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The Use of Algae for the Removal of Perfluorooctane Sulfonic Acid (pfos)

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THE USE OF ALGAE FOR THE REMOVAL OF PERFLUOROOCTANE SULFONIC ACID (PFOS)

by

Zach Henderson

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ABSTRACT

THE USE OF ALGAE FOR THE REMOVAL OF PERFLUOROOCTANE SULFONIC ACID (PFOS)

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Zach Henderson

The University of Wisconsin-Milwaukee, 2022

Under the Supervision of Professor Shangping Xu

Per- and polyfluoroalkyl substances (PFAS) are a class of artificially manufactured compounds, being extensively used for different industrial and commercial products since the mid-twentieth century. There are growing public health concerns about these chemicals due to their toxicity in humans and wildlife, being known carcinogens and disruptors of the endocrine system. Current remediation processes for PFAS often have limited efficiency under conditions of high natural dissolved organic carbon (DOC), high or low solution pH, initial levels of competing pollutants, high dissolved salt concentrations and so on. The main hypothesis for this research was selected algae strains may be able to remove PFAS from contaminated water sources such as wastewater treatment plants and industrial effluents. The main goal of this project was to quantify the effectiveness of several selected fresh water algal species on the removal of PFOS from water media in a laboratory setting.

I would like to thank for my professor and advisor, Dr. Shangping Xu, for all the help with preparing and completing this project. Staring your masters during a pandemic was a tricky situation and he helped me a lot along the way. I would also like to thank the many people who helped me on the project along the way: Nate Thorngate-Rein, Dr. Erica Young, Dr. Yin Wang and Dr. John Berges. Without their knowledge, I would not have been able to complete the project. I would also like to thank my office mates, Cullen and Kendyl for always being there and helping when times get tough. Finally, I would like to thank my friends, family and girlfriend for helping me along the way and always letting me know there is a light at the end of the tunnel! Thanks to each and everyone of you, I have completed my thesis and have completed my educational journey. From the bottom of my heart, thank you all.

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LIST OF ABBREVIATIONS

AFFF: Aqueous film-forming foams

Bioaccumulation Factor: BAF

DOC: Dissolved organic carbon

CI-PFESA: Chlorinated polyfuoroalkyl ether sulfonic acid

HDPE: High density polyethylene

PFAS: Per- and polyfluoroalkyl substances

PFOS: Perfluorooctane sulfonic acid

PP: Polypropylene

WW: Wet weight

WWTPs: Wastewater treatment plants

µg: Microgram

µL: Microliter

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a class of artificially manufactured compounds, being extensively used for different industrial and commercial products since the mid-twentieth century (Wang et al., 2017). PFAS compounds are synthetic chemicals consisting of hydrophobic and lipophobic fluorinated carbon chains and hydrophilic functional group, having a high thermal and chemical stability due to the strong C-F bonds (Mulabagal et al., 2018). Because of the unique combination of chemical and physical properties, PFAS has been used for many consumer, commercial and industrial applications such as water proofing applications, non-stick pans, electronic manufacturing, industrial surfactants, and firefighting foams. (Kissa 2001; OECD 2015; Organisation for Economic Co-operation and Development 2002; Paul et al. 2008; Pontius 2019; US Environmental Protection Agency 2019). Due to their wide range of applications, the recalcitrant nature and relatively high aqueous solubility's, PFAS are now found in soil, groundwater, surface water and aquatic biota. (Giesy and Kannan 2001; Giesy and Kannan 2002; Guelfo Jennifer et al. 2018; Haag 2020; Kaboré et al. 2018; Kunacheva et al. 2012; Wisconsin Department of Natural Resources 2020). Because the substances are found to be carcinogenic, disruptors of the endocrine system, as well as being linked to several other adverse health effects (Blum et al. 2015), there are growing concerns about the health impacts in humans as well as wildlife.

Wastewater treatment plants (WWTPs), airports, landfills and industrial facility effluents have been found to be major sources of PFAS substances (Meegoda et al.,2020). For instance, airports regularly conduct firefighting trainings. During these

trainings, firefighting foams, specifically class B aqueous film-forming foams (AFFF) are often used. Both surface water runoff and groundwater infiltration can allow for PFAS compounds to contaminate the surrounding waters and sediments (Meegoda et al.,2020). PFAS compounds are also used in many household products. Once these products are disposed of, they typically end up at the landfill. The leachate is often disposed and processed through WWTPs, which will lead to the release of PFAS through the WWTPs effluent. Other sources of contamination from landfills may be due to groundwater infiltration from unlined landfills, as well as leaky landfill linings (ITRC PFAS Team, 2020). Many WWTPs, airports and industrial facilities, however, do not have systems set in place to remove persistent organic contaminates from the effluent before releasing the treated water back into the environment (Stroski et al., 2020; Chen et al., 2018). Furthermore, the bioaccumulation of PFAS in surface waters has been found within the food web (e.g., fish and plankton) (Rossmann 2005).

The most widely used treatment techniques for the removal of PFAS from water include activated carbon adsorption, resin ion exchange, and membrane filtrations (American Water Works Association, 2020). The efficiency of PFAS removal using the previous techniques can be significantly impaired by the presence of natural dissolved organic carbon (DOC), solution pH, initial levels of pollutants, and the adsorbent type (Omo-O koro et al., 2018; Darlington et al., 2019; Du et al., 2014). There is a need for the development of alternative PFAS removal techniques, which can be low-cost, and can process large volumes of contaminated water under challenging conditions (e.g., high DOC)

Recent studies reported that bioaccumulation of PFAS could occur within both freshwater and marine algal species. For instance, it was shown that the bioaccumulation factor for perfluorooctanesulfonoic (PFOS) by marine algae could be ~20,000 ($10^{4.3}$) (Zhang et al. 2019). The aim of this study is to determine the potential use of selected algal species for the removal of PFOS, which is a common and representative PFAS substance, from the water media.

2. OBJECTIVE

Algae has been used for the removal of chemical contaminants such as heavy metals, nitrogen and phosphorus removal, volatile organic compounds (VOCs), and chemical contaminates (Sabariswaran et al., 2021; W. H. Adey and Loveland 2007). For instance, the Algal Turf Scrubber^{TM} , a simulated stream system, uses periphytic algae for nitrogen and phosphorus removal. Algae has also been used for the remediation of volatile organic compounds (VOCs) (W. H. Adey and Loveland 2007). Limited amount of research has been conducted to determine if algal species are able to remove or degrade PFAS from contaminated waters.

Recent studies reported the bioaccumulation of PFAS could occur within both freshwater and marine algal species. For instance, it was shown the bioaccumulation factor for perfluorooctanesulfonoic (PFOS) by marine algae could be ~20,000 ($10^{4.3}$) (Zhang et al. 2019). Its hypothesized algae would be able to remove PFOS from contaminated water. The specific goal of this study is to determine the potential use of selected algal species for the removal of PFOS, which is a common and representative PFAS substance.

3. METHOD AND MATERIALS

Algal Specie Selection

The algal seed culture was purchased from The University of Texas Austin (UTEX). All algal cells were grown in separate 1-liter round flask, with bubbling air, stirring with stir bar/magnet, sampling, and exhaust exports (Figure 1). All materials used for the growth of the algae were autoclaved to ensure no contaminated, as well as all experiments and algal transfers took place in the sterile hood. The algae were grown at 18 °C, with bubbling air and high light (16-hour light: 8-hour dark cycle) to create a high biomass culture. Once the cells reached optimal density, 5-mL of the culture was aliquoted into smaller 60 mL tubes for the growth experiments. The remaining culture was left as a main culture, used for future experiments. The main culture was refreshed with new media every two weeks (~200 mL), as well as 200 µL of $NaNO_3$ (46.8 g/L) and $KH2PO_4$ (3.05 g/L) (each) to replenish any nutrients used by the culture.

The ideal algal species that are suited for PFOS removal should be fast growing and can tolerate PFOS concentrations that are comparable to the high range of naturally observed concentrations and can effectively remove PFOS under challenging chemical conditions (e.g., the presence of high DOC concentrations). Exploratory experiments were first performed to screen for a series of algae species that maintain similar growth kinetics under the presence of PFOS. A total of three main cultures, consisting of *Chlamydomonas reinhardtii, Chlorella vulgaris* and *Raphidocelis subcapitata,* were then selected based on the results of the exploratory experiments and continually sustained in the growth chamber.

