

A PFAS Water Treatment Lab Incorporating Scaffolded Active Learning to Strengthen Undergraduate Learning

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Abstract

The undergraduate Environmental Engineering curriculum introduces students to the fundamentals of water chemistry and treatment. However, traditional water treatment is often less effective in removing emerging contaminants of concern, such as per- and polyfluoroalkyl substances (PFAS). To address this need, we designed and implemented a laboratory module for Environmental Engineering sophomores focused on PFAS removal during water treatment. The module was developed iteratively through two implementation cycles in separate semesters. For the first cohort, we observed a disconnect between class discussions and lab reports. We hypothesized that students may need additional support to build from one lab exercise to the next and to improve their scientific writing. To address this in the second cycle, we implemented scaffolded active learning by providing students with pre- and post-lab activities, followed by engaging in-class small-group discussions. We also increased transparency in the second semester by redesigning the rubric to clarify expectations for the lab report and specifying the requirements for full, partial, and zero credit. The pedagogical intervention in the second semester resulted in a significant increase in student performance in their reports, specifically in analyzing and discussing laboratory results, and in drawing conclusions and real-world implications for PFAS water treatment. Students in the second semester also demonstrated greater self-efficacy in data analysis and in writing laboratory reports. At the end of the treatment semester, students provided positive feedback, mentioning they benefited from personalized instruction, learning new laboratory skills, and appreciated the relevance of the PFAS topic. Our work demonstrated that new lab activities focused on contemporary issues can generate greater student enthusiasm. Combined with additional pedagogical support, our new PFAS lab led to improved student outcomes. These teaching changes are not uniquely applicable to our field (civil and environmental engineering); our teaching methodology, which emphasizes active learning with pedagogical scaffolding and transparent expectations, can be readily adapted by other engineering educators. All laboratory handouts, procedures, and rubrics are attached in the Appendix of this work.

Introduction

Environmental Engineering undergraduates study water chemistry and treatment operations to remove contaminants from water, including organic carbon, suspended solids, hardness, heavy metals, and pathogens. However, emerging contaminants challenge traditional water treatment processes because they can persist in treated waste and drinking water. Emerging contaminants are not always emphasized in the traditional curriculum, though they can be highly relevant to the careers of undergraduate students enrolled in environmental engineering programs.

One class of emerging contaminants, per- and polyfluoroalkyl substances (PFAS), has been particularly important in recent years due to their environmental persistence and toxicity [1], [2]. PFAS are human-made fluorinated chemicals that are being regulated in the United States and throughout the world, but are not readily removed by traditional water treatment [3]. The US EPA has established stringent, legally enforceable levels in drinking water, called Maximum Contaminant Levels (MCLs), for several PFAS in the nanogram per liter (ng/L) range, which must be met within a decade [4].

The Environmental Engineering curricula should prepare students to tackle PFAS removal from water. One pedagogical paper has explored the introduction of PFAS to undergraduate students in a laboratory setting by having them synthesize a novel metal–organic framework (MOF) to remove PFAS from water [5]. Although this study engages students in material synthesis, analytical techniques, and the research process, the use of novel materials such as MOFs may not be applicable or relevant to Environmental Engineering students due to scalability concerns [5]. Instead, separation techniques, such as activated carbon, are widely used at the field scale for PFAS removal from drinking water and groundwater at impacted sites [6]. Therefore, there remains a need for an environmentally relevant pedagogical course that reflects the growing importance of PFAS removal from drinking water.

Here, we employed evidence-based pedagogical practices to develop a lab that introduces undergraduate Environmental Engineering students to the challenges and potential real-world solutions in PFAS water treatment, preparing future engineers to tackle this challenge. We created the PFAS lab with the intention of including it in our Environmental Engineering Laboratory course, a required course for Environmental Engineering majors. Given the unique opportunity to create a new lab module, we wanted to understand how implementing different pedagogical strategies, such as scaffolding and active learning, could affect student engagement, course outcomes, and self-efficacy during our PFAS lab.

Educational research supports that increased scaffolding and transparency can enhance student engagement and retention of critical course materials. Belland [7] defines instructional scaffolding as the support provided by an instructor that enables students to meaningfully participate in and acquire a skill for a task they would be unable to complete without it [7]. Scaffolding is fundamentally different from other instructional resources, such as job aids, because it is designed to be provided temporarily while students are learning or acquiring a skill, then removed or faded so that students can use their acquired skill independently [7]. Rubrics can be used as scaffolding to help students meet teaching objectives [8], [9]. Active learning, defined as an instructional technique that intentionally engages students in the learning process [10], is also well-supported as a means of improving student performance [11]. Laboratories are a form of active learning because students directly engage in knowledge acquisition. We wanted to

understand whether these pedagogical techniques help students retain and apply new PFAS knowledge through laboratory activities and report writing.

Through these interventions, we also aimed to enhance students' self-efficacy, which is an individual's perception of their ability to successfully complete a task and has been positively correlated with students' academic and career achievement [12]. Although similar, self-efficacy differs from academic self-confidence in that it is domain-specific and can change due to successful performance and feedback [13], [14]. Moreover, self-efficacy positively correlates with engineering student retention and success in their specific field [15]. Because feedback is important for self-efficacy development, effective instruction and pedagogy can have a direct role in enhancing it.

Given the urgent need for environmental engineers at water treatment plants across the United States to meet upcoming stringent PFAS regulations, we aimed to prepare Environmental Engineering undergraduates to tackle this issue through a Laboratory course with increased scaffolding, added transparency of expectations, and more active learning. Specifically, we (1) increased the detail of rubrics to add transparency, (2) scaffolded instruction before and after lab activities, and (3) employed active learning prior to the lab to reactivate key knowledge. We used pre- and post-surveys to measure self-efficacy and gauge students' confidence in their lab report abilities. This paper will address the following two research questions:

- (1) To what extent does our new pedagogical approach impact student performance on the PFAS lab report?
- (2) How does student self-efficacy in their skills vary after the completion of the PFAS lab?

