The Mitigation of Eutrophication Using Microporous Polymer Membranes to Control Algae Growth

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June 2, 2012

Approval Page

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Acknowledgements

I would like to thank my family and friends for their support and encouragement throughout my collegiate endeavors. I would like to thank Dr. Vanasupa for her guidance on this project and for her inspiration in seeking sustainable solutions to environmental issues. This field has become the focus of what I hope will be my future career. I would also like to thank all of the Materials Engineering professors for their guidance and teaching which have continually both challenged and inspired me to become a better student and as well as to actively pursue learning experiences.

I would like to thank Dr. Hampson for his expertise in microalgae cultivation, as well as his generous donation of time, lab equipment and supplies, without which I would not have been able to pursue this project. I would also like to thank Christina Brox for her help in operating lab equipment and preparing algae culturing medium for the experiments. Additionally, I would like to thank Max Martin for his assistance in SEMing samples of the polycarbonate membrane used in this project.

Abstract

A system was designed to mitigate the accelerated process of anthropogenic eutrophication. This system aimed to contain Chlorella Vulgaris microalgae cells within an enclosed polymer membrane pouch while allowing for water and nutrients to diffuse through the pouch. As a test model, a 10 gallon aquarium was partitioned into three sections using polycarbonate membranes with 1 micron pore diameters. Each section was then gradually filled with a deionized water and Bristol solution recommended for microalgae growth. Phosphate and nitrate were added to Section A of the aquarium and allowed to diffuse throughout the tank. A water pump was used to agitate the solution and increase the diffusion rates of the nutrients. Samples were drawn periodically from section A and section C. A spectrophotometer was then used to analyze the phosphate and nitrate concentration of the samples. The resulting diffusion rates were graphed for trials with and without Chlorella Vulgaris cells present in section B of the tank to quantify the rate and overall amount of nutrient absorption by this microalgae.

The membrane was shown to successfully contain the microalgae cells within section B, so long as it was properly adhered to the aquarium. Both nitrate and phosphate were readily able to permeate the polycarbonate membrane and diffuse throughout the tank. Quantitative analysis of chlorella cell population failed to yield representative data. However, qualitative observations found that microalgae growth had occurred within Section B. Nutrient diffusion trends were highly linear. With the exception of two data sets that had substantially lower values, all data sets demonstrated R^2 values of at least 0.9059 and 0.985 at the highest. This behavior was contrary to that predicted prior to conducting the experiment.

As anticipated, rates of nutrient concentration change into Section C were lower when chlorella was present in Section B than when it was not added to the system. For phosphate, the rate at which this concentration increased in Section C was 1.4 µg/L/min lower when chlorella was added to Section B. For nitrate, the rate of concentration increase in Section C was 11 µg/L/min lower when chlorella was added to Section B. These results suggest that microalgae within the tank was successful in absorbing both nitrate and phosphate as they diffused throughout the tank.

Overall, results suggest that the proposed system would be able to absorb excess nutrients present within a eutrophic water system, thereby mitigating the ill effects of this biological state. However, collected results were based on a limited number of trials and thus were not robust. Further investigation should be undertaken to confirm the quantitative results obtained in this project.

Key Words

Materials Engineering, eutrophication, microalgae, microporous, polymer, membrane, Chlorella Vulgaris, polycarbonate, algal growth, diffusion

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I. INTRODUCTION

1.0 The Problem of Eutrophication

Eutrophication has emerged as an environmentally destructive phenomenon in water bodies throughout the world. While eutrophication occurs naturally, anthropogenic effects have both increased the occurrence and accelerated the rate of this biological process. Through the organization of scientific studies, this harmful process has become better understood. However, much is still unclear about the dynamics of eutrophication. One thing is clear, though. Unless measures are developed to control this phenomenon, eutrophication will continue to have devastating impacts for both the environment and society.

1.1 Background Information

In many clear water bodies, there exists a balance between the growth of plant life and the levels of plant nutrients, most notably nitrogen and phosphorus, present within the water. In aquatic environments, these elements are typically supplied through animal and microbial metabolism.[1] Though, there is a wide variety of factors that contribute to the concentrations of these nutrients in aquatic ecosystems. Among these factors are the physiographic characteristics of the water body, salinity, temperature, tides, stratification characteristics and nutrient loading events.[2] Thus, external processes may disrupt the rate of nutrient loading present in these environments. In some instances, this occurs as a natural aging process of the aquatic environment. However, human activity has been linked to accelerated rates of nutrient loading, resulting in excessive concentrations of plant nutrients within the water.

Figure 1. Satelight image of Lake Atitlan in Guatemala. Algae blooms promoted by excessive nitrogen levels are visible within the water.

Eutrophication is defined as the enrichment of water bodies by nutrients.[3] While these nutrients promote plant life and, in turn, provide a base food source to some aquatic animal life, excessive concentrations can be destructive to these ecosystems. High levels of nitrates and phosphates present within water bodies promote the growth of algae blooms. **Figure 1** shows a satellite image of Lake Atitlan in Guatemala in which excessive levels of nitrogen within the water have spurred the growth of algae blooms. Additionally, **Figure 2** shows a common case of algae growing in pond water Although the presence of nutrients within water bodies increases their fertility and productivity, eutrophic nutrient levels can have a damaging impact on both the aquatic ecosystem and society.

Figure 2. Algae blooms growing in a recreational pond.[4]

1.2 Eutrophic Environments

The level of nitrogen and phosphorus present within an aquatic environment is typically a good indicator of its potential to foster algae blooms. However, since phosphorus is typically the limiting factor for algae growth, more emphasis is placed on studying this nutrient. While water bodies with low concentrations of these nutrients tend to be devoid of plant and animal life, waters rich in nitrogen and phosphorus offer one of the important qualifications in becoming productive environments. However, the presence of these nutrients is not always directly correlated to plant and animal population. Depending on the characteristics of a given aquatic system, its productivity may change overtime. However, several factors have been accepted as indicators of water quality.

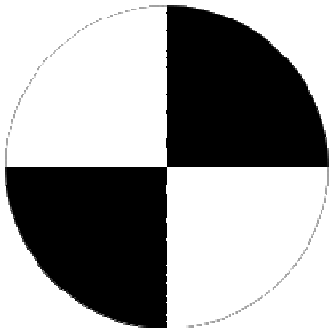


Figure 3. A Secchi disk used to measure water transparecy.[5]

In 1977, R.E. Carlson developed a method of characterizing water quality based on three criteria. The resulting measurement was termed a "trophic state index." According to Carlson's method, a water body's trophic state index depends on the amount of phosphorus and chlorophyll (the green pigment found in photosynthetic organisms) present and the Secchi depth of the water.[5] The Secchi Depth is a measure of water transparency that uses a black and white patterned disk, as shown in **Figure 3**. The disk is lowered into the water using a calibrated cord or pole, and the Secchi depth is recorded once the pattern is no longer distinguishable.[6] **Figure 4** depicts a Secchi disk in use. A low Secchi depth corresponds to a high concentration of particulate matter within the water. Additionally, this typically indicates a higher level of biomass (organic matter) present. Using these three criteria, water bodies are commonly classified as oligotrophic, mesotrophic, eutrophic or hypereutrophic. **Table I** lists the trophic index and ranges of the three criteria that correspond to each classification. Additionally, **Table II** summarizes the water characteristics that may be observed within each trophic class.

Figure 4. A Secchi disk in use.[7]

The three criteria used in Carlson's tropic indices tend to correlate within aquatic environments. However, it is somewhat difficult to say with certainty that a given water body within the three criteria ranges for a single trophic class will necessarily fall under that classification. The amount of biomass and phosphorus present and the Secchi depth of a given water body may be a function of season or may show a progression of increase or decrease over time.

Table I. Trophic index and tropic class characterizations [8]

Trophic Index	Chlorophyll (µg/L)	P (µg/L)	Secchi Depth (m)	Trophic Class
<30—40	0—2.6	0—12	>8—4	Oligotrophic
40—50	2.6—20	12—24	4—2	Mesotrophic
50—70	20—56	24—96	2—0.5	Eutrophic
70—100+	56—155+	96—384+	0.5—<0.25	Hypereutrophic

In addition to the dynamic quality of these measurements, water bodies vary greatly in terms of physical dimensions, currents, nutrient loading sources and geographic features. For instance, stagnant water may become eutrophic more readily, as it tends to collect and retain nutrients over time. Flowing systems in which water is replenished in the system tend to accumulate less nutrients. Because of the complex and interconnected role that these limnological factors play in a water body's characteristics, the distinctions between trophic classes are somewhat nebulous. Through their research in the matter, the Organization for Economic Cooperation and Development (OECD) has concluded that a more appropriate method for defining trophic class may be to see these categories as having a probabilistic nature. **Figure 5** displays a series of probability distribution curves generated from OECD research in average phosphate concentrations. Here, a water body with a given average phosphate concentration may be thought of as having a certain probability of existing in each of the trophic classes.

Table II. Typical water attributes in various trophic water bodies

Trophic Class	Typical Water Attributes
Oligotrophic	• Clear, deep, cold • Dissolved oxygen available at bottom of water body throughout the year
Mesotrophic	• Moderately clear, might be greener in more productive months • No dissolved oxygen available at bottom of water body • Incompatible for some aquatic species
Eutrophic	• Low water clarity • Green most of the year due to excessive algae and plant growth • Domination of certain aquatic species
Hypereutrophic	• Dense algae growth on water surface • Productivity limited by light availability • Domination of algae and rough aquatic species

Likewise, **Figure 6** shows a probability distribution curve for each trophic class with average chlorophyll concentration as the criteria of interest. Thus, it is somewhat difficult to put a clear-cut definition on a water body's trophic state. However, it may be readily surmised that higher nutrient concentrations typically lead to eutrophic waters heavily populated by algae and plant growth. This in turn impacts water clarity and Secchi depth. Put together, these observations yield an understanding of the trophic state and the resulting characteristics of a given aquatic environment.

