Advanced treatments for PFAS removal from landfill leachate: evaluating biological and ozone based chemical approaches

Abstract

The increasing waste generation driven by industrialization, urbanization, and population growth has reinforced the reliance on landfilling, which remains a dominant waste management practice. Landfill leachate is a highly complex matrix containing both biodegradable and recalcitrant compounds, including micropollutants such as per- and polyfluoroalkyl substances (PFAS). These synthetic fluorinated compounds, characterized by their high chemical stability and widespread use, are released into leachate at concentrations reaching up to thousands of µg/L, posing significant challenges for conventional treatment technologies.

This study investigates the removal of PFAS from landfill leachate using two bench-scale treatment schemes: (1) a biological treatment in a Sequencing Batch Biofilter Granular Reactor (SBBGR) and (2) a hybrid ozone-enhanced biological treatment. The SBBGR operated in sequential cycles (8 h), and in the integrated scheme, an ozonation phase (4.0–5.5 g/L) was incorporated to oxidize recalcitrant compounds. The experimental phases included biomass acclimation to high salinity (23 mS/cm), steady-state biological treatment in SBBGR mode, ozone-enhanced treatment, and biological treatment with leachate enriched with higher PFAS concentrations compared to the previous phases.

The SBBGR biological treatment effectively reduced key pollutants, including nitrogen and long-chain PFAS. While ozone integration enhanced some removal aspects, it proved less effective than expected for PFAS removal. The findings highlight the potential of the SBBGR biological system and the need for further optimization to address persistent contaminants.

Photos of PFAS sources, landfill leachate, and the SBBGR reactor.

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Advanced treatments for PFAS removal from landfill leachate: evaluating biological and ozone based chemical approaches

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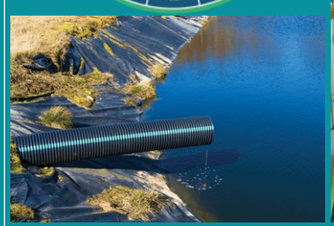

Politecnico di Bari

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Al Magnifico Rettore
del Politecnico di Bari

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Advanced treatments for PFAS removal from landfill leachate: evaluating biological and ozone based chemical approaches

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		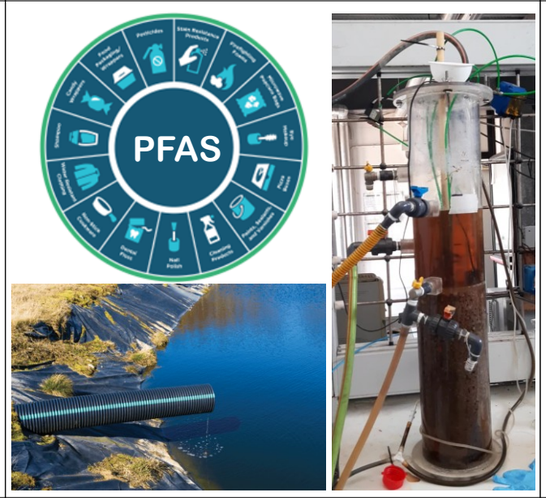	

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		Trattamenti avanzati per la rimozione dei PFAS dal percolato di discarica: valutazione di approcci biologici e chimici con ozono.	
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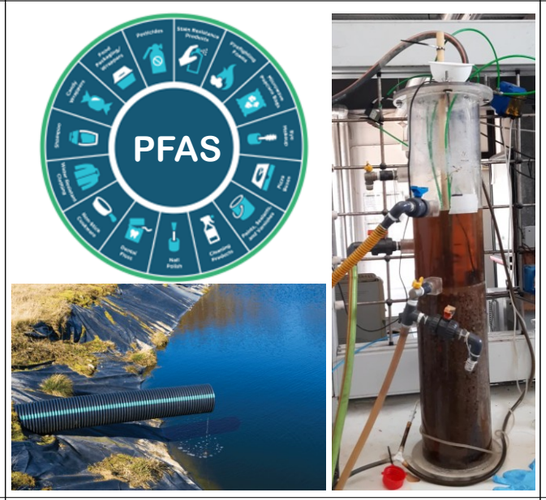
			

To my parents,

for their loving support.

EXTENDED ABSTRACT

In the last decades, the rapid industrialization, the population growth and the urbanization led to the increase in waste generation. The practices of reuse, recycling, energy and matter recovery from waste are not sufficient to cope with this rise. Therefore, landfilling still remains a widely used practice. Within landfills, waste undergoes a number of physical, chemical and biological changes and releases micropollutants within the landfill leachate, making this matrix one of the most difficult to treat (Kumar et al., 2023).

Leachate is a complex mixture containing very high concentrations of biodegradable and recalcitrant toxic compounds (Qian et al., 2024). It contains a variety of micropollutants, such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), pharmaceuticals, personal care products, pesticides, microplastics (MPs), per- and polyfluoroalkyl substances (PFASs), and many more. Although micropollutants are present in very low concentrations they have significant impacts on the ecosystems, economy, human health.

The subject of this study are per- and polyfluoroalkyl substances, better known as PFAS, substances synthesized since the 1950s and entered the composition of a great many commercial products because of their outstanding hydro-, oleo-repellency and high stability characteristics. PFASare found in a wide range of products, from fertilizers to food packaging, from personal hygiene products to fire-fighting foams. These, at the end of their life cycle, sent to landfills release fluorinated substances into leachate, where the concentrations can reach up to thousands of µg/L (Gallen et al., 2017).

This study stems from the need to identify a solution for PFAS removal in landfill leachate and aims to evaluate the removal efficacy of PFAS and other key chemical parameters of two bench-scale treatment schemes: a biological treatment conducted in a Sequencing Batch Biofilter Granula Reactor (SBBGR) - and an ozone-enhanced biological treatment. Given the matrix complexity and

the high chemical stability of PFAS, conventional treatments are inadequate for the removal of fluorinated substances. Hence the need to investigate an integrated approach combining biological degradation with chemical oxidation. Biological treatment was conducted in an SBBGR, an advanced biological treatment system, which is an upflow reactor in which leachate was fed, treated, and extracted sequentially. The plant operated in sequential mode with 8-hour treatment cycles. Each cycle featured a fill, reaction, and discharge phase. The chemical upgrading included an additional phase, the integration of biological degradation with chemical oxidation, performed with ozone at two different doses (4.0 g/L and 5.5 g/L). The discontinuity of the SBBGR system allowed oxidative treatment with ozone to be used in a specific and controlled manner. The ozonation step, following biological treatment, was specific for resistant biological degradation compounds. Ozone, dosed in a controlled manner, allowed for the partial oxidation of recalcitrant substances before returning them back to the biomass action.

The experimentation consisted of four phases: a start-up phase, a second phase with a steady state biological , a third phase in which biological treatment was enhanced with ozone at two different dosages, and a final phase in which reactor worked in biological mode fed with leachate at high PFAS concentration. During the preliminary start-up phase, an appropriate feeding program was used: gradual dilutions with water at decreasing ratios to acclimate the biomass to high salinity values (approximately 23 mS/cm) and to stimulate the growth of the species involved in the process.

The influent and effluent of all treatment schemes were characterized in terms of traditional parameters and PFAS. Per- and polyfluoroalkyl substances were analyzed by a mass spectrometer interfaced with very high-pressure liquid chromatography.

Keywords: landfill leachate, PFAS, biological treatment, advanced oxidation, ozone.

EXTENDED ABSTRACT (ITA)

La rapida industrializzazione, la crescita della popolazione e l'urbanizzazione hanno determinato negli ultimi decenni un aumento della produzione dei rifiuti. L'incremento delle pratiche di riuso, riciclo e recupero di energia e materia dai rifiuti non sono sufficienti a fronteggiare questo incremento. Pertanto il conferimento in discarica rimane ancora una pratica ampiamente utilizzata. All'interno delle discariche, i rifiuti subiscono una serie di cambiamenti fisici, chimici e biologici e liberano microinquinanti all'interno del percolato da discarica, rendondo questa matrice una delle più difficili da trattare (Kumar et al., 2023). Il percolato è una miscela complessa contenente concentrazioni molto elevate di composti biodegradabili e composti tossici recalcitranti (Qian et al., 2024). Contiene diversi microinquinanti, come i policlorurati bifenili (PCB), gli idrocarburi policiclici aromatici (IPA), i farmaci, i prodotti per la cura della persona, i pesticidi, le microplastiche (MP), le sostanze per- e polifluoroalchiliche (PFAS) e molte altre ancora. Nonostante i microinquinanti siano presenti in concentrazioni molto basse hanno un significativo impatto sugli ecosistemie sull'uomo.

Oggetto di questo studio sono le sostanze per- e polifluoroalchiliche, meglio note come PFAS, sostanze sintetizzate a partire dagli anni '50 ed entrate nella composizione di moltissimi prodotti commerciali per le loro straordinarie caratteristiche di idro, oleo repellenza ed elevata stabilità. I PFAS sono presenti in una vasta gamma di prodotti: dai fertilizzanti agli imballaggi alimentari, dai prodotti per l'igiene personale alle schiume antincendio. Questi, al termine del ciclo di vita, conferiti in discarica rilasciano nel percolato sostanze fluorurate, le cui concentrazioni possono raggiungere fino a migliaia di μg/L (Gallen et al., 2017).

Questo studio nasce dalla necessità di individuare una soluzione per la rimozione dei PFAS dal percolato da discarica ed ha lo scopo di valutare l'efficacia di

rimozione di queste sostanze e dei principali parametri chimici in due schemi di trattamento a scala da banco: un trattamento biologico - condotto in un reattore granulare Sequencing Batch Biofilter (SBBGR) - e un trattamento biologico potenziato con ozono. Data la complessità della matrice e l'elevata stabilità chimica dei PFAS, i trattamenti convenzionali risultano inadeguati per la rimozione delle sostanze fluorurate. Da qui l'esigenza di studiare un approccio integrato che combina la degradazione biologica con l'ossidazione chimica. Il trattamento biologico è stato condotto in un SBBGR, un reattore upflow in cui il percolato è stato alimentato, trattato ed estratto in sequenza. L'impianto ha funzionato in modalità sequenziale con cicli di trattamento di 8 ore. Ogni ciclo è stato caratterizzato da una fase di riempimento, reazione e scarico. Il potenziamento chimico ha previsto una fase aggiuntiva durante la quale si è realizzata l'integrazione della degradazione biologica con l'ossidazione chimica, eseguita con ozono a due diverse dosi (4.0 g/L e 5.5 g/L). La discontinuità del sistema SBBGR ha permesso di utilizzare il trattamento ossidativo con ozono in maniera specifica e controllata. La fase di ozonizzazione, successiva al trattamento biologico, è risultata specifica per i composti resistenti alla degradazione biologica. L'ozono, dosato in maniera controllata, ha permesso di ottenere una parziale ossidazione delle sostanze recalcitranti prima di restituirle nuovamente all'azione della biomassa.

La sperimentazione si è articolata in 4 fasi: una fase di avvio, una seconda fase con solo trattamento biologico, una terza fase in cui il trattamento biologico è stato potenziato con ozono a due diversi dosaggi, ed un'ultima fase in cui il reattore ha lavorato in modalità biologica alimentato con percolato ad alta concentrazione di PFAS. Durante la fase preliminare di avvio è stato utilizzato un appropriato programma di alimentazione: diluizioni graduali con acqua a rapporti decrescenti per acclimatare la biomassa ad alti valori di salinità (circa 23 mS/cm) e allo stesso tempo stimolare la crescita delle specie coinvolte nel processo.

L'influente e l'effluente di tutti gli schemi di trattamento sono stati caratterizzati in termini di parametri tradizionali e PFAS; questi ultimi analizzati con uno

spettrometro di massa interfacciato con una cromatografia liquida ad altissima pressione.

***Keywords**:* percolato da discarica, PFAS, trattamento biologico, ossidazione avanzata, ozono.

INDEX

INTRODUCTION

It is estimated that municipal solid waste (MSW) volumes will reach 3.6 billion tons by 2050(Chen et al., 2020). Landfills function as the main disposal sites for vast amounts of consumer products once they have reached the end of their useful life. As the water moves through the waste, it dissolves and mobilizes contaminants present in the landfill. Chemical reactions between leachate and waste materials occur leading to leachate formation. Thus landfill leachate results as a complex mixture of pollutants containing a broad spectrum of contaminants, including heavy metals, inorganic salts, nitrogen compounds, as well as other toxic and emerging pollutants (Carvajal-Flórez & Santiago-Alonso Cardona-Gallo, 2019). The persistent contamination by per- and polyfluoroalkyl substances (PFAS) regarding all environmental matricies represents a significant challenge for the environmental protection. Their presence in landfill leachate is of particular concern due to the potential for leachate to contaminate surrounding environments and water sources.

Per- and polyfluoroalkyl substances (PFAS) are a large group of synthetic chemicals distinguished by the presence of carbon-fluorine (C-F) bonds. Fluorine is characterised by high electronegativity, high ionization potential and low polarizability; when bonded to carbon, forms one of the strongest and the most inert single bonds found in organic compounds (Leung et al., 2023). The strength and polarity of the C-F bond make PFAS highly resistant to various chemical reactions, including hydrolysis, oxidation, and reduction (Wei et al., 2019).These substances do not readily degrade, leading to their accumulation in soil, water, and biota.

Due to their unique properties PFAS have a broad range of applications. In the last decades they have been used in firefighting foams, non-stick coatings, stain-resistant treatments, and industrial processes. Currently, while some of these applications are being phased out in favor of safer alternatives, PFAS are still used

in specialized areas such as advanced firefighting foams, electronics manufacturing and water-repellent treatments.

PFAS exposure is linked to a range of health effects, including developmental issues, liver damage, immune system disruption, and an increased risk of certain cancers (Dickman & Aga, 2022). The persistence of PFAS in the environment exacerbates the hazard, as their bioaccumulation in the food chain can lead to prolonged human exposure.

Their ubiquitous presence in the environment is due to the chemical stability. They are found in water sources, soil, sediments, and air, landfill leachate. Recent studies identified landfill leachate as one of the main sources of emerging contaminants (EC) including PFAS (Ramakrishnan et al., 2015).

Once PFAS-containing materials enter a landfill, several mechanisms contribute to their presence in leachate. As waste decomposes, the breakdown of materials containing PFAS can lead to their leaching into the surrounding liquid (Hamid et al., 2018). The percolation of water through the landfill mass can mobilize PFAS from disposed materials into the leachate. This water dissolves PFAS and carries them through the landfill to the leachate collection system (Stoiber et al., 2020)

Landfill leachate management assumes a priority role in the context of waste disposal for several reasons, such as the large quantities produced, the large number of both active and decommissioned landfills, and the 30-year duration of a landfill's post-management. Effective landfill leachate management and treatment strategies are key to mitigating the impact of PFAS pollution.

In comparison with a vast literature about PFAS removal from water, very few studies exist regarding the treatment for removing PFAS from landfill leachate. The reasons are to be found in the inherent characteristics of this matrix, such as the high salinity, the high level of contamination, the heterogeneity of its composition, the presence of biorefractory compounds, the color and turbidity, which make treatment a complex challenge. Busch et al. (Busch et al., 2010a) and Zhang et al., 2022 studied the efficiency of PFAS removal in existing leachate treatment plants highlighting how reverse osmosis (RO) or nanofiltration (NF)

plants only are able to remove PFAS with an efficiency of more than 90 %, while other treatment methods, such as biological treatment, sand filtration are ineffective for this purpose. Reverse osmosis may achieve a high removal efficiency of 90–100% based on full-scale data, which, however, is vulnerable to the organic fouling and requires additional disposal of the concentrate (Zhang et al., 2022). Several studies have been conducted on the emerging technology of foam fractionation(Burns et al., 2022; McCleaf et al., 2021; Smith et al., 2022), showing good results for the removal of long-chain PFASs, but low removal efficiency for those with a short chain. Singh et al., 2021 tested plasma reactor technology which indicated good results for long-chain PFASs, but the technology is still immature for large-scale implementation and the energy consumption is still quite high. The removal of PFAS by granular activated carbon (GAC) full-scale leachate treatment plants was reported to be initially good but with the breakthrough much faster compared to the studies on the treatment of drinking water (Belkouteb et al., 2020). It was also observed that anion exchange resins (AIX) work well for the removal of PFAS from drinking water (McCleaf et al., 2017), but there is no evidence on leachate. Similarly, the removal of PFAS by ozonation has been demonstrated for drinking water [16], but there are no studies regarding PFAS removal from landfill leachate (Franke et al., 2019).

Currently, landfill leachate is treated in waste water treatment plant (WWTP) , which are not designed to remove PFAS. Among conventional treatment technologies, reverse osmosis (RO) has proven to be very effective in removing most PFAS from leachate. However, there is a strong membrane fouling effect and the need for further treatment of PFAS-rich concentrate.

Adsorption on activated carbon (AC) has variable efficiency for different PFAS. It is more effective in removing long-chain PFAS and sulfonates (PFSA) than acidic PFASs (PFCA). Activated carbon tends to saturate rapidly at high levels of contamination, requiring frequent regeneration or replacement, which contributes to the high operating costs of this technology. (Zhang et al., 2022)

Emerging treatment technologies for removing PFAS from landfill leachate include adsorptive photo-catalysts, plasma treatment, foam fractionation, and advanced oxidation processes. These emerging systems are being explored for PFAS removal and destruction but, excluding foam fractionation, most of these technologies are still limited to the laboratory or pilot scale, with very limited data available regarding potential capital and operational costs.

An effective treatment system must be flexible to address both conventional and emerging contaminants, to adapt to different contaminant loads over time, and to accommodate future regulatory changes that may require more stringent removal of specific pollutants. A leachate treatment system is also required to achieve high removal efficiencies for a range of contaminants (organic contaminants, heavy metals and ammonia, PFAS, and emerging pollutants) and to be economically and energy sustainable.

This study attempts to offer an answer to these scientific questions by proposing two approaches.

CHAPTER 1: THEORETICAL FRAME

1.1 OVERVIEW OF PFAS

Per- and polyfluoroalkyl substances (PFAS) are a large group of human-made chemicals that have gained significant attention due to their persistence in the environment and potential risks to human health. These compounds contain carbon-fluorine bonds, one of the strongest chemical bonds in nature, which makes them highly resistant to degradation. As a result, PFAS are often referred to as "forever chemicals" because they can remain in the environment for extended periods. Initially developed in the mid-20th century for a wide range of industrial applications, PFAS are now present in numerous consumer products, such as non-stick cookware, water-resistant fabrics, and firefighting foams. Their widespread use and environmental persistence have raised concerns regarding their accumulation in ecosystems and living organisms, including humans. As scientific research advances, there is increasing recognition of the potential harmful effects of PFAS exposure, leading to more stringent regulations and greater public awareness. This chapter provides an overview of the fundamental aspects of PFAS, including their definitions, physical and chemical properties, and the impact they have on human health and ecosystems.

1.1.1 Definitions and classification of PFAS

PFAS (per- and polyfluoroalkyl substances) are a class of chemicals unified by the presence of fluorinated carbon chains, known as perfluoroalkyl moieties. Despite their diverse structures and properties, the common characteristic across PFAS is the fluorination of carbon atoms. Their classification can vary depending on the definition used.

According to Buck et al. (Buck et al., 2011), PFAS are described as “highly fluorinated aliphatic substances containing one or more carbon atoms, where all hydrogen atoms have been replaced by fluorine, resulting in a perfluoroalkyl moiety (C_nF_{2n+1}–).” This definition emphasizes fully fluorinated aliphatic chains. In 2021, the OECD (Organisation for Economic Co-operation and Development) (Reconciling Terminology of the Universe of Per-and Polyfluoroalkyl Substances: Recommendations and Practical Guidance Series on Risk Management No.61 JT03479350 OFDE, 2021)expanded the definition by moving beyond strictly aliphatic structures. The OECD highlights the presence of fully fluorinated groups, such as –CF_3 and –CF_2–, making the classification more inclusive. According to the OECD, PFAS are defined as "fluorinated substances containing at least one fully fluorinated methyl (–CF_3) or methylene (–CF_2–) carbon atom, without any other attached atoms like hydrogen, chlorine, bromine, or iodine." Under this broader classification, a substance may be classified as a PFAS even if only a small portion of it is fully fluorinated, and the molecule does not need to consist entirely of a fully fluorinated aliphatic chain.

The classification of PFAS is complex due to their broad chemical diversity. This complexity influences how PFAS are regulated and managed in the environment. PFAS compounds exhibit a range of physical and chemical properties, including variations in molecular weight, structure, and environmental behavior. This diversity includes polymers and nonpolymers, reactive or inert substances, and substances that are soluble, insoluble, volatile, nonvolatile, mobile, immobile, bioaccumulative, or non-bioaccumulative. To

better organize this wide array of substances, PFAS are often categorized in a "PFAS family tree" (Figura 1.1) that divides them into two main classes: polymers and nonpolymers. These classes are further divided into various subclasses, groups, and subgroups, as illustrated in the figure 1.2.

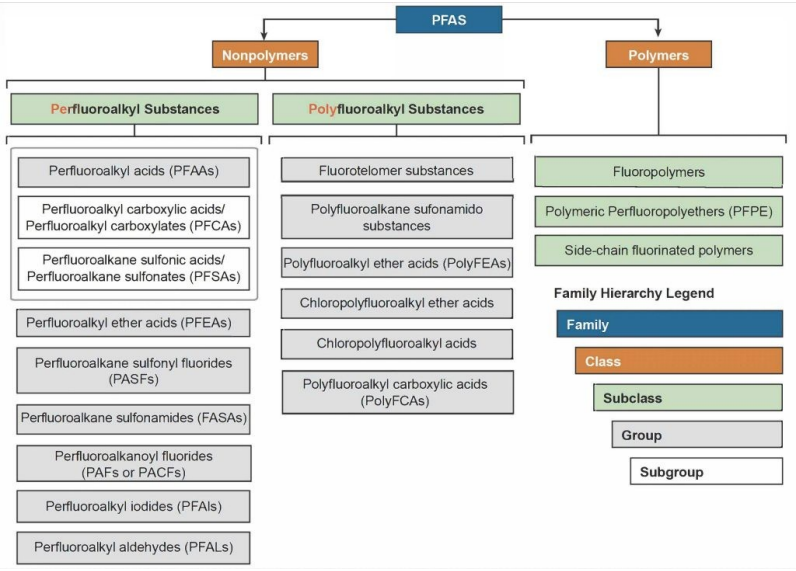


Figure 1.1 PFAS Family

The PFAS polymer class comprises fluoropolymers, polymeric perfluoropolyethers, and side-chain fluorinated polymers (Buck et al., 2011; Henry et al., 2018; Z. Wang et al., 2013) Nonpolymer PFAS are divided into two main subclasses: perfluoroalkyl substances and polyfluoroalkyl substances, which consist of multiple groups and subgroups of chemicals.

Nonpolymeric PFAS are more commonly detected in humans, wildlife, and environmental samples, and are often more prevalent at PFAS-contaminated sites. They have been studied more extensively, with a greater amount of data available regarding their environmental and health impacts.

Long-chain nonpolymeric PFAS, such as PFOA and PFOS, are particularly persistent and bioaccumulative, posing significant risks to human health and ecosystems due to their ability to accumulate in living organisms and disperse

through water systems. As a result, these compounds have been subject to increased regulatory oversight and environmental investigations.

Figure 1.2 presents a general classification, chemical structures, examples of each group and/or subgroup, and highlights the primary uses of some nonpolymeric PFAS mentioned in figure 1.1.