Method

Participants

Participants were sophomore Environmental Engineering majors enrolled in a required 14-week Environmental Engineering course during Spring 2024 (S24, $n = 11$) and Spring 2025 (S25, $n = 7$) at a mid-sized, private, R-1 institution in Pennsylvania, USA. Spring 2024 students served as the control group, while S25 students were the treatment group that received our pedagogical intervention. Each semester, students were divided into 3 teams of 2 to 4 students. Only S25 students were given the pre- and post-surveys. Six students completed the pre-survey, 85.6% response rate. Only four students completed the post-survey questions (57.1% response rate), resulting in three students having full pre-/post- survey data¹. Data were collected under an Institutional Review Board-approved protocol.

¹ One student was eliminated for responding “100” to each pre and post self-efficacy item, suggesting they were using a response set rather than thoughtfully responding to each item (a threat to construct validity).

Materials

A six-question pre-lab survey measured S25 students' confidence in their skills aligned with the learning objectives of the lab report assignment. These items were measured on a 0-100% scale, with students indicating their confidence at that moment. For example, "I am ___% confident that I can do the required analysis for a lab report" (see Table 2 for all items). The same six self-efficacy items were used on the post survey.

In both semesters, the lab activities included an introductory lecture on PFAS, a calibration curve activity, a set of traditional water-treatment methods for removing PFAS, and an adsorption modeling lab on PFAS and activated carbon (see **Appendix A**). In Spring 2025, additional active learning was implemented through four scaffolded in- and post-lab activities to reactivate key laboratory knowledge. Students were asked to critically evaluate hypotheses before the upcoming labs and to produce figures and analyses from the previous lab for their final reports. Students discussed their responses in small groups and then as a whole class. As students discussed their responses, instructors engaged with each student group to provide additional guidance and feedback. Pre- and post-lab activities are included in the handout in **Appendix A**. Students from Spring 2024 only participated in the introductory lecture on PFAS and the four weekly in-lab activities, which were not followed by out-of-lab activities or post-lab reviews.

In both semesters, rubrics were provided before students completed the PFAS lab report. The Spring 2024 rubric only included the desired deliverables and the points for each section (see **Appendix D**). The Spring 2025 rubric was more detailed by including transparent performance levels for each criterion (see **Appendix B**). There were six criteria (e.g., introduction, analysis), each worth 10-30 points, with explicit details on how to earn full, partial, or no credit for each criterion (see **Appendix B** for full rubric). For this study, both years' lab reports (S24 and S25) were evaluated after the S25 semester was over using the S25 rubric (**Appendix B**). This allows us to assess student performance against the desired learning objectives and to directly compare across semesters.

Procedure

Teams of students in both Spring 2024 and Spring 2025 completed their PFAS lab during the last five weeks of the semester. For Spring 2024 students, prior to the lab, they were given the rubric for evaluating their lab reports. These students did not complete the pre- and post-survey measuring self-efficacy.

Students in Spring 2025 completed the pre-survey during week 3 or 4 of the semester, prior to the PFAS lab. Teams of students then completed the four scaffolded active learning activities leading up to the PFAS lab. Following this, they were given the detailed evaluation rubric, completed their PFAS lab during the second-to-last week of the semester, and prepared and

submitted the related lab report. Finally, after the semester ended, S25 students completed the post-survey.

Students from both Spring 2024 and Spring 2025 were given a 45-minute lecture on per- and polyfluoroalkyl substances (PFAS) prior to beginning the PFAS lab. The lecture included background on sources of contamination, the chemical properties that make them concerning, current regulatory standards for drinking water, and a brief overview of analytical chemistry concepts such as chromatography and mass spectrometry. Additionally, the lecture included an in-class discussion to gauge students' awareness and understanding of PFAS.

Groups from both semesters then selected water treatment strategies such as chlorination, filtration, and UV for PFAS treatment experiments. Two types of PFAS were selected: perfluorooctanoic acid (PFOA), a more hydrophobic long-chain PFAS; and perfluorobutanoic acid (PFBA), a less hydrophobic short-chain PFAS (see supplier and specifics in **Appendix C**). Each semester, groups conducted a literature review for each treatment process to identify experimental conditions and formulate hypotheses about treatment efficacy. Literature searches were assigned to encourage students to develop an understanding of current research in the PFAS field.

Following the introductory lecture, students in each semester conducted laboratory experiments for 80 minutes, 1 day per week for 3 weeks. The detailed lab procedure handout is included in **Appendix A**. In-lab experiments required student participation in both semesters. Post-lab assignments were given in S25 to ensure continued student participation between labs (part of the scaffolding intervention). The following is a brief summary of each weekly laboratory activity completed by both Spring 2024 and 2025 students.

The first activity instructed students to perform serial PFAS dilutions to establish a calibration curve for liquid chromatography tandem triple quadrupole (LC-QqQ) mass spectrometry analysis to quantify the concentration of an unknown PFAS sample. The following week, students tested the effectiveness of traditional water treatment processes (chlorination, sand filtration, and UV irradiation) for removing PFAS from water. These experiments are meant to demonstrate to students the persistence of PFAS throughout these traditional water treatments. In the final week, students perform adsorption isotherms of PFAS on granular activated carbon (GAC) to determine the amount of GAC required to remove PFAS to the levels required for drinking water.

All PFAS quantification was performed using an LC-QqQ method adapted from Santiago-Cruz et al. [16], described in **Appendix C**. A graduate research assistant operated the LC-QqQ. Analytical methods and quality control procedures are detailed in **Appendix C**. Laboratory safety practices were emphasized before and during the lab activities. Personal protective equipment, including safety goggles, gloves, and aprons, was provided and required for students at all times during the laboratory.