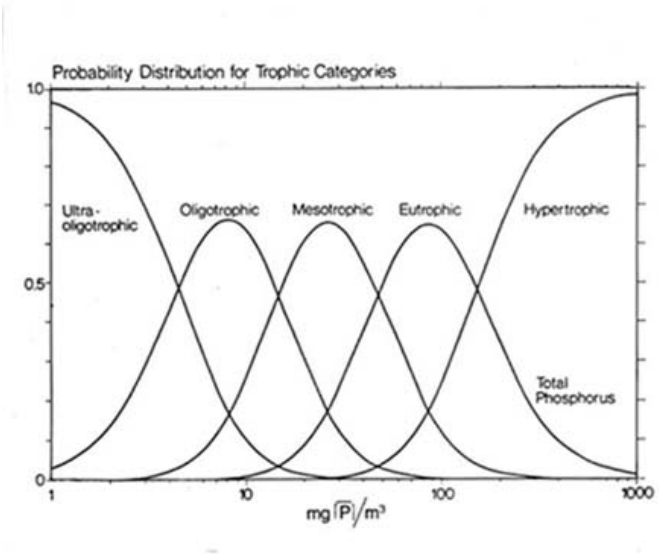


Figure 5. Probability distribution curves for each trophic class with respect to average phosphorus concentration.[9]

1.3 Environmental Impact

Thus far, the characteristics of eutrophication have been discussed. The next necessary step in evaluating the problematic nature of this biological process is to understand its impact on aquatic environments. As water bodies receive amounts of nutrients beyond their naturally capacity, microalgae blooms tend to flourish. This resulting growth tends to affect plant and animal life in several ways. First, microalgae cells accumulate near the surface of water bodies. This directly inhibits the passage of sunlight from reaching the lower depths of the water body. As a result, photosynthetic life beneath the blooms suffers.

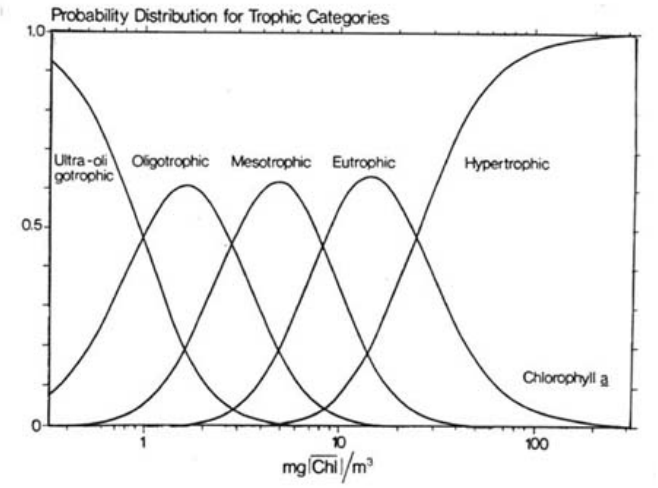


Figure 6. Probability distribution curves for each trophic class with respect to average chlorophyll concentration.[9]

Secondly, microalgae cells tend to clump together within the blooms, forming particulates. These particulates may then sink to the lower depths of the water body. When the sunken algae cells die, oxygen-consuming bacteria decompose the dead cells. In doing so, they drain the surrounding water of its dissolved oxygen. Aquatic animals in the vicinity can suffocate in this hypoxic environment.[9] This condition often leads to decreased biodiversity and a resulting dominance of a few adaptable aquatic plant and animal species. As algae continually die and are decomposed, sediment accumulates at the bottom of the water body. If given enough time, this process raises the floor, decreasing water depth. If the system is shallow to begin with, the water body may eventually disappear altogether.

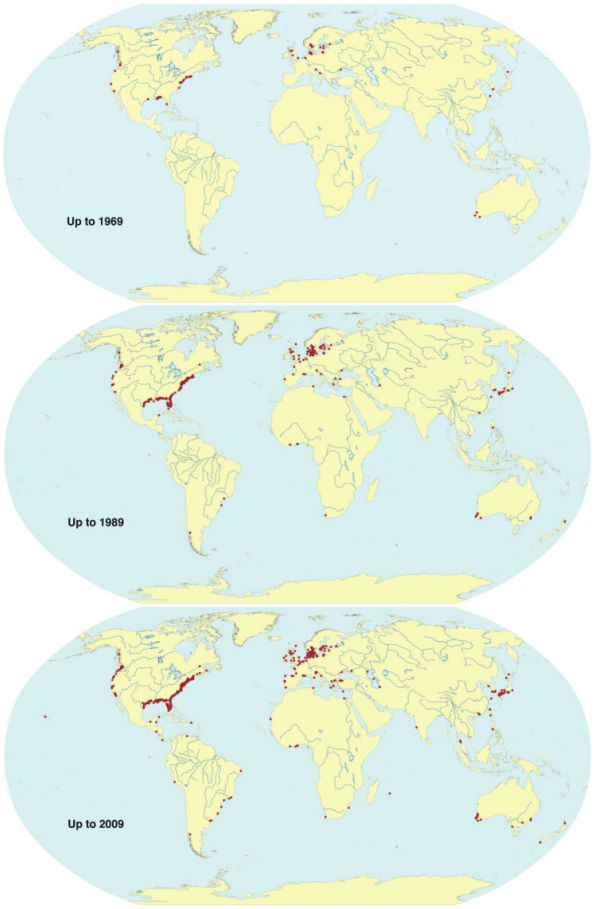


Figure 7. Recorded hypoxia cases in marine waters caused by human activity. From top to bottom, the maps correspond to the years 1969, 1989 and 2009.[11]

Natural eutrophication can occur over the course of centuries, giving aquatic environments time to adjust to changing conditions. However, anthropogenic activities can greatly accelerate the process of eutrophication. An example of this is the environment of Lake Erie during the 1960s and 1970s. Agricultural and urban development surrounding the lake dumped significant amounts of nutrients into the water. Plant life overran the aquatic environment and a majority of the other species present within it. Rotting algae that washed onto the lake's shore made its beaches unusable, and it was pronounced a "dead lake." However, improved sewage treatment and agricultural practices greatly mitigated the

nutrient load received by Lake Erie. Presently, it is once again a biologically healthy environment.[10]

Although eutrophication may be reversed, mitigation methods have not been enforced in many water systems throughout the world. In water systems, decreasing levels of dissolved oxygen have increasingly negative effects on inhabiting animal life. **Figure 7** shows the progression of hypoxia cases due to human activity from 1969 to 2009 for marine waters alone. Additionally, **Figure 8** lists some of the impacts related to diminishing levels of dissolved oxygen in marine environments. The condition of hypoxia also causes the release of hydrogen sulfide from water body sediments, which creates a lethal environment for most animal life.[12] These conditions have caused occurrences of massive fish killings. This not only disrupts the food web of the ecosystem for aquatic species, but is also costly for coastal human communities who rely on fish as a major food source. Between 1985 and 1986, it is estimated that 625,000 fish died in the Pecos River. Algae was attributed to creating the lethal environment for these fish.[13]

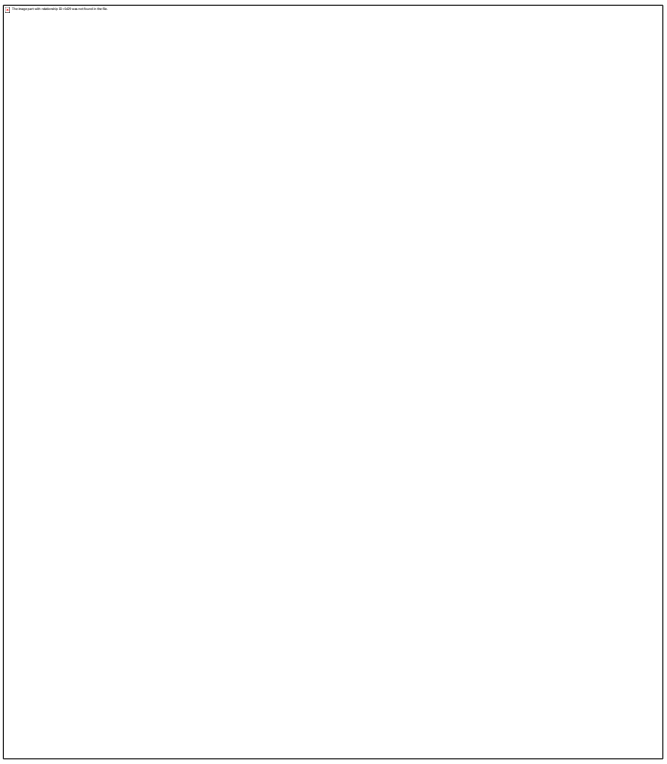


Figure 8. A representation of the effects of decreasing dissolved oxygen levels in marine environments.[11]

An interesting case of eutrophication also occurs in Lake Victoria, Tanzania. It is the second largest freshwater lake in the world and houses the largest freshwater fishery. When the lake initially became eutrophic, its nutrient-rich state was exploited, as it offered more available food to Nile perch. While this proved beneficial to local fishing communities, the ecological conditions of the lake are undoubtedly shifting. However, it is believed that fish populations will not be able to keep algal growth in check. The recognition of eutrophication as a more imminent problem than fishing pressure has prompted studies of Lake Victoria to gain a better understanding of its ecological responses.[14] Through this example, it is clear how the environmental impacts of eutrophication are not far removed from its societal impacts for humankind.

