Evaluation of an Algal Turf Scrubber™ (ATS) Approach for the Remediation of Perfluoroalkyl Substances (PFAS)

by

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Abstract

Per- and polyfluoroalkyl substances are a large group of synthetic organic chemicals ubiquitous and persistent in the environment due to their fluorinated alkyl chains. Existing methods for remediation of mixtures of PFAS in water media are limited and many times not applicable for shorter-chain PFAS. Algal Turf Scrubber™ (ATS) systems have been widely used for biomass production and pollutant removal through periphytic algae, but have not been yet investigated for PFAS. This project evaluated the suitability of an ATS approach for the remediation of a mixture of PFAS (PFOS, PFOA, PDHA, and HFPO-DA), and quantified the mechanisms of remediation. A mixed community of periphytic algae obtained from Chewacla Creek (32.5480°, -85.4806°) was cultured during 14 harvests in four flow-lane microcosms under a 16:8 light:dark regime. The dominant species identified through light microscopy were primarily composed of filamentous forms from the genera *Spirogyra, Oscillatoria, Stigeoclonium*, *Mougeotia, Ulotrix,* and *Oedogonium*. The experimental design consisted of four treatment channels and four controls (two positive and two negative), in which algal cultures where exposed to the contaminant mixture at 2 µg L^{-1} for a period of 72h. Water and algal biomass were repeatedly sampled from each channel, from which 66 water and 4 biomass samples were analyzed using ultra-high performance liquid chromatography coupled to triple quadrupole mass spectrometry (UHPLC-QqQ-MS). The amount of contaminants found in the biomass was equal to 1.24 ± 0.40% for PFOA, 1.21 ± 0.41% for PFOS, 0.26 ± 0.16% for HFPO-DA, and 0.76 ± 0.27% for PDHA, based on their initial concentration. In contrast, it was observed that between 35-92% of the initial concentration of PFOS and PFOA remained unaccounted for after a mass balance was performed. Sorption into the materials used to build the ATS system was hypothesized to be responsible for this mass loss, according to results from preliminary experiments. Finally, results from this project indicate that ATS might not be an effective alternative for PFAS remediation, due to the low removal rates.

Nonetheless, results from this analysis can contribute to the growing understanding on the bioaccumulation potential of these compounds. Periphytic algae are in the bottom of several food chains and can potentially biomagnify PFAS into upper levels. Furthermore, findings in this study are in agreement with reports that suggest shorter chain compounds are less bioaccumulative and present lower half-lives in organisms, when compared to legacy PFAS.

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"Alone we can do so little, together we can do so much" – Helen Keller

Table of Contents

List of Tables

List of Figures

List of Abbreviations

6:2 FTOH	6:2 Fluorotelomer Alcohols
6:2 FTSA	6:2 Fluorotelomer Sulfonic Acid
8:2 FTOH	8:2 Fluorotelomer Alcohols
AFDW	Ash Free Dry Weight
AFFF	Aqueous Film Forming Foam (firefighting foams)
ANOVA	Analysis of Variance
ATS	Algal Turf Scrubber ™
BAF	Bioaccumulation Factor
BBM	Bolds Basal Media
CF	Concentration Factor
Cl-PFESA	Chlorinated polyfluorinated ether sulfonate
Danish EPA	Danish Environmental Protection Agency
DF	Dilution Factor
DI	Deionized Water
dw	Dry Weight
GAC	Granulated Activated Carbon
HDPE	High Density Polyethylene
HFPO-DA	Undecafluoro-2-methyl-3-oxahexanoic Acid

OECD	Organization for Economic Co-operation and Development
PDHA	Nonafluoro-3,6-dioxaheptanoic Acid
PFAS	Per- and polyfluoroalkyl Substances
PFBA	Perfluorobutanoic Acid
PFBS	Perfluorobutane Sulfonic Acid
PFCAs	Perfluoroalkyl Carboxylic Acids
PFDA	Perfluorodecanoic Acid
PFDoA	Perfluorododecanoic Acid
PFDS	Perfluorodecane Sulfonic Acid
PFHpA	Perfluoroheptanoic Acid
PFHpA	Perfluoroheptanoic Acid
PFHxA	Perfluorohexanoic Acid
PFHxS	Perfluorohexane Sulfonic Acid
PFNA	Perfluorononanoic Acid
PFOA	Perfluoro-n-octanoic Acid
PFOS	Perfluorooctanesulfonic Acid
PFOSA	Perfluorooctane Sulphonamide
PFPeA	Perfluoropentanoic Acid
PFUnA	Perfluoroundecanoic Acid
POSF	Perfluorooctane Sulfonyl Fluoride
PTFE	Polytetrafluoroethylene
PWS	Public Water System
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals

UCMR 3	Third Unregulated Contaminant Rule
US EPA	United Sates Environmental Protection Agency
US FDA	United Sates Food and Drug Administration
ww	Wet Weight
WWTP	Wastewater Treatment Plant

1. INTRODUCTION

1.1 The PFAS Issue

Technological advancements have benefited society to a great extent, from the Internet to an increase in life quality. Despite the benefits, in many cases such advancements did not come without repercussions to the environment, humans and wildlife. That is the case of per- and polyfluoroalkyl substances, collectively known as PFAS. These substances present great stability and have particular properties that made them very attractive to industries. PFAS started being produced in large scale in the late 1940's (Lindstrom, Strynar, and Libelo 2011), in a time when regulations were even less enforced than nowadays. Since then, PFAS have been used in a variety of products and sectors, including everyday life products such as non-stick cookware and stain-repellent products (KEMI 2015).

Lack of appropriate regulations permitted the irresponsible usage and consequent disposal of these substances into the environment. These substances have been found in every environment globally, even in polar bears in the Artic (Tartu et al. 2018). Because of their stability, they are likely to stay in these environments for a long time. Their ubiquity would be less of a concern if it were not for their reported toxicity in humans and wildlife. These substances have been observed to be carcinogenic, act as endocrine disruptors, and have been linked to a number of other adverse health issues (Blum et al. 2015). Finally, in the early 2000's governmental agencies all over the world started to implement sanctions in the usage and production of PFOS, PFOA and other so-called legacy PFAS (Danish EPA 2015). However, manufacturing facilities that produced or used legacy PFAS started the development of shorter-chain fluorinated alternatives. As a result, it is estimated that at least 3,000 PFAS are currently being used in the global market (Wang et al. 2017).

Because of their diversity and stability, PFAS are extremely difficult to be removed from contaminated matrices. Granulated Activated Carbon (GAC) is the current method employed to treat PFAS-contaminated drinking water but it has been shown to be expensive, generate waste, and ineffective for shorter chain PFAS (Appleman et al. 2013). Several studies have been conducted in efforts to find alternative remediation methodologies. However, these approaches are either costly (Lampert, Frisch, and Speitel 2006), inefficient (Park et al. 2016), produce undesired byproducts (Bruton and Sedlak 2018; Zhang et al. 2013), or present kinetic limitations (Luo et al. 2015; Yamamoto et al. 2007; McGregor 2018).

1.2 Targeted Analytes

When released into the environment, polyfluoroalkyl substances can breakdown into several persistent perfluoroalkyl compounds. PFASs are also present in the air, surface water, groundwater and soils, and can present high mobility. These facts make it unlikely that an environment will be contaminated with only one PFAS (Guelfo and Adamson 2018). With that in mind, four perfluoroalkyl substances were chosen to be analyzed in this study. The legacy PFOS and PFOA are the most studied PFAS and were chosen because of their ubiquity in the environment, as well as their observed toxicity in humans and wildlife. In contrast, the emerging shorter-chain replacements HFPO-DA and PDHA were chosen due to the lack of information on their behavior and fate, and to establish comparisons between emerging and legacy PFAS.

PFOA, a perfluoroalkyl carboxylic acid (PFCA) and PFOS, a perfluoroalkyl sulfonic acid (PFSA), have been widely studied and are the subject of several regulations. They are both hydrophobic, where PFCAs are strong acids and PFSAs present more polar characteristics (Danish EPA 2015). PFOS and PFOA are not degradable under environmental conditions, but are endpoints from the degradation of many other PFAS precursors. Despite regulatory efforts, these two substances are still widely distributed in the environment. For instance, PFOS and PFOA were, respectively, the most frequent PFAS detected in a study conducted by EPA in American public water systems (Hu et al. 2016). High quantities of these compounds

have also been found in the blood serum of the US population, reaching up to 4,751.5 μg L^{-1} near a contaminated area (Hoffman et al. 2010).

On another hand, HFPO-DA is a short-chain perfluoroether carboxylic acid. Its ammonium salt, trademarked GenX, has been used as a PFOA alternative since 2010. Even though HFPO-DA is a fairly new substance, it has already proven to be ubiquitous in the environment, reaching concentrations as high as 3830 ng L^{-1} in a river in China (Pan et al. 2018). In Europe, GenX is regulated under REACH and has a reported yearly production in the European area between 10 and 100 tons (ECHA 2019). Even less information is known about PDHA, a perfluoroether acid that presents ether linkages that reduce the length of the perfluoroalkyl chain (Hori et al. 2012).

1.3 A green alternative for PFAS?

As a result of an exponential human population growth, an increase in waste from rural and urban activities has been threatening clean water supplies all around the world. This ongoing issue led to the development of new ecological technologies that have been used as alternatives in the treatment of water. One of the most successful examples of ecological engineered technologies is the Algal Turf Scrubber™ (ATS), a simulated stream system that treats water using periphytic algae. The majority of the ATS studies have been related to the removal of nitrogen and phosphorus, since algae naturally need these nutrients for their growth. ATS systems have been explored to be used in the treatment of animal manure (Kebede-Westhead, Pizarro, and Mulbry 2006; Walter Mulbry et al. 2008), surface waters (Sindelar et al. 2015; W. Mulbry, Kangas, and Kondrad 2010; Chen et al. 2015), wastewater from aquaculture (Ray, Terlizzi, and Kangas 2015; Huang et al. 2013; Valeta and Verdegem 2015) and agriculture (D'Aiuto et al. 2015; HydroMentia Inc. 2005), volatile organic compounds (VOCs) and other industrial contaminants (W. H. Adey and Loveland 2007), among others. Algae has also been reported to uptake heavy metals (S. H. Paul 2014).

Furthermore, growth inhibition effects of PFAS in algae have been studied for a few species, which have shown to be tolerant under environmentally-relevant concentrations (Latała, Nedzi, and Stepnowski 2009; W. Liu et al. 2018). In fact, a recent study reported that the concentration of CI-PFESA in a planktonic green algae averaged around 130 μg gww^{-1} (W. Liu et al. 2018), indicating that algae have the capability of absorb and adsorb PFASs.

1.4 Goal and Objectives

The main goal of this study is to assess if an algal turf scrubber approach can be effectively employed as a novel strategy in the treatment of PFAS-contaminated surface water. The laboratory-scaled ATS used in this study is believed to be representative of a typical full-scale system, and removal rates could be potentially extrapolated. A mixed algal community acquired from a local stream was cultured under simulated-environmental conditions until constant growth was reached. At that point, the community populating the ATS was exposed to a mixture of the four targeted analytes for 72 hours. Water samples were taken over time to assess rates of sequestration, whereas biomass samples were taken at the end of the experiment to assess the mechanisms of remediation. Furthermore, the following specific objectives were outlined:

1 – To optimize growth parameters to yield high biomass production rates;

2 – To quantify the mechanisms of sequestration and compare them among contaminants through biomass analysis. It is hypothesized that sequestration will take place either by absorption into algae cells or adsorption onto cell's surface;

3 – To compare absorption and adsorption rates of legacy (PFOS and PFOA) and emerging (PDHA and HFPO-DA) substances. Legacy compounds are likely to present higher sorption rates based on what it is available in the literature;

4 – To evaluate possible abiotic transformation of PFAS in the positive control channels.

2. BIBLIOGRAPHIC REVIEW

2.1 Per- and Polyfluoroalkyl Substances (PFAS)

2.1.1 Overview - Groups, history, and applications

Per- and polyfluoroalkyl substances, collectively known as PFAS, represent a large group of synthetic organic surfactants in which one or more hydrogen atoms have been replaced by fluoride atoms, presenting the moiety C_nF_{2n+1-} (Buck et al. 2011; Guelfo and Adamson 2018). Perfluoroalkyl substances have fully fluorinated chains, with a non-polar tail and a polar head (Lampert, Frisch, and Speitel 2006). Due to the strength of their fluorine-carbon bonds, perfluoroalkyl substances are chemically and thermally stable, being referred to as terminal endpoints once they are released into the environment. In contrast, polyfluoroalkyl substances still contain some C-H bonds, which makes them prone to be transformed through biotic or abiotic processes into perfluoroalkyl substances (Buck et al. 2011; Guelfo and Adamson 2018; Newton et al. 2017). As a result, the release of a single precursor can lead to the formation of several perfluoroalkyl products (Guelfo and Adamson 2018). Furthermore, PFAS present high thermal and electrical stability, are fire resistant, and have film-forming properties (KEMI 2015; Guelfo and Adamson 2018; Kaboré et al. 2017).

In general, fluorinated substances are based on two components: a hydrophobic tail (fully fluorinated) and a hydrophilic head. Some substances also present a group in between those two structures, referred to as a spacer (KEMI 2015). Based on that, several chemicals that have similar configurations were grouped together in families and subgroups. Figure 2.1 illustrates a hierarchic classification of per- and polyfluorinated substances for nonpolymeric and polymeric substances. The characteristic chemical

formula and most common substances are shown for each of the nonpolymeric groups.

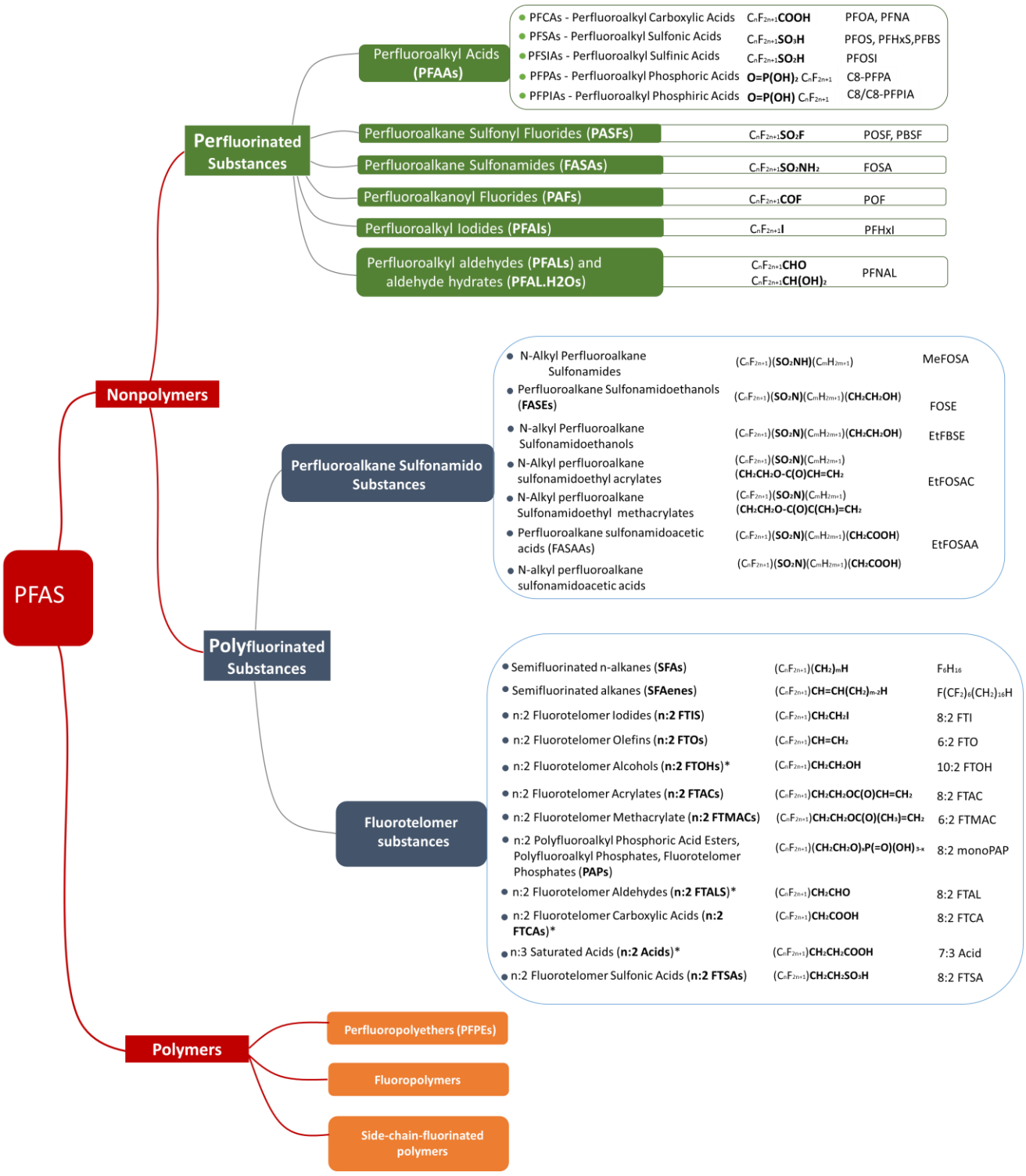


*Figure 2.1 - Hierarchic Classification of polymeric and nonpolymeric PFAS. Substances marked with an * also present unsaturated versions. This illustration was developed based on information from Buck et al. 2011; Danish EPA 2015; and KEMI 2015.*

Because of their unique characteristics, PFAS have been produced in large scale since the late 1940's and used in the formulation of a variety of products. In 1949, 3M started the production of POSF, a now known PFOS precursor, used in the formulation of PTFE. In that same year, DuPont introduced their PTFE-based Teflon® brand, which was approved to be used in non-stick cookware by the US FDA in 1962. It did not take long until 3M introduced their stain repellent products (Scotchgard™) containing PFAS substances, in 1956 (Lindstrom, Strynar, and Libelo 2011; OECD 2015). Around that time, in 1960, the US Navy started to employ AFFF firefighting extinguishers, which contained several PFAS substances in its formulation (Vecitis et al. 2010). In 1967, US FDA approved the use of DuPont's PFAS-based Zonyl® products in food packaging. The employment of these compounds was expanded to many other applications, leading to the identification of PFOS in human blood serum in two major studies, conducted in 1976 and 1978. 3M reported to EPA in 1998 evidence of widespread occurrence of fluorochemicals in human blood bank samples, leading to the complete phase out of POSF/PFOS-based substances in 2002 by the company (Lindstrom, Strynar, and Libelo 2011; OECD 2015).

Since first discovery and production, the number of PFAS related products has increased substantially. As mentioned, due to their hydrophobic and lipophobic properties, PFAS have been used as an additive in the production of PTFE, which was employed in the coating of non-stick cookware. In addition, these substances have been used in the protective coating of carpets, flame retardant and waterproof clothing, umbrellas, bags, car seat covers, and shoes to provide soil, water, and oil repelling. They have also been used as paper treatment in food packaging to produce oil- and water-repellent materials. Furthermore, they have been applied to sunscreens, body lotions, shampoos, and conditioners as emulsifiers and oil- and water-repellents. Their aqueous surface tension-lowering properties made PFAS suitable to be used in the production of AFFF (Aqueous film forming foam) firefighting foams, paints, printing ink, as well as mist suppressants in metal plating. They have also been used in the semiconductors industry as photoacid generators, in photography as anti-static agents, and in aviation as fire-resistant fluids in aircrafts. Their usage extends to building materials, medical devices, oil and mining, ski wax, cleaning

agents and pesticides. It is estimated that over 3,000 PFAS are currently used in the global market (Pan et al. 2018; Z. Wang et al. 2017; Guelfo and Adamson 2018; Johns and Stead 2000; Kaboré et al. 2017; KEMI 2015; Schaider et al. 2017; Pérez et al. 2014; Brooke, Footitt, and Nwaogu 2004).

As it can be inferred from the previous paragraph, these substances and their precursors have been used in a myriad of sectors, and consequently directly and indirectly discharged into the environment. In light of human and wildlife exposure reports, regulations regarding PFAS production and discharge started to take place. In 2006 the US EPA invited eight major producers of PFAS to join a global stewardship program to reduce the emissions of PFOA and its precursors by 95% by 2010 and 100% by 2015 (US EPA 2006). In addition, PFOS and its related substances were included in the Persistent Organic Pollutants (POP) list in the 2009 Stockholm Convention, leading to the restriction (rather than phase-out) in their use in over 100 countries (OECD 2015; Z. Wang et al. 2013). Furthermore, PFOA and its related substances are currently being evaluated for inclusion in the POP list (Pan et al. 2018). In 2016, the US EPA released a non-enforceable Health Advisory for the total levels of PFOS and PFOA of 70 ng L^{-1} in drinking water (US EPA 2016b). Several other countries such as Australia, Canada, and Denmark have also issued guidelines for PFAS usage and phase-out (OECD 2015). The European Commission also amended the REACH regulation to include PFOA and its related chemicals in their regulatory list in 2017 (Pan et al. 2018). In the US, PFOS and PFOA have been added to the Contaminant Candidate List (CCL4), but no guidelines on Maximum Contaminant Levels (MCLs) have been reported for these substances (US EPA 2016a).

2.1.2 Shorter-chain Replacements for Legacy PFAS

Regulatory efforts successfully led to a decrease in production and discharge of legacy PFAS (Ahrens and Bundschuh 2014). However, rather than developing non-fluorinated alternatives, manufacturing industries replaced legacy substances with shorter-chain PFAS with similar structures or ether linkages (Z. Wang et al. 2017; Blum et al. 2015). For example, since C8 phase-out, 3M started to use PFBS derivatives on their Scotchgard™ line of products. Similarly, DuPont Capstone® products are now

based on short-chain substances that cannot break down to PFOA, but to other short-chain PFAS (Danish EPA 2015). In some cases, not even the chain length is changed; fluorine atoms are replaced by hydrogens or part of the chain is substituted by oxygen atoms. For instance, a recent study in Decatur, AL identified nine new polyfluorinated carboxylic acids in the water samples, as well as two other substances that seemed structurally similar to legacy PFAS, with the only change being one fluorine atom replaced by a hydrogen atom (Newton et al. 2017).

Some studies have suggested that shorter-chain substances are less bioaccumulative and present a shorter half-life than longer-chain PFAS (Buck et al. 2011). In agreement to that assumption, Olsen et al. (2008) evaluated the elimination rates of PFBS in the serum of six human subjects, reporting that PFBS elimination occurred at a greater rate than PFOS and PFHxS. That indicates that PFBS has a lower potential for bioaccumulation when compared to legacy PFAS (Olsen et al. 2008). However, toxicity of shorter-chain replacements has not been studied nearly as extensively as that of legacy PFAS (Danish EPA 2015), and comparative conclusions must be drawn with caution. In fact, there is evidence that Fluorotelomeric Alcohols (FTOH) replacements have a higher endocrine disrupting potential than their long-chain analogues (Danish EPA 2015).

Shorter-chain replacements substances are also ubiquitous and recalcitrant in the environment. Since the alternative compounds are often less effective than the legacy substances they replaced, shorter-chain PFAS are used in larger quantities to obtain the same performance (Blum et al. 2015). Shorter-chain PFAS are also known for being more water soluble then their analogues, representing a higher aqueous transport potential (Danish EPA 2015). Replacement substances accounted for over 19% of the total PFAS concentration in 160 water samples collected in the US, China, UK, South Korea, Germany, and Sweden in an effort to obtain a global estimative of novel short-chain PFAS (Pan et al. 2018). In fact, some scientists suggest that the use of such replacements is leading to an increase in stable perfluorinated products, elevating the risk of human and wildlife exposure (Blum et al. 2015). Furthermore, once in the environment, shorter-chain substances are not likely to be removed, since the main method available for PFAS removal

(Granulated Activated Carbon) is not as effective for shorter PFAS, due to their low adsorption potential (Brendel et al. 2018).

2.1.3 Sources and Occurrence in the Environment

As a result from the large scale use and production of PFAS, these substances have been emitted into the environment, many times without the appropriate treatment. The chemical stability that made PFAS so attractive to the industry also makes them very persistent in the environment (Danish EPA 2015; Newton et al. 2017). As stated in Buck et al. (2011), a distinction must be made between direct and indirect sources of PFAS. Direct sources are related to the emissions of a specific PFAS, during its manufacturing, usage, or disposal. In this case, emission from the usage of PFAS-based products would be counted as a direct source. Indirect emissions are associated with the formation of PFAS through the transformation of their precursors. For instance, the formation of PFOA from the transformation of 8:2 FTOH would be categorized as indirect emission (Buck et al. 2011).

PFAS are primarily inserted into the environment through direct emissions from fluorochemical manufacturing facilities and industries that used PFAS-based substances in their products (Hu et al. 2016). 3M was the main producer of PFOS-related substances in the world until 2002 (Ahrens et al. 2009), when the company voluntarily ceased out the production due to toxicological reports and identification of PFOS in the serum of the US population. It is estimated that 3M produced 96,000 t of POSF (a PFOS-related substance) between 1970 and 2002, from which 42,250 t are estimated to have been released to air and water through direct and indirect sources (Paul, Jones, and Sweetman 2008). One expressive example of possible contamination from industries is Decatur, AL, home of several facilities that manufacture or use PFAS (Newton et al. 2017). A 2002 study conducted by 3M identified PFOS and PFOA in the Tennessee River at concentrations up to 144 and 598 ngL^{-1}, respectively (Hansen et al. 2002). In addition, a local wastewater treatment plant accidentally distributed, between 1995 and 2008, over 34,000 metric tons of biosolids contaminated with PFAS to local farmers who used it as a soil amendment. As a result, PFOS was

found to be present at up to 11,000 ng L^{-1} in groundwater samples taken from a well near these sites, indicating that the biosolids influenced the contamination of the surrounding water resources (Lindstrom et al. 2011). A more recent study, conducted in 2017, revealed a hotspot in concentration of PFOS, PFOA, and PFBS in the surface water of the Tennessee River downstream manufacturing facilities, where the sum of PFAS concentrations reached up to 750 ng L^{-1} (Newton et al. 2017). Another study conducted in groundwater from wells close to a DuPont facility in Parkersburg, WV reported that PFOS was present at concentrations as high as 13,300 ng L^{-1} (Hoffman et al. 2010).

These compounds are also often discharged into the environment through Wastewater Treatment Plants, since conventional treatments are not effective at removing PFAS from wastewater (Ahrens et al. 2009; Schultz, Barofsky, and Field 2006). Schultz et al. (2006a) studied the difference in concentrations of selected PFAS in the influent and effluent of ten WWTP across the US, and no systematic increase or decrease was observed (Schultz, Barofsky, and Field 2006). For instance, Schultz et al. (2006b) observed no significant decrease in the mass flow of 6:2 fluorotelomer sulfonate and perfluoroctanoate after conventional wastewater treatment process. In fact, an increase in PFAS concentration in the effluent of WWTP compared to the influent was observed in several studies, probably due to the degradation of labile precursors (Loganathan et al. 2007; Schultz et al. 2006). Furthermore, rivers and oceans are usually wastewater endpoints. Even though oceans were once thought to be a sink for these compounds, new evidence suggests that they can also act as sources of PFAS to the atmosphere (Mcmurdo et al. 2008). In agreement to that, WWTP and landfills were also found to be sources of PFAS to the atmosphere, especially in the aeration stage in conventional WWTP (Ahrens et al. 2011).

Military bases, fire training facilities, and airports are also a possible candidate for PFAS emissions from the use of aqueous film-forming foams (AFFF) (Hu et al. 2016). Both hydrocarbon and fluorocarbon surfactants are used in the composition of AFFFs, where fluorinated surfactants work by reducing the water-air interface and hydrocarbon by reducing the aqueous-hydrocarbon phase (Moody and Field 2000). According to Houtz et al. (2013), the PFAS chemicals used in the production of AFFFs varies by year and

manufacturer, which makes it harder to link contamination to AFFF. The same study identified that AFFFs have been produced with polyfluorinated substances that can degrade to perfluorinated carboxylates and perfluorinated sulfonates (Houtz et al. 2013). Finally, AFFF emissions can contaminate groundwater systems by infiltration and atmospheric deposition, and surface water through runoff. For example, Ahrens et al. (2015) reported a total concentration of PFAS of 4,000 ng L^{-1} near a fire training facility close to the Stockholm Arlanda Airport, Sweden (Ahrens et al. 2014).

Consequently, these compounds have been found in water, sediment, air, humans, and wildlife, in a variety of countries and environments (Zareitalabad et al. 2013; Blum et al. 2015; OECD 2015; Danish EPA 2015; Newton et al. 2017; Gebbink et al. 2016; X. C. Hu et al. 2016; Hoffman et al. 2010; Place and Field 2012; Ahrens et al. 2009, 2014). Between 2013 and 2015, the US EPA collected and analyzed water samples across the US for six perfluorinated compounds (PFOS, PFOA, PFNA, PFHxS, PFHpA, PFBS), among other contaminants, as part of their third Unregulated Contaminant Rule (UCMR 3) program. These water samples were collected at entry points of the water distribution systems of all Public Water Systems (PWS) serving more than 10,000 people, and a selected number of PWS serving less or 10,000 people (US EPA 2016c). PFAS were identified in 194 of the 4864 PWS analyzed, which are estimated to provide drinking water to 16.5 million residents from 33 US states, where concentrations reached up to 349 ng L^{-1} for PFOA and 1800 ng L^{-1} for PFOS (Hu et al. 2016).

2.1.4 PFAS in humans and wildlife

As previously stated, PFAS-based products have been in the market since 1949, and have been reported to cause adverse effects in humans. A study conducted between 2003 and 2004 reported that PFASs were found to be present in the serum of 98% of a representative sample of the US population, with geometric mean concentration of 20.7 μg L^{-1} for PFOS and 3.9 f μg L^{-1} for PFOA (Calafat et al. 2007). As expected, studies have shown that PFAS concentration in human serum was found to be substantially higher in impacted areas. That is the case of the residents of Washington, WV, where PFOS concentration in

human serum was found to be as high as 4,751.5 μg L^{-1} (with a median of 75.7 μgL^{-1}) (Hoffman et al. 2010). Studies have shown that levels of PFOS, PFOA, and other legacy compounds in human serum are decreasing since their phase-out (Hoffman et al. 2010; Calafat et al. 2007). In contrast, levels of shorter-chain and legacy-replacements have shown a substantial increase (Danish EPA 2015).

Studies with animals suggested that inhalation and oral intake are the most likely pathways for PFAS into the body, and contamination from skin absorption is less likely to occur (Danish EPA 2015). Furthermore, PFOS, PFOA, PFHxS, and PFHpS have shown to be transferred from mother to newborns through transplacental transfer, prenatally, and breastfeeding (Papadopoulou et al. 2016). Unlike other persistent organic chemicals, PFAS are not lipophilic; they tend to bind to proteins and are more likely to be present in highly perfused tissues, such as the ones in the liver, kidney and heart (Danish EPA 2015). Perez et al. (2013) evaluated the concentration of 21 PFAS in a *post-mortem* study and discovered high levels of these substances in the liver, bones, brain, lungs, and kidney (Pérez et al. 2013). PFAS half-lives in human serum are substance-dependent, ranging from hours to years, and are usually eliminated through the urine (Danish EPA 2015).

PFAS have been linked to several diseases in humans, either through toxicological studies in animals or empirical associations. The Madrid Statement on PFAS reviewed that these compounds were linked to disruption of the endocrine system, testicular and kidney cancers, high cholesterol, obesity, decrease in birth weight and size of newborns, liver malfunction, among many others (Blum et al. 2015).

Studies have also been conducted to evaluate adverse effects of PFAS in wildlife, as well as bioaccumulation and biomagnification potentials. PFAS have been identified in dolphins, algae, seals, fish, and even polar bears (Ahrens and Bundschuh 2014; Brooke, Footitt, and Nwaogu 2004; Danish EPA 2015). PFAS was also observed to accumulate in terrestrial plants irrigated with PFAS-contaminated water (McCarthy, Kappleman, and DiGuiseppi 2017). Algae response to PFAS contamination has been previously evaluated (Liu et al. 2018; Latała, Nedzi, and Stepnowski 2009), since algae represent the first step to bioaccumulation in several food webs (Correa-Reyes et al. 2007). Latala et al. (2019) observed the

sensitivity of selected species of green, diatom, and blue-green algae to PFCAs, reporting that blue-green and diatom species were far more sensitive (Latała, Nedzi, and Stepnowski 2009). Xu et al. (2014) observed that C9-C12 PFCAs were significantly biomagnified in an eutrophic freshwater food web study, where PFAS were detected in more than 60% of phytoplankton, zooplankton, fish, shrimp, and egret samples (Xu et al. 2014). In contrast, Lescord et al. (2015) did not find evidence of biomagnification in lake food webs from the Canadian high Artic, but concluded that habitat and sources of contamination were important on determining PFAS contamination in biota (Lescord et al. 2015). Even though many studies have been done in this area, results are not consistent and are not reported in a standardized notation (McCarthy, Kappleman, and DiGuiseppi 2017).

2.1.5 Existing remediation strategies

Several studies indicated that current wastewater treatment plants are not adequate for treating PFAS-contaminated waste (Hu et al. 2016; Loganathan et al. 2007; Ahrens et al. 2011; Kucharzyk et al. 2017; Schultz, Barofsky, and Field 2006; Schultz et al. 2006). The current technology used in the treatment of PFAS-contaminated drinking water is Granulated Activated Carbon (GAC) (Eschauzier et al. 2012; Kucharzyk et al. 2017; McNamara et al. 2018). PFAS sorption onto activated carbon has shown to be as effective as 90% (Kucharzyk et al. 2017), and GAC plants have been successfully implemented to treat surface and groundwater in several locations across the US, such as Little Hocking (OH), and Decatur (AL) (McNamara et al. 2018). However, GAC systems are extremely costly, generate solid waste, have shown to present a time-dependent efficiency, and to be inadequate for short-chain PFAS (Appleman et al. 2013; Eschauzier et al. 2012). Therefore, several studies have been conducted in efforts to find remedial alternatives to remove PFAS from surface and groundwater, wastewater, and sludge. A summary of a few selected studies is presented in Table 2.1.

Table 2.1 – Selected studies regarding new alternatives for the remediation of legacy and emerging PFAS. Targeted compounds, methods, highlights and observed challenges are presented for each study.

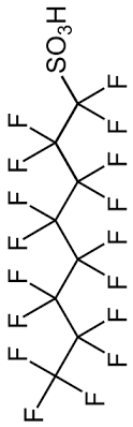
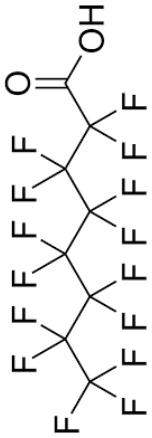
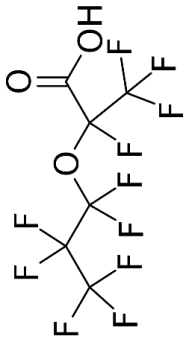
PFAS	Methods	Highlights and Results	Challenges	Reference
PFOA, PFOS, 6:2 FTSA	Heat-activated oxidation	- Methodology was not able to oxidize PFOS; - PFOA and 6:2 FTSA were oxidized at low temperatures (30-60 °C) with $S_2O_8^{2-}$, producing shorter chains PFAS and F^-.	Temperature, production of short-chains, not applicable for PFOS	(Park et al. 2016)
PFOA	Enzyme-catalyzed oxidative humification reactions (ECOHRs) with Laccase	- ECOHR was able to partially transform PFOS into F and partially fluorinated products in the presence of HBT. Rate of 28.2% of defluorination after 157 days	Kinetic limitations, low defluorination rates	(Luo et al. 2015)
PFOS and PFOA	Adsorption onto Calcium Fluoride (CAF_2) solids	- Decrease in 44-62% for PFOA and 58-76% to PFOS initial concentration.	Unrealistic concentration, second treatment required	(Lampert, Frisch, and Speitel 2006)
PFOS and PFOA	Evaporation	- Methodology was able to reduce concentration to bellow detection limits (1 mgL^{-1})	Cost, feasibility, unrealistic concentrations, use of foam controllers.	(Lampert, Frisch, and Speitel 2006)
PFOS and PFOA	Ion Exchange	- Reduction in concentration from 4320 to 13 mgL^{-1} for PFOA, and 950 to <1 mgL^{-1} for PFOS after 25 hours.	Kinetic and applicability limitations	(Lampert, Frisch, and Speitel 2006)
PFHxA, PFHpA, PFOA, PFOS, PFNA, PFDA, PFUnA, PFDoA	Electrocoagulation	- Sum of PFAS decreased by 70.8% after 45 min reaction time in landfill samples.	Increase in concentration of PFUnA and PFDoA by up to 20%.	(Zhang et al. 2013)
PFOA, PFOS, PFBS, PFHxS, PFDS, PFOSA, PFBA, PFPeA, PFHxA, PFHpA,	Colloidal Activated Carbon (*in situ*)	- PFOS concentration was reduced from 1,450 to 40 ngL^{-1} after a period of 18 months; - Concentration of all the other PFASs were reduced to bellow detection limit (30 ngL^{-1})	Kinetic and applicability limitations	(McGregor 2018)

PFNA, PFDA, PFUnA, PFDoA				
PFOS, PFOA, and other 20 PFCAs, PFSA, Emerging PFASs, and PFAS precursors.	Poly(ethylenimine)-Functionalized Cellulose Microcrystals	- Near instant removal of PFOA (70-80%) within 100s; - High removal rates for other long chain and precursors at pH 6.5 after 2 h contact time.	Low sorption rates for short-chain PFAS	(Ateia et al. 2018)
PFOS and PFOA	Heat-activated persulfate under conditions representative of in situ chemical oxidation	- pH had a great influence on PFOA removal; -PFOS was not transformed in any configuration analyzed; - $S_2O_8^{2-}$ decomposition resulted in a 98% loss of PFOA.	pH, production of hazardous byproducts (short chain PFAS, ClO_3^-, and HF)	(Bruton and Sedlak 2018)
PFOS and PFOA	Electrochemical treatment using a nanocrystalline boron-doped diamond (BDD) anode.	- Both compounds were successfully removed at a current density of 50 $mAcm^{-2}$.	Second Treatment required, unrealistic concentration used, and generation of short chain PFAS.	(Schaefer et al. 2017)
PFOS	Photodegradation by UV Irradiation in Water and Alkaline 2-Propanol	- Reduction in 8 and 68% in PFOS concentration, after 1 and 10 days exposure, respectively. - Reduction in 76 and 92% after 1 and 10 days exposure, respectively, in alkaline 2-propanol.	Kinetics and Applicability	(Yamamoto et al. 2007)
PFOS, PFOA, PFNA, PFDA	Mg-aminoclay coated nanoscale zero valent iron (nZVI)	- Removal of 38 – 96% of initial concentration with 1 gL^{-1} of Mg-aminoclay coated nZVI at pH 3 after 1h reaction	Low yields for older nZVI, degradation products	(Arvaniti et al. 2015)

2.1.6 A closer look at PFOS, PFOA, HFPO-DA, and PDHA

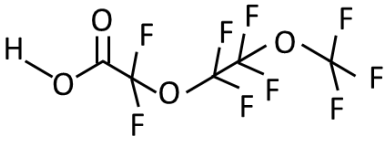
As mentioned in the introductory section, this study targeted four perfluorinated compounds: PFOS, PFOA, HFPO-DA, and PDHA. These compounds were chosen based on their ubiquity, toxicity, and lack of information and studies on the shorter-chain replacements. Tables 2.2 and 2.3 summarize the chemical properties and molecular structures of these substances.

Table 2.2 - PFOS, PFOA, and HFPO-DA Properties and Chemical Structure. Sources: (a) MSDS sheets for respective substance (Synquest Laboratories 2016, 2015); (b) (US EPA 2017); (c) (Kucharzyk et al. 2017); (d) (Zareitalabad et al. 2013); (Latała, Nedzi, and Stepnowski 2009); (f) Technical Information and image from the PubChem website for each substance; (g) (Hopkins et al. 2018). N/a stands for not available.

Properties	PFOS	PFOA	HFPO-DA
Chemical Structure and Name	 Perfluorooctanesulfonic acid[f]	 Perfluoro-n-octanoic acid[f]	 Undecafluoro-2-methyl-3-oxahexanoic acid[f]
CAS no.	1763-23-1[a]	335-67-1[a]	13252-13-6[a]
Molecular Weight (g/mol)	500[a]	414[a]	330[a]
Water Solubility (mg/L)	680[b]	9.5x10^3[b]	n/a
Boiling Point (°C)	258-260[b]; 145[a]	192[b]; 189[a]	60[b]
Melting Point (°C)	>400[c]	59-60[a]	n/a
pKa	<1[f]	2.8 [d]	-0.77[g]

Vapor Pressure (mm Hg)	0.002 (a) $2.48x10^{-6}$ (c)	0.525[a] 0.017[c]	n/a
log K_{OW}	Not Measurable[c]	6.30[e]	3.21-8.12[g]
Organic Carbon Partition Coefficient (K_{OC})	2.57[a]	2.06[a]	n/a
Half-Lives	Atmosphere: 114 days Water: >41 years[c]	Atmosphere: 114 days Water: >92 years[c]	n/a

Table 2.3 - PDHA Properties and Chemical Structure. Sources: (a) MSDS sheet ((Synquest Laboratories 2015); (b) Technical Information and image from the PubChem website for PDHA.

Abbreviation	Chemical Formula and Name	CAS no.	Molecular Weight (g/mol)	Boiling Point (°C)
PDHA	 Nonafluoro-3,6-dioxaheptanoic acid [b]	151772-58-6 [a]	296 [a]	145-146 [a]

2.2 Algal Turf Scrubber

2.2.1 ATS and Periphytic Algae

The Algal Turf Scrubber™ (ATS) is an algal-based mini-ecosystem designed to improve a variety of water quality parameters by growing algal biomass (Ray, Terlizzi, and Kangas 2015; Kangas and Mulbry 2014; Pizarro, Kebede-Westhead, and Mulbry 2002; W. H. Adey et al. 2013). This system consists of a slightly inclined channel in which water runs through continuously or in a pulse (wave) motion (D'Aiuto et al. 2015). The wave motion is usually used to increase algal growth, by facilitating water exchange and

nutrients availability (Mayr, Jerney, and Schagerl 2015). The flow can be re-circulated with the help of a pump system, or discharged back into the natural environment after the desired amount of nutrients are removed (Ray, Terlizzi, and Kangas 2015).

ATS systems are usually shallow floways (1-2 cm water depth) illuminated with high intensity sunlight, and are frequently harvested for algal community regeneration (Adey and Bannon 2008; Adey, Kangas, and Mulbry 2011). Unlike ponds that use mono-cultures of planktonic algae, ATS systems employ poly-cultures of attached algae, usually referred to as periphytic (Bohutskyi et al. 2016). To support that, ATS systems are equipped with a netting-like substratum on the bottom of the channels, allowing attachment of the desired species (Gross, Jarboe, and Wen 2015; Adey, Kangas, and Mulbry 2011). The mixed algal community that populates these systems are usually indigenous to that environment, and are resilient to grazers and variation in environmental conditions (Bohutskyi et al. 2016). Perhaps the main advantage of using periphytic algae is that they can be easily harvested from the system through either suction or scraping, in contrast to the time-demanding and expensive techniques used in the harvesting of planktonic algae (Kangas et al. 2017; Bohutskyi et al. 2016). Figure 2.2 illustrates a representation of a typical periphytic turf-mat, obtained from Adey, Kangas, and Mulbry (2011).

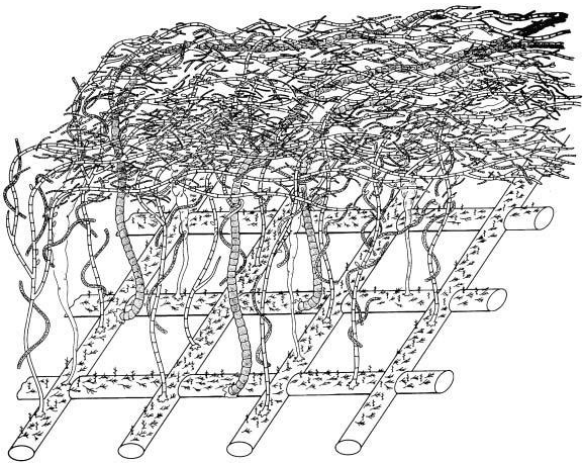


Figure 2.2 - Attachment on a screen substratum. Source: Adey, Kangas, and Mulbry (2011).

