REMOVING BPA USING *CHLORELLA VULGARIS* FOR WASTEWATER TREATMENT

Sophie Bermudez
Lauren Chaleff
Sasha Johnson-Freyd
Joseph Lanzillo
Reid Bergsund

ES 165: Water Engineering
Professor Chad Vecitis
TF: Andrea Weber
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INTRODUCTION

Introduction Part 1- Sophie Bermudez

From a broader perspective, drinking water treatment is important because water is essential to life. Any water that is not purified for humans to consume can result in disease or death. It is very important to understand our water quality so we have reliable access to this resource so we can function properly. The United Nations estimates that each person requires access to a minimum of 20 to 50 liters of water per day. Universal access to clean drinking water is considered a basic human right. Lack of clean water causes health problems, economic loss, disease, and death of about 1.8 million people every year due to water-borne illness.

It is important to understand why contaminated water causes illness. The most common reason is due to microbial contamination occurring during any step from the water source to the consumer tap. In general, the greatest microbial risks come from water that is contaminated with animal or human feces. If left untreated, the water may cause outbreaks of intestinal illness, neurological disorders, and reproductive problems. The top causes of disease outbreaks are Giardia, Legionella, Norovirus, and Shigella. Other pathogens, such as guinea worm (Dracunculus medinensis), toxic cyanobacteria and Legionella, are also important to monitor to prevent disease.

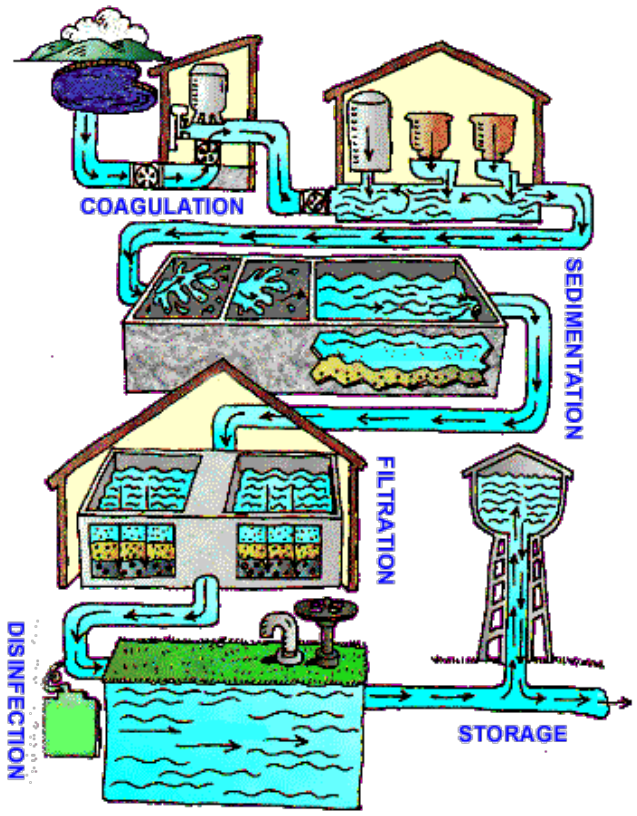
The Safe Drinking Water Act sets standards that our water must comply with in order to protect us from natural or human-made toxins. The US EPA, individual states, and water systems all work together to ensure the national health-based standards are met. The threats to drinking water safety in the US include improperly disposed of chemicals, animal and human wastes, pesticides, and natural substances in the ground that could contaminate surface or

groundwater . If this water is not properly treated, or if it travels through a poorly maintained water distribution system, the health of large communities could be threatened.

Depending on the source of a particular drinking water facility, influent water will have varying levels of quality. It is important to keep the source water as uncontaminated as possible so less costly treatment is needed. Public water comes from surface water or groundwater. In general, populated urban areas depend on surface water, while rural areas rely on groundwater. Surface water collects in lakes, rivers, and reservoirs. It is supplied by precipitation, and replaced through evaporation and infiltration into groundwater. The EPA estimates that 68% of community water users obtain their water from a surface source. Groundwater is located in the pores and spaces in rocks, and is obtained by drilling wells underground.

In particular, Cambridge drinking water comes from surface water in Fresh Pond reservoir. The pond is fed by upland reservoirs including Hobbs Brook Reservoir in Lincoln, Lexington, and Waltham, and Stony Brook Reservoir in Weston and Waltham. Since this water source is located in an urban environment, it is surrounded and protected by 162 acres of open space. Because of the higher contamination levels in urban water sources, this water undergoes a series of steps to provide safe drinking water to the citizens of Cambridge.

The Centers for Disease Control and Prevention describe the most common methods of water treatment.



The first step is coagulation and flocculation. In this process, positive-charged chemicals are added to the water containing negatively charged dirt and other dissolved particles in the water. This allows the particles to form larger flocs, as they bind with the chemicals. The next step is sedimentation. During this step, the floc settles to the bottom. Once the floc settles, this leaves clear water above that can pass through the next step, of filtration. The filters are made of varying pore sizes in order to remove bacteria, chemicals, and viruses using sand, gravel, and charcoal. The last step is disinfection. Chlorine, or another chemical is added to the water to kill any remaining pathogens or bacteria.

At the Cambridge Drinking Water Treatment Facility, many steps must be done to ensure safe drinking water quality. Depending on the source, the steps of treatment will vary. The following is a description of the treatment process at the Fresh Pond Facility in Cambridge, in which the source water comes from an urban environment. Thus, this process may have more

steps than other facilities, but this process outlines the general steps needed to ensure quality drinking water, and will provide the basis for our understanding. The approach at Fresh Pond is to have a series of steps that can prevent contamination. Each step is not enough on its own, but when combined with all the others, the highest drinking water quality is provided.

During the first step, coagulant aluminum sulfate is added to the water. Next, the water is mixed rapidly causing aluminum hydroxide to form. This is a precipitate that is mixed in the water, causing small particles to collide with it. When the small particles stick to the precipitate, this creates floc. After flocculation, Dissolved Air Flotation (DAF) occurs, to create many tiny bubbles in the water stick to the solid floc. These small bubbles float solid particles to the surface of the water. There, the particles are removed by skimming.

Primary disinfection occurs using ozone. This ozone is generated at the treatment plant, where fine bubbles are dissolved into the water. Disinfection occurs as the ozone comes into contact and mixes with the water, killing bacteria and viruses. Once the pathogens are dead, the ozone is removed from the water. Disinfection is very important to the supply of safe drinking-water, and should be used for both surface waters and groundwater. Chemical disinfection of drinking water that is contaminated with feces may not necessarily make the water safe, although it reduces the overall risk of disease.

If natural organic matter (NOM) is present in drinking water, this can produce conditions that cause microorganisms to regrow or NOM can react with disinfection to form by-products. It is important to have biofiltration to remove the biodegradable NOM. For this process, there are 6 filters made of granular activated carbon. After filtering through these 6 filters, the combined filter effluent enters into the next step, where chlorine is added through a 15% solution of Sodium hypochlorite at a concentration of 2.5 mg/L. The water is detained in a tank until the

water is disinfected again. Next, the excess chlorine must be removed because regulations require a minimum of a 0.2 mg/L concentration of disinfectant in the distribution system. During this process, ammonia is added to the chlorinated water. This reduces the taste and odor of the chlorine.

In Cambridge, one issue with drinking water transportation is lead and copper that may be present in the pipes. As a result, the National Primary Drinking Water Regulations limit the amount of these elements that can be present at the tap. In order to meet this requirement, the pH must be adjusted to a pH of 9 to reduce the corrosiveness of the water. This is done using a 50% solution of Sodium Hydroxide at a concentration of 14 mg/L. This prevents the lead and copper in the pipes from leaching into the water supply.

The last step of the process is the addition of a 23% solution of Hydrofluocylicic acid at a concentration of 1 mg/L. This is because the Massachusetts Department of Health requires fluoridation of the drinking water to prevent of dental cavities.