1.4 Societal Impacts

Eutrophication has multiple implications for human society which tend to fall into three categories. These are water quality, aquatic resources and recreation and tourism. All of these have economic repercussions. As eutrophication directly impacts the appearance of a water system, the quality of water is perhaps the most readily recognizable of these societal impacts. Eutrophication has been named as one of the major obstacles to providing clean drinking water. Filters used in water treatment plants may become clogged with algae cells, making for an expensive preparation process. Because of this, it has been suggested that taking measures to prevent eutrophication before it develops may be a less expensive course of action than attempting to reverse it once it has developed. Even with the removal of

algae cells from the water, problems may persist in regard to water quality. Treated eutrophic water may retain an upleasant smell or taste and may contain unfavorable levels of ammonia, iron, manganese and other impurities. Additionally, these waters tend to have a higher risk of fostering bacteria.[15]

In treating eutrophic waters for human consumption, it is often necessary to use high levels of chlorine to cleanse the water. However, mixing chlorine and organic material can create organochlorinated substances considered to be carcinogenic. Even in waters where excessive algal growth is not present, high levels of nitrates present in drinking water can be harmful for humans. A maximum safe consumption concentration of 10 mg/L of nitrate has been specified by the United States Public Health Service.[16] Thus, treating eutrophic waters, or simply waters of high nutrient concentrations, for human consumption is a costly, difficult and potentially hazardous process.

Another societal implication of eutrophication lies in the quality of water bodies for recreational and touristic purposes, most commonly of lakes and coastal waters. This should come as no surprise, as eutrophic waters often have thick films of algae on their surface, high turbidity and possibly a foul odor. These characteristics make water bodies unsightly and discourage activities such as swimming, snorkeling, boating and fishing. Businesses centered around these water bodies have suffered as a result of reduced tourism. Additionally, value of shoreline property has greatly diminished around affected water bodies. A study conducted in the U.S. examined 14 ecological regions for their prevalence of eutrophication and the resulting economic losses in terms of reduced tourism and property value. **Figure 9** shows the percentage increase of water systems to a hypereutrophic classification and the resulting annual losses to fishing and boating industries for 1 and 3 month periods of lake closure in each region. **Table III** lists each ecological region represented in the study.

Figure 9. The increasing prevalence of hypereutrophic conditions in U.S. water systems and the resulting economic losses to fishing and boating industries in each region of study.[17]

Table III. Ecological Regions studied[17]

Number of Region in Study	Ecological Region	Number of Region in Study	Ecological Region
I	Willamette and Central Valleys	VIII	Nutrient poor glaciated upper Midwest and Northeast
II	Western Forested mountains	IX	Southeastern temperate forested plains and hills
III	Xeric west	X	Texas-Louisiana costal and Mississippi alluvial plains
IV	Great Plains grass and shrublands	XI	Central and Eastern Forested uplands
V	Central cultivated Great Plains	XII	Southern coastal plain
VI	Corn belt and Northern Great Plains	XIII	Southern Florida coastal plain
VII	Mostly glaciated dairy region	XIV	Eastern coastal plain

As is shown in **Figure 9**, ecoregions V and VI (the central cultivated great plains and the corn belt and northern great plains, respectively) showed the greatest percentage increase in hypereutrophic classification. This makes sense, as these agricultural lands are heavily fertilized. Excess fertilizer is rich in nitrates and phosphates, as will be discussed later in this report. It is also shown that within the corn belt and northern great plains, eutrophication related lake closures may result in annual property losses close to 600 million dollars annually for 3 month closure periods. **Figure 10** shows the annual property value losses for each ecological region due to changes in Secchi depth assuming varying percentages of the land surrounding the water bodies is available for private ownership. Even assuming that only 25 percent of surrounding land is available, annual value losses may be as high as 400 million dollars for a single ecological region. It is clear from these figures that eutrophication is damaging to not only the environment but also to the economy. An example of the great effort and expenses required to combat eutrophication occurred in Chinese coastal waters before the 2008 Olympics. **Figure 11** shows soldiers desperately trying to remove accumulated algae off the coast of Qingdao, which threatened a sailing event. Over 10,000 people and 1,200 boats were dispatched to tackle the problem.[18] If measures are not taken to prevent this process, especially its accelerated forms, shoreline communities will continue to suffer losses. Water alone proves a crucial resource in these environments. However, there are other resources contained within them that have been lost and may continue to disappear with the increasing prevalence of eutrophication.

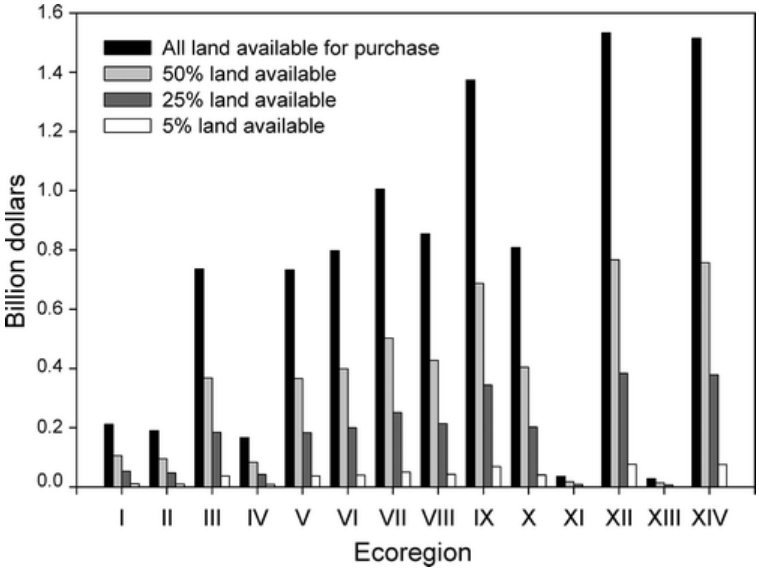


Figure 10. Annual economic losses through reduced value of shoreline property by ecological region assuming various percentages of land surrounding water bodies available to private ownership.[17]

Figure 11. Soldiers on the coast of Qingdao, China removing algae from the water for an Olympic sailing event.[18]

Coral reefs constitute a valuable resource to both human society and marine ecosystems for a variety of reasons. First, coral reefs are among the most diverse and oldest ecosystems, housing approximately one-third of Earth's marine biodiversity.[19] Their structure contains thousands of years of ecological development. Hundreds of thousands of marine animals are supported by the reefs and in turn provide a valuable food source to thousands of coastal communities throughout the world. For example, these environments provide American Samoa with 50 percent of its fish for human consumption.[20] However, the benefits coral reefs offer do not stop here. Economically, coral reefs harbor fisheries and promote tourist activities, which together generate billions of dollars annually. These activities, among

others, include scuba diving, sport fishing and snorkeling. Over four million tourists travelled to the Florida Keys each year in the 1990s. This contributed approximately 1.2 billion dollars of revenue annually to the area. Additionally, more than 90 percent of the economies of the Northern Mariana Islands and Guam from newer development rely on tourism of their reefs and coastal waters.[20] In addition to promoting tourism, coral reefs also offer a wide variety of plants and animals receiving increased attention from medical industries. Chemicals produced by these organisms are being developed for use in new medicines to combat heart disease, cancer, infections, viruses and arthritis.[20] However, the availability of these substances has been diminishing with increasing coral reef destruction.

Coral reefs do not only generate economically beneficial industries, they also prevent economic losses to coastal communities. Coral reefs in coastal waters provide an effective barrier to large waves and flooding. Without them, millions of people living in U.S. coast regions could potentially suffer from land erosion, property damage and loss of life.[20] The existence of coral reefs provides crucial support to marine ecosystems and human coastal communities. However, coral reefs are suffering greatly from human activity.

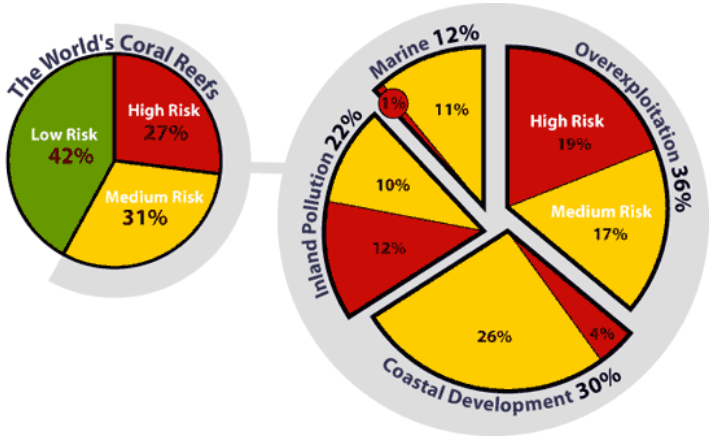


Figure 12. Percentage of world's coral reefs at a relative risk level and sources of coral reef destruction.[21]

Approximately 35 million acres of coral reefs have been lost in the last several decades, and more will disappear if action is not taken to prevent it.[21] Coral reefs, while incredibly productive, are delicate environments that require plenty of oxygen and sunlight, steady temperature and salinity, low concentrations of nutrients and clear water. Thus, there are many factors that, if disturbed, may damage the reefs. **Figure 12** demonstrates the percentage of coral reefs at a relative level of risk and the sources of coral reef destruction on a percentage basis. As shown, human activity poses the largest threat to these ecosystems. Although there are many anthropogenic factors that contribute to coral reef destruction, those that pertain to eutrophication include deforestation, air and water pollution and urban development. In each of these, excessive amounts of nutrients are allowed to flow into marine waters. Coral reefs, which rely on extremely low nitrate concentrations, become choked with algae in the excessive presence of this nutrient. Algae blooms cloud the surrounding water and prevent a sufficient amount of sunlight from reaching the reefs. As a result, zooxanthellae (protozoan organisms within the coral) are unable to perform photosynthesis, and the coral reefs deteriorate. **Figure 13** shows the erosion of nutrient-rich sediment into the ocean off the coast of Costa Rica, contributing to the over-enrichment of marine waters. Unless preventive actions are taken, marine ecosystems will continue to suffer. In order to remedy this problem, however, it is

Figure 13. Nutrient-rich sediment flowing into the coastal waters of Costa Rica.[21]

necessary to understand where excess nutrients come from and how they are received into aquatic environments.

