Removal of Estrone (E1) in Water:

A Comparative Study of UV, Natural Light, and Algal Treatments

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Abstract

Estrone (E1) is an environmental estrogen that threatens both man's health and the ecosystem due to its endocrine-disrupting action. This research compared and evaluated three treatment methods - ultraviolet (UV) irradiation, natural sunlight exposure, and algal treatment with Spirogyra, based on the removal efficiency of E1 under controlled laboratory conditions. Because LC–MS identification of the first-round samples is still pending, a second round of experiments was conducted using UV–Vis spectrophotometry to track E1 degradation at higher concentrations (0–10 mg/L). The results demonstrated that natural sunlight achieved the highest E1 removal efficiency, with the 10 mg/L samples showing an 84.86% decline in absorbance by the second day. The UV group showed consistent but less degradation in E1 removal, while the algal group showed the least removal in E1. Further analyses, including light-field mapping, short-term reproducibility tests, pH condition experiments, and concentration-time curves, also provided mechanistic explanations and reinforced the robustness of these findings.

This study gives proof that the non-catalytic photodegradation of E1 in natural provides experimental support for the development of energy-saving and green water treatment processes.

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Chapter 1: Introduction

1.1 Background

Environmental estrogens, specific natural and synthetic steroidal estrogens, have been traced consistently in water systems during the last twenty years and have become an environmental issue globally due to their endocrine-disrupting functionality. These chemicals, known under the colloquial term endocrine-disrupting chemicals (EDCs), might mimic, potentiate, or block hormonal messages both in wildlife and in humans (Ropero et al., 2006). Estradiol (E2), estrone (E1), and estriol (E3) are biologically produced in animals and in humans and are excreted in the feces and the urine and eventually reach the environmental water body in the form of municipal wastewater (Khanal et al., 2006). Synthetic estrogens, like the example of the chemical compound ethinylestradiol (EE2) and the chemical compound diethylstilbestrol (DES), are of pharmaceutical production and animal agriculture and represent an even bigger environmental threat due to their persistence and their strong receptor binding (Kidd et al., 2007).

Since their entry into water courses, the estrogens have been discovered to be detectable in surface water, sediment, and groundwater in non-agricultural and agricultural areas (Colucci et al., 2001). Estrogen molecules have the attribute of showing bioactivity at very miniscule concentrations (ng/L levels), and it has been demonstrated to elicit undesired developmental and reproduction effects amongst water organisms. Kidd et al. (2007) demonstrated nearly extinction in fathead minnows that had been subjected to trace concentrations of EE2, and Jobling et al. (1995) reported intersex expressions amongst natural fish that had resided downstream of waste water waste treatment plants. This evidence has, thus, invited better regulation and scientific interest in the estrogens as an essential class of micropollutants. While partially removed via conventional activated sludge processes, residual estrogens frequently appear in wastewater effluents (Andersen et al., 2003). Furthermore, it has been revealed that the estrogens may reversibly absorb onto the matrix of the soil and sediment, undergo transformation via microbial processes, or survive under specific configurations of the redox and hydrology (Bonin & Simpson, 2007). These environmental properties significantly complicate their fate and transport and pose threat not only to the aquatic ecosystems but, via the contamination of drinking water, to the health of the humans too.

Because of their wide prevalence, stability, and strong biological activity, environmental estrogens, especially the estrone (E1), have attracted increasing interest. Given the need for new, economic, and environmentally friendly treatment technology, it is very significant to speak about estrogen contamination in water management processes of the world in the modern age.

1.2 Hazards and Importance of Estrone, E1

Among the vast number of environmental estrogens, there has been particular interest in the compound estrone (E1) due to its prevalence, durability, and endocrine-disruption capability. Despite being weakly bound at the receptor compared to 17 β -estradiol (E2), it is present in higher concentration present in treated effluents, owing to its release, making it a noteworthy contaminant of interest (Ankley et al., 2017).

E1 is commonplace in surface water, sediments, and aquifers due to agriculture, and it is an environmental indicator of its persistence and mobility (Colucci et al., 2001). Sorption is determined by the properties of the soils, namely, the clay and the organic carbon, and change the capture and redeposition under different hydrological regimes (Bonin & Simpson, 2007). This permits the possibilities of long-term, low-level exposures of the aquatic systems.

It is ecotoxicologically proven that exposure to E1 inhibits the endocrinology of reproduction among water organisms. Martinović et al. (2007) proved that E1 inhibits the level of the reproduction hormone and masculine behavior among fathead minnows (Pimephales promelas). Furthermore, Tapper et al. (2020) proved that E1 is transformed in water organisms and, subsequently, transformed back into estrogens of higher potency, including that of the estradiol (E2), which increases its endocrine activity.

And, besides, E1 tends to be the prevailing estrogenic compound in secondary effluents of sewage treatment plants, despite the various treatment undertaken (Andersen et al., 2003). Based on this, E1 has featured in the list ofpriority pollutants among the environmental protection agencies and scientists, and it has been important to research its fate, behavior, and removal in water ecosystems (Ankley et al., 2017).

1.3 Technologies of Present-Day Treatments and Gaps

To counteract the excretion of environmental estrogen, the series of remediation methods has been used, which include physical, chemical, and biological processes. Activated carbon adsorption is able to decrease the estrogen level, however, it is limited due to the limitation of adsorbent saturation and regeneration difficulty (Zhang et al., 2008). The chemical oxidative processes, that is, ozonation and advanced oxidative processes (AOPs), have experienced sufficient elimination effectiveness of steroidal estrogens such as E1, however, frequently produce by-products and are required to be strictly regulated during operation (Zhang et al., 2008). These biological treatment processes, namely activated sludge and membrane bioreactors, are used extensively in sewage treatment plants. E1, however, is most frequently encountered under hydraulic retention time or at lowered temperatures, in effluents (Andersen et al., 2003; Clara et al., 2005).

Additionally, estrogen removal via biological processes is very sensitive to microbial components and environmental factors like pH and dissolved oxygen, which render stable removal challenging (Andersen et al., 2003). While the traditionally used processes remained successful, the latter become inefficient during removal of E1 under environmentally relevant concentrations (Clara et al., 2005). Most of the work is based on controlled laboratory-scale systems, and limited work has assessed the efficiency of treatment under natural surface water with complex matrix systems (Schröder et al., 2010). It is quite limited work on hybrid systems of photodegradation and biological uptake processes. Despite the probable synergistic benefits, systematic investigations under natural illumination intensity and natural algal environments are still lacking (Schröder et al., 2010).

1.4 Integrative Potential of Photodegradation and Algal Technologies

Photodegradation and algae-based treatment are the green removal processes of estrogen compounds like estrone (E1) (Yang et al., 2016). The photodegradation under UV or simulated solar irradiation may cleave the aromatic structure of E1 and produce secondary products with rings cleaved and hydroxylated (Zhang et al., 2008). The photodegradation is, however, limited due to poor quantum efficiency, limited penetration of the light into natural lakes and seas, and the disturbance caused by dissolved organic components (Zhang et al., 2008; Schröder et al., 2010).

It was said that algae take in E1 via the activity of biosorption and metabolism under phototrophic growth. The algae systems could produce oxygen and provide shading, and hence, adjust water photochemical reactions (Schröder et al., 2010). While theoretical synergistic interactions between photodegradation and biological uptake have been hypothesized, however, very little research has investigated the former under natural illumination and algal community regimes, and that is indeed one real gap in the literature (Schröder et al., 2010).

1.5 Research Objectives

The paper describes an experiment that compared and evaluated the removal efficiency of the natural steroidal estrogen estrone (E1) under three environmentally plausible treatment regimes: sunlight exposure, ultraviolet irradiation, and algal treatment. Differing from the account of cumulative or synergistic processes, the work registers the separate impact of each process on the removal of E1.

There are three treatment groups, natural light exposure, UV light exposure, and algal treatment with Spirogyra, and their aim was to contrast their removal efficiencies of the compound estrone (E1).

Introduction

Samples under natural light group remained under the exposure of natural sunlight under laboratory windows. UV group remained under the biosafety cabin and treated with UV light with 254 nm wavelength. Algal group seeded with pre-cultured Spirogyra and remained under laboratory windows. All groups remained under individual operation.

The samples were taken at pre-set timings, and the UV–visible spectrophotometer quantified the concentrations of the E1 at 290 nm to assess the efficiency of the treatment under the individual setup. Also, further experiments were conducted to evaluate the distribution of the light, to evaluate short-term reproducibility when the pH was 7, to explore the effect played by different pH levels on the efficiency of removal, and to explore concentration-time curves when dealing with kinetic studies. All the other studies were directed towards acquiring mechanistic insights and validating the significance of the principal findings.

The specific objectives are:

(1) to determine the removal efficiency of E1 under natural sunlight exposure, UV irradiation, and algae treatment separately,

(2) to contrast the trends of temporal degradation and the efficiencies between the three treatment conditions,

(3) provide experimental evidence to guide the choosing of adequate E1 elimination methods in water sources,

(4) to evaluate further factors, including light distribution, short-term experiment, pH conditions, and kinetics, that may influence or explain the observed removal performance.

Chapter 2: Literature Review

Environmental estrogens, specifically estrone (E1), are becoming widely popular as micropollutants that are dangerous to human health as well as aquatic systems. To put this research into perspective, this chapter places under review the prevalence, sources, as well as environmentally relevant dynamics of oestrogens, whereby E1 is portrayed as the primary contaminant. An examination of state-of-the-art wastewater treatment technologies, all involving physical, chemical, as well as biological means, shows their efficiency as well as inadequacies. There is significant interest in photodegradation as well as algal-based treatment due to their promising, long-lasting nature that can potentially complement one another. This review incorporates the findings from most current studies as well as identifies gaps in knowledge, thereby providing a theoretical base as well as research justification for the experimental studies discussed in the subsequent chapters.

2.1 Estrogen's presence and role

2.1.1 Definition of Estrogen

2.1.1.1 Basic Definition

2.1.1.1.1 Endogenous and Environmental Estrogens

Horst (2010) highlighted estrogen is a class of steroid hormones that are the primary sex hormones in female. Estrogen, which is one of the most important hormones within a few biological processes, is a group of compounds essential to endocrine function. 17beta-estradiol is the most popular form of estrogen, which is produced by the ovaries and is essential for the maintenance of reproductive health. However, "estrogen" also encompasses environmental estrogens, or external compounds, that result in interference with normal hormonal function. Environmental estrogens, also referred to as endocrine disrupting chemicals (EDCs), replicate the activity of endogenous estrogens and can produce adverse health effects (Ropero et al., 2006).

2.1.1.1.2 Clinical Application and Associated Risks

Estrogen-based treatments have been extensively studied, especially hormone replacement therapy (HRT). Writing Group for the Women's Health Initiative Investigators et al. (2002) reported that findings from the Women's Health Initiative randomized controlled trial indicated that the risks of combined estrogen plus progestin therapy, as they pertained to coronary heart disease and breast cancer, exceeded the benefits for healthy postmenopausal women. Likewise, Hulley et al. (1998), in the Heart and Estrogen/progestin Replacement Study (HERS), concluded that estrogen plus progestin therapy did not reduce the overall rate of coronary heart disease events in postmenopausal women with existing coronary heart disease and was associated with increased thromboembolic events.

2.1.1.1.3 Broader Physiological and Carcinogenic Effects

The impacts of environmental estrogens are not limited to reproductive health alone but can affect several physiological processes and are even involved in causing diseases like uterine leiomyomas, in which growth factors and their corresponding receptors are controlled by ovarian hormones (Yu et al., 2010). Morgan et al. (2017), in their review, addressed the possible carcinogenic nature of endocrine-disrupting estrogen mimics like polychlorinated biphenyls (PCBs), phthalates, and bisphenol A (BPA), and their involvement in the initiation as well as growth of breast cancer. Complexity in estrogenic activity, such as the interaction among various compounds and their interference with endocrine function, calls for cautious risk assessments and regulatory measures for avoiding the impact of these compounds on human health (Endocrine Disruptors, 2019).

2.1.1.1.4 Molecular Mechanisms of Estrogen Receptor Interaction

Environmental estrogens are of specific concern since they are capable of binding to estrogen receptors (ERs), i.e., ERalpha and ERbeta. The binding activity triggers a cascade of biological reactions by functioning as transcription factors that interact with estrogen response elements (EREs) in the DNA structure. Classical estrogenicity analyses have mainly addressed this specific mechanism; however, emerging evidence indicates that the concept of estrogenicity requires a broader interpretation. It is now understood that environmental estrogens could activate several signalizing pathways and possibly interact with estrogen receptors found outside the nuclear compartment, including those found in the plasma membrane and mitochondria (Ropero et al., 2006). Subsequent research on environmental estrogens has explored their molecular interactions with estrogen receptors.

2.1.1.1.5 Importance of Estrone (E1) as a Priority Pollutant

Ankley et al. (2017) reexamined the environmental significance of estrone (E1) and concluded that despite its lesser receptor-binding affinity relative to estradiol, its prevalence in effluents renders it a priority pollutant. The health effects of environmental estrogens have been observed in fields like carcinogenesis and immune dysfunctions.

2.1.1.2 Natural Estrogen

2.1.1.2.1 Structural Characteristics and Physiological Roles

Estrogens have a wide range of biological effects, and some natural phytoestrogens can mimic some of the effects of endogenous estrogens (Gruber et al., 2002). Gruber et al. (2002) say that the naturally occurring estrogens 17β-estradiol (E2), estrone (E1), and estriol (E3) are C18 steroids that come from cholesterol (Figure 1). Estradiol (E2), estrone (E1), and estriol (E3) are the main endogenous steroid hormones, often known as natural estrogens. They are very important for reproductive and metabolic functions (Figure 2). Estradiol is the most powerful and active form, and it affects systemic functioning through both central and peripheral pathways (Zhu, Zhou, Jin, & Shu, 2023). Estradiol (E2) is the one that binds most strongly to estrogen receptors (ER). It is mostly found in the ovarian granulosa cells of women who have not yet reached menopause. Estrone (E1) and estriol (E3) are E2 metabolites, but they are much weaker than E2 (Barros & Gustafsson, 2011). Estrogens do not just pollute water; they also raise worries about the health of people and the ecosystem in general.

2.1.1.2.2 Environmental Occurrence and Removal Potential

Adeel et al. (2016) wrote a review that looked at what happens to estrogens in the environment, such as how they are released through wastewater, how they move about in different sections of an ecosystem, and how modern farming and waste management systems make these hazards worse. Their research says that we need to understand how estrogens are spread and changed in the environment to protect people, animals, and plants from the risks that come with them. Studies of the environment have shown that traditional ways of treating wastewater can get rid of natural estrogens in water. Andersen et al. (2003) said that municipal sewage treatment plants using activated sludge systems can get rid of more than 98% of natural estrogens, such as estrone (E1) and estradiol (E2).

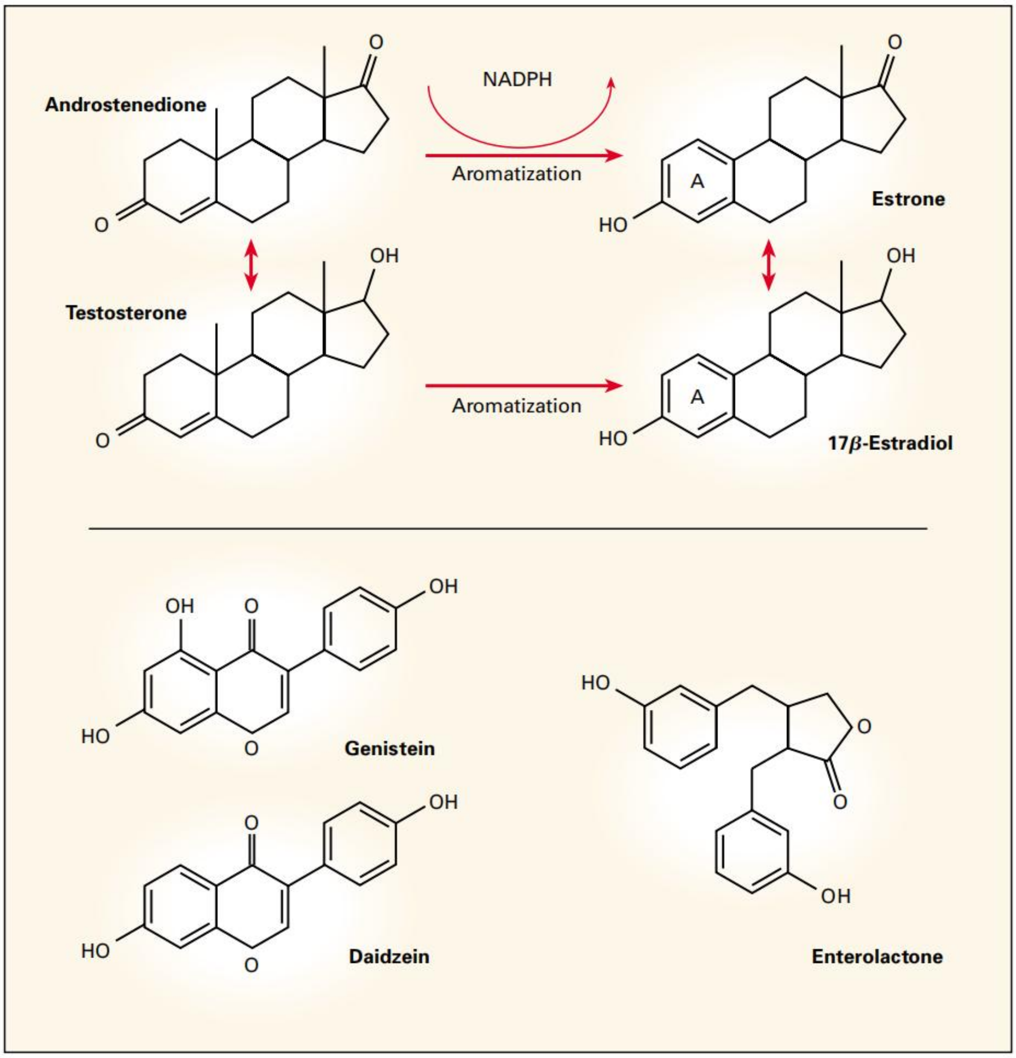


Figure 2.1. Structure and Production of Endogenous Estrogens and Structure of Phytoestrogens (Gruber et al., 2002).

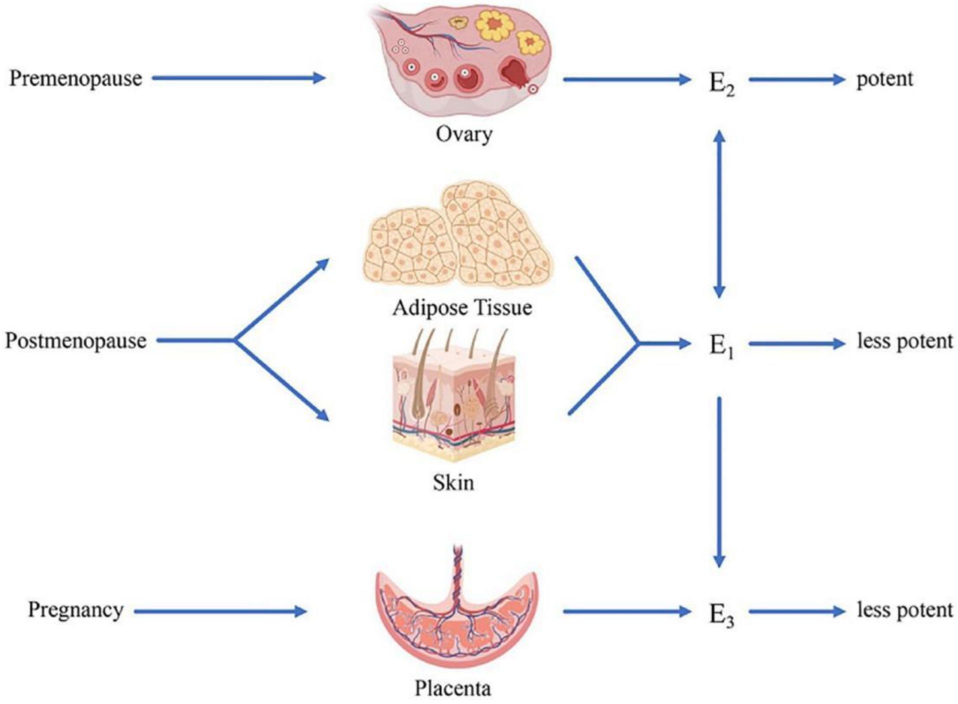


Figure 2.2. Schematic representation of biosynthesis of estradiol (E2), estriol (E3), and estrone (E1) (Zhu, Zhou, Jin, & Shu, 2023).

2.1.1.3 Synthetic Estrogen

2.1.1.3.1 Development and Structural Characteristics of Synthetic Estrogens

Santen and Simpson (2019) talked about how the nonsteroidal estrogens, such diethylstilbestrol (DES), came to be. DES was originally used in the late 1930s. Sato et al. (1987) showed that synthetic estrogens can stop microtubule polymerization, which leads to the creation of an unusual ribbon-like shape. This suggests that the way these molecules are arranged can affect how they work in the body. This structural feature might be linked to their ability to connect with parts of cells in ways other than the usual receptor contact. Ma et al. (1998) showed that exposure to synthetic estrogens like diethylstilbestrol (DES) during pregnancy can cause reproductive organs to develop abnormally. This shows how synthetic estrogens can affect reproductive development.

2.1.1.3.2 Receptor Binding and Biological Behavior

Fang et al. (2001) looked at a wide range of natural, synthetic, and environmental estrogens and found that they have comparable structures that affect how well they bind to estrogen receptors. Zhu et al. (2006) also looked at how different natural and synthetic estrogens attach to the ER α and β isoforms. They found that numerous metabolites had varied binding preferences, which could affect how they work in the body.

2.1.1.3.3 Environmental Persistence and Ecotoxicological Risk

Andersen et al. (2003) said that municipal wastewater treatment systems can remove synthetic estrogens such estrone, estradiol, and ethinylestradiol, but there are still traces of them in the effluents. Kidd et al. (2007) showed that even very small amounts of synthetic estrogens can do terrible damage to aquatic life. Researchers have found that exposing fish to synthetic estrogen can have detrimental effects, such as making male fathead minnows more feminine, which has led to the species near extinction.

2.1.1.4 Environmental Estrogens

2.1.1.4.1 Endocrine Disruption Potential

Environmental estrogens are known for their ability to mess with the endocrine system and create a wide range of health problems.

2.1.1.4.2 Sources and Estrogenic Activity

Jobling et al. (1995) showed that many substances in the environment, like long-lasting pollutants and plasticizers, have mild estrogenic action. Nimrod and Benson (1996) said that alkylphenol ethoxylates have been shown to have estrogenic action, which raises concerns about their effects on wildlife and human health.

2.1.1.4.3 Combined Effects and Risk Amplification

Arnold et al. (1996) investigated the possibility of synergistic or combinatorial effects of mixtures of chemicals and found that the interactions of environmental chemicals stimulated estrogen receptors more strongly than the chemicals alone. This suggests that there are either additive or synergistic risks.

2.1.2 Role of Estrogen

2.1.2.1 Role in Reproductive System

Kayo et al. (2019) stressed how crucial estrogen receptor β1 (Esr2a) is for female reproduction. It has also been established that estrogen is important for keeping women fertile. Yu et al. (2024) investigated how estrogen signaling works in the hypothalamus and find that it is very important for keeping prolactin levels stable and for female reproductive function. Their study shows how complicated and important the estrogen pathway is for reproductive physiology. The study by Wan et al. (2023) showed how estrogen affects hormonal balance and the ability of the uterus to accept an embryo. This shows how important estrogen is for female fertility.

2.1.2.2 Role in other biological processes

Aubead (2021) expanded on this by researching the extensive function of sex hormones, that specifically estrogen, in containing optimal physiological processes in the human body and describing their crucial significance to the entire system. Rehman et al. (2005) studied the influence of estrogen on cognitive processes, including verbal memory, abstract thinking, and information processing. This indicates that estrogen has effects that extend beyond reproductive physiology. Horst (2010) considered the presence of estrogen receptors within the limbic system, the part of the brain that controls mood and emotion and indicated that estrogen influences women's responses to stress and also their emotional responses, which also linked hormonal activity with women's mental health and stress problems. Control of the menstrual cycle, or estrus, is just one of many roles carried out by estrogen. It also manages bone development, the functioning of cardiovascular and vascular systems, and cognitive function, among many others.

2.1.3 Sources of Estrogen

Shore and Shemesh (2003) affirm that the primary biogenic source of natural estrogens present in the environment is through urinary and fecal excretion by humans and animals. Khanal et al. (2006) also point out that in agricultural systems, livestock production—especially concentrated animal feeding operations (CAFOs)—produce enormous amounts of manure that is rich in estrogens.

Khanal et al. (2006) provide a critical examination of the biological processes of natural estrogens, which are mostly plant- and animal-derived, and performed detailed work on their behavior, mobility, and biodegradation in environmental systems, and it is known that these natural estrogens, such as estradiol, estrone, and estriol, are endogenously synthesized by plants and animals and that their occurrence in environmental systems is usually linked with biological activities and natural biological cycles.

Khanal et al. (2006) emphasized the paramount importance of monitoring estrogenic compounds in diverse environmental matrices across nations, with the point raised that these compounds may either be biodegradable or bioaccumulative and hence impact ecosystems and possibly wildlife and human health as well.

Klein et al.'s (2003) work illustrates that some herbs, like red clover and soy, are rich in estrogenic bioactivity, which is quantifiable through the application of bioassays.

2.1.4 Environmental Behavior and Effects of Estrogen E1

2.1.4.1 Environmental Behavior of E1

Colucci, Bork, and Topp (2001) indicated that estrone (E1), a major naturally occurring estrogen released by humans and animals, has been ubiquitous in surface water, sediments, and soils in the vicinity of agricultural and urban regions.

Arnon et al. (2008) discovered that estrogens, such as E1, were transferred from waste lagoons of dairy farms to aquifers below, raising concerns about the potential groundwater contamination.

In the same manner, Johnson et al. (2006) recapitulated the steroid hormone inputs to farm animals, underlining their significant presence in aquatic environments.

Bonin and Simpson (2007) investigated adsorption of steroid estrogens onto soil constituents and noted that E1 adsorbs differently according to organic matter and clay content.

Bowman, Zhou, and Readman (2002) examined sediment – water interactions and established that E1 reversibly adsorbs sediments with desorption being regulated by salinity and particulate matter.

2.1.4.2 Environmental Effects of E1

Martinovic et al. (2007) discovered that exposure to E1 repressed levels of reproductive hormones and changed the behavior of male fathead minnows.

Tapper et al. (2020) reported that E1 can be transformed into E2 by aquatic marine organisms, thereby amplifying its endocrine effect.

In an estuarine monitoring context, Noppe et al. (2007) reported the frequent detection of E1, again highlighting its position as a widespread and important contaminant in aquatic environments.

Sarmah, Northcott, and Scherr (2008) investigated the adsorption capacity of estrone (E1) in various New Zealand soils and reported that E1 was extensively retained in several soils, particularly in high organic matter and clay-containing soils. The adsorption process might greatly restrict the short-term mobility of E1; however, it may also lead to possible accumulation in the soil with subsequent irregular release under certain hydrological conditions. This ongoing release can continue to impact the ecosystem of nearby aquatic systems, thus emphasizing the role of soil texture in controlling migration pathways and evaluating the risks of estrogen pollution.

2.2 Estrogen Removal Technology in Wastewater Treatment

2.2.1 Physical Treatment

2.2.1.1 Membrane Filtration and Adsorption

Nasrollahi et al. (2022) point out the significance of membrane technologies for the removal of antibiotics that tend to co-occur with estrogens and stress the prospects of membrane systems — especially when combined with other strategies such as adsorption or advanced oxidation.

Rowsell et al. (2009) made a comparative study on the effectiveness of virgin and regenerated granular activated carbon (GAC) in the removal of steroidal estrogens from municipal wastewater, which showed that the regenerated carbon (C401) had better performance (up to 81%) in removing compounds such as 17 β -estradiol (E2) and estrone (E1), compared to the virgin carbon (F400), which had about 65% effectiveness.

Ifelebuegu et al. (2015) posted an extensive investigation on various biosorbents, like black tea waste, and establish its efficiency in the removal of E2 and EE2, that are comparable to that of commercially utilized activated carbon, thereby presenting it as a viable and environmentally friendly substitute.

2.2.1.2 UV-Based Photodegradation

Huang et al. (2022) examined photodegradation of four common estrogens (E1, E2, E3, and EE2) using low-pressure UV light at 253.7 nm, and they found that the fastest degradation was noted with E1 due to its higher molar absorptivity and quantum yield. Furthermore, continuous-flow operation enhanced degradation over batch mode, because of higher generation of reactive oxygen species (ROS) in oxygen-rich media such as lake water.

In a study of the effects of UV treatment on antibiotic pollutants, Avisar et al. (2010) found alkaline conditions that can improve the efficiency of photolytic degradation due to enhanced ionization and reactivity of target analytes. Although this work was primarily concerned with antibiotics, the principles can be extended to estrogens, which also undergo pH-dependent speciation.

Gabet-Giraud et al. (2010) compared estrogen elimination in 14 full-scale wastewater treatment plants throughout France. UV alone was found to result in moderate removal efficiencies (30 – 60%) of E2 and EE2, depending on operational intensity and water quality.

Köhler et al. (2012) confirmed the efficient applicability of advanced UV systems for biologically pretreated hospital wastewater. Through comparison, they proved significant reductions in pharmaceutical residues, including substances of estrogenic activity.

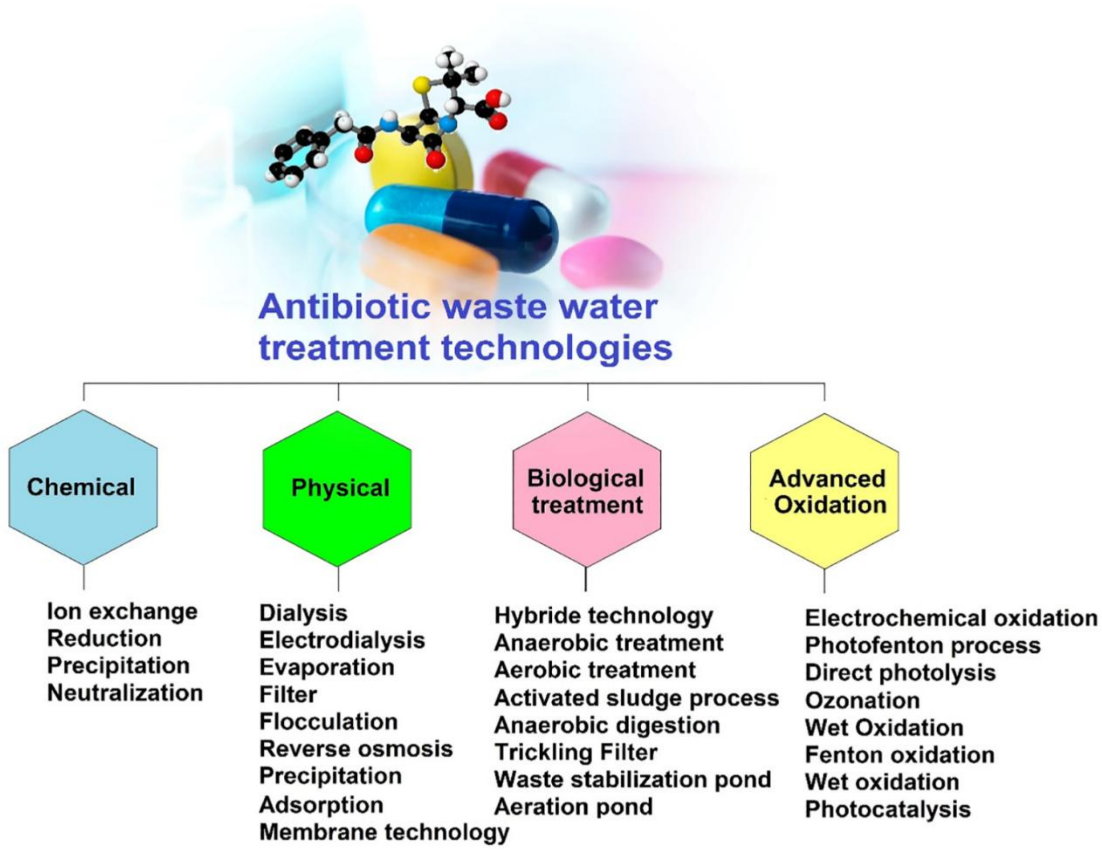


Figure 2.3. Common techniques for antibiotics removal from wastewater (Nasrollahi et al. 2022)

2.2.1.3 Advanced Oxidation Processes (AOPs)

Madsen and Søgaard (2012) demonstrated that the coupling of UV with TiO_2 photocatalysis significantly improved the photodegradation of 17α-ethinylestradiol in municipal wastewater. This study demonstrates the synergistic effect of UV and catalytic surfaces on degrading recalcitrant estrogens.

Moreira et al. (2022) examined UV/H_2O_2 and reverse osmosis for treating EE2 and BPA. Even though UV/H_2O_2 is an advanced oxidation process (AOP), UV remains the central activator of oxidative degradation, which further increases its importance in EDC removal.

2.2.2 Chemical Treatment

2.2.2.1 Mechanism of Chemical Oxidation

Oxidation reactions involving strong oxidants like ozone and chlorine have been shown to degrade endocrine-disrupting chemicals (EDCs), including estrogens, in diverse wastewater matrices. Such technologies are inclined to target electron-rich structural groups, such as phenolic groups and aromatic rings, to achieve molecular degradation or mineralization (Hua et al., 2006; Deblonde et al., 2011; Bertanza et al., 2010).

2.2.2.2 Effectiveness of Ozonation

Bertanza et al. (2010) reported a study on the use of advanced oxidation processes (AOPs), as showed in Figure 4, such as ozonation, for the elimination of endocrine-disrupting chemicals (EDCs) from wastewater treatment plant effluents, and the investigation revealed that single ozonation (without UV or catalysts) was capable of removing over 90% of compounds such as nonylphenol (NP) and bisphenol A (BPA), which contain structural similarities with estrogens, and removal efficiency was found to be enhanced by greater ozone dosage and increased contact time.

2.2.2.3 Comparison with Chlorination

Hua, Bennett, and Letcher (2006) investigated the removal of some pharmaceuticals and the herbicide atrazine from drinking water through ozone treatment. Although this study was with pharmaceuticals, the findings point to the relative reactivity of ozone compared to chlorine. Ozone was much more effective than chlorine in the elimination of detectable quantities of the test compounds. These findings indirectly confirm ozone's superiority over chlorine when it comes to the oxidation of estrogen-like structures.

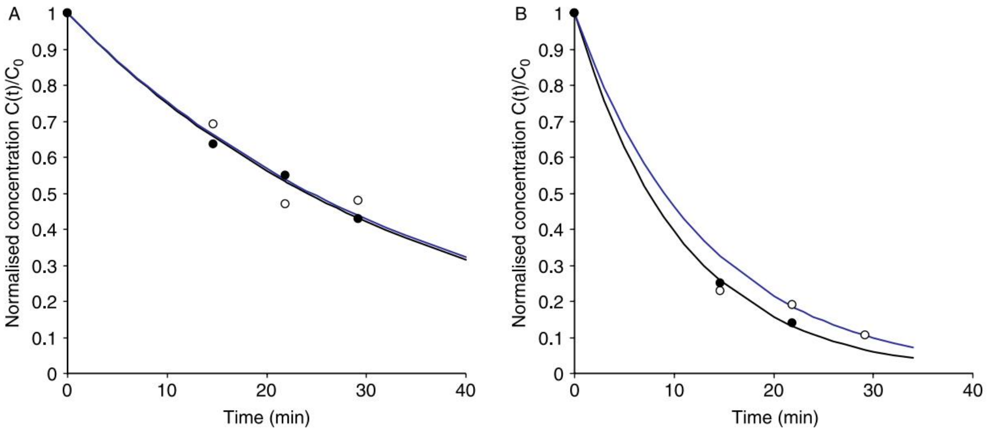


Figure 2.4. Ozonation: Target pollutants – normalised concentration vs. time profile. (Bertanza et al. 2010)

2.2.2.4 Potential By-products and Challenges

Deblonde, Cossu-Leguille, and Hartemann (2011) talked about the treatment of emerging pollutants in wastewater and noted that chemical oxidation is efficient but is associated with the potential to form toxic by-products. Chlorination, for example, may form trihalomethanes (THMs), and ozonation in waters containing bromide may form bromates. These issues point to the necessity of process optimization and post-treatment monitoring.

2.2.2.5 Residual Estrogens and Detection

Schröder, Gebhardt, and Thevis (2010) used high-resolution liquid chromatography-tandem mass spectrometry (LC-MS/MS) to identify trace quantities of anabolic steroids and estrogens in wastewater and surface water samples, both before and after treatment. Their results showed that residual estrogens were detectable at low nanogram per liter (ng/L) levels even after treatment procedures, that also emphasizes the requirement for very sensitive analytical methods and the potential necessity for more polishing treatments for completely remove estrogenic activity.

2.2.3 Biological treatment

2.2.3.1 Performance of Activated Sludge and Membrane Systems

Clara et al. (2005) compared the removal efficiency of membrane bioreactor systems and conventional activated sludge systems for the removal of micropollutants and observed membrane bioreactor systems to exhibit greater removal efficiencies for estrogens when operated at extended sludge retention times.

Joss et al. (2004) found out that activated sludge systems achieved removal efficiencies greater than 90% for 17β-estradiol (E2) under aerobic conditions. However, the efficiency in synthetic estrogens such as ethinylestradiol (EE2) is relatively low and may require the application of additional technologies.

2.2.3.2 Use of Fungi in Estrogen Degradation

Shreve et al. (2016) illustrated that fungi such as white-rot fungi Trametes versicolor can produce laccases to break down different estrogens in wastewater with up to 98% removal. But despite its effectiveness, its actual use is hampered by the difficulty in cultivation conditions.

2.2.3.3 Algal Degradation Capabilities

Liu et al. (2018), in a study, demonstrated that freshwater green alga Raphidocelis subcapitata is capable of efficiently removing natural estrogen 17β-estradiol (E2) and synthetic estrogen diethylstilbestrol (DES). Under axenic conditions, within 96 hours,

R. subcapitata removed 74.6%–89.9% of E2 and 54.1%–80.4% of DES predominantly by biodegradation rather than simple adsorption and further revealed that metabolites were detected within the algal cells, thereby establishing the fact of active biodegradation.

2.2.3.4 Microalgae–Bacteria Synergistic Systems

Nagarajan et al. (2022) presented a comprehensive review about the advantages of microalgae–bacteria consortia for the treatment of estrogen-contaminated wastewater. In these kinds of systems, microalgae supply oxygen through photosynthesis, and bacteria degrade organic pollutants, forming synergistic metabolic interactions that enhance treatment efficiency overall.

2.3. Application of Photodegradation in E1 Removal from Wastewater

2.3.1 Basic Principles of Photodegradation

Hassaan et al. (2023) confirmed that photocatalysis is the transfer of an electron from the valence band to the conduction band, which generated electron-hole pairs enabling the oxidation-reduction reactions, and they emphasized the significance of photocatalysis in environmental remediation owing to its potential to degrade a broad array of recalcitrant organic pollutants.

Farooq et al. (2023) investigated the potential of hexagonal copper selenide (CuSe) nanoflakes for the degradation of estrone (E1) under visible light. The results of their research indicate that CuSe facilitates electron-hole pair generation under visible light. These couples respond with water and dissolved oxygen to yield reactive oxygen species (ROS), including hydroxyl radicals (•OH) and superoxide anions ($O_2^{\bullet -}$). ROS attacks the aromatic moiety of E1 to degrade through hydroxylation and subsequent ring-opening oxidation. The degradation was pseudo-first order in kinetics and was strongly parameter-dependent on pH, catalyst amount used, and the extent of illumination.

Liu et al. (2024) acknowledged that environmental parameters such as salinity, light intensity, and pH exert pronounced influences on degradation processes.

2.3.2 Selection and Application of Catalysts in E1 Photodegradation

2.3.2.1 Activated Carbon-Doped TiO_2 Catalysts

Pastre et al. (2024) synthesized a TiO_2 photocatalyst that was doped with activated carbon (AC) to degrade E1, E2, EE2, and levonorgestrel. The photocatalyst performance of the system was enhanced due to the extensive surface area of AC, which facilitated the trapping of impurities and electrons. Upon optimal conditions, over 95% of E1 was degraded, causing a significant reduction in overall estrogenicity.

2.3.2.2 Gold Nanoparticle-Modified TiO_2 Catalysts

Sornalingam et al. (2018) explored the prospects of Au–TiO_2 nanocomposites in degrading E1. The addition of gold nanoparticles enabled the absorption of visible light via surface plasmon resonance. The efficiency of E1 removal was achieved at a level up to 87%, which was higher than that of pure TiO_2. In addition, it was observed that the generation of intermediate by-products was less likely to cause appreciable toxicity.

Sornalingam et al. (2018) established that scanning electron microscopy images revealed the presence of spherical gold nanoparticles deposited across the surface of spherical titanium dioxide nanoparticles (Figure 5a-c). Figure 5d also shows the energy-dispersive spectroscopy spectrum of 8 wt% Au-TiO_2. The analysis showed the presence of titanium, oxygen, and gold but not chloride ions, which were utilized in the gold precursor solution. These ions may inhibit photodegradation by blocking the surface or quenching hydroxyl radicals (Katz et al., 2015).

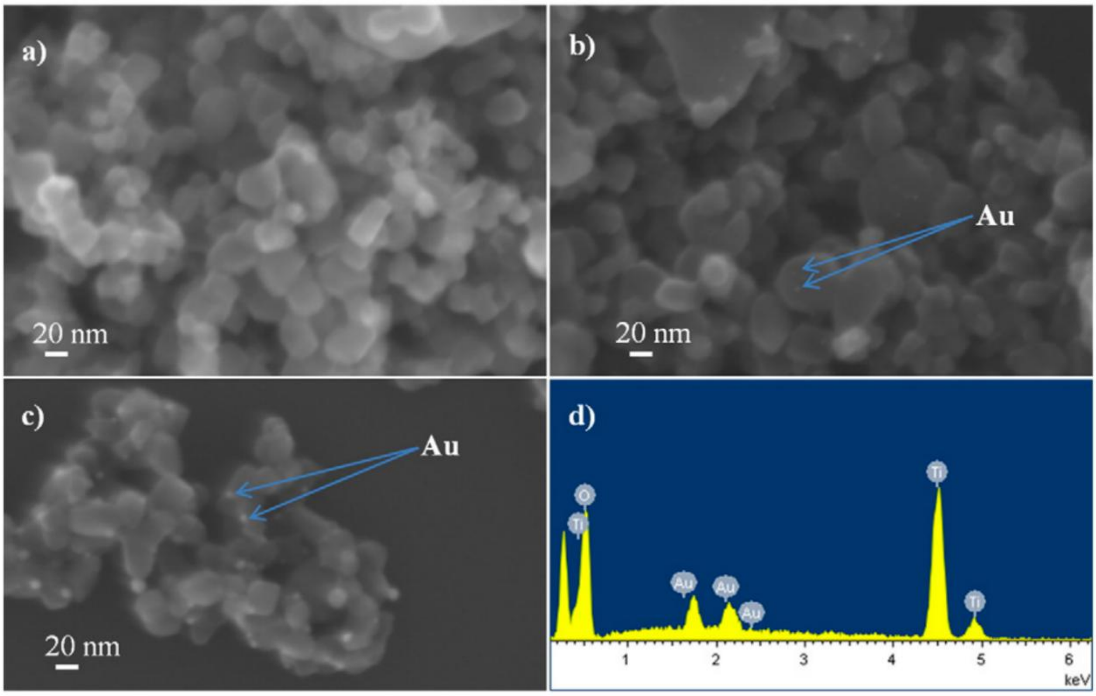


Figure 2.5. SEM images of a) P25 TiO2, b) 2 wt% Au-TiO2 and c) 8 wt% Au-TiO2 and d) EDS of 8 wt% Au-TiO2. (Sornalingam et al. 2018)

2.3.2.3 Copper Selenide (CuSe) Nanoflakes

To enhance degradation of E1 under visible light, Farooq et al. (2023) synthesized hexagonal CuSe nanoflakes. The material had a narrow bandgap (1.4 – 1.8 eV), was an extremely effective carrier-separation material for photogenerated carriers and was stable through multiple reuse cycles. It accounted for the degradation of more than 93% of E1.

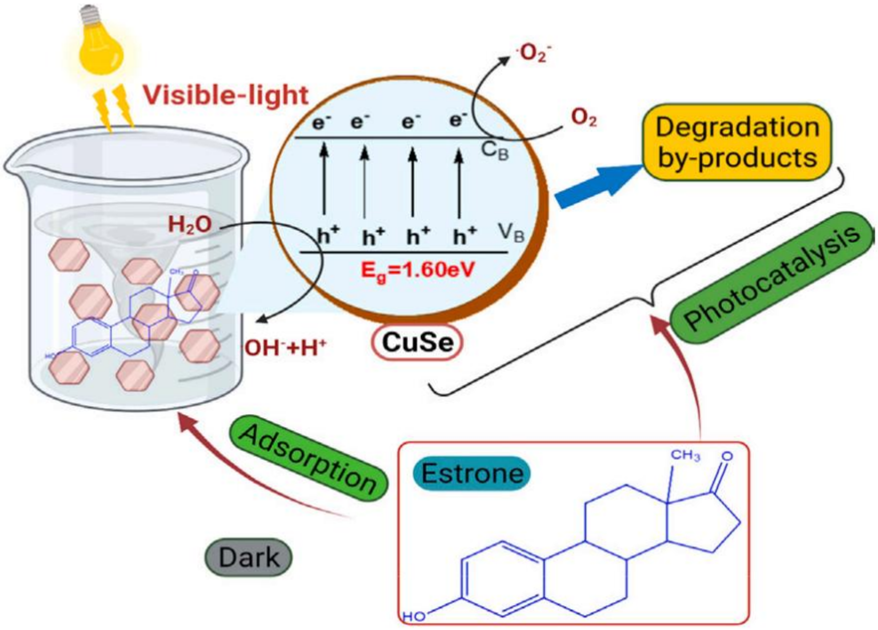


Figure 2.6. Schematic-layout of processes taking place at the interface of CuSe nanoflakes and E1 in the solution in the absence/presence of light irradiation. (Farooq et al. 2023)

2.3.2.4 Catalyst Performance Factors and Material Considerations

Wang et al. (2020) focused on photocatalytic EDC degradation, considering catalysts, water chemistry, and performance mechanisms. In the case of phenolic EDCs like E1, oxygen vacancies in the semiconductor and surface acidity are controlling factors. Zatloukalová et al. (2017) studied immobilized TiO_2 photocatalysts on ceramic and glass fiber supports for EDC removal. While not quite as efficient as suspended systems, the catalysts were more stable and reusable, making them suitable for practical applications.

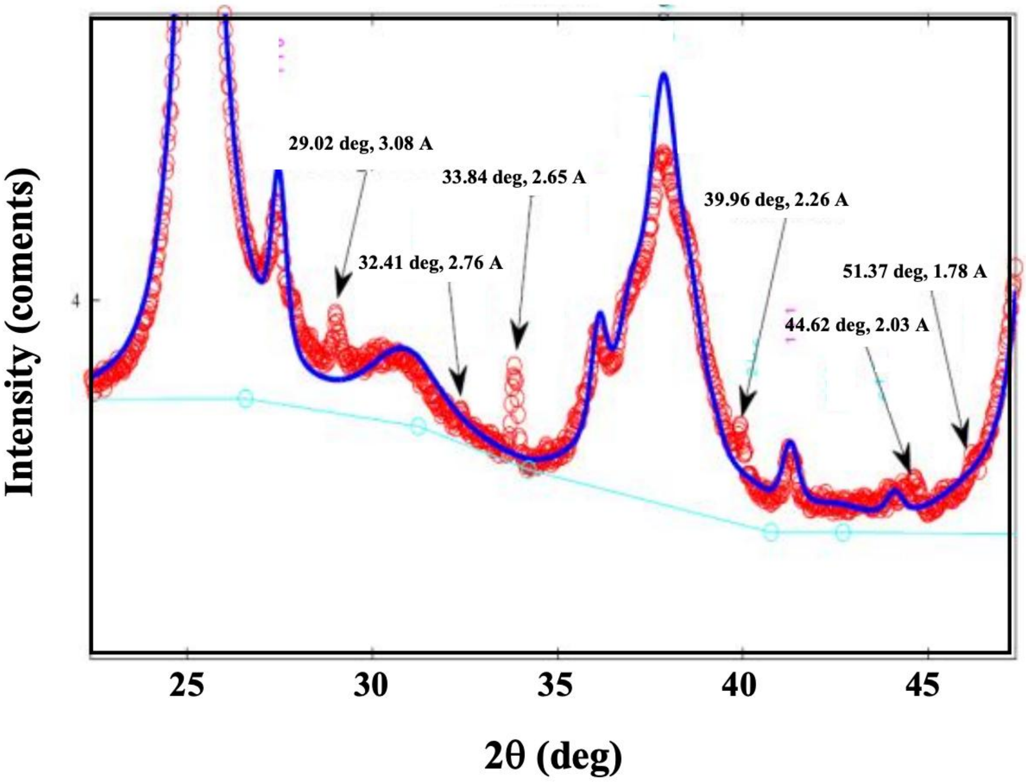


Figure 2.7. XRD patterns of NnF Ceram TiO2 catalyst. (Zatloukalová et al. 2017)

2.3.3 Status and Challenges of Photodegradation

2.3.3.1 Mechanisms and Laboratory Effectiveness

Photodegradation is a promising technique for E1 elimination via direct or catalyst-facilitated breakdown of its molecular framework upon exposure to simulated or natural light (Zhang et al., 2007). It entails direct photolysis and indirect oxidation via reactive oxygen species (e.g., ·OH, O_2 ·⁻) generated from photocatalysts like TiO_2 (Han et al., 2012; Sornalingam et al., 2016). Zhang et al. (2007) established that E1 achieved 98% degradation in 16 minutes under 15 W UV lamps and that slightly alkaline to neutral pH (pH ≈ 7–8) favors photodegradation.