In practice, polluted water is pumped into the ATS system, where the mature algal turf biologically uptakes nutrients such as nitrogen and phosphorus, which are required for their growth (Ray, Terlizzi, and

Kangas 2015). In addition, organic matter, planktonic algae, and other suspended or dissolved substances can be physically captured by the vertical turf structure formed from the substratum (Mayr, Jerney, and Schagerl 2015; Valeta and Verdegem 2015). Through photosynthesis, algae can remove carbon dioxide and increase the concentration of dissolved oxygen in the finished water (Chen et al. 2015). The captured nutrients and particles are removed from the system when the turf is harvested, which is usually conducted every 5 to 15 days (Kangas et al. 2017; Adey and Bannon 2008). Finally, ATS are highly-productive systems, and consequently can remove nutrients at a rapid rate, while using solar energy (Kangas et al. 2017; D'Aiuto et al. 2015).

2.2.2 History and Applications

The idea of using an attached algal turf was first employed by Dr. Water Adey from the Smithsonian Institution on a study in coral reefs in the Caribbean Sea. The same author patented the on-land methodology in 1983 (US Patent 4333263A), referring to it as an Algal Turf Scrubber (Adey 1983). Since then, the ATS methodology has been expanded to many other applications.

One of the main areas of study is the employment of Algal Turf Scrubbers™ in the treatment of wastewater from animal manure. The most common method for manure wastewater disposal is through direct application into the soil, which can contaminate water bodies through runoff and atmosphere by the volatilization of ammonia (Kebede-Westhead, Pizarro, and Mulbry 2006; Walter Mulbry et al. 2005). Kebede-Westhead et al. (2006) explored the usage of ATS systems to remove P and N from swine manure effluents. Their systems were mainly composed of species from the genera *Microspora, Ulothrix, Rhizoclonium*, and *Oedogonium*, and were able to reduce up to 98 and 77% of N and P concentration, respectively, while producing biomass at a rate of 9.4 gdw m^{-2} d^{-1} (Kebede-Westhead, Pizarro, and Mulbry 2006). Mulbry et al. (2008) used a similar approach to test the applicability of ATS to treat dairy manure effluents, reporting a reduction in N and P concentration in the effluent wastewater by up to 89 and 91%, respectively (Walter Mulbry et al. 2008).

ATS methodology has also been applied in the treatment of impaired natural waters (Sindelar et al. 2015; W. Mulbry, Kangas, and Kondrad 2010) and a drinking water reservoir in China (Chen et al. 2015). For instance, a pilot-scale study evaluated the suitability of an ATS to be used in the restoration of Chesapeake Bay, where elimination rates for nitrogen and phosphorus were observed to be as high as 250 mgN m^{-2} d^{-1} and 45 mgP m^{-2} d^{-1}, respectively (W. Mulbry, Kangas, and Kondrad 2010).

Furthermore, these systems have been tested for wastewater treatment at an oyster facility with removal rates of 12.2 gN m^{-2} d^{-1} and 0.25 gP m^{-2} d^{-1} (Ray, Terlizzi, and Kangas 2015), to improve water quality for freshwater mussel production (Huang et al. 2013), to remove nitrogen from catfish effluents (Valeta and Verdegem 2015), as well as to remove nutrients from horticultural wastewater (Liu et al. 2016). This methodology has also been tested to be used in the treatment of runoff water from a citrus orchard in South Florida, where elimination rates averaged 16% PO_4-P and 49%, for NO_3-N (D'Aiuto et al. 2015). In 2002, the ATS methodology was successfully scaled up by HydroMentia Inc., who started manufacturing units up to 100 million-lpd in south Florida for nutrient removal from agricultural wastewater. For instance, an ATS unit located in the Lake Okeechobee watershed (Figure 2.3), was able to remove around 6400 lb-N ha^{-1} yr^{-1} and 4,000 lb-P ha^{-1} yr^{-1} from stormwater runoff (HydroMentia Inc. 2005; W. H. Adey, Kangas, and Mulbry 2011).

Figure 2.3 - Aerial view a full scale Algal Turf Scrubber System located in the Lake Okeechobee Watershed, Central Florida. Source: HydroMentia Inc. 2005.

2.2.3 ATS Byproducts

Algal biomass produced from the mentioned applications is rich in nutrients and fatty acids, and has been studied to be used as a slow-release fertilizer, as a feedstock for biofuel and bioplastics, as well as a nutraceutical supply (Kesaano and Sims 2014).

For instance, Mulbry et al. (2005) explored the possibility of using the harvested biomass as a slow-release fertilizer in a growth chamber study with cucumber and corn seedlings. The authors reported that 3% of the total algal N was available at mineral N at day zero and up to 30% at day 21. At that rate, the biomass used to treat dairy effluents from 100 cows would provide enough phosphorus and nitrogen to fertilize 4 ha and 6 ha of corn, respectively. In addition, as a contrast to what happens in manure fertilizers, ammonia does not volatilize from slow-release algal fertilizers (Mulbry et al. 2005).

Biofuels using algal biomass have been extensively studied and shown to be a feasible application, especially for planktonic microalgae (Savage and Hestekin 2013; W. H. Adey, Kangas, and Mulbry 2011). A small percentage of algal cells material, like phospholipid membranes and oil, can be converted into biodiesel; diatoms and certain planktonic algae have been shown to present a high fatty acid content, which is desirable for this application (W. H. Adey, Kangas, and Mulbry 2011). In contrast, studies with algal species from ATS systems reported very low concentration of fatty acids (0.2-10% of dry biomass), indicating their usage in biodiesel production is limited (Bohutskyi et al. 2016; W. H. Adey, Kangas, and Mulbry 2011). However, Bohutskyi et al. (2016) observed that biomass material from ATS systems could be used in the production of methane through an anaerobic digestion process, at a rate of 0.2 L methane per gram of biomass VS (Bohutskyi et al. 2016).

3. METHODS

3.1 ATS System

The algal turf scrubber system used in this project is an adaptation from existing channels located in the CASIC building (Mike Hubbard Center for Advanced Science, Innovation and Commerce) at Auburn University. The new system is comprised of eight 12:165:7 cm (w:l:h) PVC channels built from residential rain gutters. Four of these reactors were equipped with a 1/8 in nylon mesh that was used as the substratum material for algal growth, with an effective growth area of 1300 cm^2 (10:130 cm w:l). Nylon was chosen based on a recent study that suggested that nylon and polyethylene meshes yielded the best results for early algal colonization and long-term attachment, when compared to other 26 materials (Gross et al. 2016).

Each channel was continuously supplied by Pondmaster pumps (Danner Pondmaster Magnetic Drive Pump Model 7 (700 gph) and 5 (500 gph)), submerged in eight separate 38 L (10 U.S. gal) reservoirs (Sterilite, 10 Gal/38 L Tote Box). The pipeline structure is comprised of 0.5 in PVC pipes (600-PSI Schedule 40 PVC Plain End Pipe) with several fittings and a PVC ball valve for flow adjustment (see Figure 3.3 for details).

Furthermore, each reactor was equipped with an individual light fixture (Lithonia Lighting 2-Light White T8 Fluorescent Residential Shop Light), placed 20 cm above the bottom of the reactors. Blackout fabric (Roc-lon Blackout Drapery Lining White Fabric) was placed along the length of the fixtures to avoid light contamination between channels. Figure 3.1 and Figure 3.2 illustrate a schematic view of the system. A front image of the system is presented in Figure 3.3. Additionally, a structure composed of blackout

curtains supported on a PVC structure hanged from the ceiling was built around the ATS system to avoid UV light exposure to laboratory users (Figure 3.4).

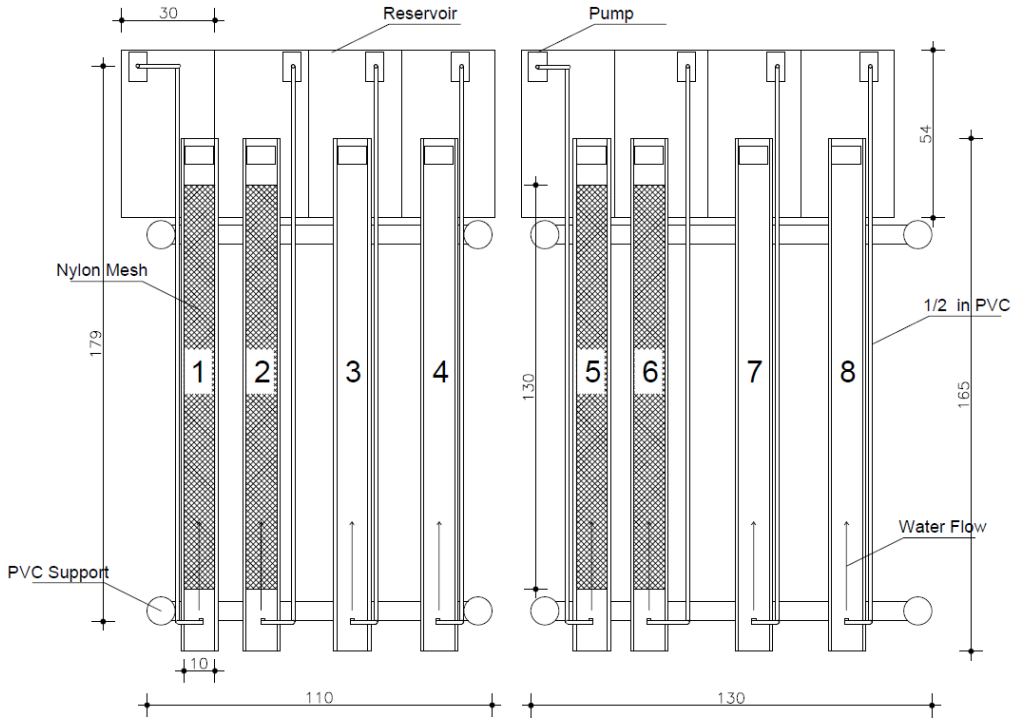


Figure 3.1 - Schematic top view of the ATS system. All dimensions are in centimeters

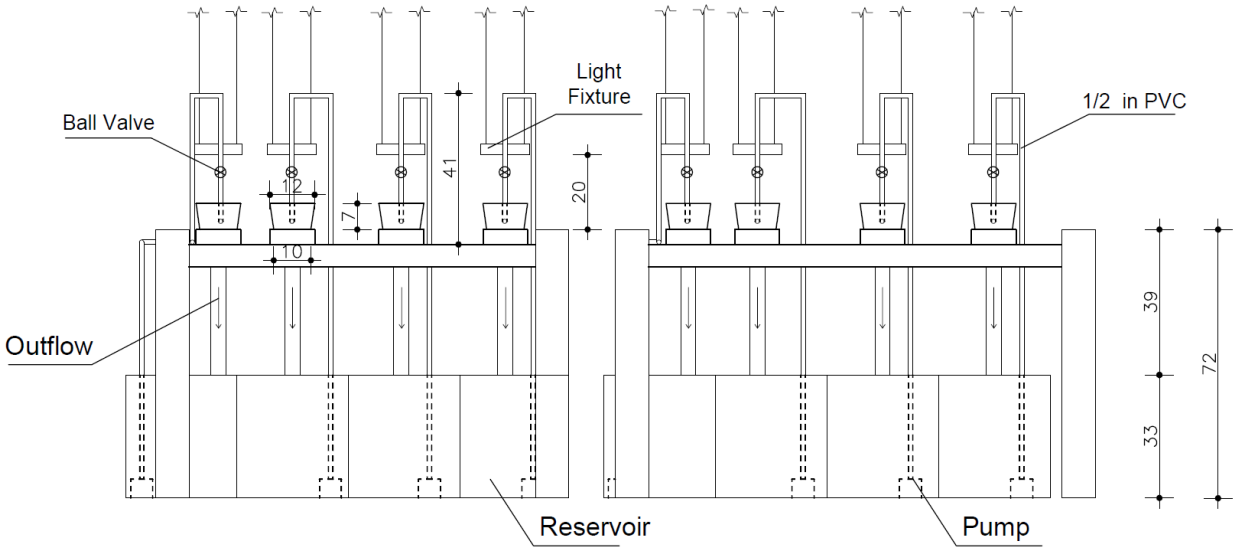


Figure 3.2 - Schematic front view of the ATS systems. All dimensions are in centimeters

(a) *(b)*

Figure 3.3 - Front view of the ATS system. (a) illustrates a longitudinal overview of all channels and (b) a more detailed front view of Channels 1-4.

Figure 3.4 - UV Light Protection Structure

3.2 Algal Culture

3.2.1 Algal Collection and Growth Parameters

A mixed community of periphytic algae was obtained from Chewacla Creek (State Park permit number 180603), located at Chewacla State Park, Alabama on May 27th of 2018. Rocks containing the desired community were collected in three points along the width of a section of Chewacla Creek, indicated in Figure 3.5. The rocks were then transported to the laboratory in plastic containers filled with natural water.

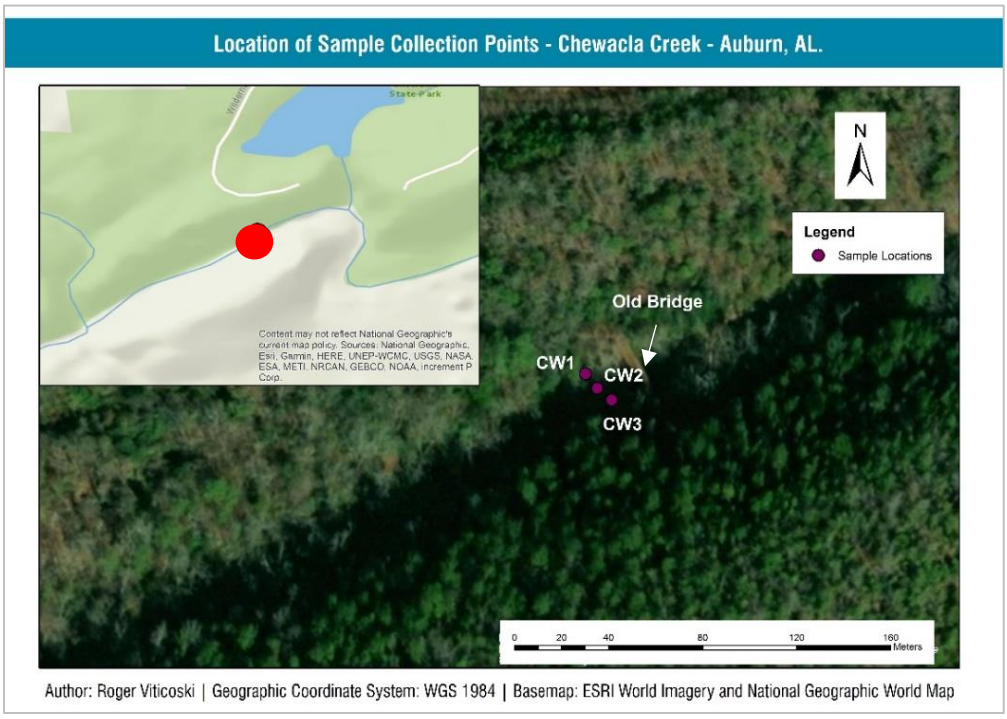


Figure 3.5 - Sample Collection Points for Rocks and Water. Samples were collected downstream of the confluence of Chewacla Creek and Moores Mill Creek, downstream of the old bridge at the end of Wrights Mill Road

Water samples from Chewacla Creek were also collected to assess the environmental conditions in which the community was exposed. Temperature and pH of the natural water were analyzed *in situ* using a Hannah HI 98130 meter. Phosphate and Nitrate concentration were analyzed in the laboratory using an YSI

9300 photometer (YSI, Inc.) with a ±0.01 mg L^{-1} accuracy. Both tests were conducted following the instructions in the manual provided by the manufacturer. Table 3.1 shows the results from those analyses.

Table 3.1 - Natural Water Characteristics

Sample	Temperature (°C)	pH	Nitrate (mg L^{-1} NO_3)	Phosphate (mg L^{-1} PO_4)
CW1	25.2	7.70	0.89	0.38
CW2	25.1	7.62	1.06	0.37
CW3	25.3	7.68	0.87	0.27

After collection, the rocks containing the attached algae were placed in the reservoirs of four (Channels 1, 2, 5, and 6) of the eight channels described in section 3.1. The collected algae were inoculated using an adapted version of Bolds Basal Medium (Anderson 2005). Bolds Basal Medium (BBM) was developed by Bischoff and Bold in 1963 and has been widely applied in the culture of freshwater green algae. Each stock solution was prepared by dissolving the desired components in 1 liter of deionized water, following the recipe presented in Table 3.2. After complete dissolution, stock solutions were transferred to glass bottles. Media solutions were prepared by adding the recommended amounts of each stock solution to 936 mL of deionized water. Each media solution was autoclaved and stored in the refrigerator at 4 °C. A 10% dilution of the original recipe was used to better represent the conditions to which the community was being exposed in its natural environment.

Table 3.2 - Bolds Basal Media Recipe – Original vs. Diluted Concentrations

Component	Stock Solution (g L^{-1})	Quantity	Molar Concentration in Original Media	Molar Concentration in Diluted Media
Macronutrients				
$NaNO_3$	25.00	10 mL	$2.94x10^{-3}$ M	$0.294x10^{-3}$ M
$CaCl_2 \cdot 2H_2O$	2.50	10 mL	$1.70x10^{-4}$ M	$0.170x10^{-4}$ M
$MgSO_4 \cdot 7H_2O$	7.50	10 mL	$3.04x10^{-4}$ M	$0.304x10^{-4}$ M
K_2HPO_4	7.50	10 mL	$4.31x10^{-4}$ M	$0.431x10^{-4}$ M
KH_2PO_4	17.50	10 mL	$1.29x10^{-3}$ M	$0.129x10^{-3}$ M
NaCl	2.50	10 mL	$4.28x10^{-4}$ M	$0.428x10^{-4}$ M
Alkaline EDTA Solution				
EDTA	50.00	1 mL	$1.71x10^{-4}$ M	$0.171x10^{-4}$ M
KOH	31.00		$5.53x10^{-4}$ M	$0.553x10^{-4}$ M
Acidified Iron Solution				
$FeSO_4.7H_2O$	4.98	1 mL	$1.79x10^{-5}$ M	$0.179x10^{-5}$ M
H_2SO_4	1 mL		-	

Boron Solution				
H_3BO_3	11.42	1 mL	$1.85x10^{-4}$ M	$0.185x10^{-4}$ M
Trace Metals Solution				
$ZnSO_4.7H_2O$	8.82	1 mL	$3.07x10^{-5}$ M	$0.307x10^{-5}$ M
$MnCl_2.4H_2O$	1.44		$7.28x10^{-6}$ M	$0.728x10^{-6}$ M
MoO_3	0.71		$4.93x10^{-6}$ M	$0.493x10^{-6}$ M
$CuSO_4.5H_2O$	1.57		$6.29x10^{-6}$ M	$0.629x10^{-6}$ M
$Co(NO_3)_2.6H_2O$	0.49		$1.68x10^{-6}$ M	$0.168x10^{-6}$ M

The system was continuously operated by recirculating 25 L of a mixture of deionized water and culture media under a flow rate of 0.1398 ± 0.0012 L s^{-1} (137 gph). The flow rate was measured in each channel by recording the time to fill up certain volume in a 2 L plastic bucket. The procedure was performed five times for each channel and the final flow rate was considered as being equal to the average flow rate of the five trials.

3.2.2 Light Configuration

Three light configurations were used during this study, based on the presence or absence of UVA and UVB rays, as shown in Table 3.3. Configuration #C1 was used in all channels during inoculation, whereas #C2 and #C3 were alternated during the harvesting process, as it will be further explained in section 3.3.2.

Table 3.3 - Light Configurations used throughout culturing and harvesting process

#	Configuration
C1	Two 48 inch Cool White Phillips Alto II fluorescent lightbulbs
C2	One Phillips Alto II fluorescent lightbulb and one Sunsource Helio-Vite UVA-UVB fluorescent lightbulb
C3	One Phillips Alto II fluorescent lightbulb and one Sunsource Helio-Vite UVA-UVB fluorescent UV with one UVA-UVB Filter Tube (no UV rays)

In configuration #C1, channels were illuminated with two 48 inch Cool White Phillips Alto II fluorescent lightbulbs under a 16:8 h light:dark cycle. Cool white fluorescent lightbulbs are widely used for culturing algae and plants, because of their emitted radiance being similar to the daylight spectrum

(Kommareddy and Anderson 2003). The light fixtures were connected to two digital timer power strips (Defiant 15 Amp 7-Day Indoor Digital Timer with 8-Outlet Power Strip) to control the light:dark cycle. Light intensity of each set was measured using an Apogee MQ-200 PAR meter (Apogee Instruments, Inc.) at 12 points throughout each channel, resulting in an average light intensity of 338 ± 73 μmol m^{-2} s^{-1}.

In configuration #C2, the channels were illuminated with a combination of one Phillips Alto II fluorescent lightbulb and one Sunsource Helio-Vite UVA-UVB fluorescent lightbulb under a 16:8h light:dark cycle. This configuration is believed to better represent the light inputs required for algal growth. The spectral irradiance obtained from the manufacturer for the Sunsource Helio-Vite is shown in Figure 3.6.

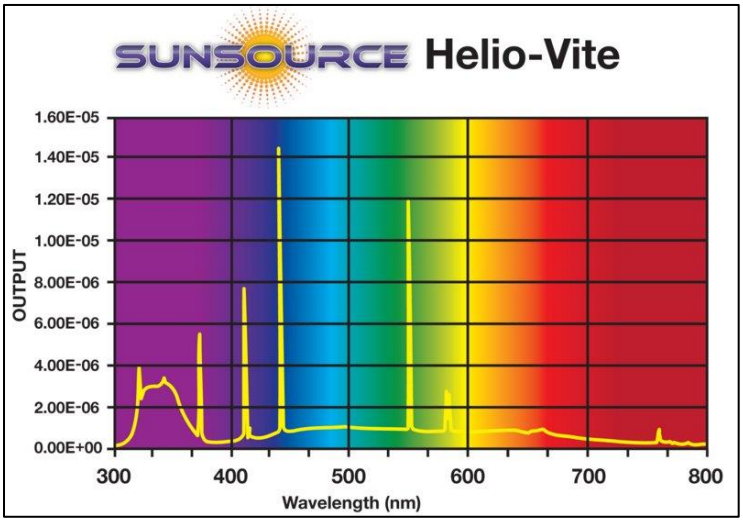


Figure 3.6 - Spectral Irradiance of Sunsource Helio-Vite UVA-UVB fluorescent lightbulb. Source: Everyday Green LLC

Configuration #C3 is similar to #C2, with the only change being a UV Filter Tube (FS10 UV light filter; Ergomart, Dallas, TX) placed around the Helio-Vite lightbulb to remove UVA and UVB rays. In addition to the spectral irradiance obtained from the manufacturers, the spectral irradiance of both configurations (with and without filter) were measured using a Stellarnet Black-Comet UV-VIS spectrometer (Stellarnet, Inc.). Figure 3.7 illustrates the combined spectral irradiance of one Cool White bulb and one Helio-vite UVA-UVB bulb. As it can be seen, the filter placed around the Helio-Vite bulb was successfully able to filter most of UVA (λ = 315 to 400 nm) and UVB (λ = 280 to 315 nm) rays.

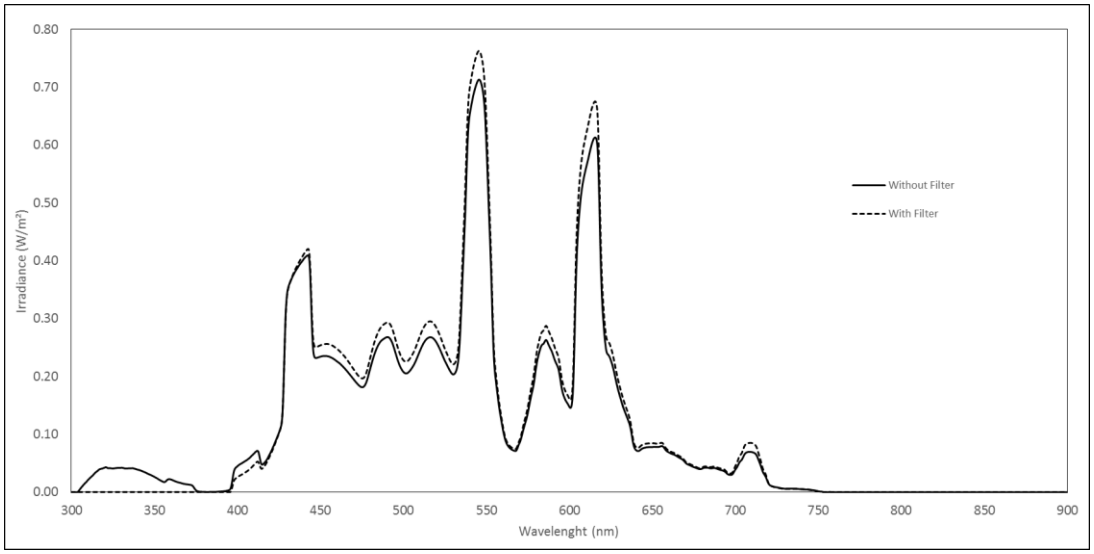


Figure 3.7 - Spectral Irradiance of Sunsource Helio-Vite UVA-UVB fluorescent lightbulb and Phillips Alto II fluorescent lightbulbs. Dashed line represents the configuration with UV Filter and solid line without filter.

3.2.3 Harvest and Biomass Quantification

Harvesting is an important step in the ATS maintenance because it promotes renovation of the community, diminishes the effect of microgazers, and increases biomass yields (W. H. Adey, Kangas, and Mulbry 2011). Thus, after the culture was first established, and then, after every eight days, each channel was harvested using an Erlenmeyer flask attached to a vacuum pump at a maximum pressure of 10 in Hg, as illustrated on Figure 3.8. This process consisted of carefully removing algal biomass from the nylon mesh through suction, until all material was removed.

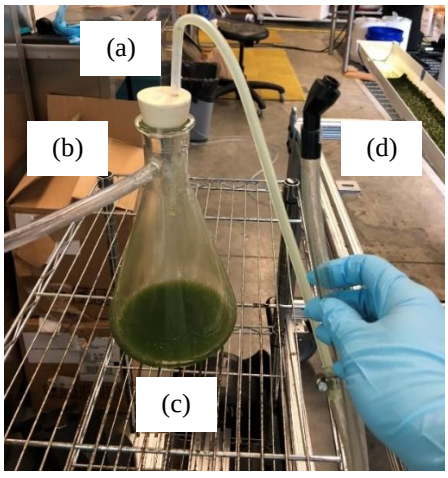


Figure 3.8 - Equipment setup: (a) biomass inflow; (b) vacuum inflow; (c) collected biomass; (d) vacuum head.

The harvested biomass presents a high water content that can vary from harvest to harvest, as well as a varying ash content, depending on species and growth media. With that in mind, an approach based on the Ash Free Dry Weight Method (Wychen and Laurens 2015) was used to quantify algal biomass. This technique allowed comparisons since it standardized the weights. A flow chart of the process is illustrated on Figure 3.9.

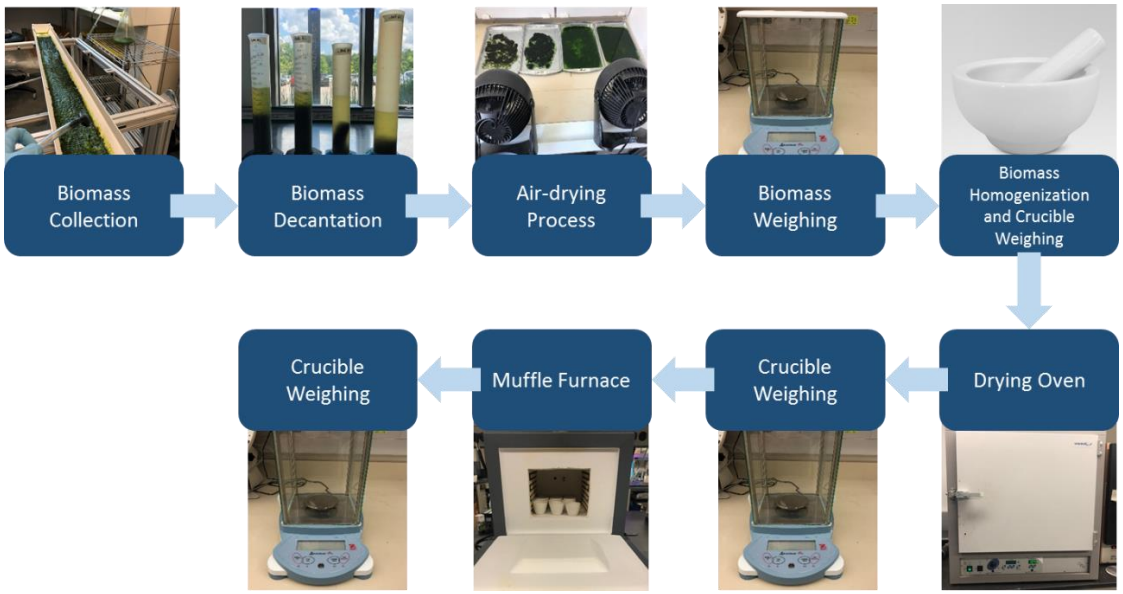


Figure 3.9 - Ash Free Dry Weight Flow Chart

First, the collected material was transferred from the Erlenmeyer glass into a graduated cylinder, to promote decantation of solids (biomass). After 12 hours, the liquid material was discarded and biomass was air dried on a drying sheet covered with plastic wrapping with the help of two mechanic fans. The air dried biomass was then weighted to assess overall production. Furthermore, the air-dried biomass was homogenized using a porcelain mortar and pestle. Three samples of approximately 100 ± 5 mg from each channel were transferred to porcelain crucibles and placed in a drying oven for 24 hours at 60 °C. The average difference in weight between the air-dried and oven-dried samples was considered to be equal to the amount of water present in the biomass, as shown in the equation below.

$$\%\, Water\ Content = 100\% - \left(\frac{W_{crucible+dry\ weight} - W_{crucible}}{W_{wet\ sample}}\right) * 100\%$$

Finally, the oven-dried samples were then place on a muffle furnace for 5 hours. A temperature ramping program was used to achieve the desired temperature of 550 °C, to avoid immediate combustion of material. The average difference in weight between oven-dried and muffle-dried samples was considered to be equal to the biomass ash content.

$$\%\ Ash\ Content = \left(\frac{W_{crucible+ash} - W_{crucible}}{(1 - water\ content) * W_{wet\ sample}}\right) * 100\%$$

3.2.4 Daily Measurements and Nutrient Control

The 16:8 light:dark cycle from the lightbulbs was observed to directly interfere with pH and temperature in the channels. That happened because during the light period, dissolved CO_2 is removed from the water through photosynthesis and pH becomes more basic. On another hand, during the dark period, CO_2 is produced through respiration and pH becomes more acidic. During each harvest, temperature, pH, and conductivity were monitored three times per day using a Hannah HI 98130 meter, based on this cycle. The first set of measurements was conducted around 8 AM, right after the lights were turned back on, representing the lowest pH on that day due to the highest concentration of CO_2. The second set was taken around 4 PM, halfway through the 16h-light period. Finally, the third set of measurements was usually conducted around 11 PM, right before the lights would be turned off, representing the highest pH of the day due to a low concentration of dissolved CO_2.

Figure 3.10 represents a typical pH profile during an in-between harvests period. As it can be seen, the initial pH is fairly low, around 6.0, and it tends to approach a more basic state once the community starts to grow again after the harvesting process. This trend is expected since the increase in biomass leads to a higher dissolved CO_2 usage, increasing the media pH.

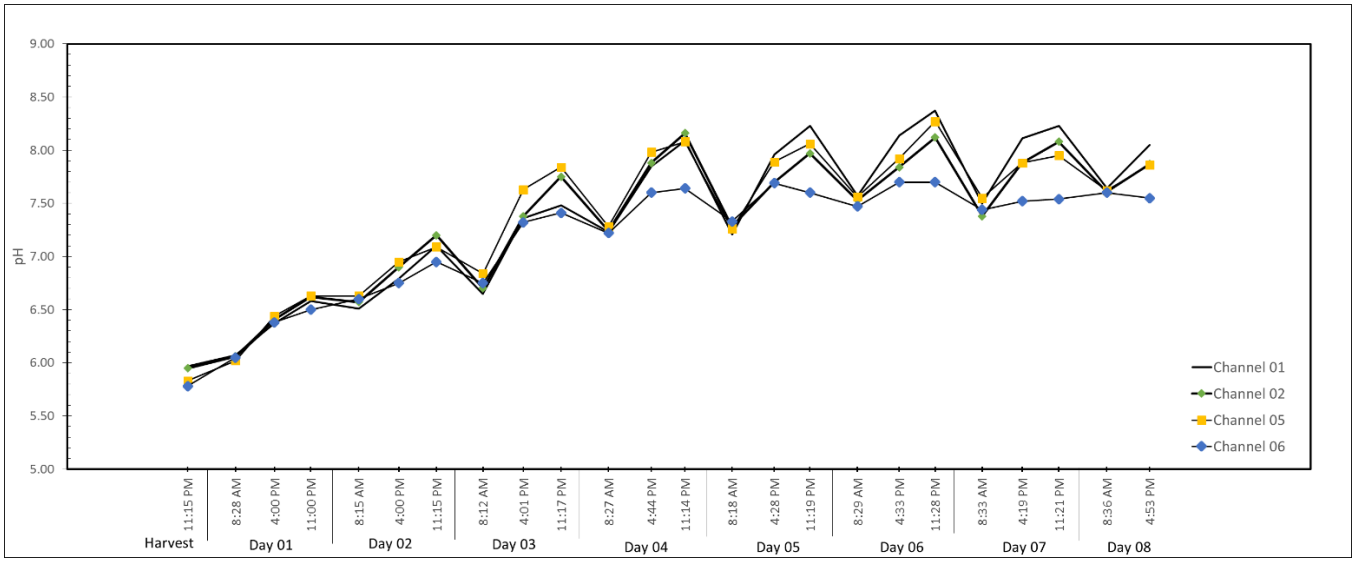


Figure 3.10 - pH profile for Harvest #6

On week 6 of the harvesting process, HOBO temperature data loggers (HOBO Pendant Temperature/Light Data Logger, 8k) with an accuracy of ± 0.5 °C were added to the reservoirs of channels 1, 2, 5, and 6. Each temperature logger was set to acquire readings every 30 minutes. In addition, to have a better understanding of pH variation, a data logger (USB-6009 Multifunction I/O, National Instruments) used to acquire pH measurements was also added to these same reservoirs.

The USB-6009 NI data logger is a multi-channel input for a Labview computer-based system and it is composed of eight analog channels. It works by acquiring voltage measurements, which then can be transformed to pH through a conversion curve/equation. The first step to set up this data logger was to create a Labview (National Instruments) program to acquire and save voltage information in an Excel spreadsheet. Figure 3.11 illustrates the block diagram and front panel interface for the Labview application created for this project.

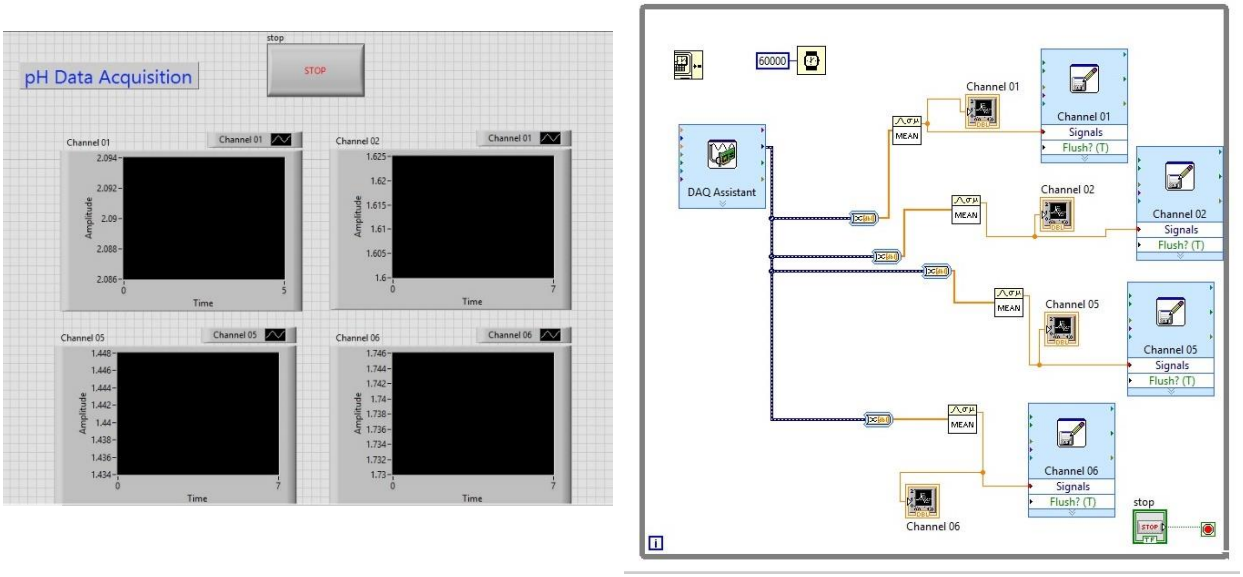


Figure 3.11 - Block Diagram and User Interface – Labview. User interface displays a voltage chart for each channel. In the block diagram, data is acquired through the DAQ Assistant for Voltage measurements. Data is processed and stored in an Excel file for each channel.

The pH controller receives the information in terms of voltage and pH but outputs the readings in terms of current. Thus, a resistor was added in the back of the controller's output channel, to transform current readings into voltage. Among the resistors tested, the 220 Ω resistor was found to acquire pH readings closer to the ones displayed in the pH controller. Next, four pH probes individually coupled to pH controllers (Jenco pH/ORP Controller 3672) were used to elaborate the conversion curves/equations. Both the pH probes and controllers were previously calibrated using standard solutions (Buffer Solution HACH Company pH 4.01 and pH 7.0). The conversion curves were elaborated for each probe + controller system by measuring the voltage response for each of the standard pH solution (Buffer Solution HACH Company pH 4.01, 7.0 and 10.01). A graph of the voltage vs. pH measurements was elaborated for each of the probes and a linear trendline was used to create the conversion equations. Finally, the pH probes were submerged into the reservoirs of the four channels growing algae and the data acquisition process started. Figure 3.12 illustrates a flowchart of the data acquisition process.

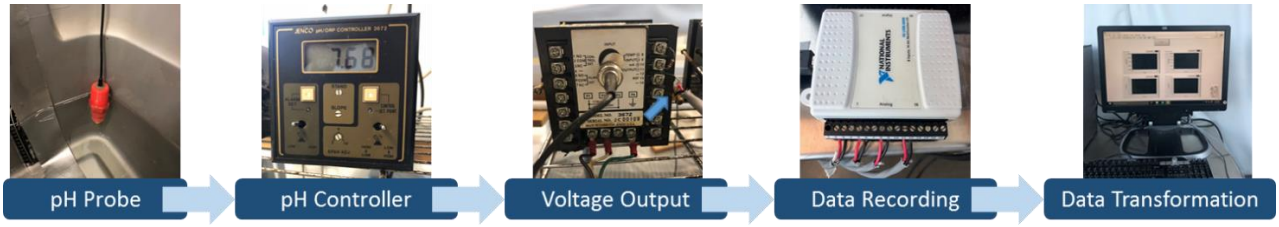


Figure 3.12 - Data Acquisition Process Flowchart. The pH probe send pH information to the pH controller, which outputs it as voltage. The data logger then reads and stores the voltage information in an excel file, which will be transformed to pH measurements through conversion equations

Furthermore, Nitrate and Phosphate levels were daily monitored using an RqFlex plus 10 Reflectrometer (EMD Millipore) with an accuracy of ±1 mg L^{-1}. The tests were conducted following the procedure in the instructions manual provided by the manufacturer. Test strips (EMD Milipore) with a detection range between 3 and 90 mg L^{-1} were used for the nitrate test, as well as 5 and 120 mg L^{-1} for the phosphate test.

Nutrients were daily replenished by replacing 20% of the total volume of each reservoir with fresh media. Briefly, after the first morning measurement, losses due to evaporation were replaced with deionized water, followed by the discard of 5 L (20%) of the media solution in each reservoir. Finally, 5 L of fresh media solution were added back to each reservoir. After this process, rocks and approximately 2 L of media solution were exchanged in between reservoirs, to promote a more homogeneous algal community throughout the channels. In addition, the entire volume was replenished after every harvesting process, to ensure less dependency between harvests.

3.2.5 Water Temperature Control

From the measurements discussed in the previous section, it was observed that water temperature had a high variation in each channel throughout the day, as well as in between channels, as shown in Figure 3.13. Such variation could be explained by a slight variation in heat output from the lightbulbs, as well as inconsistencies in the air temperature in the room. To decrease this variation, 100 W aquarium heaters

(Odyssea Aquarium Heater Submersible) were added to the reservoirs and set to 25 °C (± 1 °C). As shown in Figure 3.14, the variation significantly decreased after the heaters were added to the reservoirs.

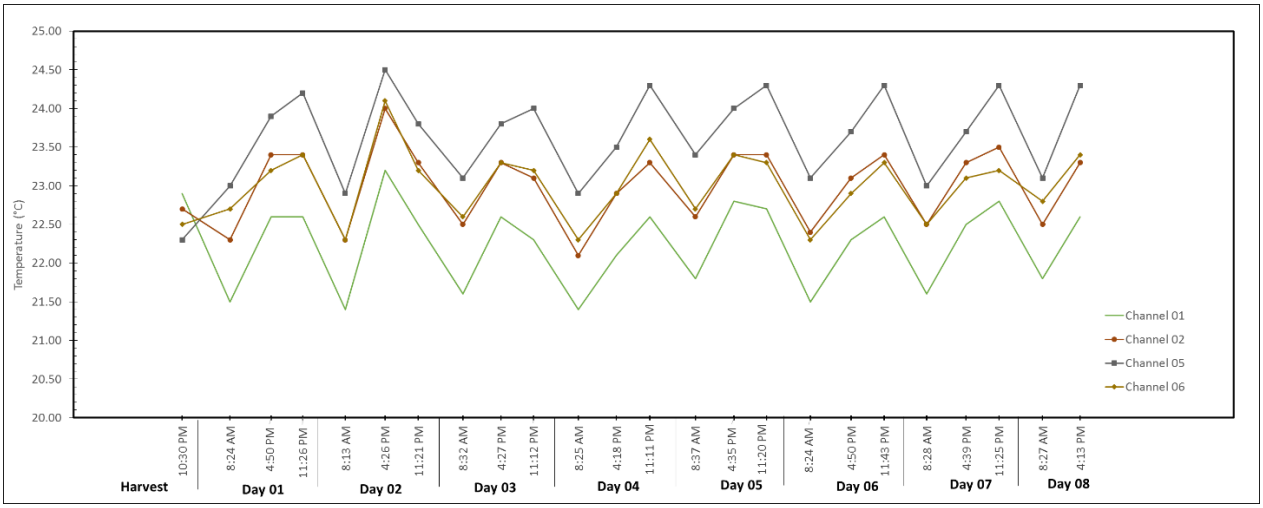


Figure 3.13 - Variation in water temperature in all the channels during Harvest #2 – before adding the heaters. The average in daily variation in between and within channels were equal to 1.2 °C and 1.3 °C, respectively

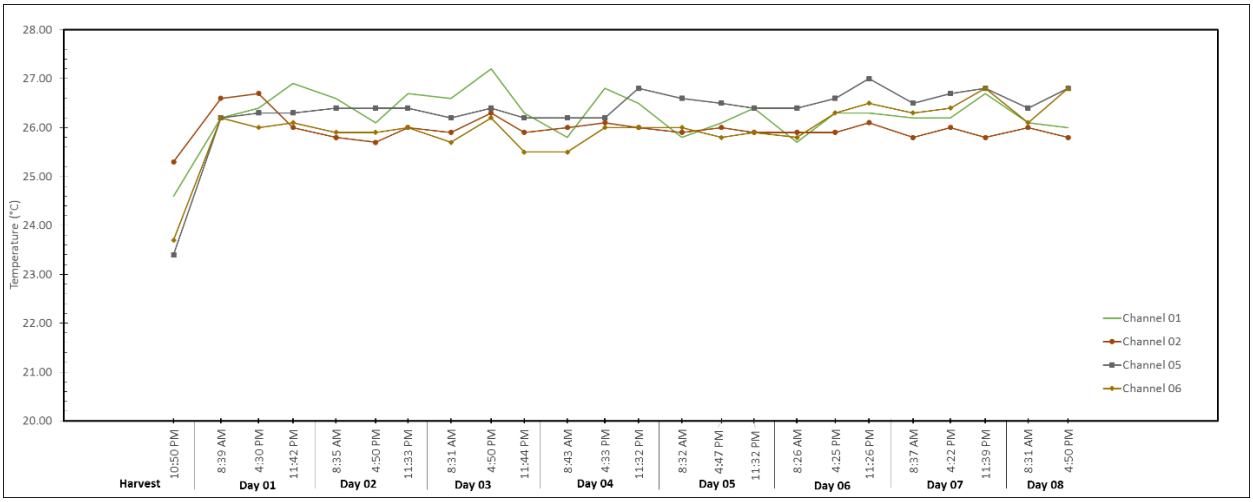


Figure 3.14 - Variation in water temperature in all the channels during Harvest #5 – after adding the aquarium heaters. Daily variation in between and within channels were decreased to 0.4 °C (-67%) and 0.6 °C (-54%), respectively

3.2.6 Species Identification

Prior to harvest and biomass collection, a small amount of the biomass was sampled and stored at 4 °C for species identification analysis. The major filamentous algae were identified and semi-quantified through light microscopy using a biological microscope (Motic B3 Professional Series). The major species

were identified using two guides for freshwater identification (Bellinger and Sigee 2010; Prescott 1954) as well as online keys (Algal Web; Manaaki Whenua Landcare Research). Filamentous green algae from the *genera Stigeoclonium, Spirogyra, Klebsormidium, Mougeotia, Oedogonium, Unidentified A, Ulotrix, and Microspora*, were observed to be the most abundant green algae present in the channels. The presence of two species of filamentous blue green algae from the genus *Oscillatoria* were also observed in the mixed community. In addition, planktonic algae and diatoms were also observed in the samples in a smaller amount, but were not identified nor quantified. Figure 3.15 presents microscopic images for the main species observed in the community, as well as their respective classification.