2.0 Sources of Excess Nutrients

There are many different sources of nutrients in water systems. The nutrients in eutrophic environments are typically in large part (yet likely not completely) supplied from anthropogenic activity. However, it is essential in treating the problem of eutrophication to understand that every water system has a natural level of nutrients. Some water bodies, especially shallow estuaries, are naturally eutrophic, and it may not be appropriate to remove nutrients from these waters, or other waters for that matter, beyond their naturally occurring concentrations. Doing so may disrupt the delicate balance of these environments.

2.1 Point and Diffuse Sources

Excess nutrients can come from a wide range of sources which may be categorized as point or diffuse. Point sources have a readily discernible and confined, yet potentially discrete, location or mode of transportation.[22] Some general examples of point sources include smokestacks, pipes and ditches. Diffuse sources alternatively do not exist in specific or readily identifiable locations. Some examples include atmospheric deposition and agricultural fertilizer leaching.[23] Both point and diffuse sources can cause eutrophication, and both may contribute simultaneously to this condition in a given water system. **Figure 14** shows some of the many point and diffuse sources that may contribute to eutrophication and how they may be mitigated.

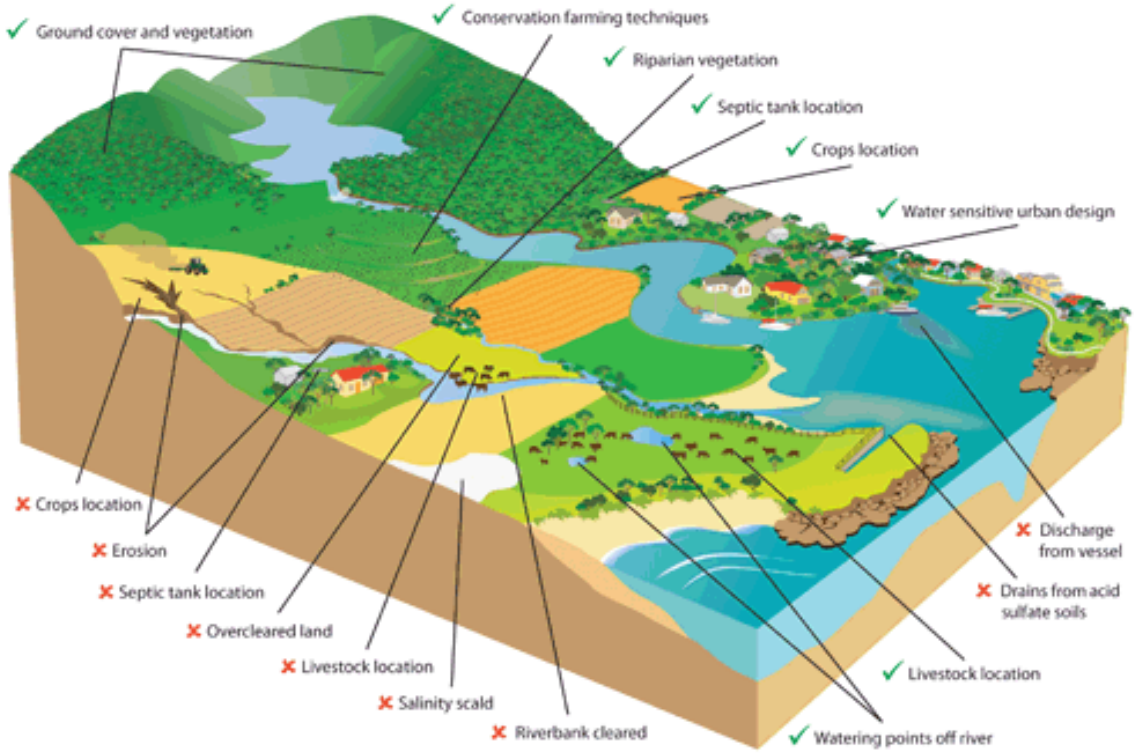


Figure 14. A depiction of various point and diffuse sources of water pollution and measures that may mitigate their environmental impacts.[23]

2.2 Natural Nutrient Sources

Without human activity, nutrients are cycled through water systems through natural processes. These processes incorporate atmospheric, geological, plant and animal aspects. Animal and plant waste decomposes and deposits nutrients into soil. From here, the nutrients may then erode or leach into surrounding water systems. These nutrients are absorbed by plant life, which is then consumed by animals. Animals and plants within a water body may both decompose nutrients into sediments. **Figure 15** and **Figure 16** represent these naturally occurring cycles for phosphorus and nitrogen, respectively. As shown, the processes that cycle these two nutrients are quite similar. It may be noted that the atmosphere constitutes a near inescapable diffuse source of nitrogen, though, due to the large portion of nitrogen within its composition. Although these factors play an important role in the natural existence of phosphorus and nitrogen within water systems, physical and climatic characteristics of a water body may impact which natural sources dominate in nutrient delivery.

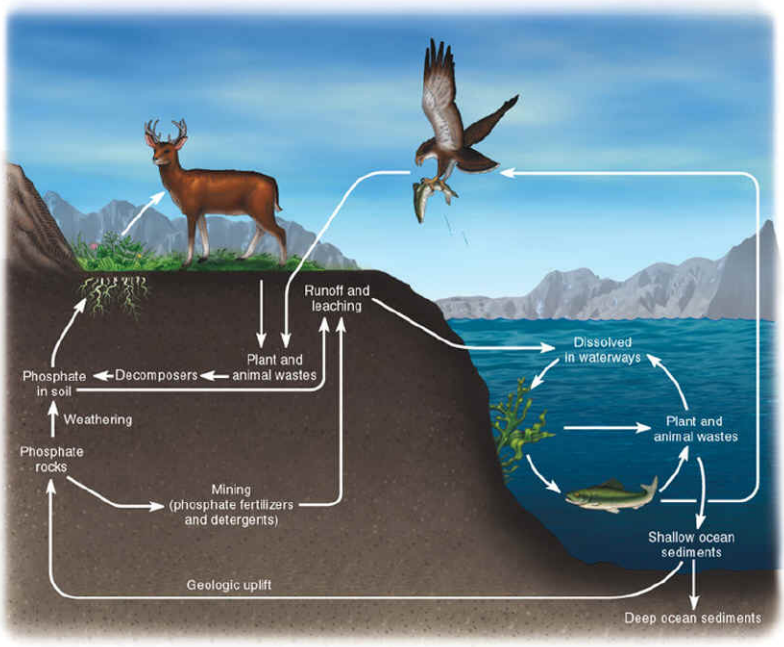


Figure 15. A diagram of the natural phosphorus cycle.[24]

The size, number and flow rate of inlets and outlets to a water system may influence the rate of nutrient loading. Additionally, precipitation, water current and wind may serve to agitate the water body and, as a result, the nutrients held within it. As a result, undisturbed waters tend to have lower rates of nutrient turnover. An example of these effects occurs in the shallower sections of the Hoover Reservoir in which wind currents were found to have a particularly significant impact on the distribution of nutrients due to sediment mixing.[25]

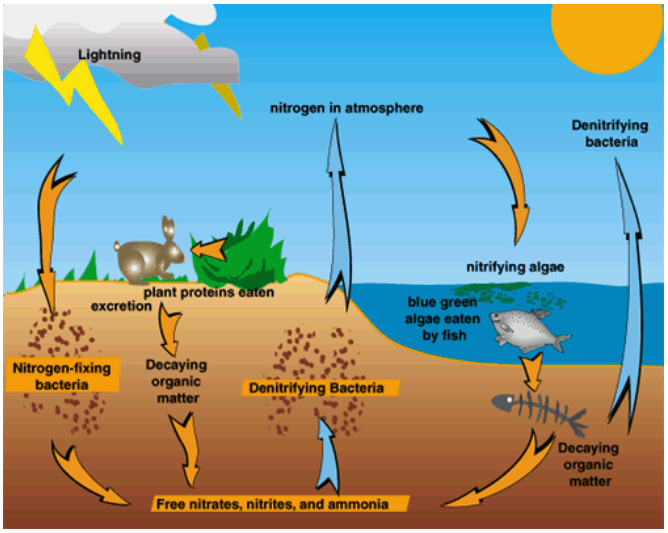


Figure 16. A diagram of the natural nitrogen cycle.[26]

Because the naturally occurring processes that supply nutrients to a water body typically exist as diffuse sources, they are typically judged as impractical to mitigate. However, it is important to study these sources so that the natural trophic state for a given water body may be established. From here, anthropogenic contributions to eutrophication may be controlled accordingly.[27]

2.3 Anthropogenic Nutrient Sources

As previously discussed, anthropogenic activities exacerbate trophic index. These activities are in large part a result of urban growth, land development, industrialization and agricultural practices. Although it is not always the case, anthropogenic sources exist as point sources more commonly than do natural sources. Thus, even though they may be more threatening to water bodies than natural sources, anthropogenic sources may be more readily mitigated.[27] Additionally, these sources represent human activity and are often easier to alter than naturally occurring processes. However, whether these activities are actually altered in practice is another matter.

Urban development has increased levels of land, water and air pollution, all of which pose a threat to aquatic ecosystems. Sewage and waste water runoff, if improperly contained, carries with it large supplies of nutrients. This phenomenon has become evident in highly urbanized coastal areas. **Figure 17** shows proportionally the large amounts of untreated waste water runoff received in coastal waters.

