological decomposition occurs faster in hotter climates due to increased microbial activity. This, in turn, helps the landfill to convert from the transition to acid formation and methanogenic phases at a quicker rate (Khattabi *et al.*, 2002; Kamaruddin *et al.*, 2017).

2.11.2 Waste composition

The composition of MSW and its subsequent decomposition greatly influences the quality and quantity of LFL. During this process, the various breakdown products of waste infuse moisture present in the landfill to produce LFL. In particular, waste with both high organic and moisture content produce high strength leachates due to the amount of water available and the organic and inorganic compounds produced during waste degradation (Moody and Townsend, 2017). However, several studies have found that waste minimization measures such as the segregation of the organic matter fraction of MSW (compostable waste), waste pre-treatment, and recycling can have a significant impact on LFL quality (Kamaruddin *et al.,* 2017). As such, in order to minimize the generation of high strength LFL based on waste composition, it is recommended that landfill operators review individual site acceptance practices (Christensen, 2011).

2.12 Leachate treatment

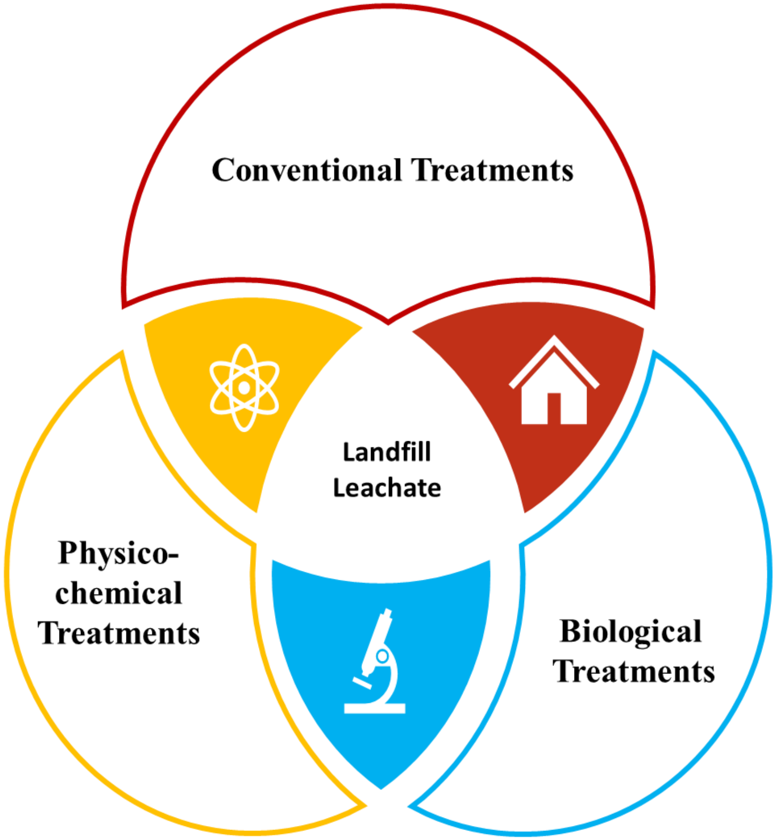
Currently, many different methods are employed for the treatment of LFL, most of which are adapted from WW treatment methods (Raghab *et al.,* 2013). In general, a combination of both biological and physiochemical methods are effective for the treatment of LFL, as it can be difficult to obtain satisfactory results with just one method (Figure 2.9; Kargi and Pamukoglu, 2004). In addition, the LFL generated in different facilities often vary greatly in composition and require different types of treatments. For example, LFL that has a high organic content can be effectively treated using biological processes, whereas LFL with a low organic content is more suited to treatment via physicochemical methods (Kheradmand *et al.,* 2010; Gao *et al.,* 2015; Torretta *et al.,* 2017). The following section aims to describe; (1) an overview of the current treatment options for LFL; (2) biological treatment methods including both aerobic and anaerobic treatments; and (3) physiochemical treatments processes such as adsorption and reverse osmosis. These treatment options are summarised Tables 1.2, 1.3 and 1.4. In addition, Figure 2.10 highlights the number of papers relating to this topic that have been published since the beginning of 2014.

Conventional Treatments

Recycling/Recirculation
Treatment with Domestic Sludge (WWTPs)

Physicochemical Treatments

Adsorption
Membrane Filtration
Flocculation-Coagulation
Air Stripping
Chemical Precipitation
Ion Exchange
Chemical Oxidation



Biological Treatments

(1)Aerobic
Sequence Batch Reactors
Trickling filters
Aerated Lagoons
Constructed Wetlands
Rotating Biological Contactors
Moving Bed Reactors

(2)Anaerobic
Anammox
Up-Flow Sludge Blanket
Anaerobic filters
Submerged Anaerobic Membrane Reactor

Figure 2.9. The current treatment options for landfill leachate.

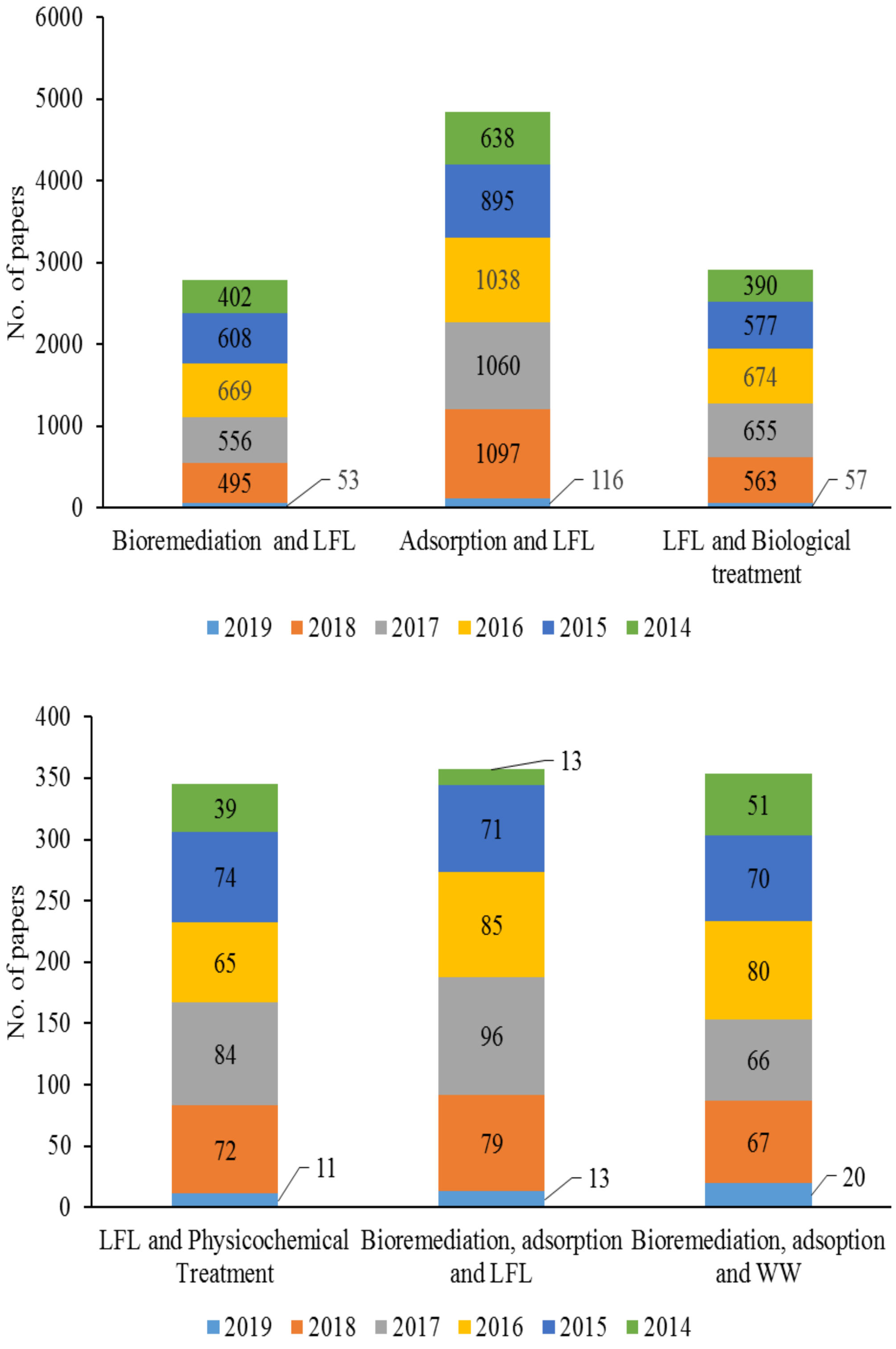


Figure 2.10. The number of papers published (Pubmed) between the year 2014 to the start of 2019, relating to key topic highlighted on the x-axis.

2.12.1 Current treatment options for LFL

2.12.1.1 Recycling

LFL recirculation/recycling within landfill sites has been employed since the 1990s for the purpose of LFL management, landfill gas generation and recovery, and improving the overall stability and sustainability of the landfill (White *et al.*, 2011; Jones-Lee and Fred Lee, 2018). In general, the process involves controlling the landfill biological, chemical and physical process through the addition of effluent LFL back into the landfill, which is now referred to as a landfill bioreactor (Lema *et al.*, 1988; Warith, 2002). Benson *et al.* (2007) assessed the composition of LFL generated in a landfill bioreactor system and observed a reduction in the BOD/COD ratio from 0.5-0.7 to 0.1 after a four-year period of LFL recirculation. Wraith (2002) also studied leachate recirculation in an MSW landfill, in Toronto, Canada. The objectives of this study were to enhance organic waste degradation over a six-month period, with leachate samples being collected for analysis weekly. Wraith (2002) observed decreases in LFL COD concentrations from 9910 mg.L^{-1} to 200 mg.L^{-1} and BOD concentrations from 2055 mg.L^{-1} to 200 mg.L^{-1}. Chugh *et al* . (1998) investigated the effect of LFL degradation in a 200 L bioreactor containing two tonnes of MSW waste, with an applied LFL recirculation rate of 2.2 L.min^{-1}. The study concluded that high recirculation rates improved the solubilization of fresh waste and established a methanogenic microbial culture quicker, allowing for the stabilisation of waste within the reactor. Similar results were observed by Šan and Onay (2001) and Reinhart and Al-Yousfi (1996). However, although the recirculation of LFL at landfill sites has recorded positive results, increasing the time LFL is retained within landfill sites also increases the potential for ground and surface infiltration and pollution, which are of major concern. The summary of this treatment option is highlight in Table 2.4

Table 2.2. Summary of leachate recycling as a treatment

		Removal rate		
Treatment options	**Study**	**COD**	**BOD**	**COD:BOD**
Leachate Recycling	Benson *et a.,* (2007)			0.5-0.7 decrease 0.1 after 4 years
	Wraith (2002)	9910 mg.l^{-1} decrease 200 mg.l^{-1}	2055 mg.l^{-1} decrease 200 mg.l^{-1}	

2.12.1.2 Wastewater treatment plants

One of the most common solutions for LFL is its co-treatment with domestic WW in WWTPs (Renou *et al.,* 2008; Abbas *et al.,* 2009; Gao *et al.,* 2015). This process involves the drip feeding or shock loading of LFL into the influent of the WWTP. Theoretically, this is an economical and practical solution where LFL contributes to the nitrogenous fraction of this waste stream, while sewage contributes to the WWTPs phosphorus requirements (Borghi *et al.,* 2003; Abbas *et al.,* 2009; Gao *et al.,* 2015). However, many critics have questioned reliance on this option due to the presence of organic compounds with low biodegradability (PAHs PCBs, humic- and fulvic- acids) and heavy metals in LFL that may reduce treatment efficiency and therefore increase the effluent concentrations of these facilities (Renou *et al.,* 2008; Gao *et al.,* 2015). The co-treatment of LFL and its suitability to be treated in WWTPs has recently been reviewed in Ireland (Brennan *et al.,* 2016; Brennan *et al.,* 2017a; Brennan *et al.,* 2017b). These reviews have indicated that 99% of all LFL is managed either by direct discharge to sewer or tankered to WWTPs, with just 1% receiving some type of treatment onsite. This 1% consists of six landfill sites, of which only three facilities are achieving the standards that allow direct discharge to receiving bodies (Brennan *et al.*, 2016; Brennan *et al.*, 2017b). The same reports also indicate that the supplementation of domestic WW with LFL places stress on already overburdened treatment plants, putting pressure on them to reach the stringent discharge limits set by the EPA, with the main advantage and disadvantages highlighted in Table 2.4. This point is also highlighted by the fact that 30% of Irish WWTPs refused to accept LFL during the period 2010-2014 (EPA, 2015; Brennan *et al.*, 2016). As such, the treatment of LFL within WWTPs is deemed inappropriate for this waste stream and alternative solutions must be sought. The advantages and disadvantages of employing WWTPs for the treatment of LFL as described previously (Renou *et al.*, 2008; Teixeirae Junior and Marinheiros, 2014; Brennan *et al.*, 2017b; Lippi *et al.*, 2019) are outlined in Table 2.3

Table 2.3.. Advantage and disadvantage of treating LFL in a WWTPs.

Advantages	Disadvantages
Effects a reduction of the COD / BOD ratio of LFL, thus increasing its biodegradability due to synergistic effect when it is mixed with sewage.	Many WWTP's can become over loaded by the addition of LFL to influent as they are normally designed for specific organic loading rates. This overloading can have a negative impact on the efficiency and performance of these processes.
Greater amount of biologically available organic matter encourages the degradation and stabilization of microorganisms.	Conventional WWTPs are not suitable for the treatment of recalcitrant compounds including heavy metals which may be present in LFL.
The alkalinity of LFL produced in the methanogenic phase, favours anaerobic treatments, eliminating the need for external means of pH correction.	Heavy metals can have inhibitory action towards the nitrifying and heterotrophic bacteria present in these largely aerobic systems.
Operational simplicity for landfills operators as LFL only requires transportation off site.	High ammonia levels can put pressure on the WWTPs to reach national guidelines.
	High transportation and storage costs are associated with treating LFL in WWTPs.
	LFL can increase the production of sludge as well as the alteration of its composition preventing its reuse.
	Within Ireland, LFL can only be added to WWTP at a maximum of 4% of the hydraulic load which results in a storage issue for LFL awaiting treatment.

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2.13 Biological treatments

Due to their reliability, simplicity, and cost-effectiveness, biological methods are commonly employed for the treatment of LFL (Renou *et al.,* 2008). Biological treatment methods are particularly effective for the reduction of high strength biodegradable organics present in LFL, specifically COD and BOD. It is well documented that young LFL (< 5 years) which are characterised by COD/BOD ratios of 0.4-0.6 are more suited to biological treatment than older LFLs, due to the abundance of biodegradable organics (Renou *et al.,* 2008; Torretta *et al.,* 2017). As the landfill mature the COD/BOD ratio reduces to ≤ 0.2, and it becomes less amenable to biodegradable due to the present of humic and fulvic acid, alongside other recalcitrant organic compounds (Kreith and Tchobanoglous, 2002; Lee and Nikraz, 2014; Contrera *et al.,* 2015). Biodegradation is carried out by microorganisms, mainly aerobic and anaerobic bacteria, which degrade organic compounds to CO_2 and CH_4 (Song *et al.,* 2015a; Wang *et al.,* 2017). Thus, biological treatment options can be divided into two categories; aerobic and anaerobic processes (Figure 2.9). In general, aerobic treatment options are inexpensive and effective at degrading organic pollutants alongside BOD and COD (Bove *et al.,* 2015). Most biological treatment options are based on suspended growth system, such as sequence batch reactors (SBR), activated sludge processes, biofilm reactors, and aerated lagoons, with numerous reports within the literature on the use of these to treat LFL (Diamadopoulos *et al.*, 1997; Lin and Chang, 2000; Loukidou and Zouboulis, 2001; Xu *et al.,* 2010b). In contrast, anaerobic options are more suited for the treatment of high strength LFL.

Unlike aerobic treatments, anaerobic treatments are less energy intensive, produce fewer solids and can operate at lower/ambient temperatures (Lin and Chang, 2000; Christensen, 2011). In addition, anaerobic treatment options can be utilised to transform waste into a valuable renewable energy source in the form of biogas (CH_4, CO_2, H_2, N_2, O_2 and H_2S). Some of these anaerobic treatment options include the up-flow anaerobic sludge bed (UASB), suspended growth biomass process, some SBR and anaerobic filters such as hybrid bed filter and fluidized bed reactors. (Kennedy, 2000; Lin *et al.,* 2000; Liang and Liu, 2008; Sun *et al.*, 2010; Miao *et al.*, 2015). For the purpose of this thesis, the treatment of LFL by both aerobic and anaerobic options has been reviewed. The advantages and disadvantages of both systems as described previously (Lin and Chang, 2000; Christensen, 2011) are outlined below in Table 2.4.

2.13.1 *Aerobic treatment*

Table 2.4 Advantages and disadvantages of aerobic treatment

Advantages	Disadvantages
Low hydraulic retention time (HRT) and high sludge retention time (SRT).	Forced aeration and mixing add to high energy requirement.
Low volumes of sludge production.	Biofilm reactors requires high concentrations of dissolved oxygen for denitrification.
Increased resistant to toxic shock.	Congestion problems associated with fixed bed systems.
Active biological biomass retained within the system.	
Low sensitivity to temperature changes.	
Nitrification achievable at low temperatures (approx. 5°C).	
Nutrient removal.	
Simultaneous nitrification and denitrification on the exterior and interior of microbial biofilm.	

2.13.2 *Anaerobic treatment*

Table 2.5. Advantages and disadvantages of anaerobic treatment

Advantages	Disadvantages
Lower dosage of phosphorus required as a growth factor for anaerobic bacteria.	Sensitive to changes in temperature and pH, as well as various toxic substances, including heavy metals and ammonia.
Low energy requirements, can act as a net producer of energy via the production of CH_4 rich biogas.	Longer start-up periods.
Low biomass production.	
High organic loading rates coupled with high removal rates for both BOD and COD.	

2.13.3 Sequence batch reactors

SBRs represent an activated sludge treatment technology that involves one or more steps under non-stationary conditions (Spagni *et al.,* 2008). The system typically employs aerobic conditions and is composed of four sequential steps; (1) fill, (2) react, (3) settle, and (4) discharge. This treatment method is particularly effective for nitrification and denitrification of LFL, as it provides an operational regime compatible with concurrent organic carbon oxidation and nitrification (Renou *et al.,* 2008). For example, a study carried out combining the treatment of LFL and municipal sewage in an SBR, reported removal rates of 80%, 95% and 85% for ammonia, COD and BOD, respectively (Diamadopoulos *et al.,* 1997). Laitinen *et al* . (2006) combined SBR and membrane reactor technology for the treatment of LFL from a Finnish MSW landfill, achieving removal rates of 94%, 99% and 82% for BOD, ammonia and phosphorus, respectively. In addition, the treatment of LFL using SBRs under anaerobic conditions has also been investigated. For example, Timur and Özturk (1999), employed such a system for the treatment of LFL sourced from an MSW landfill, with COD concentrations ranging between 3,800 -15,940 mg.L^{-1} and a pH of 7.4-8. The study reports COD removal rates of between 64-84% with conversion to CH_4 calculated at 84% (Table 2.2). In general, the these studies indicate that SBR treatment is robust and less affected by frequent variation of organic load or ammonia nitrogen (Laitinen *et al.,*2006). This is important in terms of LFL treatment as these concentrations can vary greatly over time. However, this system is not without its drawbacks. One such issue is carbon consummation in the aeration stage, which can lead to insufficient carbon sources in the following anoxic stage resulting in low TKN removal rates (Wei *et al.,* 2012). Ammonia levels have also been shown to impact and decrease the organic biodegradation rates of these systems, suggesting that it may be necessary to modify the SBR to add a pre/post-treatment step to achieve higher removal rates (Wei *et al.*, 2012).

2.13.4 Rotating biological contactors

Rotating biological contactors (RBC) are aerobic fixed-film biological treatment process, that consists of a series of closely spaced discs on a horizontal shaft composed of plastic materials, generally polyvinyl chloride (PVC) or polystyrene. The discs, which have a large surface area on which biofilms can form, are submerged via rotation in the system liquor. The applied hydraulic retention time (HRT), rotational speed, temperature and the submerged surface area of the disc govern the efficiency of this treatment option (Castillo *et al.,* 2007a; Cortez *et al.,* 2008). RBC systems have been used widely to treat WW that are suitable for biological treatments including industrial WW (Torkian *et al.,* 2003; Hanhan *et al.,* 2005; Cortez *et al.,* 2008). The main advantages of RBC systems are their ease of operation and maintenance, however, they require substantial initial investment and have high running costs (Tchobanoglous *et al.,* 2003; Spuhler, 2018). Several studies have evaluated the use of RBCs for the treatment of LFL. Wang *et al* . (2009) reviewed the literature and stated that the "long retention time of biomass in RBC and significant thickness of biofilm facilitates nitrogen removal in addition to nitrification". This is the oxidation process of ammonia to nitrate and then to nitrite which has been observed in LFL with low biodegradable organic matter (Wang *et al.,* 2009). The study also recorded a total nitrogen removal rate of 70%. Cema *et al* (2007) operated an RBC anammox process over a six-month trial period investigating the removal of ammonia and nitrate from LFL. The study recorded maximum ammonium and nitrate removal rates of 0.56 kg N m^{-3} and 0.76 kg N m^{-3} d^{-1}, respectively, while the maximum inorganic nitrogen removal rates were 0.93 kg N m^{-3} d^{-1}. This study indicates that RBC can be used to operate anammox processes for the successful reduction of nitrogen compounds in LFL.

In addition, RBC can be employed for the removal of COD from LFL. One such study described the use of perforated acetate disc covered with a polyester mesh for COD removal under different rotational speeds, flow rates and HRTs (Castillo *et al.*, 2007a). High COD removal rates of 69% and 74% were achieved, however operational issued related to disc detachment significantly impacted these rates. Nevertheless, both studies indicate the potential for RBC to be employed for the treatment of LFL, however, in order to achieve optimal removal rates, RBC systems should be combined with additional processes to remove both inorganic and organic compounds (Cema *et al.*, 2007; Cortez *et al.*, 2008; Hassard *et al.*, 2015).

2.13.5 Moving bed biofilm reactors

Moving bed biofilm reactors (MBBR) are typically aeration tanks containing suspended porous polymeric carriers with high specific surface areas onto which microbial biofilms can develop. This carrier material usually occupies c. 50% of the reactor volume and is maintained in a fluidized state within the bioreactor through aeration (Chen *et al.,* 2008; Bove *et al.,* 2015). The advantages of these systems over other conventional suspended-growth processes are summarised in the literature as; (1) MBBR utilises the whole tank volume for biomass growth and as such have a very low head-loss; (2) unlike activated sludge processes, they do not require sludge recycling as biomass is kept within the system by a sieve at the reactor outlet; (3) lower sensitivity to toxic compounds; (4) organic and high ammonia removals in a single process and; (5) the reactor may be used for aerobic, anoxic or anaerobic processes (Welander *et al.*, 1998; Loukidou and Zouboulis, 2001; Chen *et al.*, 2008; Renou *et al.*, 2008). Several studies describe the use of MBBR for the treatment of LFL with varying results. One study, Welander *et al* . (1998) describes the removal of biological nitrogen from LFL in a two-stage aerobic and denitrification process, which resulted in a 90% removal efficiency of both inorganic and total nitrogen. Chen *et al* . (2008) employed an MBBR system with an anaerobic-aerobic configuration for the treatment of LFL. The research concluded that high COD removal efficiencies of >90% were achieved for applied organic loading rates (OLRs) of 4 -15 kg COD m^3d^{-1} within the anaerobic system, while the aerobic phase acted as a COD-polishing and ammonium removal step. Furthermore, Loukidou and Zouboulis (2001) investigated the effect of different carrier materials, free-floating polyurethane particles and granular activated carbon (GAC), in MBBR used for the treatment of LFL. The GAC offered both a porous media onto which both organic matter and ammonia could be adsorbed and a suitable surface for microbial biofilm formation. Both methods were used in the degradation of nitrogen as well as the removal of organic compounds. GAC outperformed the polyurethane particles, having a higher COD removal rate of 81% compared to 65%, while little difference was observed between BOD and nitrogen removal efficiencies.

2.13.6 Anaerobic ammonium oxidation

Anaerobic ammonium oxidation (anammox) is defined by Jetten *et al* ., (2009) as a microbiological process carried out by strictly anoxic autotrophs primarily using ammonium and nitrite as substrates for their catabolism (Jetten *et al.,* 2009). The process has been harnessed in several of the applications previously described including SBR, MBBR and up-flow anaerobic sludge bed (UASB) (Xiong *et al.,* 2013; Vega De Lille *et al.,* 2015; Klaus *et al.,* 2017) for the treatment of high strength ammonium-rich WW including LFL (Zhang and Zhou, 2006; Gao *et al.,* 2015). For example, Ruscalleda *et al* ., (2010) described the successful removal of ammonium and nitrogen from LFL using both an anammox and a heterotrophic denitrification process. LFL was initially treated via a partial nitrification process before being fed into an anammox SBR. Overall the results of the study indicated an 87% nitrogen removal efficiency, with the anammox process contributing to approximately 85% of this removal. In comparison Selic *et al.* (2007) employed an SBR for the treatment of LFL from both Chinese and German landfill facilities and recorded a c.90% reduction in both COD and ammonia concentrations. Similar results were observed by Wang *et al.* (2011) who recorded a 21-45 % COD removal efficiency with a simultaneous 62-80% reduction of total nitrogen while treating LFL in a combined SBR and anammox process. Anammox is a suitable and sustainable treatment option for LFL which can be coupled with other treatment options, as described above. In addition, the anammox process is also suitable for the treatment of LFL from older landfills which are characterised by high ammonia concentrations and low levels of organic compounds such as COD and BOD (Gao *et al.,* 2015; Torretta *et al.,* 2017).

2.13.7 Up-flow anaerobic sludge bed

The UASB is a single tank process into which WW enters via an inlet at the base of the bioreactor and is forced upwards via hydraulic pressure. UASB bioreactors contain metabolically active anaerobic granular sludge which is retained within the system enabling the treatment of WW at high organic loading rates (Renou *et al.*, 2008; Torretta *et al.*, 2017). The anaerobic granules are composed of bacteria and archaea (methanogens) which reduce influent WW via anaerobic digestion (AD). AD is the bacterial breakdown of organic materials in the absence of oxygen (Rogoff and Rogoff, 2014). This biological process produces a gas, sometimes called biogas, principally composed of CH_4 and CO_2 (Rogoff and Rogoff, 2014). Kettunen and Rintala (1998) employed UASB bioreactors inoculated with mesophilic biomass from a sewage treatment plant for the treatment of MSW LFL at low temperature (13-20°C). The LFL used in the study had a COD concentration of 630-2,200 mg.L^{-1} and the bioreactors achieved COD removal efficiencies ranging between 50% and 75%, with the highest removal rates observed at temperatures ≥18°C. Sun *et al* . (2010) also investigated the removal of COD from LFL using two UASB bioreactor operated at low temperature (10-15°C) and an SBR system. Both UASB bioreactors were inoculated with activated sludge from a WWTP treating a brewery WW in Heilongjiang, China. The influent COD concentration ranged between 7,856-22,500 mg.L^{-1}. The research reported a high removal efficiency (≥77%) within the first USAB bioreactor, with reduced COD removal in the subsequent treatment systems. Furthermore, Lin *et al* . (2000) also employed USAB bioreactors to treat LFL combined with septage (septic tank sludge) in the ratio of 1:1. These bioreactors had an applied ORL of 7.73 kg COD $m^3.d^{-1}$ and a HRT of 1.5 d. Percentage removal efficiencies for total COD, ammonia and phosphorus were 42%, 47%, and 44%, respectively. As outlined through the examples above the use of USAB bioreactors for the treatment of LFL has been proven feasible. However, the sensitivity of the AD process to high concentrations of ammonia remains a concern and makes this treatment option unsuitable as a standalone treatment for LFL (Renou *et al.*, 2008; Torretta *et al.*, 2017).

Overall biological treatments are particularly effective for the removal of ammonia, BOD and COD from LFL. A summary of the previously outlined biological treatment options is provided in Table 2.2. In summary, the biological treatments described above have individual advantages and disadvantages. Specifically, the main disadvantage of most commercially available biological treatment options, with the exception of anaerobic systems, is their high operational cost, mainly caused by the forced aeration and mixing which is required for large scale aerobic processes. In addition, many biological treatment options are not effective for the removal of inorganic compounds, such as heavy metals from WW. As such, for biological treatment to be effective, they must successfully treat the wide range of compounds found within LFL, including heavy metals, phosphate and sulphate. In some instance, pre/post treatments options may be required to treat LFL to the national discharge standards required for each country.

Table 2.6. Summary of biological treatment options for LFL.

Treatment Option	**Study**	**Removal Rate %**				
		BOD	**COD**	**Ammonia**	**Phosphate**	**Nitrogen**
Sequence batch reactors	(Diamadopoulos *et al.*,1997)	80	98	85	-	-
	(Laitinen *et al.*,2006)	94	-	99	94	-
	(Timur and Özturk 1999)	-	64-84	-	-	-
Rotating Biological Contactors	(Cema *et al.*,2007)	-	-	88	95	-
	(Castillo *et al.*,2007a)	-	67-94	-	-	-
Moving Bed Biofilm Reactor	(Welander *et al.*,1998)	-	-	-	-	90
	(Chen *et al.*,2008)	-	86-91	97	-	-
		-		-		-
Anammox	(Blauvelt 2009)	-	15	-	-	87
	(Selic *et al.*,2007)	-	90	90	-	-
Up-Flow Anaerobic Sludge Bed	(Kettunen and Rintala 1998)	95	60-75	-	-	-
	(Sun *et al.*,2010)	-	77	-	-	-
	(Lin *et al.*,2000)	-	42	47	44	-

2.14 Physicochemical treatment

The physicochemical treatment of LFL is often necessary when the concentration of polluting compounds greatly exceed their national discharge standards. These treatment options are commonly employed for the removal of non-biodegradable organics such as humic and fulvic acids, heavy metals and absorbable organic halogens (AOXs) (Renou *et al.,* 2008; Abbas *et al.,* 2009) and are also effective for the reduction of colour and colloidal particles (Gao *et al.*, 2015; Torretta *et al.*, 2017). Physicochemical methods are usually hampered by the presence of bio-refractory compounds and as such are best employed as pre- or post-treatment steps. Physicochemical treatments are commonly used for the treatment of mature LFL (> 10 years), characterised by a BOD: COD ratio $\leq$0.2 with low amounts of biodegradable compounds (Kjeldsen *et al.*, 2002; Lee and Nikraz, 2014). The primary physicochemical options that have been successfully used for the treatment of LFL, including adsorption, membrane filtration, chemical precipitation and chemical oxidation, are described below.

2.14.1 Adsorption

Adsorption is the process of accumulating substances that are in a solution (gas or liquid) on a suitable interface (Tchobanoglous *et al.*, 2003; Worch, 2012). Fixed bed adsorption has become a frequently used physiochemical treatment option for many different WW streams largely due to ease of use and cost (Karunarathne and Amarasinghe, 2013; Jahangiri-Rad *et al.*, 2014; Biswas and Mishra, 2015). Adsorption can be carried out using activated carbon (AC), or other absorbents, such as zeolite, pumice stone and calcareous shells (Tsai *et al.*, 2006; Renou *et al.*, 2008; Halim *et al.*, 2010; Melegari and Matias, 2012; Asgari and Rahmani, 2013; Chansuvarn, 2018). Several studies have been conducted testing a range of adsorbent on different WW (Song and Gao, 2013; Lakdawala and Patel, 2015; Huong *et al.,* 2016), with a few investigating the treatment of LFL. Martins *et al.*(2017) examined the use of clinoptilolite (zeolite) to remove ammonium from LFL in batch studies. The study employed the Langmuir isotherm to predict adsorption parameters and reported q_{max} (maximum theoretical adsorption capacity per g of adsorbent) values ranging between 2.61-17.68 $mg.L^{-1}$ NH_4-N under different operational parameters including pH, the quantity of adsorbent and concentration of the LFL. Langmuir isotherm depicts monolayer adsorption and is widely used in adsorption assays to determine the type and amount of ions being adsorbed. In addition, Halim *et al* . (2010) analysed the capability of AC, zeolite and a composite material containing zeolite, limestone, AC and rice husk carbon, to remove COD and ammonia from LFL in batch studies. The Langmuir isotherm was also applied to the data and revealed that COD and ammonia adsorption rates varied greatly between the different adsorbent materials tested. However, it is important to remember that research carried out using batch studies as described above, can give errors over longer time frames (Callery *et al.*, 2016). As such and ideally, adsorption materials should also be examined in fixed bed columns systems to determine if LFL can be treated over longer time periods. Further to this, Aziz *et al.*(2010) enhanced an SBR treatment option with adsorption, using powdered activated carbon (PAC) for LFL treatment. The SBR consisted of six 2 L beaker containing 1.08 L of sludge and 120 ml of LFL from Kulim Landfill, Kedah, Malaysia and had 1.2 g of PAC added, with a standalone SBR control. Removal efficiencies of COD and ammoniacal nitrogen (NH_4-N) were monitored throughout the experiment. COD (influent 1655 mg.L^{-1}) had removal rate of 69.8%, while, ammonia (influent 600 mg.L^{-1}), had a removal rate of 74% in the SBR, but increases to 76% with the addition of PAC.

Adsorption has been proven effective for the treatment of LFL, but removal rates depend on the adsorption materials utilised. These systems also have the potential to be cost-effective and easily operated. This technology also has the added benefit of being implemented as an isolated treatment or in combination with biological treatment options as described above.

2.14.2 Air Stripping

High levels of ammonium nitrogen are often found in LFL (Renou *et al.*, 2008; Christensen, 2011; Brennan *et al.*, 2017b). Air stripping, a process of separating the organic constituents from a waste stream via exposure to air, can be successfully used for eliminating this pollutant, known to increase WW toxicity(Renou *et al.*, 2008; Christensen, 2011)During the process, volatile organic compounds (VOCs) are freed from the aqueous phase to a gas phase until a state of equilibrium is reached. This process occurs in a stripping tower, containing aerated media, into which the LFL is filled. Treated leachate is collected at the bottom of the tower and the gases rise to the top. The air that is polluted with ammonium is then treated with sulphuric or chloric acid before release. However, treated leachate does not always reach the discharge limit for ammonia and often requires re-treatment before release into a biological treatment facility (Renou *et al.*, 2008). A study conducted by Ferraz *et al.* (2013) investigated the pre-treatment of LFL using air stripping and ammonia recovery by absorption. The aerated packed tower treated 100 L of LFL and achieved an ammonia removal rate of 88% over 72 hrs. Further to this, 80% of the stripped ammonia was recovered in bench scales absorption units containing 0.4 mol.l^{-1} H_2SO_4 solution with deionized water. In addition to being effective for the removal of ammonia from LFL, this treatment facilitated the recovery of ammonia which is an added benefit to the process. Further to this, Marttinen *et al* . (2002) investigated the air stripping of ammonia from LFL with a basic pH (11) at temperatures ranging between 6-20°C. The study concluded that ammonia removal via air stripping was reduced by 30% at lower temperatures. The study also highlighted that regardless of pH or temperature COD removal by air stripping was between 4–21%, indicating the treatment was ineffective for the removal of biodegradable organics. In addition, Silva *et al* . (2004) investigated the removal of ammonia from an ammonia rich LFL, 750-800 mg.L^{-1}, which had received pre-treatments of coagulation and flocculation, as well as membrane fractionation, though neither had affected the initial concentration of ammonia. The results indicated that a 72% ammonia removal rate was achieved at pH 12 with a 2 hrs aeration, however, a further 84 hrs aeration was required to obtain a 5 mg.L^{-1}ammonium concentration.

This treatment option presents some disadvantages and limitations. Of particular concern is the control and destruction of exhaust air (such as ammonia gas) from the stripping processes. These off-gases, if directly released into the atmosphere, have the potential to cause severe air pollution, thus subsequent treatment or ammonia recovery steps are required (Renou *et al.,* 2008; Abbas *et al.,* 2009; Song and Gao, 2013). Furthermore, the success of this treatment option can vary between LFLs of differing compositions and may require additional optimisation.

In general, this treatment option shows potential for the treatment of LFL but the technology requires further advancements to achieve higher removal rates of other compounds present.

2.14.3 Chemical precipitation

Chemical precipitation is used for the removal of non-biodegradable organic compounds, ammonium and heavy metals from LFL because of the simplicity of the process and the inexpensive equipment employed (Calli *et al.,* 2005; Blauvelt, 2009). During the process, dissolved ions are converted to an insoluble solid phase by chemical reactions. Different pH values are needed in order for the precipitation of different compounds to proceed (Kurniawan *et al.,* 2010). Struvite production, the most common chemical precipitation method, is also governed by pH, and the ratios of magnesium, ammonium, and phosphate (MAPs) present in the WW. Struvite production is extremely efficient for the removal of ammonia from WW, however, the process is less efficient for the treatment of LFL as the concentrations of magnesium and phosphorus required to form crystalline MAPs are lower. As such, supplementation of LFL with these compounds is required for successful MAPs production to occur. Li and Zhao (2001) used struvite production for the treatment of LFL and investigated the effect of three different MAPs combinations (i) $MgCl_2.6H_2O$ + Na_2HPO_4; (ii) MgO + 85% H_3PO_4 and; (iii) $Ca(H_2PO_4)_2.H_2O$ + $MgSO_4.7H_2O$ on effective precipitation. The initial concentration of NH_4-N in LFL was 5618 mg.L^{-1}. When $MgCl_2.6H_2O$ + Na_2HPO_4 was added to leachate samples, a decrease in NH_4-N to 112 mg.L^{-1} was observed, with Mg_2^+: NH_4^+: PO_4^{3-} having a molecular ratio 1:1:1 and achieved crystalline MAPs in 15 min. While Di Iaconi *et al* . (2010) evaluated the effectiveness of magnesium, ammonium and phosphate (1:1:1) precipitation for the removal of ammonia from a mature LFL. This was achieved through the addition of phosphoric acid (H_3PO_4) and magnesium oxide (MgO) as external sources of phosphorus and magnesium. The authors suggest that the low solubility of magnesium oxide resulted in a low ammonia removal efficiency of 67%, which was increased to 95% when the ratio of magnesium oxide in the reaction was doubled (2:1:1). It is important to note that although this treatment option works well for ammonia removal it is not sufficient for the removal of compounds such as heavy metals, leaving effluent LFL requiring further treatment (Renou *et al.,* 2008; Abbas *et al.,* 2009; Song and Gao, 2013). Further to this, when LFL is used for the production of struvite, the resulting product is not in a pure form and requires further purification before it can be utilised.

2.14.4 Chemical oxidation

The introduction of strong chemical oxidizers directly onto a contaminated medium for the destruction of a variety of organic compounds is commonly employed for the treatment of contaminated soil and groundwater (Abbas *et al.,* 2009; Torretta *et al.,* 2017). Typically, chemical oxidation-reduction (redox) reactions oxidises one of the reactants while the other becomes reduced or gains electrons. Thus, the oxidizing compound alters the contaminants into a less harmful compound. Chemical oxidation is a widely studied method for the treatment of WW effluents, including LFL (Haapea *et al.,* 2002; Oturan and Aaron, 2014; Hilles *et al.,* 2016; Rajasulochana and Preethy, 2016). Recent interest has focused on advanced oxidation processes (AOP). Most, except simple ozonation (O_3), use a combination of strong oxidants, e.g. hydrogen peroxide

(H_2O_2) and irradiation, e.g. ultraviolet (UV) or electron beam (EB). However, a major drawback of AOP is the high energy required to power UV lamps and ultrasonic devices, resulting in increases in treatment costs. In addition, for the complete degradation of the pollutants to occur, high oxidant doses are also required (Renou *et al.,* 2008; EPA, 2012; Gao *et al.,* 2015; Torretta *et al.,* 2017). The catalysation of hydrogen peroxide by ferrous sulphate, Fenton's reagent, is one of the most common AOPs. The Fenton process, which can remove toxic organic compounds and increase the biodegradability of organic compounds, is commonly used as a pre- or post-treatment option for LFL (Torretta *et al.,* 2017). The Fenton treatment of LFL generally includes steps for oxidation, neutralization, flocculation and solid-liquid separation. Specifically, the process aids with the reduction of organic content, odour, and colour (Zhang *et al.,* 2005; Zhang *et al.,* 2006; Deng and Englehardt, 2007). Zhang *et al* ., (2006) successfully employed the Fenton process for the treatment of a dilute LFL, with a COD value of 1000 $mg.L^{-1}$ and recorded average COD removal efficiencies of 79% throughout the treatment process. Lin and Chang (2000) investigated the use of an electro-Fenton method, a combination of an electrochemical process and Fenton’s oxidation for the treatment of LFL generated within a Northern Taiwan MSW landfill. Briefly, the process employed two electrodes, anodic and cathodic, placed into LFL samples, with hydrogen peroxide (500 and 1500 mg l^{-1}) supplemented to the electrolytic cell before the electrical current was turned on. Fenton's reagent was created via the continuous addition of H_2O_2 to LFL after which a COD removal efficiency of >85% was observed c.67% of which was attributed to the electro-Fenton process. The main advantage of chemical oxidation is the complete mineralization of organic matter, which is achieved rapidly and without the generation of harmful by-products. However, disadvantages include the requirement to reduce the pH of the LFL to ≤ 6 to achieve effective treatment and the high operational cost.

In general, research conducted using physicochemical methods for the treatment of LFL has shown promise. However, these treatment options are generally suited to LFL generated in older landfills characterised by lower BOD: COD ratios and which contain low levels of biodegradable organics. Specifically, physiochemical treatment options are best suited for the removal of ammonia, phosphate, nitrate and heavy metals from LFL and have been proven ineffective for the removal of BOD and COD (Renou *et al.,* 2008; Christensen, 2010; Kamaruddin *et al.,* 2017). As such these processes are best implemented as pre/post-treatment options to biological treatment processes. A summary of the results achieved by the physiochemical treatment options described above is provided in Table 2.3.

Table 2.7. Summary of physicochemical treatment of LFL.

Treatment Option	**Study**	**COD (q_{eq} mg.L-1)**	**Ammonia (q_{eq} mg.L-1)**
Adsorption	(Halim *et al.*, 2010)	Zeolite 2.34 Activated Carbon -37.88 Composite Material-22.99	Zeolite 17.34 Activated Carbon -7.07 Composite Material-24.34
	(Martins *et al.*, 2017)		
		COD (% Removed)	**Ammonia (% Removed)**
	(Halim *et al.*, 2010)	69.8	74-76
Air Stripping	(Ferraz *et al.*, 2013)	-	72-99
	(Marttinen *et al.*, 2002)	-	64-89
	(Ozturk *et al.*, 2003)	-	72-95
Chemical Precipitation	(Di Iaconi *et al.*, 2010)	-	95
	(Li *et al.*, 1999)	-	98
Chemical Oxidation	(Zhang *et al.*, 2006)	79	-
	(Lin and Chang, 2000)	85	-

Table 2.8. Overview of all LFL treatment processes.