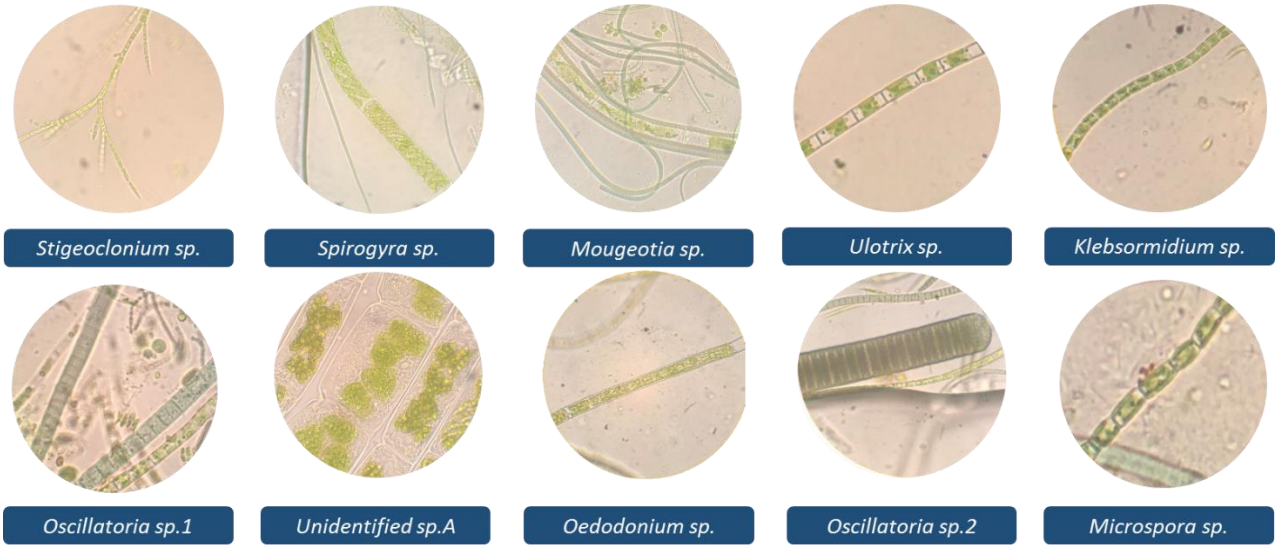


Figure 3.15 - Microscopic Image and Classification for the main filamentous green and blue green algae

A semi-quantitative analysis was used to classify the species based on their abundance into four classes:

- Dominant: correspondent to the most abundant specie observed in the samples during light microscopy analysis;
- Present: related to the species that are observed to have a high occurrence;
- Rare: correspondent to the species that are observed to have a low occurrence;
- Absent: corresponding to the species not observed in the analysis.

3.3 Preliminary Experiments

3.3.1 Growth Curves

Growth curves were prepared for each channel to compare productivity rates and evaluate if the communities were under exponential growth at the time of the harvest. This experiment consisted of collecting algal material on a known area over time and compute changes in mass. Each channel was divided in three areas, since small changes in growth were observed along the length of the reactors. Each section was divided in smaller areas and numbered, as shown in Figure 3.16. Prior to biomass collection, Excel was used to generate a random number between 1 and 39 for each section in each channel. The area corresponding to that number was assigned as to be the one to be harvested. Thus, each channel was sampled three times for each respective sampling date, once in each region. In addition, biomass located in the gap in between regions was also collected.

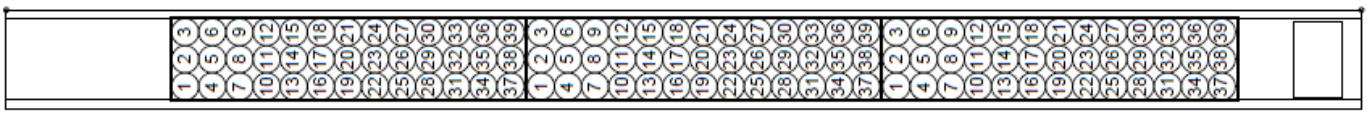


Figure 3.16 - Channel division scheme. The gap region adjacent to the number to be sampled was also randomly sampled

Algal collection was performed following the methods presented in Hauer & Lamberti (2007). A 0.5 in PVC tube coupled with a rubber sealant at its base was used as a chamber for collection. For each number generated, the chamber was placed in the area to be collected and a brush (3/8 in. and 3/4 in. Stencil Brush) was used to remove the attached algae from the substratum. The chamber was then eluted with deionized water and a syringe was used to remove the scrapped biomass. The biomass slurry was stored in a 60 mL HDPE storage bottle (Square Storage Bottles, Wide Mouth, HDPE Material, Screw Cap, Karter Scientific) at 4 °C in the dark.

The samples were then filtered using a porcelain Buchner funnel coupled to an Erlenmeyer cylindrical flask and a vacuum pump. The filters (1.1 µm VWR Glass Microfiber Filter, 693, 4.25cm) used in the filtration process were previously weighted on aluminum weighing boats, and oven-dried at 105 °C

for 24h. In some cases more than one filter had to be used for the same sample, depending on the amount of material to be processed. The same process was repeated five times for each channel at days 1, 3, 5, 7, and 8 after the previous harvest. Figure 3.17 presents the filtered samples for each of the sampling day.

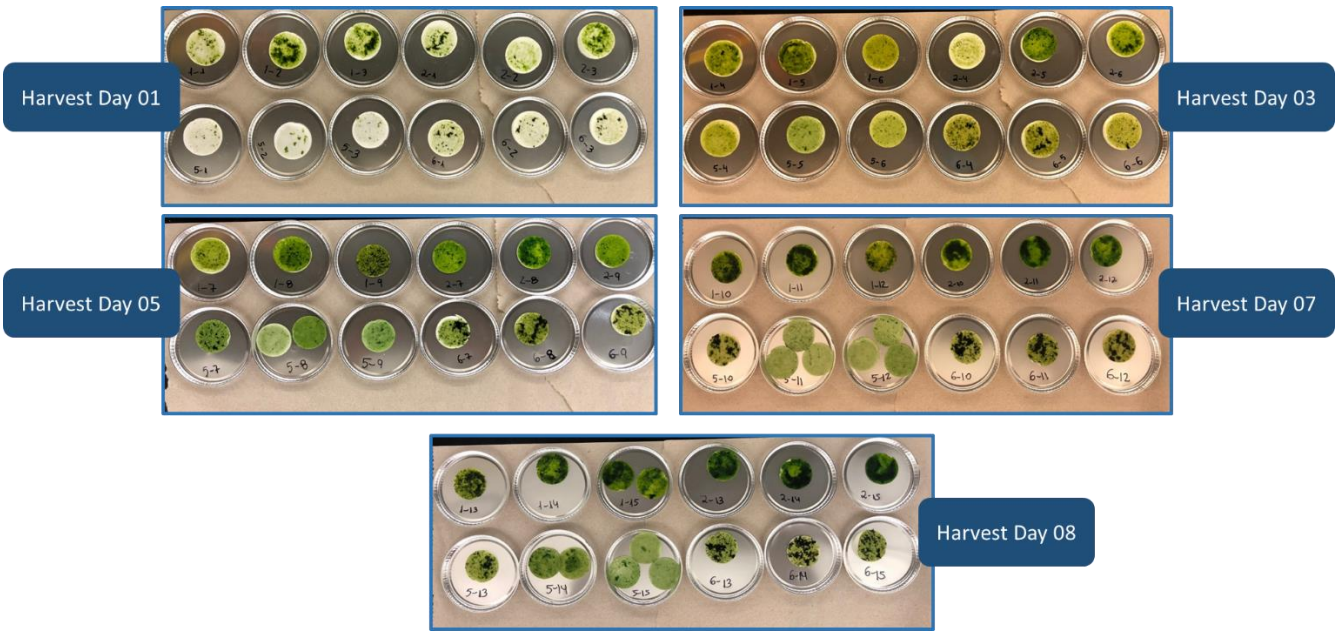


Figure 3.17 - Growth Curves - Filtrated Samples

Finally, the filtered samples were oven-dried at 105 °C for 24h. The final biomass was found by decreasing the dry weight of the filter and aluminum boat from the oven-dried sample.

3.3.2 Growth Parameters Analysis

A preliminary study was conducted to identify the optimum parameters to culture the mixed periphytic algal community by analyzing the effects of UV exposure, light intensity, and N/P ratio on biomass production and species dominance.

3.3.2.1 UV Exposure and Light Intensity

Light configuration and light intensity were modified throughout the fourteen harvests to evaluate which configuration would yield the desired community and high biomass production rates. Figure 3.18 describes the variation in light configuration among the channels during the 14 harvests period.

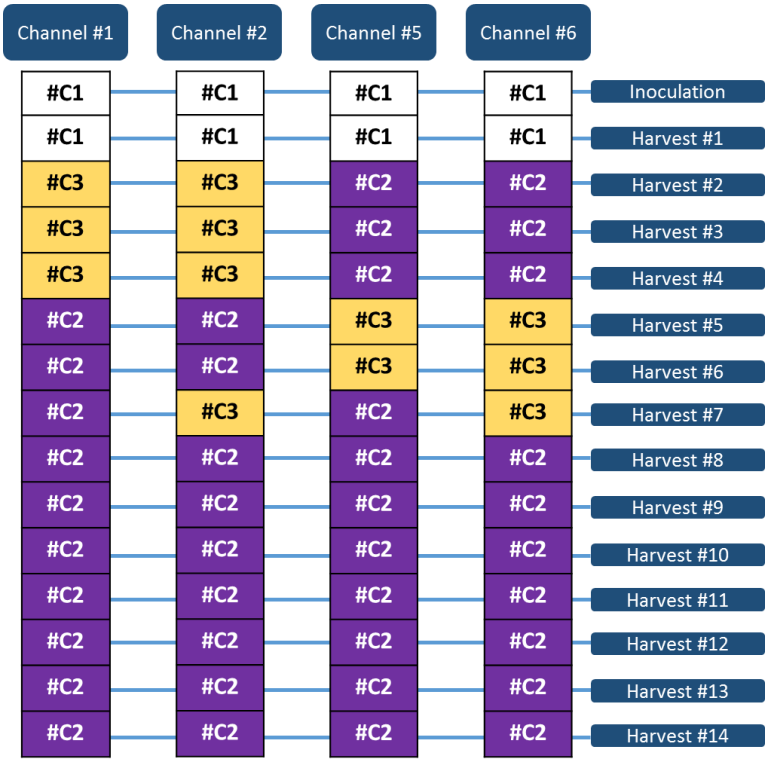


Figure 3.18 - Light configuration throughout the experiment. In the figure, #C1 stands for two Cool White Phillips Alto II fluorescent, #C2 for one Cool White Phillips Alto II and one Sunsource Helio-Vite UVA-UVB, and #C3 for one Cool White Phillips Alto II and one Sunsource Helio-Vite UVA-UVB with UV filter

As it can be seen, during inoculation and first harvest, all of the channels were exposed to the same light configuration (two 48 inch Phillips Alto II fluorescent lightbulbs, configuration #1). After this period, channels 5 and 6 were illuminated with a combination of one 48 inch Phillips Alto II fluorescent and one Sunsource Helio-Vite UVA-UVB fluorescent lightbulb (configuration #2). At the same time, channels 1 and 2 were illuminated with a similar configuration, with the only change being the placement of an UV Filter Tube around the Heliovite lightbulb (configuration #3). The initial plan was to maintain this configuration throughout the duration of the experiment. However, a shift in the algal community towards

an *Oscillatoria spp.* dominance on the channels exposed to UVA and UVB rays (5 and 6) was observed after the initial 4 harvests. With the intention to verify if this shift was caused by exposure to UVA and UVB rays, light configuration was switched among channels during Harvests #5 and #7, as shown in Figure 3.18. Since the efforts to shift the community back to a green algae dominance were not successful, all four channels were exposed to UVA and UVB rays for the remaining harvests (configuration #3).

Furthermore, light intensity was altered throughout the harvesting process to assess its impact on biomass production and species dominance. Light fixtures were raised 5 cm, being placed 25 cm from the bottom of the channels, during harvests 8 and 11.

3.3.2.2 Nitrate Concentration

During the early harvests, it was observed that the levels of Nitrogen (measured as Nitrate) were drastically dropping to below measurement levels as biomass production increased (Figure 3.19). Since Nitrogen is one of the most important macronutrients needed for algal growth, nitrogen depletion could be an important limiting factor on biomass production. To overcome this issue, Nitrate levels in the media was increased three folds. This alternative recipe, usually referred as 3N BBM, has been widely used to enhance algal growth (Griffiths et al. 2011; Griffiths, van Hille, and Harrison 2012). Figure 3.20 illustrates a typical Nitrate profile after this change was made. As it can be seen, even though nitrate levels are steadily dropping throughout the eight day period, there is still a considerable amount left in the media.

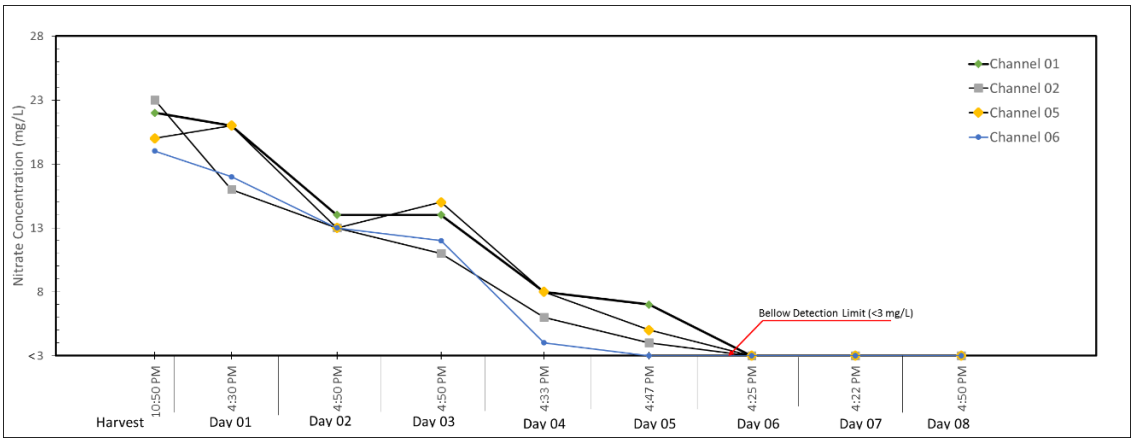


Figure 3.19 - Harvest #5 Nitrate Concentration profile. Nitrate levels drop to below the detection limit of 3 mg L^{-1} on Day 05 for Channel 06, and Day 06 for the other three channels

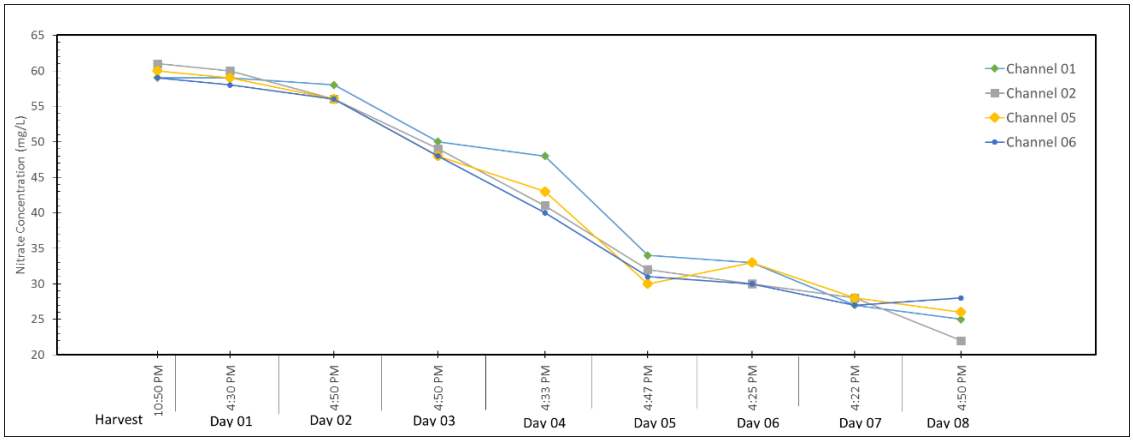


Figure 3.20 - Harvest #6 Nitrate Concentration profile

3.4 Exposure Study

The main exposure study was designed to assess the suitability of the Algal Turf Scrubber (ATS) described in this project to be used as a novel remedial alternative for PFAS. To do so, the periphytic algal community was exposed to a mixture of four PFAS: PFOS, PFOA, PDHA, and HFPO-DA at 2 µg L^{-1} for a period of 72 hours. Such concentration was chosen based on environmental levels in contaminated areas (Hoffman et al. 2010; X. C. Hu et al. 2016; Ahrens et al. 2014), instrument capability, and to be below known toxic levels to algae (Latała, Nedzi, and Stepnowski 2009; W. Liu et al. 2018).

3.4.1 Experimental Design

From the eight channels built for this project, four (Channels 1, 2, 5, 6) were used as experimental units, where the sorption of PFAS by algae was observed (Figure 3.21). Two of the remaining channels (Channels 3 and 7) were used as positive controls, to identify possible losses to the system or abiotic transformation. Channel 4 and 8 were used as negative controls, where possible release of PFAS from the materials used to build the system was observed.

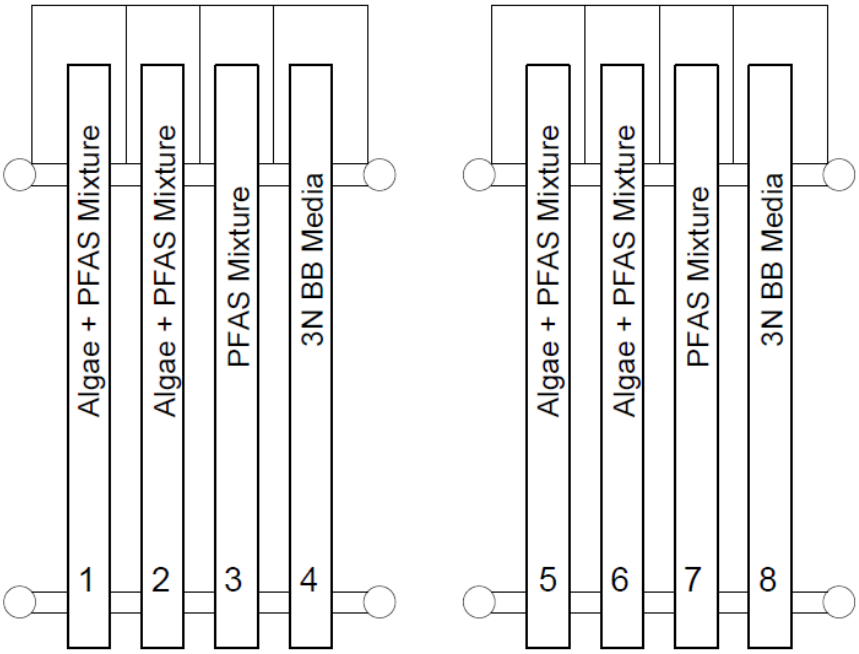


Figure 3.21 - Experimental Design

Growth parameters during the exposure experiment were similar to the ones adopted during culturing. Each channel was illuminated with a combination of one Phillips Alto II fluorescent lightbulb and one Sunsource Helio-Vite UVA-UVB fluorescent lightbulb under a 16:8 h light:dark cycle. The average flow rate was equal to 0.1398 ± 0.0012 L s^{-1}. Data loggers for pH and temperature were added to experimental channels (1, 2, 3, 4), as well as aquarium heaters adjusted to 25 °C.

Prior to exposure, the system was turned off and the reservoirs from all the channels were emptied and filled with 15 L of a 10% 3N Bolds Basal Media (13.5 L of DI Water and 1.5 L of Growth media), to ensure that the algal community would have enough nutrients during the experiment. Temperature, pH, conductivity, as well as NO_3 and PO_4 levels were recorded for each channel following the methods presented in section 3.2.4. A 50 mL sample was taken from all reservoirs prior to contamination, to analyze possible background concentration of the targeted substances. In addition, biomass samples were taken from Channels 1, 2, 5, and 6 for species identification and pigments analysis. Species identification analysis was conducted through light microscopy, as discussed in section 3.2.6. Pigments Analysis will be further discussed in section 3.7.

The contaminant mixture was spiked directly into the reservoirs of channels 1-3 and 5-7 and thoroughly mixed. To achieve the desired concentration of 2 $\mu g\ L^{-1}$, 1 mL from a stock solution of a 1:1:1 PFOS:PFOA:PDHA mixture and 0.6 mL from a HPFO-DA stock solution were diluted in each of the mentioned reservoirs. Six 50 mL water samples were collected from each reservoir immediately after they were spiked with the contaminant's mixture, which was assumed to be equal to the contaminant's initial concentration. The same amount (50 mL) of uncontaminated media solution was added back to the reactors. Each reservoir was spiked within a 5-minute interval from each other.

Water samples were collected using 10 mL Eppendorf pipettes with disposable pipette tips. Since a frequent sampling took place during the first hour, each pump was turned on within a 2-minute interval from each other. A 50 mL water sample was taken immediately from experimental units after the flow was turned back on, to assess the instantaneous uptake of PFAS by the algae. After that, 50 mL from experimental and positive control channels were sampled every ten minutes during the first hour, every two hours during the remaining first 24 hours, and every 4 hours for the remaining 48 hours. Additionally, 50 mL samples from negative controls were collected every 24 hours. The same volume sampled was added back to the system with uncontaminated media solution, to maintain the volume in the channels unchanged. Table 3.4 presents the sampling time schedule.

Table 3.4 - Water Sampling Time Schedule during main experiment. Sample 0 refers to the sample taken after the reservoirs were spiked and 0+ taken immediately after the channels were turned back on.

	Channel #							
Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
0	8:33	8:38	8:43		8:48	8:53	8:58	
0+	9:00	9:02	9:04	9:06	9:08	9:10	9:12	9:14
10 min	9:10	9:12	9:14		9:18	9:20	9:22	
20 min	9:20	9:22	9:24		9:28	9:30	9:32	
30 min	9:30	9:32	9:34		9:38	9:40	9:42	
40 min	9:40	9:42	9:44		9:48	9:50	9:52	
50 min	9:50	9:52	9:54		9:58	10:00	10:02	
60 min	10:00	10:02	10:04		10:08	10:10	10:12	
2h	11:00	11:02	11:04		11:08	11:10	11:12	
4h	13:00	13:02	13:04		13:08	13:10	13:12	
6h	15:00	15:02	15:04		15:08	15:10	15:12	
8h	17:00	17:02	17:04		17:08	17:10	17:12	
10h	19:00	19:02	19:04		19:08	19:10	19:12	
12h	21:00	21:02	21:04		21:08	21:10	21:12	
14h	23:00	23:02	23:04		23:08	23:10	23:12	
16h	1:00	1:02	1:04		1:08	1:10	1:12	
18h	3:00	3:02	3:04		3:08	3:10	3:12	
20h	5:00	5:02	5:04		5:08	5:10	5:12	
22h	7:00	7:02	7:04		7:08	7:10	7:12	
24h	9:00	9:02	9:04	9:06	9:08	9:10	9:12	9:14
28h	13:00	13:02	13:04		13:08	13:10	13:12	
32h	17:00	17:02	17:04		17:08	17:10	17:12	
36h	21:00	21:02	21:04		21:08	21:10	21:12	
40h	1:00	1:02	1:04		1:08	1:10	1:12	
44h	5:00	5:02	5:04		5:08	5:10	5:12	
48h	9:00	9:02	9:04	9:06	9:08	9:10	9:12	9:14
52h	13:00	13:02	13:04		13:08	13:10	13:12	
56h	17:00	17:02	17:04		17:08	17:10	17:12	
60h	21:00	21:02	21:04		21:08	21:10	21:12	
64h	1:00	1:02	1:04		1:08	1:10	1:12	
68h	5:00	5:02	5:04		5:08	5:10	5:12	
72h	9:00	9:02	9:04	9:06	9:08	9:10	9:12	9:14

Water samples were stored in 60 mL HDPE storage bottles (Square Storage Bottles, Wide Mouth, HDPE Material, Screw Cap, Karter Scientific) at -20°C in the dark until analysis. At each of the sampling times shown in Table 3.4, temperature and pH were automatically recorded for each of the experimental

units. Temperature and pH were also manually measured in the negative control channels using a Hannah HI 98130 meter. Positive controls did not have their temperature and pH measured to avoid cross contamination. Since evaporation is an important factor, all channels had the volume of their tanks recorded at all sampling times using a rubber ruler previously installed in each reservoir. In addition, to make the controls as similar as possible to experimental units, pH from controls were adjusted using the required amount of a 0.1 M NaOH acid solution to match the pH in the experimental channels.

After the 72-hour exposure, the pumps were turned off, marking the end of the experiment. A small portion of the biomass was sampled and stored at -20 °C in the dark for pigments analysis. The remaining biomass was harvested following the methods presented in section 3.2.3, and transferred to 500 mL HDPE bottles and stored at -20 °C in the dark until analysis.

3.4.2 Safety Considerations

Due to the high concentration and volume of contaminants used in this project, several measures were taken place to ensure the safety of researchers and environment.

General laboratory protection measures were taken to ensure common safety and avoid any possible contamination. The grate under the channels was covered with a plastic sheeting (HDX 10 ft. x 25 ft. clear 3.5 mil plastic sheeting) for the containment of possible spills. In addition, absorbent pads (New Pig Absorbent Mat Pad 12 oz Absorbency, 13" L x 10" W, MAT251) were placed on top of the plastic sheeting for immediate absorption of eventual spills. Finally, absorbent socks (Evolution Sorbent 12GS34SB Poly-Cellulose Universal Super Absorbent Flake Sock, 21 gal Absorbency, 3" Diameter x 48" Length) were placed around the drains under the grate, as well as on the corners of the experimental area. In addition, danger signs and a safety protocol were elaborated and placed around the laboratory. A safety binder containing MSDS (Material Safety Data Sheets) for the contaminants as well as additional safety considerations was also manufactured and placed near a Spill Kit. Details of the safety measures can be seen in Figure 3.22.

(a) *(b)*

Figure 3.22 - (a) illustrates the mentioned common protection measures; (b) illustrates signs and safety protocol placed on the experimental area entrance.

The use of personal protective equipment was also enforced. Laboratory personnel were required to wear personal protection equipment, including closed-toed shoes and long pants, face shields, goggles, mask, as well as chemical resistant gloves and a lab coat. After the 72-hour exposure period, the solution remaining in the reservoirs was transferred to waste containers as per guidelines of the Auburn University Risk Management and Safety Department for proper disposal. A 5% methanol solution was cycled through the system for 20 minutes to remove any traces of contaminants in the system.

3.5 Complementary Studies

Three additional experiments were conducted to complement the main exposure study. As it will be further discussed in the results section, a large quantity of the contaminant's initial mass remained unaccounted for after a mass balance equation was performed. Such trend was observed especially for PFOS and PFOA, and less expressively for HFPO-DA and PDHA. Thus, these additional experiments were designed in efforts to identify the mechanism(s) responsible for such loss in mass, focusing on PFOS and PFOA.

3.5.1 Media and UV Effects on Abiotic Transformation of PFOS and PFOA

At first, it was hypothesized that these substances were being transformed by UV light or binding to the salts from the BBM. To assess that, an experiment was performed to identify the individual effects of UV light, pH adjustment (NaOH), and growth media on the transformation or elimination of PFOS and PFOA. To do so, HDPE bottles were used as experimental units, to avoid sorption of the contaminants into the bottle's walls. Bottles 1 to 3 were exposed to UV light and 4 to 6 were kept in the dark. UVA and UVB rays were introduced to the mentioned units from the top through an opening in the bottles. Experimental design is shown in Figure 3.23. As it can be seen, bottles 3 and 6 were used to analyze the direct impact of UV light on the transformation of these compounds, whereas bottle 2 was used to analyze the combined impact of media and UV light. Experimental unit 5 was used to assess the growth media contribution on PFAS transformation. Units 1 and 4 were used to assess the contribution of NaOH (used for pH adjustment during the main study) on PFOS and PFOA transformation under UV and no UV exposure, respectively.

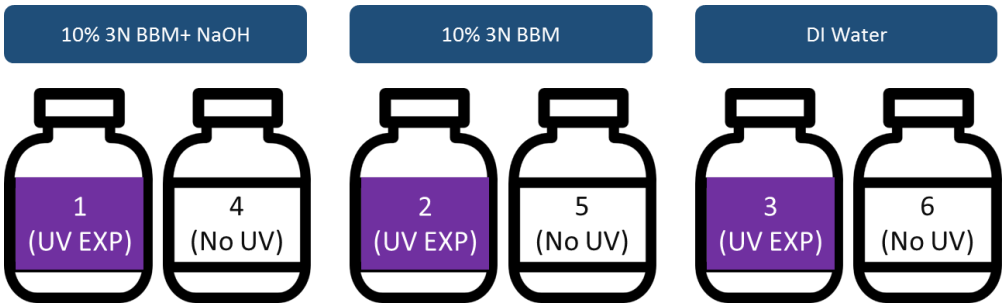


Figure 3.23 - Experimental Design for Abiotic Transformation Study

Each 1L bottle was filled with 200 mL of 10% 3N BB media (units 1, 2, 4, 5) or DI water (units 3 and 6), and spiked with a small volume of PFOS and PFOA to reach the desired concentration of 2 μg L^{-1}. A 25 mL aliquot was sampled from each bottle at time zero to assess the initial concentration. The same volume was replaced with growth media for units 1, 2, 4 and 5, and with DI Water for units 3 and 6. Bottles 1 and 4 were spiked with 210 μL of a 0.1 M NaOH solution at time 8h, mimicking what was done in the main study. All units were sampled again at time 24 h, marking the end of the experiment. No volumetric losses to evaporation were observed during the duration of the experiment.

3.5.2 *Volatilization of PFOS and PFOA*

It was also hypothesized that the volatilization of PFOS and PFOA could have played a major role in the loss in mass observed in the main study. Furthermore, an experiment was conducted to evaluate possible losses in the mass of PFOS and PFOA to volatilization. To do that, eight 1.9 L HDPE containers were filled with 500 mL of either 10% 3N BBM or DI water solutions (Table 3.5), previously prepared and spiked with PFOS and PFOA at 2 μgL^{-1}. Similarly to the sorption study, two 45 mL samples were taken from each stock solution (10% 3N BBM and DI Water) using 20 mL disposable pipettes to confirm the initial concentration of the contaminants in the mixture.

Table 3.5 - Description of experimental units used in the evaporation study

#	Description
1	10% 3N BBM
2	10% 3N BBM
3	DI Water
4	DI Water
5	10% 3N BBM (covered)
6	10% 3N BBM (covered)
7	DI Water (covered)
8	DI Water (covered)

The potential volatilization and sorption of PFOS and PFOA into the walls of the containers were assessed in containers 1 to 4. Containers 3 and 4 were used as controls to 1 and 2, to evaluate if the salts from the Bolds Basal Media were affecting sorption or evaporation of these compounds. In addition, the sorption into the walls of the containers was directly observed in containers 5 to 8, which were covered to avoid evaporation. Containers 1-4 were placed on an orbital shaker (VWR Advanced Orbital Shaker, Model 5000) to maintain consistency between experiments. In contrast, Containers 5 to 8 were not placed on the shaker to avoid losses to evaporation. The shaker was turned off at the 72 hours mark. A 45 mL sampled was collected from each container and placed in the refrigerator at 4 °C. The final volume in each container was measured using a graduated cylinder to assess evaporation losses.

The remaining solutions from both experiments were transferred to waste containers that were sent to the Auburn University Risk Management and Safety Department for proper disposal. Water samples were analyzed following the methods presented in Section 3.6.

3.5.3 Sorption of PFOS and PFOA onto ATS Materials Study

Finally, it was also theorized that the materials used to build the ATS system could also have played a major role in the loss of mass by sequestering PFOS and PFOA, probably through sorption. This study consisted of individually exposing the ATS materials to a mixture of PFOS and PFOA for 72 hours. A list of the materials used is shown in Table 3.6.

Table 3.6 - List of materials used in the Sorption study

#	Material
1	PVC Valve
2	Rubber Ruler
3	Duct Tape
4	Rubber Sealant
5	PVC Pipe + Connections
6	Nylon Mesh + Silicone
7	Vinyl (Channels)
8	Mixed Material Pump (mainly ABS plastic)
9	Plastic Reservoir
10	Blank

Six liters of a 10% 3N Bolds Basal Solution were prepared by mixing 5400 mL of deionized water and 600 mL of media concentrate. The media solution was spiked with the appropriate amount of PFOS and PFOA to reach the desired concentration of 2 μg L^{-1}. A 45 mL sample was taken using a 20 mL disposable pipette to determine the initial concentration of the contaminants.

The mentioned materials were individually transferred to nine labeled 1.9 L HDPE Containers (VWR® HDPE Multipurpose Containers 16.5dia. x 11.4H cm), which were then placed on an orbital shaker (VWR Advanced Orbital Shaker, Model 5000). A volume of 500 mL from the contaminated media solution was transferred to each container and the orbital shaker was turned on, marking the beginning of the experiment. The containers were placed on the orbital shaker to mimic the water motion from the exposure study. Additionally, a blank container was placed on the shaker with the intention to assess possible cross contamination between samples. Experimental set up is shown in Figure 3.24.

Figure 3.24 - Sorption Study Experimental Setup

The shaker was turned off at the 72 hours mark. A 45 mL sampled was collected from each container and placed in the refrigerator at 4 °C. The final volume in each container was measured using a graduated cylinder to assess evaporation losses.

3.6 Quantitative Analysis

3.6.1 Chemicals and reagents

LC grade solvents (methanol, acetonitrile, and water) were purchased from VWR International, Suwanee, GA. Analytical grade ammonium hydroxide was purchased from Sigma-Aldrich, St. Louis, MO. LC grade ammonium formate and ammonium acetate reagents were procured from Agilent Technologies (Wilmington, DE). Captiva Glass fiber nylon syringe filters (0.2µm), UHPLC analytical column (Agilent ZORBAX RRHD Eclipse Plus C18, 2.1×100mm, 1.8µm, Part No. 959758-902) and guard column (Agilent ZORBAX Eclipse Plus C18, 2.1, 1.8µm, Part No. 821725-901) were also purchased from Agilent Technologies (Wilmington, DE). Oasis WAX (6cc, 150 mg) extraction cartridges, and 20-Position vacuum

manifold were supplied by Waters Corporation (Milford, MA, USA). Analytical grade standards (purity > 98%, LC/MS analysis) used in the current study were obtained from Wellington Laboratories, Ontario, Canada.

3.6.2 Water samples preparation

Prior to cleanup, experimental samples were brought to room temperature and subjected to solid phase extraction (SPE) method using Oasis WAX cartridges. Cartridges were placed on a 20-position vacuum manifold unit and pre-conditioned with 0.1% ammonium hydroxide in methanol (4 mL), methanol (4 mL), and LC grade water (4 mL) at a flow rate of 1 drop/sec. Subsequently, the 50 mL samples were loaded into these pre-conditioned cartridges and eluted at the same flow rate. The cartridges were then washed with a 25 mM ammonium acetate buffer (pH 4.0) in LC grade water (4 mL) and dried under vacuum. Finally, the target analytes were recovered from the SPE cartridges by elution with methanol (3 mL) followed by 0.1% ammonium hydroxide in methanol (3 mL). Both methanol and 0.1% ammonium hydroxide in methanol fractions were combined and filtered through 0.2µm Agilent glass fiber nylon syringe filters.

3.6.3 *Biomass samples preparation*

After harvesting, biomass samples were washed thoroughly for three times with deionized water and decanted. The wash water was assumed to be equal to the amount of contaminants adsorbed onto the cells, and samples were processed through SPE as discussed in the previous section. The solid portion of the biomass was freeze-dried at -80 °C for 24 hours prior to analysis. After that, the freeze-dried samples were dissolved with 50 mL of a 5% methanol solution in water and sonicated at 60 Hz for 2 hours to allow complete extraction. Extracted samples were centrifuged at 6000 rpm at 4 °C for 15 minutes and the supernatant was collected and concentrated using a rotary evaporator. Samples were then processed through SPE as discussed in the previous section.

3.6.4 UHPLC-MS/MS Analysis

Quantitative analysis of target PFAS water and biomass samples was performed by an Agilent UHPLC-MS/MS instrument, composed of a 1290 Infinity II high-speed pump (model G7120) coupled to a triple quadrupole mass spectrometry (MODEL g6460C) and Jet-Stream Electrospray Ionization source. Targeted analytes were quantified using a modification from the methods presented at Mulabagal et al. (2018). The chromatography of PFAS was altered by changing the composition in the mobile phase of solvents over time to achieve a stable column backpressure. Samples were analyzed in multiple reaction monitoring (MRM) mode, with parameters from Mulabagal et al. (2018). PFAS chromatographic separation was achieved with 5 mM ammonium formate in water/acetonitrile, 95:5, v/v (solvent A) and acetonitrile/ water, 95:5, v/v (solvent B) as mobile phase solvents. Mobile phase gradient method conditions for PFAS separation were programmed as follow: 0 to 0.2 min (5%B); 0.5 min (50% B); 2 min (60% B); 4 min (80% B); 5 min (90% B); 5.5 min (95% B); 6.5 min (99% B); 7 min (20% B), resulting a total run time analysis of 7 min. Samples were analyzed three times to assess instrument accuracy. A sample injection volume of 5 µL was used, and each sample was spiked with internal standard (MPFOS) at 1 ng/mL concentration prior to analysis. Quantitative experiments were setup by preparing calibration solutions for target analytes to achieve concentrations in the range of 0.1 and 0.01µg/mL.

3.7 Photosynthetic Pigments Analysis

Pigments analysis was conducted in pre- and post-exposure samples to assess changes in algal community, observe if the community was under stress, and identify overall structure. The collected biomass was freeze-dried at -80 °C for 24 hours prior to analysis. Each sample was weighed and placed in two microcentrifuge tubes. Pigments were extracted using 1.2 mL of a previously sonicated extraction solution containing acetone, methanol, and water in an 80:15:5 ratio. Microcentrifuge tubes were then vortexed for ten seconds to ensure complete mixing and placed in a -20 °C freezer for 16 hours. After

extraction, samples were vortexed again and centrifuged for 10 minutes. Finally, the supernatants were filtered through a 0.22 μm syringe filter to remove any remaining particles prior to HPLC analysis.

After filtration, samples were placed in an autosampler tray, where 100 μL aliquots at a 3:1 (sample:ion-pairing agent) ratio were analyzed for pigments using a Shimadzu HPLC system. A solution of 0.75 g tetrabutyl ammonium acetate and 7.7 g ammonium acetate in 100 mL HPLC-grade water was used as the ion-pairing agent. Mobile phase and time sequence were used according to the methods presented in Leavitt & Hodgson (2001). Chlorophylls and carotenoids pigments were separated by using a Rainin Model 200 Microsorb C18 column and measured using a photodiode array detector. Pigments were identified based on retention time and peak shapes, and integrated by comparing peak areas with standards of known concentration. Pigment concentrations are expressed as nmol OM^{-1} to allow comparisons between chlorophylls and carotenoids.

4. RESULTS

4.1 Algal Culture

4.1.1 Biomass Production

After inoculation, the attached biomass was harvested fourteen times, once every eight days. Biomass production was quantified through the Ash Free Dry Weight method, and results are shown in Figure 4.1. Throughout the harvesting process, several variables were adjusted to find optimum parameters for algal culture and a more detailed analysis is presented in section 4.2.2. Additional biomass data is presented in Appendix A.

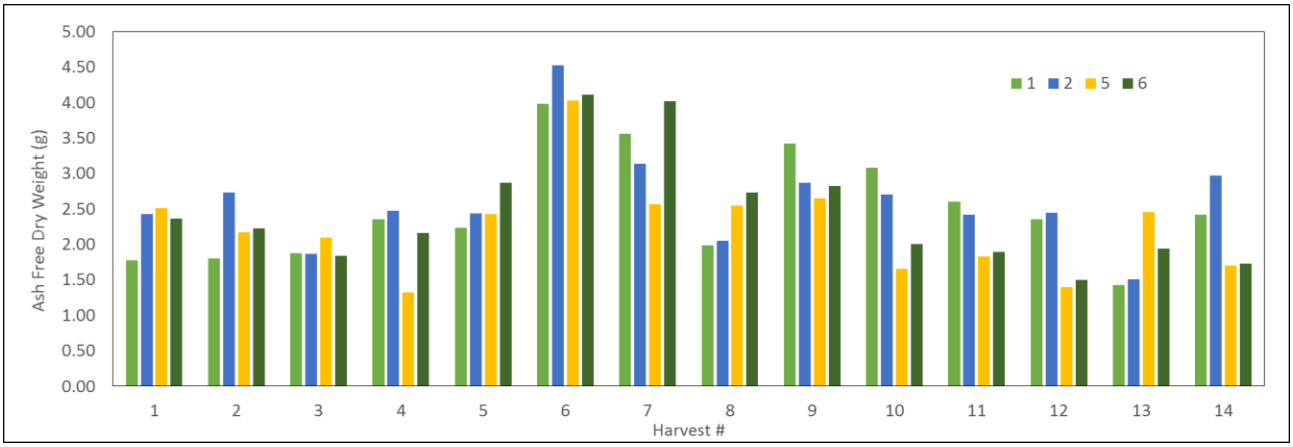


Figure 4.1 - Ash Free Dry Weight Biomass for Channels 1, 2, 5, and 6 during fourteen 8-day harvesting period

Figure 4.2 displays side-by-side boxplots for the distribution of biomass production (AFDW) per channel over fourteen harvests. Even though the distributions are not symmetric, there is enough evidence to suggest that the overall mean growth was the same among the four channels (SAS 9.4, PROC GLM,

ANOVA, p-value 0.4794, α 0.05). A log transformation (natural log) was used to correct skewness prior to ANOVA analysis.