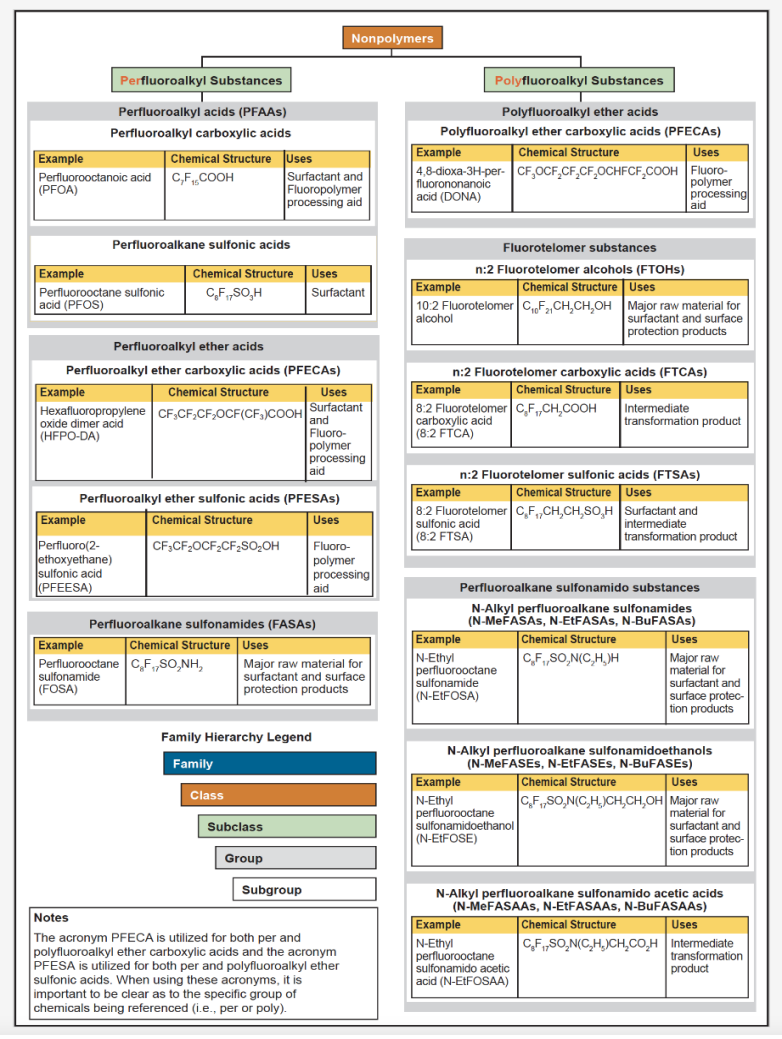


Figure 1.2 Nonpolymer PFAS subclasses

Source: Interstate Technology & Regulatory Council (ITRC), Chemistry, Terminology, and Acronyms – PFAS — Per- and Polyfluoroalkyl Substances (itrcweb.org), September 2023

In the class of nonpolymer PFAS, perfluoroalkyl substances are fully fluorinated alkane molecules that include, but are not limited to, perfluoroalkyl acids (PFAAs). These molecules have a basic chemical structure consisting of a carbon chain (or "tail") with two or more carbon atoms, and a charged functional group (or "head") attached at one end. Common functional groups include carboxylates or sulfonates, though other forms have also been detected in the environment.

Fluorine atoms are bonded to every available site along the carbon chain, except for the final carbon where the functional group head is attached. This structure, as shown in figure 1.3 for PFOS and PFOA, can be represented by the formula:

C_nF_{2n+1}-R

where "C_nF_{2n+1}" defines the length of the perfluoroalkyl chain tail, "n" is >2, and "R" represents the functional group head. It's important to note that the functional group may include one or more carbon atoms, and these carbons are counted when naming the compound.

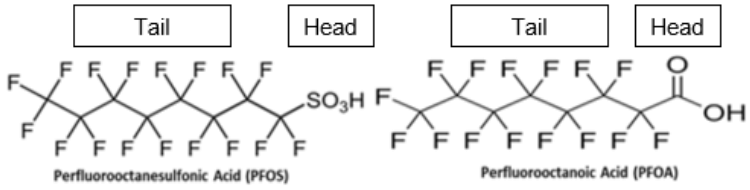


Figure 1.3 PFOA e PFOS chemical structure

Perfluoroalkyl acids (PFAAs) are a subgroup of perfluoroalkyl substances and are characterized by having a fully fluorinated carbon chain attached to an acidic functional group. These compounds are essentially non-degradable under normal environmental conditions. The biotic and abiotic transformation of many polyfluoroalkyl substances can lead to the formation of PFAAs, which is why they are often referred to as "terminal PFAS" or "terminal transformation products." This means that no further transformation occurs from them under

environmental conditions. Polyfluoroalkyl substances that transform to create terminal PFAAs are called "precursors."

The PFAA group is further divided into two main subgroups: perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkane sulfonic acids (PFSAs). PFCAs are used in commercial applications and can be produced as terminal transformation products of specific polyfluoroalkyl precursors, such as fluorotelomer alcohols (FTOHs). A well-known example of a PFCA is PFOA. Similarly, PFSAs are also used in commercial applications and can be generated as terminal products from precursors like perfluoroalkane sulfonamido ethanols (FASEs). A common example of a PFSA is PFOS.

PFAS, particularly PFAAs, are sometimes described as long-chain or short-chain as a way to categorize PFCAs and PFSAs that may exhibit similar environmental behaviors. However, it's essential not to oversimplify the behavior of PFAAs based solely on chain length. Recent research suggests that factors other than chain length may influence the bioaccumulation potential of PFAS. (Ng & Hungerbühler, 2014)

According to the OECD (2013), long-chain refers to PFCAs with eight or more carbon atoms (seven or more of which are perfluorinated), and PFSAs with six or more carbon atoms (all of which are perfluorinated). Short-chain refers to PFCAs with seven or fewer carbon atoms (six or fewer of which are perfluorinated), and PFSAs with five or fewer carbon atoms (all of which are perfluorinated).

1.1.2 Physical and chemical properties of PFAS

Understanding the physical and chemical properties of PFAS is important for the prediction of the PFAS fate and transport in the environment.

The physicochemical characteristics of PFAS are heavily influenced by the properties of fluorine and the abundance of C-F bonds within the compounds. Fluorine is characterized by high electronegativity, high ionization potential, and low polarizability. It is the most electronegative element in the periodic table, and when bonded to carbon, it forms one of the strongest and most inert single bonds found in organic compounds (Krafft & Riess, 2015). PFAS benefit from the optimal overlap between the 2s and 2p orbitals of fluorine with the carbon orbitals in the C-F bonds, forming multiple dipolar resonance structures along the perfluoroalkyl chain (Kirsch, 2004) The strength of the C-F bonds further increases as the number of fluorine atoms bonded to the central carbon atom rises. Consequently, PFAS exhibit significantly higher chemical and physical stability compared to their hydrocarbon counterparts.

The low polarizability of fluorine further leads to weak intermolecular interactions, such as Van der Waals interactions and hydrogen bonding. The reduced intermolecular forces contribute to the exceptionally low surface tension of PFAS, which in turn enhances their surface wettability. The absence of van der Waals forces also imparts an oleophobic nature to these compounds (Van Oss, et al., 1986).

PFAS exhibit complex behavior in the environment due to their functional groups, which can significantly influence their fate and transport properties. These functional groups—such as carboxylates, sulfonates, sulfates, phosphates, and amines—play a critical role in determining whether a PFAS molecule will dissociate into ions and what impact this dissociation will have.

The ionic state of PFAS compounds governs key characteristics like electrical charge, volatility, bioaccumulative potential, and solubility. For instance, most perfluoroalkyl acids (PFAAs), like perfluorooctanoic acid (PFOA),

dissociate into anionic forms under typical environmental conditions due to their low acid dissociation constants (Ka). As a result, PFAAs are generally found in their anionic forms except in highly acidic environments (e.g., pH < 3), where undissociated forms might occur. Different PFAS can be classified based on their functional groups and resulting ionic behavior:

Anionic PFAS: These compounds contain acidic functional groups such as carboxylic acids, sulfonic acids, sulfates, or phosphates. They dissociate in water, releasing a hydrogen ion (H^+) and forming anions. For example, perfluorobutanoic acid (PFBA) dissociates into a perfluorobutanoate anion and a hydrogen ion as shown in 1.4.

Cationic PFAS: These compounds feature basic functional groups like amines, which can accept a hydrogen ion and form cations. Some cationic PFAS have permanent positive charges.

Zwitterionic PFAS: These molecules contain both acidic and basic groups, allowing them to exist as both anions and cations simultaneously. They typically exhibit a balance between positive and negative charges in different parts of the molecule.

Nonionic PFAS: These compounds do not dissociate into ions in water and include functional groups like alcohols, which are neutral and non-ionic.

This classification system highlights the diversity of PFAS and explains their varied environmental behaviors. The specific dissociation properties of each PFAS determine how they interact with soils, water, and living organisms, influencing their persistence and potential toxicity.

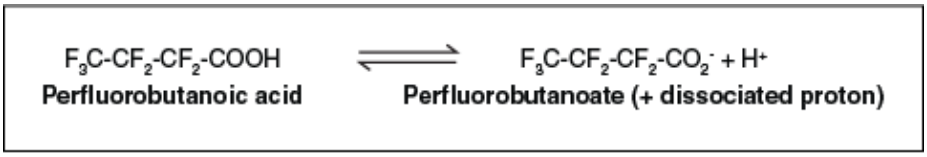


Figure 1.4 Dissociation of PFBA

Cationic PFAS are anticipated to have different transport behaviors in the environment compared to anionic PFAS (Place and Field 2012). For instance, the sorption of organic anions like PFAA anions is usually reduced at higher pH levels due to electrostatic repulsion from the increasingly negative charge of deprotonated oxides and other functional groups on soil surfaces (Lee & Mabury, 2017). In contrast, cations tend to bind strongly to soils, which generally have a net negative charge across a range of environmentally relevant pH levels. For example, cationic fluorotelomer-based PFAS, found in AFFF products, have been observed to strongly adsorb to soils and sediments (Barzen-Hanson et al., 2017). Zwitterionic PFAS are expected to adsorb more strongly to soils and sediments than anionic PFAS but less strongly than cationic PFAS due to the presence of both positive and negative charges on their functional groups. The transport characteristics of specific PFAS are also highly influenced by their interactions with the surrounding environment, meaning site-specific information is crucial for accurately predicting their movement (Guelfo & Higgins, 2013)

For acids like PFAAs, the acid dissociation constant (K_a) measures the equilibrium between the dissociated anion and hydrogen ion in water and is defined by the equation:

$K_a = [anion–]*[H+]/[acid]$

Equation 1

where [acid] is the concentration of the undissociated acid form, [anion–] is the concentration of the anion, and [H+] is the concentration of the hydrogen ion at equilibrium. The dissociation constant is also commonly expressed as its negative logarithm, pKa, where:

$pK_a = – \log_{10}(Ka)$

Equation 2

Higher pK_a values indicate that an acid will dissociate less in water at a given pH than will an acid with a lower pK_a. When the pH of a solution equals the pK_a for a particular constituent, then one half of the constituent molecules will exist as the undissociated acid and one half will exist as the dissociated anion.

PFAS with pK_a values of 4 or less will exist in aqueous solutions at neutral pH (7) almost entirely as the dissociated acid. This is important because the physical and chemical properties of the undissociated acid and its anionic form can differ significantly.

Thermal stability is a key factor in determining how long a chemical will last in the environment. PFAAs, such as PFOA and PFOS, are both thermally and chemically stable, resisting degradation and oxidation. This stability is largely due to the strength of the carbon-fluorine (C-F) bonds in the fluoroalkyl tail.

Understanding chemical stability helps predict a compound's persistence in the environment. PFCAs and PFSAs, for example, are highly resistant to environmental oxidation. Under extreme pressure, PFCAs can be transformed in the presence of oxidants, but under normal conditions, their C-F bonds remain highly stable. This is due to the protective effects of fluorine, such as shielding carbon atoms and the inductive effects of fluorine's electronegativity. Nucleophiles, which are electron-rich species, would typically be attracted to the partial positive charge of carbon, but the size of fluorine atoms prevents nucleophiles from approaching the carbon closely enough to react. This makes hydrolysis, a process that removes fluorine atoms, ineffective at degrading PFAA tails. Likewise, PFAAs are resistant to oxidative processes that rely on electron loss and reductive processes that involve gaining electrons (Park et al., 2009).

While the perfluorinated tails of PFAAs are highly stable, the polar functional groups and polyfluorinated regions may be more susceptible to chemical transformations.

Regarding the physical properties of PFAS, they are key aspects that reflect their unique characteristics and their widespread use in various applications. Most PFAS are solids, often crystalline or powdery in form, at room temperature; however, shorter-chain compounds (the acid forms of PFCA and PFSA, FTS and FTOH with a 4-6 carbon tail) tend to be liquid at room temperature.

Melting and boiling point information refers to the temperature of phase transitions of pure compounds. These properties determine whether a specific

pure PFAS compound will exist as a liquid, solid, or gas at typical environmental temperatures. These data vary among sources. Available data indicate that the melting and boiling points of PFAS tend to increase as the fluorinated chain lengthens.

Another relevant aspect of PFAS behavior is micelle formation. Given the difference in behavior between the hydrophilic "head" and the hydrophobic "tail," when immersed in water, PFAS tend to form micelles (spherical structures with the hydrophobic part of the molecules inside) when a certain concentration is exceeded. Early studies on PFAAs demonstrated that they behave like traditional surfactants, aggregating into simple and mixed micelles (Downer et al., 1999; Pedone et al., 1997). The theoretical concentrations at which aggregation occurs, commonly known as critical micelle concentrations (CMCs), are well documented. However, some observed properties of PFAS do not align with the traditional model of micelle formation. These anomalies have yet to be fully explained in scientific literature. Some researchers suggest that PFAA supramolecular aggregations in the environment are more complex than simple micelle formation at concentrations above the CMC, which is based on single-compound systems. It is hypothesized that such aggregations may occur at concentrations lower than the CMC in groundwater, due to interactions with particles, co-contaminants, hemi-micelle formation, or variable concentrations within soil matrices (Johnson et al., 2007; Yu et al., 2009).

The amphiphilic structure of PFAAs (a hydrophobic tail and a hydrophilic head) suggests that they behave like classical surfactants, accumulating at fluid interfaces (such as air-water or NAPL-water) and orienting themselves along the boundary, with the hydrophobic tails facing the air and the hydrophilic heads in the water (Krafft & Riess, 2015). Although extensive research confirms surface aggregation, the precise molecular structure of these aggregations is still unclear. However, studies like those by (Hasegawa, 2017) suggest that the aggregational shape of PFAS may be more complex than that of traditional surfactants and depend on the length of the fluorinated tail.

Due to the low polarizability of the C-F bond compared to the C-H bond, PFAAs exhibit a stronger affinity for interfaces than traditional hydrocarbon surfactants (Brusseau, 2018).This strong self-assembly tendency and surface-active properties make PFAAs highly effective and widely used in various applications, such as water- and grease-repellent coatings and in firefighting foams (AFFF).

As one might expect from a heterogeneous group of hundreds or thousands of substances, PFAS can exhibit different characteristics even within the same physical form. For example, their physicochemical properties can vary significantly, even when they are structurally similar (Krafft & Riess, 2015). provide an example of this variability among homologous compounds, showing that in a series of fluorotelomer alcohols with carbon chains ranging from 4 to 10, the water solubility, air/water partition coefficient, octanol/water partition coefficient, and other partitioning parameters, such as those between air, water, and organic carbon, can vary by 2 to 5 orders of magnitude. This variability in physicochemical properties is also observed in other PFAS classes, leading to significant differences in their transport and environmental fate.

In addition to affecting the transport and fate of substances released into the environment, these physicochemical differences also influence how PFAS are absorbed by aquatic and terrestrial organisms. One of the major concerns regarding the environmental presence of PFAS is that they are highly unlikely to undergo biodegradation or final mineralization (Blum et al., 2015).Some PFAS do not degrade at all, while others degrade into terminal products that do not further break down, leading to potential accumulation in various environmental matrices. Although the bioaccumulation profiles of these terminal degradation products may differ (Conder et al., 2008), the primary concern lies in the increasing environmental concentrations, which could reach levels that cause negative ecological effects.

1.1.3 Impact of PFAS on human health and ecosystem

Per- and polyfluoroalkyl substances (PFAS) represent a broad and diverse group of chemical compounds, whose presence raises increasing concerns for both human health and the environment. However, the lack of adequate data for most of these compounds makes it difficult to accurately assess the risks in both prospective and retrospective studies.

Exposure to PFAS has been associated with a wide range of adverse effects, including physiological, morphological, behavioral, and genetic alterations. The mechanisms and modes of action of these substances remain unclear. Conducting epidemiological studies to assess the effects of PFAS exposure is a complex challenge, influenced by various factors such as the coexistence of multiple contaminants, the difficulty of analyzing the effects of pollutant mixtures, the absence of control groups made up of unexposed populations, and the inherent complexity of the human biological system.

The primary route of human exposure to PFAS is through drinking water, and the risk of exposure persists throughout different stages of life. Infants and children are particularly vulnerable to PFAS compared to adolescents, adults, and the elderly, especially due to exposure during early life stages through placental transfer and breastfeeding. This early exposure can lead to cumulative PFAS accumulation(Blake & Fenton, 2020; Mondal et al., 2014). In early life, higher water consumption relative to body weight further contributes to elevated exposure levels (Yang et al., 2019). Prenatal exposure to PFAS has been linked to several adverse effects, including reduced birth weight, delayed fetal growth, and impaired neurological development, manifesting in long-term cognitive and motor deficits. A study conducted on children aged 4 to 6 found an association between PFAS levels and lower scores in cognitive development tests (Stein et al., 2014).

PFAS have a high affinity for blood proteins, particularly albumin, the main plasma protein. This interaction has significant consequences for the circulatory

system, affecting the transport, distribution, and effects of substances on other organs (Forsthuber et al., 2020). Several studies have associated PFAS exposure with chronic cardiovascular diseases, metabolic, neurological, and infectious diseases (Fenton et al., 2021).

Furthermore, PFAS exposure has been linked to insulin resistance, a contributing factor in the development of type 2 diabetes. These chemicals can alter lipid and carbohydrate metabolism, increasing the risk of metabolic syndrome (Cardenas et al., 2018). Changes in the synthesis and metabolism of fatty acids can cause liver toxicity and activate oncogenic signaling pathways, particularly in fetuses exposed to PFOS during pregnancy (Lai et al., 2017).

Elevated levels of PFOA and PFOS in the serum have been associated with negative effects on the immune system, particularly through the modulation of immunoglobulin G (IgG) glycosylation, a glycoprotein essential for cellular interactions and the regulation of protein functions. These effects can influence the onset and progression of various diseases (G. Liu et al., 2020). In addition to IgG modulation, PFAS can impair the overall immune response. A reduced vaccine response has been observed in exposed children, suggesting potential immune system suppression that increases vulnerability to infections (Grandjean et al., 2012).Research by (Barry et al., 2013) highlighted a positive correlation between PFOA exposure and the development of kidney and testicular cancers, Additionally, some studies have suggested an association between PFAS exposure and an increased risk of other types of cancer, such as liver and pancreatic cancer. PFAS have also been associated with chronic kidney failure and the deterioration of renal function, showing significant correlations with an increased risk of nephropathy (Shankar et al., 2011).

Some studies have highlighted a connection between PFAS exposure and reduced bone density, suggesting that these compounds may impact skeletal health, increasing the risk of osteoporosis and fractures. An analysis of a sample of postmenopausal women revealed that PFAS exposure is correlated with

reduced bone mineral density, increasing the risk of osteoporosis (Khalil et al., 2016)

At the same time, environmental pollution caused by PFAS has a significant impact on aquatic and terrestrial ecosystems, affecting organism health and compromising biodiversity. PFAS are known for their persistence in the environment and their ability to accumulate in water bodies, contaminating both groundwater and surface water. This contamination has long-term effects on aquatic wildlife and water quality. Aquatic organisms, such as fish and crustaceans, can absorb PFAS through water and sediment, leading to bioaccumulation—the buildup of PFAS in the tissues of organisms. Consequently, predators that feed on these organisms may undergo a process of biomagnification, further increasing PFAS concentrations in their bodies.

Recent studies have shown that PFAS can alter the behavior, reproduction, and development of fish and invertebrates. Beyond aquatic ecosystems, PFAS can contaminate soil, wastewater, and atmospheric deposition. This contamination directly impacts soil biodiversity and plant health. Terrestrial organisms, including insects and mammals, can be exposed to PFAS through contaminated soil and vegetation. PFAS contamination poses a threat to human health and has serious implications for biodiversity and the balance of both aquatic and terrestrial ecosystems.

1.2 PFAS CONTAMINATION IN LANDFILL LEACHATE

1.2.1 Sources and dynamics of PFAS in landfill leanchate

Landfills are significant environmental sources of per- and polyfluoroalkyl substances (PFAS), as these compounds are commonly found in various consumer products and industrial waste. As these waste materials decompose, PFAS can be released and migrate into the leachate due to their high water-solubility. The composition of leachate is characterized by four main groups of pollutants: dissolved organic matter, inorganic macrocomponents, heavy metals, and xenobiotic.

Around the 2000s, PFAS (perfluoroalkyl substances) were first detected in landfill leachate. Lang et al., 2017 highlighted the potential of landfills as a continuous source of PFAS in the environment.

Multiple factors contribute to the presence of PFAS in landfills. Key sources include perfluoroalkyl acids (PFAAs), fluorinated telomers, perfluoroalkyl sulfonamide derivatives, and polyalkyl phosphate (PAP) compounds. Despite some restrictions on their use, PFOA remains one of the most abundant PFAS in landfill leachate. Additionally, short-chain variants (C4–C7) are more prevalent due to their increased mobility and ability to transform into more stable PFAS. A critical aspect is the biodegradation of fluorotelomer compounds (FTC), which include fluorotelomer alcohols (FTOHs) and fluorotelomer sulfonic acids (FTSs). These compounds can undergo abiotic and biotic transformations within landfills, releasing fluorotelomer monomers and generating additional PFAS through oxidative biotransformation. For instance, the aerobic biotransformation of precursors like 6:2 FTS can lead to the formation of perfluoroalkyl carboxylic acids (PFCA) and saturated fluorotelomer carboxylic acids (FTCA) (Field & Seow, 2017).

International studies have reported elevated concentrations of PFAS in landfill leachate from various regions. For example, (Busch et al., 2010b) noted a minimum concentration of PFAS at 146.1 ng/L in raw leachate from a landfill in

Germany. In China, significantly higher concentrations have been recorded, ranging from 7.280 ng/L to 292.000 ng/L (Yan et al., 2015a). Compared to other sources of contamination, such as coastal and surface waters, the concentrations of PFAS in landfill leachate are exceedingly high and vary based on waste type, landfill management, and treatment practices (Hamid et al., 2018; Solo-Gabriele et al., 2020a).

Research on PFAS in landfill leachate remains in its early stages, yet it is crucial for understanding their environmental behavior and developing effective mitigation strategies. Future efforts should focus on optimizing treatment technologies, implementing continuous monitoring of PFAS concentrations, and assessing long-term risks to human health and the environment.

1.2.2 Chemical and biological challenges in landfill leachate treatment

The challenges related to the matrix mainly concern the high salinity, to which many microorganisms are not adapted. High salinity negatively affects the growth and activity of numerous microorganisms, compromising the effectiveness of biological treatment processes. Elevated salinity levels alter cellular osmosis, reducing the ability of microorganisms to absorb nutrients and inducing osmotic stress, which can lead to cell death or a significant reduction in their metabolic activity.

Another important challenge concerns the ammonia (N-ammoniacal) levels present in the leachate, which are often very high. Ammonia is a problematic contaminant for biological treatment, as it can inhibit the nitrification activity of nitrifying bacteria at high concentrations. Effective ammonia treatment requires oxidation processes, which can be negatively influenced by the presence of high salt levels and humic acids.

Furthermore, the presence of humic acids presents a significant difficulty. These compounds are the result of organic material decomposition, and although they are naturally present in leachates, their complex chemical structure makes them difficult to degrade biologically.

The low biodegradability index is another critical factor. The biodegradability index measures the ability of an organic substrate to be degraded by microorganisms. A low index indicates that a substantial portion of the compounds in the leachate is resistant to biodegradation, requiring the application of advanced technologies such as advanced oxidation processes or the addition of chemical additives to enhance the degradation process. Additionally, the low C/N (carbon/nitrogen) ratio in the leachate implies that the available organic substrate is insufficient to support effective biological nitrification. In this case, adding an external carbon source, such as acetate, is necessary to stimulate denitrifying bacteria and promote denitrification, an essential process for the removal of excess nitrogen.

On the other hand, the difficulties related to PFAS are primarily due to the stability of the C-F (carbon-fluorine) bond. Carbon-fluorine bonds are among the strongest in nature, which gives these compounds significant resistance to both chemical and biological degradation. PFAS are present in various matrices at concentrations in the order of nanograms per liter. This low concentration makes both accurate determination and the implementation of effective treatments difficult.

Overcoming the challenges in landfill leachate treatment requires the integration of advanced technologies, innovation in biological and chemical processes, and continuous research to optimize the effectiveness of existing solutions.