	Leachate			**Pollutants**				**Performance**			
	Young	**Medium**	**Old**	**Compounds**	**BOD**	**COD**	**TKN**	**Cost-effective**	**Pre-treatment**	**Post-treatment**	**Remarks**
Conventional Treatment											
WWTPs	Good	Fair	Poor		Good	Good	Fair	Yes	Often	Often	Put pressure on stringent discharge limits.
Leachate Recirculation	Good	Fair	Poor					Yes	No	No	Expensive to transport leachate, Least expensive and low efficiency
Biological Treatment											
SBR	Good	Fair	Poor	Organics	Good	Good	Fair	Yes	No	Often	Hampered by refractory compound and excess biomass. Post-treatment needed for the removal of heavy metals etc.
RBC	Good	Fair	Poor	Organics	Fair	Fair	Fair	No	No	Often	Hampered by the refractory compound,
MBBR	Good	Fair	Poor	Organics	Fair	Fair	Fair	No	No	Often	High energy demand, Costly, hampered by refractory compound and excess biomass
Anammox	Good	Good	Poor	Organics	Fair	Fair	Good	Yes	No	Often	Pre/Post treatment needed if not combined with alternative treatment, effective at TKN removal

Table 2.8. Continued from previous page

	Leachate			**Pollutants**				**Performance**			
	Young	**Medium**	**Old**	**Compounds**	**BOD**	**COD**	**TKN**	**Cost-effective**	**Pre-treatment**	**Post-treatment**	**Remarks**
UASB	Good	Fair	Poor	Organics	Fair	Fair	Fair	Yes	No	Often	Hampered by refractory compound, long reaction time
<u>Chemical treatment</u>											
Air Stripping	Poor	Fair	Good	Ammonia	Poor	Poor	Good	No	Often	No	Air Pollution, poor removal of biodegradable organics, pre-treatment required
Chemical Precipitation	Poor	Fair	Good	Ammonia, Heavy metals Organics	Poor	Poor	Good	No	Often	No	Produces sludge, possibly requires disposal as hazardous waste, purification of Struvite is needed before use
Chemical Oxidation	Poor	Fair	Good	Organics and Inorganics	Poor	Poor	Good	Yes	Often	No	Works best on diluted waste stream Use of chlorine can result in the formation of chlorinated hydrocarbons, high energy consumption

2.15 Combined treatments

In some instances, the treatment of LFL using a combination of biological and physiochemical options has been proven effective (Renou *et al.,* 2008; Gao *et al.*, 2015; Torretta *et al.,* 2017). Research suggests that the removal of different compounds by each process results in greater removal efficiencies overall (Renou *et al.,* 2008; Gao *et al.*, 2015). As the composition of LFL is strongly influenced by numerous factors including the age of the landfill, the number of phases/sections in the landfill, types of waste and moisture content, the generated LFL could fall into two or more categories. As such, different treatments options are more suited than others, therefore a combination of treatments are usually required. This section will focus on some of the different treatments that have shown promising results.

2.15.1 Aerobic SBR and adsorption

Lim *et al* . (2016) examined the treatment of LFL using anaerobic SBR (ASBR) combined with adsorption onto zeolite. The influent LFL used in this study was obtained from an MSW landfill site located in Johor, Malaysia, and had an initial ammoniacal-nitrogen and COD concentration of 1800 and 3200 mg.L^{-1}, respectively. The ASRB was inoculated with *Brevibacillus panacihumi* strain ZB1 and aerated with an up-flow velocity of 1.0–1.2 cm/s. The ASRB was operated for 7 d after which the bioreactor effluent was applied to a 10 % W/V zeolite column. The results of the study indicate a 65% and 30% removal efficiency for ammoniacal-nitrogen and COD, respectively, during the 7 d ASRB trial. Further, a reduction of 96% and 43% of ammoniacal nitrogen and COD, respectively was observed through adsorption. Furthermore, this combined biological–physical treatment system proved effective for the removal of heavy metals from LFL, including aluminium, vanadium, chromium, and magnesium.

2.15.2 *Constructed wetland and adsorption*

Mojiri *et al* . (2016) researched a method for the co-treatment of LFL with municipal WW (LFL to WW 1:5) using a constructed wetland (CW) and two adsorbent; ZELIAC (a composite material composed of zeolite, AC, limestone, rice husk ash, and Portland cement) and zeolite. Three fresh, young, and healthy plants (*Typha domingensis*) were transplanted into the CW, which contained two substrate layers of adsorbent. Influent LFL samples contained high concentrations of COD (2301 $mg.L^{-1}$), NH_4-N (627 $mg.L^{-1}$), Ni (4.6 $mg.L^{-1}$), and Cd (2.5 $mg.L^{-1}$). The influent BOD_5 was 461 $mg.L^{-1}$ and a low biodegradability ratio (BOD_5/COD = 0.20) was observed. The influent LFL and WW mixture was poured onto the CW and effluent samples were collected after different contact times (12, 42 and 74 hrs). High removal rates, between 88-99%, were achieved for all components tested, including Ni, Cd, COD and ammonia over reaction times of c. 50 hrs. This system was effective in the treatment of LFL combined with WW, however, it was noted that as the leachate concentration increased the removal rates were not as effective. This system shows the ability to treat low strength leachate and would not perform as effectively with higher strength LFL.

2.15.3 *Combined sequence batch reactor, coagulation, fenton oxidation and biological aerated filter technology*

Wu *et al* . (2011) researched the combination of SBR, coagulation, Fenton oxidation, and biological aerated filtering (BAF) in series for the removal of pollutants from LFL. The characteristics of raw leachate were outlined as follows:

COD 6722 mg.L-1, BOD_5 672 mg.L-1, NH4-N 850 mg.L-1, and total phosphorus (TP) 8.3 mg.L-1. The researchers reported significant percentage removal of COD (93 %) after the SBR process which in turn showed a reduction in BOD_5/COD ratio from 0.19 to 0.01. In addition, a >97% removal efficiency was achieved for TP and NH4-N by the SBR process. Overall the combined treatments achieved total removal efficiencies of between 98-99% for all components analysed in the final effluent, indicating the effectiveness of the combined process. Furthermore, the authors highlight the major role played by the SBR system in the removal of organic pollutants and the importance of coagulation and Fenton oxidation, both of which reduced the organic load and enhanced biodegradability. Although this treatment method recorded similar removal efficiencies for LFL to other combined treatment methods it is a more costly treatment option. Further to this, the process failed to reduce the heavy metals and inorganic anions concentration of the influent LFL, meaning it would require additional treatment before discharge.

2.15.4 Sequential membrane bioreactor and electro-oxidation processes

Zolfaghari *et al* ., (2016) investigated the treatment of mature LFL with a BOD: COD ratio range of 0.14-0.3 using membrane bioreactors (MBR) and ultrafiltration combined with electrochemical oxidation with a boron-doped diamond electrode. The MBR consisted of submerged hollow-fibre ultrafiltration with a pore size of 0.04 µm and a total filtration surface area of 0.047 m^2. The electrochemical oxidation unit was equipped with niobium coated boron-doped diamond rectangular anode and a titanium cathode with an inter-electrode gap of 2 cm. The MBR was operated with an applied OLR of 1.2 $g.L^{l-1}$ COD and a sludge

RT of 80 d, at 17.5°C. The MBR achieved COD, TOC, NH_4-N and phosphorous removal efficiencies of 63%, 35%, 98% and 52%, respectively. The electrochemical oxidation was operated with an intensity of 3 A and had a reaction time of 120 mins. The electrochemical oxidation increased removal efficiencies of the combined system to 94%, 97%, 99% and 77.8% for COD, BOD, NH_4-N, phosphorous, respectively. The main issue with this treatment was electrochemical oxidation before MBR had a noxious effect on the residual radical on nitrification. However, this issue may be overcome by keeping electrooxidized LFL in a storage tank before introduction into the aeration basin. However, although the process recorded high percentage removal rates and it may not be as cost-effective as other treatment options.

2.16 Conclusion

The major drawback of MSW landfilling is the production of high strength LFL, a chemical cocktail which if left untreated poses a risk to environmental and public health. Traditional treatment of LFL in urban WWTP does not represent a sustainable or cost-effective method of dealing with this waste stream. The supplementation of domestic WW with LFL, even at low volumetric loading rates, places pressure on the ability of these facilities to meet the stringent national and EU discharge limits set for many compounds including COD, BOD and ammonia.

Recent research has proven that a combination of biological and physiochemical treatments methods can be used for the successful treatment of LFL. Ideally, these systems need to reduce the polluting constituents of LFL to below the national discharge limits set for each individual compound in a timely and cost-effective manner. Many of the methods described above consist of multi-step systems, which employ aerobic and energy-intensive processes that may not be realistic options for implementation on-site at landfill facilities. This coupled with the fact that as currently operational landfills mature and are subsequently decommissioned they will continue to produce more stabilised LFL which may be more resistant to biodegradation.

Considering the above, further research efforts are now required to develop treatments that are suitable for all types of LFL and adaptable to individual landfills requirement. As such, an effective treatment option should take into consideration, the initial influent concentration and characteristics, the final effluent discharge requirements and the stage of waste decomposition. Combination biological and physiochemical treatment methods seem to offer the most suitable alternative to traditional practices and can be employed for the effective removal of both biodegradable organics such as COD and BOD, and inorganics, heavy metals, and ammonia.

In order to determine the best combination of treatment processes, first, the age of the landfill needs to be considered alongside which leachate samples need to be analysed to correlate the data with the stage of waste degradation. This will enable the researcher to determine which treatment option is required and in which order. Once the stage of waste degradation is known, the BOD: COD ratio can be used to determine how well the leachate will perform under both biological and physiochemical treatment. Secondly, a small-scale trial should be conducted on proposed biological and physicochemical treatments to assess their suitability, after which pilot studies should be conducted to assess the ability of both treatments to performing together. If successful removal rates are achieved during the pilot study, larger more comprehensive trials should be performed to assess the whole treatment option at reducing the pollutant power of LFL, taking into account additional variables such a seasonal variation, and variation in leachate composition.

Overall LFL, is a major problem associated with landfilling and more research needs to be conducted into suitable treatment options. With that being said, the main objectives of the proposed research include; (1) Assess the ability of a range of microbial species to bioremediate LFL, (2) Design and optimise a physiochemical treatment based on the adsorption of xenobiotic compounds and (3) Develop a treatment option combining both biological and physiochemical treatments, that has the potential to treat LFL on-site within an Irish landfill.

2.17 Summary

This chapter highlights the major issues with LFL.LFL is a highly variable WW, that has the potential to cause environmental damage , if contamination was to occur. LFL varying composition require different treatment methods, with a combination of both biological and physiochemical treatment showing promising for achieve high removal rates for compounds such as, ammonia, phosphate, BOD and COD. Research will now be conducted to look at the potential to use bioremediation and adsorption, as a single treatment option for LFL.

Chapter 3
Bioremediation of Landfill Leachate Using Isolated Bacterial Strains

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Bioremediation of Landfill Leachate Using Isolated Bacterial Strains

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***Authors Contributions**

Sinead Morris conceived and designed the experiments. Sinead Morris acquired the samples, performed the experiments and analysed data. Sinead Morris, Guiomar Garcia-Cabellos, David Ryan, Deirdre Enright and Anne-Marie Enright interpreted the data. Guiomar Garcia-Cabellos and Anne-Marie Enright contributed reagents/materials/analysis tools to the work. Sinead Morris wrote the paper which was revised critically by Anne-Marie Enright.

3.1 Overview

This chapter looks at the potential of microbes to be used in leachate treatment. In particular it will focus on isolating microbes from leachate. After which, they will be characterised and identified before being used in leachate treatment. The main objectives of this chapter are:

- Isolate microbes that are capable of degrading toxic compounds with leachate.
- Assess bacterial isolates in relation to heavy metal residence, ammonia, phosphate and nitrate resistance and salt tolerance.
- Phylogenetically identify bacterial strains using 16rRNA gene sequence analysis.
- To access the bioremediation potential of isolates microbes to treat LFL

3.2 Abstract

Landfilling is one of the most common and widely accepted practices for the disposal of waste throughout the world. Leachate, a major drawback of landfilling, continues to be produced at vast rates and current treatment options are costly and often inadequate. The management of leachate is of economic and environmental importance, due to its potential to cause contamination to ground and surface water. This research focuses on treating leachate in a cost-effective manner through bioremediation. Microorganisms were isolated from landfill leachate (LFL) and screened to determine their ability to remediate a wide range of compounds found in leachate, such as ammonia, phosphate and nitrate. Selected isolates, identified as belonging to the phylums Firmicutes, Actinobacteria, and Proteobacteria, were inoculated into soil contained in a fixed

bed column system. The column system was optimised and used for the treatment of LFL over a 10 hr period. High percentage removal rates were achieved for ammonia (>90%), nitrate and phosphate (>60%). Although Environmental Protection Agency (EPA) discharge limits were not achieved, bioremediation using selected microbial strains represents a cost effective treatment option when compared to conventional methods. Research is now required to further optimise this system to achieve discharge limits for all compounds tested.

3.3 Introduction

The generation of municipal solid waste (MSW) continues to rise, largely due to global population increase, industrial activities and modern lifestyle (Ahmed and Lan, 2012; Torres-socías *et al.,* 2014). Landfilling is the most commonly used method of MSW disposal which results in the production of large volumes of landfill leachate (LFL), the product of water that has percolated through waste picking up the products of degradation. This chemical cocktail can be produced for hundreds of years after the landfill is decommissioned (Crowley *et al.,* 2003; Torres-socías *et al.,* 2014). As such, the management of landfill leachate is essential for the protection of the surrounding environment, specifically ground and surface water.

The waste industry within Europe has changed dramatically over the past 20 years, due to the implementation of the Landfill Directive 1999/31/ EC (EU, 2001) and waste management legislation. The Landfill Directive and the Waste Framework Directives 2008/98/EC (EU, 2008b) directly influence leachate management practices, such as leachate collection, while the Water Framework Directive 2000/60/EC (EU, 2000) and the Urban Wastewater Treatment

Regulation Council Directives 99/31/EC (EU, 1991) govern and sets discharge limits for wastewater treatment plants (WWTPs), where leachate is often treated. The implementation of these directives has led to a reduction in the amount of waste being sent to landfills. As a result, there has been a dramatic reduction in the number of landfills within Ireland from two hundred in the mid-nineties to six in current operation (McCarthy *et al.,* 2010; EPA, 2015; Brennan *et al.*, 2017b). This significant reduction can be accredited to the closure of smaller landfills directly leading to the production of large volumes of leachate with higher concentrations of pollutants at remaining facilities (McCarthy *et al.,* 2010; Brennan *et al.,* 2017b; Brennan *et al.*, 2017a).

The remediation of LFL is difficult due to the recalcitrant nature of some of its constituents and usually involves the combination of both biological and physiochemical methods. In addition, the varying range of LFL composition also complicates treatment options. For example, biological treatment is hampered by toxic substances and biorefractory compounds, traditional physicochemical treatment, including air stripping and coagulation-flocculation are costly, while treatments such as reverse osmosis only transfer rather than treat the pollution. Biological treatments are more effective at treating leachate from a young landfill (≤ 10 years old), while physiochemical works best for intermediate or mature landfill (≥ 10 years of age; Kargi and Pamukoglu, 2004; Kheradmand *et al.,* 2010; Raghab *et al.,* 2013). Current biological onsite treatment options for LFL include constructed wetlands (CW), sequence batch reactor (SBR) systems, and aerated lagoons (Nivala *et al.,* 2007; Mehmood *et al.,* 2009; Kadlec and Zmarthie, 2010; Mojiri *et al.,* 2016). However, in Ireland, the most common treatment practice is to discharge leachate to sewers (51%) or removal by tanker for treatment in WWTPs (48%) with less than 1% being treated on-site. This compares unfavourably to other European Union (EU) countries, such as, France, where 79% of all leachate is treated onsite (Renou *et al.*, 2008; Brennan *et al.*, 2016). The main problem with the implementation of onsite treatment is the high capital cost, which can range from €260,000 for constructed wetlands to €500,000 for SBR systems, and operational costs (Renou *et al.*, 2008; Brennan *et al.*, 2016; Brennan *et al.*, 2017b). These options represent significant investment requiring correct planning or retrofitting into existing facilities.

Bioremediation, the process of biologically removing a pollutant from the environment (Tiwari and Singh, 2014; Azubuike *et al.*, 2016; Ojuederie and Babalola, 2017), is a cost-effective and environmentally friendly method of mineralising most organic compounds in LFL. It is carried out by naturally occurring microorganisms which contribute to degradation and stabilisation of waste in landfill sites by reducing organic compounds to CO_2 and CH_4 under anaerobic conditions (Wang *et al.*, 2010). To enhance and improve bioremediation rates it is necessary to isolate and identify microorganisms from landfill locations capable of remediating LFL, particularly its varying toxic pollutants. A study by Latorre *et al* (2012), isolated *Chryseomicrobium imtechense, Lysinibacillus fusiformis* and *Acinetobacter* capable of degrading Di-(2-ethylhexyl) phthalate. Work carried out by Xie *et al.* (2012) used an aged refuse bioreactor and achieved a reduction in BOD of 95% and total nitrogen of 70%. The results of their pyrosequencing analysis indicated that bacteria from *Pseudomonas, Lysobacter, Bacillus* and *Delta-proteobacteria*, *Flexibacteraceae* were abundant in their samples, and contributed to these reduction rates. Work by Liang & Liu (2008) showed that anammox (ammonia-oxidising) bacteria in a bioreactor can reduce ammonia and nitrogen by >60%, while research carried out by Zhang *et al* (2016) describes microbial communities and their important biological function in treating LFL.

Isolating microorganisms capable of bioremediating the most common components of LFL, ammonia, phosphate, nitrates, biological oxygen demand (BOD) and chemical oxygen demand (COD) and growing them as pure cultures may result in these strains being harnessed as a cost-effective, natural method of LFL treatment (Tiwari and Singh, 2014; Azubuike *et al.,* 2016; Ojuederie and Babalola, 2017). The use of microorganisms in bioreactors to treat landfill leachate has been widely reported in the literature particularly in rotating biological contractors, SBR and moving-bed biofilm reactors, where they have been used for the bioremediation of pollutants including xenobiotic organic compounds (XOCs) and polycyclic aromatic hydrocarbons (PAHs), as well as removing ammonia (Wiszniowski *et al.,* 2006; Renou *et al.,* 2008; Chan *et al.,* 2009; Zhang *et al.,* 2016). Zhang *et al* (2016), studied the functional microbial ecology of these reactors in LFL treatment process highlighting the need to have a wide range of microbial species within reactors to achieved optimum removal efficiency.

The aims of the current study were; (1) the isolation and characterisation of microorganism with the potential to bioremediate LFL; (2) the identification of isolates using molecular biology techniques and; (3) to determine if these isolates can be used in the treatment of LFL through bioremediation, in a fixed bed column system.

3.4 Materials and methods

3.4.1 Site description and leachate collection

LFL used in this study was sourced from Powerstown Landfill, Co. Carlow, Ireland (52°45’58.46’’ N, 6°57’20.13’’ W). The landfill is located 8 km south-east of Carlow Town in a rural setting and has been operational since 1977. The site consists of three different phases; phase 1 (P1) which operated from 1975-1990, phase 2 (P2) which operated from 1991-2006, and phase 3 (P3) opened in 2006 and is due to close before the end of 2018. Powerstown landfill accepted MSW from both local authority county council and privatised companies. In 2015, the landfill received 56000 tonnes of MSW which had decreased to just over 52,000 tonnes by 2017 (Powerstown Landfill 2015; Powerstown Landfill 2017). Powerstown Landfill was decommissioned in December 2018 and the final capping is currently being placed on phase 3.

It was decided to use LFL generated in P3 as it is currently in operation and generates a higher concentrated leachate than the other phases. Leachate samples were collected mid-November 2015 from the leachate tank (LT) and cell 11 (C11). Further sampling occurred between October 2016 and February 2017. All samples were stored at 4°C prior to analysis.

3.4.2 Isolation and characterisation of bacterial isolates

Ten-fold serial dilutions of LFL samples LT and C11 were made using sterile distilled (d)H_20. From these dilutions, 0.1 ml aliquots were spread onto nutrient agar (NA) plates and incubated at 30 °C for 2-7 days. Once growth was observed, single colonies were transferred to fresh NA plates, to isolate unique pure cultures for further analysis. These were further characterised using the Gram stain, oxidase test and catalase test (Cappuccino and Sherman 2014).

Isolates were assessed for halotolerance using both NA and minimal media (MM) plates supplemented with NaCl (1, 5 and 10%). Isolate resistance to and ability to tolerate heavy metals was also determined using, CuCl, ZnCl, CdCl, NiCl, $As(NO_3)_3$ and FeCl. NA and MM were supplemented with either 5, 10, 25 and 100 $mg.L^{-1}$ of each metal. Isolates were further characterised by assessing their resistance and ability to tolerate NH_4, PO_4 and SO_4. This was carried out by supplementing NA and MM plates with NH_4Cl, KH_2PO_4, and $MgSO_4$, in stepwise concentrations of 10 $mg.L^{-1}$ to 1000 $mg.L^{-1}$. All culture assays were performed in triplicate and incubated at 30ºC for 2-7 d. All reagents and chemicals used were supplied by Sigma Aldrich, unless otherwise stated.

3.4.3 DNA Extraction, Bacterial 16S rDNA PCR, Amplified rDNA Restriction Analysis and phylogenetic analysis of isolates

Isolates were grown overnight in LB Broth (LAB M) and 3 ml of culture was centrifuged at 13000 rpm for 10 min. The resultant supernatant was removed, and the pellet was retained for DNA extraction using Omega E.N.Z.A® Bacterial extraction kit (VWR Ireland) as per kit protocol. Extracted DNA was visualised by UV excitation after electrophoresis in 1% agarose gels (w/v) 1× TAE (40 mM Tris-base, 1 mM EDTA, 1.14 mM glacial acetic acid; pH 8) gel containing 1 $\mu g.ml^{-1}$ GelRed™ (Bioscience) with Hyperladder IV (Bioline) as a molecular weight marker.

Bacterial 16S rRNA genes were amplified with the forward primer 27F (5'-GAGTTTGATCCTGGTCAG-3' (Delong, 1992) and reverse primer 1329R (5'-ACGGGCGGTGTGTRC-3 (Lane *et al.,* 1985). All PCR reactions (50 µl) were carried out using the GoTaq™ G2 (Promega) kit and contained; 50mM Tris-HCl (pH 9.0); 50mM NaCl; 5mM $MgCl_2$; 200µM each of dNTP (dATP, dGTP, dCTP, dTTP), 12.5 pmol of each primer, 200 ng template DNA and 1.25 U Taq DNA polymerase. A 'touchdown' PCR was used to specifically amplify bacterial 16S rRNA genes, with the following conditions: denaturation at 95°C for 10 min followed by 10 cycles of 94°C for 60 s, annealing at 63°C for 60 s and extension at 72°C for 120 s, where the annealing temperature was reduced by 1°C for each cycle; this was followed by 20 cycles of denaturation at 94°C for 60 s, annealing at 52°C for 60 s and extension at 72°C for 120 s, which were in turn followed by a 10-min final extension at 72°C. Negative controls containing no DNA were used, while *E.coli* DNA was used as a positive control. PCR products were visualised as described above.

Amplified rDNA Restriction Analysis (ARDA) was carried out as follows; 5 µl of PCR product was digested with 1 U of the restriction endonuclease HaeIII (Thermo Fisher, Ireland) for 3 hrs at 37°C. The resulting DNA fragments were resolved by electrophoresis on 3.5% high-resolution agarose, containing 1 $\mu g.ml^{-1}$ GelRed™ (Bioscience). Banding patterns were compared by visualisation and grouped into operational taxonomic units (OTUs) as previously described (Moyer *et al.,* 1996)

PCR products were sequenced by Eurofins Genomics, Wolverhampton. Resultant sequences were analysed using BLASTn searches on NCBI basic local alignment search tool (Appendix 1) and tool and the Ribosomal Database Project (RDP) (Version 11.5) (classifier function). Similar sequences were downloaded and used for phylogenetic analyses. Evolutionary history was inferred using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei 1993; Kumar *et al.*,2016). Phylogenetic trees were constructed using MEGA 7 (Version 7.0.14). Bootstraps were set at 100, with all other parameters being set as default. Bootstraps indicated the confidence levels for phylogenetic trees. Sequences were deposited in Genbank and assigned the accession numbers MG880063-MG880077.

3.4.4 Preliminary study into the bioremediation potential of microbial isolates

3.4.4.1 Influent and effluent analysis

Leachate samples were analysed before, during and after all treatments for ammonia, phosphate, nitrate, BOD and COD. All reagent used where of analytical grade and made with deionised water. Ammonia (NH_3) was analysed using the phenate method and the concentration read on Shimadzu UV1800 spectrophotometer (Rice *et al.*,2017). BOD was tested over 5 days (BOD_5) and analysed according to standard methods using a Hanna dissolved oxygen meter (Rice *et al.*,2017). COD was analysed using HACH Lange COD vials. Phosphate (PO_4^{3-}) was analysed using molybdovanadate reagent (HACH Lange). Nitrate (NO_3^-) was analysed using NitraVer® 5 reagent power pillows (HACH Lange). All HACH products were used according to manufactures instruction and measured on HACH DR 6000 UV- spectrophotometer. Percentage removal for each compound was calculated using quation 2.1 for all three trials.

$$\frac{\text{Co-Ceff}}{\text{Co}} \text{ X } 100 = \%$$ **Equation 2.1**

Where, c_o is the initial concentration (mg.L^{-1}), c_{eff} is the effluent concentration (mg.L^{-1})

3.4.4.2 Trial 1: Effect of the carrier matrix

Three PVC columns, Column 1 (C1), Column 2 (C2) and Column 3 (C3) (11 cm ø, 30 cm height, and IC 2850 cm^3 each (Figure 3.1) were utilised in this study. C1 and C2 were packed with c. 1 kg of soil to a height of 20 cm. C3 was packed with sterile soil, autoclaved at 15 psi for 30 min at 121°C, as described for C1-C2. Columns C2 and C3 were then spiked with a 500 ml overnight culture of 15 previously isolated leachate degrading microorganisms (microbial mastermix) in nutrient broth. The soil mixture was left to incubate for 48 hrs at room temperature (25°C) after which the liquid was allowed to drain off. Leachate (2 L) was then passed through each of the three columns at 10 ml.min^{-1} over 3.5 hrs with a retention time of 45 min. The composition of the influent leachate is described in Table 2.1. Effluent samples were collected at 20 min intervals and stored at 4°C. All experiments were conducted in triplicate.

3.4.4.3 Trial 2: Effect of flow rate

Column operation was further optimised by determining the flow rate which affected the highest percentage pollutant removal efficiency from LFL. Two PVC columns C4 and C5 (dimensions and set up as described above for C1) were utilised in this study. Leachate (2 L) was passed through C4 and C5 at 5 and 10 ml.min^{-1}, respectively. Column operation and influent and effluent concentrations of ammonia, phosphate and nitrate were determined as described previously. All experiments were conducted in triplicate.

3.4.4.4 Trial 3: Optimised column operation

The most effective carrier material and flow rate as determined in trials 1 and 2 were then combined in a further trial (Trial 3) which was operated over a 10 hrs period, with a retention time of 50 min for the treatment of 3 L of leachate. C6 was operated with soil inoculated with the microbial mastermix, while the control C7 contained uninoculated soil. The resulting influent and effluent samples collected were analysed as described above. All experiments were conducted in triplicate.

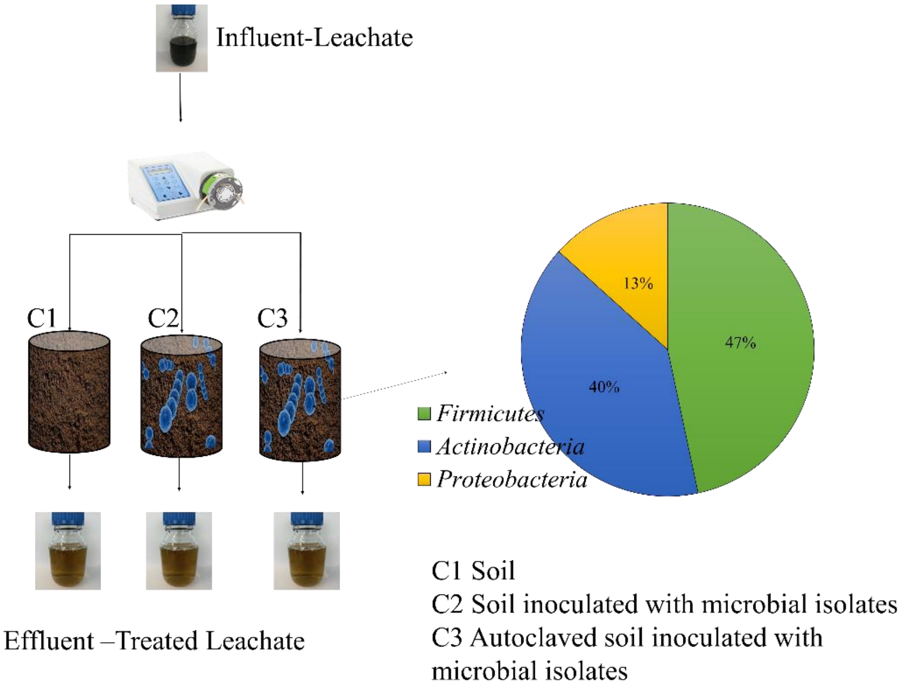

Figure 3.1. Schematic outline of the column system for C1, C2 and C3 utilised in trial 1.

3.5 Results and discussion

3.5.1 Leachate composition and characterisation

Leachate from Powerstown was analysed to determine potential treatment options, as well as evaluating the phase of decomposition at the landfill. The results were compared against both previous LFL compositions as determined by Carlow Co. Council during the period 2009-2017 and the EPA discharge limits for each of the individual components (Table 3.1). During the sampling (November 2015-February 2017), BOD_5 from Powerstown varied from 112-180 $mg.L^{-1}$. The current EPA limit for BOD_5 is set at 5 $mg.L^{-1}$ (EPA, 2001). COD ranged from 450-650 mg.L^{-1}, with EPA limits set at 40 mg.L^{-1}. According to both Christensen *et al* (2001) and Jokela *et al* (2002), this leachate would classify the landfill in the methanogenic phase, which is determined by a COD range of 500-4500 mg.L^{-1}. The BOD$_5$/COD ratio is used to determine the organic composition of leachate, as it is a good representation of waste stabilisation, and the transition from early acetogenic phase to the mature methanogenic phase. Ratios between 0.4 and 0.6 are an indicator that the organic matter in the leachate is biodegradable. In mature landfills, this ratio is often in the range of 0.05 to 0.2 as leachate typically contains humic- and fluvic- acids as well as recalcitrant organic compound, which are not biodegradable (Christensen *et al.,* 2001; Halim *et al.,* 2010; Lee and Nikraz, 2014; Contrera *et al.,* 2015). The leachate in this study had a BOD$_5$/COD ratio ranging from 0.18-0.26, indicating stable leachate which may prove difficult to treat biologically but which should respond well to physicochemical treatments (Kjeldsen *et al.,* 2002; Lee and Nikraz, 2014; Contrera *et al.,* 2015).

Ammonia is a common component of LFL which can promote algae growth and accelerate eutrophication in receiving water bodies. In addition, high concentrations of ammonia can persist for up to 50 years after landfill decommission (Chu *et al.,* 1994; Wang Yu, 2013; Brennan *et al.,* 2017a) and can decrease the effectiveness of biological treatments such as those employed in wastewater treatment plants (WWTPs) (Aziz *et al.,* 2010; Bashir *et al.,* 2010). The leachate used in this study recorded an ammonia level range between 790-1010 mg.L^{-1} (Table 3.1), these high levels correspond to the methanogenic phase of waste decomposition (Gao *et al.,* 2015; Kamaruddin, 2015; Torretta *et al.,* 2017). High levels of ammonia, can impact on biological treatment, resulting in low removal levels for both BOD and COD. To improve the efficiency of treatment, pre-treatment may be necessary to lower the BOD and COD levels.

Nitrates and phosphate were also analysed within this study, as a number of publications discuss these in relation to determining the age/ stage of LFL. Nitrate concentrations, in Powerstown leachate, were above the discharge limit set by the EPA. Nitrate levels can fluctuate depending on the concentration of ammonia within the leachate. Leachate with high concentrations of ammonia, often have high nitrate levels due to the conversion of ammonia to nitrate during the aerobic process occurring in the landfill. Phosphate level was also determined to be above the EPA discharge limit of 0.4 $mg.L^{-1}P$ (Table 3.1). Phosphate levels are considering to be high when compared to the discharge limit as there is a greater than tenfold increase in the level of phosphate in LFL. Nitrate and phosphate can cause contamination to both ground and surface water, and an imbalance in the nutrient cycling process and eutrophication.

Table 3.1. The composition of Powerstown Landfill leachate from 2009-2015, leachates used in this study and the discharge limits set by the EPA.

Compounds	**Powerstown Landfill 2009-2017**[a]	**Leachate used in this study**	**EPA DL**[d]
Ammonia	360-960	790- 1040	≤4
Arsenic	25-64	NM	≤0.05
BOD_5	46-1332	112-170	≤5
Cadmium	0.2-0.5	NM	≤0.005
COD	539-3005	450- 650	≤40
Copper	5-40	NM	≤0.05
Iron	2700-11190	NM	≤0.2
Nickel	60-180	NM	List II substance[b]
Sodium	510-1280	NM	≤200
Sulphate	61-390	NM	≤200
Zinc	30-260	NM	≤3
Nitrate	NM[c]	89-120	≤50
Phosphate	1.2-7.4	3.6-7.25	≤0.4
BOD_5: COD	0.07-0.62	0.18- 0.26	

Where;
All results are presented as $mg.L^{-1}$
[a] Sampling didn't occur in 2010 or 2015
[b] have harmful effects on the environment
[c] not measured
[d] Discharge Limit

3.5.2 Isolation and characterisation of microbes

A total of 96 candidate strains were isolated from initial screening on NA; 52 from LT (LCT) and 46 from C11 (LCC). These were selected based on their differing colony morphology, Gram staining and oxidase and catalase test results.

3.5.2.1 NaCl tolerance

The sodium levels recorded from Powerstown LFL had a mean concentration of 696 mg.L^{-1} which is equivalent to 1.74 g.L^{-1} of NaCl, well above the discharge limit set by the EPA of 200 mg.L^{-1} (EPA, 2001). To ensure the survival of selected isolates in the bioremediation process, it was deemed essential to use halotolerant microorganisms. The results obtained from this screening indicated that 40 of the 96 isolates were halotolerant i.e. capable of growth on NA supplemented with 10% NaCl. These 40 isolates were screened further against other compounds.

3.5.2.2 Heavy metal resistance and utilisation

In order to achieve successful bioremediation of LFL, it was decided to screen isolates for both their resistance to and ability to tolerate heavy metals. Results indicated 15 out of 40 isolates were capable of growth in the presence of the selected heavy metals tested at concentrations ≤100 mg.l^{-1}. Isolates showed varied results in their ability to grow on MM supplemented with heavy metals (Table 3.2) all isolates screened showing growth on one or more of the metals tested at concentrations ≤100 mg.L^{-1}. In particular, LCC32 was capable of growing on all five metals at concentrations of ≤100 mg.L^{-1}, while three isolates, LCT24, LCT33 and LCC31, displayed growth on concentrations of ≤100 mg.L^{-1} on three or more metals (Table 3.2).

3.5.2.3 Ammonia, phosphate and nitrate utilisation

All 15 strains were resistant to NH_4, NO_3 and PO_4 at concentrations of ≤100 mg.L^{-1} on NA. In addition, all strains were capable of growth on MM plates containing varying concentrations of NH_3, NO_3 and PO_4. In particular five isolates, LCT12, LCT33, LCC18, LCC19 and LCT33, were capable of growth at a concentration of ≤100 mg.L^{-1} for all three compounds.

Table 3.2. Isolates ability to tolerant heavy metals on minimal media.

Strain ID	**Copper**	**Nickel**	**Iron**	**Cadmium**	**Arsenic**
LCT 10	+	-	+	++	+++
LCT11	+	++	+	++++	++++
LCT12	+	++	-	++++	++++
LCT22	++++	++++	++++	++	++
LCT24	-	++++	++++	+++	++++
LCT26	-	++	++++	++++	++++
LCT33	-	++++	++++	++++	++++
LCT42	+	++++	-	-	+++
LCT43	+	++++	++++	++	++++
LCT48	+	-	-	-	+++
LCC18	++++	++++	-	+++	+
LCC19	++++	++++	-	++++	++++
LCC29	-	++++	++++	++	++
LCC31	++++	++++	++++	++	++++
LCC32	++++	++++	++++	++++	++++

Key*:* Growth on + ≤10 mg.L^{-1}, ++ ≤ 25 mg.L^{-1}, +++ ≤ 50 mg.L^{-1} and ++++ ≤ 100 mg.L^{-1}

3.5.2.4 16s rRNA gene sequencing and phylogenetic analysis

A total of seven bacterial isolates belonged to the phylum *Firmicutes,* containing six *Bacillus* spp. and one *Lysinibacillus* spp. Phylogenetic analysis of these sequences resulted in the formation of two distinct clades (Figure 3.2) both belonging to the order *Bacillaceae.* The order *Bacillaceae* is a diverse group of Gram-positive bacteria within which there are 14 distinct *Bacillaceae* groupings

(Priest *et al.*, 1988). The two families observed in Figure 3.2 are the *Bacilli* (first clade) and the *Planococcaceae* (second clade). Manchola and Dussán (2014) and Sharma and Saharan (2015) observed that *Lysinibacillus* spp. are both phosphate solubilisers and ammonia oxidizers. *Bacillus* species are well known for their bioremediation potential in soil. A study carried out by Safitri *et al.*(2015) used a microbial consortium containing *Bacillus pumilus, Bacillus subtilis, Bacillus coagulans, Nitrosomonas sp.,* and *Pseudomonas putida* to treat WW. This was achieved by inoculating these organisms into the WW using 10% of the bacterium to the original volume of WW being treated. The results showed that this consortium were effective at treating WW, resulting in removal rates of 71%, 64%, 94% and 95% for BOD, total suspended solids (TSS) and ammonia, respectively. All *Firmicutes* isolates used in this study showed great potential for the remediation of heavy metals (Table 3.2). In particular, isolates LCT 24 and 43 showed resistance to As, Ni, and Fe at high concentrations (Table 3.2). Leachate from Powerstown Landfill is known for having high levels of nickel (60-180 mg.L^{-1}) and copper (5-40 mg.L^{-1}), therefore it is important to have a range of microorganisms within the consortium that are resistant to these metals at high concentrations.

Isolates LCT 11, 33 and 48, as well as LCC 19, 29 and 32, belonged to the phylum *Actinobacteria*, a group of microorganisms found in soils with high metabolic versatility and potential for bioremediation (Polti *et al.*, 2014). In particular, several studies have indicated their potential for the remediation of heavy metals (Albarracín *et al.*, 2005; Egli *et al.*, 2001; Polti *et al.*, 2007, 2011, 2014). Specifically, Verma & Singh (2013), found that *Brevibacterium casie* was capable of reducing 78% Cr_{6+} and 82% polychlorinated biphenyls in an LB broth medium, indicating that these strains may have the potential to be used in bioremediation. These findings concur with the results of this study (Table 3.2) which revealed that strains LCT 11,33 and 48, as well as LCC 19, 29 and 32, show resistance to heavy metals, as well as, ammonia, phosphate and sulphate.

For *Proteobacteria* there were two isolates, *Bruvundimonas diminutas* and *Brevundimonas naejangsanensis* (LCT 12 and LCC 31). *B. diminutas*, previously classified as *Pseudomonas* (Segers *et al.,* 1994), are well known for their bioremediation potential for a wide range of compound including arsenic, nickel, organophosphorus triesters and thioesters and fluorophosphate compounds such as maps and methyl parathion, alongside oil-contaminated WW (Boone *et al.*, 2001; Koukkou and Vandera, 2011; Wang *et al.,* 2016b; Das and Dash, 2017). Other studies have shown *Bruvundimonas* sp. can bioremediate Polycyclic aromatic hydrocarbons and oil (Antizar-Ladislao *et al.,* 2004; Wang *et al.,* 2016b; Basuki, 2017). In addition, isolate LCT 12 was able to tolerate Cd, As, NH_3, PO_4 and SO_4 at concentrations of ≤100 $mg.L^{-1}$, while LCC 31 could tolerate Cu, Ni, Pb, As, PO_4 and NO_3 to concentrations of ≤100 $mg .l^{-1}$

A further characterisation of the isolates described above is presented in Appendix 1.

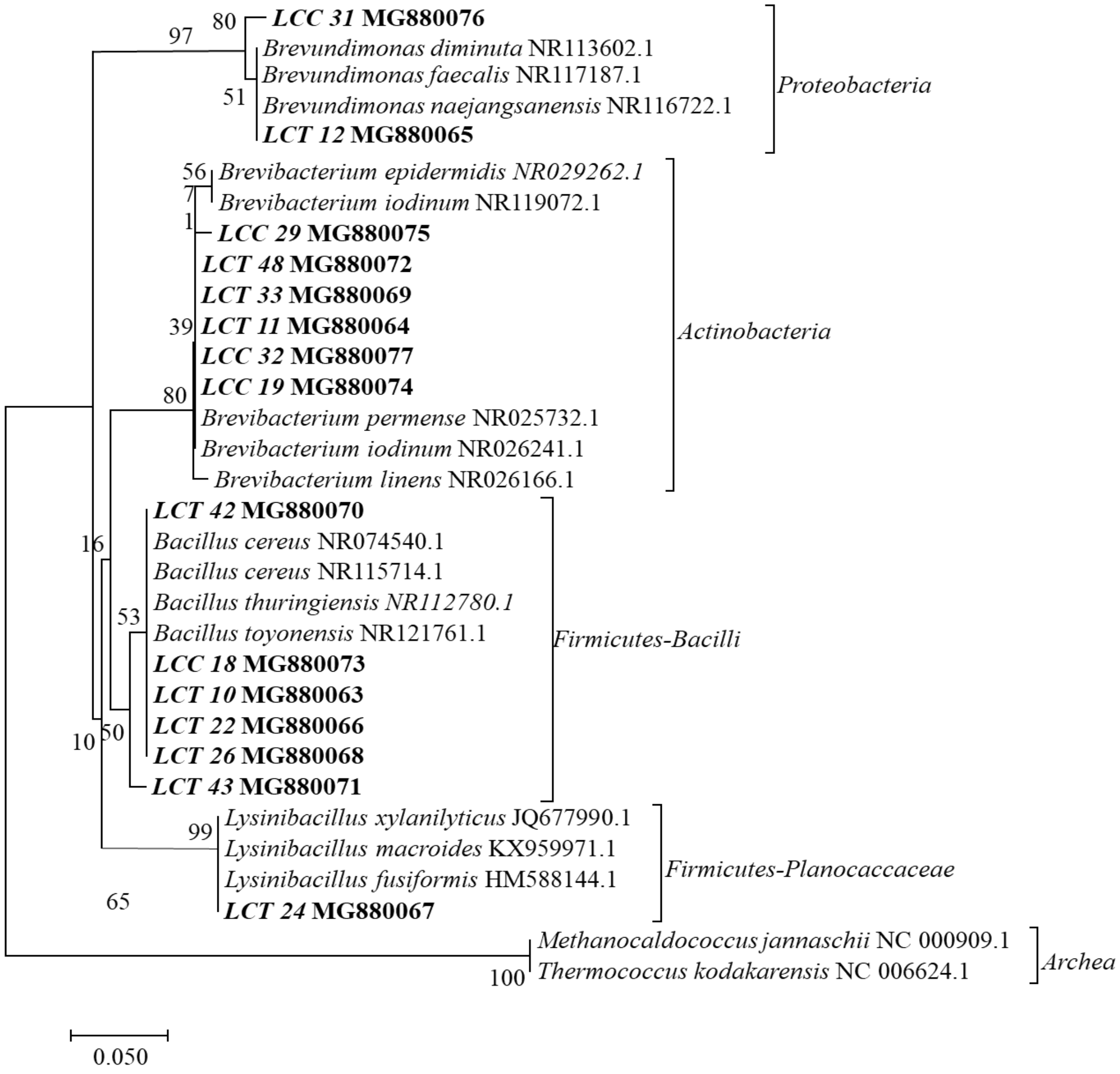

Figure 3.2. Molecular Phylogenetic analysis by Maximum Likelihood method using MEGA 7 (Version 7.0.14).