2.3.3.2 Catalyst Comparison and Performance

Han et al. (2012) compared ZnO and P25 TiO_2 under UVA and sunlight and determined that ZnO degraded E1 faster under the same conditions because of better electron–hole separation. Sornalingam et al. (2016) added that although UV photodegradation is effective, it takes tens of days for E1 half-life in natural waters, and most experiments are under optimum conditions with minimal real-world validation.

2.3.3.3 Application under Visible Light

To enhance the utilization of natural (visible) light, Al-Hajji et al. (2021) prepared a 0.1 wt% Au–TiO_2 photocatalyst that completely degraded various estrogens like E1 within 75 minutes under visible light.

2.3.3.4 Environmental Factors and Real-Water Constraints

The presence of DOM, organic/inorganic ions, and suspended solids in wastewater significantly reduces light penetration and radical generation, decreasing degradation efficiency compared to laboratory conditions (Han et al., 2012; Sornalingam et al., 2016). In realistic wastewater scenarios, the existence of dissolved organic matter (DOM), organic and inorganic ions, and suspended solids greatly hinders transmission of light and the production of radicals, then lowering degradation efficiency relative to laboratory conditions (Han et al., 2012; Sornalingam et al., 2016).

2.3.3.5 Toxic By-products and Risk

Furthermore, the process frequently produces degradation by-products such as lumiestrone, which display estrogenic activity or toxicity, and the complete toxicological implication of such by-products is yet to be intensively studied (Al-Hajji et al., 2021; Zhang et al., 2007).

2.3.3.6 Lack of Long-Term Evaluation

Sornalingam et al. (2016) said that current studies mainly focus on short-term performance with a significant lack of investigations related to long-term stability, catalyst deactivation, and reusability potential.

In summary, although photodegradation exhibits significant removal efficiency for E1 under controlled laboratory conditions, there are major challenges related to visible light responsiveness, applicability to real water matrices, ecotoxicity of byproducts, cost control, and engineering application.

2.4. Application of High-rate filamentous algal ponds (HRFAP) in E1 Removal from Wastewater

2.4.1 Adsorption and Degradation Mechanism of HRFAP

2.4.1.1 Contribution of Adsorption and Biodegradation

Microalgae photosynthesis in high-rate filamentous algal pond (HRFAP) systems creates a lot of dissolved oxygen and sets up algae-bacteria symbiosis to enable estrogens like E1 to stick to and break down (Liyanage et al., 2024). Liyanage et al. (2024) also observed that algal adsorption makes up around 30 – 35% of HRFAP, while microbial degradation takes away the rest. This means that the overall efficiency is between 85% and 92%.

2.4.1.2 Role of Microalgal Species and EPS

Prosenc et al. (2021) found that the extracellular polymeric substances (EPS) help E1 stick to surfaces and minerals oxidize when they are under a bacterial culture of Chlorella vulgaris. Ruksrithong and Phattarapattamawong (2019) said that Scenedesmus obliquus absorbs around 25% of E1 in 60 minutes and subsequently breaks down according to a pseudo-second-order kinetic model. Wang et al. (2019) documented that four microalgae, including Haematococcus pluvialis and Scenedesmus obliquus, can degrade E1, E2, and EE2 and produce hydroxylated and ring-cleavage intermediates.

2.4.1.3 Synergistic Environmental Conditions

Liyanage et al. (2024) also emphasized that diel pH (6.8 – 9.2) and DO (3 – 8 mg/L) variations in HRFAP construct a synergistic condition for photochemical and biological degradation, enhancing removal efficiency. HRFAP efficiently eliminates E1 through a three-tier synergistic mechanism of quick adsorption, algae – bacteria co-metabolism, and photobiological mineralization.

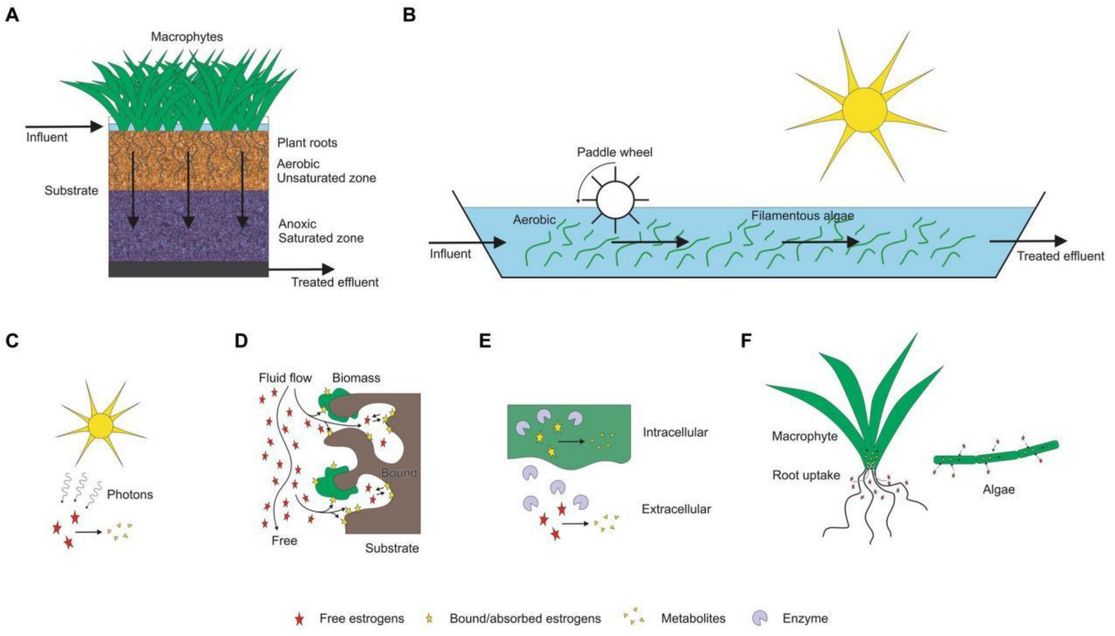


Figure 2.8. Estrogen removal mechanisms associated with TWs and HRAPs. (A) Treatment wetland (TW; B) HRAP, (C) Photodegradation, (D) Adsorption, (E) Biodegradation, (F) Plant uptake and bioadsorption. (Liyanage et al. 2024)

2.4.2 Optimization of HRFAP system

2.4.2.1 Operational Parameters: Light Cycles and Retention Time

Liyanage et al. (2024) found that diel light–dark cycles effectively control pH and DO fluctuations in HRFAP, twin-well promoting photosynthesis and biodegradation. The optimum light-dark ratio of approximately 12 h/12 h achieved approximately 90% estrogen removal. Ruksrithong and Phattarapattamawong (2019) found that the optimal hydraulic retention time (HRT) of 2–4 days enables the removal of E1 to be maximized in HRFAP, in which degradation kinetics follows a pseudo-second-order model, thus highlighting the need for adequate contact and transformation.

2.4.2.2 Microbial Community Balance and Synergy

Prosenc et al. (2021) found that setting the algal-to-bacterial ratio to approximately 3:1 promotes extracellular polymeric substance (EPS) production, vastly stimulating E1 adsorption and biological mineralization. Wang et al. (2019) found Scenedesmus obliquus to have higher ability in ring-cleavage, whereas Haematococcus pluvialis to have higher capacity in hydroxylation, co-culturing of which could bring synergistic degradation pathways.

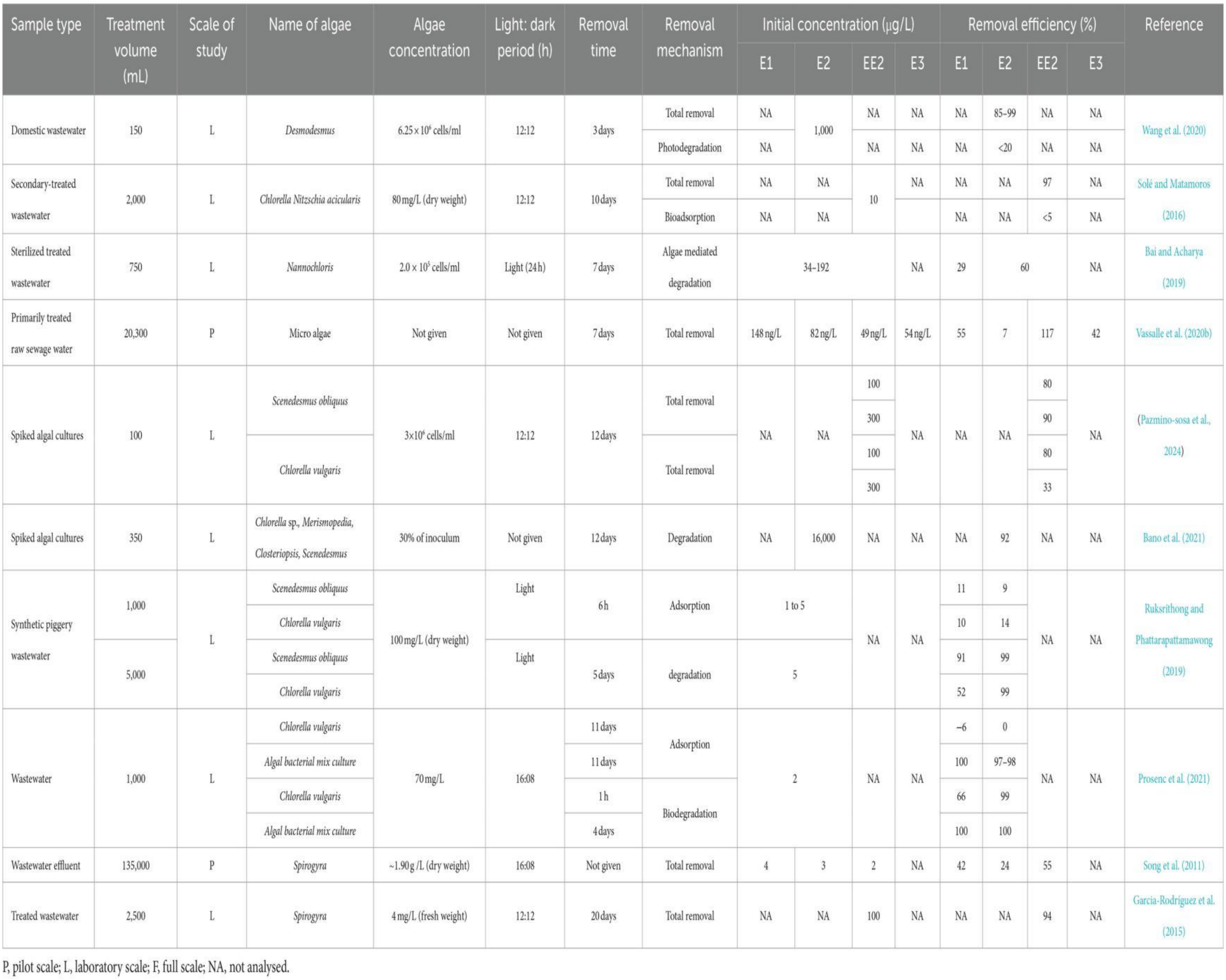
2.4.3 HRFAP Design and Practical Application

2.4.3.1 Pond Configuration and System Layout

Effective utilization of high-rate filamentous algal ponds (HRFAPs) is dependent on a reasonable structural design, practicable operational modes, and effective biomass harvesting methods. Some of the essential factors are pond configuration, mode of operation, and harvesting method (Liyanage et al., 2024). HRFAPs generally consist of shallow raceway-like ponds with depths ranging from 0.15 to 0.25 meters, which enable sufficient light penetration and minimize anaerobic zones (Liyanage et al., 2024).

2.4.3.2 Algal Properties and Operational Resilience

It was discovered by Trochine et al. (2010) that filamentous green algae, mainly Cladophora spp., possess excellent adhesive qualities and increased biomass growth under high temperature in nutrient-rich conditions, in situations where the efficiency of fixed-bed systems is increased. Prosenc et al. (2021) further demonstrated that algae–bacterial co-cultures are also more resistant to environmental variation and could be applied in outdoor high-rate algal pond (HRFAP) systems. Filamentous algae are easier to separate than suspended microalgae systems. Wang et al. (2019) stated that Scenedesmus obliquus has inherent settling capacity which facilitates the dewatering process and minimizes energy requirements.



Sample type	Treatment volume (mL)	Scale of study	Name of algae	Algae concentration	Light: dark period (h)	Removal time	Removal mechanism	Initial concentration (μg/L)				Removal efficiency (%)				Reference
								E1	E2	EE2	E3	E1	E2	EE2	E3	
Domestic wastewater	150	L	*Desmodesmus*	6.25×10^6 cells/ml	12:12	3 days	Total removal	NA	1,000	NA	NA	NA	85–99	NA	NA	Wang et al. (2020)
							Photodegradation	NA		NA	NA	NA	<20	NA	NA	
Secondary-treated wastewater	2,000	L	*Chlorella Nitzschia acicularis*	80 mg/L (dry weight)	12:12	10 days	Total removal	NA	NA	10	NA	NA	NA	97	NA	Solé and Matamoros (2016)
							Bioadsorption	NA	NA			NA	NA	<5	NA	
Sterilized treated wastewater	750	L	*Nannochloris*	2.0×10^5 cells/ml	Light (24 h)	7 days	Algae mediated degradation	34–192			NA	29	60		NA	Bai and Acharya (2019)
Primarily treated raw sewage water	20,300	P	Micro algae	Not given	Not given	7 days	Total removal	148 ng/L	82 ng/L	49 ng/L	54 ng/L	55	7	117	42	Vassalle et al. (2020b)
Spiked algal cultures	100	L	*Scenedesmus obliquus*	3×10^6 cells/ml	12:12	12 days	Total removal	NA	NA	100	NA	NA	NA	80	NA	(Pazmino-sosa et al., 2024)
										300				90		
			Chlorella vulgaris				Total removal			100				80		
										300				33		
Spiked algal cultures	350	L	*Chlorella* sp., *Merismopedia*, *Closteriopsis*, *Scenedesmus*	30% of inoculum	Not given	12 days	Degradation	NA	16,000	NA	NA	NA	92	NA	NA	Bano et al. (2021)
Synthetic piggery wastewater	1,000	L	*Scenedesmus obliquus*	100 mg/L (dry weight)	Light	6 h	Adsorption	1 to 5		NA	NA	11	9	NA	NA	Ruksrithong and Phattarapattamawong (2019)
			Chlorella vulgaris									10	14			
	5,000		*Scenedesmus obliquus*		Light	5 days	degradation	5				91	99			
			Chlorella vulgaris									52	99			
Wastewater	1,000	L	*Chlorella vulgaris*	70 mg/L	16:08	11 days	Adsorption	2		NA	NA	−6	0	NA	NA	Prosenc et al. (2021)
			Algal bacterial mix culture			11 days						100	97–98			
			Chlorella vulgaris			1 h	Biodegradation					66	99			
			Algal bacterial mix culture			4 days						100	100			
Wastewater effluent	135,000	P	*Spirogyra*	~1.90 g /L (dry weight)	16:08	Not given	Total removal	4	3	2	NA	42	24	55	NA	Song et al. (2011)
Treated wastewater	2,500	L	*Spirogyra*	4 mg/L (fresh weight)	12:12	20 days	Total removal	NA	NA	100	NA	NA	NA	94	NA	Garcia-Rodríguez et al. (2015)

P, pilot scale; L, laboratory scale; F, full scale; NA, not analysed.

Table 2.1. Estrogen removal efficiency of freshwater algae. (Liyanage et al. 2024)

2.4.3.3 Practical Applications and Limitations

Liyanage et al. (2024) highlighted the feasibility of HRFAP for surface and rural wastewater treatment while, meanwhile, suggesting drawbacks like light efficiency, high land requirements, and stability of algal strains.

2.5. Potential Integration of Photodegradation and HRFAP

2.5.1 Theoretical foundations of the integrated treatment

Filamentous algae and their symbiotic bacteria in HRFAP systems biodegrade pollutants via adsorption, biotransformation, and enzymatic degradation; coupled with free radicals from photocatalysis ($\cdot OH$, $\cdot O_2^{-}$), it forms a synergistic "adsorption–photochemical oxidation–biomineralization" pathway (Liyanage et al., 2024).

HRFAPs receive natural sunlight in the hybrid system, which triggers photocatalytic reactions, and algal photosynthesis promotes dynamic DO and pH fluctuations. The natural light energy aspect supports ongoing photodegradation (Liyanage et al., 2024). The advanced composites, like Ni–Al LDH/LM-TiO_2 , possess superior adsorption and charge separation capabilities. Combined with filamentous algal biofilms, the materials effectively seize pollutants and augment oxidation processes as the major foundation of a "material + biological" synergistic approach (Zhang et al., 2023).

Filamentous algae, like representatives of the genus Cladophora, possess high growth rates under temperate, nutrient-sufficient conditions and have a great adaptability to light and temperature variation, indicating their applicability in outdoor integrated systems (Trochine et al., 2010)

2.5.2 Insights from Existing Research

Norvill, Shilton, and Guieysse (2016) looked at all the way that algal pond reactors could low the new contaminants like estrogens, so they aimed to hydrolysis, sorption, biodegradation, and photodegradation as important mechanisms that work together to get rid of these pollutants. The most important things that affected these processes were changes in pH and dissolved oxygen over the course of the day.

Zhang et al. (2014) tested a model algal system called Scenedesmus dimorphus to see if it could get rid of four steroidal estrogens. It got rid of around 85% of E1 and E2 and about 95% of E3 in 8 days. Sorption and algal-mediated biotransformation were the main processes, followed by secondary photodegradation.

Furthermore, the life cycle analysis has shown that the algal co-treatment is more energy-efficient and has greater environmental advantages compared to ozone and activated carbon systems, thereby demonstrating its potential for sustainability (Zhang et al., 2014).

Norvill et al. (2016) also added that most research is confined to the laboratory, without measuring photodegradation contributions under continuous-flow or real-world conditions. Cooperative effects of light on biological systems under dynamic diel cycles are still poorly understood and should be researched more.

2.5.3 Future Integration Perspectives

Bobirică et al. (2024) introduced modular photocatalytic reactors that are integrable into HRFAP systems as and when required, enabling scalable deployment, simple maintenance, as well as incremental upgrading.

Oruganti et al. (2022) did a detailed examination of mutualistic interactions in algae-bacteria systems, specifically in relation to micropollutant and nutrient removal; they pointed out the potential for incorporating photosensitive particle carriers or catalysts into future uses of the systems to enhance the efficiency of the adsorption and oxidation processes.

Lu et al. (2022) compared integration approaches in a systematic manner, ranging from sequential to concurrent operations, and concluded that co-located processes substantially enhance the removal efficiency of recalcitrant organic pollutants.

Leng et al. (2020) demonstrated widespread elimination of antibiotics and hormones in algae-bacteria systems with light-driven processes being involved and predicted future use of photocatalysts in facilitating greater removal at low pollutant concentrations.

Liyanage et al. (2024) noted the necessity for life-cycle assessments of whole systems in a quest to measure energy, land, as well as operational sustainability to supply vital information for scale-up by policy and engineering.

2.6. Research Gaps and Future Prospects

2.6.1 Shortcomings of Existing Studies

Norvill et al. (2016) said that the biggest problem with HRAP/HRFAP systems is that they don't have any continuous-flow tests done in real-world situations, including changing light levels during the day. In addition, Norvill et al. (2016) stressed the importance of making a distinction between photodegradation and algae absorption and microbial transformation in outdoor pond habitats.

Liyanage et al. (2024) emphasized how a holistic system-level analysis needs to be conducted to determine land use, energy production, and overall feasibility, especially in comparing HRFAP with traditional treatment processes. Liyanage et al. (2024) applied the concept of circular bioeconomy in the realm of algal valorization but indicated the lack of any concrete uses.

Oruganti et al. (2022) identified [the need for such system-level clarity] as one of the elements making scaling harder and leading to inconsistency in performance.

2.6.2 Future Research Directions

HRFAP field experiments coupled with photocatalytic systems must be conducted to simulate actual diurnal and seasonal conditions (Norvill et al., 2016).

The combination of sensor arrays and machine learning has the potential to optimize HRT, nutrient loading, and photoreactor operation in real time (Oruganti et al., 2022; Liyanage et al., 2024).

Research needs to establish how photocatalysts like Ni–Al LDH/TiO_2 composites influence microbial and algal activity at the microlevel and whether they promote co-metabolic processes (Zhang et al., 2023).

Economic and life-cycle analysis consideration is pivotal in evaluating environmental trade-offs, carbon yield, and return on investment (Liyanage et al., 2024; Oruganti et al., 2022). Algae harvested from High-Rate Algal-Facilitated Aquaculture Processes should be analyzed for their prospective applications in bioenergy generation, nutrient recovery, or as a supplement to secondary treatment in a closed-loop system (Leng et al., 2020).

Chapter 3: Methodology

This chapter shows the experimental methods to evaluate and compare three ways to decrease E1 with exposure to natural sunlight, UV light, and treatment by algae through Spirogyra. These experiments were conducted on the gaps that were shown in the literature review. The methodological template was designed to capture degradation trends in artificial controlled laboratory settings, while systematically exploring the implications of environmental parameters like pH, light distribution, and concentration effects. The first-round experimental phase worked with environmentally relevant concentrations held back for LC- MS characterization, while later experiments utilized higher concentrations quantified by UV- Vis spectrophotometry to supplement quantitative measurement. Repeat short-term validation experiments are also included, and mechanism experiments to verify that the findings can be replicated, as well as to obtain insight into the treatment's mechanism.

3.1 First-Round Experiments (Awaiting LC-MS Analysis)

This experiment series aimed to assess the removal behavior of low-level estrone (E1) in a distilled water matrix, under the coupled effect of algal activity and exposure to natural/artificial light. The work sought to examine both the direct effect of algae on E1, as well as light regimes increased its breakdown, with a view to investigating possible synergistic removal processes.

Three test treatments were set up:

(1) an algal exposure group under natural sunlight for evaluating the algal removal ability under light-exposed conditions;

(2) a control blank group under natural sunlight (free of algae) for monitoring light-induced degradation;

(3) a UV exposure group (without algae) to assess photolytic degradation under artificial ultraviolet light.

Methodology

At first, each group had E1 levels of 0, 2, 5, and 10 μ g/L. Water samples were collected at predetermined intervals to generate time-resolved datasets for subsequent concentration trend analysis.

After sampling, all samples went through the same pretreatment procedures (which are explained in more depth in the next sections), were put in glass vials, and were kept at 4 ° C to keep them from breaking down or changing before they were analyzed. All samples are still in cold storage awaiting LC-MS quantitation.

Due to a temporary malfunction of the LC-MS instrument, the samples have not yet been analyzed. Future quantification will proceed once the instrument is repaired, or alternative analysis facilities are secured.

3.1.1 The Algal Cultivation Phase (Natural Light, around 1.5 Months)

The Spirogyra algal strain used in this study came from the Ruakura Laboratory of the New Zealand National Institute of Water and Atmospheric Research (NIWA) as a stable, pure laboratory strain. The cultivation began on 23 September 2024 and took around one and a half months to complete, to produce enough and consistent biomass for the following experimental stages.

The cultivation initiation consisted of three 2-liter lab-grade borosilicate glass bottles (Bottles 1, 2, and 3). Bottles 1 and 2 were initiated together, with each being inoculated with 10 g dry weight of algae on 23 September 2024. Bottle 3 was added on 4 October 2024, seeded with biomass from Bottles 1 and 2 at the same 10 g dry weight. This incremental expansion permitted gradual growth while ensuring biomass uniformity.

Figure 3.1. The first algal growing arrangement with 2-L glass bottles (Bottles No. 1 and 2) attached to an air pump.

Figure 3.2. Algal propagation stage with the introduction of a third 2-L glass bottle (Bottle No. 3).

The culture was inoculated on November 4, 2024, into four 1-liter experimental bottles, which were then exposed to sunlight and estrone experiment interaction. The inoculum of these four experimental bottles was prepared using a mixed culture from Bottles 1, 2, and 3. To ensure that all treatments begin on the same basis, each 1-liter bottle was filled with 10 grams of dry algae.

Figure 3.3. Stabilized algal cultures before experimental trials, grown in four 1-L experimental bottles with continuous aeration.

The experiment was conducted indoors at the Environmental Laboratory C.3.11, University of Waikato. The culture was grown in a laboratory with large windows that received natural light. The early spring to early summer season in the Southern Hemisphere provided favorable growing conditions in Hamilton. Day lengths ranged from approximately 11.0 to 12.5 hours daily. Air temperatures maintained a seasonal pattern: late September was 14–16 °C average, mid-October was 11–14 °C, and early November was 16–18 °C. Overnight minimums ranged from 8 to 10 °C. These conditions are suitable for prolonged photosynthetic activity for freshwater microalgae.

Each culture vessel was sealed with an air-tight lid and connected to a continuous air pump system. Bubbling provided oxygenation, in addition to gentle agitation of the medium, thus maintaining homogeneity and preventing sedimentation or surface biofilm formation. There was no orbital shaking or mechanical stirring.

The growth medium was distilled water and was weekly supplemented with Varicon® commercial algal fertilizer, which was supplied by NIWA. The concentration of the fertilizer in the stock was 10 g in 100 mL. 2 mL of fertilizer was added weekly to each 2-liter bottle and 1 mL weekly to each 1-liter bottle from this stock. The changes were carried out using sterilized syringes under clean conditions to reduce the risk of contamination.

Nitrate (NO_3^-) concentration was tested by Palin Test colorimetric reagent on 23 September, 30 September, 1 October, 4 October, and 10 October 2024 to track culture conditions and nutrient utilization. The measurements were taken to establish nitrate depletion trends and to determine when fertilizer supplementation was required.

Algal biomass was qualitatively determined through visual observation. The biomass was reported to have achieved a stable growing stage when the culture solution was densely green and green flocculation was observed at the bottle bottom. No dry weight or optical density readings were made at this time.

This regulated and staged approach to plant growth has developed a consistent and reproducible biomass platform for future research on how to eliminate estrone via photodegradation and algal activity.

3.1.2 Algal–Estrone Exposure to Natural Light Conditions

This study was performed to explore the potential for algal elimination of the environmental estrogen estrone (E1) under natural light. To mimic different contamination levels, four treatment groups were set up with initial E1 concentrations of 0, 2, 5, and 10 µg/L. E1 was introduced to the culture bottles on 11 November 2024. All bottles had been inoculated with 10 g dry weight of Spirogyra algae on 4 November 2024. At E1 addition on 11 November, the algal biomass had risen through continued growth, although the precise biomass was not measured.Experiments were conducted in 1-L borosilicate glass bottles, each filled with 1000 mL of distilled water to prevent background interference from organic matter.

Bottles were stopped firmly and aerated continuously via tubing linked to air pumps, replicating dissolved oxygen levels in natural waters without mechanical agitation from stirring. Bottles were left on a laboratory windowsill to receive daylight but were not put outside, and no artificial light was supplemented. This setup maximized experimental control and environmental realism.

The experiment was conducted indoors in the Environmental Laboratory (Room C.3.11), University of Waikato, Hamilton, New Zealand, which is in a temperate oceanic climate zone of the Southern Hemisphere. It had an average of 13–14 hours of sunlight per day, with daytime temperatures generally ranging from 18 to 23 °C and minimal nocturnal deviation. The windows in the laboratory were single-glazed, providing adequate light entry while preserving constant indoor conditions, which protected the setup from inclement weather.

Sampling commenced one day following E1 addition (12 November 2024) and was carried out for 12 time points. Phase I comprised daily sampling between 12 and 22 November (9 working days), while Phase II consisted of less frequent sampling on 29 November, 4 December, and 6 December. At every time point, 20 mL of sample was taken from every bottle, sealed in glass autosampler vials, and refrigerated at 4 °C, awaiting batch processing.

Figure 3.4. Sampling vials sealed.

In pre- treatment, filtration with 0.45 µm syringe filters (Minisart®, Sartorius, Germany) was performed in all samples to remove suspended particles and algal biomass. This filtration was not only performed in algal treatment groups, but also in algal-free groups in the following natural light and UV exposure experiments to ensure consistency in sample handling. All samples collected were analyzed in batch from 19 February 2025 to prevent analytical bias.

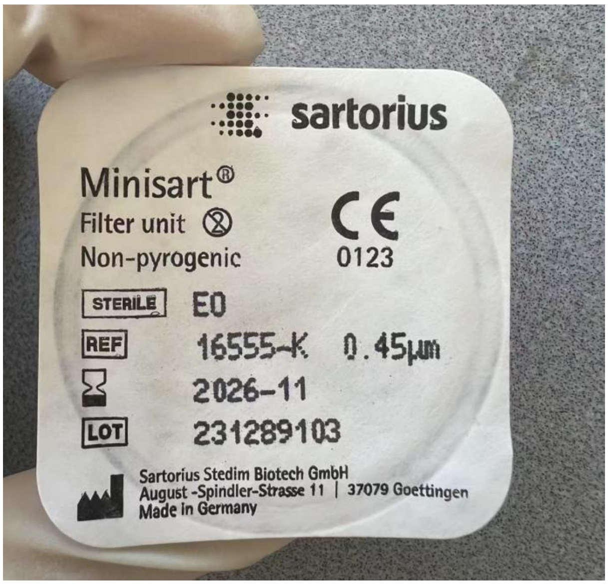


Figure 3.5. 0.45 µm Minisart® syringe filter (Sartorius, Germany) used for algae removal.

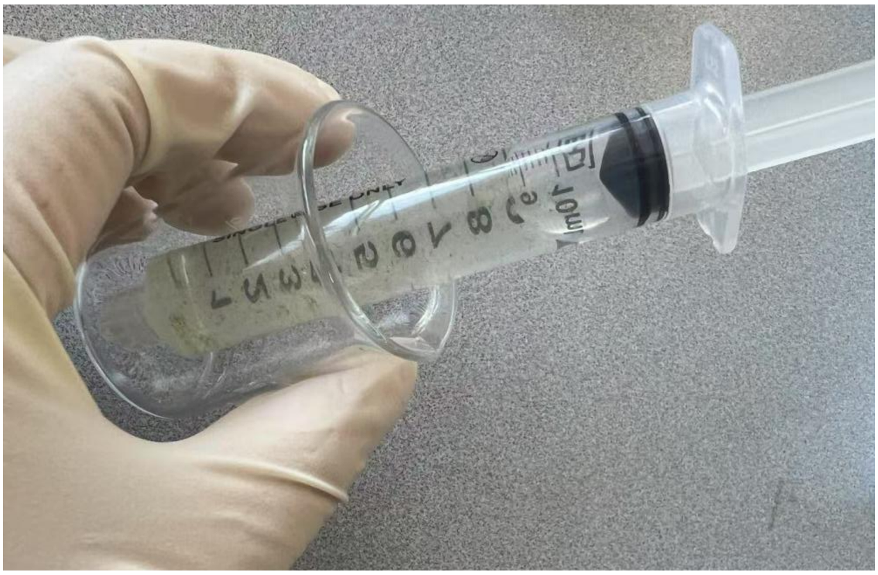


Figure 3.6. Filtration of samples using 10 mL syringe and filter setup.

Pre- treatment included Solid Phase Extraction (SPE) steps to concentrate on the target estrone for the subsequent LC-MS analysis. The process followed in-house laboratory protocols that were developed and established by S. Liyanage (personal communication, 17 February 2025).

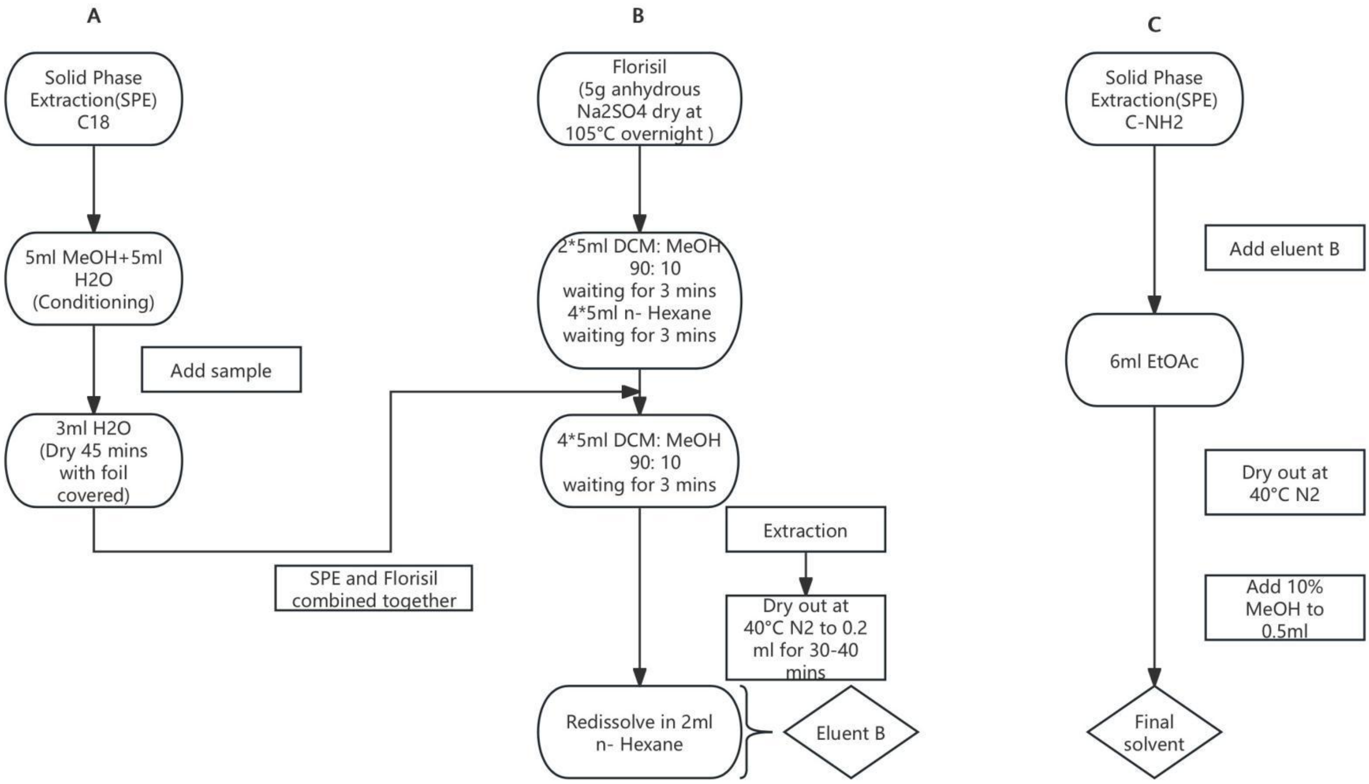


Figure 3.7. LCMS sample pre-treatment process.

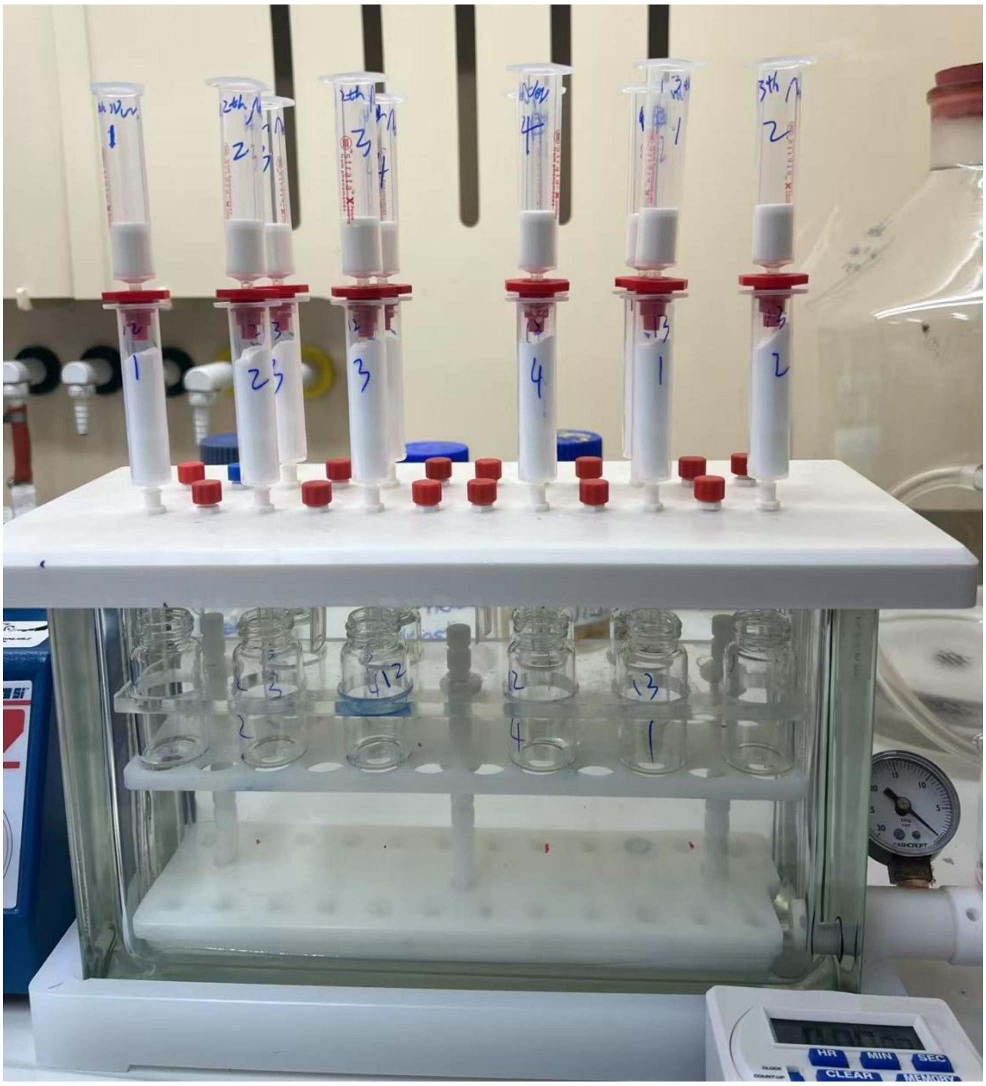


Figure 3.8. Solid-phase extraction of filtered samples.

3.1.3 Algal-Free Estrone Removal under Natural Light and UV Exposure

To further investigate the role of abiotic factors (i.e., light exposure) in the degradation of the environmental estrogen estrone (E1), two algal-free control tests were conducted under natural sunlight and artificial ultraviolet (UV) light conditions. By removing algal influence, these tests directly assessed the role of photodegradation and other physicochemical processes in E1 removal.

Natural Light Exposure

This experiment was conducted from 28 January to 3 February 2025 in Room C.3.11

of the Environmental Laboratory at the University of Waikato. The vials contained only distilled water and E1 at initial concentrations of 0, 2, 5, and 10 µg/L without any algae. Bottles were left on a laboratory windowsill and in natural light for 7 consecutive days. On Day 7, 20 mL of sample was removed from each bottle, closed in autosampler vials, and stored at 4 °C for future analysis.

UV Exposure

The experiment of UV exposure was carried out on 21 January 2025 in EG.01 Lab at the University of Waikato. A biosafety cabinet (Heraeus KS 12) with 254 nm UV lamps was used for the experiment. The samples consisted of distilled water spiked with E1 at levels of 0, 2, 5, and 10 µg/L with no algae. Exposure times to UV were 0, 15, 30, 45, 60, 75, and 90 minutes to test short-term photodegradation. At each time interval, a sample of 20 mL was taken and refrigerated at 4 °C for future analysis.

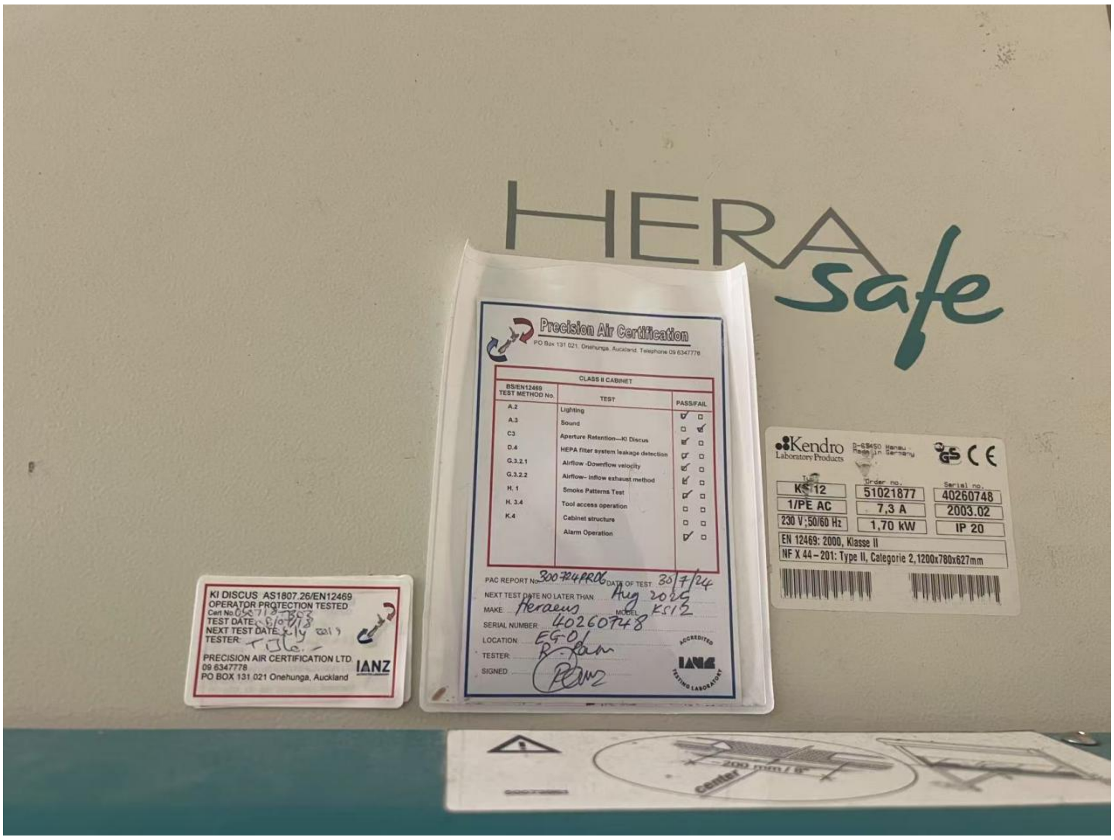


Figure 3.9. UV biosafety cabinet

Consistency and Pre-treatment Protocol

The UV and natural light control groups had the same sampling volume, vial, filtration, and storage treatment as the algal treatment groups for consistency in the experiment. All samples were pretreated starting on 19 February 2025 to minimize analytical bias. The pre-treatment consisted of solid-phase extraction (SPE) of estrone concentration before LC-MS analysis. This method (Figure 3.7) was established and conducted by S. Liyanage (personal communication, 17 February 2025).

3.2 Second-Round Experiments: Absorbance Monitoring of E1 Removal

To bridge the data gap caused by LC-MS analysis delays from the first-round experiments, the second round of experiments was initiated in April 2025. The aim was to have real-time data on the removal of estrone (E1) under different treatment conditions using UV–Vis's spectrophotometry as a substitute for LC-MS. This round was made consistent with the first-round design by maintaining the same three experimental pathways: algal treatment, natural light photodegradation, and artificial UV exposure.

Figure 3.10. UV–Vis's spectrophotometer (Shimadzu UV-1900)

Methodology

The algae used for this round were drawn from the original Spirogyra culture used in Round 1 to ensure biological continuity and were all stabilized for seven days prior to E1 dosing, under normal conditions with continuous aeration and nutrient addition. The therapies began on 28 April 2025.

One of the primary modifications in this round involved tweaking the concentration levels of E1. While the first round experimented with environmentally applicable concentrations (0–10 µg/L), the second round spiked E1 dosing to 0, 2, 5, and 10 mg/L. The intention was to try to maximize the sensitivity and analytic precision of UV–Vis's detection. At low µg/L levels, E1 absorbance tends to be indistinct and difficult to quantify without preconcentration. Increasing concentration allowed for the recording of more defined signals and improved kinetic resolution for time-series monitoring.

In addition, the second round eliminated the need for cold storage or solid-phase extraction. The absorbance measurements were all performed directly on the sampling day, streamlining procedures without sacrificing the integrity of the in-situ system. For consistency in all experimental avenues, all samples were filtered through 0.45 μm syringe filters before measurement to eliminate suspended particles and interferences. The methods for each of the three segments of the second round are covered in the sections that follow.

3.2.1 Algal–Estrone Exposure to Natural Light Conditions (Second Round)

To investigate the possibility of degradation of algae on high concentrations of estrone (E1), the second-round algal experiment was established at the Environmental Laboratory (Room C.3.11) in the University of Waikato. Measurement by absorbance with a UV–Vis's spectrophotometer was employed as a proxy for observation of E1 concentration changes. The arrangement followed the template of the first round with refinements in the range of concentration and immediacy of measurements.

The laboratory is in Hamilton, New Zealand. According to historical meteorological data, the mean daily high for April–May 2025 was approximately 20 °C with a low of approximately 12 °C and a mean of 11 hours of daylight per day. This promoted consistent algal growth and even photochemical activity throughout the experiment.

On 14 April 2025, Spirogyra algae that had been previously cultured from the first-round experiment were re-established into four 1000 mL borosilicate glass bottles. Each bottle was filled with 10 g dry weight of algae and 1000 mL of distilled water. There was constant aeration using air pumps to prevent hypoxia and sedimentation. Varicon® algal fertilizer (10 g/100 mL) was added weekly at 1 mL per bottle. Bottles were capped and left on a laboratory windowsill where they were exposed to natural light without shading.

E1 was introduced into each bottle on April 28, 2025, which signaled the beginning of the experiment. Four treatment groups with initial E1 concentrations of 0, 2, 5, and 10 mg/L were created to depict varying contamination levels. To ensure thorough mixing, E1 was first dissolved in high-purity methanol before being volumetrically added into each bottle, whereupon the bottles were shaken gently. Absorbance Monitoring and Sampling Schedule Absorbance measurements were taken at 12 time points between 28 April and 16 May 2025: 28, 29, and 30 April; 1, 2, 5, 6, 8, 9, 12, 13, and 16 May. On each sampling day, 3 mL of water was withdrawn from each bottle and analyzed immediately on a Shimadzu UV-1900 UV–Vis's spectrophotometer (Figure 3.9). The detection wavelength was 290 nm, corresponding to a known absorbance peak of E1.

No refrigeration or pretreatment of the samples was required so that in-situ measurements could reflect actual E1 behavior in the algal system. The data obtained are plotted to draw removal curves and to analyze the data.

3.2.2 Algal-Free Estrone Removal under Natural Light Exposure (Second Round)

To examine the photodegradation behavior of environmental estrogen estrone (E1) under abiotic conditions, an algae-free control treatment was established. The experiment simulates the removal of E1 in aquatic environments with sunlight availability but without uptake or biological degradation. The experiment was conducted in parallel with the algal treatment group (Section 3.2.1) to enable direct comparability of treatment pathways.

The experiment was initiated on 28 April 2025 and was carried out in Environmental Laboratory C.3.11 at the University of Waikato. Each treatment was established in 20 mL glass autosampler vials with 20 mL of distilled water and the necessary concentration of E1 (0, 2, 5, and 10 mg/L). No algae were added. E1 was first dissolved in high-purity methanol and added to the vials to achieve even distribution. All vials were closed and left on a laboratory windowsill in ambient natural light, with no artificial light or temperature control.

The experiment was carried out indoors in natural daylight through single-pane windows with good penetration of sunlight. Hamilton received about 11 h of daily sunshine and daytime temperatures of 18–22 °C in April–May 2025, based on NIWA meteorological records, which provided steady and good conditions for prolonged photochemical activity.

The sampling schedule was identical to that of the algal treatment group (Section 3.2.1), with twelve time points: 28, 29, and 30 April; 1, 2, 5, 6, 8, 9, 12, 13, and 16 May 2025. For every time point, 3 mL of sample was extracted from each vial and read immediately using a Shimadzu UV-1900 UV–Vis's spectrophotometer at 290 nm (Figure 3.10). To maintain the volume and to prevent dilution effects, the samples read were returned to their corresponding vials after reading.

Figure 3.11. Sunset lighting conditions on the windowsill (April 2025)

3.2.3 Algal-Free Estrone Removal under UV Exposure (Second Round)

The objective of this experiment was to assess the direct photodegradation of the environmental estrogen estrone (E1) upon exposure to controlled laboratory UV light, and to further investigate its stability under artificial high-intensity irradiation. As part of the experimental design for the second round, this group is complementary to the natural light experiment that allows comparison between varying light regimes.

The trial was conducted on 5 June 2025 at the University of Waikato, Laboratory EG.01. The artificial UV source was a biosafety cabinet (Heraeus KS 12) with a central emission wavelength of 254 nm.

The samples were placed in 20 mL glass autosampler vials, which contained 20 mL of distilled water and the desired E1 concentration (0, 2, 5, and 10 mg/L). The E1 was first dissolved in high-purity methanol, then diluted. The vials were all sealed and statically exposed to the UV light with no agitation or aeration.

Exposure times were 0 (control), 15, 30, 45, 60, 75, 90, 105, and 120 minutes, for a total of eight time points. At each time point, 3 mL of solution was removed from the respective vial and promptly analyzed for absorbance at 290 nm via UV–Vis's spectrophotometry. All analyses were conducted on the same day without refrigeration or storage over the long term.

Figure 3.12. Experimental setup for UV exposure within the biosafety cabinet

3.3 Short-Term Experiment

3.3.1 Light Intensity Distribution under Algal vs. Non-Algal Conditions

This experiment was performed in an effort to determine if and how the existence of the algae in the culture bottles would have an impact on light transmission and dispersion in the daylight conditions indoors. The experiment was carried out on the 14th day of July 2025 at 10:30 a.m. in Environmental Laboratory C.3.11, University of Waikato, Hamilton, New Zealand. Vessels used for testing were two identical make 1-L borosilicate glass bottles filled with an aqueous solution of estrone (E1) at the same concentration of 2 mg/L for the sake of uniformity in the treatments.

The light intensity was determined by the reading of a mobile luxmeter app (iOS/Android device), taking full advantage of the device's internal photometric sensor. The sensor of the phone was located near the beverage bottle at the measuring point. The field of view was from 0 ° to 180 ° while the measurement was conducted at 5 ° intervals so that the direct and the diffuse light parts could be measured. The external variability was controlled by the test group and the control group being tested in the same light ambient condition.