For Spring 2025 students, the scaffolded post-lab questions and activities were designed to prompt students to consider how lab assignments would contribute to the lab report (see **Appendix A**). This included student comprehension of liquid chromatography capabilities, hypothesis testing results based on the current literature, reflection on the hypothesis following data results, treatment mechanisms, and isotherm modeling. Students' responses were discussed in class each week before the next in-lab experiment (part of the active learning intervention). Revised responses were intended to be incorporated into the final lab reports to incentivize distribution of the workload.

To determine student outcomes for this study, teams' PFAS lab reports from Spring 2024 (S24, $n = 3$ teams) and Spring 2025 (S25, $n = 3$ teams) were evaluated using the same 115-point rubric (see **Appendix B**). The course instructor marked both semesters' reports. The S25 reports were scored during the course of the semester. The S24 reports were scored at the end of S25. The instructor was aware of which semester he was scoring, though using the detailed rubric ensured consistency in the approach.

Results

Lab Report Performance

Both control (S24) and treatment (S25) groups were evaluated using the detailed rubric (**Appendix B**) developed during the spring semester of 2025. Mean semester results for each rubric section and total report score are reported in Figure 1 and Table 1. An independent-samples t -test revealed a significant increase in the mean total report grades from 62% in S24 to 85.7% in S25 ($t(4) = -4.06$, $p = .02$, Hedge's $g = -2.64$). This result suggests that the pedagogical intervention of increased scaffolding, transparency, and active learning allowed S25 students to produce stronger overall lab reports and achieve learning objectives.

Individual rubric criteria were also analyzed to determine where the shift in performance was most pronounced. Two report sections showed significant improvements in the S25 treatment group ($ps < .05$), specifically *Analysis & Discussion* (Mean_{Control} = 17.3; Mean_{Treatment} = 32.0 points out of 40 possible points) and *Conclusion & Implications* (Mean_{Control} = 5.3; Mean_{Treatment} = 8.7 points out of 10 possible points). The *Results* and *Citation* sections showed marginally significant improvements for the treatment semester. However, the *Introduction* section showed a marginally significant decrease in the S25 treatment group. All other rubric sections showed no statistically significant difference between the S24 and S25 cohorts (see Figure 1).

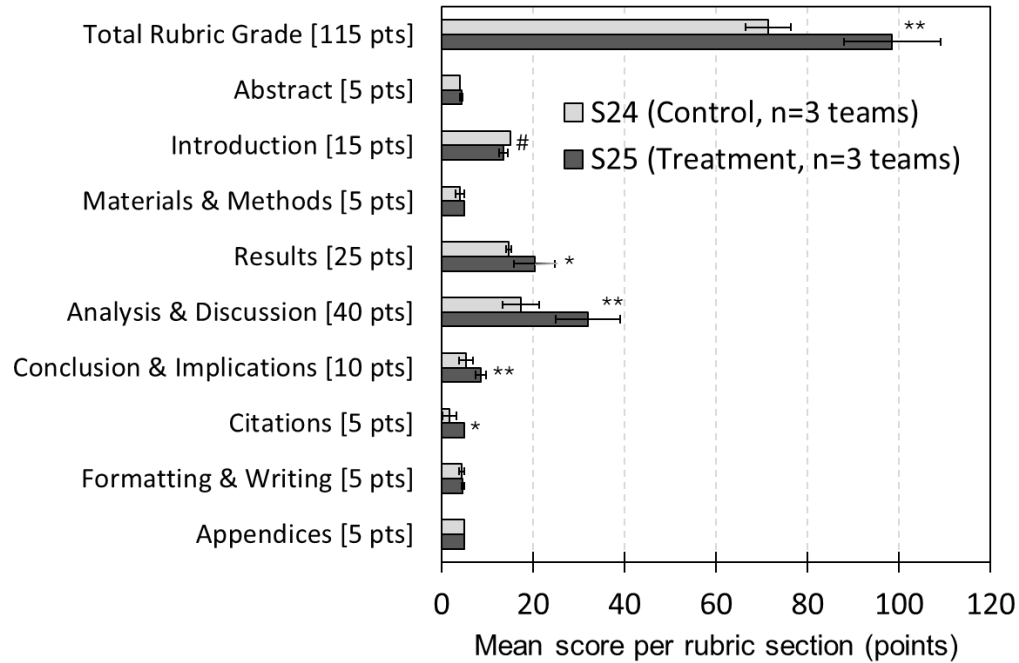


Figure 1. Mean point results for each rubric section for Spring 2024 (S24, light gray) and Spring 2025 (S25, dark gray) groups. Error bars represent one standard deviation.

Notes:

** Indicates significantly higher score ($p < .05$) for Spring 2025.

* Indicates marginally significantly higher score ($p = .05$ to $.10$) for Spring 2025.

Indicates marginally significant decrease ($p = .06$) for Spring 2025 compared to Spring 2024.

Table 1. Rubric criteria used to calculate the average grade for lab reports of students from Spring 2024 and Spring 2025, and the statistics between semesters.

Rubric Criteria	S24 (n = 3 teams) Mean	S25 (n = 3 teams) Mean	t-test	p-value	Hedge's g
Abstract [5 pt]	4.0	4.3	$t(2.00)^2 = -2.00$	0.18	-1.30
Introduction [15 pt]	15.0	13.5	$t(4) = 2.60$	0.06	1.69
Materials & Methods [5 pt]	4.0	5.0	$t(4) = -1.73$	0.16	-1.13
Results [25 pt]	14.7	20.3	$t(4) = -2.16$	0.10	-1.41
Analysis & Discussion [40 pt]	17.3	32.0	$t(4) = -3.14$	0.04	-2.05

² The t-test was adjusted due to unequal variances between the two groups.