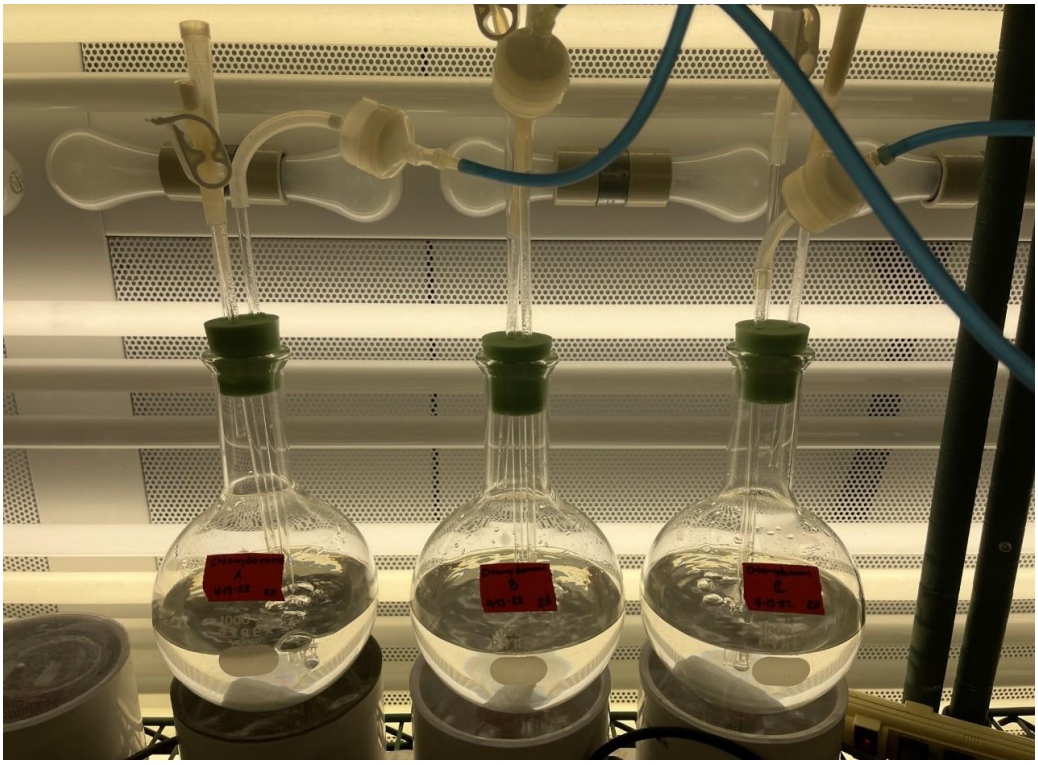

Figure 1: Image of algae growth 1-liter round flask set up with bubbling air (filtered), sampling and exhaust ports.

During the growth phase of the algae culture, fluorescence measurements were taken daily using TD- 700 Fluorometer. A media blank was run first to measure any background fluorescence in the algal media and the background measurement was subtracted from the algal measurements. The 60 mL tubes with the algal cells were inverted 10 times (by hand) to homogenize the sample, then placed in in the fluorometer and the F0 value was recorded once the values stabilized. Fluorescence measurements were continually taken daily until the values began to plateau, indicating the algae was at its max biomass.

Flow cytometry was also used to count the density of algae cells (i.e., number of algae cells in unit volume of aqueous phase) and when needed, to quantify the number

of live and dead algae cells. The flow cytometry measurements were carried out using a BD Accuri™ C6 flow cytometer. A volume of 200 µL was taken from the 60 mL growing tubes and ran for cell density. The measurement was conducted in the beginning, middle and end of the algal growth periods. The flow of the flow cytometer was set at 65 µL/minute and ran for 30 seconds, for an average of 32.5 µL cell count analysis. The algal cells were then gated in the software to ensure no noise (dust and other small particles) were counted in the cell count.

High Density Algal Sub-Culture Creation

Using the previous main cultures, sub-cultures were grown to be used for the high cell density PFOS removal experiments. All algal cells were grown in separate 1-liter round flask, with bubbling air, stirring with stir bar/magnet, sampling, and exhaust exports (Figure 2). Similarly, all materials used for the growth of the algae were autoclaved to ensure no contamination, as well as all experiments and algal transfers took place in the sterile hood. The algae were grown at 18 °C, with bubbling air and high light (16-hour light: 8-hour dark cycle) to create a high biomass culture. Five separate 1-liter round flasks were used to grow five sperate algae cultures of the same species for triplicate PFOS removal experiments and additional DOC experiments.

Each of the algal species were grown in DYV media (see appendix B for media concentrations). 500 mL of DYV media were added to the 1-liter round flask, as well as an additional 500 µL of NaNO3 (46.8 g/L) and KH2PO4 (3.05 g/L) (each) for extra nutrients. 10 mL of algal cells were aliquoted from the main cultures into the new flasks,

creating the sub-cultures. Depending on the algal species, the growth time to reach optimal cell density was 1-2 weeks.

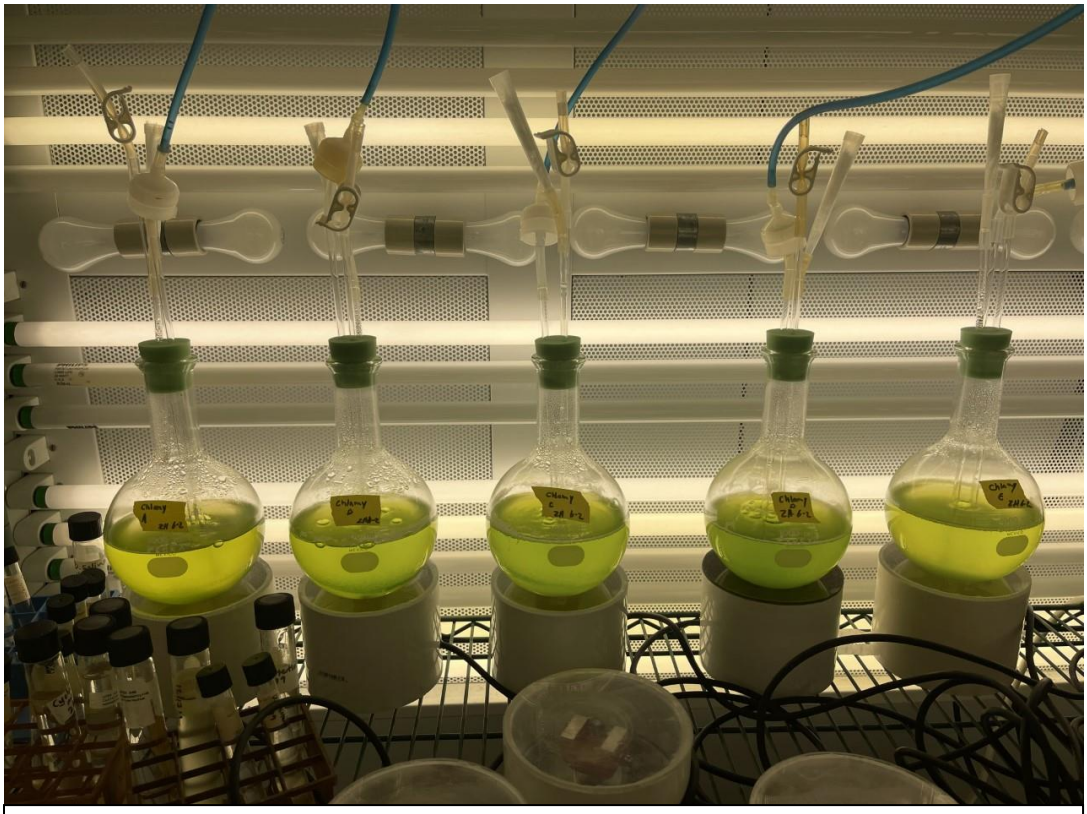

Figure 2: Image of sub-culture for high density PFOS removal experiment in the growth chamber with bubbling air (filtered), sampling and exhaust ports.

PFOS Removal/DOC Experimental Set Up

Once the cells reached stationary phase, 250 mL of the total 500 mL in each flask were distributed between two 250 mL centrifuge bottles (total of 10 bottles). The bottles were centrifuged at 2500 rpm, for a total of 30 minutes. The supernatant waste was gently poured off, leaving a “pellet” of algal cells at the bottom of the bottle. This step was repeated four separate times, for each of the five flasks. The algae pellets were then reconsolidated and resuspended in a separate flask for later distributions. This process was done to ensure cell density was kept consistent for each triplicated and additional experiments.

The experimental set up consisted of nine 60 mL PP tubes. Three of the tubes were used for PFOS removal from algal cells, another two used to quantify the concentrations of DOC within the algae suspension (Figure 3), and the remaining four tubes were used for PFOS control triplicates, as well as a PFOS blank to ensure no contamination is occurring.

