BIODEGRADATION OF PERFLUOROCARBOXYLIC ACID IN LANDFILL LEACHATE

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Abstract

Long-chain perfluoro carboxylic acids (LC-PFCAs), a subset of perfluorochemicals (PFCs), were identified in landfill leachate during the sampling period from June 2022 to July 2023. In this thesis, the exclusive focus is on the chemical degradation of PFCAs, omitting biological degradation experiments due to their persistent nature. The leachate analysis revealed a seasonal fluctuation in its chemical characteristics including nitrogen, phosphate, alkalinity, pH, and chemical oxygen demand (COD). Interestingly, the selected C9 and C10 LC-PFCAs were consistently present in the leachate but did not show any significant seasonal variation. Concentrations of LC-PFCAs ranged from 100 ng/L to 800 ng/L. To assess the biodegradability of C9 and C10 PFCA in the leachate using native microorganisms, a bench-scale experiment was conducted. The microbial community exhibited biphasic growth when exposed to the specified PFCAs (10-100 mg/L) for 6 days. Notably, the C9 and C10 PFCAs displayed a biodegradation efficiency of up to 58% within this 6-day period.

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Abbreviation(s)

ACS: American Chemical Society

CNP ratio: Carbon nitrogen phosphate ratio

COD: Chemical oxygen demand

FTAC: Fluorotelomer acrylate

FTCA: Fluorotelomer carboxylates

FTOH: Fluorotelomer alcohols

FTUCA: Fluorotelomer unsaturated carboxylates

HLB: Hydrophillic-Lypophillic balance

HPLC: High-performance liquid chromatography

ICP-AES: Inductively coupled plasma–atomic emission spectrometry

LC-MS: Liquid chromatography–mass spectrometry

LOD: Limit of detection

LOQ: Limit of quantification

MgAC: Magnesium-aminoclay

nZVI: Nanoscale zero-valent iron

OD: Optical density

PAPs: Polyfluoroalkyl phosphates

PFAS: Per-and polyfluoroalkyl

PFBA: Perfluorobutanoic acid

PFCA: Perfluorinated carboxylic acids

PFCs: Perfluoro chemicals

PFDA: Perfluorodecanoic acid

PFHpA: Perfluoroheptanoic acid

PFHxA: Perfluorohexanoic acid

PFNA: Perfluorononanoic acid

PFOA: Perfluorooctanoic acid

PFOS: Perfluorooctanesulfonic acid

PFPeA: Perfluoropentanoic acid

PFSA: Perfluorinated sulfonic acids

RfDs: Reference dose

RO: Reverse osmosis

SNR: Signal noise ratio

SPE: Solid phase extraction

STD: Standard deviation

WWTP: Wastewater treatment process

Chapter 1: Introduction, literature review and proposed research

1.1 Introduction

From 1997 to 2000, 1000 - 100,000 kg of perfluoro carboxylic acid (PFCA) and its precursors were imported by the Canadian industry (Health Canada 2012). These compounds have been commonly found in cleaning reagents, firefighting foams, and a non-stick layer on cooking pans (Phong Vo et al. 2020). These compounds find ways into wastewater treatment systems or landfill sites. PFCA is persistent during wastewater treatment processes (WWTP) and cannot degrade completely in the WWTP. These contaminants eventually leach into the natural environment, especially the surface water system (Phong Vo et al. 2020). PFCA can accumulate in the food chain and eventually affect human health by food intake and drinking water. This raises a big concern about PFCA as an emerging contaminant in Canada.

PFCAs are a type of perfluorochemicals (PFCs) which is a man-made chemical compound that was first discovered in the late 1930 (ITRC 2020). Due to the high bonding energy of the C-F bonds (around 485 kJ/mol), perfluoro chemicals are chemically and thermally stable, which makes them forever compounds in the natural environment and even in animal bodies (Yi et al. 2016). This thesis focuses on measuring the concentration and degradation of long-chain PFCA, especially perfluorononanoic acid (PFNA) and perfluorodecanoic acid (PFDA) in landfill leachate produced in Hamilton Glanbrook Landfill site. These compounds have a solubility of 1.3 g/L (PFNA) and 0.46 g/L (PFDA) at room temperature (Health Canada 2012). They are mainly generated by the degradation of their prosecutor, such as fluorotelomer alcohols (PTOHs), fluorotelomer unsaturated carboxylates (FTUCA), and fluorotelomer carboxylic acid (FTCA) (RISK PROFILE 2022). Conventional approaches for treating PFCA involve physical removal techniques, including methods such as granular activated carbon adsorption, ion exchange resin, and membrane filtration, as per reported information. (Lenka, Kah, and Padhye 2021). However, these methods only concentrate contaminants from one environmental compartment to another without degradation. The most popular degradation method that has been widely studied is chemical degradation compared to biological degradation. Chemical degradation requires energy consumption and generates toxic degradation by-products (Trojanowicz et al. 2018). As an alternative solution, biological degradation has been investigated as a cutting-edge technology. It uses bacteria to consume PFCA as a carbon source and perform degradation during metabolic

pathways (Liou et al. 2010). However, most of the literature on biological degradation has only been performed by mono-culturing using isolated bacteria strains. In addition, no bio-degradation studies have been conducted for long-chain PFCA so far (Ruiz-Urigüen et al. 2022). To fill the research gap, this thesis offers a potential biological degradation method for long-chain PFCAs.

The background research helps readers understand the relationship between different types of PFCs. It provides more information about long-chain PFCAs, including sources, toxicity, and persistence. This section also compares the current chemical degradation methods on the long-chain PFCAs (Panchangam et al. 2009). Landfill is one of the primary sources of PFCAs and inherently is a complex matrix. Hence, in Chapter 1, as a comprehensive study, a seasonal variability of leachate has been investigated. Different chemical properties including pH, alkalinity, chemical oxygen demand, nitrogen, and phosphate have been analyzed throughout the year. Also, the solids test has been performed to test total solids, volatile solids, dissolved solids, and suspended solids. Characterizing long-chain PFCA in the leachate is difficult because of its low concentration. Solid phase extraction (SPE) has been used as a pretreatment method and cleaning up processes before liquid chromatography–mass spectrometry (LC-MS) analysis. After the SPE method has been optimized, a periodical long-chain PFCA analysis has been performed to determine its seasonal variation in the landfill leachate. In Chapter 2, a flask experiment has been performed to determine the biodegradability of long-chain PFCA in landfill leachate. Phosphate and glucose have been added to the leachate according to the chemical characterization of leachate. It provides an ideal culturing condition for the microbial communities in the leachate to perform long-chain PFCA degradation. Finally, the outlook section discusses the future improvements and research aspects based on the experimental result. It also provides the researcher's ideas and suggestions about further application of biological degradation method on the long-chain PFCA treatment.

1.2 Literature review

1.2.1 Background research of PFCs

PFCs have been widely used in commercial and industrial products since 1950 (ITRC 2020). In 1970, PFCs were first detected in workers' blood; in 1990, it was detected in blood serums worldwide (Buck et al. 2011). Due to their toxicity to human health, researchers have started to pay more attention to these compounds and treat them as emerging contaminants. The main group of compounds in PFCs are per-and polyfluoroalkyl (PFAS) (USEPA, 2017.) They contain a variable length of fluorinated alkyl chain followed by a function group, as shown in Figure 1. When an alkyl chain is longer than 8 carbons, it is considered a long-chain PFAS; when it is shorter than 8 carbons, it is regarded as short-chain PFAS. Long-chain PFAS break down during wastewater treatment into short-chain products(Health Canada 2012). In the natural water system, 88.8% of PFCs contamination is short-chain PFAS(Chambers, Hopkins, and Richards 2021). The half-life of these compounds can vary depending on the molecular structure and surrounding environment. For example, PFOS has a half-life longer than 41 years in the water system and long-chain PFAS has a half-life of approximately 3 years in human serum (Nicole 2020; Government of Canada 2010). There are several ways for people to get exposed to these contaminants. These contaminants exist widely in the surface water system and can eventually enter the drinking water system. They can be consumed through daily water intake. PFAS can also exist in daily consumer products as a cleaning reagent or waterproof coating. When touching these products, people risk exposing themselves to PFAS. PFAS can accumulate through the food chain and eventually enter the human body through food intake (ATSDR 2023; Phong Vo et al. 2020). When PFAS enters the human body, it will cause endocrine disruption, liver disease, and even cause cancer (Fenton et al. 2021). The United States Environmental Protection Agency (USEPA) established a non-cancer reference dose (RfDs) of PFAS in 2021 from 10^{-7} – 10^{-9}mg/kg/day (USEPA 2022). Canadian Government also established a drinking water standard of PFAS with a concentration of 30 ng/L (Government of Canada 2023).

Figure 1 shows that there are mainly 2 types of PFAS: perfluorinated sulfonic acids (PFSAs) and perfluorinated carboxylic acids (PFCAs). They are usually produced by other polymers, such as fluorotelomer alcohol (FTOH), polyfluoroalkyl phosphates (PAPs), 8:2 fluorotelomer acrylate (8:2 FTAC), and fluorotelomer carboxylates (FTCAs) (Dinglasan et al. 2004). PFAS is a variable length of fluorinated alkyl chain followed by a sulfonic acid functional

group. The most popular compound is perfluorooctanesulfonic acid (PFOS), which contains 8 carbons on the alkyl chain. Another type of PFAS is PFCA, formed by various alkyl chains connected to a carboxylic function group. The back alkyl chain length can be varied from 4 to 18 carbons. The most popular PFCA is C8 perfluorooctanoic acid (PFOA)(Phong Vo et al. 2020).

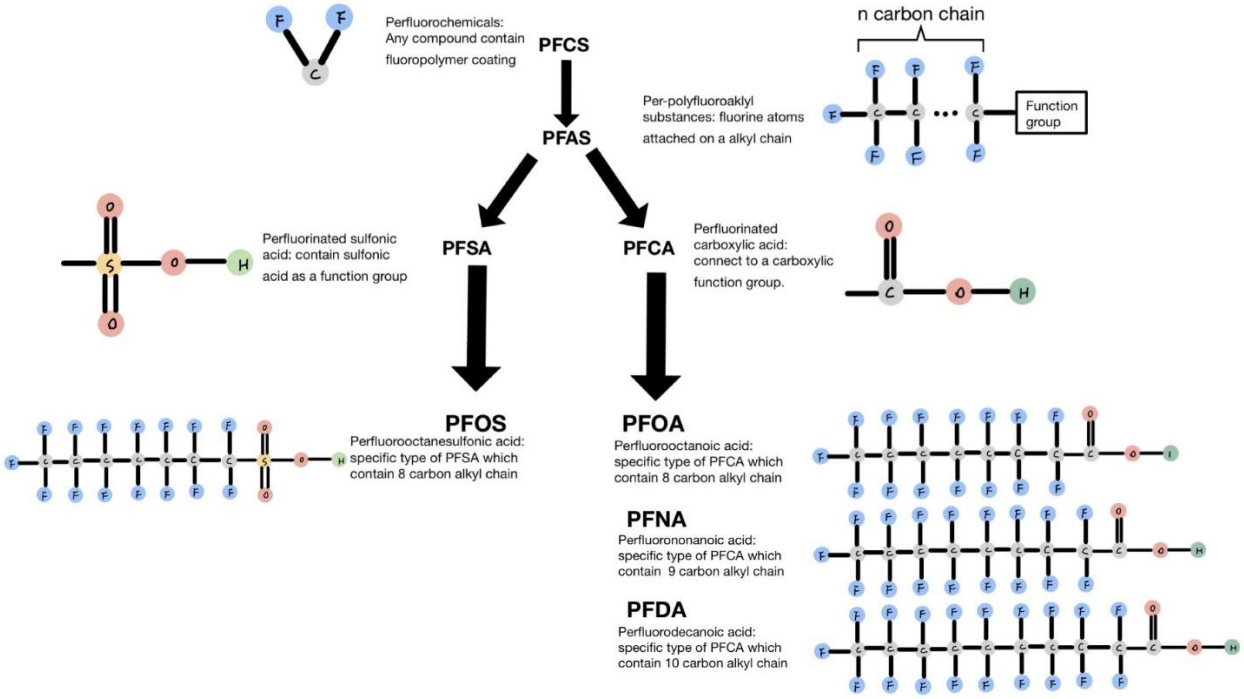


Figure 1: Classification and characteristics of each compound.

During the degradation processes, the long-chain PFCA can degrade into short-chain PFCA. Even though short-chain PFCA is more prevalent in the natural water system, long-chain PFCA is more toxic than short-chain PFCA (Staff 2021). Also, the research shows that with the increase in chain length; it bioaccumulates in the human body and has a longer half-life in blood serum (3.5 years half-life of PFOA compared to up to 12 years of PFDA) (Xu et al. 2020; RISK PROFILE 2022; Nicole 2020). Long-chain PFCA has a hydrophobic structure which makes it more attractive and reactive to protein-rich organs such as the liver and blood. They can cause damage to the organs by replacing the original lipids (RISK PROFILE 2022). According to previous research, the PFOA is the most common PFCA that researcher have targeted. However, its stable structure makes it difficult to perform degradation (Liou et al. 2010; Trojanowicz et al. 2018). According to the kinetic study, the reaction rate of C8 PFOA is lower than the C9 PFNA and C10 PFDA(Gong et al. 2014). PFCA normally goes through stepwise degradation, which

means PFNA and PFDA are the prosecutors of the PFOA (Deng et al. 2021). Hence, it is important to develop technology to remove PFNA and PFDA as precursors to prevent PFOA formation and solve the PFCA contamination problem.

Prominent source of these long-chain PFCA compounds is landfill leachate, which consistently contains concentrations ranging from 15-450 ng/L of PFNA and 56-1100 ng/L of PFDA throughout the year, as reported by (Benskin et al. 2012). When considering the yearly production rate of landfill leachate, it can contribute approximately 232 g of PFNA and 473 g of PFDA annually (Benskin et al. 2012). Another significant source of long-chain PFCA is wastewater treatment systems. Schultz et al. (2008) have documented the presence of both PFNA and PFDA in secondary effluent and final effluent wastewater, with concentrations ranging from 3.4-6.6 ng/L for PFNA and 2.3-2.7 ng/L for PFDA (Becker, Gerstmann, and Frank 2008). These compounds have also been detected in other sources, such as drinking water systems and groundwaters worldwide, although the concentration data varies significantly, with levels potentially as low as 6 ng/L or as high as 830 ng/L (Xu et al. 2020; Kurwadkar et al. 2022; Health Canada. 2012).

Due to the strong C-F bonding energy, long-chain PFCA compounds exhibit thermal and chemical stability, similar to their shorter-chain counterparts. While data regarding their persistence in natural water systems is limited, research indicates long half-lives of 2.5-4.3 years for PFNA and significantly longer half-lives of approximately 4.5-12 years for PFDA in humans (RISK PROFILE 2022). These compounds are not only detected in human serum. Still, they are also found in aquatic animals through food chain accumulation, with concentrations in marine animals varying from below the detection limit to 480 ng/g body weight (Health Canada 2012).

Limited resources are available to demonstrate the toxicity of long-chain PFCA in humans. However, researchers have indicated potential adverse effects, such as liver toxicity and disruption of the endocrine system (Land et al. 2015). Furthermore, Environment Canada has shown acute toxicity of long-chain PFCA compounds towards aquatic organisms, ranging from 6.6 to 265 mg/L (Health Canada 2012). Given the potential health and environmental risks associated with long-chain PFCA, developing methods for their degradation is imperative.

1.2.2 Degradation methods of long-chain PFCA

While research on long-chain PFCA is limited, it suggests that PFNA and PFDA undergo stepwise degradation similar to PFOA. In PFCA degradation, three primary methods are available: electrochemical degradation, photocatalysis degradation, and zero-valence ion-catalyzed degradation.

Among these methods, photocatalysis is the most widely adopted. It involves using 254 nm UVC light as an energy source for degradation. Researchers have employed TiO_2 or Fe_0 nanoparticles as catalysts to enhance the reaction rate (McQueen et al. 2022). Table 1 demonstrates that the reactions occur at room temperature, typically around 20-25°C. The intensity of UV dosing varies from 0.45 mW/cm^2 to 5.4 mW/cm^2, depending on the UV light power (Xia et al. 2023; Panchangam et al. 2009). The removal rate of PFCA compounds ranges from 90% to nearly 100%, although the defluorination rate can differ due to initial concentration and UV dosing intensity. Panchangam et al. (2009) for example, achieved a lower defluorination rate, ranging from 38% to 54%, when using a high initial concentration of long-chain PFCA (55 mg/L) and low UV intensity (0.45 mW/cm^2). In comparison, Xia et al. (2023), achieved a higher defluorination rate, nearly 98%, by using 5.4 mW/cm^2 intensity to treat a 1 mg/L PFCA solution. The primary degradation products and intermediate compounds observed in these processes include CO_2, free fluoride ions, and various short-chain PFCAs such as perfluorooctanoic acid (PFOA), perfluoroheptanoic acid (PFHpA), perfluorohexanoic acid (PFHxA), perfluoropentanoic acid (PFPeA), and perfluorobutanoic acid (PFBA) (Xia et al. 2023). Photocatalysis degradation can occur in both aerobic and anaerobic conditions. In the absence of oxygen, only PFHpA is detected as a degradation product of PFNA. This is because greater defluorination occurs in anaerobic conditions, resulting in fewer short-chain PFCA intermediate compounds (Xia et al. 2023). Additionally, PFHpA is less reactive than other short-chain PFCA compounds, leading to its accumulation as a final degradation product (Gong et al. 2014).

Table 1: Comparison between different degradation methods of long-chain PFCA

Mechanism	**Degradation device**	**Initial concentration**	**Degradation environment**	**Degradation efficiency**	**Detection**	**Reference**
Electrochemical degradation	Ti/SnO_2−Sb−Ce (SnO_2), Ti/SnO_2−Sb/Ce−PbO_2(PbO_2), and/BDD (BDD) anodes	128 mg/L	25 °C 1.5cm distance 10 mA/cm^2 3 pH 1224 mg/L $NaClO_4$	98% -97% PFNA 96-92% PFDA 180 min	HPLC 20 mM NaH_2PO_4 1ml/min UV 210 nm	(Lin et al. 2013)
Electrochemical degradation	Porous Ti/SnO_2-Sb membrane anode	50 mg/L	25°C 3 cm distance 20mA/cm^2 2.26 pH 800 rpm 6120 mg/L $NaClO_4$ 1500 mg/L peroxymonosulfate	90-95% removal 85-95% defluorination 90min	HPLC WpH C18 column 5 μm acetonitrile and 20 mM sodium dihydrogen phosphate (pH = 2) 1mL/min	(Wang et al. 2021)
Photocatalysis	660mg/L TiO_2 16w, 254nm LP UV	55mg/L	25°C 0.45mW/cm^2 1-1.7pH Aerobic	99% removal Defluorination: 38% PFDA 54% PFNA	LC-MS ZORBAX XDB C18 20Ul	(Panchangam et al. 2009)

Mechanism	Degradation device	Initial concentration	Degradation environment	Degradation efficiency	Detection	Reference
				8h	Water + ammonium acetate 1ml/min 8min	
Photocatalysis	100mg/L Fe^0 nano particle 112W, 254nm UV	1 mg/L	Room temperature 5.4mW/cm^2 Anaerobic	100% removal 98% defluorination 72h	LC-MS ZORBAX C18 3.5um, 80A, 5uL 2mM NH4Ac in water & methanol 0.2ml/min	(Xia et al. 2023)
Photocatalysis	660mg/L TiO_2 600W, 254nm UV	0.000029mg/L PFDA 0.000066mg/L PFNA	1mW/cm^2 7.1-7.6 pH Aerobic	90% PFDA 87% PFNA	LC-MS C18 column (2.1 x 150 mm) 5um 0.3ml/min	(McQueen et al. 2022)
Nanoscale zero-valent iron (nZVI)	1000mg/L Mg-aminoclay (MgAC) coated nZVI	0.2mg/L	3pH 20°C 130rpm Anaerobic	80% PFNA 96% PFDA 1h	LC-MS C18 column (3.5um)	(Arvaniti et al. 2015)

Researchers employed Ti/SnO_2-Sb anodes with $NaClO_4$ as the electrolyte in electrochemical degradation. The experiment detailed in Table 1 was conducted at room temperature, using a current density ranging from 10-20 mA/cm^2 in an acidic environment (Lin et al. 2013). The initial concentration of PFCA varied between 50-128 mg/L. The degradation experiment resulted in the removal of 90-98% of PFCA and up to 95% defluorination within a reaction time of 90-180 minutes (Wang et al. 2021). Long-chain PFCA degradation during electrochemical treatment followed a first-order reaction rate, producing shorter-chain PFCA products such as PFBA, PFOA, PFHxA, and PFHpA. Wang et al. (2021) noted a conspicuous peak in the degradation intermediate product C7 PFCA (PFHpA), although its concentration decreased after the peak. (Wang et al. 2021). Lin et al. (2013) observed that the degradation rate of C10 PFDA was lower than that of C9 PFNA, possibly due to longer-chain PFCA being less reactive than shorter-chain PFCA. However, the precise mechanism behind this phenomenon remains unclear. During the mineralization reaction, chain shortening processes occurred, resulting in the removal of CF_2 as CO_2 and HF. Nevertheless, electrochemical degradation has a few drawbacks. It demands high energy input, and the efficiency of degradation can be affected by the electrode area as a reaction site (Wang et al. 2021). Furthermore, PbO_2 and SnO_2 electrodes may release heavy metals into the water system, posing unexpected hazards when applied to large-scale reactors (Lin et al. 2013).

Apart from electrochemical and photocatalytic degradation, another method is available for removing long-chain PFCA. It involves using Mg-aminoclay (MgAC) coated nanoscale zero-valent iron (nZVI). Arvaniti et al. (2015) utilized MgAC-coated nZVI to treat long-chain PFCA under anaerobic conditions and achieved up to 96% removal of PFCA within 1 hour (Arvaniti et al. 2015). The experiment revealed that PFNA is less reactive than PFDA but more reactive than PFOA (Arvaniti et al. 2015). The degradation product in that experiment remained unclear, with only F- detected in the final degradation solution, indicating defluorination occurred (Arvaniti et al. 2015). Notably, unlike other electrochemical or photocatalytic degradation methods, nZVI does not require additional energy input. Additionally, MgAC is less toxic than anodes that may release heavy metals in large-scale applications.