In order to make sure water is aesthetically pleasing, turbidity, or the measurement of suspended particles in the water, must be measured. It is determined by measuring the scattering of light at right angles in a water sample. Turbidity is quantified using nephelometric turbidity units (NTU's), and the higher amount of light that is scattered, the higher the turbidity levels. No one would like to drink water that appears discolored or cloudy, so this parameter can ensure aesthetically pleasing water.

Additionally, turbidity is also used throughout the treatment process as a measure of treatment effectiveness. The particles contributing to the discoloration of water include clay, silt, inorganic matter, algae, and microorganisms. While many of these particles are harmless, high

levels of particulate matter can protect bacteria or pathogens from chlorine and ozone. As a result, it is important to have low turbidity levels for both safety and aesthetics.

Introduction Part 2 – Lauren Chaleff

We started this project with a desire to investigate the effectiveness of phytoremediation. According to the US Environmental Protection Agency (EPA), phytoremediation is "the use of plants to partially or substantially remediate selected contaminated soil, sludge, sediment, ground water, surface water, and wastewater." Phytoremediation can function through degradation of pollutants; accumulation of pollutants for later removal; dissipation of pollutants from water or soil into the atmosphere; and immobilization to contain pollutants to one location. [1] These different types of treatment have been used to treat for metals, radionuclides, non-metals;[2] organic and inorganic compounds,[3] and even unfavorable soil pH.[4] Although not technically plants, different forms of algae are often considered viable agents of phytoremediation since they require similar conditions to grow, utilize photosynthesis, and produce many similar enzymes as aquatic plants.[5]

Many plants used for phytoremediation have long germination and growth times, and were thus not feasible options for use in this short-term project. However, algae grow relatively quickly. Therefore we decided to use algae to investigate phytoremediation. Next we had to decide what pollutant to remediate, and how to devise our treatment process. We initially

[1] Bruce E. Pivetz, "Phytoremediation of Contaminated Soil an Ground Water at Hazardous Waste Sites, *United States Environmental Protection Agency Office of Research and Development* (2011): 1, accessed December 16, 2014, doi: http://www.epa.gov/superfund/remedytech/tsp/download/epa_540_s01_500.pdf
[2] ibid 3
[3] ibid 6
[4] ibid 5
[5] ibid 8

decided on estradiol. Estradiol is not normally referred to as a pollutant since it plays an important role in many key bodily functions, such as the menstrual cycle and puberty. However, it has become a concern as a pollutant due to its release into aquatic ecosystems through undigested birth-control pills and other media. According to the EPA's Drinking Water Treatability Database, estradiol is an endocrine disrupting compound that can have adverse effects on humans and other animals. Different artificial forms of estradiol have differing impacts on aquatic ecosystems. For example, ethynyl estradiol can impact fish reproduction at concentrations that are typical of effluent from municipal wastewater treatment facilities (2 ng/L).[6] It is somewhat difficult to study the impacts of these substances, however, because they interact with other natural and synthetic compounds in synergistic and complex ways that are difficult to observe. There are also numerous estrogen-mimickers that can have similar impacts on aquatic as well as land-bound wildlife, including many organic chemicals used in agriculture, industry, household products, and other pharmaceuticals.[7]

There are a number of methods available to deal with estrogen and estrogen-mimickers, but it is not yet clear which method will be economically feasible or desirable for widespread usage in the US. Some treatments used so far for estrogen-mimickers and other persistent organic pollutants include ozonation; treatment with H2O2 and ultraviolet radiation; photocatalysis; and photolysis.[8] However, many of these treatments are very expensive. One area of research that may prove to be just as effective and less expensive is phytoremediation of

[6] "17a-ethynyl estradiol," *Drinking Water Treatability Database* (2007), accessed December 16, 2014, doi: *http*://iaspub.epa.gov/tdb/pages/contaminant/contaminantOverview.do?contaminantId=-1013719332

[7] Amber Wise, Kacie O'Brien, and Tracey Woodruff, "Are Oral Contraceptives a Significant Contributor to the Estrogenicity of Drinking Water?," *Environmental Science Technology* (2010): 1, accessed December 16, 2014, doi: http://coe.ucsf.edu/coe/spotlight/env_hlth+wm/contraceptives_water.pdf

[8] Santiago Esplugas et al., "Ozonation and advanced oxidation techonologies to remove endocrine disrupting chemicals (EDCs) and pharmaceuticals and personal care products (PPCPs) in water effluents," *Elsevier* (2007) 149: 637-638.

estrogens and estrogen-mimickers with algae. In one study published in *Applied and Environmental Microbiology*, Lai et al. found that Chlorella vulgaris was an effective phytoremediator of estradiol valerate, biotransforming and sorbing it out of solution.[9]

We decided to base our project on the results of this study. Could we perhaps replicate the results of the study, or use the results of the study to conduct an even more successful experiment? For example, in the Lai et al. study, the removal of estradiol from solution was found to be more effective when solutions were shaken for the shortest amount of time during incubation. We could use this knowledge to inform the design of our project.

However, we encountered a problem when designing our project. We had decided to use Ultraviolet and Visible Spectroscopy (UV-vis) to measure the concentration of estradiol in our algae-estradiol solutions since this was the simplest and cheapest way we had available. But we could not detect the estradiol using this technology. This is most likely because estradiol valerate has a very low solubility in water (3.6 mg/L at 27 degrees C)[10] and so was not present at a high enough concentration to impact the absorbance of the light measured in the UV-vis machine. UV-vis can be an accurate measurement of concentration of a substance in solution, but only if there is enough to have a measurable absorbance. UV-vis works as follows: light of specified wavelengths is separated into rays of different wavelengths by a prism. Each beam is then split into two beams using small mirrors. One of these two beams passes through a clear cuvette containing the solution being studies, and the other beam passes through a reference cuvette. The latter beam results in little to no light absorption, and is labeled I_0. The intensity of the beam that passes through the sample in question is defined as I. This process occurs for all wavelengths

[9] K.M. Lai, M.D. Scrimshaw, and J.N. Lester, "Biotransformation and Bioconcentration of Steroid Estrogens by Chlorella vulgaris," *Applied Environmental Microbiology* (2002) 68: 859.
[10] "Substance: Estradiol," *Royal Society of Chemistry* (2011): accessed December 16, 2014, doi: *http*://www.rsc.org/learn-chemistry/wiki/Substance:Estradiol

being studied. Absorbance is measured as $A = \log I_0/I$. The absorbance for a particular compound tends to resemble a mound with a single peak at a particular wavelength, named λ_{max}.[11]

Rather than dissolve our estradiol in alcohol or another substance first and then mixing with water, which could have negatively impacted the growth of the algae, we decided to change our contaminant to another organic pollutant.

One widespread organic pollutant of interest is bisphenol A (BPA). BPA is a polycarbonate used in plastic and epoxy resins that can be found in food packaging, water and milk bottles, impact-resistant safety equipment, water pipes, and dental sealants.[12] It is difficult to ascertain what the exact impacts of are of BPA on human and environmental health, since BPA interacts with so many other chemicals in our environment and is present at such low levels. However, according to the Food and Drug Administration's Department of Health and Human Services, there are three main areas of potential concern regarding BPA and human health: developmental neurotoxicity, cardiovascular disease, and sperm/testicular/hormone related illnesses.[13] Specifically, BPA may also be an estrogen-mimicking, endocrine-disrupting compound.[14] We also thought BPA would be an appropriate pollutant to study since it is a popular topic of discussion in the media, with BPA-free water bottles and other household items becoming more and more common. BPA also seemed like a better pollutant to study since it is

[11] "UV-Visible Spectroscopy," accessed December 16, 2014, doi: https://www2.chemistry.msu.edu/faculty/reusch/virttxtjml/Spectrpy/UV-Vis/uvspec.htm
[12] "Bisphenol A (BPA)," *National Institute of Environmental Heal Sciences,* accessed December 16, 2014, doi: http://www.niehs.nih.gov/health/topics/agents/sya-bpa/
[13] Jason Aungst and Steven Anderson, "final report for the review of literature and data on BPA," *Department of Health and Human Services* (2014), accessed December 16, 2014, doi: http://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/FoodAdditivesIngredients/UCM424011.pdf
[14] Y. Zhang et al., "Removal of bisphenol A by nanofiltration membrane in view of drinking water production," accessed December 16, 2014, doi: http://oatao.univ-toulouse.fr/1379/1/Zhang_1379.pdf

more soluble in water. According to the Bisphenol A Global Industry Group, BPA's solubility in water at ambient temperatures ranges from 120-300 milligrams per liter, two orders of magnitude greater than the solubility of estradiol. We were pleased to find that BPA in solution was detectable with our UV-vis machine, with absorbance peaking at about 280 nm.