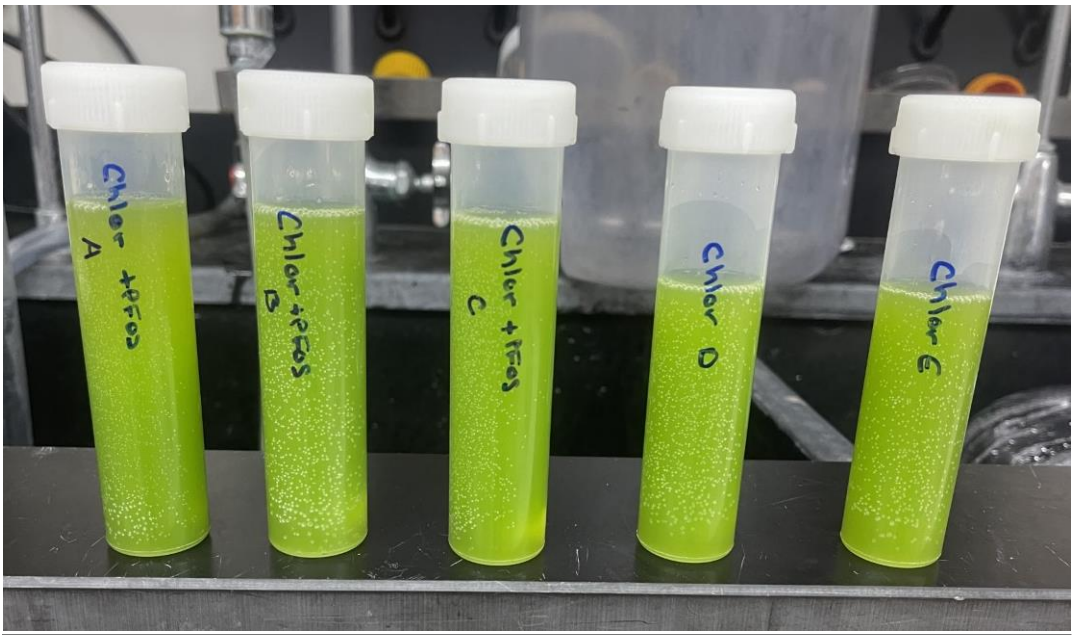

Figure 3: Example 60 mL PP tubes with algal cells. Tubes labeled A-C contain PFOS, tubes labeled D and E are for DOC measurements.

Before any addition of algal cells, 20 mL of DYV media was added to all 60 mL PP tubes, as well as a magnetic stir bar. The additional nutrients were added to all nine of the tubes, as well as 60 µL of 100 ppm PFOS potassium salt stock (Sigma-Aldrich INC, ≥98% purity) added to the triplicate algal cells and PFOS control tubes. The PFOS potassium salt stock was dissolved in LC-MS grade methanol. The tubes were then mixed on magnetic stir plate for a total of five minutes to ensure all nutrients and PFOS stock was homogenized in the PP tube. After mixing, algal cells were added to the five PP tubes with algal cells, as well as additional DYV media to the remaining three PFOS control PP tubes and single blank control tube to a total volume of 60 mL. The initial PFOS concentration in the tubes is 100 ppb. The tubes were then placed on the magnetic stir plate for another two minutes to homogenize.

Sample collection was performed following the mixing step. Briefly, each 60 mL PP tube was inverted ten times by hand to ensure homogenization. 3 mL aqueous sample was then extracted from the 60 mL PP tubes with algal cells and PFOS, as well as the PFOS control and blank tubes. The samples were filtered using glass fiber AE filters to catch the algal cells. The sampling times were the following: ~0, 0.5, 1, 2, 3, and 6 hours. Time 0 was considered approximate because the completion of the mixing step would take a few minutes. The actual sampling time was recorded. 5 mL were extracted from the remaining two algal cell positive tubes which would be used for DOC measurement. After each sampling period, the PP tubes were placed back in the growth chamber and mixed on the magnetic stir magnets.

Biomass Measurement

Upon the completion of the sample collection (~6 hours), the remaining volume of samples were used to quantify the total algae biomass in the tubes. ~10 mL of algal cells from tubes were extracted and vacuum filtered through a A/E filter. The filters were dried for a total of three days. The filters were then weighed to quantify the dry mass of the algae cells.

Flow Cytometry Measurement

For each algae sample, an additional 250 µL sample was taken for the quantification of live and dead algal cell using flow cytometry. Each sample was firstly diluted by a ratio of 1:10 (v/v) with algal DYV media to assure optimal density of algal cells suited for flow cytometry measurements. 100 µL of the diluted sample was placed in a new tube and mixed with 10 µL of sytox green solution which would dye any dead

algal cells that had compromised cell walls. The samples with sytox green were then placed in a dark box for 15 minutes of reaction time. Upon the completion of the reaction step, the samples were immediately run through the flow cytometer.

The parameters set for *Chlamydomonas reinhardtii* start with creating a threshold of only recording events greater than 10^3 FL3-H (log based) forward red fluorescence. The remaining events were then distributed into two separate gates (Figure 4), P1 being the healthier cells, and P2 are the less healthy cells. The overall health can be seen by having less red fluorescence than P1 and smaller in size (FSC-H). The total count of P1 and P2 was added together to get the total count of algal cell. The cells with green dye, which indicated compromised cell walls, would be counted as dead cells (Figure 5).

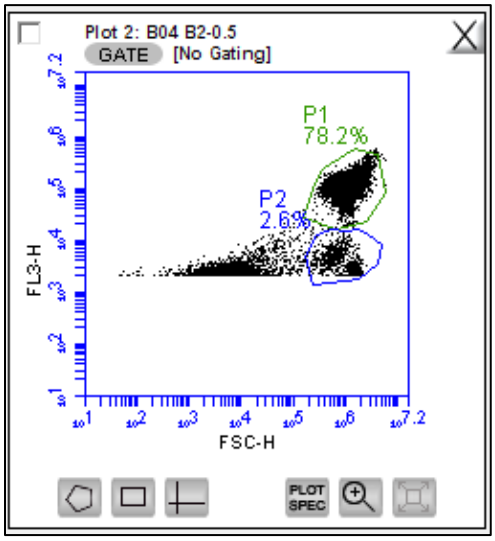

Figure 4: Example of gating plot for algal cells.

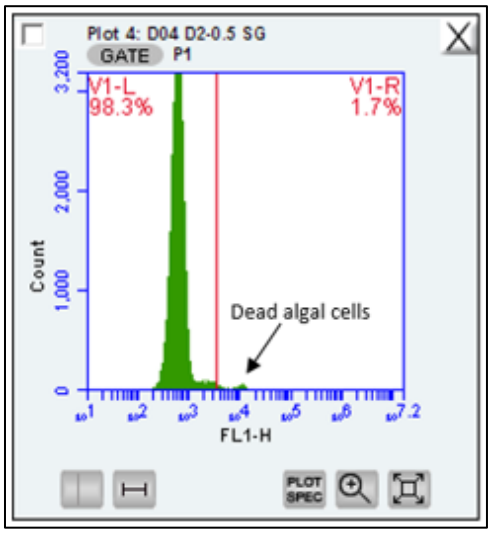

Figure 5: Example of analysis for dead algal cells.

PFOS Concentration Measurements Using LC-MS-2020

The concentrations of PFOS were determined at the University of Wisconsin Milwaukee (UWM) Kenwood Interdisciplinary Research Center (IRC) using the Shimadzu Single Quadrupole High-Performance Liquid Chromatography - Mass Spectrometer (LC-MS) 2020. Samples were prepped for the Shimazu LC-MS 2020 by diluting with LCMS grade MeOH 1:1 (v:v) as well as having an internal standard of M8PFOS 10 ppb (Wellington Laboratories, ≥98% purity) to correct for any signal drift in the analysis. The internal standard SOP was developed based on the modification of U.S EPA Validated Method 8327. All samples used for the LC-MS analysis were filtered using a 0.22 µm Millex syringe filters with a PES membrane.

Approximately 1 mL was pushed through the filters before the sample was collected. 0.5 mL of the sample was extracted and placed into 1.5 mL PP auto sampler tubes. 0.5 mL of LC-MS grade MeOH, containing M8PFOS internal standard, was also added to the auto sampler tubes. The tubes were then mixed via vortex mixed for 1 minute. The sample were then analyzed with the LC-MS 2020.

The mobile phases used for the analysis were LC-MS grade H_2O + 0.1% formic acid and acetonitrile. The column used for the analysis was a Shimadzu Nexcol C18 1.8 um 50 x 2.1 mm. The flow rate was set at 4 mL/minute and the volume of the sample was 2 µL. One example calibration curve for PFOS is shown in Figure 6 with a R^2 value >0. 99.