Overall, long-chain PFCA degradation can occur under both aerobic and anaerobic conditions. In anaerobic conditions, low pH generates more H^+ ions, which facilitate redox

reactions of PFNA and PFDA, leading to a higher defluorination rate (Arvaniti et al. 2015). When comparing the degradation efficiency among different PFCA, all exhibited a first-order reaction rate, with both PFNA and PFDA consistently being more reactive than PFOA (Panchangam et al. 2009). However, some studies showed PFNA to be less reactive than PFDA, while others reported the opposite results (Lin et al. 2013; Arvaniti et al. 2015). This discrepancy may be attributed to chain length, although a detailed explanation needs to be provided. The use of fluoride balance to calculate defluorination during the reaction may be influenced by the evaporation of F^- (Xia et al. 2023). Due to the high C-F bonding energy, the PFCA removal rate consistently exceeded the defluorination rate. As the reaction time and energy input increased, defluorination rates as high as 98% were achieved (Xia et al. 2023). Research on the degradation mechanism of long-chain PFCA is limited. However, according to the degradation products, PFNA and PFDA undergo stepwise degradation similar to PFOA. As shown in Table 1, when PFCA dissolves in water, it releases H and forms $C_nF_{2n+1}COO$ radicals. Subsequent decarboxylation reactions produce CO_2 and C_nF_{2n+1} radicals. During defluorination, free OH radicals in the water attach to F on the alkyl chain and replace it with H (Panchangam et al. 2009; Xia et al. 2023; Lin et al. 2013). These processes result in alkyl chain shortening and the production of CO_2 and F^- as degradation products.

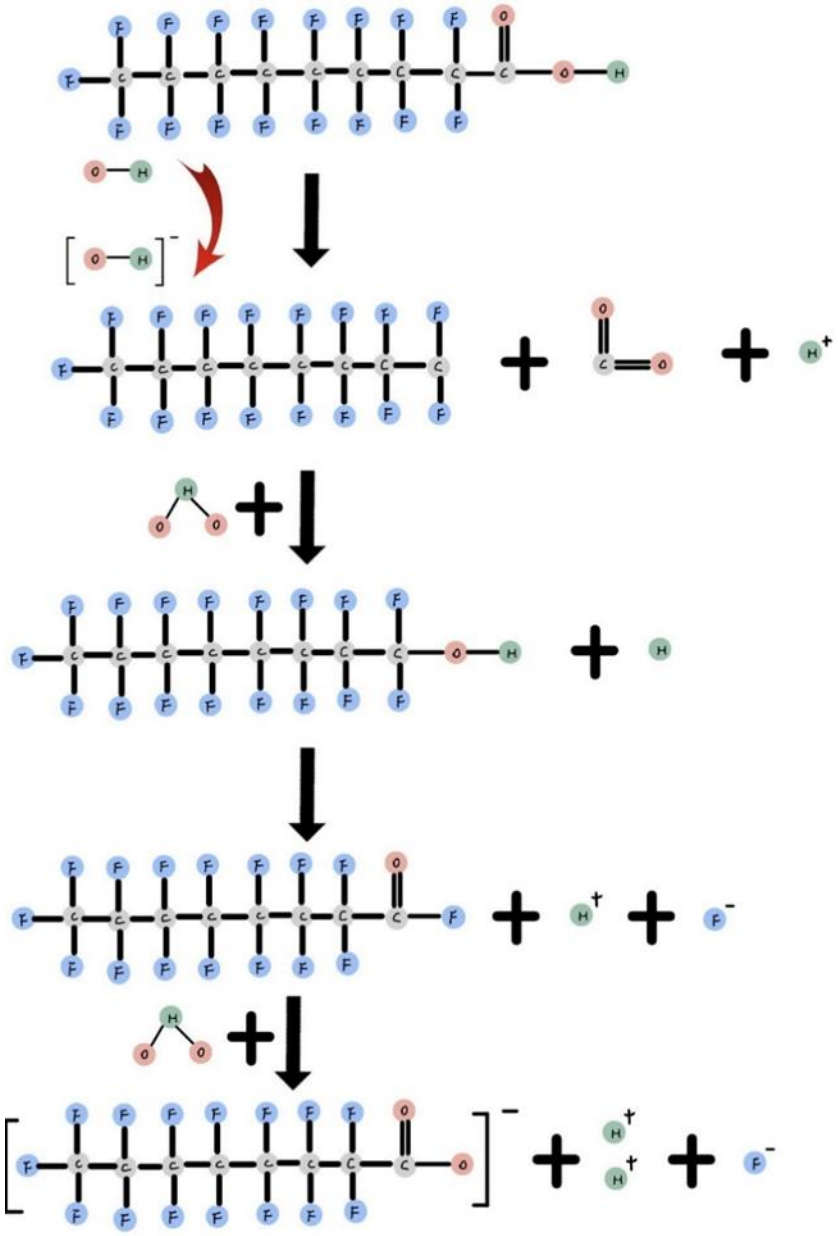


Figure 2: Degradation mechanism of long-chain PFCA

1.3 Proposed research

1.3.1 Research Gaps

As drawn from the literature published so far, the following research gaps (RG) are drawn:

RG1. Seasonal variation of contaminants in the landfill leachate has not been studied.

RG2. Research on long-chain PFCA is limited, especially the detection method in the landfill leachate and its seasonal variation.

RG3. No previous research performed mixed culturing of microorganisms for biodegradation on the long-chain PFCA in a leachate matrix.

1.3.2 Hypotheses

Following Hypotheses (H) can be drawn for the thesis:

H1. The contaminants in the landfill leachate have seasonal variations throughout the year due to leachate dilution and human activity.

H2. PFNA and PFDA exist in the landfill leachate at low concentrations around 0.5 ng/L and the concentration of PFNA and PFDA can be affected by seasonal variation.

H3. The microbial community from the landfill leachate can consume the dissolved long-chain PFCA as carbon sources and degrade the PFDA and PFNA under aerobic conditions.

1.3.3 Objectives

The thesis comprised the following objectives:

a) Determine the seasonal variation of different contaminant parameters in the landfill leachate.
b) Optimize the SPE processes of PFNA and PFDA from landfill leachate, then use LC-MS to determine the concentration of these contaminants in the leachate.
c) Perform biodegradation of PFNA and PFDA in the flask experiment using landfill leachate as a culture media.

1.3.4 Novelty

The novelty of the thesis is “Understanding the seasonal variation of C9/C10 PFCA compounds in a typical Canadian landfill leachate and investigating its bioremediation using a novel mixed-culture indigenous consortium”.

Chapter 2: Method development and assessment of the seasonal fluctuations of PFCAs in landfill leachate samples

2.1 Abstract

A periodic characterization of landfill leachate has been conducted to test nitrogen, phosphate, COD, alkalinity, pH, and different levels of solids. The results highlight most of the contaminant parameters that exhibit seasonal variations throughout the year and can be influenced by human activity. Ammonia constitutes 95% of the total nitrogen in the leachate, and 99% of the total solids in the leachate are dissolved solids. The pH is stabilized around 8 due to the age of the landfill. During the summer, high evaporation reduces leachate production and increases the concentration of contaminants. In winter, a decrease in temperature produces diluted leachate with a low concentration. When workers pump rainwater or pond water into wet wells, it dilutes the leachate and causes fluctuations in contaminant concentration. Long-chain PFAC has been extracted from the leachate with a recovery rate of 103% for PFNA and 80% for PFDA. The concentration of PFNA and PFDA in the leachate is around 0.1 µm/L. Both long-chain PFCAs do not exhibit high seasonal variation and have a high standard deviation of up to 26%

2.2 Introduction

Landfills stand out as primary sources of perfluorocarboxylic acids (PFCAs) in the surrounding environment. This is because landfills accept disposed household goods and commercial products containing PFCs, with these compounds present in building materials, electronics, textiles, carpets, and packaging. PFCAs may undergo degradation in landfills, transforming into both long-chain and short-chain forms, eventually entering landfill leachate or the local ambient system (Li et al. 2012). The concentration of PFCAs in Canadian landfill leachate can vary from 10 to 10,000 ng/L, with long-chain PFCAs contributing around 27% due to their lower solubility compared to short-chain PFCAs (Benskin et al. 2012). Two significant factors influence PFCA concentration in landfill leachate. Firstly, the age of landfill sites plays a crucial role, as older sites exhibit a decrease in overall long-chain PFCA due to slow biodegradation transforming them into short-chain PFCAs. Secondly, climate change has an impact, with seasonal variations in rainfall altering the landfill's moisture levels, subsequently affecting microbial metabolism and PFCA degradation (Hamid, Li, and Grace 2018). In anaerobic landfill reactor models, the abiotic control group produces only 0.1 nmol/L of long-chain PFCA from a carpet landfill reactor over 552 days. However, in a live reactor,

the production rate of long-chain PFCA increases to 0.96 nmol/L, nearly ten times that of the abiotic condition (Lang et al. 2016). Additionally, the overall rainfall or evaporation.

of the landfill can either dilute or intensify PFCA levels. Consequently, studying the seasonal variation of PFCAs and determining their contribution from landfill leachate sites becomes essential for a comprehensive understanding of this environmental concern.

The concentration of long-chain PFCA in landfill leachate typically remains at trace levels, often less than 1 μg/L, as reported by Gallen et al. 2017; Lang et al. 2017. Analyzing such low concentrations poses challenges due to the low signal-to-noise ratio (SNR) in LC-MS analysis, as highlighted by (Yan et al. 2015). To address this, researchers employ filtration or centrifugation to remove potential solids in the samples. (Yan et al. 2015). Given the varying pH of landfill leachate, it is adjusted to 7-8 using hydrochloric acid or sodium hydroxide, as noted by (Lang et al. 2016; 2017). Solid-phase extraction, using Hydrophilic-Lipophilic balance (HLB) or Oasis Wax cartridges, is a common method for extracting long-chain PFCA, as observed in studies (Yan et al. 2015; Benskin et al. 2012; Gallen et al. 2017). Additionally, the Micro-Liquid-Liquid extraction method, utilizing trifluoroethanol and ethyl acetate, has been employed (Lang et al. 2016). Due to the low concentrations of PFNA and PFDA, recovery rates in testing results can vary between 66% and 110% (Yan et al. 2015). Most studies report a recovery rate of around 120%, with a standard deviation of up to 28% (Lang et al. 2016; Benskin et al. 2012). Notably, Simmons (2019) presents a PFCA reading of 0.79 μg/L with a 0.76 μg/L standard deviation, approaching a 100% standard deviation (Simmons 2019). In research, the quantification limit is commonly defined as an SNR of 3, but some experiments use an SNR of 19 as the quantification limit and SNR of 3 as the detection limit (Lang et al. 2017; Yan et al. 2015; Benskin et al. 2012). The limit of detection (LOD) in previous studies ranges from 0.01 to 0.5 μg/L, and the limit of quantification (LOQ) is typically around 2-5 μg/L (Gallen et al. 2017; Simmons 2019).

In this research, periodical landfill leachate samples from Hamilton were collected, and leachate characterization included tests for solids, nitrogen, phosphate, pH, alkalinity, and COD. PFNA and PFDA extraction using solid-phase extraction (SPE) was followed by analysis using LC-MS. The SPE processes were optimized to enhance testing accuracy and improve recovery rates. The study also investigated seasonal variations in different contaminant parameters, including long-chain PFCA.

2.3 Material and methods

2.3.1 Landfill leachate sampling and storage

The landfill leachate samples are being collected from the Glanbrook–Hamilton Landfill site at the address 1500 Haldibrook Road, Caledonia, Ontario N3W1T2 (Figure 4 (a)). The landfill was originally built in 1980 and has continuously collected all municipal garbage from the Hamilton area (Detail picuture of landfill site available in Appendix 1). Even though the landfill has been built for over 40 years, it is still under usage and also expanding. From Figure 3, it could be observed that the landfill is under construction on the west-south side. On the bottom of the landfill site, there is 20 feet of natural clay which prevents the potential leaking of leachate into the underground water system. Above the natural clay, the contracture applied a geotextile which is an impermeable fiber. The below satellite picture shows the color difference between natural clay and geotextile in the constructing landfill site and Supporting Figure 3 shows a closer look for the geotextile. Stone and pipe systems are set above the geotextile which collect leachate from the landfill and eventually drain into the wet well. There are a few ponds near the landfill site, which collect rainwater and other surface water in the landfill. It performs as a natural surface water storage and can used to monitor the potential leakage of landfill leachate.

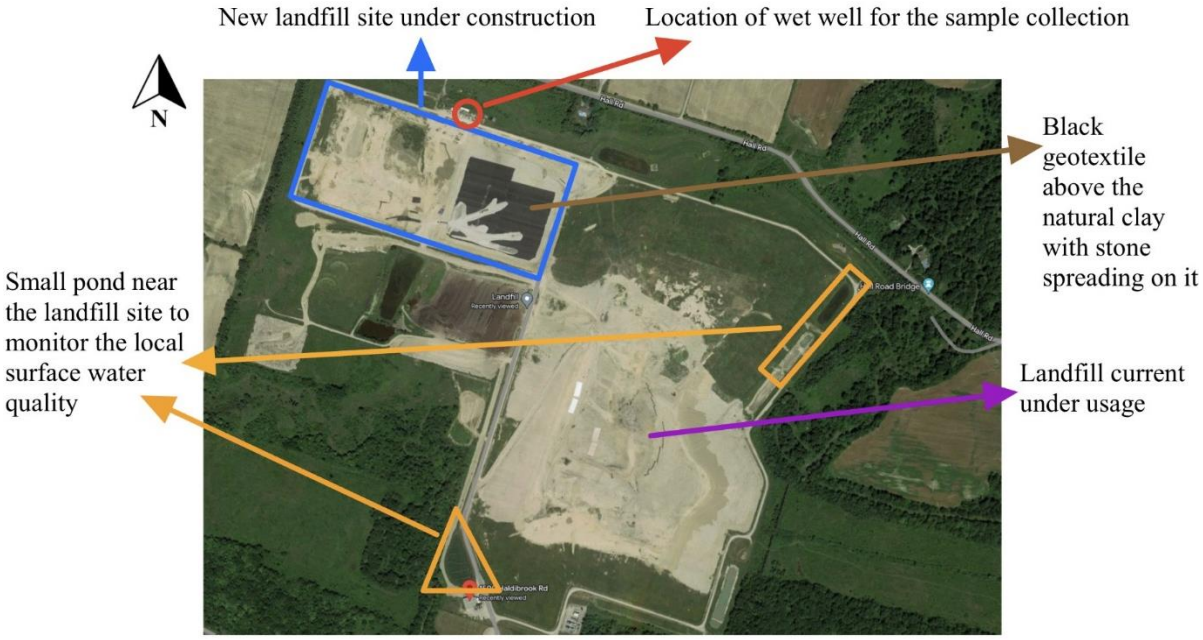


Figure 3: Satellite picture for the landfill site

There are primarily three wet wells responsible for gathering all the leachate from the entire landfill via the plumbing system. The leachate collected in these three wet wells is then routed to

Wet Well 3 and subsequently pumped out of the landfill for periodic treatment, determined by the water level in Wet Well 3 (as illustrated in Figure 4 (b)). Since the landfill is not subjected to any pretreatment before being released into the wastewater treatment plant, the leachate gathered in this wet well will reflect the general properties of the unprocessed leachate from the entire landfill.

(a) (b)

Figure 4*: (a)The landfill position shown on google map. (b) The wet well for the sample collection*

Since the wet well is approximately 10 meters deep and may contain toxic gases, it is unsafe to descend into the well to collect the leachate. The method employed in this research project involves using a cut plastic bottle fastened with a long nylon string, suspended to the water level for leachate collection. The samples have been collected in cleaned plastic bottles, filled to the top, and sealed with caps. Collection occurred every two weeks from July 12th, 2022, to July 17th, 2023. Upon delivery to the university, the samples were stored in a cold room at 4 °C. Prior to each experiment, the samples were taken out and allowed to warm to room temperature before any analysis was performed.

Figure 5: Leachate stored in the environment lab

2.3.2 Chemical reagent and test kits list

In this experiment, the following chemical reagents are used: Millipore Sigma 97% CAS grade Perfluorononanoic acid (PFNA), Millipore Sigma 98% CAS grade Perfluorodecanoic acid (PFDA), Fisher Chemical HPLC Grade methanol, Alfa Aesar 97% pure ACS grade ammonium acetate, Alfa Aesar 99.7% pure ACS grade acetic acid, Honeywell 5 M ammonia hydroxide solution, Fisher Chemical 88% Laboratory Grade formic acid, and Fisher Chemical ACS Grade sulfuric acid. Milli-Q water is produced using the Milli-Q® Advantage A10 Water Purification System with Oasis MAX 6cc cartridge 150 mg, 30 µm used for the SPE of PFCA. The leachate characterization employs a test kit obtained from the Hach Canada website, as shown in Table 2.

Table 2: Testing kits used in the leachate characterization.

Name of test kit	**Target compound**	**Testing range**
TNT Plus 836 HR	Nitrate	5-35 mg/L NO^-_3-N
NitriVer 3 TNT reagent set LR	Nitrite	0.003-0.5 mg/L NO_2-N
TNT plus 831 LR	ammonia	1-12 mg/L NH_3-N
TNT plus 828 UHR	Total nitrogen	20-100 mg/L N
TNT plus 843 LR	Total phosphorus	005-1.5 mg/L PO_4-P
COD digestion vials LR	Chemical oxygen demand (COD)	3-150 mg/L

2.3.3 Solids analysis

The solids test has been performed according to the Standard Methods for the Examination of Water and Wastewater. Both the aluminum dish and Whatman 934-AH glass microfiber filters are dried in the furnace at 550 °C as pretreatment. Subsequently, 300 ml of leachate is filtered through a glass fiber filter paper using a vacuum filter. The raw leachate and filter paper are then dried in an oven at 110 °C overnight to determine the total suspended and dissolved solids. Finally, both the dried aluminum dishes and filter paper are placed in the furnace at 550 °C for 18 minutes. The weight loss during these processes is used to calculate the total volatile solids, volatile suspended solids, and dissolved volatile solids according to the equation in Appendix 2. Figure 6 provides a detailed description of the experimental procedure and measurement for the solids test. The equation for the solids calculation is found in the appendix.

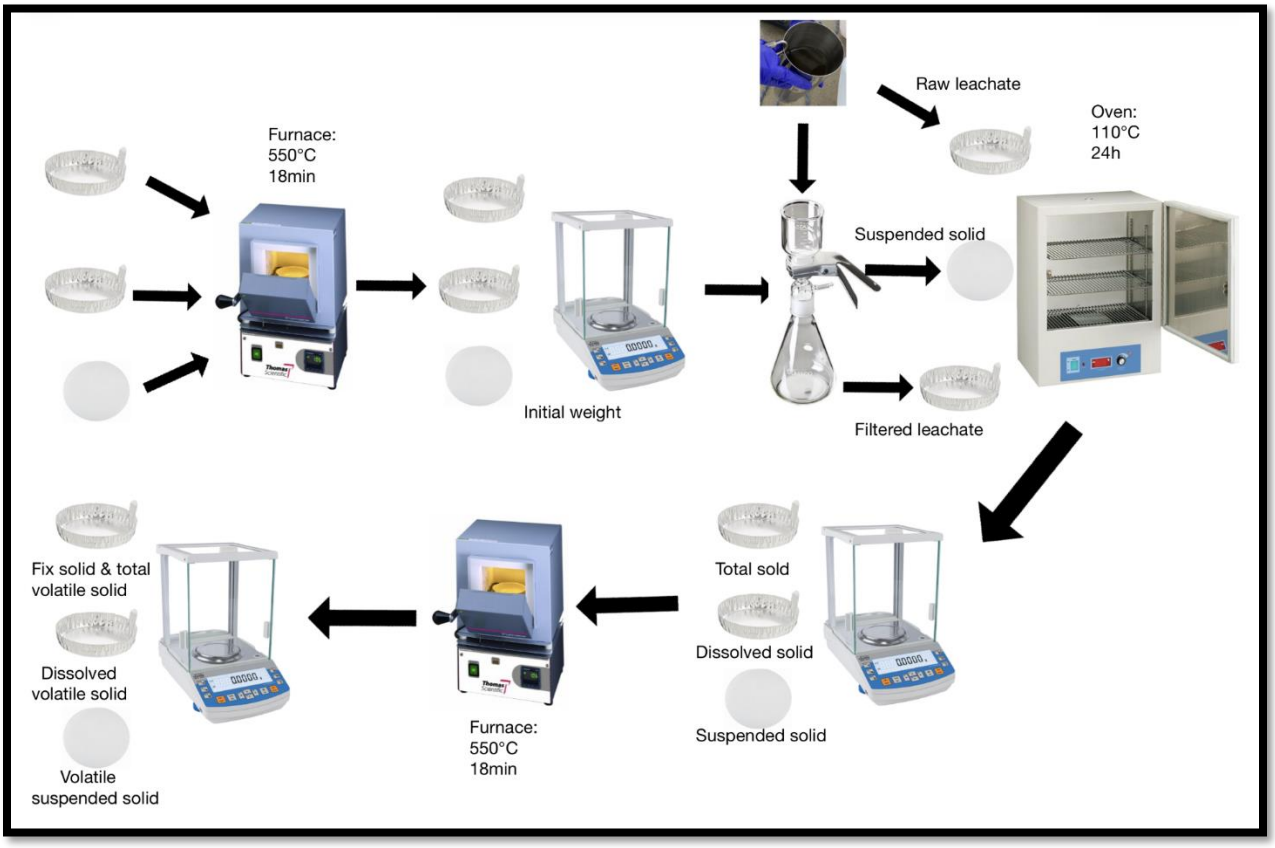


***Figure 6**: Analysis procedure for the landfill leachate solids characterization*

2.3.4 Alkalinity test

The leachate was warmed to room temperature and a pH meter to test the pH value. Then, using 0.1 N H_2SO_4 solution and 50 ml burette to titrate the leachate to pH 4.5 (EPA 2012). At this pH, all the alkalinities have been consumed and the following equation 1 can be used to calculate the total alkalinity in the leachate sample:

$$Alkalinity\ (mg/L) = \frac{volume\ of\ sulfuric\ acid\ (ml) * 0.1\ N * 50000}{volume\ of\ sample\ (ml)} \quad (1)$$

Equation 1: Alkalinity calculation for the samples

2.3.5 Heavy metal analysis

Inductively coupled plasma–atomic emission spectrometry (ICP-AES) was conducted to test for heavy metals in the landfill leachate. The raw leachate sample was sent to the Eau Terre Environnement Research Centre for further analysis, following method #200.7 of the United States Environmental Protection Agency (U. EPA 1994). The samples were filtered through a 0.45 µm pore diameter filter paper and then subjected to acidification and digestion using 0.2% HNO_3 before being injected into the ICP machine. The system is capable of both qualitative and quantitative analyses depending on the light emission signal.