3.3.2 Behavior of E1 under Algal Conditions at pH 7 (15 – 16 Jul 2025)

The experiment was conducted to establish the short-term response of algal–estrone (E1) systems in the environment at pH 7, to establish the stability of the buffer system and the correctness of the method used for measurement, to serve as control for the subsequent study at a prolonged time. The pH was controlled by an anhydrous phosphate buffer system accurately measured in ratios between anhydrous KH_2PO_4 and anhydrous Na_2HPO_4 to constitute a buffer solution at pH 7 to keep the system in a stable condition for incubation at night.

Experiments were conducted in University of Waikato Environmental Laboratory C.3.11, Hamilton, New Zealand. Those samples were prepared in 50 mL Falcon tubes with 500 µg dry weight Sperioga algae and up to 50 mL volume. Estrone was added from methanolic stock solutions and brought to desired concentrations for 0, 2, 5, and 10 mg/L as a set. This experiment included 5 sets. Baselines were taken on 15 July 2025 shortly after samples had been prepared: pH by DIGITECH pH Meter Pen (QM-1670, New Zealand), and absorbance by Shimadzu UV-1900 spectrophotometer, 290 nm. The Falcon tubes were shaken for around 12–16 hours at room temperature and domestic light conditions. The same readings were then taken on 16 July 2025. To maintain consistency throughout the whole procedures for the experiments, the samples were filtered through a 0.45 μm syringe filter before absorbance measurement to eliminate particulates in suspensions and interferences.

Figure 3.13. DIGITECH pH Meter Pen (QM-1670)

The important parameters were ΔpH ((pre- vs. post-)-pH difference) and ΔA_{290} ((change in) absorbance at 290 nm), and they were utilized to determine buffer stability at pH 7 and to determine if there were any short-term effects by algal adsorption or transformation on the E1. The above validation served as the methodological control foundation and the standard for the one-month, multi-pH duration-long-term experiment conducted subsequently.

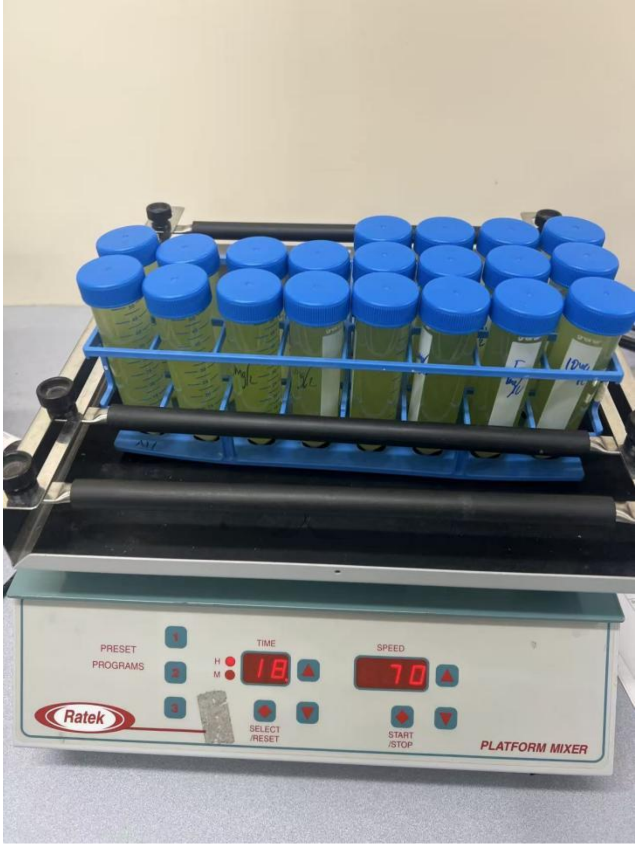


Figure 3.14. 20 experimental tubes were incubated overnight on a Ratek platform stirrer at 70 rpm during a short-term validation experiment at pH 7

3.4 Effect of pH on E1 Removal at 10 mg/L under Algal and Natural Light Conditions (16 Jul – 15 Aug 2025)

This longer-term experiment would then seek to systematically evaluate removal activity at different levels of pH by comparing the influence of the presence and absence of algae. Given that pH was known to be an important environmental parameter in regulating the degradation of organic contaminants as well as algal metabolism, four different levels of the parameter were selected to span the aqueous environment ranges, i.e., levels 5, 6, 7, and 8. Experimental sets for each of the selected pH included algal and natural light sets to model aqueous systems from acid to slightly basic. Carried out in Environmental Laboratory C.3.11, University of Waikato, Hamilton, the experiment utilized eight 1-L borosilicate glass vessels continuously aerated to provide dissolved oxygen. The algal set consisted of the use of four bottles in total for 27.8858 g dry weight Sperioga algae distributed about evenly at approximately 6.9715 g per vessel. The natural light control sets of 4 bottles consisted of only the E1 solution. The 8 bottles were started with a concentration of 10 mg/L E1 to facilitate easy observation of degradation during the one-month study.

Figure 3.15. Four 1 L glass bottles for the algal treatment groups, under continuous aeration, in the initial pH buffered by phosphate buffers at 5, 6, 7, and 8. Estrone (E1) at an initial concentration of 10 mg/L

Figure 3.16. Four 1 L glass bottles for the non-algal treatment groups, under continuous aeration, in the initial pH buffered by phosphate buffers at 5, 6, 7, and 8. Estrone (E1) at an initial concentration of 10 mg/L

pH control was done by utilizing phosphate buffer system, blend of anhydrous KH_2PO_4 and anhydrous Na_2HPO_4 in pre-calculated proportion to offer pH 5, 6, 7, and 8 conditions. The pH was daily checked by a DIGITECH pH Meter Pen (Model QM-1670, New Zealand), a glass-electrode meter standardized against buffer standards at pH 7.00. At the same time, the absorbance at 290 nm was recorded in a Shimadzu UV-1900 spectrophotometer and the analysis was conducted immediately under the non-refrigerated condition. The experiment ran between 16 July and 15 August 2025, covering late winter until early spring at Hamilton. The temperature ranged from 10 – 14 °C, and the lab temperature ranged from 10 – 20 °C. The duration of the day light was roughly 10 hours per day. The bottles were kept near a one-pane lab window to get the natural light, thereby achieving the realistic environment for the photodegradation without the assistance of any light artifact. To maintain uniformity throughout the complete experimental protocol, samples went through a 0.45 μm syringe filter before absorbance measurement in an effort to eliminate suspended matter and interfering components.

This long-term design allowed for assessment of E1 degradation in various pH regimes over long times, elucidating the distinction between the algal and natural light systems and enhancing knowledge of E1 stability and transformation in environmental settings.

3.5 Summary

This research conceived and conducted a series of experiments to study the removal of E1 under various light and environmental conditions. The first series studied lower concentrations of E1 under natural solar irradiance, UV irradiance, and algal culturing and prepared samples for LC – MS analysis. The second series expanded the study to elevated concentrations and used UV – Vis spectrophotometry to estimate changes in absorbance directly. To detail the research, three experiments were conducted: a light intensity distribution test by comparing the algal and non-algal conditions, an overnight validation study to verify buffer stability and short-term algal response, and a pH experiment to study the degradation of the E1. Across the series, the pretreatment processes were kept identical in detail, including using syringes with 0.45 μm syringe filters, to allow direct comparisons.

Chapter 4: Results

This chapter summarizes its experimental results for estrone (E1) elimination within varied treatment settings such as algal exposure, solar exposure, and ultraviolet irradiation. Data are organized so that raw absorbance values (A_{290}) for second-round experimentation are first shown, followed by removal efficiency calculations. Then, light intensity distributions and pH-dependent experiments are brought in to clarify observed patterns, while kinetic evaluations are conducted for further interpretation in support of its degradative behavior. A_{290} is the absorbance observed at each subsequent point.

4.1 Raw Absorbance (A_{290}) Measurements of Second-Round Experiments

4.1.1 Algal Group: Biodegradation and Natural Light

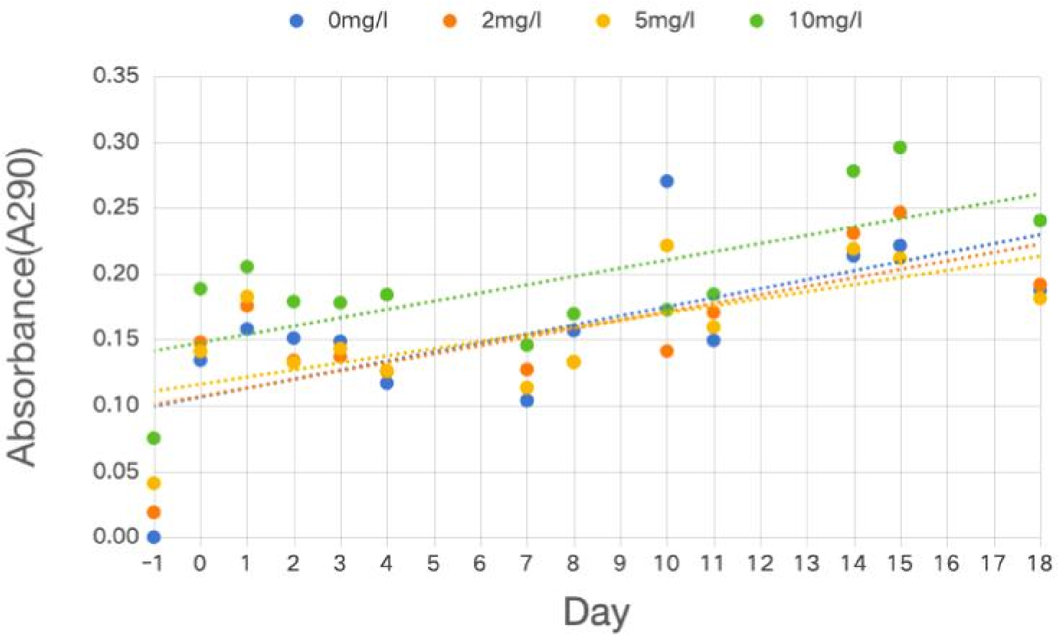


Figure 4.1. Temporal variation of absorbance (A_{290}) of E1 solutions with different initial concentrations under algal treatment conditions

For this cohort, E1 was inoculated with green algae under natural light. There was daily sampling over the course of 18 days (28 April to 16 May 2025). Table A1. monitors A_{290} values by concentration over the course of the experiment. Blank (non-algae, non-light) absorbance values appear at the top of the table and were used as the baseline (A_0) for removal rate calculation.

For A_{290} values of the algal group, they kept on increasing with time throughout all exposures. It is due to the fact that organic absorbing ultraviolet compounds had accumulated in the media.

When the group held 0 mg/L, it began at 0.0000 (base line) and remained until 0.1344 on day 0. It held a maximum of 0.2703 on day 10 after which it reduced to 0.1872 by day 18. It indicates that metabolism of algae leads to by-products which absorb ultraviolet light irrespective of the amount of E1 that remains.

Its initial absorbance of 2 mg/L on day 0 dropped from 0.0189 to 0.1481. It further increased until it showed a maximum of 0.2465 on day 15 and dropped to 0.1917 on day 18. There are two phases of growth of the curve: one sharp maximum in initial 1–2 days, and one sharp increase thereafter starting from day 8.

A_{290} at 5 mg/L group increased from 0.0410 to 0.1411 on day 0, and further increased to 0.2214 on day 10, but decreased rapidly to 0.2117 on day 15 and 0.1814 on day 18. Compared to that of the 2 mg/L group, this group had a more stable and smoother trend.

Group 10 mg/L had the original highest (0.0753) and overall highest A_{290} readings of all groups. Its absorbance also continued to increase throughout, starting at 0.1886 on Day 0 to 0.2780 on Day 14, but dropping somewhat to 0.2404 on Day 18. It had the most stable curve of all groups, which just meant that it continued to increase with very little deviation.

Results

4.1.2 Natural Light Group: Abiotic Photolysis Control

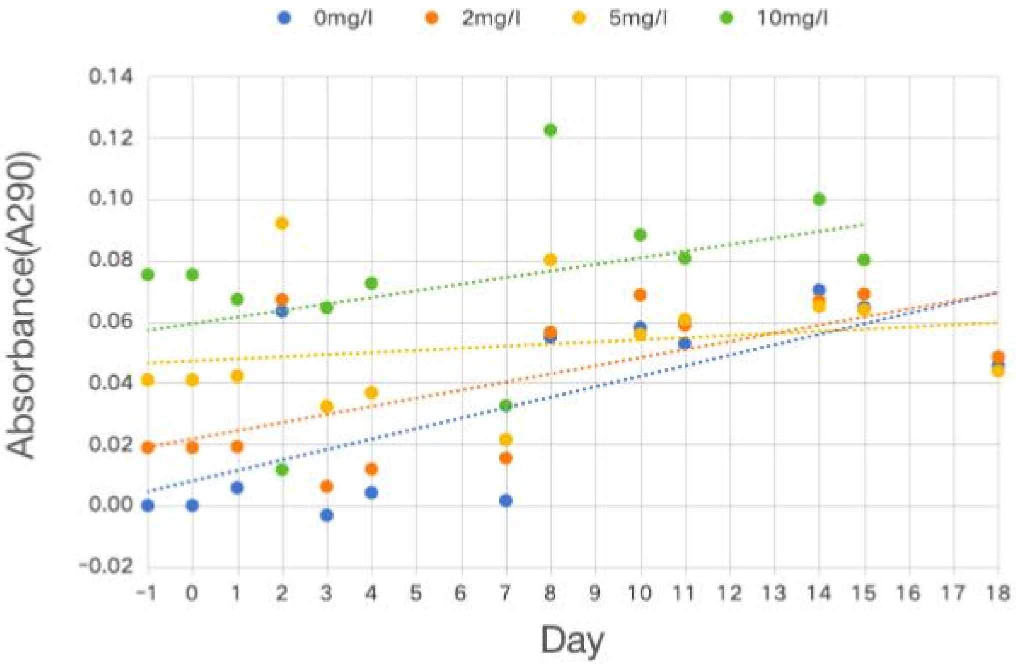


Figure 4.2. Temporal variation of absorbance (A_{290}) of E1 solutions with different initial concentrations under natural light treatment conditions

This was an abiotic control in natural light with no algae. It was run in parallel with the algae group, using the same concentrations and sampling times. Table A2. gives A_{290} readings at each time point under natural light. Baseline readings before any treatment were used as A_0 for consistency.

Under daylight, A_{290} values were comparatively low in all cases but showed occasional short-term maxima suggestive of light-induced by-product formation:

In the 0 mg/L group, A_{290} rose from 0.0000 to 0.0634 on Day 2, then fluctuated slightly, peaking again at 0.0703 on Day 14 before declining to 0.0457 on Day 18.

There was also a sharp rise at Day 2 (0.0672) in the 2 mg/L group, which subsequently dropped to 0.0155 on Day 5, and a peak of 0.0687 on Day 10. Terminal reading was 0.0485 at Day 18.

A_{290} in Group 5 mg/L had an early peak on Day 2 (0.0921) — maximum for this group — and thereafter gradually decreased to 0.0439 on Day 18.

There was a distinct trend in Group 10 mg/L: Steep decline to 0.0117 on Day 2, then steady rise to a maximum of 0.1225 on Day 8, then steady decline to 0.0569 on Day 18.

Also, on Day 2, there were typical peak days for A_{290} for all groups (especially 2 and 5 mg/L) suggestive of initial product synthesis of photoreaction upon irradiation. But there was a peak lag for the 10 mg/L group, which could reflect slower dynamics of buildup.

4.1.3 UV Light Group: Response to Short-Term UV-C Irradiation

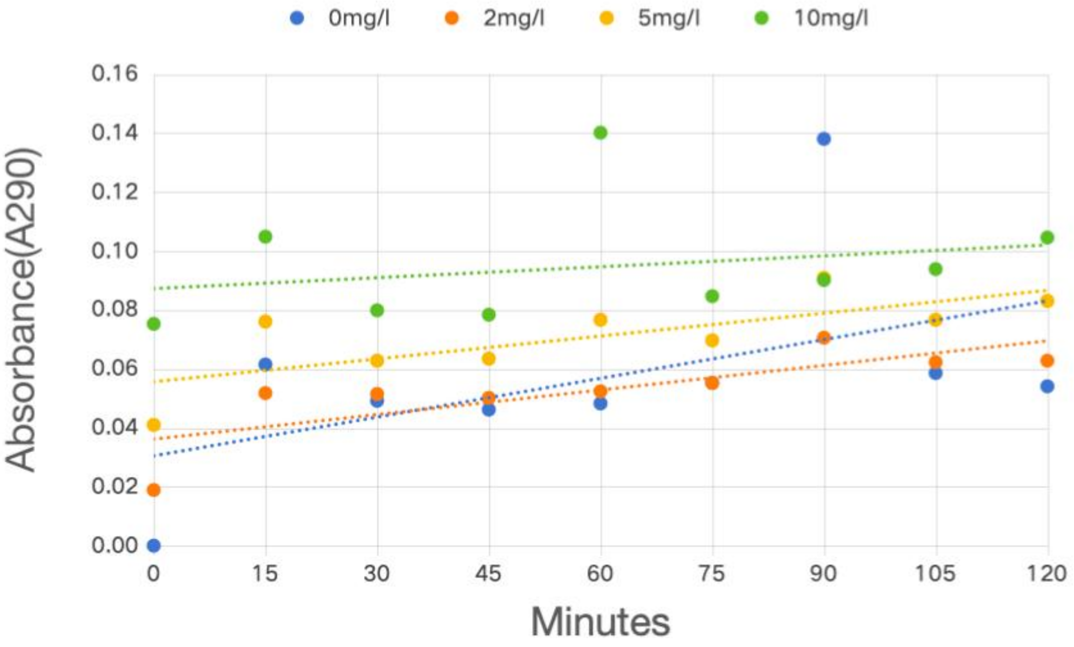


Figure 4.3. Temporal variation of absorbance (A_{290}) of E1 solutions with different initial concentrations under UV light conditions

For the UV group, E1 solutions were subjected to artificial UV-C light to simulate accelerated degradation. Sampling was done every 15 minutes for a duration of 120 minutes. Initial absorbance values (A_0) were taken prior to UV exposure and used throughout concentrations. Table A3. shows A_{290} trends for all concentrations under UV exposure and serves as a basis for the comparison of photodegradation efficiency.

Results

The raw absorbance data provided in this section are the basis for subsequent E1 removal efficiency calculations. These data are used in the next section to discuss the degradation behavior of E1 under different treatment conditions and describe the temporal trends with graphical plots.

Under UV irradiance, absorbance at 290 nm (A_{290}) at all E1 concentrations generally exhibited an initial increase at 15 min, a gradual decrease until 45 min, and a second increase or fluctuation after 60 min.

In the 0 mg/L group, A_{290} rose from 0.0000 to 0.0615 at 15 min, declined to a minimum of 0.0462 at 45 min, then increased sharply, peaking at 0.1381 at 90 min.

In the 2 mg/L group, starting at 0.0189, peaked at 0.0518 (15 min), dropped to 0.0502 (45 min), and reached 0.0628 at 120 min.

In the 5 mg/L group, an initial value of 0.0410, peaked at 0.0761 (15 min), decreased to 0.0635 (45 min), then fluctuated slightly to reach 0.0831 at 120 min.

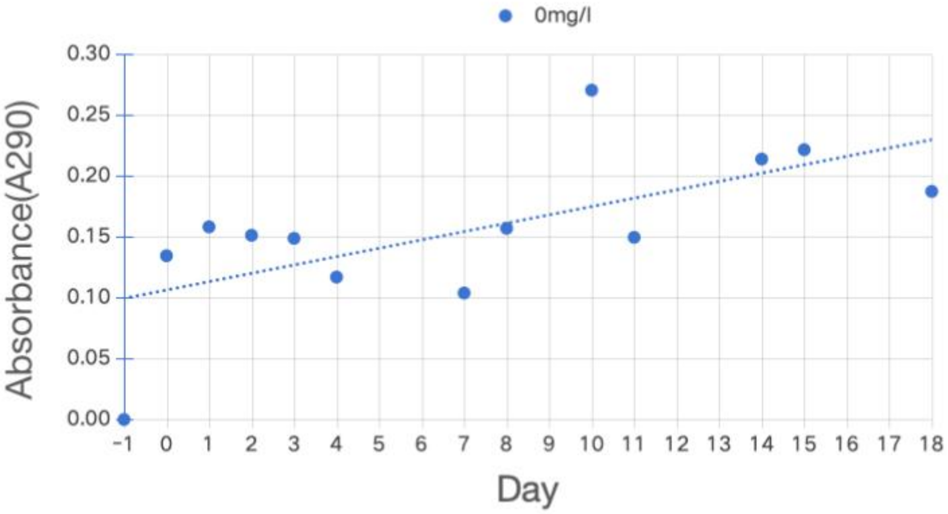
In the 10 mg/L group, from 0.0753 to a small peak of 0.1049 at 15 min, dropped to 0.0784 (45 min), reached another high of 0.1402 at 60 min, and ended at 0.1046.

In general, all groups showed a rise – fall – fluctuation profile of A_{290} after irradiation with UV light. At all-time points, 15 min always corresponded to the initial maximum, and minimum values were always measured approximately 45 min afterwards, before various degrees of secondary rise.

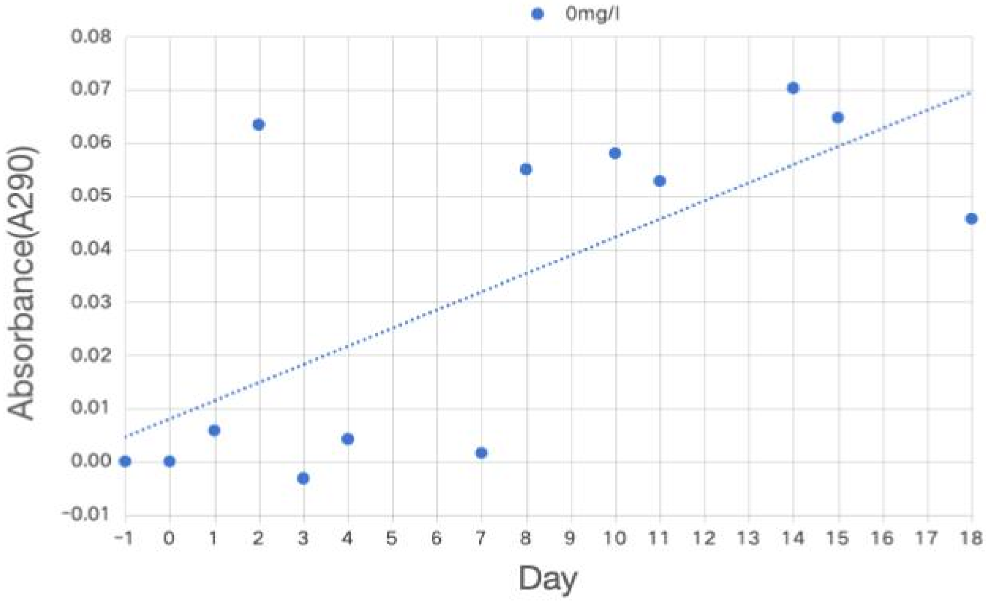
Results

4.1.4 Comparative Analysis of Absorbance Trends Across Treatments

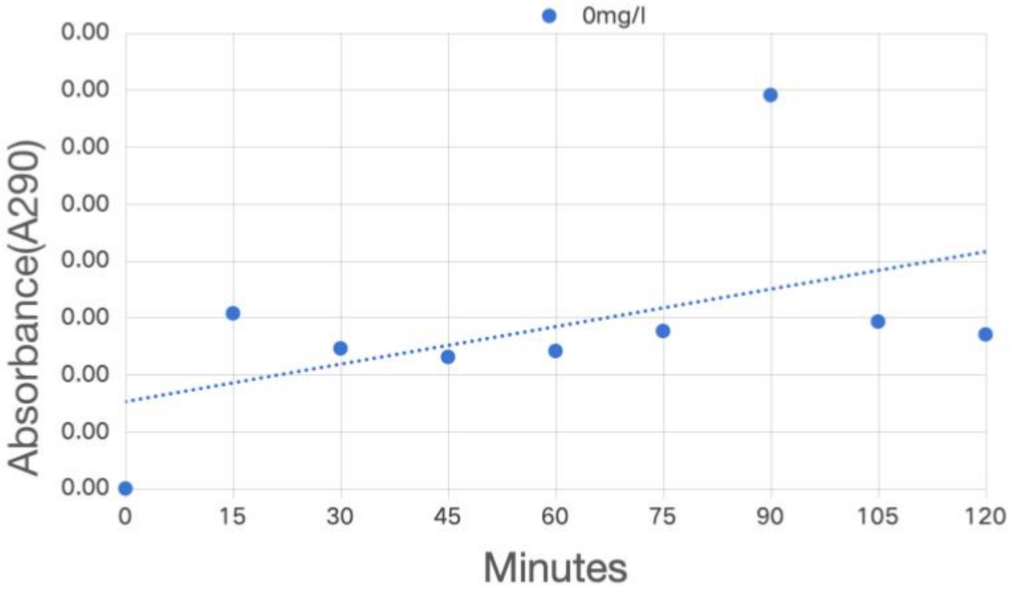
4.1.4.0 Comparative Trends of 0 mg/L E1 Absorbance Under Various Treatments



a. Algal group



b. Natural light group



c. UV light group

Figure 4.4. Comparative absorbance variation of 0 mg/L E1 solutions under three treatment conditions

Under the 0 mg/L E1 treatment level, there were three treatment groups that exhibited different trends of absorbance (A_{290}) against time:

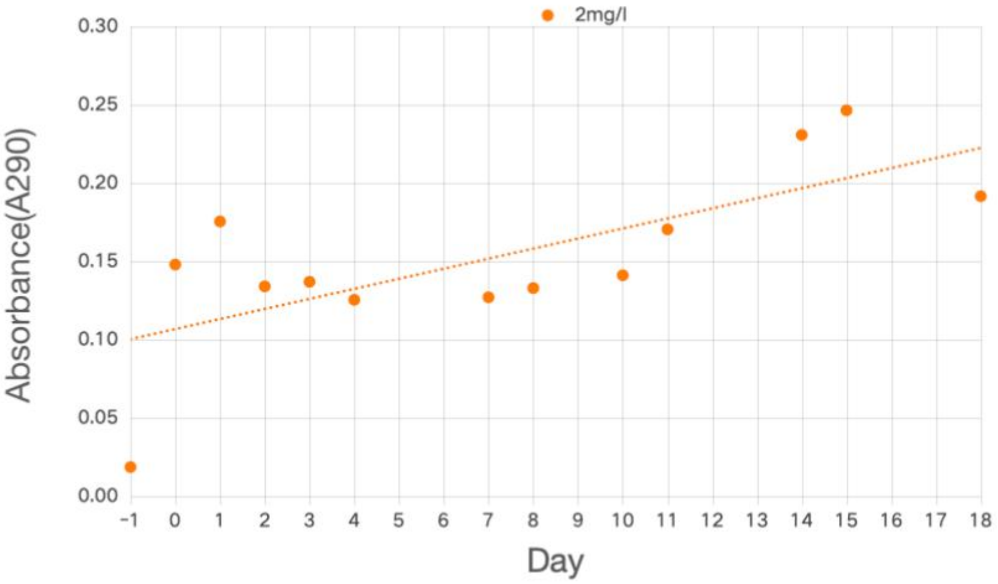
Among the algae group, A_{290} grew significantly from 0.1344 on Day 0 to 0.1581 on Day 1, then dropped slightly to 0.1512 on Day 2 and continued a downward trend to 0.1487 on Day 3 and 0.1169 on Day 4. The value reached its lowest point at 0.1038 on Day 7. Then A_{290} started to increase, peaking at 0.2214 on Day 15, before decreasing slightly to 0.1872 on Day 18. There was a typical decreasing phase between Day 1 and Day 7.

For the natural light group, A_{290} was kept at 0.0000 on Day 0, went up a little to 0.0058 ion Day 1, and shot up to 0.0634 on Day 2. The absorbance went down to –0.0032 on Day 3. After the temporary recovery to 0.0042 on Day 4, the second low point was observed on Day 7 with 0.0016. Subsequent measurements fluctuated between 0.0550 and 0.0703. Decreasing phases were observed between Day 2 and Day 3, and between Day 4 and Day 7.

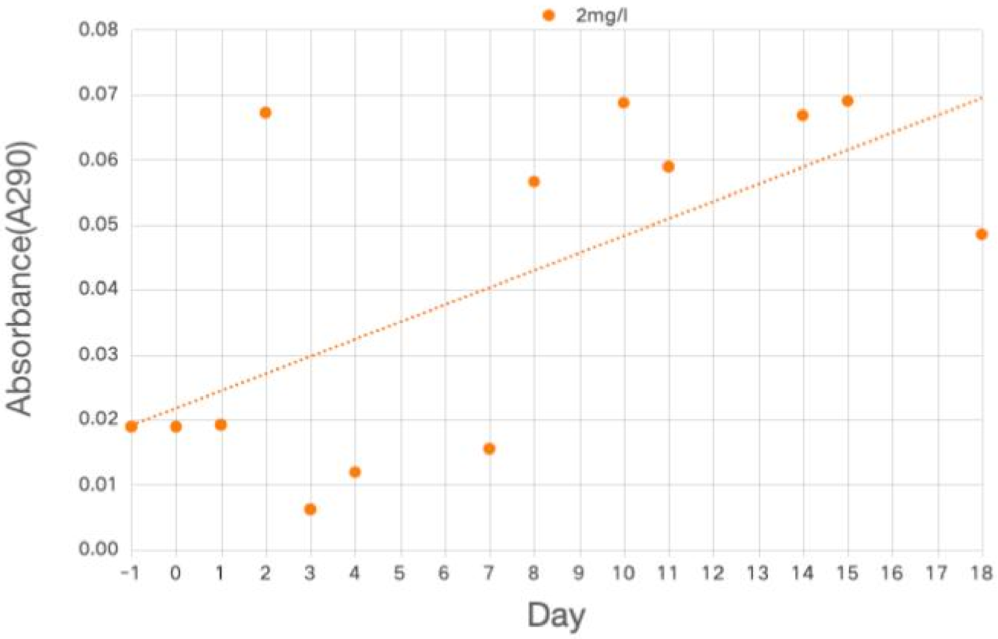
Results

In the UV light group, A_{290} was 0.0000 at 0 minutes, rose to 0.0615 at 15 minutes, then dropped slightly to 0.0492 at 30 minutes and 0.0462 at 45 minutes. The reading decreased further to 0.0483 at 60 minutes. Then, A_{290} rose steadily, peaking at 0.1381 at 90 minutes, then dropping again to 0.0541 at 120 minutes. There was a sharply decreasing section between 15 and 60 minutes.

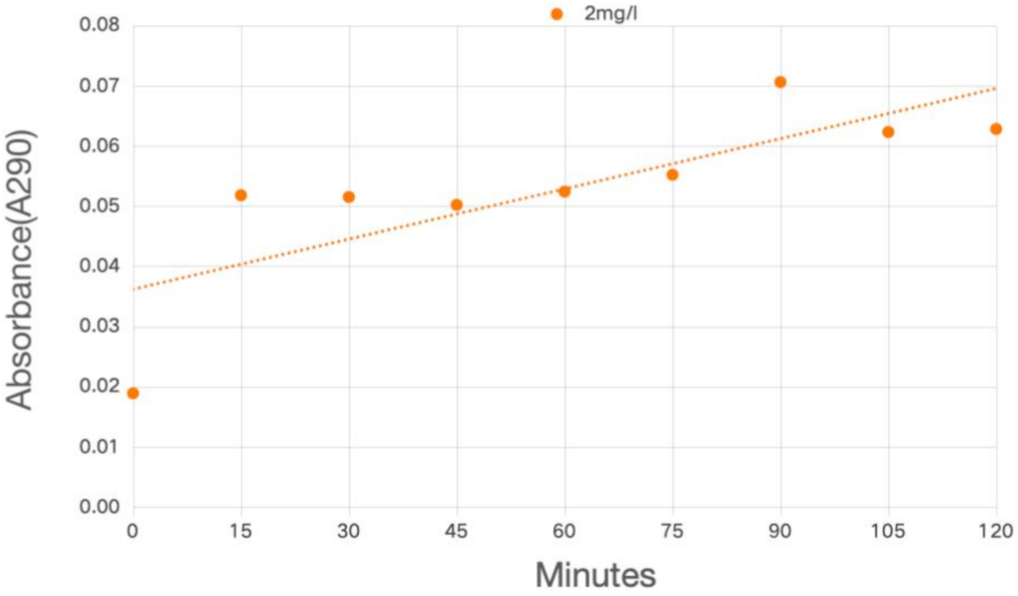
4.1.4.1 Comparative Trends of 2 mg/L E1 Absorbance Under Various Treatments



a. Algal group



b. Natural light group



c. UV light group

Figure 4.5. Comparative absorbance variation of 2 mg/L E1 solutions under three treatment conditions

A very different pattern of absorbance (A_{290}) versus time was observed in the algal exposure, natural light, and UV light treatment groups at the E1 concentration of 2 mg/L.

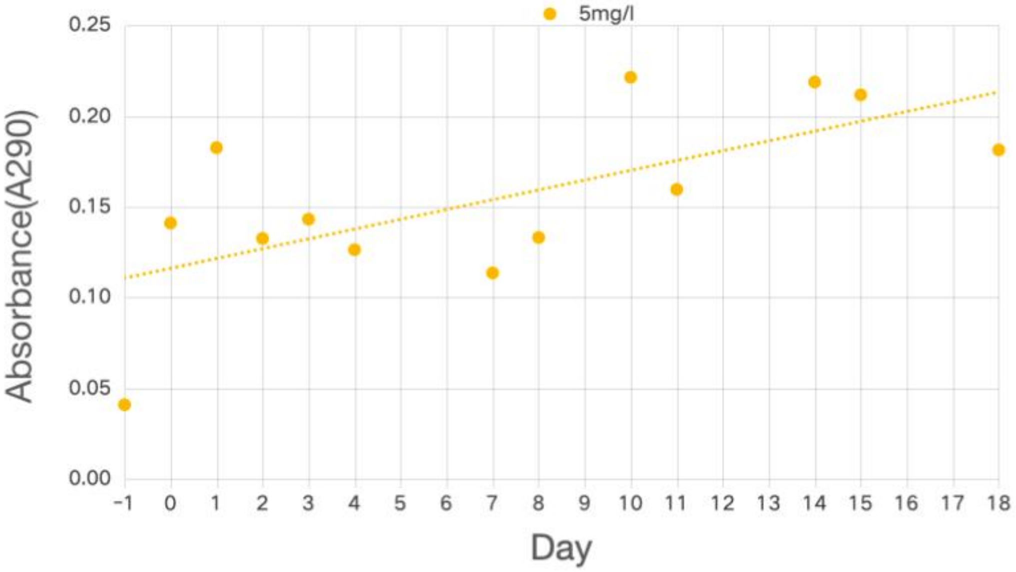
Results

In the algae group, A_{290} increased from 0.1481 on Day 0 to 0.1756 on Day 1. It then had a temporary decrease, with values decreasing to 0.1342 on Day 2, 0.1371 on Day 3, and down to 0.1256 on Day 4. From Day 7, the absorbance slightly fluctuated and rose again and reached its peak at 0.2465 on Day 15, then dropped to 0.1917 on Day 18. A period of decline was observed between Days 1 and 4.

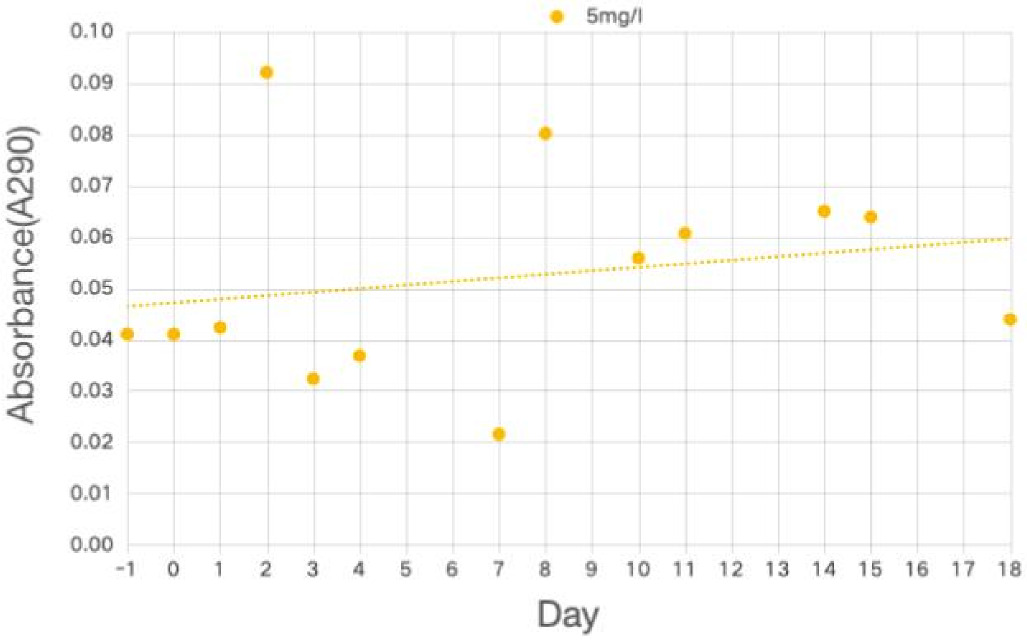
For the natural light treatment, A_{290} was low at 0.0189 from Day 0 to Day 1, then increased sharply to 0.0672 on Day 2, followed by a sharp decrease to 0.0062 on Day 3. A second increase started after Day 7, to 0.0687 at Day 10, then fluctuated slowly between 0.065 and 0.069 until Day 15, before decreasing slightly to 0.0485 by Day 18. The most extreme drop was from Day 2 to Day 3.

In the UV group, A_{290} began at 0.0189 and increased to 0.0518. It had little fluctuation with readings of 0.0515 at 30 min, 0.0502 at 45 min, and a slight increase to 0.0524 at 60 min. A peak in absorbance occurred at 0.0706 at 90 minutes, and then there was a slight decrease to 0.0628 at 120 minutes. There was an overall rise from 0 to 90 minutes.

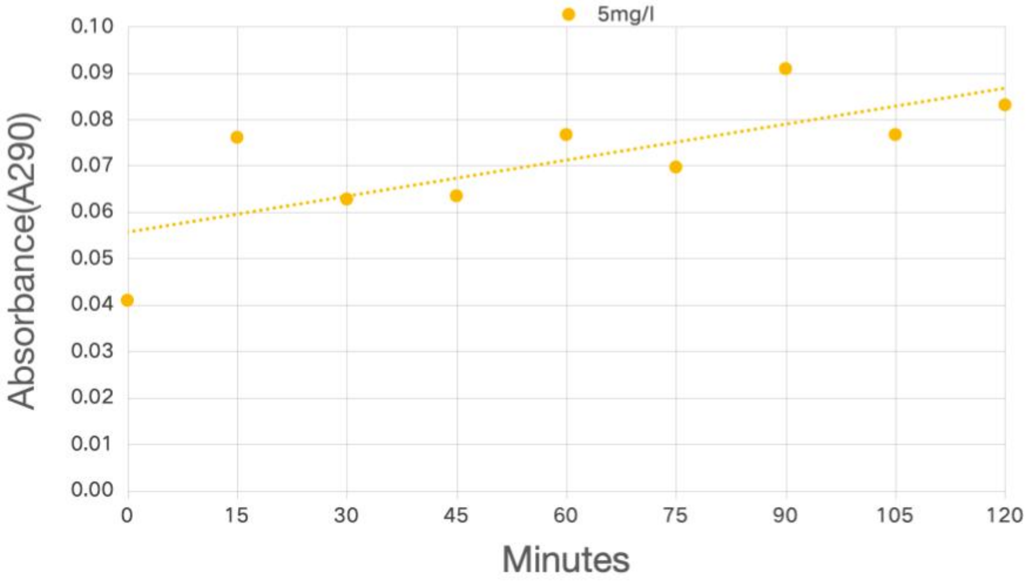
4.1.4.2 Comparative Trends of 5 mg/L E1 Absorbance Under Various Treatments



a. Algal group



b. Natural light group



c. UV light group

Figure 4.6. Comparative absorbance variation of 5 mg/L E1 solutions under three treatment conditions

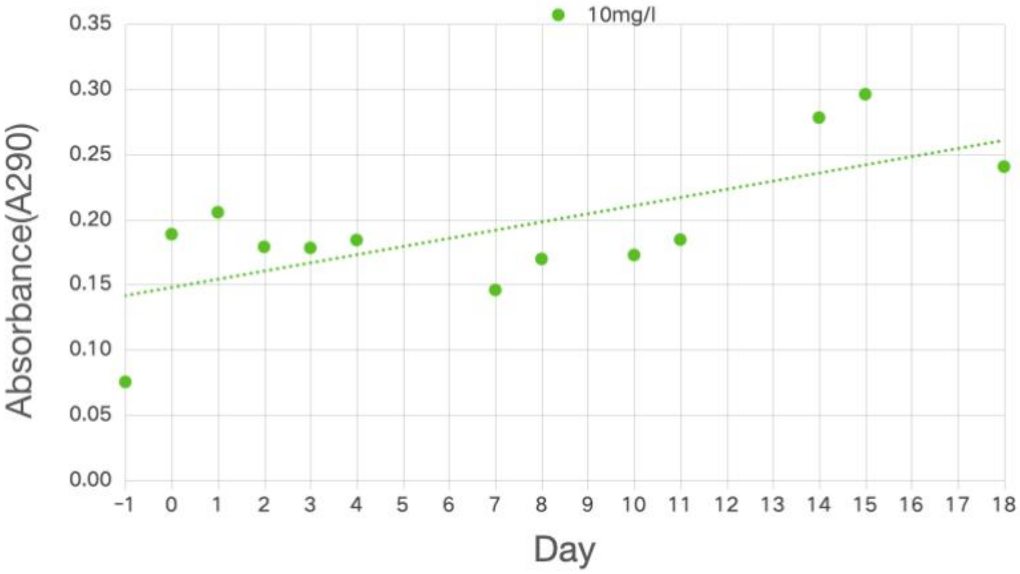
At a concentration of 5 mg/L E1, the A_{290} absorbance values showed different time trends under the three treatment conditions.

Within the algae group, At Day 0, A_{290} was 0.1411 and rose to 0.1826 on Day 1. It dropped to 0.1326 on Day 2, then slightly increased to 0.1432 on Day 3. A continuous decline followed, reaching 0.1136 on Day 7. After that, absorbance values increased again, peaking at 0.2214 on Day 10, and then gradually decreased to 0.1814 on Day 18. A clear downward trend occurred between Days 1 and 7 (0.1826 to 0.1136)

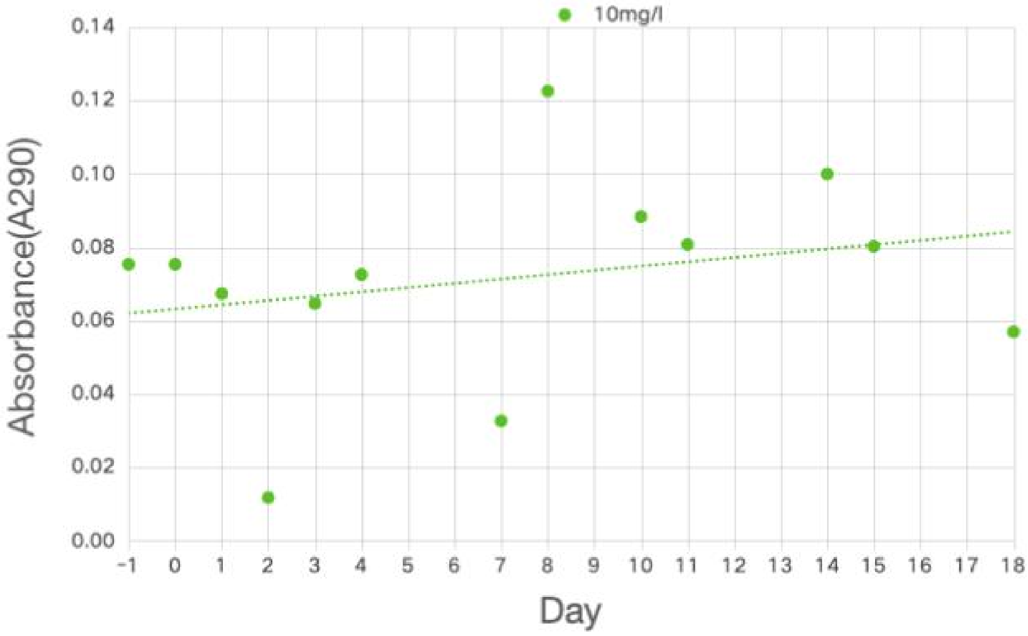
For the natural light group, starting at 0.0410 on Day 0, A_{290} slightly increased to 0.0423 on Day 1 and rose sharply to 0.0921 on Day 2. It then declined to 0.0323 on Day 3, followed by a minor increase to 0.0368 on Day 4. By Day 7, the value dropped further to 0.0215. Subsequent readings gradually increased to 0.0639 on Day 15 and slightly fell to 0.0439 by Day 18. Significant decreases were observed between Days 2 and 3 (0.0921 to 0.0323) and Days 4 to 7 (0.0368 to 0.0215).

In the UV group, A_{290} started at 0.0410 (0 min) and increased to 0.0761 at 15 min. A gradual decrease followed, reaching 0.0628 at 30 min and 0.0635 at 45 min. Afterward, values rose to 0.0767 at both 60 min and 105 min. A peak of 0.0909 was recorded at 90 min, with a slight decline to 0.0831 at 120 min. A steady decreasing trend was observed between 15 and 45 minutes (0.0761 to 0.0635).

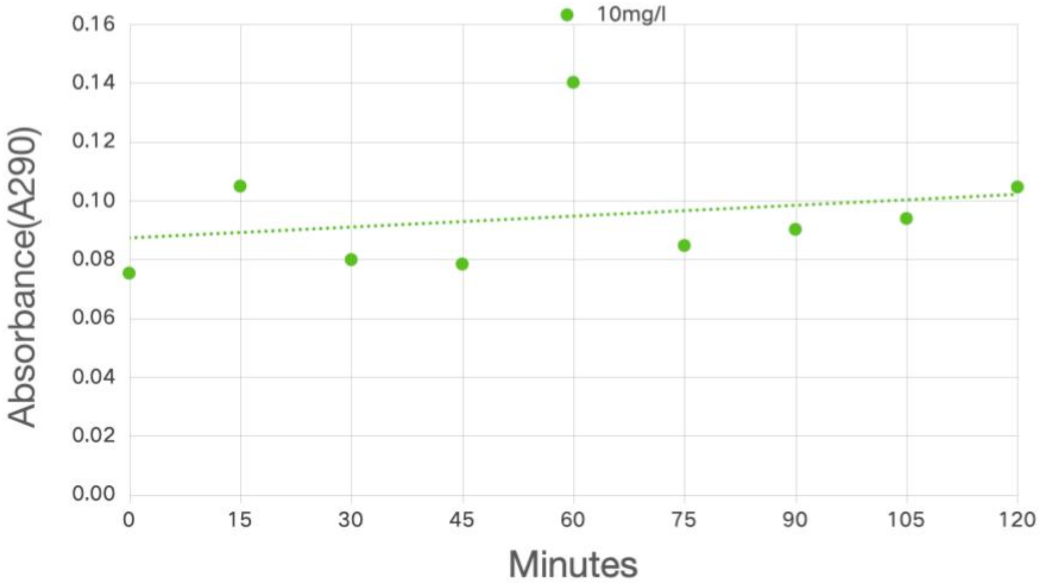
4.1.4.3 Comparative Trends of 10 mg/L E1 Absorbance Under Various Treatments



a. Algal group



b. Natural light group



c. UV light group

Figure 4.7. Comparative absorbance variation of 10 mg/L E1 solutions under three treatment conditions

At 10 mg/L concentration, lower absorbance values were recorded throughout all three doses, but they were registered at different time intervals and had different intensities.

The given graph showcasing algae treatment asserts that maximum absorbance at A_{290}

was noted on Day 1, which stood at 0.2017. After that, this reading dropped to 0.1785 on Day 2 and kept on decreasing, standing at a reading of 0.1458 on Day 7. After that time, there was a trend towards improvement, where A_{290} registered 0.1875 on Day 10, peaking high at 0.2967 on Day 15. This, however, was later followed by a downslide, where A_{290} stood at 0.2391 on Day 18. The data, therefore, show a decreasing trend from Day 1 until Day 7, followed by a downslide after peaking on Day 15.

Under natural lighting conditions, absorbance at A_{290} showed some stability between Day -1 and Day 1, with measured values ranging between 0.0758 and 0.0697. By comparison, between Day 1 and Day 2, there was a sharp fall, since absorbance dropped dramatically to 0.0113. From this nadir, absorbance values rose again, peaking at a reading of 0.1235 by Day 8, and holding at a comparatively high range throughout Day 13, ranging between 0.0877 and 0.0987. From Day 14 (0.0997) until Day 18 (0.0573), there was a sharp, progressive fall, here indicating a later phase of decline throughout the experiment course.

In the UV treatment plot, absorbance started at 0.1049, going down to 0.0784. Then it had a sudden increase, hitting 0.1402 at 60 minutes—the peak under UV. This was followed by a progressive decline: 0.0871 at 75 minutes and 0.0847 at 120 minutes. There are two decreasing phases: one between 15–45 minutes and another from 60–120 minutes.

4.1.5 Baseline-Corrected A_{290} Variation After 0 mg/L Control Subtraction

To eliminate the influence of background variation of absorbance present in the 0 mg/L control group, the raw data of A_{290} were corrected for the baseline. For a given point in time, the A_{290} reading at the 0 mg/L control (in the same state of treatment: algal, natural light, or UV light) was subtracted from the measured value at the treatment group. Baseline correction allows a clearer expression of the change of estrone (E1) absorbance occasioned specifically by the treatment manipulations.

This corrected A_{290} data via baseline was thereafter plotted to visualize and compare the net responses of E1's absorbance for different treatments and concentrations. The outcome helped better perceive the treatment-specific effect.