Rubric Criteria	S24 (n = 3 teams) Mean	S25 (n = 3 teams) Mean	t-test	p-value	Hedge's g
Conclusion & Implications [10 pt]	5.3	8.7	$t(4) = -3.02$	0.04	-1.96
Citations [5 pt]	1.7	5.0	$t(2.00)^3 = -3.78$	0.06	-2.46
Formatting & Writing [5 pt]	4.3	4.7	$t(4) = -.89$	0.42	-0.58
Appendices [5 pt]	5	5	N/A	N/A	N/A
Total Rubric Grade [115 pt] Mean (standard deviation)	71.3 pt (4.9 pt) 62.0%	98.5 pt (10.5 pt) 85.7%	$t(4) = -4.06$	0.02	-2.64

S25 Pre- and Post- Surveys

Six self-efficacy items aligned with the lab report's learning objectives were completed by S25 students at the beginning and end of the semester (see Table 2). A series of dependent-means *t*-tests was run for each item and the total self-efficacy score to determine whether there was a change from pre to post in the three students who had complete survey data. While there was no significant change in total self-efficacy ($t(2) = -2.09$, $p = .17$, Hedge's $g = -.68$), two specific items did significantly improve across the duration of the course (see Table 2). Specifically, items 1 and 2 which both measured skills directly related to preparing a lab report increased by the end of the semester ($ps < .05$). Importantly, item 4 ("apply statistical tests to data") was a skill not targeted in the lab nor practiced by students and this item failed to significantly change ($p = .40$).

Table 2. Mean survey responses and statistics from Spring 2025 students.

Survey Item Scale: 0-100% confidence that I can...	S25 Pre Mean	S25 Post Mean	T-test t(2)	p-value	Hedge's g
1) complete a lab report	68.7	79.0	-5.10	0.04	-1.66
2) do the required analysis for a lab report	49.7	73.0	-10.68	0.01	-3.48
3) Conduct an experiment	64.0	87.7	-2.58	0.12	-0.84
4) Apply statistical tests to data	54.7	59.7	-1.06	0.40	-0.35

³ The *t*-test was adjusted due to unequal variances between the two groups.

Survey Item Scale: 0-100% confidence that I can...	S25 Pre Mean	S25 Post Mean	T-test t(2)	p-value	Hedge's g
5) Combine analysis of lab data and conceptual understanding to make relevant conclusions	73.3	66.3	0.61	0.61	0.20
6) Document the procedure of an experiment on a lab report	86.0	85.3	0.07	0.95	0.02
Total Self-Efficacy Mean (standard deviation)	66.1 (7.4)	75.2 (12.2)	-2.09	0.17	-68

Discussion

The present study focused on evaluating the impact of pedagogical interventions to teach undergraduate students about PFAS, a class of emerging contaminants with a multitude of environmental and human health impacts [1]. Environmental regulatory agencies in the US and around the world are acting to minimize human exposure to PFAS by setting limits on exposure routes, such as drinking water [3]. In preparation for careers in which future environmental engineers are likely to encounter PFAS-related projects, undergraduate environmental engineering curricula should engage students in addressing this impactful and pervasive group of chemicals.

Therefore, we designed an undergraduate lab focused on the chemistry and water treatment of PFAS. To investigate the most effective way to implement the lab, we planned an intervention to test how improvements in scaffolding, transparency, and active learning could enhance students' learning. After our intervention in the second year, we compared students' performance on the lab report between the first and second year. In the second year, we also surveyed students' self-efficacy before and after the lab.

The interventions for the second year (S25) were designed to address areas for improvement identified in the first year (S24). During S24, we noticed that although students demonstrated the ability to discuss the subject during weekly office hours, this knowledge was not explicitly reflected in the final report deliverable. Thus, the scaffolding was targeted to improve students' analytical aptitude. Our scaffolding intervention relied on weekly pre- and post-lab questions, which were then discussed in class using the active learning strategy of small-group discussions. The increase in rubric transparency helped manage expectations for writing the report, explicitly outlining the details and discussions to include. The updated rubric detailed the deliverables and format that allowed for Full Credit, Partial Credit, and No Credit for each section of the report (**Appendix B**). The results in Figure 1 suggest that our intervention approach indeed improved

the targeted sections on Analysis & Discussion and Conclusions & Implications. Interestingly, the use of detailed rubric did not significantly affect other sections of the report that were not explicitly targeted by our scaffolding intervention though it should be noted that our study is underpowered by comparing three teams performance in each semester.

Marginal improvements in Results and Citations may have been due to clearer formatting expectations explicitly stated in the rubric. For example, the S25 rubric (**Appendix B**) was explicit about the expectation that figures had to be properly labeled, captioned, and include data points, while the S24 rubric (**Appendix D**) mentioned which figures were expected but not the format or how to build them. Therefore, S25 students received clearer instructions for writing the lab report than S24 students. Additionally, for S24 students, citation use was included as a criterion in the “Overall quality of writing” section rather than in its own section of the rubric (**Appendix D**). Thus, the expectation for S24 students to include citations in the lab report were not as explicit and transparent. This indicates that an explicit Citation section in a rubric may encourage students to include proper references in lab reports.

The observed marginal decrease in performance in the introduction may indicate better delivery of expectations in S24 groups. One way could have been through the Introduction section of the S24 rubric. Notice how the S24 rubric details the desired information to include in each paragraph of the introduction (**Appendix D**) while the S25 rubric removes the scaffolding for each paragraph and instead lists the desired topics that should be addressed in the introduction as a whole. This suggests that students may perform better when details of each paragraph are explicitly stated in the rubric, reinforcing the value of transparency in rubrics. All the results discussed provide important feedback on the rubric and scaffolding design.

Self-efficacy measurements were limited by the sample size and the lack of a comparison between the two semesters. Even with data from only three participants, a couple of effects were observed, specifically students’ confidence in completing a lab report and analyzing data. Based on these limited data, applying statistical tests and combining analysis with conceptual understanding to draw conclusions could be a good focus for the Laboratory's next iteration. Students' self-efficacy with key concepts and skills (e.g., modeling adsorption isotherms, performing calibration curves, predicting novel PFAS treatment) can also be measured in future work.