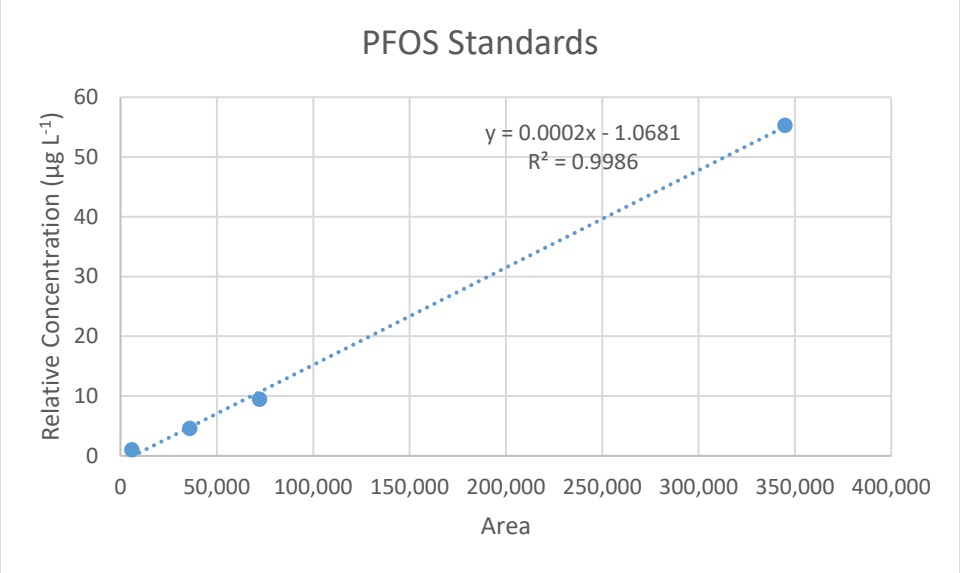

Figure 6: Calibration curve for target PFOS detection.

4. RESULTS AND DISCUSSION

Cell Count/Viability Results

The total, live and dead algae cell counts are shown in Figures 7-9. On average, each PFOS positive algal specie's (samples A-C) total cell count was within 5-10% of the algal species (samples D-E) without PFOS. This result indicated that the survival of the algal species used for the PFOS removal experiments was not influenced by PFOS at a concentration of 100 µg L^{-1}. This was also supported by the observation that the number of dead algae cells was usually >2 orders of magnitude lower than the live cells. Relatively speaking, *Chlamydomonas reinhardtii* had the most cell death for both PFOS positive and PFOS negative samples, over the total sampling time, then the other two species. Further research will be needed to elucidate potential underlying causes.

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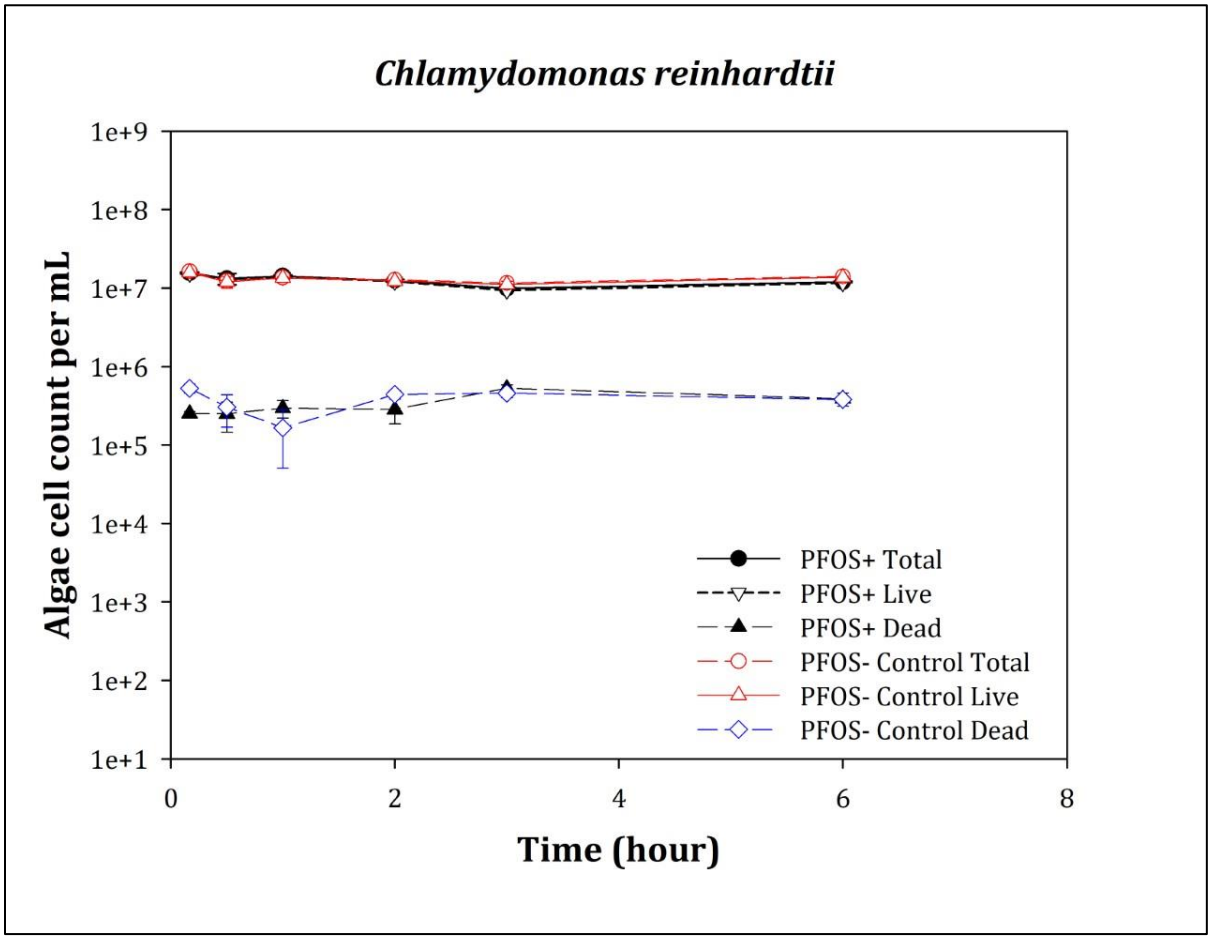

Figure 7: *Chlamydomonas reinhartii* cell count per mL. Y-axis depicted in logarithmic base ten.

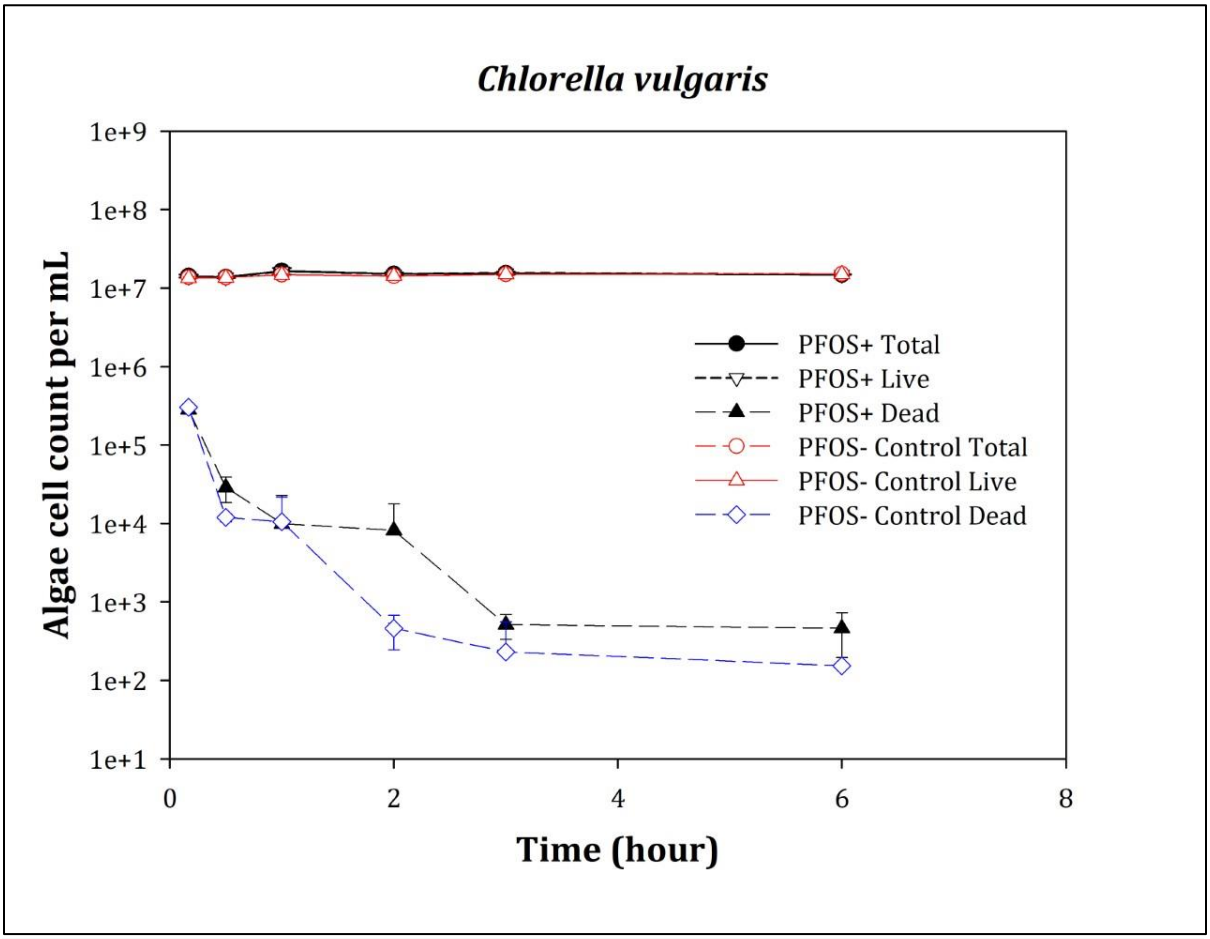

Figure 8: *Chlorella vulgaris* cell count per mL. Y-axis depicted in logarithmic base ten.