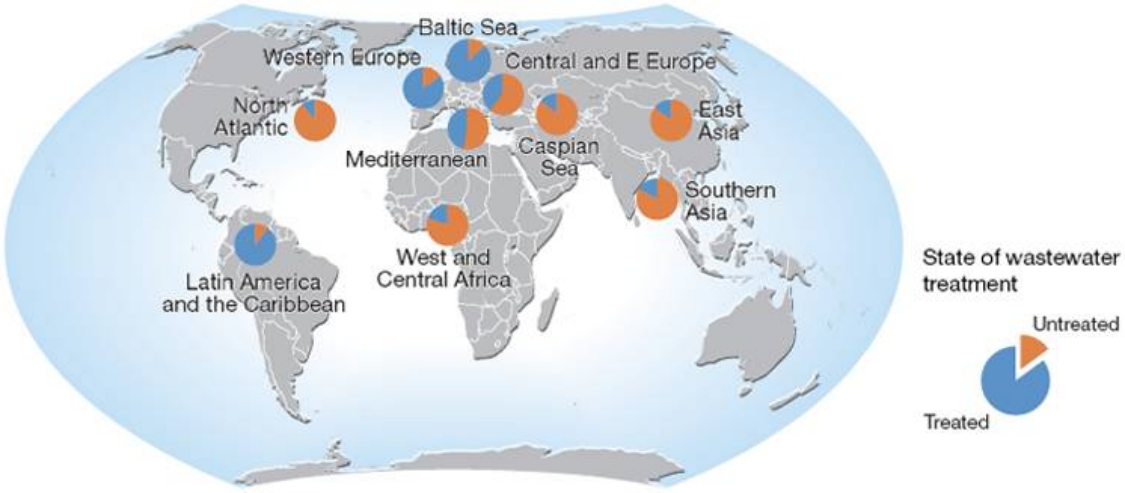


Figure 17. Proportion of waste water treated near coastal waters in various regions.[28]

In the 1970s, detergents were found to be a significant contributor within this waste due to their high phosphorus content.[29] Additionally, increasing urbanization and land development has spurred deforestation. This practice loosens soil which may then erode into water systems, bringing nutrients with it. The growth of industrial factories and use of internal combustion vehicles pollutes the air with nitrogen oxides which may in turn be deposited atmospherically into water systems.[30]

Agricultural practices have become recognized as a huge contributor to eutrophication. Among other reasons, this may be attributed to the fact that the practices of this industrial sector aim to maximize plant productivity through the extensive use of phosphates and nitrates. To ensure maximum crop production, over-fertilization has become a common agricultural practice. However, crops often cannot absorb all of these nutrients available to them. As a result, the nutrients remain in the soil where they may eventually erode into water streams or leach into ground water. Depending on the crop under consideration and seasonal yields, 30 to 50 percent of nitrogen applied might remain unabsorbed.[31] Data from a North Carolina crop study (**Table IV**) demonstrates the potential contribution of nitrogen in drainage water due to fertilizer runoff. Since levels of 10 milligrams per liter of nitrate nitrogen has been deemed unsafe for human consumption, it is easy to see how, in conjunction with other sources, this contamination could pose a threat to health as well as water systems if allowed to reach aquifers. **Figure 18** displays the

relative contamination risk within the U.S. It is shown that several of the areas at the highest risk (the Midwest and central California) are known for their large expanses of agricultural land.

Table IV. Nitrogen concentrations in runoff agricultural water [31]

Crop uptake (% N)	Predicted average concentration in runoff water (ppm N)	Predicted average concentration in runoff water (mg N / L H_2O)
0	4.6	3.58
25	3.4	2.64
50	2.3	1.79
75	1.2	0.93
90	0.5	0.39

Through the multitude of anthropogenic sources of runoff nitrogen and phosphorus, it is not surprising that eutrophication has overtaken aquatic environments around the world. Modern lifestyle, food production and industrial practices have become such polluting aspects of human society that these behaviors have left their mark, and will continue to do so, on a global scale. It is interesting to observe how this contamination has lead to destruction of some species while allowing others to thrive. Thus, it is necessary to discuss the characteristics of algae and their adaptive nature in eutrophic systems.

3.0 Microalgae

In discussing algae, particularly microalgae when dealing with eutrophic systems, it is important to note that these organisms play a crucial role in their environments; despite its destructive impact when allowed to dominate, moderate amounts of microalgae provide an important food source to animal life. Human society is finding increasing use for microalgae as well. Thus, eradication of microalgae from eutrophic environments is neither a logical nor feasible goal in mitigating this biological problem. Understanding the functions and naturally occurring concentrations of these organisms in aquatic ecosystems should be central to any action taken to counteract the presence of both nutrient and algal overabundance.

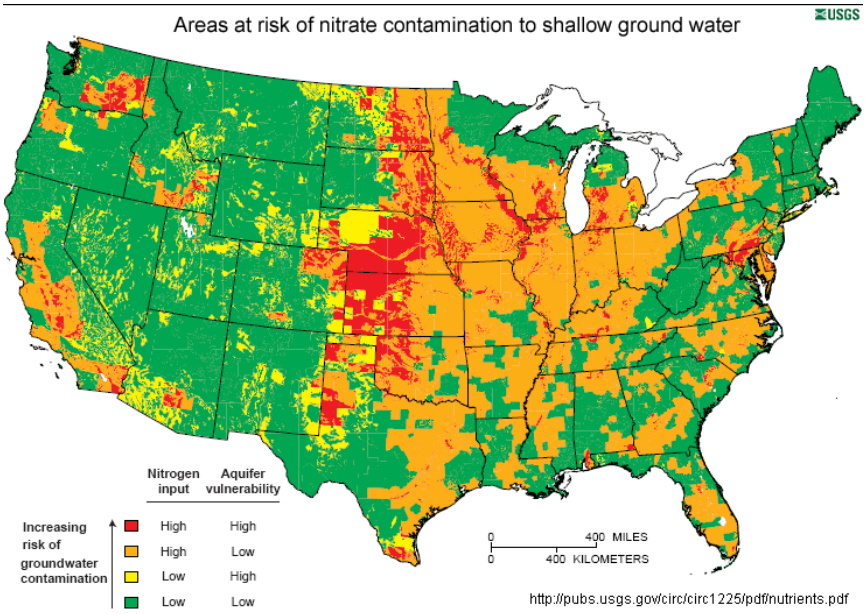


Figure 18. The relative risk of U.S. land to nitrogen ground water contamination.[32]

3.1 Background Information

Algae are diverse organisms, ranging from ocean kelps many feet tall to single cells. Microalgae are, of course, the latter. Though, these cells are known for their ability to clump together, creating a variety of

conglomerated forms, such as particulates, webs and filaments. Microalgae are photosynthetic organisms, yet they do not exactly fit into the category of plants. Unlike plants, they lack stem, leaf and root systems. In fact, blue-green algae are also categorized as cyanobacteria, and there is some debate as to whether this group may even be called algae.[33] This is due to the prokaryotic structure (lacking membrane bound nuclei) of blue-green algae cells. Most other algae cells are eukaryotic in structure (having a membrane that encloses nuclei).[34]

Other classifications of algae include green algae, red algae, brown algae, dinoflagellates and diatoms. While these algae groups have many similarities, there are tens of thousands of documented algae species (their true number is believed to be between 200,000 to 800,000 species). Red algae, which are nearly all found in marine waters, are used in agar and carageenan (food thickeners). On the other hand, dinoflagellates may be found in both fresh and salt water, and some are responsible for poisoned shellfish and red tides. Diatoms, constituting the largest algae populations in open marine waters, create about 25 percent of oxygen produced on Earth annually. In this process, they simultaneously absorb harmful green house gas. Green algae, a category that includes some 17,000 species, are incredibly adaptable organisms; these algae have been found living in both marine and freshwaters as well as on other animals, lichens and even on snow. Green algae are the closest in character to plants, having photosynthetic pigments (chlorophyll a and b), carotenoids and cellulose cell walls.[33]

Microalgae may range from a single micrometer to several hundred micrometers in size.[35] Additionally, they exist in many different shapes. Chlorella Vulgaris cells (**Figure 19**) are spherical, while those of Spirulina have a rod like structure (**Figure 20**). Dinoflagellates (**Figure 21**) are so named for their flagella that aid them in moving through their environment.

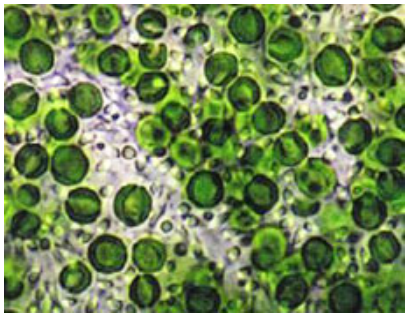


Figure 19. Chlorella Vulgaris cells[36]

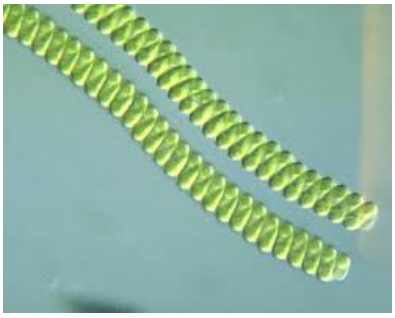


Figure 20. Chains of Spirulina cells [37]

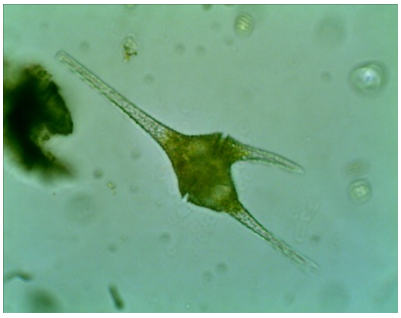


Figure 21. A Ceratium Dinoflagellate cell [38]

3.2 Living Conditions

As already discussed, microalgae are capable of living in a variety of different environments. However, there are limitations to the living conditions in which these organisms may survive. The needs of algal species vary, and parameters of nutrient intake, temperature, pH, water salinity, light intensity and photoperiod are important factors in supporting a thriving algae population. The level of compatibility of an algae species with these environmental factors may in turn dictate the growth rate and allowed population of the algae. **Table V** lists the recommended ranges of these factors for culturing a variety of microalgae species. It should be noted though that these generalized ranges are chosen to maximize microalgae growth and do not necessarily represent the conditions required for their health or survival.