4.1.5.1 Algal group

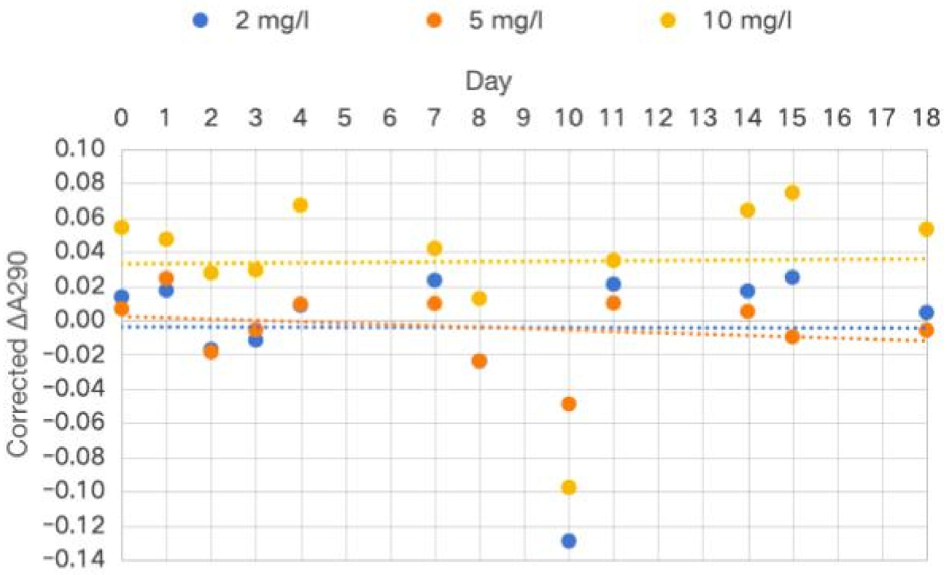


Figure 4.8. Scatter plot of corrected Δ A_{290} values after subtraction of absorbance from 0 mg/L E1 solution in the algal group

In a bid to reduce base-line interference of the 0 mg/L control, the ΔA_{290} values were corrected by subtracting the A_{290} of the control group from the relevant treatment values. Data are presented in Table A4. and shown in Figure 4.8.

Corrected ΔA_{290} values for the 2 mg/L group fluctuated near zero, with a slight increase for Days 0, 1, 7, and 11 (e.g., Day 1 = 0.0175; Day 11 = 0.0211) but were negative for Days 2, 3, 8, and 10 (e.g., Day 10 = –0.1290) showing sporadic changes in absorbance.

At the 5 mg/L group, corrected ΔA_{290} was comparatively lower, with near-zero values at Day 0 and Day 3 (0.0067 and –0.0055) and considerable reductions at Day 2 (–0.0186) and Day 10 (–0.0489) trending towards a slightly decreasing, gradual trend.

In contrast, the 10 mg/L group showed continuously-positive corrected ΔA_{290} values for most sampling days, with their maximal values higher on Days 14 and 15 (0.0643 and 0.0745) as an indicator of a sustained rise of the A_{290} absorbance relative to the control baseline.

These trends are reinforced when the individual group's linear trendlines are applied, as the 10 mg/L group demonstrates the general upward trend, the 5 mg/L group trends slightly down, and the 2 mg/L group trends sporadically with no real direction.

4.1.5.2 Natural Light Group

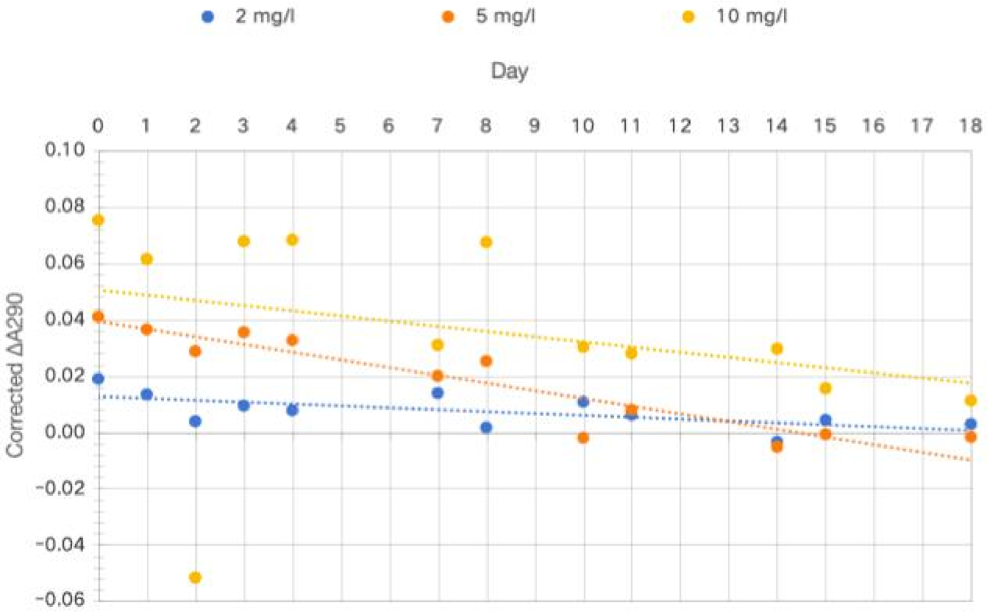


Figure 4.9. Scatter plot of corrected ΔA_{290} values after subtraction of absorbance from 0 mg/L E1 solution in the natural light group

In an attempt to lower baseline interference by the 0 mg/L control, the corrected ΔA_{290} values were found by subtracting the control group's A_{290} values from all matching treatment values. Values are listed in Table A5. and are plotted in Figure 4.9.

Corrected ΔA_{290} of the 2 mg/L group was rather stable and low, with the readings mostly remaining within the 0.01 – 0.02 interval over the observation course. Day 1 and Day 7 exhibited slight increases (0.0134 and 0.0139, respectively) while Day 10 showed a slight decline (0.0107), reflecting slight yet sustained variation of the absorbance.

In the 5 mg/L group, corrected ΔA_{290} varied considerably. While Day 0 started at 0.0410, others, such as Day 2 and Day 14, had major decreases (0.0287 and −0.0053, respectively), and Day 10 became a negative number, −0.0021. As such, a weak trend of decreasing corrected absorbance with the progression of time is found.

The 10 mg/L group, meanwhile, showed higher and longer-lasting positive values. Though a decrease was recorded on Day 2 (−0.0517), the values were mostly greater than 0.02, with considerable increments on Days 4 and 15 (0.0683 and 0.0155, respectively), which indicate stronger absorbance signals than the baseline.

The individual linear trendlines substantiate these observations: the 10 mg/L group shows a decreasing trend, the 5 mg/L group shows a gradual decline as well, while the 2 mg/L group shows relatively stable values with slight variation.

4.1.5.3 UV Light Group

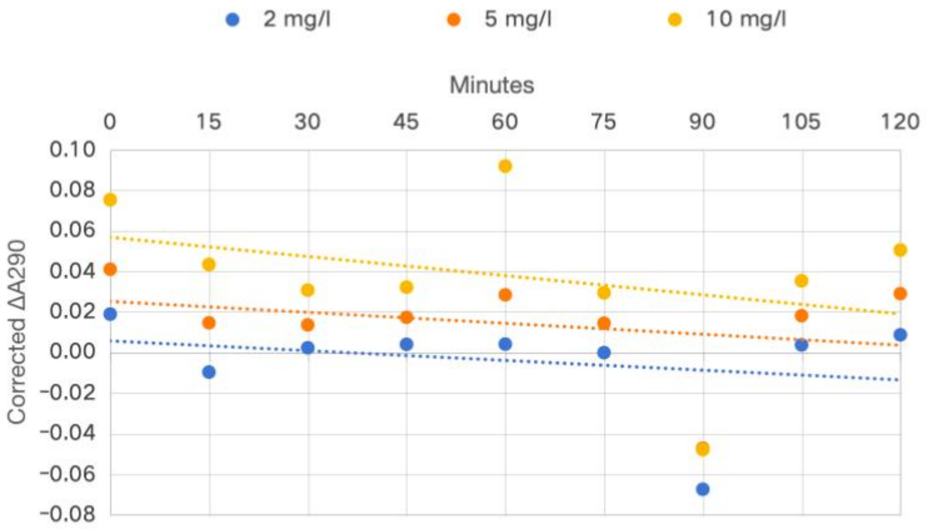


Figure 4.10. Scatter plot of corrected ΔA_{290} values after subtraction of absorbance from 0 mg/L E1 solution in the UV light group

In a bid to remove baseline interference imposed by the 0 mg/L control group, corrected Δ A_{290} values were obtained as the difference of the A_{290} of the control and the corresponding treatment group at the corresponding time point. The values are tabulated in Table A6. and a visual representation of the result can be found in Figure 4.10.

In the 2 mg/L group, the corrected Δ A_{290} values were unstable near zero, ranging from −0.0097 at 15 min to 0.0189 at 0 min. Small positive values re-appeared at 60 min (0.0041) and 120 min (0.0087), whereas slightly negative shifts were found at 15 and 30 min, indicating unstable absorbance changes compared to the

Group 5 mg/L had broadly favorable corrected Δ A_{290} values, increasing as high as 0.0284 at 60 min. There was a temporary dip at 90 min (−0.0472) but returned to 0.0290 by 120 min. This fluctuation reflects a nonlinear and somewhat oscillatory response to UV irradiance.

The 10 mg/L group had overwhelmingly positive corrected Δ A_{290} values through the time points, peaking at 60 min (0.0919). There was a negative shift at 90 min (−0.0479) with a recovery to 0.0505 by 120 min. While these shifts were noteworthy, the net change of absorbance compared with baseline remained significant.

Also, all three concentration group linear trendlines were negatively sloped, which meant that, though temporarily increasing, UV irradiation actually produced a net A_{290} decrease with time.

4.2 Estrone (E1) Removal Efficiency of Second-Round Experiments

To compare the degradation or removal efficiency of estrone (E1) when subjected to various environmental exposure conditions, this section provides a quantitative comparison using absorbance data (A_{290}) over time for three different treatment groups: the algal group, the natural light group, and the UV light group.

The absorbance at 290 nm was taken as an indirect measure of E1 concentration. For every group, the measured absorbance on Day –1 (before algae addition or light exposure) was set as the baseline value (A_0). The relative removal efficiency was then calculated by comparing subsequent absorbance values at each sampling time (A_{290}) to A_0 using the following equation:

$$\text{Removal}(\%) = \left(1 - \frac{A_{290}}{A_0}\right) \times 100\%$$

Where:

- A_0 is the initial absorbance (Day –1 baseline)

- A_{290} is the absorbance observed at each subsequent time point

This percentage is the extent to which E1 was removed under each treatment condition and provides a basis for performance comparison.

For the 0 mg/L group, in which $A_0 = 0$, percent removal is not applicable. Instead, the change in absorbance ($\Delta A_{290} = A_{290} - A_0$) provides a representative system background fluctuation measure.

The results are explained in the following sub-tables:

Table A7.: Algal group

Table A8.: Natural light group

Table A9.: UV light group

Each table includes the initial absorbance (A_0), absorbance reading (A_{290}), change in absorbance (ΔA_{290}), and removal efficiency calculated (wherever relevant).

Results

4.2.1 E1 Removal in the Algal Group

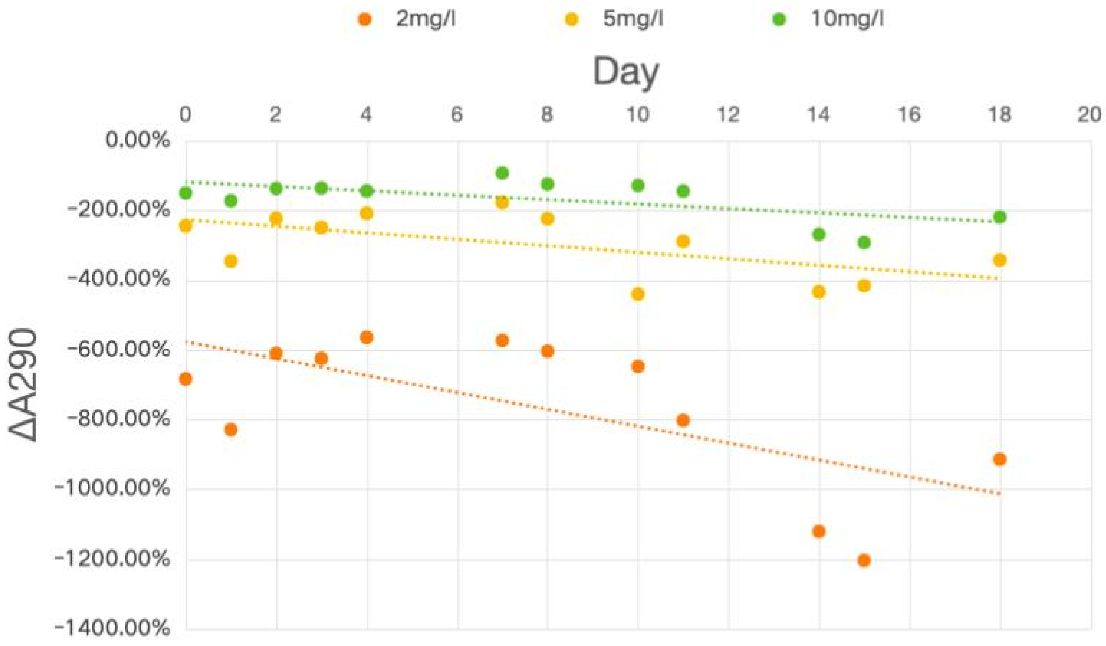


Figure 4.11. Time-course of E1 removal (%) at different initial concentrations under algal treatment

0 mg/L Group (Blank Control)

On Day 0, initial absorbance (A_0) was 0.0000. A_{290} continuously increased over the 18-day duration, peaking at 0.2703 on Day 10. Then it decreased slightly to 0.2214 (Day 15) and 0.1872 (Day 18). ΔA_{290} varied from +0.1344 to +0.2703. As $A_0 = 0$, removal rate is not applicable ("—") in this group. The continuous rise in A_{290} suggests the build-up of UV-absorbing substances from background and algal metabolites, even in the absence of E1 addition.

2 mg/L Group

$A_0 = 0.0189$. A_{290} increased immediately on Day 0 to 0.1481 ($\Delta A_{290} = +0.1292$, removal = −683.60%). Absorbance continued to increase to 0.2308 on Day 14 (removal = −1121.16%) and reached its peak on Day 15 at 0.2465 (removal = −1204.23%). The most negative removal rate (i.e., best removal) was on Day 7, with $A_{290} = 0.1272$, $\Delta A_{290} = +0.1083$, removal = −573.02%.

5 mg/L Group

Results

A_0 = 0.0410. A_{290} on Day 0 was 0.1411 (ΔA_{290} = +0.1001, removal = −244.15%). A_{290} fluctuated between 0.1136 and 0.2599 over time. Maximum removal (least negative) was on Day 7, A_{290} = 0.1136 (ΔA_{290} = +0.0726), removal = −177.07%. The highest A_{290} was on Day 15 (0.2599, removal = −533.90%).

10 mg/L Group

Initial A_0 = 0.0753. On Day 0, A_{290} = 0.1886 (removal = −150.46%). The most negative removal rate was on Day 7, where A_{290} = 0.1458 (ΔA_{290} = +0.0705), removal = −93.63%, the least negative value in this group. Then, absorbance was at its peak on Day 14 at 0.2780 (removal = −269.19%) and fell slightly to 0.2404 on Day 18 (removal = −219.26%).

In all concentration groups (2, 5, 10 mg/L), the optimal removal effect (i.e., lowest negative removal rate) was achieved on Day 7 (2025/5/5). Although all removal values were negative, this signifies a momentary slowdown in absorbance accumulation. The 10 mg/L group exhibited the largest relative removal (−93.63%), followed by 5 mg/L (−177.07%) and 2 mg/L (−573.02%)

Throughout all three levels of concentration in the algal treat group, readings of ΔA_{290} always remained adverse throughout all experiments, indicating a continued rise in absorbance of A_{290} throughout all experimental treatements. This finding indicates a limited efficiency towards abolishing E1 in algal interaction.

Maximum negative deviation, and hence minimum removal efficiency compared to other tested concentrations, occurred at a concentration of 2 mg/L, where ΔA_{290} reduced from –683.60% on day 0 to –1204.23% on day 15.

In Group 5 mg/L, reduction was much less evident, starting at a ΔA_{290} of –244.15% on Day 0 and then increasing to –416.34% on Day 15. The process states a more rapid increase in absorbance.

A variation of ΔA_{290} between – 150.46% and –292.96% at certain time points, that declined constantly to –93.63% on Day 7, were exhibited by the group exposed to a concentration of 10 mg/L. The finding states a relatively stable tendency under high exposure doses.

4.2.2 E1 Removal in the Natural light Group

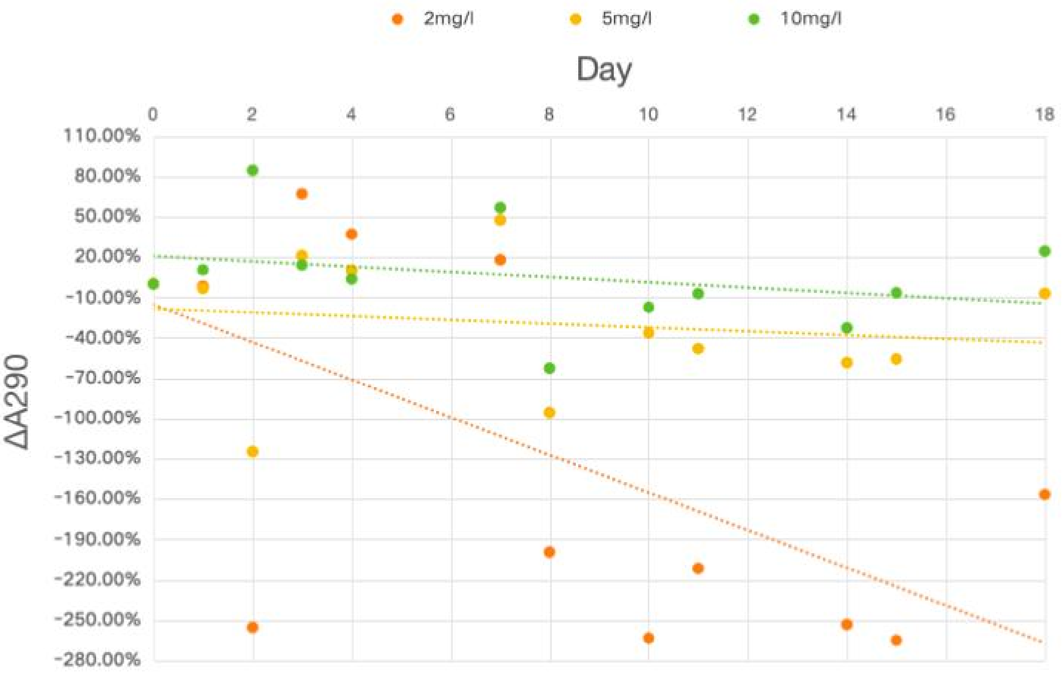


Figure 4.12. Time-course of E1 removal (%) at different initial concentrations under natural light treatment

0 mg/L Group （Blank Control）

The A_0 (initial absorbance) was 0.0000. A_{290} increased steadily with time to 0.0703 on Day 14 and decreased slightly to 0.0457 on Day 18. ΔA_{290} was positive throughout the test and ranged from +0.0042 to +0.0703. Removal rate was not calculated due to an undefined denominator. This trend shows the gradual accumulation of UV-absorbing material from the algal matrix or from external environmental influence.

2 mg/L Group

Initial A_0 = 0.0189. A_{290} initially increased to 0.0672 on Day 2 (removal = −255.56%) and then dropped sharply to 0.0062 on Day 3, providing the peak removal rate of +67.20%. A_{290} then recovered and remained variable thereafter: 0.0119 on Day 4 (removal = 37.04%), 0.0566 on Day 8 (removal = −199.47%), and 0.0485 on Day 18 (removal = −156.61%). Overall, absorbance readings fluctuated from 0.0062 to 0.0687.

Results

5 mg/L Group

A_0 = 0.0410. A_{290} rose initially on Day 1 to 0.0423 (removal = −3.17%) and fluctuated thereafter. The lowest A_{290} (0.0215) was observed on Day 7, which translates to a maximum removal of +47.56%. The absorbance rose significantly later to 0.0802 on Day 13 (removal = −95.61%) prior to falling marginally to 0.0439 on Day 18. ΔA_{290} ranged from −0.0195 to +0.0392.

10 mg/L Group

A_0 = 0.0753. A_{290} reduced on Day 2 to the minimum value of 0.0117, with the maximum removal rate of +84.46%, the best among all groups. A_{290} then increased and peaked on Day 10 at 0.0883 (removal = −17.26%) and ended at 0.0569 on Day 18 (removal = +24.44%). Removal rates were also positive on Day 3 (+14.21%) and Day 18 (+24.44%).

The maximum removal efficiency was at Day 3 of 2 mg/L (67.20%), Day 7 of 5 mg/L (47.56%), and Day 2 of 10 mg/L (84.46%). Positive removal was observed at specific time points in all concentration groups.

Under natural light, significant changes of ΔA_{290} values of the three concentrations of E1 were found, which in normal conditions always were negative, confirming the A_{290} recovery of absorption with a low efficiency of E1 removal.

Below 2 mg/L concentration, it was seen that a highly fluctuating pattern of removal was followed. ΔA_{290} reduced to –255.56% on Day 2, reached +67.20% on Day 3, then reduced again, ultimately reaching –265.08% on Day 15.

At 5 mg/L, ΔA_{290} varied from –124.63% (Day 2) to +47.56% (Day 7). But from Day 7, values slowly settled between –40.00% and –80.00%, indicating a trend of moderate removal.

At 10 mg/L, ΔA_{290} values were most fluctuated, from –62.86% on Day 8 to +84.46% on Day 2. Even though phase I is high variation, follow-up readings did indicate stabilization in the rather narrow range of about –5% to –30%, which means there is a stabilization effect that occurs with some time lag.

4.2.3 E1 Removal in the UV light Group

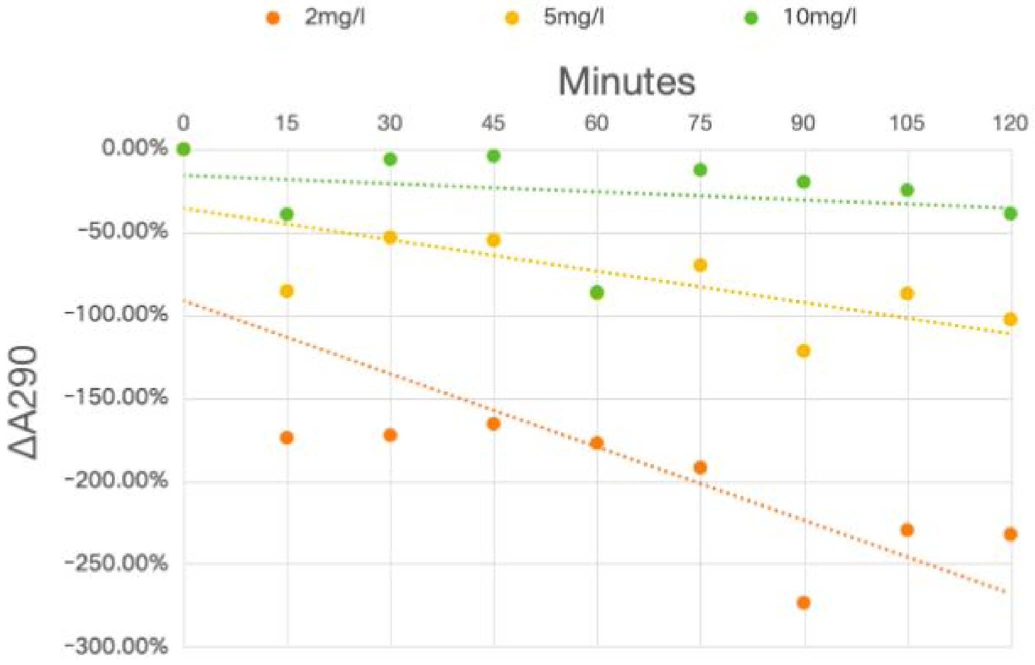


Figure 4.13. Time-course of E1 removal (%) at different initial concentrations under UV light treatment

0 mg/L (Blank Control)

A_0 = 0.0000. A_{290} rose gradually throughout the 120 min interval with a maximum of 0.1381 at 90 min. ΔA_{290} was positive throughout, and removal rate was calculated because of zero initial absorbance.

2 mg/L group

A_0 = 0.0189. A_{290} rose steadily throughout all time points, reaching a maximum of 0.0706 at 90 min (ΔA_{290} = +0.0517, Removal = −273.54%). The least negative removal was at 45 min, when A_{290} = 0.0502 (ΔA_{290} = +0.0313, Removal = −165.61%).

5 mg/L group

A_0 = 0.0410. A_{290} rose over time to the highest value of 0.0847 at 75 min (ΔA_{290} = +0.0437, Removal = −106.59%). The least negative removal was at 30 min, where A_{290} = 0.0606 (ΔA_{290} = +0.0196, Removal = −47.80%), which was the best removal result in this group.

Results

10 mg/L group

A_0 = 0.0753. A_{290} reached a peak of 0.1402 at 60 min (ΔA_{290} = +0.0649, Removal = −86.19%). The minimum negative removal was at 45 min, with A_{290} = 0.0784 (ΔA_{290} = +0.0031, Removal = −4.12%), being the best in all groups.

All of the UV-treated samples had positive ΔA_{290} values and negative removal rates. The most (least negative) removal was observed in the 10 mg/L group at 45 min (−4.12%), followed by the 5 mg/L group at 30 min (−47.80%), and the 2 mg/L group at 45 min (−165.61%). A_{290} values generally peaked between 60–90 min.

Ultraviolet irradiation always yielded negative values for ΔA_{290} for all three concentration regimes, indicating increased absorbance and retardation of degradation of E1. However, there was no clear trend evident; instead, there were increasingly decreasing ΔA_{290} values within the time interval of 15 to 45 minutes, indicating there exists a transient phase in the degradation process.

ΔA_{290} of sample 2 mg/L showed a rise in increase from − 174.07% after 15 minutes to − 165.61% after 45 minutes, showing improvement in removal efficiency for this time interval.

For the 5 mg/L group, ΔA_{290}, which had -85.61% at 15 minutes, that had risen to -54.88% at 45 minutes, and that rise became statistically significant.

10 mg/L group: ΔA_{290} varied from − 39.31% at 15 minutes to − 4.12% at 45 minutes, with the most apparent improvement in removal at higher concentration.

Overall, the 15- 45- minute window reflects a common period of enhanced photolytic degradation across all E1 levels under UV exposure. After this interval, ΔA_{290} values began to drop again, suggesting the reaccumulation of intermediate products or decreased reaction efficiency.

4.2.4 Comparative Analysis of Maximum E1 Removal through Various Treatments

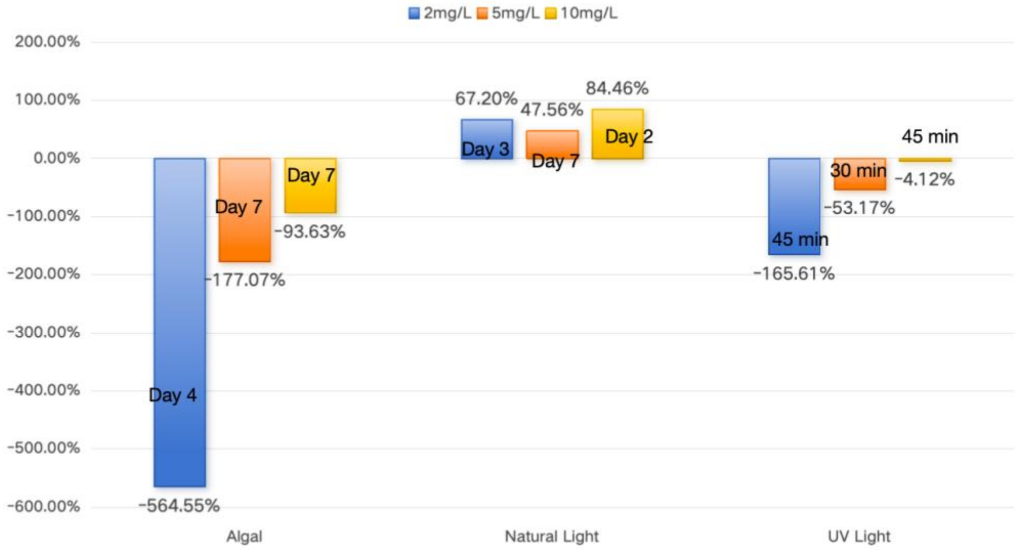


Figure 4.14. Maximum removal efficiencies (%) of E1 solutions at different initial concentrations and corresponding time points under algal, natural light, and UV treatments

Table A10. and Figure 4.14 illustrate maximum calculated removal efficiency for E1 in three types of treatments (algal, natural light, and UV light) for 2, 5, and 10 mg/L. The maximum results are in the form of highest A_{290} reduction in each group, expressed as percent removals in comparative bar chart form.

The maximum removals in the natural light group were during the initial phase (Day 2 – 7), with:

2 mg/L achieving up to 67.20% on Day 3

5 mg/L at the peak value of 47.56% on Day 7, and

10mg/L at the maximum result of 84.46% on Day 2.

The UV trials, with their much- reduced timescale, also indicated definite peak removal values:

2 mg/L at – 165.61% after 45 minutes,

5 mg/L at −53.17% after 30 minutes, and

10 mg/L at −4.12% at 45 minutes, the most consistent of the UV tests.

Among the algal community, removal maxima were all negative and showed a consistent rise in A_{290} absorbance during exposure:

2 mg/L attained −564.55% on Day 4,

5 mg/L attained −177.07% on Day 7, and

10 mg/L was −93.63%, again on Day 7.

Removal values for all were calculated in terms of A_{290} absorbance trends.

4.3 Light Intensity Distribution

The environmental conditions controlling the removal of estrone (E1) were also elucidated through measurements of light intensity around experimental bottles under algal and non-algal growth conditions. Analysis of the manner by which algae manage light penetration and angular dispersion is required so that the efficacies of removal seen are understood. Lux measurements have been made at angles of 0 ˚ through 180 ˚ and path lengths of up to 10 cm. Results are shown separately for bottles containing algae (Section 4.3.1) and algae-free bottles (Section 4.3.2) so that comparisons of light attenuation can be made directly.

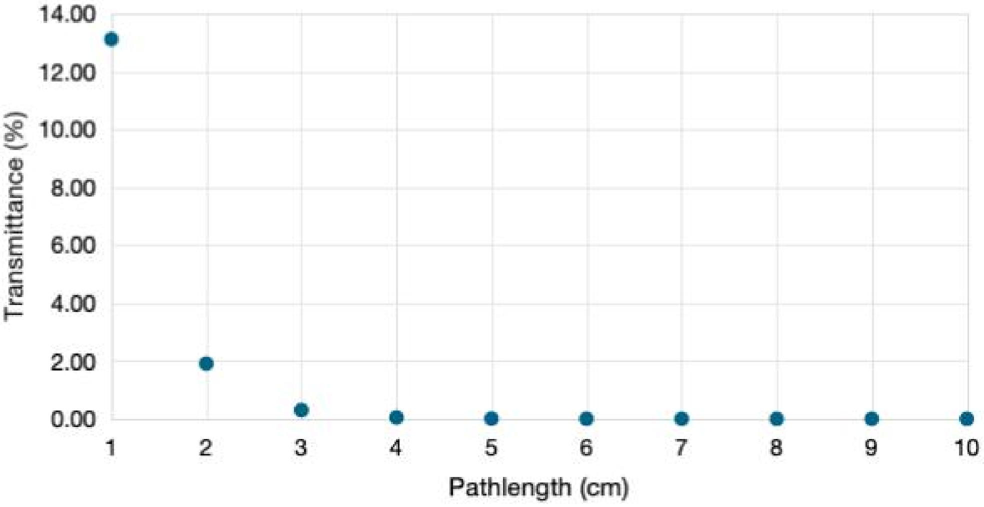


Figure 4.15. Light transmittance (%) as a function of optical path length

Prior to analyzing the angular distribution of light, total transmittance through various pathlengths was determined (Table A11.). The results indicate that light penetration decreased substantially with increasing depth, inasmuch as transmittance decreased from 13.11% at 1 cm to less than 1% at 3 cm and approximately 0% at more than 5 cm. Because values were appreciably comparable between algal and non-algal bottles, only one of the data sets appears this chapter.

Results

4.3.1 With Algal

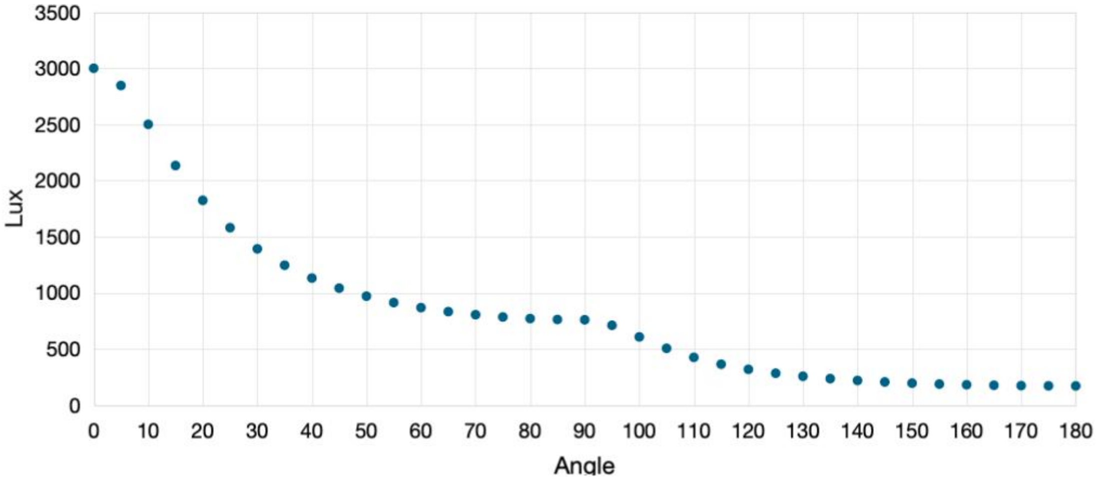


Figure 4.16. Angular distribution of light intensity (lux) under algal treatment

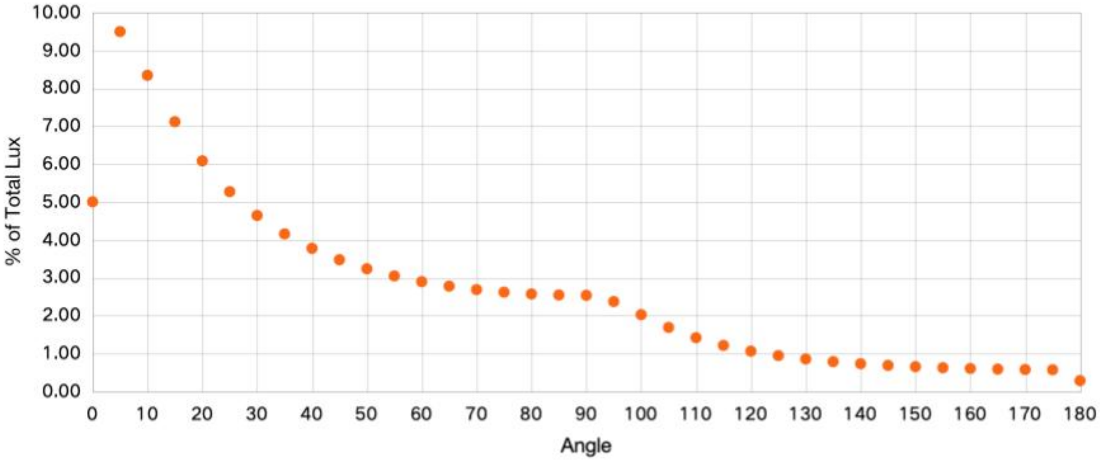


Figure 4.17. Percentage contribution of light intensity to total lux as a function of angle under algal treatment

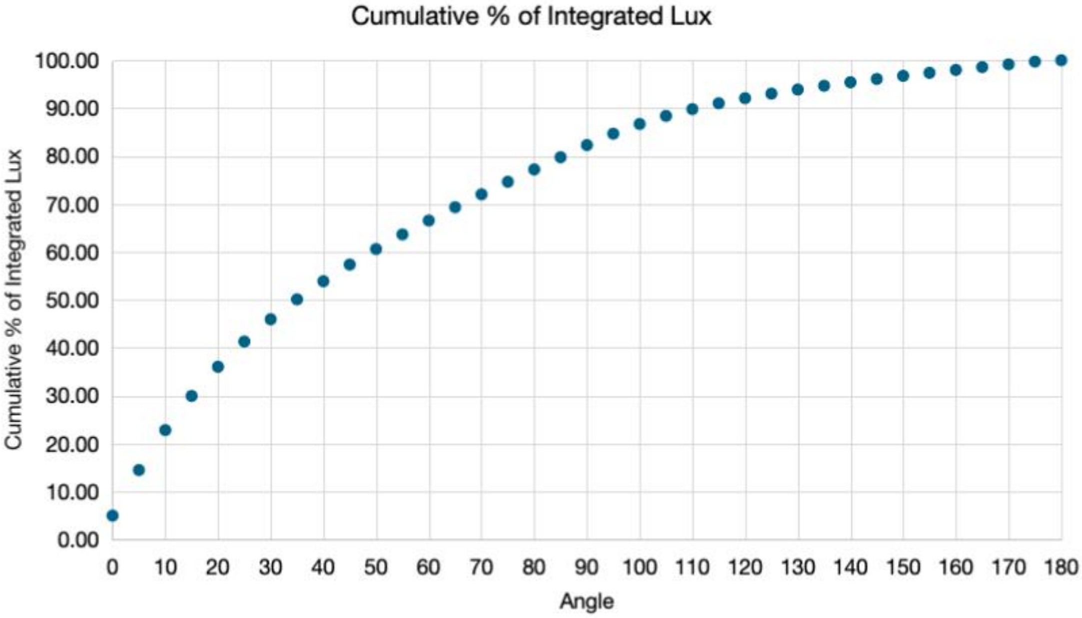


Figure 4.18. Cumulative percentage distribution of integrated light intensity as a function of angle under algal treatment

Table A12. and Figures 4.16-4.18 presents light intensity angular distribution around algal bottles. The highest reading of lux was at 0° (3000), and the intensity declined steadily with the increased angle, to 171 at 180°. Pair symmetry of lux values is typical of paired contribution at opposite angles, the sum of total integrated lux at all angles aggregating 59,946, and of mean lux contributing 833. Percentage contribution of each angle to the integrated lux was at 5.00% at 0°, declined below 1% at angles greater than 125°, and at 180° was at its lowest of 0.29%. Cumulative percent rose linearly with angle, at 35° at 50% and at 75° at 75%, at 110° at 90%, and at 175–180° at 100%. The finding indicates that the majority of the integrated light intensity was found at the lower angular range, with the contribution of the higher angles progressively minor.

The contribution of integrated intensity increased monotonically with angle. 50% integrated lux was reached at 35°; 75% was the ratio at 75°, and 90% was reached at about 110°. The curve then leveled out to almost 100% at 175–180°.

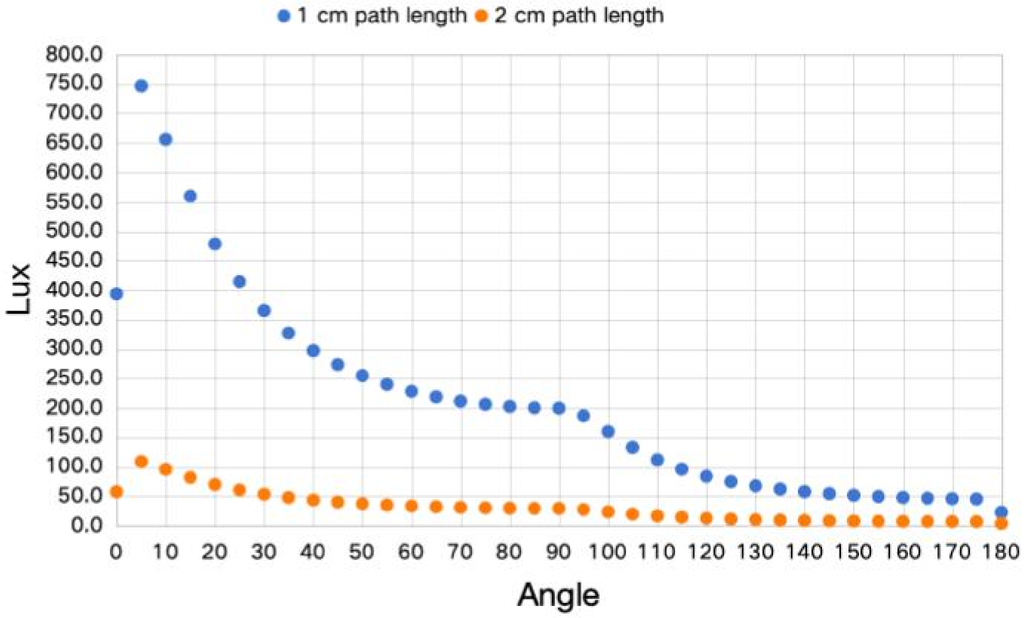


Figure 4.19. Angular distribution of light intensity (lux) at 1 cm and 2 cm optical path lengths in the algal system

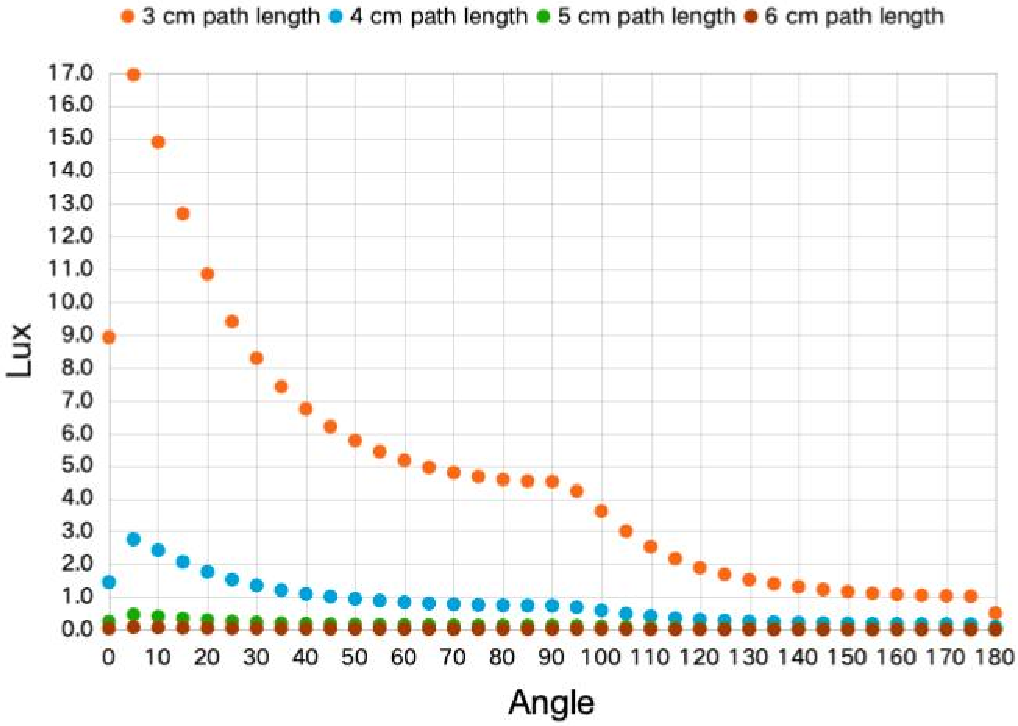


Figure 4.20. Angular distribution of light intensity (lux) at 3-6 cm optical path lengths in the algal system

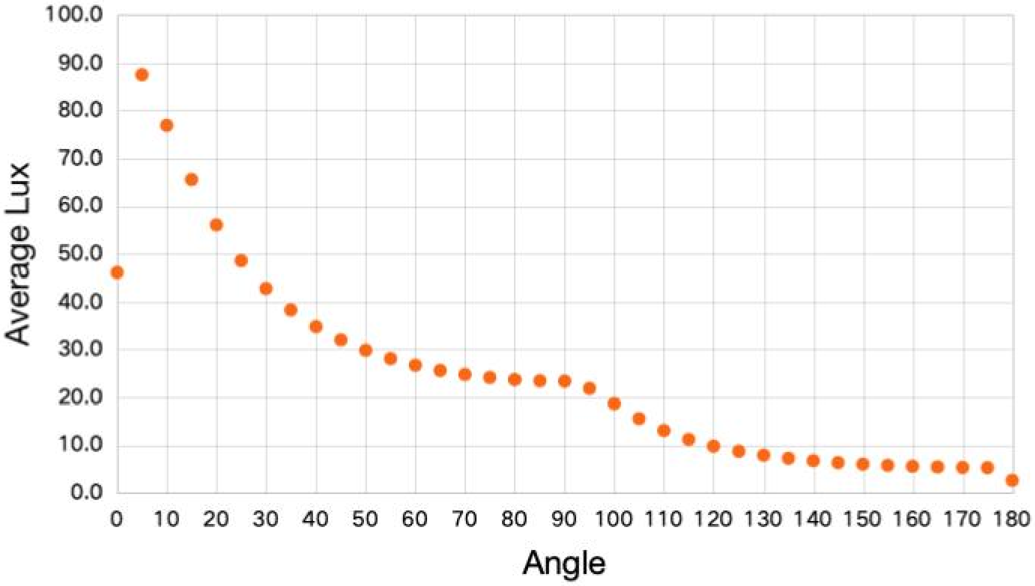


Figure 4.21. Average angular distribution of light intensity (lux) across all path lengths in the algal system

As Table A13. and Figures 4.19-21 shows that lux readings of the algal bottles dropped with increasing pathlength. At 1 cm, they spanned from 22.4 lux at 180° to 746.5 lux at 5°, with maximum values always at small forward angles. By 3 cm, values were always <20 lux, while at 5 cm, readings were typically ≤1 lux. Weighings of >6 cm path lengths were always negligible at all angles. The average lux by angle (or row mean by depths) dropped from 87.5 lux at 5° to 2.6 lux at 180°. When averaged across all angles and path lengths, the mean intensity was 24.9 lux across all.

4.3.2 Without Algal

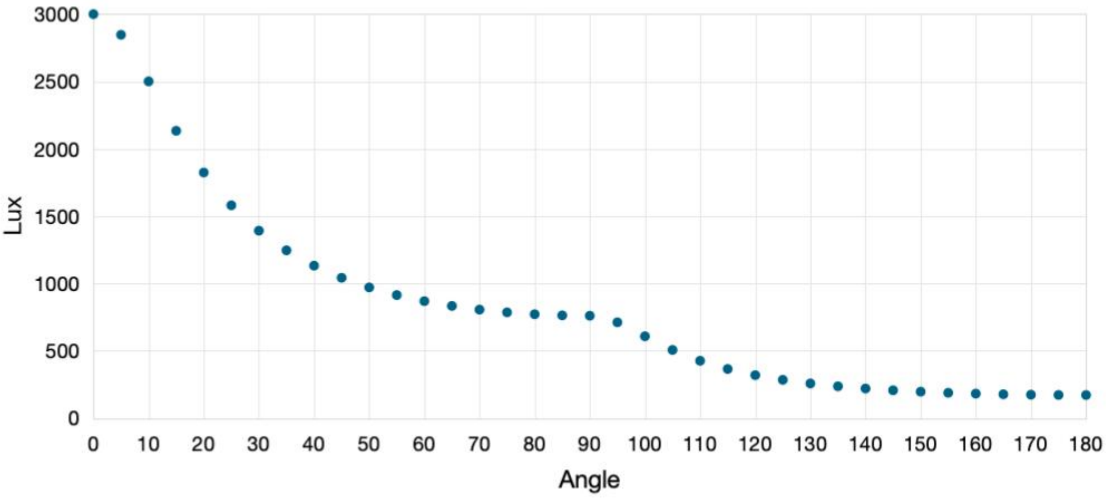


Figure 4.22. Angular distribution of light intensity (lux) without algal

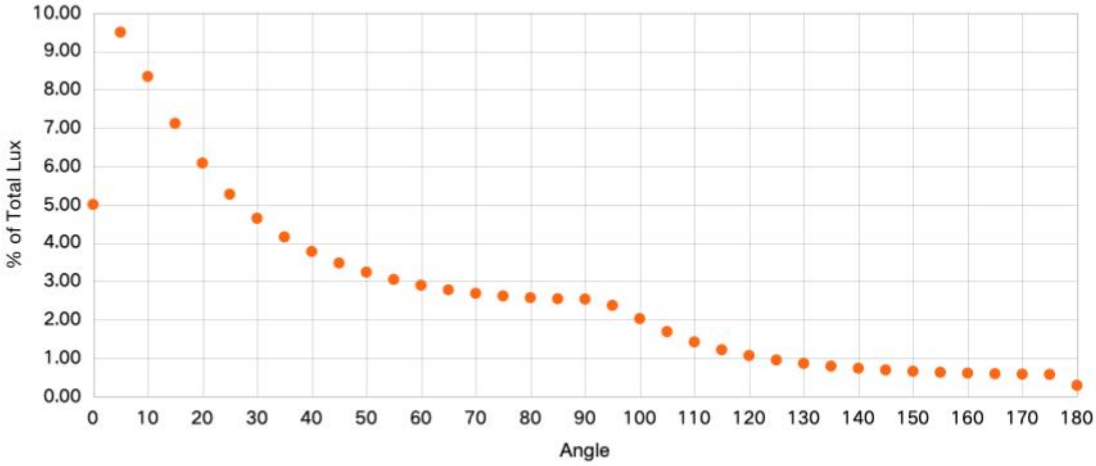


Figure 4.23. Percentage contribution of light intensity to total lux as a function of angle without algal

The non-algal bottles' peak angular light distributions are as follows in Table A14. and Figures 4.22-4.23. The peak intensity was a maximum at 0° at 3000 lux, and values went down gradually with angle to 171 lux at 180°. Symmetrical Lux values, i.e., the sums of angle pairs, summed to a sum of integrated lux of 59946 lux with a mean of 833 lux over all angles. Contributions by percent by angle ranged from 9.50% at 5° down through less than 1% at angles greater than 125°, with the minimum contribution of 0.29% at 180°. The cumulative percent of integrated lux summed up gradually with angle, 50% at 35°, 75% at 75°, 90% at 110°, and 100% at

175–180°.