3.5.3 Bioremediation potential of isolates in a fixed bed column system-optimisation and overall results

3.5.3.1 Trial 1: Optimisation of the fixed bed system- Carrier matrix

Soil was chosen as a carrier matrix for microorganisms as it is low cost and has been previously used in bioremediation studies treating toxic compounds (Azubuike *et al.*, 2016; Ojuederie and Babalola, 2017). Columns containing un-inoculated soil and soil containing the mastermix of microorganisms were compared. Leachate was passed through the column systems and their effluent was analysed. A significant difference of 69% was observed between both soil and soil inoculated with microorganisms in the final percentage removal of ammonia (Table 3.3). Likewise, for phosphate and nitrate (Table 3.3) there was a difference in the final percentage removal of 26% and 15%, respectively. In addition, in order to determine the effect, the indigenous microorganisms may have on bioremediation it was decided to compare soil inoculated with microorganisms against autoclaved soil inoculated with microorganisms. The removal rate between soil and microbial mastermix and autoclaved soil and microbial mastermix is significant. The final percentage removal achieved for ammonia, phosphate and nitrate were 88%, 55% and 35%, respectively (Table 3.3), while the autoclaved soil inoculated with the microbial mastermix recorded slightly reduced removal rates of 81% for ammonia, 46% for phosphate and 31% for nitrates. This deviation was not entirely unexpected as it has been previously reported that autoclaving soil affects its chemical properties, altering pH and affecting the availability of macro-compounds within the soil, while also affecting the physical structure by destroying soil aggregates. It is believed for these reasons the autoclaved soil was not able to hold the inoculum as well as the non-sterile soil (Williams-Linera and Ewel, 1984; Berns *et al.*, 2008). Overall from the three possible options the soil inoculated with microbial mastermix (C2) was the most effective for the removal of ammonia, phosphate and nitrates (Table 3.3).

3.5.3.2 Trial 2: Optimisation of the fixed bed System-Flow rate

Flow rate is an important parameter that influences the removal capacity of fixed bed columns. It is a common hypothesis that the greater the flow rate the lower overall removal in these systems as it determines the pollutant/microorganism contact time (Aksu and Gönen, 2004; Burgess *et al.*, 2004; Lim and Aris, 2014). In Table 3.3 it can be seen that the 5 ml.min^{-1} flow rate achieved a greater percentage removal compared to that of the 10 ml.min^{-1} flow rate. The overall percentage removals at 5 ml.min^{-1} were 76%, 64% and 36% for ammonia, phosphate and nitrate, respectively (Table 3.3). The retention time of 5 ml.min^{-1} was 50 mins compared to 35 min for 10 ml.min^{-1}, which effected a longer pollutant/microorganism contact time. As there was a significant difference between 5 ml.min^{-1} and 10 ml.min^{-1} for both ammonia and phosphate, it was decided that a flow rate of 5 ml.min^{-1} would be used in the optimised trial.

Table 3.3. Influent, effluent and percentage removal achieved by trial 1(C1, C2 and C3: effects of soil and autoclaved soil) and trial 2 (C4 and C5: effects of flow rate) for ammonia, phosphate and nitrate.

Trial 1	**Influent**	**C1 Effluent (Soil Only)**	**% Removed**	**C2 Effluent (Soil with microbes)**	**% Removed**	**C3 Effluent (Autoclaved Soil with microbes)**	**% Removed**
Ammonia	820 ± 0.2	658 ± 0.2	19.7 ± 1.2	92.8 ± 0.3	88.7 ± 1.5	148 ± 0.6	81.9 ± 0.5
Phosphate	4.4 ± 0.2	4.4 ± 0.2	22.3 ± 1.5	2.5 ± 0.3	57.7 ± 1.2	3.0 ± 0.5	47.5 ± 0.5
Nitrate	102 ± 2.3	92.2 ± 0.4	9.7 ± 3.4	67.5 ± 0.6	37.8 ± 2.3	70.2 ± 0.5	31.2 ± 1.2
Trial 2	**Influent**	**C4 Effluent (Soil with microbes at 5 ml.min^{-1})**	**% Removed**	**C5 Effluent (Soil with microbes at 10 ml.min^{-1})**	**% Removed**	-	-
Ammonia	869 ± 0.5	208. ± 0.6	77.5 ± 1.2	321.1 ± 0.6	63.6 ± 1.5	-	-
Phosphate	4.3 ± 0.2	1.5 ± 0.6	64.1 ± 1.2	1.9 ± 0.5	54.7 ± 1.6	-	-
Nitrate	115 ± 1.5	72.5 ± 1.5	37.9 ± 1.2	79.7 ± 1.6	30.6 ± 2.3	-	-

Where;
Performance values are the trial period mean ± standard deviation and are expressed as mg.L^{-1};

3.5.3.3 Trial 3: Overall bioremediation potential

The use of an optimised fixed bed system to bioremediate ammonia, nitrate and phosphate over 10 hrs was investigated. As previously described ammonia is commonly present at high concentrations in both young and mature LFL (Renou *et al.*, 2008; Aziz *et al.*, 2010; Brennan *et al.*, 2017a; Naveen *et al.*, 2017). Bashir *et al* ., (2010) notes that ammonia removal is an important concern as its level continues to rise as the landfill ages. In Ireland and the EU, the Water Framework Directive and Urban Wastewater Treatment Directive have stringent regulations for the discharge of WW to receiving bodies. These stringent emission limits have caused increasing concern to WWTPs that treat leachate with high ammonia levels and is one of the main reasons why WWTPs are reluctant to treat LFL. This has resulted in over 30% of WWTPs in Ireland rejecting leachate during the period 2010-2015 (EPA, 2015a; Brennan *et al.,* 2016; Brennan *et al.,* 2017b). Leachate used in this trial contained ammonia concentrations of 1040 mg.L^{-1} which was reduced to 95 mg.L^{-1}, in the final effluent. However, despite the final effluent not reaching the discharge limits (4 mg.L^{-1} N), there was a significant overall ammonia reduction of 95 % (Table 3.3). To achieve ammonia levels that are acceptable further studies would need to be carried out including recirculating leachate back through the column system or by using this step as a pre-treatment followed by a physiochemical treatment, such as ammonia air stripping or reverse osmosis. As previously discussed, leachate from a methanogenic stage of the landfill may not respond well to biological treatment and the need for a physiochemical treatment has been shown. It is known that ammonia in the methanogenic phase is quite toxic to microorganisms and can inhibit the biological degradation process. Therefore, it is essential to have a microbial consortium that can deal with high levels of ammonia.

Phosphate is found at a very low levels in leachate from Powerstown Landfill when compared to ammonia and nitrate levels (1.2-8 mg.L^{-1}P;Table 3.1). However, levels are still above the EPA set discharge limits of 0.4 mg.L^{-1} P. The main problem associated with phosphate is the contamination of ground and surface waters. When phosphate contaminates ground and surface waters it can cause an imbalance in the nutrient cycling process, eutrophication and blooms of cyanobacteria. Even though phosphate is a concern when it comes to contamination, it is not a major concern for WWTPs in Ireland, in fact, some WWTPs may require the addition of phosphorous as a nutrient for bacterial growth (EPA, 2000b). The microbial consortia used in this study has shown the ability to be resistant to and capable of utilising phosphate at concentrations ≤ 100 mg.L^{-1} P. In particular, two isolates LCT 24 (*Bacillus vitnamensis*) and LCC32 (*Brevibacterium iodinum)* have the ability to survive in medium to high level of phosphates (<100 mg.L^{-1}). A study carried out by Riazanova *et al* ., (2007) showed that *B. casei, B. linens,* and *B. epidermidis* were able to reduce phosphate concentration by 90 %. In total, seven isolates (Figure 3.2) that cluster within the *Brevibacterium* phylum have the ability to utilise phosphate and achieve high removal rates. Phosphate levels of the initial leachate samples were 7.25 mg.L^{-1} P. Overall phosphate was reduced by 67%, however, discharge limits were not reached (Table .4) indicating further treatment is needed similar to those previously described for ammonia.

Nitrates are the result of the nitrification process of microorganisms, in which biological oxidation occurs to convert ammonia to nitrite and then to the nitrate. The influent concentration was 460 mg.L^{-1} N which was reduced by 63 % overall. This was the lowest reduction rate achieved, but similarly ammonia and phosphate discharge limits were not achieved. A final effluent concentration of 166 mg.L^{-1} N, was recorded which is above the discharge limit (50 mg.L^{-1} N; Table 3.4). It should be noted that nitrate readings fluctuated thought-out the trial. This may be due to the nitrification process as when ammonia is treated in aerobic processes it is accompanied by a concomitant increase in nitrate concentrations. Other treatment methods such as physicochemical treatment may be needed for nitrates to counteract this problem.

Table 3.4. Overall results for the removal of ammonia, phosphate and nitrates from soil inoculated with microorganism.

	Influent Concentration	**Effluent Concentration**	**% Removed**
Ammonia	1040 ± 1.3	95 ± 0.9	90.9 ± 1.3
Phosphate	7.25 ± 0.5	2.36 ± 0.5	67.5 ± 0.9
Nitrate	460 ± 1.2	166 ± 1.3	63.9 ± 1.3

Where;
Performance values are the trial period mean ± standard deviation and are expressed as mg.L^{-1}

3.6 Conclusion

LFL from Powerstown landfill in Carlow, Ireland contains a wide range of bacteria, including *Firmicutes, Proteobacteria* and *Actinobacteria,* which displayed resistance to a wide range of compounds found within leachate. Bioremediation is a promising treatment option for LFL. The system described and optimised in the current study achieved high percentage removal rates for ammonia (90%), while for both phosphate and nitrate lower percentage removal rates were recorded (67% and 63%, respectively). Overall these reduction rates are promising, but further work is needed to achieve regulatory discharge limits. The findings of this study are as follows, (1) LFL from Powerstown is in the methanogenic phase of decomposition and contains high levels of COD, BOD and ammonia; (2) Microorganisms isolated from leachate have the potential to utilise a range of heavy metals, ammonia, phosphate and nitrate, common constituents of leachate; (3) The microbial consortium used in this study was capable of reducing ammonia, phosphate and nitrate by 90, 67 and 63%, respectively.

3.7 Summary

This chapter identified 15 microbes that show potential to be used in the bioremediation of LFL. Overall, high percentage removal rates were achieved for all compounds tested (COD, BOD, ammonia, phosphate and nitrate). However, even though high percentage removal rates were achieved, EPA discharge standards for receiving bodies were not achieved. This indicated that further treatment is needed. The research will now focus on physicochemical treatment in term of adsorption for the removal of pollutants form LFL. Adsorption studies have shown to be beneficial in the removal of high level of pollutants within WW.

Chapter 4

Low-cost physicochemical treatment for removal of ammonia, phosphate and nitrate contaminants from landfill leachate

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Low-cost physicochemical treatment for removal of ammonia, phosphate and nitrate contaminants from landfill leachate.

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***Authors Contributions**

Sinead Morris conceived and designed the experiments. Sinead Morris acquired the samples, performed the experiments and analysed data. Sinead Morris, Guiomar Garcia-Cabellos, David Ryan, Deirdre Enright and Anne-Marie Enright interpreted the data. Guiomar Garcia-Cabellos and Anne-Marie Enright contributed reagents/materials/analysis tools to the work. Sinead Morris wrote the paper which was revised critically by Anne-Marie Enright.

4.1 Overview

Adsorption, the process of accumulating substances that are in a solution on a suitable interface, is a widely used physiochemical treatment option for WW (Tchobanoglous *et al.*, 2003; Worch, 2012). Fixed bed adsorption shows potential to remove pollutants form WW, and may be a beneficial treatment for LFL.

The main objectives of this chapter are:

To determine if adsorption is a successful treatment option for landfill leachate.

To study the best adsorption materials to treat landfill leachate.

To determine the removal efficiency of adsorption materials in a fixed bed column

To analyse the best bed height and flow rate for a fixed bed column system.

To treat LFL to national discharge standards using a fixed bed column

4.2 Abstract

Four low-cost materials, oyster shells (OS), pumice stone (PS), sand and zeolite were employed as adsorbents in an adsorption batch assays investigating the removal of ammonia, phosphate and nitrate from an aqueous solution. These compounds were chosen as they represent typical compounds found in landfill leachate (LFL). Assay performance was evaluated by the Langmuir and Freundlich adsorption isotherms and the adsorbent materials were ranked in terms of their adsorption capacity for each test compound. The top two materials, OS and PS, were then employed as adsorbents in a fixed-bed column optimisation trial that examined the effect of bed height and flow rate on the treatment of a synthetic LFL containing ammonia, phosphate and nitrate at an flow rate of 5 ml.min^{-1}. The optimisation trail was evaluated by both adsorption isotherms and the percentage removal efficiency of each compound. The trial concluded that the highest rates of adsorption were achieved using bed heights of 20 cm with an applied flow rate of 5 ml.min^{-1}. After optimisation, the system was employed for the treatment of LFL from Powerstown landfill, Carlow, Ireland. Column performance was evaluated by the percentage removal efficiency of each compound. Ammonia and nitrate were effectively removed by both adsorption materials resulting in their reduction to below the national discharge limits set for these compounds of ≤4 mg.L^{-1} and ≤50 mg.L^{-1}, respectively. In contrast, although similar high removal efficiencies were observed for phosphate these rates were not maintained during the test period, with overall results indicating reduced phosphate adsorption in comparison to the other compounds tested.

4.3 Introduction

Nutrient pollution, largely caused by the deliberate release of excess nitrogen and phosphorus into the atmosphere and waterbodies, is one of the more costly and challenging environmental problems of the industrialised world. Nutrient pollution of the aquatic environment is of particular concern as it often results in eutrophication and increased algal blooms within these ecosystems which reduces their stability (Baker, 1973; EPA, 2000a; Oram, 2014). One potential source of nutrient pollution is landfill leachate (LFL) which may contain, among other pollutants, particularly high concentrations of ammonia, nitrate and phosphate

(EPA, 2000a; EPA, 2015a; Brennan *et al.,* 2017a). LFL is produced when water percolates through a landfill picking up the by-products of waste degradation (McCarthy *et al.,* 2010). Its composition changes dramatically over the lifespan of the landfill, as such in-depth knowledge of the stages of waste degradation is required to determine the most effective treatment options (Figure 2.4). Such investigations have revealed that mature LFL, i.e. >5 years, characterised by BOD: COD ratios of ≤0.2, low concentrations of biodegradable compounds and elevated levels of ammonia, can be successfully treated physicochemically (Kjeldsen *et al.,* 2002; Lee and Nikraz, 2014). In particular, physiochemical treatments are effective for the removal of non-biodegradable organics such as humic and fulvic acids, heavy metals and absorbable organic halogens (AOXs) from LFL (Renou *et al.,* 2008; Abbas *et al.,* 2009).

Despite this, within the European Union (EU) the co-treatment of LFL with domestic wastewater (WW) in wastewater treatment plants (WWTPs) (Renou *et al.,*2008; EPA 2015a; Brennan *et al.,*2016) is the preferred option due to maintenance cost and the ease of treatment for landfill operators. However, the inclusion of LFL into the influent of these facilities can negatively affect their ability to reach the stringent discharge limits set for receiving bodies, often resulting in fines when breaches occur. Consequently, the supplementation of a WWTP with LFL is often restricted to 4% of its hydraulic load in an effort to reduce shock loading regimes (EPA, 1997; Brennan *et al.,* 2017a). As a consequence, many WWTPs refuse to accept this waste stream entirely, as was the case in Ireland during the period 2010-14, due to the burden it was placing on these plants (Brennan *et al.,* 2017b). Further to this LFL, treatment in WWTPs poses a significant cost to landfill operators due to the storage of LFL and its transportation to these facilities (Environment Agency UK, 2007; McCarthy *et al.*, 2010; Brennan *et al.*, 2017b; Brennan *et al.*, 2017a). Thus, it is essential that research continues into a cost-effective treatment option that can treat LFL to acceptable limits.

Physicochemical treatments are commonly applied to a variety of WWs including industrial, swine, aquaculture, municipal and dairy WW (Kushwaha *et al.*, 2010; Cappuccino and Sherman, 2014; Mojiri *et al.*, 2016; Bernardi *et al.*, 2018; Cao *et al.*, 2018; Mojiri *et al.*, 2018). In particular, they are important tools for the reduction of suspended solids, colloidal particles, colour and toxic compounds from these waste streams. Several physiochemical treatment options are currently available, including adsorption, reverse osmosis, air stripping, chemical precipitation and oxidation (Renou *et al.*, 2008; Gao *et al.*, 2015; Torretta *et al.*, 2017), all of which vary in both cost and ease of use. Adsorption, the process of accumulating substances that are in a solution on a suitable interface, is a widely used physiochemical treatment option for WW (Tchobanoglous *et al.*, 2003; Worch, 2012). The adsorbent, a solid, liquid or gas-phase on which the adsorbate accumulates, can vary greatly depending on the processes required (Laidler *et al.*, 2003; Tchobanoglous *et al.*, 2003). For example, studies have reported the use of black (carboniferous) shale to adsorb methane (CH_4), waste coconut shells for the adsorption of carbon dioxide (CO_2), and zeolite for the adsorption of hydrogen sulphide (H_2S) (Abdullah *et al.*, 2018; Yue *et al.*, 2018; Zhu *et al.*, 2018). Additionally, several studies have described the adsorption of a wide range of compound including ammonia, phosphate and heavy metals from different influents using zeolite (clinoptilolite), pumice, and oyster shells (Namasivayam *et al.*, 2005; Tsai *et al.*, 2009; Asgari and Rahmani, 2013; Chansuvarn, 2018).

Fixed bed adsorption, where a solid adsorption material is packed into a fixed column, has also been utilised for the treatment of several WWs (Karunarathne and Amarasinghe 2013; Jahangiri-Rad *et al.*,2014; Biswas and Mishra 2015). Within these systems, activated carbon (AC) remains the most common adsorbent employed industrially (Foo and Hameed, 2009). However, the use of AC has many disadvantages including; the high cost associated with the generation and regeneration of carbons fibres, due to the brittle nature of carbons used for the removal of organic species (Lin and Juang, 2009; Nwabanne and Igob okwe, 2012). With a view to reducing the use of AC in these systems several studies have been carried out employing novel adsorbent materials, such as zeolite, bentonite, PS and OS (Futalan *et al.*, 2011; Guler and Sarioglu, 2014; Han *et al.*, 2016; Yuangsawad and Na-ranong, 2017). For example, many researchers have reported the effective use of zeolite for the adsorption of heavy metals, ammonia and phosphate (Song and Gao, 2013; Lim and Aris, 2014; Martins *et al.*, 2017; Yuangsawad and Na-ranong, 2017). Martins *et al.* (2017) examined the use of zeolite to remove ammonia from LFL through batch study experiments, applying the Langmuir isotherm to predict adsorption parameters. The q_{max} value obtained in this study was 14.27 $mg.g^{-1}$ NH_4-N indicating this material may be useful for ammonia removal from LFL. Similarly, several studies have used calcareous shells, a low-cost by-product of the aqua industry, as an adsorbent. Results for these studies also recorded high removal rates for ammonia, phosphate and heavy metals, indicating that they may be suitable for the treatment of LFL rich in these compounds (Song and Gao, 2013; Lim and Aris, 2014; Yuangsawad and Na-ranong, 2017). In addition, these adsorbents show the potential to be regenerated using a range of chemical such as HCl and NaOH, allowing them to be reused (Xu *et al.,* 2012; Lata *et al.,* 2015; Helard *et al.,* 2018).

Despite the considerable volume of research carried out in this area, little emphasis has been placed on the treatment of LFL using fixed bed column systems. Furthermore, research that has been conducted tend to focus on the determination of the adsorption capacity of adsorbents with little concern of the longevity of the process (Rodríguez *et al.,* 2004; Foo and Hameed, 2009; Halim *et al.,* 2010; Couto *et al.,* 2017). In order for a material to be suitable for LFL/WW treatment, it needs to show high adsorption capacity and have the ability to be used on a continuous basis (Çifçi and Meriç, 2015; Grace *et al.,* 2016).

This research focuses on the removal of ammonia, phosphate and nitrate from LFL. Ammonia is one of the main reason why WWTPs in Ireland refuse to treat LFL (Brennan *et al.,* 2017b; Brennan *et al.,* 2017a). Ammonia can cause environmental pollution, promoting algae growth and accelerate eutrophication in receiving water bodies. In addition, high concentrations of ammonia can persist for up to 50 years after a landfill is decommissioned (Chu *et al.,* 1994; Wang Yu, 2013; Brennan *et al.,* 2017b) and can decrease the effectiveness of biological treatments such as those employed in WWTPs (Aziz *et al.,* 2010; Bashir *et al.,* 2010). Phosphate and nitrate are found in low concentration within receiving bodies, but when elevated can cause a nutrient imbalance in receiving bodies, leading to the overproduction of plankton and aquatic plants, which in turn decrease oxygen causing eutrophication and reduced stability of the ecosystem (Mann, 2000; Khan and Ansari, 2005; Oram, 2014).

In light of the above, the aims of this paper may be summarised as follows: (1) adsorption materials vary greatly in relation to their adsorption capacity for different waste streams. As such, we evaluated the efficacy of four different adsorbent materials, PS, zeolite, OS and sand, for the adsorption of ammonia, phosphate and nitrate using batch adsorption assays; (2) through this screening process the best performing adsorbents were chosen and employed in a small scale fixed bed column study treating synthetic LFL, which investigated the effect of flow rate and bed height; (3) this optimised column system was then employed for the treatment of LFL to the discharge limits for receiving bodies set by the Environmental Protection Agency (EPA) Ireland.

4.4 Theory

Adsorption batch assays are used to determine the effectiveness of adsorbents by calculating the quantity of analyte adsorbed per g of adsorption material. Such assays are commonly employed due to their low cost and ease of use but are poor predictors of adsorbent ability over large timeframes or their performance in real-world scenarios (Ali and Gupta, 2007; Crini and Badot, 2008; Callery *et al.*, 2016). Despite these drawbacks, data obtained from batch assays can be used to determine adsorption isotherms, which are essential for the design and optimisation of the adsorption process (Crini, 2006; Ali and Gupta, 2007; Behnamfard and Salarirad, 2009; Callery *et al.*, 2016). The relationship between the adsorbent and the influent and effluent concentration is considered as:

$$\mathbf{q_e = \frac{(c_o - c_e).v}{m}} \qquad \textbf{Equation 4.1}$$

Where q_e, is the mass of contaminating adsorbed per g of adsorbent, c_o is the influent concentration (mg.L^{-1}), c_e is the effluent concentration (mg.L^{-1}), v is the volume of liquid used (L) and m is the mass of adsorbent used (g).

The simplest isotherm was first obtained in 1916 by Irving Langmuir and is based on all part of the surface behaving in the same way as far as adsorption is concerned. The Langmuir model represents monolayer adsorption onto surfaces with a finite number of identical sites. This model assumes; (1) a fixed number of vacant or adsorption sites are available on the surface of the solid; (2) all the vacant sites are of equal size and shape on the surface of the adsorbent; (3) each site can hold a maximum of one molecule and a constant amount of heat energy is released during this process and finally; (4) a dynamic equilibrium exists between adsorbed and free molecules (Tang *et al.*, 2012; Enright, 2015; Han *et al.*, 2016). The Langmuir isotherm is represented in the following form:

$$\mathbf{\frac{c_e}{q_e} = \frac{c_e}{q_{max}} + \frac{1}{K_L q_{max}}} \qquad \textbf{Equation 4.2}$$

Where, q_e is the adsorbent phase concentration after equilibrium (Equation 4-1), c_e is the final effluent concentration, q_{max} is the maximum adsorption at monolayer coverage (mg.g^{-1}) and K_l is the Langmuir adsorption equilibrium constant (L mmol^{-1}). The plot of the graph (c_e/q_e against $c_{e,}$) yields both the q_{max} and K_L from the slope and intercept (Reed and Matsumoto, 1993; Tang *et al.*, 2012; Han *et al.*, 2016). The essential characteristics of the Langmuir isotherm can be expressed in terms of a dimensionless constant separation factor, R_L, which is given by the following equation:

$$R_L = \frac{1}{1+K_L c_o}$$ **Equation 4.3**

There are four probabilities for the R_L value: favourable sorption, $0<R_L<1$; unfavourable sorption, $R_L>1$; linear sorption, $R_L=1$; and irreversible sorption, $R_L=0$ (Dada *et al.*, 2012; Enright, 2015).

Nonideal systems can be applied to adsorption isotherms due to the work of Herbert Max Finlay Freundlich. This method is commonly used to describe the adsorption characteristics of the heterogeneous surface (Huong *et al.*, 2016). In particular, Freundlich demonstrated that the ratio of the amount of solute adsorbed onto a given mass of an adsorbent to the concentration of the solute in the solution was not constant at different solution concentrations. The Freundlich isotherm assumes that if the concentration of solute in the solution at equilibrium, c_e, is raised to the power n, the amount of solute adsorbed being q_e, then c_e/q_e is constant at a given temperature. Freundlich offered the following linear equation (Rawajfih and Nsour, 2006):

$$\mathrm{Inq_e} = \mathrm{InK_f} + \frac{1}{n}\mathrm{Inc_e}$$ **Equation 4.4**

The constant K_f is an approximate indicator of adsorption capacity, while n is a function of the strength of adsorption in the adsorption process. The slope and the intercept of both equations are used to determine the isotherms constants (n) which is related to the sorption intensity of the sorbent where adsorption is favourable between 1< n <10, while a greater value for K_f indicates a higher adsorption capacity (Dada *et al.,* 2012; Tang *et al.,* 2012; Enright, 2015).

In addition to the batch assays described above, column studies are often employed to investigate the long-term performance of adsorbents. Ideally, these should be carried out over long time periods, but in practice, shorter small-scale column studies are often performed to generate more realistic data onto which several models can be applied (Callery *et al.,* 2016). These models attempt to predict the performance of the adsorbent by studying the relationship between the effluent concentration and the loading of the chosen adsorbents. Some of the most commonly used models are described below.

The performance of packed bed columns is described through the concept of the breakthrough curve, specifically breakthrough appearance and the shape of the breakthrough curve. The position of the breakthrough curve along the volume axis depends on the capacity of the column with respect to the feed concentration and flow rate (Aksu *et al.,* 2002; Rao and Viraraghavan, 2002; Aksu and Gönen, 2004). The curve is expressed as c_{eff}/c_o against the contact time, where c_{eff} and c_o are the effluent and influent concentrations (mg.L^{-1}). The breakthrough point is set at 10% of the influent concentration, while the exhaustion point is set at 90% of the influent concentration (both mg.g^{-1}) (Futalan *et al.,* 2011). From this breakthrough graph, the total adsorbed ions can be obtained by integrating the plot of the adsorbed concentration (C_{ad}) versus the flow time (t). This plot is necessary to obtain the area (A) which is used to determine the $q_{total,}$ the total amount of ions adsorbed by the adsorbent:

$$q_{total\,(mg)} = \frac{QA}{100} = \frac{Q}{1000}\int_{t=0}^{t=t_{total}} C_{ad}dt$$ **Equation 4.5**

The total amount of ions delivered to the system, m_{total}, is determined by the following:

$$m_{total}\,(mg.L^{-1}) = \frac{c_o Q t_{total}}{1000}$$ **Equation 4.6**

In this equation, the Q and the t_{total} represent the flow rate (ml.min^{-1}) and the total flow time (min), respectively. Both equation 6 and 7 are needed to evaluate the removal efficiency of the column, while equation 4 and 5 are required to determine the percentage removal efficiency of the test compound:

$$\text{Total removal}\,(\%) = \frac{q_{total}}{m_{total}}\,x\,100$$ **Equation 4.7**

The adsorption capacity can then be determined for the whole system using equation 4 and the mass of the adsorbent, presented as analyte removal per g of material in each column (mg.g^{-1}):

$$q_{eq}(mg.g^{-1}) = \frac{q_{total}}{x}$$ **Equation 4.8**

Where *x* is the unit mass (g) of adsorbent packed into the column.

The Thomas model and the Adam-Bohart model can be used to analyse the data generated in column studies. The Thomas model used to describe the behaviour of adsorption in fixed-bed columns can be applied to data following the Langmuir isotherm of adsorption-desorption and second-order kinetics (Aksu *et al.,* 2007; Taylor *et al.,* 2014; Djomgoue *et al.,* 2015). The Thomas rate constant (k_{TH}) is dependent on flow rate, initial ion concentration and bed height, assuming that adsorption is controlled by the surface reaction between the adsorbate and the unused capacity of the adsorbent. The Thomas model is suitable to estimate the adsorption process, where the internal and external diffusion resistance is extremely small (Aksu and Gönen 2004). The linear form of the Thomas model is expressed as:

$$\mathrm{In}\left(\frac{c_o}{c_t}-1\right)=\frac{k_{Th}q_o m}{Q}-k_{Th}c_o t$$ **Equation 4.9**

Where, k_{Th} is the Thomas kinetic coefficient (mg.L^{-1}), t is the total flow time (min), m is mass (g) and Q is the volumetric flow rate (ml.min^{-1}). The adsorption capacity and the mass of adsorbent used are q_o (mg.g^{-1}) and m (g), respectively. The plot of ln(co/c_t-1) versus t gives the values k_{Th} and q_o which are calculated from the slope and the intercept of the line (Lim and Aris, 2014; Biswas and Mishra, 2015).

Bohart and Adams (1920) developed the model through the analysis of a typical chlorine charcoal transmission curves, hypothesising that the uptake rate of chlorine is proportional to the concentration of the chlorine existing in the bulk fluid and the residual adsorptive capacity of charcoal. Although originally designed for adsorption of gases this approach can be applied successfully to the description of other systems including adsorption of compounds within the liquid solution (Aksu *et al.,* 2007; Xu *et al.,* 2013). In particular, the Adam-Bohart model is used to describe both the dynamic behaviour of the column systems and the delineation of a breakthrough point. This model is based on the surface reaction theory which assumes that equilibrium is not instantaneous, therefore, the rate of adsorption will be proportional to the residual capacity of the adsorbent and the concentration of the compound being adsorbed (Chowdhury *et al.*, 2013). This equation is expressed as (Lim and Aris, 2014; Biswas and Mishra, 2015):

$$\text{In}\left(\frac{c_t}{c_o}\right) = k_{AB}c_o t - k_{AB}N_o\left(\frac{Z}{U_o}\right) \qquad \textbf{Equation 4.10}$$

Where; k_{AB} is the Adam-Bohart kinetics constant (L mg.min^{-1}), N_o is the saturation concentration (mg.L^{-1}); Z is the bed depth (cm) and U_o represent the linear velocity, which is determined from the calculation of the volumetric flow rate over the bed section. The values of k_{AB} and N_o are obtained from the linear plot of In(C_t/C_o) versus t.

4.5 Materials and methods

4.5.1 Adsorbents and influents

OS (⌀5-10 mm), PS (⌀ 2-5 mm), sand (⌀ 1-3 mm), and zeolite (⌀ 3-8 mm) were employed as adsorbents in this study. All adsorbent materials where triple washed with deionised (d) H_20 and dried at 80°C for 12 hrs prior to use. All chemicals were supplied by Sigma Aldrich (Ireland) unless otherwise stated. Individual stock solutions of ammonia ($(NH_4)_2SO_4$), phosphate (KH_2PO_4) and nitrate (KNO_3) were prepared at a concentration of 1000 mg. l^{-1} in dH_20. Final working standards were made from these stocks for immediate use. LFL used in this study was obtained from Powerstown Landfill (52°45’58.46’’ N, 6°57’20.13’’ W), previously described by the authors (Section 2.3.1). LFL samples were collected in January 2016 and stored at 4 °C prior to use.

4.5.2 Batch adsorption assay

Batch adsorption assays were carried out in 100 ml glass beakers containing 1 g of prepared adsorbent and 20 ml of standard solutions of the individual test compounds within the concentration ranges of 100-1000 mg. l^{-1}. Beakers were shaken at 350 RPM for 16 hrs period at 20°C. Beakers containing a known initial concentration of each compound without adsorbent served as controls. Both controls and test assays were performed in triplicate.

4.5.3 Column optimisation trial

As PS and OS proved the most suitable adsorbents for all compounds tested, based on both the Langmuir and Freundlich isotherms, it was decided to employ them in the subsequent column studies. PVC, laboratory-scale (height 30 cm, Ø 11 cm and an internal capacity of 2850 cm^3) columns with mesh bases, were each packed with one of the prepared adsorbent materials. Prior to use dH_20 was passed through the columns in a downflow direction to remove trapped air. All columns were employed for the treatment of a synthetic LFL containing ammonia, phosphate and nitrate all at a concentration of 1000 $mg.L^{-1}$. In an effort to optimise the process the following parameters were investigated; (1) the effect of different bed heights and (2) the effect of flow rate. Column prefixes and operating parameters are described in Table 4.1.

Table 4.1. Parameter for optimisation of columns study trial, for both bed height and flow rate.

Column	Bed height[a]	Flow rate[b]	RT[c]	Column	Bed Height	Flow rate	RT
OS[d] 1	7	10	12.5	**OS 4**	20	5	39.5
OS 2	15	10	22.5	**OS 5**	20	10	35
OS 3	20	10	35	**OS 6**	20	15	24.5
PS[e] 1	7	10	17.5	**PS 4**	20	5	42.5
PS 2	15	10	23.5	**PS 5**	20	10	35
PS 3	20	10	37.5	**PS 6**	20	15	29.5

Where;

[a] is bed height in cm

[b] flow rate in ml.min^{-1}

[c] the retention time in min

[d] Oyster Shells

[e] Pumice Stone

4.5.4 Leachate treatment

After column optimisation, two new columns, as described above, were established with fresh adsorbent material (pumice stone and oyster shells) to a bed height of 20 cm. LFL (Table 4.2) was applied to the columns at 5 ml.min^{-1} and the resultant RT was 42.5 min and 39.5 min for PS and OS, respectively. Both columns were operated over a 10 hrs period with each column treating approximately 3 l of leachate. The effluent was sampled every twenty min until all leachate had passed through the systems and stored at 4°C prior to analysis.

Table 4.2. Composition and concentration of leachate used in this trial

Compound	Influent concentration [a]
Ammonia	750 ± 12
Phosphate	12.6 ± 0.5
Nitrate	256 ± 1.6

Where; [a] is the concentration in $mg.L^{-1}$
All results are average ± standard deviation, where n=3

4.5.5 Chemical analysis

All reagent used where of analytical grade and made with dH_20. Ammonia was analysed using the phenate method and analysed on Shimadzu UV1800 spectrophotometer (Rice *et al.*, 2017). Ortho-Phosphate was analysed using molybdovanadate reagent (HACH Lange) and Nitrate analysis was carried out using NitraVer® 5 reagent power pillows (HACH Lange). All HACH products were used according to manufactures instruction and measured on HACH DR 6000 UV- spectrophotometer.

4.5.6 Determination of the model of error

Generally, isotherm models are selected based on the determination of the correlation coefficient (R^2) value, where high R^2 values represent the best fit. However, since the non-linear forms of an isotherm are converted to linear equations, errors and distribution can be altered, influencing error variance and normal R^2 values. Due to this bias in the transformation of the equation, mathematical error functions have been applied to determine the model of best fit. As such, accurate models are those with high R^2 values and low values of error function indicating a stronger linear relationship. The error models chosen for this study were the hybrid fractional error function (HYBRID), the mean percentage error (MPE) and non-linear chi-squares (Foo and Hameed, 2010; Krishni *et al.*, 2014; Hamid Reza *et al.*, 2017).

4.5.6.1 Hybrid fractional error function (HYBRID)

This error model was developed by Porter *et al* ., (1999), in an attempt to improve the fit of the sum of the squares of the errors. This error function takes into account the number of degrees of freedom and the number of data points minus the number of parameters in each isotherm. It was used as a metric of the goodness of model fit of the experimental data from batch studies. This function is supplied below (Porter *et al.*, 1999; Callery *et al.*, 2016):

$$\frac{100}{n-p}\sum_{i=1}^{n}\frac{(q_{e.}exp-q_{e.}cal)^2}{q_{e.}exp} \qquad \textbf{Equation 4.11}$$

Where; n is the number of data points, p is the number of constants in the proposed isotherm, q_e.exp is obtained from equation 1 and the q_e.cal is obtained from equation 2 or 4 depending on the isotherm being used.

4.5.6.2 Mean percentage error

The mean percentage error (MPE) was used to determine the over- or underestimation of the experimental data and attempts to reduce fractional error across the study concentration range. Minus values indicate that the model is underestimating the experimental adsorption, while positive values indicate an overestimation. The MPE was calculated using the following equation:

$$\frac{100\%}{n}\sum_{i=1}^{n}\frac{q_{e.}exp-q_{e.}cal}{q_{e.}exp}$$ **Equation 4.12**

4.5.6.3 Nonlinear Chi-Square

The nonlinear chi-square (χ^2) test is a statistical tool used to determine the best fit of an adsorption system from the data. This is obtained by judging the sum of the square difference between both the experimental and theoretical data, where each squared difference is divided by the corresponding values (from the calculated model). Small χ^2 values indicate a better fit than higher χ^2 values. The χ^2 was calculated using the following equation:

$$\sum_{i=1}^{n}\frac{(q_{e.}exp-q_{e.}cal)^2}{q_{e.}exp}$$ **Equation 4.13**

4.6 Results and discussion

4.6.1 Batch studies

Both Langmuir and Freundlich isotherm were applied to batch studies data obtain during each trial.

4.6.1.1 Langmuir isotherm

The Langmuir isotherm was applied to the data sets generated by all batch assay combinations to determine the performance of each adsorbent. Before applying this linear model, the plot of c_e against q_e was used to depict the types of adsorption that were occurring based on the system proposed by Brunauer *et al* ., (1940). The adsorption of ammonia, phosphate and nitrate on the four adsorbent materials are thus described in Figure 4.1. The shape of the curve depicts type one adsorption, monolayer adsorption easily described by the Langmuir isotherm, while the correlation coefficients (R^2) were all above 0.95 (Table 4.3), indicating this isotherm is suited to the data sets. These graphs are also useful for determining the maximum adsorption capacity (q_{max}) of an adsorbent, which can be defined as both a theoretical maximum capacity (q_{max}.th) and an experimental value (q_{max}.exp) based on the Langmuir linear equation (Equation 4.2). All adsorbent materials tested showed great potential in their ability to remove ammonia with OS and PS giving the greatest q_{max}.th and q_{max}.exp (Table 4.3). In particular, OS and PS gave the greatest q_{max}.th and q_{max}.exp for both phosphate and nitrate (Table 4.3).

The MPE for this model was negative for all adsorbent and compound combinations tested (Table 4.3), indicating that the experimental system is underperforming in relation to achievable theoretical values. In addition, PS had the lowest HYBRID error and χ^2 values for all compounds tested (Table 4.3), indicating that the Langmuir isotherm is best suited to this adsorbent.

Additional optimisation of batch assays to investigate the effects of pH, temperature and adsorbent modifications could now be carried out to determine the full potential of each adsorbent. However, as the scope of the study was to evaluate adsorbents for the treatment of LFL rich in ammonia, phosphate and nitrate these parameters were not investigated.

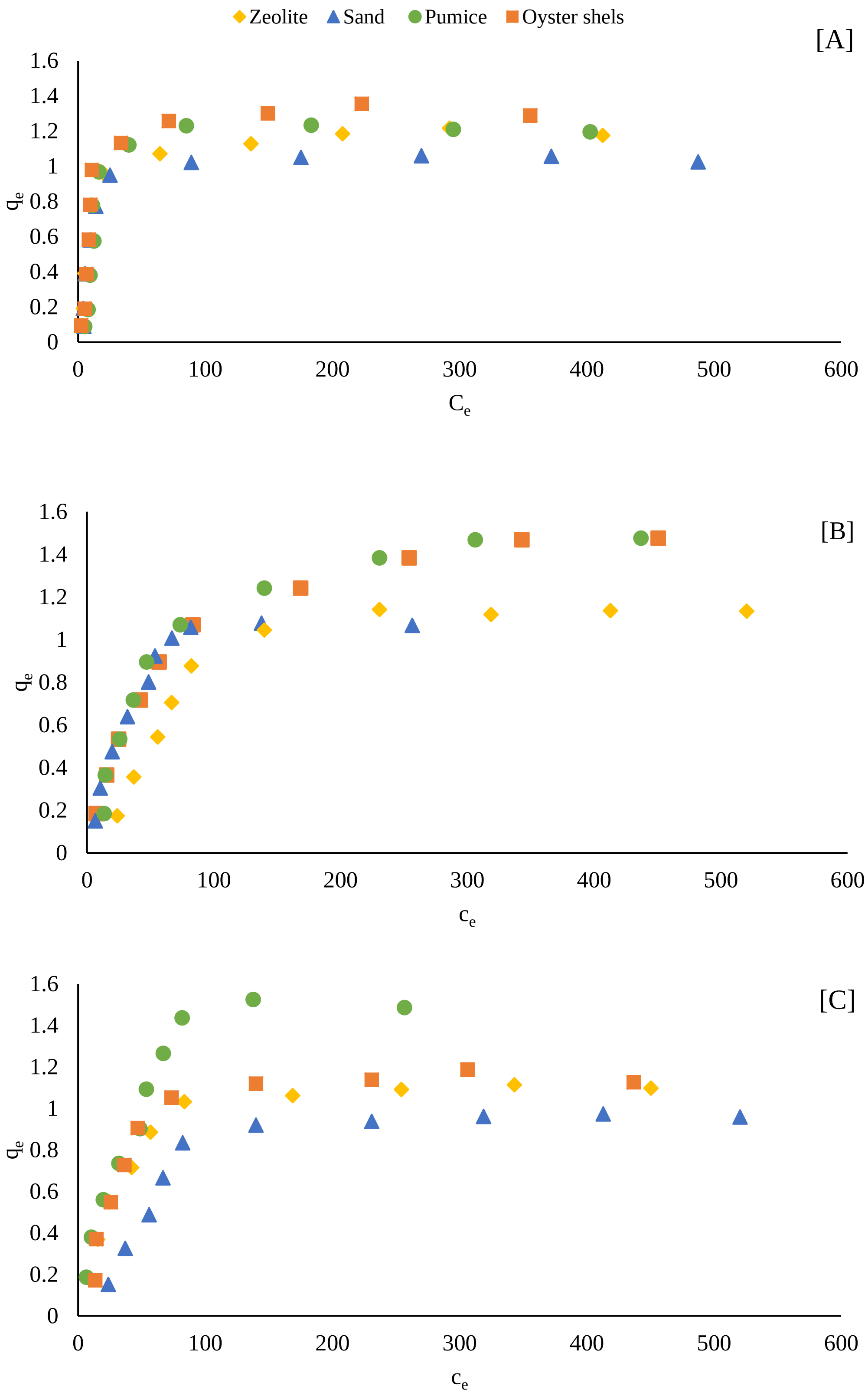

Figure 4.1. The adsorption isotherm for ammonia [A], phosphate [B] and nitrate [C] on the indicated adsorbents materials at a varying initial concentration between 1000 mg.L^{-1}

4.6.1.2 Freundlich Isotherm

The Freundlich isotherm was also applied to the data sets where n, the function of the strength of adsorption, remained within the favourable range ($2 \leq n \leq 3$) for all adsorbents tested (Table 4.3). However, poor correlation was observed in the linear graphs for all adsorbents ($0.75 \leq R^2 \leq 0.86$; Table 4.3), with the exception of PS and zeolite whose performance was greater for nitrate and phosphate. Nevertheless, it can be considered that the Freundlich isotherm is a poor fit for this data set. In addition, the APE for this model was negative for all adsorbent and compound combinations tested (Table 4.3), again indicating an underperformance of the experimental system in relation to the achievable theoretical values. PS, however, had the lowest MPE, with experimental values comparable to theoretical values, ranging from $-1.84 \leq \text{APE} \leq -3.61$. Similarly, HYBRID error and nonlinear chi-square tests also indicate a poor fit to the Freundlich isotherm when compared to the Langmuir isotherm (Table 4.3). High HYBRID scores where obtain, while $\chi 2$ values were also high for each adsorbent and compound tested (Table 4.3). When comparing both isotherms to the data sets obtained through the experiment, is it clear that Langmuir's isotherm fits best (Table 4.3).