1.2.3 Regulatory framework for PFAS in water and leachate

PFAS have become emerging contaminants of concern since the early 2000s, quickly drawing the attention of the scientific community due to their hazardous nature. Many countries are working to develop stricter regulations in response to growing public health and environmental concerns.

In recent years, state and international authorities have established a series of regulatory values, but the regulatory process is at different stages depending on the country, with values and criteria that are not uniform. This applies to all environmental matrices, including water and landfill leachate. Limits for PFAS vary significantly, mainly due to the challenges in monitoring and removing these substances.

In December 2020, the European Union updated the Drinking Water Directive (Directive 2020/2184), introducing limits for PFAS for the first time, aiming to reduce contamination in drinking water:

- Total PFAS: limit of 0.5 μg/L.
- Sum of 20 specific PFAS: limit of 0.1 μg/L (for long-chain PFAS like PFOA and PFOS).

In the United States, the EPA (Environmental Protection Agency) has set provisional guidelines for PFAS in drinking water. As of 2022, it has proposed very stringent limits for the two most well-known PFAS:

- PFOA: proposed limit of 4 parts per trillion (ppt) or 0.004 μg/L.
- PFOS: proposed limit of 4 ppt or 0.004 μg/L.

Previously, the EPA had established a limit of 70 ppt (0.07 μg/L) for the sum of PFOA and PFOS, but the new proposals aim for much lower values, nearly at the detection threshold.

In Denmark, in June 2021, the Danish Environmental Protection Agency (Miljøstyrelsen) set a strict limit for drinking water, stating that the total concentration of four specific PFAS—PFOA, PFOS, PFNA (perfluorononanoic acid), and PFHxS (perfluorohexanesulfonic acid)—must not exceed 2 nanograms

per liter (ng/L). This regulation reflects Denmark's commitment to reducing exposure to harmful substances and protecting public health, as part of a broader effort to manage the risks associated with PFAS, known for their persistence in the environment and potential health effects.

Regarding the management of PFAS in landfill leachate, the issue is evolving. In Italy, Legislative Decree 152/2006 on waste management states that local authorities can set specific limits for hazardous substances, including PFAS. However, such limits are not explicitly defined at the national level and may vary based on regional regulations or specific management plans.

At the European level, Directive 2010/75/EU on industrial emissions requires the monitoring and limitation of hazardous substances, while the REACH Regulation establishes limits for certain chemicals but does not specify direct limits for leachate. In the United States, the EPA has issued guidelines, but there are no uniform federal limits for PFAS in landfill leachate.

In general, the challenge remains to define limits that are both realistic concerning the available treatment technology and effective in protecting human health and the environment.

1.3 TECHNOLOGIES FOR PFA REMOVAL FROM LANDFILL LEACHATE

1.3.1 Overview of treatment approaches

Landfill leachate is a significant source of PFAS release into the environment. Currently, most leachate is treated in public treatment plants (POTWs) designed to remove conventional constituents such as organics and nutrients, but they are inadequate for PFAS removal. Only a few landfills treat or pre-treat leachate specifically for PFAS removal, but this situation may evolve with the introduction of national and state regulations regarding surface waters and pre-treatment processes. In countries such as the United States and Europe, discharge standards for PFAS are becoming increasingly stringent.

Therefore, it is crucial to develop specialized technologies for the treatment of PFAS in landfill leachate. Table 1.1 provides a summary of the most relevant technologies, including granular activated carbon (GAC) (Q. Yu et al., 2009), ion exchange resins adsorption (Malovanyy et al., 2023), photocatalysis (Lu et al., 2023), electrochemical oxidation (EO) (Zhuo et al., 2020a; Appleman, Dickenson, et al., 2013), ultrafiltration (UF) (Appleman, Higgins, et al., 2013), and reverse osmosis (RO) (Tang et al., 2006). Emerging technologies such as ultrasound and plasma treatment are also being studied for PFAS removal from landfill leachate (Singh et al., 2021b).

The treatment of landfill leachate for PFAS removal faces numerous challenges. First, PFAS concentrations in leachate are often significantly higher than in other pollution sources (Solo-Gabriele et al., 2020b), requiring highly effective and specific treatment technologies (Hamid et al., 2020; X. Yu et al., 2018). Additionally, the chemical complexity of the leachate, which contains a variety of organic and inorganic substances, can reduce the efficiency of removal technologies.

The pH and other chemical properties of leachate can also negatively impact the effectiveness of conventional treatments, making the development

of more advanced and targeted techniques necessary. Furthermore, the costs associated with emerging technologies present additional challenges for large-scale implementation.

One of the most common methods for removing PFAS, particularly long-chain PFAS, is **adsorption on activated carbon**. Thanks to its high specific surface area, this porous material can effectively adsorb PFAS molecules. However, its effectiveness depends on the characteristics of the leachate and the concentration of PFAS present. Activated carbon (AC) provides numerous pores and hydrophobic surfaces that attract the fluorinated chains of PFAS (Q. Yu et al., 2009). Modifications to AC primarily focus on introducing alkaline features to attract PFAS anions through electrostatic and chemical interactions, thus improving adsorption.

The adsorption mechanism of PFAS onto activated carbon involves both physical and chemical processes. Physical adsorption is driven by van der Waals forces between PFAS molecules and the carbon surface, a reversible process that allows desorption of PFAS through heating. In contrast, chemical adsorption is irreversible and involves chemical bonding between PFAS and the activated carbon, ensuring more effective removal. Various forms of activated carbon, such as powdered activated carbon (PAC), activated carbon fiber (ACF), and granular activated carbon (GAC), have been used to remove PFAS, With GAC showing removal efficiencies higher than 95% for PFOS, while for shorter-chain PFCs (such as PFBS and PFBA), removal was between 40% and 70% (Zhao et al., 2011). PAC and ACF, due to their higher surface area, can offer better performance compared to GAC. It has also been demonstrated that using ultrasound can enhance the diffusion of PFAS into GAC's nanopores, increasing its adsorption capacity (Zhao et al., 2011). Optimized thermal regeneration of GAC breaks the PFAS cycle by destroying the adsorbed molecules and recovering the spent activated carbon.

Anion exchange resin (AER) treatment has proven to be effective in removing PFAS from water, with higher efficiency in removing long-chain PFAS compared to short-chain ones (Gagliano et al., 2020). This approach can also be employed to treat landfill leachate, which contains a complex mixture of contaminants. Anion exchange resins work by capturing PFAS ions from solutions and binding them to the active sites of the resin, making them useful for purifying both drinking water and wastewater.

However, a key issue with this method is the regeneration of the adsorbent. Once the resin has adsorbed a significant amount of PFAS, it needs to be regenerated to restore its effectiveness. This regeneration process is usually carried out using saline solutions, which release PFAS from the resin, allowing it to be reused. However, this generates a new source of contaminated waste, as the saline solutions used for regeneration contain high concentrations of PFAS, creating an additional environmental challenge. Therefore, finding effective ways to manage this waste is critical to preventing it from becoming a secondary source of pollution.

Reverse osmosis (RO) and nanofiltration (NF) are membrane separation technologies that retain PFAS, allowing only clean water to pass through. NF membranes, with pores smaller than 0.001 μm, remove low molecular weight organic compounds and microorganisms (Yan et al., 2015b; Q. Yu et al., 2009), while RO membranes, using pressure, separate water from the solution and remove most dissolved organic and inorganic compounds (Yan et al., 2015b). Studies have shown the effectiveness of membrane technologies in eliminating PFAS from leachate, with membrane bioreactors followed by RO or NF capable of removing over 95% of PFAS from biologically or conventionally treated leachate (Yan et al., 2015b). Ultrafiltration (UF) and NF have also shown success rates above 90% in PFAS removal from leachate (Fuertes et al., 2017).

RO membranes have shown positive results in PFAS removal, thanks to a polyamide barrier layer supported by a polysulfone or polyethersulfone backing (Toure & Anwar Sadmani, 2019). (Dharupaneedi et al., 2019) Membrane filtration

systems offer advantages such as low energy consumption and continuous separation, but they may encounter challenges related to high levels of salinity, heavy metals, and suspended solids, which can reduce permeate flow and increase energy consumption (Dharupaneedi et al., 2019; Huang et al., 2021). Pre-treatment is often required to prevent membrane fouling. Additionally, in membrane systems, PFAS are retained but concentrated in the waste stream, generating a highly contaminated residue that requires further treatment.

Advanced Oxidation Processes (AOP) are emerging technologies that have proven effective for the removal of PFAS from landfill leachate. These processes rely on the generation of reactive species, such as hydroxyl radicals (•OH), capable of degrading resistant organic compounds like PFAS through oxidation reactions. Among the various AOPs, the main methods used to treat PFAS-contaminated leachate include electrochemical oxidation (EO), photocatalysis, and ozonation.

Electrochemical oxidation (EO) is one of the most promising techniques for removing PFAS, particularly compounds like PFOA and PFOS. It operates by applying an electric current that generates oxidizing species, such as hydroxyl radicals, directly on the surface of an electrode. However, there are few studies on the application of EO to PFAS, especially in complex environments like landfill leachate, where most research has focused on the oxidation of PFOS in pure water (Y. Liu et al., 2019; Zhuo et al., 2020). Pierpaoli et al. 2021, proposed a multi-phase reaction model for the anodic degradation of PFOA and PFOS, which includes electron transfer, radical reactions, Kolbe decarboxylation, hydrolysis, and hydroxylation. Their results showed that a higher current density (75 mA/cm^2) led to removal rates of 80% for PFOA and 78% for PFOS, while lower current densities (25 mA/cm^2) reduced these values by about half.

Despite its effectiveness, the complex conditions of leachate resulted in high energy consumption, reaching up to 256 kWh/m^3 after 10 hours of EO treatment. While the high effectiveness in removing PFAS (up to 99.74%) is

notable, reducingenergy consumption remains a critical challenge to make the process more sustainable and practical at scale.

Turning our attention to photocatalysis, this method utilizes semiconductor materials (such as titanium dioxide, TiO_2) activated by UV radiation to generate free radicals that degrade PFAS. This process is effective in leachate, although it is hindered by the presence of other organic substances competing for active sites on the catalyst. The photocatalytic degradation of PFAS in leachate is a complex process requiring the assessment of various factors. For instance, the high content of organic matter in the leachate can compete with PFAS for the active sites of the adsorbent or photocatalyst, thereby reducing process efficiency. Furthermore, high concentrations of PFAS can inhibit the activity of photocatalysts or adsorbents, further diminishing treatment effectiveness. Additionally, the pH of the leachate significantly impacts PFAS removal: maximum efficiency is observed at pH values between 4.0 and 5.3 (Saien et al., 2011; Tian et al., 2021). Extreme pH values can alter both the stability and catalytic activity of photocatalysts, limiting their overall effectiveness (Travar et al., 2020).

Finally, ozonation involves the use of ozone (O_3) as an oxidizing agent to degrade or remove contaminants. Ozone can react directly with contaminants like PFAS, causing the breaking of chemical bonds and degradation of molecules, or generate free radicals. In the presence of water, ozone decomposes and produces hydroxyl radicals (•OH), which are extremely reactive and can attack a wide range of polluting compounds.

Previous studies have shown that ozonation of drinking water has limited effectiveness in removing PFAS, often requiring high doses, prolonged treatment times, and sometimes the use of catalysts or the addition of persulfate (Flores et al., n.d.; Franke et al., 2019b). In the context of leachate, the high concentration of carbonates acts as a "scavenger," interfering with ozone and hydroxyl radicals (•OH), thus reducing treatment effectiveness.

Leachate is characterized by high concentrations of dissolved organic matter (DOC), which can play a complex role in the ozonation process. DOC can act both as an initiator and promoter of radical formation and as an inhibitor of ozone degradation, complicating treatment further. In experiments conducted with high doses of ozone (up to 173 mg/L, corresponding to 4.2 g O_3/g DOC), concentrations of PFOS, PFOA, and other long-chain PFAS decreased by over 86%, even with lower doses, such as 28 mg/L. However, the removal of short-chain PFAS was significantly less effective, with a maximum reduction of 45% for ΣPFAS (Yong & Lin, 2013).

It's crucial to note that PFAS may not be completely degraded but transformed into potentially toxic fluorinated by-products. Moreover, leachate typically contains high concentrations of bromide, chlorate, and chromium, which can react during ozonation, generating toxic by-products such as bromate, chlorate, perchlorate, and Cr (VI) (van der Merwe et al., 2012; Von Gunten & Hoigne, 1994). These compounds pose a significant risk to human health and the environment.

In summary, ozonation can significantly reduce PFAS concentrations in leachate, but its effectiveness strongly depends on operational conditions and the chemical composition of the leachate itself.

Finally, let’s examine biological treatments, which present a promising option for treating PFAS in landfill leachate by leveraging microorganisms for the biodegradation of fluorinated compounds (Busch et al., 2010c; Fuertes et al., 2017; Solo-Gabriele et al., 2020b; Yan et al., 2015b). However, an increase in PFAS compounds has been observed in some bio-treated leachate matrices using membrane bioreactors and aerobic reactors (Busch et al., 2010c; Yan et al., 2015b). This phenomenon is attributed to the partial degradation of fluorinated precursors, such as the transformation of FTOH 8:2 into PFOA, FTOH into PFHxA, and FTCA into PFPeA 5:3 (Xiao et al., 2012). The biodegradation of such precursors generates more stable and resistant compounds, like PFOA and PFOS, which are more difficult to remove.

Several key factors influence the biological degradation of PFAS:

- Microbial Species and Quantity: The diversity and adaptability of microorganisms play a crucial role. Some microbial strains are more efficient in degrading PFAS than others, and the abundance of these organisms can enhance the overall effectiveness of the process.
- Temperature and pH: Environmental conditions, such as temperature and pH, significantly influence microbial activity. Appropriate temperatures and pH levels favor the growth and metabolic efficiency of
- microorganisms, optimizing the degradation process. However, it is important to adapt these parameters based on the specific characteristics of the microorganisms used.

In conclusion, biotechnological treatment, which leverages the ability of specific microorganisms to degrade PFAS, could be used to integrate physicochemical technologies to optimize the total removal of PFAS from landfill leachate.

Table 1.1 Summary of the most relevant technologies

Technology	Description	Efficiency	Limitations	References
Activated Carbon (AC)	Adsorption of PFAS onto porous carbon surfaces, including GAC, PAC, and ACF forms	>95% for long-chain PFAS like PFOS; 40-70% for short-chain PFAS like PFBS, PFBA	Efficiency varies by leachate composition and PFAS concentration; requires regeneration	Q. Yu et al., 2009; Zhao et al., 2011
Anion Exchange Resins (AER)	Ion exchange process that captures PFAS ions on resin surfaces	High for long-chain PFAS	Requires regeneration, leading to saline PFAS-contaminated waste	Gagliano et al., 2020
Reverse Osmosis (RO)	Membrane filtration that retains PFAS while allowing clean water to pass	Up to 99%	Requires pre-treatment to prevent fouling; produces concentrated PFAS waste	Yan et al., 2015b; Toure & Sadmani, 2019
Nanofiltration (NF)	Membranes with ultra-fine pores to filter out low molecular weight PFAS and microorganisms	>90%	Reduced efficiency for short-chain PFAS; high energy consumption	Yan et al., 2015b; Q. Yu et al., 2009
Electrochemical Oxidation (EO)	Uses electric current to generate oxidants that degrade PFAS	Up to 99%	High energy consumption; limited effectiveness in complex environments	Y. Liu et al., 2019; Zhuo et al., 2020b
Photocatalysis	UV-activated catalysts produce radicals that degrade PFAS in leachate	Variable	Low efficiency in leachate with high organic content	Saien et al., 2011; Tian et al., 2021
Ozonation	Uses ozone as an oxidant to break down PFAS in water	>86% for long-chain PFAS (PFOS, PFOA)	Reacts with other substances in leachate; requires high doses and extended treatment times	Yong & Lin, 2013; van der Merwe et al., 2012
Biodegradation	Microorganisms degrade PFAS	Variable, limited for certain PFAS	Slow process; formation of resistant compounds like PFOA and PFOS	Busch et al., 2010c; Xiao et al., 2012
Ultrasound and Plasma	High-energy methods (vibration and ionization) for enhancing PFAS degradation	In study phase	High cost and limited large-scale application	Singh et al., 2021b

To make landfill leachate treatment technologies a viable solution for reducing the environmental risk of PFAS, it is essential to lower both their costs and environmental impact. A promising approach is the integration of multiple processes. For example, biological treatments could be combined with chemical or physical methods to achieve a more comprehensive and sustainable removal. Additionally, the use of membrane filtration followed by electro-oxidation or ozonation could provide more effective solutions than standalone methods. These advancements have the potential to make treatment technologies more sustainable and scalable, addressing the challenges posed by the chemical complexity of leachate and the persistent toxicity of PFAS.

1.3.2 Biological Processes

Biological treatment is known for its reliability, simplicity, and high economic efficiency. Biodegradation is carried out by microorganisms, which can degrade organic compounds into carbon dioxide and sludge under aerobic conditions, and into biogas (a mixture primarily composed of CO_2 and CH_4) under anaerobic conditions. Biological processes have proven to be highly effective in removing organic and nitrogenous substances from immature leachates when the BOD_5/COD ratio is high (>0.5). Over time, the increasing presence of refractory compounds (mainly humic and fulvic acids) tends to limit the effectiveness of the process (Renou et al., 2008).

Biological processes used in the treatment of wastewater and landfill leachates can be distinguished based on several factors, including the presence of oxygen, the type of biomass involved, and technological advancements that have improved the efficiency of these processes.

A fundamental distinction concerns the oxidative condition of the biological process. Aerobic processes require the presence of oxygen to promote the growth and activity of microorganisms, which break down organic matter in the presence of oxygen, producing mainly carbon dioxide and water. These processes are ideal for treating wastewater with moderate organic loads and are widely used in treatment plants to remove biochemical oxygen demand (BOD_5) and, in some cases, for nitrification, the process that oxidizes ammonia to nitrates. The most common aerobic systems include activated sludge treatment, aerated biofilters, and moving bed bioreactors (MBBR). Another example of an aerobic process is the Sequencing Batch Reactor (SBR), which operates in cycles, alternating aeration and sedimentation phases, and can be adapted to variable wastewater flows.

On the other hand, anaerobic processes occur in the absence of oxygen and are mainly used to treat waters with high organic matter concentrations. In these systems, microorganisms degrade organic matter, producing biogas,

mainly methane, which can be used as an energy source. Anaerobic processes are advantageous because they do not require aeration, thus reducing energy consumption. Key anaerobic systems include anaerobic digestion, used for treating sludges and organic wastes, and anaerobic sludge bed reactors, such as the Upflow Anaerobic Sludge Blanket (UASB), which use a column of active sludge for the treatment of incoming liquids.

Another important difference between various biological processes concerns the type of biomass used, which can be suspended or attached to supports. In suspended biomass processes, microorganisms grow freely in the fluid, without binding to solid supports. A classic example of this type of process is activated sludge treatment, where microorganisms break down organic matter directly in the water through aeration. Suspended biomass is particularly suitable for plants that treat large volumes of water but can be difficult to separate from the treated liquid during sedimentation phases, requiring advanced separation systems.

In contrast, in attached biomass systems, microorganisms grow on solid supports, forming a biofilm that provides a larger surface area for biological activity. These systems are generally more stable, less subject to load fluctuations, and easier to manage, as the separation of biomass from the treated liquid is simpler. Biofilters and trickling filters are examples of biofilm systems used for aerobic treatments, while in Moving Bed Biofilm Reactors (MBBR) and Sequencing Batch Biofilm Granular Reactors (SBBGR), attached biomass develops on granular particles, typically supported by plastic media, which in some systems are kept in motion in the liquid through water flow, and in others are trapped between two plates through which the liquid phase to be treated flows.

Over the years, biological processes have evolved due to the introduction of advanced technologies that combine various approaches to improve treatment efficiency and reduce operating costs. One of the main advancements involves the combination of aerobic and anaerobic processes, leveraging the benefits of both. Hybrid systems have become a well-established reality, where

aerobic processes, used for the removal of easily biodegradable substances, alternate with anaerobic treatments to handle waters with high organic matter concentrations and to produce biogas. For instance, the integration of an anaerobic reactor (UASB) followed by an aerobic treatment optimizes the process, reducing energy costs and sludge production.

A notable innovation is represented by Membrane Bioreactors (MBR), which combine biological treatment (aerobic or anaerobic) with a membrane filtration system to separate solids from the treated liquid. These systems are particularly useful in advanced treatments, where high effluent quality is required. Membrane Aerated Biofilm Reactors (MABR) are a more recent version, in which oxygen is directly diffused into the biofilm, improving the efficiency of the nitrification process and reducing energy consumption.

Another significant advancement concerns the use of biomass granules, which enhance biomass density and the separation of solids from liquids. Granular biofilm reactors like the SBBGR (Sequencing Batch Biofilm Granular Reactor) represent a technology that combines the benefits of aerobic biofilm treatments with sequential batch systems, potentially offering a more efficient and robust solution compared to other systems, such as traditional activated sludge reactors or biofilters. The ability of the SBBGR to effectively treat landfill leachate while maintaining high efficiency and flexibility makes it an attractive choice in this context.

1.3.2.1 Aerobic granulation and feast and famine dynamics in Sequencing Batch Reactors

Aerobic granular sludge represents an advanced and efficient technology for wastewater treatment, characterized by the formation of a compact biomass with high sedimentability. This technology primarily utilizes Sequencing Batch Reactors (SBR), which operate through discontinuous cycles of feeding, aeration, sedimentation, and effluent discharge. Figure 1.5 illustrates the phases of an SBR, highlighting the sequence of operations within each cycle. These cyclic operations create varying environmental conditions, promoting the emergence of microbial communities specialized in the simultaneous removal of organic matter, nitrogen, and phosphorus (Tay et al., 2001). The alternation between nutrient-rich (Feast) and nutrient-limited (Famine) phases plays a crucial role in the stabilization of granules, which develop and consolidate through specific ecological mechanisms.

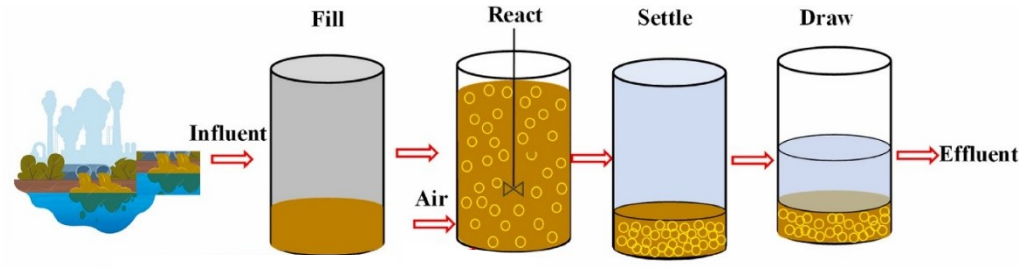


Figure 1.5 AGS SBR operating stages (Hussain et al., 2024)

The Feast and Famine cycle is a distinguishing feature of wastewater treatment systems based on aerobic granular sludge, particularly in SBR reactors. During the cycle, microorganisms are exposed to alternating periods of nutrient abundance (Feast) and scarcity (Famine), such as carbon and nitrogen. This alternation plays a crucial role in shaping the selection and growth of specific microbial populations, driving the development of densely aggregated and highly

sedimentable granules.During the Feast phase, which follows reactor feeding, nutrients are abundantly available. In this period, heterotrophic bacteria, which use organic carbon as a nutrient source, become highly active and consume the available substrate. The high nutrient availability stimulates the exponential growth of these microorganisms, fostering a rapid biomass increase. Specifically, bacteria capable of efficiently utilizing resources grow faster than others, gaining a competitive advantage. However, when nutrient levels reach optimal levels, bacterial growth slows, preparing the system for the transition to the Famine phase.

In the Famine phase, nutrients become depleted or are scarce. In this scenario, bacteria that have accumulated intracellular resources during the previous phase are those that survive best. The nutrient scarcity leads to a reduction in bacterial growth rates but stimulates the production of extracellular polymeric substances (EPS), which promote cohesion among microorganisms and the consolidation of biomass. EPS production not only enhances the stability of granules but also promotes the formation of microbial aggregates that exhibit greater resistance to dispersion compared to conventional activated sludge flocs.Furthermore, the nutrient scarcity during the Famine phase increases bacterial hydrophobicity, facilitating microorganism adhesion and the formation of compact granular structures (Tay et al., 2001). This process promotes the stabilization of granules, making them less vulnerable to environmental changes and physical and chemical stress.