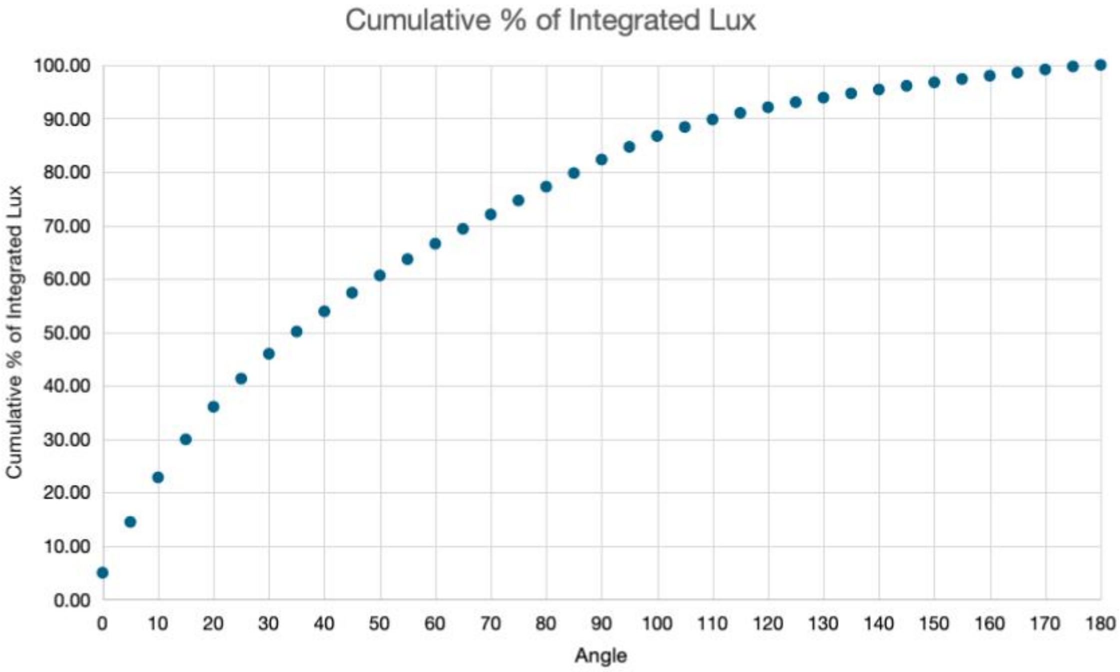


Figure 4.24. Cumulative % of integrated lux without algae

Figure 4.24. illustrates the percent accumulated contribution of integrated lux of algae-free bottles. The accumulated contribution was steeper at the low angles, near 50% at 35° and near 75% at 75°. At 110°, just above 90% of the integrated total lux was explained, and the curve was at a plateau thereafter, near 100% at 175–180°.

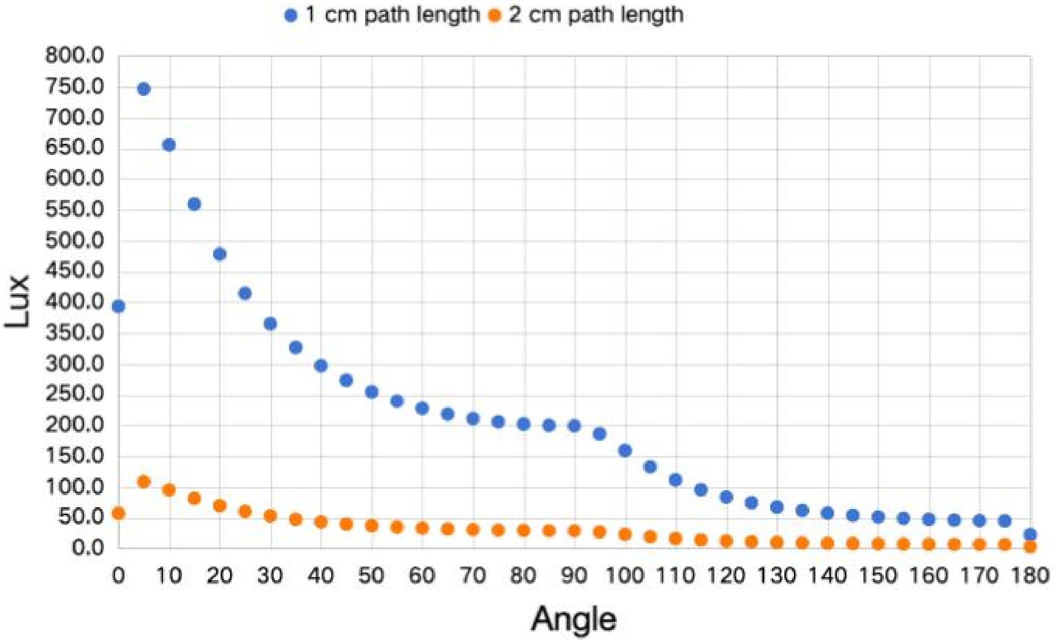


Figure 4.25. Angular distribution of light intensity (lux) at 1 cm and 2 cm optical path lengths in the non- algal system

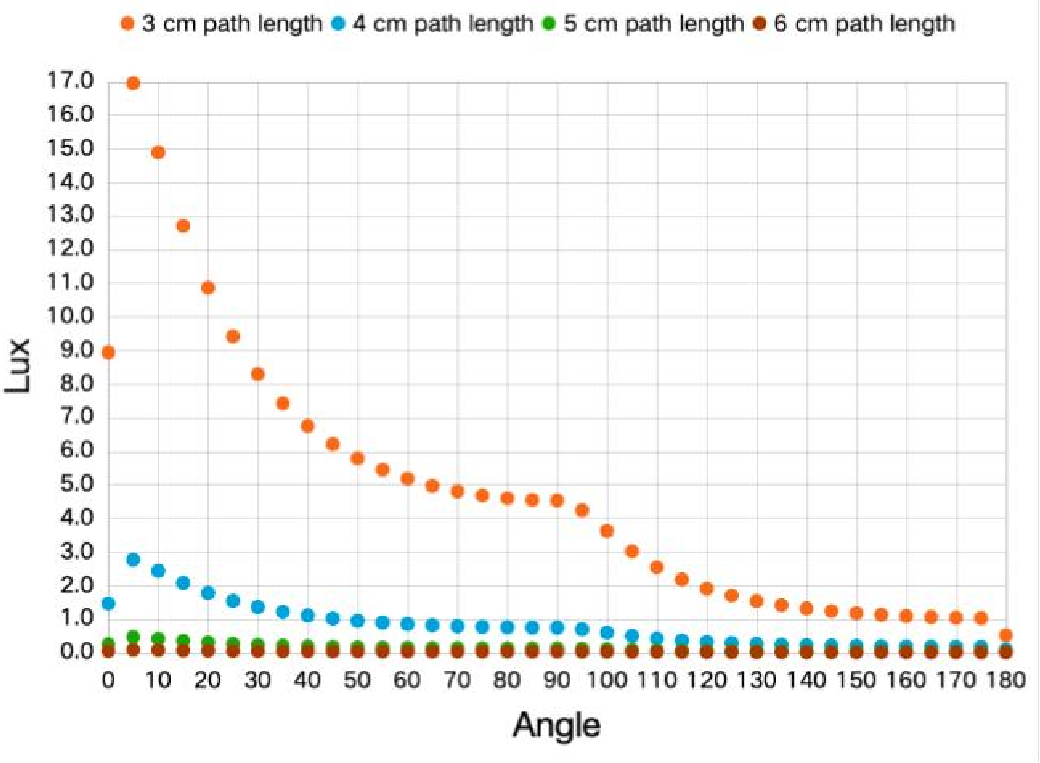


Figure 4.26. Angular distribution of light intensity (lux) at 3-6 cm optical path lengths in the non- algal system

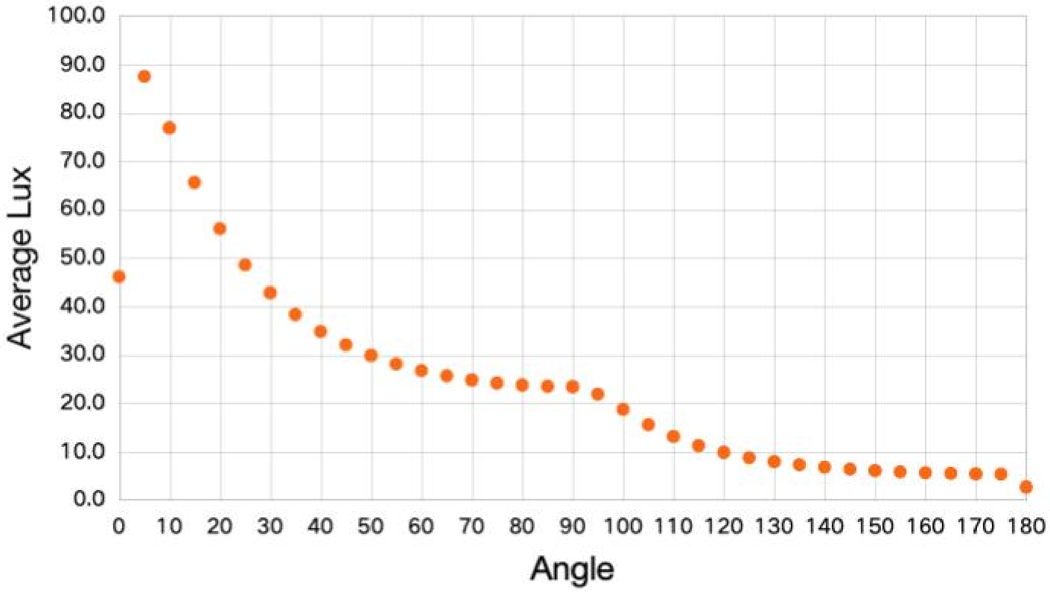


Figure 4.27. Average angular distribution of light intensity (lux) across all path lengths in the non- algal system

Lux in non-algal bottles fell systematically with pathlength. At 1 cm, there were between 22.4 lux at 180 ° and 746.5 lux at 5 ° , with maximum values at angles close to forwards. At 3 cm, most of the values were <20 lux, and at 5 cm, nearly all values were ⩽1 lux. At >6 cm, light intensities were low in all angles. Row means fell from 87.5 lux at 5 ° through 2.6 lux at 180 ° , with general mean of 24.9 lux at all angles and depths.

4.4 Short-term Behavior of E1 under Algal Conditions at pH 7

To gain more details of the short-term reactions of E1 under algal conditions, more experiments were conducted based on the design laid out in Section 3.3.2. The tests were conducted at pH 7 On 15 July, one reading of absorbance was taken at each of the different initial concentrations of E1, and pH was simultaneously measured. On 16 July, replicates of absorbance reading were taken 5 times at each sample, while pH was simultaneously measured once at each sample.

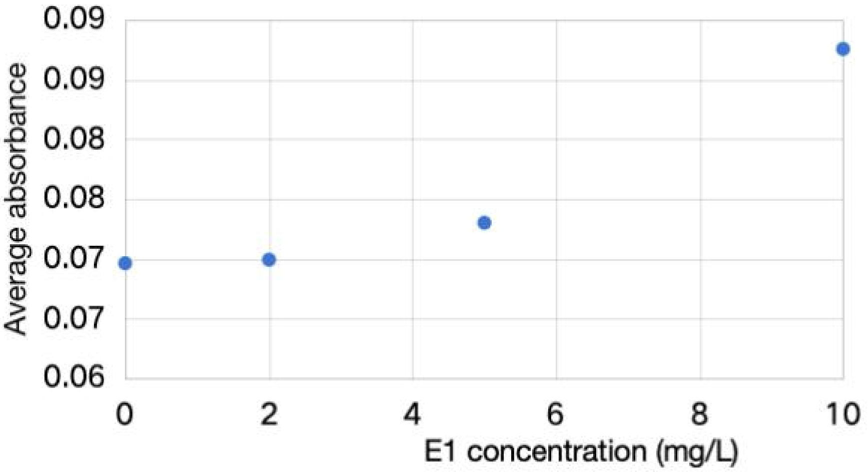


Figure 4.28. Average absorbance (A_{290}) of E1 solutions with different initial concentrations on 15 July 2025

Table A16. lists absorbance (A_{290}) values that were quantified after measuring estrone (E1) solutions at pH 7 on 15 July. Five sets of samples were tested. Each set included four E1 solutions with 0mg/L, 2 mg/L, 5mg/L, and 10mg/L concentrations. One reading was taken at each of the initial concentrations through five sets of experiments. Figure 4.28. shows that the mean absorbance values that were found increased with growing concentrations of E1, from 0.0696 at 0 mg/L up to 0.0876 at 10 mg/L.

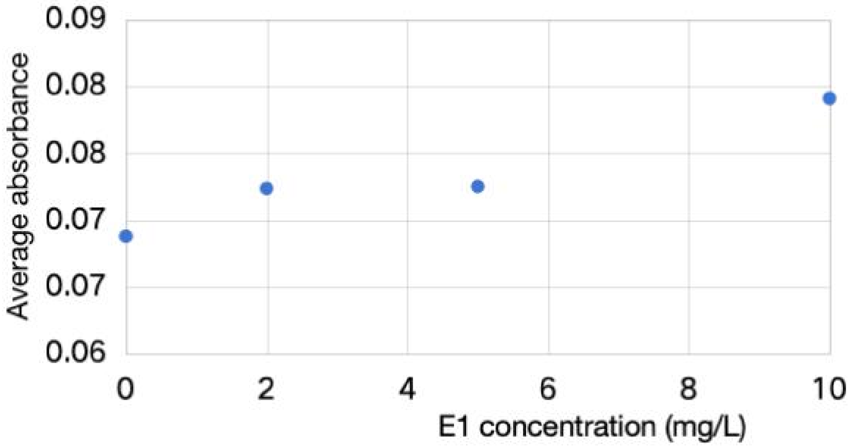


Figure 4.29. Average absorbance (A_{290}) of E1 algal solutions with different initial concentrations after overnight incubation (16 July)

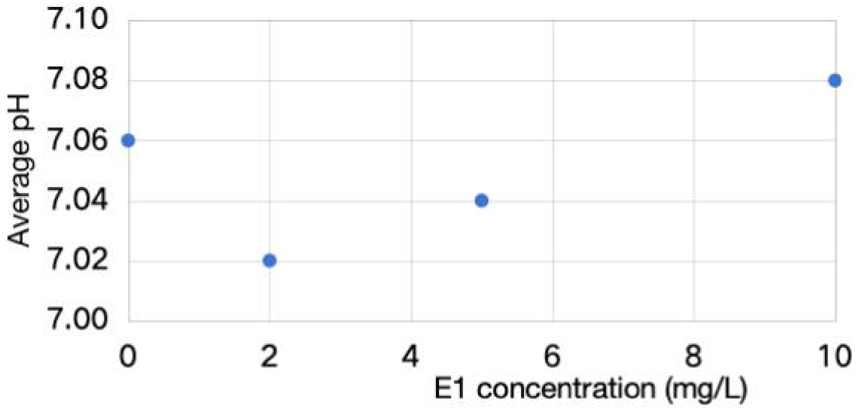


Figure 4.30. Average pH of E1 algal solutions with different initial concentrations after overnight incubation (16 July)

Results

Table A17. includes overnight absorbance measurements, absorbance measurements at 290nm, and pH of solutions of estrone (E1), which had been prepared initially at pH 7. There were 5 sample sets, which were run with each of the sets having four solutions of E1 at 0mg/L, 2 mg/L, 5mg/L, and 10mg/L concentrations. Comparatively with the individual measurements, which were made on 15 July, duplicate measurements made on 16 July had five replicate absorbance measurements and one pH reading of each of the samples. The average absorbance values increased slightly with E1 concentration of E1 from 0.0688 at 0 mg/L concentration of E1 up through 0.0791 at 10 mg/L concentration of E1. The pH was near neutral but variable slightly across sets from 7.02 to 7.08. These are short-term changes in the data that arose after overnight standing.

Absorbances at 15 July (Table A16.) were 0.0696 at 0 mg/L and 0.0876 at 10 mg/L. The replicate measurements after overnight standing on 16 July (Table A17.) had slightly higher means at the lower levels and more sets that agree with one another. The mean absorbance values were 0.0688 at 0 mg/L, 0.0724 at 2 mg/L, 0.0725 at 5 mg/L, and 0.0791 at 10 mg/L. As with absorbance, pH was stable near neutral over the two days but shifted slightly: pH was 7.0 on 15 July, but on 16 July, values ran slightly from 7.02 through 7.08, depending upon sets. These data suggest lightly overnight changes both in absorbance and pH, but steadily trending values through those concentrations.

4.5 Effect of pH on E1 Removal at 10 mg/L under Algal and Natural Light Conditions

To eliminate the impact of pH at constant contaminant concentration, time-series tests have been conducted at an initial concentration of E1 of 10 mg/L at four regimes of pH values of 5.0, 6.0, 7.0, and 8.0, and two treatability types, namely algae-contained bottles and light-exposure bottles without algae. The absorbance at the wavelength of 290nm (A_{290}) and fluctuations of pH have been observed in 31 days.

4.5.1 Algal Group

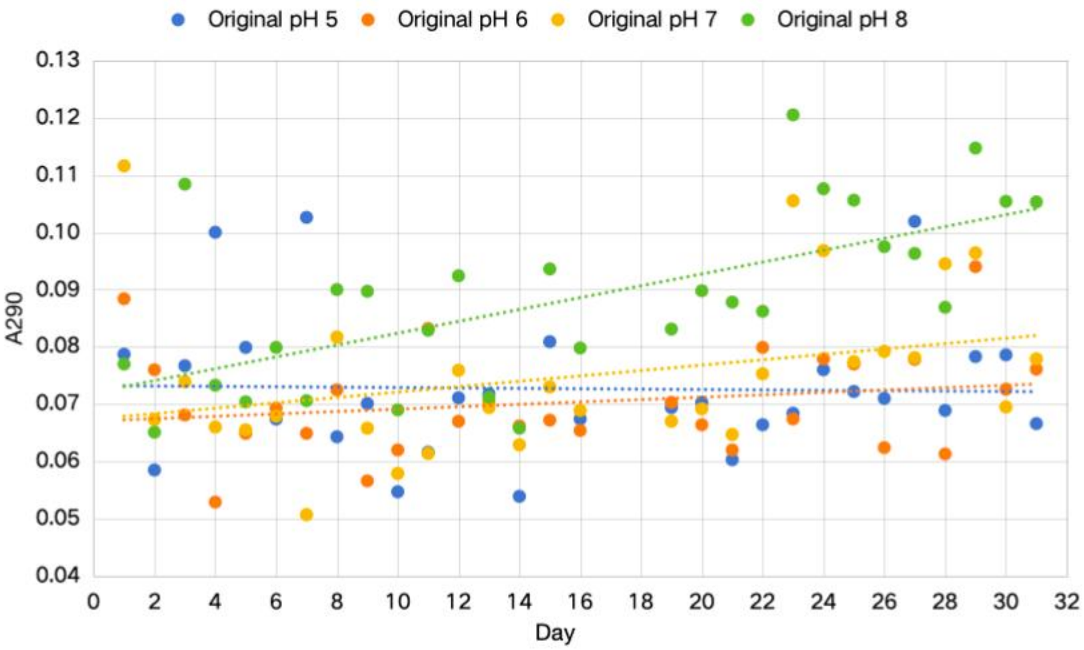


Figure 4.31. Temporal variation of absorbance (A_{290}) in the algal group under different initial pH conditions

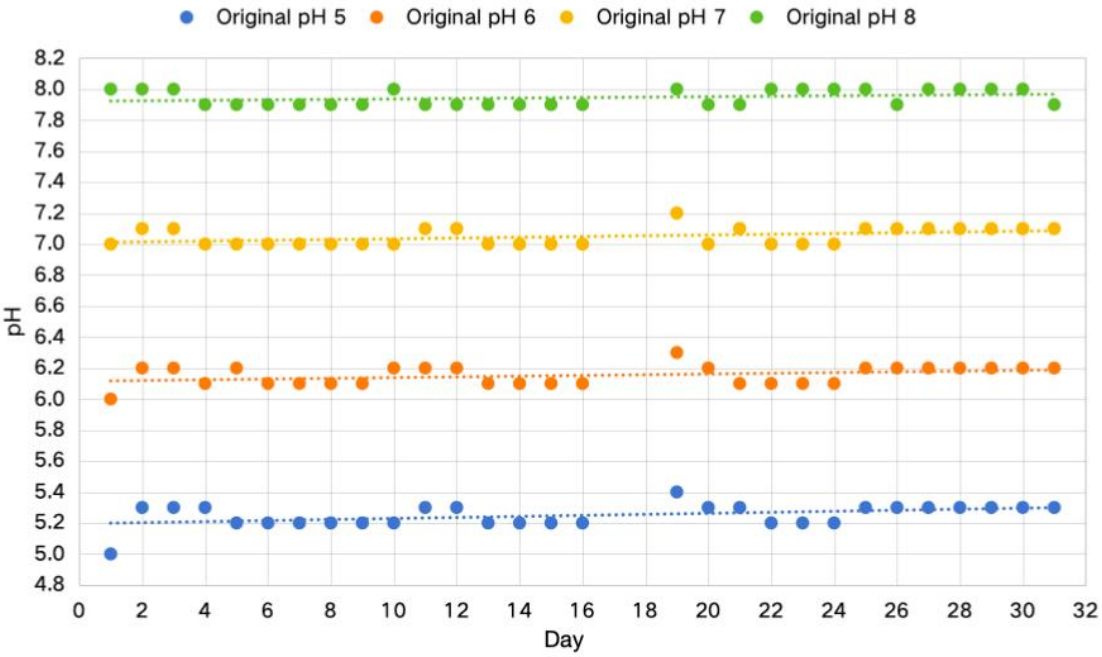


Figure 4.32. Temporal variation of pH in the algal group under different initial pH conditions

For the algal treatment, measurements of A_{290} and pH were made daily, also for 31 days, and demonstrated sharp time trends among the four treatments (Table A18.). Day 1 measurements of absorbance were different among the treatments, with the original pH 7 group having the highest starting A_{290} at 0.1116, followed by the original pH 6 group at 0.0884, while the original pH 5 group and the original pH 8 group began closest together at 0.0787 and 0.0770, respectively. Measurements of pH equaled the target starting settings, given at 5.0, 6.0, 7.0, and 8.0.

In the first week, day 1 – 7, all groups declined in A_{290} to 0.06 – 0.07 except the original pH 5 group. As illustrated by the example of Day 4, absorbance was down to 0.0529 for the original pH 6 group and 0.0507 – 0.0706 for the original pH 7 group and original pH 8 group but up to about 0.1026 for the original pH 5 group. Meanwhile, pH measured during the corresponding time interval still was close to the starting level of each group, with the original pH 5 group at 5.2 – 5.3, the original pH 6 group at 6.0 – 6.2, the original pH 7 group at 7.0 – 7.1, and the original pH 8 group at 7.9 – 8.0.

From day 8 – 20, absorbance across all groups was stable, typically 0.06 – 0.09, especially for the control original pH 6 and 7 group. At this middle phase, pH readings were also stable, typically ± 0.2 units of initial readings: the original pH 5 group at 5.2 – 5.4, the original pH 6 group at 6.1 – 6.3, the original pH 7 group at 7.0 – 7.1, and the original pH 8 group at 7.9 – 8.0.

During the later stage, day 21 – 31, groups started to vary significantly. The original pH 5 group, original pH 6 group, and original pH 7 group remained variable between 0.06 – 0.08, with the majority below 0.080. The original pH 8 group, however, included some of the high values above 0.10, like 0.1076 on Day 24, 0.1205 on Day 23, and 0.1053 on Day 31. At this stage, pH values remained steady with the original setting values, with little deviation (the original pH 5 group: 5.2 – 5.3, the original pH 6 group: 6.1 – 6.2, the original pH 7 group: 7.0 – 7.1, the original pH 8 group: 7.9 – 8.0).

In general, the dataset for the algal group showed, between the treatments, one overall trend of the reading of the absorbance: it fell at week one precisely, then plateaued at the mid-experiment mark, and varied a little at the subsequent date, significantly at the original pH 8 group. The similar measurements of the corresponding pH values indicated that the conditions were close to the initial settings throughout the course of the 31-day experiment, with some slight variations.

4.5.2 Natural Light Group

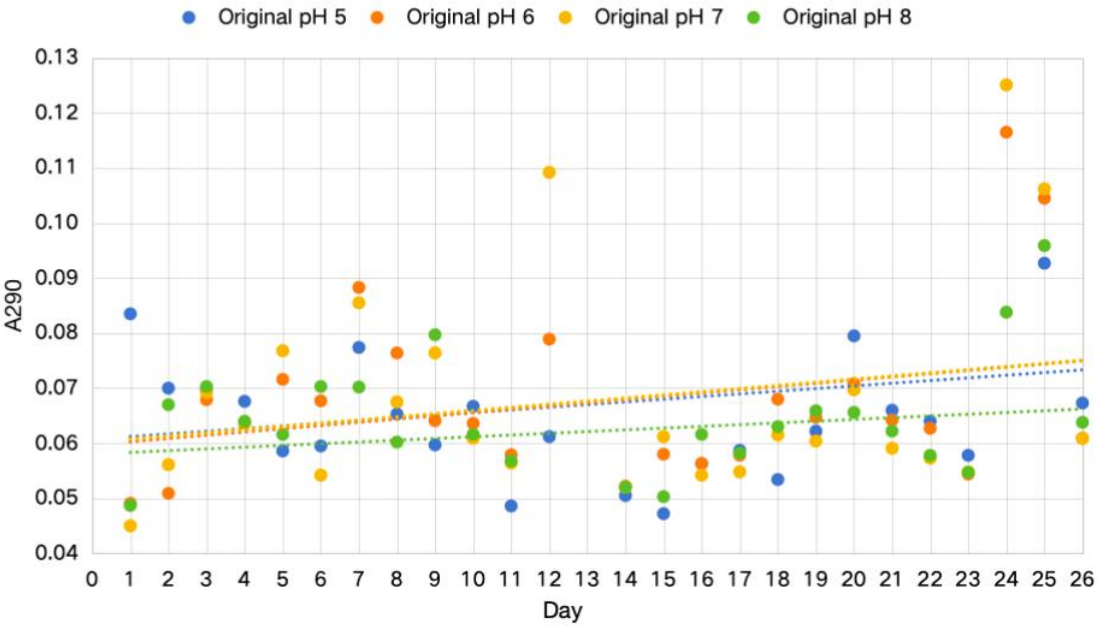


Figure 4.33. Temporal variation of absorbance (A_{290}) in the natural light group under different initial pH conditions

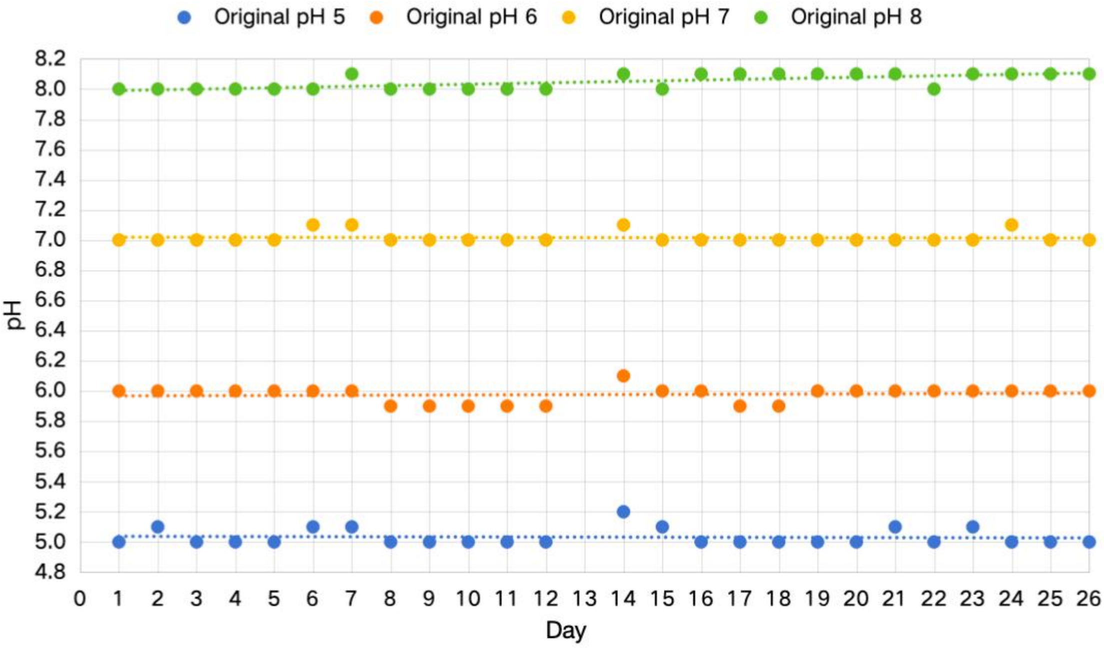


Figure 4.34. Temporal variation of pH in the natural light group under different initial pH conditions

A_{290} and pH were checked for 26 days regarding the natural light conditions(Table A19.). Day 1 A_{290} readings were the lowest in the original pH 7 (0.0450) and in the original pH 8 (0.0487) groups, with originally higher readings in the original pH 5 group (0.0835). Original pH 8 was 0.0487, meanwhile, pH was within the target settings of 5.0, 6.0, 7.0, 8.0. In the days 2 – 4 period, there were four groups of series within the same range close to 0.06 – 0.07 (for example, at Day 4: 0.0676, 0.0637, 0.0636, 0.0640).

Between Days 5 – 11, absorbance fluctuated by about 0.05 – 0.08. Day 7 and Day 11 are typical samples: (0.0774, 0.0883, 0.0855, 0.0702) and (0.0486, 0.0579, 0.0564, 0.0568), respectively, for the original pH5/6/7/8 groups.

In the mid-period (Days 12 – 22), the majority of daily readings fell within the same range (around 0.055 – 0.080) with the occasional single-day outlier: Day 12 experienced the original pH7 group with a high reading of 0.1092 and the original pH8 group with a high reading of 0.0117. On Day 16, readings had returned to 0.054 – 0.062 within groups.

There was a recurring late-period peak on Day 24 – 25 in all the treatments. A_{290} on Day 24 was 0.1382 (original pH 5 group), 0.1165 (original pH 6 group), 0.1251 (original pH 7 group), and 0.0838 (original pH 8 group). On Day 25, four original pH5/6/7/8 groups were still in high states (0.0927, 0.1045, 0.1062, 0.0959). On Day 26, values again dropped to 0.0673, 0.0609, 0.0609, and 0.0638, respectively, to the earlier mid-range.

Over the 26-day period, pH oscillated around the initial values with little day-to-day change: the original pH5 group was generally 5.0 – 5.2, the original pH 6 group was 5.9 – 6.1, the original pH 7 group was 7.0 – 7.1, and the original pH 8 group was 8.0 – 8.1. Overall, there was an early A_{290} decrease to around 0.06 – 0.07, a mid-experiment plateau with minor oscillations, and a transient late-period rise and return to earlier levels, with pH near the initial target values for the four treatments.

4.6 Kinetic Analysis of E1 Degradation under Long-Term Experiments

For further study on the degradation characteristics of estrone (E1) under varied treatment conditions, the kinetic study was conducted on the basis of the long-term experiment. This section does not try to develop an overall prediction model, but rather aims to study whether observed elimination patterns were explicable by traditional kinetic mechanisms, and assess apparent rate differences among treatments. On the basis of the requirements above, the study was separated into two stages: first, the second-round experiment outcomes (Section 3.2) on algae, natural light, and UV irradiance were used individually, second, the pH long-term experiments (Section 3.4) were analyzed to assess the solution chemistry impacts on kinetic properties.

4.6.1 Kinetic Analysis of Second-Round Experiments

4.6.1.1 Algal group

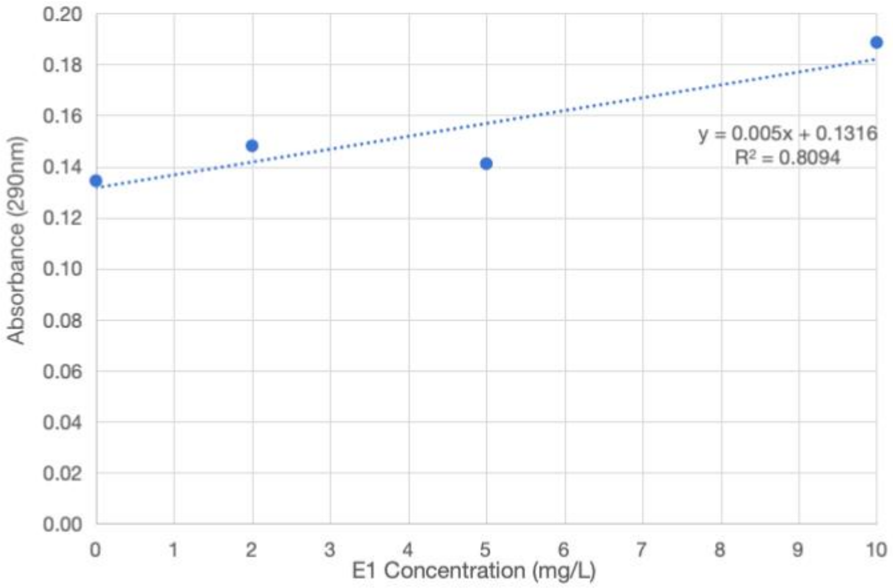


Figure 4.35. Calibration curve of E1 in the algal group at 290 nm

Based on the absorbance of 0mg /L, 2mg /L, 5mg /L, and 10 mg /L E1 at Day 0 (Table A4.), a calibration curve was constructed (Figure 4.35). Linear regression yielded the equation:

Results

$$A = 0.005C + 0.1316 \ (R^2 = 0.8094)$$

where A is the absorbance at 290 nm, and C is the E1 concentration (mg /L).

This constant (slope K=0.005) was then applied to convert absorbance values into concentrations in subsequent time-series measurements. The conversion followed:

$$C = \frac{A - b}{K}$$

where b=0.1316 is the intercept from the regression. When blank-corrected absorbance values (ΔA) were used, the formula simplified to:

$$C = \frac{\Delta A}{K}$$

The levels of E1 obtained from the treatment of the algae, as calculated by the calibration constant (K=0.005), are given in the Table A20. The time dependency of the E1, following the first-order kinetic model, would be:

$$-\ln\left(\frac{C}{C_0}\right) = kt$$

where C is the concentration at time t, C_0 is the Day 0 initial concentration, and k is the apparent first-order rate constant.

These adjusted concentrations varied widely over the course of time points, and there were even negative values. The values are not linearly increasing with time and include negative and positive entries. This scatter shows that the data set was not explained by a first-order kinetic scheme, and an apparent rate constant was not obtained for this group.

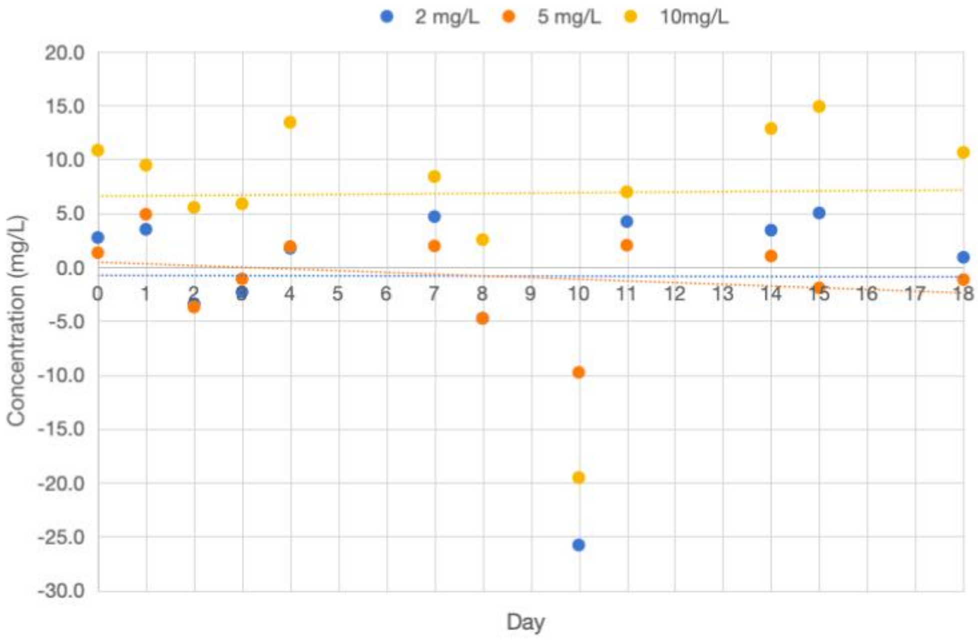


Figure 4.36. Corrected concentrations of E1 over 18 days in the algal group

Figure 4.36. shows the corrected concentrations of E1 in the algal treatment for the initial concentrations of 2 mg/L, 5 mg/L, and 10 mg/L across an 18-day period. The data display substantial fluctuations, with several values falling below zero. No consistent decreasing trend was observed, and the concentration profiles did not follow a first-order decay pattern.

4.6.1.2 Natural Light Group

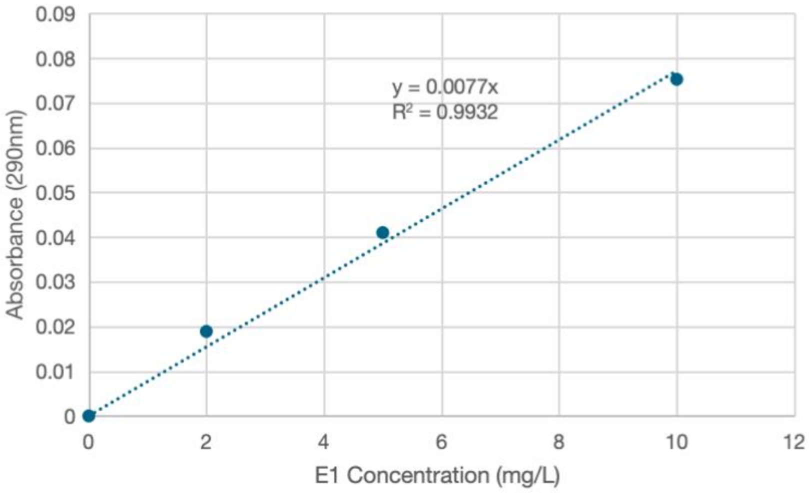


Figure 4.37. Calibration curve of E1 in the natural light group at 290 nm

The calibration curve was constructed from the Day 0 natural light treatment absorbance (Table A5.). Linear regression produced a slope of 0.0077 ($R^2 = 0.9932$), used for converting the absorbance to concentrations for natural light experiments.

$$A = 0.0077C \ (R^2 = 0.9932)$$

where A is the 290 nm absorbance and C is the estrone (E1) concentration in mg /L. Since the intercept was near zero, the calibration was forced through the origin. Therefore, the E1 value at any particular time point was calculated from the absorbance with:

$$C = \frac{A}{K}$$

Table A21. shows the E1 levels from the natural light treatments calculated by using the calibration constant (K = 0.0077). These concentrations were then used for kinetic analysis according to the first-order expression:

$$-\ln\left(\frac{C}{C_0}\right) = kt$$

with C is the concentration at time t, C_0 is the Day 0 initial concentration, and k is the apparent first-order rate constant.

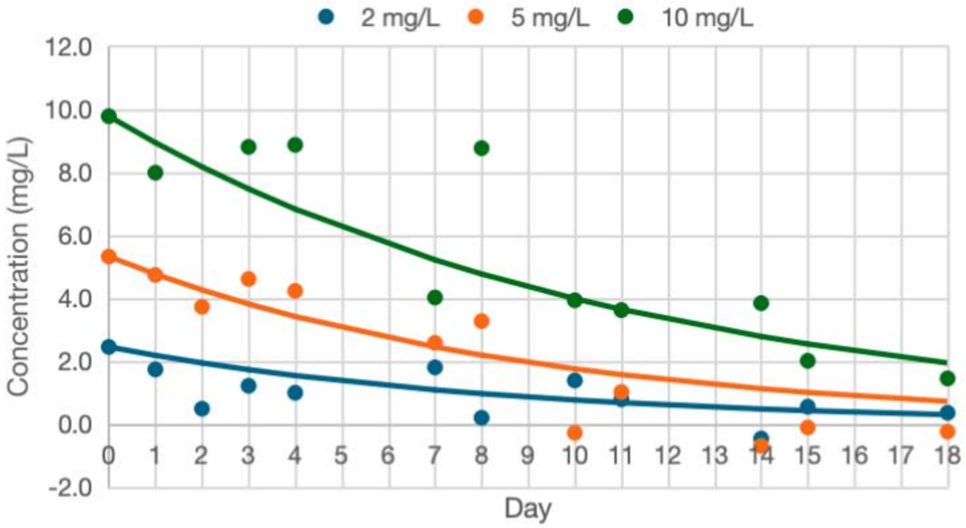


Figure 4.38. Corrected concentrations of E1 over 18 days in the natural light group

Table A21. and Figure 4.38. show the adjusted levels of E1 after the natural light treatment for the initial levels of 2 mg/L, 5 mg/L, and 10 mg/L, as well as the corresponding transformation kinetic values.

Over the 18-day course period, the concentrations in all three groups were generally in decline. For Group 2 mg/L, the concentrations decreased from 2.45 mg/L at Day 0 to 0.36 mg/L on Day 18 with intermediate fluctuations. For Group 5 mg/L, the concentrations decreased from 5.32 mg/L at Day 0 to a value of near zero or less at Day 10 onwards. For Group 10 mg/L, the concentrations decreased from 9.78 mg/L at Day 0 to 1.45 mg/L at Day 18.

The $-\ln\left(\frac{C}{C_0}\right) = kt$ individual values rose over time, and there was more regular first-order kinetic behaviour than was the case for the algal system. However, there were again some instances of fluctuation, especially the 5 mg/L system mid-period readings.

4.6.1.3 UV Light Group

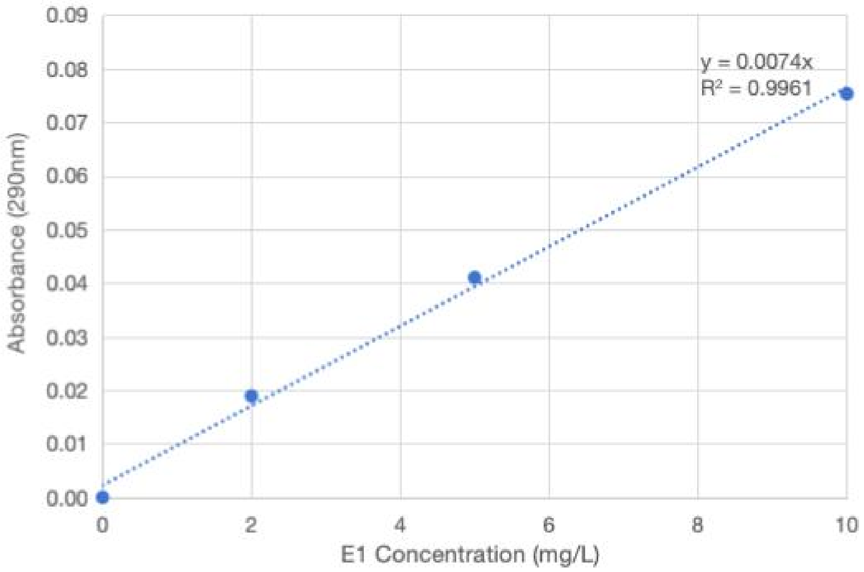


Figure 4.39. Calibration curve of E1 in the UV light group at 290 nm

Based on the 0 minute absorbance values in the UV group (Table A6.), a calibration curve was constructed (Figure 4.39.). Linear regression produced the equation:

$$A = 0.0074C \ (R^2 = 0.9961)$$

where A is the absorbance at 290 nm and C is the estrone (E1) concentration (mg/L). The regression intercept was approximately zero, and the calibration was therefore forced through the origin.

Accordingly, E1 concentrations during the UV irradiation experiments were obtained using:

$$C = \frac{A}{K}$$

For kinetic analysis, the time-dependent concentrations were transformed according to the first-order expression:

Results

$$-\ln\left(\frac{C}{C_0}\right) = kt$$

with C is the concentration at time t, C_0 is the 0 minute initial concentration, and k is the apparent first-order rate constant.

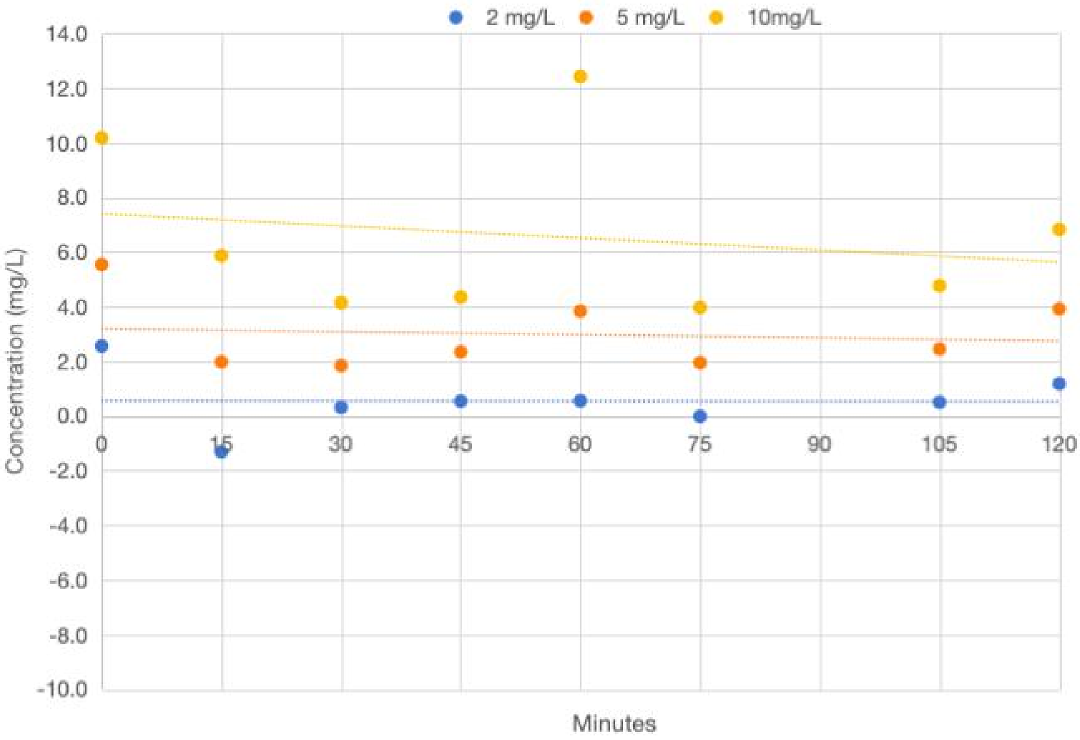


Figure 4.40. Corrected concentrations of E1 over 120 minutes in the UV light group

The corrected E1 concentrations after UV irradiation starting from the initial 2 mg/L, 5 mg/L, 10 mg/L after 120 minutes, and the corresponding kinetic transformation values are presented in Table A22. and Figure 4.40.

For the 2 mg/L group, the level decreased from 2.55 mg/L at 0 min to 1.18 mg/L at 120 min with intermediate variations and sporadic negative values at some points. In the 5 mg/L group decreased from 5.54 mg/L at 0 min to 3.92 mg/L at 120 min with occasional negative values. In the 10 mg/L group decreased from 10.18 mg/L at 0 min to 6.82 mg/L at 120 min with sporadic transient peaks.

The modified $-\ln\left(\frac{C}{C_0}\right) = kt$ values tend to increase as a function of irradiation time, with an overall decreasing trend of the E1 level on UV irradiation. Random variations were, however, observed especially among the low-concentration group, thus compromising the reproducibility of the kinetic profiles.

4.6.2 Kinetic Analysis under pH-Adjusted Conditions

Since the pH experiments were run on a single E1 starting concentration as 10 mg/L with different pH 5.0–8.0, the new calibration constants were unable to be calculated. Conversion from absorbance to concentration thus employed preestablished constants. 0.0077 for the natural light group and 0.0050 for the algal group.

The conversion from absorbance to concentration followed the calibration equation:

$$C = \frac{A}{K}$$

and the kinetic analysis was performed according to the first-order expression:

$$-\ln\left(\frac{C}{C_0}\right) = kt$$

with C is the concentration at time t, C_0 is the Day 1 starting concentration, and k is the apparent first-order rate constant.

4.6.2.1 Algal Group

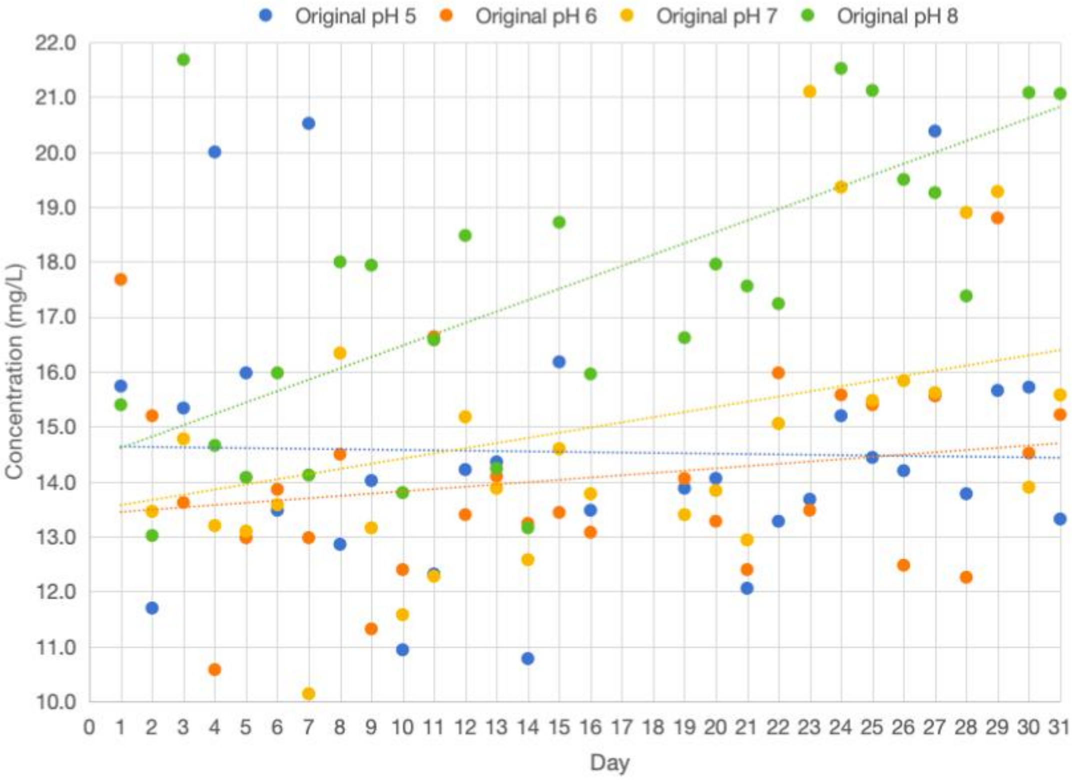


Figure 4.41. Concentrations of estrone (E1) under algal treatment with different pH levels

The levels of the corrected estrone (E1) were listed in Table A23. and Figure 4.41. for four pH levels (5.0, 6.0, 7.0, 8.0) during the course of the 31-day incub

The initial levels on Day 1 were 15mg/L to 22 mg/L, significantly greater than the 10 mg/L originally specified. This difference held across all pH treatment levels and thus resulted in the effective initial levels being quite different from the level intended.