Table 4.3. Parameters for Langmuir and Freundlich isotherms, based on the linear model of these isotherms, alongside the dimensionless constant and error models.

		Langmuir Isotherm				**Error Model**				**Freundlich Isotherm**			**Error model**		
Parameter	**Absorbent**	**q_{max}.th** (mg.g^{-1})	**q_{max}. Exp** (mg.g^{-1})	**K_L** (d m^{3}g^{-1})	**R_L**	**R^2**	**HYBRID**	**MPE**	**$\chi 2$**	**N^f** (mg.g^{-1})	**K_F** (d m^{3}g^{-1})	**R^2**	**HYBRID**	**MPE**	**$\chi 2$**
Ammonia	Z	1.25	1.18	0.1	0.91	0.95	8.78	-7.03	0.63	2.58	0.26	0.86	7.61	-3.10	0.6
	PS	1.28	1.19	0.09	0.92	0.98	4.53	-1.42	0.32	2.89	0.17	0.78	7.93	-3.61	0.62
	S	1.08	1.03	0.13	0.88	0.99	9.09	-9.15	0.88	2.34	0.17	0.81	17.95	-13.57	1.44
	OS	1.38	1.29	0.13	0.93	0.99	7.75	-9.46	0.55	2.35	0.14	0.75	11.97	-8.17	1.08
Nitrate	Z	1.19	1.11	0.05	0.95	0.99	3.82	-3.94	0.47	2.56	0.13	0.91	4.46	-2.73	0.4
	PS	1.59	1.52	0.08	0.92	0.99	1.33	-2.52	0.11	2.73	0.09	0.95	7.35	-2.17	0.57
	S	1.07	0.97	0.05	0.95	0.99	3.71	-7.85	0.24	2.03	0.12	0.84	7.88	-4.68	0.53
	OS	1.27	1.03	0.05	0.98	0.98	2.03	-3.94	0.3	2.37	0.06	0.85	7.51	-7.56	0.68
Phosphate	Z	1.23	1.08	0.03	0.98	0.99	4.78	-3.35	0.59	2.58	0.07	0.94	7.58	-2.32	0.5
	PS	1.49	1.48	0.02	0.98	0.95	1.66	-2.14	0.14	2.89	0.03	0.91	7.94	-1.84	0.71
	S	1.28	1.14	0.04	0.96	0.99	4.64	-4.97	0.3	2.34	0.1	0.86	7.35	-3.98	0.66
	OS	1.38	1.34	0.04	0.95	0.98	2.54	-3.35	0.38	2.35	0.06	0.96	9.39	-4.73	0.85

Where;
Z-Zeolite , PS- Pumice stone, S-Sand and OS- Oyster shells

4.6.2 Fixed-bed column optimisation

4.6.2.1 Effects of bed height and flow rate

Percentage removal of the test compounds was used to determine the most effective bed height and flow rate for a synthetic LFL over a 10 hrs trial period (Figure 4.2). In general, increases in percentage removal were concurrent with increases in bed height due to the increase of adsorbent mass and longer RT (Table 4.1; Figure 4.2). Specifically, for ammonia, both PS3 and OS3 recorded a high percentage removal of 89% and 92%, respectively for ammonia at a bed height of 20 cm. While lower bed heights for both OS1 and PS1 with shorter RTs exhibited a c.10% reduction in percentage removal of ammonia for each adsorbent tested (Table 4.1; Figure 4.2). Similar, results were observed for phosphate and nitrate removal with the 20 cm bed height (OS3 and P3) recording a 10-16% increase in % removal for all compounds tested in comparison to the lower bed heights (Figure 4.2).

Adsorption isotherms indicated that the higher the bed height the overall greater maximum adsorption capacity achieved, due to the increased RT. The Thomas model indicated q_{eq} values of between 2.05-2.78 mg.L^{-1} for ammonia, phosphate and nitrate, which was a 20-30% increase in the values achieved for the lower bed heights for each adsorbent (Appendix 2). Likewise, the Adam-Bohart model displayed similar finding for the exhaustion point (N_o) where higher bed heights achieved exhaustion points of 2.01-3.08 mg.L^{-1} for all compounds tested for both adsorbents. This represents a 20% increase on values obtained from the lower bed heights indicating that the 20 cm bed height is more sustainable and can be used to treat a greater volume of LFL.

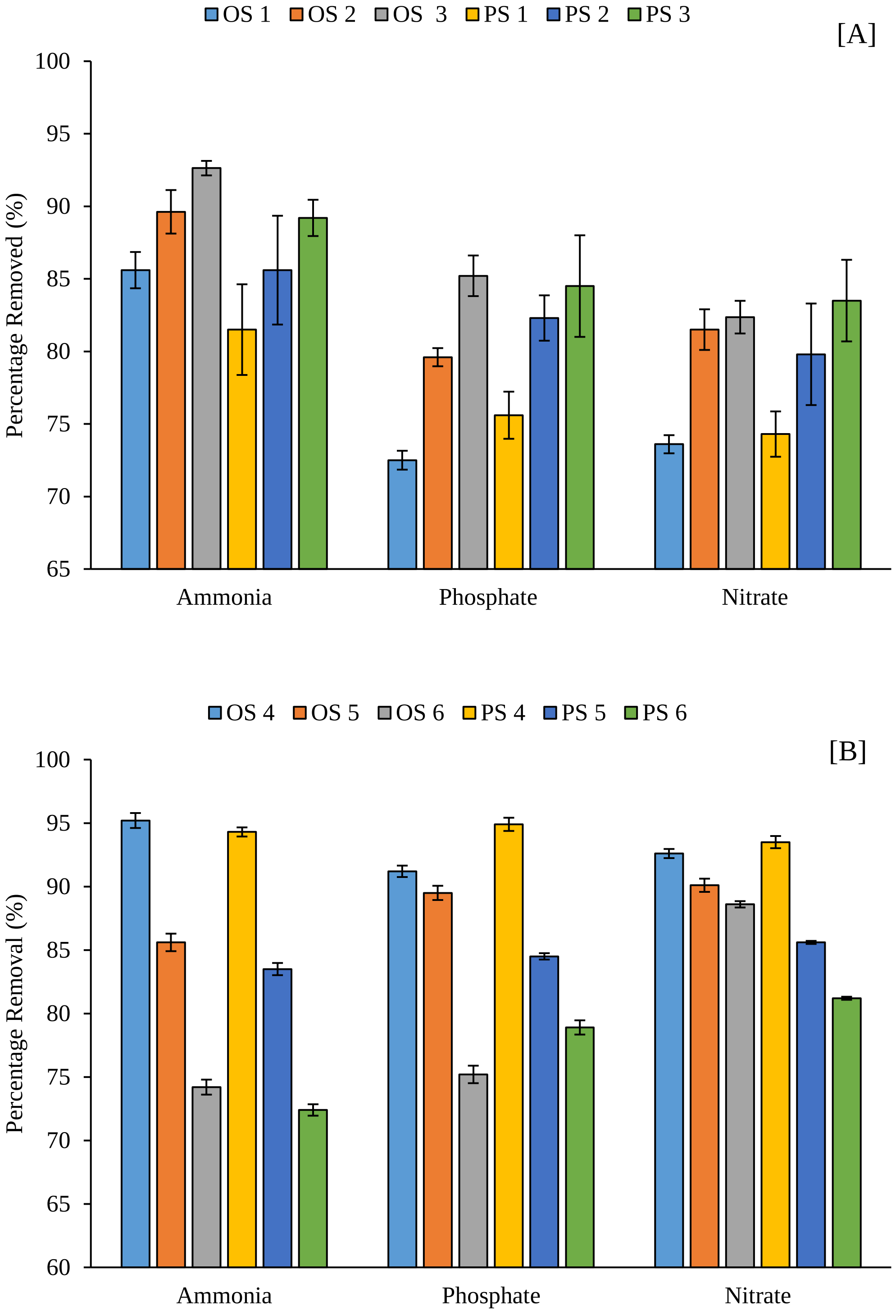

Figure 4.2. Effects of [A] bed height and [B] flowrate on ammonia, phosphate and nitrate removal within column studies. Where, the effects of bed height was ran at a flowrate of 10 ml.min^{-1}, with the following bed heights: OS/PS – 7cm,

OS/PS2-15cm and OS/PS3- 20cm. Where, effects of flow rate had a bed heaight of 20 cm, with flowrates as follows:OS/PS4- 5 ml.min^{-1}, OS/PS5- 10 ml.min^{-1} and OS/PS6 – 20 ml.min^{-1}.

Similar results were observed by other researchers, for example, Jahangiri *et al* ., (2014) found nitrate removal from an aqueous solution by adsorption was greater at a bed height of 15 cm compared to that of 5 cm and attributed it to the availability of more adsorption sites within the columns. Likewise, Nguyen *et al* ., (2017) observed ammonia removal from an aqueous solution, where bed heights of 24 cm achieved greater removal efficiencies and had a higher exhaustion point than bed heights of 8 cm.

The effects of increasing flow rate were also examined using 20 cm bed height columns containing PS and OS. In contrast to changes in bed height, increases to the applied flow rate affect a reduction in RT as greater pressure was applied to the liquor to exit the column. This reduced RT resulted in a decrease in the observed percentage removal efficiency for all compounds tested (Figure 4.2). As such, high percentage removal efficiencies of >94% for both PS4 and OS4, were achieved for the lowest flow rate, 5 ml.min^{-1}(Figure 4.2). Similar results were observed for phosphate and nitrates where a 10-20% increase in removal efficiency was observed when the applied flow rate was reduced from 15 to 5ml.min^{-1} (Figure 4.2).

Adsorption isotherms also indicated that the lower the flow rate the higher the adsorption capacity and the exhaustion points. In particular, the Thomas model indicated that as the flow rate decrease the adsorption capacity increased within each column resulting in a 30% increase in this capacity for both adsorbents between the 15 to 5ml.min^{-1} flow rates. Furthermore, the Adam-Bohart model showed the lower the flow rate the greater the exhaustion point, indicating that by applying a 15 ml.min^{-1} flow rate the adsorbent will become saturated at a quicker rate. This was observed in all columns tested as a 10-20% increase in the exhaustion points at the lower flow rate of 5ml.min^{-1} (Appendix 2).

Furthermore, several studies have described the effect lower flow rates has on increasing residence/contact time, therefore, allowing the compounds to diffuse into the pores of the adsorbent material. One such study showed that the time to reach the exhaustion point decrease from 9 hrs to 2 hrs when the flow rate of 2ml.min^{-1} was increased to 7 ml.min^{-1} (Jahangiri-Rad *et al.*, 2014). While Nguyen *et al.*, (2017) describes a 70% increase in exhaustion point when the flow rate of 9 ml.min^{-1} was decreased to 1 ml.min^{-1} while treating an aqueous solution of ammonia.

4.6.3 Leachate treatment

In general, the ammonia concentration of LFL ranges between 500 to 2000 mg.L^{-1} (Kjeldsen *et al.,* 2002). This is of particular concern for its subsequent treatment in WWTPs as ammonia concentrations > 600 mg.L^{-1} can negatively affect microbial growth within these mainly aerobic systems thus reducing treatment efficiencies (Li *et al.,* 1999). Consequently, refusal to accept this waste stream at the point of entry into WWTPs is not uncommon, resulting in increased cost to the landfill operator (Renou *et al.,* 2008; Brennan *et al.,* 2016; Brennan *et al.,* 2017a). As such, the reduction of ammonia concentrations from ≥ 750 mg.L^{-1} to below the national discharge limits of ≤ 4 mg.L^{-1} (EPA, 2001) for both adsorbents over the trial period represents a significant removal efficiency (>98%; Figure 4.4). In terms of adsorption values, the q_{eq} for ammonia adsorption from LFL was 1.92 and 1.94 mg.g^{-1} for PS and OS, respectively (Table 4.4). These q_{eq} values are well below the exhaustion points (N_o) obtained from the Adam-Bohart model for this data set, of 3.61 and 2.85 mg.g^{-1} for PS and OS, respectively (Table 4.4) indicating that neither of the adsorption columns had reached saturation and could continue to meet the ammonia discharge limit. Further to this, from the equation of the line of each plot [PS;$f(x); 0.0054x - 0.3364$ and OS; $f(x); 0.0056x - 0.116$], a prediction of this longevity was determined as 13.5 and 12.5 hrs, for PS and OS respectively (Table 4.4; Figure 4.3). In contrast, lower q_{eq} values for ammonia adsorption from LFL of 1.74, 0.75 and 1.04 mg.g^{-1} has previously been recorded for clinoptilolite (Zeolite), bentonite-chitin and natural bentonite, respectively (Wang *et al.,* 2006b; Gaouar Yadi *et al.,* 2016). As such, ammonia reduction from LFL has been proven effective for both adsorbents, further research is now required to determine the ability of these adsorbent to treat LFLs containing higher concentrations of ammonia.

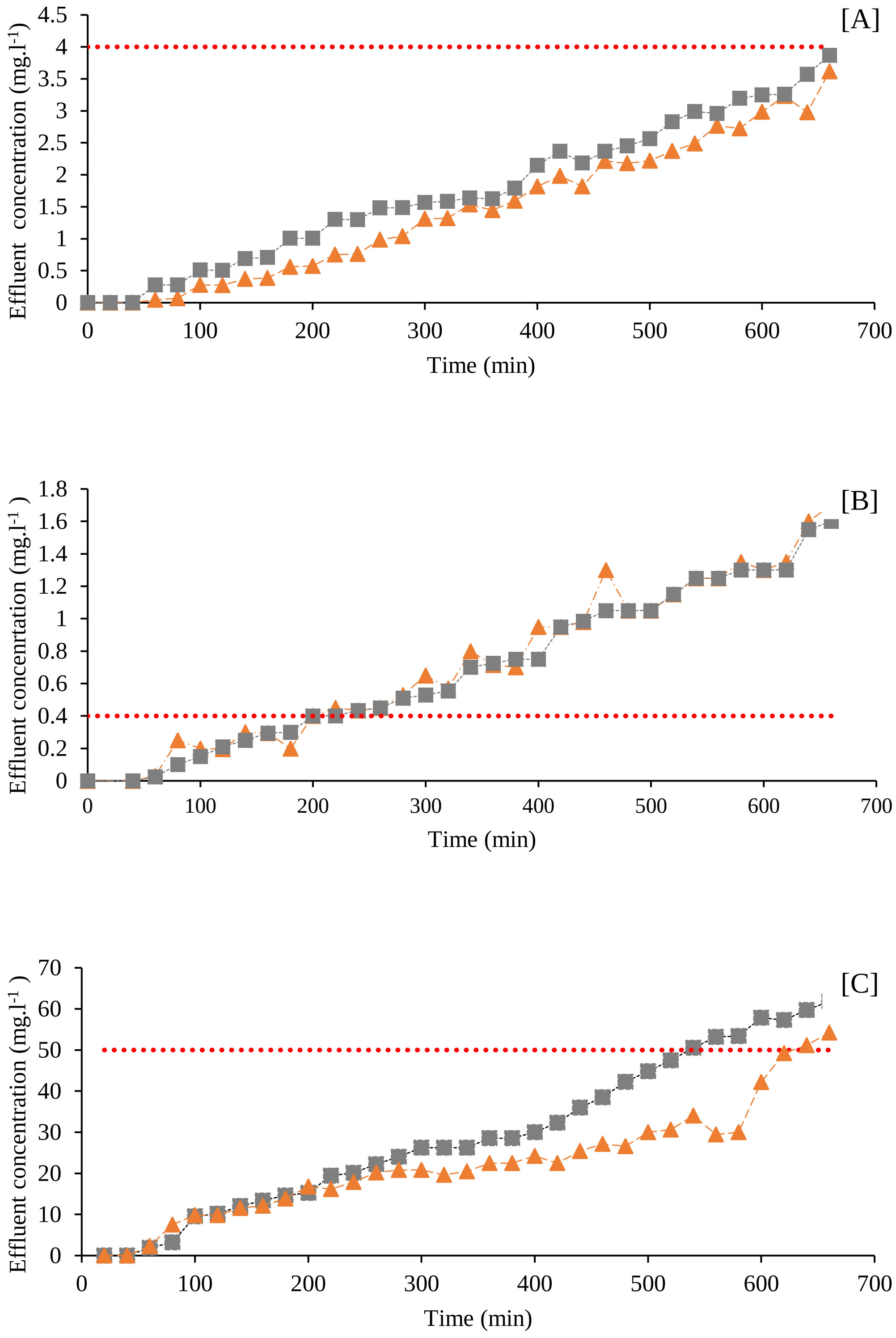

Figure 4.3. Final effluent concentration of [A] ammonia, [B] phosphate and [C] nitrate after adsorption onto oyster shells (▲), Pumice (■) and their respected limits EPA Discharge limit (EPA 2001) (- -)

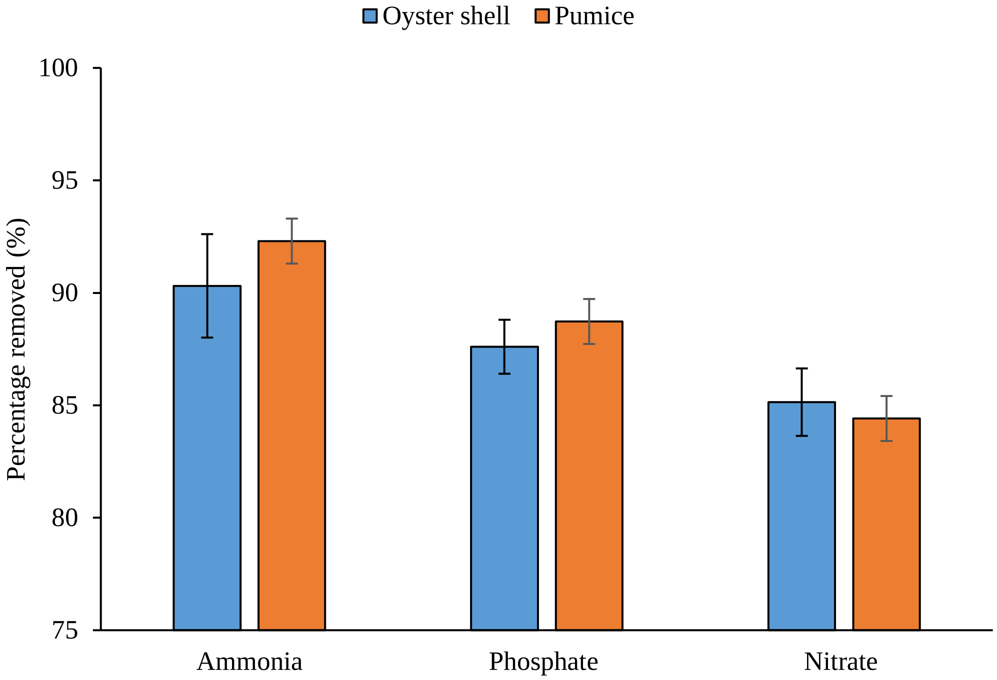

Figure 4.4. Percentage removal of ammonia, phosphate and nitrate from LFL.

Table 4.4. Column adsorption isotherm for LFL treatment

		Thomas Model				**Adam-Bohart Model**		
	Adsorbent	q_{eq} (mg.g^{-1})	q_o (mg.g^{-1})	K_{TH} (d m^{3}g^{-1})	R^{2d}	N_O (mg.g^{-1})	K_{AB} (d m^{3}g^{-1})	R^2
Ammonia	OS	1.94	2.34	0.03	0.94	2.85	0.05	0.99
	PS	1.92	2.98	0.02	0.95	3.61	0.02	0.98
Phosphate	OS	2.64	2.78	0.04	0.96	2.98	0.06	0.97
	PS	2.54	2.74	0.05	0.98	2.86	0.05	0.98
Nitrate	OS	2.36	2.78	0.02	0.95	2.98	0.05	0.95
	PS	2.21	2.76	0.03	0.93	2.82	0.02	0.94

Where;

[a] Theoretical adsorption capacity mg.g^{-1}

[b] Experimental adsorption capacity mg.ml^{-1}

[c] Thomas adsorption constant (mg.g^{-1})

[d] Correlation Coefficient

[e] Exhaustion point (mg.g^{-1})

[f] Adam-Bohart constant

Environmental nitrate pollution is of particular concern for two reasons. Firstly, high nitrate coupled with high phosphate levels are known to accelerate eutrophication causing damage to aquatic ecosystems (Xu *et al.,* 2010; European Environment Agency, 2015). Secondly, because of their high solubility in water nitrates can move easily through soil into drinking water supplies where they represent a public health risk (World Health Organization, 2011; European Environment Agency, 2015). Similar to ammonia, nitrates are often found in high concentrations in LFL (≥ 100 mg.L^{-1}) specifically from MSW landfills and are greatly affected by the presence of oxidising conditions, which can result in volatilisation and subsequent nitrification reactions. Volatilisation within these systems results in the production of free ammonia which is then converted to nitrate via nitrification (Hassan and Ramadan, 2005). However, when reducing (anaerobic) conditions persist the reduction of nitrate to ammonia or N_2 via dissimilatory nitrate reduction may result, thus decreasing nitrate concentrations and increasing ammonia concentrations (Hassan and Ramadan, 2005). The analysis of the influent LFL used in this study revealed higher concentrations of ammonia than nitrates. As such, it is possible that this process of ammonia reduction is occurring within the landfill.

Interestingly, nitrate levels are not as damaging to WWTPs as high concentrations of ammonia. The discharge of WW containing nitrate has been considered acceptable, however, in sensitive areas where high nitrate concentrations already exist in receiving bodies this may be restricted (Fanning *et al.,*2017). Discharge limits for nitrate are <50 mg.L^{-1}, with most WWTPs achieving this (EPA, 1997, 2012, 2015b). Both columns were effective for the reduction of nitrate to below the national discharge limit of ≤50 mg.L^{-1} (EPA 2001), with overall percentage removal rates of 85% and 84% achieved for both OS and PS, respectively (Figure 4.4). In addition, the reduction below the discharge limit was sustained for >550 min for both adsorbents tested with OS maintaining removal ≤50 mg.L^{-1} for a further 100 min (Figure 4.3). Furthermore, both adsorbents recorded q_{eq} values (Table 4.4) that were lower than their N_o values indicating the columns could continue to treat LFL. Overall nitrate removal from LFL is positive with both material showing great potential, however further work is needed in order to increase the length of time the discharge limit is achieved.

As phosphate is one of the main contributing factors causing the eutrophication of receiving bodies, great emphasis is placed on its reduction in WWTPs (Tchobanoglous *et al.,* 2003; Gray, 2005). The discharge limit for effluent phosphate is <0.4 mg.L^{-1} and receiving bodies are classified under the headings good (≤ 0.035 mg.L^{-1} P) and high (0.025 mg.L^{-1} P) quality (EPA, 2001; Caroline, 2013; Fanning *et al.,* 2017). Phosphate levels within LFL can vary greatly from 0.1 to 30 mg.L^{-1} and are strongly influenced by the stage of waste degradation within the landfill, the type of waste present and seasonal variation (Christensen *et al.,* 2001; Kjeldsen *et al.,* 2002; Christensen, 2011; Lee and Nikraz, 2014; Paskuliakova *et al.,* 2016). A rapid and successful removal of phosphate was achieved for both columns tested (>80%, Figure 4.4) in the first 100 min of operation. However, despite the high q_{eq} values recorded for PS and OS, of 2.54 and 2.64 mg. l^{-1}, respectively this removal rate was not maintained and the EPA discharge limit of ≤0.4 mg. l^{-1} phosphate was breached by both systems after 200 min (Table 4.4, Figure 4.3). Moreover, the N_o values determined by the Adam-

Bohart model for both adsorbents of c. 2.86 -2.98 mg.L^{-1} were close to the q_{eq} values recorded for these adsorbents, indicating the columns were reaching exhaustion point, thus become less effective at phosphate removal (Table 4.4).

Consequently, the results of the trial indicate reduced phosphate adsorption in comparison to the other compounds tested (Figure 4.4). One reason for this reduction may be the fact that phosphate adsorption occurs more effectively in acidic condition (pH 4-5; Shang *et al.,* 1992) and at temperatures >20°C (Yang *et al.,* 2014; Luo *et al.,* 2016). The leachate used within this study had a pH of 7.6 and the trial was conducted at c.17-18 °C. As such in an effort to increase phosphate removal it may be necessary to reduce the pH of the influent LFL, however, this would have to be investigated carefully as an overly acidic influent may not be compatible with biological treatments processes downstream.

Furthermore, it is important to note that effective adsorption is also dependent on numerous factors including the molecular weight of a compound, adsorption affinities and the concentration of the compound found in solution (Tchobanoglous *et al.,* 2003). As such, it is probable that inefficient and unstable phosphate adsorption may be a direct result of the considerably higher ammonia and nitrate concentrations of the LFL (Table 4.4). In batch studies it was noted that phosphate adsorption, occurred at a rate of 1.49 and 1.38 mg.g^{-1} for both adsorbents (Table 4.3) while in columns studies the maximum capacity achieved was between 2.54 and 2.64 mg.g^{-1} (Table 4.4), thus further indicating phosphate is failing to adsorb when present at a lower concentration in LFL.

Finally, although adsorption was shown to be effective for the treatment of LFL over the 10 hrs trial period research is now required to scale-up this process, with the aim of treating LFL to discharge limits over a longer timescale. In particular, as the LFL in this study was applied directly onto the columns, further research is also required to determine the potential of suspended solids and other particles present in LFL clogging the adsorption materials, as well as the potential for microbial biofilms forming. However, this may not be an issue if a biological treatment is employed prior to adsorption. In addition, as all adsorbents utilised regardless of their efficiency will reach exhaustion point at some point in the process, research needs to be conducted into the most suitable method for adsorbent regeneration to ensure the cost-effectiveness of the process.

4.7 Conclusion

Adsorption of LFL using low-cost and sustainable materials is an effective treatment option for this waste stream. The main findings of this study are;

1. All adsorbents tested, PS, OS, zeolite and sand, followed the Langmuir model and were effective for the removal of ammonia, phosphate and nitrate, displaying high q_e.th and q_e.exp values.
2. The data obtained from this study does not fit Freundlich isotherm, due to poor R^2, HYBRID error, high APE and χ^2 values.
3. PS and OS proved the most effective adsorbents for the treatment of LFL based on the data obtained from batch studies.
4. The most effective bed height for the removal of ammonia in the column system was 20 cm, with an applied flow rate of 5 ml.min^{-1}.

5. Significant ammonia removal efficiencies (98%) were observed for both OS and PS, reducing the LFL influent ammonia concentration from 750 ml.l^{-1} to below the national discharge limit of ≤4 mg.L^{-1}.
6. Nitrate was reduced to below its national discharge limit of ≤50 mg.L^{-1}, with percentage removal rates of 85% and 84% achieved for both OS and PS, respectively.
7. A reduced phosphate adsorption in comparison to the other compounds tested was observed during the trial.

In conclusion, adsorption is an effective, low-cost physiochemical treatment option for LFL, that has the potential to be utilised as an alternative to some of the current treatment options for this waste stream, mainly WWTPs. Further research is now required to optimise the fixed bed columns to achieve discharge limits for all compounds tested over the larger time frame, and evaluate their performance under different operational conditions such as pH and temperature while treating different strength LFL.

4.8 Summary

This chapter indicated that both oyster shells and pumice stone have the potential to treat LFL. However , it indicated that further treatment may be required in order to achieve national discharge standards. Therefore next chapter (5) will look at combining both chapter 3 and 4 into a single treatment option to access their ability as a combine treatment.

Chapter 5

Leachate treatment using a novel sustainable fixed bed-based method

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Leachate treatment using a novel sustainable fixed bed based method

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***Authors Contributions**

Sinead Morris conceived and designed the experiments. Sinead Morris acquired the samples, performed the experiments and analysed data. Sinead Morris, Guiomar Garcia-Cabellos, David Ryan, Deirdre Enright and Anne-Marie Enright interpreted the data. Guiomar Garcia-Cabellos and Anne-Marie Enright contributed reagents/materials/analysis tools to the work. Sinead Morris wrote the paper which was revised critically by Anne-Marie Enright.

5.1 Overview

The results of chapter 3 and 4 have revealed some important insights into the bioremediation and adsorption-based treatment of LFL. The main aim of this chapter is to develop a commercial unit that combined both the bioremediation and physicochemical treatments elements into a single treatment option. The overall aim of this treatment unit should be to deliver an efficient and cost effective method treating LFL to the discharge limits set by the EPA. In addition, ideally this unit should be amenable to scale up for use onsite in landfills. The objectives of the study were twofold:

(1) to evaluate the effectiveness of the fixed bed system for the removal of ammonia, phosphate, nitrates, biological oxygen demand (BOD) and chemical oxygen demand (COD),

(2) to treat LFL to discharge limits set by the Environmental Protection Agency (EPA), Ireland for the discharge of WW to receiving bodies

5.2 Abstract

Toxic and environmentally damaging leachate is the product of municipal solid waste (MSW) disposal in landfill systems. Currently, 51% of landfill leachate produced in Irish landfill sites is discharged directly into sewer mains with 48% being treated in increasingly overloaded regional wastewater treatment plants (WWTPs). These discharge and treatment options are inadequate, costly and pose risks for both public and environmental health. Unlike other European Union (EU) countries onsite treatment of leachate in Ireland is uncommon (<1%), but could represent a viable and sustainable alternative to current practices.

This study utilises a fixed bed column system, combining both bioremediation and adsorption, to treat landfill leachate (LFL). This research has shown that low-cost adsorption material, such as oyster shells (OS) and pumice (PS), are capable of reducing the concentration of ammonia, phosphate and nitrate from leachates. In addition, microbial isolates from leachate have demonstrated the ability to reduce compounds, such as biological oxygen demand (BOD) and chemical oxygen demand (COD). This treatment has the ability to reduce LFL to below the acceptable limits set by the EPA (Ireland) for the discharge of leachate into receiving bodies. High percentage removal rates of >90% were achieved by the column system for ammonia, phosphate and nitrate, reducing each to below their respective national discharge limit. However, despite high percentage removal rates of 91% and 96% for BOD and COD, respectively neither reached their national discharge limit. These results demonstrate that leachate can be treated effectively by bioremediation and adsorption in a combined column system, which has the potential to be implemented as a novel cost-effective onsite treatment method for LFL in Irish landfill. Further research is now required to test this system with larger volumes of LFL which vary in composition and concentration.

5.3 Introduction

Landfill leachate (LFL) production and management are one of the greatest problems associated with Municipal Solid Waste (MSW) landfills. The generation of MSW waste continues to grow due to population growth, industrial activities and lifestyle changes (Ahmed and Lan, 2012; Torres-Socías *et al.,* 2014). While there has been a decline in the number of landfills in recent years, the generation of leachate is a legacy problem and its treatment is a major management issue for landfills operators within the European Union (EU) (Zhang *et al.,* 2009; Brennan *et al.,* 2016; Brennan *et al.,* 2017a) . LFL is defined by McCarthy *et al* ., (2010) as 'liquid, which has percolated through the waste, picking up suspended and soluble materials that originate from or are products of the degradation of the waste'. As liquid penetrates through the solid matrix it assists with biochemical, chemical and physical reactions, directly influencing the quality and quantity of the leachate produced (Kamaruddin, 2015). Leachate, a chemical cocktail, is a major drawback of MSW landfills, as they continue to produce leachate for hundreds of years after the landfill is decommissioned (Crowley *et al.,* 2003; Torres-Socías *et al.,* 2014; Brennan *et al.,* 2017a). The correct treatment of LFL is essential for the protection of the surrounding environments, as uncontrolled discharge has the potential to impact negatively on ground and surface water resources.

Many methods are used to treat LFL, however, most are adapted from wastewater (WW) treatment methods. Usually, a combination of both biological and physiochemical methods are effective as it can be difficult obtain satisfactory results with just one method due to the diverse quality of LFL (Kargi and Pamukoglu, 2004; Raghab *et al.,* 2013). For example, LFL with high organic content is best treated using biological methods, whereas LFL with a low organic content is best treated by physicochemical methods. (Kheradmand *et al.*, 2010). A number of treatment options have been successfully employed to treat LFL. A study carried out by Paskuliakova *et al* ., (2016) applied chlorophytes to reduce the total ammonia nitrogen and organic nitrogen of LFL. Zayen *et al.*, (2016) combined processes of anaerobic digestion, lime precipitation microfiltration and reverse osmosis to treat LFL, while Kaur *et al* ., (2016) used cow-dung ash as an adsorbent material to assess for the removal of organic material. Despite the success of these treatments, it is important to investigate other options, especially those that are low cost and can be implemented onsite in Irish landfills.

Currently, in Ireland, over fifty urban wastewater treatment plants (WWTPs) receive and treat MSW LFL, requiring transport and costly aerobic biological treatment. Volumes and composition of LFL collected at these sites varies greatly depending on the content, size, and age of the specific landfill (McCoole *et al.*, 2010). In 2013, there were approximately 1.1 million m^3 of LFL collected in Ireland, which was discharged directly either to sewers (51%) or transported to WWTPs (48%) for final treatment, with only 1% receiving any onsite treatment (EPA, 2015). Out of the six current onsite treatments in Ireland, only three are directly discharging to receiving bodies. Treatment of leachate in WWTPs is not effective, as the systems employed in these treatment centres are often inadequate and do not effectively treat leachate to the discharge limits. Another drawback for WWTPs is the stringent emission limits. Non-compliance with ammonia and total nitrogen emission values in WWTPs has been attributed to leachate loading at these plants, resulting in the discontinuation of leachate acceptance by these facilities. This has resulted in a 30% decrease in the number of WWTPs treating leachate from 2010 to 2015 (McCarthy *et al.*, 2010; EPA, 2015; Brennan *et al.*, 2016). As such, it is of economic and environmental importance to investigate the best way to treat LFL, in order to develop a cost-effective, suitable treatment, that will ultimately reduce LFL constituents to required discharged limits.

The main purpose of this study is to combine both biological, in terms of bioremediation, and the physicochemical treatment by adsorption, into a novel cost-effective system to treat LFL. This study utilised low-cost adsorption material, and microorganisms isolated from leachate to treat LFL from an Irish landfill. Both treatment processes were combined into a continuous fixed bed system The objectives of the study were twofold: (1) to evaluate the effectiveness of the fixed bed system for the removal of ammonia, phosphate, nitrates, biological oxygen demand (BOD) and chemical oxygen demand (COD), and (2) to treat LFL to discharge limits set by the Environmental Protection Agency (EPA), Ireland for the discharge of WW to receiving bodies.

5.4 Materials and methods

5.4.1 Site description and sampling

LFL used in this study was sourced from Powerstown Landfill, Co. Carlow, Ireland (52°45’58.46’’ N, 6°57’20.13’’ W). The landfill is located 8 km south-east of Carlow town in a rural setting and has been operational since 1977. The site consists of three different phases; Phase 1 which operated from 1975-1990, Phase 2 which operated from 1991-2006, and Phase 3 opened in 2006 and is due to close in late 2018. Phase 3 consists of four lined cells, surface water settlement pond, leachate tank and green waste composting area. Leachate collection systems are in operation in both Phase 2 and Phase 3. It was decided to use LFL generated in Phase 3 as it is currently in operation and generates a higher concentrated leachate than the other phases. LFL samples were collected in January and February 2017 from the leachate tank and stored at 4°C until use within 48 hrs.

5.4.2 Experimental setup

5.4.2.1 Continuous fixed bed system set up

Three sequential Polyvinyl chloride (PVC) columns (11 cm internal diameter, 30 cm height, and IC 2850 cm^3) were utilised in this study (Figure 5.1). The first column (C1) was packed with c. 1 kg of soil (Westland Topsoil) to a height of 20 cm. This soil was inoculated with a microbial mastermix (OD600 = 0.8) containing 15 previously isolated leachate degrading microorganisms (GenBank accession numbers: MG880063– MG880077) in nutrient broth (Lab M, United Kingdom). These organisms belong to the Firmicutes, Actinobacteria and Proteobacteria phylums, which were previously isolated from LFL. The soil/microbial isolate mixture was left to incubate for 48 hrs at room temperature (20°C) after which the excess liquid was allowed to drain off. The second column (C2) was packed with c. 1.3 kg of crushed oyster shells (OS; particle size 5 – 10 mm) (Harty Oyster Farm, Dungarvan Waterford, Ireland) to a height of 20 cm. The final column (C3) was packed with 0.65 kg of pumice stone (PS; particle size 2- 5 mm) (Lennox, Ireland) to a height of 20 cm. Both adsorption materials were prepared by triple washing with deionized water and drying at 100°C for 24 hrs (Namasivayam *et al.*, 2005; Melegari and Matias, 2012). Before commencement of the experiment deionized water was washed through the column in a downflow direction to withdraw trapped air between the materials. Prior to this trial, batch studies were carried out using adsorbent materials, over a range of concentrations, to determine whether both materials were suitable for adsorption (Chapter 3).

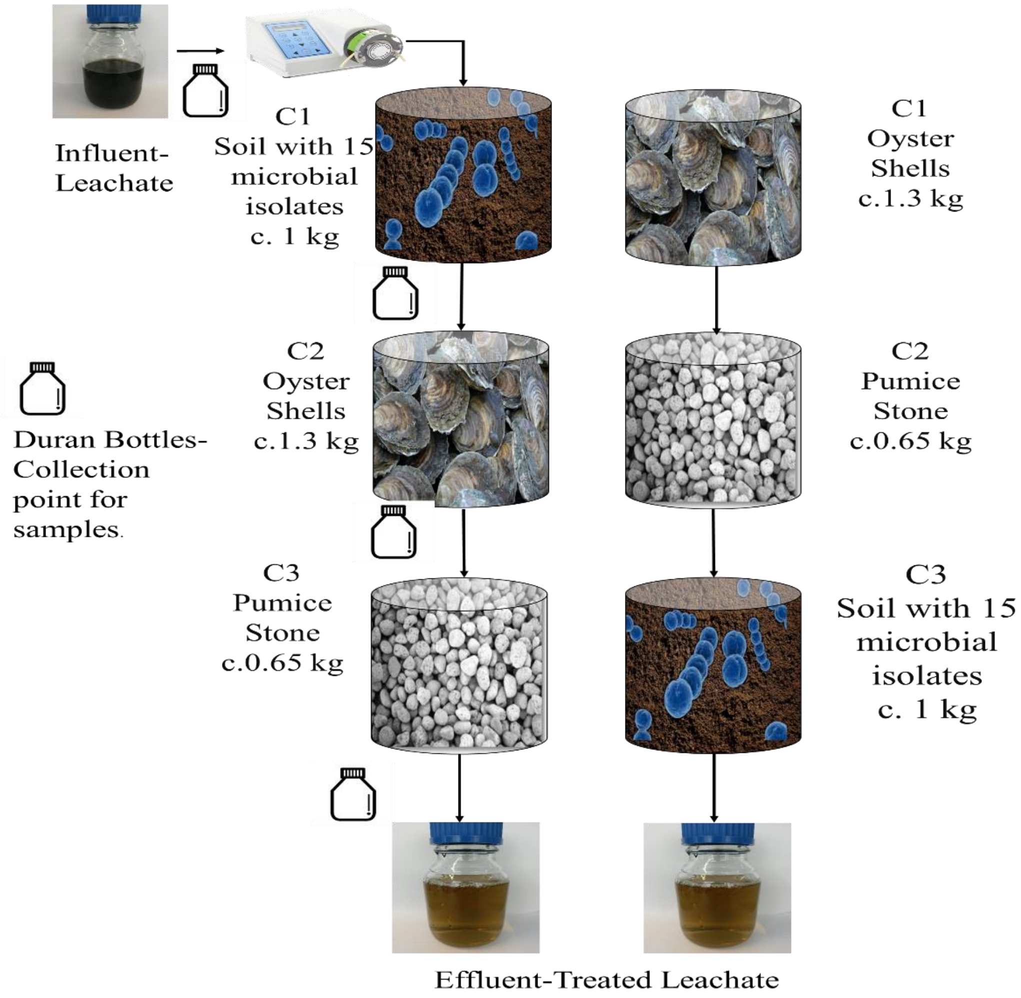

Figure 5.1. Fixed bed system configuration

5.4.3 Mathematical description of a continuous fixed bed system

The performance of a packed bed is often described using the concept of a breakthrough curve (BTC). The time until the sorbed molecule is detected in the effluent, and the shape of the BTC curve are very important characteristics for operation and process design of a biosorption column (Aksu *et al.,* 2007; Agrawal and Bajpai, 2011; Taylor *et al.,* 2014).

Experimental determination of these parameters is very dependent on column operating conditions such as influent concentration and flow rate. A BTC is expressed as the influent/effluent (C/C_o against time (T)). The area (A) under the BTC can be obtained from this plot using trapezoidal rule. From this we can then calculate the overall percentage removal of ammonia, phosphate, nitrates, as well as BOD and COD using the following equations (Aksu *et al.,* 2002; Lim and Aris, 2014):

The q_{total} demines the total amount of pollutant adsorbed by the column:

$$q_{\text{total}}\ (\text{mg}) = \frac{Q.A}{100} = \frac{Q}{1000}\int_{t=0}^{t=t_{\text{total}}} C_{\text{ad}}\,dt \qquad \textbf{Equation 5.1}$$

The total amount of ions delivered to the system (M_{total}) is determined by the following equation:

$$m_{\text{total}}\ (\text{mg}) = \frac{C_o.Q.T_{\text{total}}}{1000} \qquad \textbf{Equation 5.2}$$

In this equation, the Q and the T_{total} represent the flow rate (ml.min^{-1}) and the total flow time (min), while c_o is the effluent concentration. Both equations are required to evaluate the removal efficiency of the column. Total percentage removal was calculated as follows;

$$\text{Total removal (\%)} = \frac{q_{\text{total}}}{m_{\text{total}}} \text{x } 100 \qquad \textbf{Equation 5.3}$$

5.4.4 Influent and effluent analysis

Influent (LFL) and effluent samples were analysed before and after each sampling point as indicated in Figure 5.1. All reagent used were of analytical grade and supplied by Sigma Aldrich, Ireland unless otherwise stated. Ammonia (NH_3-N) was analysed using the phenate method (Rice *et al.,* 2017) and analyzed on

Shimadzu UV1800 Spectrophotometer. BOD was tested over 5 d (BOD_5) using a Hanna Dissolved oxygen meter (Rice *et al.*, 2017). COD was analysed using HACH Lange COD vials. Phosphate (PO_3) was analysed using molybdovanadate reagent (HACH Lange Ireland) and nitrate (NO_3-) analysis was carried out using NitraVer® 5 reagent power pillows (HACH Lange Ireland). All HACH products were used according to manufacture's instruction and measured on HACH DR 6000 UV- Spectrophotometer.

5.4.5 Optimisation and operation of the column system

Optimisation of the fixed bed system was carried out by determining the best flow rate and order in which the columns should be placed. Two flow rates, 10 $ml.min^{-1}$ and 5 $ml.min^{-1}$, were initially utilised in batch experiments to determine the optimum conditions for pollutant removal. The surface loading rate where 7.3 $L.Day^{-1}$ for 10 $ml.min^{-1}$ and 2.65 $L.Day^{-1}$ for 5 $ml.min^{-1}$. The column was optimised further by changing the order in which the columns were placed. Option 1 (OP 1)- consisted of C1-soil, followed by C2- OS and a final C3- PS column (Figure 5.1). Option 2 (OP 2)- consisted of C1- OS, followed by C2- PS and a final C3 soil column (Figure 5.1). Both trials were carried out over a 3 hrs period. For the purpose of optimising the fixed bed system and determining the effectiveness of each option, ammonia, phosphate and nitrate concentrations of effluent samples were analysed. Once the system was optimised, 5 L of LFL was actively pumped into the column at the determined optimal flow rate and allowed to filter via gravity into C1, C2, and C3 sequentially over a 16 hrs period. All trials were carried out at room temp (20°C ± 2).

