Two - Stage AnMBR for Removal of UV-Quenching Organic Carbon from Landfill Leachates: Feasibility and Microbial Community Analyses

Ankit Pathak

Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy
In
Civil Engineering

Amy J. Pruden
John T. Novak
Zhen He
Charles D. Goldsmith

January 17, 2017

Blacksburg, Virginia

Keywords: anaerobic membrane bioreactor (AnMBR), landfill leachate, wastewater treatment, UV disinfection, UV absorbance, humic substances, dissolved organic matter (DOM), anaerobic treatment, DNA sequencing, antibiotic resistance genes (ARGs)

Two - Stage AnMBR for Removal of UV Quenching Organic Carbon from Landfill Leachates: Feasibility and Microbial Community Analyses

Ankit Pathak

ABSTRACT

Landfilling is the most widely used method for the disposal of municipal solid wastes (MSW) in the United States due to its simplicity and low cost. According to the 2014 report on Advancing Sustainable Materials Management by the USEPA, only 34% of the total MSW generated in the US was recycled, while 13% was combusted for energy recovery. In 2014, 53% of the MSW generated, (i.e. 136 million tons) in the US was landfilled. The treatment of landfill leachates, generated by percolation of water through the landfill, primarily due to precipitation, has been found to be one of the major challenges associated with landfill operation and management. Currently, leachates from most landfills are discharged into wastewater treatment plants, where they get treated along with domestic sewage.

Issues associated with treatment of landfill leachates due to their high nitrogen and heavy metal content have been widely studied. Recently, it has been observed that the organic carbon in landfill leachates, specifically humic and fulvic acids (together referred to as "humic substances") contain aromatic groups that can absorb large amounts of ultraviolet (UV) light, greatly reducing the UV transmissivity in wastewater plants using UV disinfection as the final treatment step. This interference with UV disinfection is observed even when landfill leachates account for a very small fraction (of the order of 1%) of the total volumetric flow into wastewater treatment plants. Humic substances are present as dissolved organic matter (DOM) and typically show very low biodegradability. Removing these substances using chemical treatment or membrane processes is an expensive proposition. However, the concentrations of

humic substances are found to be reduced in leachates from landfill cells that have aged for several years, suggesting that these substances may be degraded under the conditions of long-term landfilling.

The primary objective of this research was to use a two-stage process employing thermophilic pretreatment followed by a mesophilic anaerobic membrane bioreactor (AnMBR) to mimic the conditions of long-term landfilling. The AnMBR was designed to keep biomass inside the reactor and accelerate degradation of biologically recalcitrant organic carbon such as humic substances. The treatment goal was to reduce UV absorbance in raw landfill leachates, potentially providing landfills with an innovative on-site biological treatment option prior to discharging leachates into wastewater treatment plants. The system was operated over 14 months, during which time over 50% of UV-quenching organic carbon and 45% of UV absorbance was consistently removed. To the best of our knowledge, these removal values are higher than any reported using biological treatment in the literature. Comparative studies were also performed to evaluate the performance of this system in treating young leachates versus aged leachates.

Next-generation DNA sequencing and quantitative PCR (qPCR) were used to characterize the microbial community in raw landfill leachates and the bioreactors treating landfill leachate. Analysis of microbial community structure and function revealed the presence of known degraders of humic substances in raw as well as treated landfill leachates. The total numbers of organisms in the bioreactors were found to be higher than in raw leachate. Gene markers corresponding to pathogenic bacteria and a variety of antibiotic resistance genes (ARGs) were detected in raw landfill leachates and the also in the reactors treating leachate, which makes it necessary to compare these ARG levels with wastewater treatment in order to determine if leachates can act as sources of ARG addition into wastewater treatment plants. In addition, the high UV absorbance of leachates could hinder the removal of ARBs and ARGs by UV disinfection, allowing their release into surface water bodies and aiding their proliferation in natural and engineered systems.

Two - Stage AnMBR for Removal of UV Quenching Organic Carbon from Landfill Leachates: Feasibility and Microbial Community Analyses

Ankit Pathak

GENERAL AUDIENCE ABSTRACT

Municipal solid waste is most often disposed by dumping it in landfills. Percolation of water through these landfills due to precipitation or the intrusion of surface or groundwater, results in the formation of landfill leachate, a mixture of organic and inorganic contaminants, at the bottom of the landfill. Landfills are therefore lined with special materials to prevent leachate from seeping into soil or groundwater and have sophisticated collection systems to periodically extract and dispose leachate.

Perhaps the most commonly used method for the disposal of landfill leachates is discharge into wastewater treatment plants, where leachates can cause toxicity to biological processes due to their high organic load as well as their substantial heavy metal content. In the last decade or so, it has been established that leachates can absorb UV light considerably by virtue of aromatic organic compounds present in them, causing inhibition of UV disinfection in wastewater treatment. Thus, leachates must be appropriately treated to reduce their capacity to absorb UV light prior to discharge into wastewater treatment plants.

This study employed a novel two – stage reactor system to treat landfill leachates in order to reduce their UV-quenching ability. The system was successfully operated over 14 months and was able to remove more than half of the UV light absorbing organic carbon from landfill leachate. Additionally, samples of biomass isolated from untreated landfill leachates and the reactors treating them revealed the potential presence of pathogenic bacteria and antibiotic resistance genes. Preliminary data suggests that landfill leachates might have large antibiotic resistance content, higher than that observed in wastewater and other engineered systems.

DEDICATION

This dissertation is dedicated to the memory of my uncle, Vinit S. Pathak, who encouraged me to follow my passion and made me realize that loving my work was the easiest way to be good at it. You continue to shape the person I am to this day, and I hope I never let you down.

I also dedicate it to my grandparents, Sarla P. Trivedi and Prataprai H. Trivedi, both of whom passed away in late 2016. Thank you both for 25 years full of beautiful memories.

ACKNOWLEDGEMENTS

This work was funded via account number 443357 at Virginia Tech by the Environmental Research and Education Foundation (EREF), an organization dedicated to promote research in the field of solid waste management.

I would like to thank my advisers, Dr. Amy Pruden and Dr. John Novak. Working with both of them has been a privilege. I have learned more from them than any class has ever been able to teach me, and I am very grateful for their approachability and promptness, especially during these last few months, when time was of essence. I would like to express my heartfelt appreciation for the insights and inputs provided by my PhD committee members Dr. Zhen He and Dr. C. D. Goldsmith. Having them on my committee has proven to be invaluable. I would also like to thank Dr. Gregory D. Boardman, who served on my committee through most of the duration of my PhD but had to drop out towards the end.

I want to acknowledge my colleagues in the Pruden Lab, for their support and friendship. In particular, I would like to thank Pan Ji and Emily Garner for spending hours of their time helping an engineer like me find my bearings in the field of metagenomics and DNA sequencing. I also want to thank Dongjuan Dai, who helped and is continuing to help with analyzing the data obtained from DNA sequencing, and is listed as a co – author on two manuscripts. I sincerely thank Jody Smiley, Jeff Parks and Julie Petruska for training me in all the physico – chemical analyses. Their professionalism and expertise in the lab is second to none and their diligence is exemplary.

I want to thank my parents and my brother for being the best support system anyone could hope for. Having a family that understands and unconditionally supports me is a gift I do not take for granted. Finally, over the course of my time at Virginia Tech, I developed enduring friendships and relationships, and I thank every friend, acquaintance, and colleague that made my 5 years in Blacksburg, Virginia more memorable than I could have ever thought possible.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER 3

CHAPTER 4

CHAPTER 5

APPENDICES

LIST OF TABLES

ATTRIBUTION

Each author is duly credited for her / his contribution to the work. Dr. Amy Pruden served as principal investigator on this project, while Dr. John Novak served as the co – principal investigator.

Amy Pruden, PhD.
W. Thomas Rice Professor and Associate Dean of the Graduate School
Civil and Environmental Engineering
Virginia Polytechnic Institute and State University. Blacksburg, VA 24061
Coauthor: Chapter 3, 4, 5, Appendix A

John T. Novak, PhD.
Professor Emeritus
Civil and Environmental Engineering
Virginia Polytechnic Institute and State University. Blacksburg, VA 24061
Coauthor: Chapter 3, 4, 5, Appendix A

Dongjuan Dai, PhD.
W. Thomas Rice Professor and Associate Dean of the Graduate School
Civil and Environmental Engineering
Virginia Polytechnic Institute and State University. Blacksburg, VA 24061
Coauthor: Chapter 4,5

CHAPTER 1

Introduction

BACKGROUND

According to the US Environmental Protection Agency (USEPA), in 2013, the United States generated over 254 million tons of Municipal Solid Waste (MSW). This waste generation corresponded to roughly 4.4 pounds of solid waste per person per day, of which only 1.51 pounds per person per day was recycled / composted. Over 134 million tons of waste is being disposed of in landfills while 33 million tons is incinerated according to the USEPA report on Advancing Sustainable Materials Management (2014). Despite an emphasis on reducing, reusing and recycling solid waste, the amount of municipal waste being put into landfills in 2013 was higher than it had been in 3 years. In the same report, the EPA estimates that there are 1,908 active landfills in the US. While the number of landfills has been reduced over the last decade, there is an increase in the average area occupied by individual landfills. This dependence on landfills results in the generation of large quantities of landfill leachate, a solution formed by rainwater percolating through the landfill or by water intrusion into the landfill (USEPA, 2005). It is estimated that every cubic meter of waste put into a landfill generates about 0.2 m^3 of landfill leachate (Kurniawan *et al.*, 2009). Precipitation is usually in the biggest driver of leachate formation, although the quantity and nature of leachate generated in a landfill can be a function of several parameters including the water content of the wastes, location of the landfill, climatic conditions and also the temperature and pressure within the landfills by virtue of the landfill design and the kinds of wastes put into them (Renou *et al.*, 2008).

Landfill leachate can pollute soil and water bodies in the vicinity of a landfill and also cause odor problems. Landfills, therefore, must have liners to prevent leachate percolation into the surrounding soil and pumps and collection systems for periodically extracting leachate from the depths of the landfill (kurniawan *et al.*, 2006). The leachate that is removed from the landfill then needs to be treated and disposed. Several methods for leachate treatment and disposal exist.

While on – site biological treatment in lagoons, constructed wetlands or engineered systems followed by direct discharge into streams, rivers and other surface waters is practiced, leachate quality post on – site treatment is seldom good enough to allow for direct discharge into water bodies. The most commonly used option for the disposal of landfill leachate is therefore discharge into nearby wastewater treatment plants with or without on – site pretreatment (Uygur *et al.*, 2004), where it gets biologically treated and eventually discharged into surface waters.

Over almost a decade, several studies performed by our research group at Virginia Tech have shown that the addition of landfill leachates into wastewater treatment plants could drastically reduce the transmissivity of UV disinfection owing to the ability of landfill leachates to quench (i.e. absorb) UV light at a wavelength of 254 nm (Zhao *et al.*, 2013, Gupta *et al.*, 2014). The organic carbon present in leachate, primarily humic and fulvic acids (which together are commonly called humic substances) were found to be responsible for this quenching. These studies also found that as landfills got older, the humic substances in the leachates extracted from the landfills were reduced, suggesting that these substances were degraded under the conditions of long term landfilling.

So far, both aerobic and anaerobic treatment of landfill leachates have been found to be ineffective for the removal of humic substances because of their high resistance to biodegradation. This research sought to bring about accelerated biodegradation of UV quenching organic carbon in landfill leachates by employing a two – stage, thermophilic – mesophilic AnMBR system to mimic long term landfilling. Studies were also performed to compare the performance of the two – stage system with a single – stage AnMBR. A brief outline of the dissertation highlighting the focus areas of each chapter has been provided in the following section.

DISSERTATION OUTLINE

Chapter 1: Introduction

This chapter covers key background information on the research topic and a brief summary of each chapter in the dissertation.

Chapter 2: Literature Review

Chapter 2 provides a comprehensive and exhaustive review of published, peer – reviewed research and review articles focusing on landfill leachate management. The goal of the literature review was to shed light on the currently available treatment strategies for the treatment of landfill leachates prior to discharge into wastewater treatment plants or surface waters and identify the merits and limitations of each process. Both types of treatment processes, i.e., physico–chemical processes and biological processes were reviewed. This chapter is likely to be included as part of a review paper, to be written in collaboration with former students that have also worked on this topic, that summarizes all the work done at Virginia Tech over the last decade in the area of landfill leachate characterization and treatment.

Chapter 3: Two–Stage Anaerobic Membrane Bioreactor (AnMBR) System to Reduce UV Absorbance in Landfill Leachates

This study analyzes the removal of UV-quenching organic carbon and UV absorbance in a two-stage system treating a young landfill leachate by mimicking long-term landfilling. The two-stage system was operated and data were collected over a period of 14 months to evaluate the feasibility of using the process as a long – term, on - site biological treatment option for landfills prior to discharging leachates into the sewers where they are routed to wastewater treatment plants. This study was able to achieve over 50% reduction in UV quenching organic carbon (greater than reported in literature using any form of biological treatment) and sustained it over a year of operation. The long-term applicability of this treatment strategy provides landfills with a new treatment process that may be tested at larger scales. This manuscript has been submitted to Water Research (manuscript number WR37893) and is under review. This data was also presented at the Virginia Water Environment Association (VWEA) Joint Annual Meeting (WaterJAM 2016, Virginia Beach, Virginia) and at the AEESP special sessions at WEFTEC 2016, New Orleans, Louisiana.

Chapter 4: Two – Stage vs Single Stage Anaerobic Membrane Bioreactor (AnMBR) for removal of UV – Quenching Organics from Landfill Leachate

In order to validate the superiority of the two-stage AnMBR, a single-stage AnMBR was set up using inoculum from the two-stage system and operated for 6 months. Both the AnMBRs were identical in terms of operational parameters, the only difference being that the two AnMBR configurations were compared both with regard to the removal of UV-quenching organics and UV absorbance achieved in each, and the differences in microbial community structure and function. The data obtained during this study showed that the two-stage AnMBR removed almost 3 times as much UV-quenching organic carbon and UV absorbance as compared to the single – stage AnMBR. Subtle differences were also observed in microbial community structure and function, and the two-stage process also showed greater abundance of 16s rRNA gene copies. This manuscript will be submitted to *Water Research*.

Chapter 5: Pathogens and Antibiotic Resistance Genes (ARGs) in Untreated and Biologically-Treated Landfill Leachates

This manuscript focuses on the data obtained from next-generation DNA sequencing performed on raw (i.e., untreated) landfill leachates, as well as samples collected from reactors treating landfill leachate (both, the two – stage and single stage AnMBR configurations). The sequenced genomes were annotated against the SILVA rRNA database to identify microbial species and against the CARD database to detect ARGs. Matches to DNA sequences corresponding to several genera of bacteria containing pathogens were identified; including *Salmonella*, *Staphylococcus*, *Pseudomonas* and *Legionella*, were detected in the raw leachates as well as the bioreactors treating leachate. ARGs conferring resistance to over 15 different antibiotics were also detected in both untreated and treated leachates. It was found that the abundance of these potentially pathogenic organisms and ARGs did not significantly reduce during biological treatment, suggesting that landfills might act as sources of pathogen and ARG addition to wastewater treatment plants receiving both untreated as well as biologically pretreated landfill leachates. However, given that this study was DNA sequence based, it will be important for future work to validate the presence of true, viable pathogens and also compare the influent estimated pathogen and ARG content to that of typical influent sewage. This manuscript is planned to be submitted as a concise, letter – style paper to *Environmental Science & Technology Letters*.

Chapter 6: Appendices: Comparison of UV-quenching organic carbon removal in young versus mature landfill leachates using a two – stage AnMBR (Outline and Data)

The appendices contain the outline of a fourth manuscript and additional data not included in the main body of the dissertation. This manuscript is being submitted as an outline, and covers the data obtained from a short study comparing the performance of the two – stage system when treating young versus mature landfill leachates. One leachate used was from an active landfill cell and was high in refractory, UV-quenching organic carbon, while the other was from a landfill cell that had been closed over 12 years ago and was considerably lower in organic carbon. It was found that the mature leachate, which was low in organic carbon, was not very effectively treated with the two – stage system. With the younger leachate, sustained removals of over 50% of UV-quenching organics was observed. Previous studies indicate that for this level of attenuation to naturally occur in landfills, the time required may be of the order of 5 – 10 years or more (Zhao *et al*., 2013). This data will be published, with the journal yet to be decided.

Chapter 7: Concluding Remarks

This chapter encapsulates the lessons learnt from all the studies done and explains the broader engineering significance of this research.

REFERENCES

Gupta, A., Zhao, R., Novak, J. T., Goldsmith, C. D., 2014. Variation in organic matter characteristics of landfill leachates in different stabilization stages. Waste Management & Research 32 (12), 1 – 8.

Kurniawan, T.A., Lo, W., 2009. Removal of refractory compounds from stabilized landfill leachate using an integrated H2O2 oxidation and granular activated carbon (GAC) adsorption treatment. Water Research 43 (16), 4079 – 4091.

Kurniawan, T.A., Lo, W., Chan, G.Y.S., 2006. Radicals-catalyzed oxidation reactions for degradation of recalcitrant compounds from landfill leachate. Chemical Engineering Journal, Volume 125 (1), 35 – 57.

Renou, S., Givaudan, J.G., Poulain, S., Dirassouyan, F., Moulin, P., 2008. Landfill leachate treatment: review and opportunity. Journal of Hazardous Materials 150 (3), 468 – 493.

United States Environmental Protection Agency (USEPA), 2010. Municipal Solid Waste Generation, Recycling, and Disposal in the United States: Facts and Figures for 2009. EPA-530-F-010-012, Washington, DC.

United States Environmental Protection Agency (USEPA), 2013. Advancing Sustainable Materials Management: Facts and Figures 2013 - Assessing Trends in Material Generation, Recycling and Disposal in the United States. EPA530-R-15-002, Washington, DC.

Uygur, A., Kargı, F., 2004. Biological nutrient removal from pre-treated landfill leachate in a sequencing batch reactor. Journal of Environmental Management (71), 9–14.

Zhao, R., Gupta, A., Novak, J. T., Goldsmith, C. D., Driskill, N., 2013. Characterization and treatment of organic constituents in landfill leachates that influence the UV disinfection in the publicly owned treatment works (POTWs). Journal of Hazardous Materials (258 – 259), 1 – 9.

Chapter 2

Literature Review

LANDFILL LEACHATES AND THEIR INTERFERENCE WITH WASTEWATER DISINFECTION

The routing of landfill leachates to wastewater treatment plants has been found to present several issues, notably the impact of large quantities of ammonia and heavy metals on treatment effectiveness or cost (He *et al.*, 2006a). However, recent studies have shown that a major impact that landfill leachates have on wastewater treatment processes is due to their ability to absorb (or quench) ultraviolet (UV) light (Zhao *et al.*, 2012; Zhao *et al.*, 2013; Gupta *et al.*, 2014b). This ability of leachates to absorb UV light is thought to be due to the presence of aromatic carbon containing organics in leachate that are usually not readily biodegradable. These organics resist degradation during wastewater treatment, and end up in the disinfection step. This is especially problematic for treatment plants that use UV light to disinfect treated wastewater. The organics present in landfill leachate tend to greatly reduce the transmissivity of the water in the disinfection step to levels below those required for effective removal of microorganisms (Zhao *et al.*, 2013; Reinhart *et al.*, 2015). The capacity of landfill leachates to absorb UV light is substantial, so much so that it can interfere with UV disinfection even when making up as low as 1% or less of the total flow into a wastewater treatment facility (Zhao *et al.*, 2012). These findings have been supported by independent studies that showed that leachate discharged into wastewater treatment plants from nearby landfills was able to reduce transmittance to levels that were below the regulated requirement for effective disinfection, even when it made up only 1% of the total flow (Reinhart *et al.*, 2015). While it may be agreed that the nature and characteristics of landfill leachates can vary widely, the need to address the issue of UV-quenching by landfill leachates in wastewater treatment plants is urgent. A USEPA report from 2000 found that over 50% of landfills in the United States discharged their leachate into wastewater treatment plants, usually without pretreatment. According to the EPA, this percentage has since increased due to the implementation of more stringent regulations for direct discharge of landfill leachates into surface water and the lack of a need for capital investment by landfills to discharge into wastewater utilities.

Both the hydrophobic and the hydrophilic fractions of the organic carbon present in landfill leachate contribute to UV quenching. The hydrophobic organic substances that absorb UV light are primarily humic acids and fulvic acids (He *et al.*, 2006b). Humic acids, which have been found to have a very high capacity to absorb UV light, are present in landfills in significant amounts as they are products or intermediates formed during the natural biodegradation of lignins in a landfill (Xiaoli *et al.*, 2008). Lignins are abundantly present in paper and board, wood, leaves and other vegetation that may be put in a landfill. In 2013, paper, wood and yard trimmings collectively made up 46.7% of the total waste disposed of via landfills across the US. The process of humic acid formation may also be aided by the proteins, carbohydrates and fatty acids present in food wastes, which constituted 14.6% of the total MSW put into landfills in 2013. As a result, landfills provide conditions conducive to the formation of UV quenching organic compounds which can then interfere with disinfection processes at wastewater treatment facilities.

While wastewater treatment processes using UV disinfection are vulnerable to the addition of landfill leachate, the presence of recalcitrant organic carbon species in chlorine – based disinfection systems can also be problematic due to an increase in the potential for the formation of carcinogenic disinfection by – products (DBPs). It has been shown that the interaction of humic substances with free chlorine in disinfection systems can greatly increase the potential for formation of trihalomethanes (THMs) in processes using chlorine gas as well as chloramines (Kim and Yu, 2007; Wu *et al.*, 2003). Both humic acids and fulvic acids have been found to be precursors in THM formation (Nikolaou *et al.*, 2003; Zhang et al., 2005), presenting issues for both drinking water and wastewater disinfection. In fact, the potential for DBP formation was among the drivers that pushed wastewater treatment plants towards employing UV disinfection over chlorine-based processes. In addition to the formation of DBPs, cheaper electricity costs, safety issues with handling chlorine gas or chemicals and the deleterious effect of residual disinfectants on the natural biota in surface water bodies that receive the effluent from wastewater treatment also make the case for UV disinfection to be used at WWTPs.

The capacity of landfill leachates to absorb UV light in quantities sufficient to compromise disinfection goals is a fairly recent discovery. Most on – site treatment processes for landfill leachate, therefore, have focused on removal of COD and to some extent nutrients, specifically nitrogen. The issue of UV-quenching by landfill leachates and its potential impact on wastewater disinfection systems only came to light in the late 2000s (Alkan *et al.*, 2007; Zhao *et al.*, 2012; Zhao *et al.*, 2013; Gupta *et al.*, 2014). There have been only a limited number of studies in this area since it has only recently begun to receive attention. Efforts to degrade the organic compounds responsible for UV quenching in landfill leachate have been impeded by their low biodegradability, making biological treatment challenging (Zhao *et al.*, 2012). Membrane processes and advanced oxidation, though effective, prove to be expensive due to the costs involved in set up and issues with operation and maintenance (Trebouet *et al.*, 2001; Rautenbach *et al.*, 2000). Other forms of physico – chemical treatment such as coagulation – flocculation, ion exchange, chemical precipitation etc. have not been proven to be effective for removal of UV quenching organics from leachates.

Overall, it is evident that UV absorbance by landfill leachates is an issue that is yet to be widely studied, and can tend to get ignored since organic carbon species as well as UV absorbance / transmittance in wastewater disinfection are not regulated at the federal or state level. There is a need for the development of treatment processes that can mitigate if not completely resolve the issue of UV quenching by landfill leachates to minimize their impact on wastewater disinfection systems.

CHARACTRIZATION OF ORGANIC CARBON IN LANDFILL LEACHATE

The substances in landfill leachate that cause most of the UV absorption are typically present in the form of dissolved organic matter (DOM), and are not readily amenable to biodegradation (He *et al.*, 2006b; Seo *et al.*, 2007). These substances, therefore tend to persist in the leachate even when it undergoes biological treatment at wastewater treatment plants and reach the disinfection step unchanged.

Considerable progress has been made in quantifying and characterizing DOM in natural as well as engineered systems. It is worthwhile to note that DOM refers to a group of several different categories of organic compounds which includes amino acids, carbohydrates and high molecular weight organic compounds (Kalbitz *et al.*, 2000). DOM in aquatic systems or landfill leachates is typically purified and separated into humic acids, fulvic acids and hydrophilic substances for analysis (Zhang *et al.*, 2009). The humic acids and fulvic acids are hydrophobic and together constitute what are known as 'humic substances'. These have been described as 'polar, straw - colored organic acids'. Humic substances have a tendency to form complexes with metal ions. They may therefore play a role in imparting color to landfill leachates, which can have high concentrations of metal ions available for complexation (Thurman and Malcolm, 1981; Christensen *et al.*, 1998).

Though there is no standard method to isolate humic acids, fulvic acids and hydrophilic substances from landfill leachate, the methods described by Thurman and Malcolm (1981), Christensen *et al.* (1998) and Leenheer (1981) have been widely accepted and used to characterize DOM in aquatic samples. The humic acids are first isolated by precipitating them out of the solution via acidification. The remaining mixture, which contains fulvic acids and hydrophilic substances, is passed through a column packed with XAD-8® ion – exchange resin. The fulvic acids, which get adsorbed on to the surface of the resin are extracted by elution with sodium hydroxide, while the hydrophilic substances pass right through the column.

Analyses of humic and fulvic acids using pyrolysis – GC/MS have provided insight into the structure and make – up of these substances. When humic and fulvic acid isolates from landfill seepage were broken down in the presence of heat, at temperatures of as high as 770 °C, three types of compounds were detected (Göbbels and Püttman, 1997). The first of these were aromatic hydrocarbons such as benzene, naphthalene and alkylbenzenes, which suggest the presence of humic substances similar in structure to those found in soil (Schulten and Plage,1991). The second group are phenols and aromatic acids that are known to be pyrolysis products of lignin and lignin derivatives. Finally, the third group of pyrolysis products are aromatic and heterocyclic nitrogen containing compounds that are likely to be protein derivatives. These results suggest that humic and fulvic acids are a broad group of compounds, containing several different types of organics. However, it may be worth noting that humics and fulvics isolated from the seepage of different landfills did not show significant structural differences (Göbbels and Püttman, 1997).

Even though humic substances are essentially composed of similar compounds in different landfills, there can be large variations in the relative abundances of these molecules, causing leachates to vary considerably in terms of their strength in terms of refractory carbon and their treatability using biological processes. A comparison of humic acid isolates from landfill leachates in Korea with commercially purchased humic acids supplied by Aldrich co. suggested that the humic acids found in landfill leachates are lower in molecular weight and aromaticity (Kang *et al.*, 2002). The same study also found that as landfills got older, the humic acids in the leachates had increased aromaticity and were larger in molecular weight, suggesting that continuing humification occurs within the landfill as its age increases. These findings are supported by other similar studies that have shown that humic acids derived from landfill leachates tend to be higher in aliphatic content, and therefore lower in aromatic carbon as compared with humic substances obtained from natural sources such as soil (Nanny and Ratasuk, 2002). The study also found that humic substances (i.e. humic acids and fulvic acids) can account for 50 – 80% of the non – purgable organic carbon (NPOC) in municipal landfill leachates, and these substances can be highly branched aliphatic or cyclic species, that are not necessarily aromatic. In studying landfill leachates, it has been observed that humic acids tend to be high molecular weight (i.e. bulk of the organic matter present as humic acids has a molecular weight >10,000 Da), while fulvic acids and hydrophilic compounds were found to be lower in molecular weight (He et al., 2006b).

The capacity of humic acids, fulvic acids and hydrophilic substances to quench UV light may be quantified using their specific ultraviolet absorbance at 254 nm i.e. $SUVA_{254}$ value. For bioreactor landfills employing leachate recirculation, it has been observed that the $SUVA_{254}$ values of humic acids and fulvic acids were considerably higher than those of hydrophilic substances isolated from the same leachate (He *et al.*, 2006b). It must, however be noted that these results were for laboratory scale landfills. While only a handful of studies have investigated the organic make up of landfill leachate and its UV quenching capacity, there is data to suggest that it is the humic acids usually absorb significant quantities of UV light. Analyses of landfill leachate samples drawn from multiple landfills in three states in the US showed that in most cases, even when the humic acids made up a small fraction (<25%) of the total organic carbon present in the leachate, they were responsible for over 50% of the UV_{254} absorbance of the sample (Zhao *et al.*, 2013). This has been attributed to the fact that humic acids had the high $SUVA_{254}$ values, considerably greater than those for fulvic acids or hydrophilic compounds. This was supported by another study that found that humic acids were responsible for the most UV absorbance, even though hydrophilics formed the bulk of the DOM in leachates (Gupta *et al.*, 2014b).

Even though the DOM in landfill leachates has usually been found to be highly resistant to biodegradation, it has been observed that in leachates extracted from older cells of a landfill, the concentrations of humic acids, fulvic acids and hydrophilics were considerably lower (Gupta *et al.*, 2014b). This suggests that natural biodegradation of DOM occurs under the conditions of long term landfilling, although the time period required for significant biodegradation through natural pathways may be as high as a few decades. It may be noted, however, that older leachates were found to contain almost no biodegradable organic carbon, and had higher $SUVA_{254}$ values even though UV_{254} absorbance of the leachate as a whole was greatly reduced, which was supported by the increase in $SUVA_{254}$ reported for a lab scale bioreactor landfill after biodegradable organic carbon had been removed (He *et al.*, 2006b). This suggests that DOM in a landfill becomes more bio refractory with increasing age, even though it is greatly reduced in concentration, causing an overall decrease in the total UV_{254} absorbance.

TREATMENT METHODS FOR LANDFILL LEACHATES

Physical / chemical treatment

Several forms of physico – chemical treatment have been utilized to treat landfill leachate in order to make it suitable for discharge into surface water or wastewater treatment plants. These methods have been used by themselves or in conjunction with biological treatment, which is usually a cheaper treatment option.

Coagulation – flocculation is a technique frequently used to remove polar or charged species, and is used to remove heavy metals from landfill leachates, although efforts have been made to employ it for the removal of organic carbon. A study of several different types of polymers at different doses to remove COD from landfill leachate showed that with appropriate testing and optimization, up to 80% of the total COD in landfill leachate could be removed by coagulation – flocculation (Tatsi et al., 2003). Another study using different coagulants reported that up to 57% of the total COD and 73% of the TOC could be removed using coagulation – flocculation (Amokrane *et al.*, 1997). However, this study only used coagulation – flocculation as a pretreatment option for leachate treatment by reverse osmosis (RO), since coagulation – flocculation by itself was not enough to provide an effluent that could be discharged into sewers. Membrane fouling in the RO process was found to be reduced as a result of the coagulation – flocculation pretreatment. Studies employing coagulants to remove COD and TOC from landfill leachate have typically reported that iron based coagulants are more effective than aluminum based coagulants (Diamadopolous, 1994; Amokrane *et al.*, 1997). While COD and a portion of the TOC may be removed by coagulation – flocculation, large, low – polarity organic molecules such as those that cause UV quenching are difficult to remove. For old, mature leachates, coagulation flocculation is a good treatment option since they are deprived of most of their biodegradable organic carbon content, rendering biological treatment ineffective (Monje – Ramirez and Velasquez, 2004; Kang and Hwang, 2000).

Adsorption using activated carbon or other media has also been tested for the purpose of removing organics from leachates. Since activated carbon has a strong affinity for organic carbon, it could serve as a good pretreatment option prior to discharging leachates into sewers or other on – site biological treatment (Çeçen and Aktas, 2004). There has also been a report of powdered activated carbon (PAC) addition to activated sludge processes at wastewater treatment plants receiving landfill leachate to allow for enhanced removal of organics and also provide nucleation sites for better floc formation and sludge dewatering characteristics (Çeçen *et al.*, 2003). Laboratory scale tests of landfill leachate run through adsorption columns packed with activated carbon showed good removal of organics. The removal of organics was higher than those achieved by tests that involved chemical addition to the leachates, such as chemical precipitation or coagulation – flocculation (Morawe *et al.*, 1995). PAC addition to aerobic biological treatment processes to treat landfill leachate showed promising results for removal of COD and ammonia, with 86% and 26% removal respectively (Kargi and Pamukoglu, 2003). Adsorption using PAC has been used as a pretreatment as well as post treatment step in landfill leachate treatment using biological methods (Çeçen et al., 2003, Morawe *et al.*, 1995). Most of the aforementioned studies employing activated carbon adsorption, cite frequent column breakthrough and regeneration and large carbon demand as the drawbacks of using PAC for large scale leachate treatment.

Advanced oxidation is another strategy that has been tested for landfill leachate treatment. These treatment processes typically involve the use of strong oxidizing agents to remove COD and organics present in leachates. The oxidants work by generating chemically unstable hydroxyl radicals (•OH) which are highly reactive and the strongest known oxidizing species (Metcalf and Eddy Inc. *et al.*, 2003). Advanced oxidation is effective in the case of landfill leachate due to its capacity to oxidize substances that are biologically recalcitrant. It is especially an attractive option for the treatment of leachate from mature landfills that is devoid of biodegradable organic carbon, and therefore unsuitable to be treated biologically (Renou *et al.*, 2008). It could also be used to pretreat young leachates prior to biological treatment to degrade the organic carbon into simpler, more bioavailable forms. Several different chemical combinations can be used to carry out advanced oxidation. Commonly used oxidants for wastewater and leachate treatment include ozone (O_3), O_3 coupled with UV light (O_3/UV), O_3 coupled with hydrogen peroxide (O_3/H_2O_2), H_2O_2/UV and Ferrous Iron coupled with H_2O_2 (Fe^{2+}/H_2O_2). Combinations of different oxidants may be used simultaneously for enhanced oxidation (Wang *et al.*, 2003). Treatment processes employing ozone for advanced oxidation of the COD content of landfill leachates reported 30% removal of the total COD after 1 hour of ozonation. When this was followed up by treatment with activated carbon, a net 90% COD removal was achieved (Rivas *et al.*, 2003). When using combinations of oxidants such as O_3/H_2O_2, COD removal efficiencies as high as 90% were observed, while the corresponding values for H_2O_2/Fe^{2+} and H_2O_2/UV were 50% and 87% respectively (Schulte et al., 1995).

The use of ***Fenton's reagent*** for advanced oxidation of landfill leachates has received considerable interest. Not only was treatment of landfill leachate using Fenton's reagent and H_2O_2 able to remove 60% of the total COD in the leachate, but it increased the BOD_5/COD ratio from 0.2 to 0.5, suggesting that while removing COD, some of the COD content is converted to biodegradable species (Lopez *et al.*, 2004). The drawback of using advanced oxidation processes such as this is the rigorous process control required for effective use of the oxidant. The parameters that need to be controlled include reaction time, H_2O_2 dosing, Fe^{2+} concentration and pH. Fenton's reagent is reported to be very effective for treatment of old leachates that cannot be treated biologically, with COD removal efficiencies >90% reported by several studies. Importantly, Fenton's reagent is known to be effective at removing UV_{254} quenching organic carbon from landfill leachates with reductions in UV_{254} absorbance as high as 97% reported for some leachates (Gupta et al., 2014a). Despite the effectiveness of Fenton's reagent in removing UV quenching organics, it is seldom used at large scale by landfills for treatment of leachates due to several drawbacks. In addition to the high cost of the oxidant itself, the costs involved in using large quantities of acid required for pH adjustment to use the Fenton process, the safety, corrosion and operational challenges involved, the slow kinetics of the reaction below 18 °C and the production of large quantities of ferric sludge as a waste product that needs to be treated and disposed greatly limit the use of Fenton's reagent at full scale (Deng and Englehardt, 2006).

Another array of treatment methods widely tested for the purpose of landfill leachate treatment are ***membrane separation processes***. Ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) have been used to remove COD, nitrogen species and organic carbon from landfill leachates. Ultrafiltration has been used both as a polishing step post biological treatment and as a pretreatment step to prevent membrane fouling in RO (Bohdziewicz *et al.*, 2001). UF is able to remove about 50% of the total organic carbon present in landfill leachates (Renou *et al.*, 2008), although there is little evidence to suggest that it brings about any significant reduction in the UV absorbance of the leachates. UF membranes are also frequently used in MBRs, as discussed in a following section. NF and RO are much more effective for removal of COD and UV quenching organic carbon than UF but cannot be used without physico-chemical pretreatment to mitigate membrane fouling. NF was reported to remove 60 – 70% of the COD content and approximately 50% of ammonia (Marttinen *et al.*, 2002). When combined with physico – chemical pretreatment, the COD removal using NF was found to be 80% (Trebouet *et al.*, 2001). Despite the fact that NF works well to remove DOM from leachates, it is rarely used for leachate treatment since NF membranes are extremely sensitive to pH and ionic concentrations and can start adsorbing humic acids on their surface, inducing rapid fouling. Thus, not only does the pH need to be adjusted, but the choice of pretreatment method and the dosage of chemicals applied in the pretreatment step must be rigidly monitored (Trebouet *et al.*, 2001). This drives up the operation and maintenance costs of the process, reducing its appeal for full scale application.