Table V. Recommended parameter ranges for microalgae culturing[39]

Parameter	Range	Optimum
Temperature (^{o}C)	16 - 27	18 - 24
Salinity (g/L)	12 - 40	20 - 24
Light intensity (lux)	1,000 - 10,000	2,500 - 5,000
Photoperiod (light:dark, hours)		16:8 - 24:0
pH	7 - 9	8.2 - 8.7

Microalgae have been found capable of living in close to freezing temperatures as well as in waters near boiling.[40] However, lower temperatures typically decrease their growth rates, and temperatures higher than 35 ^{o}C are lethal for certain species.[41] Additionally, some microalgae may have trouble adjusting to certain levels of water salinity, and it is believed that rapidly altering this parameter may solve problematic algal blooms for some species. However, marine microalgae are incredibly well suited to changes in this factor. **Table VI** indicates the amount of salt present in various water-salt solutions as well as for the range and optimum amounts listed in **Table V** with the assumption that NaCl is the only salt present within each solution. From **Table VI**, the relative levels of salinity used to culture algae are shown. Generally, water of higher salinity than irrigation water yet of lower salinity than sea water provides a healthy environment for microalgae.

Table VI. Salinity of various water-salt solutions[42]

Water-salt solution	Salinity (ppm)	Salinity (g/L)
Average drinking water	100	0.32
Restricted drinking water	500	1.62
Upper limit of irrigation water	2000	6.49
Algae culturing solution (range)	3700 - 12330	12 - 40
Algae culturing solution (optimum)	6165 - 7400	20 -24
Sea water	30,000 - 50,000	97.32 - 162.2
Brine	> 50,000	> 162.2

As with the previously discussed parameters, microalgae prefer a certain range of water pH values. Like other photosynthetic organisms, microalgae absorb carbon dioxide. In conjunction with the microalgae, this gas serves to regulate the pH of their environment. Carbon and oxygen become available to these organisms through the formation of carbonic acid when carbon dioxide is dissolved into water. This acts to lower the pH of the water. During the day, photosynthesis is allowed to occur. In the process, carbonic acid is absorbed by microalgae and the pH rises. In the absence of light, carbonic acid accumulates in the water, and the pH is lowered again.[43] For this balance to occur, the photoperiod (duration of sunlight exposure) must be sufficiently long. As well, the intensity of light received impacts photosynthesis and growth rates of microalgae, as will be discussed shortly.

Numerous environmental parameters in microalgae sustainment have been discussed so far. One key factor remaining is nutrient availability. Microalgae can only grow where they may obtain these crucial elements, and their population may be controlled through nutrient supply. **Table VII** displays nutrients crucial to microalgae and plant life. As shown, the list is broken up into primary, secondary and micro nutrient categories. Primary nutrients are required in the largest amounts while micro nutrients are needed in the smallest quantities. However, it should not be implied that micro nutrients are necessarily less important that the other nutrients listed. In fact, photosynthesis could not occur without the presence of these trace heavy metals.[43]

Table VII. Microalgae nutrient classifications [44, 45]

	Nutrient	Useful Forms	Purpose of Nutrient
Primary Nutrients	Carbon	CO_2 , HCO_3^-	Forms backbone of biomolecules
	Hydrogen	H_2O	Role in electron transport and building of sugars
	Oxygen	H_2O, O_2	Necessary for cellular respiration
	Nitrogen	NH_4^+ , NO_3^-	Used in structure of proteins, chlorophyll, nucleic acids and coenzymes
	Phosphorus	H_2PO_4 , HPO_4^{2-}	Role in energy transfer through adenosine triphosphate (ATP)
	Potassium	K^+	Role in regulation of photosynthesis and protein synthesis
Secondary Nutrients	Calcium	Ca^{2+}	Constituent of cell walls and structure of membranes
	Magnesium	Mg^{2+}	Contained in chlorophyll and used as an enzyme activator
	Sulfur	SO_4^{2-}	Contained in plant proteins
Micro Nutrients	Iron	Fe^{2+} , Fe^{3+} , chelate	Role in electron transfer and chlorophyll synthesis
	Zinc	Zn^{2+} , $Zn(OH)_2^0$, chelate	Role in regulation of metabolic activity
	Manganese	Mn^{2+} , chelate	Controls reduction/oxidation systems
	Copper	Cu^{2+} , chelate	Respiration catalyst
	Boron	$B(OH)_3^0$	Role in carbohydrate metabolism
	Molybdenum	MoO_4^-	Required for nitrogen fixation
	Chlorine	Cl^-	Activates production of gaseous oxygen in photosynthesis

These nutrients all play an important role within microalgae and other photosynthetic organisms. Thus, it is observed that their growth rate is subject to availability of the most limiting nutrient. To overcome such deficiencies, some microalgae species are capable of storing nutrients like phosphorus within their cells in periods of excess availability.[43] However, both low and high nutrient concentrations may hinder microalgae growth or even be fatal to these organisms in extreme cases. **Figure 22** shows a graphical depiction of this principle.

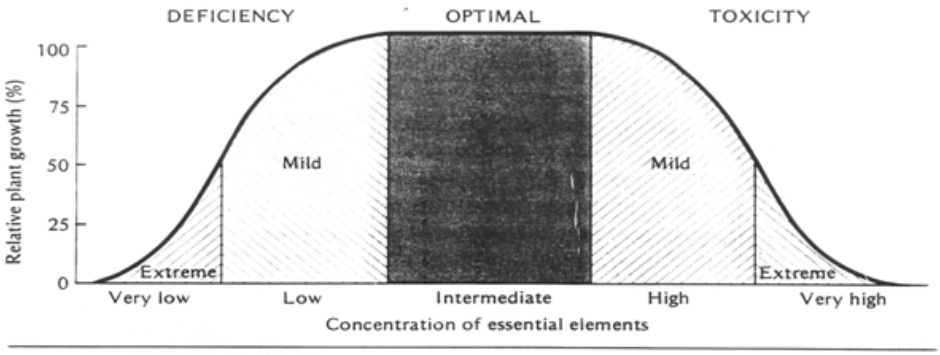


Figure 22. A graph depicting the relation between nutrient availability and organism growth.[44]

3.3 Photosynthesis

The previous discussion examined environmental inputs, such as nutrients and sunlight, that were necessary to microalgae growth. Photosynthesis is the process through which microalgae and plant life convert these inputs into food and energy. In doing such, photosynthetic organisms convert water, carbon dioxide and light energy into oxygen, water and carbohydrates, or sugars used for energy within the organism. **Figure 23** shows the general chemical equation for photosynthesis.

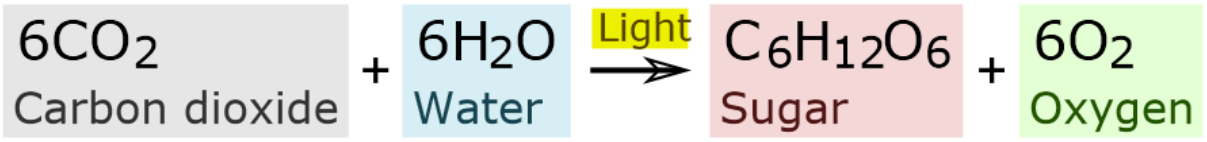


Figure 23. The balanced chemical equation for photosynthesis.[46]

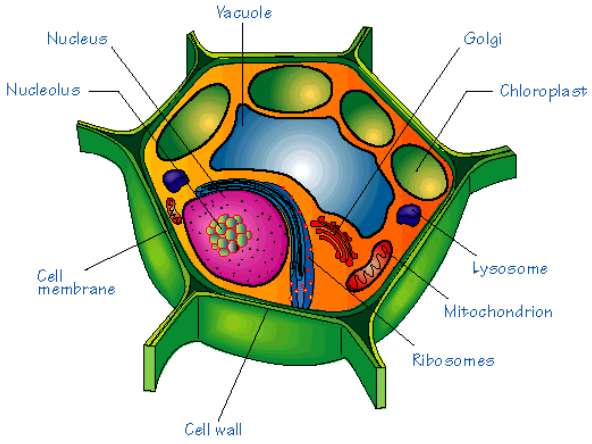


Figure 24. A depiction of a eukaryotic cell showing the chloroplast organelles responsible for photosynthesis.[49]

Photosynthesis provides for the creation of gaseous oxygen and for the absorption of carbon dioxide on Earth. From this equation, it is clear that water, carbon dioxide and light energy are crucial inputs. However, photosynthesis would not be able to occur without green chlorophyll pigments present within photosynthetic organisms. For eukaryotic cells, photosynthesis takes place in organelles called chloroplasts. These organelles house chlorophyll pigments which are capable of absorbing light energy received by the cell. **Figure 24** depicts a eukaryotic cell. The chloroplasts may be seen as green disk-like structures within the cell. For prokaryotic cells, such as blue-green algae, photosynthesis occurs in cytoplasm, a somewhat transparent fluid found throughout the cells.[47,48]

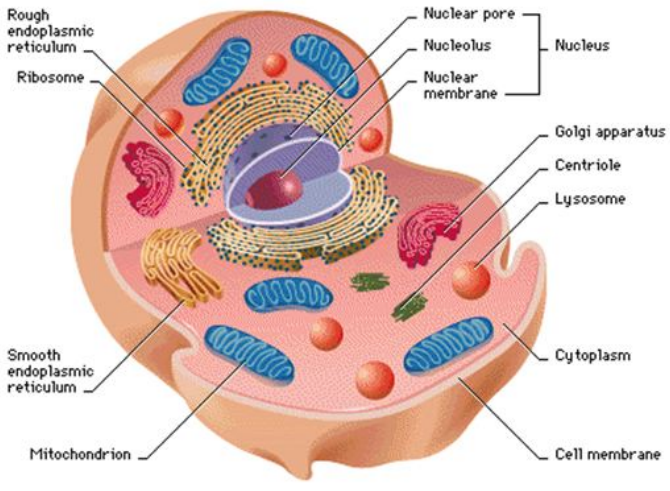


Figure 25. A depiction of a prokaryotic cell showing the cytoplasm fluid in which photosynthesis occurs.[50]

Figure 25 shows a diagram of a prokaryotic cell with cytoplasm surrounding the cell's organelles.