BPA's widespread presence, potential involvement in nefarious health conditions, and high solubility made it an ideal candidate for our study of the phytoremediative capabilities of alga. We were encouraged to find that one type of algae, though different from the kind that we had begun to culture with estradiol in mind, had been found to effectively remove BPA from solution. According to one study published in Elsevier, Monoraphidium braunii removed BPA from solution at a statistically significant level.[15] Removal of BPA using algae could prove to be an efficient and effective method, and potentially less expensive than other methods. For example, it has been shown possible (and expensive) to remove BPA from water using nanofiltration with a polysulofane-based membrane, in which BPA sorbs or diffuses onto the membrane and fouls it in the process,[16] or electrochemical oxidation of BPA using a carbon fiber electrode, also fouling the electrode.[17] One aspect of a simple algae phytoremediation treatment process is that it is potentially very low-tech. Simple batch reactors containing algae, BPA, and water could suffice for the removal of BPA.

There were a number of conditions that we could choose to vary in our algae-BPA batch reactors, including shaking time, incubation temperature and length, amount of light reaching the

[15] CE Gattulo et al., "Removal of bisphenol A by the freshwater green alga Monoraphidium braunii and the role of natural organic matter," *Elsevier* (2011): accessed December 16, 2014, doi: http://www.ncbi.nlm.nih.gov/pubmed/22209372
[16] Y. Zhang et al., "Removal of bisphenol A by nanofiltration membrane in view of drinking water production," accessed December 16, 2014, doi: http://oatao.univ-toulouse.fr/1379/1/Zhang_1379.pdf
[17] Hideki Kuramitz et al., "Electrochemical oxidation of bisphenol A. Application to the removal of bisphenol A using a carbon fiber electrode," (2001): 45, p. 37-42.

algae, the presence or absence of natural organic matter, concentration of algae, etc. We largely based our decisions on the study conducted around Chlorella vulgaris and estradiol, assuming that the ideal study conditions in this experiment may be ideal for the growth (and phytoremediation capabilities) of Chlorella vulgaris as opposed to being ideal for the specific uptake of estradiol.

Once these variables were figured out, we could envision what possible treatment technologies could look like using our experimental data. If our project showed a high removal of BPA from solution, it would be successful to some extent. However, if we were to expand this treatment to a complete treatment process, we would have to have a plan for what to do with the algae after it was used to remove the BPA from solution. Understanding the optimum steps to take in this process would require us to have more knowledge than we would obtain in this experiment, because we would have to know whether the BPA was being primarily sorbed by the algae or transformed by it to another chemical. If BPA was mostly being sorbed by the algae, we would have to investigate a way of disposing of the algae that would not result in BPA leaching back into the environment. If, however, the BPA were transformed into a less harmful compound, then the algae could theoretically be collected and used as biofuel. There are already numerous instances of algae successfully being converted into bio-diesel, bio-oil, bioethanol, bio-hydrogen, and biomethane.[18] If the chlorella were to biotransform BPA into a harmless substance, it may even be possible to sell the algae as food. According to the American Cancer

[18] Ayhan Demirbas, "Use of algae as biofuel source," *Elsevier* (2010): 51:12, 2738-2749.

Society, Chlorella vulgaris is commonly sold as a health supplement in pill and powder form, and many people consume 2-3 grams per day.[19]

Our analysis used UV-vis to measure relative concentrations of chlorophyll (indicative of Chlorella) and BPA in solution. Chlorophyll has a peak absorbance at about 650 nm, while BPA has a peak absorbance at about 280 nm. We tested our batch reactors (which were conveniently housed in vials that fit in the UV-vis machine) primarily for BPA, but we also noted peaks around 650 nm as an indication that algae were present. Initially, we had no way of knowing whether increases or decreases in absorbance at 280 nm was from a change in BPA concentration or from the addition/decrease of a compound produced by the algae that coincidentally absorbed light with the same wavelength as the light absorbed by BPA. For this experiment we attributed changes in absorbance at 280 nm to BPA rather than to unknown products of the algae. No other wavelength of light peaked in absorbance other than those around 280 nm and 650 nm, so it is reasonable to assume that any by-products of the algae were not responsible for the 280 nm peak. It would be quite a large coincidence if the only by-product of the algae absorbed light at the same wavelength as BPA. In order to be sure, we also conducted some trials with just algae to measure changes in absorbance at 280 nm, to see If those theoretical by-products were present in these trials or not. However, if these by-products were created only in the presence of BPA, then they could mask decreased in BPA concentration by absorbing light at the same wavelength as BPA. This should be parsed out in future studies.

If we had wanted to measure quantitative changes in concentration of BPA (as opposed to relative changes), we would have to create a detailed UV-vis calibration curve of BPA

[19] "Chlorella," *Ameircan Cancer Society* (2011): accessed December 16, 2014, doi: http://www.cancer.org/treatment/treatmentsandsideeffects/complementaryandalternativemedicine/herbsvitaminsandminerals/chlorella

concentration vs. absorbance. We were not able to conduct such a detailed calibration curve for the purposes of this experiment. Although our research methodology did not allow us to know exactly how much the BPA concentration had decreased, it did show relative decrease. This is the key piece of information we were interested in. As far as we were aware, there had been very few studies done regarding the ability of Chlorella vulgaris to remove BPA from water at the time this experiment was conducted. If our experiment did show that Chlorella vulgaris removed BPA from solution, that would indicate to other scientists that this is a promising area of research. This research would prompt other researchers to investigate the ideal conditions in which Chlorella will uptake BPA, as had been done by Lai. et al for the specific conditions at which Chlorella vulgaris uptakes estradiol.

HYPOTHESIS

Hypothesis-- Sasha Johnson-Freyd with additions by Joseph Lanzillo

In this study, we investigated the following question: Can algae be used to remove BPA from waste water? We examined this question using the species *Chlorella vulgaris* in a simplified batch reactor model of the process (batch reactors contained only algae, BPA, and distilled water). Assuming algae is effective at removing BPA from water, what are the ideal reactor conditions for removal?

We will operationalize BPA quantity using spectroscopy (see *Methods*). We will vary two incubation variables: time incubated (for zero, 24, or 48 hours) and light vs. dark incubation.

We hypothesize that *Chlorella vulgaris* will be effective at removing BPA from wastewater. In specific, we hypothesize that the decrease in 280 nm absorbance (peak absorbance of BPA) during incubation will be larger among reactors with algae than among reactors containing only BPA and no algae. Furthermore, we hypothesize that reactors incubated

in the light for a longer period of time (48 hours) will show the largest decrease in BPA among our experimental conditions.

We will run two control groups - one with only BPA without algae, and one with algae and no BPA. We are including these controls to understand how much of a change in absorbance in the experimental groups can be accounted for absorbance change by either BPA or algae on its own. We predict no discernable changes in observance for the either control group.