1.3.2.2 Nitrification and Denitrification in Aerobic Granular Systems

Nitrification is an aerobic process that occurs in two phases, according the Eqs. 3 and 4. The first phase converts ammonia (NH_3) to nitrite (NO_2^-) through bacteria such as Nitrosomonas:

$$NH_3 + O_2 \rightarrow NO_2^- + H_20 + H^+$$

Equation 3

The second phase converts nitrite (NO_2^-) to nitrate (NO_3^-) through bacteria such as Nitrobacter:

$$NO_2^- + O_2 \rightarrow NO3^-$$

Equation 4

Denitrification, on the other hand, occurs in the absence of oxygen, where denitrifying bacteria, such as Pseudomonas and Paracoccus, reduce nitrate to nitrogen gas (N_2), which is then released into the atmosphere:

$$NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$$

Equation 5

Figure 1.6 depicts the diagram representing nitrification and subsequent denitrification, detailing the biochemical pathways involved.

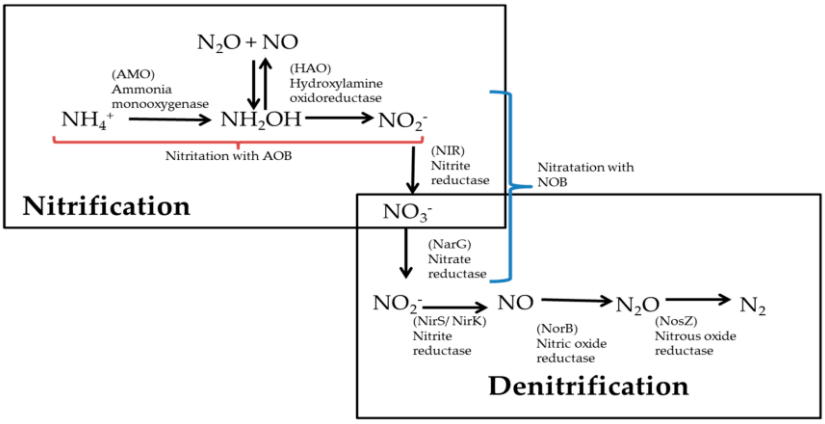


Figure 1.6 Diagram representing nitrification and denitrification. (Mpongwana et al., 2019)

One of the distinctive features of aerobic granular sludge is its stratified structure, which allows the coexistence of microorganisms with different redox

requirements. The outer layers of the granules are exposed to high oxygen concentrations, promoting the growth of heterotrophic bacteria that remove organic matter through nitrification. The intermediate layers house autotrophic bacteria that, in the presence of oxygen, convert ammonia (NH_3) to nitrite (NO_2^-) and then to nitrate (NO_3^-). In the inner part of the granules, where conditions become anoxic or anaerobic, denitrifying bacteria reduce nitrate to nitrogen gas (N_2), completing the nitrogen cycle (De Kreuk et al., 2005)

Figure 1.7 provides a graphical representation of the distribution of (a) microorganisms and (b) nitrogen removal pathways, illustrating how each process occurs within specific granule zones.

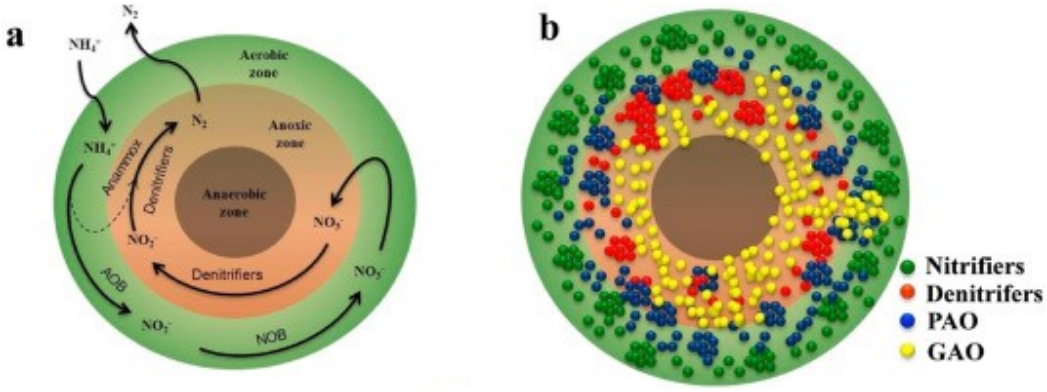


Figure 1.7 Graphical representation of the distribution of (a) microorganisms, (b) nitrogen removal pathways. Adapted fron (Nancharaiah & Kiran Kumar Reddy, 2018)

The coexistence of these redox conditions within a single granule enables the simultaneous execution of nitrification and denitrification, enhancing the overall efficiency of nitrogen removal in wastewater treatment (Nancharaiah et al., 2016). The granular sludge approach reduces the system's vulnerability to toxic substances and heavy metals, providing the granules with superior sedimentability compared to traditional activated sludge flocs, due to their spherical shape, defined contours, and high density (De Kreuk et al., 2005).

1.3.3 Advanced Oxidation Processes

Advanced oxidation processes (AOPs) are defined as techniques that use the in situ generation of highly reactive radicals, primarily the hydroxyl radical (•OH), for the oxidative degradation of complex organic contaminants. These processes may also involve other reactive oxygen species (ROS), such as singlet oxygen and superoxide, as well as radicals derived from persulfate, carbonate, or nitrate, influencing reaction kinetics, reaction mechanisms, and the formation of by-products (Wang & Wang, 2021). AOPs are applied in a wide range of areas, including drinking water and wastewater treatment, water reuse, brine and leachate treatment, and groundwater remediation (Sillanpää et al., 2018).

AOPs are distinguished by their ability to generate radicals through chemical, photochemical, catalytic, and other high-energy methods. Special attention has been given to the Fenton process, where •OH is produced by the activation of hydrogen peroxide (H_2O_2) with ferrous ions (Fe^{2+}), and its enhanced version through UV or solar radiation (Fischbacher et al., 2013). Other well-documented approaches include heterogeneous photocatalysis (e.g., TiO_2, ZnO) and ozone-based systems, which can generate •OH radicals either directly or indirectly through interactions with the aqueous matrix (Pocostales et al., 2010).

In addition to the AOPs already established at the industrial scale, there has been growing interest in emerging technologies such as sulfate radical ($SO_4^{\bullet-}$) oxidation, plasma treatments, and catalytic ozonation. These systems, often tested at pilot or laboratory scale, aim to improve process efficiency and sustainability, reducing energy consumption and costs associated with traditional AOPs (Guerra-Rodríguez et al., 2018; Khan et al., 2020). For example, heterogeneous catalysis in ozone decomposition utilizes advanced materials like metal oxides and activated carbon to promote interfacial reactions that enhance contaminant removal without introducing metallic ions into the solution.

This technological diversity and the broad range of applications make AOPs crucial tools for addressing challenges related to the management of

persistent organic contaminants, which pose serious threats to aquatic ecosystems and human health (Priyadarshini et al., 2022).

1.3.3.1 Ozone-Based Chemical Oxidation

Growing interest has been driven by their ability to degrade persistent pollutants through the in situ generation of highly reactive oxidizing agents, ensuring effective pollutant mineralization and the absence of secondary waste. Among the various AOP technologies, ozone-based processes have proven particularly promising due to their accessibility, high reactivity, and capability to purify final effluents.

Ozone, with a redox potential of 2.07 V, operates mainly through two mechanisms. The first is direct oxidation, where ozone attacks electron-rich sites in organic contaminants, such as double bonds or aromatic groups, breaking down molecules and forming simpler intermediates (Von Gunten, 2003). The second is indirect oxidation, in which ozone generates highly reactive hydroxyl radicals (•OH) through dismutation reactions or metal-catalyzed reactions (Eq. 6-7). Hydroxyl radicals, characterized by a high redox potential (~2.8 V), react rapidly and non-selectively with a wide range of contaminants, converting them into more biodegradable byproducts or into carbon dioxide and water (Andreozzi et al., 1999).

$$O_3 + H_2O \rightarrow 2\ \bullet OH + O_2$$

Equation 6

$$O_3 + M^{n+} \rightarrow M^{n+1} + \bullet OH$$

Equation 7

Despite its potential, ozonation has limitations such as low solubility and instability of ozone in water, as well as high generation costs[13]. Innovative

techniques, including ozonation at high pH, the O_3/H_2O_2 system, UV irradiation, and the use of catalysts, enhance process efficiency by promoting the formation of •OH radicals (Buffle et al., 2006). For instance, ozonation at pH levels above 8 leverages the interaction between ozone and hydroxide ions to generate superoxide anion radicals ($O_2•^-$), which further contribute to •OH production (Tizaoui et al., 2007).

In the context of landfill leachate treatment, ozonation processes are an effective option for removing color and refractory contaminants. Systems such as O_3/OH^- or O_3/H_2O_2 can oxidize large refractory organic molecules (up to 10^4 g/mol), converting them into more biodegradable compounds that can subsequently be removed through biological treatments. However, the presence of inhibitors such as carbonates, chlorides, and sulfates can reduce the oxidative efficiency of hydroxyl radicals, necessitating careful process design.

These characteristics make ozonation a versatile and promising technology for treating complex effluents, combining high oxidative performance with a significant reduction in the toxicity and recalcitrance of pollutants.

CHAPTER 2: RESEARCH PURPOSES AND PLAN

2.1 RESEARCH PURPOSES

Landfills represent a significant source of contamination from per- and polyfluoroalkyl substances (PFAS) in the environment, as these substances are found in numerous consumer products and industrial waste. International studies have documented high PFAS concentrations in landfill leachate, highlighting the urgency to develop effective removal solutions. The complexity of the matrix to be treated and the exceptional chemical stability of PFAS make traditional treatment methods insufficient. For this reason, in recent years, technologies combining chemical, physical, and biological treatments have been developed and applied to optimize results.

This study aims to validate innovative treatment schemes for removing PFAS from landfill leachate. The focus is on analyzing the removal performance of gross parameters and PFAS through an integrated approach that combines biological treatment, carried out in a Sequencing Batch Biofilm Granular Reactor (SBBGR), and chemical oxidation with ozone (O_3). The experimental activity will take place at the laboratories of the Water Research Institute (IRSA-CNR) in Bari, and the leachate to be treated, from non-hazardous waste landfills in northern Italy, will be provided by the project's partner company, Erica S.r.l.

The challenges to be addressed are related to two main aspects: the intrinsic characteristics of the leachate matrix and the complexity of PFAS as a chemical target. The matrix to be treated presents difficulties such as high salinity, elevated ammonia nitrogen values, the presence of humic acids, low BOD_5/NH_4 values, heterogeneity, and low biodegradability of pollutants. Regarding PFAS, the stability of the C-F bond and their presence in concentrations in the nanogram range make both determination and treatment complex.

Two laboratory-scale approaches will be tested: biological treatment (SBBGR) and biological treatment enhanced with ozone (BIO&CHEM). The experimental design includes four phases: an initial system start-up phase, a second phase dedicated to biological treatment in the SBBGR, a third phase in which the biological treatment will be enhanced with ozone, administered at two different doses, and a fourth phase in which the biological treatment was fed with leachate containing high concentrations of PFAS.The SBBGR will operate as an up-flow reactor, where the leachate will be sequentially fed, treated, and extracted. The influent and effluent quality parameters will be monitored through traditional chemical-physical analyses and PFAS concentration will be monitoring through an high-pressure liquid chromatography (HPLC) coupled with mass spectrometry.

The main research objectives are summarized in Table 2.1 and include: the chemical-physical characterization of leachate from non-hazardous waste landfills, the start-up of the biological process and achievement of steady-state conditions, and finally, enhancement of the treatment with ozone and its monitoring. COD, BOD_5, suspended solids, nitrogen compounds, and the concentrations of a set of target PFAS will be determined upstream and downstream of the two treatment schemes to evaluate the removal efficiency. Furthermore, sludge production for both treatment methods will be calculated.

The results of this research could have significant scientific and social impacts. From a scientific perspective, the study will contribute to advancing knowledge on innovative treatments for PFAS removal, a group of emerging contaminants that pose serious risks to human health and the environment. The integrated approach, combining biological and chemical treatments, could offer an effective and sustainable alternative to conventional methods, representing a significant step forward in managing contaminated water. From a social perspective, the research findings could lead to a review of existing regulations, considering potential risks to public health and the environment. Landfill and

leachate management is a crucial challenge for collective health and environmental protection, and the innovative solutions proposed by this study could play a fundamental role in improving waste disposal practices and preventing long-term contamination.

Table 2.1 Summary of the research purposes

Objective	Description
Chemical-Physical Characterization of Leachate	Determination of conductivity, pH, COD, BOD_5, nitrogen forms, TSS and VSS.
Activation of the Biological System (SBBGR)	Determination of the concentrations of a selected set of PFAS.
Enhancement of the SBBGR System with Ozone (BIO&CHEM)	Gradual achievement of steady-state conditions through an appropriate feeding system.
Evaluation of Performance in Terms of Gross Parameters for the Two Treatment Schemes	Introduction of an ozonation phase into the treatment cycle.
Evaluation of PFAS Removal Efficiency in SBBGR and BIO&CHEM	Determination of the efficiency of the two treatment schemes in terms of removal of COD, BOD_5, suspended solids, nitrogen, and color.

2.2 RESEARCH PLAN

The research plan aimed to evaluate, at a laboratory scale, the effectiveness of an ozone-enhanced SBBGR system for landfill leachate treatment, with a particular focus on PFAS removal. The study analyzed the performance of biological treatment (SBBGR) and the combined BIO&CHEM treatment to assess the feasibility and benefits of an integrated approach. The main research questions that guided the investigation were:

I. What is the effectiveness of biological treatment in removing PFAS and other significant parameters from leachate?
II. What is the effect of ozone on PFAS removal?
III. What compositional differences are observed in the effluent with and without ozone chemical treatment?
IV. How does the biomass respond when the PFAS concentration in leachate increases by approximately five times?

The project followed a four-phase structure. In the initial phase, the biomass was acclimated to a high salinity level (around 22 mS/cm) through gradual dilution of the leachate with water, encouraging the growth of the involved biological species. Subsequently, biological treatment was carried out in an SBBGR (Sequencing Batch Biofilm Granular Reactor), with a biofilter immobilizing the biomass on a porous plastic support, operating in eight-hour sequential treatment cycles (filling, reaction, discharge). The treatment was then enhanced with ozone by activating a chemical oxidation unit. In this experimental setup, an additional phase integrated biological degradation with chemical oxidation via ozone, allowing selective removal of biodegradable and refractory compounds. The intermittent use of ozone aimed to optimize treatment effectiveness by dosing it only on the refractory component to prepare it for subsequent microbial activity.

An additional phase was implemented with biological treatment using leachate with high PFAS concentrations to study the behavior and response of the biomass to elevated PFAS levels.

Influent and effluent samples from the treatments were analyzed using mass spectrometry and ultra-high-pressure liquid chromatography (UHPLC) to detect 13 PFAS (acids and sulfonates).

The project timeline included the following phases: start-up, biological treatment, ozone enhancement, biological treatment with leachate containing high PFAS concentrations, and data analysis, spanning a total duration of 15 months.

Experimental trials were conducted at the IRSA-CNR laboratories in Bari, using leachate provided by the ERICA s.r.l. company. The equipment used for the experimental activities included an SBBGR plant (geometric volume 25 L) consisting of a reactor, feed and recirculation pumps, aerator, washing system, effluent storage system, and process control and management system; an ozone chemical oxidation unit, equipped with an extraction pump, ozone generator, a reactor (volume: 5 L), and a residual ozone destructor.

Throughout the experimental period, key parameters such as temperature, pH, and dissolved oxygen were continuously monitored, as these factors could influence both biomass activity and PFAS removal efficiency.

This study allowed for a comparison of the effectiveness of two treatment schemes: biological treatment conducted in SBBGR mode and its integration with ozone-enhanced chemical oxidation. The results of this work pave the way for future research aimed at refining existing technologies to address environmental challenges related to PFAS and other difficult-to-treat contaminants.

CHAPTER 3: MATERIALS AND METHODS

3.1 DESCRIPTION OF TREATED LEACHATE

The experiment, lasting a total of 440 days, involved feeding the plant with six distinct batches of leachate derived from municipal waste landfills located in Lombardia, each characterized by specific physicochemical compositions and varying concentrations of PFAS. Each leachate sample was analyzed for conventional physicochemical parameters and for the presence of a targeted selection of PFAS.

The physicochemical characterization data of the six leachates used provided significant insights into the composition and treatability of these matrices, as detailed in Table 3.1. The COD values ranged from 1.850 g/L to 3.625 g/L, with elevated values across all samples, indicating a substantial presence of non-biodegradable organic matter.

The total nitrogen (TN) values in the leachates were particularly high, ranging from 689 mg/L to 1.685 g/L. The C/N (carbon/nitrogen) ratio was imbalanced, with excess nitrogen, which inhibited the denitrification process. This imbalance highlighted the necessity of adding external carbon sources, like acetate, to facilitate the full completion of the biological degradation process. The BOD_5/COD ratio, indicative of the biodegradability of the leachate, was low across all samples, suggesting limited biological degradation potential. A low BOD value relative to COD indicated that a significant portion of the organic matter in the leachate was resistant to biological degradation.

The electrical conductivity of the leachate samples was high, ranging from 15 mS/cm to 24 mS/cm, with consistently elevated values, indicating a significant concentration of dissolved solids and minerals.

Starting from the tenth month of experimentation, a new experimental strategy was introduced, involving feeding the system with ultraconcentrated leachate, characterized by high concentrations of PFAS. The total concentration of monitored PFAS in this type of leachate was approximately five times higher

than in previous samples. For certain compounds, such as PFOA, the concentration increased by a factor of 24, while for others, like PFOS and PFHxS, the values were about 30 times higher compared to standard leachate samples. The concentration of PFAS in the influent leachate showed significant variability for each compound, with the highest concentrations observed for PFOS, PFHxS, and PFOA. For example, the average concentration of PFOA in the standard leachate was 10.472 ng/L, while in the ultraconcentrated leachate, it reached an average concentration of 254.067 ng/L (Fig. 3.1, 3.2). Some PFAS, such as PFUdA and PFDoDA, were not detected in the analyzed samples.

The choice to use ultraconcentrated leachate was driven by the need to achieve sufficiently high PFAS concentrations, ensuring accurate measurements despite the instrumental challenges associated with quantifying compounds at nanogram-per-liter levels.

Furthermore, the use of high-concentration leachate allowed for monitoring the system's response more rapidly, providing valuable data on the treatment process's effectiveness under high PFAS load conditions.

This initial phase of characterizing the influent leachate samples provided a fundamental basis for understanding the variability of PFAS concentrations entering the plant and for tracking the evolution of the treatment process over time.

Table 3.1 Gross parameters analysis in the six leachate stocks used throughout the entire experimental period.

Parameter	Stock 1	Stock 2	Stock 3	Stock U4	Stock U5	Stock U6
pH	8.1	8.0	8.6	8.4	8.7	8.3
Cond. (mS/cm)	23.8	24	18.2	17.4	23.8	15.0
COD (mg/L)	3625	3420	2610	2447	2993	1850
sCOD (mg/L)	3295	3260	2580	2355	2887	1766
BOD_5 (mg/L)	416	226	-	122	244	71
TN (mg/L)	1685	1558	965	864	1237	689
NO_2 (mgN/L)	0.1	0	0.2	0.3	0.2	0.2
NO_3 (mgN/L)	9.3	11	9.1	6.9	10.9	5.4
NH3 (mgN/L)	1465	1470	882	827	1200	715
P (mg/L)	12.8	12	8.1	5.9	11.1	4.9
TSS (mg/L)	618	203	82.1	73.8	77.2	46.2
VSS (mg/L)	350	108	82.1	62.6	72.4	42.5
TS (g/L)	15.3	14	16.0	16.3	13.7	9.1
VS (g/L)	4.1	4	5.4	4.9	3.6	2.3

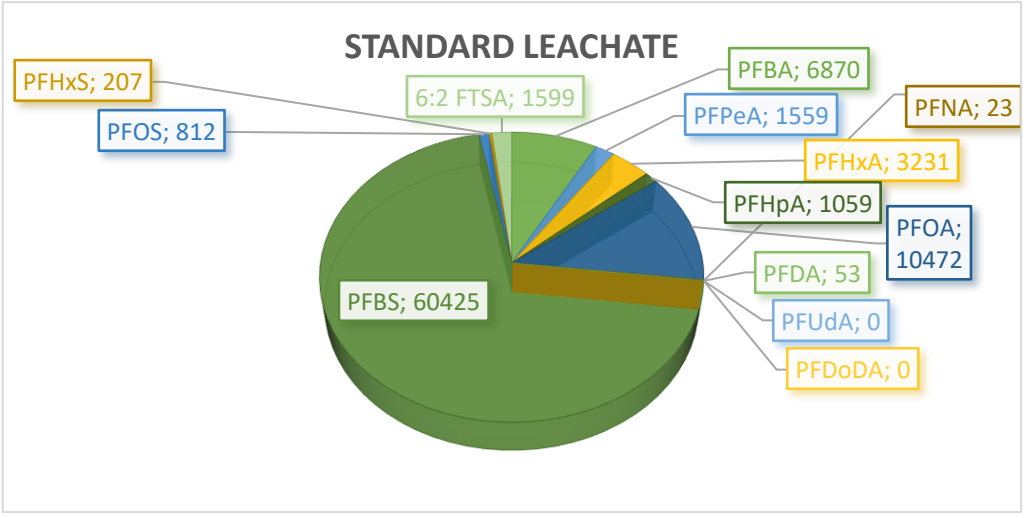
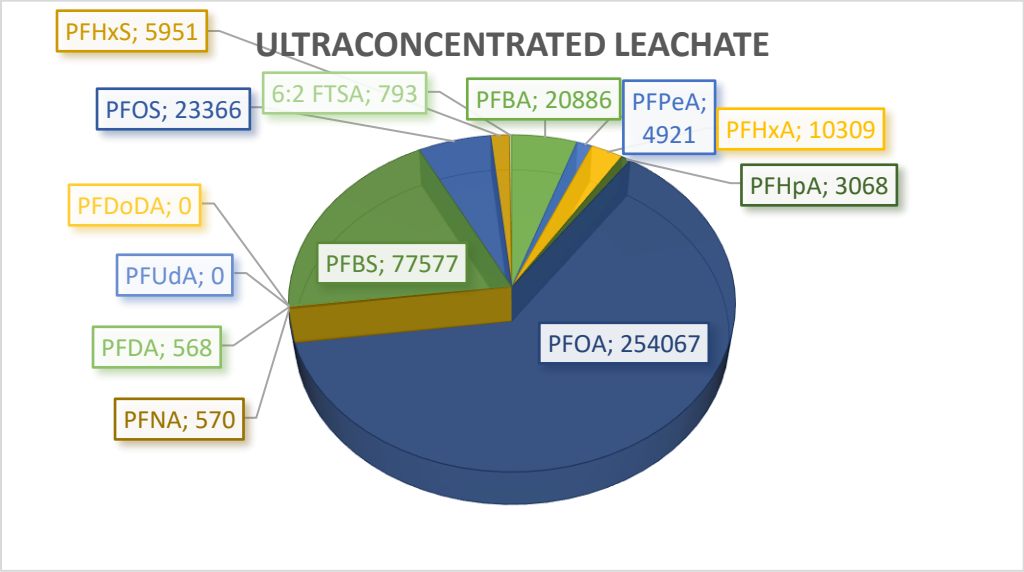


Figure 3.1 Average composition of target PFAS in stocks 1,2, and 3.



3.2 Average composition of target PFAS in stocks 4,5,and 6.

3.2 TREATMENT TECHNOLOGIES

Addressing PFAS contamination is an urgent environmental concern that threatens animal and human health. The complexity of the matrix to be treated and high chemical stability of PFAS makes conventional treatments inadequate for their effective removal. In recent years, technologies based on combination of chemical, physical and biological treatments have been used to maximize the outcome.

The experimental campaign presented in this study compares two treatment schemes: one with biological treatment only and its integration with chemical oxidation. The following sections provide a detailed description of the two proposed treatment schemes and their respective operating modes.

3.2.1 Sequencing batch biofilm granular reactor (SBBGR)

The SBBGR (Sequencing Batch Biofilm Granular Reactor) technology is an advanced system for the biological treatment of wastewater, particularly effective in treating complex liquids such as landfill leachates. The core of the system lies in the use of biological granules and biofilms, which form a dense, compact structure, maximizing the available surface area for bacterial proliferation and thus facilitating the removal of organic substances and nutrients such as nitrogen and phosphorus.