5.5 Results and discussion

5.5.1 Leachate composition and characterisation

The chemical composition of leachate used in this study was analysed and compared to known readings from Powerstown Landfill, supplied by Carlow Co. Council, Ireland during the period 2009-2016 (Table 5.1). There are various factors that affect the parameters of leachate including; age, precipitation, seasonal weather variation, waste type and composition (Renou *et al.,* 2008; Abbas *et al.,* 2009; Xu *et al.,* 2010c; Kamaruddin, 2015). LFL was analysed before treatment to accesses the stage of waste degradation that had occurred in the landfill in order to determine the best treatment option. It is known that a landfill has different phases; hydrolysis, acidogensis, acetogenesis and methanogenesis, and leachate composition differs at each phase (Butkovskyi 2009; Schiopu and Gavrilescu 2010; EPA 2011). During each phase, the characteristic of the leachate changes significantly; leachate in the hydrolysis-acetogenesis phase (Landfill < 10years old) is known for having pH values of ≤7.5 and having high concentrations of BOD and COD, as well as, a BOD_5/COD ratio of < 0.7. While older landfill (10 + years) in the methanogenic phase are known for having COD values <4000 mg.L^{-1}, high level of ammonia- nitrogen >400 mg.L^{-1} and a low BOD_5/COD ratio of <0.01 (Christensen *et al.,* 2001; Abbas *et al.,* 2009; Kamaruddin, 2015; Torretta *et al.,* 2017). BOD_5 from Powerstown varies from 46-180 mg.L^{-1}, the current EPA limit for BOD_5 is set at 5 mg.L^{-1}. COD ranges from 450-650 mg.L^{-1}, with EPA limits set at 40 mg.L^{-1}. According to Christensen *et al* (2001) and Jokela *et al* (2002) this leachate would classify the landfill being in the methanogenic phase, which is determined by a COD range of 500-4500 mg.L^{-1}. The BOD_5/COD ratio is good at determining the organic composition of leachate and it is a good representation of waste stabilization and the transition from early acetogenic phase to the mature methanogenic phase. In young landfills, this ratio is high and falls in mature landfills. Ratios between 0.4 and 0.6 are an indicator that the organic matter in the leachate is biodegradable. In mature landfills, this ratio is often in the range of 0.05 to 0.2, reducing as leachate from mature landfills typically contains humic and fulvic acids, as well as recalcitrant organic compounds, which are not biodegradable (Christensen *et al.,* 2001; Halim *et al.,* 2010; Lee and Nikraz, 2014; Contrera *et al.,* 2015). The BOD_5/COD ratio ranges from 0.18- 0.26 indicating that leachate is stable and could be difficult to treat biologically but should respond well to physicochemical treatments.

Leachate is known for having high concentrations of ammonia. This is a critical problem as it promotes algae growth, accelerates eutrophication and decreases the effectiveness of biological treatments (Aziz *et al.,* 2010; Bashir *et al.,* 2010). In addition, ammonia can continue to leach from landfills for up to 50 years after their decommission and can be difficult to treat in WWTPs (Chu *et al.,* 1994; Wang, 2013; Brennan *et al.,* 2017b). The ammonia levels in the Powerstown leachate used in this study ranged from 790- 1010 mg.L^{-1} (Table 5.1). These high levels correspond to the methanogenic phase of waste degradation (Gao *et al.,* 2015; Kamaruddin, 2015; Torretta *et al.,* 2017). High levels of ammonia, are one of the main factors contributing to the 30% decrease in WWTPs accepting leachate during the period 2010-14 within Ireland (Brennan *et al.,* 2017b). It is therefore essential to use a treatment option, be it biological or physicochemical, that can reduce ammonia levels to discharge limits.

Table 5.1. Composition of leachate from Powerstown Landfill from 2009-2015, leachates used in this study and the discharge limits set by the EPA .

	Powerstown leachate 2009-2016[a]	**Leachate used in this study**	**EPA DL[c]**
Ammonia	360-960	790- 1040	≤4
BOD	46-1322	112-170	≤5
COD	539-3005	450- 650	≤40
Nitrate	NM[b]	89-120	≤50
Phosphate	1.2-7.4	3.6-7.25	≤0.4

Where;
Performance values are the trial period mean and expressed as $mg.L^{-1}$;
[a] Sampling did not occur in 2010 or 2013
[b]Not measured
[c]Discharge Limit in $mg.L^{-1}$

5.5.2 Optimisation of fixed bed system

Prior to the trial, adsorption material was subject to batch studies for adsorption isotherm. Both adsorption materials followed the Langmuir model, giving an adsorption capacity for ammonia of 1.38 $mg.L^{-1}$ for OS and 1.28 $mg.L^{-1}$ for PS (Chapter 3).

5.5.3 Effects of flow rate

The hypothesis is that slower flow rates which effect a higher retention time (RT) could result in a greater percentage removal of each compound analysed. In this study two flow rate were examined, 5 $ml.min^{-1}$ and 10 $ml.min^{-1}$, for which the RT was 150 and 110 min, respectively. For all three compounds examined, there was a higher percentage removal achieved at 5 $ml.min^{-1}$for phosphate and nitrate, while ammonia did not show a large deviation. There was a difference between the two flow rates for ammonia and nitrate of 0.3% and 1.26%, respectively (Figure 5.2). The mean effluent concentration of ammonia at 5 $ml.min^{-1}$ was 4.2 ± 0.5 $mg.L^{-1}$, which is slightly above the EPA limit of 4 $mg.L^{-1}$, while for the 10 $ml.min^{-1}$flow rate the mean effluent value was 7.14 ± 1.48 $mg.L^{-1}$. The mean concentration for nitrate, at both flow rates, was below the EPA limit of 50 $mg.L^{-1}$ (Figure 5.2). Phosphate showed the largest difference in percentage removal of 7.5% between each flow rate. Similar to ammonia, the mean effluent concentration was below the EPA limits for the 5 $ml.min^{–1}$ flow rate at 0.287 $mg.L^{–1}$ P, while for the 10 $ml.min^{–1}$ flow rate the mean effluent concentration remained above the EPA guidelines. Overall, the 5 $ml.min^{–1}$ flow rate showed the greatest removal for the three compounds tested, possibly due to the longer RT, and as such was used in the subsequent LFL trial.

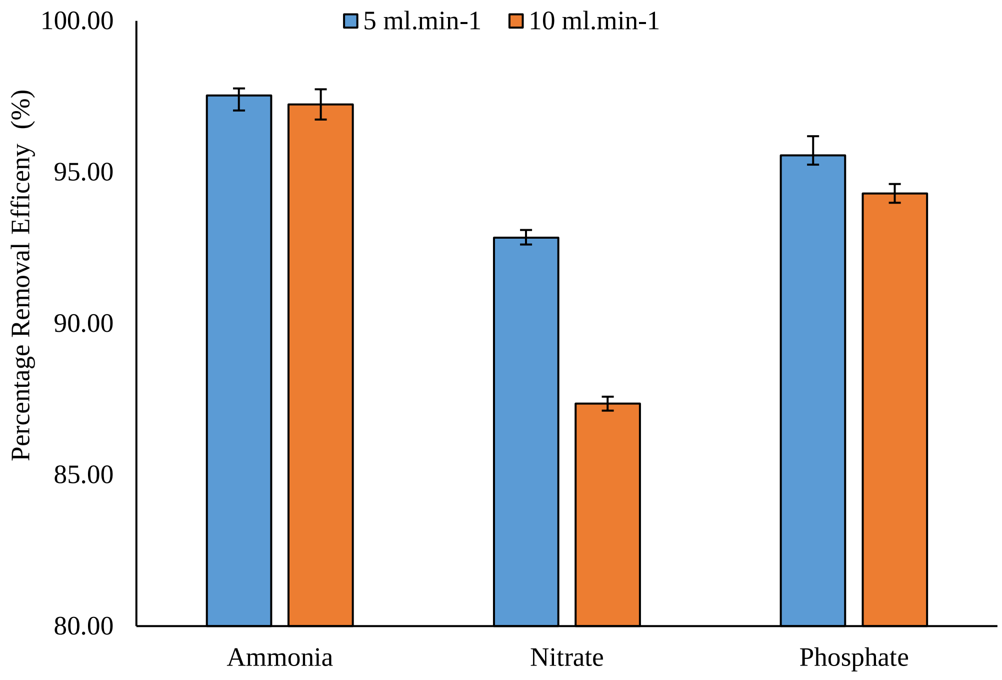

Figure 5.2. Removal Percentage efficiencies of compounds tested at 5 ml.min^{-1} and 10 ml.min^{-1} flow rates.

5.5.4 Effects of column position

In order to optimise the fixed bed system further, it was essential to look at the different order in which treatment could occur. There was a difference of 12%, in both options for phosphate. Both ammonia and nitrate show deviation between the two options of 0.3% and 1.4% (Figure 5.2), respectively. Overall, OP1 showed the greater percentage removal for each compound tested and was used in the optimised column study.

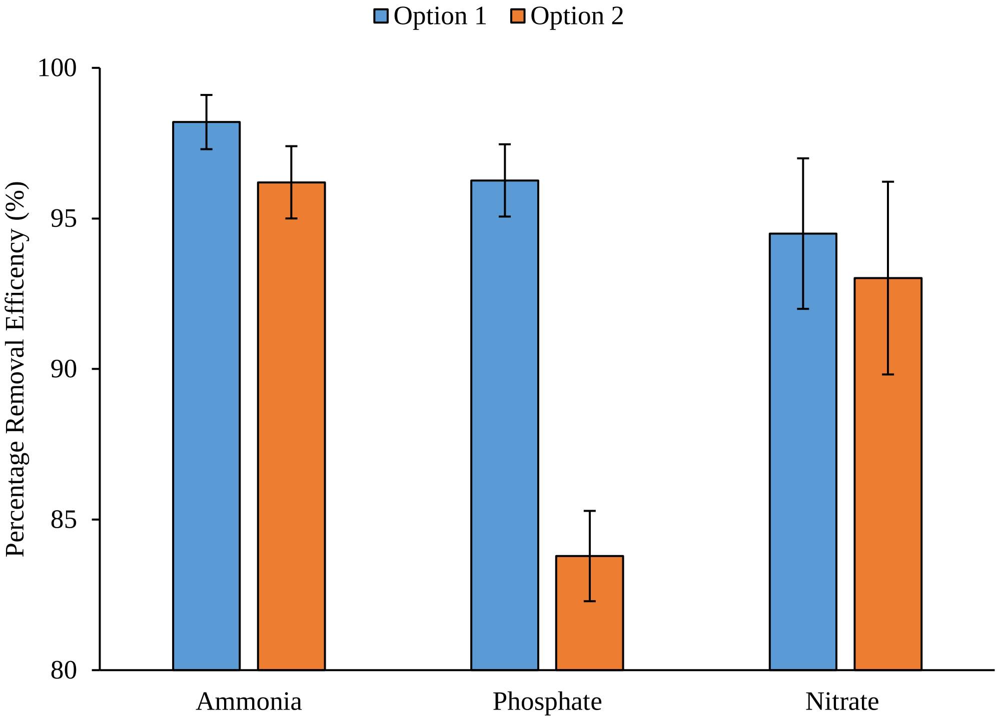

Figure 5.3. Removal Percentage, for the effects of column position for both option 1 (OP1) and option 2 (OP2).

5.5.5 Optimised column study

The system was operated using the OP1 configuration with a 5 mL min^{-1} flow rate. The system's influent and effluent were analysed routinely after each column and analysed as described above to determine the percentage removal rates of COD, BOD, ammonia, nitrate and phosphate.

5.5.5.1 C1 –Bioremediation

The results from C1 indicated that ammonia, phosphate and nitrate percentage removal efficiencies were 74%, 47% and 56%, respectively (Table 5.2). Despite this reduction, specifically for ammonia, none of the parameters measured reached the EPA discharge limits after treatment in C1, with final effluent containing 108 mg.L^{-1} N, 2.75 mg.L^{-1} P and 82 mg.L^{-1} N (Table 5.2). However, it should be noted that the RT of C1 was 90 min and the percentage removal efficiencies may be improved by increasing this to allow more contact time between the microorganisms and the influent. In addition, the application of effluent recycling within C1 could further improve bioremediation of these constituents. Interestingly, both BOD and COD showed reductions >80%. This was unexpected, as previously described Section 4.4.1 leachate from Powerstown is of a mature nature, which should not respond well to biological treatment.

Table 5.2. Influent and effluent concentrations and total percentage removal of compounds tested for column 1 (C1) containing soil and microbial isolates.

	Influent Concentration	**Effluent Concentration**	**% Removed**
Ammonia	428 ± 2.5	108 ± 2.1	74.7
Phosphate	7.2 ± 0.6	2.7 ± 0.6	47.7
Nitrate	187.3 ± 1.2	82 ± 1.6	57.2
BOD	150 ± 1.5	17.2 ± 1.5	89.8
COD	650 ± 2.3	127.3 ± 2.4	80.7

Where;
Performance values are the trial period mean ± standard deviation and are expressed as mg.l^{-1}

5.5.5.2 C2- Adsorption 1- Oyster shells

OS, a readily available waste product of the aqua industry within Ireland, were chosen as the adsorption material of C2. Results for C2 showed it was particularly effective in reducing ammonia with a $\geq$ 77% removal efficiency recorded (Table 5.3) from the previous effluent concentration (C1). Results also indicated C2 was effective in reducing phosphate and nitrates (Table 5.3). However, neither were reduced below discharge limits after this treatment with effluent levels at 1.18 mg.L^{-1} and 52.23 mg.L^{-1} (Table 5.3). Similarly, BOD and COD were reduced by 51% and 24%, respectively (Table 5.3) but did not meet the discharge limits (Table 5.3). Low percentage removal was expected; as physiochemical treatment is not known for reducing the level of organic matter. Overall the results for C2 have indicated that physicochemical treatment is an effective option for the treatment of mature leachate.

Table 5.3. Influent and effluent concentrations and total percentage removal of compounds tested for column 2 containing oyster shells.

	Influent Concentration	**Effluent Concentration**	**% Removed**
Ammonia	108 ± 2.1	24.6 ± 1.4	77.1
Phosphate	2.8 ± 0.6	1.1 ± 0.2	57.0
Nitrate	82 ± 1.6	52.2 ± 1.9	37.3
BOD	17.3 ± 1.5	7.5 ± 1.4	50.1
COD	127.4 ± 2.3	94 ± 1.3	24.8

Where;
Performance values are the trial period mean ± standard deviation and are expressed as mg.L^{-1};

5.5.5.3 C3- Adsorption 2-Pumice stone

Pumice, a volcanic stone, was used as the final low-cost adsorbent material. A study by Cifei *et al* ., (2015) highlights that PS is known for its ability to remove contaminants from WW, such as heavy metals, ammonia and phosphorus. The aim of this column was to act as a fine filter for the removal of the remaining compounds analysed. Results of the effluent analysis of C3 indicate that over a 10 hrs period, ammonia, phosphate and nitrates were treated to discharge limits. Despite this, C3 proved to be ineffective for the removal of COD and BOD with percentage removal rates of 42 and 26%, respectively (Table 5.4).

Table 5.4. Influent and effluent concentrations and total percentage removal of compounds tested for column 3 (C3) containing pumice stone.

	Influent Concentration	Effluent Concentration	% Removed
Ammonia	24.6 ± 1.4	2.3 ± 1.1	90.6
Phosphate	1.1 ± 0.2	0.2 ± 0.1	77.2
Nitrate	52.2 ± 1.9	4.2 ± 1.3	91.9
BOD	7.5 ± 1.4	7.5 ± 0.9	27.2
COD	94 ± 1.3	55 ± 1.8	41.4

Where;
Performance values are the trial period mean ± standard deviation and are expressed as mg.L^{-1}

5.5.5.4 Overall system

In general, results of the combined system were encouraging with the discharge limits set by the EPA for the compounds investigated being reached in the final effluent over a 16 hrs period (Table 5.5).

Overall, 99% of ammonia was removed (Table 5.5) from the system in a relatively short time frame with the final effluent concentration of 2.3 mg.L^{-1} well below the EPA guidelines for discharge to receiving bodies (EPA, 2001). This is a positive result, as currently in Ireland over 30% of WWTPs are refusing to treat leachate due to the high ammonia concentration and being unable to reach this limit on a regular basis. These stringent emission limits represent a significant threat to the sustainability of co-treatment of leachate with municipal wastewater in WWTPs. The variation in ammonia concentration within leachate also poses a risk to the effective treatment of other WW in WWTPs, due to the ammonia levels within leachate not being disclosed upon acceptance at WWTPs. The ammonia level within leachate can cause WWTPs hitting maximum hydraulic loading, causing a backlog and storing of leachate onsite (McCarthy *et al.*, 2010; Brennan *et al.*, 2016; Brennan *et al.*, 2017b). The combined system shows promise for the removal of ammonia and should now be tested to determine if comparable results can be obtained for LFL with varying concentrations of this compound.

Phosphate contamination of water bodies is a major environmental issue as it can result in algae blooms and eutrophication of ground and surface water. Phosphate levels in leachate from Powerstown show little variation ranging from 3.6-7.25 mg.L^{-1} (Table 5.1). The bioremediation of phosphate (C1) showed promising results, with a reduction of 41% observed after this treatment step (Table 3.2). Furthermore, treatment with both adsorption materials brought the removal of phosphate to 0.28 mg.L^{-1} (Table 5.4), within the EPA discharge limit of 0.4 mg.L^{-1}. Overall the combined system affected a 95% removal of phosphate (Table 5.5), with the greatest removal rate occurring in the adsorption columns at a combined 54%.

Similarly, to phosphates, nitrate pollution can cause eutrophication in rivers and lakes (Zhang *et al.*, 2015). Furthermore, if nitrates contaminate potable water supplies they have the potential to cause methemoglobinemia, blue baby syndrome, in infants (Savino *et al.*, 2002). Ammonia is reduced to nitrite and then to nitrate during the nitrification process. This process is generally carried out by *Nitrosomonas* spp. but other groups of heterotrophic bacteria and fungi can also carry out nitrification, although at a slower rate (EPA, 2002). Nitrate levels within leachate used in this study did not vary greatly, 89-120 mg.L^{-1} but were all above the EPA limits (Table 5.1). Results recorded for C1 effluent analysis indicated a low reduction rate of nitrate (57.2 %; Table 5.2), this may be due to bacteria nitrifying ammonia to nitrate. The OS column effected a 37.6% removal of nitrate, but the discharge limit of 50 mg L–1 N was not achieved at this stage (Table 5.3). Finally, in C3, there was a 91% removal of nitrate (Table 5.4) with the effluent reading below discharge limit of 50 mg.L N. Overall a reduction of nitrate to 4.2 mg.L^{-1} was recorded (Table 5.5), which is substantially below the discharge limit of 50 mg.L^{-1}, representing a 97% reduction rate.

Unfortunately, BOD and COD discharge limits were not achieved by this system. COD concentrations in the final effluent were 55 mg.L^{-1} which did not reach the EPA discharge limits of 40 mg.L^{-1} (Table 5.5). Similarly, BOD concentrations in the final effluent were 7.5 mg.L^{-1}, just above the EPA discharge limit of 5 mg.L^{-1}. Nevertheless, is important to note that high percentage removal rates were achieved for both BOD and COD, of 96% and 91%, respectively using this novel treatment system. These results are positive, and it is believed optimisation of the system could further improve these effluent removal rates. For example, increasing the RT in C1 or effluent recycling throughout the column, to give more contact time between the microorganisms and the liquor.

Table 5.6. Initial influent and final effluent concentration, EPA discharge limit to receiving bodies, and overall removal efficiency (%) of the combined system.

	Influent Concentration	**Effluent Concentration**	**EPA DL[b]**	**% Removed**
Ammonia	428 ± 2.5	2.3 ± 1.1	≤ 4	99.4 ± 0.5
Phosphate	7.26 ± 0.6	0.28 ± 0.1	≤ 0.4	94.6 ± 2.3
Nitrate	187.33 ± 1.2	4.2 ± 1.3	≤ 50	97.7 ± 3.2
BOD	150 ± 1.5	7.53 ± 0.9	≤ 5	97.3 ± 2.5
COD	650 ± 2.3	55 ± 1.8	≤ 40	91.5 ± 1.2

Where;
Performance values are the trial period mean ± standard deviation and are expressed as mg.L^{-1};
[b] Discharge limits

5.6 Conclusion

In conclusion, these results demonstrate that leachate can be treated effectively by bioremediation and adsorption in a combined column system. LFL represents a major problem for MSW landfills; however, this treatment system may represent a cost-effective, novel treatment option for this waste stream in Irish landfill sites. The main findings of this study are:

- Bioremediation was successfully used for the treatment of LFL, specifically ammonia which achieved a removal efficiency of ≥99%. It was also the most suitable treatment option for BOD and COD, achieving percentage removals rates of > 80%.
- Adsorption, using low-cost material, such as PS and OS can reduce the ammonia, phosphate and nitrate concentrations of LFL.
- As a whole, the system employed in this study effectively achieved discharge limits for ammonia, phosphate and nitrate.
- BOD and COD discharge limits were not reached by this system but may be achieved through leachate recirculation or by increasing the RT in C1.

Further research is now required to determine the potential of using this system on-site using larger volumes of LFL which vary in composition and concentration. This system has the potential to be a novel cost-effective treatment method for LFL

5.7 Summary

This chapter highlights that both treatment options, bioremediation and adsorption can be combined into a single treatment option for landfill leachate. This system achieved high percentage removal rates for all compounds tested, as well as , achieved national discharge standard for ammonia , phosphate and nitrate. As such, this treatment can be deemed successful in LFL treatment. Further work is now need in order to access the ability of this option onsite within an Irish landfill , taking into account the larger volumes of leachate being produced and the variation with LFL. The next research objective is to assesses this onsite with Powerstown Landfills site.

Chapter 6
The use of fixed bed column systems for on-site treatment of landfill leachate

This chapter is currently under review in the Journal of Environmental Management.

The use of fixed bed column systems for on-site treatment of landfill leachate

Sinead Morris, Guiomar Garcia-Cabellos, David Ryan, Deirdre Enright, and Anne-Marie Enright.

***Authors Contributions**

Sinead Morris conceived and designed the experiments. Sinead Morris acquired the samples, performed the experiments and analysed data. Sinead Morris, Guiomar Garcia-Cabellos, David Ryan, Deirdre Enright and Anne-Marie Enright interpreted the data. Guiomar Garcia-Cabellos and Anne-Marie Enright contributed reagents/materials/analysis tools to the work. Sinead Morris wrote the paper which was revised critically by Anne-Marie Enright.

6.1 Overview

The previous chapter highlighted that both bioremediation and adsorption can be successfully combined into a single treatment option for LFL. The aim of this chapter is to scale up the reactor design implemented in chapter 5 and optimise the system to treat LFL onsite at Powerstown Landfill, Co. Carlow. This reactor will be monitored on a continuous basis for the removal of ammonia, phosphate, nitrate, COD and BOD. Unlike experiments carried out to date the system will be exposed to fluctuations in temperature and the effect of this on both the bioremediation and adsorption processes will be determined. In addition, the cost effectiveness of the operation to treat LFL in this larger scale system will be assessed.

6.2 Abstract

Landfill leachate (LFL) is an environmentally hazardous waste characterised by elevated levels of organic and inorganic compounds, such as ammonia, phosphate and nitrate, which have the potential to cause eutrophication if discharged untreated into receiving bodies. Current treatment options for LFL, which mainly occur off-site as co-treatment in urban wastewater treatment plants (WWTP's), are often costly and inefficient. The aim of this research was 2-fold: (1) to develop a treatment option for LFL using a combination of bioremediation and adsorption in a fixed bed column system; (2) to assess the feasibility of this system on-site at an Irish landfill. Two fixed bed column systems, A1-3 and B1-3, were operated on-site at Powerstown Landfill, Co Carlow, one of six currently active landfills in Ireland, for the treatment of LFL. The systems comprised of an initial bioremediation column, containing soil inoculated with fifteen previously isolated microbial strains capable of LFL bioremediation. The soil column of the B1-3 system remained uninoculated and acted as an experimental control. Two subsequent columns, containing oyster shells (OS) and pumice stone (PS), were employed for adsorption. Both systems were operated at a hydraulic retention time (HRT) of 60 hrs at applied organic loading rates (OLR) of 0.41 g biological oxygen demand (BOD) and 0.33 g chemical oxygen demand (COD) m^3.d^{-1}. Individual and combined column performance was evaluated by BOD, COD, ammonia, phosphate and nitrate removal efficiency (%). Ammonia, phosphate and nitrate removal efficiencies of >90% were achieved by the experimental system over the 40 d trial, meeting the discharge guidelines for receiving bodies within Ireland. BOD and COD removal efficiencies of 88% and 94%, respectively, were achieved during the trial, which did not meet the required discharge limits. However, a subsequent lab trial which included an additional adsorption column (containing oyster shells) at the beginning of the trial significantly improved the combined removal efficiencies of both BOD and COD thus meeting the discharge limits. The results of both trials indicate the viability of this fixed bed column system for the treatment of LFL on-site.

6.3 Introduction

Landfilling constitutes a major method for the disposal of municipal solid waste (MSW) in both developed and developing countries. Modern landfill facilities represent highly regulated and controlled operations that strive to minimise their impact on the surrounding environment through landfill leachate (LFL) collection and biogas entrapment. LFL is a high-strength wastewater (WW), generated as water moisture and rainwater percolate through the landfill, picking up the by-products of waste degradation (McCarthy *et al.,* 2010; Gao *et al.,* 2015). LFL is produced throughout the working life of the landfill, often continuing for up to a century after the landfill is decommissioned (Wang Yu, 2013; Gao *et al.,* 2015; Brennan *et al.,* 2016). Within the European Union (EU) current environmental legislation, the Landfill Directive 1993/31/EC (EU, 2001) and the Waste Framework Directive 2008/98/EC (EU, 2008b), have deemed it essential to treat LFL due to its potential to cause environmental damage. Specifically, the discharge of untreated LFL rich in ammonia (NH_3), phosphate (PO_4^{3-}) and nitrate (NO_3^{1-}) which can cause the contamination of ground and surface water and the subsequent pollution of rivers, lakes and soil. Such pollution events often result in eutrophication, promote algal blooms, and can have a negative impact on human health (Zhang *et al.,*2015). As such, LFL must be harvested from the bottom of the landfill and stored in tanks or lagoons before treatment.

In Ireland, the treatment of LFL normally occurs off-site, often as a combined treatment with domestic sewage in wastewater treatment plants (WWTPs). Although, commonly practised this process is unsuitable due to the stringent discharge limits imposed on the treatment plants by EU directives such as Urban Wastewater Treatment Regulations Council Directive 99/31/EC (EU, 1991) and the Water Framework Directive 2000/60/EC (EU, 2000). Unlike other EU countries, the on-site treatment of LFL is uncommon in Ireland amounting to only 1% of the annually produced LFL (12,000 m^3 per annum) from the national landfills' sites. Of the six current on-site treatments within Ireland only three discharge directly to receiving bodies, with the remaining three failing to meet the effluent discharge limits for both total nitrogen and Chemical Oxygen Demand (COD) (Brennan *et al.,* 2017b). The strength of treated leachate is used to determine if it can be discharged directly to receiving bodies or to sewers. The on-site treatment options that have been successfully implemented including sequences batch reactors (SBR), reverse osmosis (10% of all leachate produced) and methane stripping (15% of all leachate produced) (McCarthy *et al.*, 2010; Brennan *et al.*, 2017b). Despite the initial high capital costs required for many of these treatment options, ranging between €260,000 to €1,000,000 depending on the types of processes employed, the overall cost of treatment per m^3 of wastewater is substantially lower at approximately €1.30 per m^3 than those associated with the energy-intensive aerobic treatments carried out at WWTPs, costing approximately €25 per m^3 (Environment Agency UK, 2007; Brennan *et al.*,2017a). For this reason, it is of environmental and economic importance to investigate alternative treatments that are cost-effective and can reach the national discharge standards set for important LFL constituents specifically BOD, COD, ammonia, phosphate, nitrate and heavy metals.

Most conventional methods employed for the treatment of LFL have been adapted from existing WW treatment methods and involve a combination of both biological and physiochemical methods (Figure 2.9) (Kargi and Pamukoglu, 2004; Raghab *et al.,* 2013). As described above these methods include the combined treatment with domestic sewage in WWTPs and leachate recycling a process whereby LFL is recirculated back into the landfill (White *et al.*, 2011; Jones-Lee and Fred Lee, 2018).

Treatment of LFL can be further divided into biological and physiochemical treatment options. Biological treatment options (Figure 2.9) including both aerobic and anaerobic processes, are commonly used to treat LFL due to their reliability, simplicity and cost-effectiveness (Renou *et al.*, 2008). These include SBR, rotating biological contractors and moving bed reactors (Diamadopoulos *et al.*, 1997; Castillo *et al.*, 2007b; Chen *et al.*, 2008) which are best suited to the treatment of LFL from younger landfill (≤5 years) due to the abundance of biodegradable organics in these waste streams (Renou *et al.*,2008; Torretta *et al.*,2017). In particular, biological treatment options show promise for the removal of BOD, COD and ammonia, which are found in elevated levels within LFL, and have been reviewed extensively in the literature (Renou *et al.*, 2008; Song and Gao, 2013; Bove *et al.*, 2015; Torretta *et al.*, 2017). In contrast physicochemical treatment options, including chemical oxidation, flocculation and coagulation and ion exchange (Renou *et al.*, 2008; Gao *et al.*, 2015) are often employed as pre- or post-treatment steps due to their ability to remove non-biodegradable organics such as humic and fulvic acids, heavy metals and absorbable organic halogens (Renou *et al.*, 2008; Abbas *et al.*, 2009). These treatment options are more commonly applied for the treatment of mature LFL characterised by BOD: COD ratios of ≤0.2, elevated ammonia levels and which contain little or no biodegradable compounds (Kjeldsen *et al.*, 2002; Lee and Nikraz, 2014).

Bioremediation is defined as a process that harnesses living organisms, mostly microorganisms and plants, to degrade, reduce or detoxify waste products and pollutants (Gouma *et al.,* 2014; Azubuike *et al.,* 2016; Ojuederie and Babalola, 2017). Several studies report the successful use of bioremediation as an economical and environmentally friendly treatment option for LFL. For example, Paskuliakova *et al.* (2018) used microalgal, *Chlamydomonas* sp. strain SW15aRL, for the bioremediation of nitrogenous compounds from LFL. This example of phycoremediation involved the removal of inorganic and organic compounds by the microalgae for their growth, while complex substances including nitrogenous compounds were biotransformed. In addition, constructed wetlands (CW), systems comprised of wetland plants, soil, and associated microorganisms have also been used successfully to remove contaminants from several wastewater effluents including LFL (Selic *et al.,* 2007; Torretta *et al.*, 2017; He *et al.,* 2018). In particular, research carried out by Bulc (2006) utilised a CW for the treatment of LFL from a sanitary landfill site accepting waste since the 1980s and achieved high percentage removal efficiencies for COD, BOD, ammonia, nitrogen and total phosphorus. Moreover, microorganisms specifically from the phylum *Proteobacteria*, *Bacteroidetes* and *Firmicutes* have also been widely reported capable of bioremediating LFL within bioreactor systems such as sequence batch reactors and moving-bed biofilm reactors (Zhang *et al.,* 2016).

Physicochemical treatment options, specifically fixed bed adsorption, the process of accumulating substances that are in a solution on a suitable interface, has become a frequently used and cost-effective application for the treatment of WW

(Song and Gao, 2013; Lakdawala and Patel, 2015; Huong *et al.*, 2016). However, although several studies have been carried out to determine the performance of a range of adsorbent for the treatment of different WW streams (Lakdawala and Patel, 2015; Huong *et al.*, 2016), little focus has been given to LFL. Nevertheless, a study carried out by Halim *et al.* (2010), which analysed the capability of activated carbon (AC), zeolite and a composite material (zeolite, limestone, AC, rice husk and cement) for the removal of COD and ammonia from LFL, has indicated the potential of this treatment option.

In light of the above discussion, the current research has investigated an alternative treatment for LFL in the form of a fixed bed column system that combines both bioremediation and adsorption into one treatment option. This treatment option harnesses microorganism previously isolated from LFL and low-cost adsorbents, including oyster shells (OS) and pumice stone (PS), both of which have been identified as suitable for the bioremediation and adsorption, respectively of common constituents of LFL (Halim *et al.*, 2010; Martins *et al.*, 2017; Morris *et al.*, 2018; Spina *et al.*, 2018) This system has been trialled over a 40 d period on-site at an Irish landfill for the continuous treatment of LFL.

6.4 Materials and methods

6.4.1 Leachate samples

Leachate samples used in this study were obtained from Powerstown Landfill located approximately 8 km from Carlow town. Powerstown landfill is a non-hazardous MSW landfill, previously described by the authors (Chapter 2, Section 2.3.1). Briefly, the landfill is composed of three phases (P1-P3). P1 and P2 are closed and final capping has been carried out. P3 consists of 4 cells (cells 15-18), this section is double lined to prevent leachate contaminating surrounding soil and ground/surface water. Leachate generated within these cells was directed to a leachate holding tank and sampled for this study during the period of January and February 2018.

6.4.2 Adsorption assay

Adsorption assays were performed to determine if the proposed materials, OS and PS, were suitable adsorbents of ammonia, a common component of LFL. All batch assays were conducted in 100 ml glass beakers containing 1 g of adsorbent material and 20 ml of an ammonia solution ranging in concentration from 100 - 1000 mg.L^{-1}. Beakers were shaken at 2500 rpm for 16 hrs at 20ºC. Beakers containing only ammonia solutions served as controls. All batch assays were performed in triplicate at 20ºC. Samples were centrifuged at 3000 rpm for 2 min and the ammonia concentration of the resulting supernatant was analysed. The adsorption phase concentration (mg.g^{-1}) was calculated in order to ascertain the type of adsorption that was occurring;

$$q_e = \frac{(c_i - c_e)V}{m}$$ **Equation 6.1**

Where, q_e is the adsorption phase concentration (mg.g^{-1}), c_i is the initial concentration (mg.L^{-1}), c_e is the effluent concentration (mg.L^{-1}), V is the volume (ml) being used and m is the mass of the adsorbent (g).

Langmuir isotherm was applied in the linear form.

$$\frac{c_e}{q_e} = \frac{c_e}{q_{max}} + \frac{1}{K_L q_{max}}$$ **Equation 6.2**

Where q_e is the adsorbent phase concentration after equilibrium ($mg.g^{-1}$), q_{max} is the maximum adsorption at monolayer coverage ($mg.g^{-1}$) and K_L is the Langmuir adsorption equilibrium constant ($d\ m^3g^{-1}$). The graph was plotted as c_e/q_e versus c_e.

The essential characteristics of the Langmuir isotherm can be expressed in terms of a dimensionless constant separation factor, R_L, which is given by the following equation:

$$R_L = \frac{1}{1+K_L Co} \qquad \textbf{Equation 6.3}$$

There are four probabilities for the R_L value: favourable sorption, $0<R_L<1$; unfavourable sorption, $R_L>1$; linear sorption, $R_L=1$; and irreversible sorption, $R_L=0$ (Lyubchik *et al.*,2011; Dada *et al.*,2012).

The mean percentage error (MPE) was calculated using the equation supplied by (Callery *et al.*, 2016). This model is used to calculate the goodness of fit of the values obtain though experimental to theoretical values and allows determination of whether the model is over or underperforming. A negative value indicates that the model is underperforming, while positive values indicate it is overperforming or overestimating (Callery *et al.*, 2016).

$$MPE = \frac{100}{n}\sum_{i=1}^{n}\frac{q_{eq.}\exp - q_{eq.}th}{q_{eq.}\exp} \qquad \textbf{Equation 6.4}$$

Where the $q_{eq}.exp$ is calculated using equation 6.1 and is the experimental adsorption capacity, while $q_{eq}.th$ is the theoretical values. This was applied to the whole data set for each adsorbent and calculated using excel solver function.

6.4.3 Trial 1- On-site treatment of LFL

Six PVC, laboratory-scale (height 50 cm, an internal diameter of 15 cm and an internal capacity of 8835 cm^3) columns, A1-A3 and B1-B3 were used for the on-site treatment of LFL. Both A1 and B1 contained 9.6 kg of topsoil. A1 was inoculated with fifteen previously isolated microbial strains shown to be capable of LFL bioremediation (Chapter 2). A2 and B2 contained 8.9 kg of OS sourced from Harty Oyster Farm, Dungarvan, Ireland, with a particle size of 10-15 mm. A3 and B3 contained 7.5 kg of PS (Lennox Ireland), with a particle size of 2-5 mm. Both adsorbents were washed three times with dH_20 and dried at 100°C prior to use. All columns had a bed height of 40 cm and were divided into test (A1- A3) and control (B1- B3) systems (Figure 6.1). Influent (leachate) was continuously applied to both A1 and B1, at 5 $ml.min^{-1}$ throughout the 40 d trial period. The effluent of both these columns was pumped sequentially into the remaining columns (Figure 6.1). The organic loading rate (OLR) applied to both systems was 0.41 g BOD and 0.33 g COD $m^{-3}d^{-1}$with a hydraulic retention time (HRT) of 60 h resulting in the treatment of 250 L of leachate during this trial.

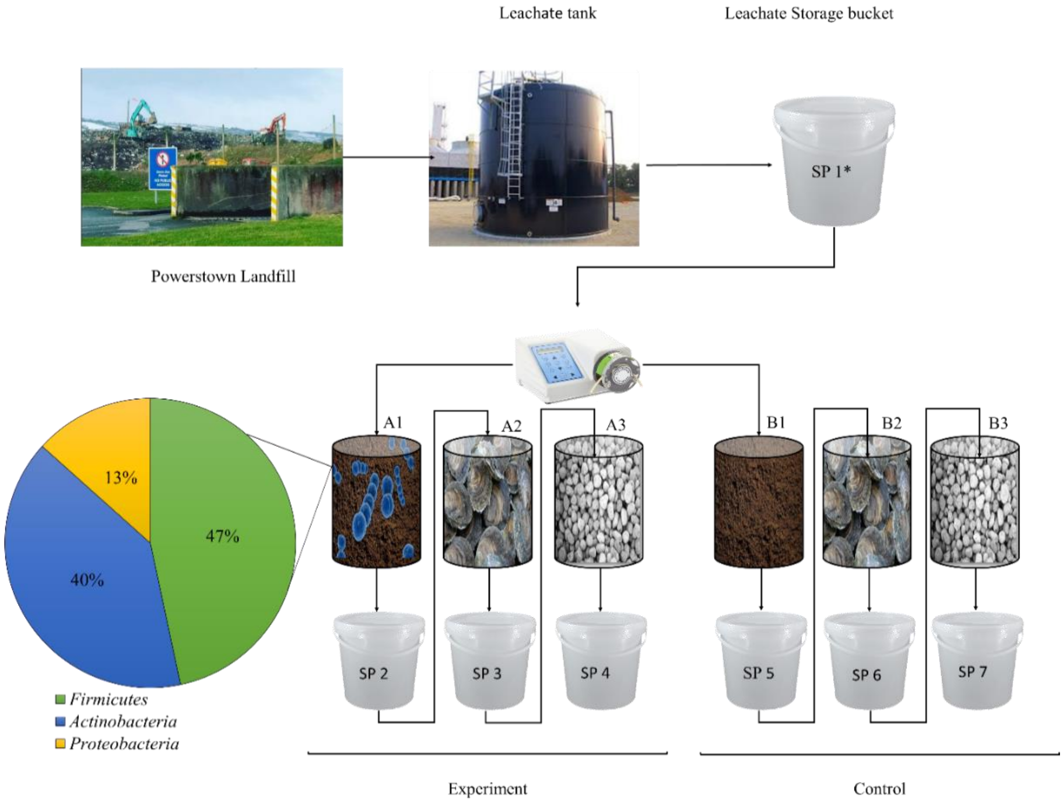

Figure 6.1. Overview of the experimental (A1-3) and control (B1-3) fixed bed column set-up for Trial 1. (*SP indicates samples points).

6.4.4 Trial 2- Optimisation trial

As discharge limits were not obtained in trial 1 for either BOD or COD a second laboratory trial (Trial 2) was carried out over a 14-d period. The trial set-up was identical to trial 1 but included an additional adsorption column, containing OS, at the beginning of the treatment option. The main aim of this second trial was to determine whether the additional adsorption column at the beginning of the treatment would help to achieve the discharge limits for BOD and COD. Adsorption was chosen as it was hoped that this column would affect a removal of inorganic compounds, such as ammonia and phosphate, thus reducing the loading rate applied to the bioremediation column (C2) allowing it to work more effectively. OS were chosen over PS firstly, because they are a waste product themselves and therefore do not have an associated economic cost and secondly because various studies have highlighted that OS are effective at the removal of a wide range of compounds, such as phosphate, heavy metals and humic acids (Namasivayam *et al.,* 2005; Luo *et al.,* 2013; Song and Gao, 2013; Radi *et al.*, 2015; Hamid Reza *et al.,* 2017). In addition, it important to note, that the influent BOD and COD were considerably lower than that of trial 1 (Table 7.3). This may be attribute to a period of colder weather which occurred during February 2018. However, although this will have an impact on removal rates regardless of the presence or absence of an additional adsorption column it reflects the variation of influent composition that can be expected within this waste stream.

Eight PVC, laboratory-scale columns (as described above), C1-C4 and D1-D4 were used for the treatment of LFL at 20°C. The setup, including bed heights, are as previously described for trial 1 (C2-C4 are the same as A1-3, while D2-4 are the same as B1-3) with the addition of the two OS-L.Daytontaining columns (C1 and D1) at the beginning of both systems. Columns were again divided into test (C1- C4) and control (D1- D4) systems and operated as described for trial 1 throughout the 14-d trial period. The OLR applied to both systems was; 0.05 g BOD and 0.25 g COD $m^{-3}d^{-1}$with an HRT of 72 h. This HRT resulted in the treatment of 72 L of leachate during this trial.

6.4.5 Microbiome analysis

Soil samples, were taking form A1,B1,C2 and D2 before and after treatment. The main objective was to access if the inoculated microbes were present in the system before treatment, and had they increased after treatment. Microbiome analysis was carried out by Novogene Ltd. Once the raw data was obtained know sequences for the isolates where BLAST-ed back into the databased, (using Bio-Linux 8 on an Ubuntu operating system) to check for the present of these samples, before and after treatment. This was carried out using command line code as displayed in appendix 3.

6.4.6 Analytical methods

Effluents from all columns were routinely sampled (Fig.2) and effluent BOD and COD concentrations were determined according to standard methods (Rice *et al.*,2017). Ammonia was analysed using the phenate method and analysed on a Shimadzu UV1800 spectrophotometer (Rice *et al.,* 2017). Ortho-Phosphate was analysed using molybdovanadate reagent (HACH Lange) and nitrate analysis was carried out using NitraVer® 5 reagent power pillows (HACH Lange). All HACH products were used according to manufactures instruction and measured on a HACH DR 6000 UV- spectrophotometer. Samples for heavy metals analysis were preserved with concentrated nitric acid to pH 2 and subsequently analysed on an Agilent AA 500, using hollow cathode lamps.