RO is easily the most effective membrane process to treat landfill leachates as it provides a quality effluent suitable for direct discharge into surface waters. Landfill leachate treated using RO showed >98% reduction in both COD as well as heavy metals concentration (Chianese *et al.*, 1998), but the treatment process required continuous, high energy input and maintenance. In fact, a study on landfill leachate treatability using RO in Japan suggested that it is possible to achieve >99% removal of all ions and organic matter, producing a "potable – level treated water" that may be suitable for direct discharge (Ushikoshi *et al.*, 2002). No data was provided with regard to operational and maintenance costs and challenges or concentrate management strategies. Another study that attempted to treat multiple leachates using RO found that even though treated effluent quality was excellent for all leachates (>98% COD and metals removal consistently) the flux through the membrane was too low for large scale application (Linde *et al.*, 1995). The study suggested that the high concentration of pollutants in landfill leachate coupled with the high rejection rate by virtue of the small pore size of RO membranes bring about quick membrane fouling, necessitating frequent shutdown and membrane restoration, which is expensive and reduces membrane life. These issues were also highlighted by a review of RO processes for landfill leachate treatment by the APEC Virtual Center for Environmental Technology Exchange (Yamada and Jung, 2005). In addition to the issues with energy costs, membrane fouling and maintenance issues, they emphasized that in treating landfill leachates using RO, the large reject fraction meant treating and disposal of significant quantities of high strength concentrates, which could account for the bulk of the cost of RO. Recycling this concentrate back into the landfill only results in the production of stronger leachate, richer in organics, nitrogen and metals, and is not a sustainable strategy.

In addition to these physico – chemical treatment methods, several other methods have been studied for leachate treatment, notably ion exchange. Removal of 91% color, 71% COD, 93% suspended solids and 92% turbidity was reported using an anion exchange resin with appropriate pH adjustment (Bashir *et al.*, 2010). However, without pH adjustment, these values were 67%, 60%, 64% and 61% respectively. This study also explicitly stated that even after treatment with ion exchange treatment, the leachate did not meet the requirements for discharge into surface water or WWTPs. Ion exchange is also reported to work well for high molecular weight humic acids such as those found in soil and aquatic systems (Fettig, 1999), although the smaller humic substances found in landfill leachate may not be amenable to removal using this process.

Biological Treatment

Biological treatment of landfill leachate is commonly used for young leachates, which have a high BOD/COD ratio (>0.5) and therefore sufficient biodegradable substrate for microbes to carry out metabolic processes. However, high concentrations of metals can be inhibitory to microbes, while refractory organics resist biodegradation. Both aerobic and anaerobic processes may be used for landfill leachate treatment. The primary reasons biological treatment is preferred over any form of physical / chemical treatment are the lower cost, ease of operations, avoidance of chemical handling and simplicity of residuals / biosolids management (Renou *et al.*, 2008).

Aerobic processes are typically used when nitrogen removal is among the primary treatment goals. Among the most widely used methods for sewage treatment is activated sludge. Activated sludge works well when landfill leachate is put into wastewater treatment plants and makes up a very small proportion of the total flow into the process. One review of leachate treatment in wastewater plants using activated sludge suggested that the process very ineffective due to toxicity by heavy metals and ammonia (Lema *et al.*, 1988), so much so that the activated sludge process can be severely inhibited when leachate concentrations are more than 5% of the total reaction volume. The report also cited corrosion issues, poor sludge settling characteristics due to ion imbalances and operational issues due to heavy metals precipitation as challenges to smooth operation. Large residence times (of the order of several days) are required for acceptable COD and nitrogen removal, leading to very high energy costs for continuous aeration (Loukidou *et al.*, 2001).

An investigation into the effectiveness of SBRs using activated sludge to reduce the total UV absorbance of landfill leachates revealed that for most leachates, activated sludge was able to remove almost none of the refractory organic matter that was >1000 Da in molecular weight, meaning that the UV absorbance of the leachates before and after activated sludge treatment were almost identical (Zhao *et al.*, 2012). This study was followed by another that demonstrated that for most leachates, the removal of UV-quenching DOM was less than 30%, and the corresponding drop in UV absorbance was less than 10% (Zhao *et al.*, 2013). It is worth noting that the HRT used for these experiments was 21 days, meaning that not only was this treatment ineffective at removing UV-quenching organics from leachates, but it had a very large energy requirement in the form of continuous aeration. The work of Zhao *et al.* concluded that UV-quenching organic compounds are "refractory in aerobic biological treatment processes", and therefore very difficult to remove by aerobic treatment.

The use of SBRs for landfill leachate treatment is well documented. A five – stage SBR process consisting of anaerobic – anoxic – aerobic – anoxic – aerobic zones removed 75% of COD and 44% each of nitrogen and phosphorus from landfill leachate when leachate was put through the process in three cycles of 7 hours each, resulting in a total residence time of 21 hours (Ugyur and Kargi, 2004). However, this performance could be achieved only by diluting the landfill leachate with wastewater and addition of PAC to the treatment process. The leachate also required pretreatment in the form of ammonia stripping, coagulation – flocculation and pH adjustment. A different study concluded that aerobic treatment for leachates was only effective when ammonia concentration in the leachate were reduced to levels below 100 mg/L and appropriate pH adjustment and metals precipitation strategies were applied (Li and Zhao, 2001).

The use of unconventional treatment methods such as nitrifying upflow clarifiers for landfill leachate treatment showed that 80 – 90% of the ammonia in landfill leachate can be nitrified, and recirculated into the landfill to remove nitrogen by denitrification (Jokela *et al.*, 2002). The use of technologies such as moving – bed biofilm reactors (MBBRs) has also shown promise with regard to nutrient removal from landfill leachates, with as much as 90% removal of total nitrogen reported, although COD removal for the same process was found to be below 20% (Welander et al., 1998).

Anaerobic processes are an attractive option for landfill leachate treatment due to the significantly lower energy requirement and the potential for generating / recovering energy in the form of methane, which is often a by – product of anaerobic respiration. Several different configurations of anaerobic reactors have been used to treat landfill leachates, and it has been found that young leachates are especially amenable to degradation by anaerobic treatment (Renou *et al.*, 2008).

Among the anaerobic reactors used for leachate treatment, conventional anaerobic digestion (AD) and Anaerobic SBRs (AnSBRs) are perhaps the most widely reported in literature. Leachates rich in degradable COD (i.e. BOD/COD > 0.6) have been successfully treated using

AD, with an average COD removal of 55% at an HRT of 20 days (Lin, 1991). However, the leachate used for this study was usually high in biodegradable COD and had an almost neutral pH, which is usually not the case with young landfill leachates. It was also diluted with distilled water to make it more treatable, which is typically not an option at full scale since it causes an increase in the reaction volume, which requires bigger reactors and higher energy input. Several reports are available of AnSBRs used for co – digestion of landfill leachate with septage, sewage as well as food wastes. When landfill leachates were co – digested with septage, over 80% COD reduction and 60% nitrogen reduction were observed using a 20 day HRT, although the system performance deteriorated when the leachate made up more than one third of the reaction volume (Lin and Chou, 1999). A follow – up to this study that made use of an upflow anaerobic sludge blanket (UASB) reactor to treat landfill leachate reported 42% COD removal, 70% solids removal and 47% nitrogen removal (Lin *et al.*, 2000). A similar effort attempting to perform co – digestion of landfill leachates with food wastes showed that controlled addition of landfill leachates to anaerobic digesters treating wastes rich in biodegradable organic carbon could bring about an increase in the methane yield, although methane production was inhibited when leachate concentrations in the feed increased (Liao *et al.*, 2014).

In the field of biological treatment of landfill leachates, there is considerable interest in the evaluation of enhanced biological treatment processes such as bioelectrochemical systems (i.e. BES or microbial fuel cells) or membrane bioreactors (MBRs) as treatment options for landfill leachate. Since no single biological process has provided a robust, reliable treatment option, biological treatment of landfill leachate is a hot topic for research.

Enhanced Biological Treatment: MBRs

Several innovative biological treatment processes are being explored for landfill leachate treatment. Among the most promising of these treatment processes are membrane bioreactors, or MBRs. MBRs are usually aerobic, but can also be operated under anaerobic conditions (AnMBRs). An MBR contains two parts: a bioreactor for biodegradation of the waste stream and a membrane module that filters the effluent as it is withdrawn from the reactor. This greatly reduces the solids content in the effluent, eliminating the need for clarifiers or filters and also helps keep biomass in the reactor, thereby increasing the rate of biodegradation of organic carbon fed into the system (Ahmed and Lan, 2012). The configuration of the membrane module can be external i.e. outside the reactor or submerged i.e. inside the reactor.

MBRs have typically been utilized for aerobic treatment. For landfill leachate in particular, aerobic MBRs have proved to be a useful tool for nitrogen removal. These MBRs essentially combine activated sludge with membrane separation (Alvarez-Vazquez *et al.*, 2004). Lab tests of MBRs for leachate treatment successfully reported removing 95% of the total COD from a 1:5 mixture of landfill leachate and domestic wastewater using an intermittently aerated MBR that also allowed nutrient removal (Hasar *et al.*, 2009). In another study, it was recommended that for aerobic MBRs treating landfill leachate with domestic wastewater, increasing the leachate concentration to >10% by volume in the feed causes inhibition of aerobic bioprocesses, and negatively impacts MBR performance (Puszczalo *et al.*, 2010), though at levels below 10%, up to 86% COD removal could be obtained. An aerobic MBR treating undiluted landfill leachate under strictly controlled conditions reported 54 – 78% COD removal, even for BOD_5/COD ratios lower than 0.3 (Sadri *et al.*, 2008).

Though they are not used as widely as aerobic MBRs, AnMBRs are the subject of active research and are being evaluated for landfill leachate treatment. While it is energetically favorable to use anaerobic treatment processes over their aerobic counterparts, their slower reaction kinetics and larger residence time requirements limit their applicability (Herrera-Robledo *et al.*, 2010). However, using a membrane as a polishing step to increase biomass concentrations in the system may make AnMBRs robust and resistant to fluctuations in influent characteristics. A laboratory scale AnMBR treating a mixture of 20% landfill leachate with 80% synthetic wastewater reported over 90% COD removal at an HRT of 2 days (Bohdziewicz *et al.*, 2008). While AnMBRs are being used for increasing number of applications in the wastewater industry (Huang *et al.*, 2011; Lin *et al.*, 2011; Martinez-Sosa *et al.*, 2011), there have been only a handful of studies that have looked into their applicability to the treatment of landfill leachates.

Overall, it is evident from the literature dealing with the use of aerobic MBRs as well as AnMBRs for landfill leachate treatment that the results are promising but need further research. Most studies using aerobic or anaerobic MBRs do not use undiluted landfill leachate, choosing instead to dilute the leachates with water or mix them with wastewater / synthetic wastewater.

While it is evident that MBRs are unable to provide a treated effluent good enough for direct discharge to surface water (Sadri *et al.*, 2008; Bohdziewicz *et al.*, 2008), it may be possible landfill leachates treated using MBRs can be discharged into wastewater treatment plants without causing interferences in the biological treatment processes or disinfection processes there. A review of studies that have used MBRs for landfill leachate treatment completely overlooked the issue of UV quenching by leachates, as the UV absorbance is usually not reported either before or after treatment (Ahmed and Lan, 2012), There is therefore, a lack of clarity as to whether MBRs can serve as feasible on – site treatment options for landfills to treat their leachates prior to discharging them into the sewers to send to WWTPs.

MICROBIAL COMMUNITY ANALYSIS IN LEACHATES / REACTORS TREATING LEACHATES

There is very little information available in the literature about microbial communities in landfill leachates. Most published studies focus on the microbial community present inside landfills or in untreated landfill leachate, and not on the microbial communities adapted to leachates or responsible for biological treatment of leachates.

Based on available information, the microbial communities in landfill leachates appear to be diverse, with several abundant phyla. A study of microbial communities in leachate extracted from a full-scale recirculating landfill using 16S rRNA PCR amplification and cloning identified several phyla and classes. Bacterial phyla Proteobacteria, Actinobacteria and Firmicutes were found in abundance. Organisms belonged to diverse classes, though low G + C gram positive bacteria, *Chlamydiae* / *Verrucomicrobia* group and *Cytophaga – Flexibacter – Bacteroides* (CFB) group were found to be most abundant (Huang et al., 2004). The study also noted that a very small fraction of the microbial community in landfill leachates was identified, and that such knowledge is “severely scarce” in the literature. A follow up study that analyzed the bacterial community in the leachate from a closed landfill identified the above mentioned types of bacteria in the landfill, but reiterated that the majority of bacterial species showed little similarity with known 16S rRNA gene sequences and likely belonged to unknown taxa (Huang *et al.*, 2005).

Other studies have also used the 16S rRNA gene to study prokaryotic diversity in landfill leachates. An analysis of 16S rRNA genes in leachate sediment ecosystems identified 59 archaeal and 283 bacterial 16S rRNA gene phylotypes. Most of the archaeal populations were found to be methanogens, primarily *Methanosaeta* spp., while the bacterial community was diverse, incorporating 18 distinct phyla, several of which were known pollutant-degrading and bio-transforming organisms (Liu *et al.*, 2011), including metabolizers of organic carbon and nitrogen species. This study focused only on microbial community structure and reported a large number of organisms from unknown taxa. Prior studies further indicate that microbial communities can vary significantly even within the same landfill (McDonald *et al.*, 2010). These analyses also alluded to the presence of novel, undiscovered archaeal species in the landfill. In analysis of a different landfill, the majority of the archaeal 16S rRNA gene sequences belonged to the *Euryarchaeota*, and most specifically relatives of the genus *Methanosaeta*, suggesting that a majority of methane generated by the landfill was derived from utilization of acetate by *Methanosaeta* (Mori *et al.*, 2003). Analyses of waste and soil samples drawn from different depths within a landfill call have shown that the microbial community within even the same cell of a landfill varies substantially with depth and different layers within the landfill can have different temperature, pressure and waste content, resulting in unique, and as suggested by the authors, "mutually-isolated" microbial communities (Sawamura *et al.*, 2010).

Although the precise structure and function of microbial communities in landfills is unpredictable, since it depends on several variables including conditions within the landfill and kinds of waste being put into it, generally speaking the landfill itself is dominated by anaerobic organisms, while the cover soil tends to have a mixture of aerobic and anaerobic organisms (Semrau, 2011). While two studies have been identified that have analyzed microbial community structure in MBRs treating landfill leachate, they have primarily focused on the performance of the MBRs and identification methanogenic species and specific bacterial taxa, without investigating the functional characteristics of the microbial ecosystem (Xie et al., 2014; Mnif et al., 2012).

In addition to identifying microbial taxa in landfills, antibiotic resistance genes (ARGs) in landfill leachate are of public health interest as an emerging environmental concern. Although landfills are just beginning to be studied, the high-stress, toxic environment and multiple inputs of contaminants, including metals and pharmaceuticals, create conditions that are conducive to the selection of ARGs. A recent study of a leachate treatment plant in Guangzhou, China found

15 different types of ARGs, including genes for resistance to tetracycline, sulfonamides, *ampC* β-lactamase and class 1 integrons in samples from the treatment plant as well as soil and surface water receiving the effluent from the facility (Zhang *et al.*, 2016). A similar study to look for antibiotic resistance in leachate from a landfill in Shanghai found traces of several different antibiotics as well as ARGs in the leachate, including genes for resistance to tetracycline, sulfonamides and erythromycin and macrolide efflux genes (Wu *et al.*, 2015). *E. coli* isolated from landfill leachate when treated with antibiotics have demonstrated high resistance to doxycycline, cephalothin, tetracycline and minocycline, suggesting that parameters such as availability of oxygen in landfills could have an effect on antibiotic resistance in some bacteria (Threedeach et al., 2012).

REFERENCES

Ahmed, F. N., Lan, C. Q., 2012. Treatment of landfill leachate using membrane bioreactors: A review. Desalination (287), 41–54.

Alkan, U., Teksoy, A., Atesli, A., Baskaya, H. S., 2007. Influence of humic acids on the ultraviolet disinfection of surface waters. Water Environment Journal (21), 61–68.

Alvarez-Vazquez, H., Jefferson, B., Judd, S. J., 2004. Membrane bioreactors vs conventional biological treatment of landfill leachate: a brief review. Journal of Chemical Technology and Biotechnology (79), 1043 – 1049.

Amokrane, A., Comel, C., Veron, J., 1997. Landfill Leachates Pretreatment by Coagulation-Flocculation. Water Research 31 (11), 2775 – 2782.

Bashir, M. J. K., Aziz, H. A., Yusoff, M. S., Aziz, S. Q., Mohajeri, S., 2010. Stabilized sanitary landfill leachate treatment using anionic resin: Treatment optimization by response surface methodology. Journal of Hazardous Materials 182 (1 – 3), 115 – 122.

Bodzek, M., Łobos-Moysa, E., Zamorowska, M., 2006. Removal of organic compounds from municipal landfill leachate in a membrane bioreactor. Desalination (198), 16–23.

Bohdziewicz, J., Bodzek, M., Górska, J., 2001. Application of pressure-driven membrane techniques to biological treatment of landfill leachate. Process Biochemistry 36 (7), 641 – 646.

Bohdziewicz, J., Neczaj, E., Kwarciak, A., 2008. Landfill leachate treatment by means of anaerobic membrane bioreactor. Desalination (221), 559–565.

Çeçen, F., Aktaş, Ö., 2004. Aerobic Co-Treatment of Landfill Leachate with Domestic Wastewater, Environmental Engineering Science 21, 303–312.

Çeçen, F., Erdinçler, A., Kiliç, E., 2003. Effect of powdered activated carbon addition on sludge dewaterability and substrate removal in landfill leachate treatment. Advances in Environmental Research 7 (3), 707 – 713.

Chian, E.S.K., DeWalle, F.B., 1976. Sanitary landfill leachates and their treatment. Journal of the Environmental Engineering Division 102 (2), 411 – 431.

Chianese, A., Ranauro, R., Verdone, N., 1998. Treatment of Landfill Leachate by Reverse Osmosis. Water Research 33 (3), 647 – 652.

Christensen, J. B., Jensen, D. L., Grøn, C., Filip, Z., Christensen, T. H., 1998. Characterization of the dissolved organic carbon in landfill leachate-polluted groundwater. Water Research (32), 125–135.

Dacanal, M., Beal, L. L., 2010. Anaerobic filter associated with microfiltration membrane treating landfill leachate. Engenharia Sanitaria e Ambiental (15), 11–18.

Deng, Y., Englehardt, J. D., 2006. Treatment of landfill leachate by the Fenton process. Water Research 40 (20) 3683 – 3694.

Diamadopolous, E., 1994. Characterization and Treatment of Recirculation – Stabilized Landfill Leachate. Water Research 28 (12), 2439 – 2445.

Do, A. T., Prieto, A. L., Yeh, D. H., 2009. Anaerobic membrane bioreactor (MBR) for removal of EDCs from landfill leachate: A pretreatment strategy formunicipal wastewater treatment plants, Proceedings of the Water Environment Federation, WEFTEC 2009: Session 61 - 70, vol. 19, 4220–4238.

Fettig, J., 1999. Removal of humic substances by adsorption/ion exchange. Water Science and Technology 40 (9), 173 – 182.

Göbbels, F., Püttmann, W, 1997. Structural investigation of isolated aquatic fulvic and humic acids in seepage water of waste deposits by pyrolysis gas chromatography mass spectrometry. Water Research 31 (7), 1609 – 1618.

Gupta, A., Zhao, R., Novak, J. T., Goldsmith, C. D., 2014a. Application of Fenton's reagent as a polishing step for removal of UV quenching organic constituents in biologically treated landfill leachates. Chemosphere (105), 82 – 86.

Gupta, A., Zhao, R., Novak, J. T., Goldsmith, C. D., 2014b. Variation in organic matter characteristics of landfill leachates in different stabilization stages. Waste Management & Research 32 (12), 1 – 8.

Hasar, H., Ipek, U., Kinaci, C., 2009. Joint treatment of landfill leachate with municipal wastewater by submerged membrane bioreactor, Water Science & Technology (60), 3121–3127.

He, P.J., Shao, L.M., Guo, H.D., Li, G.J., Lee, D.J., 2006a. Nitrogen removal from recycled landfill leachate by ex situ nitrification and in situ denitrification. Waste Management 26 (8), 838 – 845.

He, P. J., Xue, J. F., Shao, L. M., Li, G. J., Lee, D. J., 2006b. Dissolved organic matter (DOM) in recycled leachate of bioreactor landfill. Water Research 40 (7), 1465 – 1473.

Herrera-Robledo, M., Morgan-Sagastume, J. M., Noyola, A., 2009. Biofouling and pollutant removal during long-term operation of an anaerobic membrane bioreactor treating municipal wastewater. Biofouling 26 (1), 23 – 30.

Huang, Z., Ong, S. L., Ng, H. Y., 2011. Submerged anaerobic membrane bioreactor for low-strength wastewater treatment: Effect of HRT and SRT on treatment performance and membrane fouling. Water Research (45), 705 – 713.

Jokela, J.P.Y., Kettunen, R.H., Sormunen, K.M., Rintala, J.A., 2002. Biological nitrogen removal from municipal landfill leachate: low-cost nitrification in biofilters and laboratory scale in-situ denitrification. Water Research 36 (16), 4079 – 4087.

Kalbitz, K., Solinger, S., Park, J. H., Michalzik, B., Matzner, E, 2000. Controls on the dynamics of dissolved organic matter in soils: a review. Soil Science 165 (4), 277 – 304.

Kang, K., Shinb, H., Park. H., 2002. Characterization of humic substances present in landfill leachates with different landfill ages and its implications. Water Research (36), 4023–4032.

Kargi, F., Pamukogly, M. Y., Powdered activated carbon added biological treatment of pretreated landfill leachate in a fed-batch reactor 2003. Biotechnology Letters 25 (9), 695 – 699.

Kang, Y., Hwang, K., 2000. Effects of reaction conditions on the oxidation efficiency in the Fenton process. Water Research 34 (10), 2786 – 2790.

Kim, H., Yu, M., 2007. Characterization of aquatic humic substances to DBPs formation in advanced treatment processes for conventionally treated water. Journal of Hazardous Materials (143), 486 – 493.

Kjeldsen, P., Barlaz, M.A., Rooker, A.P., Baun, A., Ledin, A., Christensen, T.H., 2002. Present and long-term composition of MSW landfill leachate: a review. Critical Reviews in Environmental Science and Technology 32 (4), 297 – 336.

Kurniawan, T.A., Lo, W., 2009. Removal of refractory compounds from stabilized landfill leachate using an integrated H2O2 oxidation and granular activated carbon (GAC) adsorption treatment. Water Research 43 (16), 4079 – 4091.

Kurniawan, T.A., Lo, W., Chan, G.Y.S., 2006. Radicals-catalyzed oxidation reactions for degradation of recalcitrant compounds from landfill leachate. Chemical Engineering Journal, Volume 125 (1), 35 – 57.

Leenheer, J.A., 1981. Comprehensive approach to preparative isolation and fractionation of dissolved organic carbon from natural water and wastewaters. Environmental Science and Technology 15 (5), 578 – 587.

Lema, J.M., Mendez, R., Blazquez, R., 1988. Characteristics of landfill leachates and alternatives for their treatment: a review, Water Air and Soil Pollution (40), 223–250

Li, X. Z., Zhao, Q. L., 2001. Efficiency of biological treatment affected by high strength of ammonium-nitrogen in leachate and chemical precipitation of ammonium-nitrogen as pretreatment. Chemosphere (44), 37 – 43.

Liao, X., Zhu, S., Zhong, D., Zhu, J., Liao, L., 2014. Anaerobic co-digestion of food waste and landfill leachate in single-phase batch reactors. Waste Management 34 (11), 2278 – 2284.

Lin, Č. Y., 1991. Anaerobic digestion of Landfill leachate. Water SA 17 (4), 301 – 306.

Lin, Č. Y., Bian, F. Y., Chou, J., 1999. Anaerobic co-digestion of septage and landfill leachate. Bioresource technology 68 (3), 275 – 282.

Lin, Č. Y., Chang, F. Y., Chang, C. H., 2000. Co-digestion of leachate with septage using a UASB reactor. Bioresource Technology 73 (2), 175 – 178.

Lin, H., Chen, J., Wang, F., Ding, L., Hong, H., 2011. Feasibility evaluation of submerged anaerobic membrane bioreactor for municipal secondary wastewater treatment. Desalination (280), 120 – 126.

Linde, K., Jönsson, A., Wimmerstedt, R., 1995. Treatment of three types of landfill leachate with reverse osmosis. Desalination 101 (1), 21 – 30.

Lopez, A., Pagano, M., Volpe, A., Di Pinto, A. C., 2004. Fenton's pre-treatment of mature landfill leachate. Chemosphere (54), 1005 – 1010.

Loukidou, M.X., Zouboulis, A.I., 2001. Comparaison of two biological treatment process using attached-growth biomass for sanitary landfill leachate treatment. Environmental Pollution 111, 273–281.

Martinez-Sosa, D., Helmerich, B., Netter, T., Paris, S., Bischof, F., 2011. Anaerobic submerged membrane bioreactor (AnSMBR) for municipal wastewater treatment under mesophilic and psychrophilic temperature conditions. Bioresource Technology (102), 10377 – 10385.

Marttinen, S. K., Kettunen, R. H., Sormunen, K. M., Soimasuo, R. M., Rintala, J. A., 2002. Screening of physical–chemical methods for removal of organic material, nitrogen and toxicity from low strength landfill leachates. Chemosphere (46), 851–858.

Metcalf and Eddy Inc., Tchobanoglous, G., Burton, F. L., Stensel, H. D., 2003. Wastewater Engineering: Treatment and Reuse 4/e, McGraw Hill publications, ISBN: 007-41878-0

Monje – Ramirez, I., Velasquez, M. T. O., 2004. Removal and transformation of recalcitrant organic matter from stabilized saline landfill leachates by coagulation–ozonation coupling process. Water Research 38 (9), 2359 – 2367.

Morawe, B., Ramteke, D. S., Vogelpohl, A., 1995. Activated carbon column performance studies of biologically treated landfill leachate. Chemical Engineering and Processing: Process Intensification 34 (3), 299 – 303.

Nanny, M. A., Ratasuk, N., 2002. Characterization and comparison of hydrophobic neutral and hydrophobic acid dissolved organic carbon isolated from three municipal landfill leachates. Water Research 36 (6), 1572 – 1584.

Nikolaou, A. D., Golfinopolous, S. K., Lekkas, T. D., Kostopolou, M. N., 2003. DBP levels in chlorinated drinking water: Effect of humic substances. Environmental Monitoring and Assessment (93), 301 – 319.

Puszczalo, E., Bohdziewicz, J., Swierczynska, A., 2010. The influence of percentage share of municipal landfill leachates in a mixture with synthetic wastewater on the effectiveness of a treatment process with use of membrane bioreactor. Desalination and Water Treatment 14 (1 – 3), 16 – 20.

Rautenbach, R., Linn, T., Eilers, L., 2000. Treatment of severely contaminated waste water by a combination of RO, high-pressure RO and NF — potential and limits of the process. Journal of Membrane Science (174), 231 – 241.

Reinhart, D., Bolyard, S. C., 2015. Fate of Organic Matter from Leachate Discharged to Wastewater Treatment Plants (Year 1). Report #10285 submitted to the Hinkley Center for Solid and Hazardous Waste Management, Gainesville, FL.

Renou, S., Givaudan, J.G., Poulain, S., Dirassouyan, F., Moulin, P., 2008. Landfill leachate treatment: review and opportunity. Journal of Hazardous Materials 150 (3), 468 – 493.

Rivas, F. J., Beltrán, F., Gimeno, O., Acedo, B., Carvalho, F., 2003. Stabilized leachates: ozone – activated carbon treatment and kinetics. Water Research 37 (20), 4823 – 4834.

Rodriguez, J., Castrillon, L., Maranon, E., Sastre, H., Fernandez, E., 2004. Removal of nonbiodegradable organic matter from landfill leachates by adsorption. Water Research 38 (14 – 15), 3297 – 3303.

Sadri, S., Cicek, N., Van Gulck, J., 2008. Aerobic treatment of landfill leachate using a submerged membrane bioreactor - Prospects for on-site use. Environmental Technology 29 (8), 899 – 907.

Schulte, P., Bayer, A., Kuhn, F., Luy, T., Volkmer, M., 1995. H_2O_2 / O_3, H_2O_2 / UV and H_2O_2 / Fe^{2+} Processes for The Oxidation of Hazardous Wastes. Ozone: Science and Engineering 17 (2), 119 – 134.

Schulten, H. R., Plage, B., 1991. A Chemical Structure for Humic Substances. Naturwissenschaften (78), 311 – 312.

Seo, D., Kim, Y., Ham, S., Lee, D., 2007. Characterization of dissolved organic matter in leachate discharged from final disposal sites which contained municipal solid waste incineration residues. Journal of Hazardous Materials (148), 679 – 692.

Tatsi, A. A., Zouboulis, A. I., Matis, K. A., Samaras, P., 2003. Coagulation – flocculation pretreatment of sanitary landfill leachates. Chemosphere 53 (7), 737 – 744.

Trebouet, D., Schlumpf, J. P., Jaouen, P., Quemeneur, F., 2001. Stabilized landfill leachate treatment by combined physicochemical – nanofiltration processes. Water Research 35 (12), 2935 – 2942.

Thurman, E. M., Malcolm, R. L., 1981. Preparative isolation of aquatic humic acids. Environmental Science & Technology (15), 463–466.

Trebouet, D., Schlumpf, J. P., Jaouen, P., Maleriat, J. P., Quemeneur, F., 2001. Effect of Operating Conditions on the Nanofiltration of Landfill Leachates: Pilot-Scale Studies. Environmental Technology 20 (6), 587 – 596.

United States Environmental Protection Agency (USEPA), 2000. Development Document for Final Effluent Limitations Guidelines and Standards for the Landfills Point Source Category. EPA821-R-99-019, Washington, DC.

Uygur, A., Kargı, F., 2004. Biological nutrient removal from pre-treated landfill leachate in a sequencing batch reactor. Journal of Environmental Management (71), 9–14.

Ushikoshi, K., Kobayashi, T., Uematsu, K., Toji, A., Kojima, D., Matsumoto, K., 2002. Leachate treatment by the reverse osmosis system. Desalination 150 (2), 121 – 129.

Xiaoli, C., Shimaoka, T., Quiang, G., Youcai, Z., 2008. Characterization of humic and fulvic acids extracted from landfill by elemental composition, ^{13}C CP/MAS NMR and TMAH-Py-GC/MS. Waste Management 28 (5), 896 – 903.

Xu, Z.Y., Zeng, G.M., Yang, Z.H., Xiao, Y., Cao, M., Sun, H.S., Ji, L.L., Chen, Y., 2010. Biological treatment of landfill leachate with the integration of partial nitrification, anaerobic ammonium oxidation and heterotrophic denitrification. Bioresource Technology 101 (1), 79 – 86.

Wang, F., Smith, D. W., Gamal El-Din, M., 2003. Application of advanced oxidation methods for landfill leachate treatment. Journal of Environmental Engineering and Science 2 (6), 413 – 427.

Welander, U., Henrysson, T., Welander, T., 1998. Biological nitrogen removal from municipal landfill leachate in a pilot scale suspended carrier biofilm process. Water Research 32 (5), 1564 – 1570.

Wu, W. W., Chadik, P. A., Delfino, J. J., 2003. The relationship between disinfection by-product formation and structural characteristics of humic substances in chloramination. Environmental Toxicology and Chemistry 22 (12), 2845 – 2852.

Yamada, M., Jung, S., 2005. Application of reverse osmosis for landfill leachate treatment. APEC Virtual Center for Environmental Technology Exchange: http://www.apec-vc.or.jp/e/modules/tinyd00/?id=35.

Yeşiller, N., Hanson, J. L., Liu, W. L., 2005. Heat Generation in Municipal Solid Waste Landfills. Journal of Geotechnical and Geoenvironmental Engineering 131 (11), 1330 – 1344.

Zayen, A., Mnif, S., Aloui, F., Fki, F., Loukil, S., Bouaziz, M., Sayadi, S., 2010. Anaerobic membrane bioreactor for the treatment of leachates from Jebel Chakir discharge in Tunisia. Journal of Hazardous Materials (177), 918 – 923.

Zhang, L., Li, A., Lu, Y., Yan, L., Zhong, S., Deng, C., 2009. Characterization and removal of dissolved organic matter (DOM) from landfill leachate rejected by nanofiltration. Waste Management 29 (3), 1035 – 1040.

Zhang, X., Minear, R. A., Barrett, S. E., 2005. Characterization of High Molecular Weight Disinfection Byproducts from Chlorination of Humic Substances with/without Coagulation Pretreatment Using UF-SEC-ESI-MS/MS. Environmental Science and Technology 39 (4), 963 – 972.

Zhao, R., Gupta, A., Novak, J. T., Goldsmith, C. D., Driskill, N., 2013. Characterization and treatment of organic constituents in landfill leachates that influence the UV disinfection in the publicly owned treatment works (POTWs). Journal of Hazardous Materials (258 – 259), 1 – 9.

CHAPTER 3

Two–Stage Anaerobic Membrane Bioreactor (AnMBR) System to Reduce UV Absorbance in Landfill Leachates

Ankit Pathak, Amy Pruden and John T. Novak

Department of Civil and Environmental Engineering, Virginia Tech, 418 Durham Hall, Blacksburg, VA 24060, USA

ABSTRACT

Landfill leachate typically contains UV–quenching (or UV absorbing) organics, which interfere with UV disinfection at wastewater treatment plants (Zhao *et al.*, 2013). This study examined the potential for a 2-stage submerged Anaerobic Membrane Bioreactor (AnMBR) to accelerate natural processes in the reduction of UV–absorbing compounds in landfill leachate. Raw Landfill leachate was treated sequentially in a thermophilic reactor (operating at 55 ± 2°C) followed by a mesophilic AnMBR (operating at 37 ± 1°C). The hydraulic residence time for the thermophilic step was 25 ± 5 days and was 40 ± 5 days for the AnMBR, with the SRT approaching infinity to promote biomass accumulation. Total COD, pH, solids, metals, volatile fatty acids (VFAs) and nitrogen species were monitored over 13 months of operation. Substances known to quench UV, including humic acids, fulvic acids and hydrophilic matter, were found to be reduced by ~55%. Molecular weight distribution analysis using a series of ultrafiltration (UF) membranes revealed that the thermophilic reactor served to break down organic carbon >100 KDa into smaller fractions, which were amenable to degradation in the AnMBR. After an initial acclimation period, the system achieved 50% removal of the total UV absorbance. To our knowledge, this is the first report of >50% reduction in recalcitrant organic carbon using biological treatment, which may eliminate the need for landfills to resort to expensive chemical oxidation processes or energy intensive reverse osmosis. Overall, the results demonstrate the feasibility of using a

promising new biological pretreatment option for landfills prior to discharging leachate into municipal sewer systems.

Keywords: Landfill Leachate, Wastewater, UV Disinfection, Organic Carbon, Anaerobic Membrane Bioreactor (AnMBR), UV Quenching

INTRODUCTION

According to the United States Environmental Protection Agency, there are over 3000 active landfills in the United States, and over 10,000 sealed, aging landfills. As of 2010, these landfills were being used to dispose of over 135 million tons of municipal solid waste annually. This dependence on landfills as the disposal option of choice for municipal solid waste results in the formation of large quantities of landfill leachate, a potentially toxic liquid composed of organic and inorganic constituents that must be treated and disposed. On–site pretreatment followed by direct discharge into water bodies has been practiced but is declining due to more stringent regulations and the poor quality of treated leachate. Currently, the most widely used method of leachate disposal is discharging into sewers and to the nearest publicly owned treatment works, with or without pretreatment (Uygur *et al.*, 2004). The addition of landfill leachates into wastewater treatment plants, however, has been questioned due to their heavy metals content, possible inhibition of biological treatment processes, and effect on sludge settling characteristics and disinfection (Çeçen and Aktas, 2004).

The amount of leachate generated by a landfill is a function of several factors, including the moisture content of the wastes being landfilled and precipitation patterns (Renou *et al.*, 2008). The large concentration of aromatic organic compounds in landfill leachate causes it to absorb ultraviolet light at 254 nm, the wavelength used by UV disinfection units at wastewater treatment plants (Alkan *et al.*, 2007). This greatly hinders the disinfection process and is forcing wastewater utilities to limit the discharge of leachate into sewer systems. Studies have shown that landfill leachate can seriously reduce the transmissivity of the water during disinfection, even when it comprises as little as 1 – 2% of the total volume of the water entering the disinfection step (Zhao *et al.*, 2013). Studies have also tracked UV quenching in the disinfection process at wastewater treatment facilities over a duration of 24 hours and observed a spike in the UV absorbance during disinfection when the local landfill began to discharge leachate into the sewer (Reinhart *et al*., 2015). The effect has been observed as soon as the leachate reaches the disinfection process and typically persists until leachate discharge has stopped and the leachate concentration in the UV disinfection unit has subsided.

The ability of landfill leachate to absorb UV light has been attributed to the presence of significant quantities of organic carbon, specifically aromatic compounds (Alkan *et al.*, 2007). UV-quenching carbon can be classified into humic acids, fulvic acids and hydrophilic substances. Of these, humic acids exert the greatest UV absorbance per unit concentration. UV–quenching organics can be resistant to biodegradation (Kjeldsen *et al.*, 2002) and therefore not effectively removed by a typical wastewater treatment plant. Of interest, as the age of a landfill increases, the concentration of UV-quenching substances generally decreases (Gupta *et al.*, 2014). This is reportedly as a result of near-complete degradation of humic acids, which have the highest specific UV absorbance (SUVA) values among the relevant organic compounds. The reduction in organic materials and UV absorbance may be attributed to biodegradation via natural metabolic pathways inside the landfill or to dilution of the leachate over time due to a "washout" effect. Thus, it was hypothesized in the present study that a treatment process simulating long-term landfilling conditions could accelerate degradation of organic compounds responsible for UV quenching.