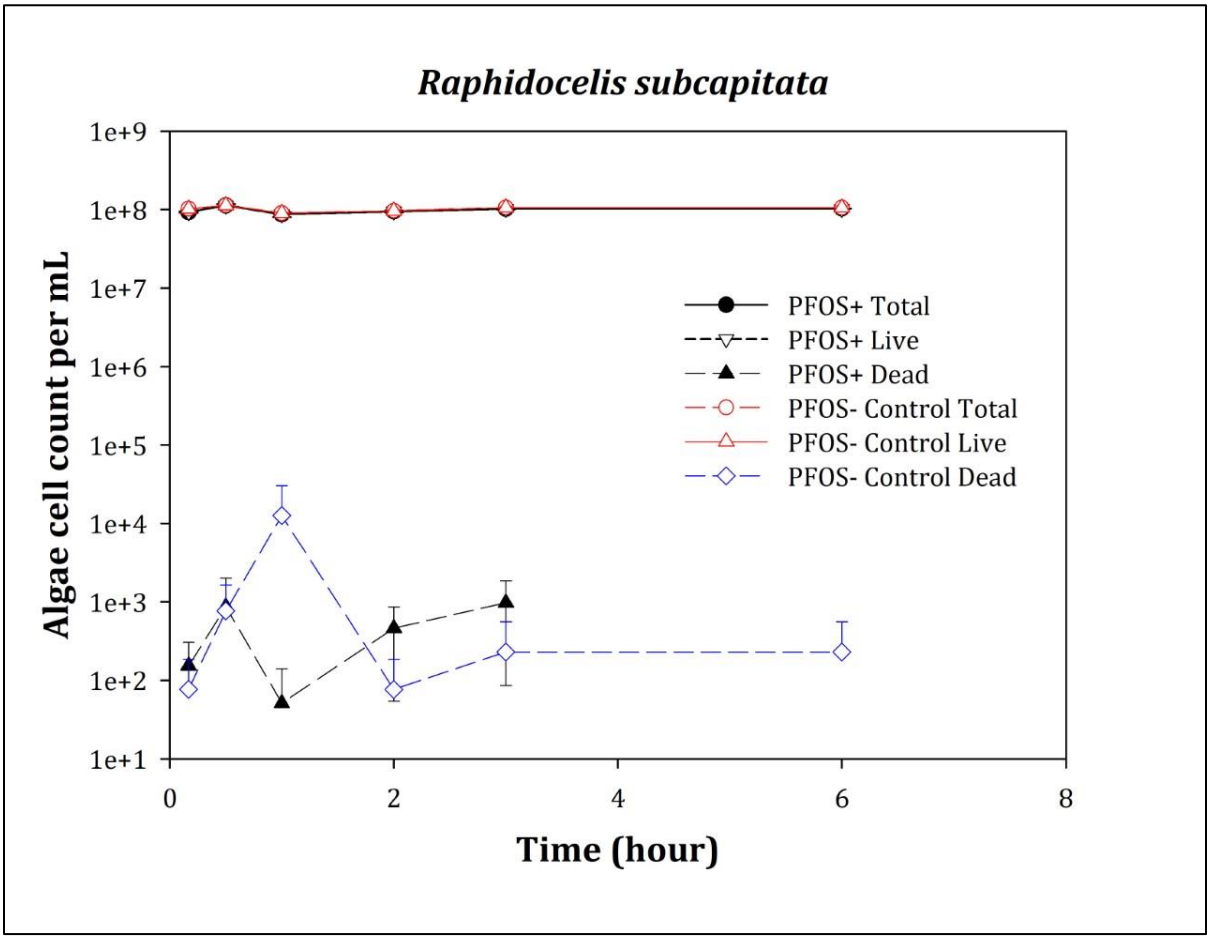

Figure 9: *Raphidocelis subcapitata* cell count per mL. Y-axis depicted in logarithmic base ten.

Biomass Results

Each algal sample's biomass was weighed and recorded to determine the total amount of algae biomass in the growth tubes. This data was then used to determine the amount of PFOS removed by the total amount of algal biomass in the tubes. See table 1 for algal biomass results.

Algal Species/Samples	Biomass (g)
Chlamydomonas reinhardtii	
Sample A	0.08220
Sample B	0.07620
Sample C	0.07980
Sample Average	0.07940
Chlorella vulgaris	
Sample A	0.13260
Sample B	0.13260
Sample C	0.13260
Sample Average	0.13260
Raphidocelis subcapitata	
Sample A	0.07680
Sample B	0.07800
Sample C	0.07680
Sample Average	0.07720

Table 1: Biomass results.

PFOS Removal Results

The algal species used were the following: *Chlamydomonas reinhardtii, Chlorella vulgaris* and *Raphidocelis subcapitata.* Triplicate replications were utilized during the experiments to ensure the results were adequate. Three algal cell, PFOS positive, three media and PFOS positive (controls), and one media blank were used in the experiment. The controls were used as a baseline for PFOS concentration.

The measured concentration of PFOS in the blank controls were below detection limit, suggesting the growth media and plastic wares used for the experiments were PFOS free.

The concentrations of PFOS in the growth tubes compared to the PFOS positive controls were shown in Figure 10 – 12. The results revealed significant removal of PFOS by the three selected algae species. For *C. reinhardtii*, the average concentration of PFOS in the control was 75.45(±13.30) ppb, while the average concentration in under the presence of algae was 30.07(±9.65) ppb, representing a 60.2% removal of PFOS. More notably, the PFOS removal seemed to occur within 10 minutes of contact time, indicating that the kinetics of the removal of PFOS by *C. reinhardtii* was fast, which could represent a major advantage in PFOS removal using *C. reinhardtii*. Similar trend was observed for *C. vulgaris* and *R. subcapitata* (Figures 11 and 12). For *C. vulgaris*, PFOS concentrations in the control vials were 86.43(±24.24) ppb, while its concentrations in the algae suspension dropped to 55.6(±7.83) ppb within 10 minutes of contact time. For *R. subcapitata*, about 36.65% of PFOS (from 107.5(±3.52) to 68.10(±4.20) ppb) was removed from the aqueous phase within 10 minutes.

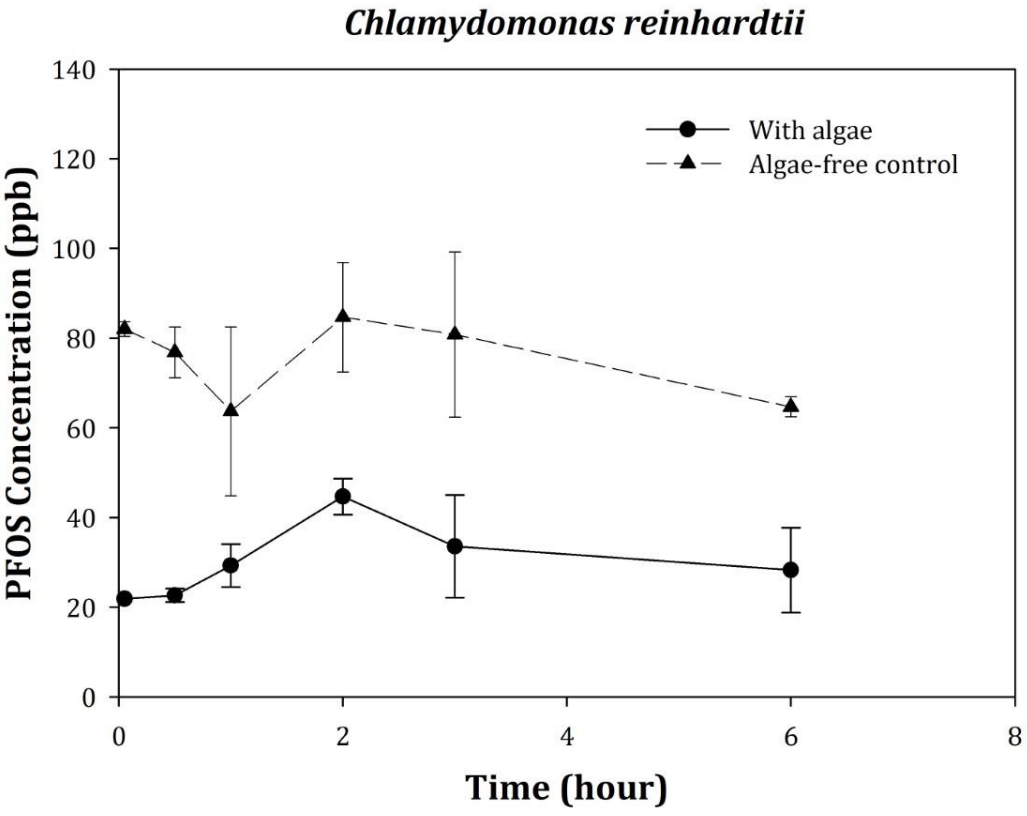

Figure 10: PFOS concentrations measured in the growth tubes for *Chlamydomonas reinhardtii*.