For more complete reasoning behind the first hypothesis, see *Introduction*. In short, there is evidence that *Chlorella vulgaris* can uptake and/or transform hormone-like chemicals in water (see, e.g., Lai et al. 2002); and other algae species are shown to uptake BPA. The second hypothesis is based of the finding of Lai et al. (2002) who studied uptake of estradiol by *Chlorella vulgaris*. They found that the highest amount of uptake and biotransformation of estradiol occurred with longer incubation times in the light. Although it is likely that *Chlorella vulgaris* uptakes and biotransforms BPA differently than it does estradiol, and therefore ideal conditions might be different, we do not have any information on how these processes differ -- therefore, our hypothesis for BPA is conservative, and based on Lai et al.'s (2002) study of estradiol. It will be valuable to determine ideal conditions not only for specific applications of algal uptake of BPA, but this research (and being wrong about our second hypothesis) could also provide us with new information pertaining to the difference in algal responses to estradiol and BPA.

If successful, this study presents a possible novel mechanism for treatment of municipal and industrial wastewater, which contain measurable and problematic levels of BPA. In this study, we create a model to test whether this method (using *Chlorella vulgaris* in simple batch reactors with BPA and water) is an effective or reasonable way to remove BPA from wastewater.

MATERIALS AND METHODS

Materials and Methods -- Reid Bergsund

This experiment was originally designed to determine the effectiveness of *Chlorella vulgaris* at taking up estradiol, an estrogen compound found in birth control, although significant changes were made to the experimental design several times when barriers to the original procedure arose. Most notable amongst these changes was the switch to testing the algae's uptake of Bisphenol A (BPA) rather than uptake of estradiol, as described below.

The original materials and procedure were inspired and heavily influenced by a paper in the Journal of Applied and Environmental Microbiology that tested, amongst other things, the ability of *Chlorella vulgaris* to take up estrogen steroids under different conditions (Lai, et al, 2002). The authors of the paper incubated solutions of algae and estradiol, varying estradiol concentrations in the solutions, shaking time, and exposure to UV radiation, to determine the ideal conditions for estradiol uptake by the algae. Their empirically determined "ideal" conditions formed the basis of this experiment, the goal of which was to test how much estradiol the algae could remove from water in a set amount of time, and whether or not the algae could be "reused," so to speak—whether or not the same algae culture could remove estradiol from water multiple times. The answers to both aforementioned questions asked in this experiment had water treatment implications, lending purpose to our experiment and meaning to our results.

The materials we needed for this experiment included mature colonies of *Chlorella vulgaris* algae, which we allowed to incubate for 21 days to ensure the cultures were fully grown. We planned dissolve solid estradiol in water, and determine the algae's estradiol uptake using ultraviolet-visible (UV-Vis) spectroscopy analysis. By comparing the amount of estradiol in the water visible on the UV-vis spectrometer before and after the addition (and incubation) of algae

in the solution, we would be able to determine how much estradiol the algae removed. However, we ran into problems almost immediately, as the estradiol would not show up on the UV-vis spectrometer. As our entire procedure was contingent on our ability to see and measure estradiol on the spectrometer, we had to make a major change; either we would need to find a different way to dissolve the estradiol such that it was visible on the spectrometer, or we'd have to choose a different chemical to use.

After much discussion and after being advised by Professor Vecitis and the rest of the ES 165 teaching staff, we decided to test *Chlorella vulgaris*'s ability to take up bisphenol-A (BPA), a chemical found in many plastics and every day items. BPA can leach into the water supply from said plastics, and can have harmful effects on human health (although these effects are not fully understood yet). Therefore, like estradiol, BPA presented an interesting opportunity for experimentation with real-world applications, as there currently exists no method for removal of BPA during water treatment. BPA dissolved in water generated visible peaks on the spectrometer, solving our original problem with estradiol, and there was also some likelihood that the algae would be able to take up the BPA as it had the estradiol in the experiment performed by Lai, et al (2002).

However, while the Lai, et al paper had given us ideal conditions for estradiol uptake by *Chlorella vulgaris,* we had no idea of the ideal conditions for BPA uptake—therefore, we redesigned our experiment to test exactly that. We would use similar procedures to those used by Lai, et al, to determine the ideal conditions for BPA uptake by *Chlorella vulgaris* as they had for estradiol.

For our new, redesigned experiment, we used the same 21-day-old colonies of *Chlorella vulgaris* algae in water, and solid BPA pellets dissolved in distilled water. We had created a 100

mg/L solution of BPA to determine it was visible on the spectrometer, and decided to use that same solution for our experiment as we were pressed for time and were unlikely to be able to complete the experiment if we took the time to create solutions with different BPA concentrations (BPA is not easily soluble in water, so creating each solution required over 24 hours of stirring to completely dissolve the BPA pellets). We planned to vary incubation time and access to UV radiation to test the effects of time and light vs. dark on the algae's ability to take up BPA, as Lai, et al had tested the same variables for estradiol uptake. We conducted four trials and four controls in batch reactors for each of the following conditions:

- No incubation time (0 hours), light
- No incubation time (0 hours), dark
- 24 hour incubation time, light
- 24 hour incubation time, dark
- 48 hour incubation time, light
- 48 hour incubation time, dark

We used 10 mL UV-vis tubes as our batch reactors for ease of analysis. Each of the four trials for each condition contained 6 mL of 100 mg/L BPA solution and 3 mL of the algae solution, for a total volume of 9 mL. Two of the controls for each condition contained 6 mL of 100 mg/L BPA solution and 3 mL of distilled water, while the other two controls for each condition contained 6 mL of distilled water and 3 mL of algae solution. For each trial, we combined the algae and BPA solutions and took preliminary UV-vis readings. By recording the absorbance of each solution on the spectrometer (peak absorbance for BPA was around 280 nm), we could determine the amount of BPA in each reactor. After incubating the algae and BPA solutions and the controls for 24 or 48 hours in the light or dark, depending on the trial, the algae had settled

out and we were able to get UV-vis readings of the algae-free water to determine how much BPA had been removed by the algae. Then, we would shake each reactor to get the algae in solution again and take another UV-vis reading, to determine the amount of BPA in solution before and after removal of the algae from the water. For the 0-hour incubation trials, we combined the algae and BPA in solution, took UV-vis readings to determine the levels of BPA in the water before algae removal, and then centrifuged each tube to remove the algae from solution so we could determine level of BPA in the water after removal of the algae. By centrifuging the tubes, we could approximate the settling that occurs naturally over time in the other conditions. From our data, we'd be able to conclude for each experimental how much BPA was removed from the water by the algae by comparing the absorbance/amount of BPA in each reactor before and after removal of the algae.

Using this procedure, it will be possible to conclude whether or not there is an "ideal" set of conditions for *Chlorella vulgaris* to take up BPA from the water, and what those conditions are. However, there are a few limitations and potential sources of error that we must keep in mind. As our entire experimental team will be helping prepare reactors and collect data, there may be some inconsistencies in methods, pipetting techniques, etc. which could result in minor variation of algae or BPA concentration in the reactor, or variation of algal BPA uptake. Likewise, as we will be preparing and testing all reactors at once, there may be some variation (on the order of a few minutes) in incubation times or in times spent in the light vs. dark, which could result in minor variations in algal BPA uptake. Nevertheless, we don't believe any variation caused by these factors will be major enough to void our results.

RESULTS AND DISCUSSION

Results– Sasha Johnson-Freyd

We hypothesized that *Chlorella vulgaris* will be effective at removing BPA from wastewater. We operationalized this general statement by hypothesizing that the decrease in 280 nm absorbance (peak absorbance of BPA) during incubation will be larger among reactors with algae than among reactors containing only BPA and no algae. Furthermore, we hypothesized that reactors incubated in the light for a longer period of time (48 hours) will show the largest decrease in BPA among our experimental conditions.

We collected data on the magnitude and location of UV-VIS peaks around 280nm (representing the relative absorbance of BPA) for each reactor across varying conditions. For each experimental condition (no incubation; Light, 24-hour; Light, 48-hour; Dark, 24-hour; and Light, 48-hour) we have data on four separate experimental replicate reactors, and two control conditions ("x" = water+BPA, no algae; "y" = water+algae, no BPA [latter not used in analyses]) with two replicate reactors each. For each reactor, we measured absorbance three times: before incubation ("initial"), right after incubation (with algae settled; "pre-shaking") and after incubation, after shaking the reactor (with algae suspended; "post shaking"). For the no-incubation condition, we performed UV-VIS twice: once directly after creating the reactor (with algae suspended, parallel to "post-shaking") and once after centrifuging the reactor to settle out the algae (parallel to "pre-shaking"). All data collected and analyzed are represented in table R1.