The experimental campaign was conducted using an SBBGR prototype at the laboratory scale (Figure 3.3).

The system includes:

- **Biofilter**: Located at the bottom, it contains a porous plastic support that retains biomass and promotes biological oxidation processes.
- **Aerator**: Positioned above the biofilter, it provides the necessary oxygen and collects the treated effluent.

- **Recirculation Circuit**: Ensures the continuous supply of substrate and oxygen along the reactor bed.

A pressure switch monitors the pressure drops in the biofilter, enabling washing interventions when necessary to maintain system efficiency.

The process operates in batch mode and consists of three consecutive phases:

- **Filling**: A pump (PA) introduces the wastewater into the reactor.
- **Reaction**: A recirculation pump (PR) distributes the wastewater and oxygen throughout the reactor bed, where pollutant degradation occurs. Oxygen can be supplied continuously or in anoxic intervals to optimize biological processes.
- **Discharge**: A motorized valve (MV) allows for the gravity discharge of the treated effluent.

These operations are automated via a microprocessor-based control system, ensuring repeatable and efficient treatment cycles.

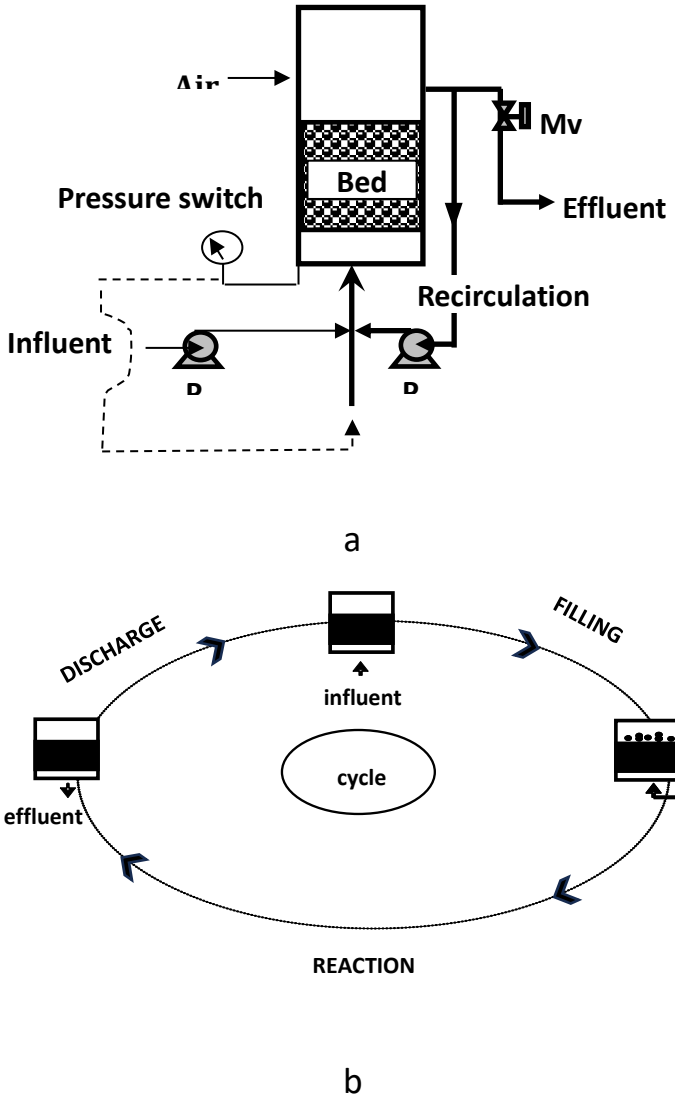


Figure 3.3 Diagram (a) and operation (b) of the SBBGR system.

Compared to traditional activated sludge reactors, the SBBGR offers superior performance with a smaller footprint. Additionally, due to the biomass retention strategy and the use of high-density biofilms and granules, sludge production is minimized.

The reduced sludge production is a result of the increased biomass concentration within the reactor, which lowers the net growth yield (Yn). This depends on the difference between the thermodynamic growth yield (Y) and the cellular decay rate (b), according to the formula:

$$\boldsymbol{Yn = Y - bX \ \left(-\frac{dS}{dt}\right)}$$

Equation 8

Where:

- Y: Yield related to substrate consumption.
- b: Biomass decay rate.
- X (-dS/dt): Substrate consumption rate per unit of biomass.

By increasing biomass, the system limits net growth, reducing excess sludge and improving the overall treatment efficiency.

3.2.2 SBBGR ozone-enhanced (BIO&CHEM)

The BIO&CHEM process integrates biological treatment (SBBGR) with chemical oxidation using ozone. The SBBGR system, with its modular and discontinuous configuration, enables the combination of biological degradation with targeted chemical oxidative treatment.

In the BIO&CHEM process, the plant operates in 8-hour treatment cycles. Compared to the traditional cycle (Figure 3.3), the enhanced cycle of the SBBGR system (Figure 3.4) includes an additional phase that integrates biological degradation with chemical oxidation.

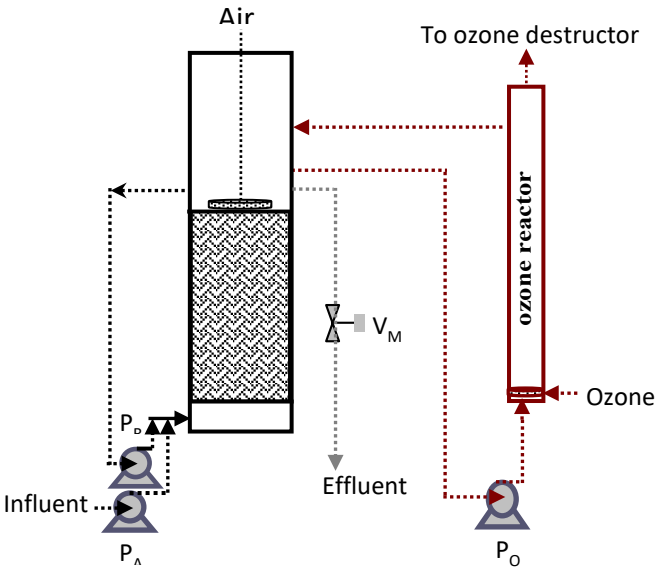
The process consists of the following steps:

1. **Loading:** Introduction of the leachate into the system.
2. **Biological degradation:** Removal of biodegradable compounds through the activation of recirculation between the aerator and the biofilter.
3. **Biological degradation + chemical oxidation:** Simultaneous activation of recirculation between the aerator and the biofilter and between the aerator and the ozonation column, where chemical oxidation occurs.
4. **Discharge:** Release of the treated effluent.

The oxidation phase occurs when the P_o pump (Figure 3.4) is activated, transferring liquid from the "aerator" zone of the SBBGR system to the ozonation column, where the oxidant is dosed.

The discontinuous nature of the SBBGR system allows the oxidizing agent to be used in a specific and controlled manner. The chemical reagent is dosed selectively, targeting only the refractory components after the initial biological degradation. Moreover, the oxidizing agent is added in a controlled way to partially oxidize the residual fraction, making it more amenable to subsequent microbial activity.

Thus, the strength of the BIO&CHEM process lies in its ability to operate selectively. The ozone-based chemical treatment exclusively targets compounds resistant to biological degradation, thereby minimizing the consumption of chemical reagents.



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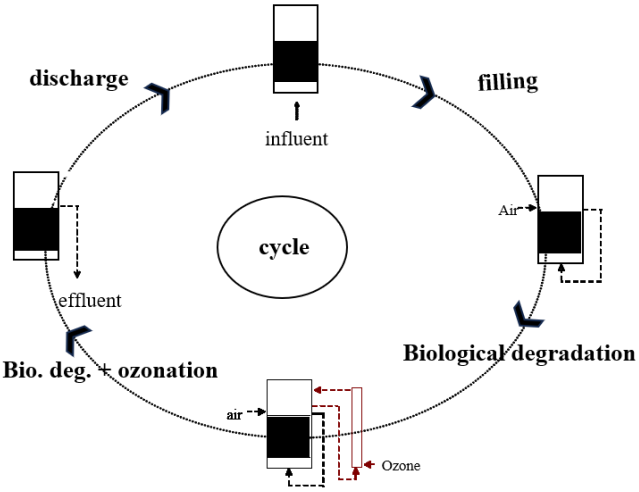

b

Figure 3.4 Diagram (a) and operation (b) of the BIO&CHEM process.

3.2.3 Experimental laboratory-scale plant

The experimental laboratory-scale plant consists of a biological unit and a chemical oxidation unit.

The biological unit (Figure 3.5) is an SBBGR (Sequencing Batch Biofilm Granular Reactor) system comprising:

- A cylindrical reactor (diameter: 19 cm, height: 100 cm; geometric volume: 28 liters) made of plexiglass, partially filled with loose plastic media (Kaldness wheel-shaped plastic cylinders, height: 7 mm; diameter: 8 mm; specific surface area: 690 m^2/m^3; density: 0.95 g/cm^3; porosity: 0.75) confined between two plates (bed volume: 13 L).
- A volumetric pump for recirculating liquid from the "aerator" zone to the biofilter (flow rate: 90 L/h).
- Two peristaltic pumps for loading leachate and acetate (used as an external substrate to support nitrogen removal).
- Three diffusers for supplying process oxygen via pure gaseous oxygen (from cylinders).
- A motorized valve for gravity discharge of the treated effluent.
- A probe for measuring head loss, with a corresponding display.
- A PLC for system automation.

In the present experiment, the SBBGR system was operated with a geometric working volume of 20 liters, 13 of which were occupied by the bed.

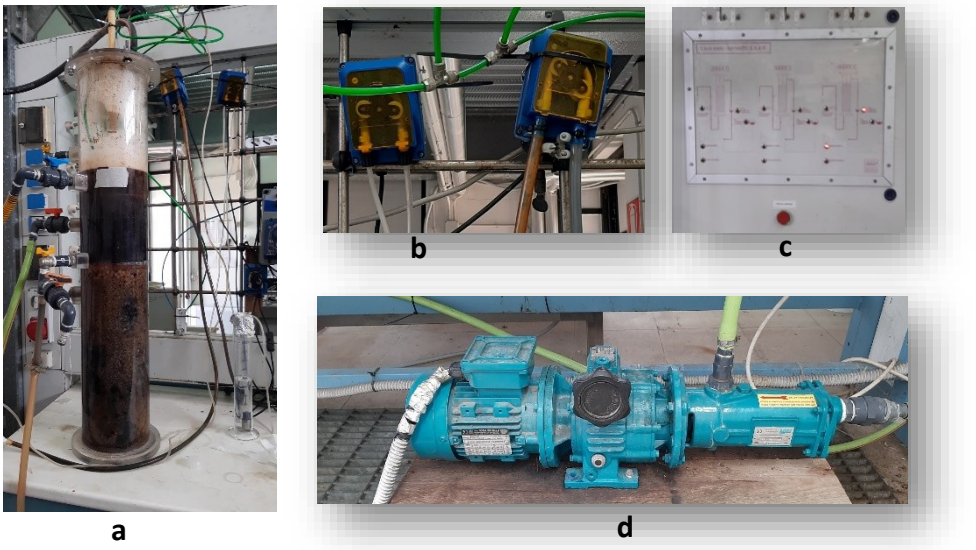

Figure 3.5 Photo of the biological unit of the experimental plant. a. Biological reactor; b. Peristaltic pumps for loading leachate and acetate; c. Programmable Logic Controller (PLC); d. Recirculation pump from the aerator to the biofilter.

The chemical oxidation unit (Figure 3.6) consists of:

- A peristaltic pump for extracting liquid from the SBBGR system (flow rate: 70 L/h).
- A cylindrical glass reactor (diameter: 85 mm; height: 90 cm; geometric volume: 5 L).
- An ozone generator (capacity: 0.5–8 gO_3/h; model: Modular 8HC, WEDECO, Germany) fed with pure gaseous oxygen.
- A thermocatalytic residual ozone destructor.
- A UV ozone analyzer for gas-phase ozone (model: BMT 964, WEDECO, Germany) to measure ozone concentration in the gas stream entering and exiting the ozonation reactor.

- An ambient ozone meter.

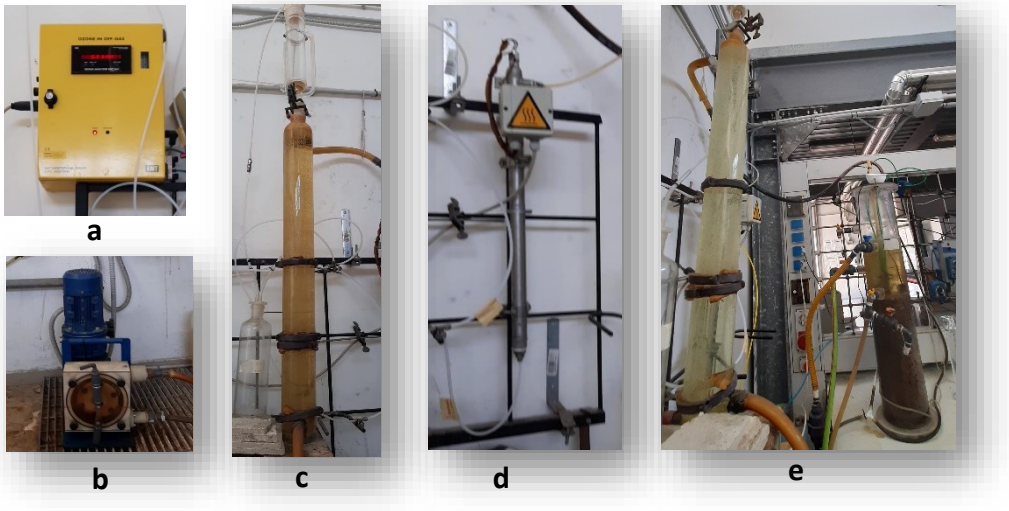

Figure 3.6 Photo of the chemical oxidation unit of the experimental plant. a. Ozone analyzer; b. Extraction pump; c. Reactor, ozonation column; d. Ozone destroyer; e. Ozonation column (L), biological reactor (S).

3.3 ANALYSIS METHODS

The influent and effluent of the two treatment schemes studied were characterized in terms of traditional parameters and PFAS. Chemical analyses enabled the monitoring of both the main quality indicators and PFAS. For PFAS identification, monitoring was carried out using advanced analytical techniques, such as high-resolution mass spectrometry coupled with liquid chromatography (LC-HRMS/MS). A total of 13 PFASs were detected, some acidic and others sulfonic. As for traditional contaminants, parameters such as chemical oxygen demand (COD), biological oxygen demand (BOD_5), total and volatile suspended solids (TSS and VSS), total nitrogen (TN), various forms of nitrogen (NH_3, NO_3^-, NO_2^-), and total phosphorus (P_{tot}) were measured using standardized methods.

Additionally, the ozone dose transferred during the chemical treatment of the BIO&CHEM process was measured to optimize the system's operating conditions.

The following sections will provide a detailed overview of the monitored parameters and the methods used for their determination.

3.3.1 Gross parameters analysis

The performance of the experimental plant was evaluated throughout the entire operational period by monitoring traditional parameters. Table 3.2 presents the list of traditional parameters along with their respective monitoring frequency and detection methods.

The transferred ozone dose per volume of percolate fed into the system (TOD transferred ozone dose), preferred over the dosed ozone to avoid the "scale" factor of the experiment, was calculated using the following equation:

$$TOD = \frac{F_{gas}}{V_{inf}} \int_0^t \left(C_{O_3 IN} - C_{O_3 OUT}\right) dt \qquad \text{Equation 9}$$

Where:

- F_{gas} (L/min) is the flow rate of the gas (oxygen-ozone) entering the ozonation reactor, controlled by the flowmeter of the ozone generator and set at 70 L/h during the entire experimental period.
- $C_{O3\ IN}$ and $C_{O3\ OUT}$ (mg O_3/L gas) represent the concentrations of ozone in the gas stream entering and exiting the ozone reactor, respectively, during the ozonation phase (i.e., the "biological degradation + ozonation" phase).
- V inf (L) is the volume of percolate fed per treatment cycle.
- $C_{O3\ IN}$ (adjusted by varying the power of the generator) and $C_{O3\ OUT}$ were measured using a UV analyzer (model BMT 964, WEDECO, Germany).

Table 3.2 Traditional parameters monitored, frequency, and detection methods used.

Parameter	**Monitoring Frequency**	**Method**
pH	2–3 times per week	Ion-selective electrode
Conductivity	2–3 times per week	Electrode
COD	2 times per week	Dichromate method (ISO 6060-1989, DIN 38409-H41-H44)
BOD_5	Once per month	EN 1899-1
TSS (Total Suspended Solids)	2 times per week	Gravimetric method (filtration at 1.2 µm and drying at 105 °C)
TN (Total Nitrogen)	2 times per week	Koroleff Digestion (Peroxodisulphate), and Photometric Detection with 2.6-Dimethylphenol (EN ISO 11905-1, ISO23697-1)
NH_3 (Ammoniacal Nitrogen)	2–3 times per week	Indophenol Blue method (ISO 7150-1, DIN 38406 E5-1)
NO_3^- (Nitrate Nitrogen)	2–3 times per week	2,6-dimethylphenol method (ISO 7890-1-2-1986, DIN 38405 D9-2)
NO_2^- (Nitrite Nitrogen)	2–3 times per week	Diazotization method (EN ISO 26777, DIN 38405 D10)
P_{tot} (Total Phosphorus)	2 times per week	Phosphomolybdenum blue method after digestion (EN ISO 6878-1-1986, DIN 38405 D11-4)
Color **(** 426, 558, and 660 nm)	1–2 times per week	Absorption in 1 cm quartz cells
O_3 in the gaseous phase	Continuous	UV analyzer (BMT 964 model, WEDECO, Germany)
Transferred ozone dose (TOD)		Refer to the description below
Head loss along the SBBGR bed	Continuous	Pressure sensor (Danfoss)

3.3.2 PFAS Analysis

For the analysis of PFAS, the analytical technique of high-resolution mass spectrometry coupled with liquid chromatography (LC-HRMS/MS) was employed. Specifically, the chromatographic system used (Ultimate 3000, Thermo Fisher Scientific) is interfaced with the TripleTOF 5600+ mass spectrometer system (AB Sciex). Analyses were conducted in ESI (-) mode.

A volume of 100 µL from each sample was injected into the LC-MS system using a ZORBAX Eclipse Plus C18 column (150 x 2.1 mm, 1.8 µm) with a flow rate of 0.300 mL/min and a temperature of 40 °C. Chromatographic separation of PFAS was achieved using 10 mM ammonium acetate (mobile phase A) and methanol (mobile phase B) with the following gradient: 0−2 min, 5% mobile phase B; 2−4 min, linear gradient from 5 to 70% mobile phase B; 4−9 min, linear gradient from 70 to 100% mobile phase B; 9−14 min, 100% mobile phase B; 14−14.5 min, from 100% to 5% mobile phase B; 14.5−20 min, reconditioning of the analytical column.

For the quantification of analytes, a calibration curve was prepared within the concentration range of 1–200 ng/L (1-5-10-20-50-100-200 ng/L). Prior to analysis, an internal standard consisting of a mix of 13C8-PFOA and 13C8-PFOS at a final concentration of 100 ng/L was added to both samples and the standards used for the calibration curve.

The following PFAS were analyzed: PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUdA, and PFDoDA, belonging to the perfluorocarboxylic acids category; PFBS, PFHxS, and PFOS, which are perfluorosulfonic acids; and 6:2 fluorotelomer sulfonic acid (6:2 FTSA).

3.4 STUDY PHASES

The experimental plant became operational in February 2023, initially running exclusively with the biological unit to evaluate the effectiveness of the SBBGR biological treatment alone. The plant treated leachate from municipal solid waste landfills located in Lombardy for a period of 440 days.

The SBBGR system was inoculated with a biomass consisting of 30% activated sludge taken from a treatment plant that processes landfill leachate, and the remaining 70% from activated sludge from a municipal wastewater treatment plant. This mixed inoculum ensured a broad variety of microbial species: the first inoculum included species already adapted to leachates, while the second provided microbial diversity to facilitate biomass acclimatization and growth.

The plant startup required a period of 103 days. During this phase, a gradual feeding program was implemented to acclimate the biomass to the high salinity of the leachate (22 mS/cm), starting from an initial conductivity of approximately 1.5 mS/cm. The feeding program involved a series of dilutions of the leachate with tap water in decreasing proportions, with intervals established based on quality checks of the effluent. During this period, two different stocks of leachate (Stock 1 and Stock 2) were treated.

Starting from the 104th day, the SBBGR system was fed with raw leachate while continuing to evaluate the biological treatment alone.

From the 137th day, the BIO&CHEM process was activated, enhancing the SBBGR system with chemical oxidation using ozone. O_3 was applied at two different dosages: 4 gO_3/L_{inf} from the 137th to the 142nd day, and 5.5 gO_3/Linf from the 143rd to the 206th day.

From the 213th day, the chemical oxidation unit was deactivated due to a biomass inhibition issue. The SBBGR system returned to full operational efficiency with biological treatment alone by the 240th day.

From the 262nd day, the plant was fed with a new stock of leachate (Stock 4), referred to as "PFAS-concentrated leachate," obtained by mixing a leachate with a high concentration of PFAS.

The operational conditions of the system during the various phases are summarized in Tables 3.3 to 3.5, which provide detailed information on dosages, daily cycles, and the characteristics of the leachates used.

Table 3.3 Operating conditions of the SBBGR system fed with leachate during the start-up phase.

Period (days)	1–103rd
Leachate stock number treated	1
Type of treatment	Biological (start-up phase)
Feeding flow rate (L/d)	Up to 4
Number of cycles per day (cycle duration in hours)	3 (8)

Table 3.4 Operating conditions of the SBBGR system fed with raw leachate from the 104th to the 136th day of operation.

Period (days)	104th–136th
Leachate stock number treated	1 and 2
Type of treatment	Biological
Feeding flow rate (L/d)	3
Number of cycles per day (cycle duration in hours)	3 (8)

Table 3.5 Operating conditions of the SBBGR system enhanced with ozone and fed with leachate from the 137th to the 206th day of operation.

Period (days)	137th–142nd	143rd–206th
Leachate stock number treated	2	2 and 3
Type of treatment	Biological + O_3	Biological + O_3
Feeding flow rate (L/d)	3	3
Number of cycles per day (cycle duration in hours)	3 (8)	3 (8)
Ozone dose applied, TOD, (gO_3/L_{inf})	4	5,5
Duration of the chemical enhancement phase per cycle (h)	1	1

Table 3.6 Operating conditions of the SBBGR system with high PFAS concentration leachate from the 262nd to the 440th day of operation.

Period (days)	262nd – 440th
Leachate stock number treated	4, 5 and 6
Type of treatment	Biological
Feeding flow rate (L/d)	1.6 to 2.4
Number of cycles per day (cycle duration in hours)	3 (8)

CHAPTER 4: RESULTS AND DISCUSSION

4.1 START-UP PHASE

The startup phase of the SBBGR system demonstrated the crucial role of acclimation in ensuring the stability and efficiency of a biological treatment plant for landfill leachate. This process allowed the microbial community to gradually adapt to the system's specific conditions, mitigating the risk of cellular shock that could otherwise compromise metabolic activity and treatment performance. The biomass used for inoculation was composed of a mixture of 30% activated sludge from a landfill leachate treatment plant and 70% active sludge from a municipal wastewater treatment plant.

Over a period of 103 days, the system underwent a carefully designed feeding program to acclimate the biomass to the high salinity of the leachate (approximately 23 mS/cm). Starting from an initial conductivity of 1.5 mS/cm, the leachate was progressively diluted with tap water in seven steps, each aimed at gradually increasing the salinity, thus avoiding overwhelming the microorganisms with sudden changes. Effluent quality checks guided the transition from one step to the next, until undiluted leachate was fed. This phase allowed the development of a specialized microbial population capable of effectively degrading pollutants such as BOD_5, COD, ammonia, and nitrates, even under the challenging conditions of high salinity and complex leachate composition.

Continuous monitoring of parameters such as dissolved oxygen, pH, temperature, electrical conductivity, organic load, solids, and nitrogen helped maintain optimal growth conditions for the microbial community. The results of

this monitored acclimation process are summarized in the figures 4.1 and 4.2, which describe the evolution of operational parameters during the startup phase.