6.4.7 Mathematical description of the fixed bed

For physiochemical treatment, the following calculations were used in order to determine the percentage removal, and the adsorption rate (q_{eq}) for each compound (Aksu *et al.,* 2007; Tang *et al.,* 2012; Enright, 2015);

The total adsorbed ions were obtained by integrating the plot of the adsorbed concentration (C_{ad}) versus the flow time (t). This plot was required to obtain the area (A) used to determine the total amount of each compound that was removed from the system (q_{total}).

$$q_{\text{total (mg)}} = \frac{QA}{100} = \frac{Q}{1000}\int_{t=0}^{t=t_{\text{total}}} C_{ad}\,dt \qquad \textbf{Equation 6.5}$$

Where, Q is the flow rate (ml.min^{-1}), A is the area of the curve. The total amount of ions delivered to the system, m_{total} is determined by the following:

$$m_{\text{total}}\ (\text{mg}) = \frac{C_o Q t_{\text{total}}}{1000} \qquad \textbf{Equation 6.6}$$

In this equation, the Q and the t_{total} represent the flow rate (ml.min^{-1}), and the total flow time (min). Both equations were required to evaluate the removal efficiency of the adsorption columns. The equations make up the total removal as a percentage:

$$\text{Total removal (\%)} = \frac{q_{\text{total}}}{m_{\text{total}}} \text{x } 100 \qquad \textbf{Equation 6.7}$$

The adsorption capacity i.e. the amount of each compound being removed per g of material used was also determined using the following equation;

$$q_{eq}(\text{mg. g}) = \frac{q_{\text{total}}}{x} \qquad \textbf{Equation 6.8}$$

Where x is the unit mass of adsorbent (grams) packed into the column.

6.4.8 Statistical analysis

Statistical analysis was carried out using IBM's statistical package SPSS (Statistical Package for Social Science, version 23.0, IBM Corp, Armonk, NY, USA) and Microsoft Excel 2017. Independent t-Test was conducted on the data. The significance level was set at $p \leq 0.05$ for all statistical analysis.

6.5 Results and discussion

6.5.1 Adsorption assay

The Langmuir isotherm is a simple isotherm, used to predict the adsorption capacity of adsorbents, based on all surfaces of the adsorbent behaving equally. The results of the adsorption assay indicate that each adsorbent followed the Langmuir isotherm in the linear form, with R_L values indicating favourable adsorption ($R^2 \leq 0.99$; Table 6.). Figure 6.2 depicts type 1 adsorption curves based on the system devised by Brunauer *et al.*, (1940). Type 1 graphs are known to follow the Langmuir isotherm and depict monolayer adsorption. The experimental adsorption capacity (q_{max}.exp) of both adsorbent materials tested were similar for ammonia (Table 6.) however, values did not reach their theoretical maximum adsorption capacity (q_{max}.th; Table 6.1). PS had the greatest q_{max}.exp 1.23 mg.g^{-1}, while the q_{max}.th of OS was 1.38 mg.g^{-1} (Table 6.1). The MPE model showed a difference of -11% and -9% for PS and OS, respectively between experimental and theoretical systems. This demonstrates that the experimental system is underperforming and greater adsorption is possible. Nevertheless, both adsorbents show favourable adsorption based on the R_L values which were 0.99 (Table 6.1), indicating that PS and OS are suitable adsorbents for the removal of ammonia from LFL.

Table 6.1. Adsorption parameters of oyster shells and pumice stone.

Adsorbent	**q_{max}.th^a** (mg.g^{-1})	**q_{max}. exp^b** (mg.g^{-1})	**K_L^{c}** (d m^{3}g^{-1})	**R_L^{d}**	**R^{2e}**	**MPE^f**
PS	1.38	1.23	0.09	0.99	0.99	-11.4
OS	1.30	1.19	0.08	0.93	0.99	-9.4

Where;

[a] theoretical adsorption capacity ;

[b] experimental adsorption capacity;

[c] Langmuir adsorption equilibrium constant ;

[d] constant separation factor;

[e] correlation coefficient;

[f] mean percentage error (%)

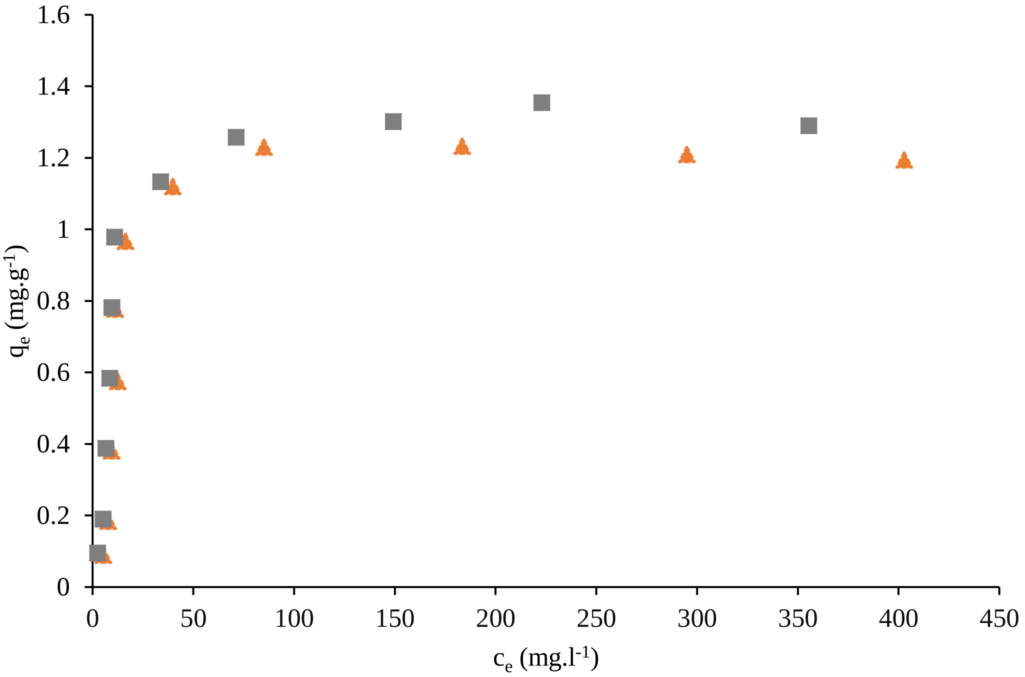

Figure 6.2. Adsorption of ammonia at varying initial concentrations (100 - 1000 mg.L^{-1}) on OS (▲) and PS (■) at 20°C. Where c_e effluent concentration and q_e is calculated from equation 6.1.

6.5.2 Leachate Composition

The chemical composition of leachate used in this study was analysed and compared with values recorded by Powerstown landfill during 2017 (Table 6.2), to determine the stage of waste degradation. Overall, the composition of LFL generated at Powerstown indicates the landfill is in the methanogenic stage, characterised by COD concentrations of ≤4000 mg.L^{-1} O_2 and a BOD: COD ratio of 0.06 (Table 6.2). However, LFL collected from the leachate tank did not correspond to that of a methanogenic landfill as described above, but that of a younger landfill in the hydrolysis- acetogenesis phase, with elevated BOD and COD concentrations and a BOD: COD ratio of ≤ 0.06 (Table 6.2) (Christensen *et al.*, 2001; Abbas *et al.*, 2009; Butkovskyi, 2009; Schiopu and Gavrilescu, 2010). This may result from the fact that although the LFL from the leachate tank was generated in P3, operational since 2006 (>10 years), the remaining cells of P3, Cell 15-18, were still in use at the time of sampling. As a result, the leachate being generated in P3 contained large amounts of organic and inorganic compounds, as waste degradation was ongoing. It should also be noted, that a permanent cap was not in place on these cells at the time of sampling, allowing precipitation to percolate through generating more leachate. Overall, the LFL used in this study was above the national discharge limits set by the Environmental Protection agency (EPA) Ireland, for all compounds tested, as well as the discharge limits set for the WWTPs that accepts leachate generated within this landfill (Table 6.2). As the LFL used in this study had a BOD: COD ratio of 0.64, it was decided that a combination of biological and physiochemical treatments would yield greater removal efficiencies than single treatment options (Butkovskyi, 2009; Connolly, 2010; Schiopu and Gavrilescu, 2010). It was expected that BOD, COD and ammonia should respond positively to biological treatments, while adsorption should remove the additional organic compounds present within this LFL (Christensen *et al.,* 2001; Lee and Nikraz, 2014; Contrera *et al.,* 2015; Gao *et al.,* 2015).

Table 6.2. Composition of leachate from Powerstown landfill from 2017, leachate used in this study and the discharge limits set by the EPA and Mortars town WWTP.

	Powerstown LFL 2017[c]	**LFL used in this study**	**EPA DL**[d]	**Mortarstown discharge limits** [e]
BOD[a]	104	735 ± 85	≤ 5	≤ 15
COD[b]	1820	970 ± 185	≤ 40	≤ 125
BOD:COD	0.06	0.64 ± 0.05	-	-
Ammonia	840	1112 ± 8	≤ 4	≤ 2
Phosphate	7.05	14.9 ± 2.1	≤ 0.4	≤ 0.8
Nitrates	-	167.5 ± 47.7	≤ 50	-
Cadmium	0.204	0.16 ± 0.001	≤ 0.005	-
Copper	0.119	0.4 ± 0.005	≤ 0.05	-
Nickel	1.42	0.35 ± 0.12	List II	-
Iron	0.44	4.56 ± 1.41	≤ 2	-
Arsenic	-	0.07 ± 0.01	≤ 0.05	-
Zinc	0.86	0.16 ± 0. 12	≤ 5	-
Lead	0.045	0.09 ± 0.04	≤ 0.05	-

All results are presented where possible as mg L-1 ± standard deviation;

[a] Biological oxygen demand;

[b] Chemical oxygen demand;

[c] Data supplied by Powerstown Landfill/Carlow County Council (Walsh 2018);

[d] Discharge Limit- Discharge limits based on A3 surface water regulation (EPA 2011);

[e] Mortarstown WWTPs water discharge limits for the River Barrow, Co. Carlow (Water 2018).

6.5.3 Trial 1- The on-site treatment of LFL

Leachate treatment was analysed by comparing the experimental system (A1-3) against the control system (B1-3) different phases, as highlighted below.

6.5.3.1 Phase 1 - Columns A1 and B1

As expected, the percentage removal of all compounds analysed was greater in A1 than B1 during the trial period (Table 6.3). Significantly, a difference in BOD and COD removal efficiency was observed between the columns, with percentage removal of both BOD and COD recorded at >60% for A1 but < 30% for B1 during the trial period (Figure 6.3a; Table 6.3). Thus supporting the conclusion that the microbial strains inoculated into A1 were assisting the breakdown of organic compounds within the system. However, although A1 achieved high removal efficiencies for BOD and COD of >60% and >80% (Table 6.3) respectively, discharge limits were not reached (Table 6.2). As previous studies have shown that biological treatment works better than physicochemical treatments for the removal of BOD and COD (Christensen *et al.,* 2001; Lee and Nikraz, 2014; Gao *et al.,* 2015) it is hoped that this rate could be improved through process optimisation.

In addition, most compounds studied were reduced in A1, with seven out of the twelve compounds analysed having percentage removals of > 60% (Figure 6.3a; Table 6.3). In contrast, B1 had percentage removal efficiencies of <25% for all twelve compounds analysed (Figure 6.3a; Table 6.3). This result again highlights the fact that the microorganisms inoculated into the soil of the A1 column had a significant impact on the removal of compounds found in LFL (Table 6.3; Figure 6.3a).

The concentration of ammonia in Powerstown leachate was high (1112 ± 8 mg .l^{-1}; Table 6.2) which can be problematic to the WWTPs that treat this waste stream (Renou *et al.,* 2008; Torres-socías *et al.,* 2014; Kamaruddin, 2015). The treatment of LFL in both columns did not reach the required national discharge limits, with the average effluent ammonia concentrations being recorded as 41.1mg.L^{-1} and 1007.84 mg.L^{-1}, for A1 and B1, respectively (Table 6.3). Nevertheless, significant percentage removal efficiencies were achieved by A1 indicating again the positive effect the microbial isolates had on the system, which performed significantly better compared to B1 (Figure 6.4).

Phosphate is naturally found in low concentrations in unpolluted waters (Quality status-good ≤ 0.035 mg.L^{-1} , High ≤ 0.025 mg.L^{-1}; (Caroline, 2013; Fanning *et al.,* 2017)). High phosphate loading by direct wastewater discharge can cause nutrient imbalance, leading to eutrophication and reduced stability of the ecosystem (Oram, 2014). As such, the reduction of phosphate below the discharge limit of ≤ 0.4 mg.L^{-1} is an important factor of any treatment option applied to LFL. High phosphate removal within A1 was at 75% (Figure 6.3a), giving an average effluent concentration of 3.7 mg.L^{-1} (Table 6.3) just below the discharge limit. In contrast, the removal in B1 was only 13% giving a final effluent phosphate concentration of 12.9 mg.L^{-1}(Table 6.3) thus not reaching the discharge limit for this compound.

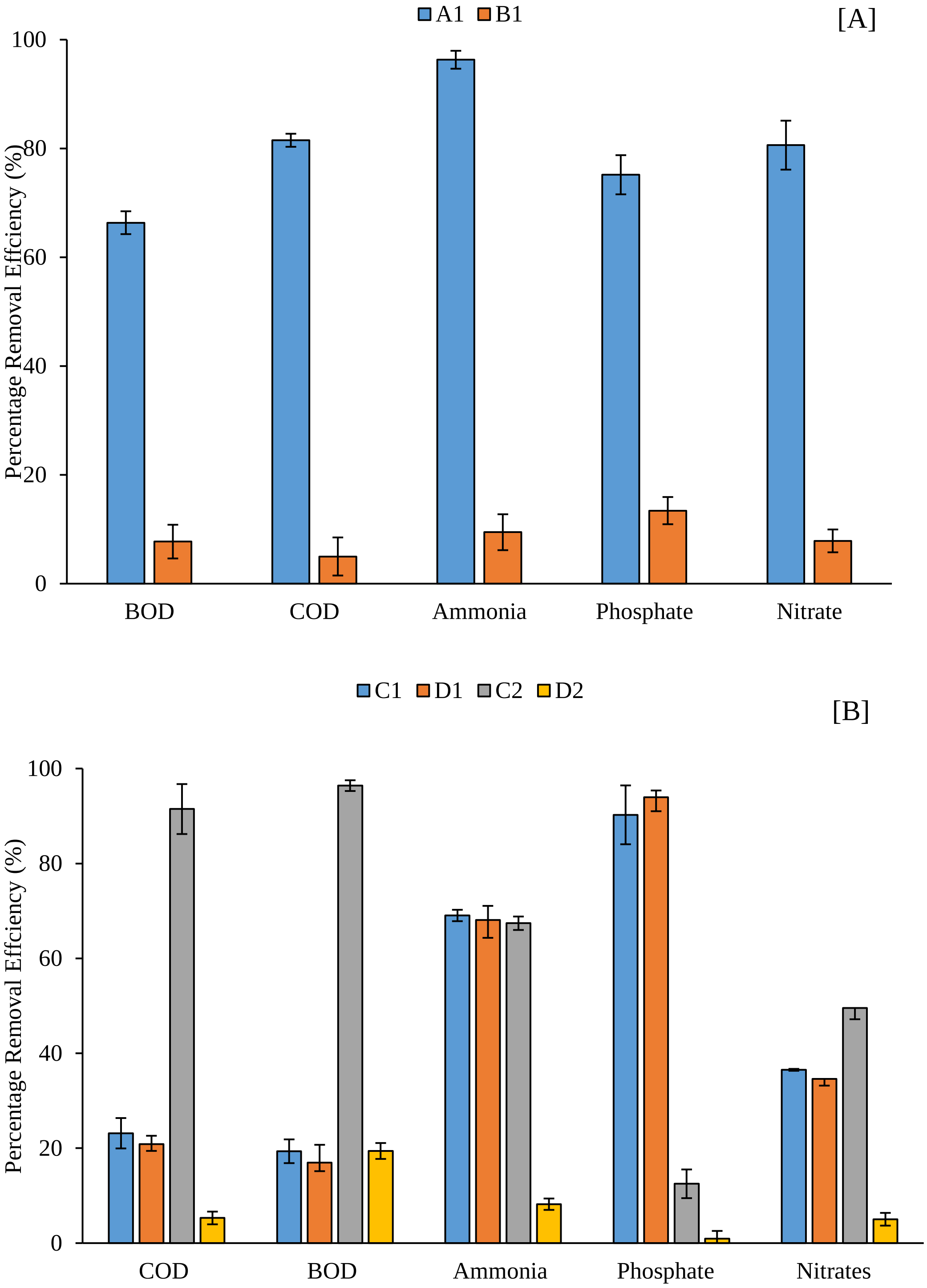

Figure 6.3. Removal efficiency (%) for the soil columns (A/B 1) in trial 1 [A] and the oyster shell (C/D1) and soil (C/D2) column in trial 2 [B].

Table 6.3. Performance data of the fixed bed systems employed in Trial 1 and Trial 2 while treating landfill leachate.

Trial 1	**Influent**	**Effluent values**									
			A1 Soil with microbes	**A2 Oyster shells**	**A3 Pumice Stone**	**% Removed**		**B1 Soil**	**B2 Oyster shells**	**B3 Pumice Stone**	**% Removed**
BOD[a]	735±85	-	247.3±61.5	153.4±50.1	81.8±22.5	88.8±2.7	-	678.32±17.1	357.52±17.2	251.61±8.2	67.76±8.2
COD[b]	970±185	-	179.4±58.5	97.1±48.1	58.3±27.2	93.9±3.9	-	921.55±67.1	511.8±61.9	361.98±2.5	62.68±3.3
Ammonia	1112±8	-	41.1±8.9	3.1±0.9	1.2±0.9	98.9±0.1	-	1007.84±14.1	28.23±13.2	3.07±1.17	97.25±0.2
Phosphate	14.9±2.1	-	3.7±1.1	0.4±0.04	0.3±0.2	97.8±3.6	-	12.9±1.98	1.74±0.65	1.51±0.17	89.86±3.5
Nitrate	167.5±47.7	-	32.1±7.7	2.2±1.1	1.5±0.4	99±0.4	-	152.49±52.21	4.7±1.06	3.18±1.05	98.01±0.8
Trial 2		**C1 Oyster shells**	**C2 Soil with microbes**	**C3 Oyster Shells**	**C4 Pumice stone**	**% Removed**	**D1 Oyster Shells**	**D2 Soil**	**D3 Oyster Shells**	**D3 Pumice Stone**	**% Removed**
BOD	196±2.5	125±2.6	4.5±2.4	4.1±2.6	3.2±3.9	97.9±1.3	123.7±12.3	103±12.9	96±11.6	87.6±14.5	44.7±1.5
COD	635±1.4	458±1.5	39.1±1.4	37.6±1.5	31.7±2.2	94.7±2.5	447.7±17.2	426±17.1	396±17.3	356±19.1	40.2±1.2
Ammonia	956±3.1	296±3.2	97.5±2.9	2.3±3.1	1.8±4.8	99.8±1.4	289.9±3.2	279±3.36	2.8±3.1	2.2±3.78	99.7±3.2
Phosphate	12.3±0.2	1.2±0.2	0.5±0.2	0.39±0.2	0.3±0.3	97.2±3.6	1.3±0.5	1.12±0.52	0.74±0.47	0.6±0.5	97.1±2.5
Nitrate	152±7.3	97.5±.7	48.7±7.2	12.3±7.4	7.1±8.3	97.9±2.3	94.4±7.2	89.67±8.1	17.3±4.2	7.6±1.2	94.9±1.2

Performance values are the trial period mean ± standard deviation and are expressed as mg.L^{-1};

[a] Biological oxygen demand;

[b] Chemical oxygen demand;

Table 6.4. The q_{eq} values for each of the adsorption columns.

	A2 Oyster Shells	**A3 Pumice Stone**	**B2 Oyster Shells**	**B3 Pumice Stone**	**C1 Oyster Shells**	**C3 Oyster Shells**	**C4 Pumice Stone**	**D1 Oyster Shells**	**D3 Oyster Shells**	**D4 Pumice Stone**
BOD	2.63	2.38	0.9	0.35	0.84	0.01	0.32	0.73	0.021	0.356
COD	2.36	1.22	1.15	0.49	3.87	0.07	0.63	3.49	0.141	0.63
Ammonia	1.06	0.74	2.74	0.84	1.85	0.778	0.163	1.82	2.64	0.021
Phosphate	0.09	0.003	0.031	0.0007	0.31	0.0005	0.012	0.32	0.009	0.006
Nitrates	0.41	0.023	0.81	0.002	1.55	0.221	2.06	1.47	1.02	2.58

Values are calculated using Equation 6.9 (Section 6.3.6) and expressed as $mg.g^{-1}$.

Nitrates are also found in low concentrations in unpolluted waters, (Quality status-good ≤ 0.8 mg.L^{-1} ; high ≤ 1.8 mg.L^{-1}; (Caroline 2013)). High nitrate levels paired with high phosphate levels are known to accelerated eutrophication (Xu *et al.*, 2010a; European Environment Agency, 2015). In addition, nitrates are extremely soluble in water and can move easily through soil into drinking water supplies where they represent a public health risk (World Health Organization, 2011; European Environment Agency, 2015). Nitrate removal was significant in A1, with the influent of 167.5 mg.L^{-1} (Table 6.3), being reduced to below national limits (≤ 50 mg.L^{-1}; Table 6.2) to 32.1 mg.L^{-1} N representing an 80% removal rate (Figure 6.3a; Table 6.3). In contrast, B1 showed little removal of nitrates with a final effluent of 152.4 mg.L^{-1} N, achieving a percentage removal of only 8% (Figure 6.3a; Table 6.3).

Microbiome analyses of A1 showed the present off all microbes (15 isolates) in the column before treatment. After treatment these had increase in numbers, indicating that the microbes remain in the system during treatment. Furthermore, increase numbers were observed after treatment indicating the leachate contributing to the increase numbers of micobes. This is backed up by the lack of isolated in B1 (soil without microbes)before treatment but shows to contain all 15 isolates after treatment (Appendix 3).

6.5.3.2 Phase 2 - Columns A2 and B2

A2 was maintained as a physicochemical treatment (adsorption) column during the trial containing OS, a waste product of the aquatic industry, which has been proven effective for the adsorption of ammonia from LFL (Section 6.4.1). The control B2 was an identical column containing OS which treated the effluent of B1, which as described previously had a higher LFL concentration due to the reduced pollutant removal rates of B1 (Figure 6.3a; Table 6.3).

BOD and COD were also adsorbed by the OS in these columns, with percentage removal efficiencies of ≤50% recorded for both A2 and B2 from the effluent of A1 and B1 over the trial period. B2 followed the control column (B1) and showed higher percentage removal efficiencies for both BOD and COD, due to having a higher initial influent concentration (Table 6.3). For example, BOD had a percentage removal rate of 38% between A1 to A2, while between B1 to B2 there was a percentage removal of 48%. Even though the percentage removal was greater for B2, the average effluent concentration of A2 was lower (153.4 mg.L^{-1}; Table 6.3) compared to B2 (357.52 mg.L-1; Table 6.3), thus resulting in greater BOD removal for the test columns (A1 and A2; Table 6.3). This trend was also observed for COD values where although slightly higher removal efficiencies were recorded in the control system overall effluent concentrations were significantly lower in the test columns (A1 and A2; Table 6.3). Despite these percentage removal efficiencies, neither of the columns (A2 and B2) in phase 2 of the on-site treatment option reached the national discharge limit for BOD or COD.

Significantly, the ammonia concentration was reduced to below the discharge limit of ≤ 4 mg.L^{-1} N (Table 6.2) for the A2 column. This was not the case for the B2 column with effluent ammonia concentration recorded at 28.23 mg .l^{-1}, representing a 97 % removal rate (Table 6.3). Achieving the discharge limit for ammonia reduces the pollutant power of LFL thus lowering its impact on receiving bodies. The ammonia adsorption capacity assays of OS showed that B2 had the highest adsorption rate (q_{eq}) adsorbing 2.74 mg.g^{-1}, while A2 adsorbed 1.06 mg.g^{-1} (Table 6.4). B2 also adsorbed more ammonia due to the higher concentrations of ammonia in the effluent from B1 (1007.84 mg.L^{-1}; Table 6.4). This indicates that as B2 had adsorbed more per gram of material it may, therefore, require replacing sooner.

Nitrate concentrations were further reduced to below the discharge limit of ≤50 mg.L^{-1}in A2 and B2, to 2.2 mg.L^{-1} and 4.7 mg.L^{-1}, respectively (Table 6.2; Table 6.3). The q_{eq} for nitrates was 0.41 and 0.81 mg.g^{-1} in A2 and B2, respectively. Like ammonia, B2 had a higher adsorption capacity due to the high level of nitrates within the B1 effluent (152.4 mg.L^{-1}; Table 6.3) and therefore may become exhausted at a faster rate than A2. Nevertheless, by achieving discharge limits in both columns, it is evident that adsorption is a suitable and reliable treatment option for LFL rich in nitrate.

The effluent phosphate concentration of A2 was reduced to 0.4 mg.L^{-1}, just on the national discharge limit (Table 6.2). High removal efficiencies were also achieved by B2 (86%) however the average effluent phosphate concentration of this system was still above the national discharge limits at 1.74 mg.L^{-1} (Table 6.3). In part, this can be explained by the higher influent concentration of phosphate into the B2 system. However, it should also be noted that the adsorption rate (q_{eq}) of phosphate by OS was relatively low (0.09 mg.g^{-1} and 0.03mg.g^{-1}, in A2 and B2 respectively when compared to that of other compounds, mainly COD, BOD, ammonia and nitrate (Table 6.4). This may be due to its low molecular weight (94.97 g.mol^{-1}), adsorption affinities and the fact that is not found in as high concentration in LFL as other compounds (Tchobanoglous *et al.*, 2003).

6.5.3.3 Phase 3- Columns A3 and B3

A3 was maintained as a physicochemical treatment (adsorption) column during the trial, containing PS, a volcanic rock which exhibits a higher adsorption capacity than OS (1.23 mg.g^{-1}; Table 6.1). PS was utilised as a fine filter for the adsorption of any remaining compounds from the influent. Similarly, to phase 2, B3 also contained PS but treated the effluent of B2, which as described previously had a higher influent concentration due to the reduced pollutant removal rates of B1 and B2 (Table 6.3).

As expected both A3 and B3 were not effective in the reduction of BOD and COD (≤ 50 % removal) and their final effluents did not reach national discharge limits (Table 6.4).

A further reduction of ammonia concentration was recorded for both A3 and B3 columns, with B3 now also reaching the national discharge limit (3.07 mg.L^{-1}). Alongside this q_{eq} values of 0.74 mg.g^{-1} and 0.84 mg.g^{-1} for A3 and B3, respectively, were lower for both columns in comparison to the values obtained in phase 2 (Table 6.4). These lower values may be attributed to the fact that the influent for A3 and B3 was substantially lower than the previous columns (Table 6.3).

Influent phosphate concentrations were also further reduced in phase 3, with average final effluent concentrations recorded at 0.3 mg.L^{-1} and 1.51 mg.L^{-1}, for A3 and B3 respectively with A3 being reduced to below acceptable limits ($\leq$.04 mg.L^{-1}; Table 6.2). Similarly, to phase two, the q_{eq} values for phosphate were relatively low when compared to that of other compounds (Table 6.4). In addition, further reductions of nitrate concentrations were observed in both columns, which were now below the national discharge limit ($\leq$50 mg.L^{-1}; Table 6.2, Table 6.3).

6.5.4 Trial 2- Optimised treatment of LFL

6.5.4.1 Phase 1: C1 and D1

As C1 and D1 were identical systems no significant differences were observed between them during the trial. In brief, both columns recorded low percentage removal efficiencies for BOD and COD (<25%: Figure 6.3b; Table 6.3). This was not unexpected as it is known from the literature that physiochemical treatment is not effective at the removal of biodegradable organics (Kjeldsen *et al.*, 2002; Lee and Nikraz, 2014).

Importantly, high percentage removal efficiencies for ammonia (>68%) and phosphate (≥90%) were recorded for both columns (Table 6.3; Figure 6.3b). However, nitrate was not removed effectively by these columns (≤36%; Table 6.3). The adsorption rate (q_{eq}; Table 6.4) did not differ greatly between C1 and D1. This was expected as both columns received the same initial leachate influent. The q_{eq} were relatively high for each compound tested, partly due to the initial high concentration of effluent. As a consequence, it is probable that these columns would become loaded at a faster rate than the adsorption columns in trial 1 as they are receiving influent with much higher pollutant concentrations.

6.5.4.2 Phase 2; C2 and D2

Importantly, the successful pre-treatment of influent LFL by the C1 and D1 columns resulted in the application of a lower strength influent to the subsequent columns. High percentage removal efficiencies (>90%) were achieved for both BOD and COD in the C2 column, resulting in influent concentrations below the discharge limits (Table 6.2). In contrast, percentage removal efficiencies of both BOD and COD in the D1 column were <17% and did not reach the national discharge limits for either compound (Table 6.2; Table 6.3). Similarly, high ammonia and phosphate removal rates of 64% and 55%, respectively, were recorded by the C2 column, while poor removal rates of 5% and 13%, were observed for the same compounds in the D2 column (Table 6.3). However, neither column (C2 or D2) reached the national discharge limit set for ammonia or phosphate. In addition, a final effluent nitrate concentration of 48.7 $mg.L^{-1}$ was recorded from the C2 column, representing a 68% removal rate, just below the national discharge limits of ≤ 50 $mg.L^{-1}$(Table 6.2). Nitrate removal in the D2 column was significantly lower at 5% and with a final nitrate effluent concentration of 89.6 $mg.L^{-1}$ did not reach the national discharge limit (Table 6.2).

As mentions in section 6.7.3.1 microbiome analysis was conducted on C2 (soil with microbes) and D2 (soil without microbes) before and after treatment. The results indicated that the 15 isolates were present before treatment in C1 and increase after treatment. Likewise, D2 had did not display any of the 15 isolates before treatment, but showed to contain some after treatment, this indicated that leachate is contributing to the microbes consortium of the column (Appendix 3).

6.5.4.3 Phase 3-4; C3-C4 and D3-D4

BOD and COD removal continued in the C3 and C4 columns, with a final average effluent concentration of 3.2 and 31.7 mg.L^{-1} for BOD and COD, respectively recorded from the C4 column (Table 6.3). In contrast and despite further removal of both BOD and COD in the D3 and D4 columns the national discharge limits were not met by this control system a fact that can be attributed to the poor performance of the D2 column which lacked LFL degrading microbial isolates.

In addition, the national discharge limits for ammonia, phosphate and nitrate were achieved by both the C3 and D3 columns, with the exception of phosphate which did not reach the national discharge limit in the control system, D1-D4 (Table 6.2; Table 6.3). Overall, the q_{eq} values for ammonia and nitrate remained high showing adsorption to be an effective treatment option for these compounds (Table 6.4). In contrast, the q_{eq} values obtain for phosphate during the trial were low for C3/4 and D3/4 when compare to C1 and D1 (Table 6.4).

As mention in section 6.7.3.1 , micobiome looked at the present of isoalted befor and after treatment. The 15 isolated wehre show to be present in C2 before treatment and increased after treatment, while they werenot found in D2 before treatment, but were after treament (Appendix 3)

6.5.5 Heavy metals

Heavy metals, which are found in abundance in LFL, are of environmental concern due to their ability to cause pollution to ground and surface water (Baun and Christensen 2004; Vaverková *et al.*,2018a). In particular, copper, nickel and cadmium, which can be bioaccumulated, pose a risk to animal and human health, often becoming non-biodegradable when they exceed certain concentrations in the environment (Baun and Christensen 2004; Vaverková *et al.*,2018a). The bioremediation of heavy metals from high strength WW has previously been proven effective, specifically, lead and titanium and to a lesser extent copper, zinc and nickel (Kamika and Momba 2013). Equally, the addition of microbial isolates to the bioremediation column of trial 1 (A1) enhanced the removal of all heavy metals tested. For example, copper and cadmium were reduced by ≥70%, representing an almost 3-fold increase in removal efficiency in comparison to the uninoculated B1 control column (≤ 25%; Table 6.5).

The removal of metals by adsorption was poor with q_{eq} values ≤ 0.01 $mg.g^{-1}$ for all adsorption columns. It is understood that adsorption rates are often affected by the concentration of compounds found in solution, their molecular weight and adsorption affinities [64]. It is thus theorised that the low concentrations of heavy metals in the LFL utilised in this study could be a contributing factor in their resultant adsorption rates. However, it is important to note that, high percentage removal efficiencies were observed specifically for cadmium, copper and nickel in both trials (>80%). Similarly, Bilardli *et al* ., (2018) achieved high percentage removal of copper and nickel from LFL using zeolite, pumice and activated granular carbon. However, and despite their high removal rates only copper and arsenic were reduced to below their respective discharge limits in both systems (Table 6.5). Furthermore, although zinc was tested throughout both trial periods and percentage removal was recorded, its influent concentration was already below the national discharge limit (Table 6.5).

Overall these results indicate that heavy metal removal from LFL is possible using the combination of bioremediation and adsorption but may vary between treatments and the strength of the applied LFL. It is possible that higher percentage removal efficiencies may be achieved through increasing the active volume of the adsorption columns and increasing the RT. Further to this, it may be necessary to apply a recirculation step in the system to achieve discharge limits for these remaining metals.

Table 6.5. Heavy metal analysis data for Trial 1 and Trial 2.

				Trial 1					**Trial 2**		
	DL[a]	**Influent**	**Effluent A1-3**	**DLA[b]**	**Effluent B1-3**	**DLA**	**Influent**	**Effluent C1-4**	**DLA**	**Effluent D1-4**	**DLA**
Arsenic	≤ 0.05	0.07±0.01	0.048±0.01	✓	0.06±0.03	×	0.06±0.002	0.04±0.002	✓	0.04±0.004	✓
Cadmium	≤ 0.005	0.16±0.001	0.01±0.009	×	0.02±0.001	×	0.19±0.03	0.06±0.04	×	0.05±0.004	×
Copper	≤ 0.05	0.4±0.005	0.008±0.005	✓	0.016±0.001	✓	0.68±0.1	0.04±0.15	✓	0.05±0.002	✓
Iron	≤ 2	4.5±1.41	4.2±1.3	×	4.2±0.9	×	7.2±0.1	3.8±0.15	×	3.9±0.9	×
Lead	≤ 0.05	0.09±0.04	0.07±0.03	×	0.08±0.03	×	8.2±0.1	0.6±0.01	×	0.69±0.12	×
Nickel	List II[c]	0.35±0.12	0.05±0.2	-	0.05±0.02	-	0.41±0.03	0.05±0.04	-	0.05±0.02	-
Zinc	≤ 5	0.16±0. 12	0.10±0.23	✓	0.14±0.15	✓	0.15±0.14	0.09±0.22	✓	0.09±0.01	×

Performance values are the trial period mean ± standard deviation and are expressed as $mg.L^{-1}$;

[a] EPA discharge limit;

[b] Discharge limit achieved;

[c] List II substance- environmentally hazardous but does not have a defined discharge limit.

6.5.6 Statistical analysis.

6.5.6.1 Trial 1

An independent-samples t-test was carried out to determine if there were differences in the final effluents of the experimental system (A1-3) and the control system (B1-3). The null hypothesis, that there were no significant differences in final average effluents between each setup, was rejected. A significant difference ($p < 0.05$) was observed for all compounds tested; BOD, COD, ammonia, phosphate and nitrates (Table 6.6), indicating the effectiveness of an initial bioremediation column on the performance of the test system (A1-A3). As such the experimental system was deemed more effective for the treatment of LFL, with many compounds reaching the national discharge limits (Table 6.2; Table 6.3).

6.5.6.2 Trial 2

An independent-samples t-test was carried out to determine if there were differences between the final effluents of the experimental system (C1-4) and the control system (D1-4). The null hypothesis, that there were no significant differences in final average effluents between each setup, was rejected. A significant difference was detected for the five compounds tested, ammonia, phosphate, nitrate, BOD and COD, where $p < 0.05$ (Table 6.6). Thus, supporting the conclusion of trial 1, that bioremediation is required to enhance percentage removal efficiencies within the system, with the experimental system reaching the discharge limits at a faster rate.

Table 6.6. Statistical analysis of Trial 1and Trial 2.

		Trial 1				Trial 2	
Compound	**Column type**	**Final Effluent**[a]	**t-stat**	**Sig**[b]	**Final Effluent**[a]	**t-stat**	**Sig**[a]
COD	Experimental	153.46	-10.29	2.62E-07	7.1	-4.61	8.52E-03
	Control	361.9			7.62		
BOD	Experimental	67.57	-4.11	1.40E-03	0.34	-7.24	3.65E-04
	Control	227.8			0.56		
Ammonia	Experimental	1.24	-3.08	4.10E-03	31.7	-3.42	2.85E-03
	Control	3.05			356		
Phosphate	Experimental	0.32	-7.14	2.99E-08	4.5	-3.51	1.25E-04
	Control	1.04			87.6		
Nitrate	Experimental	1.53	-7.98	9.19E-07	1.81	-3.59	7.23E-03
	Control	3.17			2.2		

Performance values are the trial period mean ± standard deviation and are expressed as mg.L^{-1}

[a] Mean effluent concentration

[b] Sig is when $p<0.05$

6.6 Conclusion

The following conclusions can now be drawn: (i) the on-site treatment of LFL through bioremediation and adsorption in a fixed bed column system is feasible; (ii) the experimental system was effective for the removal of ammonia, phosphate and nitrate, all of which were reduced to their respective EPA discharge limits; (iii) the performance of the bioremediation column (A1) was significantly enhanced by the addition of LFL degrading microbial isolates in comparison to the uninoculated control (B1); (iv) the addition of an OS adsorption column (Trial 2) enhanced the removal efficiencies of BOD and COD to meet discharge limits; (v) further optimisation of the process is now required, for example, to enhance the removal of heavy metals which were not removed/adsorbed effectively, with the exception of copper and arsenic, in either of the trials; (vi) furthermore, research is now required on scaling up to pilot scale for the treatment of larger volumes of LFL; (vii) in addition, the lifespan of each adsorption column needs to be evaluated in longer trials to determine when replacement/regeneration is required.

6.7 Summary

In Chapter six, the combined system trialled in Chapter five was scaled up and employed on site at Powerstown landfill for the direct and continuous treatment of LFL. Ammonia, phosphate and nitrate removal efficiencies of >90% were achieved by the experimental system over the 40 d trial, meeting the discharge guidelines for receiving bodies within Ireland (EPA, 2001). BOD and COD removal efficiencies of 88% and 94%, respectively, achieved during the trial did not meet discharge standard. However, further optimisation of the system (post trial) indicates that the addition of an additional adsorption column (containing OS) at the beginning of the trial significantly improved the combined removal efficiencies of both BOD and COD thus meeting the discharge limits. The results of both trials indicate the viability of this fixed bed column system for the treatment of LFL on-site. Further research is now needed in order to determine the use of saturated adsorbent, with a view to re-use them. This will be examining in the next chapter.

Chapter 7
The Regeneration and Reuse of Landfill Leachate Saturated Adsorbents

This chapter will be submitted to Environmental Mangement

The Regeneration and Reuse of Landfill Leachate Saturated Adsorbents

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*Authors Contributions

Sinead Morris conceived and designed the experiments. Sinead Morris acquired the samples, performed the experiments and analysed data. Sinead Morris, Guiomar Garcia-Cabellos, David Ryan, Deirdre Enright and Anne-Marie Enright interpreted the data. Guiomar Garcia-Cabellos and Anne-Marie Enright contributed reagents/materials/analysis tools to the work. Sinead Morris wrote the paper which was revised critically by Anne-Marie Enright.

7.1 Overview

The main aim and focus of this chapter is to find an alternative way to dispose of/ regenerate the adsorption materials once they have reached their maximum adsorption potential (Chapter 6). Saturated adsorbent are a by-product of the on-site treatment, and it would be usefully to determine if these have a potential to be reused again. Questions the research will address are;

1. What are the best regeneration methods available for these adsorption materials?

2. How effective are the adsorption materials after regeneration?

7.2 Abstract

Adsorption is a widely used technology for the removal of pollutants from wastewaters (WW). The process represents a low cost, effective treatment option for WW, however, the question remains as to the use of saturated adsorbents post-treatment. Currently, research is focusing on the regeneration of this material so it can be disposed of safely and effectively. However, little attention has been given to the ability of regenerated materials to be reused within treatment systems. The current study investigates the regeneration of two adsorbents, oyster shells (OS) and pumice stone (PS), previously used for the treatment of high strength leachate from an Irish landfill. Regeneration assays were conducted using a range of regeneration liquids including; acids, alkali and salts. The resulting concentrations of desorbed ammonia, phosphate and nitrate were used to calculate the desorption rate, with NaOH regeneration solutions achieving the highest rates of desorption. Further to this, batch and column assays containing regenerated OS and PS were carried out and evaluated using the Langmuir and Freundlich adsorption isotherms to determine their adsorption capacity and life span in comparison to fresh/unsaturated adsorbents. The results of both assays indicated an adsorption capacity (q_{eq}.exp) >1 mg.g^{-1} for both OS and PS for ammonia, phosphate and nitrate. After regeneration with the most effective RS, OS and PS were employed as adsorbents in a combined bioremediation and adsorption fixed-bed column trial for the treatment of landfill leachate (LFL) from Powerstown landfill, Carlow, Ireland. A replicate system containing fresh/unsaturated adsorbents was used as a control. Column performance was evaluated by percentage removal efficiency of biological oxygen demand (BOD), chemical oxygen demand (COD), ammonia, phosphate and nitrate. Overall, removal rates between 73-78% were recorded for both regenerated and control adsorbents. In conclusion, the regeneration of spent adsorbent such as OS and PS used for the treatment of LFL offer a method of reusing this waste stream.

7.3 Introduction

Adsorption, the process of accumulating substances in solution on a suitable interface, is a widely used technology for the removal of pollutants from wastewater (WW) (Laidler *et al.,* 2003; Tchobanoglous *et al.,* 2003). The pollutant (adsorbate) is the substance removed from the liquid interface and the adsorbent is the solid, liquid or gas-phase on which the adsorbate accumulates. This process represents a low cost, and effective treatment option for many WW streams including industrial, swine, municipal and landfill leachate (LFL)(Hui *et al.,* 2005; Lin and Juang, 2009; Tsai *et al.,* 2009; Ali *et al.,* 2012; Asgari and Rahmani, 2013; Guler and Sarioglu, 2014). However, the question remains as to the use/disposal of adsorbent material post-treatment, when they have become fully loaded with adsorbate. Currently, research is focusing on the removal of adsorbents from this material, in a process called regeneration in order to dispose of it in a safe manner. However, little attention has been given to the ability of regenerated materials to be reused within original treatment systems for WW.

In general, saturated adsorbents may be regenerated by two main methods; thermal regeneration and chemical modification. Thermal regeneration, which involves heating adsorbents to high temperatures (>100°C) for 24 hrs in a furnace and subsequent cooling, can be costly and time-consuming (Li *et al.,* 2011; Lashaki *et al.,* 2012; Lata *et al.*, 2015). Chemical modification offers a cost-effective alternative to thermal regeneration and can be carried out both *in-situ* and *ex-situ* (Lata *et al.,* 2015). Chemical modification can be achieved via treatment with, acids (HCl and HNO_3), alkalis (NaOH) and salts (NaCl). Specifically, desorption with acid required the following considerations; (1) low pH favours desorption (2) strong competition between H^+ ions and other cations for adsorption sites, forces displacement of cations rather that H^+ ions into the acidic solution, and (3) acid reacts with residual alkalinity and lowers adsorption capacity (Lata *et al.,* 2015). Common acids used in desorption include HCl, H_2SO_4, HNO_3, however, positive results are more often achieved with HCl regeneration (Boricha *et al.,* 2007; Lata *et al.,* 2015). In particular, various studies have assessed the regeneration of different adsorbents by HCl under a range of conditions (Iqbal and Saeed, 2002; Liu *et al.,* 2002; Ajmal *et al.,* 2003; Boricha *et al.,* 2007). Many of these studies have focused on the ability of the acid to desorbed heavy metals with recovery rates ≥85% being reported (Iqbal and Saeed, 2002; Liu *et al.,* 2002; Ajmal *et al.,* 2003; Boricha *et al.,* 2007). For example, Ajmal *et al.* (2003) examined the desorption of Cadmium (Cd(II)) from rice husk by 0.5 M HCl, recording high rates of Cd(II) recovery ≥89% *in-situ.* Desorption by common alkalis, $NaHCO_3$, Na_2CO_3, KOH and NaOH has also been widely reported (Xu *et al.,* 2002a; Thirunavukkarasu *et al.,* 2003; Hu *et al.,* 2005; Zhu *et al.,* 2009). Within this grouping, NaOH is the most commonly used and most effective at desorption. A study by Hu *et al.* (2005) employed 0.01 M NaOH to desorbed Cr(VI) from loaded maghemite (an oxide mineral), achieving a ≥87% recovery rate. Interestingly, this researcher also reported that similar results were obtained over six desorption cycles and that the maximum adsorption capacity after treatment was maintained between 15-20 $mg.g^{-1}$. In addition, salts such as NaCl and KCl can be used successfully as desorption agents (Cui *et al.,* 2006; Xu *et al.,* 2012); Xu *et al* ., (2012) examined the regeneration of zeolite and achieved a positive recovery of Zn (≥150 $mg.Kg^{-1}$) over a range of NaCl concentrations and pH's.