This study attempted to develop a simple and relatively inexpensive biological treatment process for landfill leachate treatment Studies have found that the temperature within a landfill can vary, ranging from mesophilic (~37 °C) to thermophilic (~55°C) and reaching values as high as 70 °C and above (Yeşiller *et al.*, 2005). The specific hypothesis for this study was that the biodegradation of recalcitrant, xenobiotic organic carbon can be accelerated by mimicking the thermophilic/mesophilic temperature regime of these conditions in an engineered bioprocess. To achieve this, a two stage treatment process employing a thermophilic step and a mesophilic Anaerobic Membrane Bioreactor (AnMBR) was developed. While there have been a limited number of studies that have tested MBRs for the purpose of landfill leachate treatment, most did not treat raw leachate, instead opting to blend leachates with wastewaters in varying proportions (Bodzek *et al.*, 2006; Hasar *et al.*, 2009). In this study, the reactors were operated using leachate obtained from a young landfill with high organic content and strong UV absorbance. A stand – alone MBR was also set up (i.e. mesophilic AnMBR without a preceding thermophilic step) to affirm the validity of having a two stage process.

MATERIALS AND METHODS

Leachate Properties and Set Up of Enrichment Culture

Fifty gallons of leachate was obtained from a cell being used for active landfilling at a landfill located in Waverly, Virginia. Two enrichment cultures (one mesophilic and one thermophilic) were developed using reactors constructed from PVC pipes (2.5” ID x 24” long). These cultures were essentially anaerobic digesters, each with a hydraulic retention time (HRT) of 40 days and operating volume of 1.5 L. The mesophilic culture (operating temperature = 37 ± 1°C) was seeded with anaerobic sludge from a local wastewater treatment plant (Christiansburg, Virginia) while the thermophilic culture (operating temperature = 55 ± 2°C) was seeded using thermophilic digested sludge from anaerobic digesters being operated from an ongoing laboratory study. The enrichment cultures were mixed by continuous recirculation of the headspace gas using peristaltic pumps and operated as semi batch reactors; fed with landfill leachate once every two weeks. The cultures were operated at an HRT of approximately 60 days to allow the microbial community to acclimate. The effluent drawn prior to feeding the reactors was analyzed and the reactors were operated until stable COD reduction was observed (~4 months), indicating establishment of an enrichment culture capable of thriving in landfill leachate. Table 1 summarizes some important characteristics of the leachate used in the study.

Two–Stage Treatment Process Set Up and Operation

A two–stage reactor system was set up using seed from the enrichment cultures in January 2015. The first stage was a conventional anaerobic thermophilic digester (TD) followed by a mesophilic AnMBR. The TD was operated at 55 ± 2°C while the AnMBR was maintained at 37 ± 1°C. The reactors were fabricated using locally-sourced PVC cylinders. The TD dimensions were 2.5” ID x 25” long with an operating volume of 1.8 L and a HRT of 20 days. The AnMBR dimensions were 4” ID x 27” long with an operating volume of 2 L and an HRT of 45 days. The AnMBR was fabricated to permit complete immersion of a hollow fiber membrane module. The module used was the GE ZW-1® (GE Power & Water, Ontario, Canada), containing ZeeWeed® 500 UF hollow fibers, which are commonly applied for wastewater treatment. The hollow fiber module consisted of Polyvinylidene Difluoride fibers and was chosen because of its high solids tolerance and fouling resistance. The module had a nominal pore size of 0.04 µm and an operating pH range of 5.0 to 9.5. The temperature of operation (37 °C) was within the recommended range of operation of the hollow fibers (i.e. < 40 °C). Operation of the membrane outside of these conditions can cause cracking or failure of the hollow fibers. Figure 3-1 shows a schematic diagram and photographs of the reactor set–up.

The reactors were maintained uniformly mixed using peristaltic pumps manufactured by Cole - Parmer to recirculate headspace gas back in through the bottom. A separate pump was used to feed the reactors through a dedicated opening with a visual inspection point to minimize the potential for oxygen seeping into the system during feed/draw. The TD received raw, untreated landfill leachate, while the AnMBR received the TD effluent, after passage through a 5µ filter (glass microfiber, Whatman, Pittsburgh, PA). This helped prevent solids build–up in the AnMBR, thereby diminishing the potential for membrane fouling, which was monitored by tracking the flux through the membrane at a fixed pump setting as well as measuring transmembrane pressure (TMP) for a fixed flux. The final effluent was drawn through the hollow fiber membrane module, resulting in a low–solids, filtered product with reduced organic carbon and UV absorbance. The reactor system was operated over a period of 14 months.

Analyses for Organic Carbon and UV Quenching

Samples were drawn from the reactors every two weeks and analyzed for organic carbon and UV absorbance. Percentage reduction of UV quenching organics and UV absorbance was calculated relative to the influent raw leachate. These analyses were performed on the soluble fraction of the samples. The raw leachate, TD effluent and AnMBR effluent were fractionated into humic, fulvic and hydrophilic components using previously reported methods (Christensen *et al.*, 1998; Leenheer, 1981; Thurman and Malcolm, 1981). Briefly, humic acids were separated by acidification using 5M HCl to pH < 1.5. The humic acids were precipitated out of solution at this pH, filtered and redissolved in 0.1N NaOH. The mixture of fulvic acids and hydrophilic organics was then separated by slow flow through a packed column containing DAX-8® ion–exchange resin (Supelco®, Bellefonte, PA) that adsorbed fulvic acids. The fulvic acid fraction was extracted from the resin by running 0.1 N NaOH through the column. The volume of 0.1N NaOH used to redissolve the humic acids and fulvic acids was equal to the initial volume of the sample being fractionated in order to extract them at the original concentration. The fractionated and unfractionated leachate samples were subject to TOC analysis (Shimadzu® TOC-V CSN Total Organic Carbon Analyzer) to evaluate the organic carbon content.

UV absorbance of both fractionated and unfractionated raw leachate samples and the two reactors were monitored weekly over the course of operation. UV absorbance (254 nm) was quantified using a Beckman DU® 640 spectrophotometer capable of measuring absorbance at visible as well as ultraviolet wavelengths. Absorbance values were used to calculate specific ultraviolet absorbance (SUVA) values to compare intensity of UV absorbance by humic, fulvic and hydrophilic fractions normalized with respect to their concentrations. All analyses were performed in duplicate.

Size fractionation of organic matter was done for the humic, fulvic and hydrophilic substances that were separated from the samples of raw and treated leachate from both stages. UF membranes (Millipore, Billerica, MA) were used for a series of pressurized filtrations in order to characterize the organic carbon by molecular weight. Prior to size fractionation, samples were subjected to solids removal by microfiltration using 1.5 µ Whatman® microfiltration membrane (Cole Parmer, Vernon Hills, IL) with a simple vacuum filtration assembly. A 200 mL UF cell (Amicon, Bedford, MA; Model 8200) was connected to a pressurized Nitrogen gas tank at 120 KPa to provide the pressure required to drive UF. The molecular weight cutoffs of the UF membranes used for size fractionation were 100 KDa, 3 KDa and 1 KDa. Pressurized N_2 gas was used to drive 100 mL of sample through each step of ultrafiltration.

Assessment of System Performance and Membrane Fouling

COD destruction was measured by drawing and analyzing samples from the reactors every week and comparing the effluent COD with that of the raw leachate being fed into the system. COD measurements were performed using the closed titrimetric method, in accordance with Standard Method 5520C. The detection limit was up to a maximum of 450 mg/L of COD. pH was measured using an Oakton® handheld pH meter (Cole Parmer). Volatile fatty acids (VFA) analyses were done using gas chromatography with a flame ionization detector (GC-FID, Shimadzu ®, model GC – 14A with a Nukol™ fused silica column of 15 m x 0.53 mm with 0.5 μm film thickness. C2 – C7 organic acids were detectable, with a detection limit of 0.1 mg/L. Ammonia and nitrate levels were monitored in duplicate using a NitraVer® test and tube kits (Hach, Loveland, CO).

Membrane fouling was tracked by measuring the transmembrane pressure (TMP), i.e. the pressure differential between the permeate being pulled through the membrane and the contents of the bulk reactor (i.e. the retentate), by introducing a pressure gauge into the pipe drawing effluent through the membrane. The pressure gauge was placed between the membrane and the peristaltic pump used to pull the effluent through the membrane. An Ashcroft® 1490 dual – scale pressure gauge, capable of measuring pressures from 0 – 50 KPa was used, in accordance with the manufacturer recommended operating TMP range of the membrane module (0 – 55 KPa).

Set up and Operation of a 1 – Stage AnMBR

An additional AnMBR was set up, identical to the AnMBR used in stage 2 of the 2 – stage treatment process, both in terms of dimensions and operational parameters (4” ID x 27” long, operating volume = 2 L, HRT = 45 days). The AnMBR was operated as a single stage treatment system without a preceding thermophilic step, using the same leachate that was used to feed the 2 – stage system over its last 3 months of operation. The single stage AnMBR was seeded using inoculum from the AnMBR being used in the 2 – stage treatment process so the microbial community was already adapted to landfill leachate, and was operated for a total duration of about 5 months until stable removal of organic carbon and UV_{254} absorbance were observed. This allowed for a direct comparison of the TOC and UV_{254} absorbance reduction achieved by the 2 – stage process versus the one stage AnMBR. It was hypothesized that the two stage process would show better TOC and UV_{254} absorbance reduction as compared with the single stage AnMBR, thereby validating the inclusion of the thermophilic stage 1 in the treatment strategy.

RESULTS AND DISCUSSION

Enrichment Culture Start-up and Stabilization

Mesophilic and thermophilic cultures capable of degrading organic carbon present in landfill leachate were enriched by periodically batch feeding leachate as the sole carbon source. Total COD spiked following each feeding and subsequently declined until the next feeding. This spike and subsidence was more pronounced for the mesophilic culture (Figure 3-2). An initial COD of about 19,700 mg/L was found to be reduced by 25% at the end of the first month of operation under mesophilic conditions and increased to about 55% by the end of 3 months.

The mesophilic enrichment culture demonstrated improved efficiency in COD removal with time. By four months, the COD destruction in the mesophilic culture had stabilized in the range of 50–60%, indicating an active culture flourishing on the leachate as the sole carbon and nutrient source. The thermophilic culture achieved a similar trend, but the COD destruction after four months of operation was less than 15%.

Reduction of Organic Carbon and UV_{254} Absorbance

Figure 3-3 illustrates the reduction in organic carbon and UV quenching that was achieved using the two-stage AnMBR. Comparison of the influent and effluent humic, fulvic and hydrophilic concentrations demonstrated that TOC removal in the two–stage process continually improved with time. The characteristics of the raw leachate varied as new shipments were received and stored, resulting in periodic fluctuations in the influent properties. The removal of humic acids achieved by the system ranged from as low as 28% to as high as 59%. Fulvic acids showed the greatest variability, with the AnMBR effluent exhibiting fulvic removals fluctuating from 0 – 45%. Hydrophilic substances were effectively degraded, ranging from 36% to 65% removal. However, in the TD effluent, levels of humic acids remained within ±15% of the raw leachate, while fulvic acids increased. Hydrophilic substances were reduced in in both the stages.

The TD effluent TOC, averaged over the final six months of operation when the AnMBR effluent TOC exhibited the least fluctuation (months 7 – 13), did not vary significantly relative to the TOC of the raw leachate (ANOVA, $P = 0.87$) and, in general, was within ±10 % of that of the raw leachate. However, there was a slight change in the characteristics of the organic carbon after stage 1. A small reduction in the concentration of humic acids was observed, which was offset by an almost equivalent increase in fulvic acids. In the AnMBR stage, the concentrations of humic acids, fulvic acids and hydrophilic organics decreased markedly. The levels of fulvic acids in the TD effluent, at times exceeded the fulvic acid levels in the influent, suggesting that fulvic acids might be degradation products or degradation intermediates that are formed during biodegradation of humic acids or hydrophilic substances. Humic acids (47% removal) and hydrophilic substances (60% removal), were consistently removed. Over the final 6 months of operation, the total degradation of TOC was about 50%, which exceeds the maximum TOC removal previously reported by any biological treatment using currently available methods, such as conventional anaerobic digestion (AD) or activated sludge (Zhao *et al.*, 2013).

A corresponding drop in the UV_{254} absorbance was observed concurrent with reduction in humic and fulvic acids. Notably, the raw leachate was characterized by very high UV_{254} absorbance (72 – 79 cm^{-1}). Figure 3-4 shows a plot of the variation in UV_{254} absorbance over time in treated vs untreated leachate, as well as average UV_{254} absorbance in the two stages (over final 6 months of operation). The UV_{254} absorbance exhibited by the TD stage effluent did not vary significantly relative to the raw leachate ($P = 0.14$). However, the absorbance decreased considerably following the AnMBR stage. Initially, over the first two months of operation, there was a rise in UV_{254} absorbance in the TD and the AnMBR effluents, to levels comparable to the raw leachate. This suggests that acclimation was still necessary, even though the digesters were seeded using pre-acclimated enrichment cultures. This likely reflects a higher feed rate and other distinct conditions in the digesters, as well as unavoidable exposures to oxygen during the initial set–up.

Figure 3-4(a) illustrates the overall trends in UV_{254} absorbance with time. The trends in UV_{254} absorbance closely mirrored those of TOC, i.e., the total effluent UV_{254} absorbance was within ± 15% of that of the raw leachate. As was the case with TOC, it was clear that most of the removal of UV_{254} absorbance occurred in the AnMBR stage. Figure 3-4(b) shows the average UV_{254} absorbance in the raw and treated leachate over the final 6 months of operation. The removal of UV_{254} absorbance in the reactor system was stable over a prolonged time period. Over the final six months of operation, the average reduction of UV_{254} absorbance due to degradation of humic acids was about 60%, while reduction due to degradation of fulvic acids and hydrophilic compounds were 36% and 29% respectively. The UV_{254} absorbance due to humic acids was removed most effectively, resulting in an overall UV_{254} absorbance removal of 46%. This is owing to the higher SUVA of humic acids relative to fulvic acids or hydrophilic substances. The high SUVA values observed for humic acids are in agreement with the observations of Zhao *et al.* (2013), who characterized organic UV-quenching compounds in several landfill leachates. The SUVA values of the humic acids, fulvic acids and hydrophilic substances in the raw leachate being fed into the system are summarized in Figure 3-5.

SUVA value of the humic acids were the highest, followed by the fulvic acids, with hydrophilic compounds being the lowest. Efficient biodegradation of humic acids is therefore particularly critical to achieving reduction of UV_{254} absorbance. Notably, there while there was little to no fluctuation in the SUVA values of the organic matter present in the final effluent over the final six months of operation, even when the raw and TD effluent exhibited some variation.

COD Removal and Ammonia Levels

The COD levels in the reactors with time as well as average COD removal are shown in Figure 3-6(a) and (b). The raw leachate was high in total COD, with an average value of about 12,000 mg/L. The trends for COD removal were found to be consistent with the observed removal of UV–quenching organic carbon. The COD levels in the TD were typically within ± 20% of the raw leachate COD, with most of the COD removal occurring in the AnMBR. COD removal by the overall two–stage process typically varied from 40% to 70%, with an average removal of 61% over the final six months of operation. The final effluent COD indicated little fluctuation over the final 6 months (4,130 mg/L ± 5%).

Ammonia in the reactors and raw leachate ranged from 2,000 – 3,200 mg/L. The values fluctuated within this range over the duration of the study and did not exhibit any noticeable trend.

Transmembrane Pressure Data and Membrane Fouling

Membrane fouling was assessed by measuring the TMP, as summarized in Figure 3-6(c). Membrane fouling was found to be minimal, with only a slight increase indicated by a gradual rise in TMP to approximately 10 KPa. This is much smaller than the optimal operating range of the membrane module (55 KPa). It is possible that passing the TD effluent through the 5μ filter effectively prevented the build-up of solids on the membrane surface. The gradual increase in TMP over the duration of the study suggests that extended operation without significant membrane fouling may be possible, thereby making it a candidate for scale-up and pilot-testing by the landfill and leachate management industry.

Comparison to Previously Studied Leachates

The ratio of humic acids to fulvic acids to hydrophilic substances for the leachate used in this study was different from leachates used in earlier studies and also displayed variation, even within different samples drawn from the same cell within the same landfill. This suggests that landfill leachate can be highly variable in terms of its nature and biorefractory content, not only from landfill to landfill, but also within the same landfill. Any biological treatment process that aims to treat leachate must therefore be sufficiently robust to manage fluctuations in leachate organic matter content and COD. Future efforts will focus on evaluating the range of leachate characteristics that can be accommodated by the two–stage system described here.

Role of the TD Stage and Comparison with Single stage AnMBR

The first-stage of the two stage process i.e. the TD step indicated little reduction in COD, TOC or UV_{254} absorbance. This brought to question the role of the TD step in the treatment train. Size fractionation of the organic carbon provided insight into TD function. The leachate was split into four fractions based on the molecular weight of the organics present in the sample being analyzed. Organics were sorted into four categories based on molecular weight (i.e., >100 KDa, 100 – 3 KDa, 3 – 1 KDa and <1 KDa). This analysis revealed that, even though there was only a small reduction of TOC during the TD stage, there was a major shift in the molecular weight distribution of the organic content. The two smallest fractions, i.e. 3 – 1 KDa and <1 KDa, made up only 35% of the total humic acids. However, after TD, this number increased to almost 75%. Similarly, it was observed that the percentage of the two smallest fractions of the fulvic acids increased from only 25% in the raw leachate to about 69% in the TD effluent.

Most of the hydrophilic compounds present in the raw leachate were already of low molecular weight, and were therefore degraded in the TD step to some extent (~20% removal), with more removal occurring in the AnMBR. Figure 3-7 shows the molecular weight distribution of the organic matter in the raw and treated leachates. Here it can be inferred that while the thermophilic (TD) step was unable to significantly reduce UV–quenching, it did serve to hydrolyze complex organics into smaller, chemically similar molecules. These were then effectively degraded by the AnMBR microbial community. This is consistent with previous work dealing with on–site biological treatment of landfill leachates, which suggested that most of the organic removal occurs in the molecular weight fraction of <1 KDa. We thus hypothesize that the TD step breaks up the UV – quenching organics into smaller, more bioavailable forms for the microbes in the AnMBR, thereby imparting to the system the capability to carry out the biodegradation of otherwise difficult to degrade organics.

Furthermore, analysis of organic carbon and UV_{254} absorbance of the effluent drawn from the single stage AnMBR clearly validated the inclusion of the TD step in the two stage process. It was observed that the single stage AnMBR operating without a preceding thermophilic treatment step was able to remove an average of only 15% of the total refractory organic carbon (i.e. humic acids, fulvic acids and hydrophilic substances) and 16% of the UV_{254} absorbance from the raw leachate over three months of stable operation. The corresponding values for the two – stage treatment process fed with the same leachate were 50% and 48% respectively. These data are shown in Figure 3-8. It may therefore be inferred that while the TD step (i.e. Stage 1) does not exhibit considerable removal of UV quenching organic carbon from raw leachate, it plays an important role in imparting to the two stage system the ability to degrade these compounds.

Previous research has shown that as landfills get older, the content of refractory organic compounds imparting UV–quenching decreases (Gupta *et al.*, 2014). From data obtained from analyzing samples from cells of varying ages in landfills, it is typical that the UV–quenching organic compounds are reduced to half the concentration found in a young, actively landfilling cell over approximately 9–10 years. Thus, the two–stage system could be described as accelerating the natural process of UV-quenching organics removal by simulating the sequential thermophilic, mesophilic conditions experienced in a typical landfill after several years of stabilization.

Comparison to Other Treatments

Several methods have been tested for treating landfill leachates. Most conventionally used treatment methods that work well to reduce nitrogen loading and COD are unable to remove or considerably reduce UV quenching. Coagulation, flocculation and activated adsorption have also proved ineffective for the removal of UV quenching, although they have been able to mitigate the problem of nutrient loading (Tatsi *et al.*, 2003; Rodriguez *et al.*, 2004). Ultrafiltration is reported to work well for a portion of the humic fraction, but is unable to remove the fulvic and hydrophilic content of the leachate. Reverse osmosis is the only non – oxidative form of treatment that has been shown to almost completely remove (>97% removal) UV quenching from landfill leachates but is expensive and has a large reject fraction.

Chemical oxidation with strong oxidants like peroxides, ozone and UV is somewhat effective with nitrogen species and COD, but does not work well to reduce UV quenching (Kurniawan *et al.*, 2006). Fenton's reagent has been demonstrated to remove >95% of the UV quenching in landfill leachates but is limited by high cost of the oxidant as well as the large quantity of sludge generated (Gupta *et al.*, 2014). Attempts have been made to biologically treat the organic carbon in landfill leachates (Zhao *et al.*, 2013). Using aerobic biological processes has been found to be ineffective for the removal of UV – quenching xenobiotic organic carbon. Even though a small fraction of the organic carbon is often observed to be degraded, the organics responsible for UV quenching in leachates have been found to be highly resistant to aerobic biological treatment. With the advent of advanced and enhanced biological treatment processes such as membrane bioreactors (MBRs), it may be possible to keep biomass within the reactors longer, providing a more effective form of biological treatment, with a much lower operational and maintenance cost.

Biological treatment of landfill leachate is challenging due to a variety of factors. The high ammonia concentrations typically present in landfill leachate tend to be inhibitory to metabolic pathways responsible for biodegradation of organic carbon (Berge *et al.*, 2006). The variability in the nutrient compositions (Chian and DeWalle, 1976) and low concentrations of phosphorus and bioavailable carbon also make it difficult to sustain a microbial community over a prolonged period (Berge *et al.*, 2006). In this case, phosphate concentrations in the raw leachate were below detectable levels. While stimulation of biological treatment by addition of phosphorus and organic carbon has shown promise for nitrogen removal (He *et al.*, 2006; Xu *et al.*, 2010; Jokela *et al.*, 2002), removal of UV–quenching organic carbon continues to be a challenge. Efforts to reduce UV quenching by landfill leachates using biological treatment approaches such as activated sludge and conventional anaerobic digestion have yielded poor results, illustrating that the organic carbon present in leachates is not readily amenable to biodegradation (Zhao *et al.*, 2013).

While several studies have assessed the feasibility of using MBRs for the purpose of landfill leachate treatment, it can be seen that most of them employ aerobic MBRs or aerobic – anoxic MBRs operating using a mixture of domestic or synthetic wastewater and landfill leachate (Ahmed *et al.*, 2012). Several of these studies claim to achieve over 80% COD and ammonia removal, but fail to address the issue of UV quenching since it is not one of the leachate quality parameters governed by any regulation. Some studies have also reported successful use of AnMBRs for landfill leachate treatment. As is the case for aerobic systems, most of these that reported high (>50%) COD removal, used a mixture of leachate and synthetic wastewaters (Bohdziewicz *et al.*, 2008; Dacanal *et al.*, 2010) or utilized synthetic leachates and focused on trace contaminants such as endocrine disrupting chemicals (Do *et al.*, 2009). This study is the first to address the issue of reducing the UV absorbance by landfill leachate before it is discharged to wastewater treatment plants. It is the first to achieve >50% reduction of recalcitrant organic carbon present in landfill leachate via biological treatment by employing a 2 – stage, thermophilic, mesophilic AnMBR system. While data collected over more than one year shows promise and ease of operation of the system, there is the need to test a more scaled – up, high capacity version of the process to accurately estimate operational and maintenance costs and to identify potential pitfalls and issues.

Thus, while the feasibility of having a relatively low cost process for the degradation of UV quenching recalcitrant organics has been demonstrated, there is a need to optimize the process and possibly integrate it with other forms of treatment such as the aerobic reactors that work well for nutrient removal. This will facilitate the development of a holistic, robust biological treatment process capable of removing nutrients, COD as well as organic carbon efficiently. The problem of UV quenching in landfill leachates is an emerging issue and tends to be overlooked when choosing a treatment process for leachates. Since the compounds responsible for UV absorbance as well as UV absorbance of leachate itself are not regulated by any agency, it is difficult to have clearly defined treatment goals. It is important to make landfills and wastewater treatment utilities aware that typical on – site biological treatment processes are ineffective at removing UV absorbing organic compounds from leachates, which is especially problematic when the wastewater treatment plant receiving the leachate uses UV disinfection.

CONCLUSIONS

This study evaluated the long–term performance (over 14 months) of a novel, two–stage system that used thermophilic digestion followed by an AnMBR for targeted removal of UV–quenching organic compounds. The following are the key conclusions of this study:

(1) The system was able to consistently remove 55% of the TOC present in the raw leachate over the period of operation, which is greater than the removal levels reported for any other form of biological treatment.

(2) UV absorbance in the treated leachate was reduced by 45% relative to the raw leachate. Removal of UV absorbance was attributed to effective degradation of humic acids (~50% removal) and hydrophilic substances (~60% removal). Fulvic acid concentrations were variable, suggesting that UV–quenching organics may exhibit inter–conversion during degradation.

(3) Over 60% of the total COD fed into the system was degraded.

(4) TMP data plotted over the course of AnMBR operation suggested that it was possible to operate the system for extended time periods of the order of several months without significant membrane fouling.

(5) Microbial communities required about 2 – 4 months to adapt to landfill leachate. The system performance, in terms of organics and COD removal, showed steady improvement over time, suggesting that systems designed for leachate treatment must be allowed longer acclimation periods as compared with other bioreactors (e.g., typical anaerobic digesters treating municipal sewage sludge).

The use of a two – stage AnMBR system employing sequential thermophilic and mesophilic temperatures can be considered to be a feasible, on-site pretreatment option for removal of recalcitrant UV-quenching organic carbon from landfill leachates prior to discharging into wastewater treatment plants.

REFERENCES

Ahmed, F. N., Lan, C. Q., 2012. Treatment of landfill leachate using membrane bioreactors: A review. Desalination (287), 41–54.

Alkan, U., Teksoy, A., Atesli, A., Baskaya, H. S., 2007. Influence of humic acids on the ultraviolet disinfection of surface waters. Water Environment Journal (21), 61–68.

Berge, N.D., Reinhart, D.R., Dietz, J., Townsend, T., 2006. In situ ammonia removal in bioreactor landfill leachate. Waste Management 26 (4), 334 – 343.

Bodzek, M., Łobos-Moysa, E., Zamorowska, M., 2006. Removal of organic compounds from municipal landfill leachate in a membrane bioreactor. Desalination (198), 16–23.

Bohdziewicz, J., Neczaj, E., Kwarciak, A., 2008. Landfill leachate treatment by means of anaerobic membrane bioreactor. Desalination (221), 559–565.

Çeçen, F., Aktaş, Ö., 2004. Aerobic Co-Treatment of Landfill Leachate with Domestic Wastewater, Environmental Engineering Science 21, 303–312.

Chian, E.S.K., DeWalle, F.B., 1976. Sanitary landfill leachates and their treatment. Journal of the Environmental Engineering Division 102 (2), 411 – 431.

Christensen, J. B., Jensen, D. L., Grøn, C., Filip, Z., Christensen, T. H., 1998. Characterization of the dissolved organic carbon in landfill leachate-polluted groundwater. Water Research (32), 125–135.

Dacanal, M., Beal, L. L., 2010. Anaerobic filter associated with microfiltration membrane treating landfill leachate. Engenharia Sanitaria e Ambiental (15), 11–18.

Do, A. T., Prieto, A. L., Yeh, D. H., 2009. Anaerobic membrane bioreactor (MBR) for removal of EDCs from landfill leachate: A pretreatment strategy for municipal wastewater treatment plants, Proceedings of the Water Environment Federation, WEFTEC 2009: Session 61 - 70, vol. 19, 4220–4238.

Gupta, A., Zhao, R., Novak, J. T., Goldsmith, C. D., 2014. Variation in organic matter characteristics of landfill leachates in different stabilization stages. Waste Management & Research (1 – 8).

Gupta, A., Zhao, R., Novak, J. T., Goldsmith, C. D., 2014. Application of Fenton's reagent as a polishing step for removal of UV quenching organic constituents in biologically treated landfill leachates. Chemosphere (105), 82 – 86.

Hasar, H., Ipek, U., Kinaci, C., 2009. Joint treatment of landfill leachate with municipal wastewater by submerged membrane bioreactor, Water Science & Technology (60), 3121–3127.

He, P.J., Shao, L.M., Guo, H.D., Li, G.J., Lee, D.J., 2006. Nitrogen removal from recycled landfill leachate by ex situ nitrification and in situ denitrification. Waste Management 26 (8), 838 – 845.

Jokela, J.P.Y., Kettunen, R.H., Sormunen, K.M., Rintala, J.A., 2002. Biological nitrogen removal from municipal landfill leachate: low-cost nitrification in biofilters and laboratory scale in-situ denitrification. Water Research 36 (16), 4079 – 4087.

Kjeldsen, P., Barlaz, M.A., Rooker, A.P., Baun, A., Ledin, A., Christensen, T.H., 2002. Present and long-term composition of MSW landfill leachate: a review. Critical Reviews in Environmental Science and Technology 32 (4), 297 – 336.

Kurniawan, T.A., Lo, W., Chan, G.Y.S., 2006. Radicals-catalyzed oxidation reactions for degradation of recalcitrant compounds from landfill leachate. Chemical Engineering Journal, Volume 125 (1), 35 – 57.

Leenheer, J.A., 1981. Comprehensive approach to preparative isolation and fractionation of dissolved organic carbon from natural water and wastewaters. Environmental Science and Technology 15 (5), 578 – 587.

Reinhart, D., Bolyard, S. C., 2015. Fate of Organic Matter from Leachate Discharged to Wastewater Treatment Plants (Year 1). Report #10285 submitted to the Hinkley Center for Solid and Hazardous Waste Management, Gainesville, FL.

Renou, S., Givaudan, J.G., Poulain, S., Dirassouyan, F., Moulin, P., 2008. Landfill leachate treatment: review and opportunity. Journal of Hazardous Materials 150 (3), 468 – 493.

Rodriguez, J., Castrillon, L., Maranon, E., Sastre, H., Fernandez, E., 2004. Removal of non-biodegradable organic matter from landfill leachates by adsorption. Water Research 38 (14 – 15), 3297 – 3303.

Tatsi, A. A., Zouboulis, A. I., Matis, K. A., Samaras, P., 2003. Coagulation – flocculation pretreatment of sanitary landfill leachates. Chemosphere 53 (7), 737 – 744.

Thurman, E. M., Malcolm, R. L., 1981. Preparative isolation of aquatic humic acids. Environmental Science & Technology (15), 463–466.

US EPA, 2010. Municipal Solid Waste Generation, Recycling, and Disposal in the United States: Facts and Figures for 2009. EPA-530-F-010-012, Washington, DC.

Uygur, A., Kargı, F., 2004. Biological nutrient removal from pre-treated landfill leachate in a sequencing batch reactor. Journal of Environmental Management (71), 9–14.

Xu, Z.Y., Zeng, G.M., Yang, Z.H., Xiao, Y., Cao, M., Sun, H.S., Ji, L.L., Chen, Y., 2010. Biological treatment of landfill leachate with the integration of partial nitrification, anaerobic ammonium oxidation and heterotrophic denitrification. Bioresource Technology 101 (1), 79 – 86.

Yeșiller, N., Hanson, J. L., Liu, W. L., 2005. Heat Generation in Municipal Solid Waste Landfills. Journal of Geotechnical and Geoenvironmental Engineering 131 (11), 1330 – 1344.

Zayen, A., Mnif, S., Aloui, F., Fki, F., Loukil, S., Bouaziz, M., Sayadi, S., 2010. Anaerobic membrane bioreactor for the treatment of leachates from Jebel Chakir discharge in Tunisia. Journal of Hazardous Materials (177), 918 – 923.

Zhao, R., Gupta, A., Novak, J. T., Goldsmith, C. D., Driskill, N., 2013. Characterization and treatment of organic constituents in landfill leachates that influence the UV disinfection in the publicly owned treatment works (POTWs). Journal of Hazardous Materials (258 – 259), 1 – 9.

TABLES

Table 3-1. Characteristics of Raw Landfill Leachate. Even though the leachate used over the course of the study was drawn from one landfill cell, there was a fluctuation in characteristics. Despite variations in leachate characteristics, the effluent derived from the two stage system showed low variation in terms of COD and TOC content. (BDL = Below Detectable Limit)

Parameter	***Unit***	***Range***
pH	-	8.0 – 8.6
COD	mg/L	7900 - 15800
TOC	mg/L as C	2800 - 3900
Total Solids (TS)	mg/L	12000 - 18000
Total Suspended Solids (TSS)	mg/L	2000 - 4000
BOD_5	mg/L	2500 - 5700
BOD_5/COD	-	0.31 – 0.37
Total Ammonia Nitrogen (TAN)	mg/L as N	1900 - 2300
Total Phosphates ($PO4^{3-}$)	mg/L as P	BDL
Nitrite (NO_2^-)	mg/L as N	BDL
Nitrate (NO_3^-)	mg/L as N	BDL

FIGURES

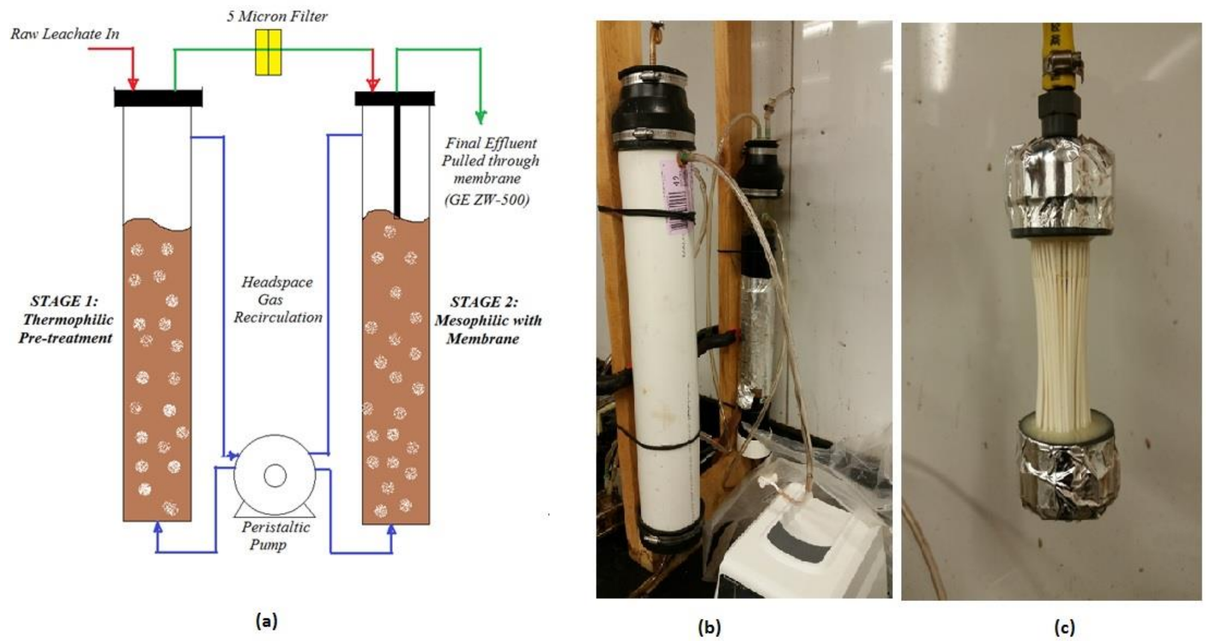

Figure 3-1. 2-stage reactor used in the study. (a) Schematic diagram illustrating the process configuration, (b) Set – up in use and (c) membrane module (ZW-1®, GE).

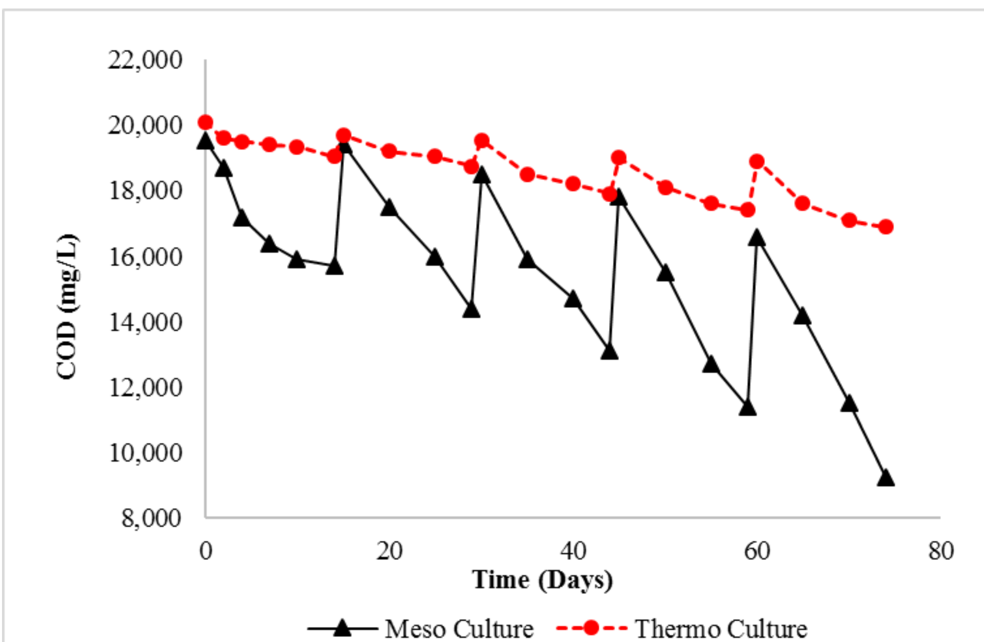

Figure 3-2. COD levels in the enrichment cultures. Spikes in COD concordant with feeding events.