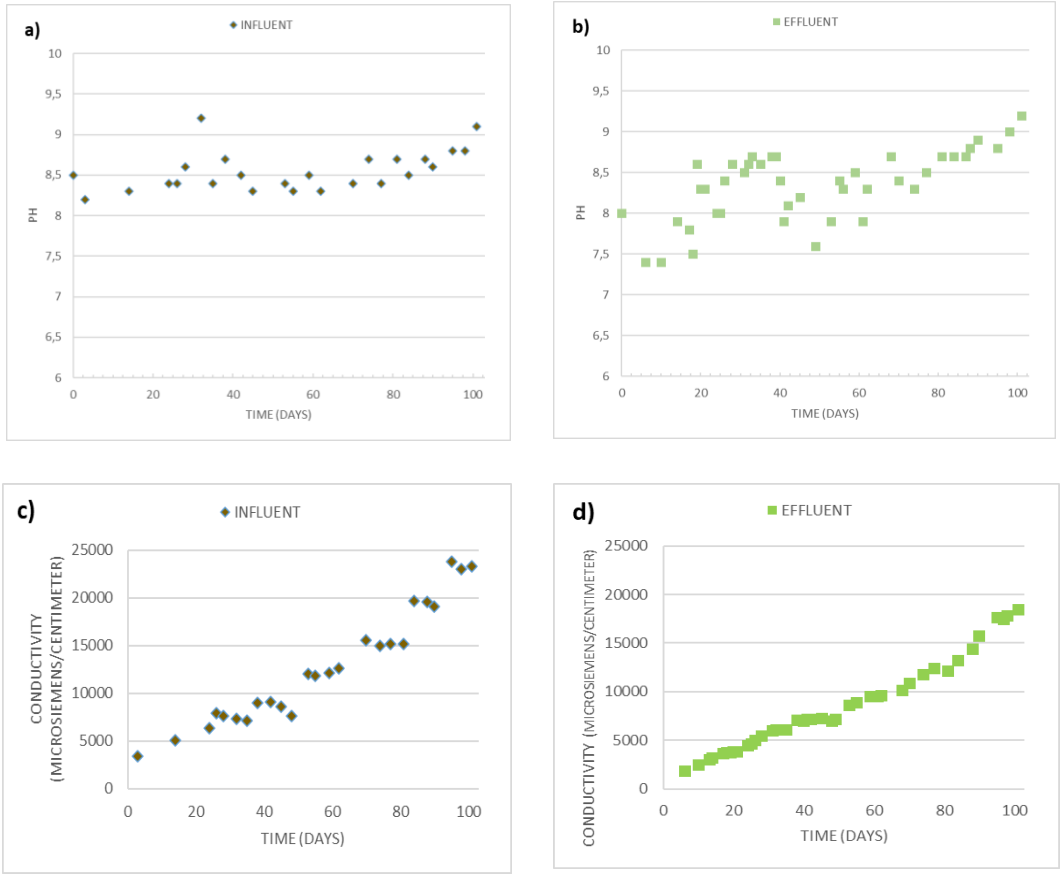

Figure 4.1 Trend of pH (a; b) and conductivity (c; d) at the inlet and outlet of the plant during the start-up phase.

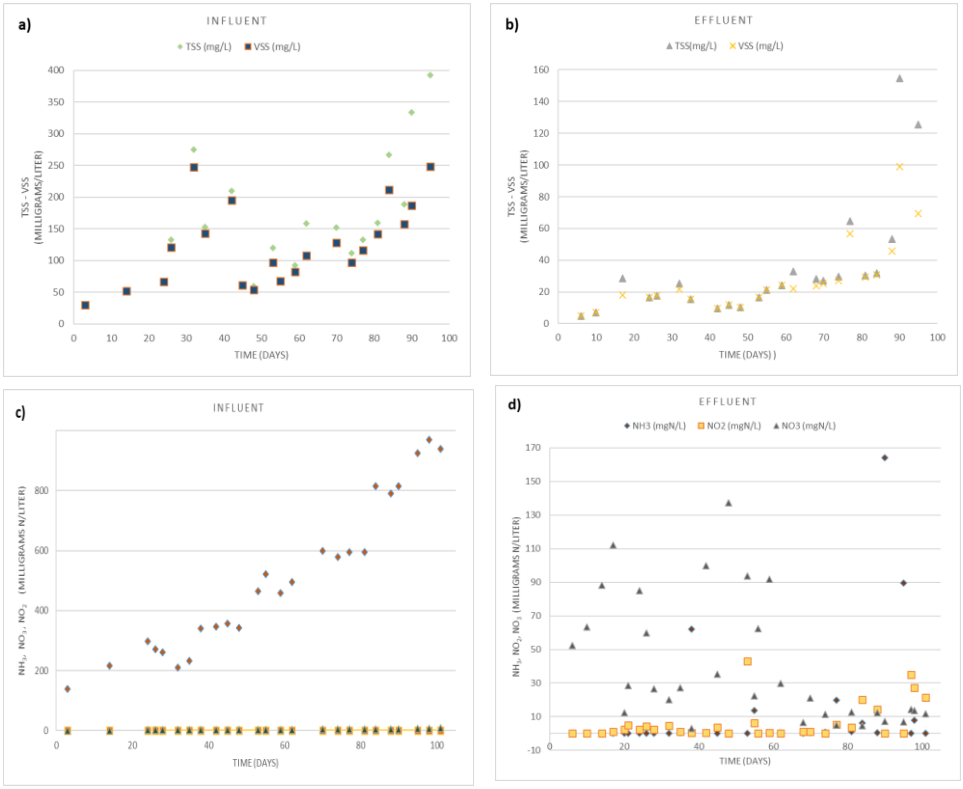

Figure 4.2 Trend of suspended solids and volatile solids (a; b) and nitrogen species (c; d) at the inlet and outlet of the plant during the start-up phase.

4.2 Biological treatment (SBBGR)

The SBBGR system was applied to the biological treatment of leachates from municipal solid waste landfills.

The startup phase required an initial acclimation period for the biomass to adapt to the leachate, lasting 103 days. Subsequently, from day 104 to day 136, the system operated in SBBGR mode.

During this phase, the chemical-physical characteristics of the influent and effluent leachates were analyzed, with particular attention to the system's efficiency in reducing traditional parameters such as COD, BOD_5, suspended solids, and nitrogen. Additionally, the study focused on the effectiveness of PFAS removal, highlighting the system's performance in treating persistent contaminants commonly found in landfill leachates.

4.2.1 Gross parameters removal efficiency

The data presented in Table 4.1 show a COD removal of 1.494 mg/L (from 3.250 to 1.756 mg/L), corresponding to an efficiency of 46% (Fig. 4.4). This relatively low efficiency is attributed to the low content of biodegradable compounds in the leachate, as indicated by the low BOD5/COD ratio, which reflects the limited biodegradability of the leachate. The high concentration of recalcitrant organic compounds in the leachate is further confirmed by the substantial residual COD in the effluent of the SBBGR system (1.756 ± 145 mg/L) and the virtually zero BOD5 value in the effluent. This suggests that the removal of biodegradable compounds was nearly complete, but the system was less effective in degrading the more persistent organic substances. Fig. 4.3 shows the values of COD, total nitrogen (TN), total suspended solids (TSS), and ammonia (NH_3) in the influent and effluent of the SBBGR system. Fig. 4.4 illustrates the removal efficiencies of the main parameters during biological treatment in the SBBGR system.

A particularly noteworthy aspect is the removal of nitrogen. The SBBGR system was able to remove 99% of the ammonia nitrogen in the treated leachate, highlighting a very effective nitrification process, despite the high salinity values that typically hinder the growth of nitrifying microbial species.

Additionally, the system achieved excellent efficiency in total nitrogen removal (91%) through an extended denitrification process, which reduced oxidized nitrogen to molecular nitrogen gas. This process was effective even though pure oxygen was supplied at concentrations ranging from 15 to 25 mg/L. The average TN values in the effluent of the SBBGR system were significantly lower than those in the influent (127 mg/L versus 1476 mg/L). This difference can primarily be attributed to the biological denitrification processes occurring in the system, considering the minimal sludge production. Indeed, during denitrification, nitrogen is converted to molecular nitrogen gas, which leaves the liquid phase and is released into the atmosphere, contributing to the reduction of TN levels in the effluent.

Table 4.1 Gross parameters in the influent and effluent of the SBBGR system during biological treatment

Parameter	Unit	Influent (mean value ± SD)	Effluent (mean value ± SD)
Cond.	mS/cm	23,9 ± 0,6	23 ± 0,8
pH	-	8,4 ± 0,2	8,7 ± 0,1
COD	mg/l	3250 ± 269	1756± 145
COD_{sol}	mg/L	3050 ± 297	1677 ± 124
BOD_5	mg/L	226	0
SST	mg/L	279 ± 151	107 ± 68
SSV	mg/L	198 ± 138	61 ± 25
TN	mgN/L	1476 ± 85	127 ± 19
NH_3	mgN/L	1423 ± 41	10 ± 16
NO_3-	mgN/L	10,4 ± 1	14,3 ± 3,3
NO_2-	mgN/L	0,13 ± 0,03	24,5 ± 10
P_{tot}	mgP/L	11,2 ± 0,3	10,7 ± 1,2

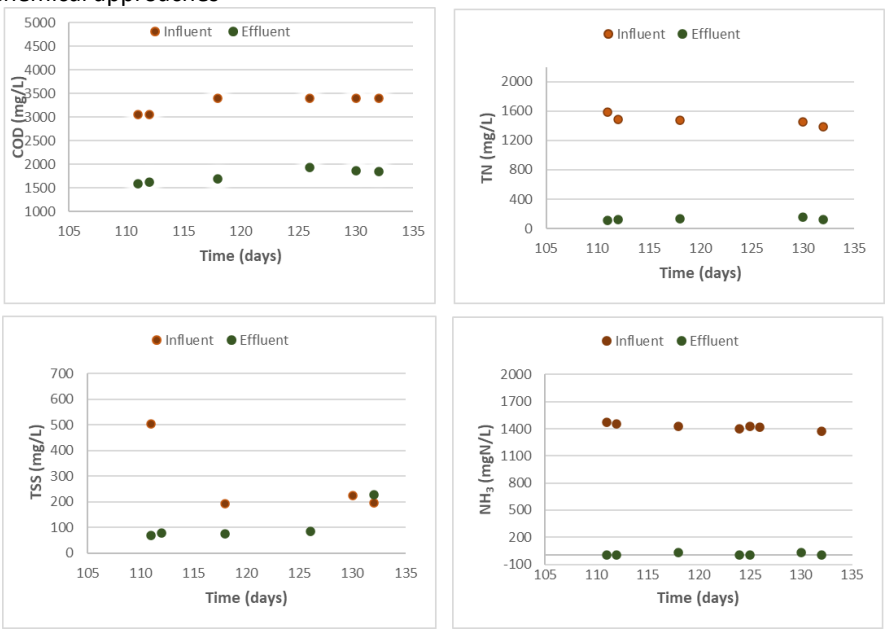

Figure 4.3 Values of Chemical Oxygen Demand (COD), total nitrogen (TN), total suspended solids (TSS), and ammonia (NH_3) in the influent and effluent of the SBBGR system during biological treatment.

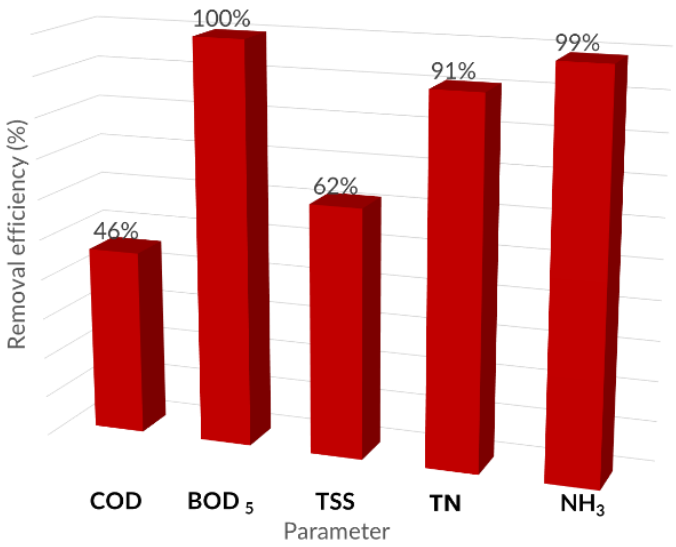

Figure 4.4 Removal efficiencies of traditional parameters, such as COD, BOD_5, TSS, TN, and NH_3, of the SBBGR system during biological treatment

4.2.2 Velocity of COD, TN and NH_3 Degradation in an SBBGR Biological Treatment cycle

During the biological treatment cycle conducted in SBBGR mode, the main pollutant parameters, namely chemical oxygen demand (COD), total nitrogen (TN), and ammonia (NH_3), were monitored at five time intervals (1, 3, 5, 7, and 8 hours) within an 8-hour treatment period. The following graphs (Figure 4.5; Figure 4.6) summarize the trends in the concentrations of COD, TN, and NH_3 over the 8 hours of biological treatment, highlighting the removal rates.

The removal efficiency of TN and ammonia was significantly high. TN showed a removal efficiency of 88.8%, decreasing from an initial concentration of 1385 mg/L to 155 mg/L by the end of the cycle. Similarly, NH_3 concentration dropped from 1370 mgN/L to 0.8 mgN/L, with a removal efficiency of 99.9%. These results suggest effective nitrification and denitrification activities within the biological system.

Regarding the removal of organic matter, expressed as COD, the removal efficiency was lower, at 41.4%. The COD concentration decreased from 3060 mg/L to 1792 mg/L, with an overall reduction of approximately 1.3 g/L, indicating partial degradation of the organic matter.

In all cases (COD, TN, and NH_3), the majority of oxidation occurs within the first hour of treatment, with substantial reductions observed at the 1-hour interval. This suggests that the oxidation process is rapid and stabilizes during the early stages of the cycle, with a slower degradation rate in the following hours.

In summary, the SBBGR biological treatment proved highly efficient in removing nitrogen, with excellent ammonia and TN removal efficiencies. However, the removal of COD, while significant, was partial, with a 41.4% efficiency. This observation suggests that an additional treatment may be necessary to complete the degradation of the remaining organic matter, such as an advanced oxidation treatment.

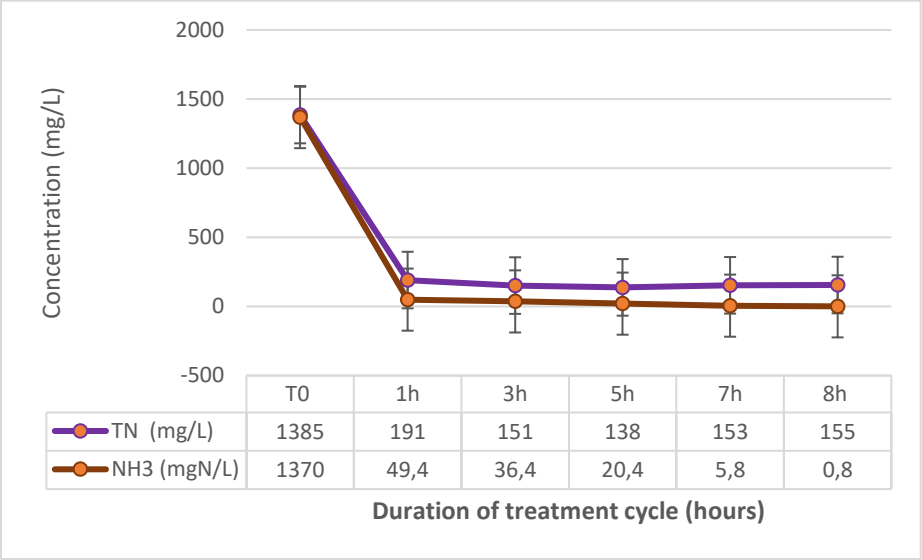

Figure 4.5 Degradation rate of TN and NH_3 within a SBBGR biological treatment cycle

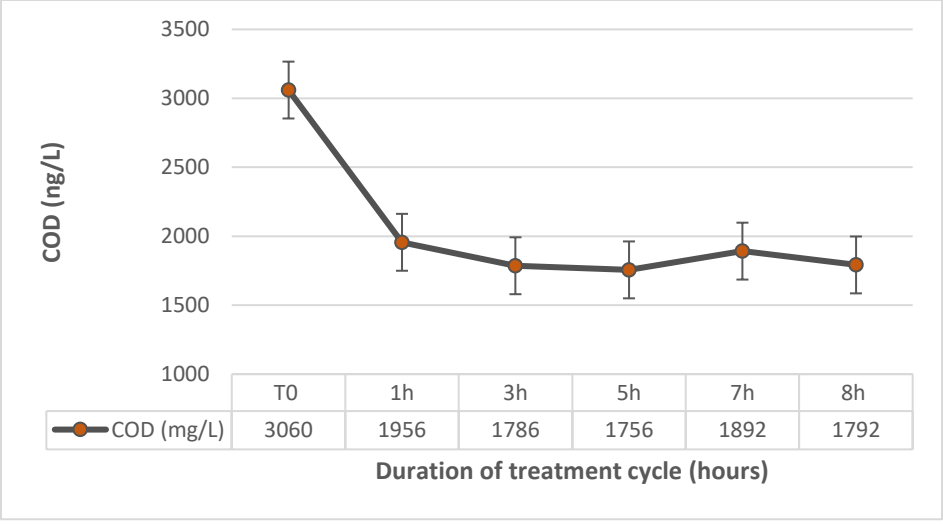

Figure 4.6 Degradation rate of COD within a SBBGR biological treatment cycle.

4.2.3 PFAS removal efficiency

The SBBGR system was evaluated for its ability to remove PFAS through an analysis of overall performance and efficiency in treating these contaminants. The data presented in tables 4.2 and figure 4.7 highlight that PFAS removal depends significantly on the chemical structure and physicochemical properties of the compounds. In particular, the average concentration values of the compounds at the system's inlet (INF) and outlet (EFF), along with the removal efficiency percentage (Removal efficiency %), suggest that the removal capacity is strongly influenced by the carbon chain length and solubility of the PFAS. Compounds with long carbon chains, such as PFHxA, PFHpA, PFOA, PFOS, PFHxS, and 6:2 FTSA, show complete removal, with a removal efficiency of 100%. These compounds, belonging to both the sulfonic PFAS class (e.g., PFOS and PFHxS) and the acid class (e.g., PFHxA, PFHpA, PFOA, and 6:2 FTSA), are particularly effectively removed by the system. In contrast, compounds with shorter chains, such as PFBA and PFPeA, characterized by higher water solubility, show limited removal, at 9% and 12%, respectively. PFBS, with a high initial concentration of 67.525 ng/L, shows a modest reduction in its concentration in the liquid phase, with a removal of 19%. Finally, for compounds such as PFNA, PFDA, PFUdA, and PFDoDA, they were not detected at either the inlet or outlet, as they were either not present in the analyzed samples or had concentrations below the detection limits of the analytical instrument used. This suggests that the SBBGR system is particularly efficient in removing long-chain PFAS, both acid and sulfonic, while being less effective for shorter-chain PFAS.

Table 4.2 PFAS concentrations at the inlet and outlet of the SBBGR system during biological treatment

PFAS Abbreviation	Influent (ng/L) ± SD	Effluent (ng/L) ± SD
PFBA	8363 ± 1805	7573 ± 513
PFPeA	1643 ± 112	1442 ± 98
PFHxA	3318 ± 244	1286 ± 308
PFHpA	1103 ± 185	< LOD
PFOA	10595 ± 827	< LOD
PFNA	< LOD	< LOD
PFDA	< LOD	< LOD
PFUdA	< LOD	< LOD
PFDoDA	< LOD	< LOD
PFBS	67525 ± 1068	54510 ± 4496
PFOS	556 ± 226	< LOD
PFHxS	207 ± 27	< LOD
6:2 FTSA	1664 ± 81	< LOD

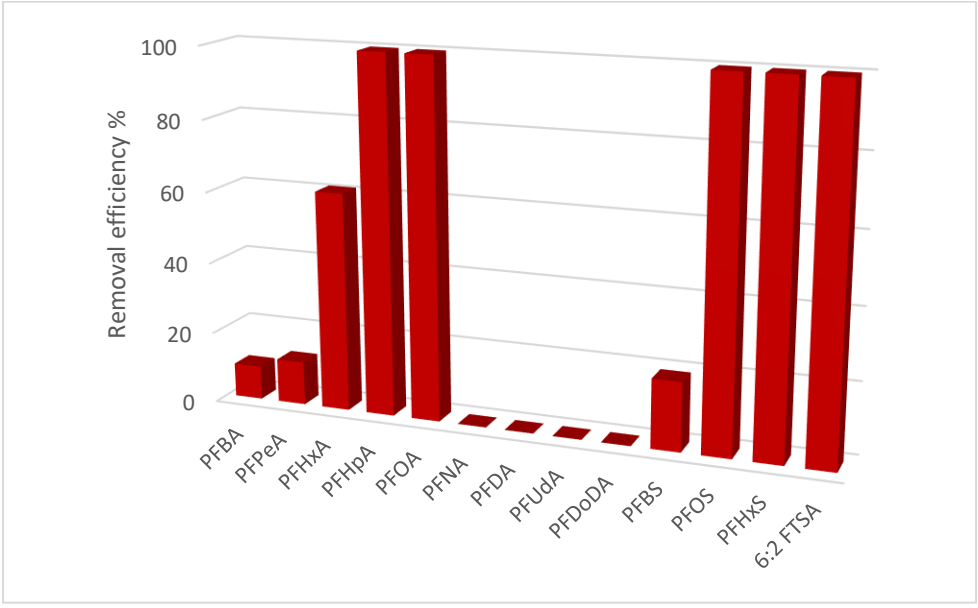

Figure 4.7 PFAS Removal Efficiency (%) in Biological Treatment.

4.3 BIOLOGICAL TREATMENT (SBBGR) OZONE – ENHANCED

The enhanced biological treatment with ozone, the BIO&CHEM process, was activated during the trial on day 137 and continued to operate until day 206. During this period, a chemical oxidation system with ozone dosing was integrated, and the plant was supplied with two stocks of leachate, Stock 2 and Stock 3. Starting from day 168, the plant began using only Stock 2.The integration of biological treatment with chemical oxidation was achieved through the application of ozone at two different doses (4 and 5.5 g/L) to a recirculating flow from the biological reactor inside an ozonation column.

4.3.1 Gross parameters removal efficiency

The treatment of leachate using the BIO&CHEM process, which integrates biological degradation with chemical oxidation via ozone, was carried out between the 137th and 206th days of the experiment. The primary goal was to improve the removal efficiency of recalcitrant COD through the oxidative action of ozone, thereby making it more accessible for subsequent biodegradation by the biomass. Two ozone dosages were used: 4 g/L and 5.5 g/L. The results for key parameters monitored in the influent and effluent during treatment with the two ozone dosages are presented in Tables 4.3 and 4.4. The obtained data were analyzed to evaluate the overall effectiveness of the process, with a particular focus on the impact of ozone on effluent quality.

With an ozone dosage of 4 g/L, the results showed a significant reduction in the monitored parameters. The COD concentration in the effluent decreased from 3420 mg/L (influent) to 1306 mg/L, corresponding to an average removal of 62%.

Regarding ammonia nitrogen (NH_3), the treatment achieved near-total removal, with NH_3 levels decreasing from 1380 mgN/L to 1 mgN/L, demonstrating the effectiveness of the biological nitrification process. Total nitrogen (TN) was

reduced from 1483 mgN/L to 196 mgN/L, with an 87% removal efficiency. (Figure 4.8)

The use of ozone at 5.5 g/L resulted in further improvements, particularly regarding COD and TSS. The COD in the effluent was significantly reduced, dropping from 3420 mg/L to 1019 mg/L, with a 70% average removal, which is higher compared to the 4 g/L dose. (Figure 4.8)

The removal of ammoniacal nitrogen was also nearly complete, with NH_3 levels reduced to 0.6 mgN/L (from 1380 mgN/L in the influent). Total nitrogen experienced a marked decrease, from 1483 mgN/L to 413 mgN/L, with a 72% removal efficiency.

Table 4.3 Gross parameters in the inlet and outlet of the SBBGR enhances with ozone 4 g/L.