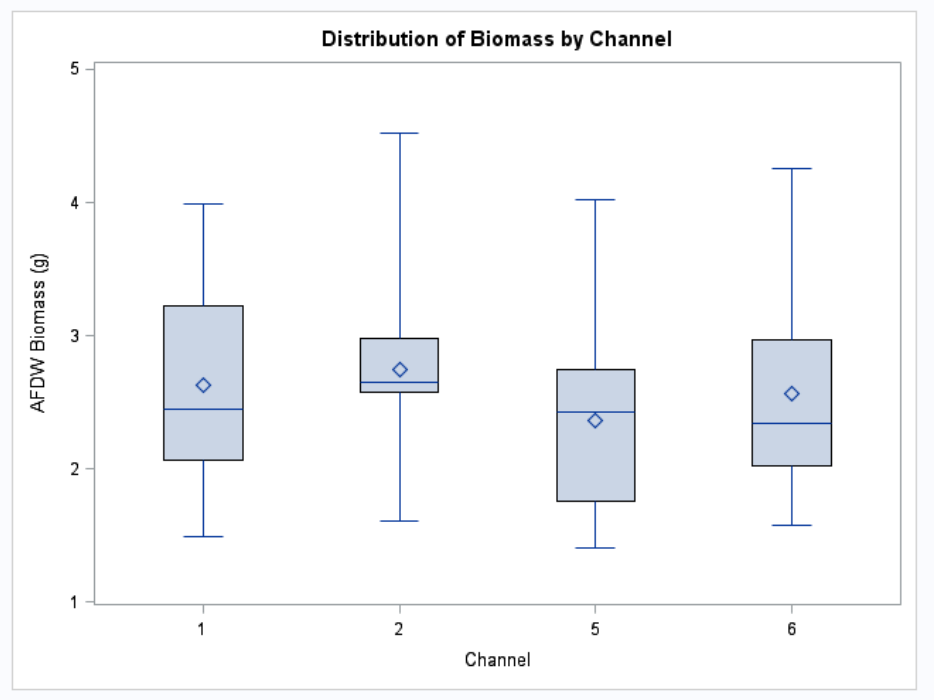


Figure 4.2 - Distribution of AFDW by channel over the fourteen 8-day harvesting period

4.1.2 Semi-Quantitative Species Dominance Analysis

Figure 4.3 and Figure 4.4 display a semi-quantitative classification for the ten most abundant species for the fourteen harvests. Species from the Chlorophyta group dominated channels 1 and 2 for the first five harvests, whereas *Oscillatoria sp.1* (blue green) was the dominant species in channel 5 for the same period. Channel 6 was dominated by species from the Green phylum during Harvests 2 and 3, followed by an *Oscillatoria* sp.1 dominance on the remaining harvests. After that, *Oscillatoria sp.1* dominated the mixed community in all the channels, with the exception of channel 1 in the last harvest.

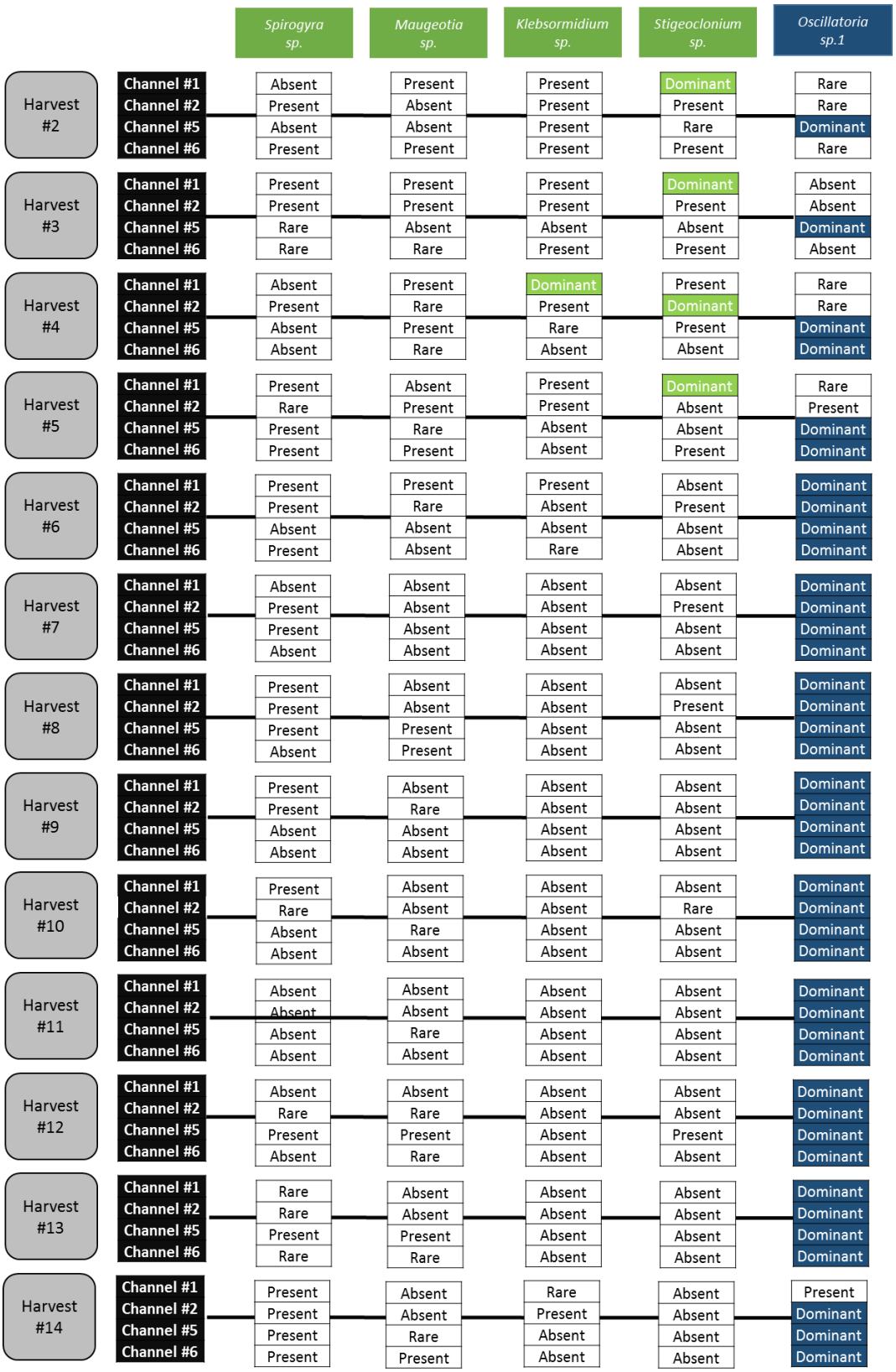


Figure 4.3 - Semi-quantitative Classification for species from the genera Spirogyra, Mougeotia, Klebsormidium, Stigeoclonium, and Oscillatoria

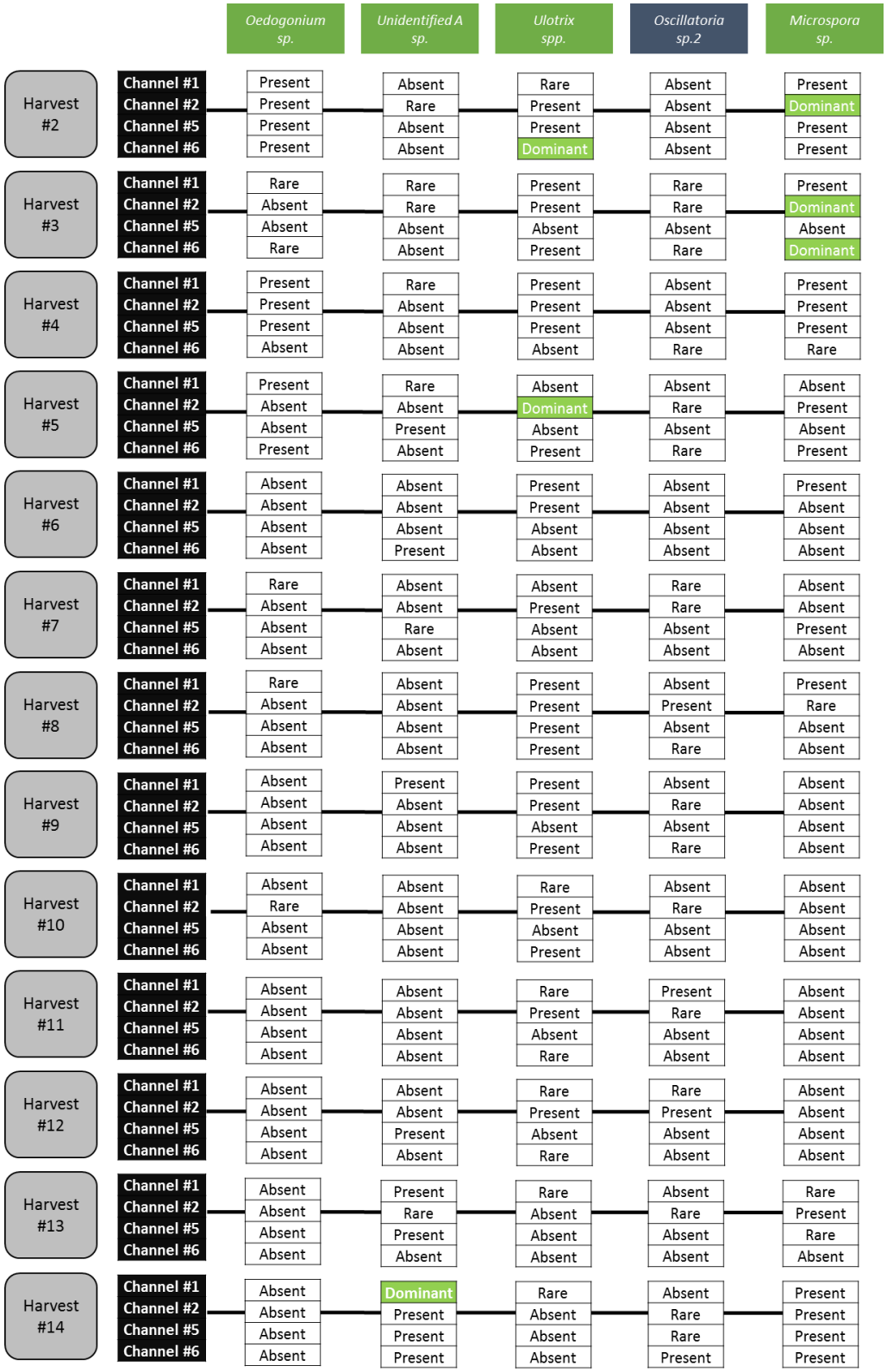


Figure 4.4 - Semi-quantitative Classification for species from the genera Oedogonium, Unidentified A, Ulotrix, Oscillatoria, and Microspora

4.2 Growth Parameters

4.2.1 Growth Curves

A plot of biomass *versus* time was elaborated in Excel and is presented in Figure 4.5. The mixed communities from Channels 1, 2, and 5 were entering the exponential growth phase at day 8. Channel 6, however, experienced a decrease in growth between days 7 and 8. Theoretically, at some point in the logistic growth curve, biomass productivity will reach a carrying capacity and growth will plateau or decline. This could explain in part the community behavior in Channel 6. This decrease could also be related to uniformities in growth along the channels in a way that samples from day 8 might not have been representative of the rest of the community.

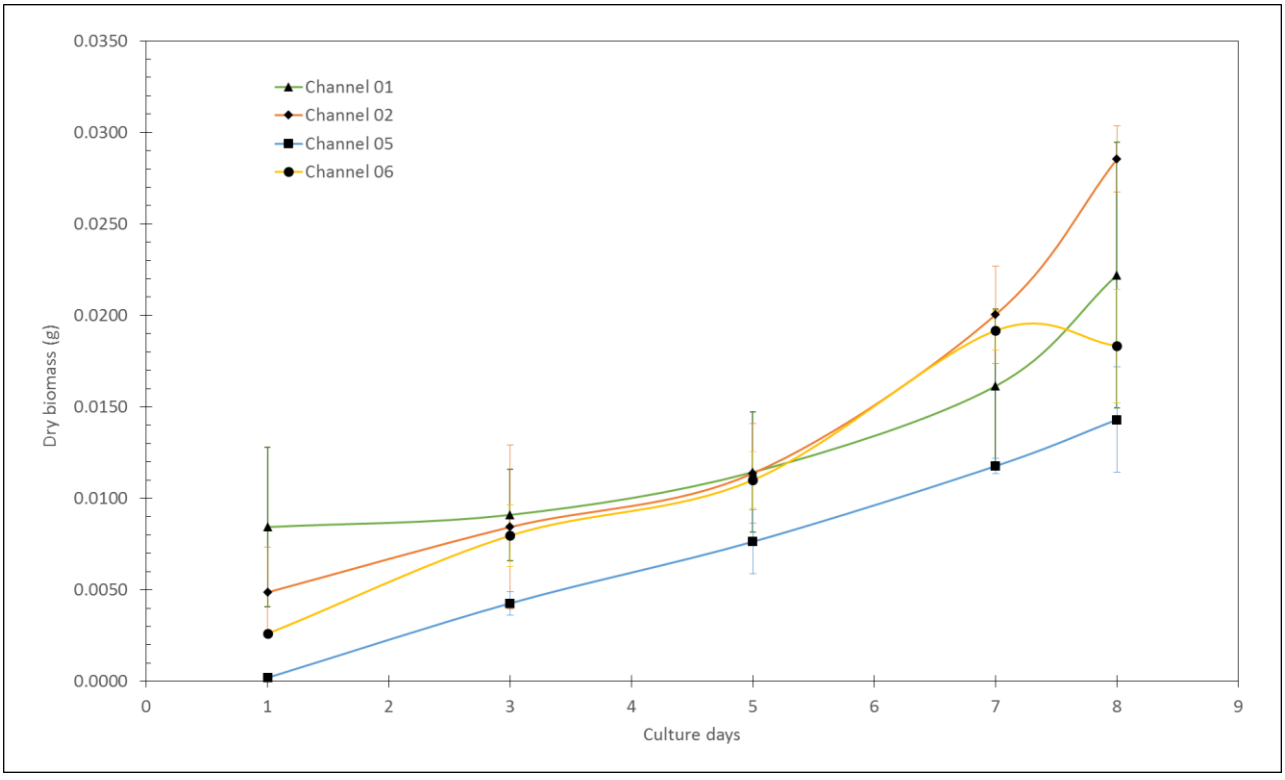


Figure 4.5 - Growth curves for Channels 1, 2, 5, and 6. Error bars represent the standard deviation of the three biomass samples obtained in each of the harvest days.

Analysis of Variance (ANOVA) was conducted with SAS 9.4 (PROC GLM, SAS Institute Inc.) to evaluate if the mean growth was the same among the channels during the described period. For ANOVA to be successfully employed, several assumptions are made, such as equality of variance and normality. The dataset used in this analysis violates one of ANOVA's primary assumptions related to the independence of measurements. That happened because the measurements in this study were taken from the same channel over time, in a way that the mass at time X+1 is dependent on the mass at time X. The "repeated" statement in PROC GLM was used to overcome such problem, since it is usually used to account for repeated measured of a response variable. Results from ANOVA suggest there is enough evidence to support that the mean biomass was statistically the same among the channels (PROC GLM, p-value: 0.4570 (accept H_0), α: 0.05).

4.2.2 Combined Effects of Nitrate, Light Intensity, and UV rays on Biomass Production and species dominance

Species dominance based on light configuration is shown in Figure 4.6. As it can be seen, filamentous species from the Green group were dominant when the community was not exposed to UVA and UVB rays. However, during the second harvest after Channels 1 and 2 were exposed to UV rays, a shift in species towards an *Oscillatoria sp.1* dominance was observed, which is similar to what happened in Channel 6 on Harvest #4. It was also noticed that even though Channels 5 and 6 were deprived of UV rays on Harvest 5 and 6, the community was not able to shift back towards a green alga dominance. Since this is an observational experiment, no causation can be inferred. However, empiric evidence in this preliminary study suggests that blue-green algae can have an advantage over green algae under UVA and UVB rays, and likely at higher temperatures too. Since the efforts to shift the community back to a green algae dominance were not successful, all four channels were exposed to UVA and UVB rays for the remaining harvests (configuration #3).

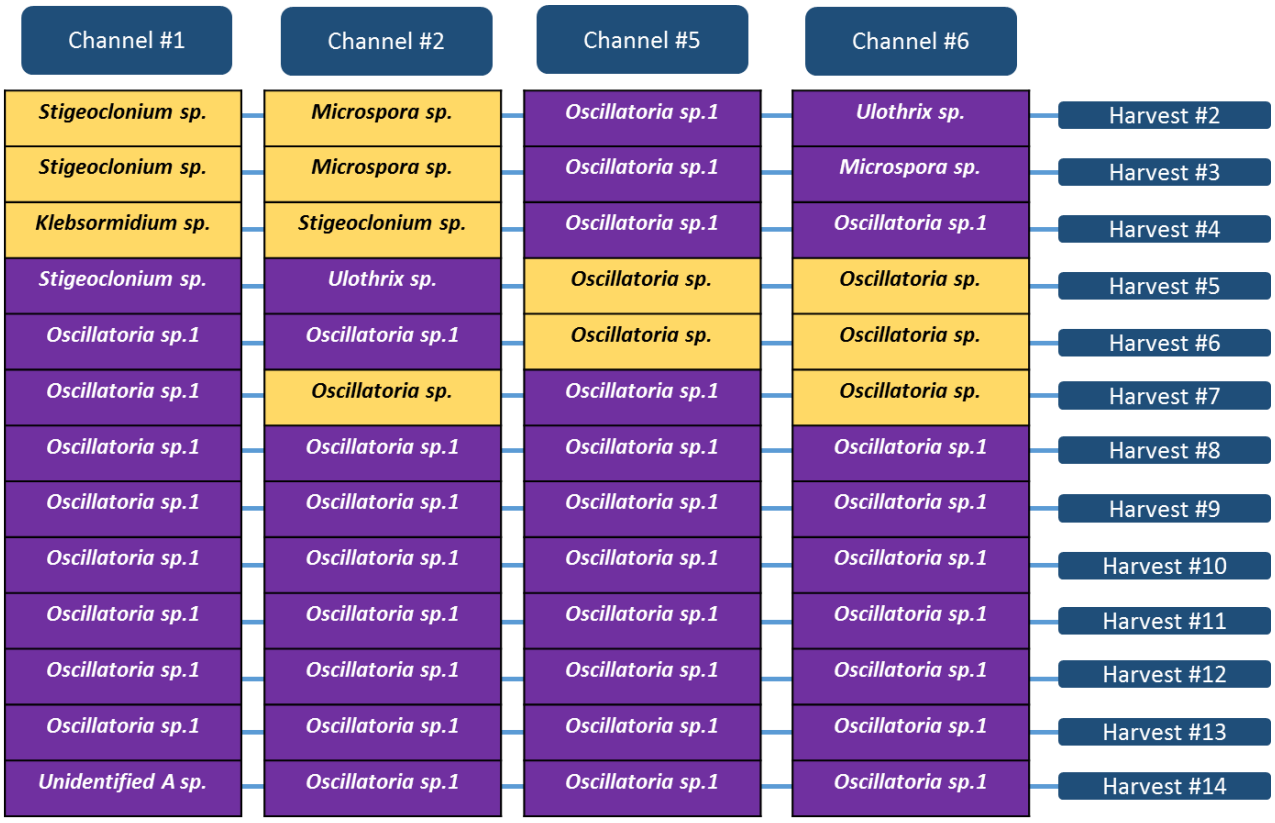


Figure 4.6 - Light Configuration Effects on Species Dominance. In the figure, purple indicates presence of UVA and UVB rays, whereas yellow indicates no UV exposure. Oscillatoria sp.1 is a blue-green alga, whereas the others are all green algae species.

Finally, no direct impact of nitrate concentration and light intensity on species dominance was observed. Since light intensity, nitrate concentration, and UV exposure were intermittently and simultaneously altered, SAS 9.4 was used to assess their combined effect on biomass production. Dummy variables were created to assign biomass data to different groups based on the parameters mentioned. A value of 0 was assigned for low light intensity, no UV exposure, and low nitrate concentration (BBM), whereas a value of 1 was assigned for biomass produced under high light intensity, UV exposure, and high nitrate concentration (3N BBM).

PROC MIXED was used to compare AFDW data to the configuration of each of the parameters throughout the fourteen harvests. PROC MIXED is comparable to the GLM procedure, with the advantage of modeling data that presents correlation and non-constant variability (SAS 2008). Biomass results from

each harvest and individual channels were assumed to be independent from each other. ANOVA results suggest that nitrate concentration in the media positively impacted biomass production (p-value: 0.0114, LSM: 2.1576 ± 0.2288 g for BBM and 2.8167 ± 0.1237 g for 3N BBM, α: 0.05). Furthermore, UV exposure was found to negatively affect biomass production (p-value: 0.0080, LSM: 2.7827 ± 0.1877 g with no UV exposure and 2.1916 ± 0.1544 g under UV exposure, α: 0.05), once exposure reduced AFDW. In contrast, light intensity was not found to significantly affect biomass production (p-value: 0.4904, α: 0.05).

4.3 Exposure Study

4.3.1 Channels and Algal Community Parameters

Figure 4.7 illustrates pH profiles of reactors during the 72 hour exposure period. As expected, pH in the experimental channels (1, 2, 5, and 6) was highly affected by the light:dark cycle, due to photosynthesis. A pH increase during the light period was observed, while a fairly quick decrease was observed when the lights were turned off. This is in agreement to what had been previously explained in section 3.2.4.

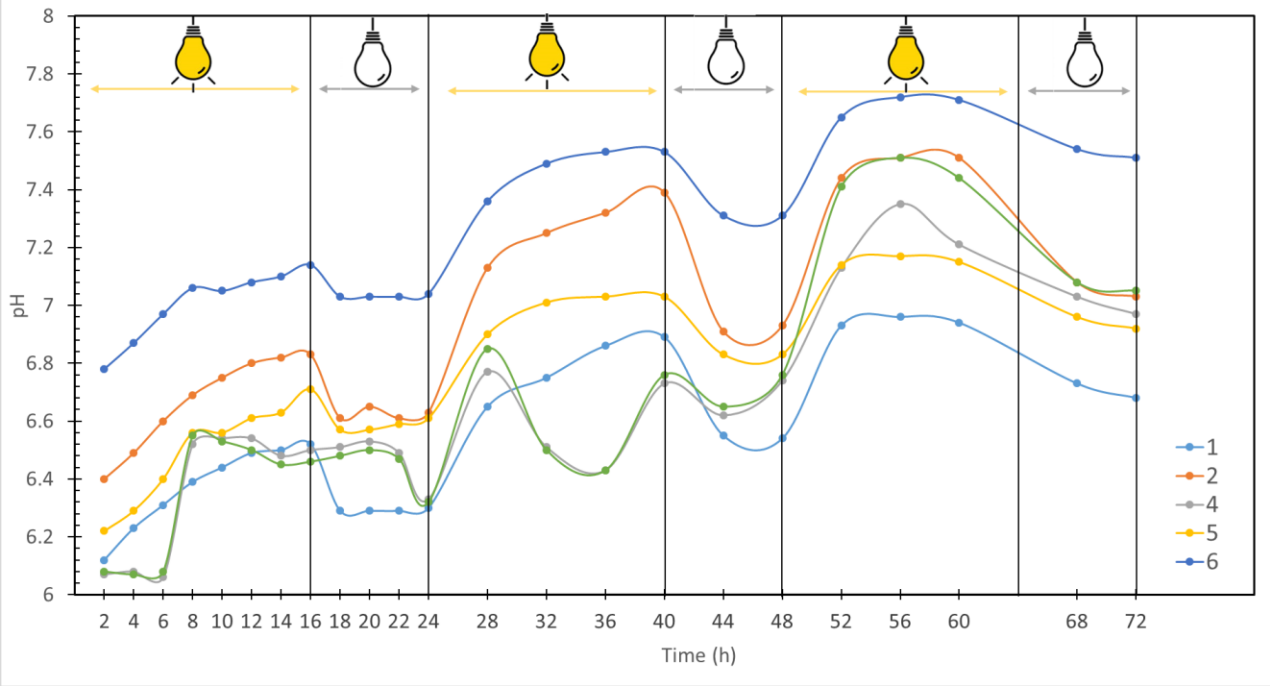


Figure 4.7 - pH profile and light:dark cycles during the 72 hour exposure experiment

Unlike pH, temperature was not affected by the light:dark cycle in the experimental channels, since these were equipped with aquarium heaters to keep the temperature constant. Negative controls, on the other hand, were directly affected by the cycle and had their temperatures decreased a few decimal degrees during the dark period. Temperature profiles are presented in Figure 4.8.

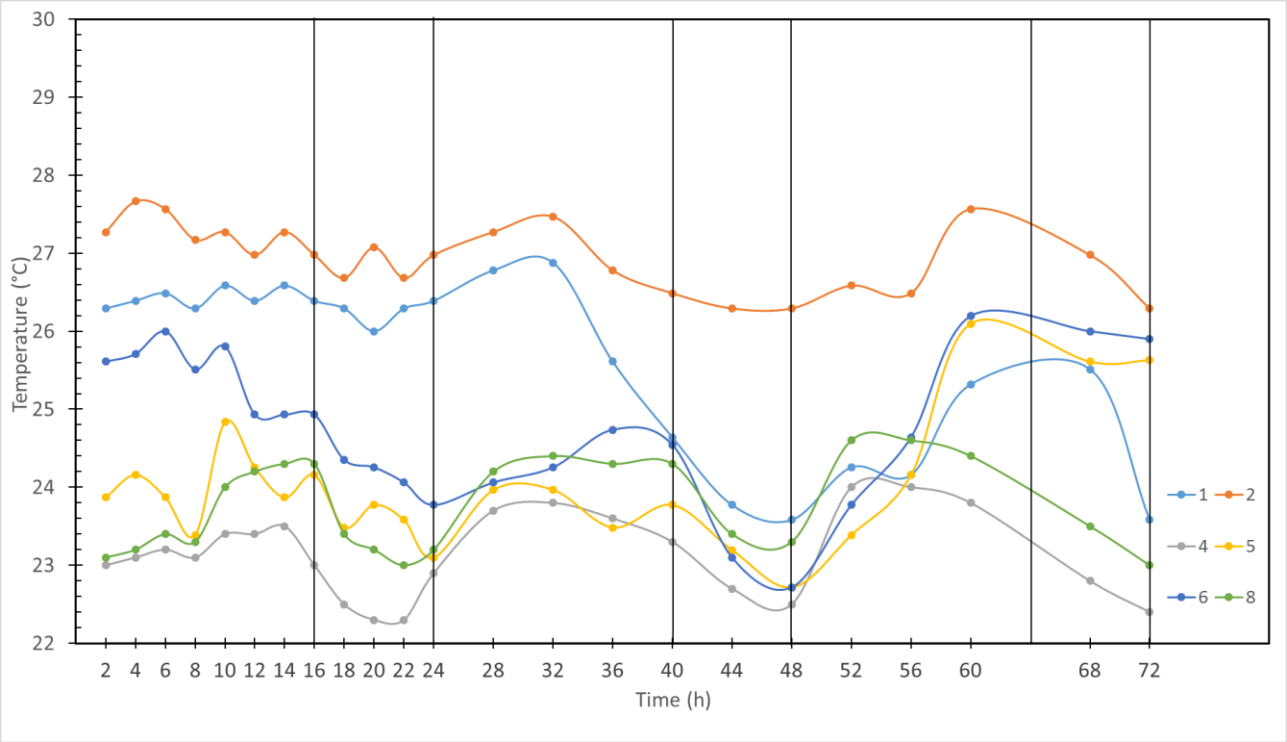


Figure 4.8 - Temperature profile and light:dark cycles during the 72 hour exposure experiment

Furthermore, evaporation occurred in all channels during the exposure experiment at similar rates from the ones observed during culturing. It can be observed from Figure 4.9 that experimental channels presented higher evaporation rates than controls, probably because of the presence of aquarium heaters in reactors with algae. For instance, 4.8 L of the initial volume was lost in Channel 6, while only 1.9 L was lost in Channel 4. It was assumed that evaporation was the only phenomena causing this loss in water since splashing was minimum and no leaking was observed.

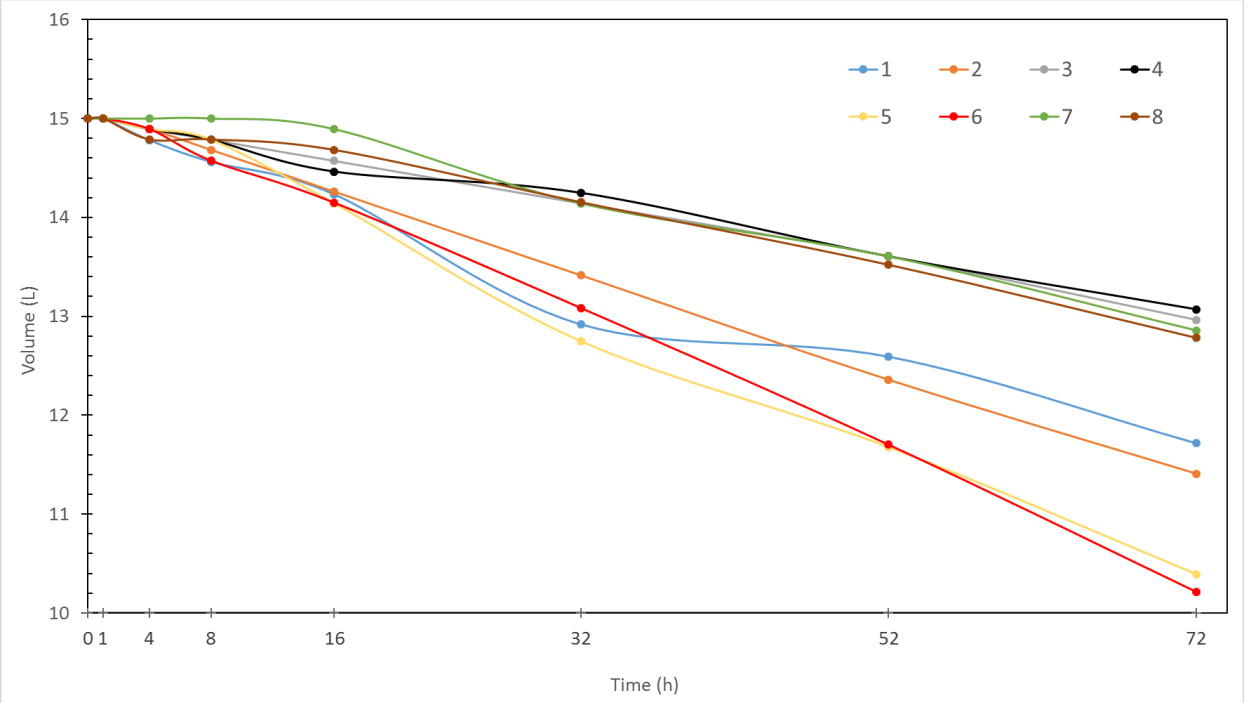


Figure 4.9 – Reservoir volume vs. time during the 72 hour exposure experiment. The slope of each curve is equal to the Evaporation Rate.

Table 4.1 presents a summary of average temperature, pH, initial nitrate concentration, and total evaporated volume for negative controls and experimental units. Results from ANOVA suggests that mean pH was not the same throughout the units (PROC MIXED, $p<0.001$, time-dependent analysis), with the exception of channels 4 and 8 (PROC MIXED, p-value 0.9048, time-dependent analysis). This was expected since pH was not controlled for experimental units. Similarly, the mean temperature was also found to be different among experimental channels (PROC MIXED, $p<0.001$, time-dependent analysis), and statistically equal between control units 4 and 8 (PROC MIXED, p-value 0.8098, time-dependent analysis). On another hand, reservoir volumes were observed to be the same among experimental units 1 and 2 and all controls (PROC MIXED, $p<0.005$, time-dependent analysis). The same trend was not observed between Channels 5 and 6 and control units (PROC MIXED, $p>0.005$, time-dependent analysis).

Table 4.1 - Summary of operational parameters during exposure experiment. Channels 1, 2, 5, and 6 are experimental units and Channel 4 and 8 are negative controls. For temperature and pH, overall average and standard deviation are also presented.

	Channel					
Parameter	1	2	4	5	6	8
Temperature	25.67 ± 1.10	26.96 ± 0.43	23.14 ± 0.54	24.01 ± 0.83	24.77 ± 0.96	23.77 ± 0.57
pH	6.55 ± 0.26	6.93 ± 0.34	6.61 ± 0.34	6.75 ± 0.27	7.25 ± 0.28	6.64 ± 0.41
Volume Evaporated (L)	3.30	3.60	1.90	4.60	4.8	2.2
Initial NO_3 (mgL^{-1})	52	51	53	52	50	52

The mixed periphytic algal community in the channels was also characterized (prior to exposure) for species dominance and biomass productivity (Figure 4.10). The community was dominated by *Oscillatoria sp.1*, but presence of filamentous species from genera *Spirogyra, Unidentified A, Klebsormidium, Oedogonium, Ulotrix, and Microspora* was also observed.

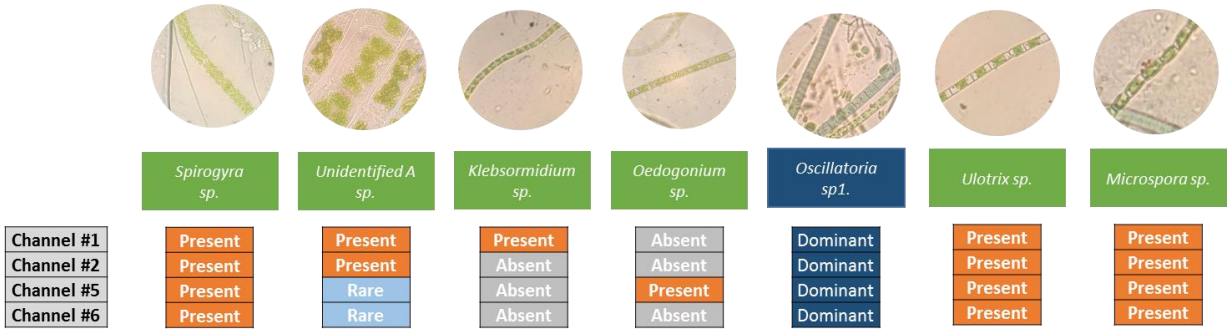


Figure 4.10 - Algal community structure prior to exposure

The total biomass displayed in Table 4.2 was measured based on the dry weight obtained from the freeze-drying process. Since only a portion of the total biomass from each channel was freeze-dried, an approximation had to be made to obtain the total dry weight. Water content was calculated for the freeze-dried samples and applied to the remaining wet portion, converting it to dry weight. Productivity was calculated by dividing the total biomass by the effective growth area (0.13 m^2) and culturing period (7 days).

Table 4.2 - Total biomass, reported in gdw (grams dried weight), and productivity (gdw m^{-2} d^{-1})

Channel	Total Biomass (gdw)	Productivity (gdw m^{-2} d^{-1})
1	3.1285	3.4379
2	2.6265	2.8863
5	3.1511	3.4627
6	1.9338	2.1251

4.3.2 Variation in aqueous concentration of contaminants over time

As previously mentioned, media samples were processed through SPE (solid phase extraction) and analyzed using ultra-high performance liquid chromatography coupled to triple quadrupole mass spectrometry (UHPLC-QqQ-MS). Two correction factors were applied to the raw data to adjust values for dilution and concentration effects. A dilution factor (DF) was applied to correct concentration for the volume of uncontaminated media added after every sampling event. As mentioned, 50 mL of media was added back after sampling, totaling 1.55 L for experimental units and positive controls. Dilution factors were calculated by dividing the adjusted volume after media addition by the initial volume (15L). For instance, at time 52 h, 1.3 L of uncontaminated media had been added into the reservoirs, and a dilution factor of 1.087 (16.3/15) was applied to the raw concentration at 52 h.

$$DF = \frac{V_{initial+added}}{V_{initial}}$$

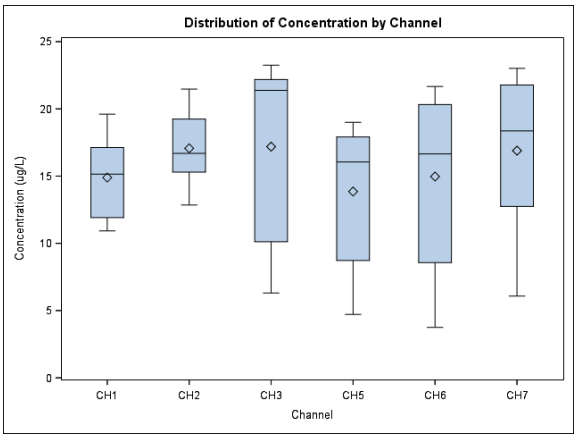
Evaporation was also observed in all channels, which increased the concentration of the contaminants over time since only the media was assumed to be evaporated. Thus, a Concentration Factor (CF) was calculated for each channel at each sampling time using the equation shown below.

$$CF = \frac{V_{initial}}{V_{inital-evaporation}}$$

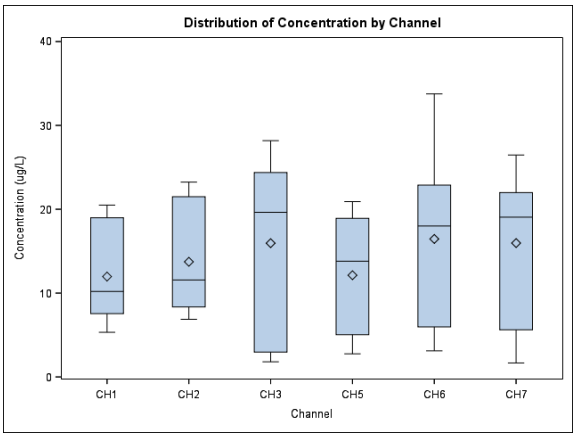
Finally, the adjusted concentration was calculated by multiplying the raw data by the dilution factor and dividing it by the concentration factor. Tabular data with raw concentration and factors are available in Appendix B.

$$Adjusted\ Concentration = (Raw\ Concentration * DF)/CF$$

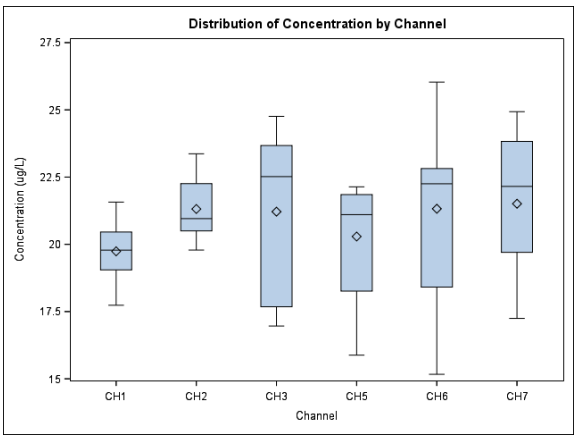
Side-by-side boxplots for the distribution of the concentration of each targeted substances by channel are displayed in Figure 4.11. It can be seen that distribution varied considerably among channels (especially for HFPO-DA and PDHA) and substances. Concentration of all substances was found to be negligible in the negative controls (Channels 4 and 8). These results suggest that the materials used to build the ATS system did not insert additional mass of PFAS into the system, indicating that cross-contamination likely did not occur.



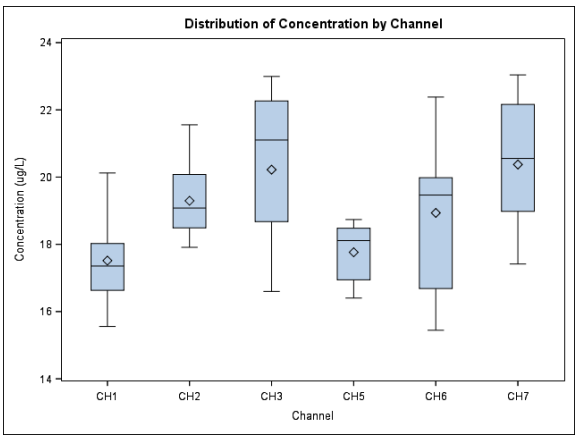
(a) PFOA



(b) PFOS



(c) HFPO-DA



(d) PDHA

Figure 4.11 - Distribution of aqueous concentration during the 72 hour exposure experiment by channels for (a) PFOA, (b) PFOS, (c) HFPO-DA, and (d) PDHA

Furthermore, Figures 4.12 to 4.15 present the variation in adjusted concentration of PFOS, PFOA, HFPO-DA, and PDHA in the media solution from positive and experimental units. An increase in aqueous concentration was observed in a few sampling times, which will be further addressed in the Discussion section. Because of that, a trendline was added to observe the overall trend in concentration, rather than a line connecting the actual points. As it can be seen, PFOS, PFOA, and HFPO-DA variation in concentration over time approximated an exponential curve, whereas PDHA was best represented by a polynomial equation. Additional graphical representations are shown in Appendix B.