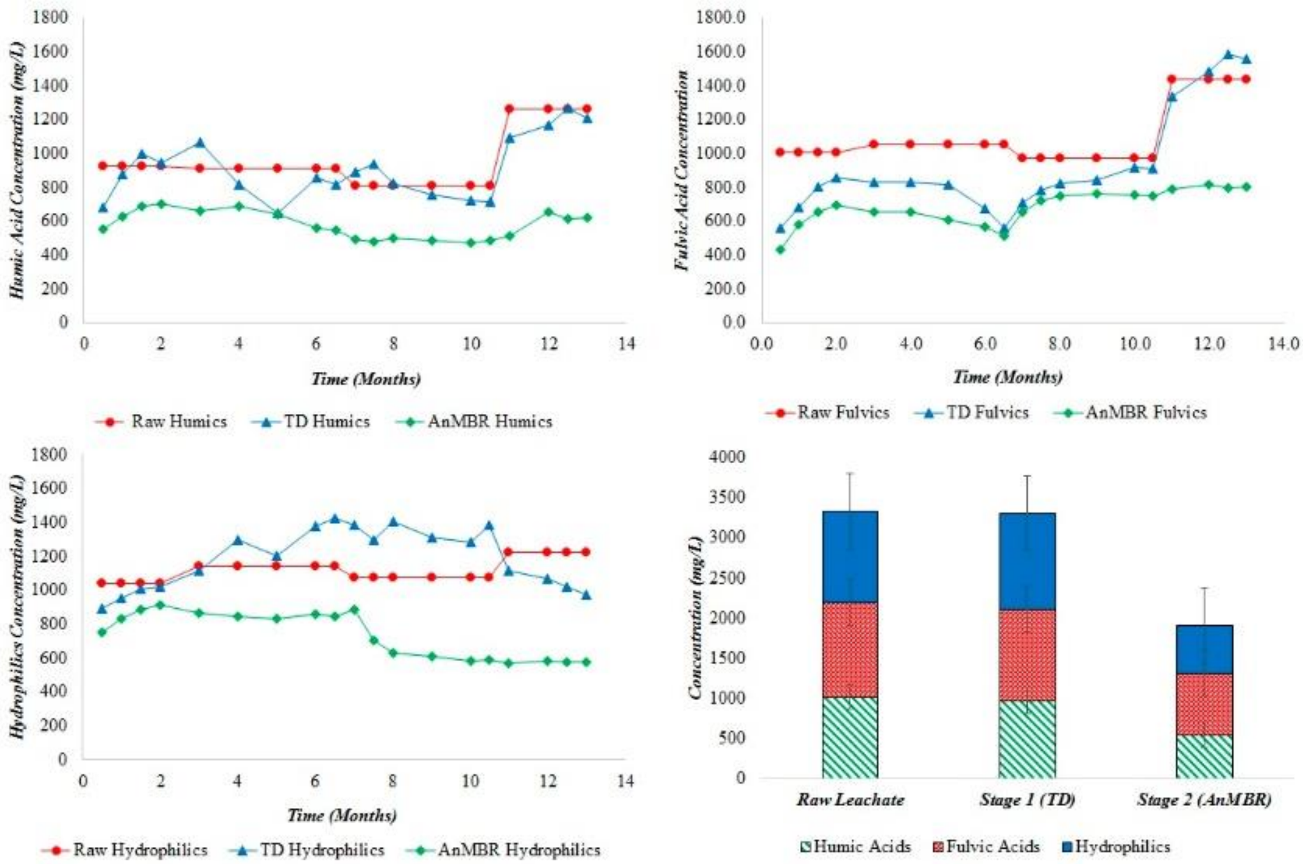

Figure 3-3. Reduction in UV – quenching organic compounds achieved by the AnMBR system. 3(a), 3(b) and 3(c) show the concentrations of humic acids, fulvic acids and hydrophilic substances respectively in the raw leachate and their corresponding effluents drawn from the first (TD) and second (AnMBR) stage of treatment. Error bars indicate standard error of the mean (n = 19). 3(d) shows the average humic, fulvic and hydrophilic destruction over the final six months (months 7 – 13) of data collection.

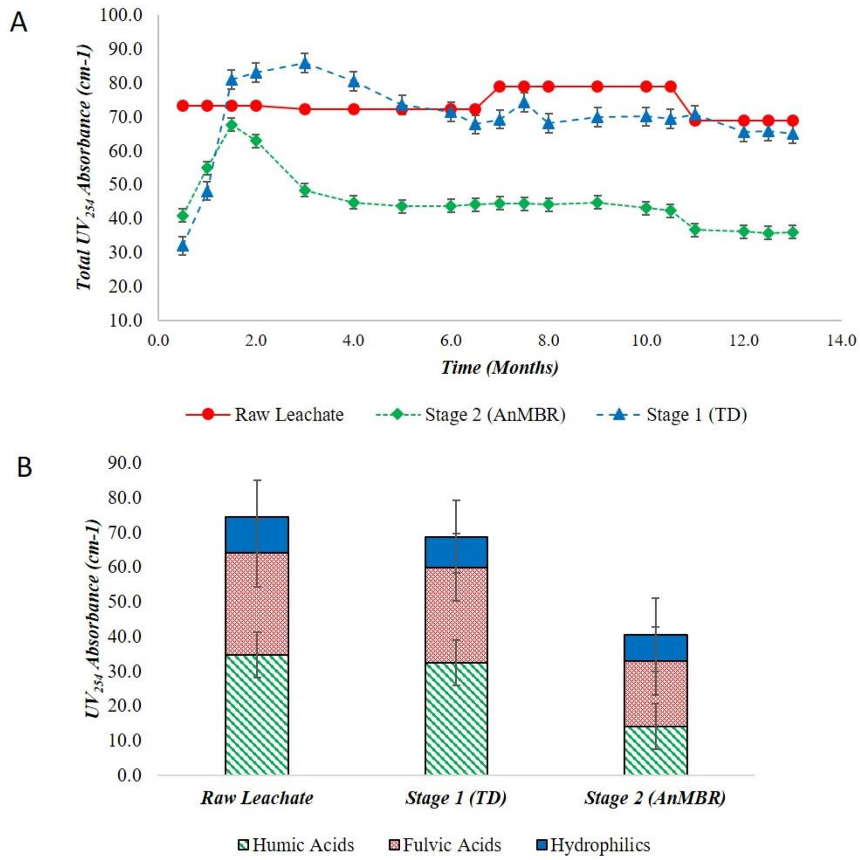

Figure 3-4. Reduction in UV absorbance in the reactor system: (a) Variation of total UV absorbance with time (b) Average UV absorbance values over the final six months of data collection (months 7 – 13), illustrating the breakdown of absorbance associated with humic, fulvic and hydrophilic substances.

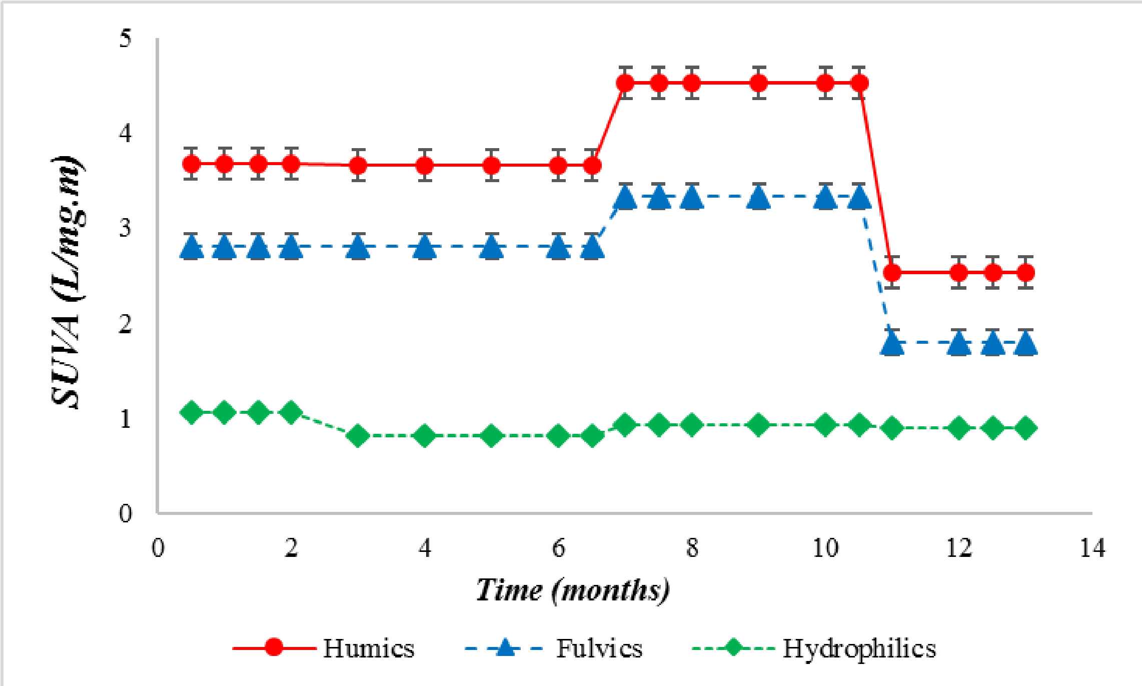

Figure 3-5. SUVA values of the organic fractions in the raw leachate with time.

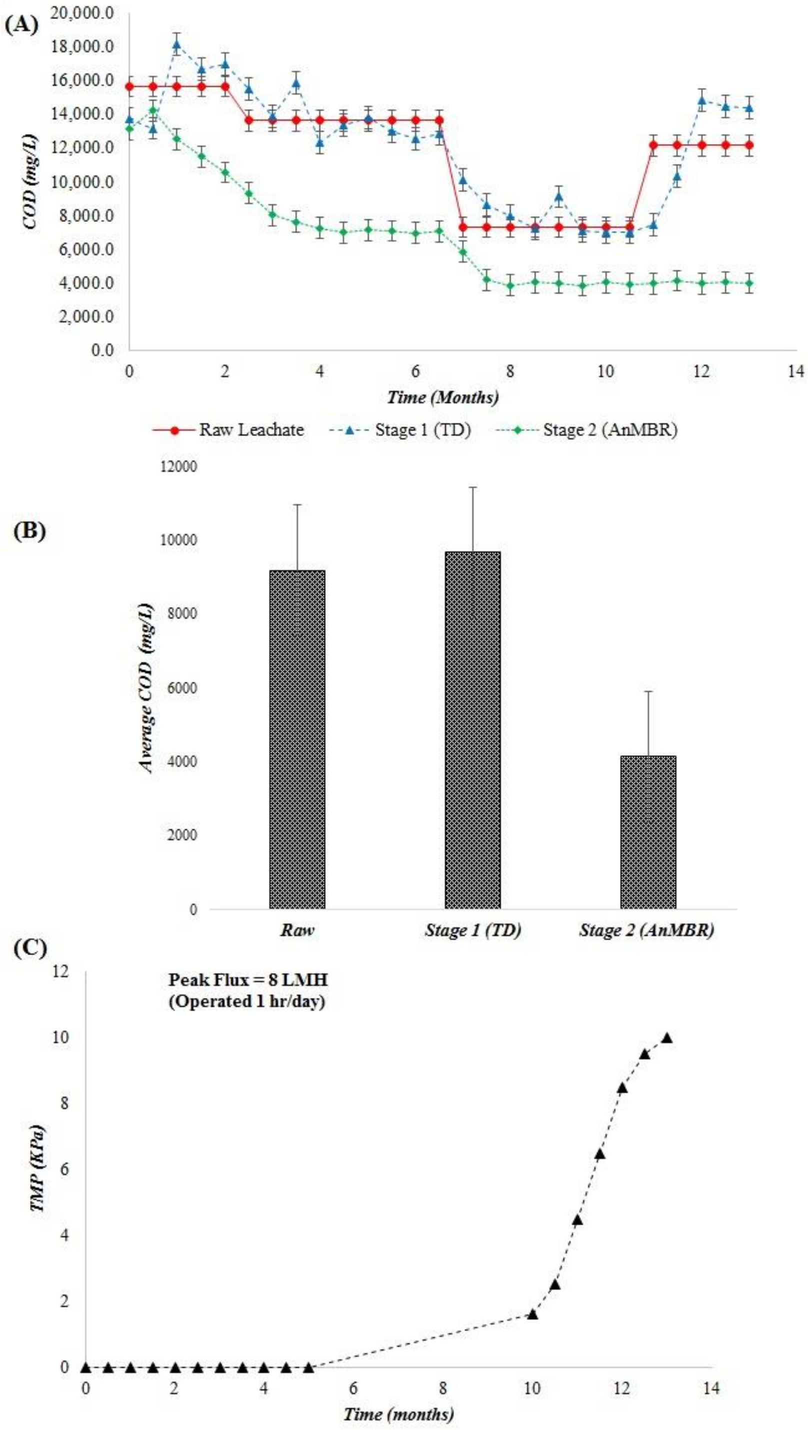

Figure 3-6. COD removal and membrane performance in the two–stage system (a) COD values in raw vs treated leachate over time; (b) COD values in raw, TD and AnMBR averaged over six months (months 7 – 13); (c) TMP values in the AnMBR over the course of the study. The effluent was only withdrawn for approximately 1 hour each day, meaning that the membrane had large resting times.

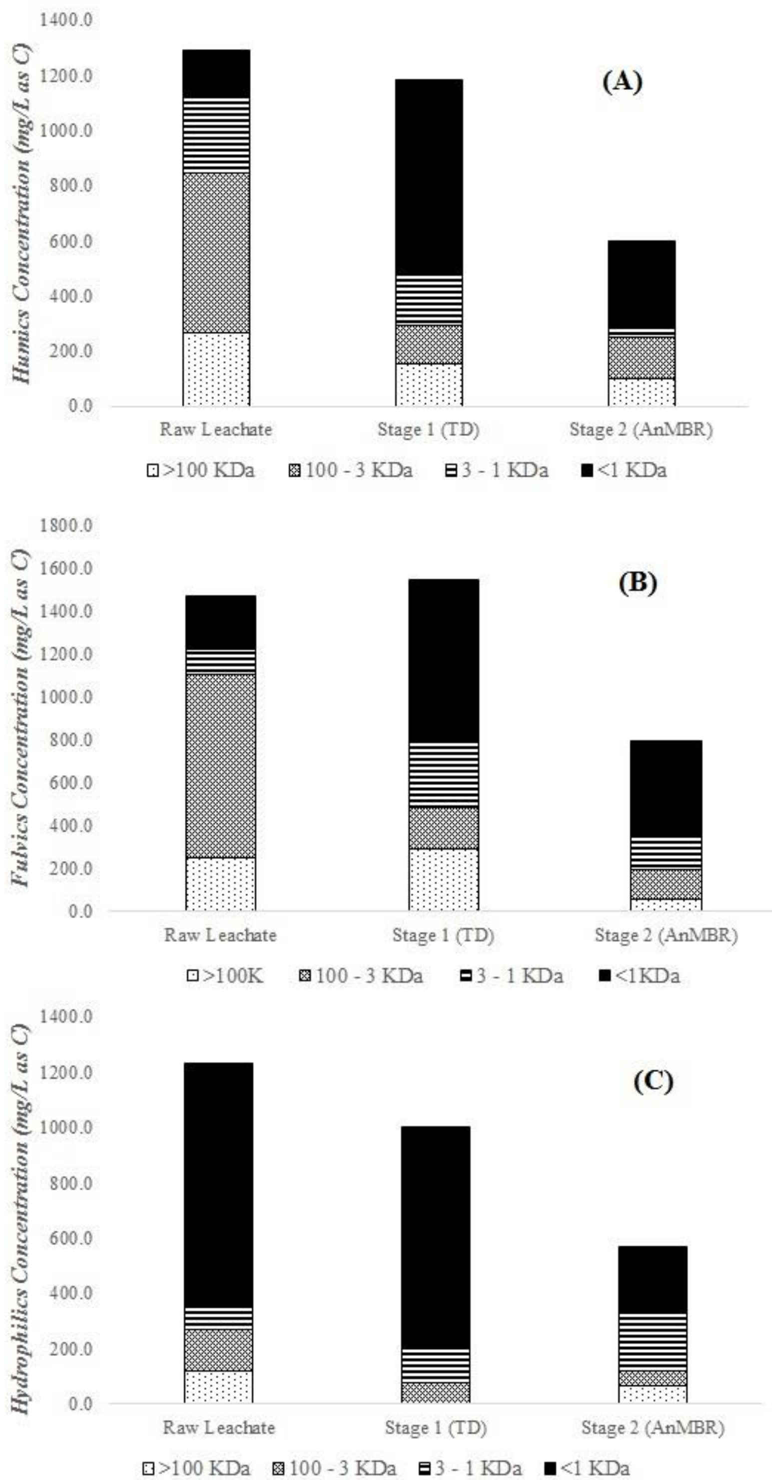

Figure 3-7. Molecular weight distribution of the organic matter in raw vs treated landfill leachate. The plots (a), (b) and (c) show the distribution of humic acids, fulvic acids and

hydrophilic substances respectively in the raw leachate, TD effluent and the final effluent drawn through the AnMBR.

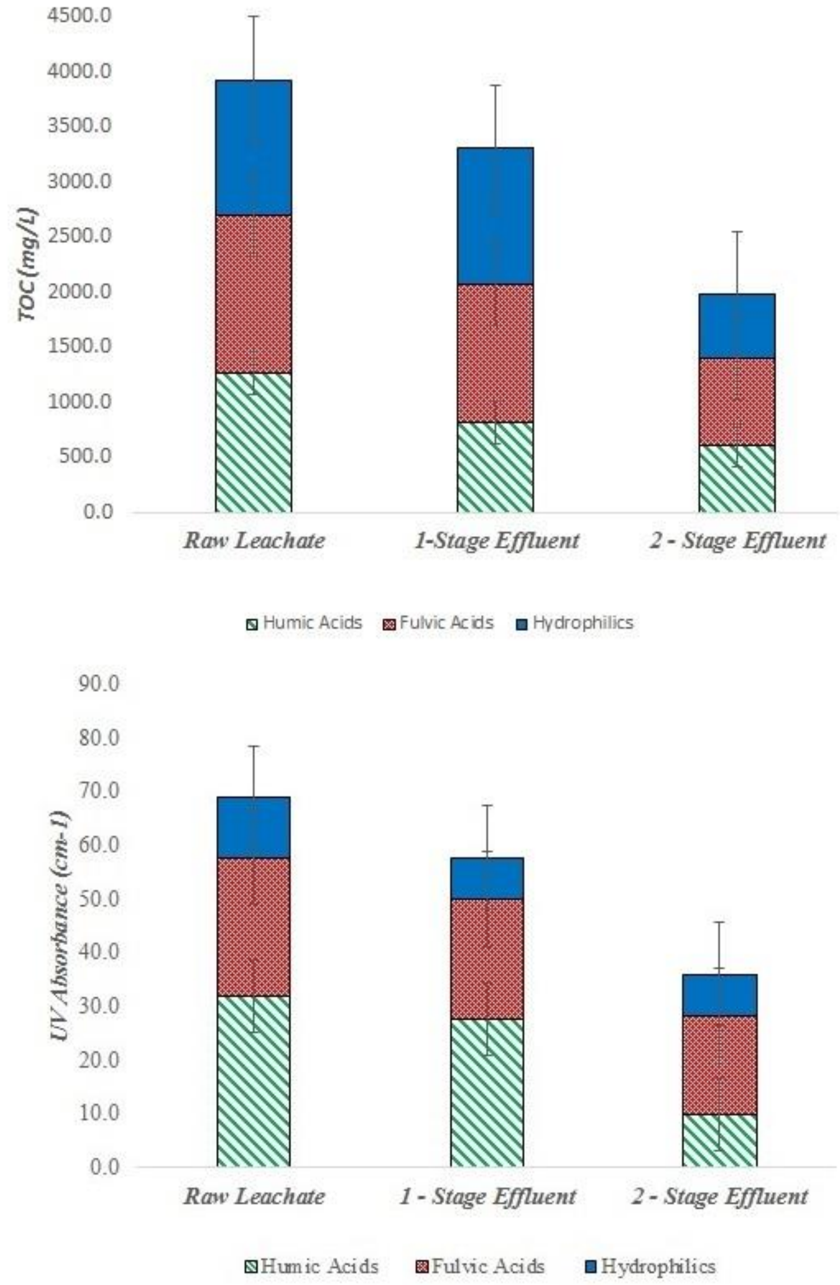

Figure 3-8. Average removal of TOC and UV absorbance observed for the 2 – stage process versus the single-stage AnMBR (1-Stage).

CHAPTER 4

Two – Stage vs Single Stage Anaerobic Membrane Bioreactor (AnMBR) for removal of UV – Quenching Organics from Landfill Leachate

Ankit Pathak, Dongjuan Dai, Amy Pruden, John T. Novak

Department of Civil and Environmental Engineering, Virginia Tech, 418 Durham Hall, Blacksburg, VA 24060, USA

ABSTRACT

Recently, a two – stage anaerobic membrane bioreactor (AnMBR) consisting of thermophilic pretreatment (55 ± 2 °C; 20 – day HRT) as Stage 1 and a mesophilic AnMBR (37 ± 1 °C; 45 – day HRT) as Stage 2 demonstrated promise for treating UV disinfection-interfering substances in landfill leachate. To gain a better understanding of the mechanistic role of each treatment step, an identical single-stage mesophilic AnMBR (37 ± 1± °C; 45 – day HRT) was compared over a 6-month period, without the preceding thermophilic step. It was found that, on average, the two – stage system removed 55% of the organic carbon present in raw landfill leachate, while the single-stage AnMBR only removed 15%. Further, the two-stage system was able to remove nearly 50% of the UV_{254} absorbance, while only 16% was removed by the single-stage AnMBR. These results support the hypothesis that mimicking the conditions of long-term landfilling in an engineered system can help improve degradation of biorefractory organic carbon compounds, particularly humic and fulvic acids responsible for UV-quenching and reduction in UV_{254} absorbance and effluent disinfection performance over time. Shotgun metagenomic DNA sequencing and qPCR analysis of microbial communities in samples of raw leachate and from each reactor stage demonstrated the presence of genera known to contain degraders of humic substances, including *Geobacter*, *Burkholderia*, *Clostridium*, *Desulfobacterium*, *Desulfovibrio*. However, the two-stage system was characterized by a higher number of matches for these organisms and overall numbers of total bacterial 16S rRNA gene copies, suggesting that higher

biomass concentrations could be maintained in the two-stage system, helping increase the rate of degradation of humic substances. While analyses for functional genes using the UniProtKB database (2016) indicated the presence of diverse functional genes across the samples, the relative abundances of functional gene subtypes did not show considerable variation.

Keywords: AnMBR, landfill leachate, DNA sequencing, Metagenomics, humic substances, UV quenching

INTRODUCTION

The 2013 USEPA report on advancing sustainable materials management states that there are over 1,900 active landfills in the US. Even though the number of landfills has seen a decline in the last decade, the average landfill area has increased. An over – reliance on landfills for the disposal of municipal waste contributes to the generation of landfill leachate, a liquid formed by percolation of rainwater through the landfill, and also due to the water content of the wastes placed in the landfill. A study by Kurniawan *et al.* (2009) estimated that the landfilling of 1 m^3 of solid waste results in the formation of 0.2 m^3 of landfill leachate. Landfill leachate can cause pollution of soil and groundwater in the vicinity of a landfill, and must therefore be regularly removed and treated (kurniawan *et al.*, 2006). Leachates may be treated and discharged directly into surface waters or into wastewater treatment plants with or without prior treatment (Ugyur *et al.*, 2004).

The treatment of landfill leachate at domestic wastewater treatment plants has been found to create problems with nutrient loading and toxicity due to heavy metals (He *et al.*, 2006a). While the treatment processes at wastewater treatment plants and the substantial dilution of landfill leachate by wastewater can overcome these issues, the capacity of leachates to absorb UV light is a problem even when leachates make up as low as 1% of the total flow (Zhao *et al.*, 2013; Gupta *et al.*, 2014; Reinhart and Bolyard, 2015). It has been found that UV quenching by landfill leachates may be attributed to the presence of aromatic and aliphatic organic carbon, specifically hydrophobic humic and fulvic acids (also known together as humic substances) and some hydrophilic compounds (He *et al.*, 2006b). While the humic substances in landfill leachates have been found to show some structural similarities with those found in soil (Göbbels and Püttman, 1997), they are different since they can also be formed by natural biodegradation of lignins that are found in paper, wood and leaves / yard wastes or by biotransformation of food wastes (Xiaoli *et al.*, 2008).

The UV – quenching compounds in landfill leachate constitute a substantial portion of the. dissolved organic matter in leachate (Seo *et al.*, 2007). Despite there being no standard method for the quantification of humic substances in leachates, the methods described by Christensen et al. (1998) and Thurman and Malcolm (1981) are frequently used. Studies of DOM in landfill leachate have found that it can be highly resistant to biodegradation (Zhao *et al.*, 2013; Gupta *et al.*, 2014) and is therefore not removed by treatment processes at wastewater treatment plants. There have been several efforts to treat landfill leachate on – site prior to discharging them into wastewater treatment plants. Both physico – chemical and biological treatment have been tested. Reverse osmosis has been shown to be effective for leachate treatment, delivering an effluent low in COD and nutrients, but is not widely used due to the high energy demand, chemicals handling requirements (for membrane cleaning and maintenance) and costs associated with treatment and disposal of the concentrate rejected by the membrane (Ushikoshi *et al.*, 2002; Yamada and Jung, 2005).

Biological treatment of landfill leachates has been challenging due to low bioavailable organic carbon concentrations. Landfill leachates have been shown to inhibit aerobic treatment processes such as activated sludge even when they make up as low as 5% of the total feed into the process (Lema *et al.*, 1988). The residence times required to effectively treat landfill leachate using activated sludge are also very high, resulting in very high aeration costs (Loukidou *et al.*, 2001). A study using activated sludge to remove UV-quenching organics from landfill leachates concluded that less than 10% of UV absorbance was removed by the process and suggested that these organics cannot be removed by aerobic treatment (Zhao *et al.*, 2013), even at HRT values as high as 21 days. Anaerobic treatment of landfill leachates has also proved to be challenging, although co – digestion of leachate with other substrates such as food wastes and septage has yielded COD removals as high as 80% (Lin *et al.*, 1999). Membrane Bioreactors (MBRs) have also been utilized for leachate treatment. Both aerobic and anaerobic MBRs have been used to treat leachates, although the treatment processes have centered around COD and nitrogen removal. Aerobic MBRs have shown promising results, but studies have typically treated mixtures of leachates diluted with wastewater (Hasar *et al.*, 2009; Puszczalo *et al.*, 2010) or synthetic leachates. Anaerobic MBRs have also been used for leachate treatment, with reports of >90% COD removal for systems treating 1:4 mixtures of leachates and wastewater at a 2 – day HRT (Bohdziewicz *et al.* 2008). The studies employing aerobic and anaerobic MBRs tend to focus on removal of COD and nitrogen, and their effectiveness for the removal of UV – quenching organic carbon from landfill leachates is unknown. A review of studies employing MBRs (aerobic and anaerobic) for landfill leachate treatment revealed that none of the studies employing MBRs for leachate treatment had reported UV quenching organic compounds or UV absorbance before and after treatment (Ahmed *et al.*, 2012). There is also a lack of information concerning the use of enhanced biological treatment methods such as MBRs for the treatment of raw, undiluted landfill leachates.

Knowledge of the structure and function of the microbial community in bioreactors treating landfill leachate is also scarce. Microbial communities in raw leachates have exhibited a variety in terms of composition. The bacterial population in raw leachates has been described as diverse (Huang *et al.*, 2004), encompassing several broad categories such as *Chlamydiae* group, CFB group, *Planctomycetales*, *Spirochaetes*, *Proteobacteria* and *Actinobacteria*. It is also noteworthy that a significant portion of 16s rRNA sequences found in landfill leachates do not display similarities with known sequences, suggesting that a significant fraction of the organisms found in landfills are unknown and the knowledge of microbial communities in leachates is incomplete (Huang *et al.*, 2005). Waste and soil samples extracted from different depths and locations within a landfill have been analyzed using 16s rRNA sequencing, showing that microbial communities can vary considerably with location and depth, even within the same landfill (Sawamura *et al.*, 2010).

While treatment of UV quenching organic carbon in landfill leachate is challenging, it is known that these substances can be naturally-degraded in landfills over a period of several years, i.e., long – term landfilling (Gupta *et al.*, 2014). Previously, we simulated the typical temperature regime of long-term landfilling through an AnMBR system employing a thermophilic stage followed by a mesophilic stage with a membrane to retain biomass and extend the solids retention time. The purpose of this study was to compare the performance of a two – stage AnMBR system with a one – stage AnMBR for the removal of UV-quenching substances in landfill leachate. Since landfills have mesophilic as well as thermophilic temperature zones under the surface, it is hypothesized that a combination of thermophilic treatment followed by a mesophilic AnMBR would degrade UV-quenching organics more efficiently and bring about a greater reduction in the UV absorbance of raw landfill leachates as compared to an identical AnMBR operated as a single – stage process. Shot-gun metagenomic DNA sequencing helped provide insight into microbial communities involved in each stage to gain mechanistic insight into the role of each stage in the removal of recalcitrant organics involved in UV-quenching.

MATERIALS AND METHODS

Set – up and Operation of 2 – Stage AnMBR System

Fifty gallons of landfill leachate were collected in 5 – gallon heavy duty polyethylene buckets (True Value, Chicago, IL) from an actively landfilling cell at a landfill in Virginia and stored at 4 °C until used. As described in the previous chapter, two anaerobic enrichment cultures were set up, one thermophilic and one mesophilic to create a microbial population that was adapted to survive in landfill leachate. The enrichment cultures were incubated at two different operating temperatures (55 ± 2 °C for thermophilic and 37 ± 1 °C for mesophilic culture), each with an HRT/SRT of 40 days. Peristaltic pumps (Cole Parmer, Vernon Hills, IL, USA) were used to keep the reactors completely mixed by continuous recirculation of the headspace gas, and the enrichment cultures were operated for a period of 4 months, until stable COD reduction was observed.

The two stage AnMBR system consisted of two tubular reactors, as described previously (Pathak et al. in review). Briefly, the first step of the process was thermophilic pretreatment (referred to as “Stage 1” henceforth), which was succeeded by a mesophilic AnMBR (referred to as “Stage 2” henceforth). Locally procured PVC pipes were used to fabricate the two reactors. Stage 1 had a reaction volume of 1.8 L with a 20 – day HRT, while the corresponding values for Stage 2 were 2 L and 45 days respectively. Stage 1 was operated at thermophilic temperatures (55 ± 2

°C) and Stage 2 was operated in the mesophilic range (37 ± 1 °C). Temperature control was achieved by placing the system in a constant temperature room at 37 °C over the course of the study. Stage 1 was maintained at thermophilic temperature using electric heating tape set to 55 °C. Both Stage 1 and Stage 2 were completely mixed. Mixing was accomplished by continuous recirculation of the headspace gas using peristaltic pumps (Cole Parmer, Vernon Hills, IL, USA). Figure 4-1 shows a schematic diagram of the reactor set – up. Sampling and feeding was done through dedicated ports that were airtight and operated by a different peristaltic pump. A separate pump was designated for this purpose so as to minimize the potential for oxygen entry into the reactors during feed or draw.

Stage 2 was designed as a submerged AnMBR i.e. the membrane module was completely immersed in the reactor contents. A pre – assembled bench – scale ZW – 1® hollow fiber membrane module supplied by GE Power and Water, Ontario, Canada, was used in Stage 2. This module was comprised of several GE ZeeWeed 500® hollow fibers, which were designed for application in wastewater treatment, and were chosen due to their higher tolerance for suspended solids. The fibers were designed for ultrafiltration (UF) (0.04 µm nominal pore size) and were made of polyvinylidene difluoride (PVDF). The optimal operating temperature range for the fibers was <40 °C, which is within the range for mesophilic processes, while the pH tolerance was 5.0 – 9.5.

Raw (i.e. untreated) landfill leachate was fed into Stage 1, and the effluent from Stage 1 was used as feed for Stage 2 after filtration to remove particulates larger than 5 µm in size. This filtration was performed by vacuum filtration using glass microfiber filters (Whatman, Pittsburgh, PA). The Stage 1 effluent was filtered prior to feeding into Stage 2 to mitigate membrane fouling, which was monitored using transmembrane pressure (TMP) as an indicator. The final effluent was drawn through the hollow fiber module. The pore size of the hollow fibers (0.04 µm) was small enough to keep most bacteria and archaea in the AnMBR (most bacterial cells range from 1 – 3 µm). Keeping biomass in the AnMBR was the primary function of the membrane, as it can be difficult to grow microorganisms in landfill leachate. Samples drawn from the reactors were primarily analyzed for UV quenching organic carbon, UV_{254} absorbance, COD, and microbial community structure and function. The system was operated for 14 months. Over the 14 months that the system was tested, the characteristics of the raw leachate did fluctuate in terms of refractory organic carbon content, UV_{254} absorbance, although these fluctuations did not have any perceptible adverse impact on removal of COD or UV adsorbing organics. The fluctuations were attributable to a lack mixing within landfills, meaning that different buckets of leachate drawn from the same cell of a landfill, had differences in characteristics.

Set – up and Operation of Single-stage Mesophilic AnMBR

A second mesophilic AnMBR was fabricated as part of this study. This reactor will be referred to as "Single-stage AnMBR". The Single-stage AnMBR was completely identical to the AnMBR that was used in the two stage process (in terms of material of construction, temperature of operation and HRT). The reactor used the ZW – 1® membrane module, identical to the one used in the two – stage system both in terms of the PVDF fibers and 0.04 µm pore size. The only difference in the operation of this AnMBR was that it was not preceded by a thermophilic pretreatment step, i.e., it was the same as the two stage system without stage 1. This AnMBR was started using inoculum from stage 2 to help acclimate the system to leachate as the carbon source.

The single-stage AnMBR was operated for a total of 6 months. During this time, the characteristics of the influent raw leachate were identical to the raw leachate that was fed to the two – stage system over its final 3 months of operation. The raw leachate was fed into the AnMBR after filtration through a 5 µm filter. As was the case with Stage 2 in the two – stage system, dedicated feeding and sampling ports were present. The feeding and sampling regime was identical to stage 2, except for the fact that Stage 2 received effluent from Stage 1 as feed as opposed to the raw leachate directly fed into the single-stage AnMBR. The TMP was also monitored to check for membrane fouling.

UV Quenching Organic Carbon and UV_{254} Absorbance Estimation

Biweekly sampling was done to analyze the raw leachate and effluents from Stage 1 and Stage 2 in the two stage system and the Single-stage AnMBR for organic carbon capable of absorbing UV light. The analyses for UV quenching organic carbon were performed in accordance with the procedure suggested by Malcolm and Thurman (1981), Leenheer (1981) and Christensen *et al.*

(1998). The first step involved precipitation of humic acids by acidification of the samples to pH values below 1.5. The humic acids were then filtered out of the solution and recovered by dissolution in 0.1 N NaOH. Once the humic acids were removed, the sample containing fulvic acids and hydrophilic substances was passed through a column packed with DAX – 8® (supplied by Supelco®, Bellefonte, PA), a highly hydrophobic ion exchange resin. The hydrophobicity of the resin allows it to adsorb fulvic acids (which are hydrophobic) on its surface, while hydrophilic compounds pass through. The fulvic acids that are adsorbed on the resin are finally recovered by elution with 0.1 N NaOH. Prior to carrying out separation of leachate into humic acids, fulvic acids and hydrophilic compounds, the resin was subjected to a rigorous cleaning procedure, as has been outlined by the studies cited above. This cleaning was done to remove traces of organic carbon already present on the resin so as to minimize error in fulvic acid and hydrophilics quantification. However, since the organic carbon content of landfill leachate is fairly high, the likelihood of traces of organics on the surface of fresh previously unused resin causing a significant deviation in the measured concentrations in the sample is low. When dissolving or extracting humic acids and fulvic acids using 0.1 N NaOH, the volume of NaOH used was the same as the initial volume of the sample to ensure that the concentration of these species does not change post elution. The separated humic acids, fulvic acids and hydrophilic substances were finally quantified by using a TOC-V CSN Total Organic Carbon Analyzer manufactured by Shimadzu®.

In addition to UV quenching organic carbon, the UV absorbance of raw leachate and the effluents from Stage 1, Stage 2 and the Single-stage AnMBR at 254 nm were also monitored on a weekly basis using a Beckman DU® 640 spectrophotometer that could measure absorbance of samples in the visible and UV range. The UV_{254} absorbance measurements were performed on the sample withdrawn from the reactors and also post separation into humic acids, fulvic acids and hydrophilics in order to better understand how these compounds quench UV light. The UV254 absorbance of each of these categories of compounds was normalized to their concentration, yielding the specific ultraviolet absorbance (i.e. SUVA) values for humic acids, fulvic acids and hydrophilic substances in the raw leachate as well as reactor effluents from both treatment processes.

Microbial Community Analysis using Illumina Sequencing and 16s rRNA Quantitative PCR

Raw landfill leachate and samples drawn from inside the reactors (i.e., not filtered through the membrane in case of Stage 2 and the single-stage AnMBR) were analyzed using next – generation DNA Illumina sequencing. Samples were collected and preserved at -80 °C until DNA extraction was done. Defined volumes of the samples were initially filtered through a cellulose acetate filtration membrane with pore size 0.2 µm (Millipore, Billerica, MA) to capture the biomass. This also helped reduce humic substances in the samples, as they can cause downstream interferences to polymerase chain reaction. DNA was extracted from the captured biomass using a FastDNA™ SPIN Kit for Soil (MP Biomedicals, Solon, OH). The filters containing captured biomass were folded and cut into pieces small enough to fit in the DNA extraction vials. DNA extracts were stored at -20 °C until use.