Our study benefited from being a field study done in a real classroom with real students. We developed a survey specifically designed for our students and our study purpose, and we included both quantitative and qualitative data. Limitations of our study include a small sample size; no psychometric testing of the survey, although it has face validity and internal consistency; less control over extraneous variables than a lab-based study, as it is a field study in a real classroom; and the lack of a comparison group for the pre- and post- self-efficacy survey. Still,

we are confident that the benefits we identified of addressing relevant topics, i.e., PFAS, improving scaffolding and transparency, and adding active learning could help other instructors.

Changes to this lab could make it more effective and more accessible to other instructors. One larger obstacle to implementation could be the equipment required to quantify PFAS. We used an LC-QqQ, which can be cost-prohibitive for many institutions and requires considerable technical expertise. To make our lab more sustainable and less dependent on graduate students with expertise using this equipment for the undergraduate students, in place of the PFAS, in the future we will use analogous anionic surfactants with similar properties to regulated PFAS but that can be quantified on more accessible equipment, such as UV-vis spectrophotometry, colometric assay, or electrode probe. Ideally, the probe molecule properties (e.g., adsorption parameters, reaction kinetics) could be calibrated to the PFAS being tested to estimate and model PFAS behavior by measuring the probe's treatment. Future work will develop protocols and select appropriate probe molecules to perform the laboratory without LC-QqQ, making it accessible to a broader range of educational institutions.

We found that our pedagogical intervention of improving scaffolding and transparency and adding more active learning to an undergraduate Environmental Engineering lab led to student gains in their lab reports. It is important to note that, because these pedagogical strategies were implemented in combination, we do not know the singular impact of each strategy on student performance and self-efficacy. We welcome colleagues at other institutions to implement our PFAS lab (described above and resources provided in the Appendix) or modify it for their teaching preferences and context. We are confident that doing so could help improve others' lab activities as it did ours.

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Appendix A. Lab Handout For Spring 2025

Lab 4: PFAS Analysis and Removal from Water

Introduction

Per- and poly-fluoroalkyl substances (PFAS) are a large group of human-made fluorinated chemicals that are being regulated in the USA. The US EPA has set established legally enforceable levels in drinking water, called Maximum Contaminant Levels (MCL), for several PFAS. The EPA established an MCL for perfluorooctanoic Acid (PFOA), a likely human carcinogen, to 4 ng/L (ppt).¹ Current regulations establish that drinking water utilities need to meet these MCLs by 2029. The objectives of this Laboratory are to (1) understand the analytical techniques used to measure these organic contaminants, (2) evaluate the efficiency of traditional and advanced water treatment techniques to remove PFAS, and (3) estimate the cost of treatment to meet current regulations.



Figure 1. Perfluorooctanoic Acid (PFOA). Molecular weight of 414.07 g/mol.

****Responses to the questions in this handout will help guide the discussion for the final report****

Week 1. Calibration curve and limit of detection

Liquid chromatography-mass spectrometry (LCMS) is traditionally used to measure organic chemicals in water. You will prepare a calibration curve with known PFOA concentrations to quantify an unknown PFOA sample. These calibration standards should be prepared through serial dilutions. The calibration must be linear with respect to the signal received from the LCMS. From this, you will also estimate the minimum detection limit (MDL) for PFOA.

Prelab questions for Week 1:

- 1) What is the minimum number of standards needed to create a calibration curve?*
- 2) List the concentrations selected for your calibration curve and provide the serial dilutions needed to achieve each concentration. The maximum concentration in your calibration should be 1 uM. (remember $C1 \cdot V1 = C2 \cdot V2$)*

Materials:

- 10 uM Stock solution of PFOA
- 10 and 25 mL Volumetric Flasks
- Milli-Q Water
- Cellulose acetate filters (0.2 microns) and syringe
- LCMS vials

Procedure:

- Prepare your calibration curve through serial dilutions starting with a 10 uM stock of PFOA. Your highest calibration should be 1uM.
- Once all calibrations are prepared, take ~1mL sample and filter it through a syringe filter into an LCMS vial. All samples entering the LCMS should be filtered through the 0.2 micron cellulose acetate filters.

Post lab questions for Week 1:

- 1) Once you receive your LCMS results in units of “Peak Area”, create and analyze your calibration curve. What are your axis? Is the fit linear? What is the R^2 value?
- 2) Determine the detection limit of PFOA based on your calibration?
- 3) Quantify the unknown sample. The value in “Peak Area” will be provided.
- 4) Would you trust this curve to run samples for: 1ppm, 1ppb, or 1ppt? Explain why?

Week 2. Traditional water treatment

Waste and drinking water can undergo several treatments such as chlorination, UV irradiation, and filtration. As discussed in the lecture, there can be many types of PFAS in water. Shortchain PFAS (less than 7 fluorinated carbons) may react differently than longchain PFAS (more than 7 fluorinated carbons). For this experiment, you will test whether these traditional water treatments are effective at removing long chain PFOA and short chain perfluorobutanoic acid (PFBA).

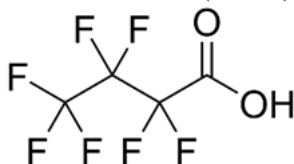


Figure 2. Perfluorobutanoic acid (PFBA). Molecular weight of 214.05 g/mol.