2.3.6 Sample clean up ---- Solid phase extraction (SPE)

According to the literature research, the PFNA and PFDA concentrations in the landfill leachate are around 150 ng/L and 300 ng/L, respectively (Benskin, et al. 2012). To obtain more accurate MS reading results and mitigate potential side effects from other organic components, a solid-phase extraction experiment was performed before LC-MS analysis. Table 3 outlines the procedures for the extraction experiment using the Oasis WAX cartridge, and Figure 7 provides an illustration of the SPE instrument (Rahman 2014).

Table 3: PFCA extraction procedure

Extraction stage	Experimental procedure
Preparation	• Rinse all the devices with methanol. • Filter leachate through Fisher P5 filter paper.

	• Dilute leachate using reverse osmosis (RO) water with 1:1 ratio. • Prepare acetate buffer: add 0.1158 g ammonium acetate in 600 ml milliQ water; add 0.5 ml acetate acid in 349 ml millQ water. Combine 50ml first solution and 200 ml second solution together for the buffer.
Cartridge activation stage	Adding the following reagent according to the order: • 12ml 1% NH_4OH in methanol • 3ml methanol • 6ml 1% Formica acid in methanol • 3ml methanol • 3ml millQ water
Sampling loading stage	Adding sample in the cartridge, let it run through the cartridge at a speed of 1 drop/sec
Cartridge washing stage	Washing the cartridge using 4 ml acetate buffer (pH 4), then vacuum for 30 min
Extraction stage	Using 5 ml 0.1% NH_4OH in methanol to extract.

Figure 7: Solid phase extraction of PFCA compound in landfill leachate

2.3.7 LC-MS analysis for the PFNA & PFDA

About 1 ml of the extracted solution has been preserved in the MS analysis vials and stored in a refrigerator at -4 °C until analysis. After preparing all the samples, they were sent to the University of Toronto for the LC-MS analysis. To be more specific, the technician used HILIC Chromatography (Thermo Scientific Ultimate 3000) coupled with the Thermo Scientific Q Exactive Mass Spectrometer, operating under 3.5 kV and 325 °C. Ten microliters of extracts were injected into a C18 column (Waters Acquity BEH HILIC, 3.0 mm x 150 mm, 1.7 µm) at a temperature of 50 °C with a flow rate of 0.6 mL/min. Two sets of solvents were applied under different gradients in the mobile phase. Solvent A was 20 mM ammonium acetate and 20 mM ammonium hydroxide in water, while solvent B was acetonitrile. The gradient conditions were as follows: 30% B for 1 min, gradient to 100% B in 4 min, maintain 100% B for 4 min, gradient to 30% B in 0.5 min, and maintain 30% B for 4.5 min. The limit of detection (LOD) for PFNA and PFDA is 0.25 µg/L, according to the library. However, the test result indicated detectability at 25 ng/L. The limit of quantification (LOQ) is 2.5 µg/L, but there is a possibility to refine this further to achieve an LOQ of around 1 µg/L.

2.3.8 Statistical analysis

For various parameters related to solids characterization, pH, and alkalinity measurements, each test has been performed triplicated to get the mean value and standard deviation for each result. For the nitrogen, phosphate and COD tests, triplicated analysis has been performed 5 times during the 1-year sampling period. The standard deviation has been calculated according to triplication analysis results and considered as validation for the remaining data during that period. For the metal analysis, the samples have been performed a triplicated analysis using the ICP-AES test. Means value and standard deviation have been calculated accordingly for each sampling point. For the PFCA seasonal variation, each sample has performed duplicated analysis and results have been used to calculate the mean and standard deviation. For every 7 sets of samples, there are 2 sets of samples that have been spiked 5 μg/L PFCA to validate the data. These 2 sets of spiked samples have been used to calculate the recovery rate of LC-MS analysis and applied to all these 7 samples to calculate the original concentration.

2.4 Result and discussion

2.4.1 Seasonal variability of leachate characteristics

According to the leachate characterization results, both solid level and chemical contaminants changed throughout the year and can also be affected by human activities. During summer, the high temperature increased the evaporation of landfill leachate, which increased the concentration of the contaminants. During winter, as the temperature dropped, more leachate was produced, and the contaminant concentrations dropped due to the leachate dilution effect. Also, when landfill sites pumped rainwater and pound water into the wet well, it brought abnormal fluctuations in characterization results.

2.4.1.1 Seasonal variation and human activity effect on solids level.

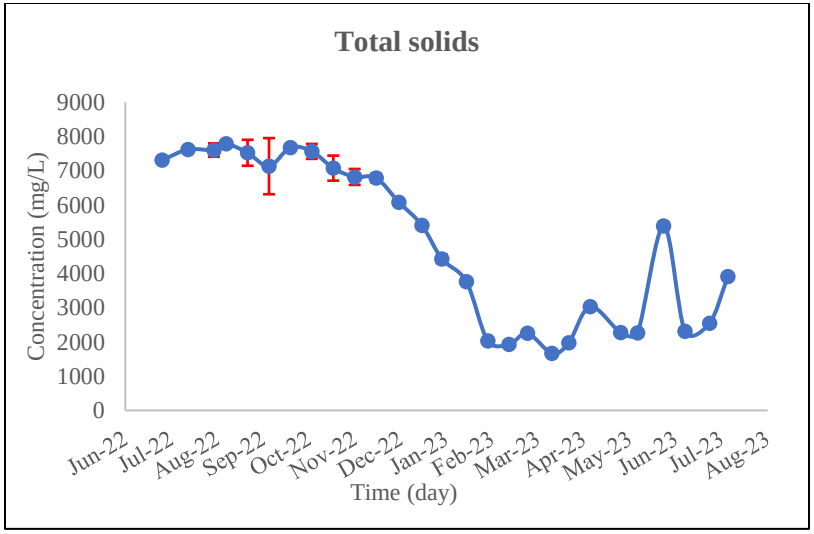
Figure 8 (a) illustrates the total solids content in the landfill leachate. High concentrations were observed during the summer, spanning from July 2022 to October 2022, with values ranging between 7000 mg/L and 8000 mg/L. However, as fall transitioned into winter, accompanied by increased rainfall and lower temperatures, the total solids concentration dropped to 2000 mg/L after October. With the return of summer and rising temperatures, the concentration increased from 2000 mg/L to 5000 mg/L between April and June. The total solids concentration experienced a decline in January and February due to two significant rainfalls in the middle of January, saturating the landfill's humidity. This led to more diluted leachate during these two months, resulting in a reduction of the total solids concentration. Additionally, solids concentration dropped in April, May, and July. During winter, water plants in the pond near the landfill site decompose, causing an increase in ammonia concentration. Since the landfill site cannot treat pond water, they pump the excess pond water into the wet well, leading to leachate dilution and a decrease in parameters from March to April. In May, the landfill sites pump rainwater into the wet wells from the newly constructed landfill, causing leachate dilution and a reduction in contaminant parameters. Pumping of rainwater stopped in June, causing a noticeable peak on June 6th. However, rainwater pumping resumed in July 2023, resulting in a parameter drop. Once rainwater pumping ceased again, the solids increased to 3904 mg/L in July, as shown in Figure 8 (a).

2.4.1.2 Comparison between different solid parameters

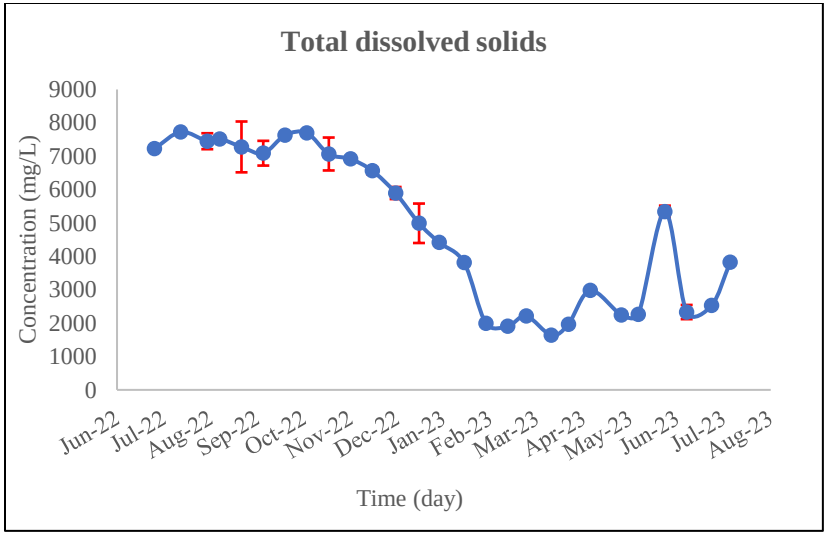
Other than total solids, the total dissolved solids have also been tested in each sample. When comparing the levels of total solids and total dissolved solids in Figure 8 (a) and Figure 8 (b), both figures show a similar trend and concentration. According to the calculations, dissolved solids constitute around 99% of the solids in the leachate. The total suspended solids concentration in landfill leachate was lower, typically ranging from 10 to 30 mg/L. It did not exhibit obvious seasonal variation, but there were two peaks in September 2022 and November 2022. An impactful rainfall before the sample collection date might have flushed solids out of the plumbing system into the wet well, causing an increase in total suspended solids concentration in the leachate samples. Total suspended solids concentration in landfill leachate was generally lower throughout the year. Volatile suspended solids in Figure 8 (d) were also low, ranging from 5 to 20 mg/L. Both suspended solids and volatile suspended solids showed fluctuations throughout the year, but the overall trend did not exhibit obvious seasonal variation. When comparing dissolved and total volatile solids, Figures 8 (e) and 7 (f) exhibited a similar trend and concentrations. Both graphs

showed the concentration of total volatile solids and dissolved volatile were around 1000 to 1400 mg/L during summer and 200 to 400 mg/L during winter. This is because dissolved solids constitute the majority of the solids in the leachate, contributing most to the volatile solids in the total volatile solids.

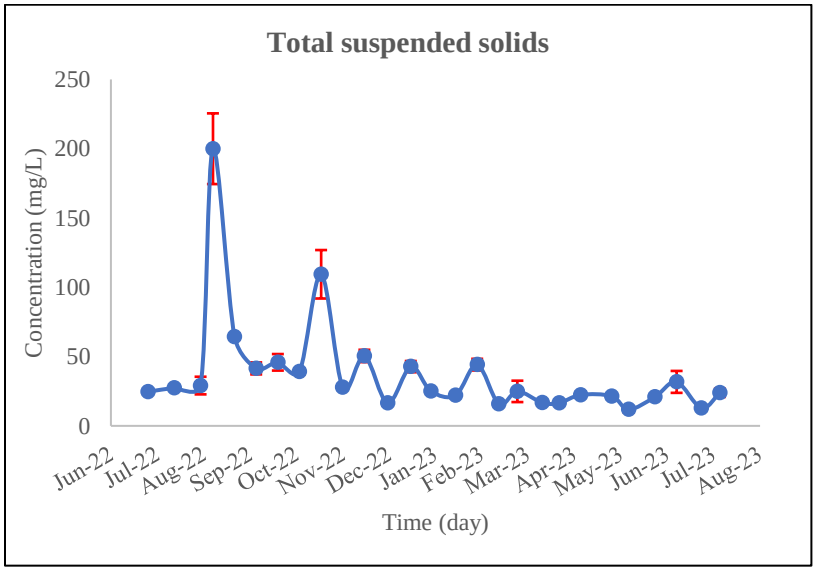
Overall, most solids levels showed seasonal variation throughout the year. During summer, from June to September, the concentration was high. The concentration started to decrease from October to February, remaining low during the winter from February to April, and increasing again after April. During the summer, high temperatures increased the evaporation of moisture in the landfill, reducing leachate production but increasing contaminant concentration. During the winter, increased rainfall with less evaporation leads to higher leachate production, ultimately diluting the leachate. Dissolved solids constitute the majority of the total solids. There were mainly two reasons for the low suspended solids level in the landfill leachate. Firstly, the landfill site applied several membrane layers between the leachate plumbing system and garbage, preventing most solid particles from entering the leachate collection system. The second reason was that only the top layer of the leachate was collected during the sampling processes, preventing the sampling processes from capturing the suspended solids settled at the bottom of the wet well.


Total solids
Concentration (mg/L)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Jun-22
Jul-22
Aug-22
Sep-22
Oct-22
Nov-22
Dec-22
Jan-23
Feb-23
Mar-23
Apr-23
May-23
Jun-23
Jul-23
Aug-23
Time (day)

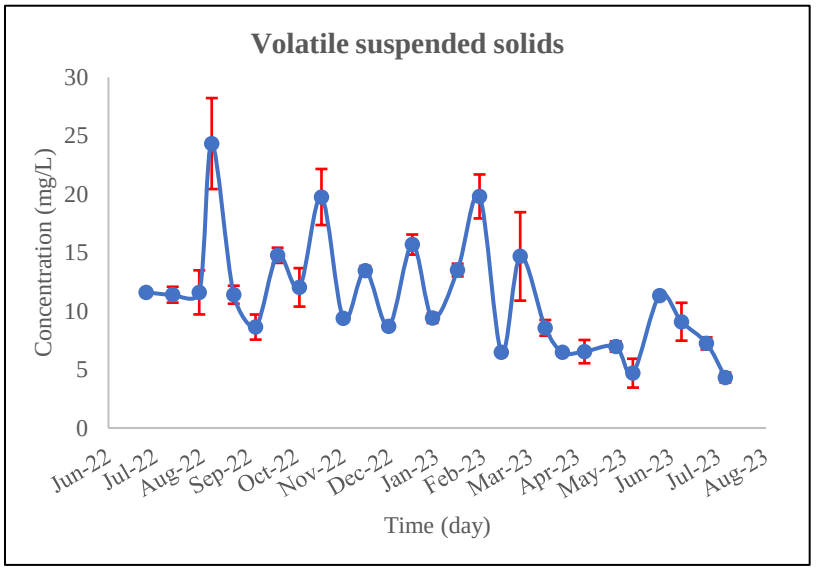
(a)


Total dissolved solids
Concentration (mg/L)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Jun-22
Jul-22
Aug-22
Sep-22
Oct-22
Nov-22
Dec-22
Jan-23
Feb-23
Mar-23
Apr-23
May-23
Jun-23
Jul-23
Aug-23
Time (day)

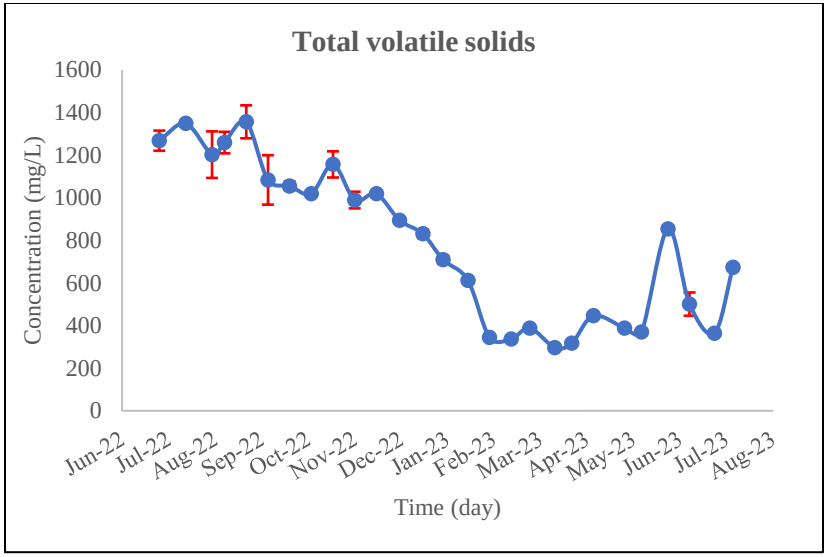
(b)


Total suspended solids
Concentration (mg/L)
250
200
150
100
50
0
Jun-22
Jul-22
Aug-22
Sep-22
Oct-22
Nov-22
Dec-22
Jan-23
Feb-23
Mar-23
Apr-23
May-23
Jun-23
Jul-23
Aug-23
Time (day)

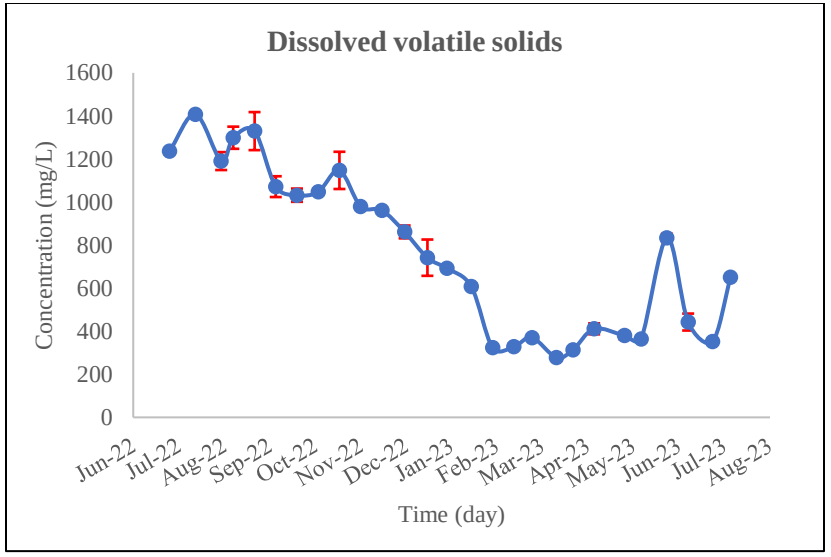
(c)


Volatile suspended solids
Concentration (mg/L)
30
25
20
15
10
5
0
Jun-22
Jul-22
Aug-22
Sep-22
Oct-22
Nov-22
Dec-22
Jan-23
Feb-23
Mar-23
Apr-23
May-23
Jun-23
Jul-23
Aug-23
Time (day)

(d)



(e)



(f)

Figure 8: Solids test of landfill leachate:. (a) Total solids, (b)Total dissolved solids, (c) Total suspended solids, (d) Volatile suspended solids, (e) Total volatile solids, (F) Dissolved volatile solids (Some standard error bars were too small to show on the Figure)

2.4.2 pH, alkalinity, COD, nitrogen, and phosphate test

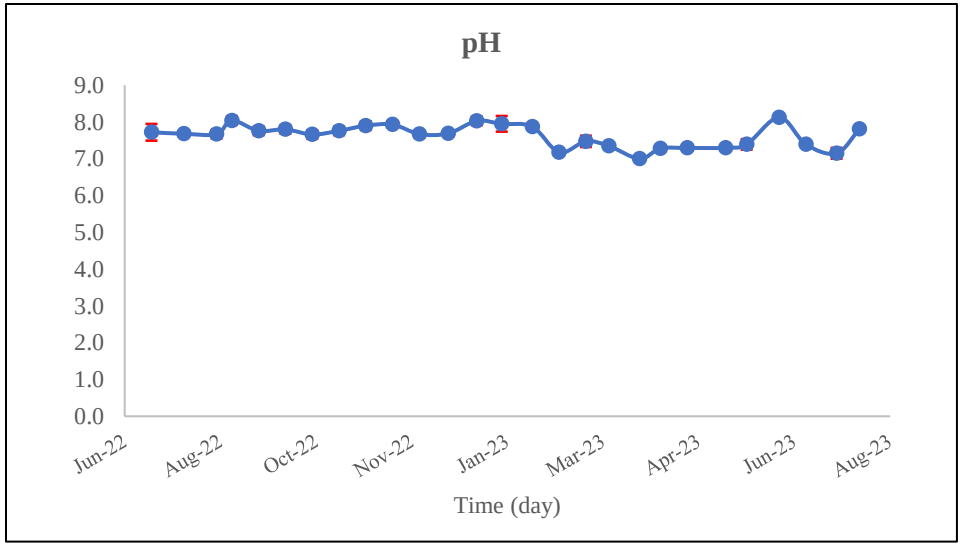
A chemical property test of leachate has been consistently conducted throughout the year. Figure 9 (a) illustrates the pH of landfill leachate, maintaining a range of 7-8. This stability was attributed to the landfill's establishment in 1980, resulting in an average leachate age exceeding 20 years. The degradation of most organic components in the leachate contributed to its alkaline pH, aligning with findings from other researchers indicating acidic leachate in younger landfills. Notably, leachate pH exceeded 7.5 when the landfill surpassed 10 years, indicating an alkaline environment (Mojiri et al. 2021).

Alkalinity, a crucial parameter monitored during leachate characterization, acts as a pH buffer during extended culturing periods. In monoculture environments, researchers introduced $NaHCO_3$ and $KHCO_3$ to augment alkalinity sources (Ruiz-Urigüen et al. 2022; Huang and Jaffé 2019). The leachate alkalinity, impacting subsequent biodegradation experiments, exhibited seasonal variation (Figure 9(b)). Summer season recorded high alkalinity (3000-4000 mg/L $CaCO_3$), while winter showed lower levels (500-1000 mg/L $CaCO_3$). Ionic organic acids from waste decomposition primarily contribute to alkalinity in landfills (Jorstad, Jankowski, and Acworth 2004). Alkalinity concentration decreased in winter due to dilution processes, resembling trends observed in solid waste tests (Somani et al. 2019).