The DNA extracted from the samples was further purified using RNAse and Ampure beads. This was done to remove traces of humic substances and organic carbon from the isolates and reduce the possibility of interference with DNA sequencing. The DNA extracts were sequenced using Illumina HiSeq 2500 Rapid Run mode (100 – cycle paired end protocol) at the Genomics Research Laboratory of the Bioinformatics Institute (BI) in Blacksburg, VA. The data obtained were analyzed using the MetaStorm server hosted by Advanced Research Computing at Virginia Tech and Metagenomics Rapid Annotations using Subsystems Technology (MG – RAST) hosted by Argonne National Laboratory, Chicago, IL. Library preparation for each sample was done using Nextera XT Library Prep (Illumina, San Diego, CA). 16S rRNA gene sequences were annotated against Greengenes (DeSantis *et al.*, 2006) and SILVA (Quast *et al.*, 2013) databases to assess the taxonomic diversity in the samples. In addition to identifying the bacterial and archaeal taxa present in the samples, functional analysis of the samples was done by annotation against the UniProtKB database (The Uniprot Consortium, 2015). This provided insight into the kinds of genes present in the biomass that code for proteins and their relative abundances.

In addition to DNA sequencing for taxonomic and functional analyses, the total 16S rRNA gene copies in each sample were quantified using qPCR. Dilution tests were used to check for the minimum dilution at which qPCR consistently estimated the maximal gene copy numbers, indicative of optimal dilution of inhibitors. Based on this test, a 1:100 dilution ratio was consistently applied across the study. Standards ranging from 10^8 – 10^2 gene copies / µL were included with every run along with samples and negative controls in triplicates.

Assessment of Membrane Fouling and Other Analyses

In order to demonstrate the feasibility of employing AnMBRs for the purpose of landfill leachate treatment, it was necessary to evaluate membrane fouling over the course of operation of both the AnMBRs i.e. Stage 2 and the Single-stage AnMBR. For both these reactors, the withdrawal of effluent from the membrane was not continuous, and the membrane was operated at a peak flux of 8 LMH for about 30 minutes a day. The membranes in both the AnMBRs, therefore, had ample resting times. Assessment of membrane fouling was done by measuring the transmembrane pressure (TMP), or the difference in pressure between the permeate and the reaction volume in the AnMBR. To continuously track TMP, a pressure gauge (Ashcroft® 1490; 0 – 50 KPa) was installed on the line drawing effluent from the membranes in both AnMBRs. The TMP range for optimal operation of the ZW – 1® membrane module was 0 – 55 KPa.

In addition to membrane fouling, several other parameters were also monitored. COD destruction was estimated by measuring COD values of the raw leachates and the reactor effluents by closed titrimetry (Method 5520C, Standard Methods for the Examination of Water and Wastewater, 2012). Since the method is incapable of detecting COD values above 450 mg/L, 1:100 dilutions of the samples were used for analysis. Other parameters monitored included pH (Oakton® handheld pH meter), C2 – C7 VFAs (Gas Chromatography with Flame Ionization Detector i.e. GC – FID; Shimadzu® GC-14A) and total ammonia nitrogen (NitraVer® Test' N Tube kits, Hach, Loveland, CO).

RESULTS AND DISCUSSION

The objective of setting up the Single-stage AnMBR was to have a direct comparison of UV_{254} absorbance and UV quenching organic carbon removal in the two stage process versus a one - stage AnMBR, which would help assess the need for Stage 1 in the two stage process. If the two – stage system performed better than the one stage, it would suggest that mimicking the conditions that exist in a landfill by having thermophilic as well as mesophilic conditions can help remove UV quenching organic compounds that are otherwise hard to biodegrade. In addition to evaluating organic carbon and UV_{254} absorbance removal, samples were analyzed using next generation DNA sequencing to analyze the microbial community from a compositional as well as a functional standpoint. Therefore, it would be possible to compare the two systems not only in terms of the quality of their effluents, but also in terms of their microbiology.

Organic Carbon Degradation and UV_{254} Absorbance Reduction

The primary treatment goal of both the two – stage AnMBR and the single-stage AnMBR was the removal of UV-quenching organic carbon and UV_{254} absorbance from the raw leachate. The two – stage system was able to remove over 50% of the organic carbon from the raw leachate consistently over a year of operation, although removals fluctuated between values as low as 40% to values as high as 75%. Throughout the duration of the study, it was noted that the bulk of this removal occurred in Stage 2, while the thermophilic Stage 1 removed less than 5% of the organic content of the raw leachate. In Stage 1, the concentration of humic acids was found to decrease slightly, which was offset by an almost equal increase in the concentration of fulvic acids, suggesting the possibility of interconversion between humic substances. The removal of humic acids, fulvic acids and hydrophilic compounds was averaged over the final 3 months of operation, since the characteristics of the raw leachate during this period were identical to the leachate fed to the Single-stage AnMBR. On average, 55% of the humic acids, 45% of the fulvic acids and 53% of the hydrophilic compounds in the raw leachate were removed by the two stage system.

The Single-stage AnMBR which was operated for a total of 6 months was found to be less efficient than the two – stage system at removing UV – quenching organic carbon. Over the first two months of operation, slight fluctuations were observed in the organic carbon removal since the biomass in the reactor was acclimating to raw landfill leachate. However, over the next four months, the removal of UV quenching organic carbon by the Single-stage AnMBR was stable, with an average removal of 15%. Over these four months the average removals of humic acids and fulvic acids were 35% and 12% respectively. However, the concentration of hydrophilic substances was almost the same as the hydrophilics concentration in the raw leachate. The hydrophilics in the treated leachate were within ±10% of the corresponding values for raw leachate. Since the treated leachates also showed higher hydrophilics concentrations than raw leachate, it is possible that hydrophobic humic acids and fulvic acids were converted into hydrophilic degradation products. A comparison of the average UV quenching organic carbon levels in the effluents from the two stage process (both Stage 1 and Stage 2) and the Single-stage AnMBR versus the raw leachate are presented in Figure 4-2.

The UV_{254} absorbance of the raw leachates was also compared to the absorbances of the effluents from the two – stage and single stage processes. The trends were found to be similar to those for UV quenching organic carbon. The reduction in UV_{254} absorbance using the two – stage and single-stage AnMBR were averaged over the same duration as the UV quenching organic carbon data. The data showed that the two – stage system was able to remove an average of 48% of the total UV_{254} absorbance from the raw leachate, while the corresponding value for the Single-stage AnMBR was only 16%. The removal of UV_{254} absorbance due to humic acids was 68% in the two stage system versus 13% in the Single-stage AnMBR, while the reductions in absorbance attributable to fulvic acids in the two – stage process and the Single-stage AnMBR were 30% and 14% respectively. The removal of UV_{254} absorbance due to hydrophilics by both configurations was approximately 30%. Figure 4-2 Shows the UV absorbance of the landfill leachate before and after treatment with the two – stage and single-stage AnMBR. UV_{254} absorbances of the raw leachates and the effluents from both reactor configurations are shown in Figure 4-2.

In studying landfill leachates, it has been found that humic acids usually have the highest SUVA values, while the SUVAs of fulvic acids and hydrophilics are relatively lower (Zhao *et al.*, 2013). SUVA is a measure of the UV quenching capacity of organic species, and not the absorbance itself or the abundance of the organic species. It was found that in the raw leachate, humic acids had the highest SUVA value, but after treatment with the two – stage system, the SUVA of the fulvic acids was higher. In the Single-stage AnMBR, SUVA values of both humic acids and fulvic acids showed an increase. This indicated that only the easily biodegradable organic carbon was removed, leaving behind refractory carbon that absorbs higher quantities of UV light per unit concentration. This is in agreement with other studies that have shown that even though the organic carbon content of landfill leachates can be reduced by biological treatment, the SUVA values of the organics in the leachates tend to increase post treatment (He *et al.*, 2006b), indicating that the compounds responsible for most of the UV quenching are difficult to biologically degrade. Figure 4-3 shows the SUVA values of humic acids, fulvic acids and hydrophilics present in raw landfill leachate in comparison with the effluents from the two treatment processes.

The removal of UV quenching organic carbon using the two stage process are higher than any reported in the literature thus far. Even though biological treatment of landfill leachates by both aerobic and anaerobic methods have been found to be effective for removal of COD, the organic carbon content of landfill leachates exhibits biorefractory characteristics. The interior of landfills can get hot due to pressure and biological activity, creating both mesophilic and thermophilic conditions inside the landfill. Typical temperatures inside landfills are in the range of 24 – 57 °C (Yeșiller *et al.*, 2005). The data obtained over the course of this study support the hypothesis that recreating the conditions that exist in a landfill in a controlled engineered system could help accelerate the biodegradation of typically recalcitrant organic carbon capable of quenching UV light. The fact that the two – stage AnMBR with a thermophilic first stage was able to remove about 3 times as much organic carbon as compared with an identical Single-stage AnMBR without thermophilic pretreatment using the exact same leachate as a carbon source suggests that the elevated temperatures inside landfills could play a role in shifting microbial communities to organisms better adapted to degrading humic substances and these organisms are likely thermophiles.

COD Removal and Membrane Fouling (TMP)

The removal of COD using both AnMBR configurations was monitored. COD values were averaged over the same time period as organic carbon and UV254 absorbance for both, the two – stage system and the single-stage AnMBR. The two – stage system showed high COD removal, with 67% of the total COD fed into the system during the final 3 months of operation removed. With the same leachate feed, the single-stage AnMBR was able to remove only 33% of the COD content. The average COD values of the raw leachate and treated effluents from Stage 1 and

Stage 2 of the two – stage process and the effluent from the Single-stage AnMBR are shown in Figure 4-4.

It is worth noting that even though the single-stage AnMBR showed 33% COD removal, the removal of organic carbon capable of UV quenching was much lower, i.e. 15%. It appears that biological treatment processes that are designed for COD removal from landfill leachates do not necessarily remove refractory, UV quenching organic carbon. COD removal, therefore, is not a reliable indicator to gauge the effectiveness of a treatment process for removal of organic carbon and UV_{254} absorbance.

TMP was monitored in order to assess fouling in the membrane. No significant fouling was observed in either AnMBR. This was presumably since the feed to both AnMBRs was filtered in order to eliminate large particulates. Also, the membrane modules were needed to be operated to collect effluents only for 30 minutes each day due to the high residence times. This allowed the membrane modules to rest most of the time, thereby mitigating fouling. Over 14 months of operation, the TMP rose to 10 KPa in the Stage 2. In the Single-stage AnMBR, TMP was found to increase to 3 KPa after 6 months of operation. These values were well within the TMP tolerance of the membrane (<55 KPa). The TMP trends in the AnMBR system may be seen in Figure 4-5.

Taxonomic and Functional Analysis of the Microbial Community

Shot-gun DNA sequencing was used to characterize the microbial community structure and function in raw landfill leachate and the two reactor systems. The number of metagenomic reads obtained per sample varied from 5,502,195 to 36,674,422 sequences and were assembled into scaffolds. Read assembly was performed using the IDBA – UD software (University of Hong Kong). The number of reads used for assembly varied from 43 – 81%, while the N50 value ranged from 842 – 1,911 bps across the samples. Gene prediction from the assembled sequences was done using the PRODIGAL software (Oak Ridge National Laboratory and the University of Tennessee).

Taxonomic diversity in the samples was assessed by annotation against Greengenes (2013) and SILVA (2015) databases. All four samples were found to be dominated mainly by bacteria, although the Stage 1 and Single-stage AnMBR showed high percentages of archaea. Archaea accounted for 5% of the total matches in Stage 1 and 25% of the matches in the Single-stage AnMBR. The relative abundances of archaea in the raw leachate and Stage 2 were 2 and 1%, respectively. Traces of eukaryotic DNA were found in Stage 1, while traces of viral DNA were detected in both Stage 1 and Stage 2.

Taxonomic profiling based on annotation against the SILVA database showed that *Proteobacteria* was the dominant phylum in all four samples, constituting more than 50% of the total matches in each. *Proteobacteria* accounted for over 90% of the total matches in raw leachate, but were not as dominant in the bioreactors. *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria* were detected in the samples. The raw leachate was found to be dominated by *Gammaproteobacteria*, which accounted for 99% of the *Proteobacteria* matches. They were also the most abundant *Proteobacteria* class in the two – stage system, accounting for 70% of *Proteobacteria* matches in Stage 1 and 53% of *Proteobacteria* matches in Stage 2, The *Proteobacteria* in the single stage AnMBR, were dominated by *Betaproteobacteria*, which constituted 87% of all *Proteobacteria* matches. Other bacterial phyla detected in the samples included *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Spirochaetes* and *Synergistetes*. The archaeal phylum Euryarchaeota was also detected in all four samples. The organisms classified in this phylum include methanogens, such as *Methanobacter*, *Methanosaeta* and *Methanosarcina*, commonly found in anaerobic systems. Figure 4-6 shows the relative abundances of bacterial and archaeal phyla in the samples.

The UniProtKB database (The UniProt Consortium, 2015) was used to compare the samples from a functional standpoint. Annotation against this database helped reveal the types of proteins represented in the microbial genomes in the samples. The proteins of interest were enzymes such as hydrolases, oxidoreductases and metal-binding enzymes that are involved in the biodegradation of humic acids. However, contrary to expectation, it was observed that the relative abundances of proteins represented in the genomes of the microbes in raw leachates were very similar to those in Stage 1, Stage 2 and the Single-stage AnMBR. This may be because there has been almost no research with a focus on enzymes capable of degrading UV – quenching organic carbon, or on the microbiology of landfill leachate treatment in general. There is, therefore the possibility that several of these proteins are undiscovered or unannotated in existing databases. Figure 4-7 shows the relative abundances of the genes for different types of proteins in raw and treated leachates.

In order to explain higher removal of UV quenching organics in the two stage system, the top 50 most abundant microbial genera in each sample were populated by annotation against RefSeq i.e. the NCBI Reference Sequence Database. The 50 most abundant microbial genera in each stage of the two stage system were compared with the 50 most abundant genera in the Single-stage AnMBR. The data obtained showed the presence of organisms known to degrade humic substances in nature in Stage 1, Stage 2 and the Single-stage AnMBR. However, the difference in the number of matches for these organisms was higher in the 2 – stage system by almost two orders of magnitude. The genera of organisms detected in Stage 1 and Stage 2 of the two stage system were fairly similar. Even though effluent from Stage 1 was filtered prior to Stage 2, the pore size was large enough to allow most prokaryotes to pass through. Although data concerning the microbiology of bioreactors treating landfill leachate and in particular degradation of UV quenching organics is unavailable, there have been studies exploring the degradation of humic and fulvic acids in natural systems such as soils, water bodies and sediments. Among the prokaryotic genera that have been shown to readily degrade humic and fulvic acids include *Geobacter* (Coates *et al.*, 2001), which has been found to use humic acids as terminal electron acceptors during dissimilatory Fe(III) reduction, *Clostridium* (Bushan *et al.*, 2006), *Enterococcus* (Benz *et al.*, 1998), *Desulfitobacterium*, *Desulfovibrio*, *Methanospirillum* (Cervantes *et al.*, 2002), *Burkholderia*, *Delftia* and *Sphingomonas* (Vacca *et al.*, 2005). Of these genera, *Clostridium*, *Burkholderia*, *Desulfovibrio* and *Geobacter* were among the 50 most abundant organisms in all three reactors i.e. Stage 1, Stage 2 and the Single-stage AnMBR. However, the number of matches for these organisms in Stage 1 and Stage 2 were almost 100 times the number of matches in the Single-stage AnMBR. Matches for *Propionibacterium* were found exclusively in the Single-stage AnMBR, while Stage 1 and Stage 2 showed the presence of *Desulfitobacterium*. Even though both Stage 1 and Stage 2 showed an abundance of known degraders of humic substances, organisms such as *Enterococcus* were found in Stage 1 exclusively. These organisms also appeared to be higher in relative abundance in Stage 1 compared with Stage 2 and the Single-stage AnMBR. Figure 4-8 shows the number of matches for bacterial genera known to degrade humic acids obtained using the RefSeq database.

From the data obtained by taxonomic analysis of the microbial community, it appeared that the thermophilic conditions in Stage 1 may have been conducive to the survival and proliferation of prokaryotic organisms capable of degrading humic substances. It is likely that many of these organisms made their way into stage 2 since the filter medium did not have a pore size large enough to filter bacterial or archaeal calls. Even though matches for several known humic acid degraders were also found in the Single-stage AnMBR, the matches were considerably fewer.

In order to quantify the total biomass in each reactor, qPCR was done for 16s rRNA genes. Analyses were performed in triplicates. Standards and negative controls were run, also in triplicates. Quantification of the total 16s rRNA genes in the DNA extracts showed that the total number of 16s rRNA copies in the two stage system were significantly larger than the corresponding gene copies in raw landfill leachate. Figure 4-9 shows the matches for 16s rRNA gene copies found in the DNA extracts from raw leachates and in the reactors treating the leachate. It was found that Stage 1 showed a one log increase in the number of 16s rRNA gene copies relative to the raw leachate, while a 500% increase was observed in Stage 2. In contrast, the Single-stage AnMBR showed a drop in 16S rRNA copies by almost two orders of magnitude in comparison to raw leachate.

The number of 16s rRNA matches detected by qPCR show that the two stage system may have been able to achieve superior removal of UV quenching organic carbon and UV_{254} absorbance by maintaining a higher population of microorganisms, and not by merely bringing about a shift in the functional characteristics of the microbial community. It may be noted that even though there is a fall in the 16s rRNA gene copies in the Single-stage AnMBR, there is still some degradation of UV quenching organics and a 33% COD removal from the raw leachate feed. This could mean that the microorganisms in the Single-stage AnMBR are “starved” due to a lack of bioavailable organic carbon. In the two – stage system, the presence of a thermophilic pretreatment step (Stage 1) helps make organic carbon more readily bioavailable by breaking larger molecular weight organic compounds into smaller, lower molecular weight fragments. This allows for the sustenance of more microorganisms in the AnMBR (Stage 2) downstream.

CONCLUSIONS

On the basis of the data obtained from analyses for UV quenching organic carbon, UV_{254} absorbance, COD removal and taxonomy and function of the microbial community, it can be seen that the a two – stage, thermophilic – mesophilic process employing an AnMBR to treat landfill leachate for removal of UV – quenching organic carbon performs much better than an identical AnMBR without thermophilic pretreatment. Using AnMBRs with higher HRTs allows for large membrane resting times, which minimizes the need for membrane cleaning or maintenance. The key conclusions of the study are summarized as follows:

(1) A two – stage, thermophilic – mesophilic treatment strategy for landfill leachate employing an AnMBR to keep biomass in the reactor was able to achieve more than 3 times the removal of UV-quenching organic carbon as an identical single-stage AnMBR without thermophilic pretreatment.

(2) The microbial communities in raw leachate and the two AnMBR configurations showed subtle differences in terms of taxonomy, but very little difference was discernable in terms of functional gene composition. Even though organisms capable of degrading UV quenching organics like humic acids and fulvic acids are present in both the treatment processes, the two – stage system shows higher matches for these organisms, and appears to sustain significantly more biomass in general.

REFERENCES

Ahmed, F. N., Lan, C. Q., 2012. Treatment of landfill leachate using membrane bioreactors: A review. Desalination (287), 41–54.

Benz, M., Schink, B., Brune, A., 1998. Humic acid reduction by *Propionibacterium freudenreichii* and other fermentative bacteria. Applied and Environmental Microbiology (64), 4507 – 4512.

Bohdziewicz, J., Neczaj, E., Kwarciak, A., 2008. Landfill leachate treatment by means of anaerobic membrane bioreactor. Desalination (221), 559–565.

Bushan, A., Halasz, A., Hawari, J., 2006. Effect of iron (III), humic acids and anthraquinone-2,6-disulfonate on biodegradation of cyclic nitramines by *Clostridiun* Sp EDB2. Journal of Applied Microbiology (100), 555 - 563.

Cervantes, F. J., deBok, F. A. M., Duong-Dac, T., Stams, A. G. M., Lettinga, G., Field, J. A., 2002. Reduction of humic substances by halorespiring, sulphate-reducing and methanogenic microorganisms. Environmental Microbiology (4), 51 – 57.

Christensen, J. B., Jensen, D. L., Grøn, C., Filip, Z., Christensen, T. H., 1998. Characterization of the dissolved organic carbon in landfill leachate-polluted groundwater. Water Research (32), 125–135.

Coates, J. D., Bhupathiraju, V. K., Achenbach, L. A., McInerney, M. J., Lovely, D. R., 2001. *Geobacter hydrogenophilus*, *Geobacter Chapellei* and *Geobacter grbiciae*, three new, strictly anaerobic, dissimilatory Fe(III) reducers. International Journal of Systematic and Evolutionary Microbiology (51), 581 – 588.

Göbbels, F., Püttmann, W, 1997. Structural investigation of isolated aquatic fulvic and humic acids in seepage water of waste deposits by pyrolysis gas chromatography mass spectrometry. Water Research 31 (7), 1609 – 1618.

Gupta, A., Zhao, R., Novak, J. T., Goldsmith, C. D., 2014b. Variation in organic matter characteristics of landfill leachates in different stabilization stages. Waste Management & Research (1 – 8).

Hasar, H., Ipek, U., Kinaci, C., 2009. Joint treatment of landfill leachate with municipal wastewater by submerged membrane bioreactor, Water Science & Technology (60), 3121–3127.

He, P.J., Shao, L.M., Guo, H.D., Li, G.J., Lee, D.J., 2006a. Nitrogen removal from recycled landfill leachate by ex situ nitrification and in situ denitrification. Waste Management 26 (8), 838 – 845.

He, P. J., Xue, J. F., Shao, L. M., Li, G. J., Lee, D. J., 2006b. Dissolved organic matter (DOM) in recycled leachate of bioreactor landfill. Water Research 40 (7), 1465 – 1473.

Huang, L. N., Zhou, H., Zhu, S., Qu, L. H., 2004. Phylogenetic diversity of bacteria in the leachate of a full-scale recirculating landfill. FEMS Microbiology Ecology (50), 175 – 183.

Huang, L. N., Zhu, S., Zhou, H., Qu, L. H., 2005. Molecular phylogenetic diversity of bacteria associated with the leachate of a closed municipal solid waste landfill. FEMS Microbiology Letters (242) 297–303.

Kurniawan, T.A., Lo, W., 2009. Removal of refractory compounds from stabilized landfill leachate using an integrated H2O2 oxidation and granular activated carbon (GAC) adsorption treatment. Water Research 43 (16), 4079 – 4091.

Kurniawan, T.A., Lo, W., Chan, G.Y.S., 2006. Radicals-catalyzed oxidation reactions for degradation of recalcitrant compounds from landfill leachate. Chemical Engineering Journal, Volume 125 (1), 35 – 57.

Leenheer, J.A., 1981. Comprehensive approach to preparative isolation and fractionation of dissolved organic carbon from natural water and wastewaters. Environmental Science and Technology 15 (5), 578 – 587.

Lema, J.M., Mendez, R., Blazquez, R., 1988. Characteristics of landfill leachates and alternatives for their treatment: a review, Water Air and Soil Pollution (40), 223–250

Lin, Č. Y., Bian, F. Y., Chou, J., 1999. Anaerobic co-digestion of septage and landfill leachate. Bioresource technology 68 (3), 275 – 282.

Loukidou, M.X., Zouboulis, A.I., 2001. Comparison of two biological treatment process using attached-growth biomass for sanitary landfill leachate treatment. Environmental Pollution 111, 273–281.

Neilson, A. H., Allard, A. S., 2012. Organic Chemicals in the Environment: Mechanisms of Degradation and Transformation, Second Edition. CRC Press, 273 – 274.

Puszczalo, E., Bohdziewicz, J., Swierczynska, A., 2010. The influence of percentage share of municipal landfill leachates in a mixture with synthetic wastewater on the effectiveness of a treatment process with use of membrane bioreactor. Desalination and Water Treatment 14 (1 – 3), 16 – 20.

Reinhart, D., Bolyard, S. C., 2015. Fate of Organic Matter from Leachate Discharged to Wastewater Treatment Plants (Year 1). Report #10285 submitted to the Hinkley Center for Solid and Hazardous Waste Management, Gainesville, FL.

Sawamura, H., Yamada, M., Endo, K., Soda, S., Ishigaki, T., Ike, M., 2010. Characterization of microorganisms at different landfill depths using carbon-utilization patterns and 16S rRNA gene based T-RFLP. Journal of Bioscience and Bioengineering 109 (2), 130–137.

Thurman, E. M., Malcolm, R. L., 1981. Preparative isolation of aquatic humic acids. Environmental Science & Technology (15), 463–466.

United States Environmental Protection Agency (USEPA), 2013. Advancing Sustainable Materials Management: Facts and Figures 2013 - Assessing Trends in Material Generation, Recycling and Disposal in the United States. EPA530-R-15-002, Washington, DC.

Ushikoshi, K., Kobayashi, T., Uematsu, K., Toji, A., Kojima, D., Matsumoto, K., 2002. Leachate treatment by the reverse osmosis system. Desalination 150 (2), 121 – 129.

Uygur, A., Kargı, F., 2004. Biological nutrient removal from pre-treated landfill leachate in a sequencing batch reactor. Journal of Environmental Management (71), 9–14.

Vacca, D. J., Bleam, W. F., Hickey, W. J., 2005. Isolation of Soil Bacteria Adapted to Degrade Humic Acid-Sorbed Phenanthrene. Applied and Environmental Microbiology 75 (7), 3797 – 3805.

Xiaoli, C., Shimaoka, T., Quiang, G., Youcai, Z., 2008. Characterization of humic and fulvic acids extracted from landfill by elemental composition, ^{13}C CP/MAS NMR and TMAH-Py-GC/MS. Waste Management 28 (5), 896 – 903.

Yamada, M., Jung, S., 2005. Application of reverse osmosis for landfill leachate treatment. APEC Virtual Center for Environmental Technology Exchange: http://www.apec-vc.or.jp/e/modules/tinyd00/?id=35.

Yesiller, N., Hanson, J. L., Liu, W. L., 2005. Heat Generation in Municipal Solid Waste Landfills. Journal of Geotechnical and Geoenvironmental Engineering 131 (11), 1330 – 1344.

Zhao, R., Gupta, A., Novak, J. T., Goldsmith, C. D., Driskill, N., 2013. Characterization and treatment of organic constituents in landfill leachates that influence the UV disinfection in the publicly owned treatment works (POTWs). Journal of Hazardous Materials (258 – 259), 1 – 9.

FIGURES

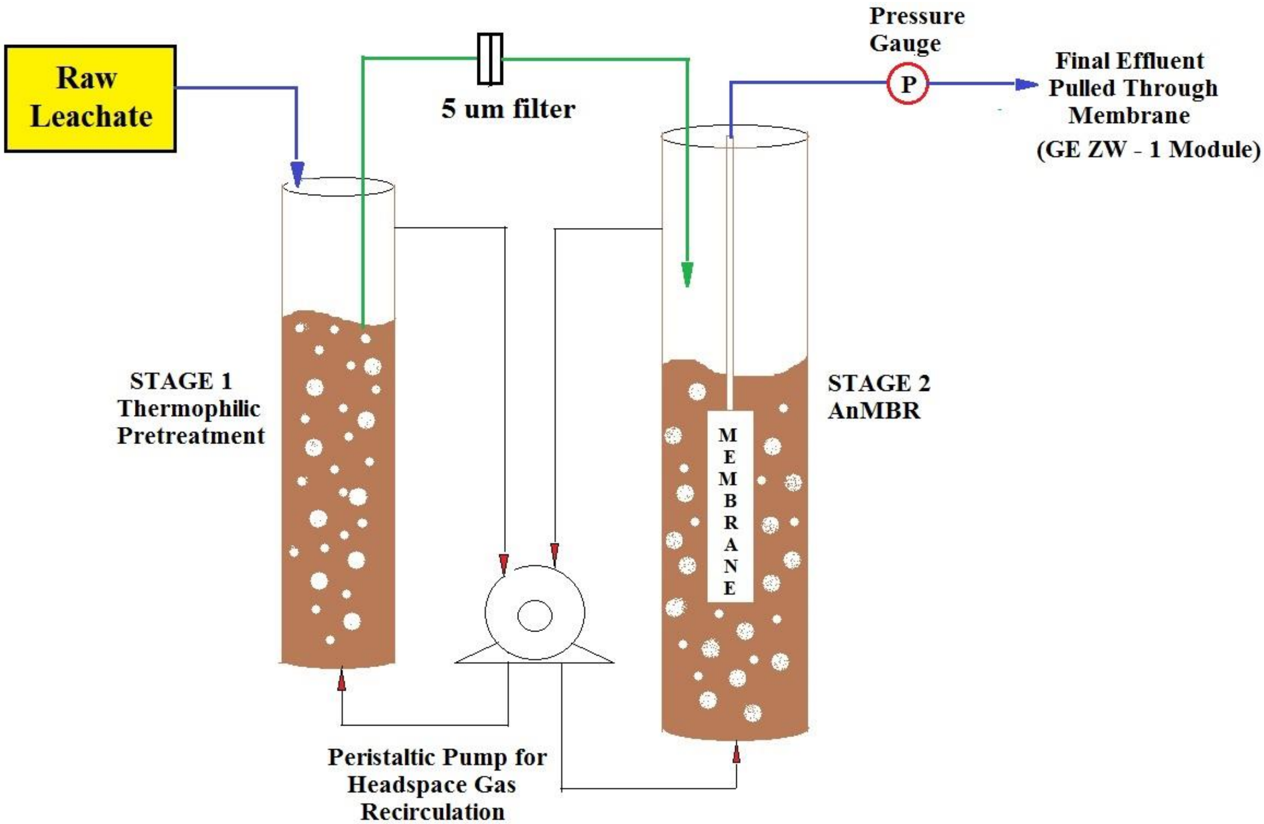

Figure 4-1. Schematic Diagram of 2 – Stage AnMBR used in the study.

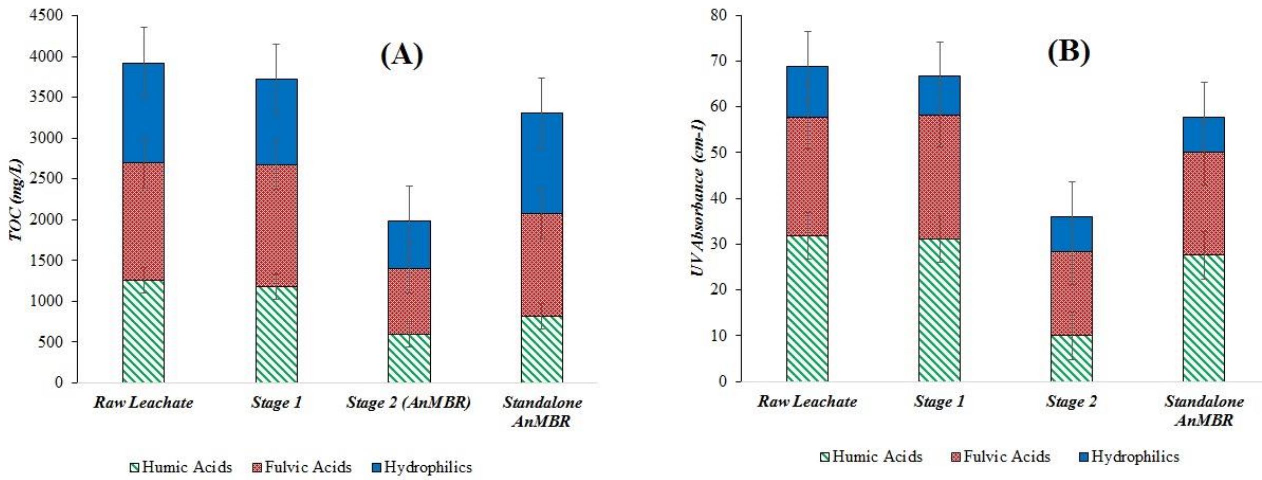

Figure 4-2. Comparison of the performance of the two – stage versus single-stage AnMBR: (a) Removal of UV – quenching organic carbon, (b) Reduction in UV_{254} absorbance post treatment

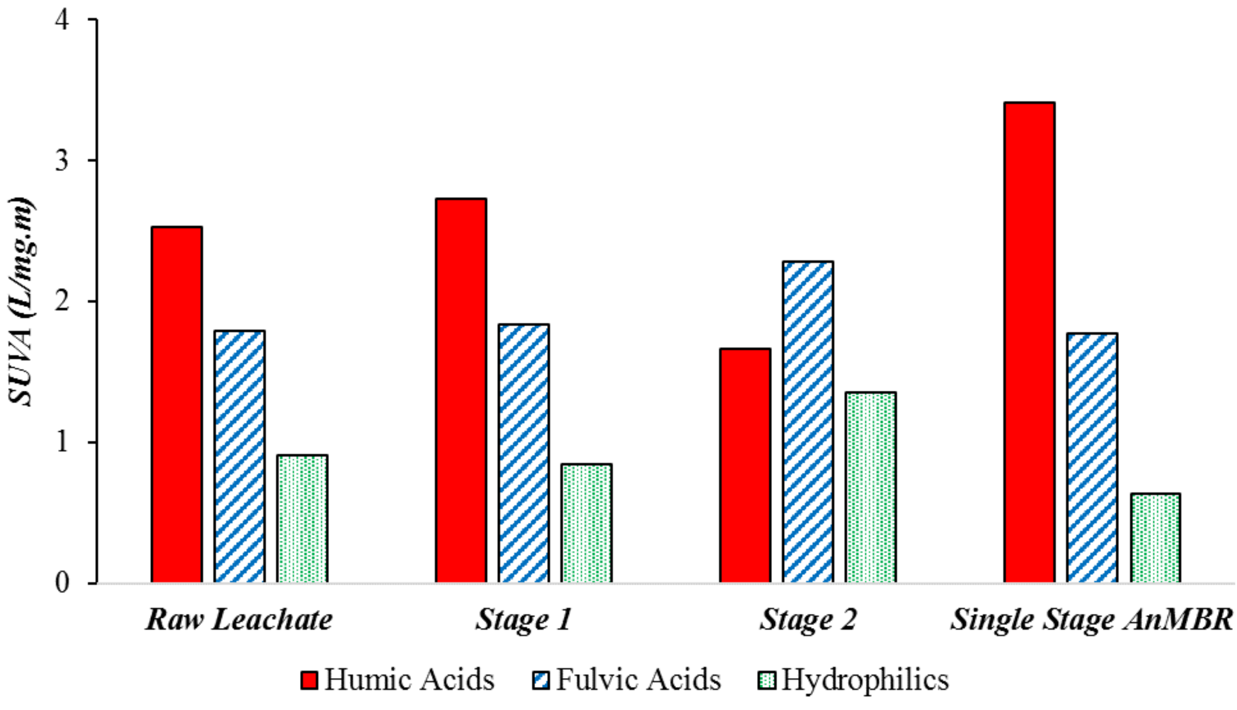

Figure 4-3. SUVA values of humic acids, fulvic acids and hydrophilic substances in raw leachate and effluents from Stage 1, Stage 2 and the Single-stage AnMBR

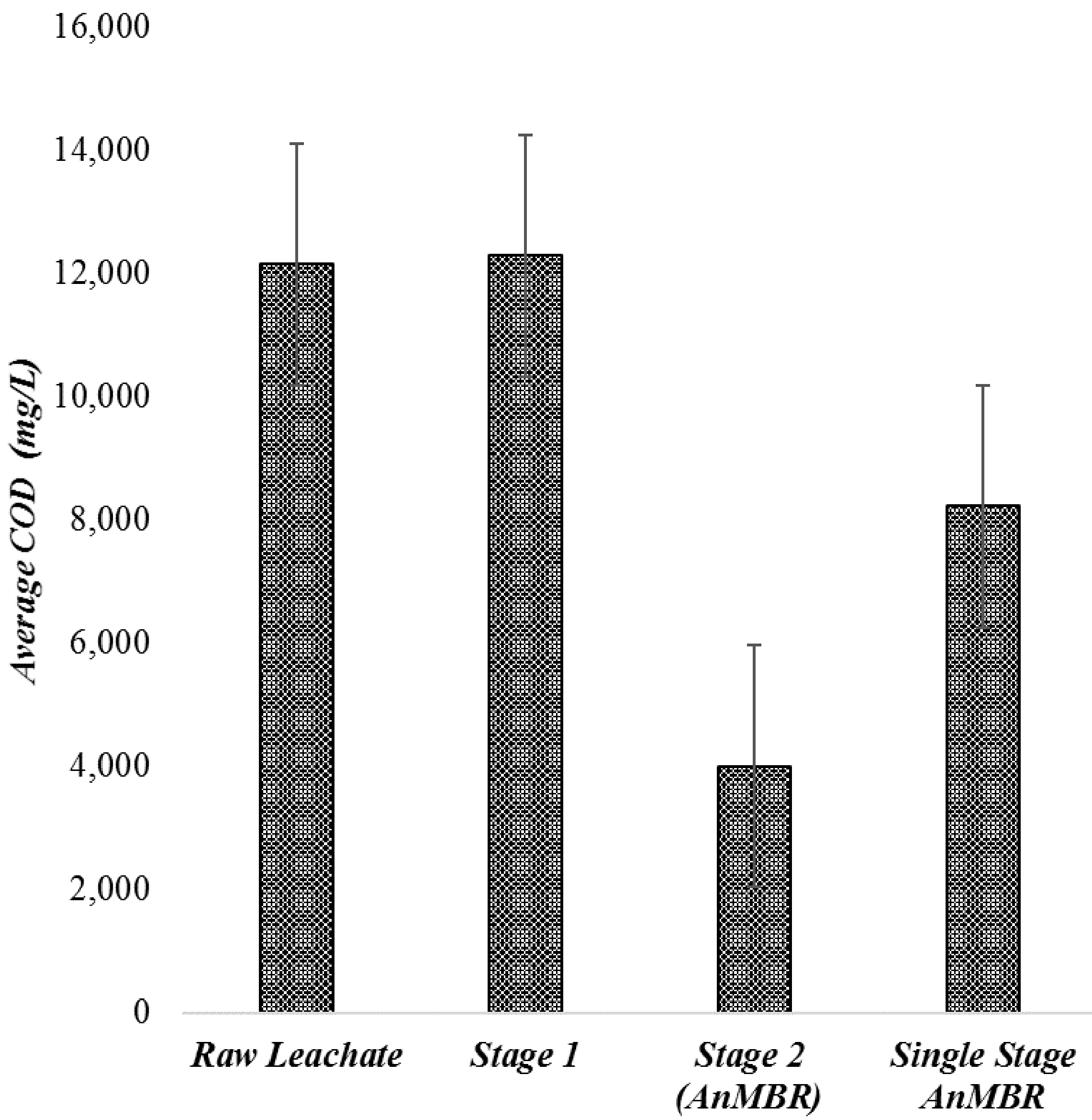

Figure 4-4. Average tCOD (i.e., total COD, soluble + particulate) values of raw landfill leachate and the different reactor effluents