In the second incubation period, the concentration profiles were undergoing large fluctuations as opposed to monotonically decreasing behavior. Recoveries were tracking the troughs temporarily, and oscillations between the four groups of pH were probable. The pH 6 treatment concentration and that of pH 7 had tracked below 12 mg/L until the first week since the beginning of the experiment, but had recovered to above 18 mg/L by the end. The pH 8 group had the most variability, whose concentration level was over 20 mg/L by mid- to late-experiment.

As a whole, there was no standard first-order type of decay relationship between the pH data of algae. Rather, the outcome was that apparent concentration variations were spread by pH among the treatments such that there were relatively narrower ranges (12–18 mg/L) for pH 5 and pH 6 relative to pH 7 and pH 8, which were always above 20 mg/L. As a consequence of the irregular dynamics, the kinetic analysis for the group of algal pH was not always possible to describe based on an uncomplicated exponential type of decay function.

4.6.2.2 Natural Light Group

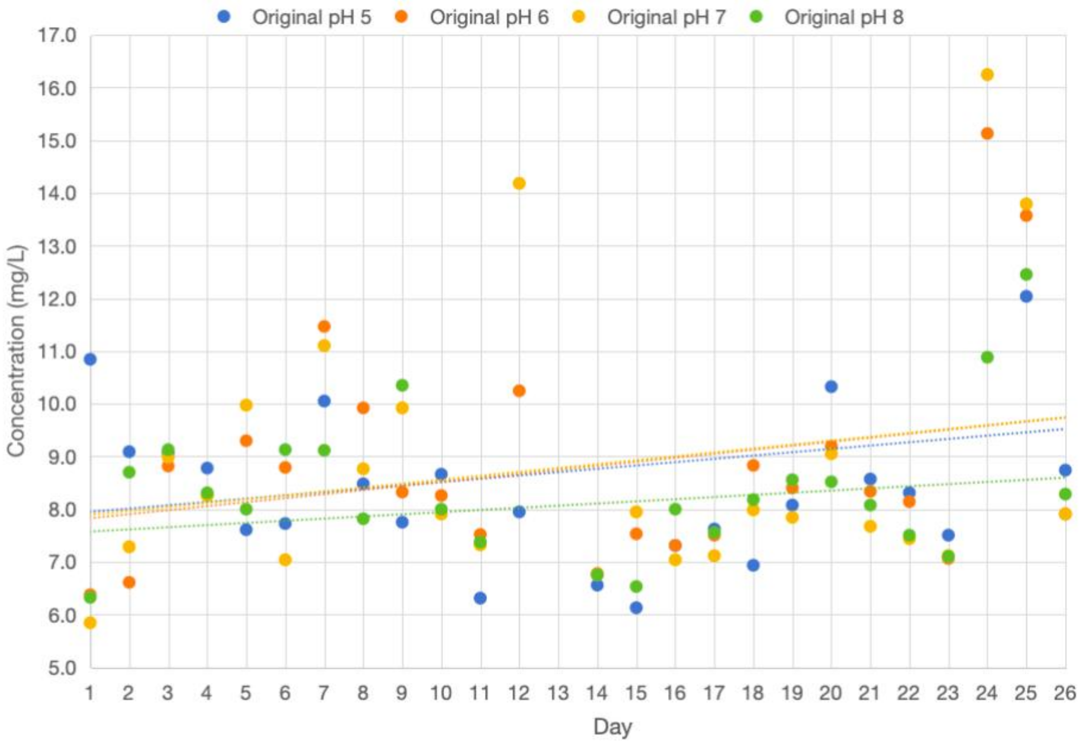


Figure 4.42. Concentrations of estrone (E1) under natural light treatment with different pH levels

The concentrations of E1 of the natural light group under four levels of pH (5.0, 6.0, 7.0, 8.0) on the 26-day incubation were shown in Table A24. and Figure 4.42.

These day 1 levels were 5.8 to 10.8 mg/L, close to the 10 mg/L nominal setup but not identical. This was the type of variation between the pH conditions that confirmed the effective initial concentrations were only slightly different from the theoretical concentrations.

Throughout the duration of the experiment, concentration profiles were oscillating and irregular, and not a straightforward exponential decline. With treatment under pH 5, for instance, concentrations fell to levels ranging around 6–7 mg/L between Days 12–14 before they rose again to levels ranging around 12–13 mg/L towards the latter half of the incubation. Similarly, the pH 6 treatment group and pH 7 treatment group exhibited initial drops to levels close to 7 mg/L before rises to levels above 14–15 mg/L towards the latter half of the duration of the incubation period. The pH 8 treatment group exhibited relatively minor fluctuations, with the concentrations oscillating between around 7 and 12 mg/L over the duration of the 26 days.

Overall, the natural light pH series exhibited relatively less extreme variation relative to the algal group, although the data failed to exhibit a first-order kinetic decline consistently. Rather, the four pH conditions exhibited non-monotonic variations in concentration in the form of decreases followed by recoveries at subsequent stages.

4.7 Summary

This chapter reports raw results for absorbance and estrone (E1) elimination due to three regimes of treatment: algal treatment, natural light exposure, and UV irradiance.

Sections 4.1 – 4.2 Second-round experiments. Jointly within the two sections, A_{290} time trends were collectively estimated at the starting 0, 2, 5, and 10 mg/L concentrations among the three treatments. Cross-comparability-corrected ΔA_{290} values among groups were found by subtracting the 0 mg L^{-1} control within the treatment. Removal efficacies of E1 were found from the resulting readings, and maxima were recorded over the concentrations and the treatments.

Section 4.3 Measuring the light intensity. Lux readings considered the light field both algae-and algae-lacking.

Section 4.4 Algal data from short-term pH 7. One-day and overnight readings gave replicate A_{290} and single-point pH data under algal conditions at pH 7.

Section 4.5 pH effect at 10 mg/L under algal and natural-light regimes. Time-series experiments were conducted on an initial concentration of E1 under four pH regimes for two parallel treatments (bottles containing algae and non-algal natural-light bottles). A_{290} and pH were checked daily for approximately 30 days.

Section 4.6. Second round experiment analysis and experiment with different pH levels. By using the found calibration constants, the concentrations were converted and re-plotted in the form of $-\ln(C/C_0)$ vs. time for the treatments.

Generally, differences were apparent at a noticeable level across treatments and concentrations. Cumulative evidence of raw absorbance, removal efficiency, characterization of light intensity, and kinetic determinations provides the empirical foundation for discussion and factors of influence to be explored in Chapter 5.

Chapter 5: Discussion

5.1 Overview of A_{290} Absorbance Trends

Second-round experiment absorbance at 290 nm (A_{290}) was an indirect indication of the existence of estrone (E1) in solution during second-round experiments. A_{290} is a reasonable temporal metric to provide an indication into the chemistry between the E1 and its probable degradates or transformates under differing conditions: natural light exposure, algal exposure, and UV irradiation. While A_{290} can reflect the presence of estrone, it is not a measurement of a specific chemical. Other substances, such as algal pigments or dissolved organic matter, can also affect A_{290} readings.

5.1.1 Algal Group: Contribution to E1 Removal Being Minimal

To better warrant the repeatability of the long-term outcome of the algae treatment, the study conducted short-term tests on repeatability when the pH was 7 (Section 4.4). Results were such that variations in A_{290} and pH when doing parallel experiments on different days and when measuring over the course of an overnight were small and considerably less than the overall decrease found when doing the long-term tests. This indicates that short-term variations in the algae treatment do little to impact the long-term interpretation of removal, thus increasing the reproducibility and stability of the long-term outcome of the algae treatment.

Although the introduction of green algae (Spirogyra) by sunlight led to discernible changes on the A_{290} spectrum over time, the background-subtracted data by subtracting the 0 mg/L control group indicate that net removal of E1 by algal activity was not significant.

In second round experiments, for the 2 mg/L treatment, the adjusted corrected ΔA_{290} never exceeded 0.030 during the whole 18-day experiment. For the 5 mg/L and 10 mg/L concentrations, the values oscillated slightly and showed no clear downtrend, and they stayed largely below 0.075. These slight variations mean that there was no significant removal of the E1 by virtue of algal processes.

This finding was further corroborated from removal efficiency data of estrone (Table A10.). For all three treatments, the greatest removal was always from the algal group, which was a mere -96.63% at 10 mg/L. Algal group showed no significant removal effect, despite there being continuous aeration and 18-day exposure time.

Based on the extracted removal rate and absorbance data, we deduce that algae following the test conditions applied were not effectively increasing removal of estrone, and its removal rate was not better or was poorer than that from the abiotic natural sunlight control.

In section 4.5, with a 10 mg/L one-month observation time and initial pH 5.0 – 8.0, the A_{290} values of the algae group decreased noticeably in the first week, after which they stabilized. In the rest of the study (days 21 – 31), the pH 8 group had relatively high concentrations (>0.10 on multiple days), while the pH was widely within the range (approximately 7.9 – 8.0 in the pH 8 group). Implications of these results in this group are that the somewhat alkaline status may have impaired general removal capacity under algae conditions, and this should be addressed in future studies with cross-treatment comparisons.

5.1.2 Natural Lighting Group: Highest E1 Removal Efficiency Observed

Among all the treatment groups, the natural lighting group, where E1 was treated by regular sunlight without biological agents, showed the highest removal efficiency on both absorbance patterns and calculated removal efficiencies.

In second round experiments, as evident from Figure 4.9, there was a definite and continual loss of corrected ΔA_{290} values throughout all concentrations. For 10 mg/L group, the corrected ΔA_{290} consistently declined from 0.0753 to nearly 0.01 on day 18. The 5 mg/L group showed a definite trend of loss, and values kept going down throughout the days. The 2 mg/L group exhibited a slower loss, yet still showed an obvious loss of absorbance.

The trend was forcefully confirmed by removal efficiencies of estrone presented in

Table A10. Maximum removal was 84.46% in 10 mg/L group on Day 2, 67.20% in 2 mg/L group on Day 3, and 47.56% in 5 mg/L group on Day 7. These values not only prove a time-dependent and quantifiable photodegradation process, but they are still higher than those of the algal and UV irradiation groups under the same conditions.

Although the natural sunlight is not rich in UV, especially while enclosed, this group still revealed that passive photodegradation efficiently assisted to remove E1 when there was sufficient exposure time.

The effectiveness of this group holds particularly true considering the fact that there was not any biological activity. Unlike the algal group, which showed removal rates that were negative, the natural light group showed sunlight was alone capable of catalyzing measurable E1 degradation, thus making it the most reliable treatment within present conditions.

In addition, in order to investigate the influence of pH on natural light treatment, control groups at pH levels of 5.0, 6.0, 7.0, and 8.0 were used in section 4.5. The outcome was that on Day 1, A_{290} values of the pH 7 group and those of the pH 8 group were comparatively low (0.0450 and 0.0487, respectively). On Days 2–11, A_{290} values of every group were comparatively steady in the range 0.05–0.08. During the mid-stage (Days 12–22), most of the observed values of the four pH conditions were again in the range of 0.055–0.080, although there were occasional small outliers on a single day. Correspondingly, pH values of all the treatments were generally maintained in the assigned ranges. This indicates an outcome that under natural light conditions, A_{290} values of the various pH groups approached to each other slowly on the short to medium term, with considerably smaller differences, such that a better general assessment of their influence was available among different treatment experiment comparisons.

5.1.3 UV Light Group: Fast Optical Response and Low Removal Efficiency

In the UV treated group, to which the E1 solutions were exposed to an indirect ultraviolet lamp for a very brief time (0 – 30 minutes), the corrected ΔA_{290} values

(Figure 4.10) showed an ultrarapid optical response, particularly at the higher concentrations.

In second round experiments, for the 10 mg/L group, the corrected Δ A_{290} decreased from roughly 0.0753 to 0.0307 within 30 minutes. The 5 mg/L group exhibited a noticeable decline, while the 2 mg/L group exhibited virtually no change within this time period.

To confirm this, all the linear regression lines from Figure 4.10 have negative slopes, which confirms an overall trend of diminishing corrected absorbance over the exposure range. This indicates that there was indeed a measurable photochemical response from the UV irradiation, despite the rather-short exposure time.

Although the corresponding actual removal efficacy of E1 was relatively not very high (Table A10.), there was a slightly positive removal rate shown by the 10 mg/L group (4.12%), and negative values by the 2 mg/L and 5 mg/L groups (−165.61% and −53.17%).

The measured results show that despite the sudden changes in absorbency that resulted from the UV irradiation, the removal of estrone was not significant under current experimental conditions, particularly for lower concentrations. Compared to the natural illumination group showing stronger and more steady performance, the UV group was not strong enough to remove E1 within the narrow irradiation window.

5.2 Comparative Treatment Group Evaluation

5.2.1 Light Field Distribution and Shielding

To further characterize the disparity in removal efficiency among the three treatment groups more accurately, this research experimentally estimated the distribution of the experimental bottles' light intensity in a quantitative manner. The light transmittance was found to decrease markedly with path length to zero at more than 3 cm and was not able to penetrate to any extent at 5 cm path length. Angular distribution experiments further demonstrated that useful light was confined within a relatively small angular window in such a way that approximately 50% of the total cumulative light intensity was confined within an angle of approximately 35 ° . Contribution below 90 ° significantly diminished. This effectively means that under experimental conditions, it was the areas close to the bottle wall and liquid surface top that were significantly illuminated, with the majority of the bottle encompassing a large "dark zone."

This phenomenon had a direct impact on the various treatment groups. The natural light group had a higher removal efficiency in general since its strength was larger and algae obstruction was less. However, the algae group, through the extra self-shading effect from suspended algae, further squeezed the effective light layer to produce a relatively low removal rate of light. The UV light source, on the other hand, since it had one wavelength and stable intensity, was able to initiate photochemical reactions within a very short amount of time. However, due to the limitations on optical path length and absorption properties, its overall removal efficiency was less than that from natural light.

Finally, the reduction of the optical path length and angle distribution gives an optical explanation of the variations among the three experimental groups in a way that geometrical conditions and the algal density of the experimental system determine the efficiency of the utilization of light and the removal rate of E1.

5.2.2 Comparative Performance of Treatment Groups

In second round experiments, in order to comparatively evaluate the effectiveness of different treatments, i.e., algal exposure, sunlight, and UV irradiation, we pooled the data from both removal rates of estrone (Table A10.) and corrected absorbance values (Figures 4.8 – 4.10). The treatment groups varied by type, degree, and time scale of the degradation.

Comparing the three, the one using natural lighting showed the most uniform and highly efficient removal of E1. Its corrected ΔA_{290} curve continuously and smoothly descended through concentrations and showed the optimal removal efficiency, up to 84.46% at 10 mg/L. The result indicates the competence of long-lasting passive photodegradation to remove E1 even without biological or external intervention.

The UV irradiation group, despite an acute initial drop in absorbance within 30 minutes post irradiation, exhibited limited removal, whereas the 10 mg/L group presented a slightly positive rate (4.12%) and lower concentrations presented negative values. The fast optically observed response that was noticed was not large scale estrone breakdown, presumably due to insufficient exposure time.

The algal group, however, performed the poorest. The corrected ΔA_{290} values exhibited fluctuations without downward-pointing trends, and all percentage removal rates were negative, up to −96.63% at 10 mg/L. This not only suggested an inability to degrade, but possible interference or background build-up by E1.

Comparing within groups for relative effectiveness, natural light > UV light > exposure by algae on both spectral and concentration grounds. The relative significance between UV and natural light further implies that longer exposure time predominates over intensity under certain conditions, and this variable warrants further discussion throughout Section 5.4.

5.3 Dose – Response Relationship and pH Effects Across Treatments

5.3.1 Dose–Response Relationship

In second round experiments, to identify how removal efficiency is impacted by the initial concentration of E1, three contamination concentrations (2 mg/L, 5 mg/L, and 10 mg/L) were tested on every treatment unit. The aim was to identify if removal capacity by every system changes proportionally or not to feeding levels of E1.

The dose – response relationship was strongest within the natural light group. The 10 mg/L (highest) concentration showed the most rapid and widespread removal, attaining a 84.46% removal efficiency on Day 2. The 2 mg/L and 5 mg/L concentrations followed, attaining 67.20% on Day 3 and 47.56% on Day 7, respectively. The trend shows substrate photodegradation occurring by ambient illumination was positively affected by substrate concentration, ostensibly due to improved photon capture and reaction propensity by contaminant concentrations that are higher. The finding supports Caupos et al. (2011), which reported acceleration of concentration-dependent E1 photolysis within illuminated dissolved organic matter simulations.

For the UV group, a similar though weak trend was observed. The 10 mg/L treatment showed a moderate positive removal rate (4.12%), while the 2 mg/L and 5 mg/L showed negative values (−165.61% and −53.17%, respectively). Although the corrected ΔA_{290} values showed an initial downward trend within the initial 30 minutes (10 mg/L from 0.0753 to 0.0307), absorbance values slowly plateaued or even recovered by 60 minutes. This shows that although UV irradiation triggered fast changes in optics, it was not able to achieve extensive breakdown throughout the whole 120-minute irradiation time period. Huang et al. (2022) showed a similar finding that by using static UV condition, free estrogens may experience fast early-phase photoreactions, yet steady removal tends to need longer irradiation time or continuous-flow system to function effectively.

All concentrations exhibited negative removal efficiencies among the algal group, however, the extent of negativity decreased with increasing initial concentration (−564.55% at 2 mg/L, −177.07% at 5 mg/L, and −93.63% at 10 mg/L). Although this might suggest minimized interferences at higher concentrations, there exists no literature to directly support such an effect from the foundation of merely E1 concentrations alone. Therefore, this result should be handled carefully and de novo work should take place to understand underlying mechanisms.

Overall, there was a dose-dependent relationship among all three groups, whereby increased concentrations of E1 tended to cause stronger absorbance and removal responses. Yet, while there was considerable and positive removal by the natural light group, there was weak or negative removal by the UV and algal groups, although there was an improving trend throughout each concentration increase.

5.3.2 pH Effects Across Treatments

In section 4.5, algae controls were conducted alongside natural light controls to investigate the effect on the elimination of E1 by pH in finer detail. Each was started at 10 mg/L under the corresponding pH conditions 5.0, 6.0, 7.0, and 8.0.

All four pH levels showed a moderate decreasing trend for A_{290} values for Days 1 – 7 of the algae treatment. However, in the later phase (Days 21 – 31), the A_{290} value of the treatment of the pH 8 was considerably higher, having a value over 0.10 for some days. The pH levels for the pH 5 – 7 were kept relatively low, and actual pH levels for each respective treatment were kept near the assigned levels. This would imply that slightly acidic to neutral conditions (namely, the pH 6 – 7) have a tendency to improve the removal of the E1 to a greater extent compared to the conditions that were basic.

For the natural light treatment, the A_{290} on Day 1 at pH 7 and pH 8 was fairly low (0.0450 and 0.0487, respectively) but was slightly greater on Day 1 at pH 5 and pH 6. But from Day 2 to Day 22, the A_{290} levels for the four levels of pH converged steadily in the 0.055-0.080 range despite day-to-day outliers. The levels of pH for the groups were held near the required level. This entails that in the short to medium term, the impact of pH isn’ t very significant under natural lighting conditions.

Briefly, there are certain differences among the pH impact on the varied treatments: under the algae system, the conditions of pH 6 – 7 showed relatively good elimination capability, whereas under the natural illumination system, the varied performances among different conditions of pH were relatively small.

5.4 Kinetics of E1 Removal

5.4.1 Kinetics in the Second-Round Experiments

For the second experiment set, transformed concentration values for the algae group (Table A20.) were significantly variable, even producing negative values at certain points. This suggests that the absorptivity of the algae blank was greater than the sample containing E1 at certain points, which implies that the absorption by algal pigment or metabolite at 290 nm caused a serious interfering effect on the signal. As a result, the transformed concentration curve had an uneven scatter plot instead of a monotonously decreasing tendency over time, and it was not consistent with the linear assumption of first-order kinetics. In other words, the signal-to-noise ratio for the algae group was low, making a valid kinetic interpretation impossible. Overall, this suggests that the actual removal effect of algae on E1 is negligible, and the absorbance signal is primarily dominated by background interference, making a reliable kinetic fit impossible. Based on the absorbance-concentration conversion results, E1 concentration curves were plotted over time to analyze the removal process of the different treatments (section 4.6).

The results showed that all three experimental groups showed a certain downward trend in concentration, but there were significant differences in removal efficiency. The concentration of the natural light group decreased most significantly during the entire experimental period, showing the highest E1 removal efficiency. This also proved the conclusion of 5.1.2. A faster decrease was also observed in the UV group within a short period of time, but the overall removal range was limited. Although the algae group also showed a certain degree of concentration decrease in the early stage, the overall trend was unstable, the curve fluctuated greatly, and the removal effect was inferior to that of the natural light group.

5.4.2 Kinetics under Different pH Conditions

From the result of the pH experiment (section 4.5, initial 10 mg/L; pH 5.0, 6.0, 7.0, 8.0), the effect of the varying pH on the degradation rate of E1 was analyzed based on transformed concentration-time graphs (section 4.6). It was observed from the result that both the algae treatment and natural light treatment exhibited a decreasing trend with respect to time, although the effect of pH varied among the two treatment processes.

Results from Figure 4.41. In the algae system, the values were significantly different between pH 6 and 7, with the strongest removal effect; the 5 pH curve was intermediate; and the 8 pH curve had significantly greater values towards the end, with the weakest removal. This was as expected from the long-term A_{290} behavior described in the 4.5 section, showing removal by algae to be sensitive to basic conditions.

In Figure 4.42, for the natural light system, the starting concentrations were rather varied across the four conditions on the starting days of the experiment (Days 1–7), although the lowest were on pH 7 and pH 8. Subsequently, the concentration curves under the four conditions continued to converge steadily after the mid-term (Days 8–22), and the curves remained relatively steady in the same ranges on most days except for small-scale day-level outliers. This shows that when natural lighting predominates, the impact that pH causes on the elimination capacity of E1 ends up being irrelevant in the short to medium term.

Overall, maximum removal of E1 occurred when the pH was 6–7 in the algal system and minimum when the pH was 8. When using the natural lighting system, the variation between the varied conditions was little, the overall removal being comparable. This confirms the result from the forward Section 5.3.2 and again shows the algal system to be most responsive to the pH, the impact being of lesser importance when using natural light.

5.5 Limitations

This study provided baseline data on the removal of estrone (E1) by UV irradiation, sunlight, and algae exposure. Certain limitations within the experimental design and data interpretation, however, should not be overlooked. These consist of effects on precision, reproducibility, and usability of the results.

(1) Static UV Exposure

The UV treatment was applied using a closed and static system, without mixing or flow of water. This was likely to lower the effectiveness of light transmittance and restrict the photoreaction.

(2) Optical Monitoring Alone, without Chemical Verification

Removal of E1 was measured by absorbance at 290 nm (A_{290}), not verified by chemical analyses such as liquid chromatography – mass spectrometry (LC-MS). For the algal group, such agents as algal metabolites may potentially interfere with A_{290} readings, which may lead to over- or underestimation.

(3) Low Time Resolution

The sampling intervals were relatively long, like 24 hours for algae and natural-light groups, and 15 minutes for the UV group.This potentially excluded sharp changes in the concentration and inhibited the monitoring reaction stages or kinetic behavior.

(4) No Measurement of Light Intensity

The true intensity of the lights was not measured throughout the experiments. For the natural light group, sunlight variability and interior location may have resulted in unequal exposures between the replicates.

(5) Lack of utilization of the Photocatalyst

While other researchers use photocatalysts like TiO_2 and ZnO to improve the removal of estrogen, this work was not outfitted with any such agent. Their effect, therefore, could not be evaluated.

(6) Potential Unknown Factors within an Algal System

In the algae group, certain unidentified entities, like extracellular matter or microorganisms attached to algae, might have interfered with measurements and disturbed readings on the A_{290} sensor.

(7) Limitations of Kinetic Analysis

Discussion

In Methodology and Results parts, this study attempted to approximate first-order kinetics for the removal of E1 using concentration-time curves. However, due to strong interference with the 290 nm absorbance of algae and their metabolites in the algal system, some converted values showed negative concentrations, and the signal-to-noise ratio was insufficient to support reliable kinetic fitting. Therefore, the kinetic conclusions are primarily applicable to natural light systems, and the results from algal systems should be interpreted with caution.

(8) Limited pH Range

The experiments only examined the pH range of 5.0–8.0. While the pH values in each group generally remained close to the set values, slight fluctuations were observed. More extreme acidic or alkaline conditions were not covered, so extrapolating conclusions regarding the effects of pH is limited.

5.6 Summary

This study revealed notable differences between the three treatment processes on the efficiency of E1 removal, with natural light treatment being the best, UV treatment showing less removal, and the algal treatment being the least. Field distribution experiments also revealed optical pathlength and angle constraints on efficient illumination, which explained the low efficiency of the algae system due to self-shading. Findings from the short-term experiment revealed that for the conditions pH 7, A_{290}, and pH fluctuations were minimal, neither significantly contributing to long-term tendencies, such that the experimental conclusion was made more robust. pH experiments revealed that under the algae system, pH 6 – 7 was most effective on E1 removal, while pH 8 was the least effective. Between the pH regimes, there was little difference observed in the natural light system. Kinetic analysis also revealed that the natural light treatment was most effective on the removal of E1, while the UV treatment was also experiencing rapid degradation in the short term. The phenomenon of data anomalies hinders efficient fitting.

Discussion

Overall, this study not only confirmed the high efficiency of the removal on natural illumination but also added further to our understanding of the differential impact displayable on the varied treatments by the mechanisms of optics, the conditions on pH, and the kinetic analysis.

Chapter 6: Conclusion

6.1 Conclusion

The removal process of estrone (E1) was assessed by taking into account three environmental settings, that is, natural sunlight, ultraviolet (UV) irradiation, and algal exposure. From absorbance readings recorded at 290 nm (A_{290}) and calculated removal efficiencies, the removal capacity was comparatively assessed for each treatment relative to three concentrations (2, 5, and 10 mg/L) of E1.

Relative to all the other treatment groups, the use of natural sunlight showed the greatest and most consistent removal capability. It showed up to 84.46% removal within the first 2 days at 10 mg/L and showed consistent downward trends throughout all the concentration levels. These outcomes appeared graphically on the line plots corrected $\Delta\ A_{290}$ (Figure 4.9.), which showed smooth and continuous decreases throughout the 18-day period.

The irradiated specimen using UV showed fast initial optical reaction, especially when concentrations of E1 are higher, yet there was not extensive removal within the entire 120-minute irradiation time. The corrective absorbance plots provided by the irradiation group using UV from Figure 4.10 showed a fast initial drop-off within 30 minutes, and a plateau or modest recovery, indicating minimal long-term breakdown. This showed that while UV induces fast photoactivation, long-duration or flow-through systems may need to offer significant photodegradation. This agreed with prior work, such as Huang et al. (2022), highlighting the limitation on the use of short-time static UV treatments.

On the other hand, the algae-exposed group showed no considerable removal. Removal rates determined by all approaches resulted in negative values, and the corrected absorbance plots from Figure 4.8 showed no steady downward slope but rather randomly oscillated. This finding was likely caused by interference from the background or feeble algal activity under the current circumstances. Though literature reports algae to have the capacity to remove effectively E1 by applying optimized circumstances, the system here was not able to replicate such effects.

Also, there was a positive correlation between initial concentration of E1 and removal efficiency in the natural light and UV groups, which suggested that higher concentrations may improve photon – substrate interaction or increase the opportunity for degradation, although more work should confirm these relationships since there was not much resolution and no chemical quantification.

Also, the plots of concentration-time verified that the natural light system attained the highest removal efficiency of E1 since this treatment exhibited an apparent, clear-cut steady drop in E1 concentration. Lux measurements have shown that light intensity decreases as path length increases. A replicate short-term under pH 7 exhibited negligible day-scale A_{290} and pH variations, hence verifying the stability of the long-term records. Under varying pH conditions, the algal system performed optimally at pH 6 – 7 and worst at pH 8, whereas natural light was relatively pH-independent. Lastly, the kinetics analysis noted that the algal group data were too randomized to allow coherent fitting by virtue of severe interference by the background, whereas the natural light system was attaining concentration behaviour consistent with first-order behaviour.

All in all, in this experiment, natural sunlight appears to hold the greatest promise among the considered systems for the removal of E1, while UV achieves fast response with inefficiency, and current algal treatment fails to function effectively. Future research should focus on improving experimental designs, condition optimizations (e.g., introduction of the catalyst, regulation of biomass, illumination level), and verification of a degradation mechanism to enable scalable environmental treatments.

6.2 Recommendations for Future Study

Apart from work extension on implementation and know-how on removal processes by E1 to other environmental treatments, further work to come will have to take on a list of methodological, analytical, and system-level improvements identified below:

(1) Combining Methods of Chemical Quantification

Although our study employed only UV absorbance 290 nm (A_{290}), secondary analyses should include chemical quantification approaches like liquid chromatography – mass spectrometry (LC-MS) such that true chemical degradations, and not shifts or optically related interferences, and, more importantly, within those very complicated matrices like algae, potentially will be differentiated from one another. The measured quantitation will enhance data quality and will allow kinetic model formulation.

(2) High Sampling Rate and High Temporal Resolution

The test could be further improved by enhanced temporal resolution, and this should be especially so during the onset exposure phase, where photoreactions are likely to occur fast. As a corollary, on first-hour sampling among UV treatments, better discrimination on onset conversion kinetics may occur, and by applying smaller interval values between exposures to sunlight and algae populations (viz., 6 or 12 hours instead of 24 hours), sharper lines to degradations may be achieved (Caupos et al., 2011).

(3) Introduction to Photocatalytic Systems

Some studies have proven the possibility of using photocatalysts like titanium dioxide (TiO_2) or zinc oxide (ZnO) to improve the removal rate of estrogens by using photodegradation. They are able to form reactive radicals by using UV or visible irradiation to improve breakdown efficiency. They, in subsequent experiments, are usable within self-contained UV setups or combined setups by using algae or sunlight to take advantage of synergic effects.

(4) Optimisation in Algal

The current system was not optimal, and this might have been triggered by poor biomass or poor growth conditions. The subsequent work will need to verify different concentrations of algae, photoperiod regimens, and concentrations of nutrients. As suggested by Liyanage et al. (2024), clearance of the E1 by help from algae was highly efficient (up to 80%) within optimal conditions within the photobioreactor that has an unlimited potential when the system was adequately optimized.

(5) System Design: Combina or Hybrid

Co-treatment by physical and biological methods might attain enhanced overall removal. Certain exemplary cases involve and simultaneously algae or UV/catalyst + algae pretreatment to attain potentially enhanced removal efficacy. Wu et al. (2020) verified this strategy, which showed that the extracellular organic matter from algae-isolated holds promise to trigger light-mediated indirect removal of indirect estrogens.

(6) Regulation and Detection of Radiant Power Intensity

The results from irradiance readings should be replicated so that there is replicate reproducibility and rate calibration on degradations. Real-time irradiance monitoring during sun testing will help to better correlate degradations from light, output vs. input. Precise measurement of lamp output and uniform delivery on exposure while using test equipment, such as UV test equipment, will increase reproducibility and knowledge.

(7) The Algal-Derived Interferences Studies

One should take into consideration that algae metabolites and co-cultures might influence microbes' development from readings on absorbance. Background absorbance by UV might increase due to reagents, and might conceal real degradation, or might cause false positive signals. Wu et al. (2020) theorized that extracellular material, which algae produce, engages in a light-dependent reaction, thus, processes within degradation are not simple.

(8) Time Extension by Irradiation and Exposure Types

While 120 minutes of UV treatment was employed within this work, there was no overall end-of-degradation termination. Huang et al. (2022) revealed that static circumstances of UV might require longer time intervals or continuously recirculating exposure reactors to obtain degradation. Future setups would therefore need longer time intervals, recirculation setups, or dynamic reactors to enhance the competence of UV treatment.

Conclusion

(9) pH Range Expansion and Control

Extend the tested pH range beyond 5.0 – 8.0 and maintain tighter setpoints to validate and refine the observed optimum (pH6 – 7) in algal systems and the relative insensitivity under natural light. This will improve the generalizability of pH - related conclusions.

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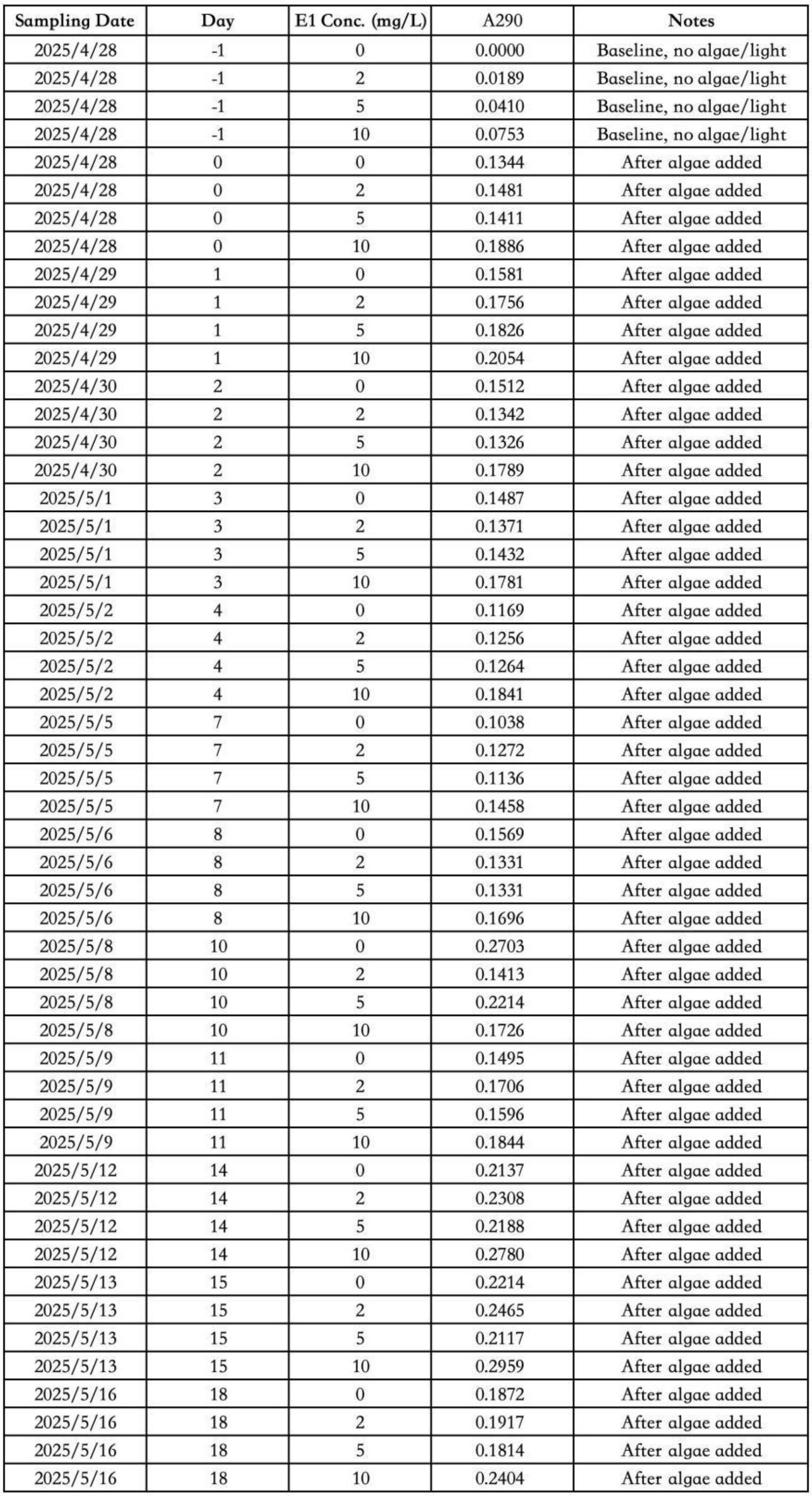
Appendix. Data Tables



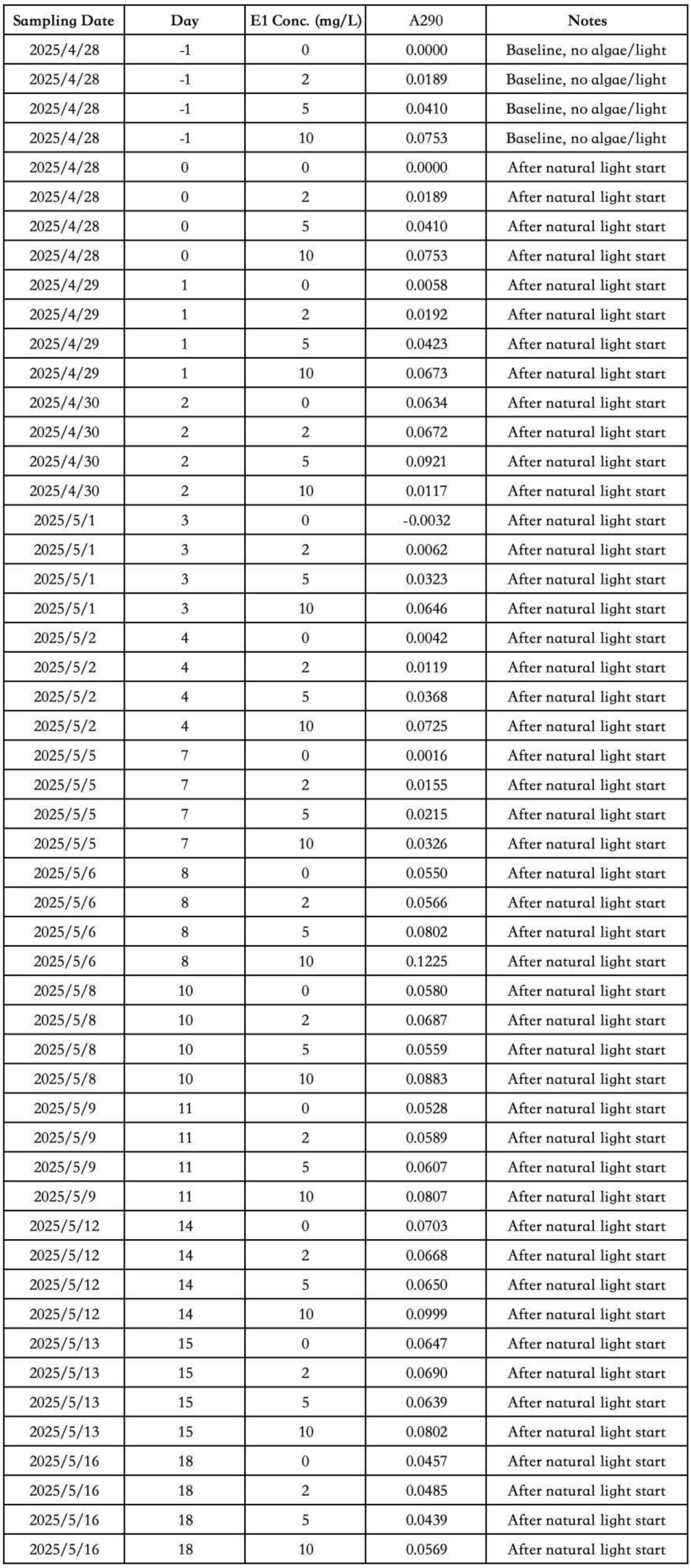
Sampling Date	Day	E1 Conc. (mg/L)	A290	Notes
2025/4/28	-1	0	0.0000	Baseline, no algae/light
2025/4/28	-1	2	0.0189	Baseline, no algae/light
2025/4/28	-1	5	0.0410	Baseline, no algae/light
2025/4/28	-1	10	0.0753	Baseline, no algae/light
2025/4/28	0	0	0.1344	After algae added
2025/4/28	0	2	0.1481	After algae added
2025/4/28	0	5	0.1411	After algae added
2025/4/28	0	10	0.1886	After algae added
2025/4/29	1	0	0.1581	After algae added
2025/4/29	1	2	0.1756	After algae added
2025/4/29	1	5	0.1826	After algae added
2025/4/29	1	10	0.2054	After algae added
2025/4/30	2	0	0.1512	After algae added
2025/4/30	2	2	0.1342	After algae added
2025/4/30	2	5	0.1326	After algae added
2025/4/30	2	10	0.1789	After algae added
2025/5/1	3	0	0.1487	After algae added
2025/5/1	3	2	0.1371	After algae added
2025/5/1	3	5	0.1432	After algae added
2025/5/1	3	10	0.1781	After algae added
2025/5/2	4	0	0.1169	After algae added
2025/5/2	4	2	0.1256	After algae added
2025/5/2	4	5	0.1264	After algae added
2025/5/2	4	10	0.1841	After algae added
2025/5/5	7	0	0.1038	After algae added
2025/5/5	7	2	0.1272	After algae added
2025/5/5	7	5	0.1136	After algae added
2025/5/5	7	10	0.1458	After algae added
2025/5/6	8	0	0.1569	After algae added
2025/5/6	8	2	0.1331	After algae added
2025/5/6	8	5	0.1331	After algae added
2025/5/6	8	10	0.1696	After algae added
2025/5/8	10	0	0.2703	After algae added
2025/5/8	10	2	0.1413	After algae added
2025/5/8	10	5	0.2214	After algae added
2025/5/8	10	10	0.1726	After algae added
2025/5/9	11	0	0.1495	After algae added
2025/5/9	11	2	0.1706	After algae added
2025/5/9	11	5	0.1596	After algae added
2025/5/9	11	10	0.1844	After algae added
2025/5/12	14	0	0.2137	After algae added
2025/5/12	14	2	0.2308	After algae added
2025/5/12	14	5	0.2188	After algae added
2025/5/12	14	10	0.2780	After algae added
2025/5/13	15	0	0.2214	After algae added
2025/5/13	15	2	0.2465	After algae added
2025/5/13	15	5	0.2117	After algae added
2025/5/13	15	10	0.2959	After algae added
2025/5/16	18	0	0.1872	After algae added
2025/5/16	18	2	0.1917	After algae added
2025/5/16	18	5	0.1814	After algae added
2025/5/16	18	10	0.2404	After algae added

Table A1. Raw absorbance (A_{290}) data of E1 solutions with different initial

Appendix

concentrations in the algal treatment group at various time points

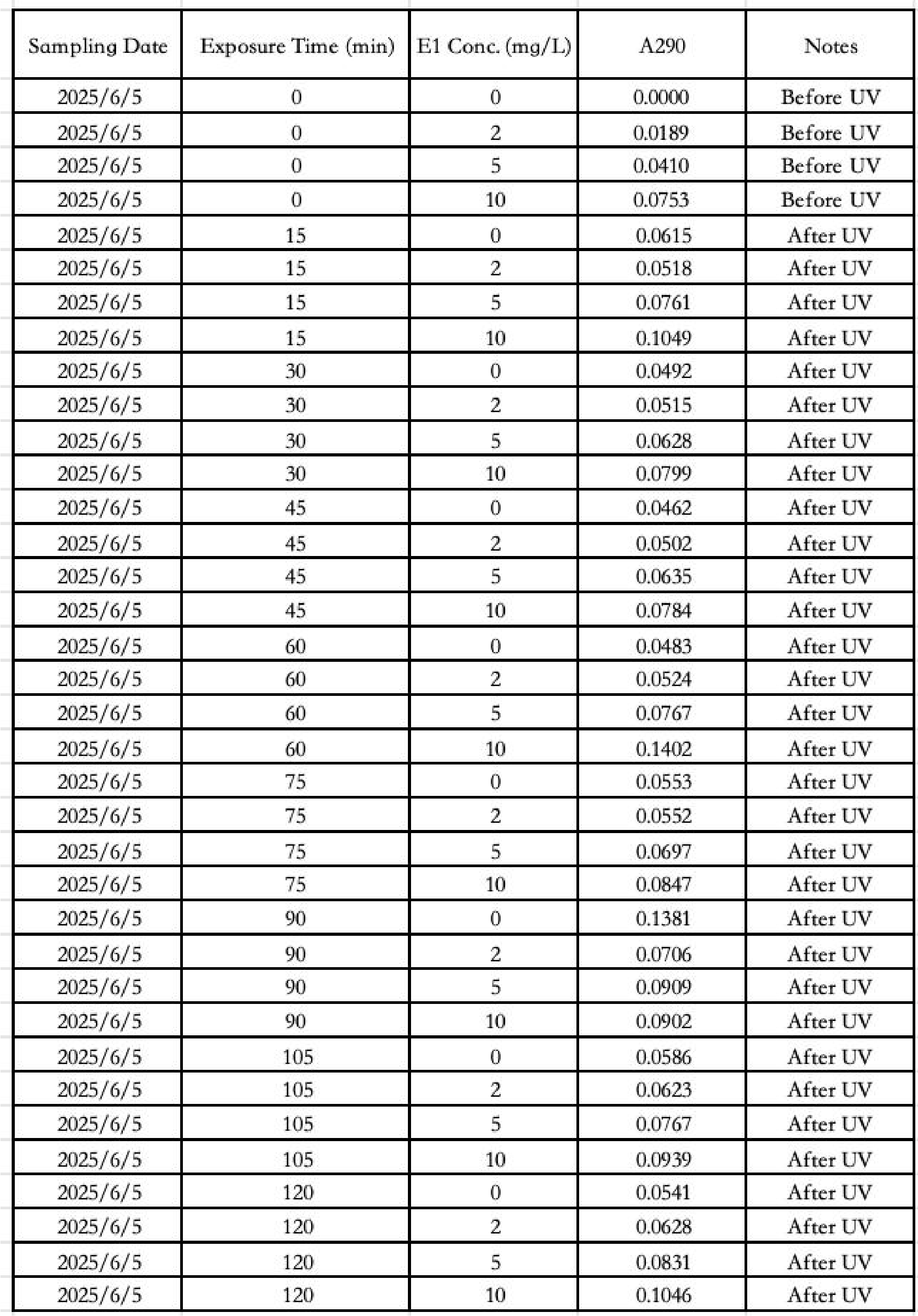
Appendix



Sampling Date	Day	E1 Conc. (mg/L)	A290	Notes
2025/4/28	-1	0	0.0000	Baseline, no algae/light
2025/4/28	-1	2	0.0189	Baseline, no algae/light
2025/4/28	-1	5	0.0410	Baseline, no algae/light
2025/4/28	-1	10	0.0753	Baseline, no algae/light
2025/4/28	0	0	0.0000	After natural light start
2025/4/28	0	2	0.0189	After natural light start
2025/4/28	0	5	0.0410	After natural light start
2025/4/28	0	10	0.0753	After natural light start
2025/4/29	1	0	0.0058	After natural light start
2025/4/29	1	2	0.0192	After natural light start
2025/4/29	1	5	0.0423	After natural light start
2025/4/29	1	10	0.0673	After natural light start
2025/4/30	2	0	0.0634	After natural light start
2025/4/30	2	2	0.0672	After natural light start
2025/4/30	2	5	0.0921	After natural light start
2025/4/30	2	10	0.0117	After natural light start
2025/5/1	3	0	-0.0032	After natural light start
2025/5/1	3	2	0.0062	After natural light start
2025/5/1	3	5	0.0323	After natural light start
2025/5/1	3	10	0.0646	After natural light start
2025/5/2	4	0	0.0042	After natural light start
2025/5/2	4	2	0.0119	After natural light start
2025/5/2	4	5	0.0368	After natural light start
2025/5/2	4	10	0.0725	After natural light start
2025/5/5	7	0	0.0016	After natural light start
2025/5/5	7	2	0.0155	After natural light start
2025/5/5	7	5	0.0215	After natural light start
2025/5/5	7	10	0.0326	After natural light start
2025/5/6	8	0	0.0550	After natural light start
2025/5/6	8	2	0.0566	After natural light start
2025/5/6	8	5	0.0802	After natural light start
2025/5/6	8	10	0.1225	After natural light start
2025/5/8	10	0	0.0580	After natural light start
2025/5/8	10	2	0.0687	After natural light start
2025/5/8	10	5	0.0559	After natural light start
2025/5/8	10	10	0.0883	After natural light start
2025/5/9	11	0	0.0528	After natural light start
2025/5/9	11	2	0.0589	After natural light start
2025/5/9	11	5	0.0607	After natural light start
2025/5/9	11	10	0.0807	After natural light start
2025/5/12	14	0	0.0703	After natural light start
2025/5/12	14	2	0.0668	After natural light start
2025/5/12	14	5	0.0650	After natural light start
2025/5/12	14	10	0.0999	After natural light start
2025/5/13	15	0	0.0647	After natural light start
2025/5/13	15	2	0.0690	After natural light start
2025/5/13	15	5	0.0639	After natural light start
2025/5/13	15	10	0.0802	After natural light start
2025/5/16	18	0	0.0457	After natural light start
2025/5/16	18	2	0.0485	After natural light start
2025/5/16	18	5	0.0439	After natural light start
2025/5/16	18	10	0.0569	After natural light start