The research outlined above has highlighted regeneration of saturated adsorbents is possible by acid, alkali and salt regeneration fluids. However, research is now required to determine if regenerated material, specifically natural adsorbents such as pumice stone (PS) and oyster shells (OS), are suitable for reuse within WW treatment systems. Specifically, knowledge is now required on the effect of regeneration on the adsorption capacity and exhaustion points of adsorbents, as most studies focus on how many regeneration cycles a material can endure, not how well they perform thereafter (Xu *et al.,* 2002b; Tchobanoglous *et al.,* 2003; Hu *et al.,* 2005; Lata *et al.,* 2015). As such, the current study attempts to addresses these questions by optimising the regeneration process of PS and OS adsorbents previously employed for the on-site treatment of high strength LFL, as described in chapter 7. LFL is a toxic WW, generated when liquid penetrates through a landfill picking up the by-products of waste degradation (Renou *et al.*, 2008; McCarthy *et al.*, 2010). LFL is composed of both organic and inorganic contaminants, which can result in environmental and public health issues if discharged untreated into ground/surface water or surrounding soils (Renou *et al.*, 2008; Gao *et al.,* 2015; Torretta *et al.*, 2017). Of particular concern, is the contamination of water bodies with ammonia, phosphate and nitrate which can result in eutrophication of these nutrient-limited environments.

In light of the above, the three-fold aims of this chapter may be summarised as follows:

(1) To evaluate the effect of acid, alkali and salt solutions for the regeneration of saturated adsorbents (PS and OS) recovered at the conclusion of an on-site fixed bed column trial as described in chapter 7.

(2) To determine the adsorption capacity (q_{eq}) and exhaustion points (N_o) of regenerated adsorbents in comparison to fresh adsorbents by batch adsorption assays and fixed bed column studies.

(3) To determine if regenerated adsorbents are suitable for re-use within a fixed bed column system employed for the treatment of LFL.

7.4 Material and methods

7.4.1 Adsorbents and leachate samples

The adsorbents, PS and OS, used in this study were recovered from a fixed-bed column system employed for the treatment of high strength LFL over a 40 d trial period at Powerstown landfill, Co Carlow, previously described by the authors (Chapter 2 and 3). The characteristics of the LFL are outlined in Table 7.2. Both materials were collected on day 40 of the trial, after treating approximately 250 L of LFL and were stored at 4°C prior to analysis.

7.4.2 Regeneration assays

All chemicals were supplied by Sigma Aldrich (Ireland) unless otherwise stated and made with deionised (d)H_2O. Regeneration assays were conducted in 2 L beakers containing 100 g of each of the saturated adsorbent materials and 1 L standard solutions of 1 M HCl, NaOH, and NaCl. Beakers were shaken at 500 RMP for 24 hrs at 20°C. Beakers containing saturated adsorbents and dH_2O served as controls. Both controls and test assays were performed in triplicate. The ammonia, phosphate and nitrate concentration of the resulting regeneration liquid (RL) was analysed using standard methods as described previously (Chapter 2/3). After regeneration, the adsorbent material was triple washed with dH_2O and dried at 80°C for 24 hrs. The regeneration solution (RS) that gave the highest rate of desorption in the above assays was then evaluated at varying concentrations, between 0.5 M- 2 M, as described above.

7.4.3 Batch studies

The newly regenerated adsorbents from all RS/adsorbent combinations where employed as adsorbents in batch assay experiments. All assays were carried out in

100 ml glass beakers containing 1 g of prepared adsorbent and 20 ml of standard solutions of ammonia, phosphate and nitrate within the concentration ranges of 100-1000 mg. l^{-1}. Beakers were shaken at 350 RPM for 16 hrs period at 20°C. Beakers containing a known initial concentration of each compound without adsorbent served as controls. Both controls and test assays were performed in triplicate.

7.4.4 Regenerated column studies

After regeneration using the most effective treatment method, a fixed bed column study was carried out using the desorbed materials. PVC, laboratory-scale (height 30 cm, Ø 11 cm and an internal capacity of 2850 cm^3), columns with mesh bases, were each packed to a bed height of 20 cm with one of the regenerated adsorbent materials (Figure 7.1). Columns containing saturated adsorbents which did not receive a regeneration treatment served as controls. Prior to use, dH_2O was passed through the columns in a downflow direction to remove trapped air. All columns were employed for the treatment of a synthetic LFL containing ammonia, phosphate and nitrate all at a concentration of 1000 $mg.L^{-1}$ at an applied flow rate of 5 $ml.min^{-1}$. The column study was conducted over 24 hrs and treated c.7 L of synthetic LFL.

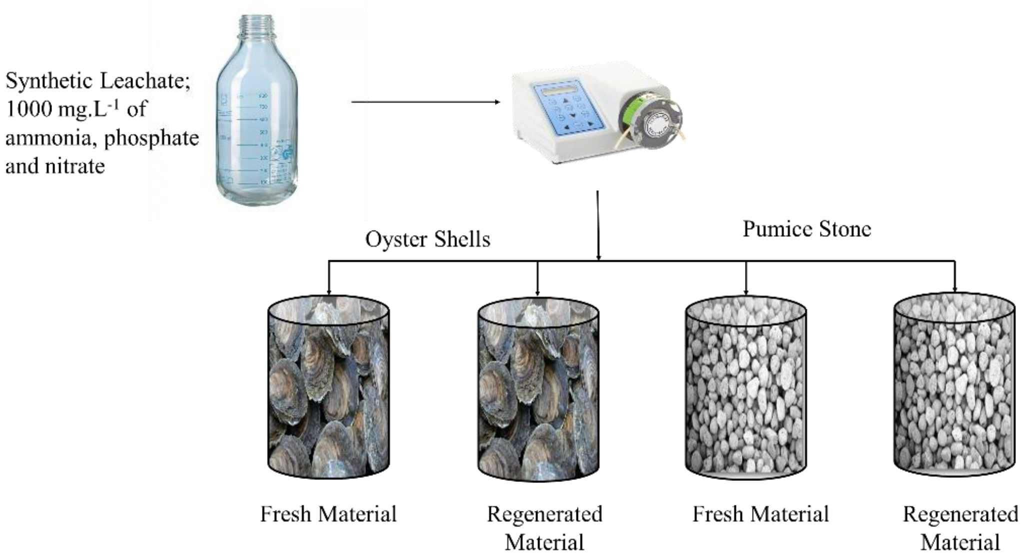

Figure 7.1. Column trial set up

7.3.5 Batch assay and column performance

The adsorption capacity of each regenerated adsorbent in both batch assays and column studies was evaluated using percentage (%) removal efficiency. In addition, batch assays were also analysed using the Langmuir isotherm. Column study data was furthered analysed using the Thomas model and Adam-Bohart model for adsorption in fixed beds. Both batch and column theory are explained in detail in Chapter 3, Section 3.3. The error models used in this study include the hybrid fractional error function (HYBRID), the mean percentage error (MPE) and non-linear chi-squares (Foo and Hameed, 2010; Krishni *et al.*, 2014; Hamid Reza *et al.*, 2017) previously described in Chapter 3, Section 3.4.7.

7.3.6 Treatment of LFL using regenerated adsorbents

After the column study, the regenerated OS and PS were employed as adsorbents in a fixed bed column system, combining both bioremediation and adsorption steps for the treatment of LFL, as previously described (Chapter 3 and 4). Six columns (A1-A3 and B1-B3), were operated as outlined in Figure 6.2 and as described in Chapter 6, Section 6.3.4, with each system being operated in triplicate. Both adsorbents were washed three times with dH_2O and dried at 100°C prior to use. All columns had a bed height of 20 cm and were divided into test (A1- A3) and control (B1- B3) systems (Figure 7.2). Influent (LFL) was continuously applied to both A1 and B1, at 5 $ml.min^{-1}$ throughout the 96 hrs trial period. The effluent of both columns was pumped sequentially into the remaining columns (Figure 7.2). The organic loading rate (OLR) applied to both systems was 0.22 g BOD and 0.45 g COD $m^{-3}d^{-1}$ with a hydraulic retention time (HRT) of 6 hrs resulting in the treatment of 10 L of leachate during this trial.

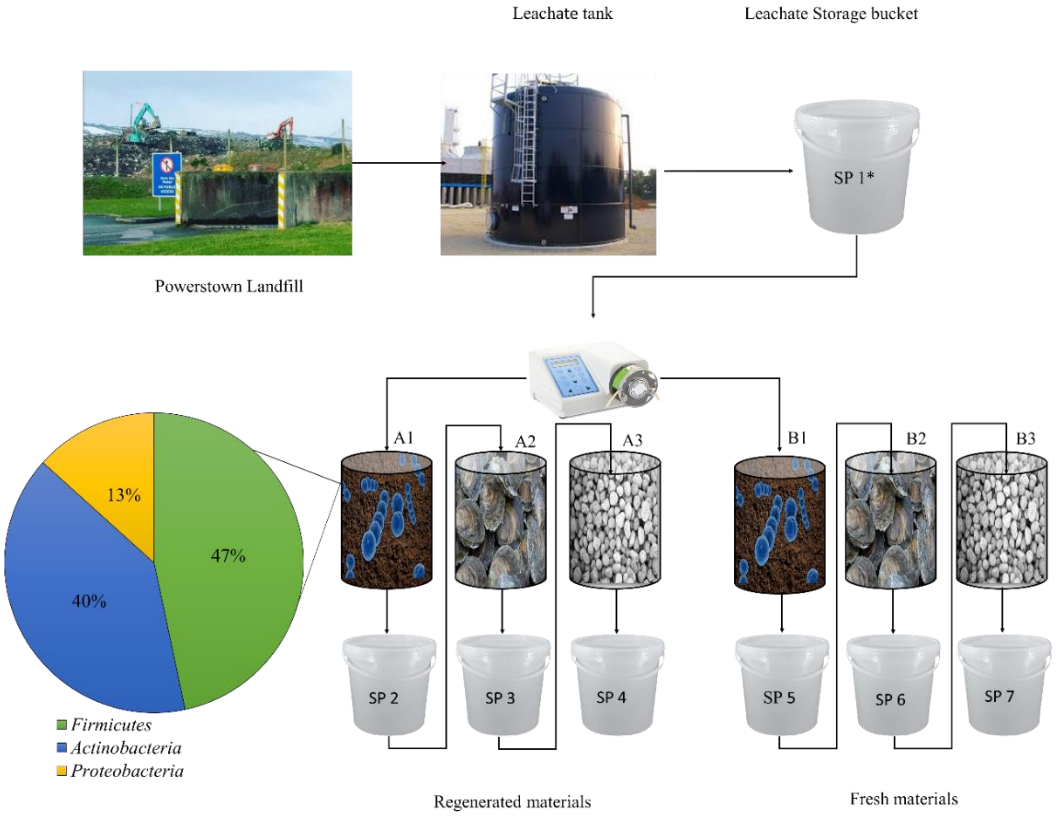

Figure 7.2. Pilot study set up. A1 and B1 contain soil inoculated with microbial isolates, while A2 and A3 contain regenerated oyster shells and pumice stone, with B2 and B3 containing fresh oyster shells and pumice stone.

7.5 Results and discussion

7.5.1 Regeneration assays 1

7.5.1.1 Desorption

The analysis of the RL from both the OS and PS assays indicate that ammonia, phosphate and nitrate can be desorbed by each of the RS tested (Table 7.1). In general, the results indicate that NaOH achieved the highest concentrations of desorbed ammonia, phosphate and nitrate in the RL of all assay combinations tested, with the exception of phosphate which had a slightly higher desorption value with NaCl (Table 7.1). In particular, high desorption values were recorded for ammonia from both OS and PS regenerated by NaOH (1052-1098 mg.L^{-1}; Table 7.1). These high concentration values were not surprising as ammonia was present in the influent LFL at higher concentrations than the other compounds tested (nitrate 526 mg.L^{-1}, phosphate 102 mg.L^{-1}, Table 7.1).

Similarly, the highest phosphate (13.45-14.3 mg.L^{-1}) and nitrate (52.3-67.2 mg.L^{-1}) desorption values were also obtained by NaOH for both adsorbents (Table 7.1). However, it should be noted that desorption values of phosphate by NaCl and HCl were only marginally lower. In contrast, a marked difference was observed for nitrate where desorption by NaOH represented an almost 2-fold increase in desorption compared to HCl and NaCl regeneration of PS (Table 7.1). This trend was also observed to a lesser extent in OS regeneration assays where c. 20-30% increases in nitrate concentrations were observed in NaOH RL compared to that of NaCl and HCl assays.

While phosphate desorption levels where lower than that achieved for ammonia and nitrate, its important to note prior to desorption this materials treated LFL with a lower concentration of phosphate than that of ammonia and nitrate. Powerstown landfill site reported phosphate level between 1.2 -7.4 mg.L^{-1} from the period of 2009-17 (Connolly, 2010; Powerstown Landfill, 2015,2017; Quirke *et al.,* 2019), while LFL that was treated during on-site trials had a phosphate value of 14.9 ± 2.1 mg.L^{-1} (Chapter 6, Section 6.4.2) The desorption rates observed for phosphate were in the region of 9.5-14.7 mg.L^{-1} (Table 7.1), with NaOH RL again recording marginally higher desorption rates than NaCl and HCl.

Table 7.1. Concentration of ammonia, phosphate and nitrate recovered from each adsorbent in regeneration trial 1 and 2.

			Oyster Shells			**Pumice Stone**	
		Ammonia	**Phosphate**	**Nitrate**	**Ammonia**	**Phosphate**	**Nitrate**
Trial 1	**d.H_2O**	52.3±2.5	0.2±2.6	2.8±3.5	69.9±2.8	1.8±3.2	7.1±4.1
	NaOH	1052.6±1.5	13.6±1.5	89.5±2.5	1098.5±2.3	14.6±2.5	97.2±2.9
	HCl	867.3±3.6	9.5±3.7	57.3±1.3	1002.8±1.1	13.7±1.2	54.5±1.5
	NaCl	987.3±7.6	14.3±7.9	67.2±7.3	958.4±7.3	13.4±7.9	42.3±7.4
Trial 2	**d.H_2O**	63.2±3.6	0.6±3.78	12.5±1.25	84.05±1.1	14.4±1.1	11.±1.4
	0.5 M NaOH	957.3±2.5	9.3±2.6	53.5±7.5	963.8±7.4	18.4±7.1	53.1±8.8
	1 M NaOH	1027.3±1.2	10.6±1.2	87.2±3.6	1058.7±3.1	28.9±3.3	97.±2.7
	1.5 M NaOH	1087.6±3.6	11.9±3.7	97.3±2.3	1098.5±1.9	17.4±2.1	114.2±7.2
	2 M NaOH	1108.5±7.8	12.3±7.1	93.5±4.5	1048.2±3.8	17.4±4.2	92.3±4.2

*Recovery rates are presented as average mg .l^{-1}± standard deviation, where n=3.

7.5.1.2 Batch assays

The Langmuir isotherm was applied to the data generated by all batch assays containing regenerated adsorbents. In general, all treatments followed the isotherm, indicated by high R^2, hybrid error and χ^2 values (Table 7.2), with the data set from the NaOH regenerated adsorbents recording the best fit to the model. The results of this analysis indicate that the regenerated OS and PS, regardless of the applied regeneration method, recorded higher adsorption capacities (q_{max}.exp and q_{max}.th) compared to the control (untreated/loaded adsorption material; Table 7.2). This indicates that these regenerated materials are capable of re-adsorption and therefore are suitable for reuse within the system. The highest q_{max}.exp and q_{max}.th values were obtained by NaOH and NaCl regenerated adsorbents for all three compounds tested (ammonia, phosphate and nitrate). For example, the NaOH regenerated OS recorded the highest q_{max}.exp for ammonia of 0.98 mg.g^{-1}, while both HCl and NaCl regenerated OS displayed values of 0.71 and 0.73 mg.g^{-1}, respectively (Table 7.2). Further to this, the q_{max}.th values for NaOH, HCl and NaCl were 1.01, 0.74 and 0.75 mg. g^{-1}, indicating that for ammonia adsorption each of the regenerated OS where performing close to their maximum limits (Table 7.2). Similarly, nitrate and phosphate recorded the highest adsorption values for both NaOH and NaCl regenerated OS with q_{max}.exp values of 0.95 and 1.13 mg.g^{-1}, respectively. While HCl recorded the lowest values at 0.82 and 0.70 mg.g^{-1} for nitrates and phosphate, respectively (Table 7.2). However, it should be noted that the MPE (Table 7.2) indicated that all regenerated OS were underperforming due to the negative values obtained for this model.

Regenerated PS exhibited similar result to that of the regenerated OS in batch assay experiments. Interestingly, high and comparable q_{max}.exp and q_{max}.th values were obtained by NaOH and NaCl regenerated adsorbents for all three compounds tested (ammonia, phosphate and nitrate). For example, NaOH and NaCl regenerated PS recorded high q_{max}.exp values when compared to the control for ammonia, nitrate and phosphate (Table 7.2). Similar to the trend observed by the regenerated OS, HCl regenerated PS recorded q_{max}.exp values that were c. 30-50% lower than that of NaOH and NaCl regenerated PS (Table 7.2). Furthermore, the MPE values for regenerated PS were negative, indicating once again the underperformance of adsorbents in batch assays. As such, it may be possible to optimise these systems further via pH and temperature adjustments (Du *et al.*, 2005; Wang *et al.*, 2006a). This underperformance was also manifested by the large difference between the q_{max} .exp and q_{max}.th for each of the regenerated PS which ranged between 4-29%. However, the NaOH regenerated PS recorded the smallest difference in q_{max}.exp and the q_{max}.th for each compound (4-10 %; Table 7.2), indicating that NaOH regenerated PS is performing close to optimally.

Overall the results of the batch assays indicate that NaOH regenerated adsorbents were capable of higher rates of adsorption compared to other regenerated adsorbents tested. As such, NaOH regeneration was chosen as the best treatment option for both PS and OS. In general, these results are in agreement with the literature where reviews by Lata *et al* ., (2015) and Kulkarnip and Kawarep (2014), conclude that alkali solutions performed better than acid solutions for the regeneration of adsorbents including zeolite, pumice and rice husks, to name a few. Moreover, Kulkarnip and Kawarep (2014) describes a high percentage desorption rates with alkali RS and higher adsorption capacity with alkali regenerated adsorbents which was maintained over successive regeneration cycles when compared to acids RS

Table 7.2. Results of the Langmuir isotherm and error models which were applied to the data set generated in regeneration trial 1

		Oyster shells								**Pumice Stone**							
		Langmuir					**Error models**			**Langmuir**					**Error models**		
		q_{max}.exp[a]	**q_{max}.th**[a]	**K_L**	**R_L**	**R^2**	**HYBRID**	**MPE**	**χ^2**	**q_{max}.exp**[a]	**q_{max}.th**[a]	**K_L**	**R_L**	**R^2**	**HYBRID**	**MPE**	**χ^2**
Ammonia	**d.H_2O**	0.06	0.13	0.01	0.99	0.95	1.17	-7.34	1.37	0.03	0.22	0	0.99	0.95	1.86	-7.34	1.02
	NaOH	0.98	1.01	0.03	0.97	0.98	1.46	-2.01	1.01	1.15	1.25	0.05	0.95	0.98	7.9	-3.83	0.96
	HCL	0.71	0.74	0.08	0.92	0.97	12.12	-4.94	1.23	0.61	0.63	0.1	0.91	0.95	7.47	-7.5	1.82
	NaCl	0.73	0.75	0.08	0.93	0.97	4.17	-7.34	2.37	1.15	1.25	0.06	0.94	0.93	11.94	-11.48	1.26
Nitrate	**d.H_2O**	0.25	0.36	0.02	0.96	0.99	1.22	-7.56	1.43	0.04	0.05	0.023	0.63	0.54	1.47	-7.73	1.73
	NaOH	0.95	1.04	0.01	0.99	0.87	7.6	-2.36	1.19	0.95	1.03	0.01	0.99	0.9	7.22	-2.25	1.13
	HCL	0.82	0.78	0.03	0.97	0.96	14.09	-7.74	1.43	0.63	0.74	0.1	0.91	0.98	17.76	-7.24	1.8
	NaCl	0.95	1.04	0.01	0.99	0.87	11.34	-7.14	2.72	0.97	1.05	0.01	0.99	0.91	2.19	-10.25	4.44
Phosphate	**d.H_2O**	0.02	0.22	0.003	0.95	0.86	1.38	-7.28	1.61	0.11	0.12	0.004	0.82	0.63	1.67	-7.6	1.95
	NaOH	1.13	1.01	0.05	0.97	0.9	7.51	-2.34	1.17	1.13	1.25	0.08	0.96	0.83	7.14	-2.22	1.12
	HCL	0.7	0.69	0.08	0.93	0.96	13.93	-7.68	1.41	0.49	0.68	0.05	0.95	0.98	17.55	-7.15	1.78
	NaCl	1.12	1.02	0.03	0.94	0.89	11.22	-7.56	2.47	1.11	1.22	0.04	0.93	0.79	1.99	-9.07	4.02

Where;

[a] mg.g^{-1}

7.5.2 Regeneration assay 2

7.5.2.1 Desorption

The analysis of the RL for various concentration of NaOH from the OS and PS assays indicate that ammonia, phosphate and nitrate can be desorbed by each of the NaOH concentration tested (Table 7.1). In general, the results showed that increased NaOH concentrations are concomitant with slight increases in desorption from OS and PS for all three compounds tested (Table 7.1). For example, assays containing 1.5 M and 2 M NaOH achieved the highest concentrations of desorped ammonia, phosphate and nitrate in the RL of all assay combinations tested, with the exception of phosphate whose desorption values peaked at 1 M NaOH for PS (Table 7.1).

7.5.2.2 Batch assay

In addition, batch assays carried out using OS regenerated with different concentrations of NaOH recorded increases in q_{max}.exp and q_{max}.th values as NaOH concentrations increased. As such the highest q_{max}.exp and q_{max}.th values were obtained for OS regenerated by 2 M NaOH for all compounds tested (Table 7.3). For example, the OS regenerated by 2 M NaOH presented the highest values for ammonia adsorption, with a q_{max}.exp of 1.35 mg.g^{-1}(Table 7.3). Similarly, high q_{max}.exp and q_{max}.th values were recorded by OS regenerated at this concentration for both nitrate and phosphate adsorption (Table 7.3). Specifically, high q_{max}.exp values of 1.68 and 1.75 mg.g^{-1} were recorded for nitrate and phosphate adsorption, respectively (Table 7.3). In addition, it should be noted that although high R_L values, indicating favourable adsorption were observed at all NaOH concentrations (Table 7.3), the lowest HYBRID error, MPE and χ^2 values were observed at 2 M NaOH, indicating that this is the best performing concentration for OS regeneration (Table 7.3).

Dissimilar to OS batch assays, PS assays revealed that regeneration with 1.5 M NaOH resulted in the highest q_{max}.exp values for ammonia, phosphate and nitrate of 1.82, 0.89 and 1.73 mg.L^{-1}, respectively (Table 7.3). However, although the R_L values obtained for all batch assay indicate favourable adsorption by PS regardless of NaOH concentration, the MPE models displayed negative values indicating the systems are underperforming. This result was expected as the q_{max}.exp values for all assays were lower than their respective q_{max}.th values. Further to this, the HYBRID error and χ^2 values were lower for the PS regenerated by 1.5 M NaOH indicating that this is the best fit (Table 7.3).

Previous studies examining the regeneration of a variety of materials via NaOH have recorded favourable desorption and subsequent reabsorption (Xu *et al.*, 2002b; Djomgoue *et al.*, 2015; Lata *et al.*, 2015). Specifically, Du *et al* ., (2005) describes the successful regeneration of clinoptilolite (Zeolite) loaded with ammonia using 0.5 M NaOH over 700 min. In addition, Tian *et al* ., (2011) examined the desorption of arsenic from wheat straw using 0.1 M L^{-1} NaOH. This process proved successful after ten cycles of regeneration with the adsorbent-retaining adsorption capacities > 80%.

Table 7.3. Results of the Langmuir isotherm and error models which were applied to the data set generated in regeneration trial 2.

		Oyster shells								**Pumice Stone**							
		Langmuir					**Error Models**			**Langmuir**					**Error models**		
		q_{max}.exp[a]	**q_{max}.th[a]**	**K_L**	**R_L**	**R^2**	**HYBRID**	**MPE**	**χ^2**	**q_{max}.exp[a]**	**q_{max}.th[a]**	**K_L**	**R_L**	**R^2**	**HYBRID**	**MPE**	**χ^2**
Ammonia	**0.5 M**	0.03	0.22	0.001	0.99	0.95	1.86	-7.34	1.2	0.65	0.65	0.003	0.97	0.93	0.6	-1.71	0.19
	1 M	1.05	1.15	0.005	0.95	0.98	7.9	-3.83	0.96	1.13	1.15	0.006	0.94	0.95	7.21	-4.03	2.48
	1.5 M	1.18	1.25	0.001	0.91	0.95	7.47	-7.5	1.82	1.82	1.92	0.002	0.98	0.8	4.25	-1.2	1.01
	2 M	1.35	1.56	0.006	0.94	0.93	1.94	-1.48	0.59	1.28	1.36	0.001	0.99	0.89	14.05	-13.51	1.48
	D.H_2O	0.12	0.11	0.004	0.82	0.63	14.245	-12.56	1.875	0.22	0.33	0.002	0.99	0.52	11.4	-10.05	1.5
Nitrate	**0.5 M**	0.41	0.57	0.002	0.95	0.93	0.6	-1.71	1.49	0.4	0.52	0.002	0.95	0.98	0.19	-0.55	0.06
	1 M	1.04	0.948	0.001	0.99	0.87	7.21	-4.03	1.01	0.55	0.95	0.005	0.92	0.85	7.54	-4.24	1.06
	1.5 M	1.59	1.4	0.003	0.97	0.88	8.8	-10.2	2.48	0.89	1.02	0.006	0.96	0.99	1.97	-1.39	0.95
	2 M	1.68	1.79	0.006	0.99	0.8	4.05	-1.28	0.19	0.79	0.99	0.002	0.99	0.95	17.53	-7.28	1.74
	d.H_2O	0.12	0.11	0.004	0.82	0.63	11.4	-10.05	1.5	0.08	0.1	0.001	0.87	0.52	9.12	-8.04	1.2
Phosphate	**0.5 M**	0.304	0.55	0.004	0.98	0.89	0.19	-0.55	1.26	0.29	0.54	0.002	0.95	0.87	0.06	-0.17	0.02
	1 M	1.01	1.128	0.007	0.95	0.9	7.54	-4.24	1.74	1.01	1.13	0.003	0.93	0.83	7.88	-4.47	1.12
	1.5 M	1.58	1.375	0.005	0.98	0.85	11.97	-13.87	3.34	1.73	1.89	0.006	0.99	0.92	2.53	-1.89	0.58
	2 M	1.75	1.98	0.001	0.98	0.86	7.53	-2.89	1.06	1.08	1.12	0.008	0.99	0.95	9.44	-8.69	2.05
	d.H_2O	0.13	0.06	0.001	0.99	0.99	9.1168	-8.04	1.2	0.02	0.25	0.001	0.96	0.99	7.29	-7.43	0.96

Where;

[a] $mg.g^{-1}$

7.5.3 Column studies

PS regenerated using 1.5 M NaOH and OS regenerated using 2 M NaOH were employed as adsorbents in a fixed bed column trial for the treatment of a synthetic LFL containing ammonia, phosphate and nitrate at a concentration of 1000 mg.L^{-1} as described in Section 7.3.4. The adsorption capacity (q_{eq}) of each regenerated adsorption material was determined using breakthrough curves over a 10 hrs period. The resulting data sets were also analysed using the Thomas and Adam-Bohart models to determine the maximum adsorption capacity (q_o) permittable during the trial and the exhaustion points (N_o) of each material. All columns were operated at a bed height of 20 cm with an applied flow rate of 5 ml.min^{-1}, parameters that had been optimised in previous studies by the authors (Chapter 3, Section 3.7.2).

7.5.3.1 Oyster shells

The results of the column study indicate that regenerated OS was capable of ammonia adsorption at a high rate. However, in comparison to the control (fresh/untreated OS), a 20% reduction in adsorption capacity was observed, with q_{eq} values for regenerated and the control materials of 0.86 and 1.08 mg.g^{-1}, respectively (Table 7.4). Similarly, the Thomas model which is used to depict the q_o of adsorbents also indicated a similar difference between regenerated and untreated OS of 1.57 mg.g^{-1} and 1.96 mg.g^{-1}, respectively (Table 7.4). However, although this c.20% reduction of adsorption capacity indicates that the regenerated material will not adsorb ammonia for the same duration as fresh material it still has a high adsorption capacity and could be successfully reused within the system. Furthermore, the Adam-Bohart model determined a significant difference (1.03 mg.L^{-1}; Table 7.4) in the N_o of both materials, this indicated that the column might not perform as successful for the same period. Alongside this, the life expectancy of the column differs greatly.

For nitrate, the q_{eq} values are similar, with a difference equating to 0.15 mg.L^{-1} (13%, Table 7.4). While the major difference between both materials is seen in the N_o values indicating a difference of 0.8 mg.g^{-1} (34%). Although the materials follow both models, once again the MPE model recorded negative values indicating that both experimental systems are underperforming in relation to their theoretical systems (Table 7.4).

The analysis of phosphate adsorption by the regenerated OS and untreated OS revealed that q_{eq}, q_o and N_o values were all lower in regenerated material, with MPE values indicating that both systems were underperforming compared to theoretical values (Table 7.4). In particular, a 56% difference in the capability of regenerated material and fresh/unloaded material to remove phosphate was observed, which is poor compared to values obtained for the removal of ammonia. Interestingly, although similar q_{eq} values were obtained for nitrate adsorption by both regenerated and control OS, a significant difference was observed in their N_o values (c.34%). Furthermore, negative MPE values were recorded, again indicating that both experimental systems are underperforming in relation to the theoretical system (Table 7.4).

The life span, i.e. how long each column will successfully adsorb each compound, can be calculated by utilising the rate of change and the equation of the line. The theoretical life span of each regenerated adsorbent was determined for ammonia, phosphate and nitrate and compared against that of fresh/unsaturated adsorbents (Table 7.5). These calculations indicate a 2-fold difference between both systems in their ability to adsorb ammonia, with regenerated material indicating an expected life span of 32 hrs, compared to 65 hrs for fresh/unloaded material (Table 7.5). Nevertheless, the fact that regenerated material is capable of achieving the discharge standards for 32 hrs indicates that it still has the potential to be used for the removal of ammonia from LFL. Additionally, similar results were observed for phosphate adsorption, with a c. 50 % reduction of the life span between unloaded and regenerated OS (25 hrs versus 53 hrs; Table 7.5). A smaller difference of 28% was observed between both systems for nitrate adsorption where regenerated materials could successfully adsorb nitrate for 47 hrs, compared to 60 hrs for fresh adsorbents (Table 7.5). Overall the regenerated adsorbents can be utilised within the system to remove the compounds for at least 32 hrs, after which time the columns should be changed out as ammonia and phosphate adsorption limits will have been reached and effluents may not be achieving discharge limits.

Table 7.4. Results of the Thomas, Adam-Bohart and error models which were applied to the data set generated during column studies using regenerated oyster shells and pumice stone.

Adsorbent		**Compound**		**Thomas model**		**Error model**			**Adam-Bohart**		**Error model**		
			q_{eq}	q_o	K_{TH}	**HYBRID**	**MPE**	χ^2	N_o	K_{th}	**HYBRID**	**MPE**	χ^2
Oyster Shells	Regenerated	Ammonia	0.86	1.57	0.00	4.63	-3.43	0.26	2.85	0.00	4.94	-1.73	0.56
		Phosphate	0.42	0.98	0.00	4.41	-2.88	0.48	1.62	0.00	1.92	-8.53	0.98
		Nitrate	0.97	1.36	0.00	4.52	-3.64	0.85	1.53	0.00	3.65	-2.52	0.52
	Fresh	Ammonia	1.08	1.96	0.004	3.70	-2.75	0.29	3.88	0.003	2.36	-1.06	0.42
		Phosphate	0.64	1.23	0.002	4.85	-3.17	0.62	2.03	0.003	2.30	-1.26	0.26
		Nitrate	1.12	1.56	0.003	3.67	-2.96	0.72	2.33	0.005	2.58	-2.36	0.42
Pumice	Regenerated	Ammonia	0.86	1.57	0.001	2.34	-4.16	0.59	2.36	0.001	7.36	-1.6	0.89
		Phosphate	0.39	0.76	0.003	4.13	-2.52	0.85	1.82	0.003	3.82	-8.84	0.98
		Nitrate	0.87	1.23	0.002	3.12	-1.25	0.58	1.45	0.002	2.12	-3.25	0.78
	Fresh	Ammonia	0.91	1.68	0.002	1.59	-2.36	0.42	3.62	0.003	4.26	-1.25	0.56
		Phosphate	0.49	0.96	0.005	2.36	-1.25	0.36	2.03	0.002	2.86	-3.62	0.63
		Nitrate	1.24	1.52	0.003	4.23	-1.86	0.42	1.95	0.001	1.96	-4.28	0.45

Table 7.5. Results of life span calculation on each adsorbent.

Adsorbent	Compound	Material type	The equation of the line	Discharge limit[s]	Life Expectancy[d]
Oyster Shells	Ammonia	RG[a]	$f(x); 737.45x - 977.53$	4	32
		F[b]	$f(x); 1470.9x - 974.3$	4	65
	Phosphate	RG	$f(x); 4227.4x - 187.12$	0.4	47
		F	$f(x); 8454.8x - 197.12$	0.4	60
	Nitrate	RG	$f(x); 60.84x - 254.24$	50	25
		F	$f(x); 78.24x - 324.27$	50	53
		RG			
Pumice Stone	Ammonia	RG	$f(x); 521.75x - 264.1$	4	30
		F	$f(x); 1043.1x - 274.3$	4	64
	Phosphate	RG	$f(x); 3789x - 172.6$	0.4	28
		F	$f(x); 5494x - 173.5$	0.4	33
	Nitrate	RG	$f(x); 59.94x - 297.4$	50	45
		F	$f(x); 62.36x - 283$	50	41

Where;

[a] Regenerated material

[b] Fresh material

[c] mg.L^{-1}

[d] Hours

7.5.3.2 Pumice stone

The results of the column study indicate that regenerated PS was capable of ammonia adsorption at a high rate compared to that of untreated PS. Specifically the q_{eq} values for regenerated and control PS were 0.86 and 0.91 $mg.g^{-1}$, respectively, indicating a 7.5% decrease in the ability of regenerated materials to remove ammonia from solution (Table 7.4). Regenerated PS also maintained a high maximum adsorption capacity value of 1.57 $mg.g^{-1}$ in comparison to the control PS (1.68 $mg.g^{-1}$; Table 7.4). However, it should be noted that the N_o values obtained from the Adam-Bohart model were significantly lower for regenerated PS in comparison to the control (2.36 $mg.g^{-1}$ and 3.62 $mg.g^{-1}$, respectively; Table 7.4) again indicating that the longevity of ammonia adsorption by regenerated PS may be reduced.

Similar results were recorded for phosphate adsorption by regenerated PS where lower q_e, q_o and N_o values were observed in comparison to the control PS. Again a difference of 20% in the q_e values for both adsorbents, which was mirrored by the q_o values obtained from the Thomas model, indicate higher adsorption capacities for the control PS. However, a smaller difference was observed for the N_o values, of 1.82 $mg.g^{-1}$ and 2.03 $mg.g^{-1}$, for regenerated and control PS (Table 7.4).

The values obtained for q_o and q_{eq} for nitrate follow the same pattern as phosphate adsorption with similar percentage differences observed between the regenerated and untreated materials. For example, the observed nitrate adsorption capacity (q_{eq}) for regenerated and control PS was 0.87 and 1.24, respectively (Table 7.4). Again, a lower exhaustion point (N_o) was recorded for the regenerated PS in comparison to the control PS of 1.45 and 1.95 $mg.g^{-1}$, respectively indicating that regenerated material can remove nitrate from solution.

7.5.4 Statistical analysis

In addition to the above analysis, a one-way multivariate analysis of variance was carried out to determine if there was a difference between regenerated and untreated OS as an adsorbent. The final effluent concentrations of ammonia, phosphate and nitrate were determined to asses performance. The difference between the treatments of the combined dependent variables was not statistically significant, $F(3, 48) = 1.074$, $p = 0.089$; Wilks' $\Lambda = 0.873$; partial $\eta^2 = 0.163$. This indicated that these regenerated materials should have the ability to perform just as well as untreated material in a full-scale trial. A similar test on the same parameters was conducted to determine if there was a difference between regenerated and untreated PS as an adsorbent. Again, the difference between the treatments of the combined dependent variables was not statistically significant, $F (3, 48) = 3.115$, $p = 0.065$; Wilks' $\Lambda = 0.089$; partial $\eta^2 = 0.911$, again highlighting that both materials should be capable of achieving the same results.

7.5.5 Fixed bed column trial

7.5.5.1 Phase 1 -A1 and B1

The focus of this experiment was to determine if the regenerated adsorption materials could achieve similar removal rates to a system which contained fresh PS and OS. As such, the initial columns A1 and B1 which contained soil inoculated with LFL degrading microbial isolates were not directly affected by this change. Nevertheless, analysis of the effluent of both columns revealed a reduction in all the compounds tested with little deviation between the two systems. However, no compound was reduced to below the national discharge standards. The characteristics of the final effluent for both A1 and B1 which became the influent for the subsequent columns are described in Table 7.7.

7.5.5.2 Phase 2- A2 and B2- Oyster Shells

The A2 column, containing regenerated OS achieved BOB and COD percentage removal rates of 35% and 22%, respectively (Table 7.6). In comparison, the B2 column, containing the fresh/untreated OS, recorded slightly higher BOB and COD percentage removal rates of 40% and 28%, respectively (Table 7.6). Despite these slight increases, neither column reduced influent BOD or COD to below the national discharge limit.

The removal of the inorganic LFL constituents, ammonia and nitrate, was successful in both the A2 and B2 columns. Specifically, both A2 and B2 effected a >98% removal efficiency for ammonia, resulting in B2 and A2 effluent ammonia concentrations of 2.3 mg.L^{-1} and 4.36 mg.L^{-1}, respectively. These comparable reductions resulted in the A2 column just falling short of the national discharge limit, which was reached by B2 (Table 7.6). Similarly, high percentage removal efficiencies, ≥94%, for nitrates were achieved by both columns resulting in their respective effluents meeting the national discharge limit set for this compound (≤ 50 mg.L^{-1}; Table 7.6). Furthermore, little or no deviation was observed between the adsorption capabilities of untreated and regenerated OS (Table 7.6). The q_{eq} for ammonia reduction between both columns did not differ greatly with A2 showing a q_{eq} of 2.22 mg.g^{-1}, while B2 had a value of 2.82 mg.g^{-1} (Table 7.7). As previously described in Chapter 5, the reduction of the ammonia concentration of LFL is required for a treatment to be deemed successful (Renou *et al.,*2008; Christensen 2010). In particular, this elevated ammonia concentration (>1000 mg.L^{-1}) is often problematic for WWTP operator, as it can put pressure on the already overloaded treatment plant (Brennan *et al.,* 2017b). Further to this, excess nitrate in discharged effluents can contribute to the eutrophication of water bodies (Horan *et al.,* 1994; European Environment Agency, 2015).

In contrast to the high percentage removal efficiencies obtained by both systems for ammonia and nitrate, phosphate removal by regenerated OS was c. 50% lower than those achieved by the control OS (Table 7.6). As such A2 recorded a final effluent phosphate concentration of 2.8 mg .L^{-1}, which was above the national discharge limit of ≤0.4 mg.L^{-1}, which was met by B2 (Table 7.6). Further to this, a difference of 61% between the q_{eq} values of A2 and B2 columns was observed, with A2 recording a value of 0.18 mg.g^{-1}, while B2 was higher at 0.29 mg.g^{-1} (Table 7.7). As described previously (Chapter 3, Section 3.5) adsorption is controlled by numerous factors including, adsorption affinity and the number and concentration of compounds within a solution (Tchobanoglous *et al.,* 2003). As such as the OS in A2 already treated phosphate and received a regeneration treatment it may not have as many available active sites, therefore making it harder for phosphate to be adsorbed. In addition, previous experiments have noted low phosphate adsorption compared to that of ammonia and nitrate due to its lower concentration in LFL, which is also a feature of the LFL used in this study. Nevertheless, the overall results of the regenerated OS column indicate, with the exception of phosphate removal, this system was comparable to the control for the treatment of LFL.

7.5.5.3 Phase 3- A3 and B3- Pumice stone

The A3 column, containing regenerated PS achieved BOD and COD percentage removal rates of 20% and 21%, respectively (Table 7.6). These results were comparable to the removal rates achieved by the control for BOD; however, this column observed a c. 13% increase in COD removal. Despite these reductions, the final effluent of A3 and B3 failed to reach the discharge limits set for BOD and COD (Table 7.6). Again, this emphasis the fact that adsorption is an ineffective method for the removal of organic matter from LFL. Likewise, the adsorption capacity of both compounds is low when compared to other compounds tested (Table 7.6).

Further moderate percentage removal efficiencies were observed by the A3 column for ammonia and nitrate, of 45% and 50%, respectively. These reductions further reduced the A3 ammonia and nitrate concentrations to below their respective discharge limits (Table 7.6). Furthermore, the overall percentage removal of ammonia by both the A3 and B3 columns were comparable at 97% and 98%, respectively. Likewise, a comparable adsorption capacity was observed between both columns with A3 and B3 recording q_{eq} values of 2.73 $mg.g^{-1}$and 2.84 $mg.g^{-1}$, respectively (Table 7.7).

In addition, particularly high phosphate removal efficiencies of 82% were achieved by the A3 column, again resulting in the final effluent of this column reaching the discharge limits for this compound (≤0.4 $mg.L^{-1}$; Table 7). Similarly, a 50% nitrate removal efficiency was recorded for the A3 influent concentration, however, it should be noted that this compound was already below the discharge limits (Table 7.6).

7.5.5.4 Combined system

Overall the regenerated adsorption materials used in this study proved effective for the treatment of LFL when compared to a replicate fixed bed column system containing fresh adsorbent material. Specifically, the percentage removal rates obtained from the final effluents of both systems for each of the five parameters tested indicate that adsorption by regenerated materials is comparable to fresh materials and thus has the potential to be reused within the system efficaciously.

Overall to optimise the removal efficiency, for application in an on-site treatment system, it would be necessary to combine both materials to maximise their adsorption potential and ensure the columns would not reach their exhaustion points as fast as a column filled fully with regenerated material. It is believed by the authors that this, in turn, would increase the longevity of the columns and aid the adsorbents to achieve the discharge limits set for each compound tested. However, a limited number of trials have been conducted using combinations of fresh and regenerated adsorbents (Lata *et al.,* 2015) so this process requires further research.