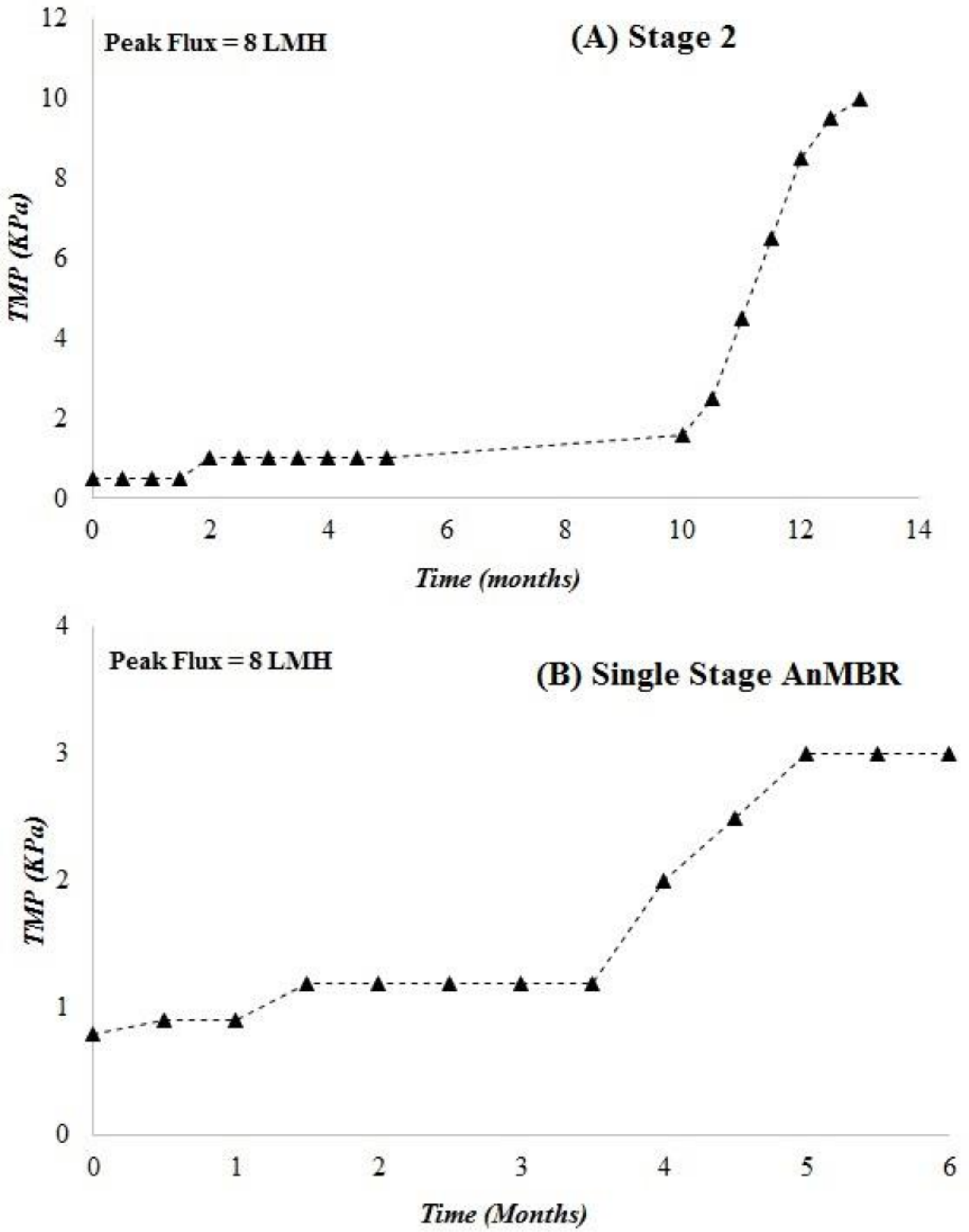

Figure 4-5. TMP across the membrane in the two AnMBRs: (a) Stage 2 in the two – stage system, (b) Single-stage AnMBR

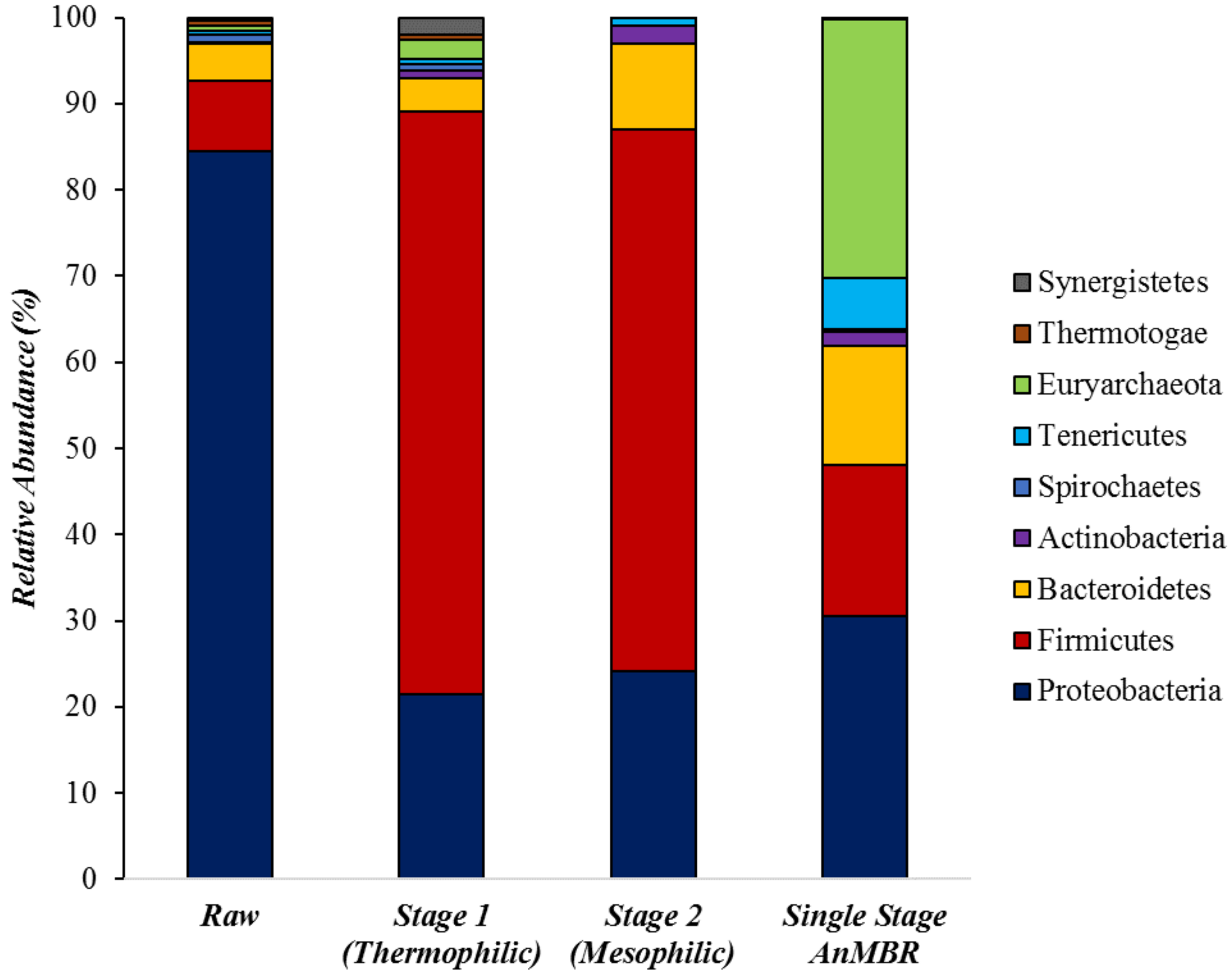

Figure 4-6. Relative abundances of bacterial and archaeal phyla in DNA extracts from raw leachate, Stage 1, Stage 2 and the Single-stage AnMBR.

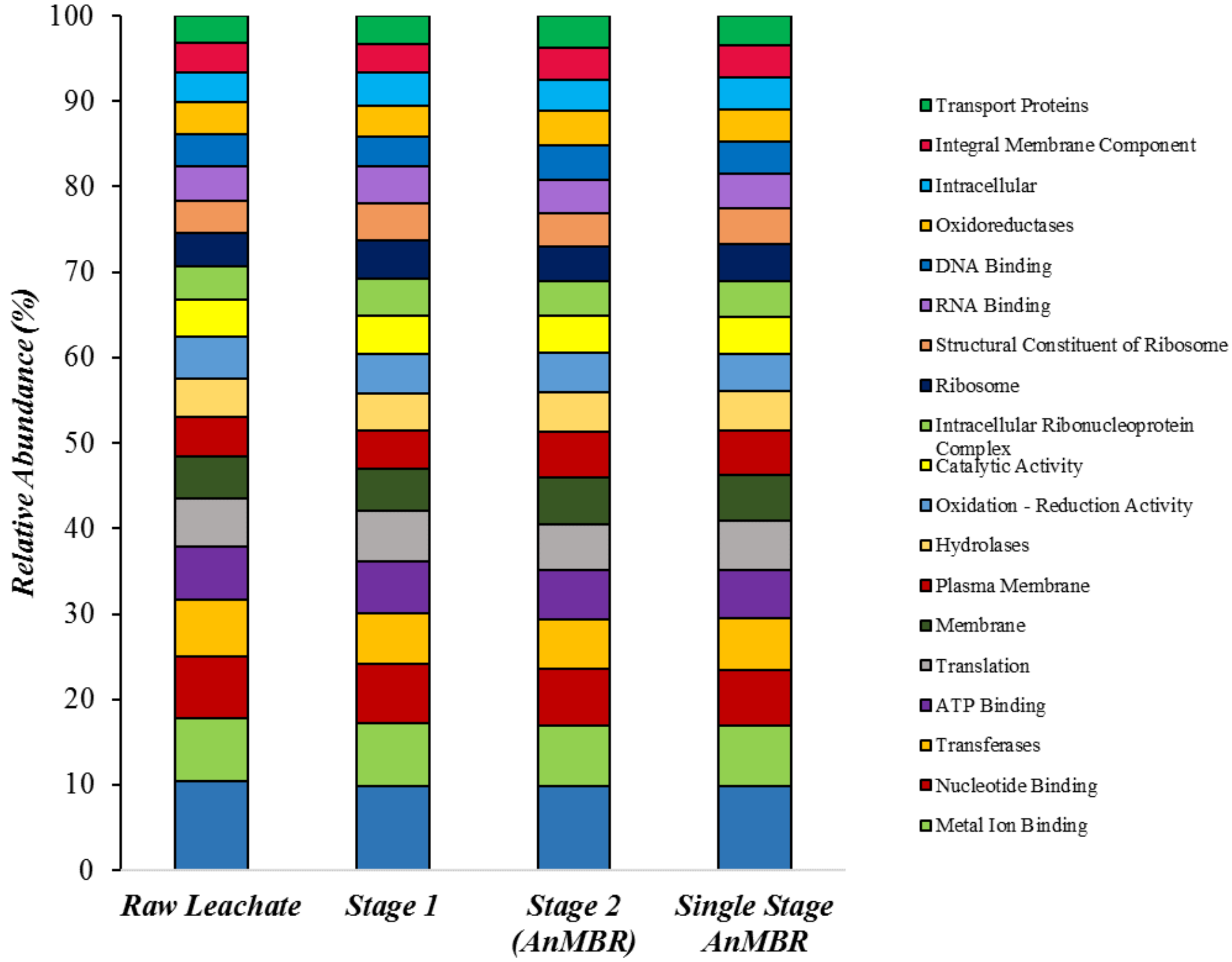

Figure 4-7. Relative abundances of functional gene matches in the UniProtKB database (2016). While no perceptible difference is seen in the samples, it is possible that the protein distribution is similar, but the organisms responsible for the matches are different. Additional functional annotation against different databases or custom databases is needed.

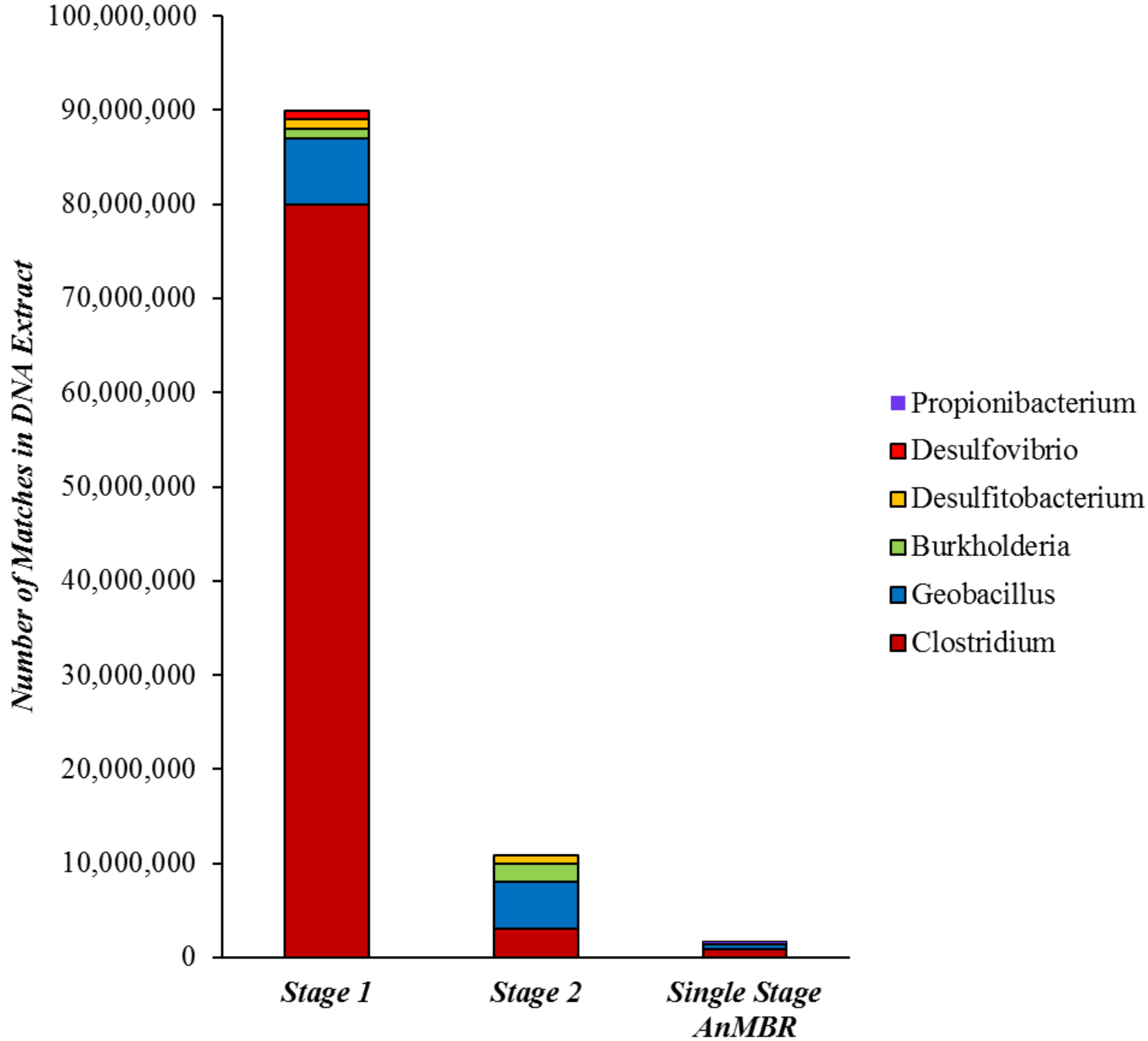

Figure 4-8. Functional gene matches for humic acid degrading organisms in Stage 1, Stage 2 and the Single-stage AnMBR using the RefSeq database. The data clearly indicate the presence of greater numbers of these organisms in the thermophilic reactor.

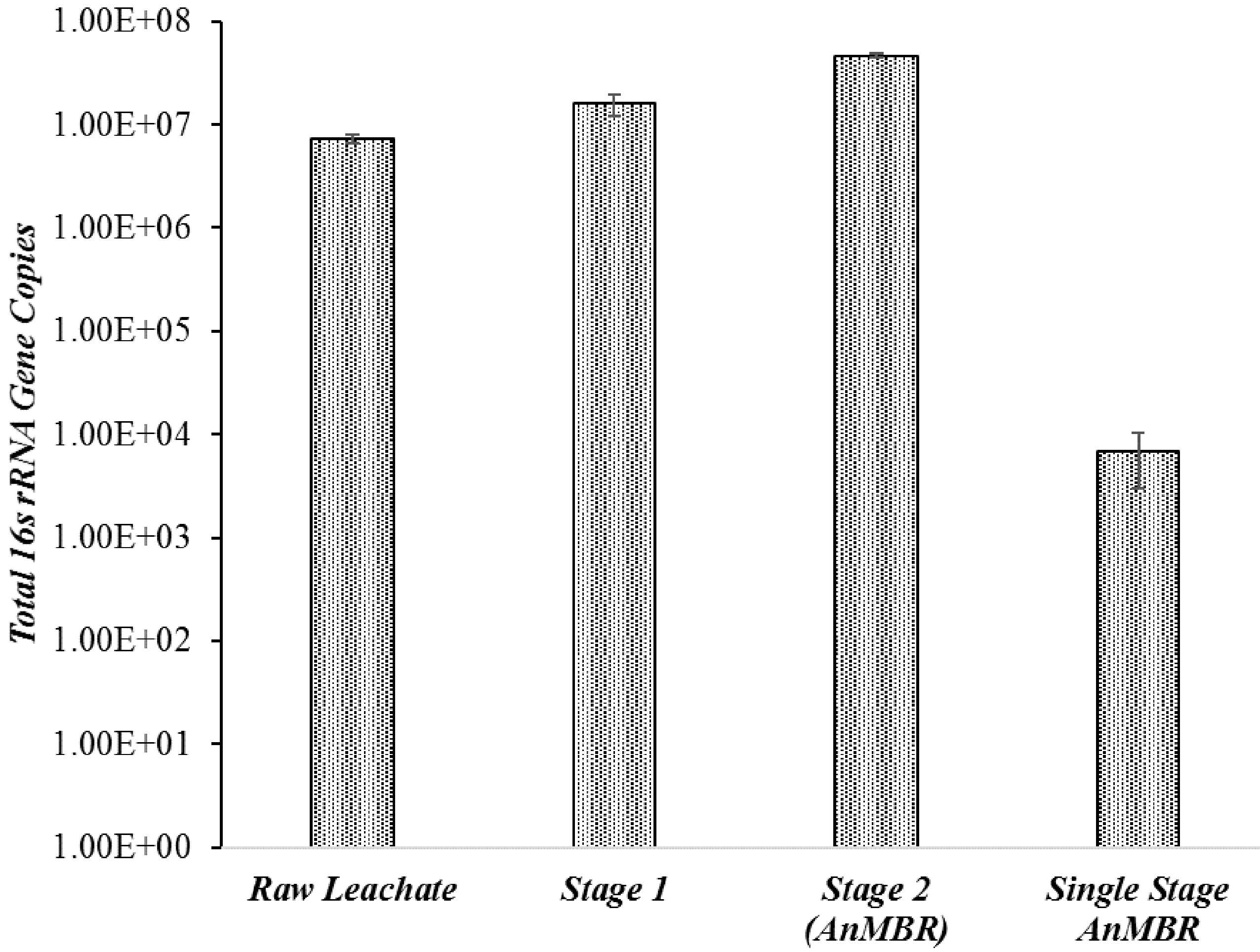

Figure 4-9. Total 16s rRNA gene copies in each sample quantified using qPCR.

CHAPTER 5

Pathogens and Antibiotic Resistance Genes (ARGs) in Untreated and Biologically-Treated Landfill Leachates

Ankit Pathak, Dongjuan Dai, Amy Pruden, John Novak

Department of Civil and Environmental Engineering, Virginia Tech, 418 Durham Hall, Blacksburg, VA 24060, USA

ABSTRACT

There is little knowledge of the microbial community composition in landfill leachates or bioreactors treating landfill leachates. While some studies have explored the structure of the microbial community inside landfills, information regarding microbial functions in leachates and biological leachate treatment is scarce. Due to the variety of wastes dumped in landfills, including food wastes, pet feces, leftover or spoiled meat products and expired medicines, there is emerging concern about the proliferation of antibiotic resistance in landfills. It is known that landfill leachates are often disposed by discharging them into sewers to be treated with sewage at wastewater treatment plants, and also that they can reduce the effectiveness of UV disinfection due to the presence of UV quenching organics such as humic and fulvic acids. This means that landfills could potentially increase levels of pathogens and antibiotic resistance genes in wastewater influents, while simultaneously compromising the capacity of wastewater treatment facilities to effectively disinfect their final effluent. This could result in the release of ARGs and pathogens into surface waters. This study used rapid – run Illumina sequencing to characterize the microbial community in four different landfill leachates and also identify ARGs present in raw and treated leachates. Annotation of sequenced genomes against the SILVA rRNA database showed matches indicating the presence of several genera known to contain pathogens, such as

Salmonella, *Staphylococcus*, *Pseudomonas* and *Legionella*. Several matches appeared to indicate the presence of known pathogenic species. Genes capable of conferring resistance to at least 18 different antibiotics in the raw leachates and 19 different antibiotics in two systems of bioreactors treating a landfill leachate. Overall, 204 different ARG subtypes were detected across the raw leachate samples, while 265 were detected across the bioreactor samples. Biological treatment of leachates was not found to bring about a significant reduction in the number of types of ARGs or pathogens identified. Thus, higher levels of pathogens and ARGs in landfill leachates could be an additional concern for blending them into wastewater treatment plant influents.

Keywords: landfill leachate, ARGs, pathogens, Illumina sequencing, wastewater treatment, UV disinfection

INTRODUCTION

The 2014 USEPA report on sustainable materials management showed that landfilling is still the most widely adopted method in the US for the disposal of municipal solid wastes (MSW). According to this report, over 52.6% of the total solid waste generated in the United States in 2014 was landfilled, amounting to 136 million tons. The amount of MSW disposed of in landfills was about 2.3 pounds per day per capita. However, despite their widespread use and cost effectiveness, the generation of landfill leachate by rainwater percolation or ground / surface water intrusion continues to pose a problem, so much so that leachate treatment and disposal is considered to be the main problem faced in landfill management (Imai *et al.*, 1995; Alkalay *et al.*, 1998).

Perhaps the most often utilized method for leachate disposal is to pay wastewater treatment plants to accept landfill leachates into the treatment process (Uygur and Kargi, 2004). These leachates may be discharged into the wastewater plants with or without on – site treatment at the landfill. As of 2000, the EPA estimated that more than half of the active landfills in the United States discharged their leachates into the nearest POTWs, in most cases without any form of on – site pretreatment. In addition to the issues presented by the nitrogen load and high concentrations of heavy metals in leachates (He et al., 2006), it is now known that landfill leachates absorb UV light at wavelengths of 254 nm, typically used for the purpose of disinfection at wastewater treatment plants (Zhao *et al.*, 2013; Gupta *et al.*, 2014). These leachates can drastically reduce the transmissivity of UV disinfection processes even when they constitute less than 1% of the flow into POTWs (Reinhart *et al.*, 2015). This could result in improper disinfection and cause the release of potentially pathogenic organisms from wastewater treatment plants into surface waters.

Over the last few decades, several methods have been tested for the purpose of landfill leachate treatment. These include physico – chemical methods such as coagulation – flocculation (Tatsi *et al.*, 2003; Amokrane *et al.*, 1997), advanced oxidation (Rivas et al., 2003), Fenton's reagent (Lopez *et al.*, 2004; Gupta *et al.*, 2014; Deng and Englehardt, 2006) and reverse osmosis (Chianese et al., 1998; Ushikoshi *et al.*, 2002; Linde *et al.*, 1995; Yamada and Jung, 2005) and also biological treatment processes like aerobic treatment (Lema *et al.*, 1988; Loukidou *et al.*, 2001; Ugyur and Kargi, 2004), anaerobic treatment (Lin, 1991; Lin and Chou, 1999; Liao *et al.*, 2014) and membrane bioreactors (Ahmed and Lan, 2012; Hasar *et al.*, 2009; Bohdziewicz *et al.*, 2008). While there has been work done with regard to treatment of landfill leachates for removal of COD and nitrogen, there is little known about the microbial community in landfill leachates and the kinds of organisms present in bioreactors treating landfill leachates.

Studies of taxonomic diversity of microorganisms in landfill leachates using 16s rRNA analyses have shown that a large fraction of the microbes found in landfill leachates are likely unknown (Huang *et al.*, 2005). However, most leachates have been shown to harbor large bacterial diversity. A study of prokaryotic diversity in landfill leachates found the presence of 59 archaeal and 283 bacterial species in a single leachate sample (Liu *et al.*, 2011). The bacterial community was found to comprise 18 phyla, showing matches for organisms known to degrade organic pollutants. The archaeal community was less diverse, with most matches coming from one phylum, *Euryarchaeota*. It is known that depending on the depth of sampling, microbial communities in a landfill can vary. The cover soils usually exhibit a mixture of aerobes, obligate anaerobes and facultative anaerobes, while the inside of landfill typically are colonized by facultative and obligate anaerobes (Semrau, 2011).

In addition to the microbial make – up of landfills, the presence of antibiotic resistance genes (ARGs) in landfill leachate is beginning to be explored. It is now known that antibiotic resistance genes are widespread in natural and anthropogenic systems (Pruden *et al.*, 2006). In addition, these genes can persist even in dead cells and pass between species through horizontal gene transfer, causing their rapid proliferation (Levy and Marshall, 2004). In landfills, the inherent variability in the nature of MSW, which potentially contains contaminants like expired medication, wastes from hospitals and clinics, animal excreta and plant or animal based food wastes treated with biocides or antibiotics (Eggen *et al.*, 2010) is coupled with a rich microbial community, providing ample opportunity for ARG proliferation through interspecies gene transfers. Landfills also tend to be rich in metal ions (Kjeldsen *et al.*, 2002), which could further accelerate the spread of ARGs by co – selection (Baker – Austin *et al.*, 2006). Although the number of published studies dealing with the persistence and proliferation of ARGs in landfill leachates is limited, it has been established that ARGs can persist in landfills for long periods of time (of the order of years) even when they are not contained in living cells (Hsu *et al.*, 2014). Studies analyzing ARGs at a leachate treatment plant found genes for resistance to 15 different antibiotics including tetracycline, sulfonamides and β - lactamase and the presence of mobile genetic elements (MGEs) like class I integrons (Zhang *et al.*, 2016).

Since it is known that landfill leachates can interfere with UV disinfection at wastewater treatment plants that accept them into their treatment processes even as a very small fraction of the total flow, it becomes important to monitor the kinds of organisms and ARGs that could potentially enter the disinfection process and get discharged into surface waters along with wastewater effluent. The fact that UV disinfection may be rendered less effective by the presence of landfill leachates means that significant number of antibiotic resistant organisms and genes could make it through the disinfection process and into the finished wastewater effluent discharged to water bodies. This study attempted to assess the variety of antibiotic resistant genes present in four different landfill leachates from separate landfill cells within the same landfill, each receiving varied wastes. One of these leachates was treated using two different anaerobic approaches, each employing an anaerobic membrane bioreactor (AnMBR). The goal was to analyze the microbial diversity and ARG profile in the untreated and anaerobically treated landfill leachates to see how ARGs can vary between leachates in order to better understand the possible effects this could have on wastewater treatment plants that add leachates into their treatment process. Next generation Illumina sequencing was used to quantify and visualize the microbial community and antibiotic resistance in the untreated and treated leachate samples.

MATERIALS AND METHODS

Sample Collection and DNA Extraction

Landfill leachates were obtained from four different cells of a landfill in Waverly, Virginia. Each of these cells varied in terms of the nature of wastes being landfilled and leachate age. These leachates were designated VA 1, VA 2, VA 3 and VA 4. While the exact nature of the wastes being added to each cell is unknown, the landfill is known to accept a large variety of wastes, even importing wastes from as far as New York. The leachates were transported to the laboratory in airtight polyethylene buckets and immediately refrigerated. Each of these leachates was analyzed for pH, total organic carbon (TOC), chemical oxygen demand (COD), and metal ions. pH was measured using an Oakton® handheld pH meter. TOC measurements were done using a Shimadzu® TOC-V CSN Total Organic Carbon Analyzer (Kyoto, Japan). COD was analyzed in accordance with Standard Method 5520C (closed titrimetric method, Standard Methods for the Examination of Water and Wastewater). Metal ions were estimated using an X – Series 2 inductively coupled plasma mass spectrometer (i.e. ICP – MS; Thermo Scientific, Waltham, MA). The leachate VA 1, which was the youngest leachate of the four collected, was used as feed for two treatment trains employing a two – stage and single – stage AnMBR, the construction and operation of which have been discussed in detail in the preceding chapters. Samples were drawn from inside these reactors (Stage 1 and Stage 2 of the two stage process and the single stage, single-stage AnMBR) for analysis as well. Like the untreated leachates, these samples were also analyzed for pH, TOC, COD and metal ions. The reactor set – up for both systems, feed and draw regimes and all the analyses listed here have been discussed in detail in previous chapters.

Both untreated and treated leachate samples were filtered using 0.2 µm cellulose acetate microfilters (Millipore, Billerica, MA), resulting in biomass getting captured onto the filter surface. The volume of the sample filtered was 10 ml. The filters with the sequestered biomass were cut into small pieces, sealed in airtight containers and frozen at -80 °C until needed for DNA extraction. Filtration and freezing were done immediately after the samples were taken to minimize errors that may be caused by exposure of anaerobic samples to oxygen. Capturing biomass onto microfilters was also done with a view to filtering out humic and fulvic acids, which are present in the soluble portion of landfill leachate, and can be inhibitory in the process of DNA extraction. A FastDNA SPIN Kit for Soil (MP Biomedicals, Solon, OH) was used to carry out DNA extraction from the stored filters.

Analyses for Taxonomic and ARG Distribution

The DNA extracts from the raw and treated leachate samples were submitted for metagenomics analysis to the Bioinformatics Institute (BI) in Blacksburg, Virginia. Prior to sequencing of the DNA extracted from the samples, treatment with RNAse and Ampure bead purification were applied to remove traces of humic substances and inhibitory organic carbon. Sequencing of DNA was done using Illumina HiSeq 2500 Rapid Run, with Nextera XT library preparation (Illumina, San Diego, CA; 100 – cycle paired end protocol) for all samples. Processing of reads from the sequenced genomes was performed using the MetaStorm server (Arango-Argoty *et al.*, 2016).

Taxonomic abundances were estimated by annotation against the SILVA rRNA database (Quast *et al.*, 2013), while ARGs were analyzed by annotation against the Comprehensive Antibiotic Resistance Database (CARD; McArthur et al., 2013). The updated CARD database was accessed (2016), which could filter out genes that undergo specific mutations in order to provide antibiotic resistance. The data from annotation against the SILVA database were used to generate relative abundances of the microbial phyla in each sample, providing a picture of the distribution of the microbial community. The top 20 genera of microorganisms across the samples were also quantified using SILVA and their relative abundances in each sample were computed. The total number of 16s rRNA gene sequences in each DNA extract were estimated by qPCR. The samples were analyzed at 1:100 dilutions, a dilution ratio selected on the basis of qPCR data obtained from serial dilution tests to check for minimum inhibition. During the dilution tests and the qPCR analyses of the diluted extracts, standards with 16s rRNA gene concentrations ranging from 10^8 to 10^2 gene copies/μL (serially diluted by a factor of 10) were run in triplicates in addition to a triplicate negative control.

ARG abundances in the DNA extracts from untreated and treated landfill leachates were normalized to 16s rRNA gene concentrations in the corresponding sample. Annotation against the CARD database was used to quantify the top 100 ARGs across all samples. In addition, 9 genes, namely *sul1*, *sul2* (sulfonamide resistance), *tetW*, *tetO*, *tetM* (tetracycline resistance), *ermF*, *ermB* (erythromycin resistance), *mefA* (macrolide resistance) and *vanG* (vancomycin resistance) were quantified across untreated leachates using CARD. PRIMER – E 6.1.13 was used to generate multidimensional scaling plots based on Bray – Curtis distances. Since only one sample of each raw leachate and one from each reactor were analyzed for ARGs, it was not possible to draw correlations between ARG and metal ion concentrations to evaluate the potential for co-selection. The focus of these analyses, was therefore to have a basic understanding of whether landfill leachates can be significant sources of ARGs for wastewater treatment plants that accept them and to underline the importance of understanding antibiotic resistance in leachates in general.

RESULTS AND DISCUSSION

The untreated landfill leachates showed considerable diversity in terms of pH, TOC, COD and metal ion concentrations, as summarized in Table 1.

Taxonomic Diversity Across Samples and qPCR Data

The number of metagenomics reads in the sequenced genomes was in the range of 5,502,195 to 58,329,814 per sample (average length of 100 bp per read). These metagenomics reads were assembled using the IDBA – UD software (University of Hong Kong). 34 – 81% of the total reads in each sample were used for assembly, with N50 values varying from 834 – 1,911 across all samples. The PRODIGAL software (Oak Ridge National Laboratory and the University of Tennessee) was used to predict genes from the assembled sequences.

Taxonomic diversity of microorganisms was analyzed in samples from four untreated landfill leachates and three bioreactors treating landfill leachate. The microbial community across all the samples, treated as well as untreated, was found to be dominated by bacteria. Bacterial 16s matches accounted for 88 – 100% of the total matches in each sample. Most samples showed high relative abundances of phyla *Firmicutes* (28 – 38% relative abundance), *Proteobacteria* (10 – 41%), *Actinobacteria* (2 – 25%) and *Bacteroidetes* (2 – 20%). The only archaeal phylum detected across the samples was *Euryarchaeota* (0 – 11% relative abundance). The difference in distribution of microbial phyla between leachates from different cells i.e. untreated leachates VA 1, VA 2, VA 3 and VA 4 and bioreactors treating VA 1 can be seen in Figure 5-1.

It may be noted that the structure of the microbial community in different leachates does show clear differences, although *Firmicutes, Bacteroidetes* and *Proteobacteria* were the most abundant phyla across all four raw leachates. It may also be noted that there are subtle changes in microbial community structure for VA 1 before and after treatment. Stage 1, operated at thermophilic temperatures, showed an increase in *Firmicutes* (38%) and *Spirochaetae* (6%), while in Stage 2 *Proteobacteria* (42%) and *Actinobacteria* (7%) were found to increase in relative abundance as compared to the VA 1 feed. The Single-stage AnMBR showed a much higher relative abundance of *Actinobacteria* (25%) relative to the raw leachate VA 1.

The total 16s rRNA genes in each sample were quantified using qPCR. The untreated landfill leachates showed large variations in 16s rRNA gene concentrations, with total numbers of these genes ranging from the order of 10^6 – 10^8 per sample. From the differences in the distribution of microbial phyla and the numbers of 16s rRNA gene copies between raw samples using qPCR, it may be inferred that microbial communities can vary significantly, even between different cells within the same landfill. Additionally, it can be seen that the number of matches for 16s rRNA genes increases in the two stage system treating the leachate VA 1. The number of matches for 16s rRNA genes in Stage 1 was 120% higher than the raw leachate VA1, while in Stage 2, the matches were 532% greater. However, in the single-stage AnMBR, the matches for 16s rRNA genes were lower than the landfill leachate by over two orders of magnitude. Figure 5-2 shows the total number of 16s rRNA gene copies in each sample obtained using qPCR.

By annotation against the SILVA database, the top 20 genera across the samples were quantified, and their relative abundances in each sample were calculated. The data showed the presence of several genera of bacteria that are known to contain pathogenic species. The most abundant genus across most samples, treated and untreated, was the bacterium *Salmonella*. In addition to *Salmonella*, other notable genera with potential pathogenicity identified across samples included

Pseudomonas, *Staphylococcus* and *Legionella*. *Salmonella* accounted for 11 – 30% of total 16s rRNA matches across samples using SILVA, while for *Pseudomonas*, *Staphylococcus* and *Legionella*, the corresponding values were 0 – 17%, 0 – 8% and 0 – 7% respectively. Interestingly, all the matches for *Salmonella* were attributed to *Salmonella enterica*, a known human pathogen. A majority of the matches for *Pseudomonas*, *Staphylococcus* and *Legionella* were also found to be associated with the pathogenic species *P. aeruginosa*, *S. aureus* and *L. pneumophila*. While it is possible that a majority of matches were associated with these species since they are well catalogued in most databases by virtue of their pathogenicity, there is enough evidence to suggest that landfill leachates are not only rich in microorganisms, but can harbor significant amounts of pathogens. While biological treatment of raw leachate VA 1 using both AnMBR trains was able to reduce the relative abundance of genera such as Salmonella from 25% to about 15%, it can be seen that pathogens can persist even in biologically treated landfill leachates and make their way into wastewater treatment plants. Figure 5-3 shows the relative abundances of the top 20 most abundant genera across samples.