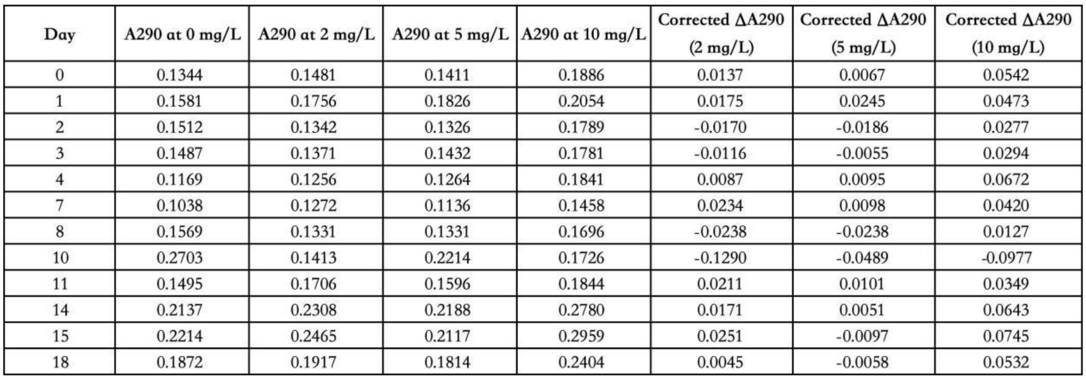
Appendix

Table A2. Raw absorbance (A_{290}) data of E1 solutions with different initial concentrations in the natural light treatment group at various time points



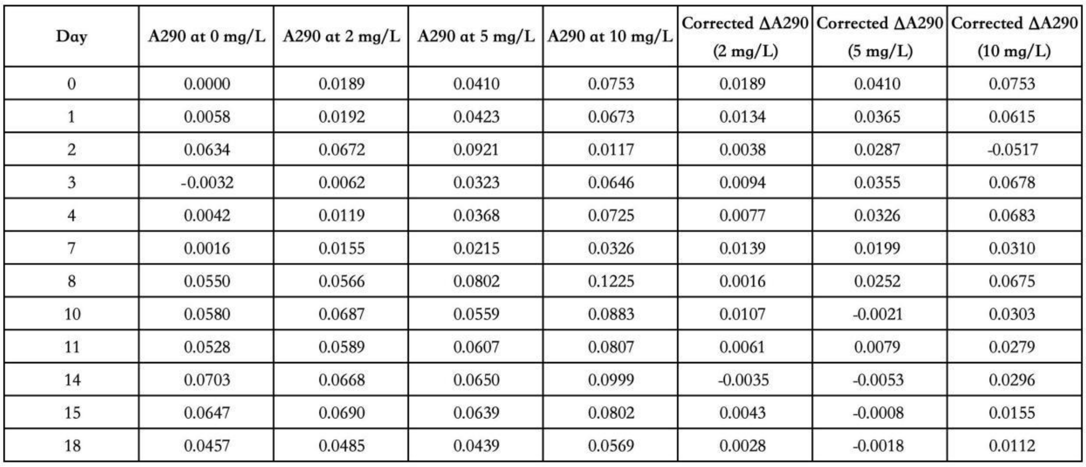
Sampling Date	Exposure Time (min)	E1 Conc. (mg/L)	A290	Notes
2025/6/5	0	0	0.0000	Before UV
2025/6/5	0	2	0.0189	Before UV
2025/6/5	0	5	0.0410	Before UV
2025/6/5	0	10	0.0753	Before UV
2025/6/5	15	0	0.0615	After UV
2025/6/5	15	2	0.0518	After UV
2025/6/5	15	5	0.0761	After UV
2025/6/5	15	10	0.1049	After UV
2025/6/5	30	0	0.0492	After UV
2025/6/5	30	2	0.0515	After UV
2025/6/5	30	5	0.0628	After UV
2025/6/5	30	10	0.0799	After UV
2025/6/5	45	0	0.0462	After UV
2025/6/5	45	2	0.0502	After UV
2025/6/5	45	5	0.0635	After UV
2025/6/5	45	10	0.0784	After UV
2025/6/5	60	0	0.0483	After UV
2025/6/5	60	2	0.0524	After UV
2025/6/5	60	5	0.0767	After UV
2025/6/5	60	10	0.1402	After UV
2025/6/5	75	0	0.0553	After UV
2025/6/5	75	2	0.0552	After UV
2025/6/5	75	5	0.0697	After UV
2025/6/5	75	10	0.0847	After UV
2025/6/5	90	0	0.1381	After UV
2025/6/5	90	2	0.0706	After UV
2025/6/5	90	5	0.0909	After UV
2025/6/5	90	10	0.0902	After UV
2025/6/5	105	0	0.0586	After UV
2025/6/5	105	2	0.0623	After UV
2025/6/5	105	5	0.0767	After UV
2025/6/5	105	10	0.0939	After UV
2025/6/5	120	0	0.0541	After UV
2025/6/5	120	2	0.0628	After UV
2025/6/5	120	5	0.0831	After UV
2025/6/5	120	10	0.1046	After UV

Table A3. Raw absorbance (A_{290}) data of E1 solutions with different initial concentrations in the UV light treatment group at various time points



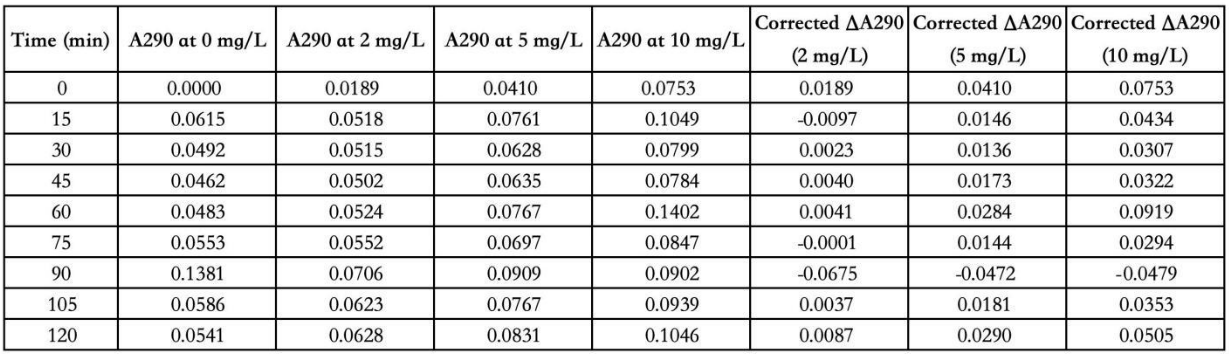
Day	A290 at 0 mg/L	A290 at 2 mg/L	A290 at 5 mg/L	A290 at 10 mg/L	Corrected ΔA290 (2 mg/L)	Corrected ΔA290 (5 mg/L)	Corrected ΔA290 (10 mg/L)
0	0.1344	0.1481	0.1411	0.1886	0.0137	0.0067	0.0542
1	0.1581	0.1756	0.1826	0.2054	0.0175	0.0245	0.0473
2	0.1512	0.1342	0.1326	0.1789	-0.0170	-0.0186	0.0277
3	0.1487	0.1371	0.1432	0.1781	-0.0116	-0.0055	0.0294
4	0.1169	0.1256	0.1264	0.1841	0.0087	0.0095	0.0672
7	0.1038	0.1272	0.1136	0.1458	0.0234	0.0098	0.0420
8	0.1569	0.1331	0.1331	0.1696	-0.0238	-0.0238	0.0127
10	0.2703	0.1413	0.2214	0.1726	-0.1290	-0.0489	-0.0977
11	0.1495	0.1706	0.1596	0.1844	0.0211	0.0101	0.0349
14	0.2137	0.2308	0.2188	0.2780	0.0171	0.0051	0.0643
15	0.2214	0.2465	0.2117	0.2959	0.0251	-0.0097	0.0745
18	0.1872	0.1917	0.1814	0.2404	0.0045	-0.0058	0.0532

Table A4. Raw absorbance (A_{290}) values and corrected ΔA_{290} values of E1 solutions with different initial concentrations after subtraction of the 0 mg/L control in the algal treatment group



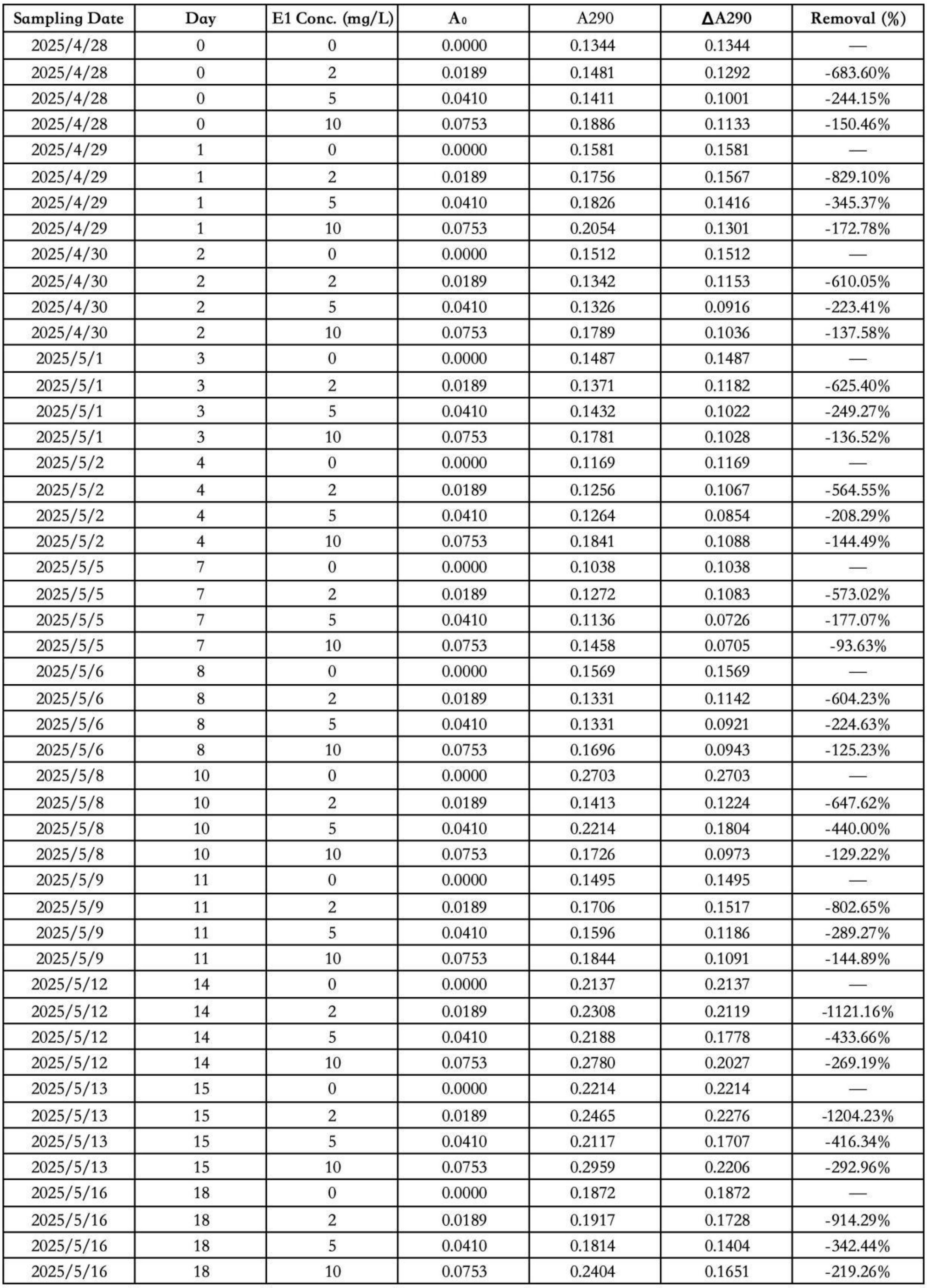
Day	A290 at 0 mg/L	A290 at 2 mg/L	A290 at 5 mg/L	A290 at 10 mg/L	Corrected ΔA290 (2 mg/L)	Corrected ΔA290 (5 mg/L)	Corrected ΔA290 (10 mg/L)
0	0.0000	0.0189	0.0410	0.0753	0.0189	0.0410	0.0753
1	0.0058	0.0192	0.0423	0.0673	0.0134	0.0365	0.0615
2	0.0634	0.0672	0.0921	0.0117	0.0038	0.0287	-0.0517
3	-0.0032	0.0062	0.0323	0.0646	0.0094	0.0355	0.0678
4	0.0042	0.0119	0.0368	0.0725	0.0077	0.0326	0.0683
7	0.0016	0.0155	0.0215	0.0326	0.0139	0.0199	0.0310
8	0.0550	0.0566	0.0802	0.1225	0.0016	0.0252	0.0675
10	0.0580	0.0687	0.0559	0.0883	0.0107	-0.0021	0.0303
11	0.0528	0.0589	0.0607	0.0807	0.0061	0.0079	0.0279
14	0.0703	0.0668	0.0650	0.0999	-0.0035	-0.0053	0.0296
15	0.0647	0.0690	0.0639	0.0802	0.0043	-0.0008	0.0155
18	0.0457	0.0485	0.0439	0.0569	0.0028	-0.0018	0.0112

Table A5. Raw absorbance (A_{290}) values and corrected ΔA_{290} values of E1 solutions with different initial concentrations after subtraction of the 0 mg/L control in the natural light treatment group



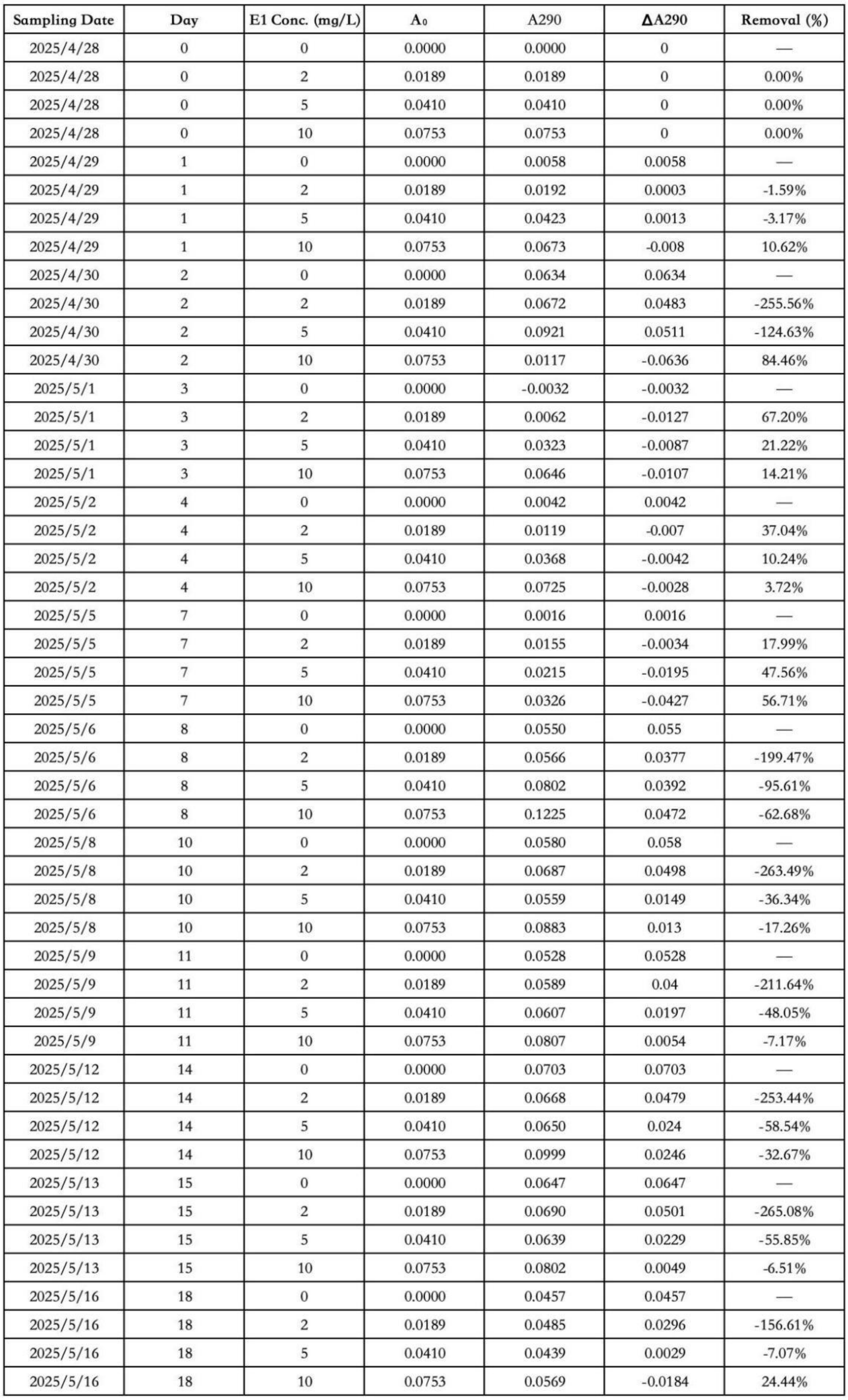
Time (min)	A290 at 0 mg/L	A290 at 2 mg/L	A290 at 5 mg/L	A290 at 10 mg/L	Corrected ΔA290 (2 mg/L)	Corrected ΔA290 (5 mg/L)	Corrected ΔA290 (10 mg/L)
0	0.0000	0.0189	0.0410	0.0753	0.0189	0.0410	0.0753
15	0.0615	0.0518	0.0761	0.1049	-0.0097	0.0146	0.0434
30	0.0492	0.0515	0.0628	0.0799	0.0023	0.0136	0.0307
45	0.0462	0.0502	0.0635	0.0784	0.0040	0.0173	0.0322
60	0.0483	0.0524	0.0767	0.1402	0.0041	0.0284	0.0919
75	0.0553	0.0552	0.0697	0.0847	-0.0001	0.0144	0.0294
90	0.1381	0.0706	0.0909	0.0902	-0.0675	-0.0472	-0.0479
105	0.0586	0.0623	0.0767	0.0939	0.0037	0.0181	0.0353
120	0.0541	0.0628	0.0831	0.1046	0.0087	0.0290	0.0505

Table A6. Raw absorbance (A_{290}) values and corrected Δ A_{290} values of E1 solutions with different initial concentrations after subtraction of the 0 mg/L control in the UV light treatment group



Sampling Date	Day	E1 Conc. (mg/L)	A_0	A290	ΔA290	Removal (%)
2025/4/28	0	0	0.0000	0.1344	0.1344	—
2025/4/28	0	2	0.0189	0.1481	0.1292	-683.60%
2025/4/28	0	5	0.0410	0.1411	0.1001	-244.15%
2025/4/28	0	10	0.0753	0.1886	0.1133	-150.46%
2025/4/29	1	0	0.0000	0.1581	0.1581	—
2025/4/29	1	2	0.0189	0.1756	0.1567	-829.10%
2025/4/29	1	5	0.0410	0.1826	0.1416	-345.37%
2025/4/29	1	10	0.0753	0.2054	0.1301	-172.78%
2025/4/30	2	0	0.0000	0.1512	0.1512	—
2025/4/30	2	2	0.0189	0.1342	0.1153	-610.05%
2025/4/30	2	5	0.0410	0.1326	0.0916	-223.41%
2025/4/30	2	10	0.0753	0.1789	0.1036	-137.58%
2025/5/1	3	0	0.0000	0.1487	0.1487	—
2025/5/1	3	2	0.0189	0.1371	0.1182	-625.40%
2025/5/1	3	5	0.0410	0.1432	0.1022	-249.27%
2025/5/1	3	10	0.0753	0.1781	0.1028	-136.52%
2025/5/2	4	0	0.0000	0.1169	0.1169	—
2025/5/2	4	2	0.0189	0.1256	0.1067	-564.55%
2025/5/2	4	5	0.0410	0.1264	0.0854	-208.29%
2025/5/2	4	10	0.0753	0.1841	0.1088	-144.49%
2025/5/5	7	0	0.0000	0.1038	0.1038	—
2025/5/5	7	2	0.0189	0.1272	0.1083	-573.02%
2025/5/5	7	5	0.0410	0.1136	0.0726	-177.07%
2025/5/5	7	10	0.0753	0.1458	0.0705	-93.63%
2025/5/6	8	0	0.0000	0.1569	0.1569	—
2025/5/6	8	2	0.0189	0.1331	0.1142	-604.23%
2025/5/6	8	5	0.0410	0.1331	0.0921	-224.63%
2025/5/6	8	10	0.0753	0.1696	0.0943	-125.23%
2025/5/8	10	0	0.0000	0.2703	0.2703	—
2025/5/8	10	2	0.0189	0.1413	0.1224	-647.62%
2025/5/8	10	5	0.0410	0.2214	0.1804	-440.00%
2025/5/8	10	10	0.0753	0.1726	0.0973	-129.22%
2025/5/9	11	0	0.0000	0.1495	0.1495	—
2025/5/9	11	2	0.0189	0.1706	0.1517	-802.65%
2025/5/9	11	5	0.0410	0.1596	0.1186	-289.27%
2025/5/9	11	10	0.0753	0.1844	0.1091	-144.89%
2025/5/12	14	0	0.0000	0.2137	0.2137	—
2025/5/12	14	2	0.0189	0.2308	0.2119	-1121.16%
2025/5/12	14	5	0.0410	0.2188	0.1778	-433.66%
2025/5/12	14	10	0.0753	0.2780	0.2027	-269.19%
2025/5/13	15	0	0.0000	0.2214	0.2214	—
2025/5/13	15	2	0.0189	0.2465	0.2276	-1204.23%
2025/5/13	15	5	0.0410	0.2117	0.1707	-416.34%
2025/5/13	15	10	0.0753	0.2959	0.2206	-292.96%
2025/5/16	18	0	0.0000	0.1872	0.1872	—
2025/5/16	18	2	0.0189	0.1917	0.1728	-914.29%
2025/5/16	18	5	0.0410	0.1814	0.1404	-342.44%
2025/5/16	18	10	0.0753	0.2404	0.1651	-219.26%

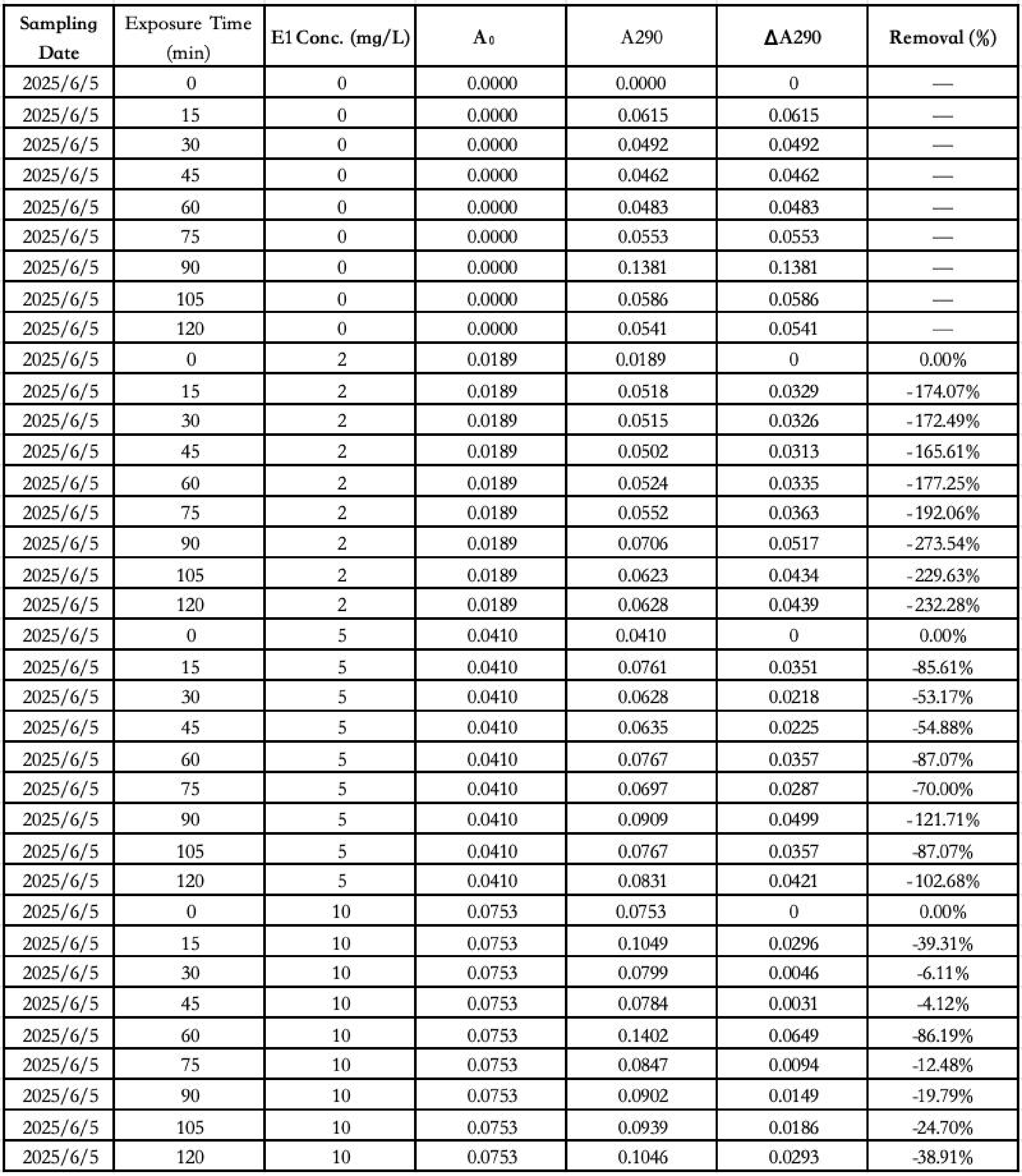
Table A7. Raw and corrected absorbance (A_{290}, ΔA_{290}) values and apparent removal rates of E1 solutions with different initial concentrations at various time points under algal treatment



Sampling Date	Day	E1 Conc. (mg/L)	A₀	A290	ΔA290	Removal (%)
2025/4/28	0	0	0.0000	0.0000	0	—
2025/4/28	0	2	0.0189	0.0189	0	0.00%
2025/4/28	0	5	0.0410	0.0410	0	0.00%
2025/4/28	0	10	0.0753	0.0753	0	0.00%
2025/4/29	1	0	0.0000	0.0058	0.0058	—
2025/4/29	1	2	0.0189	0.0192	0.0003	-1.59%
2025/4/29	1	5	0.0410	0.0423	0.0013	-3.17%
2025/4/29	1	10	0.0753	0.0673	-0.008	10.62%
2025/4/30	2	0	0.0000	0.0634	0.0634	—
2025/4/30	2	2	0.0189	0.0672	0.0483	-255.56%
2025/4/30	2	5	0.0410	0.0921	0.0511	-124.63%
2025/4/30	2	10	0.0753	0.0117	-0.0636	84.46%
2025/5/1	3	0	0.0000	-0.0032	-0.0032	—
2025/5/1	3	2	0.0189	0.0062	-0.0127	67.20%
2025/5/1	3	5	0.0410	0.0323	-0.0087	21.22%
2025/5/1	3	10	0.0753	0.0646	-0.0107	14.21%
2025/5/2	4	0	0.0000	0.0042	0.0042	—
2025/5/2	4	2	0.0189	0.0119	-0.007	37.04%
2025/5/2	4	5	0.0410	0.0368	-0.0042	10.24%
2025/5/2	4	10	0.0753	0.0725	-0.0028	3.72%
2025/5/5	7	0	0.0000	0.0016	0.0016	—
2025/5/5	7	2	0.0189	0.0155	-0.0034	17.99%
2025/5/5	7	5	0.0410	0.0215	-0.0195	47.56%
2025/5/5	7	10	0.0753	0.0326	-0.0427	56.71%
2025/5/6	8	0	0.0000	0.0550	0.055	—
2025/5/6	8	2	0.0189	0.0566	0.0377	-199.47%
2025/5/6	8	5	0.0410	0.0802	0.0392	-95.61%
2025/5/6	8	10	0.0753	0.1225	0.0472	-62.68%
2025/5/8	10	0	0.0000	0.0580	0.058	—
2025/5/8	10	2	0.0189	0.0687	0.0498	-263.49%
2025/5/8	10	5	0.0410	0.0559	0.0149	-36.34%
2025/5/8	10	10	0.0753	0.0883	0.013	-17.26%
2025/5/9	11	0	0.0000	0.0528	0.0528	—
2025/5/9	11	2	0.0189	0.0589	0.04	-211.64%
2025/5/9	11	5	0.0410	0.0607	0.0197	-48.05%
2025/5/9	11	10	0.0753	0.0807	0.0054	-7.17%
2025/5/12	14	0	0.0000	0.0703	0.0703	—
2025/5/12	14	2	0.0189	0.0668	0.0479	-253.44%
2025/5/12	14	5	0.0410	0.0650	0.024	-58.54%
2025/5/12	14	10	0.0753	0.0999	0.0246	-32.67%
2025/5/13	15	0	0.0000	0.0647	0.0647	—
2025/5/13	15	2	0.0189	0.0690	0.0501	-265.08%
2025/5/13	15	5	0.0410	0.0639	0.0229	-55.85%
2025/5/13	15	10	0.0753	0.0802	0.0049	-6.51%
2025/5/16	18	0	0.0000	0.0457	0.0457	—
2025/5/16	18	2	0.0189	0.0485	0.0296	-156.61%
2025/5/16	18	5	0.0410	0.0439	0.0029	-7.07%
2025/5/16	18	10	0.0753	0.0569	-0.0184	24.44%

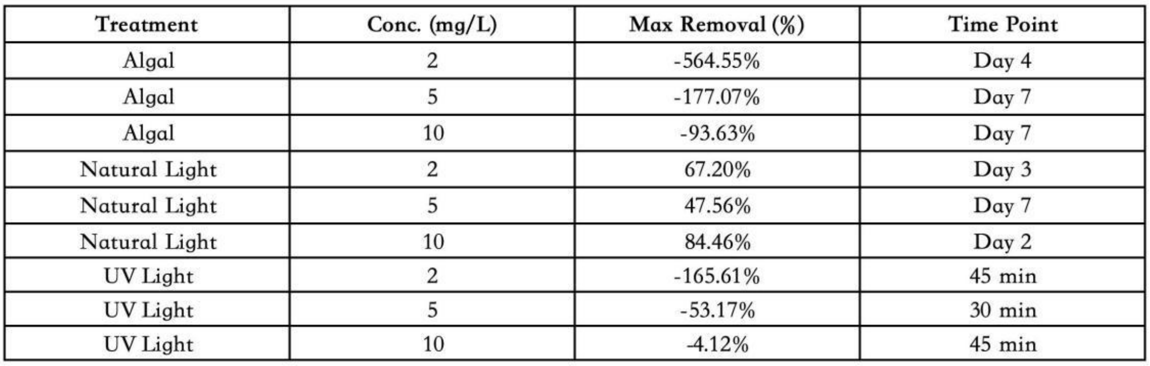
Table A8. Raw and corrected absorbance (A_{290}, ΔA_{290}) values and apparent removal rates of E1 solutions with different initial concentrations at various time points under natural light treatment

Appendix



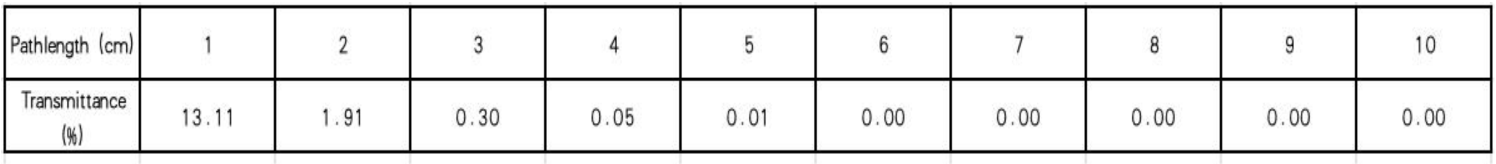
Sampling Date	Exposure Time (min)	E1 Conc. (mg/L)	A₀	A290	ΔA290	Removal (%)
2025/6/5	0	0	0.0000	0.0000	0	—
2025/6/5	15	0	0.0000	0.0615	0.0615	—
2025/6/5	30	0	0.0000	0.0492	0.0492	—
2025/6/5	45	0	0.0000	0.0462	0.0462	—
2025/6/5	60	0	0.0000	0.0483	0.0483	—
2025/6/5	75	0	0.0000	0.0553	0.0553	—
2025/6/5	90	0	0.0000	0.1381	0.1381	—
2025/6/5	105	0	0.0000	0.0586	0.0586	—
2025/6/5	120	0	0.0000	0.0541	0.0541	—
2025/6/5	0	2	0.0189	0.0189	0	0.00%
2025/6/5	15	2	0.0189	0.0518	0.0329	-174.07%
2025/6/5	30	2	0.0189	0.0515	0.0326	-172.49%
2025/6/5	45	2	0.0189	0.0502	0.0313	-165.61%
2025/6/5	60	2	0.0189	0.0524	0.0335	-177.25%
2025/6/5	75	2	0.0189	0.0552	0.0363	-192.06%
2025/6/5	90	2	0.0189	0.0706	0.0517	-273.54%
2025/6/5	105	2	0.0189	0.0623	0.0434	-229.63%
2025/6/5	120	2	0.0189	0.0628	0.0439	-232.28%
2025/6/5	0	5	0.0410	0.0410	0	0.00%
2025/6/5	15	5	0.0410	0.0761	0.0351	-85.61%
2025/6/5	30	5	0.0410	0.0628	0.0218	-53.17%
2025/6/5	45	5	0.0410	0.0635	0.0225	-54.88%
2025/6/5	60	5	0.0410	0.0767	0.0357	-87.07%
2025/6/5	75	5	0.0410	0.0697	0.0287	-70.00%
2025/6/5	90	5	0.0410	0.0909	0.0499	-121.71%
2025/6/5	105	5	0.0410	0.0767	0.0357	-87.07%
2025/6/5	120	5	0.0410	0.0831	0.0421	-102.68%
2025/6/5	0	10	0.0753	0.0753	0	0.00%
2025/6/5	15	10	0.0753	0.1049	0.0296	-39.31%
2025/6/5	30	10	0.0753	0.0799	0.0046	-6.11%
2025/6/5	45	10	0.0753	0.0784	0.0031	-4.12%
2025/6/5	60	10	0.0753	0.1402	0.0649	-86.19%
2025/6/5	75	10	0.0753	0.0847	0.0094	-12.48%
2025/6/5	90	10	0.0753	0.0902	0.0149	-19.79%
2025/6/5	105	10	0.0753	0.0939	0.0186	-24.70%
2025/6/5	120	10	0.0753	0.1046	0.0293	-38.91%

Table A9. Raw and corrected absorbance (A_{290}, ΔA_{290}) values and apparent removal rates of E1 solutions with different initial concentrations at various time points under UV light treatment



Treatment	Conc. (mg/L)	Max Removal (%)	Time Point
Algal	2	-564.55%	Day 4
Algal	5	-177.07%	Day 7
Algal	10	-93.63%	Day 7
Natural Light	2	67.20%	Day 3
Natural Light	5	47.56%	Day 7
Natural Light	10	84.46%	Day 2
UV Light	2	-165.61%	45 min
UV Light	5	-53.17%	30 min
UV Light	10	-4.12%	45 min

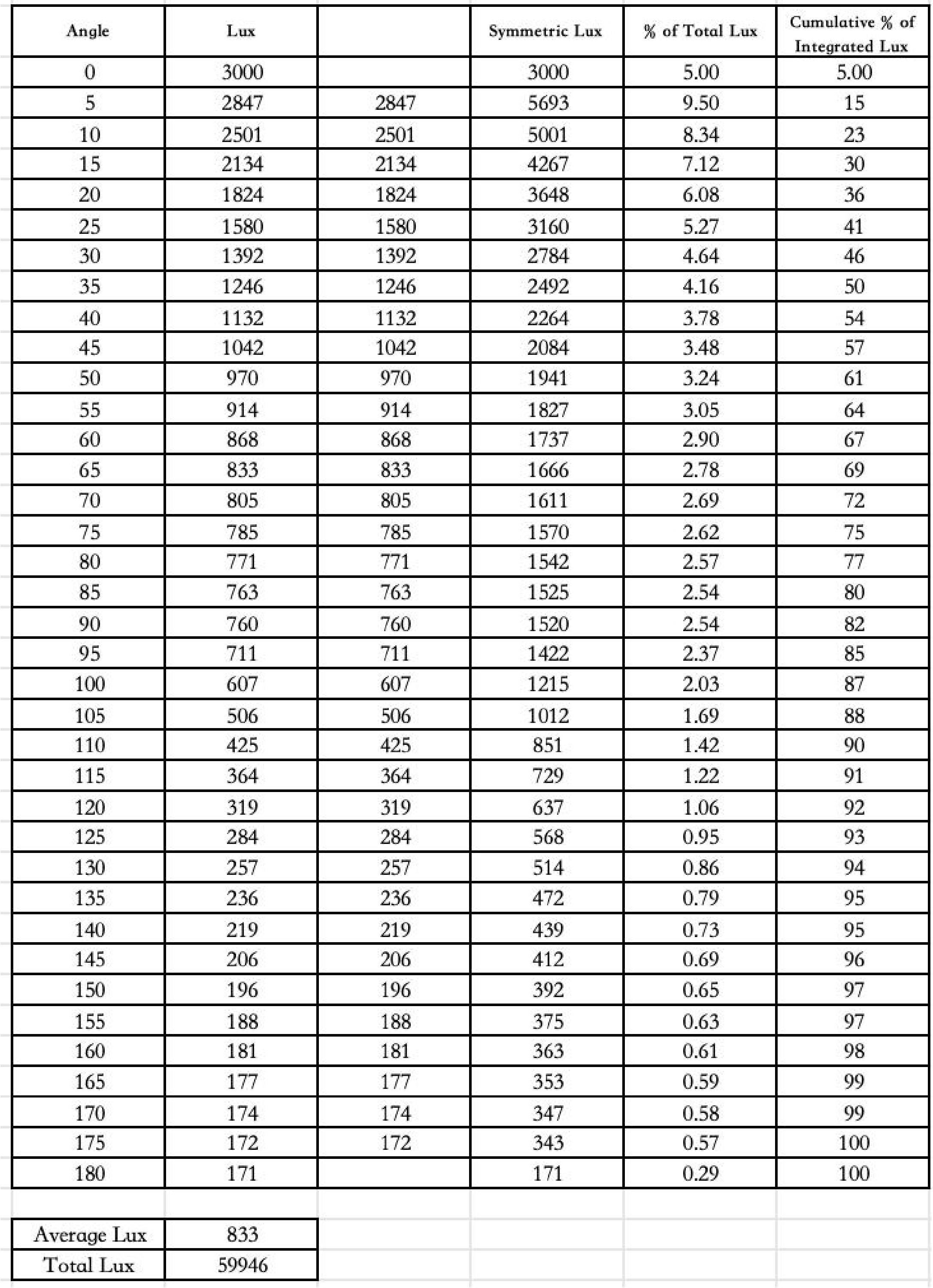
Table A10. Maximum removal efficiencies (%) of E1 solutions at different initial concentrations and corresponding time points under algal, natural light, and UV treatments



Pathlength (cm)	1	2	3	4	5	6	7	8	9	10
Transmittance (%)	13.11	1.91	0.30	0.05	0.01	0.00	0.00	0.00	0.00	0.00

Table A11. Measured light transmittance (%) at different optical path lengths

Appendix



Angle	Lux		Symmetric Lux	% of Total Lux	Cumulative % of Integrated Lux
0	3000		3000	5.00	5.00
5	2847	2847	5693	9.50	15
10	2501	2501	5001	8.34	23
15	2134	2134	4267	7.12	30
20	1824	1824	3648	6.08	36
25	1580	1580	3160	5.27	41
30	1392	1392	2784	4.64	46
35	1246	1246	2492	4.16	50
40	1132	1132	2264	3.78	54
45	1042	1042	2084	3.48	57
50	970	970	1941	3.24	61
55	914	914	1827	3.05	64
60	868	868	1737	2.90	67
65	833	833	1666	2.78	69
70	805	805	1611	2.69	72
75	785	785	1570	2.62	75
80	771	771	1542	2.57	77
85	763	763	1525	2.54	80
90	760	760	1520	2.54	82
95	711	711	1422	2.37	85
100	607	607	1215	2.03	87
105	506	506	1012	1.69	88
110	425	425	851	1.42	90
115	364	364	729	1.22	91
120	319	319	637	1.06	92
125	284	284	568	0.95	93
130	257	257	514	0.86	94
135	236	236	472	0.79	95
140	219	219	439	0.73	95
145	206	206	412	0.69	96
150	196	196	392	0.65	97
155	188	188	375	0.63	97
160	181	181	363	0.61	98
165	177	177	353	0.59	99
170	174	174	347	0.58	99
175	172	172	343	0.57	100
180	171		171	0.29	100

Average Lux	833
Total Lux	59946

Table A12. Angular distribution of light intensity (lux) and cumulative percentage of integrated lux under algal treatment

Angle	Lux of 1cm pathlength	Lux of 2cm pathlength	Lux of 3cm pathlength	Lux of 4cm pathlength	Lux of 5cm pathlength	Lux of 6cm pathlength	Lux of 7cm pathlength	Lux of 8cm pathlength	Lux of 9cm pathlength	Lux of 10cm pathlength	Average Lux
0	393.4	57.2	8.9	1.5	0.2	0.0	0.0	0.0	0.0	0.0	46.1
5	746.5	108.6	16.9	2.8	0.5	0.1	0.0	0.0	0.0	0.0	87.5
10	655.8	95.4	14.9	2.4	0.4	0.1	0.0	0.0	0.0	0.0	76.9
15	559.6	81.4	12.7	2.1	0.3	0.1	0.0	0.0	0.0	0.0	65.6
20	478.3	69.6	10.9	1.8	0.3	0.1	0.0	0.0	0.0	0.0	56.1
25	414.4	60.3	9.4	1.5	0.3	0.0	0.0	0.0	0.0	0.0	48.6
30	365.0	53.1	8.3	1.3	0.2	0.0	0.0	0.0	0.0	0.0	42.8
35	326.8	47.5	7.4	1.2	0.2	0.0	0.0	0.0	0.0	0.0	38.3
40	296.9	43.2	6.7	1.1	0.2	0.0	0.0	0.0	0.0	0.0	34.8
45	273.3	39.7	6.2	1.0	0.2	0.0	0.0	0.0	0.0	0.0	32.0
50	254.5	37.0	5.8	0.9	0.2	0.0	0.0	0.0	0.0	0.0	29.8
55	239.6	34.8	5.4	0.9	0.1	0.0	0.0	0.0	0.0	0.0	28.1
60	227.7	33.1	5.2	0.8	0.1	0.0	0.0	0.0	0.0	0.0	26.7
65	218.4	31.8	5.0	0.8	0.1	0.0	0.0	0.0	0.0	0.0	25.6
70	211.2	30.7	4.8	0.8	0.1	0.0	0.0	0.0	0.0	0.0	24.8
75	205.9	29.9	4.7	0.8	0.1	0.0	0.0	0.0	0.0	0.0	24.1
80	202.2	29.4	4.6	0.7	0.1	0.0	0.0	0.0	0.0	0.0	23.7
85	200.0	29.1	4.5	0.7	0.1	0.0	0.0	0.0	0.0	0.0	23.5
90	199.3	29.0	4.5	0.7	0.1	0.0	0.0	0.0	0.0	0.0	23.4
95	186.5	27.1	4.2	0.7	0.1	0.0	0.0	0.0	0.0	0.0	21.9
100	159.3	23.2	3.6	0.6	0.1	0.0	0.0	0.0	0.0	0.0	18.7
105	132.7	19.3	3.0	0.5	0.1	0.0	0.0	0.0	0.0	0.0	15.6
110	111.5	16.2	2.5	0.4	0.1	0.0	0.0	0.0	0.0	0.0	13.1
115	95.6	13.9	2.2	0.4	0.1	0.0	0.0	0.0	0.0	0.0	11.2
120	83.6	12.2	1.9	0.3	0.1	0.0	0.0	0.0	0.0	0.0	9.8
125	74.4	10.8	1.7	0.3	0.0	0.0	0.0	0.0	0.0	0.0	8.7
130	67.4	9.8	1.5	0.2	0.0	0.0	0.0	0.0	0.0	0.0	7.9
135	61.9	9.0	1.4	0.2	0.0	0.0	0.0	0.0	0.0	0.0	7.3
140	57.5	8.4	1.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	6.7
145	54.1	7.9	1.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	6.3
150	51.4	7.5	1.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	6.0
155	49.2	7.2	1.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	5.8
160	47.6	6.9	1.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	5.6
165	46.3	6.7	1.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	5.4
170	45.5	6.6	1.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	5.3
175	45.0	6.5	1.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	5.3
180	22.4	3.3	0.5	0.1	0.0	0.0	0.0	0.0	0.0	0.0	2.6
										Average	24.9

Table A13. Light intensity (lux) at varying optical path lengths (1–10 cm) across different angles in algal bottles

Angle	Lux		Symmetric Lux	% of Total Lux	Cumulative % of Integrated Lux
0	3000		3000	5.00	5.00
5	2847	2847	5693	9.50	15
10	2501	2501	5001	8.34	23
15	2134	2134	4267	7.12	30
20	1824	1824	3648	6.08	36
25	1580	1580	3160	5.27	41
30	1392	1392	2784	4.64	46
35	1246	1246	2492	4.16	50
40	1132	1132	2264	3.78	54
45	1042	1042	2084	3.48	57
50	970	970	1941	3.24	61
55	914	914	1827	3.05	64
60	868	868	1737	2.90	67
65	833	833	1666	2.78	69
70	805	805	1611	2.69	72
75	785	785	1570	2.62	75
80	771	771	1542	2.57	77
85	763	763	1525	2.54	80
90	760	760	1520	2.54	82
95	711	711	1422	2.37	85
100	607	607	1215	2.03	87
105	506	506	1012	1.69	88
110	425	425	851	1.42	90
115	364	364	729	1.22	91
120	319	319	637	1.06	92
125	284	284	568	0.95	93
130	257	257	514	0.86	94
135	236	236	472	0.79	95
140	219	219	439	0.73	95
145	206	206	412	0.69	96
150	196	196	392	0.65	97
155	188	188	375	0.63	97
160	181	181	363	0.61	98
165	177	177	353	0.59	99
170	174	174	347	0.58	99
175	172	172	343	0.57	100
180	171		171	0.29	100
Average	833				
Total	59946				

Table A14. Angular distribution of light intensity (lux) and cumulative percentage of integrated lux without algal

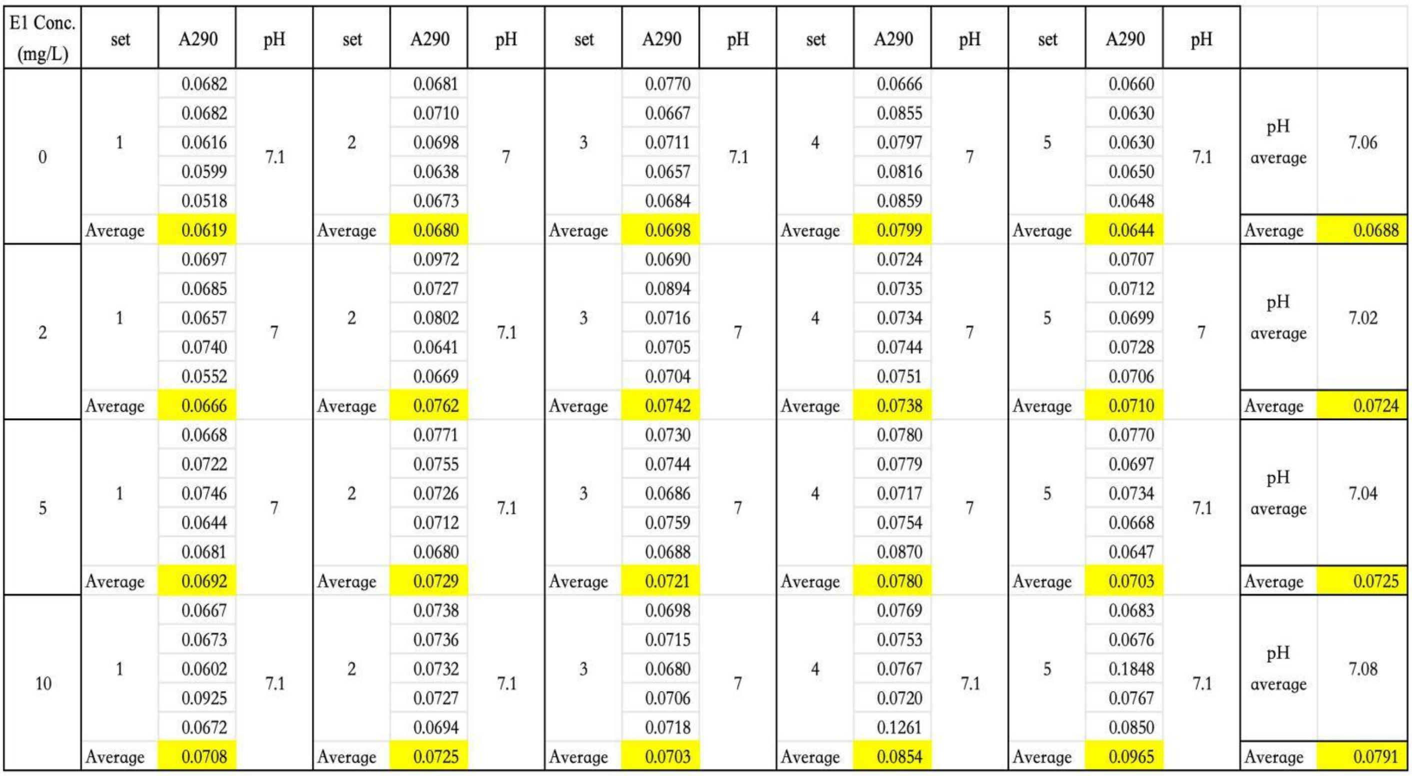
Angle	Lux of 1cm pathlength	Lux of 2cm pathlength	Lux of 3cm pathlength	Lux of 4cm pathlength	Lux of 5cm pathlength	Lux of 6cm pathlength	Lux of 7cm pathlength	Lux of 8cm pathlength	Lux of 9cm pathlength	Lux of 10cm pathlength	Average Lux
0	393.4	57.2	8.9	1.5	0.2	0.0	0.0	0.0	0.0	0.0	46.1
5	746.5	108.6	16.9	2.8	0.5	0.1	0.0	0.0	0.0	0.0	87.5
10	655.8	95.4	14.9	2.4	0.4	0.1	0.0	0.0	0.0	0.0	76.9
15	559.6	81.4	12.7	2.1	0.3	0.1	0.0	0.0	0.0	0.0	65.6
20	478.3	69.6	10.9	1.8	0.3	0.1	0.0	0.0	0.0	0.0	56.1
25	414.4	60.3	9.4	1.5	0.3	0.0	0.0	0.0	0.0	0.0	48.6
30	365.0	53.1	8.3	1.3	0.2	0.0	0.0	0.0	0.0	0.0	42.8
35	326.8	47.5	7.4	1.2	0.2	0.0	0.0	0.0	0.0	0.0	38.3
40	296.9	43.2	6.7	1.1	0.2	0.0	0.0	0.0	0.0	0.0	34.8
45	273.3	39.7	6.2	1.0	0.2	0.0	0.0	0.0	0.0	0.0	32.0
50	254.5	37.0	5.8	0.9	0.2	0.0	0.0	0.0	0.0	0.0	29.8
55	239.6	34.8	5.4	0.9	0.1	0.0	0.0	0.0	0.0	0.0	28.1
60	227.7	33.1	5.2	0.8	0.1	0.0	0.0	0.0	0.0	0.0	26.7
65	218.4	31.8	5.0	0.8	0.1	0.0	0.0	0.0	0.0	0.0	25.6
70	211.2	30.7	4.8	0.8	0.1	0.0	0.0	0.0	0.0	0.0	24.8
75	205.9	29.9	4.7	0.8	0.1	0.0	0.0	0.0	0.0	0.0	24.1
80	202.2	29.4	4.6	0.7	0.1	0.0	0.0	0.0	0.0	0.0	23.7
85	200.0	29.1	4.5	0.7	0.1	0.0	0.0	0.0	0.0	0.0	23.5
90	199.3	29.0	4.5	0.7	0.1	0.0	0.0	0.0	0.0	0.0	23.4
95	186.5	27.1	4.2	0.7	0.1	0.0	0.0	0.0	0.0	0.0	21.9
100	159.3	23.2	3.6	0.6	0.1	0.0	0.0	0.0	0.0	0.0	18.7
105	132.7	19.3	3.0	0.5	0.1	0.0	0.0	0.0	0.0	0.0	15.6
110	111.5	16.2	2.5	0.4	0.1	0.0	0.0	0.0	0.0	0.0	13.1
115	95.6	13.9	2.2	0.4	0.1	0.0	0.0	0.0	0.0	0.0	11.2
120	83.6	12.2	1.9	0.3	0.1	0.0	0.0	0.0	0.0	0.0	9.8
125	74.4	10.8	1.7	0.3	0.0	0.0	0.0	0.0	0.0	0.0	8.7
130	67.4	9.8	1.5	0.2	0.0	0.0	0.0	0.0	0.0	0.0	7.9
135	61.9	9.0	1.4	0.2	0.0	0.0	0.0	0.0	0.0	0.0	7.3
140	57.5	8.4	1.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	6.7
145	54.1	7.9	1.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	6.3
150	51.4	7.5	1.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	6.0
155	49.2	7.2	1.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	5.8
160	47.6	6.9	1.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	5.6
165	46.3	6.7	1.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	5.4
170	45.5	6.6	1.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	5.3
175	45.0	6.5	1.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	5.3
180	22.4	3.3	0.5	0.1	0.0	0.0	0.0	0.0	0.0	0.0	2.6
										Average	24.9