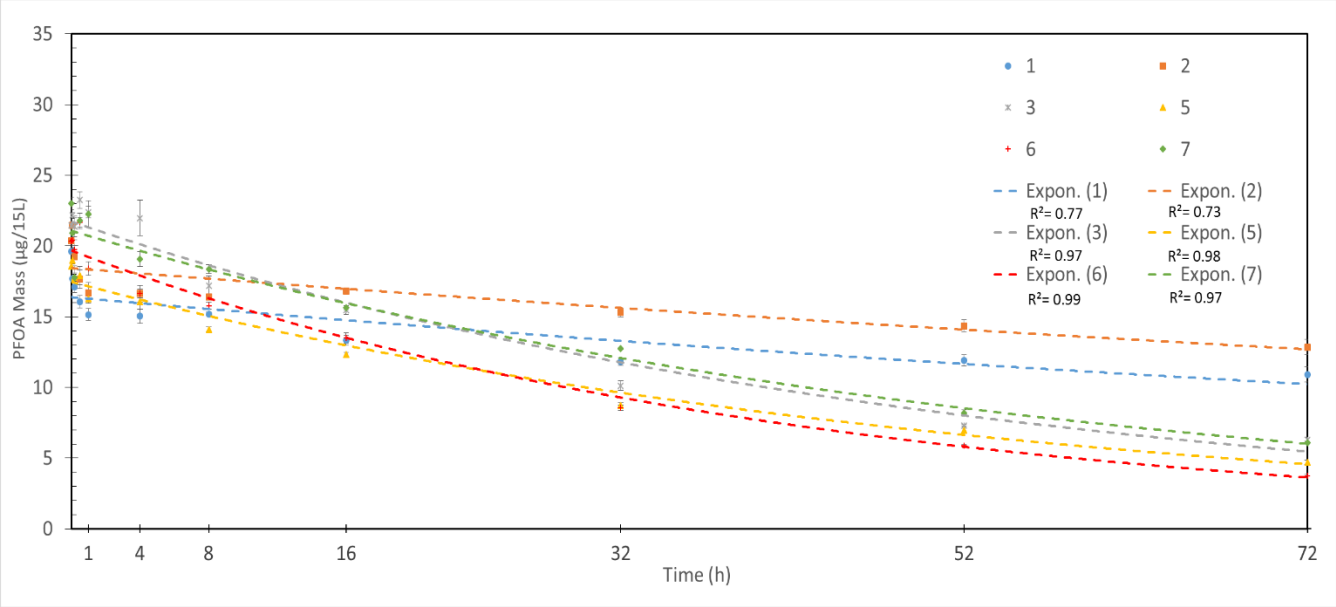


Figure 4.12 - Variation in concentration of PFOA over 72 hours for experimental and positive control channels

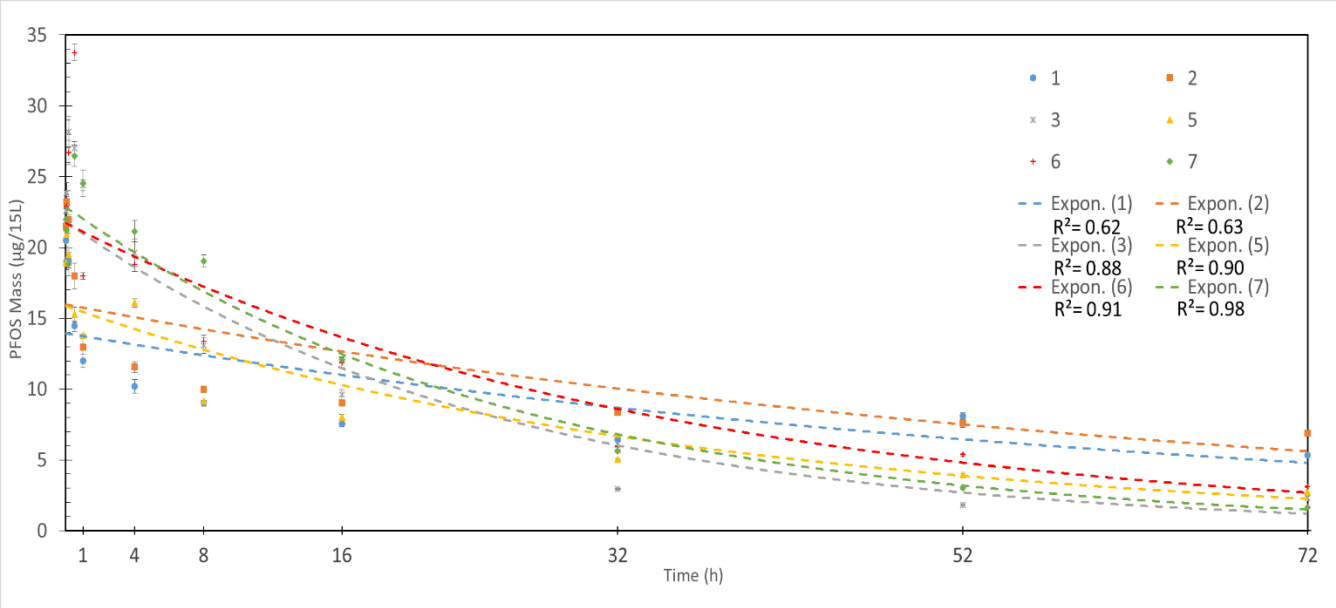


Figure 4.13 - Variation in concentration of PFOS over 72 hours for experimental and positive control channels

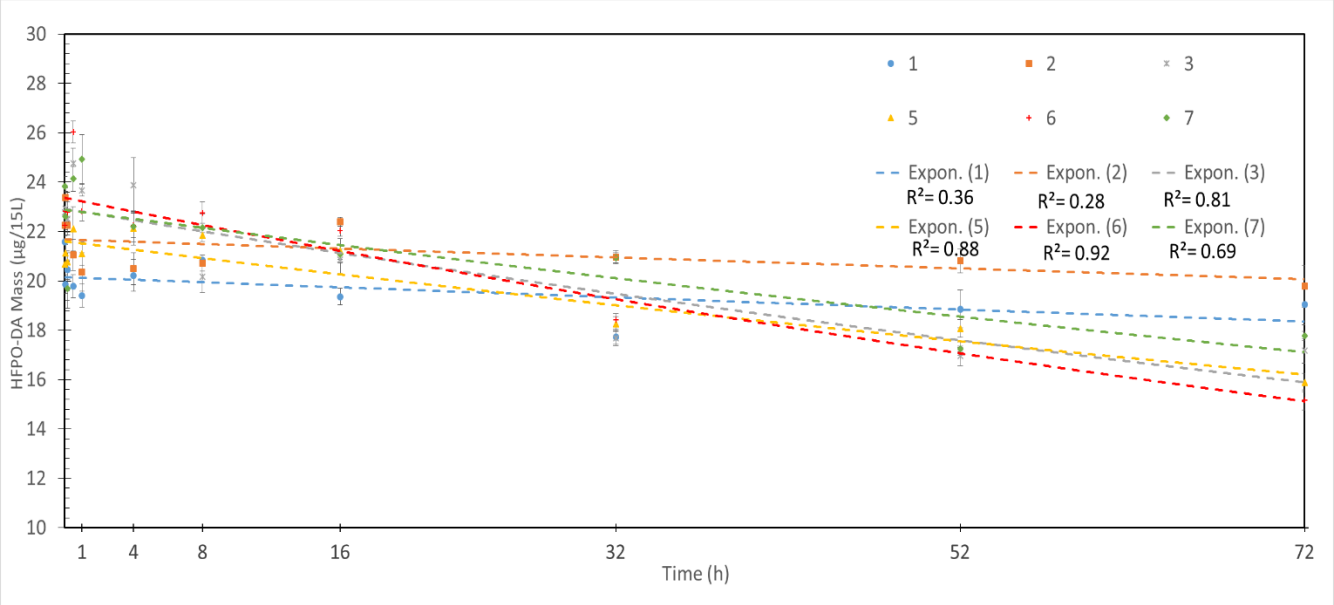


Figure 4.14 - Variation in concentration of HFPO-DA over 72 hours for experimental and positive control channels

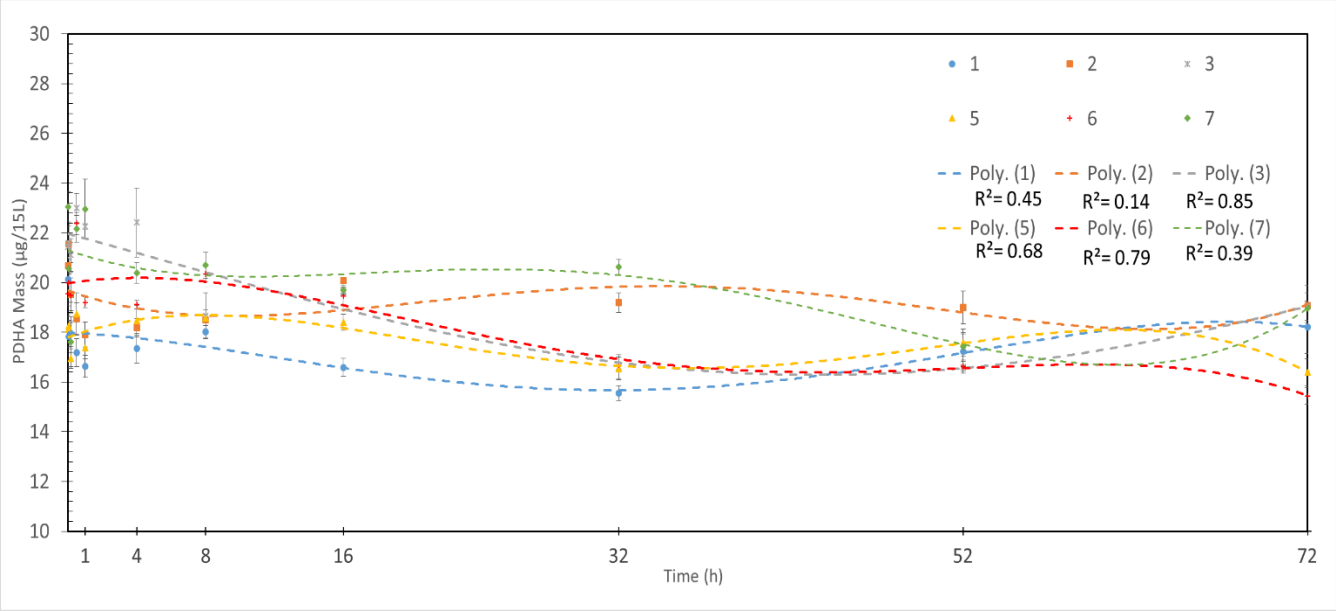


Figure 4.15 - Variation in concentration of PDHA over 72 hours for experimental and positive control channels

4.3.3 Sorption in algal cells

As previously described, algal biomass was also analyzed to assess the amount of contaminants adsorbed and absorbed into the algal cells. Biomass was filtered using a cheese cloth and samples were analyzed in two batches.

The contaminants observed in the freeze-dried portion of the biomass were assumed to have been absorbed into the cells. Results from this analysis, reported as mass of contaminants per unit of dry weight biomass, are displayed in Table 4.3. As shown, PFOS represented the highest concentration in the biomass in all channels. The highest concentration of PFOS, PFOA, and PDHA were observed in the biomass from channel 02, and HFPO-DA from channel 06.

Table 4.3- Concentration of contaminants absorbed into algal cells (ng per g dry weight)

	Channel 1 ng gdw^{-1}	**Channel 2 ng gdw^{-1}**	**Channel 5 ng gdw^{-1}**	**Channel 6 ng gdw^{-1}**
PFOA	17.27 ± 0.92	39.59 ± 1.13	10.97± 0.24	7.81 ± 0.36
PFOS	66.80 ± 1.69	92.82 ± 1.39	44.60 ± 1.26	36.1 ± 2.16
PDHA	2.78 ± 0.18	8.14 ± 0.17	2.58 ± 0.15	3.11 ± 0.13
HFPO-DA	2.65 ± 0.02	3.31 ± 0.03	2.55 ± 0.02	3.96 ± 0.01

Bioaccumulation factors (BAF) were also calculated based on the initial aqueous concentration of each contaminant and final concentration absorbed in the algae according to the equation presented below. BAF are shown in Table 4.4, represented as liters of media by kilograms of wet weight biomass.

$$BAF = \frac{Concentration\ in\ Biomass\ (ng/kg\ wwt)}{Initial\ Concentration\ in\ Solution\ (ng/L)}$$

Table 4.4 - Bioaccumulation factors (BAF), expressed in L per kg of wet weight

	Channel 1 L kgww^{-1}	**Channel 2 L kgww^{-1}**	**Channel 5 L kgww^{-1}**	**Channel 6 L kgww^{-1}**
PFOA	13.21	29.18	8.86	5.76
PFOS	48.90	64.76	35.36	25.50
PDHA	2.07	5.91	2.13	2.39
HFPO-DA	1.85	2.23	1.85	2.67

Furthermore, contaminants observed in the filtered water were assumed to be related to the amount adsorbed on the cell's surface. Since a small portion of the media was collected with the biomass, the acquired data was adjusted for background aqueous concentration. Therefore, the background concentration in the analyzed samples was proportionally calculated based on the aqueous concentration observed after the 72 hour period. Adjusted adsorbed mass was calculated by decreasing the background concentration from the raw data (see Appendix C for tabular data). Table 4.5 presents the adjusted mass of contaminants adsorbed onto the cells of the analyzed biomass.

Table 4.5 - Mass of substances (ng) in filtered solution after accounting for background concentration, reported as ng per gram of dry weight biomass.

	Channel 1 ng gdw^{-1}	**Channel 2 ng gdw^{-1}**	**Channel 5 ng gdw^{-1}**	**Channel 6 ng gdw^{-1}**
PFOA	68.87 ± 5.03	91.14 ± 3.66	59.84 ± 0.65	67.26 ± 1.20
PFOS	31.76 ± 2.38	31.55 ± 1.74	26.98 ± 0.75	29.55 ± 0.67
HFPO-DA	3.84 ± 1.74	22.00 ± 2.32	10.55 ±0.31	48.52 ±1.95
PDHA	25.48 ± 3.16	47.50 ± 2.50	45.31 ± 1.12	106.48 ± 2.21

4.3.4 Mass Balance and Summary

A mass balance equation was performed based on the initial mass spiked at time 0 ($\%M_{t=0h}$), final mass left in the media after 72 hours ($\%M_{t=72h}$), and mass absorbed ($\%M_{absorbed}$) and adsorbed ($\%M_{absorbed}$) on algal cells as shown in the equation below. A term for unknown removal mechanisms (X) was added in the case of the mass balance not being equal in both sides. Channels 3 and 7 did not present the terms related to algae ($\%M_{absorbed}$ and $\%M_{absorbed}$), since they were control channels.

$$\%M_{t=72h} = \%M_{t=0h} - \%M_{absorbed} - \%M_{absorbed} - X$$

The percent amount of contaminants absorbed by the algae was calculated using the equation displayed below. The mass of contaminants per units of dry biomass from Table 4.3 was multiplied by the total dry biomass for the channel to obtain the overall uptake amount.

$$\%M_{absorbed} = \frac{Mass\ of\ contaminants\ per\ unit\ of\ biomass\ (ng/g) * Biomass\ (g) * 100\%}{Mass\ of\ contaminants\ inittially\ added\ (ng)}$$

Similarly, values from Table 4.5 were multiplied by the total dry biomass and divided by the initial concentration of each substance to find $\%M_{adsorbed}$, using the equation shown below.

$$\%M_{adsorbed} = \frac{Adjusted\ M\ of adsorbed\ per\ unit\ of\ biomass\ (ng/g) * Biomass\ (g) * 100\%}{Mass\ of\ contaminants\ inittially\ added\ (ng)}$$

Finally, Tables 4.6 to 4.9 present the results from the mass balance analysis for experimental (1, 2, 5, and 6) and positive control (3 and 7) channels. It was observed that a high quantity of the substance's mass remained unaccounted (as high as 92.43% for PFOS in channel 7). On another hand, only a small amount of contaminants was found in the algal material in all channels, with PFOS and PFOA having the highest mass, and PDHA and HFPO-DA the lowest.

Table 4.6 - Mass balance for PFOA based on initial mass, absorbed and adsorbed quantities, and concentration remaining in the media after the 72 hours. Standard deviations are presented in ng

	Channel 01		Channel 02		Channel 05		Channel 6	
	Mass (ng)	%	Mass (ng)	%	Mass (ng)	%	Mass (ng)	%
Initial Mass	19610 ± 600	100.00	20350 ± 535	100.00	18570 ± 841	100.00	20320 ± 466	100.00
Biomass	53.86 ± 2.87	0.27	103.54 ± 2.97	0.51	34.64 ± 0.76	0.19	15.13 ± 0.69	0.07
Algal Wash	214.77 ± 15.74	1.10	238.33 ± 9.61	1.17	189.06 ± 2.05	1.02	130.38 ± 2.32	0.64
Remaining in Media	10930 ± 537	55.74	12850 ± 535	63.14	4710 ± 145	25.36	3750 ± 105	18.45
Unknown	-	42.89	-	35.18	-	73.43	-	80.83

	Channel 03		Channel 07	
	Mass (ng)	%	Mass (ng)	%
Initial Mass	21463 ± 882	100.00	23012 ± 451	100.00
Left in Media	6307 ± 192	29.39	6078 ± 110	26.41
Unkown	-	70.61	-	73.59

Table 4.7 - Mass balance for PFOS based on initial mass, absorbed and adsorbed quantities, and concentration remaining in the media after the 72 hours. Standard deviations are presented in ng

	Channel 01		Channel 02		Channel 05		Channel 6	
	Mass (ng)	%	Mass (ng)	%	Mass (ng)	%	Mass (ng)	%
Initial Mass	20490 ± 400	100.00	21500 ± 443	100.00	18920 ± 912	100.00	21230 ± 603	100.00
Biomass	208.32 ± 5.29	1.02	242.72 ± 3.65	1.13	140.90 ± 3.97	0.74	69.97 ± 4.18	0.36
Algal Wash	99.04 ± 15.74	0.48	82.48 ± 9.61	0.38	85.22 ± 2.05	0.45	57.25 ± 2.32	0.29
Remaining in Media	5430 ± 102	26.50	6870 ± 159	31.95	2760 ± 107	14.59	3110 ± 56	15.86
Unknown	-	72.00	-	66.53	-	84.22	-	83.49

	Channel 03		Channel 07	
	Mass (ng)	%	Mass (ng)	%
Initial Mass	22609 ± 1104	100.00	21986± 654	100.00
Media	2328 ± 91	10.30	1665 ± 53	7.57
Unknown	-	89.70	-	92.43

Table 4.8 - Mass balance for HFPO-DA based on initial mass, absorbed and adsorbed quantities, and concentration remaining in the media after the 72 hours. Standard deviations are presented in ng

	Channel 01		Channel 02		Channel 05		Channel 6	
	Mass (ng)	%	Mass (ng)	%	Mass (ng)	%	Mass (ng)	%
Initial Mass	21570 ± 565	100.00	22246 ± 171	100.00	20680 ± 924	100.00	22250 ± 421	100.00
Biomass	8.28 ± 0.06	0.04	8.64 ± 0.08	0.04	5.17 ± 0.06	0.03	7.72 ± 0.02	0.03
Algal Wash	11.98 ± 5.44	0.06	57.51 ± 6.09	0.26	33.35 ± 0.98	0.16	94.03 ± 3.77	0.42
Media	19049 ± 840	88.31	19788 ± 849	88.95	15880 ± 367	76.79	15167 ± 407	68.17
Unknown	-	11.59	-	10.75	-	23.03	-	31.37

	Channel 03		Channel 07	
	Mass (ng)	%	Mass (ng)	%
Initial Mass	22785 ± 599	100.00	23825 ± 401	100.00
Left in Media	17170 ± 506	75.36	17779 ± 174	74.63
Unknown	-	24.64	-	25.37

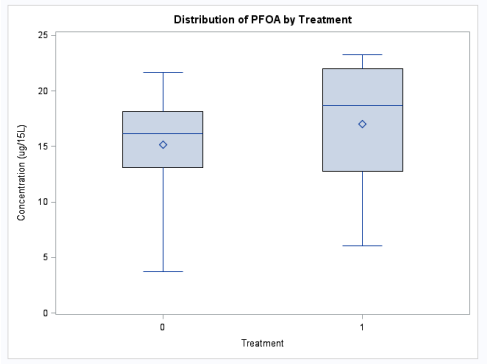
Table 4.9 - Mass balance for PHDA based on initial mass, absorbed and adsorbed quantities, and concentration remaining in the media after the 72 hours. Standard deviations are presented in ng

	Channel 01		Channel 02		Channel 05		Channel 6	
	Mass (ng)	%	Mass (ng)	%	Mass (ng)	%	Mass (ng)	%
Initial Mass	20122 ± 448	100.00	20684 ± 241	100.00	18111 ± 568	100.00	19561 ± 503	100.00
Biomass	8.67 ± 0.56	0.04	21.29 ± 0.45	0.10	8.12 ± 0.47	0.04	6.04 ± 0.25	0.03
Algal Wash	79.46 ± 9.88	0.39	124.20 ± 6.57	0.60	143.11 ± 3.53	0.79	204.94 ± 4.27	1.05
Media	18205 ± 1048	90.47	19080 ± 827	92.25	16402 ± 537	90.56	15444 ± 342	78.95
Unknown	-	9.09	-	7.05	-	8.60	-	19.97

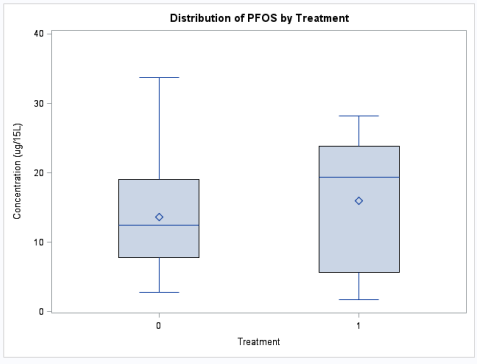
	Channel 03		Channel 07	
	Mass (ng)	%	Mass (ng)	%
Initial Mass	21454 ± 843	100.00	23040 ± 614	100.00
Left in Media	19039 ± 551	88.74	18982 ± 611	82.39
Unknown	-	11.26	-	17.61

4.3.5 Statistical Analysis for Exposure Study

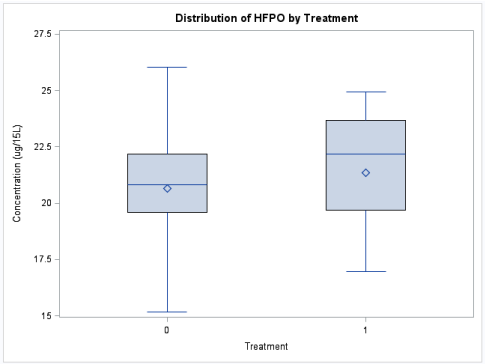
SAS 9.4 was used to conduct statistical analysis for the exposure study. All hypothesis testing consisted of comparing the Null Hypothesis, in which all means are equal, to the Alternative Hypothesis, in which at least one mean is different from the others, at a confidence level $\alpha = 0.05$. The level of significance, α, is related to Type I errors, which is the probability of rejecting the Null Hypothesis when it is actually true. Analysis of variance was performed through the MIXED procedure to compare the mean concentration of each substance in experimental units and positive control channels. Furthermore, a dummy variable was created to group the data into two treatments. A value of 0 was attributed to channels with algae (1, 2, 5, and 6), and 1 to positive control units (3 and 7). The *repeated* and *random* statements were included in the procedure to account for the repeated sampling over time. Figure 4.16 displays boxplots for each treatment and substance.



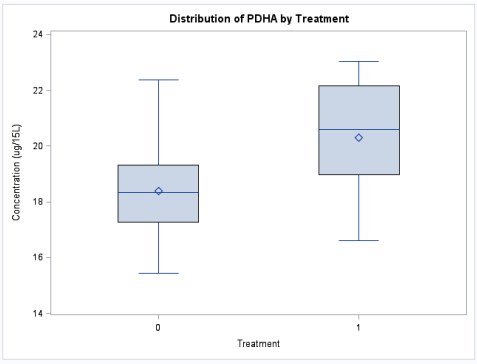
(a) PFOA



(b) PFOS



(c) HFPO-DA



(d) PDHA

Figure 4.16- Distribution of concentration by Treatment for (a) PFOA, (b) PFOS, (c) HFPO-DA, and (d) PDHA. Treatment 0 is related to experimental units and 1 to positive controls.

Therefore, the MIXED procedure was performed with *treatment* and *time* as variables in the model. As demonstrated in Table 4.10, analysis of variance suggests that the variable *treatment* is not significant in the model for PFOS and HFPO-DA (p-value 0.1346 and 0.2502, respectively), and a trend was observed for PFOA (p-value 0.0763). On the other hand, the *treatment* variable was found to be significant for PDHA (p-value 0.0055). Finally, the variable *time* was found to be significant for all substances (p-value <0.0001). It can be inferred that the presence of algae did not significantly affect the concentration of PFOS, PFOA, and HFPO-DA.

Table 4.10 - Effects of treatment and time variables in the concentration of contaminants.

Substance	Variable	p-value
PFOA	Treatment	0.0763
	Time	<0.0001
PFOS	Treatment	0.1346
	Time	<0.0001

Substance	Variable	p-value
HFPO-DA	Treatment	0.2502
	Time	<0.0001
PDHA	Treatment	0.0055
	Time	<0.0001

This conclusion is supported when analyzing the least square means from the MIXED procedure. As it can be observed in Table 4.11, concentrations of all contaminants were consistently lower in the treatment 0 (experimental units) when compared to treatment 1 (positive controls). However, 95% confidence intervals calculated based on the Standard Error (SE) for PFOA, PFOS, and HFPO-DA contained zero, indicating that the difference is not statistically significant. In contrast, difference between treatment 0 and 1 for PDHA was statistically significant, presenting a confidence interval ranging between -0.584 and 3.2518 μg $15L^{-1}$.

Table 4.11 - Least Square Means for Treatment 0 and 1. Overall mean values and 95% confidence interval are displayed.

	Least Square Means (μg $15L^{-1}$) ± CI $_{95\%}$		
	Treatment 0 (With Algae)	Treatment 1 (No Algae)	Difference
PFOA	15.1946 ± 1.1831	17.0395 ± 1.6732	-1.8449 ± 2.0494
PFOS	13.5729 ± 1.8232	15.9589 ± 2.5785	-2.3860 ± 3.1582
HFPO-DA	20.6672 ± 0.6951	21.3621 ± 0.9829	-0.6949 ± 1.2038
PDHA	18.3779 ± 0.7636	20.2972 ± 1.0797	-1.9194 ± 1.3324

Furthermore, the MIXED procedure was also used to analyze the sorption of contaminants into algal cells. Side-by-side boxplots for the distribution of absorbed concentration by substance are illustrated in Figure 4.17. For this analysis, only *concentration* was used as a variable in the model, since all samples were taken at the same time; after 72 hours of exposure. Results from ANOVA suggest that sorption of HFPO-DA and PDHA into algal cells was not significant (p-values 0.4200 and 0.2841, $CI_{95\%}$ 3.1170 ± 7.7174 and 4.1521 ± 7.1774 ng gdw^{-1} respectively, PROC MIXED). In contrast, there is enough evidence to suggest otherwise for PFOS and PFOA (p-value: <0.0001, $CI_{95\%}$ 60.0808 ± 7.7174 and 18.9078 ± 7.1774 ng gdw^{-1} respectively, PROC MIXED). In addition, the difference of least square means values were used to compare trends among the substances. As expected, only HFPO-DA and PDHA were found to have similar absorption rates (Tukey adjusted p-value 0.9975, PROC MIXED). Comparison among channels was not possible to be made due to sample size limitations.

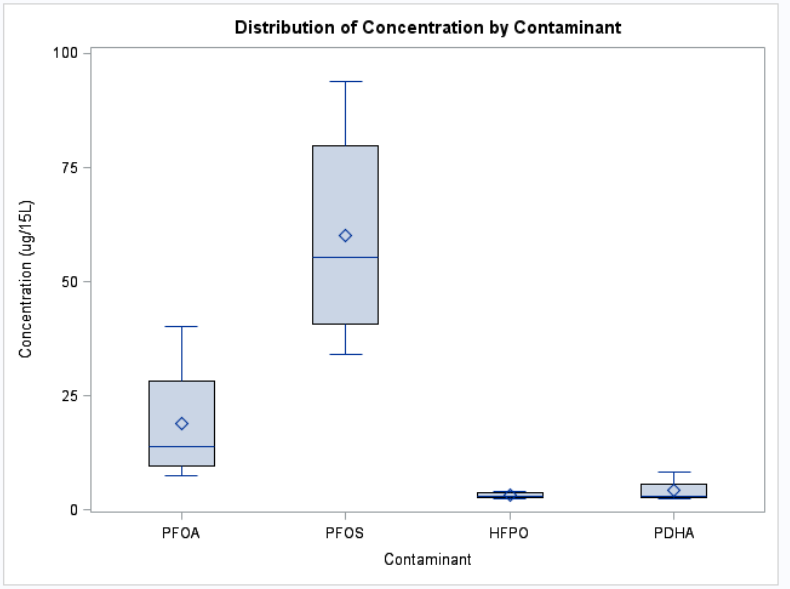


Figure 4.17 - Boxplots for the distribution of PFOA, PFOS, HFPO-DA, and PDHA in algal cells

Similarly, the same analyses were conducted to evaluate adsorption of substances on algal material. Unlike results from absorption analysis, ANOVA results suggest that adsorption was significant for all substances (p-value: 0.0004 for HFPO and <0.0001 for PFOS, PFOA and PDHA; $CI_{95\%}$ 21.2242 ± 11.1907,

56.1922 ± 11.1907, 71.7787 ± 11.1907 and 29.9568 ± 11.1907 ng gdw^{-1} for HFPO-DA, PDHA, PFOA, PFOS respectively, PROC MIXED). Furthermore, only HFPO-DA and PFOS (Tukey adjusted p-values 0.6853, PROC MIXED), and PDHA and PFOA (Tukey adjusted p-values 0.2105, PROC MIXED) were found to have similar rates when difference of least square means results were analyzed. Figure 4.18 displays side-by-side boxplots for the distribution of adsorbed concentration by substance.

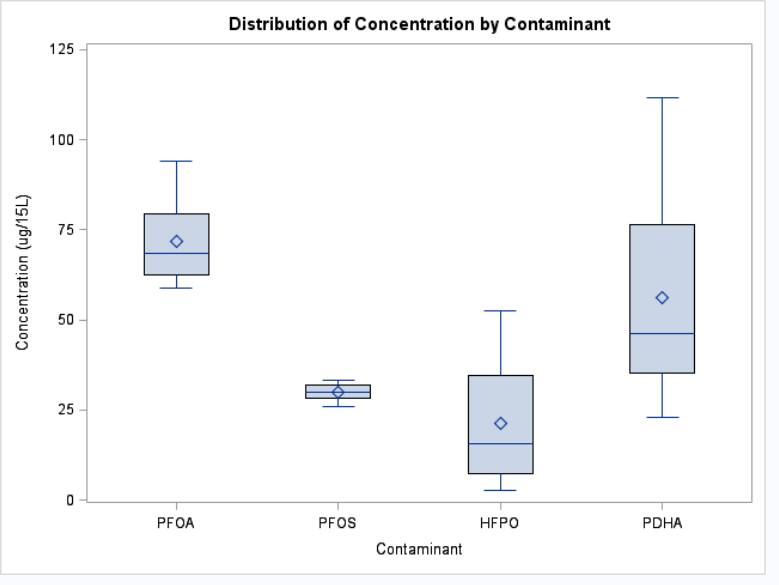


Figure 4.18 - Boxplots for the distribution of PFOA, PFOS, HFPO-DA, and PDHA in filtered wash from biomass

4.4 Complementary Studies

4.4.1 Abiotic Transformation Study

Results from the experiment conducted to assess effects of media, pH adjustment, and UV light on the abiotic transformation of PFOS and PFOA are presented in Table 4.12 and Table 4.13. A positive percent change indicates an increase in the substance's concentration, whereas a negative suggests a decrease. PFOS and PFOA concentration did not seem to follow a systematic or expressive increase or decrease in concentration in units C1, C2, C4 and C5. However, a more considerable reduction in concentration of PFOS and PFOA was observed in the units with DI Water - around 10.12 and 7.84%, respectively.

Table 4.12 - Variation in PFOS concentration over 24 hours in different media and light conditions

Light	Unit	Description	0h (ng 25mL$^{-1)}$	24h (ng 25mL$^{-1)}$	% Change
UV Light	C1	Media+pH Adj	44.23 ± 0.30	44.99 ± 0.42	1.73
	C2	Media	44.64 ± 1.63	45.10 ± 1.07	1.05
	C3	DI Water	44.01 ± 0.45	42.84 ± 1.03	-2.66
Dark	C4	Media+pH Adj	40.65 ± 0.72	42.69 ± 1.71	5.02
	C5	Media	40.14 ± 1.40	42.66 ± 0.22	6.27
	C6	DI Water	48.65 ± 1.40	43.73 ± 0.84	-10.12

Table 4.13 - Variation in PFOA concentration over 24 hours in different media and light conditions

Light	Unit	Description	0h (ng 25mL$^{-1)}$	24h (ng 25mL$^{-1)}$	% Change
UV Light	C1	Media+pH Adj	39.91 ± 0.32	38.17±1.02	-4.38
	C2	Media	37.16 ± 0.93	37.80 ± 0.56	1.72
	C3	DI Water	36.12 ± 0.73	34.96 ± 0.68	-3.2
Dark	C4	Media+pH Adj	34.35 ± 0.55	35.82 ± 1.37	4.27
	C5	Media	33.36 ±0.62	35.16 ± 0.14	5.39
	C6	DI Water	39.57 ± 1.04	36.47 ± 0.14	-7.84

4.4.2 Volatilization Study

Concentrations of PFOA and PFOS after the 72 h volatilization experiment are shown in Table 4.14 and Table 4.15, respectively. As mentioned in section 3.5, this experiment was conducted in duplicates and the results are displayed as the average of each pair of units. The standard deviation for each pair is also presented and it reflects the variability between duplicates as well as instrument accuracy, since each sample was analyzed three times. The initial concentration of PFOA in units with DI water was found to be equal to 982.14 ± 34.69 ng 500mL^{-1}, and 1033.78 ± 42.34 ng 500mL^{-1} in units with Bolds Basal Media. On another hand, the initial concentration of PFOS was observed to be equal to 1523.93 ± 44.36 ng 500 mL^{-1} and 1859.42 ± 41.19 ng 500mL^{-1} in units with DI water and BBM, respectively. The percent difference in mass was calculated based on the difference in levels of PFOS and PFOA at the beginning and end of the experiment. As it can be inferred, a systematic or significant decrease in concentration was not observed for both PFOS and PFOA. In fact, an increase in the concentration of PFOS and PFOA was observed in pairs B and C.

Table 4.14 - Concentration of PFOA in units 1-8, reported as ng/500mL. Percent difference in mass was calculated based on the initial concentration of PFOA in the mixture

#	Units	Description	Concentration (ng/500 mL) at 72 h	% Difference in Mass
A	1 and 2	10% 3N BBM	1013.74 ± 33.09	-2.04
B	3 and 4	DI Water	1089.64 ± 50.45	+ 10.95
C	5 and 6	10% 3N BBM (covered)	1091.31 ± 57.34	+ 5.86
D	7 and 8	DI Water (covered)	960.99 ± 19.92	- 2.15

Table 4.15 - Concentration of PFOS in units 1-8, reported as ng/500mL. Percent difference in mass was calculated based on the initial concentration of PFOS in the mixture

#	Units	Description	Concentration (ng/500 mL) at 72 h	% Difference in Mass
A	1 and 2	10% 3N BBM	1681.29 ± 68.68	- 9.58
B	3 and 4	DI Water	1704.25 ± 127.46	+ 11.83
C	5 and 6	10% 3N BBM (covered)	1892.94 ± 59.46	+ 1.80
D	7 and 8	DI Water (covered)	1474.06 ± 28.47	- 3.27

4.4.3 Sorption Study

Results from the sorption study are presented in Table 4.16 for PFOA and Table 4.17 for PFOS. Similarly to what happened in the exposure study, media evaporation was observed and a concentration factor was applied to the raw data. It was assumed that the change in concentration after the 72 hour period was entirely related to sorption onto the materials. Sorption rates ranged between 43-57% and 45-66% of initial concentration for PFOA and PFOA, respectively. Furthermore, PFOS and PFOA were not observed in the blank container, indicating cross-contamination likely did not occur.

Table 4.16 - Sorption of PFOA onto ATS Materials after 72 hours. Initial concentration was observed to be 92.25 ± 1.53 ng 45mL^{-1}. Percent sorption was calculated based on the change in mass over the 72 h experiment

#	Description	Average Conc ng 45mL^{-1}	Standard Deviation	% Sorption
1	PVC Valve	50.54	2.84	45
2	Ruler	52.12	3.71	43
3	Duct Tape	51.17	3.21	45
4	Rubber Sealant	48.26	2.40	48
5	PVC Pipe + Connections	52.46	1.82	43
6	Nylon Mesh + Silicone	39.74	0.68	57
7	Vinyl (Channel)	49.85	3.30	46
8	Pump	45.52	1.37	51
9	Reservoir	48.65	2.04	47

Table 4.17 - Sorption of PFOS onto ATS Materials after 72 hours. . Initial concentration was observed to be 180.35 ± 4.65 ng 45mL^{-1}. Percent sorption was calculated based on the change in mass over the 72 h experiment

#	Description	Average Conc ng 45mL^{-1}	Standard Deviation	% Sorption
1	PVC Valve	89.93	3.66	50
2	Ruler	94.32	3.68	48
3	Duct Tape	88.28	4.78	51
4	Rubber Sealant	86.92	5.18	52
5	PVC Pipe + Connections	98.51	1.43	45
6	Nylon Mesh + Silicone	72.23	2.12	60
7	Vinyl (Channel)	81.75	2.92	55
8	Pump	60.91	1.71	66
9	Reservoir	84.51	0.89	53

A graphical representation of PFOS and PFOA's sorption onto each material is shown in Figure 4.19. It can be inferred from a visual inspection that sorption rates were fairly similar, with PFOS presenting slightly higher sorption rates than PFOA. Furthermore, the highest difference in the sorption of PFOS and PFOA was observed to occur in the pump unit (around 15%).

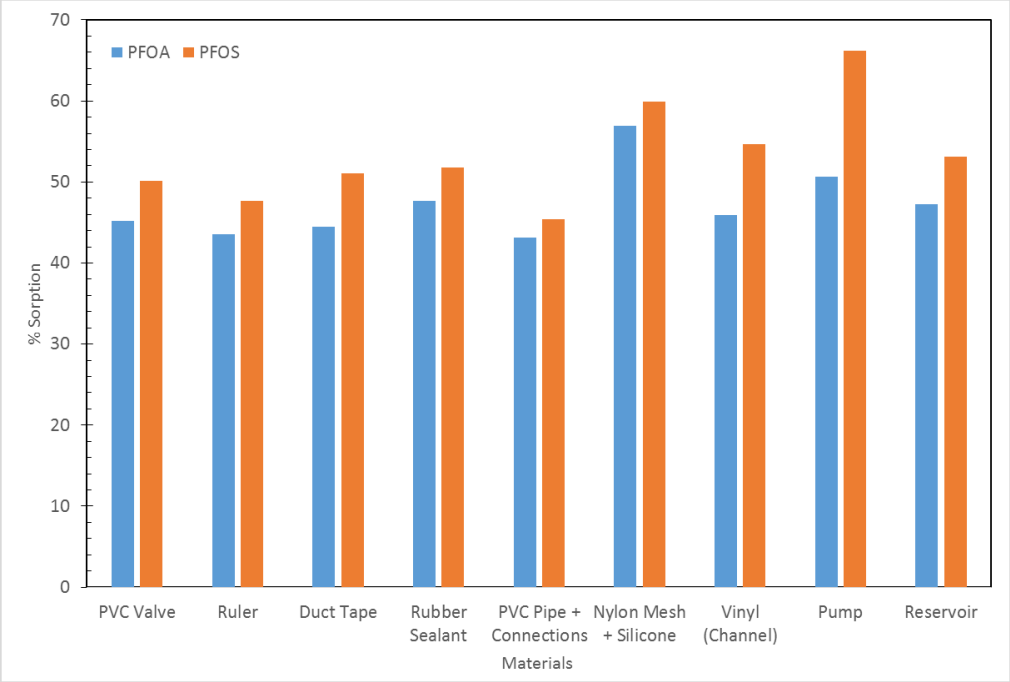


Figure 4.19 - Comparative graphical representation for the sorption of PFOS and PFA onto ATS materials

4.5 Photosynthetic Pigments

Selected pigments are displayed in Table 4.18 and Table 4.19. In this analysis, β-carotene was used as an indicator of overall abundance. Even though Chlorophyll-*a* is usually employed as a marker for overall production, Chlorophyll-*a* concentration exceeded the maximum detection limit of the instrument and could not be calculated. Zeaxanthin+ Lutein was used as indicator of blue-green algae and chlorophytes (green algae), whereas Chlorophyll-*b* was used as an indicator for green algae abundance. A cyanobacteria pigment (Retention Time 6.9) was used as a proxy for blue-green algae abundance. Since there are no available standards for this pigment, the standard value for Aphanizophyll was assigned for the 6.9 pigment. Fucoxanthin and Alloxanthin were used are proxies for siliceous algae and cryptophytes, respectively. In addition, Chlorophyllide was used as a marker for stress in algae, since it is a breakdown product from Chlorophyll-*a*. A graphical representation of selected pigments is shown in Figure 4.20.

Table 4.18 - Concentration of the photosynthetic pigments Fucoxanthin, Aphanizophyll, 6.9, and Alloxanthin, expressed as nmol per gram of organic matter

		Pigments (nmol gOM^{-1})			
	Channel	**Fucoxanthin**	**Aphanizophyll**	**6.9**	**Alloxanthin**
Time 0 (Prior to exposure)	1	12.43	54.16	338.12	0.00
	2	35.55	110.14	678.32	0.00
	5	72.47	77.21	115.67	27.33
	6	31.46	30.93	120.26	41.17
Time 72 (After Exposure)	1	9.08	47.13	329.10	0.00
	2	0.00	66.49	449.99	0.00
	5	30.02	25.74	194.32	108.10
	6	16.66	11.41	65.75	38.56

Table 4.19 - Concentration of the photosynthetic pigments Zeaxanthin+Lutein, β-carotene, Chlorophyllide, and Chlorophyll-b, expressed as nmol per gram of organic matter

		Pigments (nmol gOM^{-1})			
	Channel	**Zeaxanthin+ Lutein**	**β-carotene**	**Chlorophyllide**	**Chlorophyll-*b***
Time 0 (Prior to exposure)	1	832.34	2167.40	160.83	226.95
	2	924.82	1583.20	121.61	154.90
	5	498.06	664.79	299.49	179.74
	6	614.79	706.15	300.24	169.21
Time 72 (After Exposure)	1	748.48	544.85	176.29	132.73
	2	727.96	706.94	105.85	88.00
	5	619.55	687.98	445.51	161.18
	6	306.08	339.53	730.53	63.81

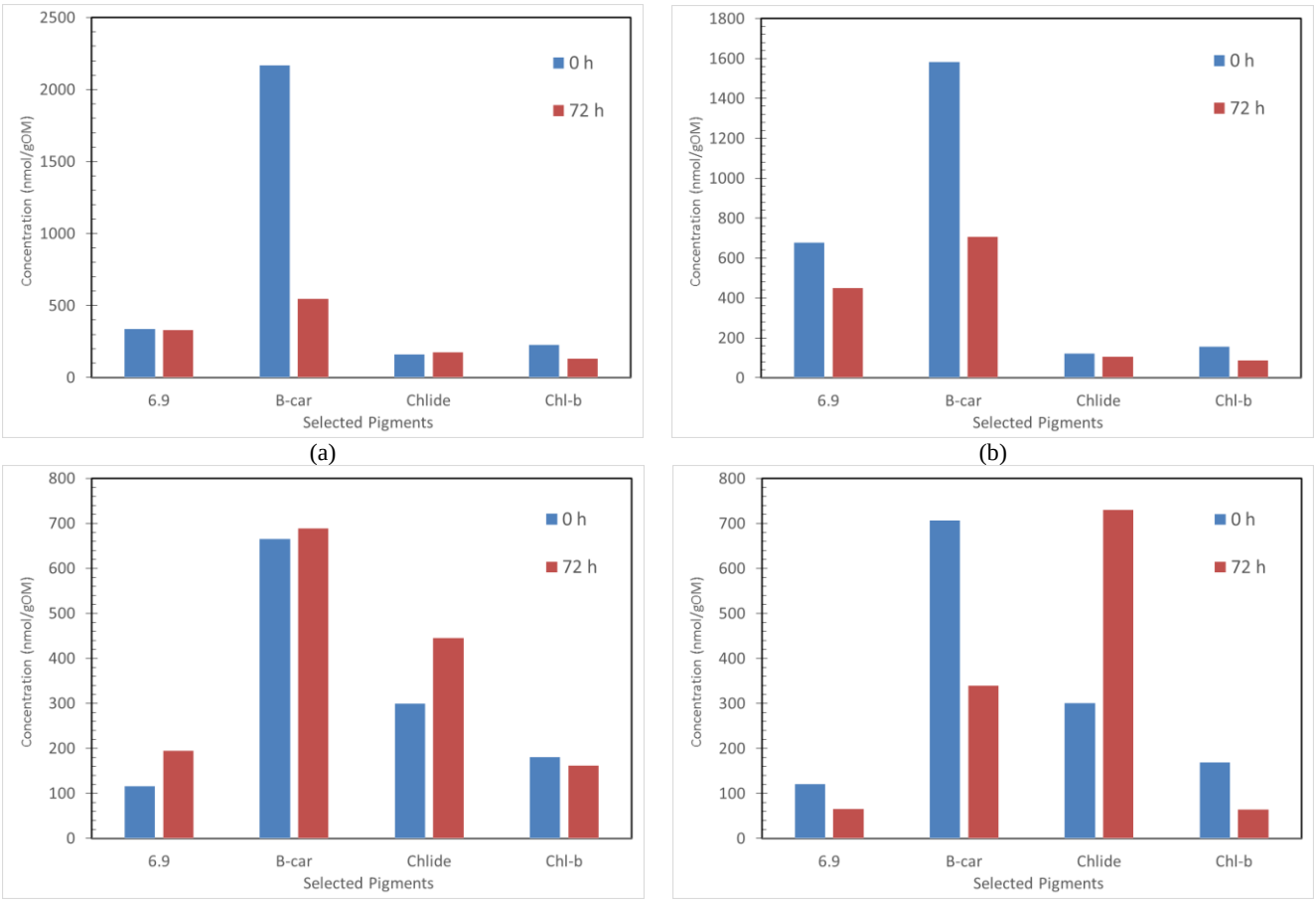


Figure 4.20 - Variation in pigments concentration before (0h) and after (72h) exposure to PFAS. Figures (a), (b), (c), and (d) are related to Channels 1, 2, 5, and 6, respectively

Furthermore, three ratios were calculated to better interpret the results from this analysis (Table 4.20). The Chlorophyll-*b*:β-carotene (Chl-b:β-car) ratio was used to describe the percentage of green algae and 6.9:β-carotene (6.9:β-car) was used to assess the percentage of blue-green algae. Furthermore, the 6.9: Chlorophyll-*b* (6.9:Chl-b) was used to assess the proportion blue-green:green algae.

Table 4.20 – Ratios used to describe community structure and overall abundance

	Channel	Chl-b:β-car (%)	6.9:β-car (%)	6.9:Chl-b
Time 0 (Prior to exposure)	1	29.01	26.12	2.03
	2	11.37	50.71	4.29
	5	23.34	16.06	0.72
	6	23.57	15.35	0.60
Time 72 (After Exposure)	1	25.86	59.54	2.47
	2	12.72	63.56	5.22
	5	23.48	29.10	1.24
	6	18.79	19.31	1.03

5. DISCUSSION

5.1 Biomass Production

Biomass productivity averaged 2.4360 ± 0.6177 gdw m^{-2} d^{-1} (n = 56, range = 1.348 to 4.0910 gdw m^{-2} d^{-1}) and 2.2985 ± 0.5909 gAFDW m^{-2} d^{-1} (n = 56, range = 1.2764 to 3.8683 gAFDW m^{-2} d^{-1}) during culturing and harvesting, and 2.9780 ± 0.6278 gdw m^{-2} d^{-1} (n = 4) during the exposure study. Other studies with ATS systems reported mean biomass production values of 5 gdw m^{-2} d^{-1} using agricultural drainage waste (Kangas and Mulbry 2014), 21.4 ± 8.5 gdw m^{-2} d^{-1} treating water from a drinking reservoir in China (Chen et al. 2015), 2 gdw m^{-2} d^{-1} filtering horticulture water (J. Liu et al. 2016), and up to 25 gdw m^{-2} d^{-1} at the highest loading rate using dairy manure (Walter Mulbry et al. 2008). Biomass productivity values are highly variable and depend on nutrient load, light regime, flow conditions, community structure, and ash content. Such variance in these properties makes it hard to draw comparisons. For instance, Blersch et al. (2013) reported productivity rates as high as 26.8 gdw m^{-2} d^{-1}, but used a higher concentration of macronutrients. Additionally, the Chinese study reported a high incidence of diatoms in the community, leading to an ash content of up to 91.4% of dry weight (Liu et al. 2016). If the reported rates were adjusted to ash content, productivity would be around 1.8404 gAFDW m^{-2} d^{-1}, which is more comparable to the values observed in this study. The study with the horticulture wastewater was the most suitable for comparisons, since it presented similar conditions to the ones used in this study, including community structure and nutrients rates. Finally, the production rate of 2 gdw m^{-2} d^{-1} observed in that study is very similar to the rates observed in this analysis.