The potential for pathogenicity in landfill leachates also makes the case for an AnMBR as a treatment step as the pore sizes used are typically small enough to keep biomass in the reactor and prevent the discharge of pathogens into sewer systems and into wastewater treatment.

ARG Profiles in Leachate Samples

The ARGs in raw and treated leachates were analyzed by annotating the sequences genomes against the CARD database. In all, 204 ARG subtypes were detected in the untreated leachate samples, while the number of ARG subtypes identified in each untreated leachate sample varied from 94 – 158. Using the data obtained from annotation against CARD, the 100 most abundant ARGs across the samples were normalized to the 16s rRNA concentrations in the corresponding sample. The data showed that raw landfill leachates contained 10 - 36 ARG copies per 16s rRNA gene copy. In the 100 most abundant ARGs alone, resistance to 18 different types of antibiotics was detected. Of these, the most number of ARG copies corresponded to multidrug resistance (2.1 – 11.7 ARG copies / 16s rRNA gene copy), followed by resistance to trimethoprim (1.3 – 6.4 ARG copies / 16s rRNA gene copy), tetracycline (0.9 – 4.34 ARG copies / 16s rRNA gene copy) and vancomycin (1.21 – 1.46 ARG copies / 16s rRNA gene copy). Resistance genes for other antibiotics such as bacitracin, erythromycin, lincomycin, novobiocin and macrolides were also detected, among others (Figure 5-4).

Samples collected from the bioreactors treating the VA 1 leachate did not show a significant difference in the relative abundances of the ARGs, but exhibited small differences in ARG concentrations relative to 16s rRNA. While the raw VA 1 leachate showed the presence of 21 ARG copies / 16s rRNA gene copies, these numbers for Stage 1, Stage 2 and the Single-stage AnMBR were 16, 26 and 27.5 respectively. This suggests that while anaerobic biological treatment may bring about subtle changes in ARG concentrations of landfill leachate, these ARGs are not appreciably removed. In the bioreactors, as in the raw leachates, multidrug resistance was found to be the most common, followed by resistance against trimethoprim, colistin and vancomycin. Overall, across the VA1 leachates and the bioreactors operated using VA 1 as feed, the top 100 ARGs conferred resistance to 19 different antibiotics. In total, 265 ARG subsets were detected, with 138 – 173 subsets present per sample. Figure 5-5 shows the shift in ARG profiles in bioreactors fed exclusively with VA 1 as carbon source.

These shifts in ARG profiles may be better visualized using a 3D multidimensional scaling plot to show similarity between the top 100 antibiotic resistance genes present in raw VA 1 leachate versus the bioreactors fed with it. The plot showed that ARG profile in untreated VA 1 landfill leachate was found to shift after anaerobic biological treatment in Stage 1, Stage 2 and the Single-stage AnMBR. The ARG profiles in the Stage 2 and the Single-stage AnMBRs were found to be similar, while Stage 1 was found to be distinct (Figure 5-6).

In addition to the visualizing the distribution of antibiotic resistance, the concentrations of 9 genes were compared across raw leachates. These genes were *sul1* and *sul2* (sulfonamide resistance), *tetW*, *tetO* and *tetM* (tetracycline resistance), *ermF* and *ermB* (macrolides, lincosamide and streptogramin A resistance), *mefA* (macrolides resistance) and *vanG* (vancomycin resistance). Data obtained from annotation against CARD showed that only one out of four raw leachates showed the presence of *sul1*, while *tetW*, *tetO* and *tetM* were detected in two leachates each. The *sul2*, *ermF*, *ermB*, *mefA* and *vanG* genes were identified in all four raw leachates. The concentrations of these genes, normalized to 16s rRNA showed considerable differences across the raw samples, underscoring the variability in the nature and concentrations of ARGs found in landfill leachates, even from different cells of the same landfill. This observation is in line with the findings of Sawamura *et al.* (2010), who suggested that the microbial community can vary significantly with depth and location not only from one landfill cell to another, but even within a single landfill cell based on conditions of temperature and pressure and the nature of the wastes landfilled. Figure 5-7 shows the concentrations of selected ARGs normalized to 16s rRNA gene copies.

Discussion and Broader Implications

The analyses of microbial community and antibiotic resistance in landfill leachates show that the structure of the microbial community can vary significantly from one landfill leachate to another. In addition, there are indications that these leachates could contain significant numbers of pathogenic organisms such as *Salmonella*, *Pseudomonas*, *Staphylococcus* and *Legionella*. In addition, landfill leachates have been shown by this and several other studies to contain high numbers and diversity of ARGs. In this study, untreated leachates showed the presence of as many as 158 different ARG subsets, reaching concentrations as high as 36 ARG copies / 16s rRNA copy.

The data obtained in this study also suggest that while biological treatment may trigger shifts in the abundances of microbial taxa and ARGs relative to each other, it does little to remove pathogenic organisms and antibiotic resistance from landfill leachates. Thus, regardless of whether leachates are treated or untreated, they can contain significant levels of pathogenicity and antibiotic resistance.

As per USEPA estimates, over 50% of landfills in the US discharge their leachates into nearby wastewater treatment plants. Therefore, landfill leachates could potentially act as sources for pathogen and ARG addition into wastewater treatment plants. It is important to note that landfill leachates quench large quantities of UV light at wastewater treatment plants using UV disinfection (Zhao *et al.*, 2013). It has also been shown that UV disinfection at wastewater treatment plants can be rendered ineffective even when leachates constitute less than 1% of the total flow coming into the plant. The addition of pathogens and ARGs by landfill leachates into wastewater, coupled with a reduction in the efficiency of disinfection by way of UV quenching could mean that wastewater treatment plants accepting landfill leachates into their treatment processes have compromised disinfection processes, resulting in insufficient log removal of pathogenic organisms, antibiotic resistant organisms and ARGs, which then get discharged into surface waters. This could result in rapid proliferation of pathogens and ARGs in water bodies, that could also potentially spill over to drinking water and groundwater.

Wastewater treatment plants have already been described as hotspots for ARG transfer and sources for the introduction of antibiotic resistant bacteria to the environment. The addition of more ARGs and a simultaneous weakening of the disinfection process could mean greater numbers of ARGs and antibiotic resistant organisms are introduced into the environment worsening an already daunting problem.

REFERENCES

Ahmed, F. N., Lan, C. Q., 2012. Treatment of landfill leachate using membrane bioreactors: A review. Desalination (287), 41–54.

Alkalay, D., Guerrero, L., Lema, J. M., Mendez, R., Chamy, R., 1998. Review: Anaerobic Treatment of Municipal Sanitary Landfill Leachates: The Problem of Refractory and Toxic Components. World Journal of Microbiology & Biotechnology (14), 309 – 320.

Amokrane, A., Comel, C., Veron, J., 1997. Landfill Leachates Pretreatment by Coagulation-Flocculation. Water Research 31 (11), 2775 – 2782.

Baker-Austin, C., Wright, M. S., Stepanauskas, R., McArthur, J.V., 2006. Co-selection of antibiotic and metal resistance. Trends in Microbiology 14 (4), 176 – 182.

Bohdziewicz, J., Neczaj, E., Kwarciak, A., 2008. Landfill leachate treatment by means of anaerobic membrane bioreactor. Desalination (221), 559–565.

Chianese, A., Ranauro, R., Verdone, N., 1998. Treatment of Landfill Leachate by Reverse Osmosis. Water Research 33 (3), 647 – 652.

Deng, Y., Englehardt, J. D., 2006. Treatment of landfill leachate by the Fenton process. Water Research 40 (20) 3683 – 3694.

Eggen, T., Moeder, M., Arukwe, A., 2010. Municipal landfill leachates: a significant source for new and emerging pollutants. Science of The Total Environment 408 (21), 5147 – 5157.

Gupta, A., Zhao, R., Novak, J. T., Goldsmith, C. D., 2014b. Variation in organic matter characteristics of landfill leachates in different stabilization stages. Waste Management & Research (1 – 8).

Hasar, H., Ipek, U., Kinaci, C., 2009. Joint treatment of landfill leachate with municipal wastewater by submerged membrane bioreactor, Water Science & Technology (60), 3121–3127.

He, P. J., Xue, J. F., Shao, L. M., Li, G. J., Lee, D. J., 2006b. Dissolved organic matter (DOM) in recycled leachate of bioreactor landfill. Water Research 40 (7), 1465 – 1473.

Hsu, J. T., Chen, C. Y., Young, C. W., Chao, W. L., Li, M. H., Liu, Y. H., Lin, C. M., Ying, C., 2014. Prevalence of sulfonamide-resistant bacteria, resistance genes and integrin – associated horizontal gene transfer in natural water bodies and soils adjacent to a swine feedlot in northern Taiwan. Journal of Hazardous Materials (277), 34 – 43.

Huang, L. N., Zhou, H., Zhu, S., Qu, L. H., 2004. Phylogenetic diversity of bacteria in the leachate of a full-scale recirculating landfill. FEMS Microbiology Ecology (50), 175 – 183.

Imai, A., Onuma, K., Inamori, Y., Sudo, R., 1995. Biodegradation and adsorption in refractory leachate treatment by the biological activated carbon fluidized bed process. Water Research 29 (2), 687 – 694.

Kjeldsen, P., Barlaz, M.A., Rooker, A.P., Baun, A., Ledin, A., Christensen, T.H., 2002. Present and long-term composition of MSW landfill leachate: a review. Critical Reviews in Environmental Science and Technology 32 (4), 297 – 336.

Lema, J.M., Mendez, R., Blazquez, R., 1988. Characteristics of landfill leachates and alternatives for their treatment: a review, Water Air and Soil Pollution (40), 223–250

Levy, S. B., Marshall, B., 2004. Antibacterial resistance worldwide: causes, challenges and responses. Nature Medicine (10) S122 – S129.

Lin, Č. Y., 1991. Anaerobic digestion of Landfill leachate. Water SA 17 (4), 301 – 306.

Lin, Č. Y., Bian, F. Y., Chou, J., 1999. Anaerobic co-digestion of septage and landfill leachate. Bioresource technology 68 (3), 275 – 282.

Linde, K., Jönsson, A., Wimmerstedt, R., 1995. Treatment of three types of landfill leachate with reverse osmosis. Desalination 101 (1), 21 – 30.

Liao, X., Zhu, S., Zhong, D., Zhu, J., Liao, L., 2014. Anaerobic co-digestion of food waste and landfill leachate in single-phase batch reactors. Waste Management 34 (11), 2278 – 2284.

Liu, J., Wu, W., Chen, C., Sun, F., Chen, Y., 2011. Prokaryotic diversity, composition structure, and phylogenetic analysis of microbial communities in leachate sediment ecosystems. Applied Microbiology and Biotechnology (91), 1659 – 1675.

Lopez, A., Pagano, M., Volpe, A., Di Pinto, A. C., 2004. Fenton's pre-treatment of mature landfill leachate. Chemosphere (54), 1005 – 1010.

Loukidou, M.X., Zouboulis, A.I., 2001. Comparaison of two biological treatment process using attached-growth biomass for sanitary landfill leachate treatment. Environmental Pollution 111, 273–281.

McArthur AG, Waglechner N, Nizam F, Yan A, Azad MA., Baylay AJ, et al. (2013). The comprehensive antibiotic resistance database. Antimicrobial Agents and Chemotherapy (57): 3348–3357.

Pal, C., Bengtsson-Palme, J., Kristiansson, K., Larsson, D. G. J., 2016. The structure and diversity of human, animal and environmental resistomes. Microbiome 4:54, 1 – 13.

Pruden, A., Pei, R., Storteboom, H., Carlson, K. H., 2006. Antibiotic Resistance Genes as Emerging Contaminants: Studies in Northern Colorado. Environmental Science & Technology (40), 7445 – 7450.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, *et al.* (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research (41), D590–D596.

Reinhart, D., Bolyard, S. C., 2015. Fate of Organic Matter from Leachate Discharged to Wastewater Treatment Plants (Year 1). Report #10285 submitted to the Hinkley Center for Solid and Hazardous Waste Management, Gainesville, FL.

Rivas, F. J., Beltrán, F., Gimeno, O., Acedo, B., Carvalho, F., 2003. Stabilized leachates: ozone – activated carbon treatment and kinetics. Water Research 37 (20), 4823 – 4834.

Semrau, J. D., 2011. Current knowledge of microbial community structures in landfills and its cover soils. Applied Microbiology and Biotechnology (89), 961 – 969.

Tatsi, A. A., Zouboulis, A. I., Matis, K. A., Samaras, P., 2003. Coagulation – flocculation pretreatment of sanitary landfill leachates. Chemosphere 53 (7), 737 – 744.

Ushikoshi, K., Kobayashi, T., Uematsu, K., Toji, A., Kojima, D., Matsumoto, K., 2002. Leachate treatment by the reverse osmosis system. Desalination 150 (2), 121 – 129.

Uygur, A., Kargı, F., 2004. Biological nutrient removal from pre-treated landfill leachate in a sequencing batch reactor. Journal of Environmental Management (71), 9–14.

Yamada, M., Jung, S., 2005. Application of reverse osmosis for landfill leachate treatment. APEC Virtual Center for Environmental Technology Exchange: http://www.apec-vc.or.jp/e/modules/tinyd00/?id=35.

Zhao, R., Gupta, A., Novak, J. T., Goldsmith, C. D., Driskill, N., 2013. Characterization and treatment of organic constituents in landfill leachates that influence the UV disinfection in the publicly owned treatment works (POTWs). Journal of Hazardous Materials (258 – 259), 1 – 9.

TABLES

Table 5-1. Characteristics of untreated landfill leachates. Only selected metal ion concentrations are shown

Parameter	***VA 1***	***VA 2***	***VA 3***	***VA 4***
pH	8.5	6.7	7.4	5.2
TOC (mg/L as C)	3,500	13,000	6,500	4,000
COD (mg/L)	14.000	22,000	17,500	11,500
^{23}Na *(ppm)*	3600	1600	1400	3950
^{54}Fe *(ppm)*	20	80	10	5
^{39}K *(ppm)*	1400	1000	850	450
^{43}Ca *(ppm)*	50	2200	25	75

FIGURES

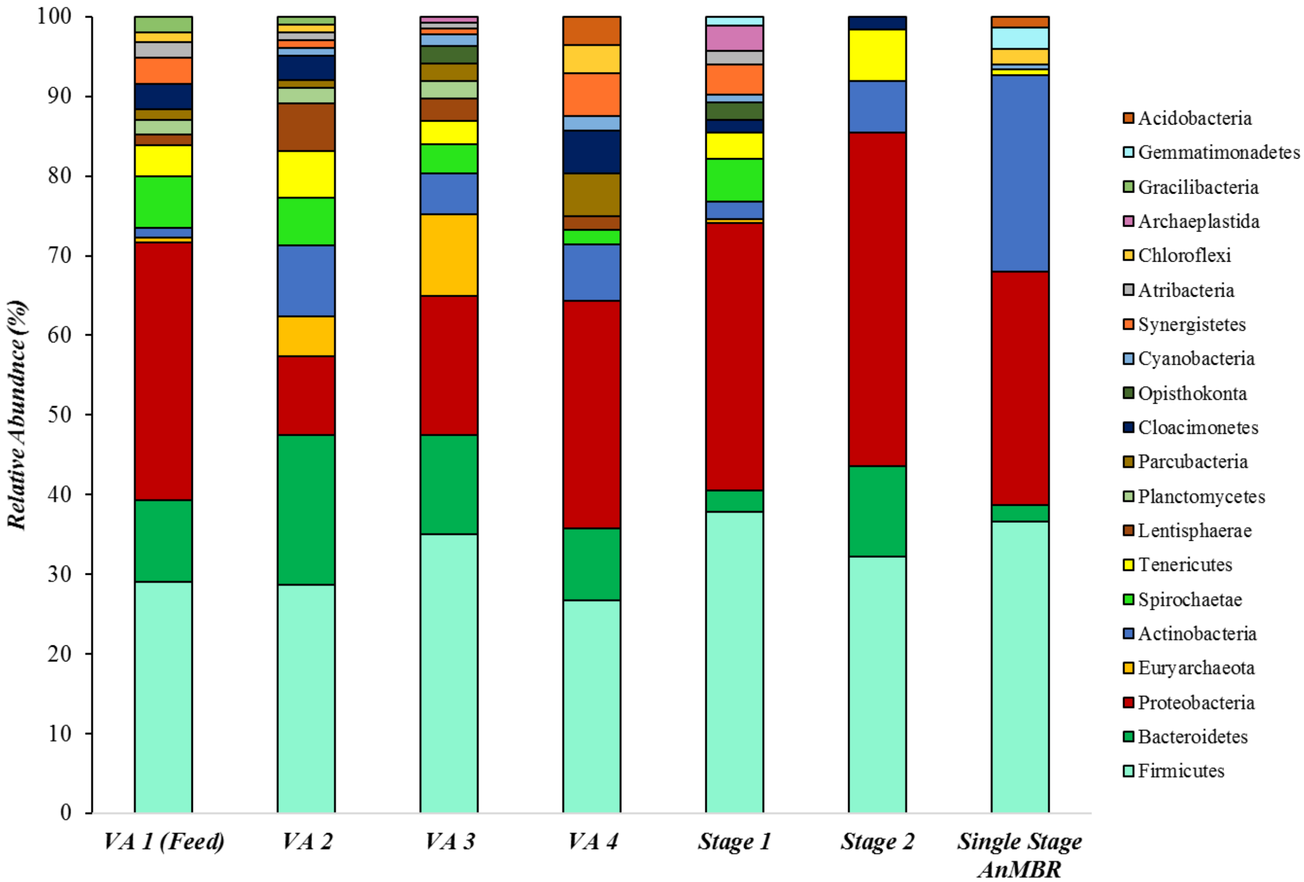

Figure 5-1. Relative abundances of dominant microbial phyla in leachate samples.

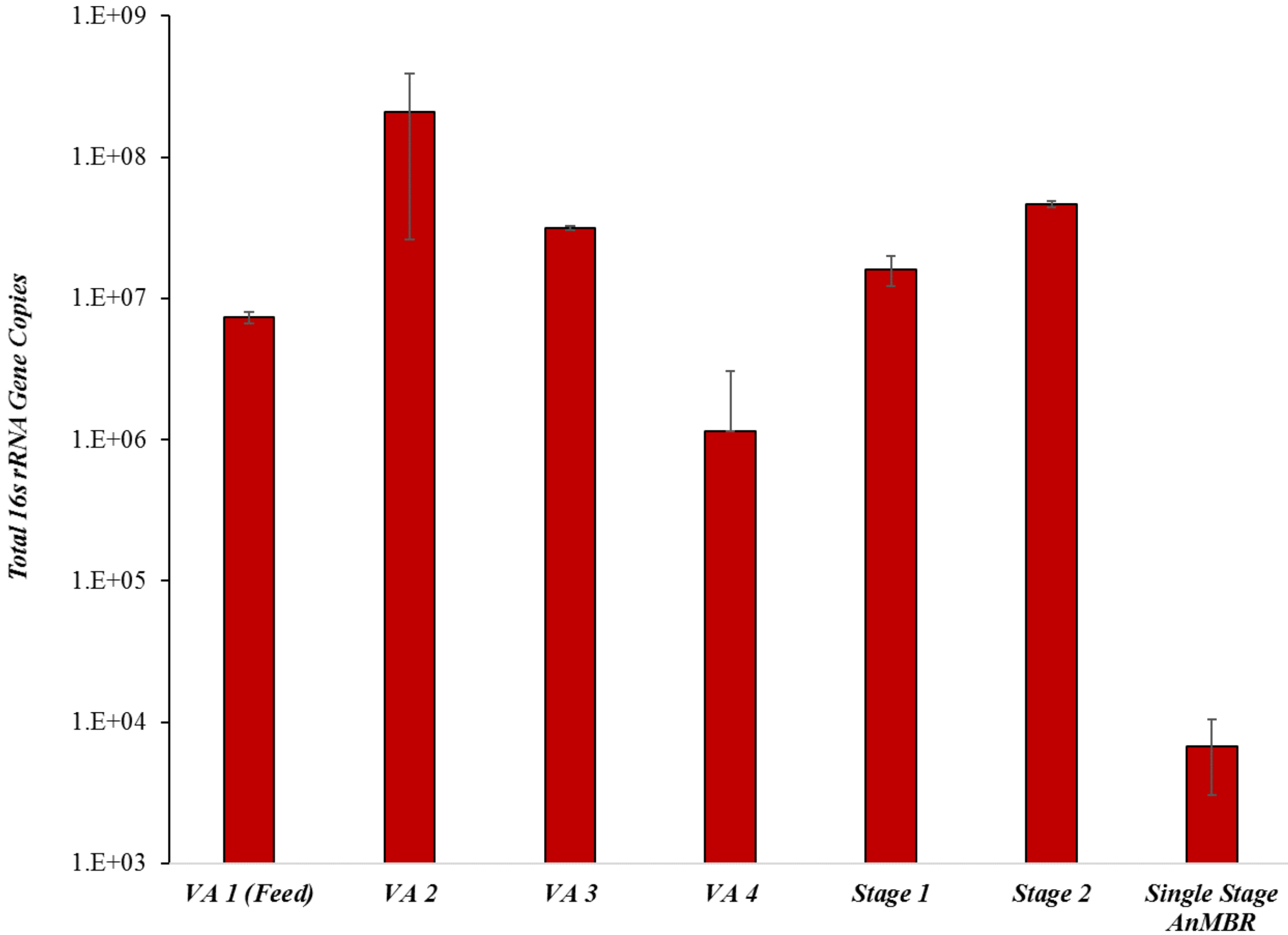

Figure 5-2. Total 16s rRNA gene copies in leachate samples using qPCR (n=3; Bars indicate standard deviation of triplicates)

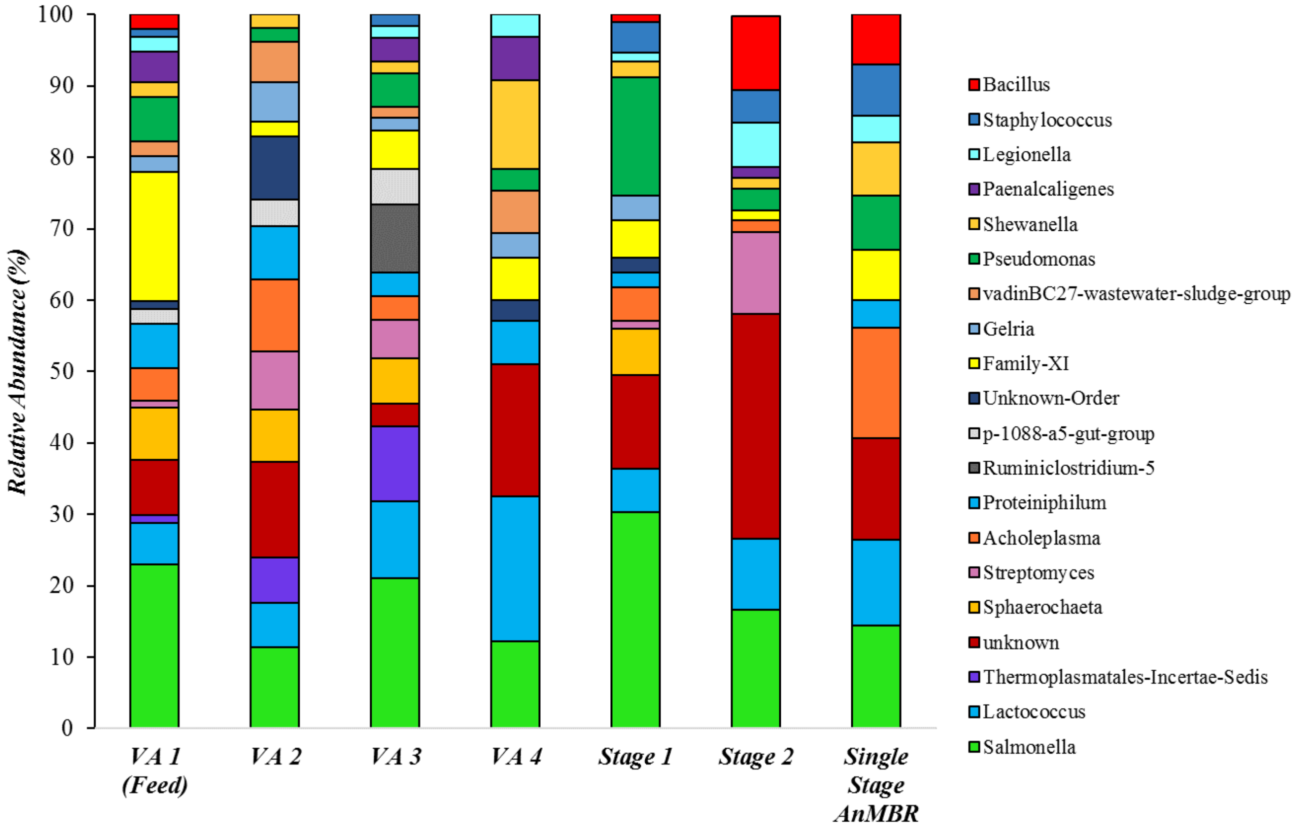

Figure 5-3. Relative abundances of the 20 most abundant genera across untreated and treated leachate samples quantified using 16s rRNA annotation against SILVA database.

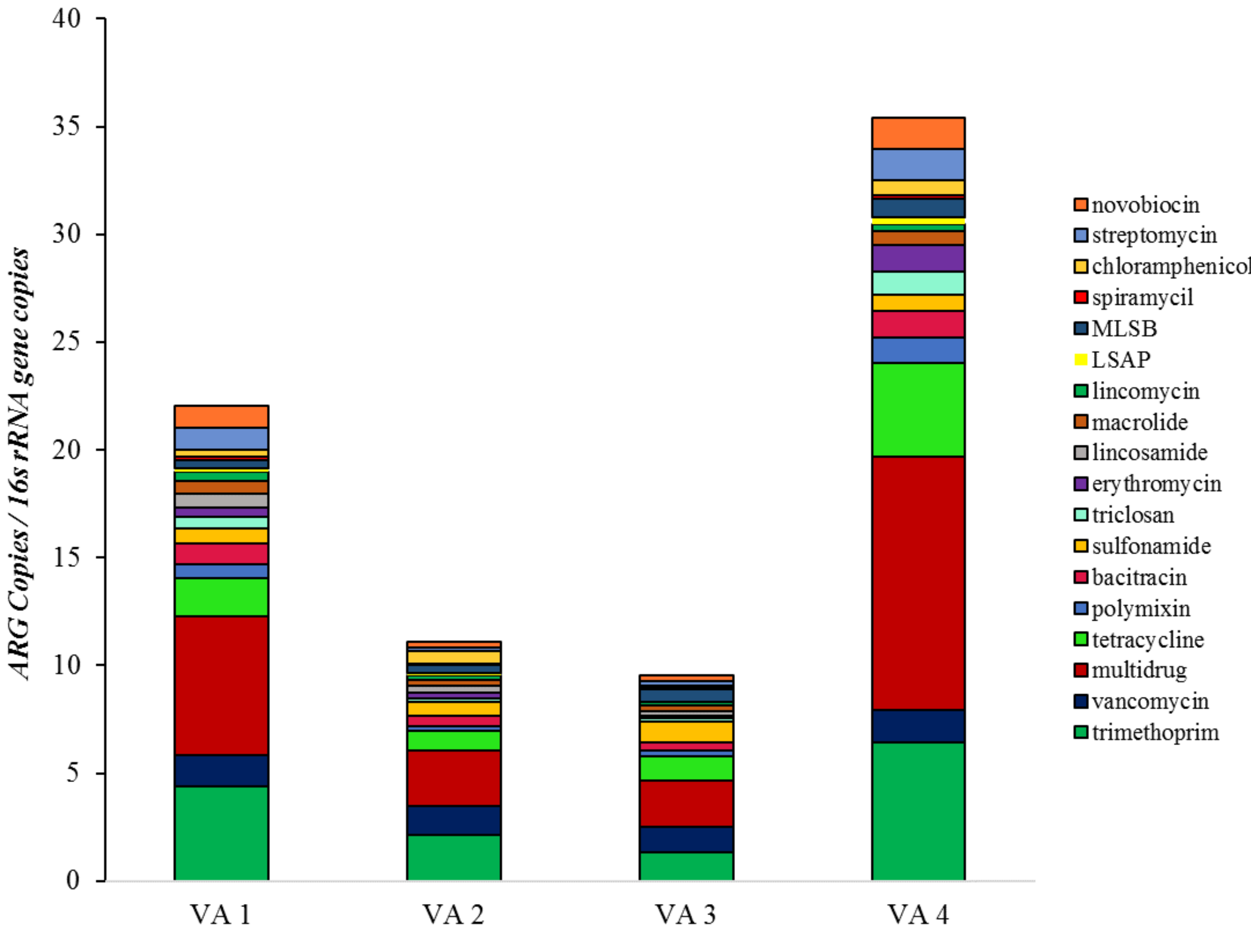

Figure 5-4. ARG concentration in untreated leachate samples normalized to 16s rRNA gene copies. These concentrations are normalized to total 16s rRNA gene copies, and are higher than levels observed in most types of environmental samples including wastewater and sludge (Pal *et al.*, 2016).

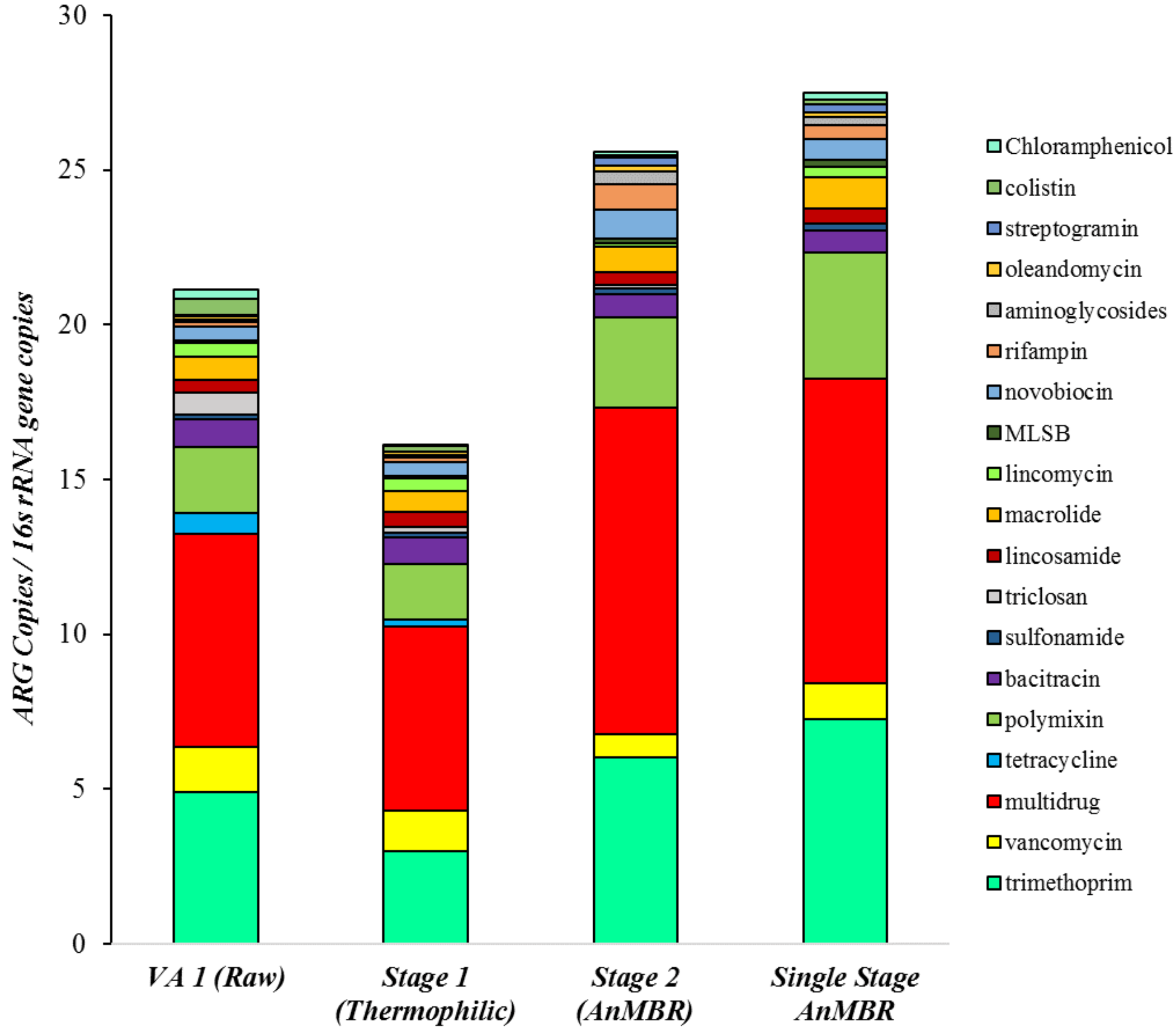

Figure 5-5. ARG concentration in Raw VA 1 leachate versus Stage 1 and Stage 2 of the two stage AnMBR and the Single-stage AnMBR fed with VA 1.

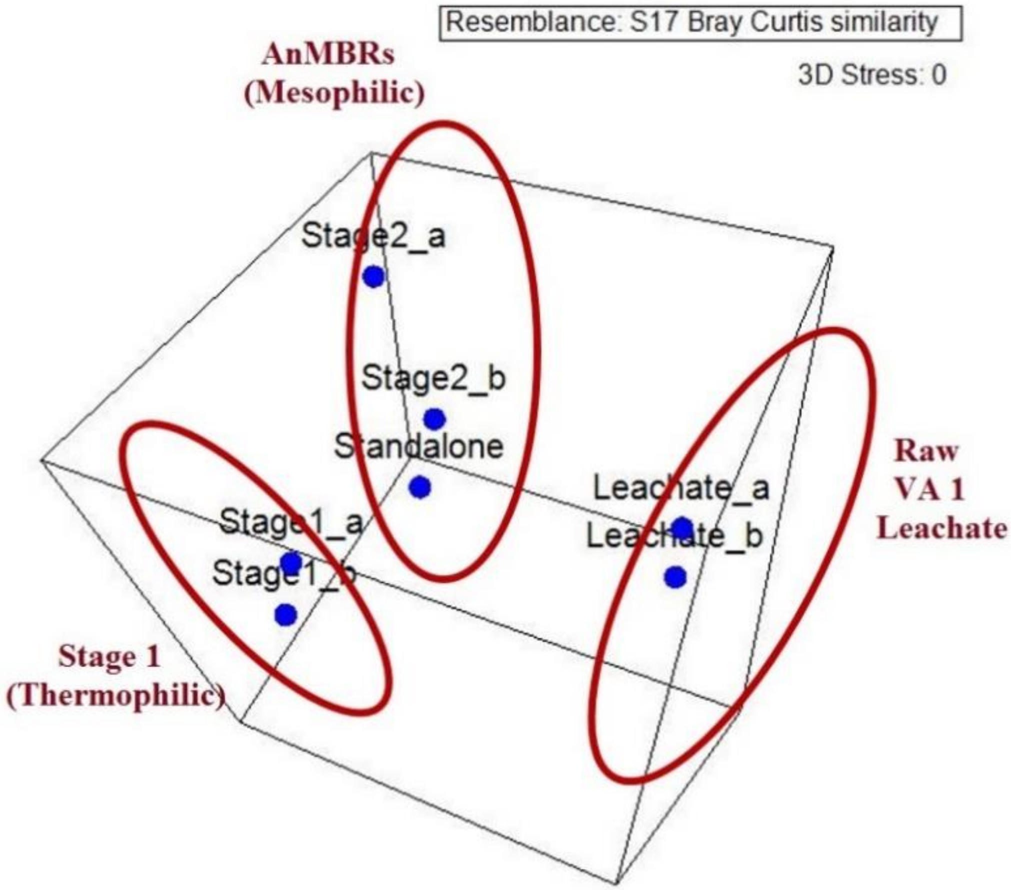

Figure 5-6. 3D multidimensional scaling plot of ARG similarity in untreated and treated leachates. Leachate_a and Leachate_b denote duplicate samples of raw VA 1 leachate. Stage 1_a, Stage 1_b, Stage 2_a and Stage 2_b denote biological replicates from stage 1 and stage 2.

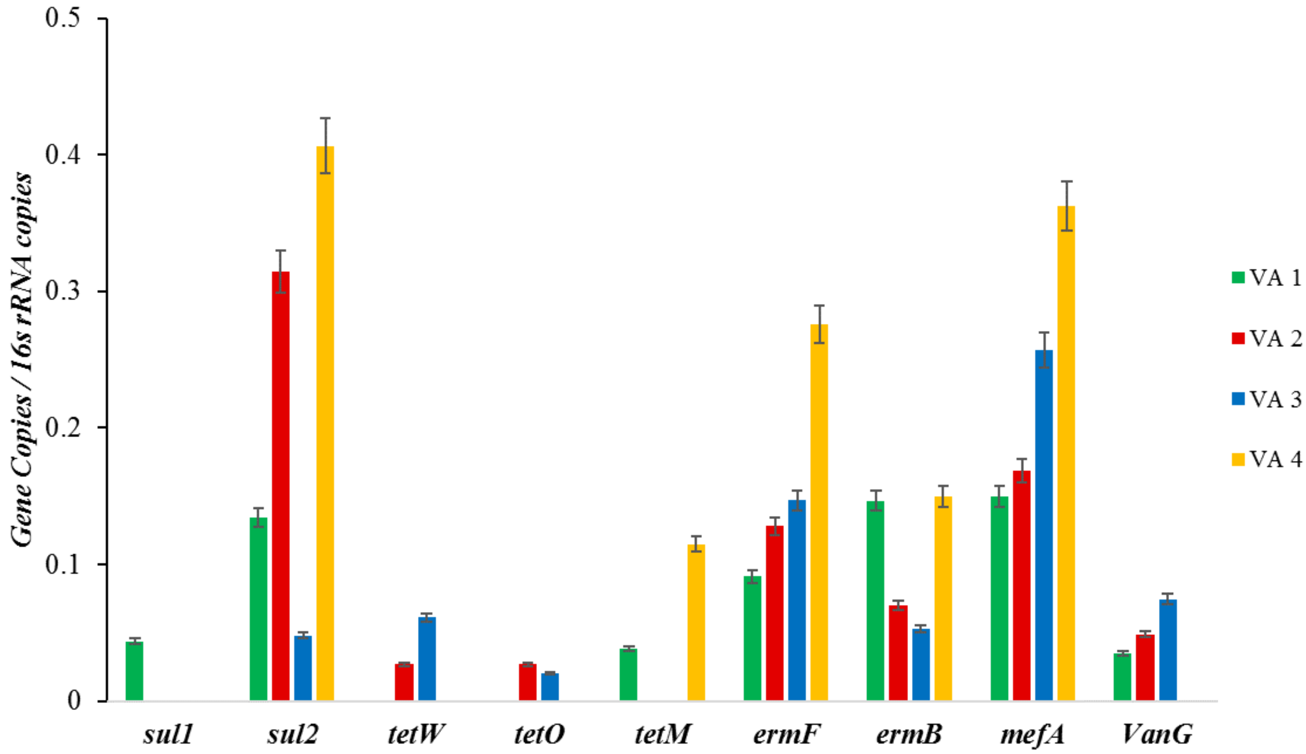

Figure 5-7. Concentration of selected ARGs in untreated landfill leachates, quantified by annotation against CARD database.