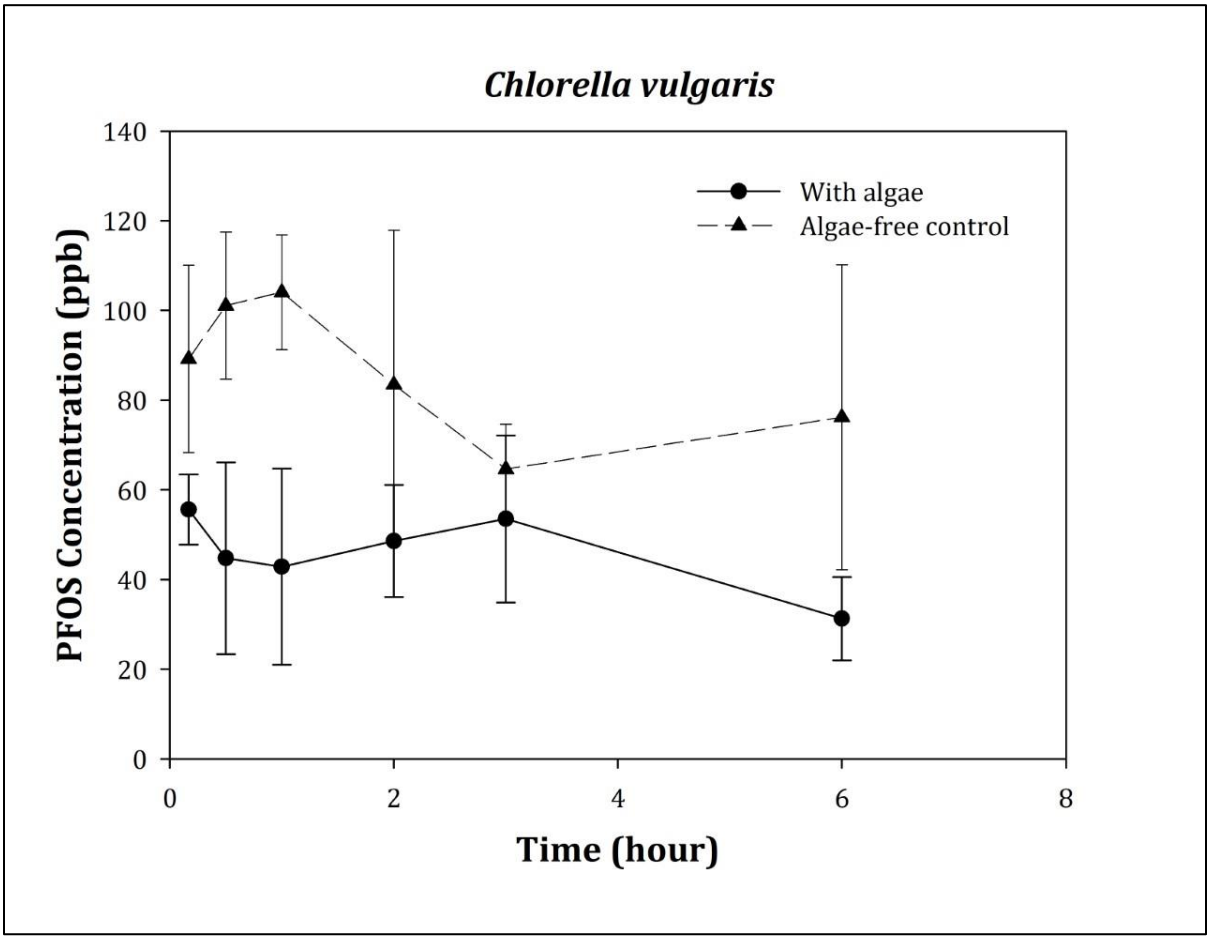

Figure 11: PFOS concentrations measured in the growth tubes for *Chlorella vulgaris.*

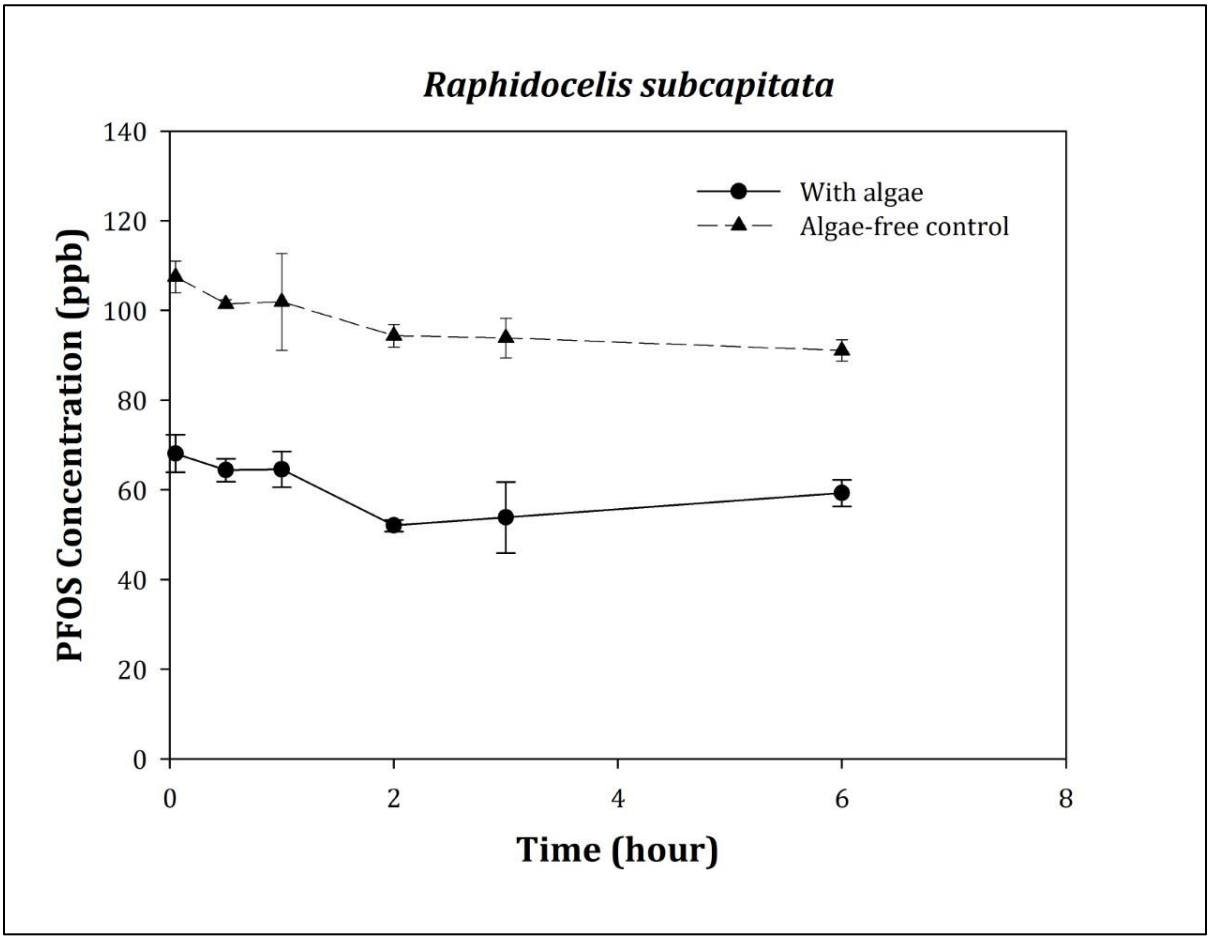

Figure 12: PFOS concentrations measured in the growth tubes for *Raphidocelis subcapitata.*

The measured PFOS concentrations and the dry algae biomass allowed us to calculate PFOS concentrations within algae. Table 2 indicated that after a total of 6 hours, *C. reinhardtii* had higher PFOS concentration (40.526 ± 7.278 µg/g) than *C. vulgaris* (26.214 ± 2.829 µg/g) and *R. subcapitata* (37.488 ± 2.813 µg/g). Consistent to the aqueous PFOS concentration results, the increase in PFOS concentrations within the algal biomass occurred rapidly (i.e, within 10 minutes).