In contrast, several studies have investigated the effect multiple cycles of regeneration has on adsorbent performance. Other studies (Thomas and Crittenden, 1998; De Gisi *et al.,* 2016; Da’na and Awad, 2017; Pathania *et al.,* 2017) investigating various adsorbent and state that a minimum of 5-10% of adsorption capacity is lost with every regeneration process with regenerated adsorbents eventually become obsolete. Similarly, Wang and Lo (2009) described the effect of multiple regeneration cycles on mesoporous magnetic γ-Fe_2O_3 for the removal of cadmium from WW, recording a 10% decrease of adsorption rate after each cycle. Furthermore, a similar reduction was observed in the adsorption capacity of PS, employed for the removal of acid red 18 azo dye from WW, after successive regeneration with 2 M NaOH (Samarghandi *et al.,* 2012). Again, these studies emphasise the fact that adsorption rates are greatly influenced by regeneration and that in order to achieve and maintain high adsorption rates, mixtures of regenerated and fresh adsorbents seem to provide the most cost-effective solution. In order to determine the most effective ratio, this would need to be examined further by both batch and column studies where the specific adsorption capacity and lifespan of each adsorbent combination could be determined.

In addition to the above concerns, the process of regeneration releases the ions and compounds originally removed from the applied WW, thus creating another high strength waste stream requiring disposal and/or treatment. Currently, little research has focused on this regeneration by-product, but alternative treatments can be sought. A common solution is to reload the RL back into the influent LFL however unless other removal processes are employed this action seems unsustainable (Thomas and Crittenden, 1998; Lata *et al.,* 2015). Struvite production, a form of chemical precipitation used for the removal of non-biodegradable organic compounds, ammonium and heavy metals from WW could offer an alternative treatment process. This simple process requires inexpensive equipment and therefore could represent a cost-efficient option (Calli *et al.,* 2005; Blauvelt, 2009). In particular, struvite production is extremely efficient for the removal of high ammonium content from WW (Kurniawan *et al.,* 2006; Renou *et al.,* 2008; Kabdaşl *et al.,* 2009). However, the process is less efficient for the removal of ammonia from LFL due to the lack of magnesium and phosphorus required for the formation of crystalline Magnesium Ammonia Phosphate (MAPs). As such, it may be necessary to supplement LFL with both compounds to overcome this issue, which in turn increases the process cost. However, it is important to note that although this treatment option is effective for the removal of ammonia it is not effective for the removal of heavy metals, and as such remaining effluent will require subsequent processing (Renou *et al.,*2008; Abbas *et al.,*2009; Song and Gao 2013). Furthermore, RL from LFL adsorbents contain a wide range of the toxic compound not just ammonia, phosphate and magnesium, therefore the struvite produced would not be pure and further purification would be needed, which may be costly and time-consuming (Kurniawan *et al.,* 2006; Kabdaşl *et al.,* 2009). As such research is now required to investigate suitable, low-cost treatment options for RL which may include chemical oxidation, air stipping and biological treatment options as outlined in Chapter 1.

Table 7.6. Performance data of the fixed bed system employed for the treatment of landfill leachate.

	Influent Concentration	**A1 Soil with microbes**	**A2 Oyster shells**	**A3 Pumice Stone**	**% Removed**	**B1 Soil**	**B2 Oyster Shells**	**B3 Pumice Stone**	**% Removed**	**EPA DL [b]**
BOD	427.5±1.7	149.2±3.6	97.3±2.5	77.5±3.5	82.024.2	143.6±2.8	87.6±2.3	67.9±1.2	84.5±2.8	≤5
COD	963.6±1.2	423±2.6	327.2±1.2	257.5±7.3	73.38±2.3	432.2±3.4	310.5±2.3	204.1±.5	78.81±2.3	≤40
Ammonia	1057.2±2.3	227.7±1.2	4.36±0.6	2.36±1.4	99.7±1.8	227.52±4.5	2.3±0.5	1.2±0.3	99.8±2.1	≤4
Phosphate	102.4±2.3	4.8±0.6	2.8±0.2	0.5±0.6	99.5±1.2	4.2±.9	0.28±0.06	0.25±0.05	99.75±3.2	≤0.4
Nitrate	527.2±1.3	134±4.2	8.5±1.2	4.23±1.5	99.23±0.25	127.20±3.2	4.2±0.15	3.2±0.12	99.3±1.1	≤50

All results are presented as average $mg.L^{-1}$± standard deviation.

A1 and B1 soil with microbes

A2 and A3 regenerated OS and PS

B2 and B3 fresh OS and PS

Where; [a]PRE is Percentage Removal Rate

[b] DL is Discharge Limit

Table 7.7. The maximum adsorption capacities (q_{eq}.max) for fresh and regenerated materials utilised in the fixed bed column system

	A2 Oyster shells	**B2 Oyster Shells**	**A3 Pumice Stone**	**B3 Pumice Stone**
BOD	0.87	1.05	1.03	1.55
COD	0.78	0.98	0.53	0.79
Ammonia	2.22	2.85	2.73	2.84
Phosphate	0.18	0.29	0.85	0.95
Nitrate	0.86	1.34	1.02	1.53

7.6 Conclusion

Regenerated OS and PS were successfully employed as adsorbents in a fixed bed column trial for the treatment of LFL. The main findings of this study are;

1. A regeneration assay concluded that the regeneration of OS and PS is possible with a range of different chemicals (acids, alkalis and salts).
2. The highest rates of desorption from both OS and PS regeneration assays were achieved by 2M and 1.5M NaOH.
3. The highest q_{eq} values were obtained from batch assays conducted using OS regenerated by 2M NaOH and PS regenerated by 1.5M NaOH.
4. All assays followed the Thomas and Adam-Bohart models and showed little deviation from fresh/ untreated materials.
5. Column studies showed that the longevity of the columns with fresh materials was higher than that of regeneration material.
6. Further to this, regenerated OS and PS performed comparably to fresh control adsorbents in a fixed bed column study which treated actual LFL over a 96 hrs trail period.

In conclusion, the regeneration of spent adsorbent such as OS and PS used for the treatment of LFL offer a method of reusing this potential waste. However, further research is now required in order to determine a solution for the WW by-product that is produced when regenerating adsorbents with alkalis. Further research is also required to optimise the fixed bed columns to achieve discharge limits for all compounds tested over longer time frames, and evaluate their performance under different operational conditions such as pH and temperature while treating different strength LFL.

7.7 Summary

The regeneration assays performed in this study concluded that the regeneration of OS and PS is possible with a range of different chemicals (acids, alkalis and salts), but the highest rates of desorption were achieved by NaOH. Adsorption batch studies conducted using NaOH regenerated OS and PS all followed the Thomas and Adam-Bohart models and showed little deviation from fresh/untreated materials. Further to this, regenerated and fresh control adsorbents were employed in a fixed bed column study for the treatment of LFL over a 96 hrs trail period. Overall the results of this trial indicate that although LFL treatment via regenerated adsorbents was comparable to that of the control (fresh adsorbents) the discharge limit was not maintained for the same time for all compounds, indicating that further optimisation of this process is required.

Chapter 8

Conclusion and Recommendation

8.1 Conclusion

Landfill leachate (LFL) is an environmentally hazardous waste characterised by elevated levels of organic and inorganic compounds. LFL is produced when water percolates through a landfill picking up the by-products of waste degradation. The discharge of this high strength wastewater (WW) to receiving bodies has the potential to cause pollution, in terms of eutrophication and promoting algal blooms (EPA, 2000a; Oram, 2014). Conventional practice involving the treatment of this waste stream in wastewater treatment plants (WWTPs) has been deemed ineffective as it places pressure on these mainly aerobic processes to maintain the required discharge standards set for these facilities (Renou *et al.,* 2008; Teixeirae Junior and Marinheiros, 2014; Brennan *et al.,* 2017b; Lippi *et al.,* 2019). In addition, as a direct consequence of the introduction of landfill regulation and other relevant EU directives (Brennan *et al.,* 2016, 2017a), a dramatic reduction in the number of operational MSW landfill sites has occurred in Ireland. Although welcome, this reduction has led to the production of much stronger LFL at these larger facilities which requires subsequent storage and treatment under the current regulations (Brennan *et al.,* 2016, 2017). In addition, the production of LFL can occur for a substantial period of time after the landfill has been decommissioned (>200 years), as such appropriate aftercare strategies must also be implemented (Wang, 2013). Furthermore, the composition and strength of LFL is subject to change based on numerous factors including; the stage of waste degradation, seasonal variation, and the type of waste being landfilled (Renou *et al.,* 2008, Christensen, 2011, Kamaruddin, 2015). As such, the development of a successful on-site treatment process for LFL should take into consideration the above factors and seek to ultimately lessen the burden of treating this waste stream in conventional wastewater treatment plants (WWTPs), thus benefiting both the environment and the economy.

The main objectives of this research were to develop cost-effective, novel methods for the treatment of LFL on-site within a landfill. This objective was achieved using the combination of biological (bioremediation) and physicochemical (adsorption) treatments. It has been noted in past literature that biological treatment is more beneficial as a pre-treatment, followed by a post physiochemical treatment. The objective of this is for biological treatment to removed biodegradable organics, while physicochemical treatment will focuses on the removal of biorefactroy and organic compounds.

- This was achieved my utilising the power of 15 microbial isolated to reduce some of the main compounds within LFL (Ammonia, phosphate, nitrate, BOD and COD). In particular it identify 15 isolates belong to the phylums of Actinobacteria, Firmicute and Proteobacteria. Bioremediation shows great potential in the treatment of LFL, however, as studies recommend (Renou *et al.*, 2008, Kamaruddin, 2015) biological treatment is more suited to younger leachate. However, it is important to note that additional work is need on the microbial consortia in order to determine the process at play, for the reduction of pollutants within LFL. These microbes show great potential, but by understanding the underlying mechanised, this process could be further enhance, maximising the potential of each isolate.

- In order to have an effective treatment for all types of LFL. For this reason adsorption studies was chosen. It highlighted (Chapter 4) that both oyster shells and pumice stone were effective at adsorbing some of the common compounds within leachate. These material show the potential to treat LFL successfully over large timeframes, however in order to determine their full potential, pore volumes should be taken into account. This wold allows a more accurate determination of the longevity of each column.
- Both treatment options combined into a fixed bed column, showed great potential when utilised on-site within Powerstown landfill. High percentage removal rates and discharge standard were achieving. However, it is important to note in order to deem this treatment suitable, a longer trial should be carried out to assess its ability over longer period of time. Further to this, if this treatment was to be implemented on-site, scale would be needed, taking into account the amount of LFL being produced on a monthly basis. Microbiome data of the microbes within system, shown them to increase in number over trial period. Microbiome analysis should be carried out more often to determine, what other microbes are at play within the system.
- Saturated adsorbent, is a by product of this treatment. Research was carried out to determine if these could be regenerated with the view reuse. This was successful and showed little difference in the longevity of the adsorption columns when treating LFL. However, spent liquid is

regenerated and research is needed in order to determine if it can be used in nutrient recycling.

- Overall, this treatment process, achieved national standard and high percentage removal rates for some of the more problematic compounds within LFL. It shows that both bioremediation and adsorption can be successful in LFL treatment, but are more effective when combined together. This research highlight a cost effective, fixed bed treatment option for LFL, that is suitable for implemented in Irish landfill site. Further work is need and this is discussed in section 8.2 below.

8.2 Recommendations

- The current study of biological and physiochemical treatment of LFL was carried out both in the laboratory and onsite at Powerstown landfill in small scale. As such, the fixed-bed column system now requires a full-scale feasibility study. This trial should fundamentally examine the effectiveness of both the bioremediation and adsorption columns over a longer time period. During this trial, the system should ideally be housed in a modular unit to limit the effect of seasonal variation and rainfall infiltration. This longer trial would also help to ascertain the life span of the individual column units which have only been theorised based on data obtained during the current study. This information is vital, as it would allow landfill operators to determine when and to what extent replacement/regeneration is required within the system.

- The current study examined the removal of ammonia, nitrate, phosphate, and selected heavy metals by the system. This remit should now be extended to determine the fate of inorganic macro compounds, such as magnesium, potassium, sodium, and polychlorinated biphenyl (PCBs) and polycyclic aromatic hydrocarbons (PAHs) which were not included in the scope of this study. In addition, specific process research questions need to be addressed: What is the effect of pH and salt composition on the stability and efficiency of the process? What are the achievable biodegradation and loading rate thresholds? What are the practical cardinal points for organic matter concentration and temperature?

- The current study examined the inoculation of a soil column with 15 microbial isolates capable of bioremediating LFL. It is assumed that these microorganisms are forming biofilm and other microbial community structures within this system, however no information exits to indicate which of these spp. are playing important roles within the bioremediation process and which are being washed out. As such, microbiome analysis of this soil column should be conducted over a longer time period. The results of such analysis would provide more solid evidence for the refinement of this “mastermix” as a potential commercial product.
- Substantial work is also required to access fully the ability of regenerated materials for use in the treatment of LFL. It is essential to determine if these materials can be used solely or if the combination of regenerated and fresh material is a more practical application of these materials. It is also important to investigate the lifespan of the regenerated material; how long will each column last? And how many regeneration cycles could be carried out before the materials are deemed loaded and unsuitable.

- The process of regenerating spent adsorbents, such as those employed for the treatment of LFL (Chapter 4), produces a WW by-product. Proposed treatment options for this waste stream, including the recirculation of WW back into the treatment system and the production of struvite, pose their own challenges. As this waste stream is rich source of nitrogen and phosphorus, it should instead become a target for nutrient recycling technologies. Of specific interest is the recovery of phosphorus, a non-renewable essential requirement for crop growth and thus the sustenance of life on earth. Of particular interest is the process of enhanced biological phosphorus removal (EBPR) which is achieved through the action of polyphosphate accumulating organisms (PAOs). The current system could be redesigned to facilitate an EPBR step for the recovery of phosphorus from this P-rich source.
- Finally, a full cost-benefit analysis of the proposed treatment system against the current aerobic or physio-chemical technologies should be carried out. This should take into account the particular benefits of coupling sustainable bioremediation with pollutant removal, and the absolute requirements for society as a whole to address the pertinent issues of global warming and diminishing fossil fuel reserves.

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Appendix 1

Related to Chapter 2

Microbial sequences of Isolates-Carried out by Eurofins, Wolverhampton, United Kingdom.

LCT- Leachate Tank LCC- Leachate Cell 11

>LCT 10

TTACAATTGTAGCGTGTGAGCATGTGAAAGAGATAGAGAGGAACAGC
AGTGGCGAAGGCGAAGTTCATGCGTCTGTGGGATAATGCTGAGGCGC
GATGCGCGTGGGGAGTGGGGAGCAATCAGATATTAGATGTAGTCCGT
AGTCGTAAACGATGAGTGATAAGTGTTAGGGGGTGTGGCCCCCTTAGT
GTAGCAGCTAACGCATTAAGCAATCCGCCTGGGGAGGGGAGTCGCAA
GACAAGAATTCAAAGGAAAGGACGGGGGGCCGCACAACACAAGGGG
CGGATGATGTAATTTAAATCGACGCGAAGAGCAAAACCAGGTCTAGC
CATGCCATGTATGGAAGTTGAGATATAGTTTAGCGTTCGGGCAAGACG
GTGACAGGTGGGGCATGGCAGTGGTCTGGTCAGGTCGTGTGATGAGA
GGTTAAGTTAAGCAACGAGCGCAGCCCATGCTTTTACATTACATCATT
TAAAAGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGT
GGGGATGACGTCAAATCATCATGCCCCTTATGGCTAGGGCTTCACACG
TCATACAATGGTCGATACAAAGGGTCGCCAACTCGCGAGAGGGAGCC
AATCCCAGAAAGTCGTTCTCAGTTCGGATTGCAGGCTGCAACTCGCTG
CATGAAAGTCGGAATCGCTAGTAATCGCGGATCAGCAGGTCGCGGAA
ACCGGTAAGGGC

>LCT 11

GGGTGTTAACCGCTTTCAGCCGGGAAGAAGCGAAAAGGACGGTACCT
CCAGAAGAAGTACCGGCTAAGTACGTGCCAGCAGCCGCGGTAATACG
TAGGGTACGAGCGTTGTCCGGAATTATTGGCGTAAAAAGCTCGTAGGT
GGTTGGTCACGTCTGCTGTGGAACGCAACGCTTAACGTTGCGCGTGCA
GTGGGTACGGGCTGACTAGAGTGCAGTAGGGGAGTCTGGAATTCCTGG
TGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAG
GCGGGACTCTGGGCTGTAACTGACACTGAGGAGCGAAAGCATGGGGA
GCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCA
CTAGGTGTGGGGGACATTCCACGTTCTCCGCTCCGTAGCTAACGCATT
AAGTGCCCCGCCTGGGGAGTACGGTCGCAAGGCTAAAACTCAAAGGA
ATTGACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGAT
GCAACGCGAAGAACCTTACCAAGGCTTGACATACACTGGACCGTTCTG
GAAACAGTTCTTCTCTTTGGAGCTGGTGTACAGGTGGTGCATGGTTGT
GGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCA
ACCCTCGTTTTATGTGGCCAGCACGTGATGGTGGGAACTCATAGGAGA
CTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAATCATCA
TGCCCTTTATGTCTTGGGCTTCACGCATGCTACAATGGCTGGTACAGAG
AGAGGCGAACCCGTGAGGGTAAGGGAATCCCTTAAAGCCAGTGTCAG
TTCGGATCGTAGTCTGCAATTTGACTACGGAAGTCGGAGTCGCTAGTA
ATCGCAGTTCAGCAACGGGGCGGCTCGGCATAAGGGCTTA

>LCT 12

CCCGCGTTAACTTCGTGGCGGCAGCCGCGGTTTTACGTAAGGGGGCGT
AGAATAGCTCGCGAATACTGGGTTTAAAGGGAGAGTAGATATGATGG
CTCGTCAGAGGTGAAATCTCAGGGCTCAACCGACGAAGTGTATTAAGA
GGGGAGATGTAGAGTGTGAGAGAGAGTATATGGAACTCGGATATGAA
CAGGAGCAACTCGGAGATAGTCGGAAGAACACCACGATAGAAGGCTC
CATATTGCGATCATTTGGGGCGCAAACAGGTGAAATATGTGGGGAGCT
TACACGATTAGATACCATGAGTAGTACACGCCGTAAACGATGCTCTTT
AGTGGTGCGGCTGCATGCAGTTCCATTCCGCAGGGGGAGCACTATGCC
ATCATTAAAAGGAATACGATTGCAAGGGGGAAAGTACAAGGAATTGA
CGGGGTGGTGCACAAGGGGGGGAGCAGGAGGTCTAATTCGAAGCAAC
GCGCAGAACCTTATCCCATTTGGGCCTGCCTGGACCTCCCCGGGGATG
GGGCTCTGCTTTCGCGGGGTAGTACTCAGGTGCTGCATGGAGATGGTC
AGATAAGGTCGGGAGAGAGTGCATTAAGTCCCGCAACGAGCCCGCAC
CTAGCCAGTATTTGCCATCAGATAGTCAGGAATTATAATGAGGAAGCC
GGGATAATTCGAGGAAGCATGGGCTGACCTCAAGTGGTCACGCCCCTT
ACACAGTGGGGTACACAGGGACACAATGGCGGGGGGGTAGACCTAAT
CCTTAAAAGTCGTCTCAGTTCGGATTGCACTCTGCAACTCGAGGGCAT
GAAGTTGGAATCGCTAGTAATCGCGGATCAGCAGCCGCCGGAATGCG
CTTGGGT

>LCT22

TCGCGGTTATACTTAGTGGCTAGGGTATCCGGAATTTTGGAGTAAGGG
GGCCACGGGGTTTATTAGTTTGATGGAAAGCCTCGGCTCAACGTGGAG
GGTCATTGAAAGTGGAGACTGAGTGCAGAAGAGGAATGTGAACTGCA
TGTGTAACGGTGAAATCGTAGAGATATGAGGATCACCAGTGAGAAGA
TGACTTTGTGGTCTGTAATTACAGTGAGGCGCGAAAGCGTGGGAGCAA
ACAGGAGTAGATACCCTGGTAGTGCACGCTGTAAACGATGAGTGTTAA
GTGATAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTTTGCATTAAGCACT
CCGCTTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACG
GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGC
GAAGAACCTTACCAGGTCTTGACATCCTCTGAAAACCCTAGAGATAGG
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BLASTn Results

Table A1. 1 BLASTn results of each sequences used. This search was carried out on 12.10.2017.

Isolate	Species ID	Percentage Identity	E value
LCT 10	*Lysinibacillus sphaericus* strain Pp10	92	7.00E-137
LCT11	*Brevibacterium* sp. strain IM9603	95	7.00E-90
LCT12	*Bruvdimonas diminutas* strain b46	86	1.00E-90
LCT22	*Bacillus thuringienis*	93	0.00E+00
LCT24	*Lysinibacillus fusiformis* strain RSNPB4	92	0.00E+00
LCT26	*Bacillus subtilis strain B29*	97	0.00E+00
LCT33	*Brevibacterium linens* strain AE038-8	98	0.00E+00
LCT42	*Bacillus vietnamensis* strain CQN-26	98	0.00E+00
LCT43	*Bacillus cereus* strain B3Vac	95	0.00E+00
LCT48	*Brevibacterium* sp. RMD 3Y-15-4	95	0.00E+00
LCC18	*Bacillus cereus* strain HB45	89	0.00E+00
LCC19	*Brevibacterium casei* strain SRKP2	89	7.00E-86
LCC29	*Brevibacterium siliguriense* strain DSM 23676	95	2.00E-75
LCC31	*Brevundimonas naejangsanensis* strain B1	90	0.00E+00
LCC32	*Brevibacterium* sp. Tc3-19	91	7.00E-86

Morphology test of isolates

Table A1. 2 Morphology test on the 15 selected strains used for bioremediation throughout each chapter.

Strain ID	Gram stain	Oxidase	Catalase
LCT 10	Positive	Positive	Positive
LCT11	Positive	Positive	Positive
LCT12	Negative	Positive	Positive
LCT22	Positive	Positive	Positive
LCT24	Positive	Positive	Positive
LCT26	Positive	Positive	Positive
LCT33	Positive	Positive	Positive
LCT42	Positive	Positive	Positive
LCT43	Positive	Positive	Positive
LCT48	Positive	Positive	Positive
LCC18	Positive	Positive	Positive
LCC19	Positive	Positive	Positive
LCC29	Positive	Positive	Positive
LCC31	Negative	Positve	Positive
LCC32	Positive	Positive	Positive

Table A1. 3 Additional information in relation to the species that were identified occurring to the BLASTn searches from table A1.1

Species	Morphology and other information	References
Lysinibacillus sphaericus	Gram-positive Rod-shaped bacterium Found in soil and aquatic environments Mesophilic, Uses organic and amino acids as carbon sources Shows potential to remediated total petroleum hydrocarbons (TPH) in contaminated soil. It has been found that these strains exhibit other unexploited biotechnological important traits, such as lactonases (quorum quenching), toxic metal resistance, and potential for aromatic compound degradation Genomic evidence suggests the resistance to arsenic, copper, tellurium, and molybdenum as well as we found genes involved in cobalt and nickel metabolism for cofactors and vitamin biosynthesis.	(Manchola and Dussán 2014; Rahman *et al.*,2014; Gomez-Garzon *et al.*,2017)
Lysinibacillus fusiformis	Gram-positive Rod-shaped bacterium Found in soil and aquatic environments Non-capsulated and motile with peritrichous flagella Oxidase positive and is an obligate aerobe Has the ability to remediate heavy metals, such as arsenic Phosphate solubilises and ammonia oxidizers Under harsh conditions, they can form dormant endospores that are resistant to heat chemicals and ultraviolet light.	(Liang *et al.*,2009; He *et al.*,2011; Mohamed and Farag 2015; Sharma and Saharan 2015; Mathivanan *et al.*,2016)

Bacillus vietnamensis	Gram-positive Found in marine environments Motile with peritrichous flagella Endospore-forming, Moderately halotolerant bacteria It shows the ability for the degradation of heavy metals, in particular arsenic It has the potential to degrade Lindane is an organochlorine pesticide belonging to persistent organic pollutants that has been widely used to treat agricultural pests	(Noguchi *et al.*,2004; Loredana *et al.*,2017; Upadhyay *et al.*,2018)
Bacillus thuringienis	Gram-positive, Soil-dwelling bacterium, Commonly used as a biological pesticide Degrades PAHs like fluoranthene and pyrene It was found to be resistant to zinc, copper, cobalt, and cadmium and nickel. Shows the potential to degrade Chlorpyrifos and 3,5,6-trichloro-2-pyridinol	(Ibrahim *et al.*,2010; Maiti *et al.*,2012; Das *et al.*,2014; Aceves-Diez *et al.*,2015)
Bacillus subtilis	Gram-positive Rod-shaped Found in soil Catalase-positive bacterium Can from protective endospore, allowing it to tolerate extreme environmental conditions Facultative anaerobe Heavily flagellated, which gives it the ability to move quickly in liquids Has the ability for crude oil degradation and accelerated the biodegradation of the aliphatic hydrocarbons. Shows the ability to remediate heavy metals from wastewater such as, copper, arsenic and cadmium.	(Cubitto *et al.*,2004; Sulaimon *et al.*,2014; Sakthipriya *et al.*,2015; de Alencar *et al.*,2017)

Bacillus cereus	Gram-positive Rod-shaped They are commonly found in soil Aerobic and facultatively anaerobic, Motile They can produce protective endospores Beta-hemolytic bacterium Has the potential to degrade heavy metals, such as copper and mercury Efficient at decolorizing wastewater effluents These bacteria also show the ability to reduce COD and BOD in wastewater. Shows the potential to remediate petroleum wastewater in particular they show the ability to biodegradate diesel, kerosene, crude oil and used engine oil. They have the tool for bioremediation of toxic hydrocarbons and to keep the environment free from PAH pollutants, such as anthracene.	(Ryan *et al.*,2004; Borah and Yadav 2014; Saleem *et al.*,2014; Dash and Surajit 2015; Banerjee and Ghoshal 2017; Rohini and Jayalakshmi 2017; Bibi *et al.*,2018)
Brevibacterium linens	Gram-positive Rod-shaped bacterium, however it exhibits a rod coccus shape on the media but as the cells enter stationary phase they become coccoid shaped They are found in soil They are non-motile and aerobic Halo-tolerant and non-spore forming Mesophilic Catalase positive and oxidative towards sugars They can produce and utilize siderophores Capable of the degradation of heavy metals, in particular capable of reducing As(V) to As(III)	(Noordman *et al.*,2006; Shabbiri *et al.*,2013; Maizel *et al.*,2015; Maizel *et al.*,2016; Zhu *et al.*,2016)

Brevibacterium casei	Gram Positive Rod Shaped Commonly found in soil and sewage Obligate aerobes Catalase positive and oxidase negative This strain shows the ability to bioremediate chromate and pentachlorophenol This strain is tolerant and bioremediate to heavy metals They also have the ability to promote plant growth and enhance Cd, Zn, and Cu uptake They are able to reduce Chromate and pentachlorophenol from wastewater tanneries. They show the ability to biodegradate polycyclic aromatic hydrocarbons, such as five and six membered ring compounds, benzo[b]fluoranthene and indenol[1,2,3-cd] Pyrene They show potential for the bioremediation of soil contaminated with crude oil	(Kerr 2003; Farahat and El-Gendy 2008; Verma and Singh 2013; Płociniczak *et al.*,2016)
Brevibacterium siliguriense	Gram-positive Rod-shaped Found in water and soil Facultatively oligotrophic Non-motile and non-spore forming Catalase-positive and oxidase-negative Limited studies have been carried out on this isolate	(Kumar *et al.*,2013)
Bruvdimonas diminutas	Gram-negative Rod Shaped Actively motile with a single polar flagellum Can survive in a wide variety of environments including different water sources Oxidase and catalase positive Does not ferment any carbohydrates and shows no haemolysis activity It oxidizes ethanol to acid	(Han and Andrade 2005; Guermouche M’rassi *et al.*,2015; Singh *et al.*,2016; Wang *et*

	The bacterium has been used as a potential bioremediator of marine oil pollution including diesels, n-alkanes and polycyclic aromatic hydrocarbons and insecticides. It has also been used to mitigate the toxic effects of heavy metals on plant growth in contaminated soils. It also possesses the ability to survive sanitisers such as Hydrogen Peroxide + Peracetic Acid	*al.*,2016a; Ryan and Pembroke 2018)
Brevundimonas naejangsanensis	Gram-negative Rod-shaped Found in both soil and water Anaerobic bacteria Motile by means of a single polar flagellum Oxidase and catalyse positive Like *Brevibacterium siliguriense* limited studies have focused on their bioremediation potential.	(Song *et al.*,2009; Ryan and Pembroke 2018)

Appendix 2

Related to Chapter 3

Table A2 1. Column adsorption isotherm for the effects of bed height

Parameter	Column	q_{eq} [a]	Thomas model k_{TH} [b]	q_o [c]	R^2	Error Model HYBRID	MPE	k_{AB}[d]	Adam-Bohart N_o[e]	R^2	Error Model HYBRID	MPE
Ammonia	OS 1	1.36	0.04	2.02	0.85	2.14	-8.25	0.03	2.08	0.97	2.25	-8.65
	OS 2	1.85	0.04	2.15	0.94	1.98	-7.52	0.03	2.19	0.96	2.08	-7.84
	OS 3	2.35	0.05	2.45	0.98	0.96	-4.25	0.03	2.35	0.97	1.01	-4.46
	P 1	1.24	0.03	1.97	0.89	3.15	-7.25	0.05	2.73	0.96	3.31	-7.61
	P 2	1.65	0.04	2.05	0.96	2.36	-7.32	0.04	2.83	0.95	2.48	-7.63
	P 3	2.04	0.04	2.13	0.95	0.81	-4.25	0.03	3.01	0.97	0.85	-4.46
Nitrate	OS 1	1.44	0.04	2.14	0.9	2.27	-8.75	0.03	2.2	1.03	2.39	-9.17
	OS 2	1.96	0.04	2.28	1	2.1	-7.91	0.03	2.32	1.02	2.2	-7.25
	OS 3	2.49	0.05	2.6	1.04	1.02	-4.51	0.03	2.49	1.03	1.07	-4.73
	P 1	1.31	0.03	2.09	0.94	3.34	-7.69	0.05	2.89	1.02	3.51	-8.07
	P 2	1.75	0.04	2.17	1.02	2.5	-7.7	0.04	3.23	1.01	2.63	-7.03
	P 3	2.16	0.04	2.26	1.01	0.86	-4.51	0.03	3.19	1.03	0.9	-4.73
Phosphate	OS 1	1.53	0.04	2.27	0.96	2.4	-9.27	0.03	2.34	1.09	2.53	-9.72
	OS 2	2.08	0.04	2.42	1.06	2.22	-7.33	0.03	2.46	1.08	2.34	-7.69
	OS 3	2.64	0.06	2.75	1.1	1.08	-4.78	0.03	2.64	1.09	1.13	-7.01
	P 1	1.39	0.03	2.21	1	3.54	-8.15	0.06	3.07	1.08	3.72	-8.55
	P 2	1.85	0.04	2.3	1.08	2.65	-7.1	0.04	3.18	1.07	2.79	-7.45
	P 3	2.29	0.04	2.39	1.07	0.91	-4.78	0.03	3.38	1.09	0.96	-7.01

Where;

[a] mg.g^{-1},[b] mg.min^{-1},[c] mg.g^{-1},[d] l.mg.min^{-1},[e] mg.L^{-1}

Table A2 2 Column adsorption isotherm for the effect of flow rate

Parameter	Column	q_{eq} [a]	Thomas model k_{TH} [b]	q_o [c]	R^2	Error Model HYBRID	MPE	k_{AB} [d]	Adam-Bohart N_o [e]	R^2	Error Model HYBRID	MPE
Ammonia	OS 4	2.49	0.06	2.55	0.99	1.46	-3.41	0.03	2.65	0.99	1.43	-3.97
	OS 5	2.25	0.05	2.45	0.96	3.02	-7.23	0.03	2.35	0.98	2.96	-7.09
	OS 6	1.78	0.05	1.83	0.87	3.26	-7.62	0.03	2.12	0.99	3.2	-7.7
	P 4	2.48	0.05	2.59	0.97	1.07	-3.41	0.03	3.41	0.99	1.38	-3.38
	P 5	2.04	0.05	2.13	0.98	3.13	-7.07	0.04	3.01	0.97	4.01	-7.03
	P 6	1.65	0.04	1.78	0.91	4.17	-7.82	0.05	2.98	0.98	7.36	-7.77
Nitrate	OS 4	2.54	0.06	2.6	1.01	1.49	-3.48	0.03	2.7	1.01	1.46	-4.05
	OS 5	2.3	0.05	2.5	0.98	3.08	-7.34	0.03	2.4	1	3.02	-7.21
	OS 6	1.82	0.05	1.87	0.89	3.33	-7.76	0.03	2.16	1.01	3.27	-7.86
	P 4	2.53	0.05	2.64	0.99	1.09	-3.48	0.03	3.48	1.01	1.41	-3.45
	P 5	2.08	0.05	2.17	1	3.19	-7.17	0.04	3.07	0.99	4.09	-7.13
	P 6	1.68	0.04	1.82	0.93	4.26	-7.94	0.05	3.04	1	7.47	-7.89
Phosphate	OS 4	2.59	0.06	2.66	1.03	1.52	-3.55	0.03	2.76	1.03	1.49	-4.13
	OS 5	2.34	0.05	2.55	1	3.14	-7.45	0.03	2.45	1.02	3.08	-7.34
	OS 6	1.85	0.05	1.91	0.91	3.39	-7.89	0.03	2.21	1.03	3.33	-8.02
	P 4	2.58	0.05	2.7	1.01	1.11	-3.55	0.03	3.55	1.03	1.44	-3.52
	P 5	2.12	0.05	2.22	1.02	3.26	-7.28	0.04	3.13	1.01	4.18	-7.24
	P 6	1.72	0.04	1.85	0.95	4.34	-7.06	0.05	3.1	1.02	7.58	-7.01

Where;

[a] $mg.g^{-1}$, [b] $mg.min^{-1}$, [c] $mg.g^{-1}$, [d] $l.mg.min^{-1}$, [e] $mg.L^{-1}$

Appendix 3

Related to Chapter 5

Table A3. 1 BLASTn results of microbiome analysis, to search for the present of the know isolates before and after treatment onsite. This was carried out using BLASTn function of Bio-Linux 8, with Blast-able databased made from the microbiome results (Carried out by Novogene Co LTD.) These results are isolates that matched with the original isolates with a percentage identity ≥ 99% and E-values of 0.00.

Isolate ID	A1 Pre-Treatment	A1-Post-Treatment	B2 Pre-Treatment	B2 Post-Treatment
LCT 10	C1_0256, C1_0002, C1_2156, C1_0321, C1_1256, C1_0265, C1_0056, C1_0887, C1_3214	C2_0065, C2_0029, C2_0106, C2_0109, C2_2006, C2_1452, C2_3125, C2_0008, C2_0105, C2_0109, C2_2354, C2_0415, C2_0687, C2_1985, C2_1256, C2_1365, C2_1478, C2_2563, C2_2456, C2_2145,	C3_256, C3_0023	C4_1126, C4_0502, C4_0046, C4_1270, C4_4308, C4_1926,
LCT 11	C1_ 0045, C1_0873, C1_1256, C1_2136, C1_3215, C1_0854, C1_0654	C2_0303, C2_0015, C2_0053, C2_0055, C2_1003, C2_1002, C2_1563, C2_0004, C2_0153, C2_0186, C2_1174, C2_0206, C2_3485, C2_2156, C2_3265	C3_802	C4_0050, C4_0126, C4_2512, C4_0082, C4_0632, C4_0512, C4_5970, C4_1738, C4_1992
LCT 22	C1_1254, C1_0758, C1_0145, C1_2415, C1_3123, C1_0086, C1_0635, C1_0025, C1_2156	C2_0875, C2_0099, C2_0045, C2_0632, C2_1258, C2_1986, C2_2986, C2_2365, C2_124, C2_4215, C2_3265, C2_2158, C2_3124, C2_0563, C2_0215,	C3_002, C3_0236	C4_1689, C4_0753, C4_0069, C4_1905, C4_6462, C4_2889,

Isolate ID	A1 Pre-Treatment	A1-Post-Treatment	B2 Pre-Treatment	B2 Post-Treatment
LCT 24	C1_0242, C1_0321, C1_0562, C1_0548, C1_1587, C1_2564, C1_3265, C1_0034	C2_0130, C2_0058, C2_0212, C2_0218, C2_4012, C2_2904, C2_2156, C2_0016, C2_0210, C2_0218, C2_4708, C2_0830, C2_1374, C2_3970, C2_2512, C2_2730, C2_2956, C2_2956, C2_4012		C4_3768, C4_0136, C4_0963, C4_0765, C4_0896, C4_2607,
LCT 26	C1_0476, C1_0635, C1_1478, C1_3246, C1_0026, C1_0048, C1_0685, C1_0785, C1_0863, C1_2145	C2_0026, C2_0023, C2_3652, C2_2561, C2_00472, C2_0050, C2_0006, C2_0912, C2_0432, C2_2956, C2_2635, C2_2153, C2_2361, C2_2365, C2_3125	C3_0569	C4_2508, C4_2156, C4_0326, C4_0825, C4_0802, C4_0472
LCT 33	C1_0064, C1_0086, C1_0347, C1_0534, C1_932, C1_1423, C1_2436, C1_3002, C1_3006, C1_3145, C1_3489	C2_0033, C2_0015, C2_0053, C2_0055, C2_1003, C2_1002, C2_1563, C2_0004, C2_0153, C2_0086, C2_1174, C2_0206	C3_2156	, C4_1850, C4_0112, C4_0168, C4_2592, C4_2490, C4_4730,

Isolate ID	A1 Pre-Treatment	A1-Post-Treatment	B2 Pre-Treatment	B2 Post-Treatment
LCT 42	C1_0241, C1_0003, C1_0016, C1_0025, C1_0087, C1_3056 C1_0387, C1_0698 C1_1487, C1_2687	C2_0260, C2_0116, C2_0424, C2_0436, C2_0256, C2_0526, C2_2154, C2_0032, C2_0420, C2_0436, C2_0416, C2_1660, C2_2748, C2_3265, C2_1254, C2_2653, C2_4586, C2_2156		C4_0178, C4_0013, C4_0018, C4_0282, C4_1078, C4_1562C4_1126, C4_0502, C4_0046, C4_1270, C4_4308, C4_1926
LCT 43	C1_0021, C1_0048, C1_0063, C1_0543, C1_0621, C1_0869 C1_1473, C1_2361	C2_0365, C2_0215, C2_0236, C2_2166, C2_2369, C2_0986, C2_00857, C2_0856, C2_0365, C2_0256, C2_0014, C2_0056, C2_0089, C2_0096, C2_0087		C4_3762, C4_0786, C4_0963, C4_0125, C4_0120, C4_0708, C4_0075, C4_00189
LCT 48	**C**1_0356 C1_25 C1_0036 C1_0564 C1_2156 C1_3125 C1_1425 C1_0036 C1_968 C1_2456 C1_3125	C2_0563, C2_0251, C2_0023, C2_0635, C2_2154, C2_0963, C2_1254 ,C2_2563 ,C2_3265 ,C2_4123 ,C2_4005 ,C2_0236 ,C2_0025 ,C2_0063		C4_2988, C4_2775, C4_0168, C4_0252, C4_3888, C4_3735, C4_0095, C4_0045, C4_0052,
Isolate ID	**A1 Pre-Treatment**	**A1-Post-Treatment**	**B2 Pre-Treatment**	**B2 Post-Treatment**

LCC 18	C1_0178, C1_0013, C1_0018, C1_0282, C1_1078, C1_1562, C1_0713, C1_0018, C1_0484, C1_1228, C1_1092, C1_0712, C1_0052, C1_0072, C1_1128	C2_1256, C2_4125, C2_3265, C2_2589, C2_2985, C2_0869, C2_0996 ,C2_0925 ,C2_0056 ,C2_0084 ,C2_1296 ,C2_1245 ,C2_2365 ,C2_3215 ,C2_4752 ,C2_1232 ,C2_1456 ,C2_0236 ,C2_0215		C4_0430, C4_0965, C4_2563, C4_2912, C4_0472, C4_0430
LCC 19	C1_296, C1_3265, C1_0021, C1_0536, C1_2212, C1_2999, C1_1987, C1_0999, C1_0942, C1_0936, C1_1278, C1_0074, C1_0816, C1_0005, C1_1341, C1_0553, C1_0749 C1_0496, C1_0249, C1_0238	C2_1126, C2_0502, C2_0046, C2_1270, C2_4308, C2_1926, C2_2508, C2_2156, C2_0326, C2_0825, C2_0802, C2_0472, C2_0050, C2_1026, C2_2512	C3_0056, C3_2563	C4_3696, C4_0063, C4_0708, C4_0645
LCC 29	C1_0526, C1_0263, C1_2150, C1_2002, C1_2015, C1_3125, C1_0065, C1_0856, C1_0958, C1_0963, C1_0456	C2_0082, C2_0632, C2_0512, C2_5900, C2_1738, C2_1992, C2_1850, C2_0112, C2_0168, C2_2592, C2_2490, C2_4730, C2_6430, C2_0965, C2_2563, C2_2912, C2_4072, C2_0430	C$_005, C3_526, C3_452	,C4_0925 ,C4_0056 ,C4_0084 ,C4_1296 ,C4_1245 ,C4_2365 ,C4_3215 ,C4_4752
Isolate ID	**A1 Pre-Treatment**	**A1-Post-Treatment**	**B2 Pre-Treatment**	**B2 Post-Treatment**

LCC 31	C1_0126, C1_0236, C1_1789, C1_1546, C1_2786, C1_3056, C1_3004, C1_3125, C1_0087, C1_0046, C1_0236, C1_0415, C1_0687, C1_1985, C1_1256, C1_1365, C1_1478	C2_1689, C2_0753, C2_0069, C2_1905, C2_646, C2_2889, C2_3762, C2_0786, C2_0963, C2_0125, C2_0120, C2_0708, C2075, C2_0189, C2_3768		C4_2156, C4_0321, C4_1256, C4_0265, C4_0056, C4_0887, C4_0018, C4_0282, C4_1078, C4_1562, C4_0713, C4_0018
LCC 32	C1_0065, C1_0029, C1_0106, C1_0109, C1_2006, C1_1452, C1_3125, C1_0008, C1_0105, C1_0109, C1_2354	C2_0136,C2_0963, C2_0765 ,C2_0896, C2_2607 ,C2_2988 C2_2775 ,C2_0168, C2_0252 ,C2_3888, C2_3735 ,C2_0095, C2_0045 ,C2_0052, C2_3696 ,C2_63 , C2_0708 ,C2_0645		C4_0109, C4_2006, C4_1452, C4_3125, C4_0008, C4_0105, C4_0109, C4_2354, C4_0415, C4_0632, C4_1258, C4_1986, C4_2986, C4_2365

Methods for BLAST-able database.

Command line code:

- The FASTA files for each microbiome were formatted. This was carried out using the command prompt from the terminal on the Bio-Linux 8 software The command that was used is:

```
formatdb -i {foldername}/{genomefilename}
```

- After this was completed there were files in each folder with the following endings:

```
{filename}.pro~

{filename}.pro.pin

{filename}.pro.phr

{filename}.pro

{filename}.pro.psq
```

- Isolates sequences were blasted I the raw microbiome file . This was achieved by entering the following:

```
Blastn –query ./{protein filename}.pro –db ./{foldername}/{genomefilename}.pro
```

- The results were then displayed and saved. This was achieved by inputting the following command:

```
blastp –query./{protein filename}.pro –db ./ {foldername}/{genomefilename}.pro –
out{filename}.odt
```