After receiving light, chlorophyll molecules act to capture and convert this energy through a process known as resonance energy transfer. Chlorophyll molecules are incredibly well adapted to absorb this energy through their molecular structures. Similar to a hemoglobin molecule, chlorophyll is composed of a porphyrin ring, which consists of alternating carbon single and double bonds. The delocalization of orbitals within this structure allows for absorption bands within the visible light

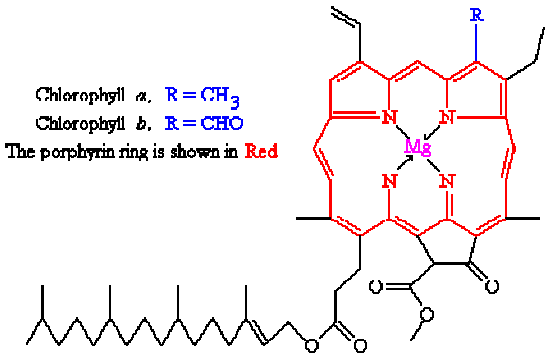


Figure 26. The molecular structure of chlorophyll. It is shown that different types of chlorophyll differ in the end group "R." [52]

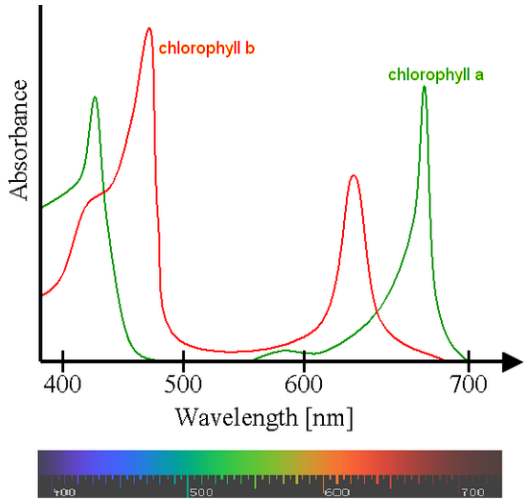


Figure 27. An absorbance graph of chlorophyll A and B. It is shown that peak absorbance values occur in blue and red light.[51]

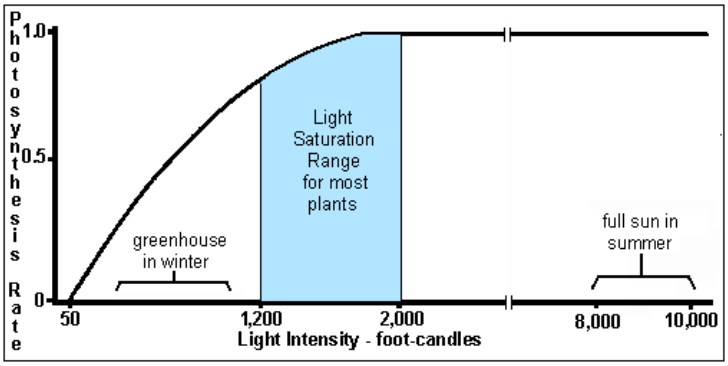


Figure 28. The rate of photosynthesis as a function of light intensity.[53]

spectrum. There are 6 different forms of chlorophyll, though chlorophyll A and B are typically focused upon in examining visible light absorption during photosynthesis.[51] The various chlorophyll structures differ with several attachments of end groups. Their basic molecular structure is shown in **Figure 26**.

Although chlorophyll A and B are adept at absorbing visible light, they do not absorb its full spectrum. As shown in **Figure 27**, these molecules demonstrate peak absorbance values of blue and red light. Green light is reflected, which accounts for the green color of photosynthetic plant pigments. Thus, the wavelength of light received has an impact on the extent to which photosynthesis may be performed. Additionally, the intensity of light received influences photosynthesis. As depicted in **Figure 28**, the rate at which photosynthesis is performed increases with increasing light intensity. However, photosynthetic organisms have a light saturation limit at which higher light intensities will not raise photosynthetic rate. In fact, sufficiently high light intensities may even be harmful to the organism. A similar trend exists with photosynthesis rate as a function of available carbon dioxide. While increasing concentrations of carbon dioxide initially increase this rate, photosynthetic organisms have a saturation limit for this gas, above which the photosynthesis rate will not increase. As photosynthesis is the process through which microalgae and plant life creates its food, the rate at which this process occurs affects the growth rate of these organisms.

3.4 Growth Characteristics

As discussed, microalgae are photosynthetic organisms and thus are autotrophic (able to create their own food from inorganic materials). The rate at which microalgae grow is in turn affected by the availability of these substances. However, microalgae observably undergo several growth phases. These phases are characterized by different rates of growth (**Figure 29**) and include a lag phase, exponential growth phase, stationary phase and death phase. In the lag phase, microalgae show little if any growth. They are adapting to the conditions of their environment. Given a large supply

of nutrients compared to cell population, microalgae then undergo an exponential growth phase. However, as cell population increases, nutrient supplies often run low. Additionally, large quantities of microalgae cells tend to accumulate near the surface of the water body they occupy. As a result, light may be unable to reach deeper water levels and microalgae growth is stunted. This is especially the case in stagnant waters, where microalgae cells are not actively circulated through their environment. This stationary phase of microalgae growth indicates that the cell population has reached a limit given its environmental conditions. If nutrients are not replenished and cells are neither removed nor circulated, the microaglae population undergoes a death phase. Thus, in microalgae culturing, it is common practice to replenish nutrient supplies and to remove an amount of cells before cultures reach a stationary phase. Additionally, containers holding the algae are placed on stir plates to continually circulate their contents. These practices maintain the culture in an exponential growth phase and maximize culture productivity.

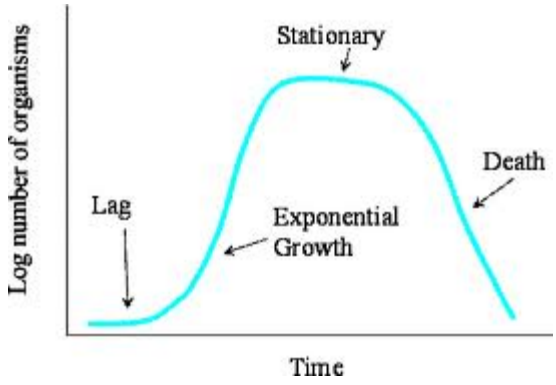


Figure 29. The stages of microalgae growth.[54]

There are several methods for characterizing the growth rate of microalgae populations. The time required for a population to double in size (the doubling time) is a common measure of microalgae growth rates. It is largely a function of species and environmental conditions. One method for determining the growth rate of a species is to monitor the levels of a nutrient present with an environment over time. **Figure 30** shows an equation adapted to experimental conditions in which a series of vessels carried the nutrient phosphorus to a tank filled with microalgae. As shown, the change in phosphorus over time is a function of the phosphorus initially present within the tank, the amount entering and exiting the tank and the rate at which cells are absorbing the nutrient. A full list of the variables used is shown in **Table VIII**.

$$\frac{dP_i}{dt} = f(P_{i-1} - 2P_i + P_{i+1}) - \frac{1}{Y_{BP}}\mu_B B_i$$

Figure 30. An experimental equation adapted to the change in phosphorus levels over time in a tank containing microalgae cells.[55]

Table VIII. Variables to Figure 30 equation.[55]

Symbol	Definition	Unit
P_i	Phosphorus in tank	μM
P_{i-1} , P_{i+1}	Entering and exiting phosphorus from neighboring vessels	μM
f	Dilution rate	$Days^{-1}$
Y_{BP}	Cell yield per unit of phosphorus	$Cells(\mu M)^{-1}$
μ_B	Nutrient dependent growth rate	$Days^{-1}$
B_i	Cell population	$Cells(mL)^{-1}$

Several alternative methods exist to determine growth rate. One is to prepare a cell sample for microscopy. Using a haemocytometer (**Figure 31)**, cells may be counted on grid patterns to determine a value of cell concentration. The advantage of this method is that actual cells within the sample may be examined. In the previously discussed method, it cannot be determined if other cells contaminating the culture account for some of the nutrient uptake. However, both methods may allow for cell viability to be determined. Another method involves the use of a spectrometer. As previously discussed, chlorophyll a and b within microalgae cells exhibit absorption peaks in red and blue light. Referring to **Figure 27**, the wavelengths of peak absorbance of chlorophyll a and b are about 680 and 630 nm, respectively. Samples collected from a cell culture may be placed into a spectrometer and analyzed for absorbance at wavelengths between these two absorbance peaks (typically wavelengths between 650 and 660 nm are used). A resulting rise in absorbance at these wavelengths corresponds to an increased amount of chlorophyll with the culture sample. The difficulty in this method lies in equating a given absorbance value to a value of cell concentration in the sample. Different microalgae species in general contain varying amounts of chlorophyll in addition to producing different amounts of chlorophyll depending on the stage of growth the culture is experiencing. These characterization and culturing methods have been applied to the study of microalgae not only because these organisms have posed a threat to water systems on a global scale, but also because algae plays a vital role in life on Earth. The most important of these roles, from a human life perspective, are the absorption of carbon dioxide and production of oxygen. However, human society is finding increasing use for microalgae, as will be discussed shortly.