5.2 Community Structure and Growth Parameters

Communities in Algal Turf Scrubber systems are usually composed of mixed species of periphytic algae. Naturally, species are competing for the same resources, and parameters such as temperature and light intensity can favor one specie over another. Georgii Gause postulated the Competitive Exclusion Principle, in which he demonstrated that two species occupying the same environment will compete for resources, resulting in the dominance of one over the other (Tilman 1977).

A shift in the algal community towards an *Oscillatoria sp.1* dominance as well as a decrease in algal diversity were observed in this work. Several studies have been conducted to characterize such shifts. First, there is evidence that blue-green algae have mechanisms that allow them to thrive at low CO_2 concentrations. As biomass production increases, dissolved CO_2 levels decrease, favoring blue-green over green algae species (Shapiro 1984). In fact, some studies reported a supplementary injection of CO_2 levels in growth media to prevent this limitation to occur (Yuvraj and Padmanabhan 2017). In addition, Havens et al. (2003) reviewed that blue-green algae were more competitive under low TN:TP ratios when compared to green algae (Havens et al. 2003). Bolds Basal Media has an N:P ratio of 1.4:1 and nitrogen limitation was observed in the channels before the three fold increase in Nitrate concentration (3N BBM). In agreement to that, Tilman et al. (1986) observed that blue-green species were dominant in a variety of N:P ratios at warmer temperatures (24 °C), but not at lower temperatures (Tilman et al. 1986), indicating temperature is also a contributing factor in species dominance.

Nonetheless, the shift in dominance in this analysis was only observed after the channels were illuminated with the Sunsource Heliovite lightbulbs, which contained UVA and UVB rays that were used to mimic sunlight irradiation. This suggests that UV exposure was the main factor responsible for this shift, since the same shift was not observed in the channels without UV exposure, despite having the same conditions. This claim is supported by Danilov and Ekelund (2008), which reported that even low levels of UV-B radiation were sufficient to shift a green algae and diatoms dominated marine community towards a

blue-green algae dominance (Danilov and Ekelund 2008). During a few harvests, the channels were deprived from UV rays on efforts to shift the community back towards a green alga dominance, which was not successful. This can be partially explained by Scheffer's alternative stable states theory, even though the algal community in this project is simpler than that to which this concept is usually applied. Scheffer observed that an ecosystem can dramatically change to a contrasting state. Because of hysteresis, additional efforts than simply bringing the system back to conditions prior to the shift must be made in order to achieve restoration (Scheffer et al. 2001).

Furthermore, results from this analysis suggested that UV light exposure reduced biomass production (see section 4.2.2). However, studies regarding UV exposure effects on biomass are not very consistent. For instance, Noyma et al. (2015) observed a significant reduction in algal cells in treatments exposed to UVA and UVB rays, whereas Santas et al. (1998) reported no change in productivity after the community was exposed to UVA rays (Noyma et al. 2015; Santas et al. 1998). Finally, these variables were not systematically changed, in a way that the dataset used in the biomass production analysis is highly unbalanced. This fact inserts a high degree of uncertainty and conclusions should be drawn with caution.

5.3 Exposure and Sorption Study

In the present study, an algal turf scrubber approach was evaluated as a remediation alternative for perfluoroalkyl substances. To observe the model's kinetics and effectiveness, water and algal material were sampled and analyzed for PFOS, PFOA, HFPO-DA, and PDHA content.

From the analyzed water samples either an exponential (PFOS, PFOA, HFPO-DA) or polynomial decrease (PDHA) trend was observed in the aqueous concentration in all channels, including the units with no algae. At the beginning of the experiment, the only removal mechanisms hypothesized were through direct uptake by the algae or adsorption onto algal cells. However, results from the mass balance indicated that a high quantity of the contaminant's initial mass was observed to be missing, especially for PFOS and PFOA. Thus, it was hypothesized that an unknown removal mechanism was responsible for this loss.

Leakage was ruled out as a possible mechanism for two reasons. First, no leaking and only minimal splashing were observed during the 72 hour experiment. Second, if the loss of mass happened due to leakage or splashing, the concentration profiles would be homogeneous and represent similar rates. However, changes in PDHA and HFPO-DA concentration due to unknown parameters were much smaller than PFOS and PFOA, weakening the leakage argument.

Additional experiments were conducted to obtain more information regarding this loss. Results from the preliminary abiotic transformation study using PFOS and PFOA suggested that media, pH adjustment and light configuration did not seem to affect their concentration. However, the limited number of samples did not make it possible for statistical analysis. This pattern was expected since PFOS and PFOA are perfluoroalkyl substances and are known to be highly stable. For instance, an OECD report described that PFOS cannot be abiotically transformed through hydrolysis or photolysis (OECD 2002), and an EPA report indicated the same for PFOA (US EPA 2002). Furthermore, results from the volatilization study indicate that PFOS and PFOA most likely did not volatilize during the 72 h experiment. That is supported by the fact that the variation in mass at the beginning and end of the experiment was not significant. This finding is in agreement with other studies in which PFOS and PFOA were not observed to volatilize under environmental conditions (KEMI 2015; Danish EPA 2015; Brooke, Footitt, and Nwaogu 2004). In addition, sorption of the contaminants onto the container's walls was also not observed, which is expected for HDPE materials (Weiss et al. 2015). This was concluded based on the fact that units C and D, which were covered to the atmosphere, did not present a significant variation in the concentration of both PFOS and PFOA. Finally, the units with Bolds Basal Media that were opened to the atmosphere presented higher volatilization rates than units with DI water. However, the opposite occurred in the units closed to the atmosphere. As a result of this inconsistency and high standard deviations, no conclusion regarding the direct effects of BBM salts in the behavior of PFOS and PFOA could be drawn from this analysis.

It was also hypothesized that the attachment of PFOS and PFOA onto the system materials could have played a role in mass loss. Results from the sorption experiment support this claim since sorption/attachment of PFOS and PFOA was observed in all the analyzed materials. Several studies have reported loss of PFASs to the container's walls. For example, Hu et al. (2014) reported that up to 34% of the initial concentration of PFOA was adsorbed to the containers walls (C. Hu, Luo, and Huang 2014). In contrast, two studies evaluated the sorption isotherms of PFOS and PFOA onto PVC solids and reported low sorption affinity (F. Wang, Shih, and Li 2015; Bakir, Rowland, and Thompson 2014). PFOS was reported to have a K_d of 100 L Kg^{-1} (F. Wang, Shih, and Li 2015), whereas PFOA was reported to have a much lower K_d of 7 L Kg^{1} (Bakir, Rowland, and Thompson 2014) . However, the study conducted by Wang and colleagues (2015) indicated that the sorption of PFOS was enhanced when salts were present in the media. The high sorption rates observed in this study could have been explained by the presence of salts from Bolds Basal Media. Furthermore, the increase in aqueous concentration observed in some samples during the main study could be related to desorption of these substances from the ATS materials.

The assumption that sorption was responsible for removing substances from the water can also help explain why the loss in mass was less considerable for HFPO-DA and PDHA. It is known that shorter chain PFAS are more soluble in water and have shown to have less binding affinities to solids (Brendel et al. 2018). Thus, these compounds would probably present lower sorption rates onto the ATS materials, as seen in this analysis.

Regarding the algal material, absorption rates were lower than 1% for PFOA, HFPO-DA, and PDHA, and ranged from 0.36-1.13% for PFOS. Even though several studies have analyzed the effects of PFAS in algal growth inhibition tests, uptake rates are not reported as often. Absorption rates from this analysis were found to be considerably lower than the ones available in the literature. For instance, Hu et al. (2014) observed that freshwater planktonic microalgae was capable of absorbing over 10% of the initial PFOA concentration; whereas Liu et al. (2018) observed an average uptake rate of 55.1% of the total Cl-PFESA concentration (W. Liu et al. 2018; C. Hu, Luo, and Huang 2014). Furthermore, PFOS and PFOA

absorption rates were considerably higher than HFPO-DA and PDHA. This is in agreement with recent reports that suggests shorter chain compounds are less bioaccumulative and present lower half lives in organisms, when compared to legacy substances (Danish EPA 2015).

As a result of low absorption rates, the calculated bioaccumulation factors (BAF) were also found to be lower than the ones previously reported in the literature. Log BAF in this analysis ranged from 0.76 to 1.47 L $kgww^{-1}$ for PFOA, 1.41 to 1.81 L $kgww^{-1}$ for PFOS, 0.32 to 0.77 L $kgww^{-1}$ for PDHA, and 0.27 to 0.43 L $kgww^{-1}$ for HFPO-DA. A study using marine plankton reported log BAF around 1.9-4.6 L $kgww^{-1}$ for PFOS and 3.1-4.6 L $kgww^{-1}$ for PFOA (Casal et al. 2017). A second study assessed the concentration of several PFAS in phytoplankton from the Taihu Lake, China and reported log BAF of 2.17 for PFOS and 1.36 for PFOA, which are more similar to the rates observed in this project (Fang et al. 2014). However, both studies were conducted in natural environments, and are more representative of a chronic exposure rather than an acute exposure like the one analyzed in this project.

Adsorption rates were also calculated and observed to range between 1.02 to 1.17 % for PFOA, 0.29 to 0.48% for PFOS, 0.06 to 0.42% for HFPO-DA, and 0.39 to 1.05% for PDHA. Adsorption rates are important because phytoplankton uptakes pollutants by the diffusion of materials adsorbed onto the surface (W. Liu et al. 2018). Similarly to what was observed in the uptake rates, adsorption values available in the literature were higher than the ones observed in this project. Hu et al. (2014) observed adsorption rates around 8% for PFOA and Liu et al. (2018) observed a rate of 44.9% of the initial Cl-PFESA concentration. Unlike in absorption, PDHA presented the highest adsorption rates among the four substances, followed by PFOA. One hypothesis to explain why this pattern occurred is that since PDHA presented the highest concentration left in the media after 72 hours, there was more available substance to be sorbed. However, more research is needed to precisely determine sorption pathways.

Results from the pigments analysis suggest that prior to exposure, Channels 1 and 2 had a higher quantity of blue-green algae, whereas green algae was more abundant in Channels 5 and 6. After the 72

hour exposure, an increase in the blue-green:green ratio was observed and blue-green algae was the most abundant in all channels. This can be explained by either one of the following hypothesis. The first alternative is that the blue-green algae species were more resilient to perfluorinated compounds than green algae species, presenting higher growth rates. This is unlikely since other study suggested that blue-green species are more sensitive to PFAS (Latała, Nedzi, and Stepnowski 2009). The second hypothesis is that the community was not highly affected by the contaminants and the cyanobacteria simply outcompeted the chlorophytes. This is more probable since the absorption of contaminants was low, and the parameters used in this study were observed to favor blue-green species over green, as shown in section 4.2.2.

The presence of diatoms and cryptophytes was also observed in the pigments analysis, which is expected since the rocks used to inoculate the channels were collected from a natural stream. It was also observed that the concentration of both Fucoxanthin and Alloxanthin decreased after the 72 hour experiment. Additionally, a significant increase of 49 and 143% in the concentration of Chlorophyllide was observed in Channels 5 and 6 after exposure, respectively. Chlorophyllide is a breakdown product from Chlorophyll-*a*, suggesting the communities from Channels 5 and 6 were under stress at the end of the experiment. However, these findings cannot be directly attributed to PFAS since a control for the algae with no compounds was not included in the experimental design. As a consequence, it can be argued that other parameters such as temperature and nutrient depletion could have contributed to the observed stress and changes in community structure.

Finally, the fact that the elimination/sequestration rates were lower than expected can be partially attributed to two factors. First and most importantly, loss in mass during the exposure study was observed to be as high as 90% of the initial concentration in some channels. Evidence from the sorption experiment suggested that attachment to the ATS materials was responsible for this decrease. This loss in mass dramatically decreased the available concentration of the substances in the media throughout the exposure experiment. Second, blue-green algae have been shown to be more sensitive to selected PFAS than green algae (Latała, Nedzi, and Stepnowski 2009), which could have directly interfered with uptake rates.

6. CONCLUSION AND RECOMMENDATIONS

To the best of the author's knowledge, this is the first study that evaluated the possibility of using an Algal Turf Scrubber™ as a remedial strategy for perfluoroalkyl substances. Biomass productivity observed in this study is comparable to others found in the literature when similar rates and community structure were used. Nonetheless, biomass production can still be optimized. Studies have shown that the presence of a wave surge device and flow turbulence rates can highly influence algal productivity (D'Aiuto et al. 2015; Blersch, Kangas, and Mulbry 2013). Light intensity can also alter algal production, either by irradiating too much light and inhibiting growth, or not emitting enough and limiting growth. Other parameters such as temperature, substratum, and nutrient levels have also been shown to affect algal production (Kesaano and Sims 2014).

Even though the removal rates were not as high as first expected, interesting indirect conclusions can be drawn from this study. It was shown that the materials used to build the ATS system severely interfered with the experiment. To overcome this issue, it is recommended that new studies should avoid the usage of PVC plastic and other materials that can interfere with uptake rates of perfluoroalkyl substances. Inert materials such as high density polyethylene (HDPE) and polypropylene (PP) should be employed instead. There is also empiric evidence that UV radiation seemed to have facilitated a shift in the algal community towards an *Oscillatoria sp.1* dominance. Future studies should focus on filamentous green algae since they have shown to be less sensitive to PFAS and could potentially display better rates.

Uptake and adsorption rates by periphytic algae have not been extensively studied, especially for HFPO-DA and PDHA. Results from this analysis can contribute to the growing understanding on the bioaccumulation potential of these compounds. Periphytic algae are in the bottom of several food chains and can potentially biomagnify PFAS into upper levels. In addition, it was observed that the algal

community employed in this study was capable of absorbing significant amounts of PFOS, PFOA, HFPO-DA, and PDHA. The parameters used in this study simulated environmental conditions, with the algal community being acquired from a local water source. Thus, findings from this research could be an indicator of what would happen in a natural environment.

Per- and polyfluoroalkyl substances are widely distributed in the environment, present toxic effects in humans and wildlife, and cannot be efficiently removed from contaminated matrices. There is still a wide gap in knowledge regarding the fate and behavior of these substances. In addition, much work needs to be done to optimize and understand algal growth as well as uptake and sorption pathways of PFAS onto algae. Future work is recommended:

1 – ATS is still a promising alternative since other studies have shown that algae can indeed uptake high quantities of certain PFAS. Different species should be evaluated for use in the ATS and conditions could be manipulated to optimize removal rates;

2 – A more comprehensive toxicity analysis should be conducted to better evaluate bioaccumulation ad biomagnification potentials;

3 – A wider range of compounds with different hydrophobicity properties should also be tested in the ATS system in mixture and individually. Synergetic and competitive effects of different PFAS have been hypothesized but not widely studied;

4 – Plastics and other similar materials should be studied to be used as a potential remedial alternative since they have shown affinity to PFAS sorption.

Finally, removal rates observed in this study were considerably lower when compared to other studies with algae and new remedial strategies. It can be concluded that the algal turf scrubber presented in this study is not suitable for the remediation of PFOS, PFOA, HFPO-DA, and PDHA.

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APPENDIX A: ASH FREE DRY WEIGHT TABULAR DATA

Table A.1 - Biomass data for Harvests 1 to 14

Channel	W crucible	W Crucible + Wet Biomass	W sample	% Water	% Ash	W biomass wet	DW	AFDW
Harvest # 1								
1	17.1730	17.2660	0.0930	13.7634	9.2269	2.7835	2.0333	1.7764
1	15.6570	15.7524	0.0954	32.5996	6.9984			
1	16.0455	16.1458	0.1003	34.4965	5.3272			
2	16.1131	16.2177	0.1046	19.4073	6.2871	3.2457	2.6276	2.4236
2	15.7470	15.8509	0.1039	22.3292	6.6914			
2	16.1829	16.2862	0.1033	15.3921	6.5217			
5	16.9283	17.0333	0.1050	15.0476	9.5291	3.6747	2.8612	2.5111
5	16.1400	16.2464	0.1064	20.7707	8.5409			
5	15.6491	15.7537	0.1046	30.5927	11.2948			
6	17.0617	17.1644	0.1027	16.6504	7.3598	3.2216	2.6005	2.3634
6	14.5899	14.6869	0.0970	15.6701	7.4572			
6	14.3793	14.5768	0.1975	25.519	7.6818			
Harvest # 2								
1	16.1121	16.2192	0.1071	10.3641	8.0208	2.2104	1.9781	1.8008
1	14.5895	14.6888	0.0993	10.3726	7.3034			
1	15.7464	15.8557	0.1093	10.796	5.1282			
2	15.6490	15.7595	0.1105	9.95475	7.3367	3.3076	2.9764	2.7338
2	15.6568	15.7648	0.1080	9.90741	6.9887			
2	17.1730	17.2762	0.1032	10.1744	7.0119			
5	16.9282	17.0312	0.1030	13.2039	4.1387	2.6037	2.2749	2.1671
5	16.0432	16.1454	0.1022	12.1331	5.0111			
5	16.1819	16.2879	0.1060	12.5472	5.0701			
6	17.0615	17.1601	0.0986	10.9533	5.8087	2.6843	2.3827	2.2268
6	14.3792	14.4858	0.1066	11.9137	5.6443			
6	16.1395	16.2410	0.1015	10.8374	6.1878			

Harvest # 3								
1	16.1394	16.2384	0.0990	11.4141	8.2098	2.3195	2.0618	1.8714
1	14.5896	14.6887	0.0991	10.8981	8.1540			
1	16.0435	16.1452	0.1017	11.0128	8.2873			
2	17.1729	17.2788	0.1059	10.1039	7.7731	2.2652	2.0402	1.8642
2	15.6566	15.7575	0.1009	9.9108	7.8108			
2	16.1122	16.2165	0.1043	9.77948	8.0765			
5	16.1819	16.2866	0.1047	11.3658	5.4957	2.5226	2.2346	2.0960
5	14.3787	14.4798	0.1011	11.3749	5.0223			
5	16.0627	16.1635	0.1008	11.5079	5.0448			
6	17.0613	17.1603	0.0990	12.1212	5.5172	2.2217	1.9579	1.8353
6	16.9281	17.0296	0.1015	11.7241	5.4687			
6	15.6490	15.7492	0.1002	11.7764	5.4299			
Harvest # 4								
1	17.1725	17.2732	0.1007	12.4131	6.4626	2.8914	2.5365	2.3497
1	16.0625	16.1634	0.1009	11.893	6.1867			
1	16.1122	16.2129	0.1007	12.5124	6.3564			
2	15.6561	15.7570	0.1009	11.3974	6.9351	3.034	2.6811	2.4707
2	16.1819	16.2826	0.1007	11.9166	6.4262			
2	14.5895	14.6905	0.1010	11.5842	6.3830			
5	15.6490	15.7503	0.1013	12.1422	5.0562	1.6131	1.4090	1.3274
5	16.9276	17.0278	0.1002	12.5749	5.4795			
5	16.0433	16.1437	0.1004	13.247	5.3961			
6	17.0611	17.1620	0.1009	12.884	4.8919	2.6338	2.2893	2.1605
6	15.7460	15.8463	0.1003	13.2602	4.9425			
6	16.1393	16.2401	0.1008	13.0952	4.4521			
Harvest # 5								
1	14.3784	14.4812	0.1028	9.24125	4.9303	2.6262	2.3647	2.2352
1	15.6487	15.7508	0.1021	10.284	5.4585			
1	17.1728	17.2733	0.1005	10.3483	4.7725			
2	15.6564	15.7565	0.1001	11.5884	4.8588	2.8923	2.5761	2.4356
2	14.5892	14.6933	0.1041	10.6628	5.0538			
2	16.0621	16.1655	0.1034	10.5416	5.2973			
5	16.0430	16.1447	0.1017	9.93117	5.2402	2.8773	2.5801	2.4293
5	16.1819	16.2834	0.1015	11.133	5.4324			
5	15.7459	15.8487	0.1028	9.92218	5.5076			
6	16.1116	16.2148	0.1032	10.9496	5.0054	3.4316	3.0406	2.8688
6	16.9276	17.0290	0.1014	11.3412	4.4494			
6	17.0611	17.1620	0.1009	11.893	4.6119			

Harvest # 6								
1	15.6489	15.7523	0.1034	14.7969	3.9728	3.9848	3.3676	3.2093
1	15.7464	15.8465	0.1001	15.8841	3.4442			
1	14.5898	14.6918	0.1020	15.7843	3.8417			
2	16.1822	16.2846	0.1024	14.3555	4.3330	4.5212	3.8545	3.6586
2	17.0613	17.1638	0.1025	14.9268	4.2431			
2	16.1124	16.2127	0.1003	14.9551	4.1032			
5	16.0435	16.1462	0.1027	14.4109	4.2093	4.0244	3.4415	3.2721
5	16.9278	17.0283	0.1005	14.7264	4.2007			
5	17.1729	17.2749	0.1020	14.3137	5.3776			
6	15.6568	15.7573	0.1005	14.4279	3.6047	4.1108	3.5260	3.3778
6	16.0628	16.1637	0.1009	14.3707	3.9352			
6	14.3785	14.4808	0.1023	13.8807	3.8593			
Harvest # 7								
1	17.1735	17.2743	0.1008	15.6746	4.0000	4.4250	3.7342	3.5572
1	16.9279	17.0295	0.1016	15.5512	4.0793			
1	16.1822	16.2828	0.1006	15.6064	4.0047			
2	16.1122	16.2125	0.1003	14.5563	4.4341	3.8501	3.3021	3.1314
2	14.3786	14.4792	0.1006	14.1153	4.1667			
2	16.0436	16.1441	0.1005	14.0299	4.0509			
5	16.1393	16.2396	0.1003	14.5563	5.6009	3.1753	2.7472	2.5694
5	16.0629	16.1642	0.1013	13.0306	5.3348			
5	14.5897	14.6916	0.1019	12.8557	5.2928			
6	17.0610	17.1625	0.1015	13.7931	4.6857	4.9439	4.2547	4.0230
6	15.6567	15.7585	0.1018	13.4578	4.5403			
6	15.7465	15.8467	0.1002	14.5709	4.5561			
Harvest # 8								
1	17.0611	17.1619	0.1008	12.7976	4.3231	2.4014	2.0903	1.9865
1	15.7464	15.8478	0.1014	13.4122	3.9863			
1	16.0628	16.1632	0.1004	12.6494	4.1049			
2	16.1393	16.2404	0.1011	13.0564	4.4369	2.4973	2.1604	2.0496
2	16.0434	16.1446	0.1012	13.834	4.4725			
2	16.1122	16.2131	0.1009	13.5778	4.3578			
5	14.5894	14.6902	0.1008	11.6071	4.7138	3.0784	2.6919	2.5468
5	16.1823	16.2837	0.1014	13.0178	4.1950			
5	16.9277	17.0297	0.1020	13.0392	4.5096			
6	14.3784	14.4784	0.1000	12.7	4.5819	3.304	2.8858	2.7345
6	15.6490	15.7495	0.1005	12.4378	4.4318			
6	15.6567	15.7588	0.1021	12.8306	4.4944			

Harvest # 9								
1	15.6569	15.7571	0.1002	11.477	4.6223	4.0955	3.6121	3.4228
1	16.0437	16.1451	0.1014	12.5247	4.6223			
1	16.1395	16.2412	0.1017	11.4061	4.6615			
2	16.9277	17.0280	0.1003	11.7647	4.2938	3.4141	3.0131	2.8665
2	17.0612	17.1624	0.1012	11.9565	4.7138			
2	14.5893	14.6900	0.1007	11.5194	4.9383			
5	15.6489	15.7495	0.1006	10.7356	4.7884	3.1447	2.8018	2.6512
5	16.1123	16.2134	0.1011	10.9792	4.7778			
5	17.1733	17.2733	0.1000	11	4.6067			
6	16.1827	16.2835	0.1008	11.6071	4.2649	3.3472	2.9679	2.8252
6	16.0628	16.1636	0.1008	11.1111	4.4643			
6	15.7463	15.8474	0.1011	11.276	4.7938			
Harvest # 10								
1	15.6569	15.7582	0.1013	10.6614	4.0884	3.5976	3.2266	3.0795
1	17.1732	17.2746	0.1014	9.86193	4.3764			
1	16.1824	16.2842	0.1018	10.4126	4.0570			
2	15.6492	15.7500	0.1008	9.82143	3.9604	3.1286	2.8304	2.7065
2	14.5896	14.6902	0.1006	9.74155	4.1850			
2	16.0627	16.1635	0.1008	9.02778	4.1439			
5	17.0615	17.1632	0.1017	9.04621	5.2973	1.9227	1.7553	1.6534
5	16.0437	16.1446	0.1009	8.12686	4.6386			
5	16.9279	17.0285	0.1006	8.94632	4.8035			
6	15.7464	15.8473	0.1009	8.42418	4.3290	2.2953	2.1052	2.0059
6	16.1395	16.2403	0.1008	8.23413	4.2162			
6	16.1126	16.2140	0.1014	8.1854	3.7594			
Harvest # 11								
1	17.0618	17.1621	0.1003	12.2632	5.2273	3.1716	2.7708	2.6050
1	16.0634	16.1652	0.1018	12.9666	5.0790			
1	16.1830	16.2847	0.1017	12.6844	6.0811			
2	15.6573	15.7589	0.1016	12.6969	5.5242	2.9547	2.5828	2.4196
2	15.6497	15.7509	0.1012	12.9447	5.6754			
2	16.0440	16.1455	0.1015	12.1182	5.6054			
5	16.9284	17.0311	0.1027	12.4635	5.0056	2.2188	1.9408	1.8297
5	14.5902	14.6916	0.1014	12.426	4.7297			
5	17.1738	17.2754	0.1016	12.6969	5.1860			
6	16.1129	16.2132	0.1003	11.2662	5.7303	2.2958	2.0260	1.8944
6	15.7469	15.8494	0.1025	12.2927	4.8943			
6	16.1400	16.2417	0.1017	11.7011	5.1225			

Harvest # 12								
1	14.5905	14.6913	0.1008	20.0397	4.0943	3.0931	2.4828	2.3562
1	16.1133	16.2139	0.1006	19.6819	4.5792			
1	15.6576	15.7593	0.1017	19.469	4.2735			
2	15.7470	15.8476	0.1006	15.7058	4.4811	3.0542	2.5837	2.4468
2	17.0617	17.1624	0.1007	15.3923	4.6948			
2	16.0442	16.1454	0.1012	15.1186	4.5402			
5	16.9283	17.0291	0.1008	16.8651	5.2506	1.784	1.4884	1.3947
5	16.1832	16.2832	0.1000	16.8	5.4087			
5	17.1738	17.2741	0.1003	16.0518	5.3444			
6	16.0637	16.1657	0.1020	14.902	4.0323	1.8454	1.5742	1.4997
6	16.1403	16.2420	0.1017	14.6509	4.1475			
6	15.6496	15.7500	0.1004	14.5418	4.3124			
Harvest # 13								
1	14.3789	14.4793	0.1004	15.0398	4.1032	1.7560	1.4927	1.4206
1	16.1832	16.2848	0.1016	14.7638	3.8106			
1	15.6497	15.7511	0.1014	15.1874	3.7209			
2	16.1403	16.2409	0.1006	14.6123	5.2386	1.8844	1.6085	1.5098
2	15.6574	15.7579	0.1005	14.8259	5.3738			
2	15.7471	15.8486	0.1015	14.4828	4.7235			
5	17.0619	17.1641	0.1022	15.362	4.5087	3.0339	2.5913	2.4545
5	14.5903	14.6906	0.1003	14.2572	4.0698			
5	16.0441	16.1452	0.1011	14.1444	4.4931			
6	16.9282	17.0293	0.1011	14.2433	3.9216	2.3763	2.0349	1.9417
6	17.1742	17.2755	0.1013	14.8075	2.8969			
6	16.0636	16.1654	0.1018	14.0472	3.6571			
Harvest # 14								
1	14.3789	14.4800	0.1011	15.0346	4.5203	2.8574	2.4200	2.2908
1	16.1830	16.2841	0.1011	15.9248	3.7984			
1	15.6497	15.7506	0.1009	14.9653	4.6523			
2	16.1402	16.2417	0.1015	15.1724	4.7958	3.5123	2.9698	2.8014
2	15.6570	15.7586	0.1016	15.1575	5.3256			
2	15.7469	15.8475	0.1006	16.004	3.9987			
5	17.0618	17.1630	0.1012	16.2055	4.2051	2.0177	1.6979	1.6130
5	16.0444	16.1457	0.1013	15.8934	4.5987			
5	14.5899	14.6915	0.1016	15.4528	4.9785			
6	16.9279	17.0301	0.1022	14.775	5.0134	2.0202	1.7306	1.6293
6	16.1133	16.2136	0.1003	14.5563	4.0687			
6	16.0632	16.1641	0.1009	13.6769	3.9875			

APPENDIX B: AQUEOUS CONCENTRATION OF TARGETED SUBSTANCES FOR EXPOSURE EXPERIMENT

B.1 Tabular Data for PFOA from experimental channels and positive controls

Table B.1 - Dilution (DF), Concentration Factors (CF) and Adj Concentration of PFOA for Channel 1

CH 1						
Sample	**µg/15L**	**DF**	**CF**	**Adj Conc µg/15L**	**Average**	**St Dev**
CH1_72h_11A_1	11.9606	1.1033	1.2804	10.3068		
CH1_72h_11A_2	13.0898	1.1033	1.2804	11.2799	10.9248	0.5372
CH1_72h_11A_3	12.9830	1.1033	1.2804	11.1878		
CH1_52h_10A_1	13.4191	1.0867	1.1913	12.2405		
CH1_52h_10A_2	12.5755	1.0867	1.1913	11.4710	11.9100	0.3961
CH1_52h_10A_3	13.1760	1.0867	1.1913	12.0187		
CH1_32h_9A_1	12.7188	1.0700	1.1610	11.7217		
CH1_32h_9A_2	13.1782	1.0700	1.1610	12.1451	11.8229	0.2853
CH1_32h_9A_3	12.5890	1.0700	1.1610	11.6021		
CH1_16h_8A_1	13.0973	1.0500	1.0538	13.0495		
CH1_16h_8A_2	13.2499	1.0500	1.0538	13.2016	13.3316	0.3650
CH1_16h_8A_3	13.7942	1.0500	1.0538	13.7439		
CH1_8h_7A_1	15.0828	1.0367	1.0301	15.1793		
CH1_8h_7A_2	14.9870	1.0367	1.0301	15.0829	15.1908	0.1141
CH1_8h_7A_3	15.2129	1.0367	1.0301	15.3102		
CH1_4h_6A_1	15.1330	1.0300	1.0148	15.3594		
CH1_4h_6A_2	15.0749	1.0300	1.0148	15.3005	15.0314	0.5180
CH1_4h_6A_3	14.2214	1.0300	1.0148	14.4342		
CH1_60m_5A_1	15.2597	1.0233	1.0000	15.6157		
CH1_60m_5A_2	14.6191	1.0233	1.0000	14.9602	15.1453	0.4105
CH1_60m_5A_3	14.5212	1.0233	1.0000	14.8600		
CH1_30m_4A_1	15.9986	1.0133	1.0000	16.2120		
CH1_30m_4A_2	16.1645	1.0133	1.0000	16.3800	16.0527	0.4297
CH1_30m_4A_3	15.3612	1.0133	1.0000	15.5660		
CH1_10m_3A_1	17.3978	1.0067	1.0000	17.5138		
CH1_10m_3A_2	16.5881	1.0067	1.0000	16.6987	17.1195	0.4082
CH1_10m_3A_3	17.0326	1.0067	1.0000	17.1461		

CH1_0+_2A_1	17.5058	1.0033	1.0000	17.5642		
CH1_0+_2A_2	17.7922	1.0033	1.0000	17.8515	17.6852	0.1489
CH1_0+_2A_3	17.5812	1.0033	1.0000	17.6398		
CH1_0_1A_1	20.2255	1.0000	1.0000	20.2255		
CH1_0_1A_2	19.0279	1.0000	1.0000	19.0279	19.6058	0.5999
CH1_0_1A_3	19.5641	1.0000	1.0000	19.5641		

Table B.2 - Dilution (DF), Concentration Factors (CF) and Adj Concentration of PFOA for Channel 2

CH 2						
Sample	**µg/15L**	**DF**	**CF**	**Adj Conc µg/15L**	**Average**	**St Dev**
CH2_72h_11B_1	14.8104	1.1033	1.3148	12.4282		
CH2_72h_11B_2	16.0296	1.1033	1.3148	13.4513	12.8495	0.5349
CH2_72h_11B_3	15.0972	1.1033	1.3148	12.6689		
CH2_52h_10B_1	15.6000	1.0867	1.2137	13.9675		
CH2_52h_10B_2	15.9698	1.0867	1.2137	14.2986	14.3560	0.4201
CH2_52h_10B_3	16.5319	1.0867	1.2137	14.8019		
CH2_32h_9B_1	16.3399	1.0700	1.1181	15.6368		
CH2_32h_9B_2	15.8599	1.0700	1.1181	15.1775	15.2973	0.2983
CH2_32h_9B_3	15.7555	1.0700	1.1181	15.0776		
CH2_16h_8B_1	16.8624	1.0500	1.0519	16.8327		
CH2_16h_8B_2	16.9610	1.0500	1.0519	16.9312	16.7934	0.1610
CH2_16h_8B_3	16.6457	1.0500	1.0519	16.6164		
CH2_8h_7B_1	16.1981	1.0367	1.0216	16.4372		
CH2_8h_7B_2	16.0001	1.0367	1.0216	16.2363	16.3891	0.1353
CH2_8h_7B_3	16.2538	1.0367	1.0216	16.4938		
CH2_4h_6B_1	15.8635	1.0300	1.0071	16.2244		
CH2_4h_6B_2	16.8994	1.0300	1.0071	17.2838	16.6272	0.5734
CH2_4h_6B_3	16.0094	1.0300	1.0071	16.3736		
CH2_60m_5B_1	15.9250	1.0233	1.0000	16.2965		
CH2_60m_5B_2	15.8782	1.0233	1.0000	16.2487	16.6887	0.7211
CH2_60m_5B_3	17.1214	1.0233	1.0000	17.5209		
CH2_30m_4B_1	17.0141	1.0133	1.0000	17.2409		
CH2_30m_4B_2	18.1392	1.0133	1.0000	18.3811	17.6488	0.6355
CH2_30m_4B_3	17.0964	1.0133	1.0000	17.3244		
CH2_10m_3B_1	18.8746	1.0067	1.0000	19.0004		
CH2_10m_3B_2	19.3452	1.0067	1.0000	19.4742	19.2380	0.2369
CH2_10m_3B_3	19.1119	1.0067	1.0000	19.2393		
CH2_0+_2B_1	21.0158	1.0033	1.0000	21.0859		
CH2_0+_2B_2	21.2520	1.0033	1.0000	21.3228	21.4717	0.4780
CH2_0+_2B_3	21.9334	1.0033	1.0000	22.0065		
CH2_0_1B_1	20.8015	1.0000	1.0000	20.8015		

CH2_0_1B_2	19.7635	1.0000	1.0000	19.7635	20.3582	0.5353
CH2_0_1B_3	20.5094	1.0000	1.0000	20.5094		

Table B.3 - Dilution (DF), Concentration Factors (CF) and Adj Concentration of PFOA for Channel 3

CH 3						
Sample	**µg/15L**	**DF**	**CF**	**Adj Conc µg/15L**	**Average**	**St Dev**
CH3_72h_11C_1	6.3850	1.1033	1.1570	6.0887		
CH3_72h_11C_2	6.6965	1.1033	1.1570	6.3857	6.3072	0.1917
CH3_72h_11C_3	6.7608	1.1033	1.1570	6.4471		
CH3_52h_10C_1	7.3747	1.0867	1.1024	7.2697		
CH3_52h_10C_2	7.4717	1.0867	1.1024	7.3653	7.3132	0.0484
CH3_52h_10C_3	7.4100	1.0867	1.1024	7.3045		
CH3_32h_9C_1	9.9674	1.0700	1.0606	10.0557		
CH3_32h_9C_2	9.7342	1.0700	1.0606	9.8204	10.1205	0.3373
CH3_32h_9C_3	10.3934	1.0700	1.0606	10.4855		
CH3_16h_8C_1	15.2866	1.0500	1.0294	15.5923		
CH3_16h_8C_2	14.8219	1.0500	1.0294	15.1184	15.4681	0.3071
CH3_16h_8C_3	15.3859	1.0500	1.0294	15.6936		
CH3_8h_7C_1	17.5886	1.0367	1.0145	17.9731		
CH3_8h_7C_2	16.5329	1.0367	1.0145	16.8942	17.2068	0.6673
CH3_8h_7C_3	16.3949	1.0367	1.0145	16.7532		
CH3_4h_6C_1	21.4728	1.0300	1.0072	21.9590		
CH3_4h_6C_2	20.2718	1.0300	1.0072	20.7309	21.9776	1.2561
CH3_4h_6C_3	22.7282	1.0300	1.0072	23.2429		
CH3_60m_5C_1	21.7207	1.0233	1.0000	22.2275		
CH3_60m_5C_2	22.3663	1.0233	1.0000	22.8882	22.3993	0.4296
CH3_60m_5C_3	21.5786	1.0233	1.0000	22.0821		
CH3_30m_4C_1	22.7772	1.0133	1.0000	23.0809		
CH3_30m_4C_2	22.4748	1.0133	1.0000	22.7745	23.2485	0.5765
CH3_30m_4C_3	23.5759	1.0133	1.0000	23.8903		
CH3_10m_3C_1	20.5433	1.0067	1.0000	20.6802		
CH3_10m_3C_2	21.1634	1.0067	1.0000	21.3045	21.3751	0.7327
CH3_10m_3C_3	21.9938	1.0067	1.0000	22.1405		
CH3_0+_2C_1	23.1228	1.0033	1.0000	23.1999		
CH3_0+_2C_2	21.8119	1.0033	1.0000	21.8846	22.1816	0.9070
CH3_0+_2C_3	21.3890	1.0033	1.0000	21.4603		
CH3_0_1C_1	20.8697	1.0000	1.0000	20.8697		
CH3_0_1C_2	22.4774	1.0000	1.0000	22.4774	21.4634	0.8825
CH3_0_1C_3	21.0430	1.0000	1.0000	21.0430		

Table B.4 - Dilution (DF), Concentration Factors (CF) and Adj Concentration of PFOA for Channel 5

CH 5						
Sample	**µg/15L**	**DF**	**CF**	**Adj Conc µg/15L**	**Average**	**St Dev**
CH5_72h_11E_1	6.1738	1.1033	1.4433	4.7195		
CH5_72h_11E_2	6.3526	1.1033	1.4433	4.8562	4.7140	0.1451
CH5_72h_11E_3	5.9731	1.1033	1.4433	4.5662		
CH5_52h_11E_1	8.1072	1.0867	1.2844	6.8591		
CH5_52h_11E_2	8.4403	1.0867	1.2844	7.1409	6.9546	0.1614
CH5_52h_11E_3	8.1127	1.0867	1.2844	6.8637		
CH5_32h_11E_1	9.3614	1.0700	1.1765	8.5142		
CH5_32h_11E_2	9.7894	1.0700	1.1765	8.9034	8.7204	0.1956
CH5_32h_11E_3	9.6134	1.0700	1.1765	8.7434		
CH5_16h_11E_1	12.2580	1.0500	1.0606	12.1354		
CH5_16h_11E_2	12.5057	1.0500	1.0606	12.3806	12.3095	0.1516
CH5_16h_11E_3	12.5378	1.0500	1.0606	12.4125		
CH5_8h_11E_1	13.7611	1.0367	1.0145	14.0619		
CH5_8h_11E_2	13.6766	1.0367	1.0145	13.9756	14.1068	0.1585
CH5_8h_11E_3	13.9774	1.0367	1.0145	14.2829		
CH5_4h_11E_1	15.1639	1.0300	1.0072	15.5073		
CH5_4h_11E_2	15.8033	1.0300	1.0072	16.1611	16.0520	0.4992
CH5_4h_11E_3	16.1225	1.0300	1.0072	16.4875		
CH5_60m_11E_1	15.9895	1.0233	1.0000	16.3626		
CH5_60m_11E_2	16.1633	1.0233	1.0000	16.5404	16.2778	0.3138
CH5_60m_11E_3	15.5671	1.0233	1.0000	15.9304		
CH5_30m_11E_1	16.9752	1.0133	1.0000	17.2015		
CH5_30m_11E_2	18.2573	1.0133	1.0000	18.5007	17.9119	0.6581
CH5_30m_11E_3	17.7962	1.0133	1.0000	18.0335		
CH5_10m_11E_1	17.3722	1.0067	1.0000	17.4880		
CH5_10m_11E_2	17.6662	1.0067	1.0000	17.7839	17.7872	0.3009
CH5_10m_11E_3	17.9700	1.0067	1.0000	18.0898		
CH5_0+_2E_1	19.1314	1.0033	1.0000	19.1951		
CH5_0+_2E_2	18.6072	1.0033	1.0000	18.6692	19.0001	0.2881
CH5_0+_2E_3	19.0723	1.0033	1.0000	19.1359		
CH5_0_1D_1	19.2667	1.0000	1.0000	19.2667		
CH5_0_1D_2	18.7783	1.0000	1.0000	18.7783	18.5579	0.8409
CH5_0_1D_3	17.6287	1.0000	1.0000	17.6287		

Table B.5 - Dilution (DF), Concentration Factors (CF) and Adj Concentration of PFOA for Channel 6

CH 6						
Sample	**µg/15L**	**DF**	**CF**	**Adj Conc µg/15L**	**Average**	**St Dev**
CH6_72h_11F_1	4.9910	1.1033	1.4688	3.7493		
CH6_72h_11F_2	5.1302	1.1033	1.4688	3.8539	3.7489	0.1052
CH6_72h_11F_3	4.8502	1.1033	1.4688	3.6435		
CH6_52h_11F_1	6.9694	1.0867	1.2818	5.9083		
CH6_52h_11F_2	7.0543	1.0867	1.2818	5.9803	5.8947	0.0931
CH6_52h_11F_3	6.8364	1.0867	1.2818	5.7956		
CH6_32h_11F_1	9.3382	1.0700	1.1463	8.7163		
CH6_32h_11F_2	9.2429	1.0700	1.1463	8.6273	8.5669	0.1870
CH6_32h_11F_3	8.9534	1.0700	1.1463	8.3572		
CH6_16h_11F_1	13.3034	1.0500	1.0602	13.1761		
CH6_16h_11F_2	13.5228	1.0500	1.0602	13.3933	13.4952	0.3805
CH6_16h_11F_3	14.0508	1.0500	1.0602	13.9163		
CH6_8h_11F_1	15.3802	1.0367	1.0292	15.4918		
CH6_8h_11F_2	15.7358	1.0367	1.0292	15.8500	15.7746	0.2537
CH6_8h_11F_3	15.8669	1.0367	1.0292	15.9820		
CH6_4h_11F_1	16.3678	1.0300	1.0071	16.7392		
CH6_4h_11F_2	16.5300	1.0300	1.0071	16.9051	16.6494	0.3106
CH6_4h_11F_3	15.9420	1.0300	1.0071	16.3038		
CH6_60m_11F_1	18.2532	1.0233	1.0000	18.6791		
CH6_60m_11F_2	18.2270	1.0233	1.0000	18.6523	18.3879	0.4814
CH6_60m_11F_3	17.4257	1.0233	1.0000	17.8323		
CH6_30m_11F_1	21.0991	1.0133	1.0000	21.3804		
CH6_30m_11F_2	21.2460	1.0133	1.0000	21.5293	21.6640	0.3698
CH6_30m_11F_3	21.7918	1.0133	1.0000	22.0823		
CH6_10m_11F_1	18.9962	1.0067	1.0000	19.1229		
CH6_10m_11F_2	19.4342	1.0067	1.0000	19.5638	19.7312	0.7071
CH6_10m_11F_3	20.3712	1.0067	1.0000	20.5070		
CH6_0+_2F_1	20.7698	1.0033	1.0000	20.8391		
CH6_0+_2F_2	19.8062	1.0033	1.0000	19.8723	20.3960	0.4884
CH6_0+_2F_3	20.4086	1.0033	1.0000	20.4767		
CH6_0_1E_1	20.0995	1.0000	1.0000	20.0995		
CH6_0_1E_2	20.8572	1.0000	1.0000	20.8572	20.3218	0.4658
CH6_0_1E_3	20.0088	1.0000	1.0000	20.0088		