In-lab Activity for Week 2:

- 1) Provide a hypothesis of what you expect will happen to PFOA and PFBA with each treatment. Explain your reasoning and search for references to cite when writing the report.
 - a. Group 1: Chlorination
 - b. Group 2: Filtration
 - i. Sand filter
 - ii. Microfiltration
 - iii. Ultrafiltration
 - c. Group 3: UV
 - i. 254nm
 - ii. 185nm

Materials:

- 1 uM solution of PFOA and PFBA
- Chlorination
 - o 10 uM PFOA and PFBA stock
 - o 500 mg/L hypochlorous acid (HOCl)
 - o 25 mL Volumetric flask
 - o Milli-Q Water
- Filtration
 - o Sand filter (~10 microns)
 - o 450 nm (0.45 microns) filter (microfiltration)
 - o 20 nm (0.02 microns) filter (ultrafiltration)
- UV
 - o 254 nm lamp
 - o 185 nm lamp
- Cellulose acetate filters (0.2 microns) and syringe
- LCMS vials

Procedures:

A) Group 1: Chlorination

- Test two chlorine doses in the form of hypochlorous acid (HOCl) in duplicate
 - o 1.5 mg/L of HOCl (water treatment conditions)
 - o 10 mg/L of HOCl (excess dose)
- Calculate and add the appropriate volume of 500 mg/L HOCl to the 25mL volumetric flask to achieve the desired dose.
- Add the appropriate volume of the 10 uM PFAS stock to achieve 1uM PFAS into the 25mL volumetric flask.
- Fill the remaining volumetric flask with Milli-Q water and mix.
- Let the solution sit for 30 minutes and grab a sample

B) Group 2: Filtration

- Filter the 1uM PFAS mixture containing PFOA and PFBA through different filtration levels. Run duplicates.
 - o Sand filter (~10 microns)
 - o 450 nm (0.45 microns) filter (microfiltration)
 - o 20 nm (0.02 microns) filter (ultrafiltration)

C) Group 3: UV

- Expose 1uM PFAS mixture containing PFOA and PFBA to 2 types of UV wavelengths: 254nm and 185nm. To get measurable rate constants, take samples at different time points: 0 min, 5 min, 10min, and 15 min.

Post Lab Questions after you receive the data:

- 1) Was your hypothesis for each treatment confirmed or rejected? Discuss why each treatment performed the way it did.
- 2) Chlorination: Did chlorine dose make a difference in removal? Why or why not? Explain by discussing how chlorination works, what reactions are possible in chlorination, and what could react with PFOA and PFBA. (Tip: Think about redox potential and thermodynamics. A single C-F bond has an energy of ~441 kJ/mol. Think about what it would take to break that bond energy. Feel free to get creative.)
- 3) Filtration: How effective was each filter? What size would be needed to remove PFOA? What size would be needed for PFBA?
- 4) UV: Explain the differences between 254 nm and 185 nm wavelength. Are there different reactive species in each one? Given the measured rate constant, how long would treatment need to be to meet PFOA regulations (4 ng/L)?

Week 3. Activated Carbon Adsorption Isotherm

Water utilities are contemplating the use of adsorbents such as granular activated carbon (GAC) to remove PFAS from water. Remember, there can be many types of PFAS in water. Shortchain PFAS (less than 7 fluorinated carbons) may adsorb differently than longchain PFAS (more than 7 fluorinated carbons) and thus may require a different dose of carbon to remove. For this experiment, long chained PFOA and the short chain PFBA will be tested for removal using GAC. To figure out the optimal dose of GAC needed to remove both PFAS types below the MDL, you need to run adsorption isotherms.

Prelab Questions for week 3:

- 1) Define an adsorption isotherm and list three common types of adsorption isotherm models. What would be the x and y axis of each isotherm model?
- 2) What type of molecular interactions allows for sorption? Provide at least two types of molecular interactions. How would you expect those interactions to differ for molecules with different chain lengths? (Hint: PFOA and PFBA are anionic in natural waters)

Materials:

- 1 uM solution of PFOA and PFBA
- Granular activate carbon (GAC)
- 50, 100, and 500 mL flasks
- Cellulose acetate filters and syringe
- LCMS vials

Procedure:

- Weigh the mass of GAC corresponding to the following concentrations. You can use either 50 mL, 100mL, or 500 mL flasks
 - o **Group 1:** 0.01g/L, 0.05 g/L, 0.1 g/L
 - o **Group 2:** 0.5 g/L, 1 g/L, 5 g/L
 - o **Group 3:** 10 g/L, 50 g/L, 100 g/L
- Add the volume of PFAS water containing PFOA and PFBA required to achieve the GAC concentrations. Perform in duplicate.
- Allow the solutions to equilibrate with the solids and grab a sample after gently stirring for 2 days.

Post Lab Questions after you receive the data:

- Which isotherm model best fits the data for PFOA and PFBA? Are they different?
- What amount of GAC is needed to remove PFOA and PFBA below 4 ng/L? You may need to extrapolate the data. Are the amounts different depending on the PFAS?
- Perfluorobutanoic sulfonic acid (PFBS) is another regulated short chained PFAS which should be below 2000 ng/L.¹ Assuming PFBS adsorbs similarly to PFBA, what amount of GAC is needed to remove PFBS below 2000 ng/L?



Figure 3. Perfluorobutanoic sulfonic acid (PFBS). Molecular weight of 300.1 g/mol.

References:

- 1) Environmental Protection Agency (EPA). PFAS National Primary Drinking Water Regulation, 2024. <https://www.epa.gov/sdwa/and-polyfluoroalkyl-substances-pfas> (Accessed March 31, 2025)

Appendix B. Rubric for Spring 2025

Section	Points
Abstract: (5 points total) Full Credit: - Summary of objectives, methods, key results, and key conclusions - Connections between topics (i.e previous labs, lectures, or discussion) are clearly and consistently made throughout the section Partial Credit: - Missing some objectives, methods, key results, or key conclusions - Occasional connections or references are made - Important connection not made No Credit: - Missing all objectives, methods, key results, and key conclusions - Connections are not made between topics	5
1. Introduction (15 points total) Full Credit: 4 paragraphs - Prepare your introduction based on the overall goal of this lab. Think of this like a funnel: starting broad with the broader water treatment topic, PFAS and its health impacts, how PFAS complicates treatment, and then narrowing to focus on the labs' objectives. It should be clear from the introduction why we settled on these objectives and why they are important to address. <u>HINT: REFER TO LAB 4 PRESENTATION</u> Partial Credit: - Less than 4 paragraphs - Mentioning some but not all key points, such as water treatment, emerging contaminants, PFAS chemistry and regulations, or objectives No Credit: - Absent or incorrect	15
2. Materials and Methods (5 points total) Full Credit: - In your own words, a description of each lab day, including a very brief summary of the goal of each activity, number of samples, types of glassware, chemical reagents, sample preparation, the instruments/apparatus used, etc. - Someone should be able to replicate your procedure just by reading this section Partial Credit: - Materials and methods are incomplete or copied and pasted from the handout No Credit: - Methods are missing or incorrect.	5