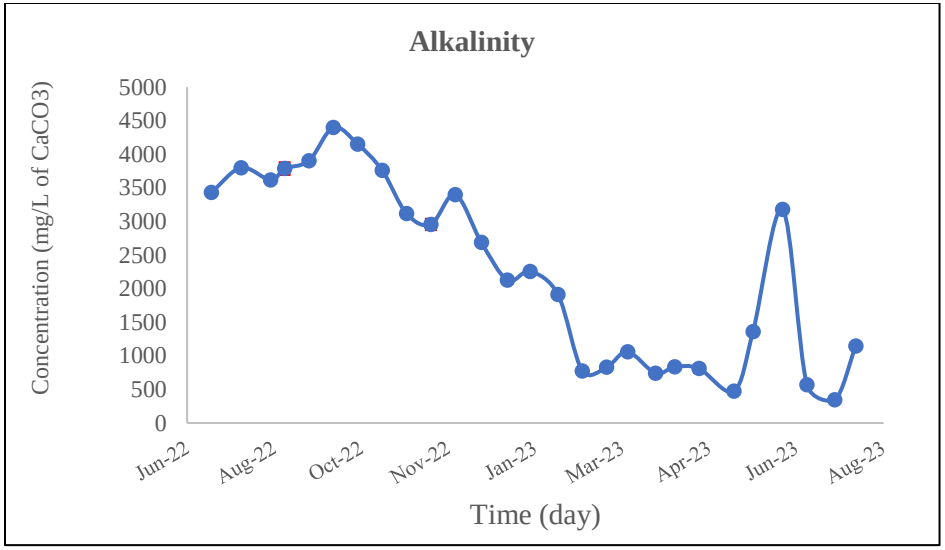
Nitrogen emerges as a key contaminant in landfill leachate, released during anaerobic decomposition. Figure 9(c) reveals high total nitrogen levels in summer (Burton and Watson-craik 2002). and lower levels in winter (around 100 mg/L), mirroring alkalinity and solid levels. This trend arose from temperature variations affecting leachate evaporation, with higher temperatures concentrating leachate in summer and dilution occurring in winter. Figure 9(c) and Figure 9(d) illustrate similar trends and concentrations for total nitrogen and ammonia. Approximately 90% of nitrogen in landfill leachate existed in ammoniacal form (NH_4-N) due to anaerobic degradation. Nitrate and nitrite, though not part of the nitrogen cycle, are detected at low concentrations during leachate characterization (Figures 9(e) and 9(f)). Nitrate and nitrite exhibit minimal seasonal variation but considerable fluctuation, attributed to the stability of nitrite and the high ammonia concentration in leachate. During leachate collection, exposure to air triggers the nitrification cycle, converting ammonia into nitrite and stabilizing it as nitrate. Thus, nitrate and nitrite are consistently detected during characterization.

COD and phosphate are two other types of contaminants that are important to monitor in landfill leachate. Figure 9 (g) depicts the seasonal variation of COD concentration in the leachate. It was high during summer, ranging from 700 to 900 mg/L, and low during winter, around 100-200 mg/L. This aligns with previous research indicating that as the age of landfill leachate increases, the COD value decreases, as the degradable components undergo decomposition over an extended period (Mojiri et al. 2021; Farquhar 1989). Monitoring phosphate in landfill leachate is crucial as it serves as a trigger parameter for microbial metabolism. Figure 9 (h) illustrates the total phosphate concentration in the leachate, varying from 13 mg/L to 1 mg/L throughout the year. The concentration is high during summer, approximately 10-14 mg/L, but low during winter, only reaching around 1 mg/L. The overall concentration remains relatively low due to the old age of the landfill. Bacterial metabolism relies on phosphate, leading to a gradual decrease and depletion of phosphate concentration throughout the extended decomposition period (Khanzada 2020).

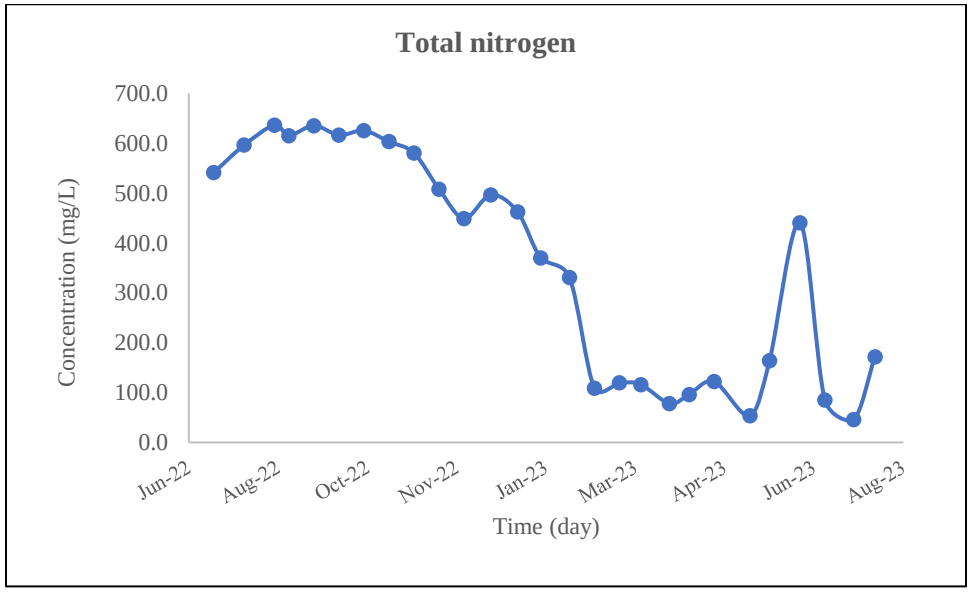
Total nitrogen, ammonia, COD, phosphate, and alkalinity exhibited noticeable seasonal variations, with higher concentrations during summer and lower concentrations during winter. In comparison to the solids test in Figure 8, most contaminants showed variability throughout the year, influenced by human activity and landfill leachate dilution. The pH remained relatively stable throughout the year due to the high alkalinity in the leachate, acting as a buffer to stabilize the pH value. Both nitrate and nitrite exhibited significant variations due to sample collection and storage processes but constituted only a small proportion of the total nitrogen in landfill leachate. Up to 95% of the nitrogen in the leachate exists in the form of ammonia.


pH
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Jun-22
Aug-22
Oct-22
Nov-22
Jan-23
Mar-23
Apr-23
Jun-23
Aug-23
Time (day)

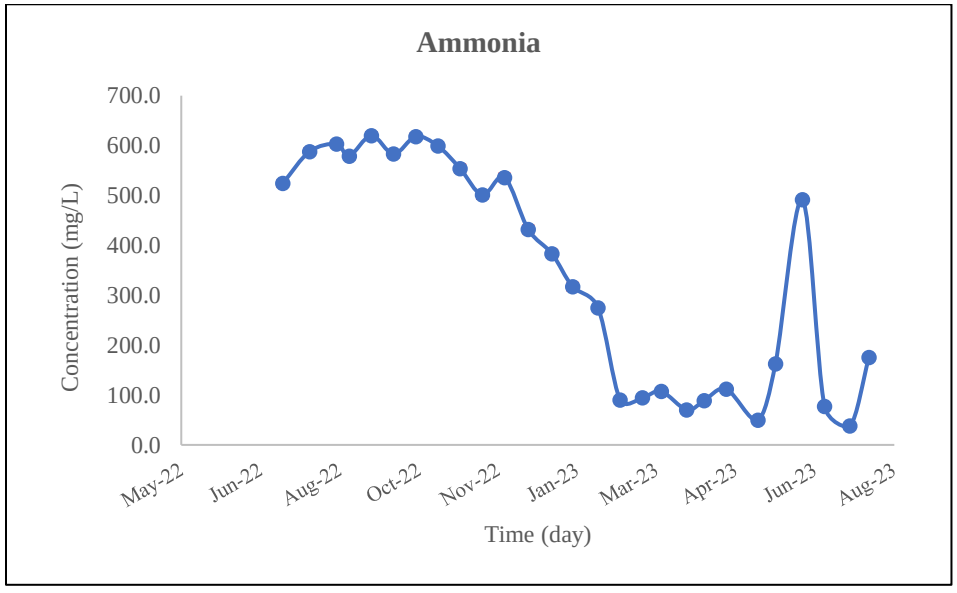
(a)


Alkalinity
Concentration (mg/L of CaCO3)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Jun-22
Aug-22
Oct-22
Nov-22
Jan-23
Mar-23
Apr-23
Jun-23
Aug-23
Time (day)

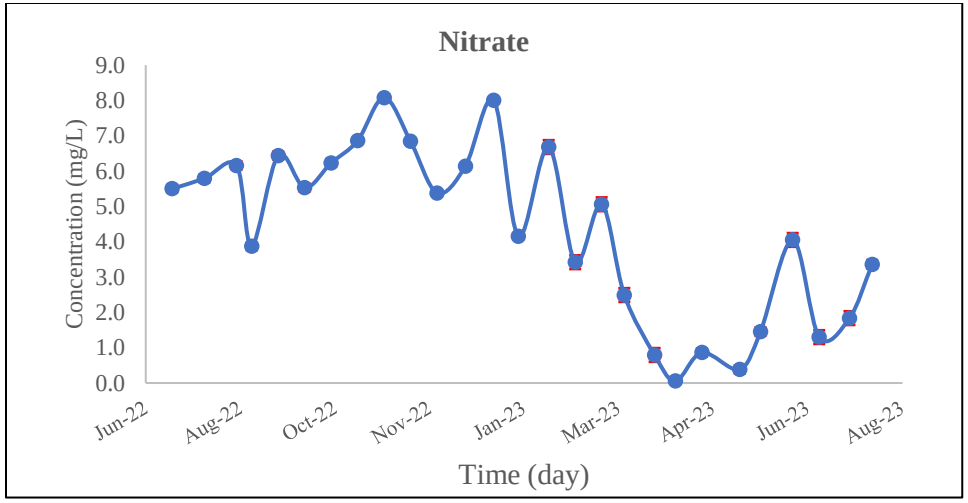
(b)


Total nitrogen
Concentration (mg/L)
700.0
600.0
500.0
400.0
300.0
200.0
100.0
0.0
Jun-22
Aug-22
Oct-22
Nov-22
Jan-23
Mar-23
Apr-23
Jun-23
Aug-23
Time (day)

(c)


Ammonia
Concentration (mg/L)
700.0
600.0
500.0
400.0
300.0
200.0
100.0
0.0
May-22
Jun-22
Aug-22
Oct-22
Nov-22
Jan-23
Mar-23
Apr-23
Jun-23
Aug-23
Time (day)

(d)



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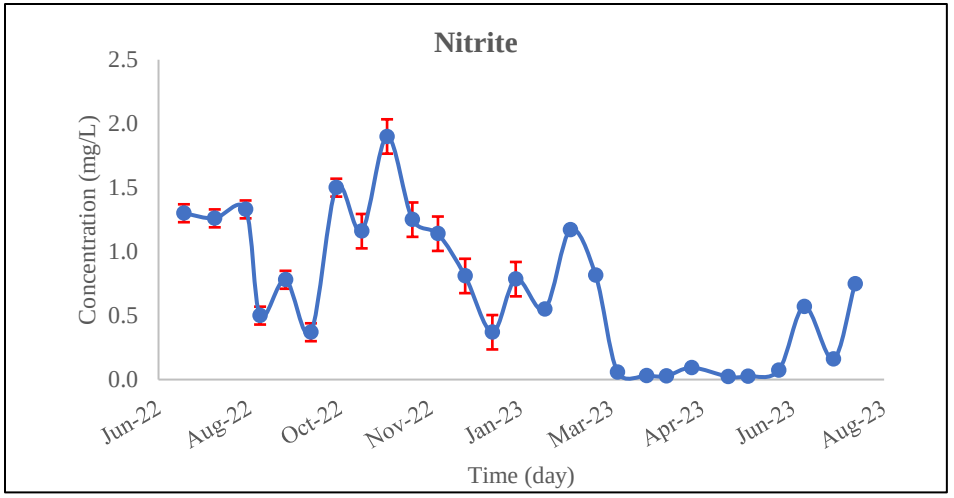

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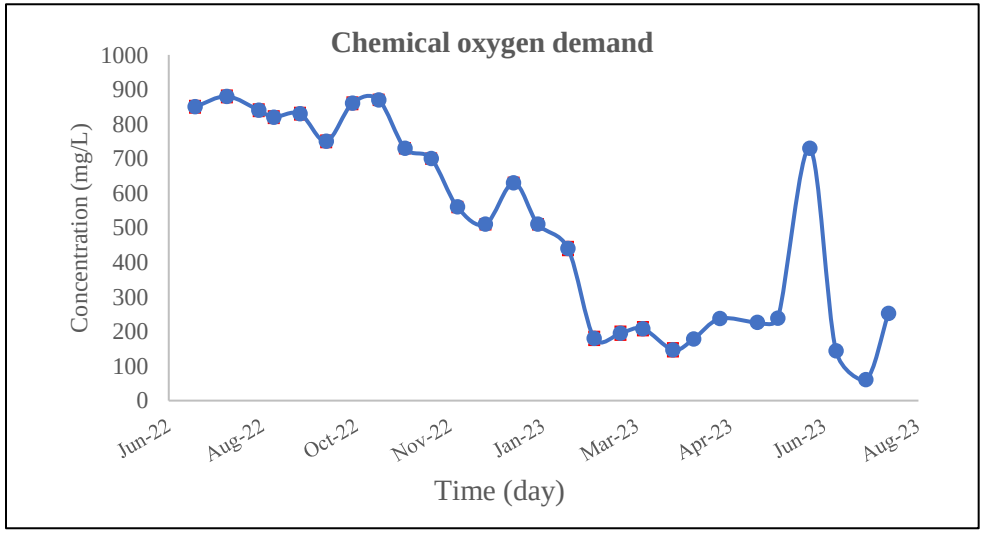
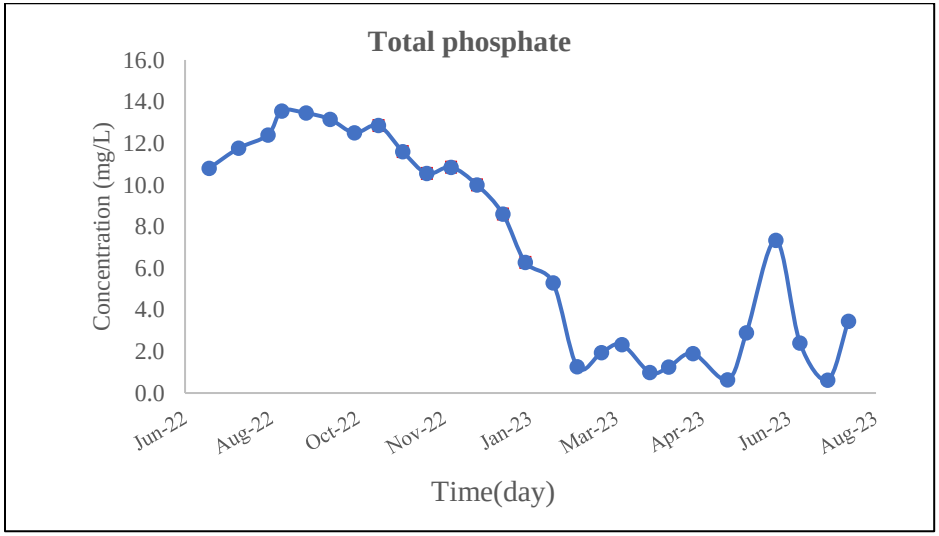


(g)



(h)

Figure 9: *Chemical property tests for (a) pH, (b) Alkalinity, (c) Total nitrogen, (d) Ammonia, (e) Nitrate, (f) Nitrite, (g) Chemical oxygen demand, (h) Total phosphate (Some standard error bar is too small to show on the Figure)*

2.4.3 Method development and quantification study for solid phase extraction (SPE) of PFCAs from landfill leachate

As the targeted compound in this research, it is crucial to scrutinize the seasonal variation of perfluorinated carboxylic acids (PFCA) in the leachate. However, due to the low concentration of PFCA in the landfill leachate and the interference of background matrix effects, direct measurement of PFCA concentration using the LC-MS method proves challenging. Consequently, a solid-phase extraction has been implemented as a clean-up pretreatment process.

To enhance the recovery rate, two sets of experiments have been conducted. The initial phase of the experiment involves a comparison of the recovery rates between water and landfill leachate, considering various spiking concentrations. A calibration curve in Appendix 4 was constructed using the standard solution, and PFCA concentrations in different samples were determined using this calibration curve. Utilizing the control group as a reference for the original PFCA concentration in the landfill leachate, the recovery rate can be calculated employing equation 2.

$$Recovery\ rate = \frac{Spiked\ sample\ concentration - \ nonspiked\ samle\ concentration}{spiked\ amount} \text{ x } 100\%$$

Equation 2: Recovery rate calculation for the SPE.

Table 4 presents the concentrations of C9 and C10 perfluorinated carboxylic acids (PFCA) in each sample, along with the corresponding calculated recovery rates. A comparison between the recovery rates of the first two sets of samples and the last two sets reveals that the leachate-based solution exhibits a lower recovery rate in contrast to the water-based solution. Notably, the recovery rate of C10 in sample 1 exceeded 100%, potentially attributable to operational or carry-over errors from the calibration solution. Excluding this anomaly, the overall recovery rates in water ranged from approximately 58% to 89%. Conversely, in the leachate-based experiment group, the recovery rates ranged only from 30% to 46%, indicative of a matrix effect in the leachate that interacts with the solid-phase extraction (SPE) cartridge, leading to diminished recovery rates.

Upon comparing sample 4 and sample 5, an interesting trend emerges: as the spiked Oconcentration of PFCA increases, so does the recovery rate. This observation suggests that a 5 µg/L spike is more effective than a 2.5 µg/L spike. This phenomenon is attributed to the

quantification limit of the machine, which is approximately 2.5 μg/L. When the spiking concentration was low, the machine reading error was higher, impacting the recovery rate. In summary, the experiment results affirmed the efficacy of the LC-MS testing method for PFCA analysis, confirming the presence of long-chain PFCAs in the leachate. Furthermore, the choice of a 5 μg/L spiking concentration proved advantageous compared to a 2.5 μg/L spiking concentration. However, it is crucial to acknowledge that the matrix effect in leachate samples diminished the recovery rate when compared to water samples.

Table 4: C9 and C10 PFCA recovery rate in leachate and water with 2.5 μg/L and 5 μg/L spike

Sample number	Sample description	C9 calculated concentration (μg/L)	C10 calculated concentration (μg/L)	Recovery rate of C9	Recovery rate of C10
1	Water, 2.5 μg/L spiked	1.83	3.23	73.28%	129.18%
2	Water, 5 μg/L spiked	2.90	4.47	57.95%	89.48%
3	diluted Leachate	1.14	2.32		
4	diluted leachate, 2.5 μg/L spiking	1.89	3.24	30%	36.8%
5	diluted leachate, 5 μg/L spiked	2.95	4.63	36.2%	46.2%

To enhance the efficiency of extraction recovery, an additional series of experiments were conducted. The study involved the use of 10 ml, 20 ml, and 30 ml of leachate, each spiked with 5 μg/L and subsequently diluted by 50% with (Reverse osmosis) RO water. To mitigate the influence of the original PFCA concentration in the leachate, three sets of unspiked leachate and one set of water-based solutions were established as the control group. Thes recovery rate was determined using Equation 1. As depicted in Table 5, an increase in sample volume during extraction corresponded to an escalation in the recovery rate of C10, rising from 71% to 87%. Notably, even water-based sample 7 exhibited the highest recovery rate for C10 at approximately 97%, while sample 6 also demonstrated an acceptable recovery rate (approximately 87%). In the case of C9, it exhibited a higher recovery rate compared to C10. Furthermore, with an increase in sample volume, the recovery rate of C9 PFCA approached 100%. Considering the recovery rate, the original concentration of PFCA in the leachate can be calculated using the following Equation 3:

$$Original\ concentration\ (ppb) = \frac{tested\ concentration\ (ppb)}{extraction\ ratio\ \text{x}\ recovery\ rate}$$

Equation 3: Original concentration calculation of PFCA in the leachate

Table 5: C9 and C10 PFCA recovery rate in leachate with different extraction volumes and 5 µg/L spiking concentration

Sample number	Sample description	C9 calculated concentration (µg/L)	C10 calculated concentration (µg/L)	Recovery rate of C9 (Percentage)	Recovery rate of C10 (Percentage)
1	10 ml leachate, 50% dilution 2.5 X extraction	0.65	0.28		
2	10 ml leachate, 5 µg/L spiked 50% dilution, 2.5 X extraction	5.19	3.85	90.9%	71.4%
3	20 ml leachate, 50% dilution 5 X extraction	1.05	0.51		
4	20 ml leachate, 5 µg/L spiked 50% dilution, 5 X extraction	6.55	4.63	110%	82%
5	30 ml leachate, 50% dilution 7.5 X extraction	1.47	0.75		
6	30 ml leachate, 5 µg/L spiked 50% dilution, 7.5X extraction	6.65	5.11	103.5%	87.2%
7	20 ml water, 5 µg/L spiked Extract into 4ml (2.5X)	5.49	4.86	109.8%	97.2%

Based on the quantification experiment results, it has been determined that a spiking amount of 5 µg/L was optimal during Solid Phase Extraction (SPE) for assessing the recovery rate of leachate. Additionally, the experiment confirmed the presence of C9 and C10 in landfill leachate,

albeit at low concentrations of approximately 0.189 μg/L for C9 and 0.115 μg/L for C10. This finding suggested the feasibility of conducting further tests to explore seasonal variations in the concentrations of C9 and C10 in landfill leachate.

2.4.4 Seasonal variation of C9 and C10 PFCA in the landfill leachate

According to the findings of the preceding quantification study, a seasonal variation characterization of PFNA and PFDA has been conducted on all leachate samples. Each set of samples has been extracted at least twice to determine the standard deviation. For every two experimental batches, two sets of samples spiked with a known concentration of 5 μg/L were utilized to calculate the recovery rate. Based on this recovery rate, Equation 2 can be applied to ascertain the original concentration of PFNA and PFDA in the leachate.