			Light						Dark					
	0-hour		Light, 24-hour			Light, 48-hour			Dark, 24-hour			48-hour		
	pre-shaking (after centrifuge)	post-shaking (before centrifuge)	initial (t=0)	pre-shaking	post-shaking	initial (t=0)	pre-shaking	post-shaking	initial (t=0)	pre-shaking	post-shaking	initial (t=0)	pre-shaking	post-shaking

	wavelength (nm)	278	278	278	280	279	281	281	280	280	280	280	282	279	279
a	absorbance	0.595	0.54	0.678	0.696	0.675	0.691	0.619	0.654	0.729	0.692	0.66	0.74	0.606	0.638
	wavelength (nm)	278	278	278	280	280	280	280	280	279	280	280	280	279	280
b	absorbance	0.598	0.555	0.667	0.645	0.668	0.57	0.618	0.654	0.635	0.638	0.661	0.701	0.619	0.654
	wavelength (nm)	278	278	278	280	280	280	280	280	277	280	280	278	279	280
c	absorbance	0.608	0.567	0.66	0.644	0.678	0.631	0.636	0.653	0.652	0.633	0.674	0.69	0.621	0.654
	wavelength (nm)	279	278	277	280	281	280			280	280	280	279	280	279
d	absorbance	0.623	0.562	0.626	0.644	0.672	0.65			0.626	0.639	0.667	0.631	0.615	0.65
	wavelength (nm)	279	280	277	280	280	280	278	279	279	280	280	279	278	278
x1	absorbance	0.643	0.714	0.634	0.624	0.627	0.635	0.608	0.609	0.634	0.608	0.615	0.623	0.599	0.597
	wavelength (nm)	278	280	279	280		277	279	279	278	280	280	280	279	279
x2	absorbance	0.61	0.6	0.641	0.622		0.634	0.609	0.602	0.632	0.611	0.615	0.643	0.615	0.616
	wavelength (nm)	271		272		271	274	669		273	280	280	272		
y1	absorbance	0.048		0.083		0.062	0.078	0.013		0.077	0.036	0.052	0.105		
	wavelength (nm)	(320)		272	271	271	272			272	280	280	269		
y2	absorbance	(0.045)		0.123	0.003	0.102	0.089			0.098	0.032	0.056	0.077		

TABLE R1: all data collected on absorbance of BPA.

UV-VIS spectroscopy is a powerful tool for rapidly analyzing the contents of a water sample. In this study, we examined the absorbance of 280nm light by our water samples, a measurement which is proportional to the concentration of BPA in the sample. Our hypotheses in this study were not about absolute amount of BPA removal, and our reactors are models that do not represent realistic concentrations of BPA in wastewater. Therefore, for this analysis we use spectroscopy data to just understand the *relative* amounts of BPA present in our samples. I will not use absorbance data to calculate total concentrations; rather, I will analyze absorbance data relative to itself to determine the relative presence, absence, and magnitude of change in BPA concentration across different experimental conditions.

We recorded the wavelength of peaks in order to ensure that we were in fact measuring BPA, rather than some other compound. I did not use wavelength data in analysis, other to ensure that the wavelengths of absorbance were consistent. All wavelengths were between 269 – 282 nm (with the large majority between 278-280nm), which is a reasonable range for BPA absorbance. One sample recorded a

wavelength of 320nm, which was excluded from analysis (however, this measurement was from a y-control, which I did not analyze anyways).

Perhaps the most non-intuitive portion of our data and analysis is that we ran UV-VIS spectroscopy on reactors before and after shaking the reactors. We did this because when the reactors incubated, the algae settled, and we wanted to measure BPA absorbance both in the clear water (with settled algae) as well as with all compounds suspended (to be parallel to the initial time=0 measurements, which have all materials well-mixed). The distinction between pre- and post-shaking might not be important; we have no reason to suspect that BPA absorbance would differ. However, it might tell us something about how the algae is uptaking the BPA: for example, if when the algae settles it brings sorbed BPA with it, but then shaking re-suspends the BPA, we would want to design our applied reactor accordingly. Having data on pre- and post-shaken reactors has the potential to reveal aspects of the mechanism by which algae removed BPA from water (if it does at all).

The data we collected are relatively messy. Figure R1 shows the averages of absorbance measures across replicates for all conditions of our experiment. From this figure, no clear pattern emerges. Most telling for the messiness of our data is the variance in the measures of initial BPA absorbance. In figure R1, these time-zero measurements are highlighted. In theory, these measurements should be equivalent, because they represent the immediate measurement of absorbance after (in theory) equivalent construction of reactors. However, as the figure shows, these initial measurements seem to capture much of the variation in all of the absorbance measures. This suggests that there is noise in our data, likely derived from either the construction of the reactors or the UV-VIS measurements.