CHAPTER 6

Conclusions and Engineering Significance

The disposal of landfill leachates, especially by discharge into wastewater treatment plants is an emerging issue with serious implications for wastewater treatment. Leachate treatment processes tend to be centered around removal of ammonia (or nitrogen loading in general) and COD, since they are governed by regulations. It is, however, becoming clear that the interference of landfill leachates with UV disinfection processes at wastewater treatment plants can greatly hinder effective disinfection, potentially causing violations of the discharge permits for microorganisms. These interferences occur even at very small dilutions. The recent cases of landfill leachate related issues at the City of Richmond Wastewater Treatment Plant in Richmond, VA showed that landfill leachates entering the plant at only a 1% v/v ratio with domestic wastewater reduced the UV transmissivity in the UV disinfection tank to less than half the value required for effective log removal of coliform bacteria, causing the plant to violate discharge permits for *E.coli* and total coliform bacteria for a period of several months.

The two – stage biological treatment process employing an AnMBR successfully brought about accelerated degradation of the organic compounds responsible for the UV quenching (humic substances) by landfill leachates by recreating the temperature profiles present inside landfills in the laboratory, and using a membrane to retain biomass within the system. While the bulk of the degradation of the humic substances occurred in the second stage i.e. the AnMBR, it was found that these substances were hydrolyzed into smaller, lower molecular weight fragments in the first (thermophilic) stage, making them more bioavailable in Stage 2. The microbial communities in the two stages also exhibited differences in relative abundances of phyla and showed matches for known degraders of humic substances.

The rationale of mimicking the conditions of long term landfilling to bring about accelerated removal of humic substances was validated by comparing the performance of the two – stage AnMBR against an identical, single - stage AnMBR without thermophilic pretreatment. The data obtained from samples drawn over 7 months showed that the two – stage process removed three times as much UV quenching organic carbon as compared to the single – stage process. While analyses of the microbial communities in the two trains showed the presence of known humic acid degraders in both, the number of matches were significantly higher in the two – stage process. The inclusion of a thermophilic first stage was therefore validated.

Over the last few years, it has been suggested that landfill leachates could be reservoirs of antibiotic resistance genes and pathogenic microorganisms due to the large amounts and variety of wastes they contain. Analyses of leachates from four different landfill cells showed the presence of genes providing resistance to 18 different antibiotics, supporting the idea that landfill leachates could act as sources of ARG addition to wastewater treatment facilities. Biological treatment using the two – stage AnMBR did not appear to reduce ARG concentrations in landfill leachate. There is therefore, a possibility that both untreated as well as biologically treated landfill leachates have significant numbers of ARGs and antibiotic resistant bacteria, further underscoring the need to ensure that leachates do not interfere with UV disinfection at wastewater treatment plants that accept them. It must be emphasized, however, that confirmatory studies are needed to affirm the presence of pathogenicity by analysis for virulence genes. Comparative analyses of ARG concentrations across different environments and landfill leachates are also needed.

A short study comparing the treatability of young and mature landfill leachates (refer to Appendix A) also revealed that young leachates are more amenable to biological treatment and can be treated for the removal of UV quenching organic carbon using the two – stage process. Biological treatment is unlikely to work well with mature leachates due to the low availability of biodegradable organic carbon, which is depleted by natural degradation that occurs during long term landfilling. Overall, it is clear that regardless of whether wastewater treatment plants receive young, old, treated or untreated landfill leachates, their characteristics must be monitored and the flow of leachates into the wastewater treatment process must be rigidly controlled. There is especially a need for vigilance during long dry spells or periods of reduced flow into wastewater treatment plants, when the addition of even small amounts of landfill leachate can have a larger impact on UV disinfection due lesser wastewater flow available for dilution.

APPENDICES

Appendix A: Comparison of UV quenching organic carbon removal in young versus mature landfill leachates using a two – stage AnMBR (Manuscript Outline)

Ankit Pathak, Amy Pruden, John T. Novak

OBJECTIVE AND RATIONALE

The objective of this manuscript is to compare the treatability of young and old leachates using biological treatment, in order to better understand the applicability of the two – stage AnMBR.

As was discussed in the literature review (Chapter 2), older leachates tend to be considerably lower in biodegradable organic carbon. This manuscript is intended as a comparative study on the effectiveness of using biological treatment (i.e. the two – stage AnMBR) for the removal of UV quenching organic carbon and UV absorbance from young versus mature landfill leachates. It will also compare microbial community distribution and function in each type of landfill leachate. Differences in ARG profiles will also be explored.

SUMMARY OF MATERIALS AND METHODS

The two – stage AnMBR set – up and operation has been described in chapters 3 and 4. Schematic diagrams of the two – stage process have been provided in preceding chapters (Figure 3-1 and Figure 4-1).

The two – stage system was operated with two different landfill leachates. The first was a young leachate collected from an actively landfilling cell at a landfill in Waverly, VA. The second was collected from an old cell at a landfill in Louisville, KY, that had been sealed for almost 12 years. The two – stage system was operated using the young leachate for a total of 14 months, following which it was transitioned to the mature leachate over 1 month. The two – stage system was then operated using the mature leachate as feed for a period of 6 months. Over the final four months of this operation, no further improvement in performance was observed in terms of removal of UV quenching organic carbon, UV_{254} absorbance and COD. The two stage system was then shut down. Samples from the reactors treating both the young and old leachates were fractionated into humic acids, fulvic acids and hydrophilic substances and each fraction was analyzed for organic carbon content, according to the methods described in the preceding chapters. Some basic differences in the characteristics of young and mature landfill leachates are shown in table A-1.

Both, the raw leachates, and samples collected from the two stage system during the treatment of each leachate were analyzed using Illumina sequencing in order to characterize the microbial community in the treatment of each raw leachate. Some of the samples from the two – stage system treating mature leachate are still in the analysis pipeline, and will be analyzed as soon as the assembly of the genomes in these samples is completed. In addition to structure and function of the microbial community in young vs old leachates, it will be possible to compare abundances and distribution of antibiotic resistance genes in each.

Available and Expected Results

Preliminary analyses of the young and mature landfill leachates showed a considerable difference in the concentrations of humic substances between the two. The young landfill leachate was found to contain approximately 4,000 mg/L of organic carbon as humic acids, fulvic acids and hydrophilics, while the corresponding number for the mature leachate was almost a quarter of that value i.e. 1,100 mg/L. The total UV_{254} absorbance of the two leachates also showed considerable differences, with the young leachate showing more than 5 times the UV absorbance as the mature leachate (68 cm^{-1} vs 13 cm^{-1}). Bar charts showing the concentrations of organic carbon and UV_{254} absorbance attributable to humic acids, fulvic acids and hydrophilic substances in the two raw leachates may be seen in Figure A-1.

Both leachates were treated using the same process, with the same operational parameters. However, the data collected over 14 months of treating the young leachate and 6 months of treating the mature leachate showed that on an average, >55% of the UV quenching organic carbon in the raw leachate was removed, while only 25% was removed in case of the two stage system. Most of this removal was found to be in the category of hydrophilic compounds. Humic acids and fulvic acids in the mature landfill leachate showed vary high resistance to degradation by the two – stage system. These data were supported by the UV_{254} absorbance measurements, which showed that the absorbance removal for the young leachate was about 50% on average, while for the mature leachate, less than 10% of the UV absorbance was removed. This was explained by the low degradation of humic and fulvic acids achieved for mature leachates. Since only hydrophilics were effectively degraded, the UV_{254} absorbance did not considerably reduce. The performance of the two – stage system treating mature landfill leachate (with regard to removal of humic substances and UV_{254} absorbance) is shown in Figure A-2. For the young leachate, the data have already been presented in Chapter 3 (Figure 3-3 and 3-4).

COD removal using the two – stage process was also assessed for young and mature leachates. It was found that when averaged over the final 3 months of operation, the two stage system removed only 23% of the total COD in the mature leachate feed, while the average COD removal for the young leachate was over 65%. Figure A-3 shows the average COD values in the treated and untreated mature leachate. The average COD removals for the young leachate were shown in Chapter 3 (Figure 3-6).

Some errors were encountered in the assembly of genomic sequences, and these sequences have been resubmitted for analysis. They will be included in the final manuscript prior to submission to a journal. It is expected that the young landfill leachate will show a higher number of matches for 16s rRNA genes relative to the mature leachate. The bioreactors treating young leachates are expected to show higher relative abundances of organisms known to degrade humic substances (as listed in Chapter 4).

CONCLUSIONS

1. From the data of UV quenching organics removal and UV absorbance reduction, it is clear that as landfills age, the leachates extracted from them are less amenable to

treatment by biological means. This means that leachates from old cells may need to be discharged into wastewater treatment plants without any biological pretreatment. However, since these leachates contain much lower concentrations of UV quenching organic carbon relative to leachates from young landfills, they may quench UV light in amounts that render the disinfection process ineffective.

2. From previous work at Virginia Tech, we know that humic substances attenuate naturally. However, a 50% removal of humic substances by natural biodegradation pathways in landfills takes over 5 years. The two – stage AnMBR is able to achieve accelerated degradation of humic substances in young leachates. However, in older leachates, most of the biodegradable humic substances are already degraded, limiting the use of this process.

TABLES

Table A-1. Comparison of characteristics of young and mature leachates

Parameter	*Unit*	*Young Leachate*	*Mature Leachate*
pH	-	8.4	6.5
TSS	*mg/L*	3,500	1,200
TDS	*mg/L*	14,000	6,300
TAN	*mg/L*	1900 - 2300	900 - 1100
COD	*mg/L*	8000 - 16000	3000 - 4000
BOD_5/COD	*mg/L*	0.30 – 0.35	0.10 – 0.15
TOC	*mg/L*	3000 - 4000	900 - 1100
Cl	*mg/L*	4500	1200
PO_4 - P	*mg/L*	BDL	BDL
SO_4	*mg/L*	40	90

FIGURES

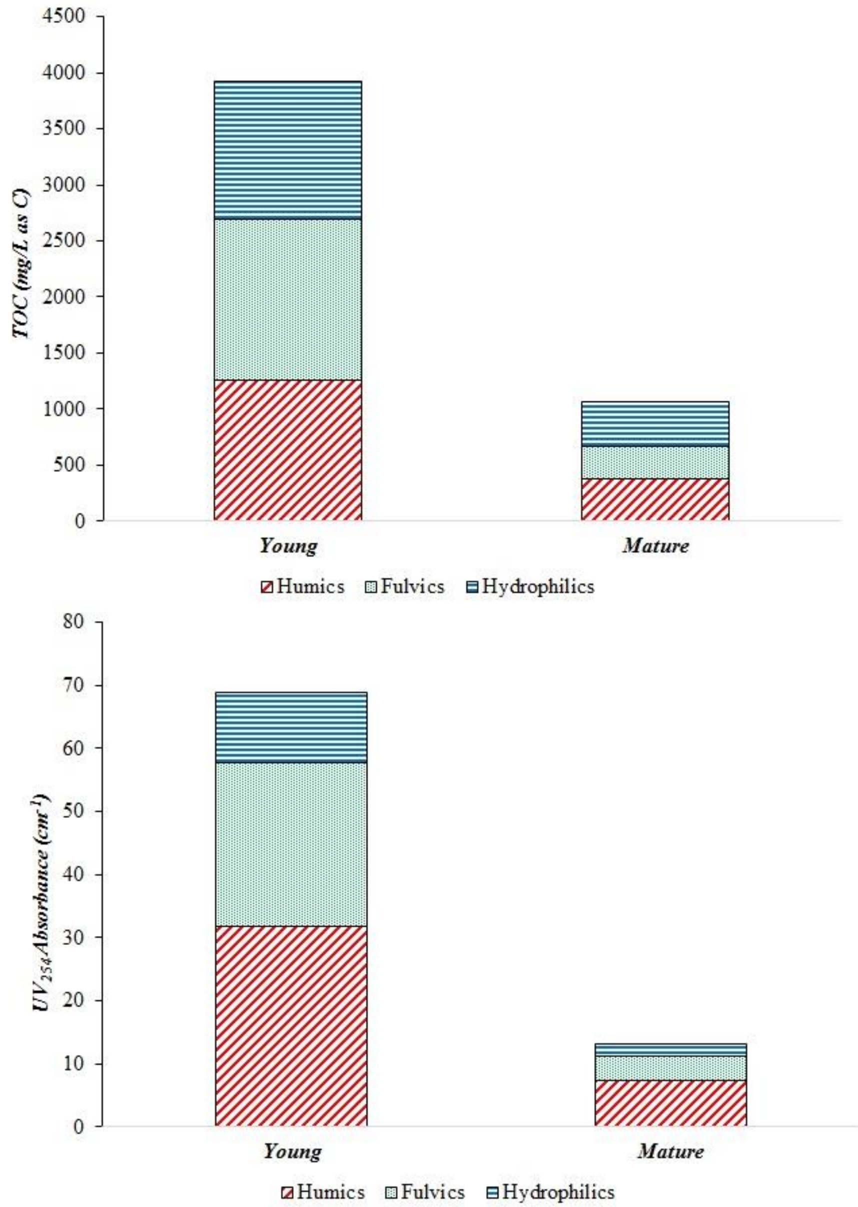

Figure A-1. Comparison of UV – Quenching Organic carbon and UV_{254} absorbance in young and mature landfill leachates

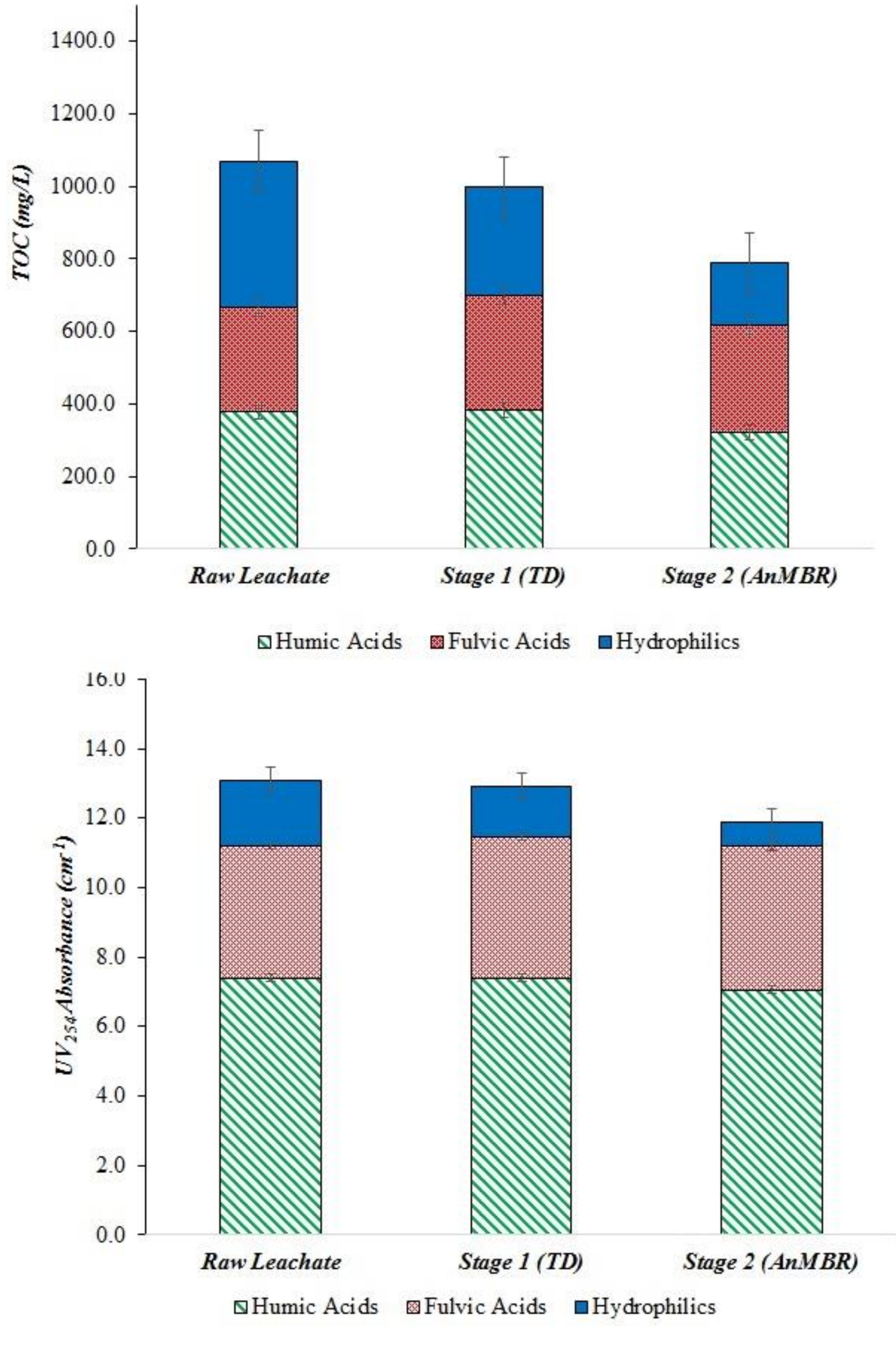

Figure A-2. Removal of UV – Quenching organic carbon and UV254 absorbance achieved by the two – stage system treating mature leachate (data averaged over final 3 months of operation to reflect steady state performance)

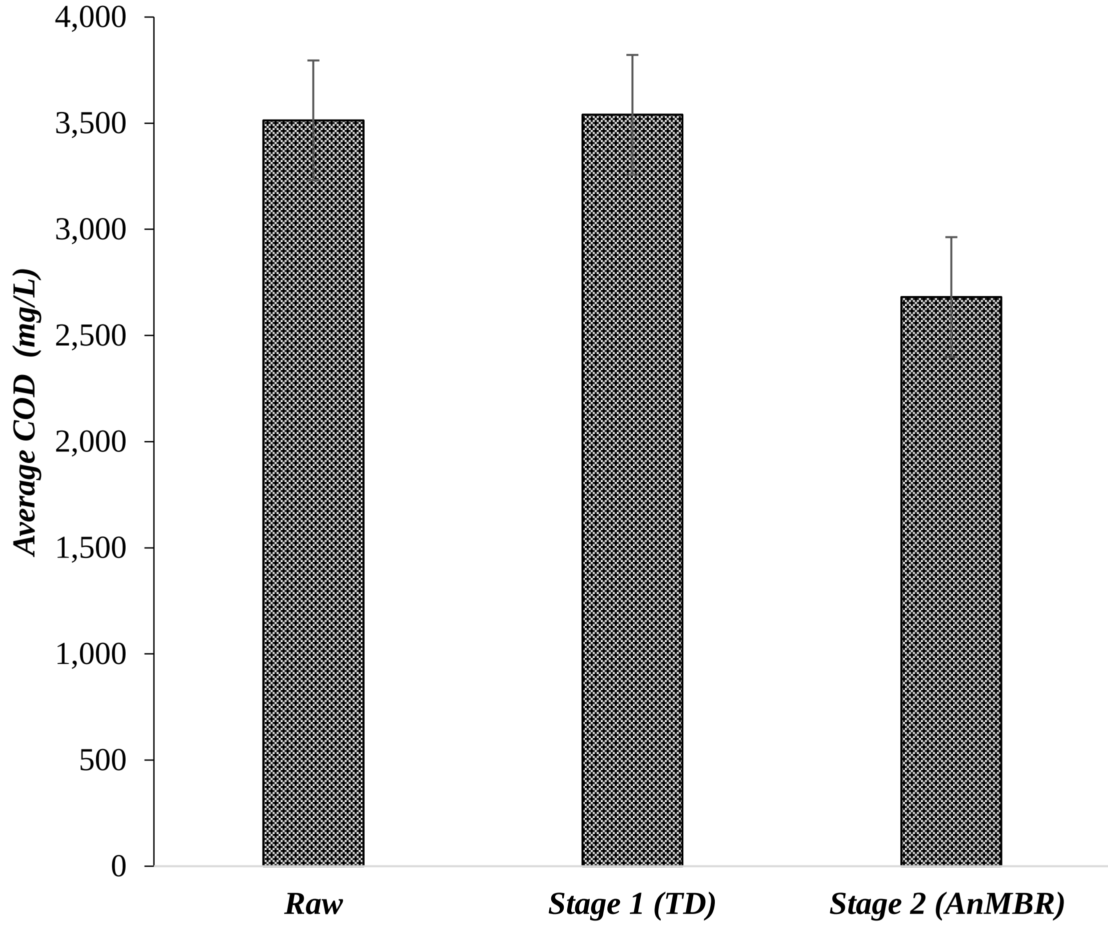

Figure A-3. COD removal in the two – stage system treating mature leachate

Appendix B: Metal Ion Content of All Raw Leachates (ppm)

Table B-2. Metal ion content of raw leachates

Species	*VA - 1 (Young)*	*VA - 2*	*VA - 3*	*VA - 4*	*KY (Mature)*
^{23}Na	3,663.5	1,643.0	1,348.5	3,950.0	1,204.5
^{2}5Mg	123.1	322.6	70.0	205.9	72.1
^{27}Al	1.5	0.5	0.2	1.1	0.2
^{28}Si	50.9	10.3	22.5	19.2	25.7
^{31}P	5.7	3.7	2.8	2.3	4.1
^{34}S	0.2	0.9	0.1	0.0	0.0
^{35}Cl	3.9	1.8	1.2	4.9	1.0
^{39}K	1,413.5	876.0	419.5	1,011.0	361.3
^{43}Ca	48.2	2,263.5	25.1	76.4	115.6
^{47}Ti	0.5	0.0	0.0	0.1	0.0
^{51}V	0.2	0.0	0.0	0.1	0.0
^{52}Cr	1.2	0.2	0.1	0.5	0.1
^{54}Fe	17.1	80.1	7.3	5.7	23.5
^{55}Mn	0.0	82.5	0.0	0.1	0.4
^{59}Co	0.1	2.8	0.0	0.1	0.0
^{60}Ni	0.3	0.6	0.3	0.2	0.2
^{65}Cu	0.4	0.2	0.3	0.2	0.2
^{66}Zn	0.5	0.4	0.4	0.4	0.2
^{75}As	0.2	0.1	0.1	0.1	0.1
^{78}Se	0.0	0.0	0.0	0.0	0.0
^{88}Sr	0.6	3.7	0.3	0.4	0.9
^{95}Mo	0.0	0.0	0.1	0.0	0.1
^{107}Ag	0.0	0.0	0.0	0.0	0.0
^{111}Cd	0.0	0.0	0.0	0.0	0.0
^{112}Sn	0.0	0.0	0.0	0.0	0.0
^{137}Ba	0.1	1.0	0.1	0.5	0.3
^{208}Pb	0.0	0.0	0.0	0.0	0.0
^{238}U	0.0	0.0	0.0	0.0	0.0

Appendix C: Time Series Plots of UV quenching organics, UV_{254} Absorbance and COD removal in Mature Leachate by the 2 – Stage System

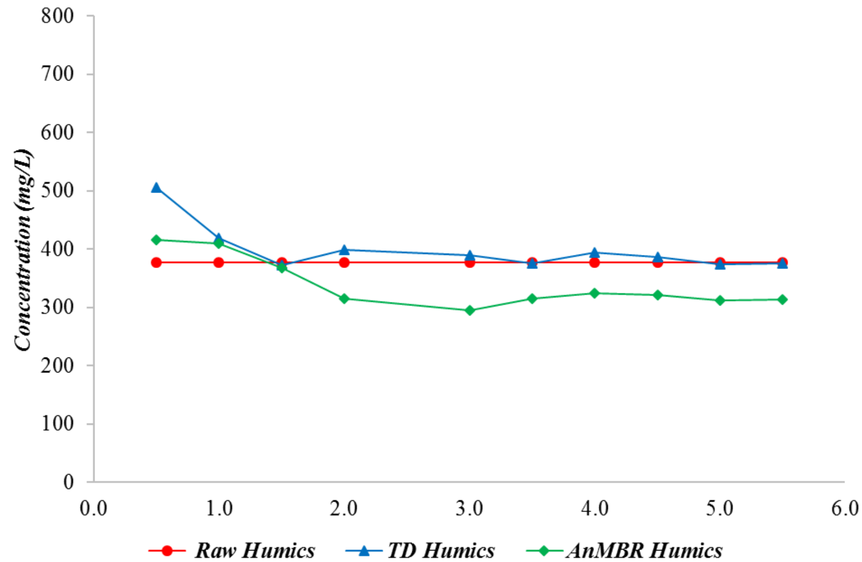

Figure C-1. Removal of humic acids over time in two stage system treating mature leachate

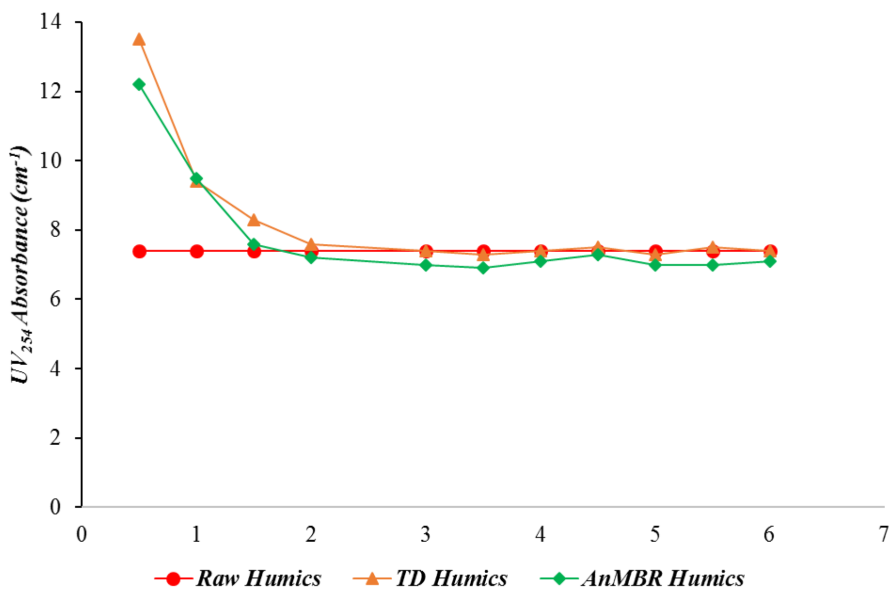

Figure C-2. Removal of UV_{254} absorbance due to humic acids over time in two stage system treating mature leachate

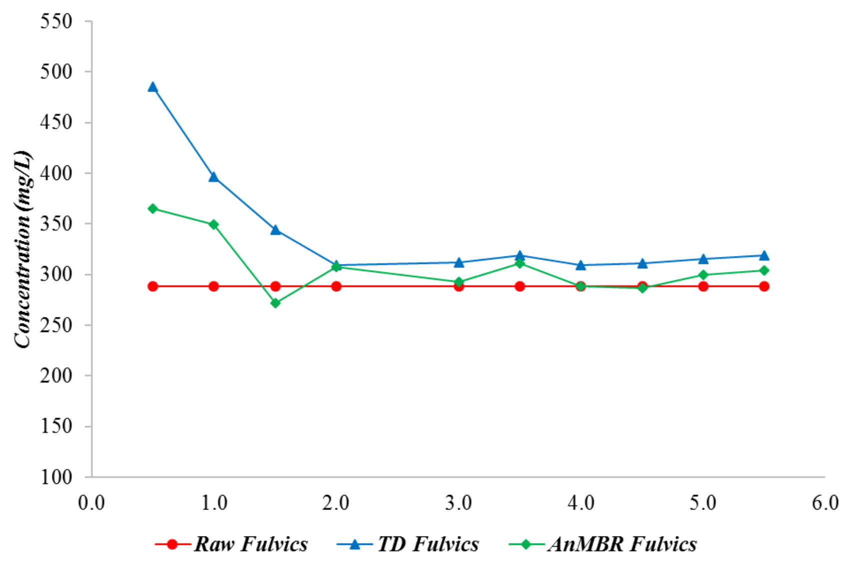

Figure C-3. Removal of fulvic acids over time in two stage system treating mature leachate

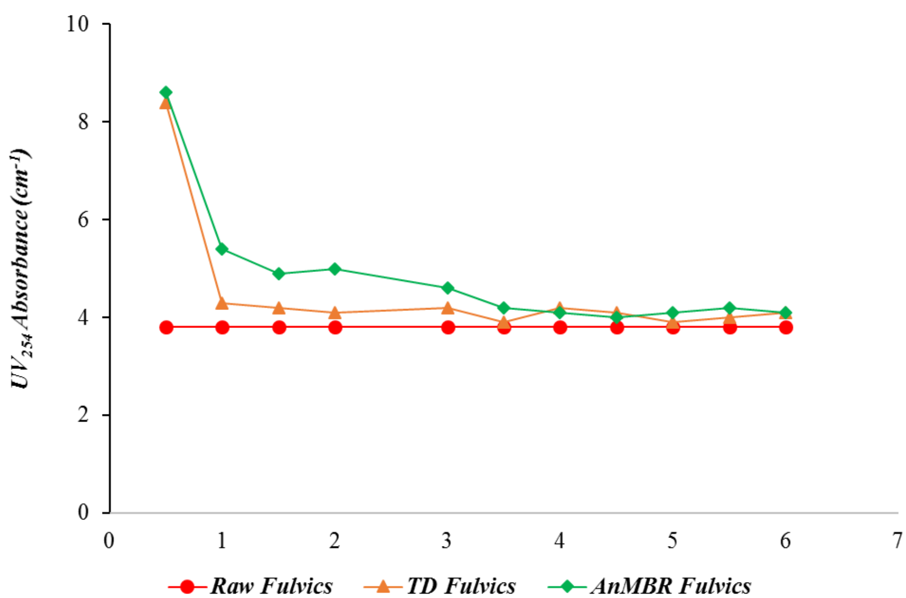

Figure C-4. Removal of UV_{254} absorbance due to fulvic acids over time in two stage system treating mature leachate

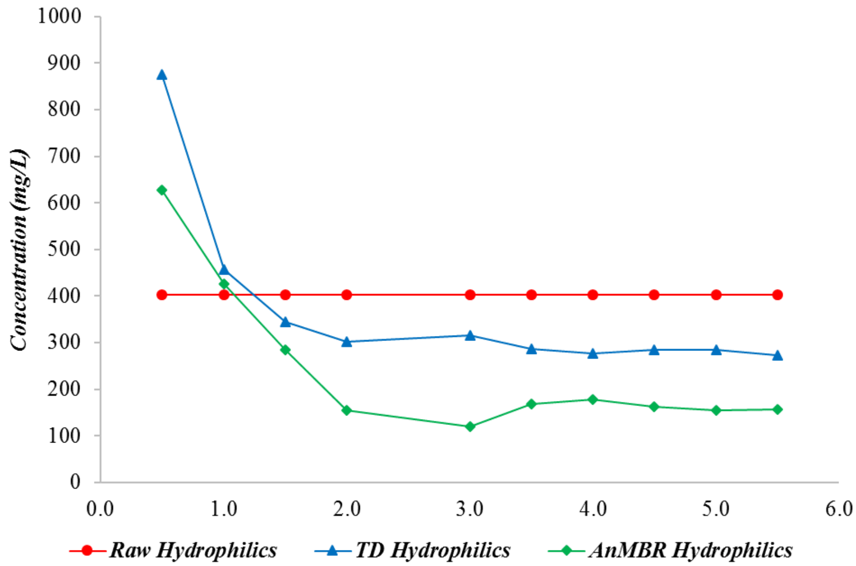

Figure C-5. Removal of hydrophilic compounds over time in two stage system treating mature leachate

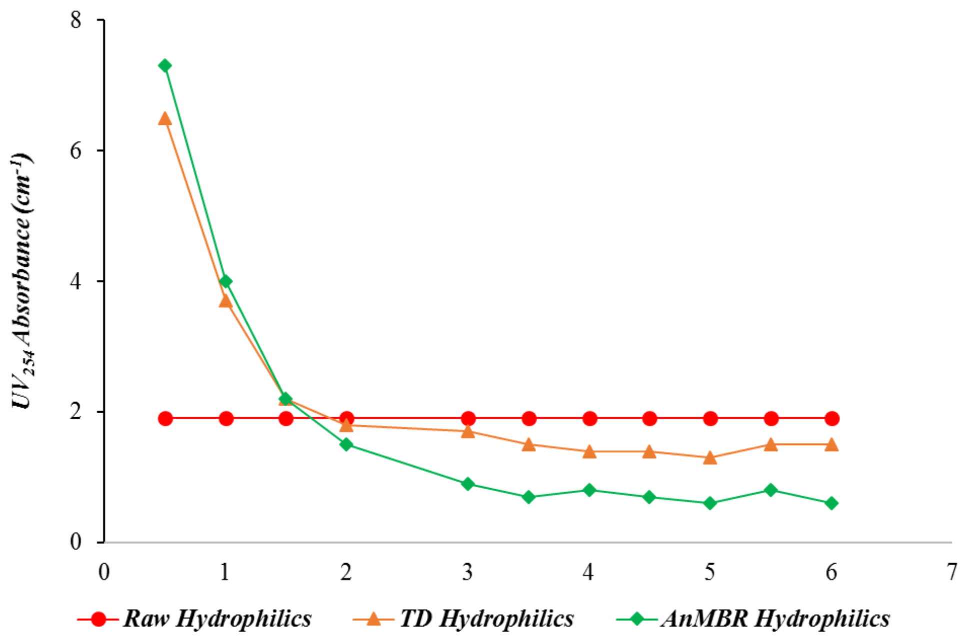

Figure C-6. Removal of UV_{254} absorbance due to hydrophilic compounds over time in two stage system treating mature leachate

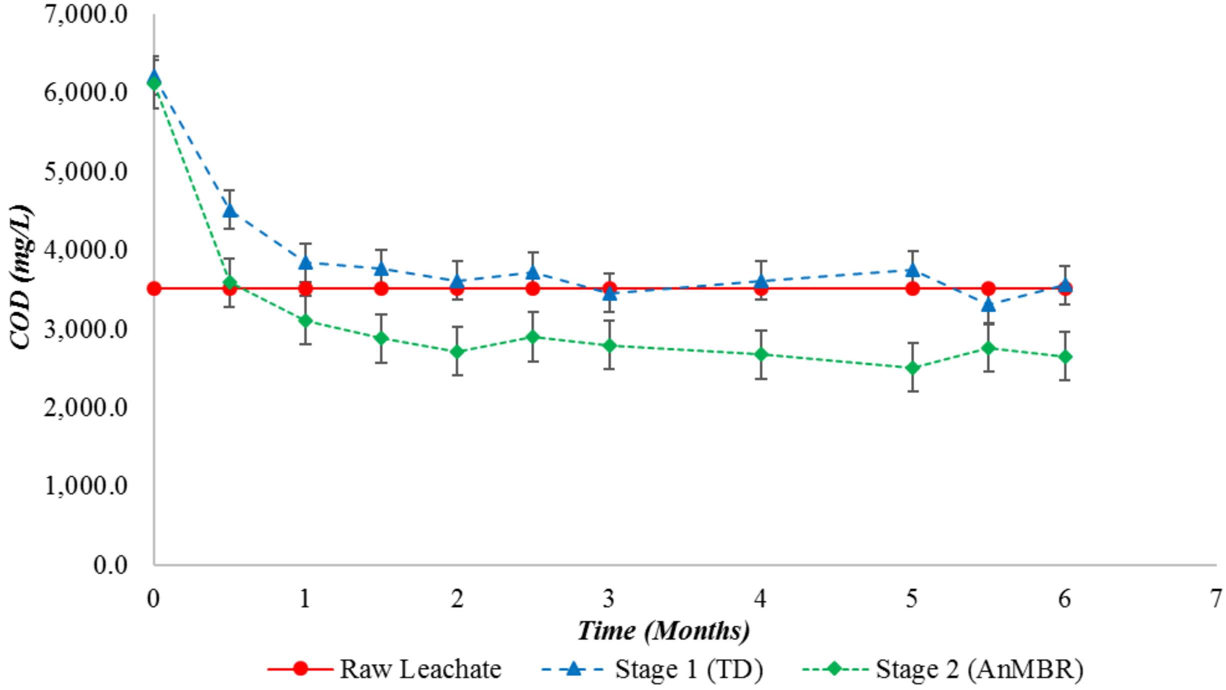

Figure C-7. Removal of COD with time in the two stage system treating mature leachate