PFOS in Algal Biomass (µg/g)						
Algal Species/Samples	Time (hours)					
	0.17	0.5	1	2	3	6
Chlamydomonas reinhardtii	±0.616	±2.026	±3.310	±2.726	±9.145	±6.375
Sample A	44.769	44.185	41.995	26.594	26.740	39.586
Sample B	45.774	47.192	41.522	26.719	38.766	34.672
Sample C	45.890	43.333	36.040	31.378	44.687	47.318
Sample Average	45.478	44.903	39.853	28.230	36.731	40.526
Chlorella vulgaris	±3.543	±9.667	±9.878	±5.646	±8.438	±4.201
Sample A	18.009	9.005	18.462	11.946	20.543	25.249
Sample B	11.222	26.561	31.855	22.489	21.493	30.814
Sample C	16.380	24.796	12.579	20.724	6.425	22.579
Sample Average	15.204	20.121	20.965	18.386	16.154	26.214
Raphidocelis subcapitata	±3.463	±1.695	±3.135	±1.102	±5.935	±2.315
Sample A	34.453	31.953	30.391	42.344	44.844	39.922
Sample B	27.692	35.308	33.077	42.615	45.385	37.231
Sample C	29.766	33.203	36.641	44.375	34.844	35.313
Sample Average	30.637	33.488	33.369	43.111	41.691	37.488

Table 2: PFOS in Algal Biomass (µg/g).

Using PFOS concentrations in the aqueous phase and the algal biomass, the values of the bioaccumulation factor (BAF) could be calculated (Table 3). For *C. reinhardtii*, the BAF values were on the order of 10^3, which for both *C. vulgaris* and *R. subcapitata*, the BAF values were about 1 order of magnitude lower. The BAF values

observed in this research was comparable to BAF values measured for similar algae species or other freshwater algae. For instance, Xu et al. reported that the average BAF for phytoplankton mixtures were $10^{2.6}$ in eutrophic lakes. For *C. reinhardtii,* the reported BAF value was ~$10^{2.7}$. Yang et al (2020), however, reported that the BAF for a mixture of marine algae was ~$10^{4.3}$. It is likely that further screening work could lead to the identification of algae species that could be significantly more effective in PFOS removal.

Algal Bioaccumulation Factor (BAF)						
Algal Species/Samples	Time (hours)					
	0.17	0.5	1	2	3	6
Chlamydomonas reinhardtii						
Sample A	2162.75	2055.11	1714.09	583.19	588.98	1423.97
Sample B	1915.24	2135.37	1417.14	555.49	1181.90	912.42
Sample C	2185.22	1775.96	1056.89	778.62	1977.29	2477.40
Sample Average	2087.74	1988.81	1396.04	639.10	1249.39	1604.60
Chlorella vulgaris						
Sample A	364.56	129.94	381.44	190.22	469.02	755.95
Sample B	174.25	870.86	1694.43	569.33	515.42	1460.40
Sample C	309.06	720.83	204.87	477.51	85.67	574.53
Sample Average	282.62	573.87	760.25	412.35	356.70	930.30
Raphidocelis subcapitata						
Sample A	543.42	479.78	443.01	794.44	895.08	707.83
Sample B	387.31	573.18	512.82	817.95	935.77	629.96
Sample C	428.90	510.82	604.63	875.25	553.95	566.81
Sample Average	453.21	521.26	520.15	829.21	794.93	634.87

Table 3: Bioaccumulation Factor (BAF) for algal species.

DOC Results

Algae could release organic materials into the aqueous phase. The concentrations of DOC were measured. The results shown in table 4 indicated that the DOC concentrations were ~100 mg/L. Previous investigations suggested that DOC could potentially affect the efficiency of PFOS removal by other remediation techniques such as activated carbon (Omo-O koro et al., 2018; Darlington et al., 2019; Du et al., 2014). The removal of PFOS by the three selected algae species thus was achieved under moderately high DOC concentrations, which could represent another advantage of the use of algae for PFOS removal.

Total Dissolved Organic Carbon (mg/L)						
Algal Species/Samples	Time (hours)					
	0.17	0.5	1	2	3	6
Chlamydomonas reinhardtii						
Sample D	125.90	128.90	128.25	130.50	165.15	141.55
Sample E	124.30	124.30	128.20	126.50	160.80	144.70
Sample Average	125.10	126.60	128.23	128.50	162.98	143.13
Chlorella vulgaris						
Sample D	120.50	126.15	123.60	110.85	112.50	115.10
Sample E	113.65	120.60	114.60	108.15	107.70	112.70
Sample Average	117.08	123.38	119.10	109.50	110.10	113.90
Raphidocelis subcapitata						
Sample D	120.15	127.60	121.15	131.90	138.95	130.75
Sample E	110.55	118.10	119.00	137.25	145.05	142.25
Sample Average	115.35	122.85	120.08	134.58	142.00	136.50

Table 4: Total Dissolved Organic Carbon (mg/L)

5. CONCLUSION

In this research, the capability of three selected algae species in removing PFOS from contaminated water was assessed. The results showed that the removal of PFOS usually occurred within 10 minutes of contact time and 35.7-60.2% of PFOS was removed. Such PFOS removal was achieved under the presence of >100 mg/L of DOC, which could significantly lower the efficiency of existing PFOS removal techniques (e.g., activated carbon). The mechanisms (e.g., adsorption) of the PFOS removal by the algae cells was not investigated in this research but should be addressed in future studies as it could lead to further improvement in the PFOS removal efficiency. The measurement of algae biomass, together with PFOS concentrations under the presence and absence of algae cells allowed for the calculation of PFOS concentration within the biomass as well as the BAF values. Overall, the observed BAF values were consistent to BAF values reported within freshwater environment. Higher BAF values, however, have been reported (Yang et al., 2020). The identification of algae species with higher BAF values could lead to further improvement in PFOS removal efficiency by algae.

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Appendix A

Fluorescence and Flow Cytometry Data

Fluorescence growth measurements (F0) for algal species *Chlorella vulgaris*

Time (days)	Chlor. A	Chlor. B	Chlor. +PFOS A	Chlor. +PFOS B	Chlor. +MeOH	PFOS control
0	48.47	41.82	45.21	48.33	48.14	2.65
1	84.66	84.85	77.5	80	90.32	2.95
2	146.44	146.13	137.14	150.1	164.53	2.84
3	213.34	140.34	149.91	206.81	207.19	1.78
4	320.16	281.25	165.11	277.19	287.73	2.96
5	431.71	340.11	240.21	381.51	358.74	3.4
6	513.13	419.49	361.99	544.73	449.02	5.3
7	607.42	589.79	579.83	570.64	583.89	5.83
8	732.45	609.84	658.01	634.56	619.35	6.29
9	739.09	571.74	739.09	659.95	695.47	6.22
10	825.07	654.48	746.11	756.67	778.48	6.17
11	720.26	779.81	913.45	869.65	823.83	6.37
12	770.75	826.67	938.24	973.27	878.44	6.44
13	713.11	812.6	999.99	859.62	857.47	6.71

Flow cytometry growth measurements (cell count) for agal species *Chlorella vulgaris.*

Time (days)	Chlor. A	Chlor. B	Chlor. +PFOS A	Chlor. +PFOS B	Chlor. +MeOH	PFOS control
0	178	142	153	260	126	0
5	19772	8402	4300	12431	13328	1
13	16634	21890	28494	26746	23258	3

Fluorescence growth measurements (F0) for algal species *Chlamydomonas reinhardtii*

Time (days)	Chlam. A	Chlam B	Chlam +PFOS A	Chlam +PFOS B	Chlam +MeOH	PFOS control
0	89.6	90.11	91.23	89.26	90.63	1.14
1	162.33	168.3	163.67	165.46	169.02	1.1
2	217.26	262.86	230.57	206.04	259.82	1.07
3	191.42	170.25	174.87	135	135.47	0
4	299.44	311.6	283.24	213.62	289.78	0.31
5	349.19	320.27	293.29	272.54	303.54	1.08
6	450.63	476.75	537.27	376.48	369.64	1.5
7	521.42	437.97	486.57	420.65	285.96	1.62
8	564.47	335.62	394.28	419.98	285.09	1.62
9	480.22	372.6	400.13	430.69	226.57	1.48

Flow cytometry growth measurements (cell count) for agal species *Chlamydomonas reinhardtii.*

Time (days)	Chlam. A	Chlam B	Chlam +PFOS A	Chlam +PFOS B	Chlam +MeOH	PFOS control
1	550	715	1191	1174	554	1
2	9279	5207	8348	6179	11101	0
9	25029	7296	7119	15544	6179	1

Appendix B

DYV Media Concentrations

Media Name	DYV
Algae Species	*Chlorella vulgaris, Chlamydomonas reinhardtii, Scenemedesmus, Selenastrum capricornutum/ R. subcapitata*
Water Type	Fresh