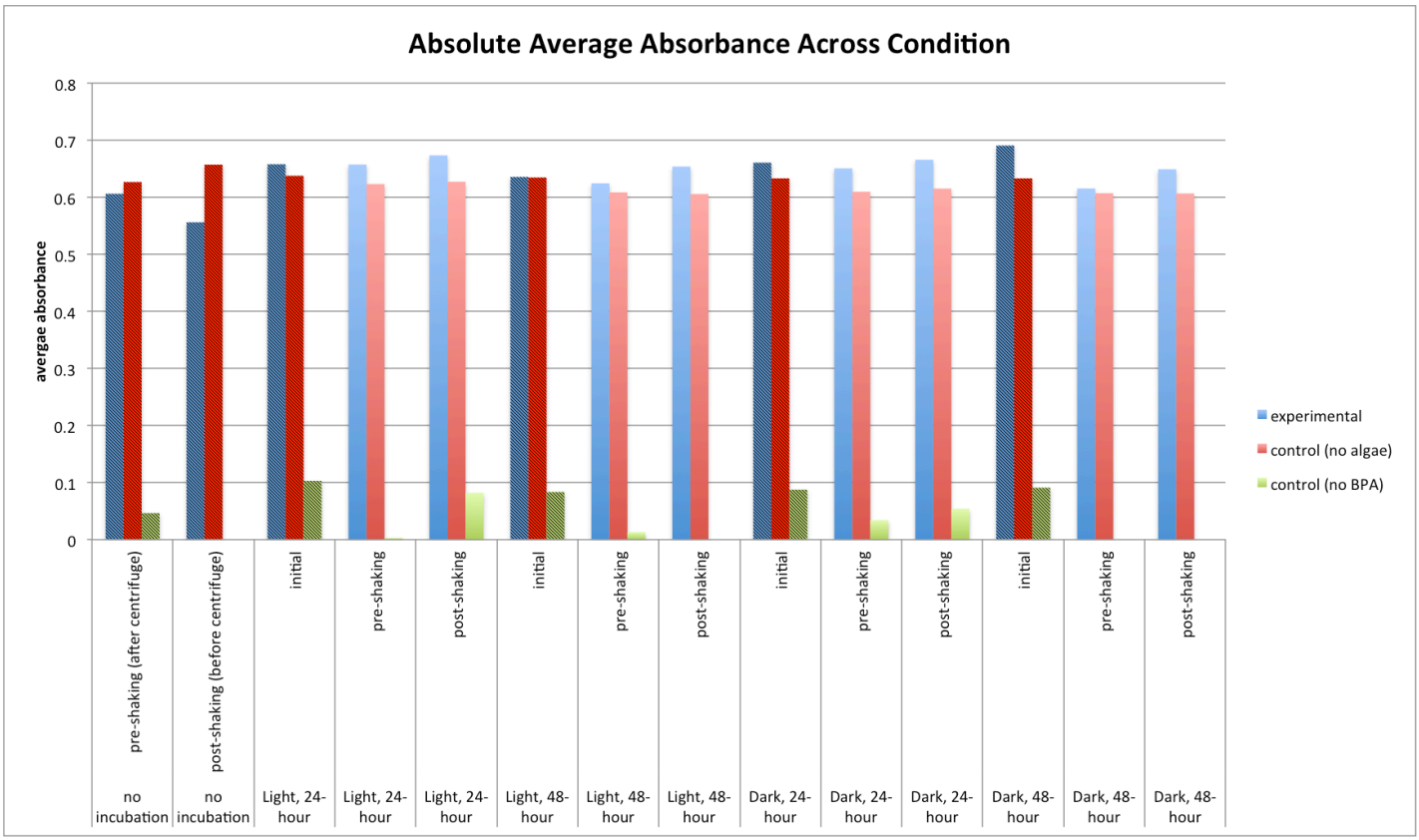


FIGURE R1: Averages of absolute absorbance measurements across all conditions. Measurements that represent no incubation (either the no incubation condition or initial measurements from other conditions' reactors) are highlighted with stripes; note the high level of variance among these zero-time measurements compared with the overall variance of all samples. This suggests that the data are messy, because we do not expect any variation in zero-time measurements: the reactors were supposedly set up equivalently, and therefore should have equivalent measurements. This suggests that there is experimental error in either the set-up of the reactors (i.e. unintentional variation in methodology) or measurement (i.e. noise in the spectroscopy data).

Another way to look at these data is by looking at the *change* in absorbance over time, rather than the absolute absorbance as I did in figure R1. For each reactor, we have initial readings (at time t=0) of absorbance. Since we are ultimately interested in the change in BPA concentrations, and since it is likely that there was an artifact in the creation of the reactors (such that they were not, in fact, equivalent), it makes sense to compare the absorbance data for each reactor to itself over time. For all subsequent analyses, I use values calculated by subtracting the time=t absorbance measurement from the initial

(time=0) absorbance measurement. Note that this change measure mean *removal* of BPA: a large positive change in BPA absorbance means that the reactor had a large decrease in BPA concentration.

The data here are still messy. Most tellingly, many change values are *negative*, which is physically impossible: a negative change value would suggest that through incubation, BPA was added to the system. Figures R2 and R3 show the average change in absorbance (change calculated for each reactor, and then averages across replicates) for each condition across both experimental replicates and control replicates ("x" replicates that contain BPA and water, but no algae). Note how in each figure, the Dark, 48-hour condition shows the highest level of positive change, suggesting that this condition might have been most effective for BPA removal. However, because of the messiness of this data, and the fact that this analysis is purely descriptive, we cannot draw firm conclusions from simple averages. Note also, though, that from these figures, pre-shaking measurements look cleaner than post-shaking measurements (many of which are negative for experimental reactors; and there is more variation here among data from control reactors). This suggests (again, non-conclusively) that either there is less BPA in the water before shaking, or that something about shaking the reactors changes the UV-VIS measurement. While these average-based analyses are not conclusive, they suggest that 1) data on this *change* measurement is still messy, 2) that the Dark, 48-hour condition might be unique, and 3) that pre-shaking might be a more robust moment of measurement than post-shaking for purposes of this analysis.

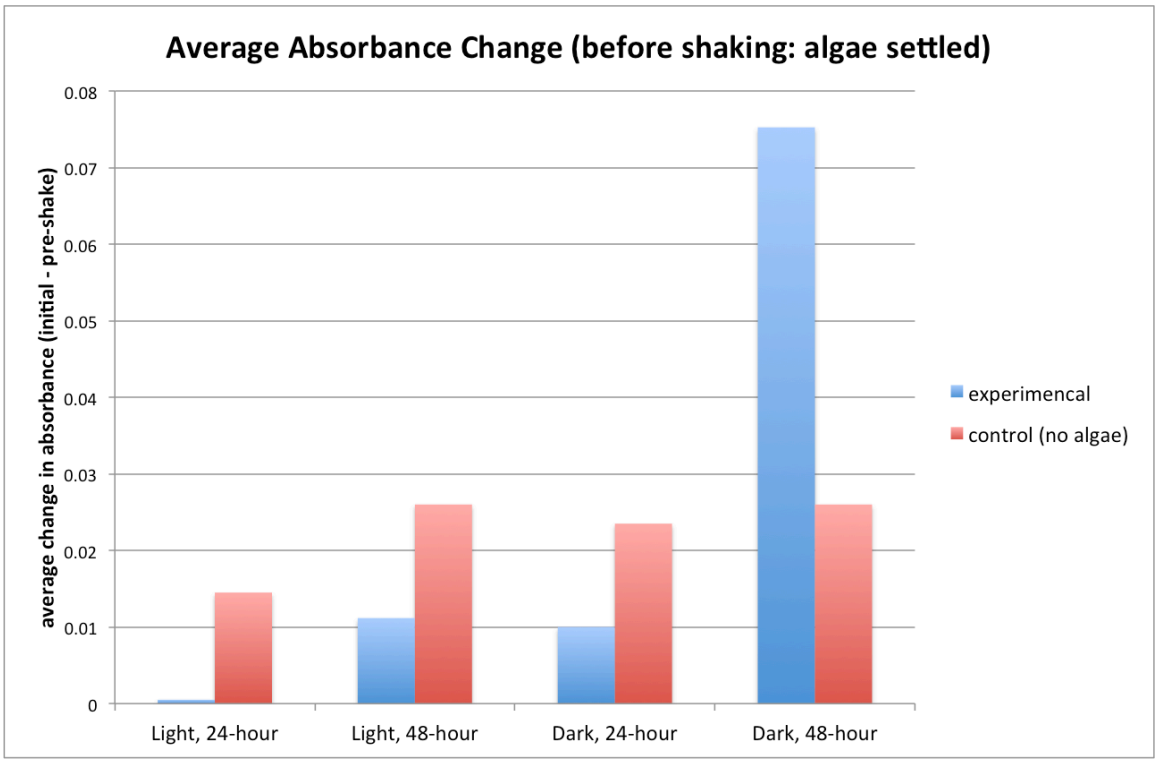


FIGURE R2: Average absorbance change (measured initial [time = 0] minus measured experimental [time = 24 or 48 hours]) of BPA (peaks ~280 nm) pre-shaking across different incubation conditions. Positive values represent decrease in BPA over time. Blue represents the average absorbance change of experimental replicates (n=4; algae and BPA); red represents the average absorbance change of control replicates (n=2; just BPA). The dark, 48-hour condition shows a much higher average difference between initial and time=t measurements.