The extracted results, as depicted in Figure 10, reveal a similar changing trend in the concentration of PFNA and PFDA within the leachate. From July 2022 to January 2023, the concentration remained low, hovering around 100 ng/L for both PFNA and PFDA in the landfill leachate. However, between February 2023 and July 2023, a significant increase was observed, with concentrations reaching approximately 0.6 μg/L for PFNA and around 0.4 μg/L for PFDA. Notably, both parameters exhibit substantial fluctuation with a high standard deviation during this period, suggesting a potential measurement or operational error.

Upon reviewing previous experimental records and supporting documentation, it was noted that samples collected from February 24th, 2023, to May 19th, 2023, were processed in the same week under the same bench of examination. Within this set of samples, specific reading errors were identified on April 4th, 2023, and March 19th, 2023, both showing elevated concentrations. These errors are attributed to carry-over issues from the calibration solution, indicating a potential source of the observed concentration fluctuation during this period. It is important to highlight that the changing PFCA concentration in the landfill leachate may not be a contributing factor to these fluctuations.

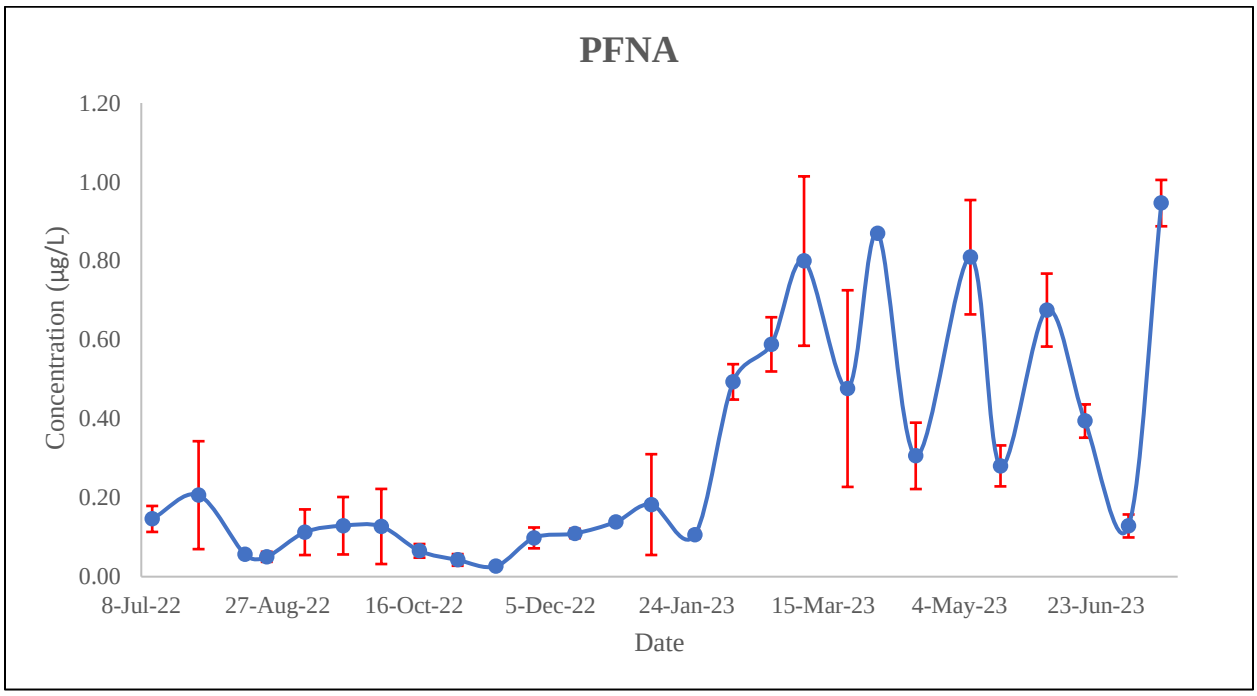
If we disregard the data with fluctuations, both PFNA and PFDA still exhibit a noticeable increase in concentration during February 2023, with decreases observed in June 2023. In Figure 8, compared to the other contaminant parameters, the concentration of long-chain PFCA shows an opposite trend. On February 10th, 2023, the concentrations of both PFNA and PFDA increased,

while all other contaminant parameters decreased on that day. A significant rainfall occurred several days before the sample collection date, leading to increased leachate production and the dilution of all other parameters. The rainfall may have elevated the production and flow rate of landfill leachate, flushing PFCA from the landfill and causing an increase in concentration. However, there is another potential reason for this opposing measurement result: rainwater dilution reduces the matrix effect, thereby increasing the machine reading of PFCA during analysis. From June 19th to July 5th, 2023, both PFNA and PFDA show a decreasing trend in concentrations. This could be attributed to the landfill pumping rainwater from construction land into wet wells, which dilutes the concentrations.

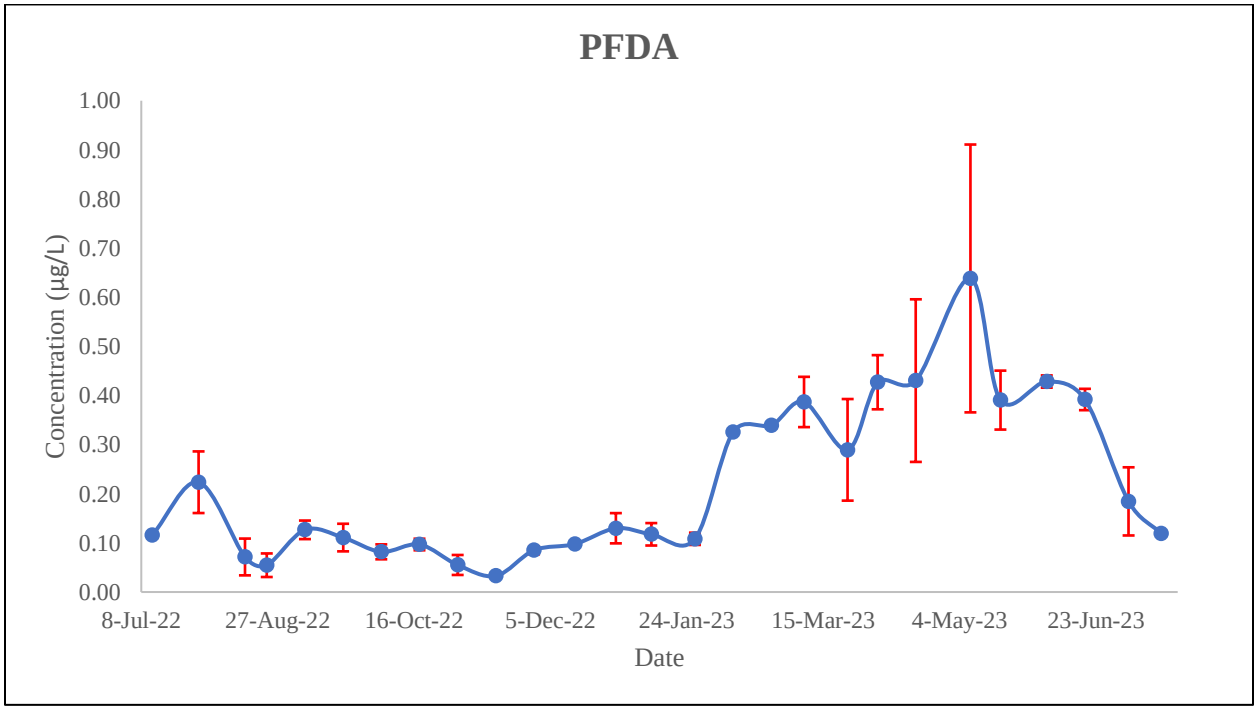
Previous research on the seasonal variability of long-chain PFCA in landfill leachate is limited. The concentrations of PFNA and PFDA in landfill leachate can vary widely, ranging from 80 ng/L to 2000 ng/L (Yan et al. 2015; Gallen et al. 2017). The concentration of long-chain PFCA depends on the waste type in the landfill site and the landfill's age (M. Zhang et al. 2023). As the landfill ages, long-chain PFCA and their precursors transform into short-chain PFCA, such as PFHxA, PFHpA, PFOA, etc. (M. Zhang et al. 2023; Benskin et al. 2012). Benskin et al. (2021) conducted a seasonal variation study of PFCA in a Canadian landfill from February 2010 to June 2010. The research indicates that both PFNA and PFDA increased from February 2010 to April 2010, reaching their highest peaks of 450 ng/L and 1100 ng/L , respectively, in April, before decreasing from April to June (Benskin et al. 2012). Although not a full-year analysis, the data was similar to the test results in this research. The specific reasons for this phenomenon are still unclear but may be related to the biodegradation of other precursors in the landfill site.

Overall, both PFNA and PFDA are present in landfill leachate, and their seasonal variation in concentrations is limited. This aligns with Benskin's research, which suggested that long-chain PFCA does not exhibit obvious seasonal variation in landfills (Benskin et al. 2012). Due to the initially low concentration of leachate, even after six extractions, the samples' concentration remained low, resulting in a high standard deviation and measuring error. This phenomenon was also evident in other researchers' work during the detection of PFCA in landfill leachate. When performing solid-phase extraction (SEP) of long-chain PFCA from landfill leachate, other researchers have shown high standard deviations (ranging from 1% to 15%) with a recovery rate of up to 120% (Lang et al. 2016; Yan et al. 2015). Considering the recovery rate, the concentration

of PFNA and PFDA in landfill leachate was estimated to be around 100 ng/L, with the concentration varying from 30 ng/L to 940 ng/L.



(a)



(b)

***Figure 10**: Seasonal variation of PFNA and PFDA in the landfill leachate*

2.5 Significance and outlook

According to the leachate characterization results, the presence of long-chain perfluorocarboxylic acid (PFCA) was identified, albeit at a low concentration. Unlike other contaminants, the PFCA did not exhibit significant fluctuations. The recovery rate of solid-phase extraction (SPE) displayed notable variability, possibly linked to the limit of quantification (LOQ) of liquid chromatography-mass spectrometry (LC-MS). Further optimization of extraction processes, such as increasing sample loading and enhancing PFCA concentration, is necessary.

The concentration of PFCA in landfill leachate was found to be lower than that of other contaminants, including nitrogen and chemical oxygen demand (COD). The coexistence of these competitors might pose challenges for PFCA degradation in the leachate. Regular leachate characterization can serve as a valuable reference for future studies on leachate treatment processes.

In flask experiments, the characterization results become crucial for calculating the carbon-to-nitrogen-to-phosphorus (CNP) ratio and optimizing bacterial culture conditions in leachate. There is, however, a need for improvement in government regulations governing water treatment at landfill sites. The current restriction on the site's ability to treat water, especially during winter when ammonia levels in pond water exceed standards, results in the unnecessary pumping of water into wet wells, leading to additional leachate with undesired dilution. Empowering landfill sites to undertake basic treatments, such as aeration, could mitigate ammonia levels and reduce leachate production, thereby alleviating the burden on wastewater treatment plants.

2.6 Conclusions

It is highlighted that various solid and chemical parameters in landfills exhibited seasonal variations, influenced by factors such as rainfall, temperature, and human activities. Some parameters, like pH, remain relatively stable, while others, including nitrate, nitrite, suspended solids, and volatile suspended solids, showed non-seasonal-related fluctuations. Anaerobic biodegradation in leachate predominantly converted nitrogen into ammonia, constituting approximately 90% of total nitrogen.

The presence of perfluorononanoic acid (PFNA) and perfluorodecanoic acid (PFDA) in landfill leachate samples was confirmed. Quantification studies revealed a recovery rate approaching 100% with increasing sample volume. Interestingly, the concentration of PFNA and PFDA did not exhibit obvious seasonal variations, unlike other contaminants. The average

standard deviations for PFNA (26%) and PFDA (19%) were attributed to the low concentration of samples and potential matrix effects in the leachate.

Chapter 3: Utilizing Indigenous Bacterial Cultures for the Biodegradation of PFCAs in Leachate Samples

3.1 Abstract

A flask experiment was performed to test the biodegradability of PFNA and PFDA in the landfill leachate. The culturing media were supplemented with phosphate and glucose to achieve a balanced CNP ratio, as indicated by leachate characterization. Long-chain PFCA was spiked in each flask with a concentration of 1 mg/L, 10 mg/L, and 100 mg/L. These flasks were cultured at 30 °C and 170 rpm in a shaking incubator. OD at 600 nm has been measured to monitor the bacteria growth in a 6-day culturing period. In a pure leachate environment, no bacterial growth was observed. However, with the inclusion of glucose in the culture medium, bacteria exhibited a log phase at 30 hours, reaching an OD 600 of up to 0.6. LC-MS analysis revealed only a modest PFCA removal of around 7%. Notably, in leachate containing both glucose and phosphate, bacteria reached an OD of 1.3, displaying diauxic growth at 30 hours and 100 hours. The bacteria demonstrated the ability to utilize PFCA as an energy source during metabolism, with both carbon source and phosphate acting as triggering elements. The maximum removal efficiency of 34% and 58% was observed for PFNA and PFDA, respectively. Results indicate that as chain length and concentration increase, PFCA becomes less stable during storage and displays higher biodegradation efficiency in flask experiments

3.2 Introduction

Biological degradation is a relatively recent technology employed for treating PFCA, an emerging contaminant. In this process, microorganisms utilize PFCA as a carbon source, resulting in lower energy consumption and the absence of toxic waste generation during the reaction (Shahsavari et al. 2021). However, the research in this field remains limited.

In recent years, scientists have successfully isolated and acclimated *Pseudomonas Parafulv* and *Acidimicrobium sp.* A6 strains from PFCA-enriched environments. This allows for the utilization of these strains in biological degradation, employing PFCA as a carbon source (Yi et al. 2016; Huang and Jaffé 2019). Researchers inoculate these acclimated bacterial strains into culturing media containing essential mineral supplements for metabolism and PFCA as a carbon source. Both aerobic and anaerobic biodegradation methods are applicable to PFCA removal, with the culturing conditions set at 30 °C and 150 rpm (Huang and Jaffé 2019). During anaerobic environments, bacteria perform degradation under an acidic pH range of 4.5-5.5, while in aerobic

environments, neutral conditions are used for degradation (Yi et al. 2016; Ruiz-Urigüen et al. 2022). Most experiments, conducted under anaerobic conditions, demonstrate high removal efficiency of PFOA (60-77%) during the 18-day reaction (Ruiz-Urigüen et al. 2022). Under aerobic conditions, the removal efficiency is limited to 48% (Yi et al. 2016) attributed to the slow reduction reaction caused by the high electronegativity of fluorine in the presence of oxygen.

All experiments were conducted on a laboratory scale in a monoculture environment, making direct application to industrial or wastewater treatment processes challenging. The initial concentration of PFCA in biodegradation experiments is critical. Research shows that as the initial PFOA concentration increases from 0.1 to 200 mg/L, the consumption efficiency of the reduction reagent remains largely unaffected (Huang and Jaffé 2019). This suggests that the reaction rate is minimally impacted by PFOA at low initial concentrations. In aerobic reactions, a concentration increase from 0 to 500 mg/L stimulates bacterial growth, but beyond 500 mg/L, the reaction rate decreases due to the toxicity of PFOA inhibiting bacterial growth (Yi et al. 2016).

During biodegradation, essential additions such as nitrogen, phosphate, and alkalinity are crucial for maintaining bacterial metabolism in a monoculture environment (Huang et al. 2022). PFOA serves as a carbon source for bacterial consumption, and glucose may act as a co-metabolism compound to accelerate bacterial metabolism (Yi et al. 2016). Calcium, magnesium, and other trace elements are introduced as supplements for biodegradation reactions (Ruiz-Urigüen et al. 2022; Huang and Jaffé 2019).

The main degradation products of PFCA include short-chain compounds such as PFHxA, PFHpA, PFPeA, and PFBA, along with detected free fluoride ions as evidence of a defluorination reaction. The concentration of these products increases as the PFCA concentration decreases during the reaction (Ruiz-Urigüen et al. 2022). The mechanisms of PFCA biodegradation remain unclear due to the complexity of metabolic processes. Some studies employ ammonium as an ion contributor and iron oxide as an ion acceptor in the Feammox process using *Acidimicrobiaceae sp.* strain A6 (Huang and Jaffé 2019). Another proposed a stepwise biodegradation mechanism based on the concentration of intermediates produced during the degradation of A6 bacteria (Ruiz-Urigüen et al. 2022).

Literature research indicates a limited number of articles exploring the biological degradation of PFCA, with a focus on PFOA (C8 PFCA). Long-chain PFCA, especially PFNA and PFDA,

has not been subjected to biological degradation experiments. All biological degradation studies thus far have been conducted in laboratory environments using specific bacteria in monoculture conditions, creating an artificial setting to eliminate potential competitors and provide an ideal environment for bacterial growth. Additionally, previous research often employs high PFCA concentrations during degradation, which may not be representative of real-world scenarios. Future research in the industrial or wastewater treatment processes should emphasize mixed culturing conditions. Exploring direct reactions in wastewater or leachate under aerobic environments could yield valuable insights. Furthermore, investigating new treatment methods to eliminate PFOA byproducts, given its stability during biodegradation, could prove advantageous in preventing the formation of PFOA.

3.3 Material and methods

To determine the biodegradability of PFCA in landfill leachate background, a flask experiment was performed. By adding a mineral solution with carbon sources, original microorganisms in the leachate are cultured to perform biodegradation of PFNA and PFDA.

3.3.1 Chemical reagent list

In this experiment, the following chemical reagents were used: Millipore Sigma 97% CAS grade perfluorononanoic acid (PFNA), Millipore Sigma 98% CAS grade Perfluorodecanoic acid (PFDA), Fisher Chemical HPLC Grade methanol, TCI 85% CAS grade beta-D-Glucose 85%, Fisher Bioreagents 99% CAS grade Potassium Phosphate Monobasic. PFCAs were purchased from Millipore Sigma website and the rest chemical reagents were purchased from Thermo Fisher website.

3.3.2 PFCA stock solution preparation

The flask experiment was performed at three different PFCA's concentrations. The high concentration was 100 mg/L, the medium concentration was 10 mg/L, and the low concentration was 1 mg/L. According to 3 different spiking concentration requirements, 3 sets of 200X stock solutions have been prepared. The original stock solution was dissolved in 0.1 g of PFNA and PFDA separately in 5 ml methanol to achieve a concentration of 20000 mg/L. Subsequently, both stock solutions were diluted tenfold, twice, yielding 2,000 mg/L and 200 mg/L stock solutions. In each flask, a systematically determined amount of 0.25 ml from the stock solution with varying concentrations is added.

3.3.3 Other element addition requirement

Different factors apart from carbon and nitrogen sources, such as trace elements and alkalinity, will affect bacteria growth in the flask experiment. During the monoculture experiment, researchers usually added calcium, magnesium, and sodium into the culturing media (Yi et al. 2016; Huang and Jaffé 2019). In a monoculture environment, calcium, magnesium and potassium concentration was around 16 mg/L, 10 mg/L 30 mg/L, respectively (Ruiz-Urigüen et al. 2022; Huang and Jaffé 2019). However, according to the ICP-OES test of landfill leachate in Appendix 3, the calcium level was around 100 mg/L, the magnesium was about 700 mg/L, and potassium was 500 mg/L. The concentration of these elements is sufficient for the essential bacteria metabolism. So, no extra mineral dosing was required for the flask experiment. The bacteria thrived in a leachate environment for a long generation and were getting used to it. Hence, no adjusting or spiking was required during the entire experiment.

3.3.4 Mineral solution spiking calculation

The leachate characterization result showed that the leachate that had been used in the flask experiment had a COD concentration of 730 mg/L, total phosphate of 7.34 mg/L, and total nitrogen of 440 mg/L.

The CNP ratio for bacteria cultivation and leachate characterization results; demonstrated that the leachate has sufficient nitrogen sources. However, the culturing solution needs extra carbon sources and phosphate to consume this nitrogen. Hence, the spiking solution for the experiment was prepared according to Table 6.

Table 6: Mineral spiking solution preparation for the flask experiments

	Leachate characterization result	Proportion in the culturing media	Stock solution concentration	Spiking amount (ml)	Working concentration
Carbon	730 mg/L of COD	100	58250 mg/L of glucose	0.8	1030 mg/L of C
Nitrogen	440 mg/L of N	5			440 mg/L of N
Phosphate	7.34 mg/L of P	1	17000 mg/L of KH_2PO_4	0.25	58 mg/L of P

3.3.5 Experimental group setup and parameter measurement

All pipette tips, Falcon tubes, and flasks underwent thorough washing and rinsing with methanol to eliminate any potential PFCA residue. Subsequently, they were sterilized via autoclave. The leachate was then meticulously filtered through Fisher P5 filter paper within a laminar flow chamber.

For the preparation of the culture media, 50 ml of leachate was introduced into a 250 ml flask. To establish three distinct experimental groups (pure leachate, glucose only, glucose and phosphate combined), mineral solution was added accordingly. PFNA and PFDA were intentionally spiked at four different concentrations (0 mg/L, 1 mg/L, 10 mg/L, 100 mg/L). Additionally, two sets of control groups, spiked at 1 mg/L and 100 mg/L in pure water, were established and stored in a refrigerator at 4°C throughout the culturing period. All flasks, including the experimental and control groups, were incubated in the Infors HT Multitron Triple shaking incubator at 30°C and 170 rpm, as illustrated in Figure 11.

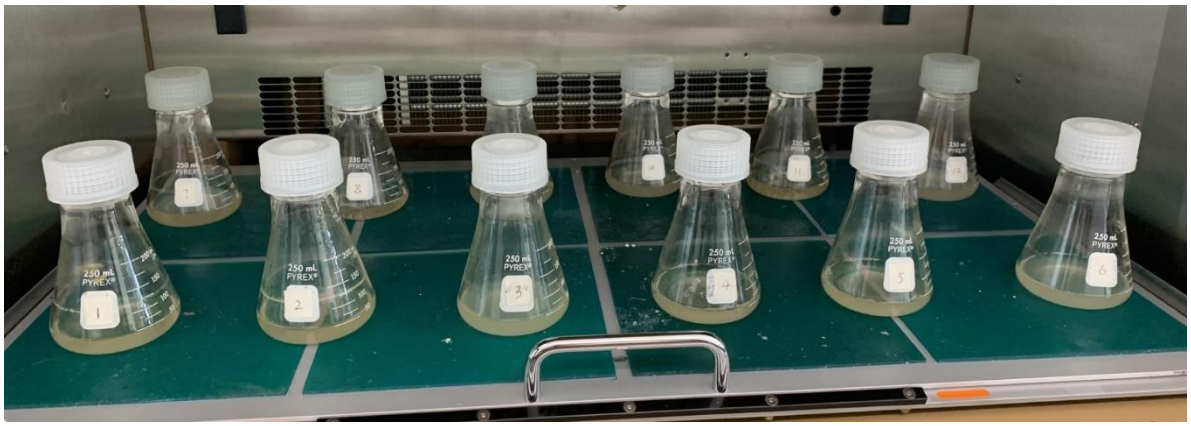


Figure 11: Bacteria were cultured in a shaking flask to perform biodegradation of long-chain PFCA.