3. Results (25 points total)

25

Full Credit:

- **Introduces all figures**
 - Include figure numbers and captions
 - All features in the figures (data points, lines, error bars) should be mentioned in the figure captions (e.g. markers represent the average value of duplicate samples, error bars represent the standard error of duplicates).
 - Design figures that visually represent the data. Feel free to try out different graph types such as scatter plots and bar graphs.
- **LC-MS Calibration Curve Graph (5 points)**
 - R^2 and linear equation are present
 - LOD is calculated — show the equation you used and where it's from.
- **LC-MS lab 2 results graphs (5 points)**
 - UV: Concentration over time for 185 nm and 254 nm
 - Chlorine: Concentration vs Dose
 - Filtration: Concentration vs filter size
- **LC-MS lab 3 isotherms (15 points)**
 - PFAS Liquid Concentration vs Mass of GAC (5 points)
 - PFAS Solid Concentration vs Liquid Concentration (5 points)
 - Equations shown so we know how you arrived to your answers
 - Fit data to an isotherm model (5 points)
 - Find isotherm parameters (show calculations)

Partial Credit:

- Does not introduce all figures or include captions
- Figures are present but incomplete (missing units, axes, error bars, wrong metrics used)
- PFAS solid concentrations for the isotherm are incorrectly calculated
- Isotherm fit or parameters are not properly determined

No Credit:

- The section is missing

Full Credit:

- **Calibration Curve (Week 1) (5 points)**
 - Discuss the linearity and uncertainty of the calibration curve using the values from the regression.
- **Traditional Treatments (Week 2) (15 points)**
 - Relate the efficiency or inefficiency of the treatment to the chemistry of PFAS and water treatment. Discuss each treatment in at least 1 paragraph (filtration, chlorination, UV). Explicitly say how these work and how PFAS treatment is challenged based on their chemistry. (6 points)
 - Include references to support your results, at least one (1) reference for each treatment (filtration, chlorination, UV). (3 points)
 - Discuss any differences between short and long-chain PFAS. (2 points)
 - Treatments that work, estimate (1) the amount of resources needed, (2) the suitability of implementing, and (3) technologies that exist already in treatment plants. (4 points)
- **Adsorption on GAC (Week 3) (20 points)**
 - Explain how the isotherms change between long chain (PFOA) vs short chain (PFBA) PFAS. (2 points)
 - Discuss by invoking **at least two (2)** molecular interactions and mechanisms of adsorption. (4 points)
 - Discuss ideas of uncertainty and error and how these impact result interpretation (2 points)
 - Calculate the amount of GAC needed to remove PFOA and PFBA below 4 ng/L and explain any differences. Evaluate the feasibility of this treatment given the cost of GAC (GAC~\$2,000 per ton, https://www.cityofimperial.org/sites/default/files/agendas/council/CC06-19-24_Item-D6.pdf) (5 points)
 - Assuming PFBS adsorbs similarly to PFBA, calculate the amount of GAC needed to remove PFBS below the regulatory limit of 2000 ng/L. (3 points)
 - Show equations and calculations (2 points)
 - Include references to support your analysis (2 points)
- **HINT: USE THE PRE AND POST LAB QUESTIONS AS A GUIDE FOR THE DISCUSSION.**

Partial Credit:

- Mentioning observations from results without explicitly explaining or discussing why these results were expected or not expected.
- Explaining the results but not directly relating them to the chemistry.
- In the right direction, but without delving deeper into key fundamental concepts like molecular interactions and PFAS chemistry.
- Some references, but not at least 1 per treatment.
- Missing discussion of treatment challenges
- Some incorrect analysis or statements.
- Data uncertainty and error are mentioned, but do not discuss how that may impact the interpretation of the results.
- Ideas are implicit but not fully developed.

No Credit:

• Ideas not implicitly or explicitly written or developed. • Mention all treatments done, but no discussion. • No references. • Consistently incorrect statements. • Incorrect interpretation or mention of the data (e.g. students mentioning an upward trend despite the data clearly showing a downward trend) • No mention of uncertainty, implicit or explicit. • No mention or explanation of the current state of PFAS removal, needed resources, or suitability of implementing technologies or incorrect.	
5. Conclusion and Implications Full Credit: • A summary of the overall and specific objectives (3 points) • Summarize the real-world implications of your work (4 points) • Future work: clearly detail a potential test to clarify scientific questions from this lab or potentially improve the experiments performed in this lab. (3 points) Partial Credit: • Mentioning some future work or implications, but not providing the reasons for it. • For future work, it does not provide the questions or hypotheses it aims to address. • For implications, not relating it back to engineering and society. No Credit: • Absent or incorrect.	10
Citations Full Credit • Formatted in a specific style (e.g. ASCE) • Citations are from reliable sources such as research articles, news articles, and/or textbook/lectures. Primary sources are more desirable. Partial Credit • Citations are lectures, news articles or research papers but did not format in a specific style (e.g. just adding the URL) No Credit • No citations or citations are not lectures, news articles, or research papers	5
Formatting and Writing Full Credit • Including consistent formatting and writing style throughout the report, consistent use of passive voice, good paragraph construction (with topic sentences), and numbering of equations.	5