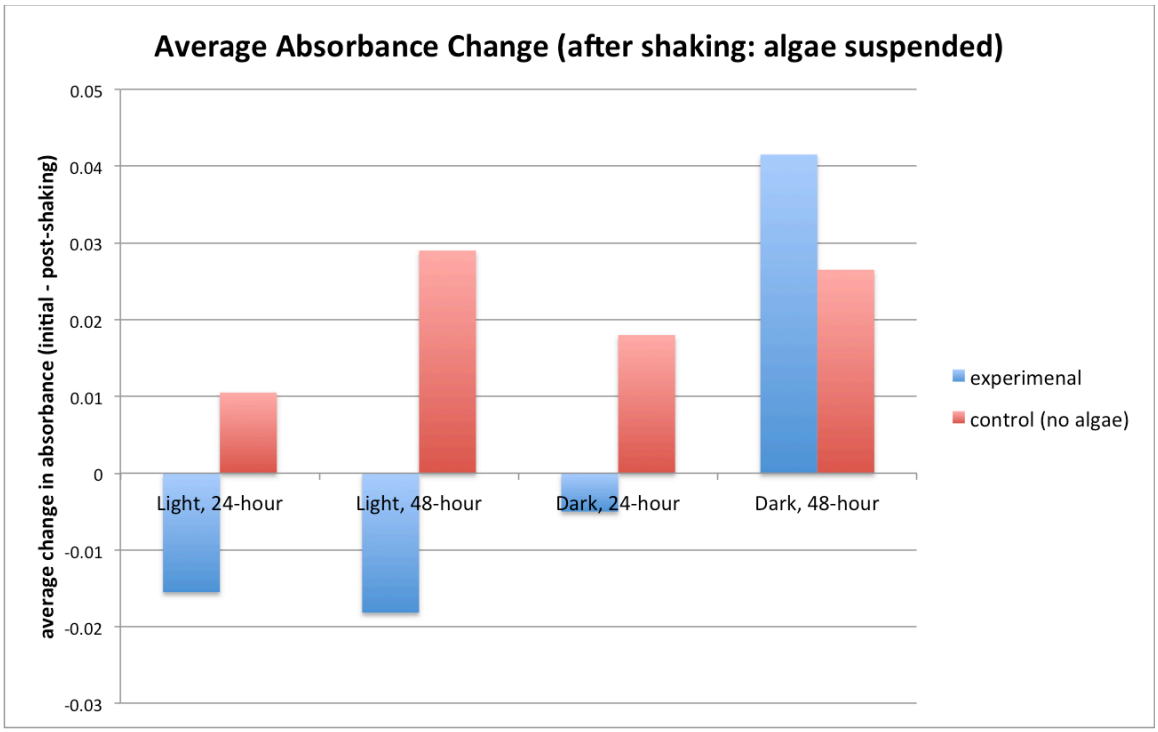


FIGURE R3: Average absorbance change (measured initial [time = 0] minus measured experimental [time = 24 or 48 hours]) of BPA (peaks ~280 nm) post-shaking across different incubation conditions. Positive values represent decrease in BPA over time. Blue represents the average absorbance change of experimental replicates (n=4; algae and BPA); red represents the average absorbance change of control replicates (n=2; just BPA). Negative values here do not make sense, because this means that absorbance was higher at time=t than at time=0, which would suggest that BPA was added to the system, which is impossible. Therefore the data are messy. The only condition that shows a positive difference value is incubation for 48-hours in the dark.

These average analyses raise the question: is all the variance in our data simply noise, or is there some effect happening behind the noise? In order to answer this question, we must use inferential statistics. In particular, I wanted to examine the variance within each condition (across replicates) to see if the distribution of results was significant or likely to be simply due to noisy data. To do this, I ran one-sample t-tests.

T-tests measure the probability of measuring a set of values assuming an even distribution around a null hypothesis. In these tests, I examine the extent to which the distribution of values we measured are likely to be due to chance. For each reactor, I calculated the change in absorbance (initial measurement –

time=t measurement). I then ran a t-test across replicates for each condition (normally with n=4, because each condition had 4 replicates [although some missing data means n sometimes = 3]) to determine the likelihood of the distributions to be produced by chance. The p-value here is the probability of measuring the distribution if the data (i.e., the replicates) were completely random. Therefore, lower p-values suggest that the data are not random, and that there was real change between time = 0 (initial) and time = t (experimental replicates). In these tests, I report both one-tailed and two-tailed p-values. While two-tailed tests are more conservative, in this case it is appropriate to use one-tailed tests because our hypothesis is directional: we are assuming that change is positive, such that time=t reactors have a lower BPA absorbance than do reactors measured at time=0. I will therefore use one-tailed p-values for this analysis.

The first two t-tests I ran (results shown in tables R2 and R3) are based on a zero null hypothesis: that there would be no change over time in BPA absorbance. These tests measure the likelihood of measuring the distribution of experimental replicate data assuming a random distribution around a mean of 0. While most conditions do not show significance, the Dark, 48-hour condition pre-shaking is significant ($p < 0.05$): this suggests that the distribution of changes in absorbance in the Dark, 48-hour experimental replicated are significantly different from a zero-mean normal distribution.

	P-value, two-tailed	P-value, one-tailed	t	Degrees of freedom
Light, 24-hour	0.9658	0.4829	0.0465	3
Light, 48-hour	0.8734	0.4367	0.1804	2
Dark, 24-hour	0.4381	0.21905	0.8921	3
Dark, 48-hour	0.0531	**0.02655 ***	3.1053	3

TABLE R2: Results from one-sample T-test for change data from experimental conditions pre-shaking (initial minus pre-shaking measurement), with null hypothesis = 0. Only the measurements from the condition incubated for 48-hours in the dark shows a significant p-value: this means that the values measured for this condition have only a 0.0531 probability of occurring by chance, suggesting that the effect is real and that the difference in absorbance between time-zero measurements and time-t measurements is meaningful for the Dark, 48-hour condition.

	P-value, two-tailed	P-value, one-tailed	t	Degrees of freedom
Light, 24-hour	0.2583	0.12915	1.3915	3
Light, 48-hour	0.5779	0.28895	0.6584	2
Dark, 24-hour	0.8543	0.42715	0.2000	3
Dark, 48-hour	0.1929	0.09645	1.6733	3

TABLE R3: Results from one-sample T-test for data from incubation conditions post-shaking, with null hypothesis = 0. No results were statistically significant.

The problem with the above t-tests is that they compare the distributions of experimental replicate measurements to a null hypothesis of zero, rather than a null hypothesis based on control data. Basing our null hypothesis on data from controls is important, because it takes into account potential artifacts and factors in our experimental design. For example, BPA might naturally degrade in water (which, from the average measurements in figures R2 and R3, seems to be the case with the consistently positive red bars), in which case we would want to examine the extent to which the presence of algae aids in the BPA removal process. We want to know if using algae to remove BPA is reasonable and effective, which requires comparing it to control conditions.

One way to compare distributions of experimental vs. control condition data is by running a two-sample t-test, to compare two distributions to each other. However, because our sample sizes are different for experimental (n=4) and control (n=2) replicates, a two-sample t-test is unlikely to work, and therefore made the conservative statistical decision not to perform it.

Instead, I ran two one-sample t-tests based on a null hypothesis derived from the control conditions (calculated as average of difference x1 and x2 for each condition: so the average change in BPA absorbance over time when there is no algae present). Similarly to before, I ran these analyses for both pre- and post- shaking measurements, the results of which are represented in tables R4 and R5, respectively. There are no significant p-values in these t-tests. Two t-tests approach significance ($p < 0.1$):

the Dark, 48-hour pre-shaking condition; and the Light, 24-hour post-shaking condition. While these results are not conclusive, the trend towards a significant change in BPA absorbance in the Dark, 48-hour condition is still present.

	P-value	P-value, one-tailed	t	Degrees of freedom
Light, 24-hour	0.2838	0.1419	1.3022	3
Light, 48-hour	0.6317	0.31585	0.5603	2
Dark, 24-hour	0.3148	0.1574	1.2043	3
Dark, 48-hour	0.1350	**0.0675**	1.0324	3

TABLE R4: Results from one-sample T-test for data from incubation conditions pre-shaking, with null hypothesis = (average of control conditions).

	P-value	P-value, one-tailed	t	Degrees of freedom
Light, 24-hour	0.1367	**0.06835**	2.0199	3
Light, 48-hour	0.2750	0.1375	1.4886	2
Dark, 24-hour	0.4255	0.21275	0.9199	3
Dark, 48-hour	0.5880	0.2940	0.6048	3

TABLE R5: Results from one-sample T-test for data from incubation conditions post-shaking, with null hypothesis = (average of control conditions).

While these analyses do not produce overwhelmingly robust results, they do support our hypothesis that *Chlorella vulgaris* can be used to remove BPA from water samples. Contrary to our second hypothesis, the Light, 48-hour condition did not show any significant removal. We found a significant change in BPA absorbance in the Dark, 48-hour condition when compared to a null hypothesis of zero removal. When compared to control conditions, a trend that approaches statistical significance is still present: the Dark, 48-hour condition removes BPA.