3.3.6 Parameter measurement

During the 6-day culturing period, samples were extracted from each flask in the laminar flow chamber for the measurement of optical density (OD600) using the Agilent Bio Tek Epoch Microplate Spectrophotometer. Optical density (OD600) readings were taken at 0 h, 6 h, 12 h, 24 h, 36 h, 54 h, 72 h, 96 h, 120 h, and 144 h. Duplicate samples were collected from each flask at every sampling point. Due to the turbidity of the original leachate samples, the OD reading was determined using Equation 4:

$$OD\ measurement\ result = OD\ reading\ of\ flask\ samples - OD\ reading\ of\ blank$$

Equation 4: OD 600 calculation for the flask experiment

The average reading and standard deviation were computed based on duplicate measurement results. pH measurement was conducted using a pH meter for both the blank sample and cultured media after the flask experiment. Subsequently, both blank and cultured samples underwent centrifugation at 9000 rpm for 25 minutes, and the resulting supernatant solution was diluted to a concentration of 100-200 μg/L using methanol for LC-MS analysis, as depicted in Figure 12. All samples were forwarded to the University of Toronto for LC-MS analysis, and the initial and final concentrations for PFNA and PFDA were determined using the calibration curve provided in Appendix 5.

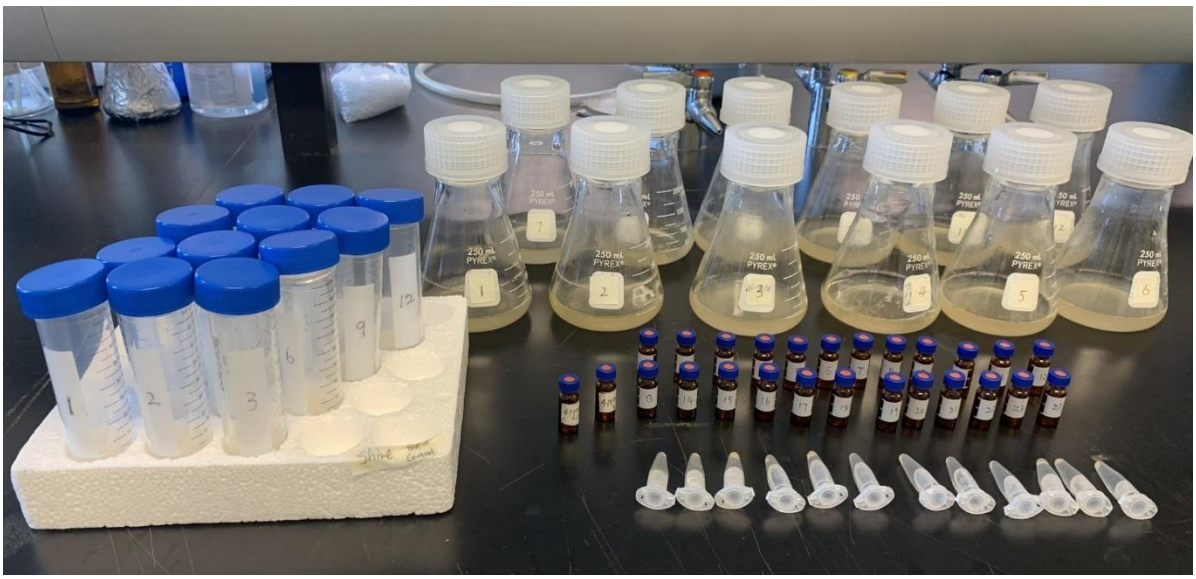


Figure 12: Sample preparation of flask experiment before LC-MS analysis

3.3.7 Statistical analysis

Duplicated measurement has been performed for both blank and flask samples for the OD600 reading. The mean and standard deviation for each sampling point have been calculated according to the measurement results. The degradation experiments have been performed 2 times with the same conditions as a cross check. One extra set of experiments was done using the bacteria strain from the previous experiment (OD graph is available in Appendix 7). For the degradation experiment, the PFCA removal efficiency has been cross-checked using the flask experiment with inoculated bacteria strain (exposed microbes) and data is available in Appendix 7, Supporting Table 3.

3.4 Result and Discussion

3.4.1 Bacterial growth in flask experiment

The optical density (OD) was measured in each flask throughout the culturing period. OD readings were plotted in Figure 13 and turbidity figures were provided in Appendix 6 to show the bacteria growth in each flask.

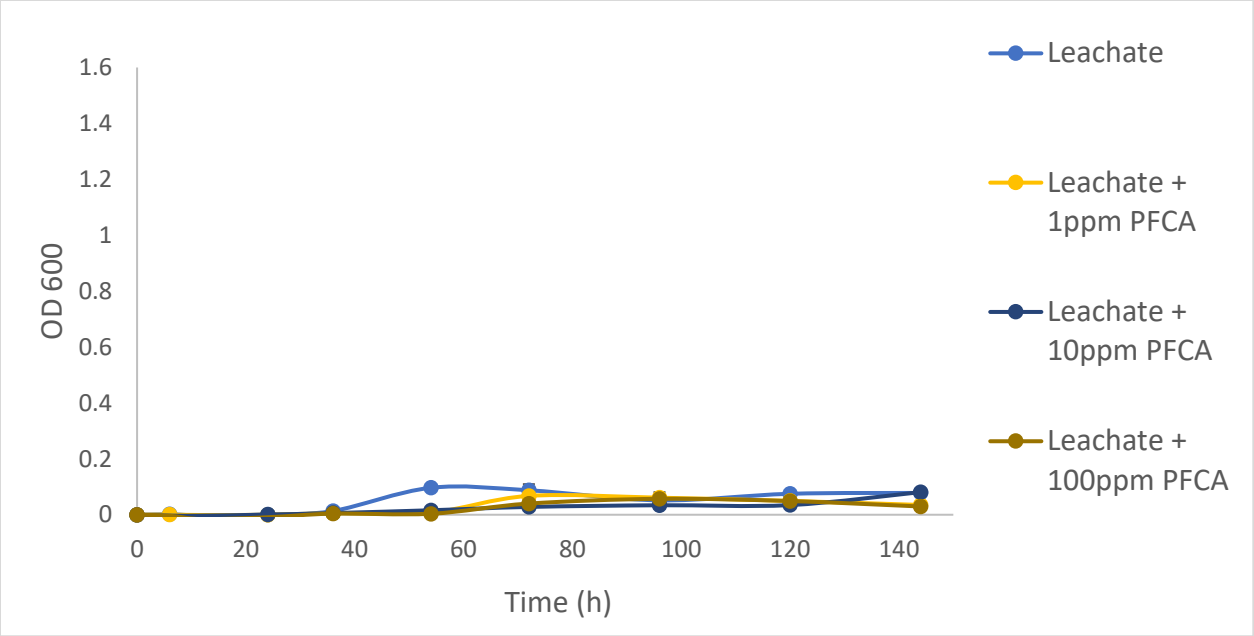
In Figure 13 (a), four flasks showed consistently a low OD reading throughout the culturing period. These flasks contain only PFCA spiked, without minerals. This observation suggests that the bacterial condition in the leachate allows for the direct consumption of PFNA and PFDA, without the presence of other mineral supplements.

Four flasks in Figure 13 (b) showed a higher OD reading when glucose was present. After experiencing a 24-hour lag phase, it showed a log phase between 24 h and 36 h, which reached the highest OD reading of 0.6. Later, the bacteria went through a decline phase after 48 h, and the OD reading decreased. All three flasks with PFCA spiked at different concentrations showed similar OD readings. The control flask, which contained pure leachate and glucose, experienced some minor fluctuation at 70 h and 120 h. However, all these flasks approached a stabilized OD reading of around 0.2. Figure 13 (b) shows that glucose can stimulate the bacteria growth in the leachate due to the presence of carbon sources. However, different PFCA concentrations did not affect the bacteria growth since the OD reading for these 3 flasks was almost identical. The control group showed fewer fluctuations when the bacteria in other flasks entered the decline phase. It can be

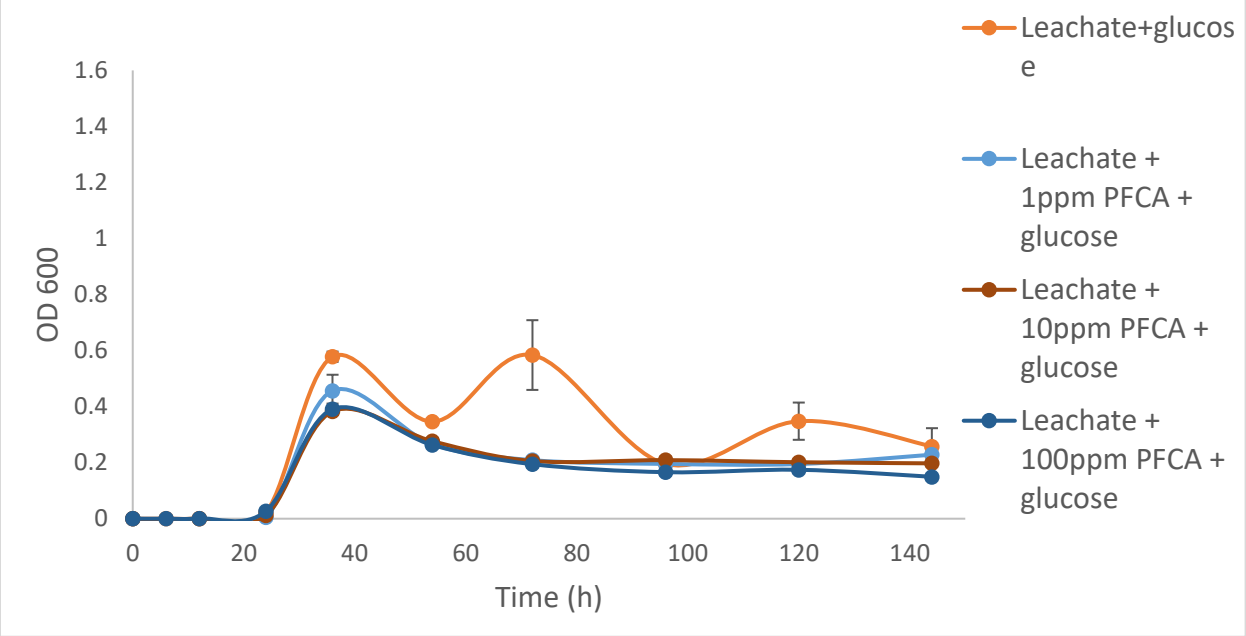
caused by some diauxic growth of bacteria in the leachate, but the trend was not apparent. Also, comparing the control group to the experiment group showed a slightly higher reading for the former. It can be caused by the toxicity of PFCA, which inhibited the bacteria metabolism in the experiment group.

In Figure 13 (c), there are 3 obvious curve with high OD readings with phosphate, glucose and PFCA in leachate culture media. The control group in the grey line, had a balanced CNP ratio by adding phosphate and glucose. However, it showed a low OD reading compared to the experiment group. The three experimental groups showed an obvious diauxic growth. After reaching an OD reading of around 0.6 at first log phase, the bacterial growth decreased, and the OD reading reduced back to 0.4 around 54h. Later, bacteria went through an obvious second growth from 54h to 110h. The second log phase brings a higher OD reading of around 1.2 and the cultivation experienced a decline phase after 110h of cultivation. Compared to the experiment group, the control group only showed the first lag phase during 24h–40h together with the experiment group. But after 40h, the OD reading decreased as a decline phase and never showed a second log phase like the experiment group.

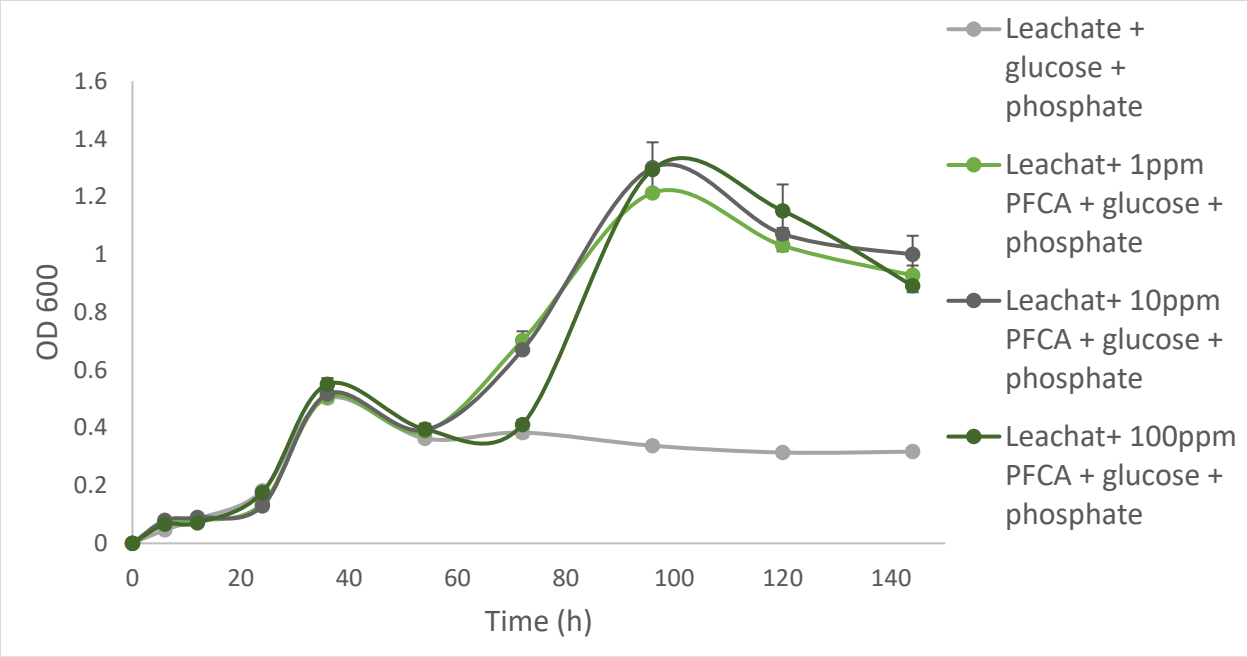
Figure 13 (c), showed that PFNA and PFDA can be consumed by the bacteria as a co-metabolism carbon source, but it needed an adequately balanced CNP ratio. There was no obvious difference in OD reading in these three experimental groups in Figure 13 (c). It meant that the concentration of PFCA in the leachate solution did not cause a big difference in the bacteria growth. Compared to all figures 13 (a), (b) and (c), it shows the first log phase during 36 h is caused by the glucose metabolism in the leachate as a carbon source. However, the diauxic growth only happened when PFCA, glucose and phosphate were all present. With a properly balanced CNP ratio, the bacteria can consume long-chain PFCA as a co-metabolism carbon source. The degradation happened in a second log phase, which achieved a much higher OD reading.


1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
OD 600
0
20
40
60
80
100
120
140
Time (h)
Leachate
Leachate + 1ppm PFCA
Leachate + 10ppm PFCA
Leachate + 100ppm PFCA

(a)


1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
OD 600
0
20
40
60
80
100
120
140
Time (h)
Leachate+glucose
Leachate + 1ppm PFCA + glucose
Leachate + 10ppm PFCA + glucose
Leachate + 100ppm PFCA + glucose

(b)



(c)

Figure 13: OD600 measurement for flask experiment with different PFCA spiking concentration (a) without glucose and phosphate. (b) with glucose but no phosphate. (c) with both glucose and phosphate

3.4.2 pH change in flask experiment

The pH was checked during the flask experiment since it is important for bacteria cultivation and metabolism; the test results are shown in Table 7. Comparing the first 3 flasks and the rest 9 flasks showed the long-chain PFCA spiking in the culture media did not affect the pH. Whether the spiking concentration was at 1 mg/L or 100 mg/L, the initial pH of the culture solution was around 8.1, which was identical to the control group or the original leachate. This is because PFCA is a weak acid, but the pH changes have been buffered since the leachate has high alkalinity. Comparing the culture media before and after spiking with phosphate showed that after KH_2PO_4 was added, the pH decreased to around 0.05. This is because the KH_2PO_4 contains hydrogen in the compound. When it dissolves in water, it releases hydrogen in the solution, which decreases the pH. However, because of the high alkalinity and low concentration of KH_2PO_4, the changing pH in the culture media is minor, which should not affect the bacteria metabolism. Overall, for the initial culturing medium, the spiking of the mineral solution, glucose, and PFCA did not cause a big change in the pH. The bacteria originally existed in the leachate for a long time; it should get used to the base environment, and no pH modification was required before the leachate cultivation. When comparing the initial and final pH, it showed an

obvious pH increase from 8 to 9 during cultivation. In other people's research, pH increased during aerobic biodegradation of landfill leachate (Zhang 2012; Sanguanpak et al. 2015). The metabolic by-product of the microbial consortia in the leachate can cause it. The final pH varies from 9.08 to 9.4 but does not show high relevance to mineral addition or PFCA addition.

Table 7: pH changes of flask experiment

Flask number	Flask description	Initial pH	Final pH
1	Leachate	8.1	9.4
2	Leachate + glucose	8.08	9.36
3	Leachate + glucose + KH_2PO4	8.04	9.35
4	Leachate + 1 mg/L C9 & C10	8.1	9.13
5	Leachate + 1 mg/L C9 & C10 + glucose	8.1	9.22
6	Leachate + 1 mg/L C9 & C10 + glucose + KH_2PO_4	8.08	9.16
7	Leachate + 10 mg/L C9 & C10	8.11	9.08
8	Leachate + 10 mg/L C9 & C10 + glucose	8.11	9.22
9	Leachate + 10 mg/L C9 & C10 + glucose + KH_2PO_4	8.07	9.11
10	Leachate + 100 mg/L C9 & C10	8.11	9.11
11	Leachate + 100 mg/L C9 & C10 + glucose	8.11	9.22
12	Leachate + 100 mg/L C9 & C10 + glucose + KH_2PO_4	8.05	9.07

3.4.3 Long-chain PFCA degradation efficiency in flask experiment

According to the LC-MS analysis result, the concentration of PFNA and PFDA has been measured in blank samples and the final solution for each flask. The difference in concentration has been used to calculate the removal of the long-chain PFCA during the flask experiment.

The result in Table 9 shows both PFNA and PFDA have a high variation of measured concentration in the blank. For 1 mg/L low-concentration spiking samples (Flask 4,5,6), the initial measured concentration of blank samples is around 0.75 mg/L for PFNA and 0.65 mg/L for PFDA. For the 10 mg/L medium concentration spiking samples (Flask 7,8,9), the measured concentration of blank was around 9.5 mg/L for PFNA and 12.5 mg/L for PFDA. However, when spiking concentrations increased to 100 mg/L, the measured concentration on the blank

was relatively low, 85 mg/L on PFNA and only around 45 mg/L on PFDA. The variation inmeasured concentration of blank in the flask experiment can be caused by the auto degradation of long-chain PFCA during the storage. Table 8 shows with a 1 mg/L low spiking concentration in water, after being stored in the refridgerator for 6 days, both PFNA and PFDA show slight auto degradation. The concentration dropped from 1 mg/L to 0.8 mg/L. When spiking 100 mg/L, the auto degradation happened more. After storing in the refrigerator for 7 days, only 57 mg/L was left for PFNA and 48 mg/L left for PFDA. According to the control group testing result, the long-chain PFCA was unstable at high concentrations, and PFDA was less stable than PFNA. Comparing the test result between Table 9 and Table 8, it showed that the low initial measured concentration in the flask experiment was caused by the instability of the compound.

Table 8: Concentration analysis for the control group

Samples condition	Original concentration of PFNA (mg/L)	Original concentration of PFDA (mg/L)
Fridge 4 °C 1 mg/L spiked	0.8	0.8
Fridge 4 °C 100 mg/L spiked	57.1	48.2

In Table 9, the final concentration is sometimes higher than the initial concentration. There are main 2 potential reasons for that result. Firstly, it can be caused by the high standard deviation in the testing error; secondly, it can be formed by the degradation of other unknown prosecutors in the leachate (Health Canada 2012). However, all these experimental groups have been marked as not applicable in Table 9, meant that there was no degradation detected in these flask experiments. However, in flasks 6, 9, and 12, both PFNA and PFDA concentrations decreased at the end of the cultivation period. In flask 6, with 1 mg/L PFCA concentration, it showed a 26% removal of PFNA and 46% removal of PFDA. When 10 mg/L was spiked, the removal efficiency of PFNA increased to 38%, and the removal efficiency of PFDA increased to 58%. In the 100 mg/L spiked flask, the removal of PFNA and PFDA were 35% and 45%, respectively. Flasks 6,9,12 were spiked with PFCA under different concentrations and balanced with CNP ratio using glucose and KH_2PO_4. In Figure 13 (c), all three 3 flasks showed high OD readings during the second log phase, which showed that the bacteria consumed PFCA as a carbon source. Another experimental group (Flask 4,5,7,8,10,11), which did not contain mineral and carbon sources spiking, offers a low OD reading and no PFCA degradation was detected. When comparing flasks 3,6 and 9, the degradation showed a little increase as the spiking

concentration increased, but the overall degradation of PFNA was around 28% and for PFDA, it was approximately 50%. It matcheed the OD graph in Figure 13 (c) that all three 3 flasks showed similar bacteria growth trends and similar peak OD values. These flasks demonstrated that the concentration of PFCA did not affect the bacterial metabolism rate and showed a minor effect on the final PFCA removal efficiency. For 100 mg/L conditions, the PFCA underwent some degradation so that the blank sample has a reading of around 88 mg/L of PFNA and 45 mg/L of PFDA. However, with biodegradation, the removal efficiency of long-chain PFCA increased even further. Comparing the removal efficiency of PFNA and PFDA under the same concentration showed that the removal efficiency of PFDA is always higher than that of PFNA. Table 9 indicated that for 100 mg/L concentration, after 6 days, the concentration of PFDA dropped by 50%, but PFNA only dropped approximately by 30%. It meant that the PFDA was less stable than PFNA and more biodegradable.