Nutrients added for Media		
Nutrient	Amount (g)	Volume (L)
CaCl 2 H2O	75	1
MgSO4 7H2O	50.99	1
MES (buffer)		
KCl	3	1
H3BO3	0.8	1
NaNO3	46.7	1
Na2SiO3 9H2O	15	1
NaH2PO4	3.094	1
Trace Metals		
ZnSO4 7H2O	0.073	1
CoSO4 7 H2O	0.016	1
MnSO4 4H2O	0.54	1
Na2MoO4 2H2O	0.00148	1
Na2SeO3	0.000173	1
NiCl2 6H2O	0.00149	
Na2EDTA 2H2O	3.086	1
Fe Metal Mix		
FeCl3 6H2O	1.77	1
Na2 EDTA 2H2O	2.44	1
Vitamin Stock		
Thiamine- HCL	0.1	1
Biotine	0.002	1
B12	0.001	1

Additional Enrichments added		
Nutrient	Amount (g)	Volume (L)
NaNO3	46.7	1
KH2PO4	3.05	1

Cultures (60 mL PP tubes)	
Fluid	Volume
DYV Media	54 mL
NaNO3	60 uL
KH2PO4	60 uL
Algae + Media	6 mL
PFOS Stock/Methanol	600 uL
Total Volume	60.720 mL

Appendix C

Cell Count/Viability Results

Chlamydomonas reinhardtii Total Cell Count						
Sample ID		A +PFOS	B +PFOS	C +PFOS	D -PFOS	E -PFOS
Time (Hours)	0.17	508,270	494,345	520,205	533,675	530,510
	0.5	465,835	348,105	467,870	448,615	350,705
	1	478,415	441,160	462,440	452,940	434,565
	2	405,050	418,195	390,130	423,280	404,825
	3	327,000	313,245	320,510	350,780	402,615
	6	381,330	398,640	386,145	469,090	447,415

Chlamydomonas reinhardtii Alive Cell Count						
Sample ID		A +PFOS	B +PFOS	C +PFOS	D -PFOS	E -PFOS
Time (Hours)	0.17	500750.2	485856.9	511733.2	518199.9	511609.7
	0.5	458325.4	343001.0	456128.7	435654.2	343950.3
	1	466513.4	434125.2	452598.7	450196.2	426533.1
	2	394596.5	406516.0	384545.2	407658.6	391823.7
	3	311530.2	294039.3	303737.5	337165.1	386424.4
	6	367860.4	384100.6	376235.0	457090.8	434726.3

Chlamydomonas reinhardtii Dead Cell Count						
Sample ID		A +PFOS	B +PFOS	C +PFOS	D -PFOS	E -PFOS
Time (Hours)	0.17	7519.8	8488.1	8471.8	15475.1	18900.3
	0.5	7509.6	5104.0	11741.3	12960.9	6754.7
	1	11901.6	7034.8	9841.3	2743.8	8031.9
	2	10453.5	11679.0	5584.8	15621.4	13001.3
	3	15469.8	19205.7	16772.5	13614.9	16190.6
	6	13469.6	14539.4	9910.0	11999.2	12688.7

Chlorella vulgaris Total Cell Count						
Sample ID		A +PFOS	B +PFOS	C +PFOS	D -PFOS	E -PFOS
Time (Hours)	0.17	484,155	458,045	448,540	460,690	427,930
	0.5	456,260	436,370	454,045	449,150	432,670
	1	591,655	511,185	496,820	468,975	491,885
	2	514,005	485,945	472,240	468,945	457,130
	3	521,425	511,690	477,730	484,475	493,190
	6	480,820	485,925	481,285	489,400	494,560

Chlorella vulgaris Alive Cell Count						
Sample ID		A +PFOS	B +PFOS	C +PFOS	D -PFOS	E -PFOS
Time (Hours)	0.17	474,530	449,600	439,025	451,225	417,670
	0.5	455,110	435,255	453,490	448,795	432,250
	1	591,575	511,100	496,015	468,380	491,795
	2	513,385	485,920	472,090	468,935	457,110
	3	521,405	511,680	477,710	484,460	493,190
	6	480,800	485,905	481,280	489,395	494,555

Chlorella vulgaris Dead Cell Count						
Sample ID		A +PFOS	B +PFOS	C +PFOS	D -PFOS	E -PFOS
Time (Hours)	0.17	9,625	8,445	9,515	9,465	10,260
	0.5	1,150	1,115	555	355	420
	1	80	85	805	595	90
	2	620	25	150	10	20
	3	20	10	20	15	0
	6	20	20	5	5	5

Raphidocelis subcapitata Total Cell Count						
Sample ID		A +PFOS	B +PFOS	C +PFOS	D -PFOS	E -PFOS
Time (Hours)	0.17	3,043,340	3,100,085	2,964,900	3,392,985	3,266,760
	0.5	3,497,700	3,723,120	3,797,860	3,708,570	3,664,240
	1	3,016,145	2,606,765	2,853,975	2,879,270	2,984,380
	2	3,013,130	3,039,605	3,175,455	3,084,075	3,194,380
	3	3,288,795	3,279,990	3,405,930	3,524,190	3,380,360
	6	3,377,490	3,324,385	3,332,830	3,291,740	3,568,335

Raphidocelis subcapitata Alive Cell Count						
Sample ID		A +PFOS	B +PFOS	C +PFOS	D -PFOS	E -PFOS
Time (Hours)	0.17	3,043,340	3,100,080	2,964,890	3,392,985	3,266,755
	0.5	3,497,700	3,723,105	3,797,790	3,708,565	3,664,195
	1	3,016,145	2,606,765	2,853,970	2,879,270	2,983,560
	2	3,013,125	3,039,595	3,175,425	3,084,075	3,194,375
	3	3,288,780	3,279,925	3,405,915	3,524,190	3,380,345
	6	3,377,490	3,324,385	3,332,830	3,291,725	3,568,335

Raphidocelis subcapitata Dead Cell Count						
Sample ID		A +PFOS	B +PFOS	C +PFOS	D -PFOS	E -PFOS
Time (Hours)	0.17	0	5	10	0	5
	0.5	0	15	70	5	45
	1	0	0	5	0	820
	2	5	10	30	0	5
	3	15	65	15	0	15
	6	0	0	0	15	0

Appendix D

PFOS Analysis Results

PFOS Control Results						
Samples	Time (hours)					
	0.17	0.5	1	2	3	6
***Chlamydomonas* PFOS Control**						
Constant A (µg L-1)	83.80	70.30	69.20	71.20	71.50	66.10
Constant B (µg L-1)	80.60	80.10	79.20	95.00	102.00	62.10
Constant C (µg L-1)	81.70	80.10	42.70	87.90	68.90	65.80
Constant Average (µg L-1)	82.03	76.83	63.70	84.70	80.80	64.67
***Chlorella* PFOS Control**						
Constant A (µg L-1)	90.90	82.30	114.20	84.50	68.90	72.10
Constant B (µg L-1)	109.20	112.70	108.30	48.50	53.20	44.40
Constant C (µg L-1)	67.50	108.20	89.70	117.40	71.80	112.00
Constant Average (µg L-1)	89.20	101.07	104.07	83.47	64.63	76.17
***Raphidocelis* PFOS Control**						
Constant A (µg L-1)	110.80	100.70	93.00	92.10	88.80	89.90
Constant B (µg L-1)	107.90	101.10	98.80	97.10	95.90	89.50
Constant C (µg L-1)	103.80	102.50	113.90	93.90	96.90	93.80
Constant Average (µg L-1)	107.50	101.43	101.90	94.37	93.87	91.07

Algae +PFOS Results						
Algal Species/Samples	Time (hours)					
	0.17	0.5	1	2	3	6
Chlamydomonas reinhardtii						
Sample A (µg L-1)	20.70	21.50	24.50	45.60	45.40	27.80
Sample B (µg L-1)	23.90	22.10	29.30	48.10	32.80	38.00
Sample C (µg L-1)	21.00	24.40	34.10	40.30	22.60	19.10
Sample Average (µg L-1)	21.87	22.67	29.30	44.67	33.60	28.30
Chlorella vulgaris						
Sample A (µg L-1)	49.40	69.30	48.40	62.80	43.80	33.40
Sample B (µg L-1)	64.40	30.50	18.80	39.50	41.70	21.10
Sample C (µg L-1)	53.00	34.40	61.40	43.40	75.00	39.30
Sample Average (µg L-1)	55.60	44.73	42.87	48.57	53.50	31.27
Raphidocelis subcapitata						
Sample A (µg L-1)	63.40	66.60	68.60	53.30	50.10	56.40
Sample B (µg L-1)	71.50	61.60	64.50	52.10	48.50	59.10
Sample C (µg L-1)	69.40	65.00	60.60	50.70	62.90	62.30
Sample Average (µg L-1)	68.10	64.40	64.57	52.03	53.83	59.27