Parameter	Unit	Influent Mean Value	SD	Effluent Mean Value
Cond.	mS/cm	23.9	0.6	23
pH	-	8.2	0.4	8.5
COD	mg/l	3420	28	1306
COD_{sol}	mg/L	3260	0.0	1276
SST	mg/L	132	68	197
SSV	mg/L	84	21	83
TN	mgN/L	1483	133	196
NH_3	mgN/L	1380	156	1
NO_{3-}	mgN/L	12.6	3.1	115.2
NO_{2-}	mgN/L	0.2	0.1	13.5
P_{tot}	mgP/L	11.6	0.7	9.6

Table 4.4 Gross parameters in the inlet and outlet of the SBBGR enhanced with 5.5 g/L of ozone.

Parameter	Unit	Influent Mean Value	SD	Effluent Mean Value	SD
Cond.	mS/cm	23.9	0.6	24	0.2
pH	-	8.2	0.4	8.5	0.1
COD	mg/l	3420	28	1019	66
COD_{sol}	mg/L	3260	0.0	1008	85
TSS	mg/L	132	68	11	10
TN	mgN/L	1483	133	413	125
NH_3	mgN/L	1380	156	0.6	0.1
NO_{3-}	mgN/L	12.6	3.1	331	96
NO_{2-}	mgN/L	0.2	0.1	13	7
P_{tot}	mgP/L	11.6	0.7	10	0.5

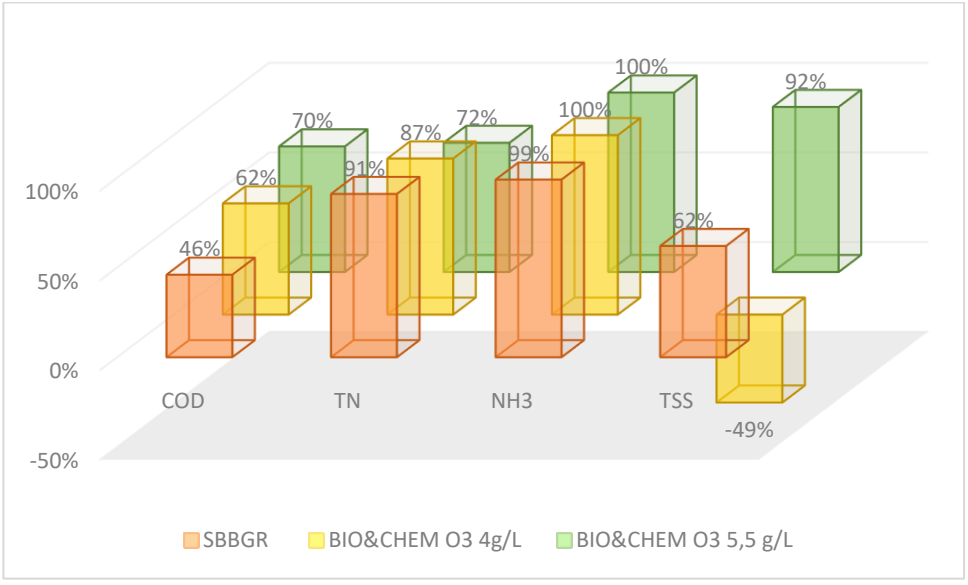

Figure 4.8 Removal efficiency of key parameters (COD, TN, NH_3, P tot) in the SBBGR and SBBGR enhanced with ozone at concentrations of 4 and 5.5 g/L.

Despite improvements in effluent quality, the modest efficiency of ozone in treating COD raises concerns about the overall effectiveness of the BIO&CHEM process integrated with ozone. The difficulty in achieving significant benefits from the integration of biological and chemical treatment is mainly due to the low efficiency of ozone, which does not fully justify the complexity and additional costs associated with combining the two processes.

For each gram of ozone used (4 gO_3/L), only 0.1 grams of COD are removed, as evidenced by the ratio between the amount of ozone applied and the COD reduction achieved compared to biological treatment (approximately 450 mg/L). The effluent from the biological treatment showed a COD value of 1756 mg/L, while the effluent from the ozone-enhanced treatment (4 g/L) presented a COD value of 1306 mg/L. The difference between these two values represents the amount of COD removed exclusively by ozone, which is 450 mg/L, corresponding to a removal efficiency of 9%, considering an initial COD of 3420 mg/L in the influent. This efficiency ratio indicates a very low overall performance. This phenomenon can be attributed to the presence of scavengers, compounds that consume oxygen without contributing to COD removal, as well as the high salinity of the leachate, which reduces the effectiveness of ozone.

A parameter that benefits significantly from the use of ozone is color removal. Figure 4.9 presents the removal efficiencies of color in the three operating conditions studied (biological treatment, BIO&CHEM with 4 g/L ozone, and BIO&CHEM with 5.5 g/L ozone). Color removal was assessed by measuring absorbance at three wavelengths (426 nm, 556 nm, and 660 nm). The results show a significant improvement in color removal with the use of ozone.

For absorbance at 426 nm, which is typical of the organic compounds responsible for color in the leachate, removal increased from 9% with the SBBGR biological treatment to 81% with the 4 g/L ozone dosage, and 92% with the 5.5 g/L dosage.

These data confirm the effectiveness of ozone in removing color from the treated leachate, resulting in a substantial improvement in effluent quality. This

is clearly demonstrated in Figure 4.10 , which compares a raw leachate sample with the effluents from biological treatment, BIO&CHEM with 4 g/L ozone, and BIO&CHEM with 5.5 g/L ozone.

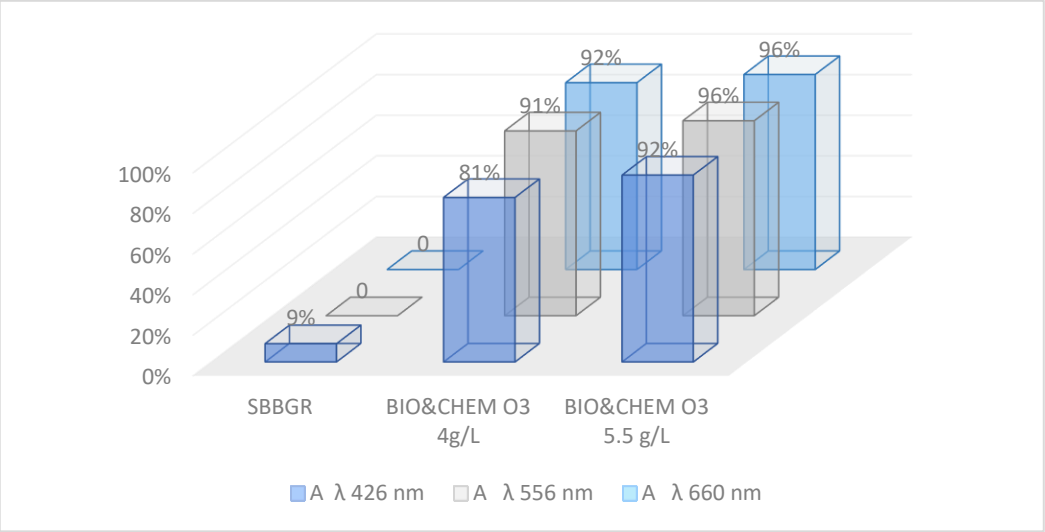

Figure 4.9 Color removal in the SBBGR and SBBGR enhanced with ozone at concentrations of 4 and 5.5 g/L.

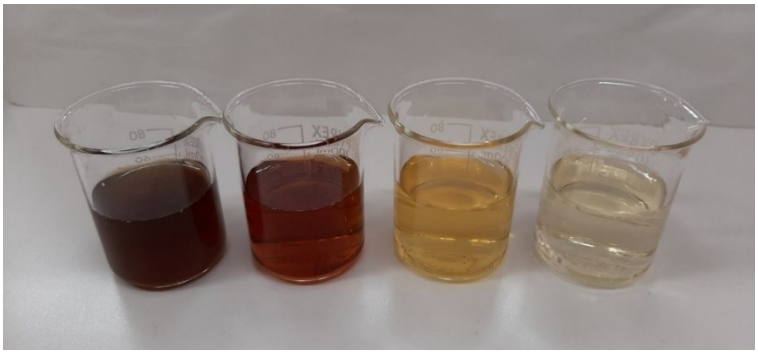

Figure 4.10 Photo of the following samples: leachate, effluent from the biological treatment, effluent from the biological treatment enhanced with ozone at 4 g/L, and effluent from the biological treatment enhanced with ozone at 5.5 g/L.

The BIO&CHEM process demonstrated effective removal of COD, nitrogen, and color.

When compared to the SBBGR biological treatment, the BIO&CHEM process exhibited higher COD removal efficiencies and similar nitrogen removal performance. Nonetheless, despite improvements in COD and color removal, the integration of ozone does not fully justify the added complexity and operational costs, given the relatively low ozone efficiency in COD removal and the high energy consumption required to achieve the observed improvement.

4.3.2 PFAS removal efficiency

The removal efficiency of perfluoroalkyl substances was evaluated in the SBBGR system enhanced with ozone at two different doses, 4 g/L and 5.5 g/L. The concentrations of PFAS were monitored at the influent and effluent for both conditions and the data presented in table 4.5 and 4.6 reveal interesting results.

Table 4.5 Concentration of PFAS in the influent and effluent of the ozone-enhanced biological treatment at 4 g/L ozone concentration.

PFAS Abbreviation	Influent (ng/L)	Effluent (ng/L)
PFBA	7086	9187
PFPeA	1564	1119
PFHxA	3490	4525
PFHpA	1234	872
PFOA	11180	1858
PFNA	0	0
PFDA	0	0
PFUdA	0	0
PFDoDA	0	0
PFBS	66770	83470
PFOS	715	30
PFHxS	188	52,6
6:2 FTSA	1606	145

Table 4.6 Concentration of PFAS in the influent and effluent of the ozone-enhanced biological treatment at 5.5 g/L ozone concentration.

PFAS Abbreviation	Influent (ng/L)	Effluent Mean Value (ng/L)	SD
PFBA	5189	8463	603
PFPeA	1340	2159	118
PFHxA	2809	4163	126
PFHpA	816	1436	58
PFOA	8626	7049	1601
PFNA	80,9	70	4
PFDA	149	76	20
PFUdA	0	0	0
PFDoDA	0	0	0
PFBS	44370	56307	4157
PFOS	986	519	18
PFHxS	217	298	32
6:2 FTSA	1696	1067	197

The introduction of ozone at a concentration of 4 g/L resulted in a significant change in the PFAS removal mechanism compared to what was observed with the biological treatment. In particular, for certain compounds such as PFBA, PFHxA, PFPeA, and PFHpA, an increase in concentration in the effluent compared to the influent was observed. For example, the concentration of PFBA increased from 7086 ng/L in the influent to 9187 ng/L in the effluent, while PFHxA increased from 3490 ng/L to 4525 ng/L. In contrast, only few compounds such as PFOA, PFOS, and 6:2 FTSA showed a reduction in concentration, although this removal was lower than that achieved by the biological treatment during the previous period. The removal efficiencies in the SBBGR enhanced with ozone are presented in figures 4.11 and 4.12.

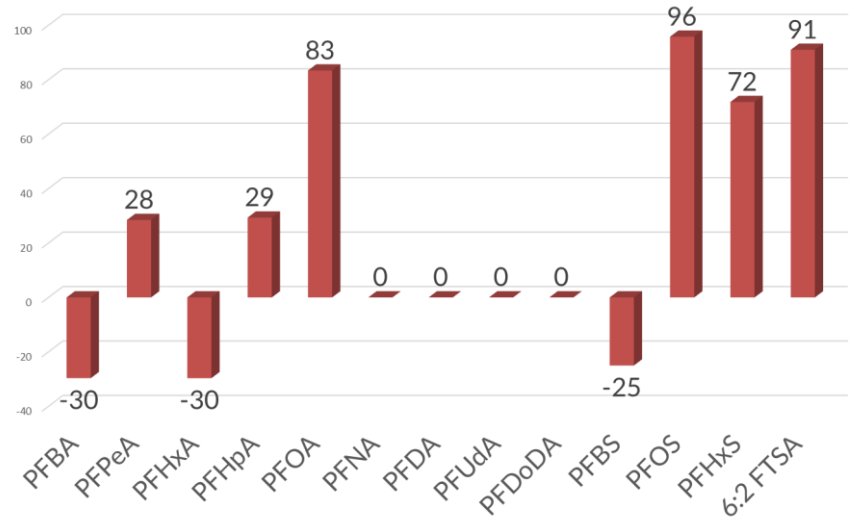

Figure 4.11 PFAS removal efficiency in ozone-enhanced biological treatment at an ozone concentration of 4 g/L.

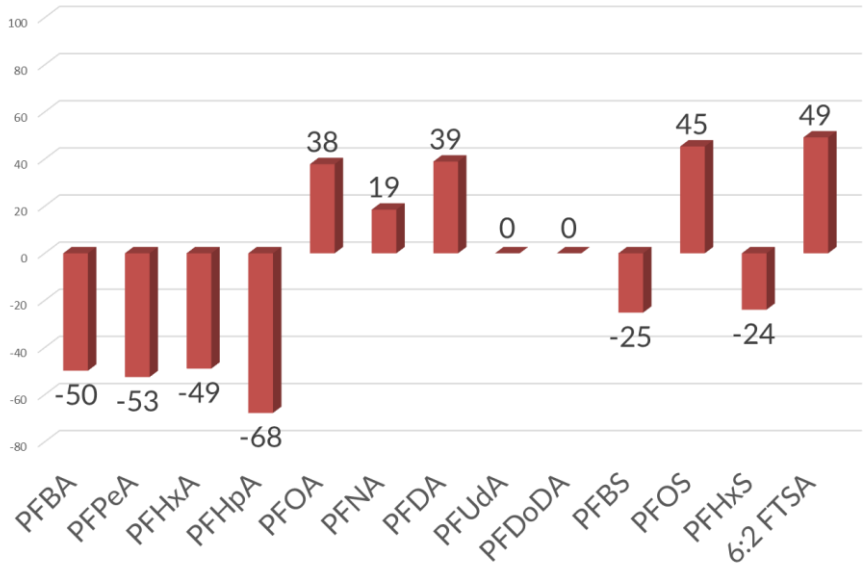

Figure 4.12 PFAS removal efficiency in ozone-enhanced biological treatment at an ozone concentration of 5.5 g/L.

The introduction of ozone at a dose of 5.5 g/L further impaired the removal efficiency for certain compounds, with negative values for PFBA, PFPeA, PFHxA, PFHpA, and PFBS. For example, the concentration of PFBA increased from 5189 ng/L in the influent to 8463 ng/L in the effluent, clearly indicating a desorption phenomenon. A similar increase was observed for PFPeA, which increased from 1340 ng/L to 2159 ng/L, and PFHxA, which rose from 2809 ng/L to 4163 ng/L. PFBS also showed a significant increase, rising from 44370 ng/L to 56307 ng/L.

While the biological treatment showed high removal efficiencies, especially for long-chain PFAS, the situation changed with the activation of ozone treatment. Removal efficiencies worsened, with the concentration of some PFAS in the effluent exceeding that in the influent. This unexpected result can be attributed to a desorption process of PFAS from the biomass. It is plausible that residual ozone in the SBBGR reactor induces slight lysis of the biomass's surface layers, leading to the release of PFAS into the liquid phase.

4.4 Biological treatment (SBBGR) with high concentration PFAS leachate

The final phase of the experimentation aimed to evaluate the performance of the biological treatment under conditions of high PFAS concentration. This operational choice was driven by the promising results obtained during the initial phase of the biological treatment study, which demonstrated high efficacy both in the removal of traditional parameters and in the reduction of PFAS, particularly for long-chain compounds.

To further investigate the system's capabilities, an ultra-concentrated leachate was employed, with the objective of assessing whether the biomass could maintain high operational performance even under increased PFAS concentrations. Additionally, the study aimed to determine whether PFAS removal was solely attributable to an adsorption mechanism or if degradation

phenomena could occur following an initial adsorption phase. To evaluate the biomass response over time, the system was maintained under these operational conditions for approximately 180 days.

The experimentation, which started on day 262 and concluded on day 440, involved feeding the system with three different leachate stocks (stock 4, stock 5, and stock 6), characterized by heterogeneous chemical-physical properties. Due to the real nature of the leachates, originating from municipal solid waste landfills, it was not possible to completely eliminate intrinsic variability. To address this variability, treatment efficiencies were analyzed separately for each of the three leachates, allowing for a comprehensive performance assessment thereafter.

The leachates used exhibited PFAS concentrations that were between 5 and 30 times higher than those in the leachates employed during earlier phases. However, as these were real leachates and not synthetic, it was not feasible to ensure a uniform concentration factor for all analyzed PFAS. Details on the concentrations and efficiencies are provided in the subsequent sections.

4.4.1 Gross parameters removal efficiency

The SBBGR biological treatment was tested using high-concentration PFAS leachate from Stocks 4, 5, and 6 to assess its performance under more challenging conditions. The gross parameters in the influent and effluent of the SBBGR during biological treatment with high-concentration PFAS leachate from Stocks 4, 5, and 6 are presented in Tables 4.7, 4.8, and 4.9, respectively.

Table 4.7 Gross parameters in the influent and effluent of the SBBGR during biological treatment with high-concentration PFAS leachate stock 4.

Parameter	Influent	SD	Effluent	SD
pH	8.4	0.3	8.7	0.1
Cond. (mS/cm)	17.4	0.7	20.0	0.6
COD (mg/L) *	2447	50	1406	86
sCOD (mg/L) *	2355	7	1387	93
BOD_5 (mg/L)	122	-	37	-
TN (mg/L)	864	101	96	15
NO_2 (mgN/L)	0.3	0.2	9.4	6.4
NO_3 (mgN/L)	6.9	0.6	11.6	2.5
NH_3 (mgN/L)	827	45	8.4	8.8
P (mg/L)	6	1.1	3.8	1.4
TSS (mg/L)	74	20	21.7	8.3
VSS (mg/L)	63	14	18.3	6.3
TS (g/L)	16.3	5.4	15.4	0.4
VS (g/L)	4.9	2.4	3.2	0.3

* The value does not take into account the COD added in the form of acetate (6.5 g/L) as an external carbon source to support the denitrification process.

Table 4.8 Gross parameters in the influent and effluent of the SBBGR during biological treatment with high-concentration PFAS leachate stock 5.

Parameter	***Influent***	***SD***	***Effluent***	***SD***
pH	8.7	0.4	8.6	0.4
Cond. (mS/cm)	23.8	1.2	23.7	2.7
COD (mg/L) *	2993	401	1838	198
sCOD (mg/L) *	2887	422	1730	91
BOD_5 (mg/L)	244	-	-	-
TN (mg/L)	1237	193	219	93
NO_2 (mgN/L)	0.2	0.0	1,4	1.2
NO_3 (mgN/L)	10.9	0.6	135	96
NH_3 (mgN/L)	1200	247	13	26
P (mg/L)	11.1	0.3	-	-
TSS (mg/L)	77.2	18.9	81.0	42
VSS (mg/L)	72.4	12.2	70.0	24
TS (g/L)	13.7	0.1	21.0	0.3
VS (g/L)	3.6	0.7	3.4	0.7

* The value does not take into account the COD added in the form of acetate (6.5 g/L) as an external carbon source to support the denitrification process.

Table 4.9 Gross parameters in the influent and effluent of the SBBGR during biological treatment with high-concentration PFAS leachate stock 6.

Parameter	*Influent*	*SD*	*Effluent*	*SD*
pH	8.3	0.6	8.3	0.2
Cond. (mS/cm)	15.0	0.0	20.7	2.0
COD (mg/L) *	1850	240	1646	174
sCOD (mg/L) *	1766	235	1344	202
BOD_5 (mg/L)	71	-	-	-
TN (mg/L)	689	8	156	35
NO_2 (mgN/L)	0.2	0.1	4.1	4.8
NO_3 (mgN/L)	5.4	0.0	55.1	76.3
NH_3 (mgN/L)	715	21	48.5	47.3
P (mg/L)	4.9	3.2	10.0	6.1
TSS (mg/L)	46.2	26.8	289	183
VSS (mg/L)	42.5	21.6	220	142
TS (g/L)	9.1	0.1	17.2	1.9
VS (g/L)	2.3	0.4	3	0.7

* The value does not take into account the COD added in the form of acetate (6.5 g/L) as an external carbon source to support the denitrification process.

The average removal efficiency for COD was 31%, and 35% for sCOD, with better values observed for Stocks 4 and 5 compared to Stock 6. In particular, the COD removal efficiency for Stock 6 was only 11%, significantly lower than for the other stocks (Table 4.10). These efficiencies were calculated without considering the contribution of external COD from acetate.For the BOD_5, a very high removal efficiency (70%) was observed based on the limited available data, indicating effective control over the biodegradable organic fraction. The average total nitrogen (TN) removal efficiency was notably high (83%), with better performance for Stocks 4 and 5, highlighting the system's strong nitrification and denitrification capabilities even under high PFAS concentrations. Ammonia (NH_3)

removal efficiency was excellent (97-99%), signaling effective ammonia oxidation into nitrites and nitrates (Table 4.10).

Stocks 4 and 5 showed positive removal for TSS and VSS (33%-37%), while Stock 6 exhibited strongly negative values, indicating system destabilization. This increase in solids in the effluent could be attributed to the higher PFAS concentration stressing the biomass and suboptimal operational conditions (Table 4.10).

Table 4.10 Removal efficiencies of the biological treatment with leachate stocks 4, 5, and 6.

Parameter	RE % Stock 4	RE % Stock 5	RE % Mean stock 4, 5	RE % Stock 6	RE % Mean stock 4,5,6
COD (mg/L)	43	39	41	11	31
sCOD (mg/L)	41	40	41	24	35
BOD_5 (mg/L)	70	-	70	-	70
TN (mg/L)	89	82	86	77	83
NH_3 (mgN/L)	99	99	99	3	97
P (mg/L)	36	-	36	-104	-34
TSS (mg/L)	71	-5	33	-526	-153
VSS (mg/L)	71	3	37	-418	-115
TS (g/L)	6	-53	-24	-89	-46
VS (g/L)	35	6	20	-30	3

In general, the use of ultra-concentrated leachates demonstrated that the system can still effectively remove certain parameters, although with reduced efficiency compared to previous tests with Stocks 1 and 2. The biological treatment showed good performance for COD, BOD_5, and NH_3 even under stressed conditions. Comparisons of key removal efficiencies in biological treatment with different PFAS concentrations indicate a decline in performance with ultra-concentrated leachates: for COD, removal dropped from 46% to 41%; for TN, from 91% to 86%; for NH_3, removal remained unchanged at 99%, while for TSS, it decreased from 62% to 33%. (Figure 4.13)

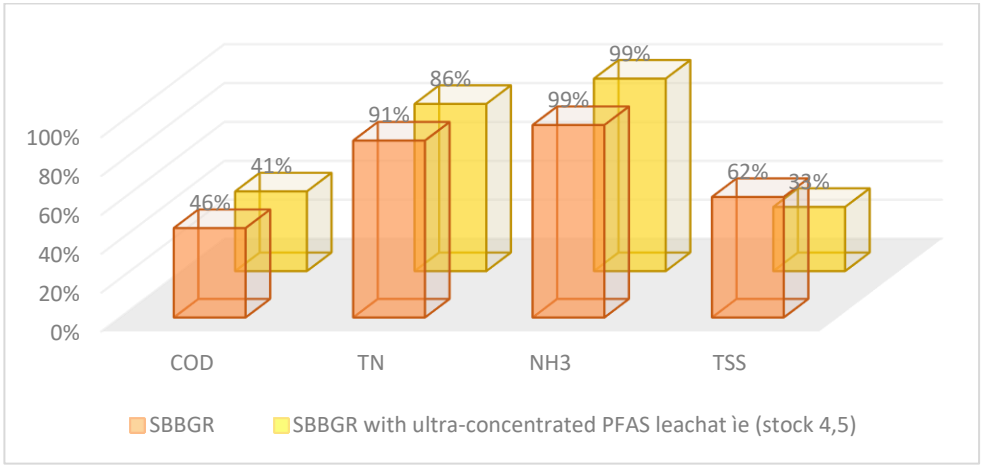

Figure 4.13 Performance of the By SBBGR biological treatment with Stock 1 and 2 and the By SBBGR biological treatment with high-concentration leachate (Stock 4, 5, and 6) in terms of COD, TN, NH_3, and TSS removal.

Regarding color removal, calculated as the variation in absorbance at three different wavelengths (426 nm, 556 nm, and 660 nm), Figure 4.14 demonstrates that, once again, the biological treatment failed to achieve substantial color removal. The results obtained with the biological treatment on high-concentration PFAS leachate samples (Stocks 4, 5, and 6) confirm the data

observed in the biological treatments of Stocks 1 and 2, which also showed a limited ability to remove color.