Table 9: Long-chain PFCA removal efficiency during flask experiment (ND means no degradation detected)

Flask number	Flask description	Initial C9 concentration (mg/L)	Final C9 concentration (mg/L)	Initial C10 concentration (mg/L)	Final C10 Concentration (mg/L)	Degradation percentage of C9	Degradation percentage of C10
1	Leachate						
2	Leachate + glucose	0.00	0.00	0.00	0.00	ND	ND
3	Leachate + glucose + KH_2PO_4						
4	Leachate + 1 mg/L C9 & C10	0.78	0.87	0.66	1.17	ND	ND
5	Leachate + 1 mg/L C9 & C10 + glucose	0.75	0.88	0.63	0.91	ND	ND
6	Leachate + 1 mg/L C9 & C10 + glucose + KH_2PO_4	0.76	0.56	0.75	0.40	25.96	46.66
7	Leachate + 10 mg/L C9 & C10	9.67	10.75	13.03	14.89	ND	ND
8	Leachate + 10 mg/L C9 & C10 + glucose	9.07	10.89	12.28	14.22	ND	ND

9	Leachate + 10 mg/L C9 & C10 + glucose + KH_2PO_4	10.22	7.32	13.50	5.63	28.35	58.27
10	Leachate + 100 mg/L C9 & C10	83.35	92.81	51.83	48.09	ND	7.22
11	Leachate + 100 mg/L C9 & C10 + glucose	81.78	91.39	45.86	57.06	ND	ND
12	Leachate + 100 mg/L C9 & C10 + glucose + KH_2PO_4	89.54	59.11	38.94	21.40	33.98	45.04

3.5 Conclusions

The flask experiment unveiled promising prospects for the biodegradation of PFNA and PFDA. The identification of a pre-existing bacterial community within PFCA-enriched landfill leachate highlighted its inherent capability to metabolize long-chain PFCA compounds as a carbon source. However, to optimize biodegradation, the crucial role of additional mineral supplementation in achieving a balanced carbon, nitrogen, and phosphorus (CNP) ratio within the leachate became apparent. This strategic adjustment fine-tuned the culturing conditions, fostering heightened microbial activity and optimizing degradation efficiency.

When PFNA and PFDA were stored at 4°C for 6 days, auto-degradation reached 20% for 1 mg/L spiking and approximately 50% for 100 mg/L spiking. In the flask experiment, testing results of PFNA and PFDA exhibited a high standard deviation of around 17% due to operational and measurement errors. However, during the optimization of degradation conditions, the removal of long-chain PFCA in the culturing media was still observed. PFNA achieved the highest removal efficiency of 34% over a 6-day culturing period with 100 mg/L spiking, while PFDA reached the highest removal efficiency of 58% throughout the culturing period with a spike of 10 mg/L.

For the same concentration, the removal efficiency of PFDA exceeded that of PFNA, demonstrating that as chain length increased, biodegradability also increased. Overall, this flask experiment demonstrated the biodegradability of PFNA and PFDA in landfill leachate, affirming the feasibility of using an aerobic mix culturing bioreactor to treat long-chain PFCA contaminants. This treatment method holds the potential for scaling up at the industrial level and could eventually be applied in wastewater treatment processes to address the PFC problem.

Chapter 4: Thesis Conclusions

A comprehensive examination of long-chain perfluorocarboxylic acids (PFCA) including PFNA and PFDA presence in landfill leachate was conducted. The biodegradability of long-chain PFCA using native microorganisms of collected leachate samples was also evaluated. The degradation experimental setup was designed to be applicable for potential upscaling in industrial settings or for incorporating hybrid treatment methods in wastewater treatment plants (WWTP).

As mentioned in Table 10, the characterization of leachate revealed that the majority of total solids consisted of total dissolved solids, and ammonia was identified as the predominant form of nitrogen in the leachate. Various solid and chemical parameters in the leachate exhibited seasonal fluctuations, with concentrations peaking in summer and decreasing in winter. PFNA and PFDA were detected in the leachate at concentrations ranging from 100 to 1000 ng/L. Despite notable fluctuations throughout the year and a high standard deviation, no discernible seasonal pattern was observed.

The biodegradation of PFNA and PFDA was successfully demonstrated in flask experiments, confirming the feasibility of leveraging the existing microbial community in the leachate to metabolize long-chain PFCA as a carbon source. The OD600 graph indicated diauxic growth at 36 h and 100 h. The second log phase occurred when phosphate, glucose, and PFCA were introduced into the flask, resulting in a higher OD reading of 1.3. PFDA exhibited a higher degradation efficiency up to 58% compared to PFNA which is 34%. Both compounds showed auto-degradation when stored at high concentrations. This implies that as the chain length increases, PFCA becomes less stable and more susceptible to biodegradation.

Table 10: Conclusion of hypothesis and outcome

Hypothesis number	Hypothesis	Outcome
1	The contaminants in the landfill leachate have seasonal variations throughout the year due to leachate dilution and human activity.	Various solid and chemical parameters have seasonal variations and can get affected by human activity.

2	PFNA and PFDA exist in the landfill leachate at low concentrations around 0.5 ng/L and the concentration of PFNA and PFDA can be affected by seasonal variation.	PFNA and PFDA exist in the leachate with concentrations from 100 ng/L-1000 ng/L with no seasonal variation.
3	The microbial community from the landfill leachate can consume the dissolved long-chain PFCA as carbon sources and degrade the PFDA and PFNA under aerobic conditions	The microbial community can perform aerobic degradation of PFNA and PFDA in the flask experiment with up to 58% removal.

Chapter 5: Thesis Outlook and Recommendations

5.1 Sampling and leachate characterization

In recent years, long-chain perfluoroalkyl carboxylic acids (PFCA) have attracted increased attention as emerging contaminants. They have been detected in various environments, with landfill leachate emerging as a primary source of PFCAs. This study undertakes leachate characterization within one week of sample collection. However, the testing results for nitrate and nitrite exhibit significant fluctuations due to their inherent instability.

Due to the high ammonia concentration and presence of bacteria in the leachate, storing samples in a refrigerator for over a week could lead to the conversion of ammonia into nitrate and nitrite through a nitrification reaction. The concentrations of nitrate and nitrite vary in the samples across different days of analysis. To address this, future protocols should involve transporting leachate samples in ice bags and conducting characterization within 48 hours. A substantial difference in nitrogen, phosphate, and COD concentrations is observed between summer and winter. For instance, ammonia and total nitrogen show variations from 600 mg/L in summer to 100 mg/L in winter, necessitating dilution factors and potentially causing reading fluctuations. Utilizing a diverse range of test kits tailored to different concentration levels can enhance measurement precision.

Throughout the sample characterization process, duplicate measurements are systematically undertaken at predetermined intervals, ensuring the reliability and reproducibility of the obtained data. For future experiments, considering duplicate or even triplicate analyses for each sampling point can provide more precise readings. Human activities during the sampling period (July 2022 to July 2023) significantly impact test results, causing fluctuations in parameters. Processes like pumping pond water into wet wells in March and May 2023, as well as pumping wastewater from the construction landfill into wet wells in July 2023, result in leachate dilution and fluctuations in total nitrogen, total solids, total phosphate, and COD. These parameters do not return to the levels observed in July 2022 due to leachate dilution. Future experiments might benefit from extending the sampling period to observe the resurgence of leachate concentration post-dilution, enabling a more comprehensive cycle analysis.

While heavy metal analysis is included in this research, it is solely used to calculate mineral spiking requirements in flask experiments. Future studies can leverage this data to explore seasonal variations in heavy metal concentrations in landfill leachate.

5.2 PFCA analysis

This thesis suggests a need for further expansion in the research on long-chain PFCA, Precisely measuring the concentration of PFCAs directly in the leachate proves challenging due to its low concentration. The use of the SPE process eliminates the matrix effect from the leachate, which leads to a more accurate signal reading. Despite undergoing the SPE process, the detected PFCA concentration in the samples still falls below the LOQ. In future studies, nitrogen evaporation can be employed to boost PFCA concentration in the samples. Additionally, the high dissolved solids of leachate block the cartridge during sample loading, restricting the loaded sample volume. Researchers may also utilize a vacuum pump to load more samples into the cartridge. Achieving sample concentrations consistently above the LOQ would contribute to more precise machine readings and measurements of PFCAs in environmental samples.

Operational errors by humans and carry-over errors by machines can impact test results. During LC-MS analysis, it is essential to perform a washing step to flush the tubing and column between different samples, particularly between the calibration standard solution and samples, to prevent carry-over errors.

5.3 Biodegradation

In the flask experiment, the initial and final concentrations of the target compound were measured. The reduction in concentration indicates the biodegradation of long-chain PFCA during the experiment. However, it could also result from bioaccumulation, which was not measured in this experiment. In future experiments, researchers should measure PFCA concentration in the biomass to determine bioaccumulation in microbes. Additionally, using a fluoride meter to measure the concentration of free fluoride in the final solution, this can be crucial for calculating the defluorination ratio.

According to previous research, long-chain PFCA undergoes stepwise degradation, and the degradation products of PFNA and PFDA can be other short-chain PFCAs such as PFOA/PFHPA (Trojanowicz et al. 2018). By tracking the degradation by-products and intermediate products, researchers can gain a better understanding of the bio-degradation mechanisms.

This study demonstrated the potential of microbial communities within landfill leachate to Degrade PFNA and PFDA. However, the specific bacteria responsible for this degradation remain unidentified, necessitating additional research to acclimate and isolate the particular bacterium involved in degradation. Inoculating the isolated and acclimated bacteria into a bioreactor can decrease the lag phase of bacterial growth. Subsequent DNA sequencing of the isolated bacteria can reveal its specific species. Building upon the findings of the flask experiment, a scaled-up study can be conducted. Throughout the biodegradation process, various parameters, such as carbon source consumption, nitrogen, and phosphate levels, can be monitored. Further investigation into the consumption rates of these compounds and the implementation of continuous feeding by subsequent researchers could lead to additional improvements in PFCA degradation. Additionally, the utilization of active sludge in wastewater treatment proves beneficial, as the microbial community within the sludge can concurrently facilitate PFCA degradation during the treatment process.

The research on the flask experiment yielded success; however, a substantial disparity persists between laboratory findings and practical wastewater treatment. The concentration of PFCA in the natural environment is typically below 1 ng/L. In contrast, the biodegradation experiments were conducted at high concentrations ranging from 1-100 mg/L, surpassing natural concentrations by 1000 to 100,000 times. The ability of bacteria to degrade PFCA under elevated concentrations does not necessarily equate to the same efficiency at lower concentrations. To address this, future research should use low-concentration of long-chain PFCA for biodegradation.

While biological degradation is a viable approach, chemical degradation stands out as a more efficient technology. Established chemical degradation methods, such as advanced oxidation processes (AOP) and photodegradation, have demonstrated effectiveness in PFCA removal. Recognizing that individual methods may fall short of ensuring efficient PFCA treatment, the

exploration of hybrid methods emerges as a promising direction for future research (Ruiz-Urigüen et al. 2022).

5.4 Real-world application

To enhance practical relevance in controlling the emerging contaminants at source in real-world scenarios, researchers should direct their treatment efforts towards pollution sources like landfill sites, industrial wastewater treatment facilities, and firefighting stations, rather than natural water systems. This emphasis is crucial because the concentration of contaminants is typically higher at the pollution source, facilitating more efficient degradation. Moreover, chemical and biological both degradation techniques can be used when dealing with higher concentrations of contaminants. However, considering the stringent drinking water standard in Canada, measured in parts per trillion (ng/L), no degradation methods are currently available for such low concentrations. Consequently, the sole viable option is the use of conventional physical removal methods, such as employing a GAC absorbent as a tertiary treatment method.

However, after contaminants have been concentrated in the absorption media, treatment can be carried out using either chemical or biological methods. This strategy enhances the efficiency of pollutant removal, particularly when dealing with high concentrations concentrated in specific locations. Moreover, it allows for the recycling of absorbent media, making the overall process more economically viable. Furthermore, tertiary treatment not only eradicates trace contaminants but also efficiently eliminates toxic by-products, playing a pivotal role in safeguarding water safety.

5.5 Recommendation

According to the experiment results from the thesis study, following recommendations has been made for the future reasrch:

a) Transport leachate samples using ice bags and characterize/analyse them within 48 hours.
b) Utilize test kits with varying ranges to assess samples with different concentration levels. Conduct duplicate and triplicate analyses at each sampling point to obtain standard deviation and average values.

c) Extend the sampling period to observe a more comprehensive cycle analysis.
d) Conduct seasonal variation studies on heavy metal content in landfill leachate.
e) Employ a vacuum pump to load additional samples into the cartridge during SPE and use a nitrogen evaporator to enhance PFCA concentration in the samples.
f) Evaluate PFCA concentration in biomass to determine the bioaccumulation of PFCA in microbes.
g) Test free fluoride ions and other short-chain PFCA to determine the defluorination ratio.
h) Isolate and acclimate bacteria, and conduct DNA sequencing tests to identify specific species. Apply the bacteria in further scaled-up bioreactor tests.
i) Investigate and optimize the biodegradability of PFCA under low concentrations.
j) Integrate chemical and biological degradation methods for a hybrid approach to PFCA treatment.

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Appendix

Appendix 1: Landfill site picture

Supporting Figure 1: Landfill site view from road

Supporting Figure 2: Close look from the top of the landfill

Supporting Figure 3: Constructing landfill with geotextile set on the bottom.

Appendix 2: Equations for the leachate solids test

$$Total\ solids = \frac{container\ dried\ weight - container\ initial\ weight}{volume\ of\ unfiltered\ leachate}$$

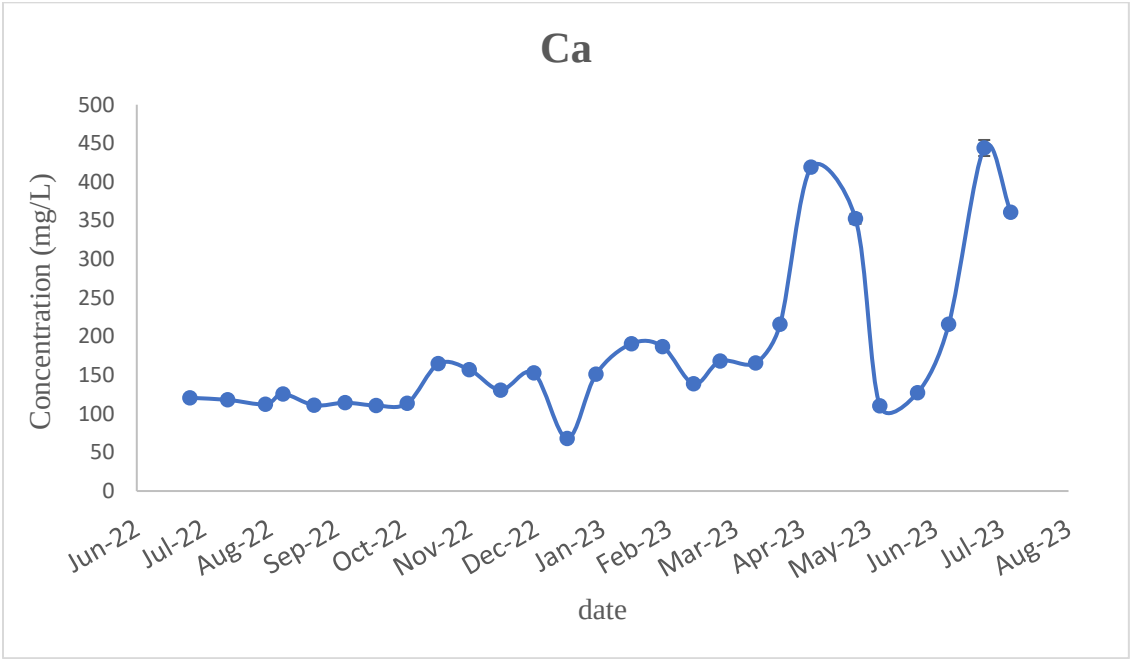
$$Total\ volatile\ solids = \frac{container\ dried\ weight\ - container\ furnaced\ weight}{volume\ of\ unfiltered\ leachate}$$

$$Total\ suspended\ solids = \frac{filter\ paper\ dried\ weight - filter\ paper\ initial\ weight}{filtered\ volume}$$

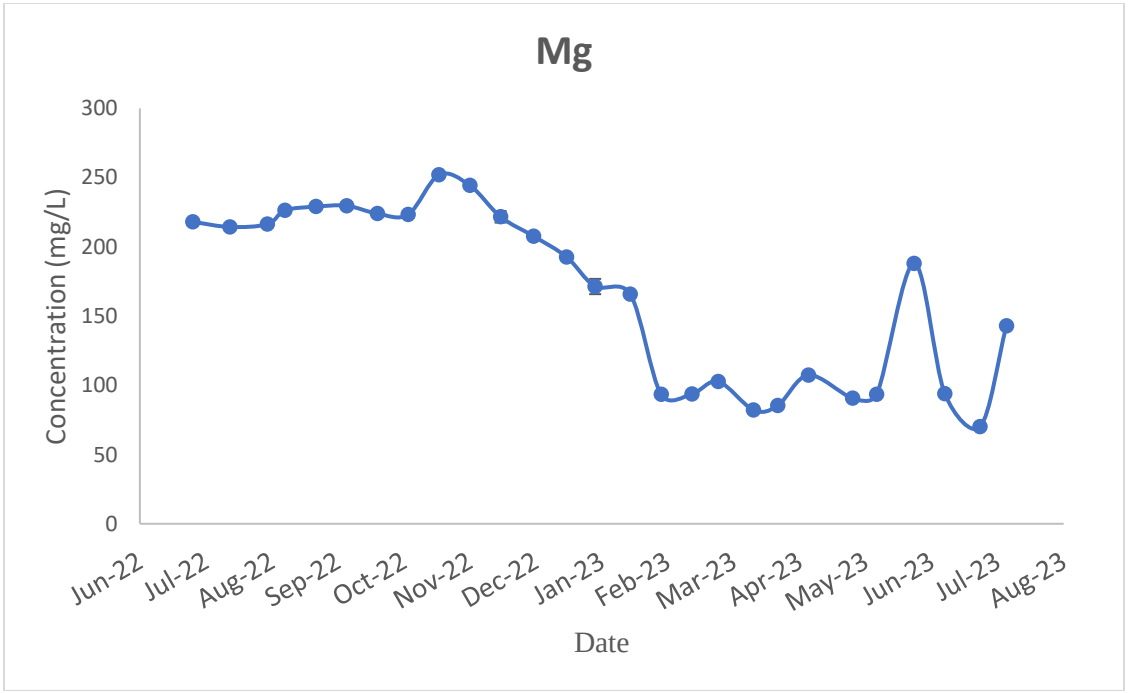
$$Volatile\ suspended\ solid = \frac{filter\ paper\ dried\ weight - filter\ paper\ furnaced\ weight}{filtered\ volume}$$

$$Total\ dissolved\ solids = \frac{container\ dried\ weight - container\ initial\ weight}{volume\ of\ filtered\ leachate}$$

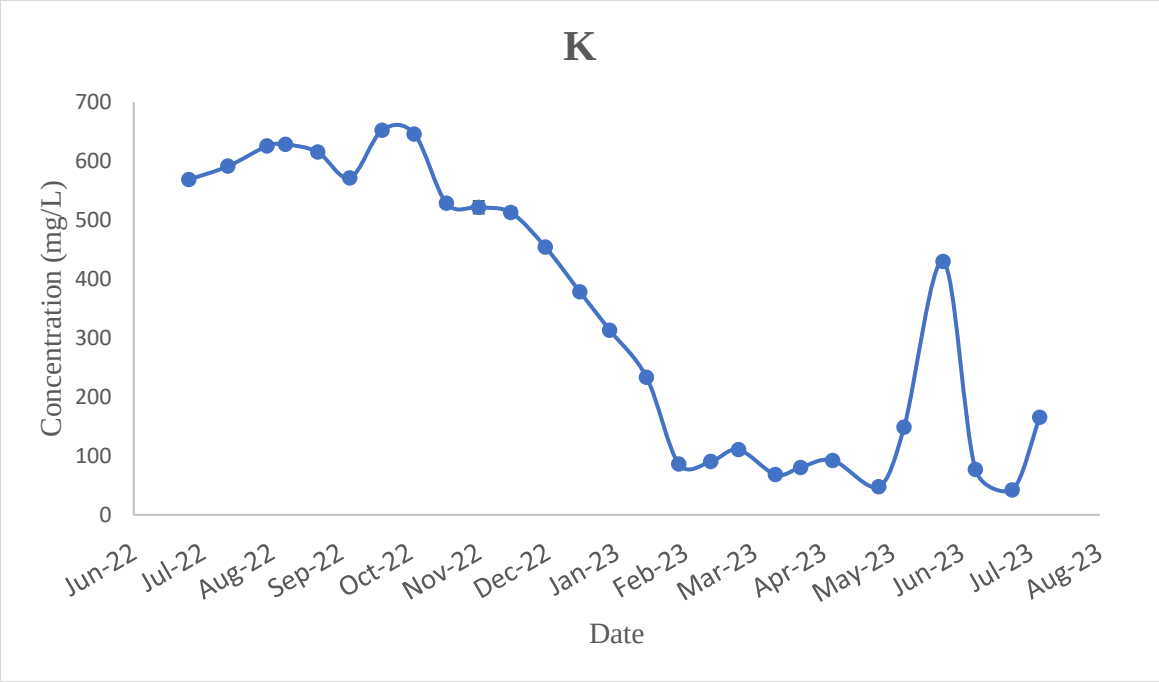
Appendix 3: Heavy metal analysis and seasonal variation