Table A15. Light intensity (lux) at varying optical path lengths (1–10 cm) across different angles in non- algal bottles

pH	E1 Conc. (mg/L)	set	A290	set	A290	set	A290	set	A290	set	A290		
7	0	1	0.0718	2	0.0659	3	0.0658	4	0.0737	5	0.0710	Average	0.0696
	2	1	0.0695	2	0.0608	3	0.0710	4	0.0742	5	0.0742	Average	0.0699
	5	1	0.0737	2	0.0690	3	0.0727	4	0.0778	5	0.0719	Average	0.0730
	10	1	0.0995	2	0.0697	3	0.1208	4	0.0764	5	0.0715	Average	0.0876

Table A16. Absorbance (A290) of E1 solutions with varying initial concentrations at pH 7, including replicate measurements (n = 5) and averages on 15 July 2025

Appendix

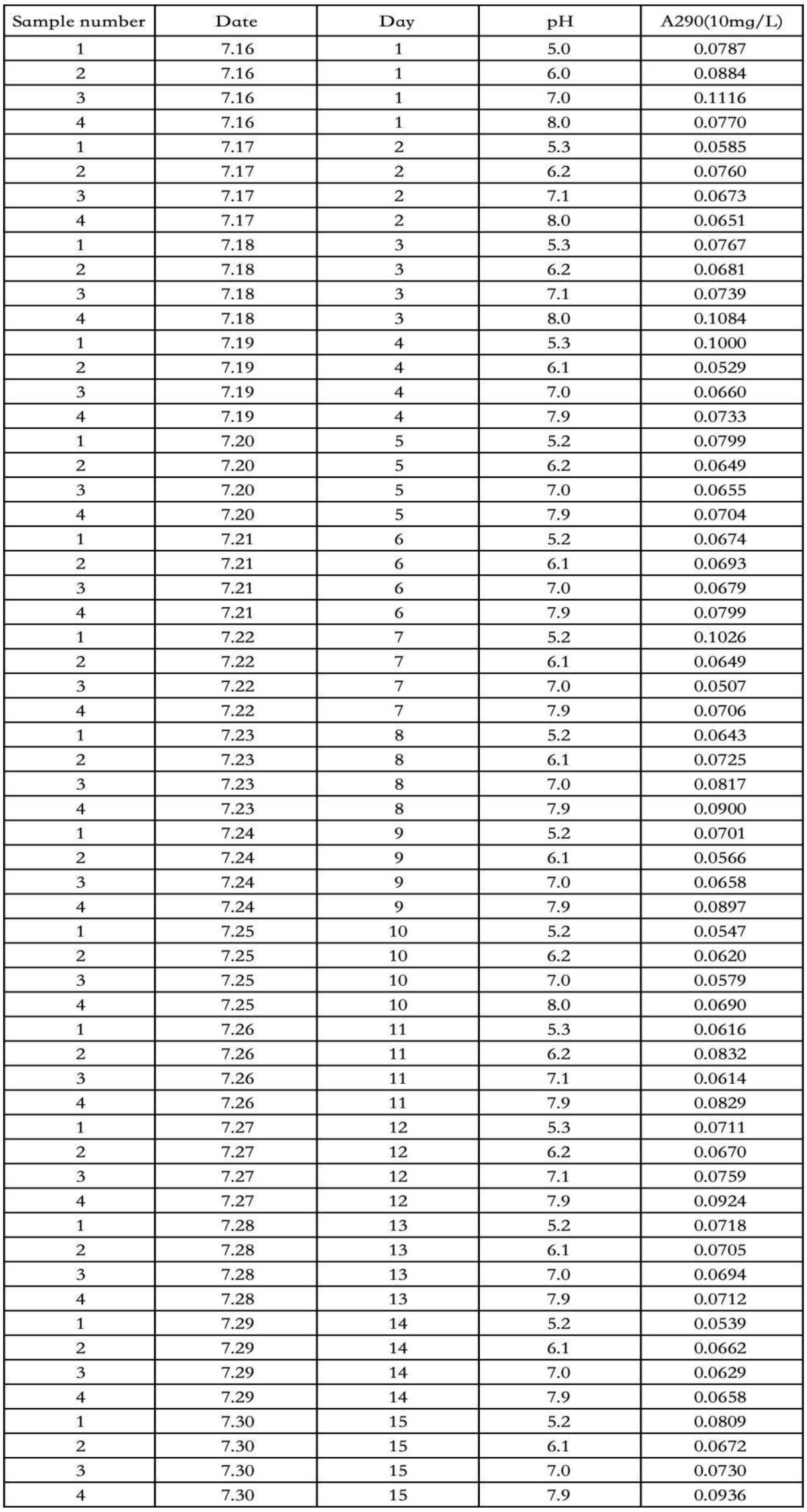


E1 Conc. (mg/L)	set	A290	pH	set	A290	pH	set	A290	pH	set	A290	pH	set	A290	pH		
0	1	0.0682	7.1	2	0.0681	7	3	0.0770	7.1	4	0.0666	7	5	0.0660	7.1	pH average	7.06
		0.0682			0.0710			0.0667			0.0855			0.0630			
		0.0616			0.0698			0.0711			0.0797			0.0630			
		0.0599			0.0638			0.0657			0.0816			0.0650			
		0.0518			0.0673			0.0684			0.0859			0.0648			
	Average	0.0619		Average	0.0680		Average	0.0698		Average	0.0799		Average	0.0644		Average	0.0688
2	1	0.0697	7	2	0.0972	7.1	3	0.0690	7	4	0.0724	7	5	0.0707	7	pH average	7.02
		0.0685			0.0727			0.0894			0.0735			0.0712			
		0.0657			0.0802			0.0716			0.0734			0.0699			
		0.0740			0.0641			0.0705			0.0744			0.0728			
		0.0552			0.0669			0.0704			0.0751			0.0706			
	Average	0.0666		Average	0.0762		Average	0.0742		Average	0.0738		Average	0.0710		Average	0.0724
5	1	0.0668	7	2	0.0771	7.1	3	0.0730	7	4	0.0780	7	5	0.0770	7.1	pH average	7.04
		0.0722			0.0755			0.0744			0.0779			0.0697			
		0.0746			0.0726			0.0686			0.0717			0.0734			
		0.0644			0.0712			0.0759			0.0754			0.0668			
		0.0681			0.0680			0.0688			0.0870			0.0647			
	Average	0.0692		Average	0.0729		Average	0.0721		Average	0.0780		Average	0.0703		Average	0.0725
10	1	0.0667	7.1	2	0.0738	7.1	3	0.0698	7	4	0.0769	7.1	5	0.0683	7.1	pH average	7.08
		0.0673			0.0736			0.0715			0.0753			0.0676			
		0.0602			0.0732			0.0680			0.0767			0.1848			
		0.0925			0.0727			0.0706			0.0720			0.0767			
		0.0672			0.0694			0.0718			0.1261			0.0850			
	Average	0.0708		Average	0.0725		Average	0.0703		Average	0.0854		Average	0.0965		Average	0.0791

Table A17. Overnight changes in absorbance (A290) and pH of E1 algal solutions on 16 July 2025

Appendix

a. Day 1 – 15



Sample number	Date	Day	pH	A290(10mg/L)
1	7.16	1	5.0	0.0787
2	7.16	1	6.0	0.0884
3	7.16	1	7.0	0.1116
4	7.16	1	8.0	0.0770
1	7.17	2	5.3	0.0585
2	7.17	2	6.2	0.0760
3	7.17	2	7.1	0.0673
4	7.17	2	8.0	0.0651
1	7.18	3	5.3	0.0767
2	7.18	3	6.2	0.0681
3	7.18	3	7.1	0.0739
4	7.18	3	8.0	0.1084
1	7.19	4	5.3	0.1000
2	7.19	4	6.1	0.0529
3	7.19	4	7.0	0.0660
4	7.19	4	7.9	0.0733
1	7.20	5	5.2	0.0799
2	7.20	5	6.2	0.0649
3	7.20	5	7.0	0.0655
4	7.20	5	7.9	0.0704
1	7.21	6	5.2	0.0674
2	7.21	6	6.1	0.0693
3	7.21	6	7.0	0.0679
4	7.21	6	7.9	0.0799
1	7.22	7	5.2	0.1026
2	7.22	7	6.1	0.0649
3	7.22	7	7.0	0.0507
4	7.22	7	7.9	0.0706
1	7.23	8	5.2	0.0643
2	7.23	8	6.1	0.0725
3	7.23	8	7.0	0.0817
4	7.23	8	7.9	0.0900
1	7.24	9	5.2	0.0701
2	7.24	9	6.1	0.0566
3	7.24	9	7.0	0.0658
4	7.24	9	7.9	0.0897
1	7.25	10	5.2	0.0547
2	7.25	10	6.2	0.0620
3	7.25	10	7.0	0.0579
4	7.25	10	8.0	0.0690
1	7.26	11	5.3	0.0616
2	7.26	11	6.2	0.0832
3	7.26	11	7.1	0.0614
4	7.26	11	7.9	0.0829
1	7.27	12	5.3	0.0711
2	7.27	12	6.2	0.0670
3	7.27	12	7.1	0.0759
4	7.27	12	7.9	0.0924
1	7.28	13	5.2	0.0718
2	7.28	13	6.1	0.0705
3	7.28	13	7.0	0.0694
4	7.28	13	7.9	0.0712
1	7.29	14	5.2	0.0539
2	7.29	14	6.1	0.0662
3	7.29	14	7.0	0.0629
4	7.29	14	7.9	0.0658
1	7.30	15	5.2	0.0809
2	7.30	15	6.1	0.0672
3	7.30	15	7.0	0.0730
4	7.30	15	7.9	0.0936

Appendix

b: Day 16 – 31

Sample number	Date	Day	pH	A290(10mg/L)
1	7.31	16	5.2	0.0674
2	7.31	16	6.1	0.0654
3	7.31	16	7.0	0.0689
4	7.31	16	7.9	0.0798
1	8.3	19	5.4	0.0694
2	8.3	19	6.3	0.0703
3	8.3	19	7.2	0.0670
4	8.3	19	8.0	0.0831
1	8.4	20	5.3	0.0703
2	8.4	20	6.2	0.0664
3	8.4	20	7.0	0.0692
4	8.4	20	7.9	0.0898
1	8.5	21	5.3	0.0603
2	8.5	21	6.1	0.0620
3	8.5	21	7.1	0.0647
4	8.5	21	7.9	0.0878
1	8.6	22	5.2	0.0664
2	8.6	22	6.1	0.0799
3	8.6	22	7.0	0.0753
4	8.6	22	8.0	0.0862
1	8.7	23	5.2	0.0684
2	8.7	23	6.1	0.0674
3	8.7	23	7.0	0.1055
4	8.7	23	8.0	0.1205
1	8.8	24	5.2	0.0760
2	8.8	24	6.1	0.0779
3	8.8	24	7.0	0.0968
4	8.8	24	8.0	0.1076
1	8.9	25	5.3	0.0722
2	8.9	25	6.2	0.0770
3	8.9	25	7.1	0.0774
4	8.9	25	8.0	0.1056
1	8.10	26	5.3	0.0710
2	8.10	26	6.2	0.0624
3	8.10	26	7.1	0.0792
4	8.10	26	7.9	0.0975
1	8.11	27	5.3	0.1019
2	8.11	27	6.2	0.0778
3	8.11	27	7.1	0.0781
4	8.11	27	8.0	0.0963
1	8.12	28	5.3	0.0689
2	8.12	28	6.2	0.0613
3	8.12	28	7.1	0.0945
4	8.12	28	8.0	0.0869
1	8.13	29	5.3	0.0783
2	8.13	29	6.2	0.0940
3	8.13	29	7.1	0.0964
4	8.13	29	8.0	0.1147
1	8.14	30	5.3	0.0786
2	8.14	30	6.2	0.0726
3	8.14	30	7.1	0.0695
4	8.14	30	8.0	0.1054
1	8.15	31	5.3	0.0666
2	8.15	31	6.2	0.0761
3	8.15	31	7.1	0.0779
4	8.15	31	7.9	0.1053

Table A18. Absorbance (A_{290}) and pH measurements of E1 solutions (10 mg/L) under algal treatment at different initial pH values (5.0 – 8.0)

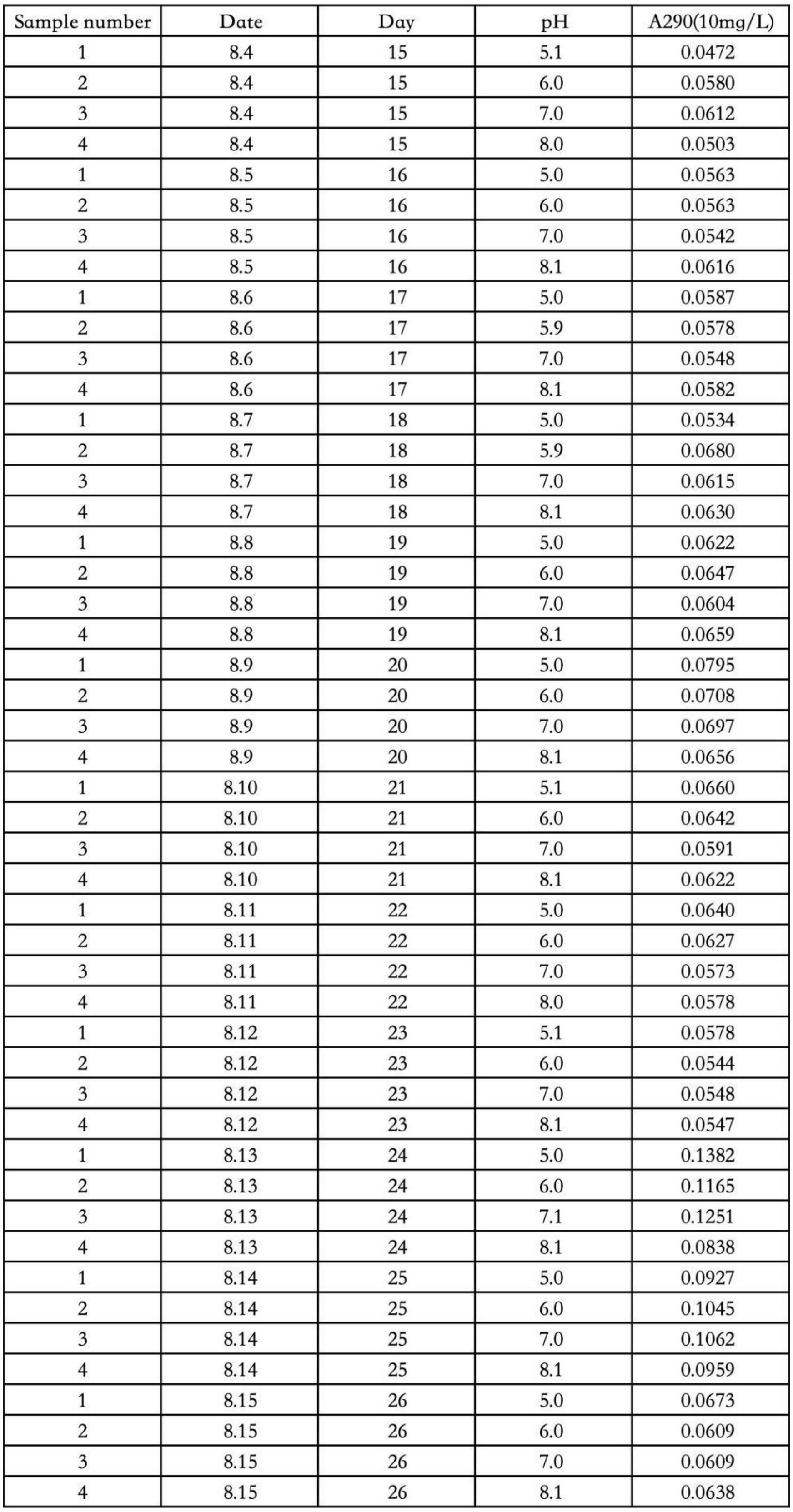
Appendix

a. Day 1 – 14

Sample number	Date	Day	pH	A290(10mg/L)
1	7.21	1	5.0	0.0835
2	7.21	1	6.0	0.0491
3	7.21	1	7.0	0.0450
4	7.21	1	8.0	0.0487
1	7.22	2	5.1	0.0700
2	7.22	2	6.0	0.0509
3	7.22	2	7.0	0.0561
4	7.22	2	8.0	0.0670
1	7.23	3	5.0	0.0698
2	7.23	3	6.0	0.0679
3	7.23	3	7.0	0.0692
4	7.23	3	8.0	0.0703
1	7.24	4	5.0	0.0676
2	7.24	4	6.0	0.0637
3	7.24	4	7.0	0.0636
4	7.24	4	8.0	0.0640
1	7.25	5	5.0	0.0586
2	7.25	5	6.0	0.0716
3	7.25	5	7.0	0.0768
4	7.25	5	8.0	0.0616
1	7.26	6	5.1	0.0595
2	7.26	6	6.0	0.0677
3	7.26	6	7.1	0.0542
4	7.26	6	8.0	0.0703
1	7.27	7	5.1	0.0774
2	7.27	7	6.0	0.0883
3	7.27	7	7.1	0.0855
4	7.27	7	8.1	0.0702
1	7.28	8	5.0	0.0653
2	7.28	8	5.9	0.0764
3	7.28	8	7.0	0.0675
4	7.28	8	8.0	0.0602
1	7.29	9	5.0	0.0597
2	7.29	9	5.9	0.0641
3	7.29	9	7.0	0.0764
4	7.29	9	8.0	0.0797
1	7.30	10	5.0	0.0667
2	7.30	10	5.9	0.0636
3	7.30	10	7.0	0.0609
4	7.30	10	8.0	0.0616
1	7.31	11	5.0	0.0486
2	7.31	11	5.9	0.0579
3	7.31	11	7.0	0.0564
4	7.31	11	8.0	0.0568
1	8.1	12	5.0	0.0612
2	8.1	12	5.9	0.0789
3	8.1	12	7.0	0.1092
4	8.1	12	8.0	0.0117
1	8.3	14	5.2	0.0505
2	8.3	14	6.1	0.0522
3	8.3	14	7.1	0.0520
4	8.3	14	8.1	0.0520

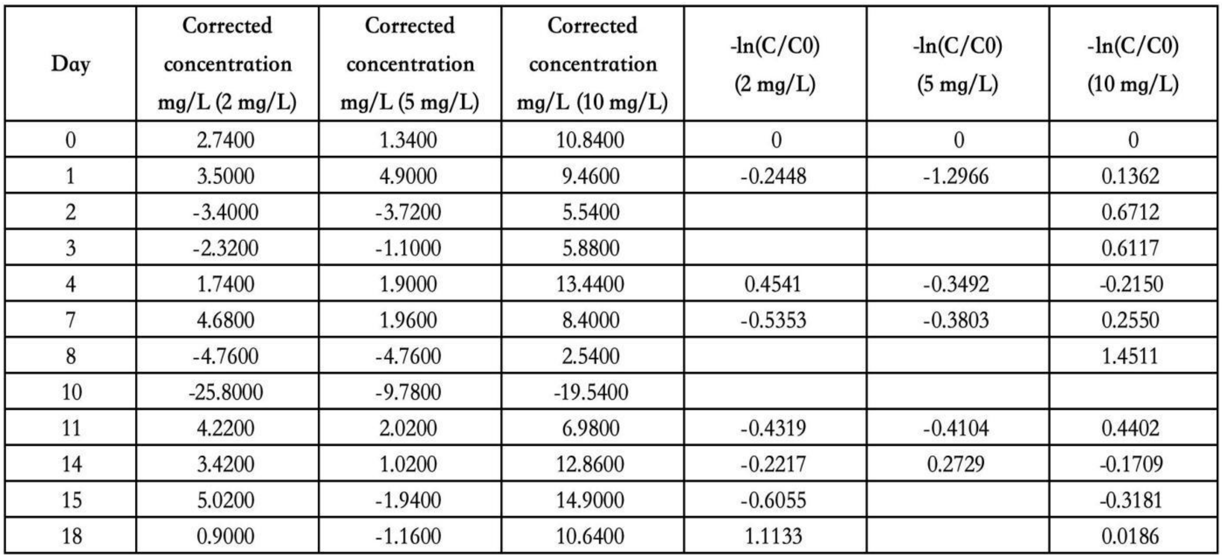
Appendix

b. Day 15 – 26



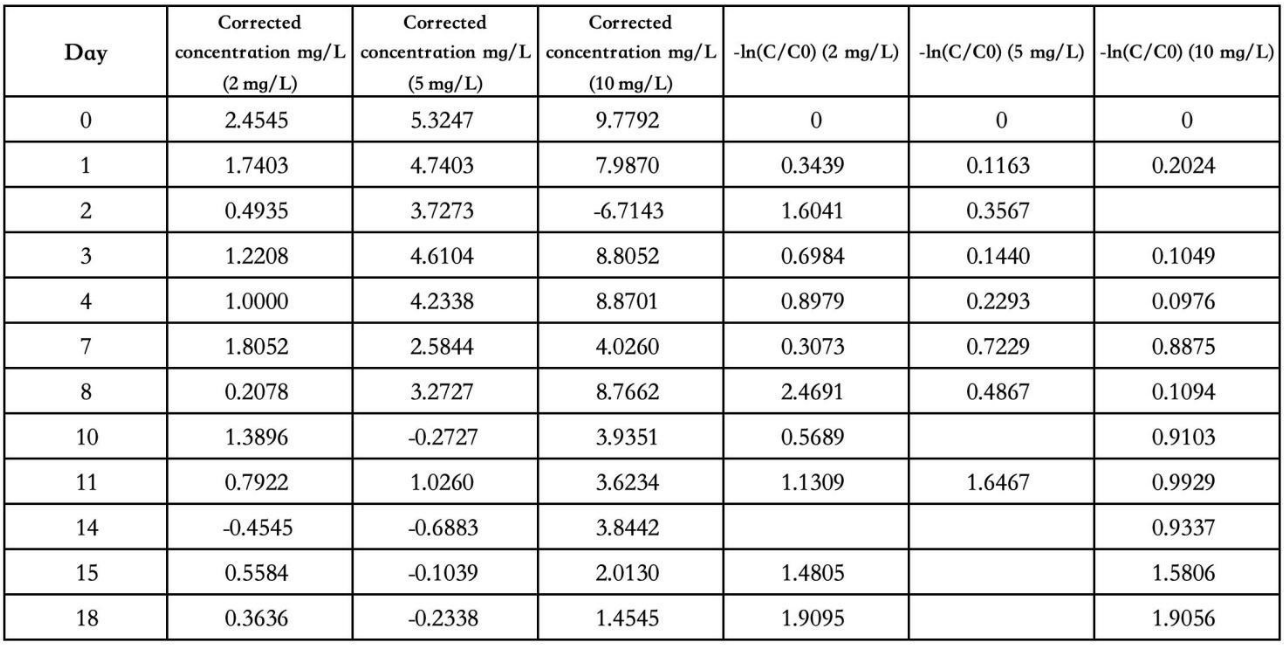
Sample number	Date	Day	pH	A290(10mg/L)
1	8.4	15	5.1	0.0472
2	8.4	15	6.0	0.0580
3	8.4	15	7.0	0.0612
4	8.4	15	8.0	0.0503
1	8.5	16	5.0	0.0563
2	8.5	16	6.0	0.0563
3	8.5	16	7.0	0.0542
4	8.5	16	8.1	0.0616
1	8.6	17	5.0	0.0587
2	8.6	17	5.9	0.0578
3	8.6	17	7.0	0.0548
4	8.6	17	8.1	0.0582
1	8.7	18	5.0	0.0534
2	8.7	18	5.9	0.0680
3	8.7	18	7.0	0.0615
4	8.7	18	8.1	0.0630
1	8.8	19	5.0	0.0622
2	8.8	19	6.0	0.0647
3	8.8	19	7.0	0.0604
4	8.8	19	8.1	0.0659
1	8.9	20	5.0	0.0795
2	8.9	20	6.0	0.0708
3	8.9	20	7.0	0.0697
4	8.9	20	8.1	0.0656
1	8.10	21	5.1	0.0660
2	8.10	21	6.0	0.0642
3	8.10	21	7.0	0.0591
4	8.10	21	8.1	0.0622
1	8.11	22	5.0	0.0640
2	8.11	22	6.0	0.0627
3	8.11	22	7.0	0.0573
4	8.11	22	8.0	0.0578
1	8.12	23	5.1	0.0578
2	8.12	23	6.0	0.0544
3	8.12	23	7.0	0.0548
4	8.12	23	8.1	0.0547
1	8.13	24	5.0	0.1382
2	8.13	24	6.0	0.1165
3	8.13	24	7.1	0.1251
4	8.13	24	8.1	0.0838
1	8.14	25	5.0	0.0927
2	8.14	25	6.0	0.1045
3	8.14	25	7.0	0.1062
4	8.14	25	8.1	0.0959
1	8.15	26	5.0	0.0673
2	8.15	26	6.0	0.0609
3	8.15	26	7.0	0.0609
4	8.15	26	8.1	0.0638

Table A19. Absorbance (A_{290}) and pH measurements of E1 solutions (10 mg/L) under natural light treatment at different initial pH values (5.0 – 8.0)



Day	Corrected concentration mg/L (2 mg/L)	Corrected concentration mg/L (5 mg/L)	Corrected concentration mg/L (10 mg/L)	-ln(C/C0) (2 mg/L)	-ln(C/C0) (5 mg/L)	-ln(C/C0) (10 mg/L)
0	2.7400	1.3400	10.8400	0	0	0
1	3.5000	4.9000	9.4600	-0.2448	-1.2966	0.1362
2	-3.4000	-3.7200	5.5400			0.6712
3	-2.3200	-1.1000	5.8800			0.6117
4	1.7400	1.9000	13.4400	0.4541	-0.3492	-0.2150
7	4.6800	1.9600	8.4000	-0.5353	-0.3803	0.2550
8	-4.7600	-4.7600	2.5400			1.4511
10	-25.8000	-9.7800	-19.5400			
11	4.2200	2.0200	6.9800	-0.4319	-0.4104	0.4402
14	3.4200	1.0200	12.8600	-0.2217	0.2729	-0.1709
15	5.0200	-1.9400	14.9000	-0.6055		-0.3181
18	0.9000	-1.1600	10.6400	1.1133		0.0186

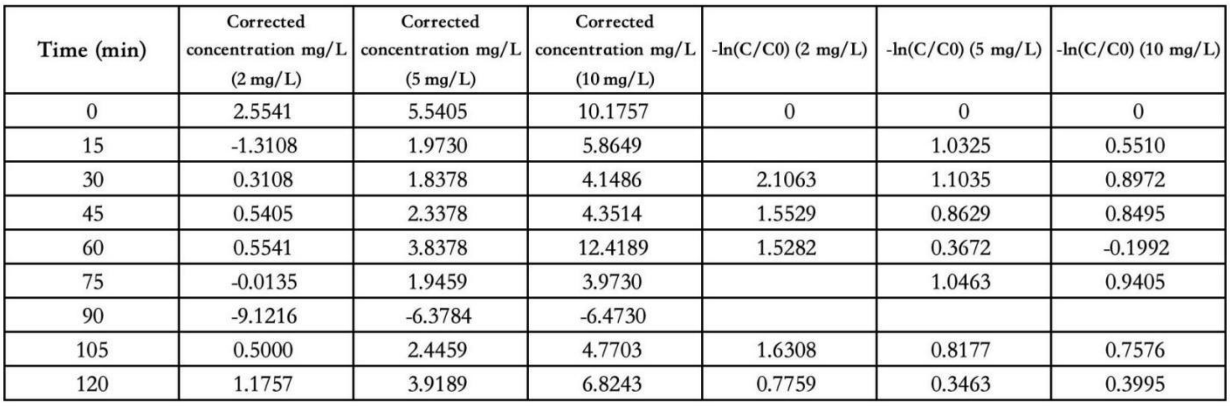
Table A20. Corrected E1 concentrations and calculated −ln(C/C_0) values in the algal group



Day	Corrected concentration mg/L (2 mg/L)	Corrected concentration mg/L (5 mg/L)	Corrected concentration mg/L (10 mg/L)	-ln(C/C0) (2 mg/L)	-ln(C/C0) (5 mg/L)	-ln(C/C0) (10 mg/L)
0	2.4545	5.3247	9.7792	0	0	0
1	1.7403	4.7403	7.9870	0.3439	0.1163	0.2024
2	0.4935	3.7273	-6.7143	1.6041	0.3567	
3	1.2208	4.6104	8.8052	0.6984	0.1440	0.1049
4	1.0000	4.2338	8.8701	0.8979	0.2293	0.0976
7	1.8052	2.5844	4.0260	0.3073	0.7229	0.8875
8	0.2078	3.2727	8.7662	2.4691	0.4867	0.1094
10	1.3896	-0.2727	3.9351	0.5689		0.9103
11	0.7922	1.0260	3.6234	1.1309	1.6467	0.9929
14	-0.4545	-0.6883	3.8442			0.9337
15	0.5584	-0.1039	2.0130	1.4805		1.5806
18	0.3636	-0.2338	1.4545	1.9095		1.9056

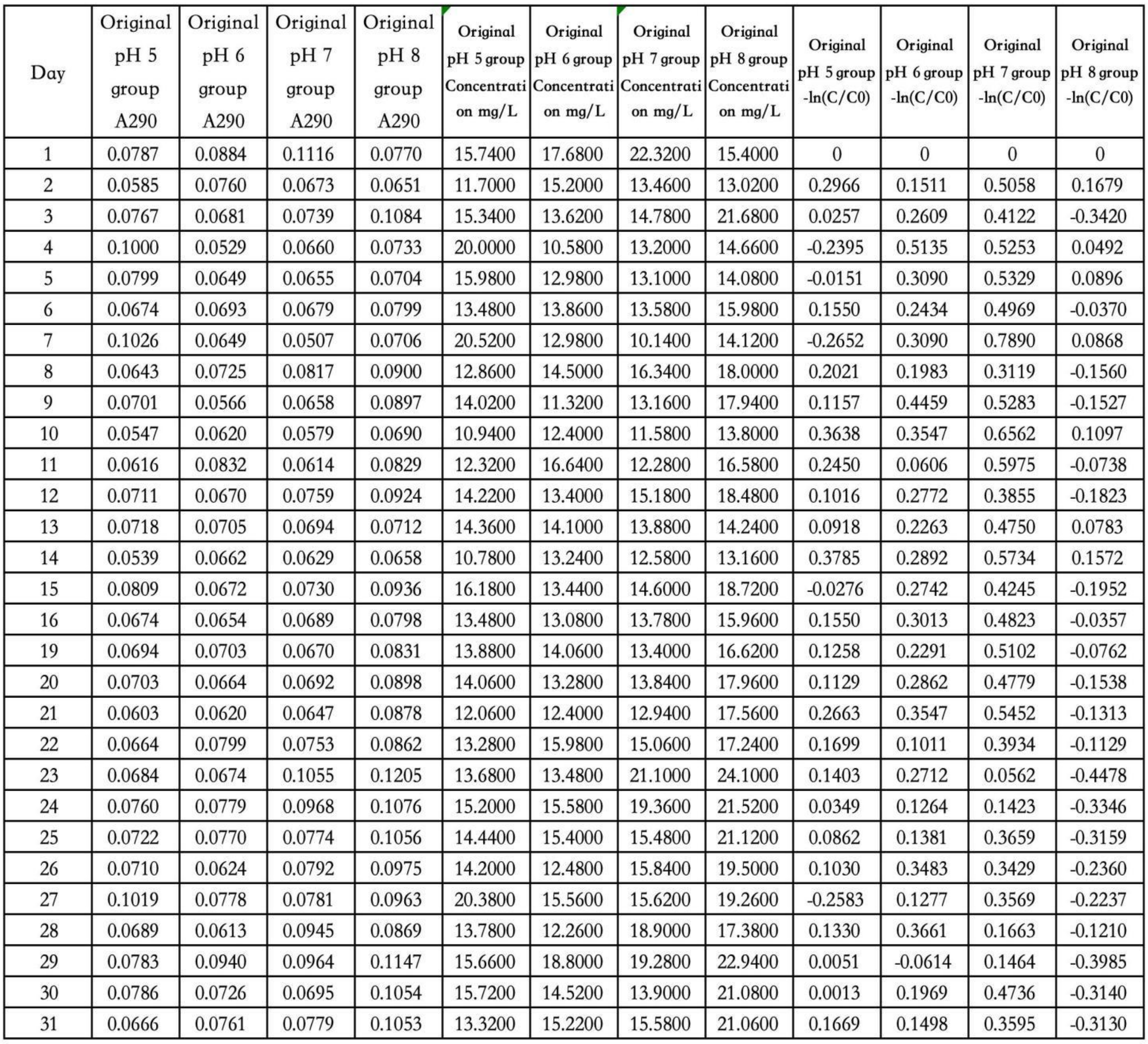
Table A21. Corrected E1 concentrations and calculated −ln(C/C_0) values in the natural light group

Appendix



Time (min)	Corrected concentration mg/L (2 mg/L)	Corrected concentration mg/L (5 mg/L)	Corrected concentration mg/L (10 mg/L)	-ln(C/C0) (2 mg/L)	-ln(C/C0) (5 mg/L)	-ln(C/C0) (10 mg/L)
0	2.5541	5.5405	10.1757	0	0	0
15	-1.3108	1.9730	5.8649		1.0325	0.5510
30	0.3108	1.8378	4.1486	2.1063	1.1035	0.8972
45	0.5405	2.3378	4.3514	1.5529	0.8629	0.8495
60	0.5541	3.8378	12.4189	1.5282	0.3672	-0.1992
75	-0.0135	1.9459	3.9730		1.0463	0.9405
90	-9.1216	-6.3784	-6.4730			
105	0.5000	2.4459	4.7703	1.6308	0.8177	0.7576
120	1.1757	3.9189	6.8243	0.7759	0.3463	0.3995

Table A22. Corrected E1 concentrations and calculated $-\ln(C/C_0)$ values in the UV light group

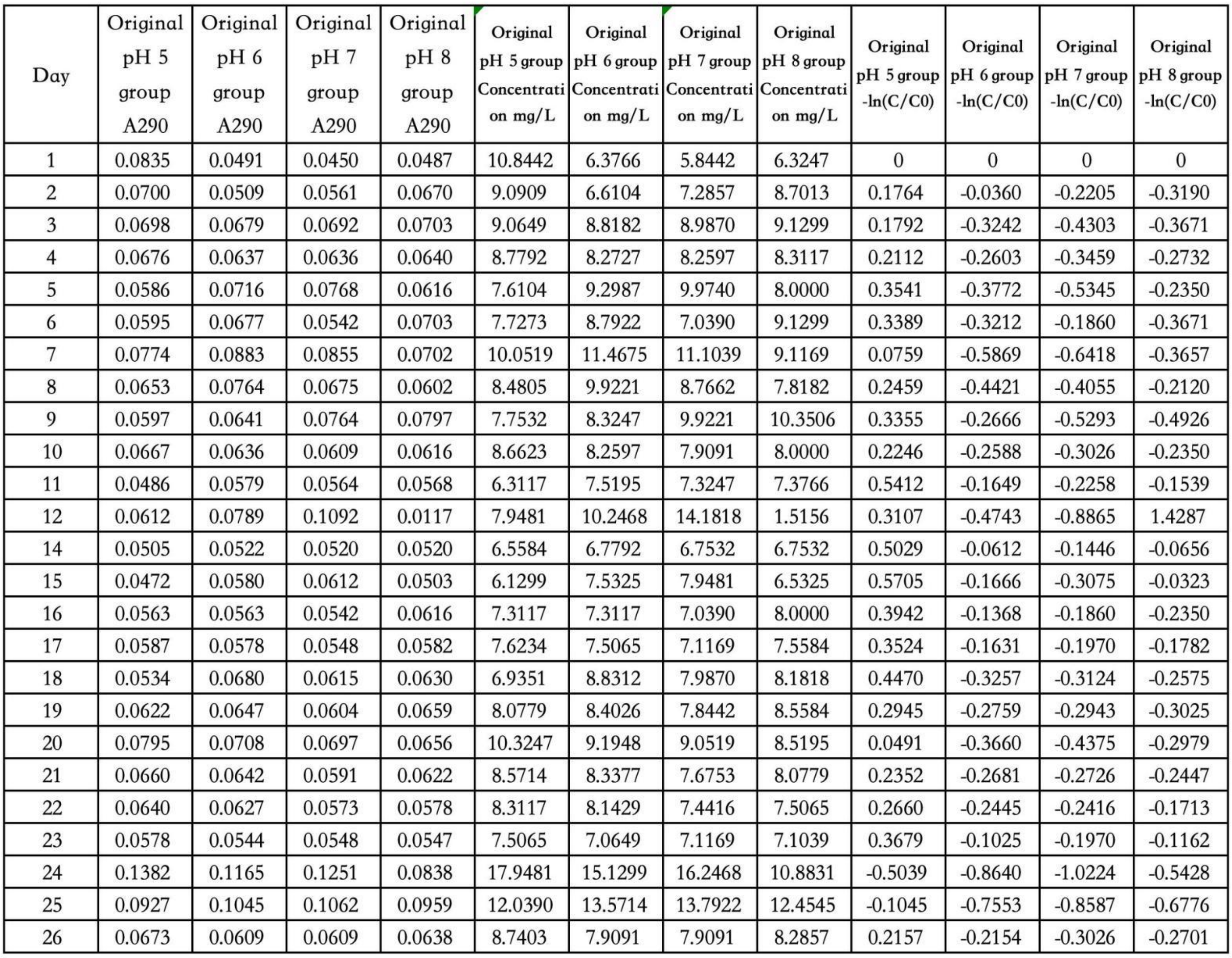


Day	Original pH 5 group A290	Original pH 6 group A290	Original pH 7 group A290	Original pH 8 group A290	Original pH 5 group Concentration mg/L	Original pH 6 group Concentration mg/L	Original pH 7 group Concentration mg/L	Original pH 8 group Concentration mg/L	Original pH 5 group -ln(C/C0)	Original pH 6 group -ln(C/C0)	Original pH 7 group -ln(C/C0)	Original pH 8 group -ln(C/C0)
1	0.0787	0.0884	0.1116	0.0770	15.7400	17.6800	22.3200	15.4000	0	0	0	0
2	0.0585	0.0760	0.0673	0.0651	11.7000	15.2000	13.4600	13.0200	0.2966	0.1511	0.5058	0.1679
3	0.0767	0.0681	0.0739	0.1084	15.3400	13.6200	14.7800	21.6800	0.0257	0.2609	0.4122	-0.3420
4	0.1000	0.0529	0.0660	0.0733	20.0000	10.5800	13.2000	14.6600	-0.2395	0.5135	0.5253	0.0492
5	0.0799	0.0649	0.0655	0.0704	15.9800	12.9800	13.1000	14.0800	-0.0151	0.3090	0.5329	0.0896
6	0.0674	0.0693	0.0679	0.0799	13.4800	13.8600	13.5800	15.9800	0.1550	0.2434	0.4969	-0.0370
7	0.1026	0.0649	0.0507	0.0706	20.5200	12.9800	10.1400	14.1200	-0.2652	0.3090	0.7890	0.0868
8	0.0643	0.0725	0.0817	0.0900	12.8600	14.5000	16.3400	18.0000	0.2021	0.1983	0.3119	-0.1560
9	0.0701	0.0566	0.0658	0.0897	14.0200	11.3200	13.1600	17.9400	0.1157	0.4459	0.5283	-0.1527
10	0.0547	0.0620	0.0579	0.0690	10.9400	12.4000	11.5800	13.8000	0.3638	0.3547	0.6562	0.1097
11	0.0616	0.0832	0.0614	0.0829	12.3200	16.6400	12.2800	16.5800	0.2450	0.0606	0.5975	-0.0738
12	0.0711	0.0670	0.0759	0.0924	14.2200	13.4000	15.1800	18.4800	0.1016	0.2772	0.3855	-0.1823
13	0.0718	0.0705	0.0694	0.0712	14.3600	14.1000	13.8800	14.2400	0.0918	0.2263	0.4750	0.0783
14	0.0539	0.0662	0.0629	0.0658	10.7800	13.2400	12.5800	13.1600	0.3785	0.2892	0.5734	0.1572
15	0.0809	0.0672	0.0730	0.0936	16.1800	13.4400	14.6000	18.7200	-0.0276	0.2742	0.4245	-0.1952
16	0.0674	0.0654	0.0689	0.0798	13.4800	13.0800	13.7800	15.9600	0.1550	0.3013	0.4823	-0.0357
19	0.0694	0.0703	0.0670	0.0831	13.8800	14.0600	13.4000	16.6200	0.1258	0.2291	0.5102	-0.0762
20	0.0703	0.0664	0.0692	0.0898	14.0600	13.2800	13.8400	17.9600	0.1129	0.2862	0.4779	-0.1538
21	0.0603	0.0620	0.0647	0.0878	12.0600	12.4000	12.9400	17.5600	0.2663	0.3547	0.5452	-0.1313
22	0.0664	0.0799	0.0753	0.0862	13.2800	15.9800	15.0600	17.2400	0.1699	0.1011	0.3934	-0.1129
23	0.0684	0.0674	0.1055	0.1205	13.6800	13.4800	21.1000	24.1000	0.1403	0.2712	0.0562	-0.4478
24	0.0760	0.0779	0.0968	0.1076	15.2000	15.5800	19.3600	21.5200	0.0349	0.1264	0.1423	-0.3346
25	0.0722	0.0770	0.0774	0.1056	14.4400	15.4000	15.4800	21.1200	0.0862	0.1381	0.3659	-0.3159
26	0.0710	0.0624	0.0792	0.0975	14.2000	12.4800	15.8400	19.5000	0.1030	0.3483	0.3429	-0.2360
27	0.1019	0.0778	0.0781	0.0963	20.3800	15.5600	15.6200	19.2600	-0.2583	0.1277	0.3569	-0.2237
28	0.0689	0.0613	0.0945	0.0869	13.7800	12.2600	18.9000	17.3800	0.1330	0.3661	0.1663	-0.1210
29	0.0783	0.0940	0.0964	0.1147	15.6600	18.8000	19.2800	22.9400	0.0051	-0.0614	0.1464	-0.3985
30	0.0786	0.0726	0.0695	0.1054	15.7200	14.5200	13.9000	21.0800	0.0013	0.1969	0.4736	-0.3140
31	0.0666	0.0761	0.0779	0.1053	13.3200	15.2200	15.5800	21.0600	0.1669	0.1498	0.3595	-0.3130

Table A23. Absorbance (A_{290}), calculated concentrations, and $-\ln(C/C_0)$ values of

Appendix

E1 in the algal group under different initial pH levels



Day	Original pH 5 group A290	Original pH 6 group A290	Original pH 7 group A290	Original pH 8 group A290	Original pH 5 group Concentration mg/L	Original pH 6 group Concentration mg/L	Original pH 7 group Concentration mg/L	Original pH 8 group Concentration mg/L	Original pH 5 group -ln(C/C0)	Original pH 6 group -ln(C/C0)	Original pH 7 group -ln(C/C0)	Original pH 8 group -ln(C/C0)
1	0.0835	0.0491	0.0450	0.0487	10.8442	6.3766	5.8442	6.3247	0	0	0	0
2	0.0700	0.0509	0.0561	0.0670	9.0909	6.6104	7.2857	8.7013	0.1764	-0.0360	-0.2205	-0.3190
3	0.0698	0.0679	0.0692	0.0703	9.0649	8.8182	8.9870	9.1299	0.1792	-0.3242	-0.4303	-0.3671
4	0.0676	0.0637	0.0636	0.0640	8.7792	8.2727	8.2597	8.3117	0.2112	-0.2603	-0.3459	-0.2732
5	0.0586	0.0716	0.0768	0.0616	7.6104	9.2987	9.9740	8.0000	0.3541	-0.3772	-0.5345	-0.2350
6	0.0595	0.0677	0.0542	0.0703	7.7273	8.7922	7.0390	9.1299	0.3389	-0.3212	-0.1860	-0.3671
7	0.0774	0.0883	0.0855	0.0702	10.0519	11.4675	11.1039	9.1169	0.0759	-0.5869	-0.6418	-0.3657
8	0.0653	0.0764	0.0675	0.0602	8.4805	9.9221	8.7662	7.8182	0.2459	-0.4421	-0.4055	-0.2120
9	0.0597	0.0641	0.0764	0.0797	7.7532	8.3247	9.9221	10.3506	0.3355	-0.2666	-0.5293	-0.4926
10	0.0667	0.0636	0.0609	0.0616	8.6623	8.2597	7.9091	8.0000	0.2246	-0.2588	-0.3026	-0.2350
11	0.0486	0.0579	0.0564	0.0568	6.3117	7.5195	7.3247	7.3766	0.5412	-0.1649	-0.2258	-0.1539
12	0.0612	0.0789	0.1092	0.0117	7.9481	10.2468	14.1818	1.5156	0.3107	-0.4743	-0.8865	1.4287
14	0.0505	0.0522	0.0520	0.0520	6.5584	6.7792	6.7532	6.7532	0.5029	-0.0612	-0.1446	-0.0656
15	0.0472	0.0580	0.0612	0.0503	6.1299	7.5325	7.9481	6.5325	0.5705	-0.1666	-0.3075	-0.0323
16	0.0563	0.0563	0.0542	0.0616	7.3117	7.3117	7.0390	8.0000	0.3942	-0.1368	-0.1860	-0.2350
17	0.0587	0.0578	0.0548	0.0582	7.6234	7.5065	7.1169	7.5584	0.3524	-0.1631	-0.1970	-0.1782
18	0.0534	0.0680	0.0615	0.0630	6.9351	8.8312	7.9870	8.1818	0.4470	-0.3257	-0.3124	-0.2575
19	0.0622	0.0647	0.0604	0.0659	8.0779	8.4026	7.8442	8.5584	0.2945	-0.2759	-0.2943	-0.3025
20	0.0795	0.0708	0.0697	0.0656	10.3247	9.1948	9.0519	8.5195	0.0491	-0.3660	-0.4375	-0.2979
21	0.0660	0.0642	0.0591	0.0622	8.5714	8.3377	7.6753	8.0779	0.2352	-0.2681	-0.2726	-0.2447
22	0.0640	0.0627	0.0573	0.0578	8.3117	8.1429	7.4416	7.5065	0.2660	-0.2445	-0.2416	-0.1713
23	0.0578	0.0544	0.0548	0.0547	7.5065	7.0649	7.1169	7.1039	0.3679	-0.1025	-0.1970	-0.1162
24	0.1382	0.1165	0.1251	0.0838	17.9481	15.1299	16.2468	10.8831	-0.5039	-0.8640	-1.0224	-0.5428
25	0.0927	0.1045	0.1062	0.0959	12.0390	13.5714	13.7922	12.4545	-0.1045	-0.7553	-0.8587	-0.6776
26	0.0673	0.0609	0.0609	0.0638	8.7403	7.9091	7.9091	8.2857	0.2157	-0.2154	-0.3026	-0.2701

Table A24. Absorbance (A_{290}), calculated concentrations, and $-\ln(C/C_0)$ values of E1 in the natural light group under different initial pH levels