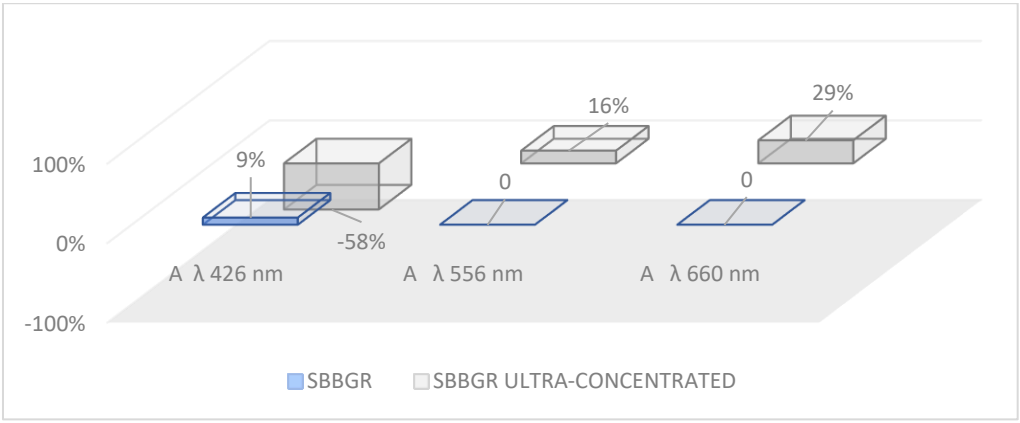

Figure 4.14 Color removal efficiency at three different wavelengths: comparison between the SBBGR treatment with Stock 1 and 2 and the SBBGR treatment with PFAS high-concentration leachate (Stock 4, 5, and 6).

4.4.2 PFAS Removal efficiency

During this phase, the concentrations of selected PFAS in the influent and effluent of the SBBGR biological treatment were evaluated to analyze the system's performance in terms of PFAS removal. The results are presented in Tables from 4.11 to 4.13.

Observing the collected data, it is evident that the effluent and influent concentrations exhibit significant variability, reflecting the chemical and physical complexity of the leachates. Regarding the compounds most effectively removed, PFOS demonstrated an average removal efficiency of 41%, reaching 70% in Stock 6. This suggests that long-chain compounds, such as PFOS, exhibit a greater tendency to undergo removal processes. Similarly, PFNA showed an average removal efficiency of 50%, with a peak of 79% in Stock 6. These results,

as highlighted in Table 4.14, indicate that, in general, long-chain compounds are more susceptible to adsorption and/or degradation processes compared to short-chain compounds.

Similarly, PFOA exhibited positive but variable removal, with an average efficiency of 23% and a peak of 56% in Stock 6. However, the treatment showed lower efficiency for compounds such as PFBA, which recorded an average removal of 31%, with a negative anomaly (-10%) in Stock 6. Additionally, PFDA showed a negative removal efficiency, with an average of -19%, but was effectively removed in Stock 6 (77%). These results are summarized in Figure 4.15.

Short-chain compounds, such as PFBA and PFPeA, demonstrated less efficient removal compared to long-chain compounds. For instance, PFBA showed good removal in some stocks (67% in Stock 5) but variable and even negative results in others (such as Stock 6, with -10% removal). Similarly, PFHxA, while showing a positive average removal efficiency of 32%, exhibited significant variability among different stocks, whereas PFBS, despite relatively high concentrations, displayed a consistent removal of 36%.

In contrast, some compounds showed resistance to the treatment, such as 6:2 FTSA, which recorded negative efficiencies in Stock 6 (-3%), suggesting potential resistance during treatment. However, the behavior of 6:2 FTSA cannot be adequately interpreted due to the low concentrations detected, which limit data reliability. It is also important to note that two PFAS, PFUdA and PFDoDA, were consistently below the detection limit in both the influent and effluent.

Overall, the results suggest that the biological treatment is effective for PFAS removal, but the efficiency significantly depends on the physicochemical characteristics of the leachates and the specificity of the compound. The removal of long-chain PFAS is favored by high hydrophobicity, while short-chain compounds, such as PFBA, pose greater challenges in biological treatment.

Table 4.11 Concentration of PFAS in the influent and effluent of the SBBGR biological treatment with leachate stock 4.

PFAS	Avg. Infl. Value (ng/L)	SD (ng/L)	Avg. Eff.Value (ng/L)	SD (ng/L)
PFBA	11939	4216	7557	1419
PFPeA	4223	1863	2679	661
PFHxA	8130	774	4372	802
PFHpA	2759	658	2044	729
PFOA	207500	36487	162288	99098
PFNA	608	217	299	207
PFDA	430	4	704	726
PFUdA	<LOQ	0	<LOQ	0
PFDoDA	<LOQ	0	<LOQ	0
PFBS	57165	39110	32143	3850
PFHxS	3981	33	3323	1829
PFHpS	3391	335	1922	1351
PFOS	19788	1821	11620	11188
6:2 FTSA	840	173	443	144

Table 4.12 Concentration of PFAS in the influent and effluent of the SBBGR biological treatment with leachate stock 5.

PFAS	Avg. Infl. Value (ng/L)	SD (ng/L)	Avg. Eff. Value (ng/L)	SD (ng/L)
PFBA	38290	523	12503	1425
PFPeA	5652	1401	4060	787
PFHxA	13778	3475	7679	1144
PFHpA	3577	274	2927	528
PFOA	203300	23759	218850	42524
PFNA	480	79	384	223
PFDA	357	505	614	536
PFUdA	<LOQ	0	<LOQ	0
PFDoDA	<LOQ	0	<LOQ	0
PFBS	111915	81013	61482	17715
PFHxS	6118	2872	4199	595
PFHpS	4239	1815	3129	668
PFOS	20670	2404	16075	7074
6:2 FTSA	802	197	647	358

4.13 Concentration of PFAS in the influent and effluent of the SBBGR biological treatment with leachate stock 6.

PFAS	Avg. Infl. Value (ng/L)	SD (ng/L)	Avg. Eff. Value (ng/L)	SD (ng/L)
PFBA	13235	1138	14546	2982
PFPeA	4840	71	5082	969
PFHxA	8748	384	8245	1869
PFHpA	2711	221	2586	745
PFOA	281300	99136	124086	49724
PFNA	608	20	131	90
PFDA	665	147	151	73
PFUdA	<LOQ	0	<LOQ	0
PFDoDA	<LOQ	0	<LOQ	0
PFBS	68645	7064	55830	10084
PFHxS	8917	1645	4344	1918
PFHpS	6385	2507	2919	1752
PFOS	22670	9857	6903	2615
6:2 FTSA	438	423	452	297

4.14 Removal efficiency of PFAS in the SBBGR biological treatment with leachate stock 4,5,6.

PFAS	RE % STOCK 4	RE % STOCK 5	RE % STOCK 6	Average RE (%) of the 3 Stocks
PFBA	37	67	-10	31
PFPeA	37	28	-5	20
PFHxA	46	44	6	32
PFHpA	26	18	5	16
PFOA	22	-8	56	23
PFNA	51	20	79	50
PFDA	-64	-72	77	-19
PFUdA	0	0	0	0
PFDoDA	0	0	0	0
PFBS	44	45	19	36
PFHxS	17	31	51	33
PFHpS	43	26	54	41
PFOS	41	22	70	44
6:2 FTSA	47	19	-3	21

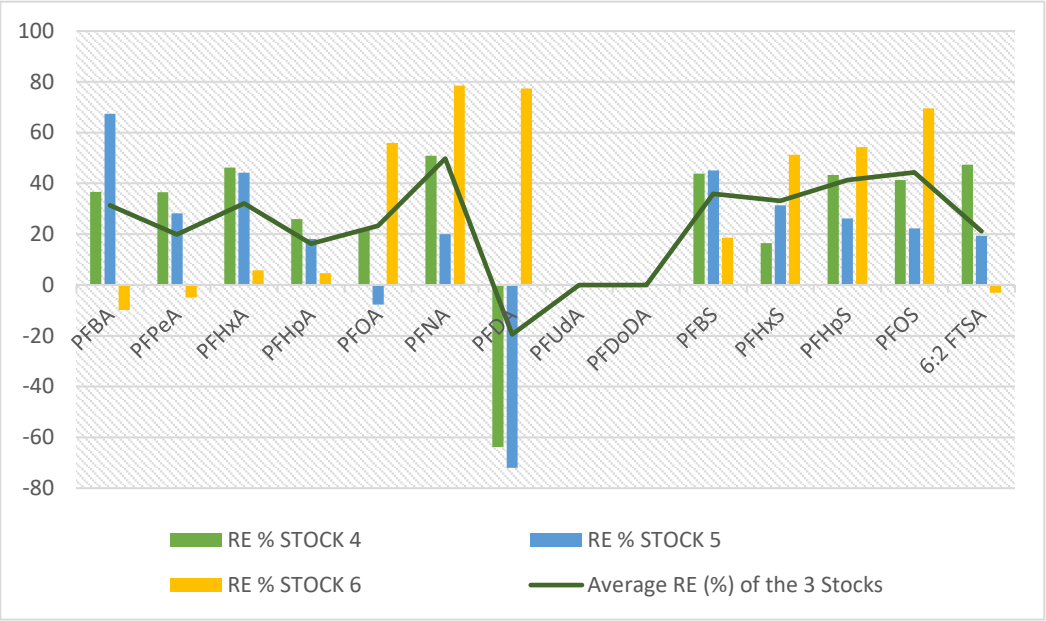

Figure 4.15 Removal efficiency of PFAS in the SBBGR biological treatment with leachate stock 4,5,6 and average removal efficiency of the 3 stocks.

Figures 4.17 to 4.20 report the concentrations (ng/L) over time (from the 262nd day to the 440th day) of the compounds PFBA, PFBS, PFOA, and PFOS. These figures provide additional insight into the dynamic behavior of these compounds throughout the treatment period, illustrating fluctuations in their concentrations and highlighting periods of greater or lesser removal efficiency.

PFBA, a short-chain compound, tends to be more mobile in the environment due to its lower molecular weight and lower hydrophobicity compared to long-chain PFAS. This makes it more difficult to remove in biological systems, as it is less likely to adsorb to surfaces or interact with microbial communities.

PFBS, another short-chain compound, showed a more stable removal performance, with a consistent efficiency of approximately 36%. Despite the relatively higher concentrations in the influent, the compound was removed at a steady rate.

PFOS, a well-known long-chain PFAS, demonstrated the highest and most consistent removal efficiency among the four compounds, with an average removal of 41%.

In conclusion, these four PFAS compounds—PFBA, PFBS, PFOA, and PFOS—highlight the complexity of PFAS removal in biological systems. While long-chain compounds like PFOS and PFOA tend to show higher removal efficiencies, the variability in the treatment of short-chain compounds such as PFBA and PFBS underscores the challenges that biological treatments face in addressing the full spectrum of PFAS contaminants. Understanding these patterns and the factors influencing their removal is essential for optimizing treatment strategies and improving the overall effectiveness of PFAS remediation.

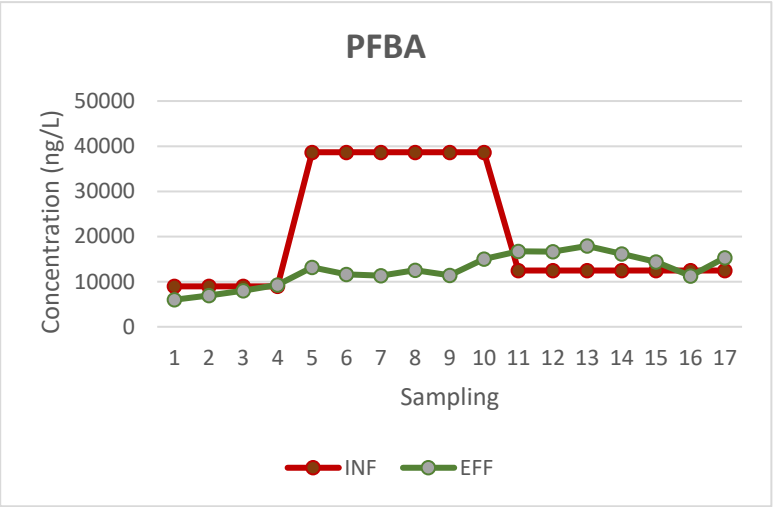

Figure 4.16 Concentrations (ng/L) over time (from the 262nd to the 440th day) of PFBA in the influent and effluent of the SBBGR biological treatment system.

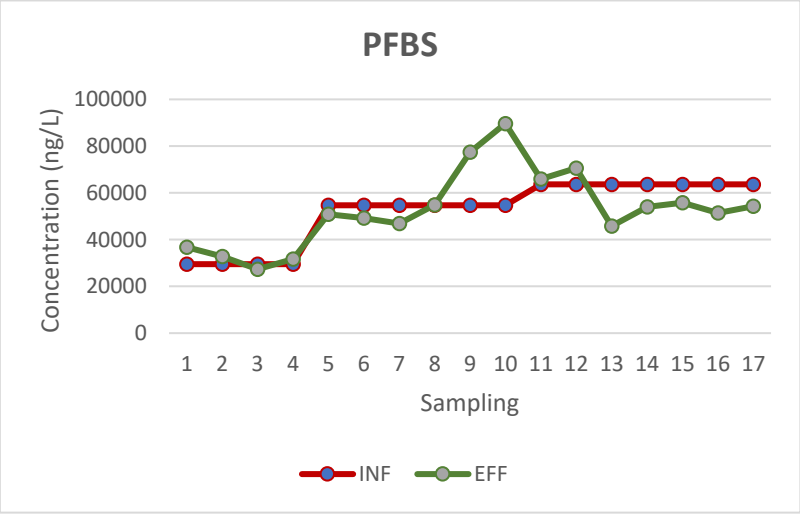

Figure 4.17 Concentrations (ng/L) over time (from the 262nd to the 440th day) of PFBS in the influent and effluent of the SBBGR biological treatment system.

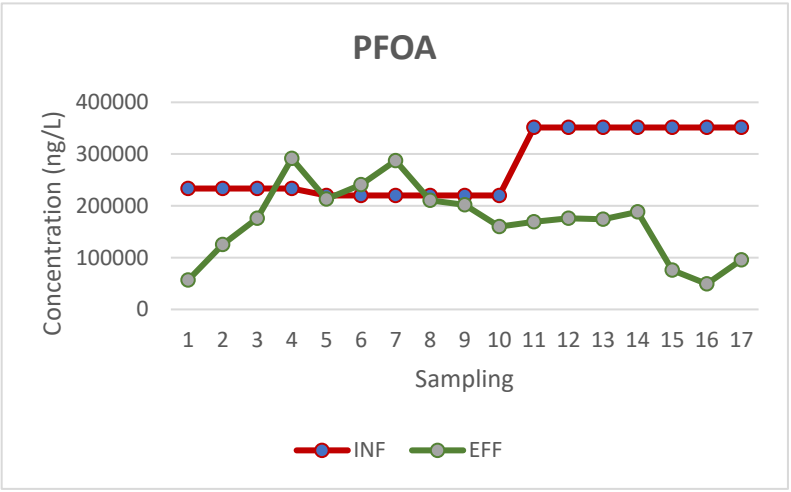

Figure 4.18 Concentrations (ng/L) over time (from the 262nd to the 440th day) of PFOA in the influent and effluent of the SBBGR biological treatment system.

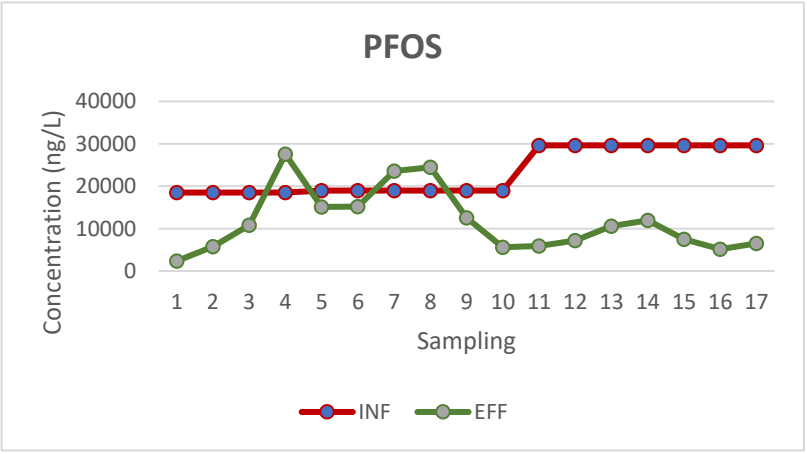

Figure 4.19 Concentrations (ng/L) over time (from the 262nd to the 440th day) of PFOS in the influent and effluent of the SBBGR biological treatment system.

CONCLUSION

This study has examined biological treatment in SBBGR mode for landfill leachates, along with an integrated scheme that includes chemical oxidation, with particular focus on its capacity to remove both traditional pollutants and perfluoroalkyl substances (PFAS). The research was driven by the need to enhance the biodegradation of complex landfill leachates, in which persistent contaminants such as PFAS are present.

In the initial phase, the startup process demonstrated the importance of acclimating the microbial community to high-salinity conditions, ensuring the system's stability and enabling it to effectively remove conventional contaminants such as COD, BOD_5, nitrogen, and ammonia. The biological treatment proved highly efficient in removing ammonia and total nitrogen, with removal efficiencies exceeding 90%, and demonstrated a significant reduction in COD, although the complex and recalcitrant nature of the organic pollutants in the leachate posed some challenges.

A particularly promising aspect of this research was the treatment of PFAS. Long-chain PFAS compounds, such as PFOS, PFHxS, and PFOA, were effectively removed by the biological treatment, achieving near-total removal in many cases. This highlighted the potential of the SBBGR system in addressing persistent organic pollutants, despite the challenges posed by the high chemical variability of landfill leachates.

In an effort to further enhance the treatment's performance, the study incorporated a combined biological and chemical (BIO&CHEM) approach, integrating ozone to oxidize recalcitrant contaminants and improve the removal of refractory COD. However, the results were unexpected. While ozone improved color removal and achieved some reduction in COD, it compromised the removal of certain PFAS, particularly shorter-chain compounds. This outcome, likely due to desorption phenomena triggered by ozone treatment, highlighted the complexity of integrating biological and chemical methods, particularly for PFAS.

The ozone treatment likely induces slight lysis of the biomass's surface layers, releasing PFAS that were previously adsorbed or bound to the biomass. This desorption effect leads to an increase in PFAS concentrations in the effluent compared to the influent, complicating the overall removal process. Thus, while ozone treatment can improve certain aspects of leachate quality, its interaction with PFAS and biomass introduces new challenges in achieving efficient removal.

Given the results from the initial phases, the study shifted focus to biological treatment using high-concentration PFAS leachate. This approach aimed to saturate the biomass and determine whether, beyond adsorption, true biological degradation was occurring. The results were promising, showing high overall contaminant removal efficiency even under these challenging conditions.

Performance varied across different PFAS compounds, with long-chain PFAS continuing to exhibit better removal than short-chain PFAS. However, the fact that the system maintained removal efficiencies for certain PFAS even after months of operation suggests that a mechanism beyond simple adsorption is at play.

In conclusion, this study demonstrates that the biological treatment approach in SBBGR mode is capable of achieving good PFAS removal efficiencies, particularly for long-chain compounds. While the system does not completely address issues such as chloride removal or meet the legal requirements for COD values, it shows promising results in treating PFAS, making it a viable option for pre-treatment of landfill leachate before it is processed in conventional wastewater treatment plants. The demonstrated effectiveness of this system in removing PFAS allows it to be considered as a treatment step before leachate reaches current treatment facilities, offering a potential solution to mitigate the impact of persistent contaminants like PFAS on the environment.

Future research should focus on several key areas to further enhance the performance of the SBBGR system for PFAS removal. Firstly, a deeper understanding of PFAS-biomass interactions is essential to better comprehend their fate within the biological system and optimize operating conditions.

Additionally, investigating the genetic composition of the biomass could provide valuable insights into the specific bacterial species involved in the process, helping to identify those most responsible for PFAS degradation.

Furthermore, large-scale applications should be explored by testing the system under real-world conditions to assess its scalability and potential for sustainable industrial use. Lastly, long-term monitoring should be conducted to evaluate the stability of the biological system over time, assessing its efficiency and adaptability to varying leachate characteristics, ensuring its continued effectiveness in the long run.

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LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AC: Activated Carbon

ACF: Activated Carbon Fiber

AER: Anion Exchange Resin

AFFF: Aqueous Film-Forming Foam

AIX: Anion Exchange Resins

AOP: Advanced Oxidation Processes

ATP: Adenosine Triphosphate

BOD: Biochemical Oxygen Demand

BOD_5: Biochemical Oxygen Demand over 5 days

BOD_5/COD: Biochemical Oxygen Demand (5 days) / Chemical Oxygen Demand

BIO&CHEM: Biological and Chemical (combined treatment approach using SBBGR and ozone)

COD: Chemical Oxygen Demand

CMCs: Critical Micelle Concentrations

DOC: Dissolved Organic Carbon

EC: Emerging Contaminants

ESI: Electrospray Ionization

EPS: Extracellular Polymeric Substances

EO: Electrochemical Oxidation

EPA: Environmental Protection Agency

FASEs: Perfluoroalkane Sulfonamido Ethanols

FTS: Fluorotelomer Sulfonate

FTOH: Fluorotelomer Alcohol

FTSA: 6:2 Fluorotelomer Sulfonic Acid

GAC: Granular Activated Carbon

GC-MS: Gas Chromatography-Mass Spectrometry

ITRC: Interstate Technology & Regulatory Council

Ka: Acid Dissociation Constant

LC-HRMS/MS: Liquid Chromatography coupled with High-Resolution Mass Spectrometry / Mass Spectrometry
LOD: Limit of Detection
MBBR: Moving Bed Biofilm Reactor
MSW: Municipal Solid Waste
NMR: Nuclear Magnetic Resonance
NF: Nanofiltration
NO_2: Nitrite
NO_3: Nitrate
OECD: Organisation for Economic Co-operation and Development
PAC: Powdered Activated Carbon
PCR: Polymerase Chain Reaction
PFAA: Perfluoroalkyl Acid
PFOA: Perfluorooctanoic Acid
PFBA: Perfluorobutanoic Acid
PFBS: Perfluorobutanesulfonic Acid
PFCA: Perfluorocarboxylic Acids
PFDoDA: Perfluorododecanoic Acid
PFHxA: Perfluorohexanoic Acid
PFHxS: Perfluorohexanesulfonic Acid
PFHpA: Perfluoroheptanoic Acid
PFHpS: Perfluoroheptanesulfonic Acid
PFNA: Perfluorononanoic Acid
PFOS: Perfluorooctane Sulfonate
PFPeA: Perfluoropentanoic Acid
PFSAs: Perfluoroalkane Sulfonic Acids
PFUdA: Perfluoroundecanoic Acid
PPM: Parts per Million
RO: Reverse Osmosis
ROS: Reactive Oxygen Species

SBR: Sequencing Batch Reactor

SBBGR: Sequencing Batch Biofilter Granular Reactor

SEM: Scanning Electron Microscopy

SST: Suspended Solids Total

SVA: Suspended Volatile Solids

TLC: Thin Layer Chromatography

TSS: Total Suspended Solids

TEM: Transmission Electron Microscopy

TS: Total Solids

UV: Ultraviolet

WHO: World Health Organization

WWTP: Waste Water Treatment Plant

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Rossella Annelio was born in Francavilla Fontana (BR), Italy, in 1985. In 2012 she earned a Master's Degree in Cellular and Molecular Biology at the University of Bari. In the same year, she was awarded a scholarship under the In.Te.R.R.A program, aimed at training experts in wastewater treatment for agricultural irrigation. She then spent several years teaching Natural Sciences at high schools and, in 2019, obtained a permanent teaching position. In 2021, she enrolled in the Environmental, Territorial, and Building Risk and Development PhD program at Politecnico di Bari. Her doctoral research focused on addressing critical environmental challenges related to landfill leachate management, proposing innovative techniques to improve the removal efficiency of persistent contaminants like PFAS. Her doctoral dissertation, titled "Advanced treatments for PFAS removal from landfill leachate: evaluating biological and ozone-based chemical approaches", provided an in-depth analysis of these challenges and proposed effective solutions. Her career reflects her commitment to environmental protection and sustainable development, particularly in water management.