Table B.6 - Dilution (DF), Concentration Factors (CF) and Adj Concentration of PFOA for Channel 7

CH 7						
Sample	**µg/15L**	**DF**	**CF**	**Adj Conc µg/15L**	**Average**	**St Dev**
CH7_72h_11D_1	6.5549	1.1033	1.1667	6.1990		
CH7_72h_11D_2	6.3955	1.1033	1.1667	6.0483	6.0778	0.1096
CH7_72h_11D_3	6.3295	1.1033	1.1667	5.9859		
CH7_52h_10D_1	8.3014	1.0867	1.1024	8.1832		
CH7_52h_10D_2	8.5298	1.0867	1.1024	8.4084	8.1929	0.2108
CH7_52h_10D_3	8.1024	1.0867	1.1024	7.9870		
CH7_32h_9D_1	12.5813	1.0700	1.0606	12.6927		
CH7_32h_9D_2	12.6576	1.0700	1.0606	12.7697	12.7429	0.0435
CH7_32h_9D_3	12.6542	1.0700	1.0606	12.7663		
CH7_16h_8D_1	14.8716	1.0500	1.0072	15.5036		
CH7_16h_8D_2	15.0305	1.0500	1.0072	15.6693	15.6560	0.1462
CH7_16h_8D_3	15.1512	1.0500	1.0072	15.7951		
CH7_8h_7D_1	17.3906	1.0367	1.0000	18.0283		
CH7_8h_7D_2	18.0079	1.0367	1.0000	18.6682	18.3608	0.3207
CH7_8h_7D_3	17.7355	1.0367	1.0000	18.3858		
CH7_4h_6D_1	18.7819	1.0300	1.0000	19.3454		
CH7_4h_6D_2	18.8270	1.0300	1.0000	19.3919	19.0702	0.5175
CH7_4h_6D_3	17.9352	1.0300	1.0000	18.4733		
CH7_60m_5D_1	22.6258	1.0233	1.0000	23.1537		
CH7_60m_5D_2	20.7862	1.0233	1.0000	21.2712	22.2476	0.9432
CH7_60m_5D_3	21.8090	1.0233	1.0000	22.3179		
CH7_30m_4D_1	21.1303	1.0133	1.0000	21.4121		
CH7_30m_4D_2	21.2256	1.0133	1.0000	21.5086	21.7759	0.5487
CH7_30m_4D_3	22.1122	1.0133	1.0000	22.4070		
CH7_10m_3D_1	17.4410	1.0067	1.0000	17.5573		
CH7_10m_3D_2	18.5484	1.0067	1.0000	18.6721	17.7720	0.8142
CH7_10m_3D_3	16.9735	1.0067	1.0000	17.0867		
CH7_0+_2D_1	20.9268	1.0033	1.0000	20.9966		
CH7_0+_2D_2	20.8390	1.0033	1.0000	20.9084	20.8995	0.1018
CH7_0+_2D_3	20.7245	1.0033	1.0000	20.7936		
CH7_0_1F_1	23.2505	1.0000	1.0000	23.2505		
CH7_0_1F_2	22.4916	1.0000	1.0000	22.4916	23.0122	0.4514
CH7_0_1F_3	23.2944	1.0000	1.0000	23.2944		

B.2 Tabular Data for PFOS from experimental channels and positive control

Table B.7 - Dilution (DF), Concentration Factors (CF) and Adj Concentration of PFOS for Channel 1

CH 1						
Sample	**µg/15L**	**DF**	**CF**	**Adj Conc µg/15L**	**Average**	**St Dev**
CH1_72h_11A_1	6.0422	1.1033	1.2804	5.2068		
CH1_72h_11A_2	6.2395	1.1033	1.2804	5.3768	5.3242	0.1019
CH1_72h_11A_3	6.2537	1.1033	1.2804	5.3890		
CH1_52h_10A_1	9.0763	1.0867	1.1913	8.2791		
CH1_52h_10A_2	8.6184	1.0867	1.1913	7.8614	8.1201	0.2260
CH1_52h_10A_3	9.0113	1.0867	1.1913	8.2198		
CH1_32h_9A_1	6.9540	1.0700	1.1610	6.4088		
CH1_32h_9A_2	7.1234	1.0700	1.1610	6.5650	6.4181	0.1424
CH1_32h_9A_3	6.8148	1.0700	1.1610	6.2806		
CH1_16h_8A_1	7.3558	1.0500	1.0538	7.3289		
CH1_16h_8A_2	7.5466	1.0500	1.0538	7.5190	7.5459	0.2316
CH1_16h_8A_3	7.8182	1.0500	1.0538	7.7897		
CH1_8h_7A_1	9.0098	1.0367	1.0301	9.0675		
CH1_8h_7A_2	8.7247	1.0367	1.0301	8.7805	9.0113	0.2084
CH1_8h_7A_3	9.1274	1.0367	1.0301	9.1858		
CH1_4h_6A_1	9.7711	1.0300	1.0148	9.9173		
CH1_4h_6A_2	10.5886	1.0300	1.0148	10.7470	10.1958	0.4774
CH1_4h_6A_3	9.7766	1.0300	1.0148	9.9229		
CH1_60m_5A_1	12.2062	1.0233	1.0000	12.4910		
CH1_60m_5A_2	11.6714	1.0233	1.0000	11.9438	11.9985	0.4676
CH1_60m_5A_3	11.2970	1.0233	1.0000	11.5606		
CH1_30m_4A_1	14.1614	1.0133	1.0000	14.3503		
CH1_30m_4A_2	14.6623	1.0133	1.0000	14.8578	14.4547	0.3624
CH1_30m_4A_3	13.9697	1.0133	1.0000	14.1559		
CH1_10m_3A_1	19.4016	1.0067	1.0000	19.5309		
CH1_10m_3A_2	18.3607	1.0067	1.0000	18.4831	19.0949	0.5455
CH1_10m_3A_3	19.1429	1.0067	1.0000	19.2705		
CH1_0+_2A_1	18.9713	1.0033	1.0000	19.0345		
CH1_0+_2A_2	19.4477	1.0033	1.0000	19.5125	18.9867	0.5513
CH1_0+_2A_3	18.3518	1.0033	1.0000	18.4130		
CH1_0_1A_1	20.9018	1.0000	1.0000	20.9018		
CH1_0_1A_2	20.1026	1.0000	1.0000	20.1026	20.4926	0.3999
CH1_0_1A_3	20.4734	1.0000	1.0000	20.4734		

Table B.8 - Dilution (DF), Concentration Factors (CF) and Adj Concentration of PFOS for Channel 2

CH 2						
Sample	**µg/15L**	**DF**	**CF**	**Adj Conc µg/15L**	**Average**	**St Dev**
CH2_72h_11B_1	8.0129	1.1033	1.3148	6.7240		
CH2_72h_11B_2	8.3906	1.1033	1.3148	7.0410	6.8731	0.1593
CH2_72h_11B_3	8.1682	1.1033	1.3148	6.8544		
CH2_52h_10B_1	8.2111	1.0867	1.2137	7.3518		
CH2_52h_10B_2	8.3717	1.0867	1.2137	7.4956	7.6023	0.3175
CH2_52h_10B_3	8.8896	1.0867	1.2137	7.9593		
CH2_32h_9B_1	8.8853	1.0700	1.1181	8.5030		
CH2_32h_9B_2	8.6770	1.0700	1.1181	8.3036	8.3437	0.1435
CH2_32h_9B_3	8.5944	1.0700	1.1181	8.2246		
CH2_16h_8B_1	9.0715	1.0500	1.0519	9.0555		
CH2_16h_8B_2	8.9914	1.0500	1.0519	8.9755	9.0347	0.0520
CH2_16h_8B_3	9.0890	1.0500	1.0519	9.0730		
CH2_8h_7B_1	9.9082	1.0367	1.0216	10.0545		
CH2_8h_7B_2	9.6298	1.0367	1.0216	9.7719	10.0010	0.2075
CH2_8h_7B_3	10.0284	1.0367	1.0216	10.1765		
CH2_4h_6B_1	11.0309	1.0300	1.0071	11.2818		
CH2_4h_6B_2	11.7355	1.0300	1.0071	12.0025	11.5625	0.3858
CH2_4h_6B_3	11.1497	1.0300	1.0071	11.4033		
CH2_60m_5B_1	12.2153	1.0233	1.0000	12.5003		
CH2_60m_5B_2	12.1577	1.0233	1.0000	12.4414	12.9701	0.8652
CH2_60m_5B_3	13.6500	1.0233	1.0000	13.9685		
CH2_30m_4B_1	17.0345	1.0133	1.0000	17.2616		
CH2_30m_4B_2	18.7462	1.0133	1.0000	18.9961	18.0009	0.8951
CH2_30m_4B_3	17.5114	1.0133	1.0000	17.7448		
CH2_10m_3B_1	21.4438	1.0067	1.0000	21.5867		
CH2_10m_3B_2	22.0735	1.0067	1.0000	22.2207	21.9677	0.3358
CH2_10m_3B_3	21.9494	1.0067	1.0000	22.0958		
CH2_0+_2B_1	23.0875	1.0033	1.0000	23.1645		
CH2_0+_2B_2	22.6457	1.0033	1.0000	22.7212	23.2374	0.5562
CH2_0+_2B_3	23.7473	1.0033	1.0000	23.8264		
CH2_0_1B_1	21.9533	1.0000	1.0000	21.9533		
CH2_0_1B_2	21.0696	1.0000	1.0000	21.0696	21.4948	0.4428
CH2_0_1B_3	21.4615	1.0000	1.0000	21.4615		

Table B.9 - Dilution (DF), Concentration Factors (CF) and Adj Concentration of PFOS for Channel 3

CH 3						
Sample	**µg/15L**	**DF**	**CF**	**Adj Conc µg/15L**	**Average**	**St Dev**
CH3_72h_11C_1	2.3755	1.1033	1.1570	2.2653		
CH3_72h_11C_2	2.5512	1.1033	1.1570	2.4328	2.3279	0.0914
CH3_72h_11C_3	2.3969	1.1033	1.1570	2.2857		
CH3_52h_10C_1	1.8192	1.0867	1.1024	1.7933		
CH3_52h_10C_2	1.8494	1.0867	1.1024	1.8231	1.8055	0.0156
CH3_52h_10C_3	1.8262	1.0867	1.1024	1.8002		
CH3_32h_9C_1	2.9678	1.0700	1.0606	2.9941		
CH3_32h_9C_2	2.9220	1.0700	1.0606	2.9479	2.9589	0.0313
CH3_32h_9C_3	2.9088	1.0700	1.0606	2.9346		
CH3_16h_8C_1	9.7306	1.0500	1.0294	9.9252		
CH3_16h_8C_2	9.0530	1.0500	1.0294	9.2341	9.6093	0.3493
CH3_16h_8C_3	9.4790	1.0500	1.0294	9.6686		
CH3_8h_7C_1	13.4470	1.0367	1.0145	13.7409		
CH3_8h_7C_2	12.3833	1.0367	1.0145	12.6539	13.0973	0.5704
CH3_8h_7C_3	12.6214	1.0367	1.0145	12.8972		
CH3_4h_6C_1	18.9106	1.0300	1.0072	19.3387		
CH3_4h_6C_2	18.4008	1.0300	1.0072	18.8174	19.6324	0.9948
CH3_4h_6C_3	20.2817	1.0300	1.0072	20.7409		
CH3_60m_5C_1	23.5217	1.0233	1.0000	24.0705		
CH3_60m_5C_2	24.2162	1.0233	1.0000	24.7813	24.3816	0.3636
CH3_60m_5C_3	23.7391	1.0233	1.0000	24.2930		
CH3_30m_4C_1	26.7650	1.0133	1.0000	27.1219		
CH3_30m_4C_2	26.1689	1.0133	1.0000	26.5178	27.0115	0.4488
CH3_30m_4C_3	27.0343	1.0133	1.0000	27.3948		
CH3_10m_3C_1	26.8318	1.0067	1.0000	27.0106		
CH3_10m_3C_2	28.1904	1.0067	1.0000	28.3783	28.1784	1.0817
CH3_10m_3C_3	28.9531	1.0067	1.0000	29.1461		
CH3_0+_2C_1	24.5746	1.0033	1.0000	24.6565		
CH3_0+_2C_2	23.5711	1.0033	1.0000	23.6497	23.8772	0.6941
CH3_0+_2C_3	23.2478	1.0033	1.0000	23.3253		
CH3_0_1C_1	21.5352	1.0000	1.0000	21.5352		
CH3_0_1C_2	23.7413	1.0000	1.0000	23.7413	22.6093	1.1042
CH3_0_1C_3	22.5514	1.0000	1.0000	22.5514		

Table B.10 - Dilution (DF), Concentration Factors (CF) and Adj Concentration of PFOS for Channel 5

CH 5						
Sample	**µg/15L**	**DF**	**CF**	**Adj Conc µg/15L**	**Average**	**St Dev**
CH5_72h_11E_1	3.6730	1.1033	1.4433	2.8078		
CH5_72h_11E_2	3.6979	1.1033	1.4433	2.8269	2.7561	0.1066
CH5_72h_11E_3	3.4450	1.1033	1.4433	2.6335		
CH5_52h_11E_1	4.5199	1.0867	1.2844	3.8241		
CH5_52h_11E_2	4.7959	1.0867	1.2844	4.0576	3.9478	0.1174
CH5_52h_11E_3	4.6826	1.0867	1.2844	3.9617		
CH5_32h_11E_1	5.5418	1.0700	1.1765	5.0403		
CH5_32h_11E_2	5.6419	1.0700	1.1765	5.1313	5.0430	0.0870
CH5_32h_11E_3	5.4506	1.0700	1.1765	4.9574		
CH5_16h_11E_1	7.9363	1.0500	1.0606	7.8570		
CH5_16h_11E_2	8.1667	1.0500	1.0606	8.0851	8.0417	0.1674
CH5_16h_11E_3	8.2658	1.0500	1.0606	8.1832		
CH5_8h_11E_1	8.9455	1.0367	1.0145	9.1410		
CH5_8h_11E_2	8.9174	1.0367	1.0145	9.1123	9.1406	0.0280
CH5_8h_11E_3	8.9722	1.0367	1.0145	9.1683		
CH5_4h_11E_1	15.4339	1.0300	1.0072	15.7834		
CH5_4h_11E_2	16.0130	1.0300	1.0072	16.3756	16.0931	0.2971
CH5_4h_11E_3	15.7634	1.0300	1.0072	16.1204		
CH5_60m_11E_1	13.5485	1.0233	1.0000	13.8646		
CH5_60m_11E_2	13.4544	1.0233	1.0000	13.7683	13.8018	0.0544
CH5_60m_11E_3	13.4585	1.0233	1.0000	13.7725		
CH5_30m_11E_1	14.6330	1.0133	1.0000	14.8281		
CH5_30m_11E_2	15.6427	1.0133	1.0000	15.8513	15.2620	0.5290
CH5_30m_11E_3	14.9078	1.0133	1.0000	15.1066		
CH5_10m_11E_1	18.8561	1.0067	1.0000	18.9818		
CH5_10m_11E_2	19.5619	1.0067	1.0000	19.6923	19.5088	0.4634
CH5_10m_11E_3	19.7208	1.0067	1.0000	19.8523		
CH5_0+_2E_1	20.6345	1.0033	1.0000	20.7033		
CH5_0+_2E_2	20.6700	1.0033	1.0000	20.7389	20.9096	0.3271
CH5_0+_2E_3	21.2160	1.0033	1.0000	21.2867		
CH5_0_1D_1	19.4045	1.0000	1.0000	19.4045		
CH5_0_1D_2	19.4810	1.0000	1.0000	19.4810	18.9165	0.9123
CH5_0_1D_3	17.8639	1.0000	1.0000	17.8639		

Table B.11 - Dilution (DF), Concentration Factors (CF) and Adj Concentration of PFOS for Channel 6

CH 6						
Sample	**µg/15L**	**DF**	**CF**	**Adj Conc µg/15L**	**Average**	**St Dev**
CH6_72h_11F_1	4.2252	1.1033	1.4688	3.1740		
CH6_72h_11F_2	4.1182	1.1033	1.4688	3.0936	3.1111	0.0563
CH6_72h_11F_3	4.0810	1.1033	1.4688	3.0656		
CH6_52h_11F_1	6.4046	1.0867	1.2818	5.4296		
CH6_52h_11F_2	6.1889	1.0867	1.2818	5.2466	5.3607	0.0995
CH6_52h_11F_3	6.3768	1.0867	1.2818	5.4060		
CH6_32h_11F_1	6.4726	1.0700	1.1463	6.0415		
CH6_32h_11F_2	6.3485	1.0700	1.1463	5.9257	5.9707	0.0621
CH6_32h_11F_3	6.3689	1.0700	1.1463	5.9447		
CH6_16h_11F_1	11.5913	1.0500	1.0602	11.4803		
CH6_16h_11F_2	12.0386	1.0500	1.0602	11.9234	11.8771	0.3758
CH6_16h_11F_3	12.3458	1.0500	1.0602	12.2276		
CH6_8h_11F_1	12.8251	1.0367	1.0292	12.9182		
CH6_8h_11F_2	13.5050	1.0367	1.0292	13.6031	13.3924	0.4115
CH6_8h_11F_3	13.5576	1.0367	1.0292	13.6560		
CH6_4h_11F_1	18.5117	1.0300	1.0071	18.9318		
CH6_4h_11F_2	18.7548	1.0300	1.0071	19.1804	18.7809	0.4927
CH6_4h_11F_3	17.8258	1.0300	1.0071	18.2303		
CH6_60m_11F_1	17.7703	1.0233	1.0000	18.1850		
CH6_60m_11F_2	17.6676	1.0233	1.0000	18.0798	18.0009	0.2337
CH6_60m_11F_3	17.3335	1.0233	1.0000	17.7380		
CH6_30m_11F_1	32.8958	1.0133	1.0000	33.3345		
CH6_30m_11F_2	33.0780	1.0133	1.0000	33.5190	33.7562	0.5780
CH6_30m_11F_3	33.9622	1.0133	1.0000	34.4150		
CH6_10m_11F_1	25.5528	1.0067	1.0000	25.7232		
CH6_10m_11F_2	26.8313	1.0067	1.0000	27.0102	26.6929	0.8564
CH6_10m_11F_3	27.1644	1.0067	1.0000	27.3455		
CH6_0+_2F_1	23.3158	1.0033	1.0000	23.3935		
CH6_0+_2F_2	21.9821	1.0033	1.0000	22.0554	22.8811	0.7220
CH6_0+_2F_3	23.1175	1.0033	1.0000	23.1946		
CH6_0_1E_1	21.0547	1.0000	1.0000	21.0547		
CH6_0_1E_2	21.9053	1.0000	1.0000	21.9053	21.2334	0.6028
CH6_0_1E_3	20.7401	1.0000	1.0000	20.7401		

Table B.12 - Dilution (DF), Concentration Factors (CF) and Adj Concentration of PFOS for Channel 7

CH 7						
Sample	**µg/15L**	**DF**	**CF**	**Adj Conc µg/15L**	**Average**	**St Dev**
CH7_72h_11D_1	1.6978	1.1033	1.1667	1.6056		
CH7_72h_11D_2	1.7782	1.1033	1.1667	1.6816	1.6648	0.0528
CH7_72h_11D_3	1.8050	1.1033	1.1667	1.7071		
CH7_52h_10D_1	2.9878	1.0867	1.1024	2.9452		
CH7_52h_10D_2	3.0823	1.0867	1.1024	3.0384	3.0455	0.1039
CH7_52h_10D_3	3.1982	1.0867	1.1024	3.1527		
CH7_32h_9D_1	5.4922	1.0700	1.0606	5.5408		
CH7_32h_9D_2	5.5469	1.0700	1.0606	5.5960	5.6238	0.0998
CH7_32h_9D_3	5.6842	1.0700	1.0606	5.7345		
CH7_16h_8D_1	11.7038	1.0500	1.0072	12.2013		
CH7_16h_8D_2	11.6045	1.0500	1.0072	12.0977	12.0799	0.1311
CH7_16h_8D_3	11.4540	1.0500	1.0072	11.9408		
CH7_8h_7D_1	17.9261	1.0367	1.0000	18.5834		
CH7_8h_7D_2	18.6386	1.0367	1.0000	19.3221	19.0544	0.4092
CH7_8h_7D_3	18.5767	1.0367	1.0000	19.2579		
CH7_4h_6D_1	21.0941	1.0300	1.0000	21.7269		
CH7_4h_6D_2	20.7991	1.0300	1.0000	21.4231	21.1460	0.7584
CH7_4h_6D_3	19.6970	1.0300	1.0000	20.2880		
CH7_60m_5D_1	24.7838	1.0233	1.0000	25.3621		
CH7_60m_5D_2	22.9519	1.0233	1.0000	23.4875	24.5142	0.9500
CH7_60m_5D_3	24.1298	1.0233	1.0000	24.6929		
CH7_30m_4D_1	25.3546	1.0133	1.0000	25.6926		
CH7_30m_4D_2	26.1401	1.0133	1.0000	26.4886	26.4670	0.7638
CH7_30m_4D_3	26.8615	1.0133	1.0000	27.2197		
CH7_10m_3D_1	18.4138	1.0067	1.0000	18.5365		
CH7_10m_3D_2	19.6006	1.0067	1.0000	19.7312	18.8132	0.8156
CH7_10m_3D_3	18.0516	1.0067	1.0000	18.1719		
CH7_0+_2D_1	20.7982	1.0033	1.0000	20.8675		
CH7_0+_2D_2	21.4015	1.0033	1.0000	21.4729	21.2115	0.3110
CH7_0+_2D_3	21.2234	1.0033	1.0000	21.2942		
CH7_0_1F_1	22.5998	1.0000	1.0000	22.5998		
CH7_0_1F_2	21.2983	1.0000	1.0000	21.2983	21.9862	0.6539
CH7_0_1F_3	22.0606	1.0000	1.0000	22.0606		

B.3 Tabular Data for HFPO-DA from experimental channels and positive controls

Table B.13 - Dilution (DF), Concentration Factors (CF) and Adj Concentration of HFPO-DA for Channel 1

CH 1						
Sample	**µg/15L**	**DF**	**CF**	**Adj Conc µg/15L**	**Average**	**St Dev**
CH1_72h_11A_1	20.9803	1.1033	1.2804	18.0793		
CH1_72h_11A_2	22.6709	1.1033	1.2804	19.5361	19.0493	0.8401
CH1_72h_11A_3	22.6668	1.1033	1.2804	19.5326		
CH1_52h_10A_1	21.3869	1.0867	1.1913	19.5084		
CH1_52h_10A_2	19.7501	1.0867	1.1913	18.0153	18.8578	0.7648
CH1_52h_10A_3	20.8841	1.0867	1.1913	19.0497		
CH1_32h_9A_1	18.8882	1.0700	1.1610	17.4075		
CH1_32h_9A_2	19.5506	1.0700	1.1610	18.0180	17.7351	0.3077
CH1_32h_9A_3	19.2922	1.0700	1.1610	17.7798		
CH1_16h_8A_1	19.0925	1.0500	1.0538	19.0228		
CH1_16h_8A_2	19.4530	1.0500	1.0538	19.3820	19.3662	0.3358
CH1_16h_8A_3	19.7659	1.0500	1.0538	19.6938		
CH1_8h_7A_1	20.7343	1.0367	1.0301	20.8670		
CH1_8h_7A_2	20.6597	1.0367	1.0301	20.7919	20.8395	0.0414
CH1_8h_7A_3	20.7271	1.0367	1.0301	20.8598		
CH1_4h_6A_1	20.1374	1.0300	1.0148	20.4388		
CH1_4h_6A_2	20.4312	1.0300	1.0148	20.7369	20.2216	0.6517
CH1_4h_6A_3	19.2017	1.0300	1.0148	19.4890		
CH1_60m_5A_1	19.4851	1.0233	1.0000	19.9398		
CH1_60m_5A_2	18.7836	1.0233	1.0000	19.2219	19.4098	0.4653
CH1_60m_5A_3	18.6331	1.0233	1.0000	19.0679		
CH1_30m_4A_1	19.9486	1.0133	1.0000	20.2145		
CH1_30m_4A_2	19.5821	1.0133	1.0000	19.8432	19.7845	0.4622
CH1_30m_4A_3	19.0418	1.0133	1.0000	19.2957		
CH1_10m_3A_1	20.6882	1.0067	1.0000	20.8262		
CH1_10m_3A_2	19.8922	1.0067	1.0000	20.0248	20.4568	0.4044
CH1_10m_3A_3	20.3837	1.0067	1.0000	20.5196		
CH1_0+_2A_1	19.5715	1.0033	1.0000	19.6368		
CH1_0+_2A_2	20.2318	1.0033	1.0000	20.2992	19.8588	0.3814
CH1_0+_2A_3	19.5751	1.0033	1.0000	19.6404		
CH1_0_1A_1	22.2050	1.0000	1.0000	22.2050		
CH1_0_1A_2	21.1231	1.0000	1.0000	21.1231	21.5700	0.5650
CH1_0_1A_3	21.3818	1.0000	1.0000	21.3818		

Table B.14 - Dilution (DF), Concentration Factors (CF) and Adj Concentration of HFPO-DA for Channel 2

CH 2						
Sample	**µg/15L**	**DF**	**CF**	**Adj Conc µg/15L**	**Average**	**St Dev**
CH2_72h_11B_1	22.7309	1.1033	1.3148	19.0747		
CH2_72h_11B_2	24.6989	1.1033	1.3148	20.7262	19.7875	0.8486
CH2_72h_11B_3	23.3110	1.1033	1.3148	19.5615		
CH2_52h_10B_1	22.8770	1.0867	1.2137	20.4830		
CH2_52h_10B_2	22.9932	1.0867	1.2137	20.5870	20.8122	0.4829
CH2_52h_10B_3	23.8639	1.0867	1.2137	21.3666		
CH2_32h_9B_1	22.2122	1.0700	1.1181	21.2565		
CH2_32h_9B_2	21.6367	1.0700	1.1181	20.7057	20.9559	0.2788
CH2_32h_9B_3	21.8455	1.0700	1.1181	20.9055		
CH2_16h_8B_1	22.4923	1.0500	1.0519	22.4527		
CH2_16h_8B_2	22.5689	1.0500	1.0519	22.5291	22.3959	0.1690
CH2_16h_8B_3	22.2449	1.0500	1.0519	22.2057		
CH2_8h_7B_1	20.5805	1.0367	1.0216	20.8844		
CH2_8h_7B_2	20.0621	1.0367	1.0216	20.3583	20.7262	0.3197
CH2_8h_7B_3	20.6314	1.0367	1.0216	20.9360		
CH2_4h_6B_1	19.3997	1.0300	1.0071	19.8410		
CH2_4h_6B_2	20.6650	1.0300	1.0071	21.1350	20.5018	0.6475
CH2_4h_6B_3	20.0729	1.0300	1.0071	20.5295		
CH2_60m_5B_1	19.7230	1.0233	1.0000	20.1832		
CH2_60m_5B_2	19.2326	1.0233	1.0000	19.6814	20.3590	0.7805
CH2_60m_5B_3	20.7288	1.0233	1.0000	21.2125		
CH2_30m_4B_1	20.3945	1.0133	1.0000	20.6664		
CH2_30m_4B_2	21.5071	1.0133	1.0000	21.7939	21.0565	0.6390
CH2_30m_4B_3	20.4367	1.0133	1.0000	20.7092		
CH2_10m_3B_1	21.8455	1.0067	1.0000	21.9912		
CH2_10m_3B_2	22.4210	1.0067	1.0000	22.5705	22.2580	0.2924
CH2_10m_3B_3	22.0651	1.0067	1.0000	22.2122		
CH2_0+_2B_1	23.1744	1.0033	1.0000	23.2516		
CH2_0+_2B_2	22.9596	1.0033	1.0000	23.0361	23.3667	0.4006
CH2_0+_2B_3	23.7331	1.0033	1.0000	23.8122		
CH2_0_1B_1	22.3817	1.0000	1.0000	22.3817		
CH2_0_1B_2	22.0536	1.0000	1.0000	22.0536	22.2459	0.1712
CH2_0_1B_3	22.3025	1.0000	1.0000	22.3025		

Table B.15 - Dilution (DF), Concentration Factors (CF) and Adj Concentration of HFPO-DA for Channel 3

CH 3						
Sample	**µg/15L**	**DF**	**CF**	**Adj Conc µg/15L**	**Average**	**St Dev**
CH3_72h_11C_1	17.4370	1.1033	1.1570	16.6278		
CH3_72h_11C_2	18.0936	1.1033	1.1570	17.2540	17.1706	0.5063
CH3_72h_11C_3	18.4879	1.1033	1.1570	17.6300		
CH3_52h_10C_1	17.0066	1.0867	1.1024	16.7645		
CH3_52h_10C_2	16.9423	1.0867	1.1024	16.7011	16.9621	0.3985
CH3_52h_10C_3	17.6724	1.0867	1.1024	17.4208		
CH3_32h_9C_1	17.5375	1.0700	1.0606	17.6929		
CH3_32h_9C_2	17.2116	1.0700	1.0606	17.3640	17.6799	0.3095
CH3_32h_9C_3	17.8248	1.0700	1.0606	17.9827		
CH3_16h_8C_1	20.9237	1.0500	1.0294	21.3422		
CH3_16h_8C_2	19.8425	1.0500	1.0294	20.2393	20.8521	0.5615
CH3_16h_8C_3	20.5634	1.0500	1.0294	20.9747		
CH3_8h_7C_1	20.4091	1.0367	1.0145	20.8552		
CH3_8h_7C_2	19.2593	1.0367	1.0145	19.6802	20.1599	0.6165
CH3_8h_7C_3	19.5175	1.0367	1.0145	19.9441		
CH3_4h_6C_1	23.0376	1.0300	1.0072	23.5592		
CH3_4h_6C_2	22.4748	1.0300	1.0072	22.9837	23.8883	1.1064
CH3_4h_6C_3	24.5657	1.0300	1.0072	25.1219		
CH3_60m_5C_1	23.1072	1.0233	1.0000	23.6464		
CH3_60m_5C_2	23.3832	1.0233	1.0000	23.9288	23.6726	0.2441
CH3_60m_5C_3	22.9082	1.0233	1.0000	23.4428		
CH3_30m_4C_1	24.2870	1.0133	1.0000	24.6109		
CH3_30m_4C_2	23.9122	1.0133	1.0000	24.2310	24.7582	0.6144
CH3_30m_4C_3	25.0982	1.0133	1.0000	25.4329		
CH3_10m_3C_1	21.8340	1.0067	1.0000	21.9796		
CH3_10m_3C_2	22.1614	1.0067	1.0000	22.3091	22.5178	0.6676
CH3_10m_3C_3	23.1108	1.0067	1.0000	23.2649		
CH3_0+_2C_1	23.6556	1.0033	1.0000	23.7345		
CH3_0+_2C_2	22.5617	1.0033	1.0000	22.6369	22.9247	0.7109
CH3_0+_2C_3	22.3284	1.0033	1.0000	22.4028		
CH3_0_1C_1	22.2934	1.0000	1.0000	22.2934		
CH3_0_1C_2	23.4521	1.0000	1.0000	23.4521	22.7851	0.5989
CH3_0_1C_3	22.6099	1.0000	1.0000	22.6099		

Table B.16 - Dilution (DF), Concentration Factors (CF) and Adj Concentration of HFPO-DA for Channel 5

CH 5						
Sample	**µg/15L**	**DF**	**CF**	**Adj Conc µg/15L**	**Average**	**St Dev**
CH5_72h_11E_1	20.8879	1.1033	1.4433	15.9678		
CH5_72h_11E_2	21.1846	1.1033	1.4433	16.1946	15.8799	0.3666
CH5_72h_11E_3	20.2464	1.1033	1.4433	15.4774		
CH5_52h_11E_1	21.2556	1.0867	1.2844	17.9832		
CH5_52h_11E_2	21.8309	1.0867	1.2844	18.4700	18.0717	0.3622
CH5_52h_11E_3	20.9940	1.0867	1.2844	17.7619		
CH5_32h_11E_1	19.7261	1.0700	1.1765	17.9409		
CH5_32h_11E_2	20.3669	1.0700	1.1765	18.5237	18.2598	0.2953
CH5_32h_11E_3	20.1372	1.0700	1.1765	18.3148		
CH5_16h_11E_1	20.8874	1.0500	1.0606	20.6786		
CH5_16h_11E_2	21.5285	1.0500	1.0606	21.3132	21.2131	0.4922
CH5_16h_11E_3	21.8662	1.0500	1.0606	21.6475		
CH5_8h_11E_1	21.4022	1.0367	1.0145	21.8700		
CH5_8h_11E_2	21.2678	1.0367	1.0145	21.7327	21.8478	0.1058
CH5_8h_11E_3	21.4714	1.0367	1.0145	21.9407		
CH5_4h_11E_1	20.8793	1.0300	1.0072	21.3520		
CH5_4h_11E_2	22.0181	1.0300	1.0072	22.5166	22.1379	0.6807
CH5_4h_11E_3	22.0459	1.0300	1.0072	22.5451		
CH5_60m_11E_1	20.4991	1.0233	1.0000	20.9774		
CH5_60m_11E_2	21.1332	1.0233	1.0000	21.6263	21.1044	0.4714
CH5_60m_11E_3	20.2373	1.0233	1.0000	20.7095		
CH5_30m_11E_1	20.8462	1.0133	1.0000	21.1241		
CH5_30m_11E_2	22.5890	1.0133	1.0000	22.8902	22.1007	0.8978
CH5_30m_11E_3	21.9946	1.0133	1.0000	22.2878		
CH5_10m_11E_1	20.3558	1.0067	1.0000	20.4915		
CH5_10m_11E_2	20.4982	1.0067	1.0000	20.6348	20.7675	0.3610
CH5_10m_11E_3	21.0358	1.0067	1.0000	21.1760		
CH5_0+_2E_1	21.1752	1.0033	1.0000	21.2458		
CH5_0+_2E_2	20.6755	1.0033	1.0000	20.7444	21.1331	0.3463
CH5_0+_2E_3	21.3379	1.0033	1.0000	21.4090		
CH5_0_1D_1	21.3526	1.0000	1.0000	21.3526		
CH5_0_1D_2	21.0614	1.0000	1.0000	21.0614	20.6802	0.9241
CH5_0_1D_3	19.6265	1.0000	1.0000	19.6265		

Table B.17 - Dilution (DF), Concentration Factors (CF) and Adj Concentration of HFPO-DA for Channel 6

CH 6						
Sample	**µg/15L**	**DF**	**CF**	**Adj Conc µg/15L**	**Average**	**St Dev**
CH6_72h_11F_1	20.1550	1.1033	1.4688	15.1405		
CH6_72h_11F_2	20.7497	1.1033	1.4688	15.5873	15.1674	0.4071
CH6_72h_11F_3	19.6675	1.1033	1.4688	14.7744		
CH6_52h_11F_1	20.4192	1.0867	1.2818	17.3105		
CH6_52h_11F_2	20.3278	1.0867	1.2818	17.2329	17.2294	0.0829
CH6_52h_11F_3	20.2238	1.0867	1.2818	17.1448		
CH6_32h_11F_1	20.0426	1.0700	1.1463	18.7079		
CH6_32h_11F_2	19.5050	1.0700	1.1463	18.2061	18.4107	0.2634
CH6_32h_11F_3	19.6250	1.0700	1.1463	18.3181		
CH6_16h_11F_1	21.9912	1.0500	1.0602	21.7806		
CH6_16h_11F_2	22.3025	1.0500	1.0602	22.0889	22.0292	0.2247
CH6_16h_11F_3	22.4328	1.0500	1.0602	22.2180		
CH6_8h_11F_1	22.0987	1.0367	1.0292	22.2591		
CH6_8h_11F_2	22.8146	1.0367	1.0292	22.9802	22.7621	0.4369
CH6_8h_11F_3	22.8809	1.0367	1.0292	23.0469		
CH6_4h_11F_1	22.0630	1.0300	1.0071	22.5637		
CH6_4h_11F_2	21.9917	1.0300	1.0071	22.4908	22.2446	0.4909
CH6_4h_11F_3	21.1982	1.0300	1.0071	21.6793		
CH6_60m_11F_1	22.3800	1.0233	1.0000	22.9022		
CH6_60m_11F_2	22.6802	1.0233	1.0000	23.2094	22.8336	0.4144
CH6_60m_11F_3	21.8786	1.0233	1.0000	22.3891		
CH6_30m_11F_1	25.5283	1.0133	1.0000	25.8687		
CH6_30m_11F_2	25.3630	1.0133	1.0000	25.7011	26.0324	0.4367
CH6_30m_11F_3	26.1782	1.0133	1.0000	26.5273		
CH6_10m_11F_1	21.8268	1.0067	1.0000	21.9723		
CH6_10m_11F_2	22.6068	1.0067	1.0000	22.7575	22.7688	0.8022
CH6_10m_11F_3	23.4204	1.0067	1.0000	23.5765		
CH6_0+_2F_1	23.2450	1.0033	1.0000	23.3224		
CH6_0+_2F_2	21.9756	1.0033	1.0000	22.0489	22.8162	0.6758
CH6_0+_2F_3	23.0006	1.0033	1.0000	23.0773		
CH6_0_1E_1	22.1405	1.0000	1.0000	22.1405		
CH6_0_1E_2	22.7174	1.0000	1.0000	22.7174	22.2514	0.4217
CH6_0_1E_3	21.8962	1.0000	1.0000	21.8962		

Table B.18 - Dilution (DF), Concentration Factors (CF) and Adj Concentration of HFPO-DA for Channel 7

CH 7						
Sample	**µg/15L**	**DF**	**CF**	**Adj Conc µg/15L**	**Average**	**St Dev**
CH7_72h_11D_1	18.9650	1.1033	1.1667	17.9355		
CH7_72h_11D_2	18.8352	1.1033	1.1667	17.8127	17.7799	0.1744
CH7_72h_11D_3	18.6012	1.1033	1.1667	17.5914		
CH7_52h_10D_1	17.5166	1.0867	1.1024	17.2672		
CH7_52h_10D_2	17.7634	1.0867	1.1024	17.5104	17.2453	0.2767
CH7_52h_10D_3	17.2032	1.0867	1.1024	16.9583		
CH7_32h_9D_1	20.5159	1.0700	1.0606	20.6976		
CH7_32h_9D_2	20.8109	1.0700	1.0606	20.9952	20.9289	0.2063
CH7_32h_9D_3	20.9088	1.0700	1.0606	21.0940		
CH7_16h_8D_1	19.9954	1.0500	1.0072	20.8452		
CH7_16h_8D_2	20.2843	1.0500	1.0072	21.1464	21.0770	0.2061
CH7_16h_8D_3	20.3736	1.0500	1.0072	21.2395		
CH7_8h_7D_1	20.7530	1.0367	1.0000	21.5140		
CH7_8h_7D_2	21.6070	1.0367	1.0000	22.3992	22.1537	0.5590
CH7_8h_7D_3	21.7505	1.0367	1.0000	22.5480		
CH7_4h_6D_1	22.0586	1.0300	1.0000	22.7204		
CH7_4h_6D_2	21.7558	1.0300	1.0000	22.4084	22.2030	0.6451
CH7_4h_6D_3	20.8546	1.0300	1.0000	21.4802		
CH7_60m_5D_1	25.0418	1.0233	1.0000	25.6261		
CH7_60m_5D_2	23.2445	1.0233	1.0000	23.7869	24.9324	0.9994
CH7_60m_5D_3	24.8054	1.0233	1.0000	25.3842		
CH7_30m_4D_1	23.4792	1.0133	1.0000	23.7923		
CH7_30m_4D_2	23.5954	1.0133	1.0000	23.9100	24.1377	0.4998
CH7_30m_4D_3	24.3857	1.0133	1.0000	24.7108		
CH7_10m_3D_1	19.3627	1.0067	1.0000	19.4918		
CH7_10m_3D_2	20.4701	1.0067	1.0000	20.6065	19.7010	0.8211
CH7_10m_3D_3	18.8789	1.0067	1.0000	19.0047		
CH7_0+_2D_1	22.7230	1.0033	1.0000	22.7987		
CH7_0+_2D_2	22.3102	1.0033	1.0000	22.3845	22.6106	0.2097
CH7_0+_2D_3	22.5732	1.0033	1.0000	22.6484		
CH7_0_1F_1	24.1193	1.0000	1.0000	24.1193		
CH7_0_1F_2	23.3681	1.0000	1.0000	23.3681	23.8254	0.4014
CH7_0_1F_3	23.9890	1.0000	1.0000	23.9890		

B.4 Tabular Data for PDHA from experimental channels and positive controls

Table B.19 - Dilution (DF), Concentration Factors (CF) and Adj Concentration of PDHA for Channel 1

CH 1						
Sample	**µg/15L**	**DF**	**CF**	**Adj Conc µg/15L**	**Average**	**St Dev**
CH1_72h_11A_1	19.7280	1.1033	1.2804	17.0002		
CH1_72h_11A_2	21.7121	1.1033	1.2804	18.7099	18.2053	1.0483
CH1_72h_11A_3	21.9396	1.1033	1.2804	18.9060		
CH1_52h_10A_1	19.5948	1.0867	1.1913	17.8737		
CH1_52h_10A_2	17.9378	1.0867	1.1913	16.3623	17.2186	0.7756
CH1_52h_10A_3	19.0973	1.0867	1.1913	17.4199		
CH1_32h_9A_1	16.9937	1.0700	1.1610	15.6615		
CH1_32h_9A_2	17.1439	1.0700	1.1610	15.7999	15.5568	0.3091
CH1_32h_9A_3	16.5026	1.0700	1.1610	15.2089		
CH1_16h_8A_1	16.2509	1.0500	1.0538	16.1916		
CH1_16h_8A_2	16.8283	1.0500	1.0538	16.7669	16.5951	0.3508
CH1_16h_8A_3	16.8886	1.0500	1.0538	16.8269		
CH1_8h_7A_1	17.8262	1.0367	1.0301	17.9403		
CH1_8h_7A_2	17.7254	1.0367	1.0301	17.8389	18.0257	0.2412
CH1_8h_7A_3	18.1817	1.0367	1.0301	18.2980		
CH1_4h_6A_1	17.1264	1.0300	1.0148	17.3827		
CH1_4h_6A_2	17.6777	1.0300	1.0148	17.9422	17.3543	0.6025
CH1_4h_6A_3	16.4914	1.0300	1.0148	16.7381		
CH1_60m_5A_1	16.7062	1.0233	1.0000	17.0960		
CH1_60m_5A_2	16.1892	1.0233	1.0000	16.5669	16.6320	0.4352
CH1_60m_5A_3	15.8628	1.0233	1.0000	16.2329		
CH1_30m_4A_1	17.2356	1.0133	1.0000	17.4654		
CH1_30m_4A_2	17.3246	1.0133	1.0000	17.5556	17.1871	0.5620
CH1_30m_4A_3	16.3226	1.0133	1.0000	16.5403		
CH1_10m_3A_1	17.9263	1.0067	1.0000	18.0458		
CH1_10m_3A_2	17.2865	1.0067	1.0000	17.4017	17.9479	0.5044
CH1_10m_3A_3	18.2743	1.0067	1.0000	18.3961		
CH1_0+_2A_1	17.3184	1.0033	1.0000	17.3761		
CH1_0+_2A_2	18.1858	1.0033	1.0000	18.2464	17.8390	0.4378
CH1_0+_2A_3	17.8351	1.0033	1.0000	17.8946		
CH1_0_1A_1	20.5834	1.0000	1.0000	20.5834		
CH1_0_1A_2	19.6872	1.0000	1.0000	19.6872	20.1224	0.4486
CH1_0_1A_3	20.0966	1.0000	1.0000	20.0966		