These results only apply to reactors with settled algae (pre-shaking). When combined with descriptive analyses of average values of absorbance change, this suggests that allowing algae to settle, and not shaking reactors after incubation, is a more robust way to remove BPA from water when compared to shaking the reactors post-incubation. This result, although not hypothesized, can perhaps be understood as consistent with previous research: Lai et al. (2002) found that experimental conditions without shaking (for them, shaking occurred during incubation, not after) showed the most removal of estradiol by *Chlorella vulgaris* from water samples.

Overall, our hypothesis that *Chlorella vulgaris* can be used to remove BPA from water was supported by our experimental study. Contrary to our second hypothesis, the most effective condition for removal was when the reactors were incubated in the dark for 48 hours. These results represent a simplified model of BPA removal; we found an effective technique for removal at a small scale with high concentrations of BPA. The question of whether this technique would be valuable to scale to a wastewater treatment scale is an open question.

Discussion -- Joseph Lanzillo

This experiment, as originally envisioned, was designed so that after incubation, each solution would be passed through a filtration system to remove the algae. Then, after running a UV-Vis test on the effluent solution, we would be able to compare it to the BPA concentration of the original 6mL of contaminated solution. At no point in the experiment would a UV-Vis test be conducted on any solution containing the algae. We would measure the BPA concentration of a solution, add an algae solution, incubate, extract (filter) the algae, and compare to the final BPA concentration. As a safety measure, however, we conducted a UV-Vis test on the tubes after the addition of the algae solution. After filtration proved too difficult to do consistently and accurately, we decided instead to run each solution through a centrifuge to extract the algae. However, an interesting development led to a further adjustment to the procedure. Returning to

the reactors after incubation, we found that all of the algae had settled to the bottom of each cuvette, essentially conducting the extraction procedure for us since the algae was no longer suspended in the region of the cuvette exposed to the spectrometer. We immediately conducted a UV-Vis test on these tubes. We then shook each cuvette to resuspend the algae, and reran the test on each, so as to have a comparison to what had been our "safety-measure test" with the algae already added.

To ensure that the settling during incubation extracted the algae to the same extent that filtration or centrifuging would have, we also centrifuged several of the first tubes, and conducted UV-Vis tests on each after centrifuging. Upon finding that all of these post-centrifuge absorbances were identical (or nearly so) to our pre-shaking absorbances, we determined that centrifuging each of the algae-infused was an unnecessary step, and that the pre-shaking measurement would be an equivalent to a post-centrifuge (post-extraction) measurement.
As a result of our "safety-measure test" we now have two sets of measurements by which to track changes in BPA concentration. The safety-measure test, conducted after the addition of the algae to the BPA solution, can be compared to the post-shaking absorbance; both of these measurements were taken with algae suspended in the cuvette. We can also compare the absorbance of the original BPA-only solution to the pre-shaking absorbance; both of these measurements are without algae.

The fact that in nearly every trial as well as the controls absorbance increased after shaking the cuvettes indicates that the resuspension of algae can account for this small increase in absorbance.

CONCLUSIONS

Conclusions -- Joseph Lanzillo

The original experiment, which set out to optimize the uptake of estradiol by a *chlorella vulgaris,* encountered issues so early that we were unable to make any substantial conclusions. We did learn that our method of measuring contaminant concentrations, a UV-Vis test, was not capable of detecting estradiol at the concentrations at which it was dissolved in water. A similar experiment could work with a different measurement technique, and the failure of this particular experiment certainly does not indicate that algae does not absorb estradiol or that estradiol does not dissolve in water. Its solubility is just too low to be detected by a UV-Vis test. Before testing began, we recast the original purpose of our experiment to instead absorb BPA.

Throughout the experiment, there were several decisions made about optimizing uptake conditions that could have influenced the scale of the results. Had these decisions been made differently, it is possible that trends in the data would be more pronounced and conclusions would be easier to determine. Decisions regarding the concentration of BPA in the contaminated solution and the concentration of algae in the algae solution are examples of this. While held consistent across the experiment, we cannot be sure that these concentrations contributed to the largest uptake of algae. We also chose to suspend the BPA in water, rather than in the algae growth solution (an agar solution) because that would be the most realistic recreation of a batch reactor in an actual water treatment facility. Of course, adding more of the agar solution to the reactors could have facilitated more uptake of BPA by the algae. It also seems likely that the uptake of BPA would have been better facilitated if the algae had been cultured within each reactor, rather than cultured as one large batch and added to the contaminated solutions. When adding algae to each cuvette, we had to scrape up algae that had settled to the bottom of the

growth beaker so that more algae was suspended and would be more readily transported via pipette to the cuvettes. This scraping and stirring process may not have allowed for the most consistent distribution of algae across cuvettes. Furthermore, our decision to eliminate the period of initial shaking after combining the algae with the contaminated solution that Lai et al had implemented in their experiment also may have limited the ability of the algae to uptake BPA. Other possibilities for optimizing the uptake could involve alternating periods of stagnant incubation with brief periods of shaking in order to prevent the settling of the algae. We also decided to leave each cuvette capped, out of concern for safety and contamination from air. However, it is also possible that leaving the cuvettes open to a clean air supply would have encouraged the algae's uptake of BPA.

While all of these conditions were consistent across every trial in the experiment, it is very likely that we did not perfectly optimize the solution compositions, concentrations, shaking and incubating times for the uptake of BPA. Had we known the exact optimal conditions and been able to replicate them, it is likely that we would have observed more marked trends in BPA uptake, allowing us to draw more firm conclusions.

There were also several minor errors in the design of the experiment, but it does not appear likely that any of them influenced the results in any significant way. For example, throughout the experiment, different individuals were responsible for pipetting solutions, and each person may have operated the pipette slightly differently. The dark solutions were kept covered in the corner of the room while the light solutions were kept in the lit fumehood; the potential temperature difference between these two environments could have affected results. While these differences were minor, so was the scale of our results, so any one of these factors could have contributed to a variation in the results. Of course, that is the purpose of running

multiple trials for each condition – eliminate the noise caused by minute, inherent variances in any experiment.

While most of the data is not significant, the results did indicate that incubating *chlorella vulgaris* with a BPA-contaminated solution in the dark for 48 hours led to the greatest decrease of BPA concentration in the water, and there is potential for use as a method of legitimate water treatment. Under those conditions, absorbance decreased by an average of .0415 across the four trials. That is only a 6% decrease from the average original absorbance (0.6905). However, the concentration of BPA in our contaminant solution was outrageously large, and only as great as it was so that it would be detectable by the UV-Vis test. Each full cuvette contained .6 mg of BPA (66 mg/L solution). A 6% reduction in absorbance, if interpreted to be equivalent to a 6% reduction in actual BPA concentration, leaves .564 mg BPA (62.666 mg/L). This means that 3 mL of our algae solution was able to absorb .036 mg of BPA. Considering that the highest concentration of BPA found is only 22 ppb[20] (.0219 mg/L), just 3 mL of an identical algae solution would be sufficient to treat over 1 L of this contaminated wastewater.

Of course, further research must be done to understand how to dispose of algae that has already absorbed BPA, and if it is possible to extract the BPA from the algae and then reuse the algae for another cycle of water treatment. If neither of those options are possible, then a treatment plant would need a fresh 3 mL batch of algae for each L of treated water. For a treatment plant with a 1 MGD capacity, appropriate for a medium-sized town, this requires 30,000 gallons/day of fresh algae, and must dispose of used algae. With the possibility of recycling / reusing algae, this demand is less. Our experiment did not rule out using algae to absorb BPA contamination in treatment waters, but ultimately, more research and engineering is

[20] Minnesota Department of Health. "Bisphenol A in Drinking Water." (n.d.): n. pag. Drinking Water Contaminants of Emerging Concern Program, July 2014. Web. 19 Dec. 2014. <http://www.health.state.mn.us/divs/eh/risk/guidance/gw/bpainfosheet.pdf>.

required to bring it from the lab to a practical implementation in a large scale water treatment facility.