● Articulating clearly (concise and little to no redundancy), correctly (based on data results, and well-cited literature), and at the expected level of depth (hit points we wanted in all sections) ● Includes table of contents, page numbers, cover page, and section titles. Partial Credit ● Some or partial clarity. Paragraphs do not have a thesis sentence and several arguments or points to support the thesis sentence. ● Missing sections No Credit ● Flow is not consistent with handout or their table of contents ● Incomplete sections/grammar ● 3 or more of the following sections are missing in the abstract: background, aim, conclusion, results, and discussion	
Appendices. Include appendices as appropriate, e.g., for raw data, sample calculations, and/or supplementary tables, figures, depictions. Do not append the measurement instructions. They should be included as citations.	5
Total	/115

Appendix C. PFAS Suppliers and Analytical Method

Materials

Sodium perfluorooctanoate (PFOA, C₇F₁₅COONa, 97%, CAS# 335-95-5) was purchased from Sigma-Aldrich. Heptafluorobutyric acid (PFBA, C₄HF₇O₂, >98.0%, CAS# 375-22-4) was purchased from Tokyo Chemical Industry (TCI). Granular activated carbon (Filtrisorb 400, denoted as GAC) was provided by Calgon Carbon.

Analytical Method

All PFAS analyses were performed as described in Santiago-Cruz et al. [16]. Briefly, PFOA and PFBA analysis was performed using direct injection liquid chromatography-tandem mass spectrometry (LC-MS/MS) on an Agilent 1100/6430 HPLC-MS (QqQ) with electrospray ionization (Agilent Technologies). To separate background PFAS present in the HPLC pumps from sample peaks, a C18 delay column (ZORBAX RR Eclipse Plus, 4.6 x 50 mm, 95 Å, 3.5 µm, Agilent Technologies, US) was installed between the binary pumps and autosampler. A C18 analytical column (Poroshell 120 EC-C18, 3.0 x 50 mm, 2.7 µm, Agilent Technologies, US) was used for analyte separation with the column temperature maintained at 40 °C. Injection volumes were 20 µL. Mobile phases were A: 20 mM ammonium acetate in Milli-Q water and B: MeOH with a flow rate of 0.4 mL/min. Mixtures of PFCA were separated using gradient elution. PFOA and PFBA were detected with multiple reaction monitoring using quantitative and qualitative ion transitions (Table S1) and quantified via external calibration.

Quality Assurance and Quality Control (QA/QC)

Laboratory reagent blanks and controls were run along with continuing calibration verifications (CCVs) at least every 10 samples to ensure calibration validity throughout sample runs. Method detection limits (MDL) were determined using the EPA MDL Procedure, Revision 2. In brief, 7 spiked samples three times the estimated MDL were prepared along with 7 method blank samples. Method blanks returned no numerical results, so the MDL was calculated as the standard deviation of the 7 prepared samples multiplied by $t(6,0.99) = 3.143$.

Table S1. Optimized MS/MS transitions and Method Detection Limits (MDL)

PFAS	MRM Transitions (<i>m/z</i>)		MS Voltages		MDL (µM)
	Precursor ion	Product ion(s)	Fragmentor	Collision Energy	
PFOA	413	368.9*, 169	92	0, 12	0.0040
PFBA	213	168.9	56	4	0.0079

*Indicates quantifier ion if more than one product ion listed

Appendix D. Spring 2024 rubric

Section	Content Points Earned
Abstract (5 points total) · Summary of objectives, methods, key results, and key conclusions	
1. Introduction (20 points total) – · In one or two paragraphs: Introduce PFAS, its sources, its benefits and risks, and most likely exposures (5 points) · In one or two paragraphs: PFAS in drinking water, relevant concentrations, and efforts to regulate PFAS (5 points) · In one or two paragraphs: Drinking water treatment (DWT) and the predicted removal of PFAS by DWT's unit processes (5 points) · In one paragraph: Give the overall and specific objectives of Laboratory 3 (5 points)	
2. Materials and Methods (10 points total) · Description of the various parameters measured, including preparation and number of samples, chemical reagents, and the detection methods used	
3. Results (20 points total) Include a brief introduction to the section and subsections (4 points) Introduce each table and figure (4 points) Include all relevant tables and figures (3 points each) · Calibration curve linear regression for PFOA · PFOA treatment results (granular filtration, chlorination, UV irradiation, aggregate samples) · Activated carbon adsorption isotherm for PFOA and PFBA · Nanofiltration for PFOA and PFBA	
4. Analysis and Discussion (30 points total) · Include an introduction (2 points), then analyze and discuss all of the data you presented in the Results section --Think about the connections among the various data you collected to transition from one topic to another (2 points) · Determine the limit of detection (2 points) · Analyze the DWT processes data and discuss the suitability of the various processes with which you experimented for PFAS removal (4 points) --Pick a location with a measured PFAS concentration; Thinking about the issues we faced with our isotherm experiment, describe an engineered treatment solution for PFAS removal; Can you estimate how much activated carbon would be required to achieve the regulatory drinking water limit? (5 points) --Comment on PFOA and PFBA and the wider range of PFAS (5 points) --Relate your results and analysis to the chemistry and structure of PFAS and PFOA and PFBA (5 points) · Future work – discuss (what is it? do you think it would work for municipal drinking water? why or why not?) one or more of the technologies discussed here (5 points): https://cen.acs.org/environment/persistent-pollutants/Competition-destroy-forever-chemicals-heats/102/i7	

5. Summary and Conclusions (10 points total) · Summary of overall objective of Laboratory 3 (5 points) · Summary of key results and conclusions (5 points)	
Appendices. Include appendices as appropriate. Do not append the measurement instructions. They should be included as citations. (5 points)	
Overall quality of writing (10 points), including consistent formatting and writing style throughout the report, consistent voice (active or passive), good paragraph construction (with topic sentences), citation of sources, numbering of equations, and use of ASCE style for citations and reference lists.	
Total	/110