(a)



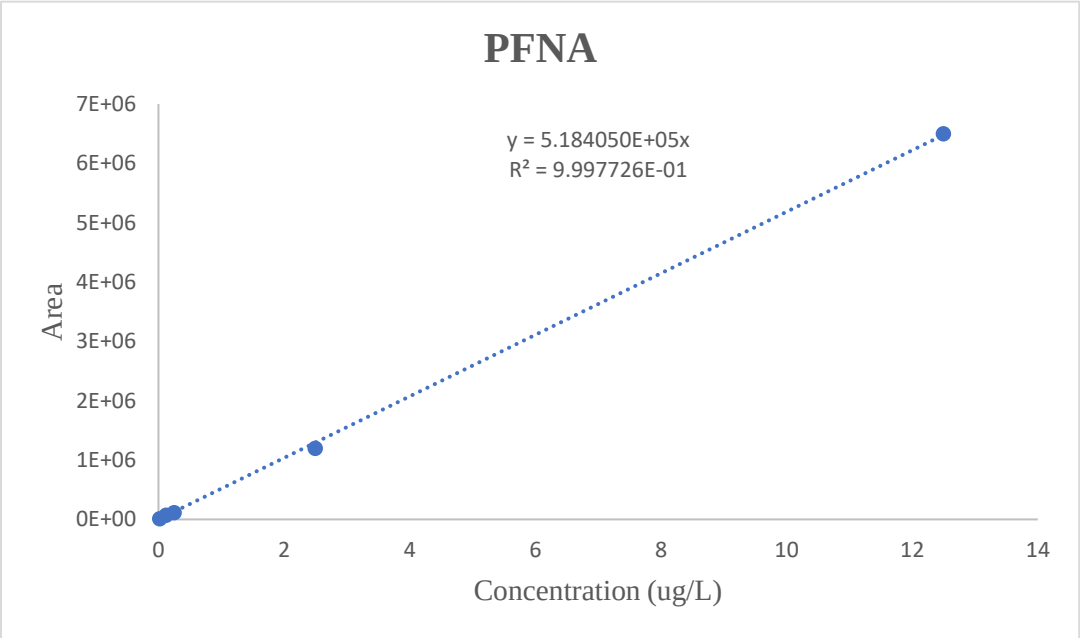
(b)



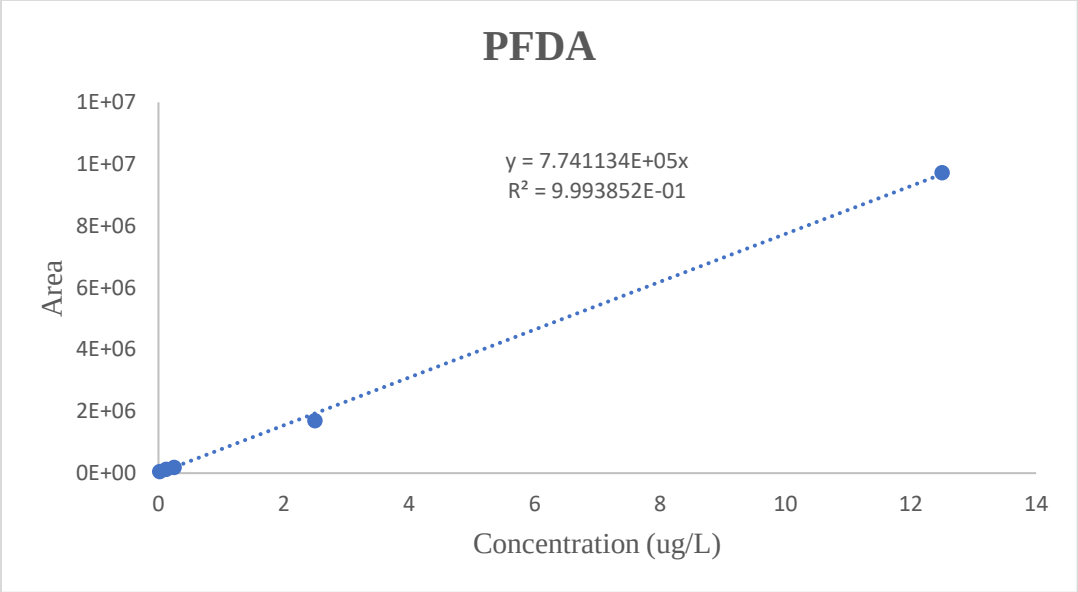
(c)

Supporting Figure 4: Heavy metal characterization result for Ca (a), Mg (b) and K (c).(The standard deviation is too small to show on the figure)

Appendix 4: Calibration curve for recovery rate calculation



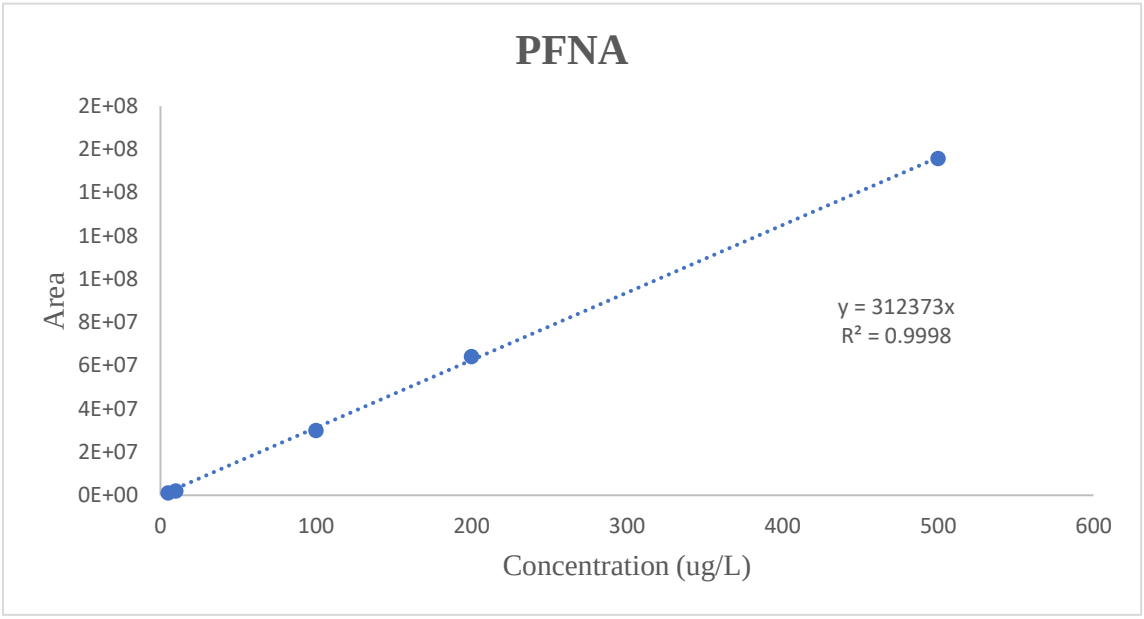
(a)



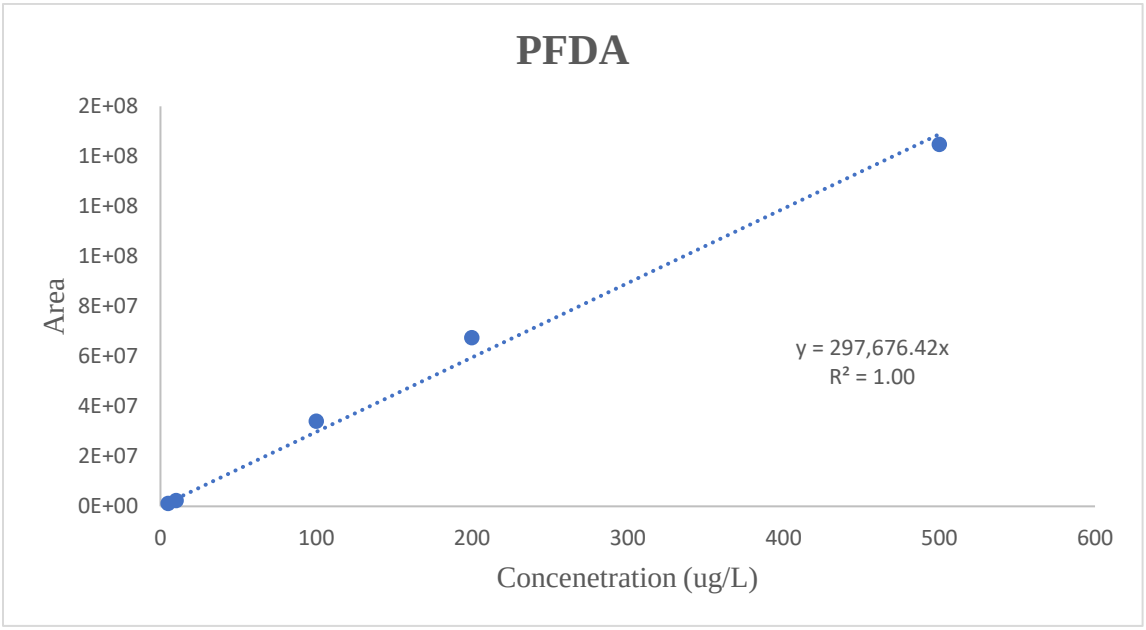
(b)

Supporting Figure 5: Calibration curve for PFNA (a) and PFDA (b) used for recovery rate calculation

Appendix 5: Calibration curve for biodegradation experiment



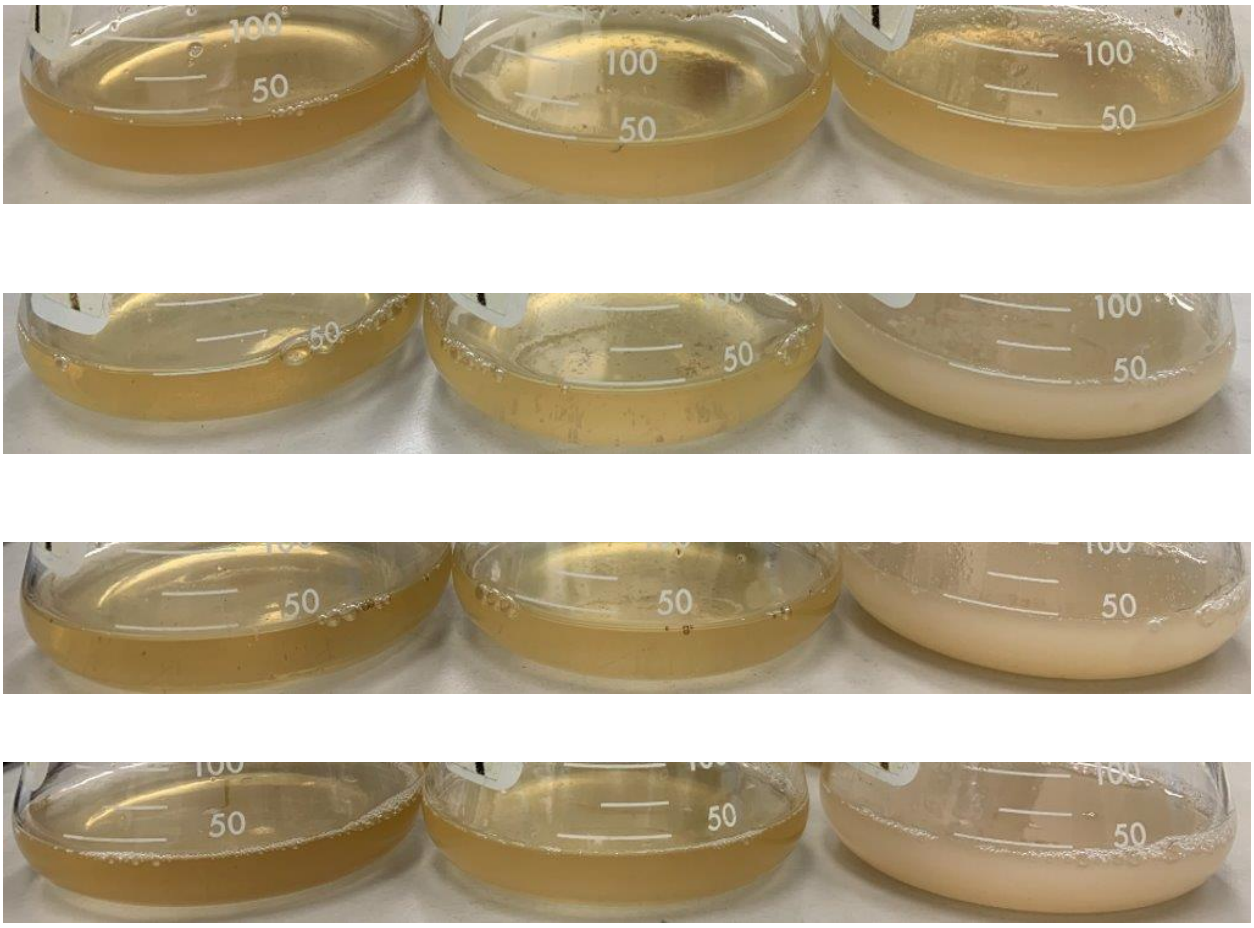
(a)



(b)

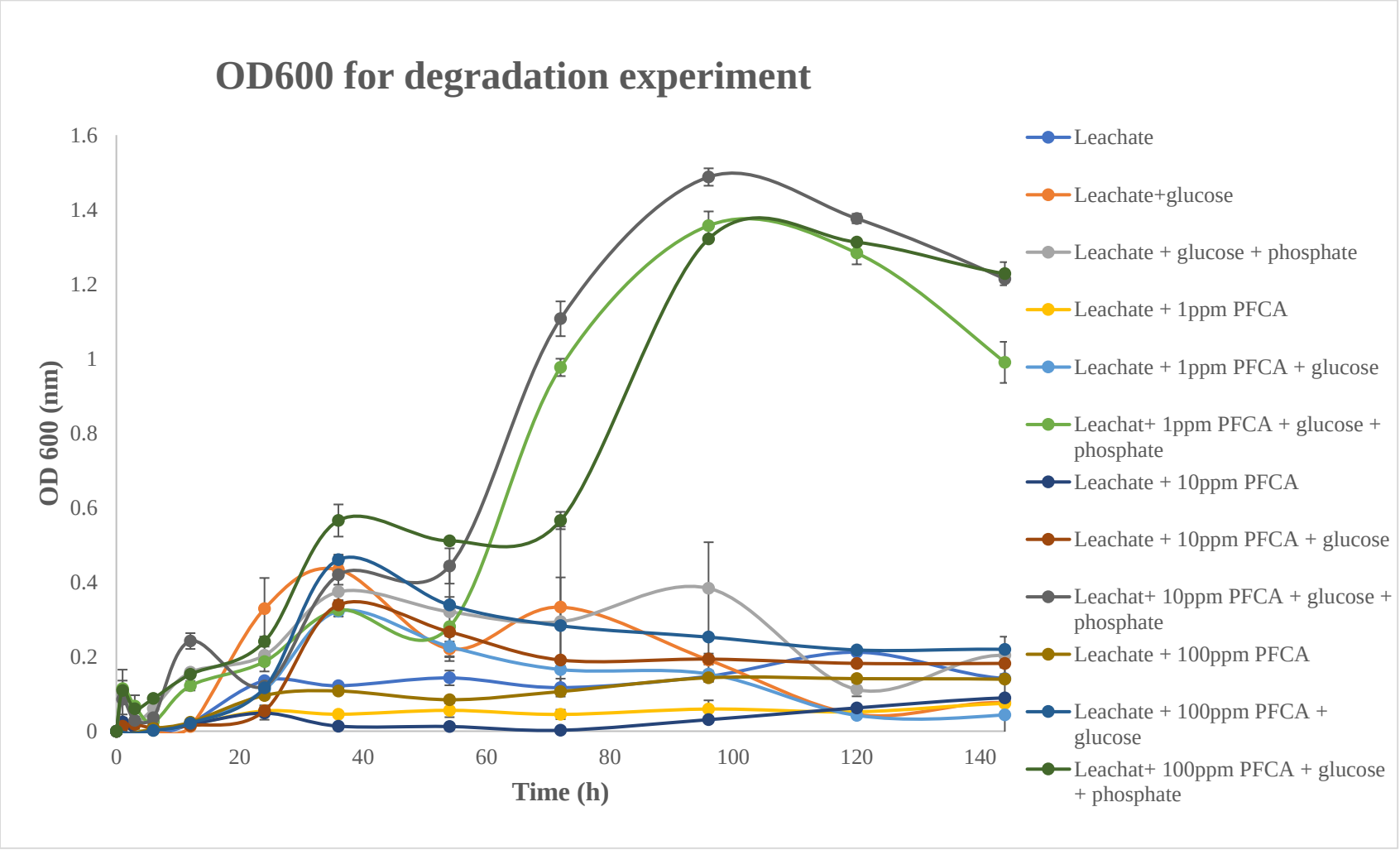
Supporting Figure 6: Calibration curve for PFNA (a) and PFDA (b) used in biodegradation experiment

Appendix 6: Turbidity picture of bacterial growing in the flask experiment

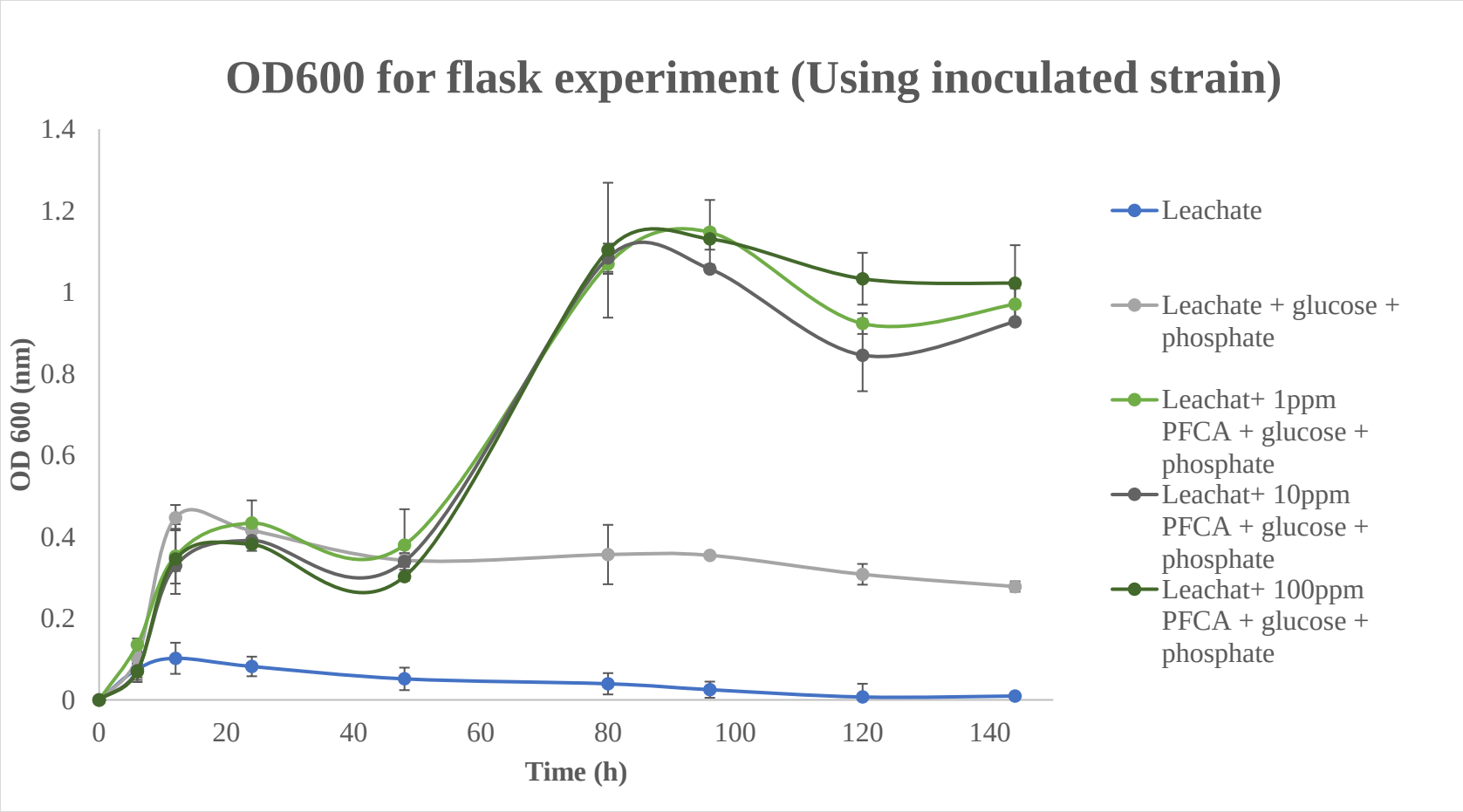


Supporting Figure 7: Turbidity of each after after culturing period. (From top to bottom are control, 1 mg/L spiked, 10 mg/L spiked, 100 mg/L spiked; from left to right are pure leachate, leachate with glucose, leachate with glucose and phosphate)

Appendix 7: Cross check of the PFCA degradation experiment



Supporting Figure 8: OD600 measurement for flask experiment with different PFCA spiking concentration (cross check)



Supporting Figure 9: OD600 measurement for flask experiment using inoculated bacteria strain from previous degradation experiment(cross check)

Supporting Table 1: Long-chain PFCA removal efficiency during flask experiment using inoculated bacteria strain

Flask number	**Flask description**	**Initial C9 concentration (mg/L)**	**Final C9 concentration (mg/L)**	**Initial C10 concentration (mg/L)**	**Final C10 Concentration (mg/L)**	**Degradation percentage of C9**	**Degradation percentage of C10**
6	Leachate + 1 mg/L C9 & C10 + glucose + KH_2PO_4	1.04	0.66	0.50	0.29	36.56	41.65
9	Leachate + 10 mg/L C9 & C10 + glucose + KH_2PO_4	7.41	4.84	6.31	2.75	34.72	56.45
12	Leachate + 100 mg/L C9 & C10 + glucose + KH_2PO_4	62.77	30.12	27.66	7.69	52.02	72.21