Table B.20 - Dilution (DF), Concentration Factors (CF) and Adj Concentration of PDHA for Channel 2

CH 2						
Sample	**µg/15L**	**DF**	**CF**	**Adj Conc µg/15L**	**Average**	**St Dev**
CH2_72h_11B_1	22.0510	1.1033	1.3148	18.5042		
CH2_72h_11B_2	23.8656	1.1033	1.3148	20.0269	19.0795	0.8268
CH2_72h_11B_3	22.2931	1.1033	1.3148	18.7074		
CH2_52h_10B_1	20.9419	1.0867	1.2137	18.7504		
CH2_52h_10B_2	20.6690	1.0867	1.2137	18.5061	19.0057	0.6651
CH2_52h_10B_3	22.0702	1.0867	1.2137	19.7606		
CH2_32h_9B_1	20.5020	1.0700	1.1181	19.6198		
CH2_32h_9B_2	19.6913	1.0700	1.1181	18.8440	19.1842	0.3966
CH2_32h_9B_3	19.9471	1.0700	1.1181	19.0888		
CH2_16h_8B_1	20.0645	1.0500	1.0519	20.0292		
CH2_16h_8B_2	20.2166	1.0500	1.0519	20.1810	20.0783	0.0890
CH2_16h_8B_3	20.0602	1.0500	1.0519	20.0248		
CH2_8h_7B_1	18.6730	1.0367	1.0216	18.9487		
CH2_8h_7B_2	17.9686	1.0367	1.0216	18.2339	18.4889	0.3990
CH2_8h_7B_3	18.0180	1.0367	1.0216	18.2840		
CH2_4h_6B_1	17.8176	1.0300	1.0071	18.2229		
CH2_4h_6B_2	18.1181	1.0300	1.0071	18.5302	18.1808	0.3722
CH2_4h_6B_3	17.3938	1.0300	1.0071	17.7894		
CH2_60m_5B_1	17.3352	1.0233	1.0000	17.7397		
CH2_60m_5B_2	17.1192	1.0233	1.0000	17.5186	17.9112	0.5008
CH2_60m_5B_3	18.0540	1.0233	1.0000	18.4753		
CH2_30m_4B_1	18.0742	1.0133	1.0000	18.3151		
CH2_30m_4B_2	19.0116	1.0133	1.0000	19.2651	18.5489	0.6326
CH2_30m_4B_3	17.8286	1.0133	1.0000	18.0664		
CH2_10m_3B_1	18.8177	1.0067	1.0000	18.9431		
CH2_10m_3B_2	20.0945	1.0067	1.0000	20.2284	19.5294	0.6500
CH2_10m_3B_3	19.2881	1.0067	1.0000	19.4167		
CH2_0+_2B_1	20.9650	1.0033	1.0000	21.0348		
CH2_0+_2B_2	21.1517	1.0033	1.0000	21.2222	21.5557	0.7458
CH2_0+_2B_3	22.3356	1.0033	1.0000	22.4101		
CH2_0_1B_1	20.8426	1.0000	1.0000	20.8426		
CH2_0_1B_2	20.4065	1.0000	1.0000	20.4065	20.6836	0.2409
CH2_0_1B_3	20.8018	1.0000	1.0000	20.8018		

Table B.21 - Dilution (DF), Concentration Factors (CF) and Adj Concentration of PDHA for Channel 3

CH 3						
Sample	**µg/15L**	**DF**	**CF**	**Adj Conc µg/15L**	**Average**	**St Dev**
CH3_72h_11C_1	19.3488	1.1033	1.1570	18.4509		
CH3_72h_11C_2	20.0542	1.1033	1.1570	19.1236	19.0391	0.5508
CH3_72h_11C_3	20.4938	1.1033	1.1570	19.5428		
CH3_52h_10C_1	16.9018	1.0867	1.1024	16.6611		
CH3_52h_10C_2	16.5730	1.0867	1.1024	16.3370	16.5986	0.2366
CH3_52h_10C_3	17.0402	1.0867	1.1024	16.7976		
CH3_32h_9C_1	16.3786	1.0700	1.0606	16.5236		
CH3_32h_9C_2	16.0366	1.0700	1.0606	16.1786	16.6100	0.4804
CH3_32h_9C_3	16.9774	1.0700	1.0606	17.1277		
CH3_16h_8C_1	19.2230	1.0500	1.0294	19.6075		
CH3_16h_8C_2	19.1978	1.0500	1.0294	19.5818	19.6825	0.1527
CH3_16h_8C_3	19.4688	1.0500	1.0294	19.8582		
CH3_8h_7C_1	19.2922	1.0367	1.0145	19.7138		
CH3_8h_7C_2	17.5706	1.0367	1.0145	17.9547	18.6761	0.9212
CH3_8h_7C_3	17.9671	1.0367	1.0145	18.3598		
CH3_4h_6C_1	21.5575	1.0300	1.0072	22.0456		
CH3_4h_6C_2	20.7907	1.0300	1.0072	21.2615	22.4231	1.3894
CH3_4h_6C_3	23.4317	1.0300	1.0072	23.9622		
CH3_60m_5C_1	21.8592	1.0233	1.0000	22.3692		
CH3_60m_5C_2	21.6559	1.0233	1.0000	22.1612	22.2644	0.1040
CH3_60m_5C_3	21.7550	1.0233	1.0000	22.2627		
CH3_30m_4C_1	22.4143	1.0133	1.0000	22.7132		
CH3_30m_4C_2	22.2982	1.0133	1.0000	22.5955	22.9941	0.5914
CH3_30m_4C_3	23.3621	1.0133	1.0000	23.6736		
CH3_10m_3C_1	20.3150	1.0067	1.0000	20.4505		
CH3_10m_3C_2	20.9474	1.0067	1.0000	21.0871	21.1029	0.6604
CH3_10m_3C_3	21.6269	1.0067	1.0000	21.7711		
CH3_0+_2C_1	22.4477	1.0033	1.0000	22.5225		
CH3_0+_2C_2	21.2863	1.0033	1.0000	21.3573	21.5786	0.8550
CH3_0+_2C_3	20.7866	1.0033	1.0000	20.8559		
CH3_0_1C_1	20.7238	1.0000	1.0000	20.7238		
CH3_0_1C_2	22.3762	1.0000	1.0000	22.3762	21.4542	0.8427
CH3_0_1C_3	21.2626	1.0000	1.0000	21.2626		

Table B.22 - Dilution (DF), Concentration Factors (CF) and Adj Concentration of PDHA for Channel 5

CH 5						
Sample	**µg/15L**	**DF**	**CF**	**Adj Conc µg/15L**	**Average**	**St Dev**
CH5_72h_11E_1	21.5942	1.1033	1.4433	16.5078		
CH5_72h_11E_2	22.0778	1.1033	1.4433	16.8775	16.4015	0.5370
CH5_72h_11E_3	20.6938	1.1033	1.4433	15.8194		
CH5_52h_11E_1	20.9086	1.0867	1.2844	17.6896		
CH5_52h_11E_2	21.3715	1.0867	1.2844	18.0813	17.5966	0.5373
CH5_52h_11E_3	20.1158	1.0867	1.2844	17.0190		
CH5_32h_11E_1	17.6393	1.0700	1.1765	16.0429		
CH5_32h_11E_2	18.6288	1.0700	1.1765	16.9429	16.5390	0.4570
CH5_32h_11E_3	18.2861	1.0700	1.1765	16.6312		
CH5_16h_11E_1	18.2057	1.0500	1.0606	18.0236		
CH5_16h_11E_2	18.7666	1.0500	1.0606	18.5789	18.3955	0.3220
CH5_16h_11E_3	18.7716	1.0500	1.0606	18.5839		
CH5_8h_11E_1	18.2508	1.0367	1.0145	18.6497		
CH5_8h_11E_2	18.0610	1.0367	1.0145	18.4557	18.5703	0.1016
CH5_8h_11E_3	18.2074	1.0367	1.0145	18.6053		
CH5_4h_11E_1	17.4910	1.0300	1.0072	17.8870		
CH5_4h_11E_2	18.6610	1.0300	1.0072	19.0835	18.4791	0.5983
CH5_4h_11E_3	18.0578	1.0300	1.0072	18.4667		
CH5_60m_11E_1	16.7921	1.0233	1.0000	17.1839		
CH5_60m_11E_2	17.4499	1.0233	1.0000	17.8571	17.3688	0.4270
CH5_60m_11E_3	16.6764	1.0233	1.0000	17.0655		
CH5_30m_11E_1	17.5538	1.0133	1.0000	17.7879		
CH5_30m_11E_2	19.1810	1.0133	1.0000	19.4368	18.7373	0.8524
CH5_30m_11E_3	18.7373	1.0133	1.0000	18.9871		
CH5_10m_11E_1	16.3685	1.0067	1.0000	16.4776		
CH5_10m_11E_2	17.0803	1.0067	1.0000	17.1942	16.9450	0.4051
CH5_10m_11E_3	17.0496	1.0067	1.0000	17.1633		
CH5_0+_2E_1	18.2750	1.0033	1.0000	18.3360		
CH5_0+_2E_2	17.9438	1.0033	1.0000	18.0037	18.2565	0.2239
CH5_0+_2E_3	18.3686	1.0033	1.0000	18.4299		
CH5_0_1D_1	18.5851	1.0000	1.0000	18.5851		
CH5_0_1D_2	18.2666	1.0000	1.0000	18.2666	18.1108	0.5685
CH5_0_1D_3	17.4806	1.0000	1.0000	17.4806		

Table B.23 - Dilution (DF), Concentration Factors (CF) and Adj Concentration of PDHA for Channel 6

CH 6						
Sample	**µg/15L**	**DF**	**CF**	**Adj Conc µg/15L**	**Average**	**St Dev**
CH6_72h_11F_1	20.4386	1.1033	1.4688	15.3536		
CH6_72h_11F_2	21.0634	1.1033	1.4688	15.8229	15.4442	0.3425
CH6_72h_11F_3	20.1758	1.1033	1.4688	15.1562		
CH6_52h_11F_1	19.6438	1.0867	1.2818	16.6531		
CH6_52h_11F_2	19.6356	1.0867	1.2818	16.6462	16.6330	0.0290
CH6_52h_11F_3	19.5809	1.0867	1.2818	16.5998		
CH6_32h_11F_1	17.9258	1.0700	1.1463	16.7321		
CH6_32h_11F_2	17.7893	1.0700	1.1463	16.6046	16.6861	0.0707
CH6_32h_11F_3	17.9146	1.0700	1.1463	16.7215		
CH6_16h_11F_1	19.3440	1.0500	1.0602	19.1588		
CH6_16h_11F_2	19.3315	1.0500	1.0602	19.1464	19.4661	0.5431
CH6_16h_11F_3	20.2874	1.0500	1.0602	20.0932		
CH6_8h_11F_1	19.8974	1.0367	1.0292	20.0418		
CH6_8h_11F_2	20.5639	1.0367	1.0292	20.7132	20.3510	0.3388
CH6_8h_11F_3	20.1518	1.0367	1.0292	20.2981		
CH6_4h_11F_1	18.7044	1.0300	1.0071	19.1289		
CH6_4h_11F_2	18.8568	1.0300	1.0071	19.2848	19.1187	0.1714
CH6_4h_11F_3	18.5220	1.0300	1.0071	18.9424		
CH6_60m_11F_1	18.8825	1.0233	1.0000	19.3231		
CH6_60m_11F_2	18.8777	1.0233	1.0000	19.3182	19.1968	0.2144
CH6_60m_11F_3	18.5172	1.0233	1.0000	18.9493		
CH6_30m_11F_1	21.9859	1.0133	1.0000	22.2791		
CH6_30m_11F_2	21.7034	1.0133	1.0000	21.9928	22.3809	0.4477
CH6_30m_11F_3	22.5698	1.0133	1.0000	22.8708		
CH6_10m_11F_1	18.5436	1.0067	1.0000	18.6672		
CH6_10m_11F_2	19.6214	1.0067	1.0000	19.7522	19.4758	0.7118
CH6_10m_11F_3	19.8754	1.0067	1.0000	20.0079		
CH6_0+_2F_1	20.3023	1.0033	1.0000	20.3700		
CH6_0+_2F_2	19.6258	1.0033	1.0000	19.6912	19.9816	0.3499
CH6_0+_2F_3	19.8175	1.0033	1.0000	19.8836		
CH6_0_1E_1	19.0630	1.0000	1.0000	19.0630		
CH6_0_1E_2	20.0695	1.0000	1.0000	20.0695	19.5610	0.5034
CH6_0_1E_3	19.5504	1.0000	1.0000	19.5504		

Table B.24 - Dilution (DF), Concentration Factors (CF) and Adj Concentration of PDHA for Channel 7

CH 7						
Sample	**µg/15L**	**DF**	**CF**	**Adj Conc µg/15L**	**Average**	**St Dev**
CH7_72h_11D_1	20.7259	1.1033	1.1667	19.6008		
CH7_72h_11D_2	20.0573	1.1033	1.1667	18.9685	18.9822	0.6118
CH7_72h_11D_3	19.4323	1.1033	1.1667	18.3774		
CH7_52h_10D_1	17.6789	1.0867	1.1024	17.4272		
CH7_52h_10D_2	18.1906	1.0867	1.1024	17.9316	17.4154	0.5221
CH7_52h_10D_3	17.1314	1.0867	1.1024	16.8875		
CH7_32h_9D_1	20.1725	1.0700	1.0606	20.3512		
CH7_32h_9D_2	20.3813	1.0700	1.0606	20.5618	20.6251	0.3104
CH7_32h_9D_3	20.7782	1.0700	1.0606	20.9623		
CH7_16h_8D_1	18.9643	1.0500	1.0072	19.7703		
CH7_16h_8D_2	18.6890	1.0500	1.0072	19.4833	19.6896	0.1800
CH7_16h_8D_3	19.0073	1.0500	1.0072	19.8151		
CH7_8h_7D_1	19.4146	1.0367	1.0000	20.1264		
CH7_8h_7D_2	20.0371	1.0367	1.0000	20.7718	20.6933	0.5319
CH7_8h_7D_3	20.4324	1.0367	1.0000	21.1816		
CH7_4h_6D_1	20.0638	1.0300	1.0000	20.6657		
CH7_4h_6D_2	19.9946	1.0300	1.0000	20.5945	20.3909	0.4158
CH7_4h_6D_3	19.3325	1.0300	1.0000	19.9125		
CH7_60m_5D_1	23.4118	1.0233	1.0000	23.9580		
CH7_60m_5D_2	21.1109	1.0233	1.0000	21.6035	22.9408	1.2095
CH7_60m_5D_3	22.7304	1.0233	1.0000	23.2608		
CH7_30m_4D_1	21.3305	1.0133	1.0000	21.6149		
CH7_30m_4D_2	21.8839	1.0133	1.0000	22.1757	22.1605	0.5382
CH7_30m_4D_3	22.3925	1.0133	1.0000	22.6910		
CH7_10m_3D_1	17.0474	1.0067	1.0000	17.1611		
CH7_10m_3D_2	18.6542	1.0067	1.0000	18.7786	17.6229	1.0075
CH7_10m_3D_3	16.8170	1.0067	1.0000	16.9292		
CH7_0+_2D_1	20.6112	1.0033	1.0000	20.6799		
CH7_0+_2D_2	20.2517	1.0033	1.0000	20.3192	20.5552	0.2045
CH7_0+_2D_3	20.5980	1.0033	1.0000	20.6667		
CH7_0_1F_1	23.5145	1.0000	1.0000	23.5145		
CH7_0_1F_2	22.3459	1.0000	1.0000	22.3459	23.0400	0.6145
CH7_0_1F_3	23.2596	1.0000	1.0000	23.2596		

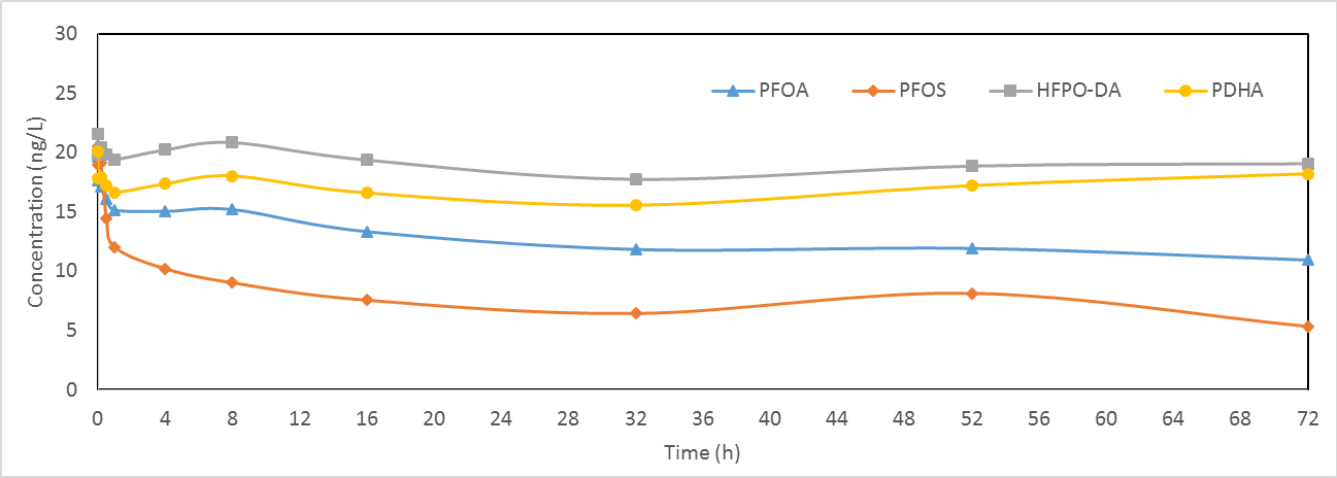

Figure B.1 - Variation in contaminants concentration over time for Channel 01

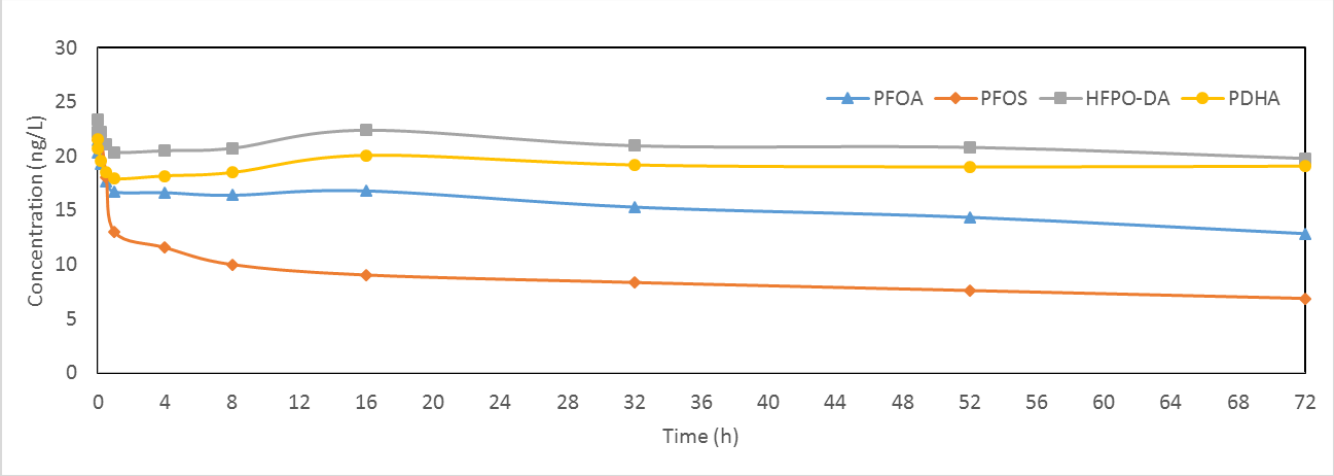

Figure B.2 - Variation in contaminants concentration over time for Channel 02

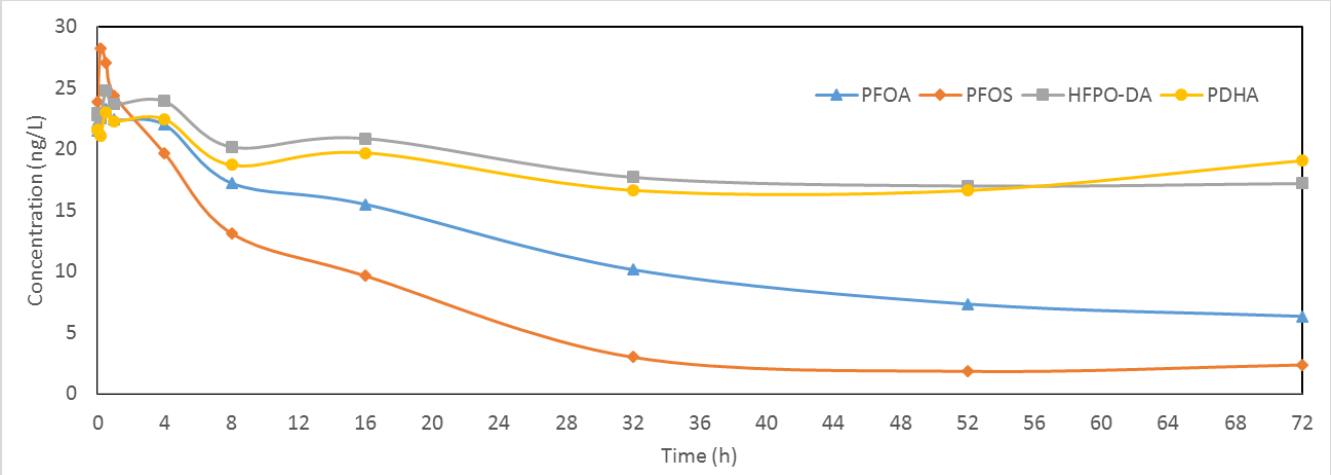

Figure B.3 - Variation in contaminants concentration over time for Channel 03

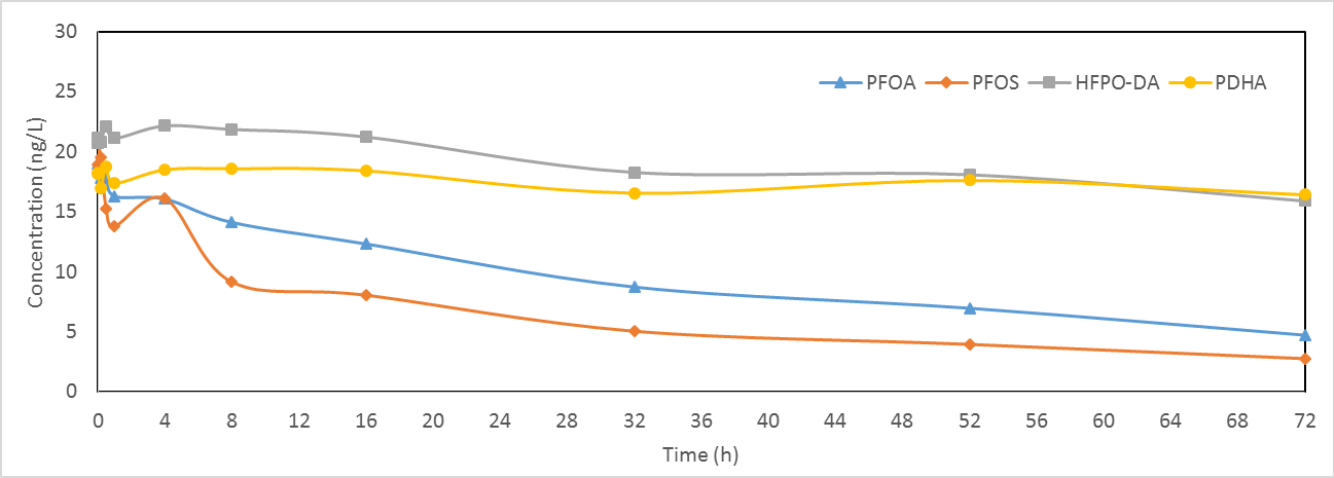

Figure B.4 - Variation in contaminants concentration over time for Channel 05

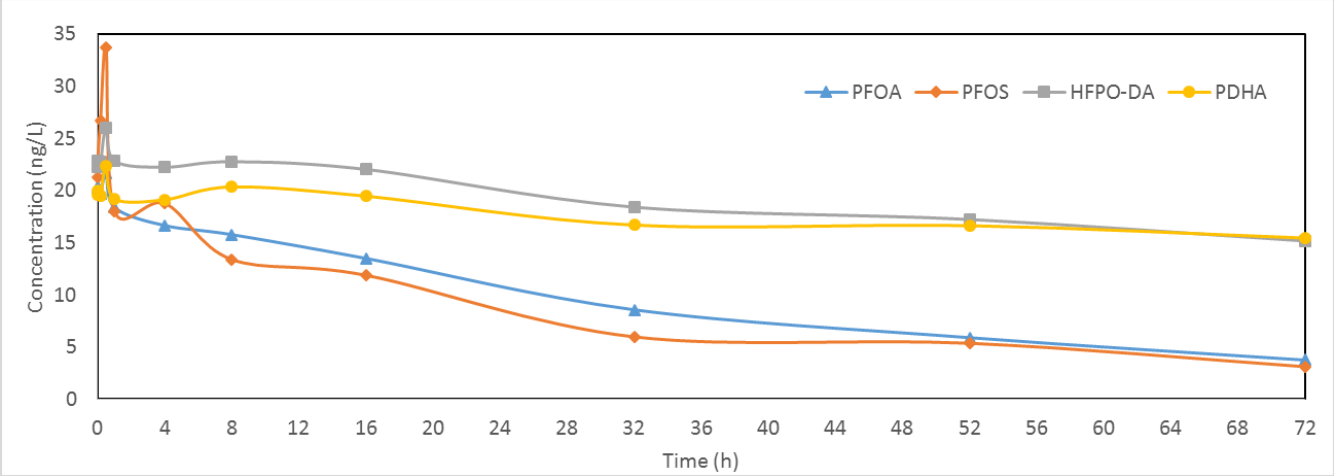

Figure B.5 - Variation in contaminants concentration over time for Channel 06

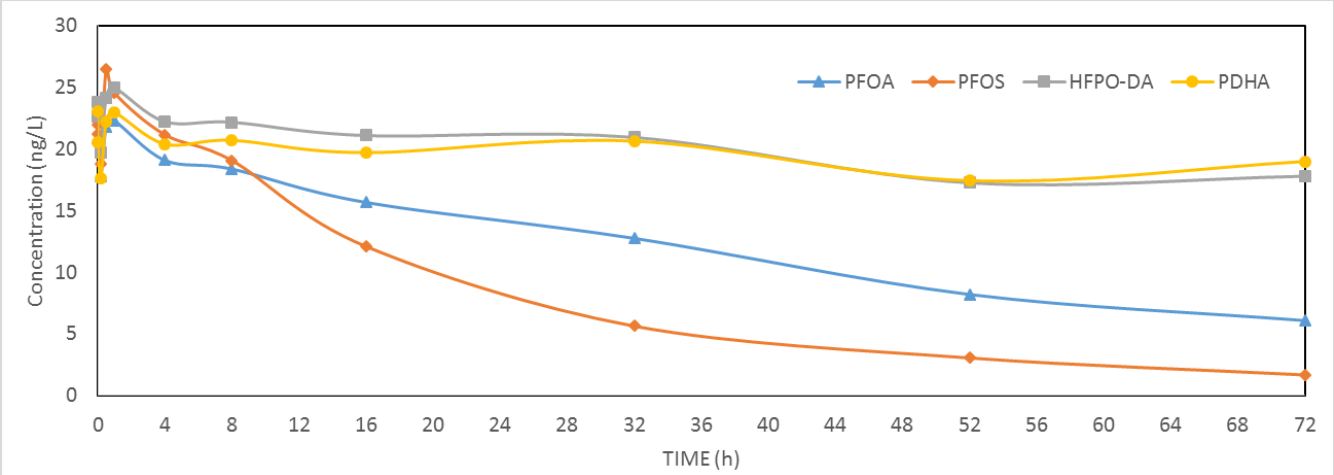

Figure B.6 - Variation in contaminants concentration over time for Channel 07

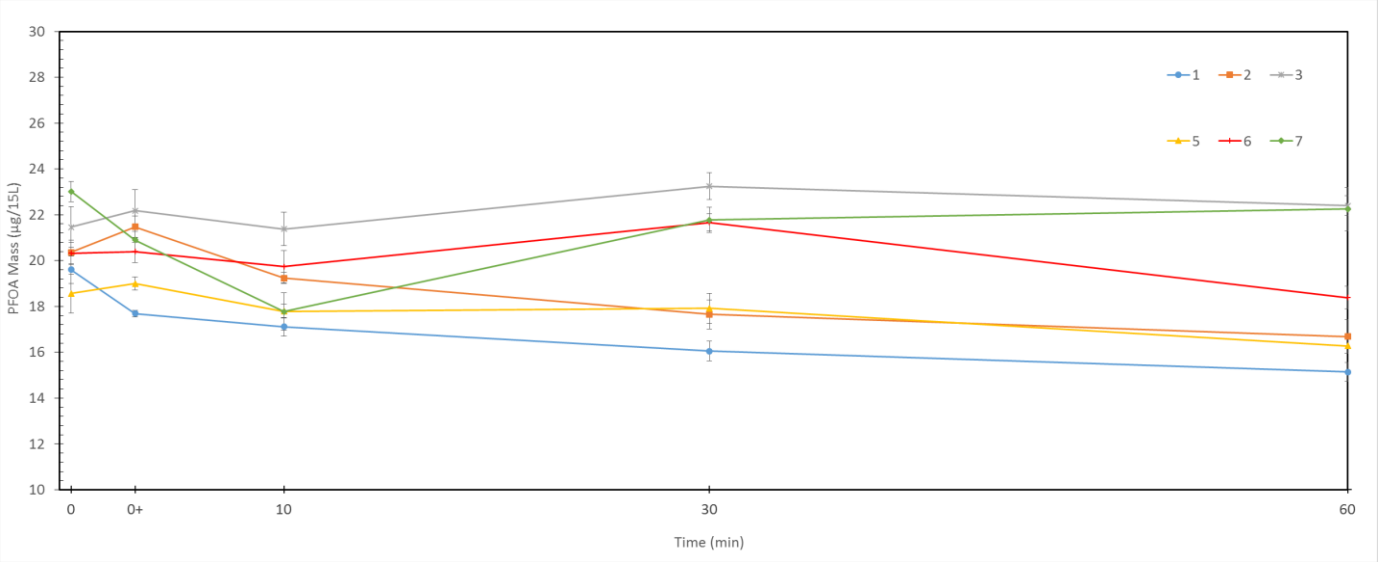

Figure B.7 - Variation in concentration of PFOA within the first hour of exposure for experimental and positive control channels

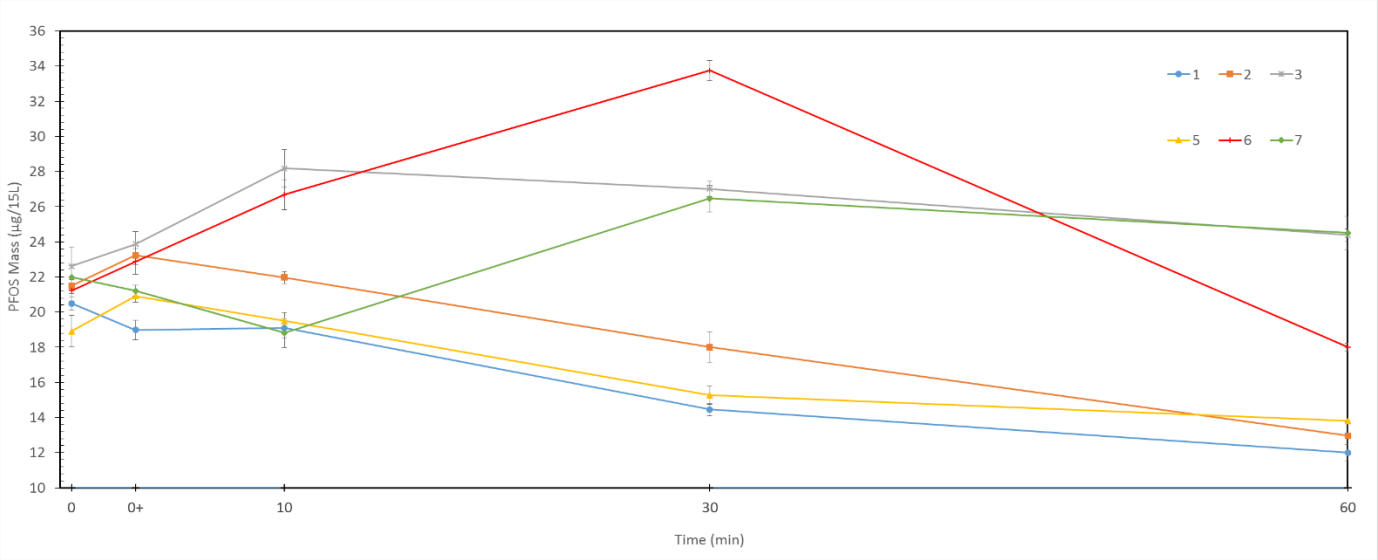

Figure B.8 - Variation in concentration of PFOS within the first hour of exposure for experimental and positive control channels

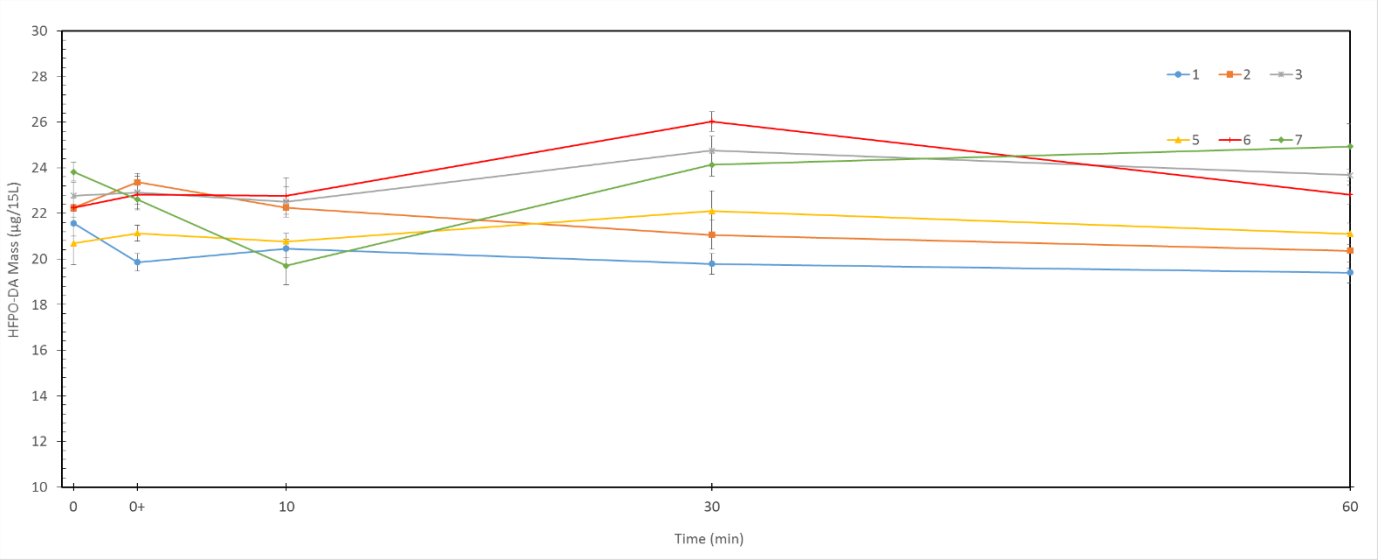

Figure B.9 - Variation in concentration of HFPO-DA within the first hour of exposure for experimental and positive control channels

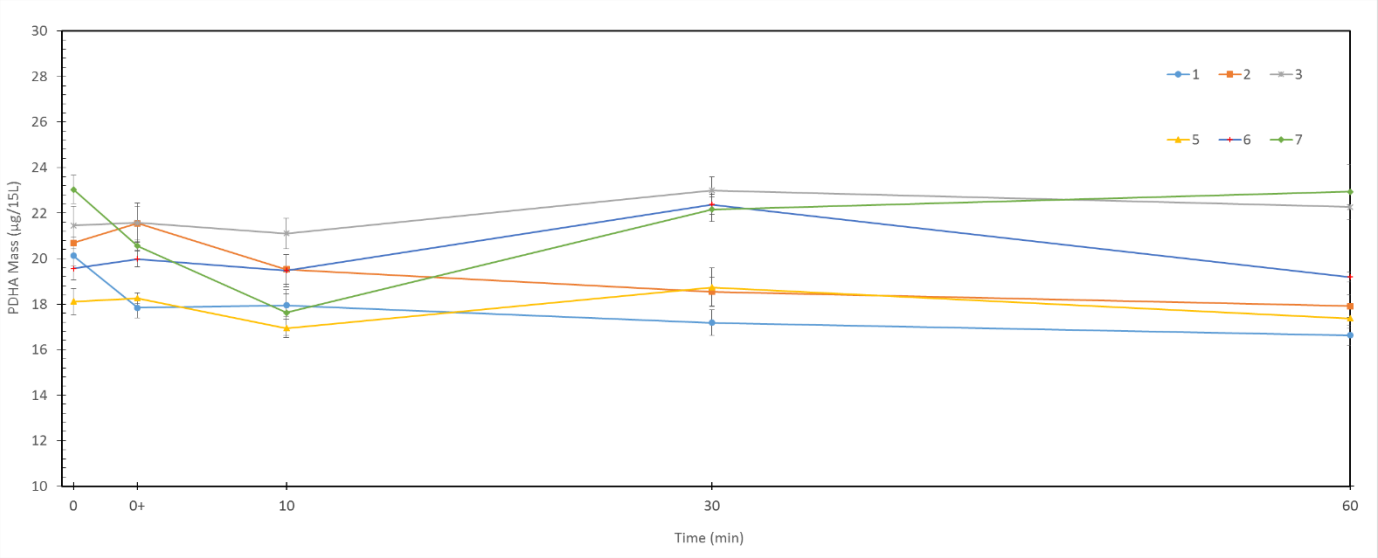

Figure B.10 - Variation in concentration of PDHA within the first hour of exposure for experimental and positive control channels

APPENDIX C – TABULAR DATA FOR TARGETED SUBSTANCES IN ALGAL BIOMASS FROM EXPOSURE STUDY

C.1 – Tabular Data from Biomass Samples – Overall mass absorbed into algal cells

Table C.1 - Complete dataset for mass of contaminants in analyzed biomass (Analyzed DW) and overall biomass (Total DW)

PFOA						
Analyzed DW (g)	**Channel**	**Concentration (ng/6mL)**	**Average**	**Std Deviation**	**Total DW (g)**	**Concentration in Overall Biomass(ng/totalDW)**
1.5287	1	24.8640	26.3958	1.3994	3.1285	54.0193
	1	26.7162				
	1	27.6072				
1.1832	2	45.3030	46.8464	1.3366	2.6265	103.9909
	2	47.6100				
	2	47.6262				
1.5561	5	16.7424	17.0630	0.3784	3.1511	34.5525
	5	16.9662				
	5	17.4804				
0.9939	6	7.6038	7.7584	0.3575	1.9338	15.0953
	6	7.5042				
	6	8.1672				
PFOS						
1.5287	1	99.5658	102.1194	2.5782	3.1285	208.9884
	1	102.0708				
	1	104.7216				
1.1832	2	107.9574	109.8286	1.6478	2.6265	243.8006
	2	111.0630				
	2	110.4654				
1.5561	5	67.2282	69.4064	1.9582	3.1511	140.5478
	5	71.0208				
	5	69.9702				
0.9939	6	35.7846	35.8754	2.1440	1.9338	69.8016
	6	33.7782				
	6	38.0634				

HFPO-DA						
1.5287	1	4.0746	4.0566	0.0276	3.1285	8.3019
	1	4.0248				
	1	4.0704				
1.1832	2	3.8994	3.9112	0.0406	2.6265	8.6822
	2	3.9564				
	2	3.8778				
1.5561	5	3.9876	3.9654	0.0344	3.1511	8.0299
	5	3.9828				
	5	3.9258				
0.9939	6	3.9426	3.9362	0.0171	1.9338	7.6585
	6	3.9168				
	6	3.9492				
PDHA						
1.5287	1	4.2456	4.2496	0.2682	3.1285	8.6968
	1	3.9834				
	1	4.5198				
1.1832	2	9.8562	9.6348	0.2012	2.6265	21.3876
	2	9.4632				
	2	9.5850				
1.5561	5	3.8640	4.0006	0.2319	3.1511	8.1012
	5	4.2684				
	5	3.8694				
0.9939	6	3.1566	3.0958	0.1316	1.9338	6.0234
	6	2.9448				
	6	3.1860				

C.2 – Tabular Data from Filtered Biomass Samples – Overall mass absorbed on algal cells

Table C. 2 - Complete dataset for mass of contaminants in analyzed filtered volume and in overall biomass

PDHA							
Channel	**Raw Conc. (ng/6mL)**	**Volume Analyzed (mL)**	**Background Mass (ng)**	**Adj. Conc. (ng/6mL)**	**Average Conc. (ng/6mL)**	**Avg Mass/unit biomass (ng/gDW)**	**Overall Mass (ng)**
1	87.8856	39	47.33	40.5556	38.953	25.48112776	38.953
1	88.3236	39		40.9936			
1	82.6398	39		35.3098			
2	120.933	49	62.33	58.603	56.1982	47.4968	56.1982
2	118.6968	49		56.3668			
2	115.9548	49		53.6248			
5	63.9816	26	56.86	71.1032	70.5036	45.3078851	70.5036
5	62.445	26		68.03			
5	64.6188	26		72.3776			
6	71.5242	20	40.46	102.5884	105.8336	106.4831472	105.8336
6	75.6678	20		110.8756			
6	72.2484	20		104.0368			
HFPO-DA							
Sample	**ng/6 ml**	**Volume Analyzed (mL)**	**Background Mass (ng)**	**Adj. Conc. (ng/6mL)**	**Average Conc. (ng/6mL)**	**Avg Mass/unit biomass (ng/gDW)**	**Overall Mass (ng)**
1	56.7258	39	49.53	7.1958	5.8684	3.838817296	5.8684
1	56.043	39		6.513			
1	53.4264	39		3.8964			
2	93.3492	49	64.64	28.7092	26.0254	21.9958	26.0254
2	89.3106	49		24.6706			
2	89.3364	49		24.6964			
5	35.9214	26	55.06	16.7828	16.4124	10.54713707	16.4124
5	35.3742	26		15.6884			
5	35.913	26		16.766			
6	44.301	20	40.44	48.162	48.2192	48.51514237	48.2192
6	46.2888	20		52.1376			
6	42.399	20		44.358			

PFOA							
Sample	ng/6 ml	Volume Analyzed (mL)	Background Mass (ng)	Adj. Conc. (ng/6mL)	Average Conc. (ng/6mL)	Avg Mass/unit biomass (ng/gDW)	Overall Mass (ng)
1	136.4544	39	28.42	108.0344	105.2816	68.87002028	105.2816
1	136.7598	39		108.3398			
1	127.8906	39		99.4706			
2	153.219	49	41.98	111.239	107.8346	91.1381	107.8346
2	150.2772	49		108.2972			
2	145.9476	49		103.9676			
5	55.1376	26	16.328	93.9472	93.124	59.844483	93.124
5	53.9796	26		91.6312			
5	55.0608	26		93.7936			
6	38.6118	40	10	67.2236	66.852	67.26230003	66.852
6	39.5232	40		69.0464			
6	37.143	40		64.286			
PFOS							
Sample	ng/6 ml	Volume Analyzed (mL)	Background Mass (ng)	Adj. Conc. (ng/6mL)	Average Conc. (ng/6mL)	Avg Mass/unit biomass (ng/gDW)	Overall Mass (ng)
1	63.0252	39	14.12	48.9052	48.545	31.75574017	48.545
1	64.8456	39		50.7256			
1	60.1242	39		46.0042			
2	61.0536	49	22.44	38.6136	37.3234	31.5445	37.3234
2	60.4518	49		38.0118			
2	57.7848	49		35.3448			
5	26.0958	26	9.56	42.6316	41.9852	26.98104235	41.9852
5	24.912	26		40.264			
5	26.31	26		43.06			
6	18.5412	40	8.2934	28.789	29.3658	29.54603079	29.3658
6	19.5984	40		30.9034			
6	18.3492	40		28.405			