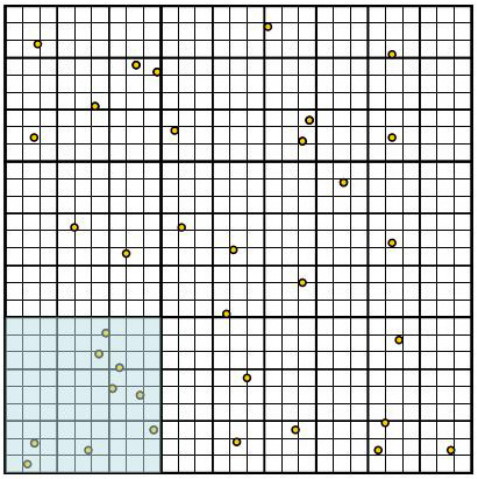


Figure 31. A microscope haemocytometer used to more effectively count cells.[56]

3.5 Nutrient Absorption Mechanisms

Despite the differences between prokaryotic and eukaryotic cells (both of which may apply to microalgae species), both are surrounded by plasma membranes and are filled with cytoplasm.[49] These membranes, which are typically semi-permeable allow for the selective passage of some substances while disallowing the passage of others. In general, there are a number of different ways in which nutrients may diffuse through membranes. Several of these are listed below:

1) the direct flow of mass through pores
2) diffusion unrelated to the structural characteristics of the membrane
3) facilitated diffusion (related to the structure of the membrane and diffusing species
4) active transport (requiring an input of energy from metabolic activities)

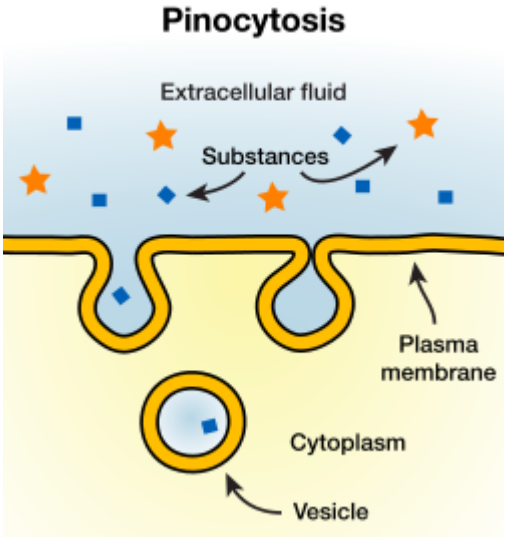


Figure 32. A diagram of the pinocytosis process through which substances may be absorbed after crossing a semi-permeable membrane. [72]

5) pinocytosis (in which cells crossing the membrane are subsequently suspended in vesicles, as shown in **Figure 32**.)

Thus, there are many different ways in which nutrients on one side of a membrane may be transported to the opposite side. In many cells, facilitated diffusion allows for nutrients from the cells' surroundings to be absorbed through their outer membranes, which are made of phospholipid bilayers (**Figure 33**). Proteins that run across these bilayers help to transport specific substances through the membrane. Once inside the cell, nutrients move through the cytoplasm to organelles where they may be metabolized.[73] Cellular functions serve to regulate when nutrients are and are not allowed to be transported into the cell through this process. One factor important in the regulation of cell content is osmotic pressure. This concept holds that higher ion concentrations in solution surrounding a cell than are present within the cell will cause water to diffuse out of the cell in an attempt to balance the concentrations. This phenomenon is shown in the wilting of leaves when there is a lack of water surrounding plant cells.

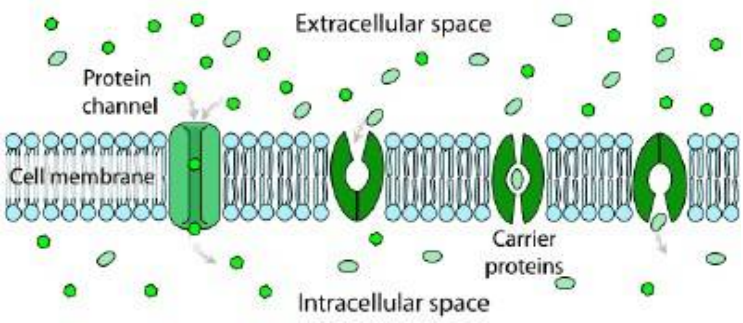


Figure 33. A schematic of the phospholipid bilayer surrounding cells and the process of facilitated diffusion through which proteins allow for the absorption of nutrients in the cell.[73]

3.6 Societal Uses

Microalgae provides a valuable role in the food chain. However, this food source is not exclusive to aquatic life forms. The Aztecs were noted to have taken advantage of spirulina, a blue-green microalgae, growing in lakes as a food source in the 14th century.[57] This custom has likewise been practiced by communities around the world. Not only is microalgae widely available, many of its species are highly nutritious. Spirulina, Chlorella and Dunaliella, among others, have all been deemed "superfood" and are grow as nutritional supplements (**Figure 34**). Spirulina has a protein content of about 50 to 70 percent (higher than beef with a content of about 20 percent), and contains all essential amino acids. In addition to its high protein content, spirulina is rich in A, B, C, D and E vitamins and essential fatty acids. Other benefits of these microalgae species include their high concentrations of antioxidants and minerals, among which are iron, magnesium, chromium, manganese, calcium, phosphorus, zinc, copper and selenium.[58]

Figure 34. Spirulina tablets for use as nutritional supplements.[58]

Beyond their excellent nutritional value, microalgae are believed useful in treating a variety of medical conditions. Though, the validity of these claims is not backed by scientific evidence in some cases. The ailments microalgae are believed to alleviate include cardiovascular disease, ADHD, cancers, diabetes and depression.[59] Despite the uncertainty in these claims, some microalgae species, such as chlorella, have been shown to stimulate the immune system, boost energy, prevent infections, reduce high cholestorol and blood pressure, reduce radiation treatment side effects and treat ulcers. Additionally, chlorella is known for its ability to bind with heavy metals. Thus, it is commonly marketed as a detoxifying agent.[60]

Because of their high nutrient content, microalgae will likely see increasing use in the treatment of malnutrition, as livestock feeds, and food supplements. However, the benefits of these organisms extend beyond their nutritional value. Microalgae have recently received great attention in the field of green energy. Microalgae are high in lipid oil content. Because of this, microalgae are thought to be an excellent crop for bio fuels. Currently used crops, such as corn and soy beans, are lower in oil content, and using them for bio fuels detracts from an important food source. Microalgae, on the other hand, is not required as heavily as a food source, requires comparatively few nutrients to grow and would require less land to produce the same amount of oil as current bio fuel crops. **Table IX** shows estimates for the number of gallons of oil that may be produced in an acre from various crops each year. The large range in microalgae oil yield is a result of the large variation in oil content between species and differences in growing conditions.[61] However, it is clear that microalgae are capable of producing a significantly greater amount of oil per acre than conventional bio fuel crops.

Figure 35. Open water systems used to cultivate microalgae in large quantities.[62]

Table IX. Oil yield of bio fuel crops[61]

Crop	Oil yield (gallons / acre / year)
Corn	18
Soybeans	48
Safflower	83
Sunflower	102
Rapeseed	127
Oil Palm	635
Microalgae	5000 - 15000

Through a pressing method, it is possible to obtain approximately 75 percent of the oil from algae cells. Once extracted, this oil may be processed into diesel fuels through a process called transesterification.[61] In order to grow large amounts of algae for this purpose, two general systems are in use. These include open and closed systems. Open systems often involve large, outdoor ponds, as shown in **Figure 35**. A popular closed system has developed called a photobioreactor. **Figure 36** shows a schematic of this device. In photobioreactors, transparent tubes house the cultured microalgae. Additional tubes feed carbon dioxide and other nutrients into the system. Monitoring devices are also often incorporated into the system to measure parameters like pH and temperature. This accurate parameter regulation provides an advantage of these closed systems over open systems. **Figure 37** shows a photobioreactor in use.

The development of such systems for producing bio fuel from microalgae on a large scale is underway. The Ohio Agricultural Research and Development Center at Ohio State University has received approximately 7 million dollars from the Department of Energy to develop an open pond system for growing bio fuel microalgae. The system is expected to yield about 2,000 gallons of fuel annually.[63] A design consideration of these systems is to locate them near a large source of carbon dioxide emission, such as coal power plants. Through this, emitted gas from the plant may be used to feed microalgae. This serves to recycle carbon dioxide emissions. Thus, if used correctly, algae have a potential to mitigate multiple of today's environmental and societal challenges.

4.0 Eutrophication Preventative Measures

As discussed, microalgae will only grow with the availability of necessary inputs. Because of this, most proposed solutions to eutrophication involve restricting these inputs. Even restricting one such input, such as available phosphorus, so that it becomes the limiting nutrient might be effective in controlling microalgae growth. Among nutrients, carbon dioxide, water and light inputs, it is typically only feasible to control nutrient inputs without disturbing the environment as a whole. Thus, current preventative measures aim to control large nutrient sources (specifically anthropogenic point sources) from contributing to water system nutrient loads. Examples of this included using just enough agricultural fertilizer to obtain maximum crop production without overloading the soil with unused nutrients, preventing erosion of soil and sediments into waterways and ensuring that urban and industrial waste water is properly treated before it flows into aquatic environments. It is also noted that preventing excessive nutrient runoff before it reaches water systems is likely more cost effective than attempting to reverse eutrophication once it has occurred. Priority should be placed on managing anthropogenic activities as well as periodically monitoring water quality of affected systems.

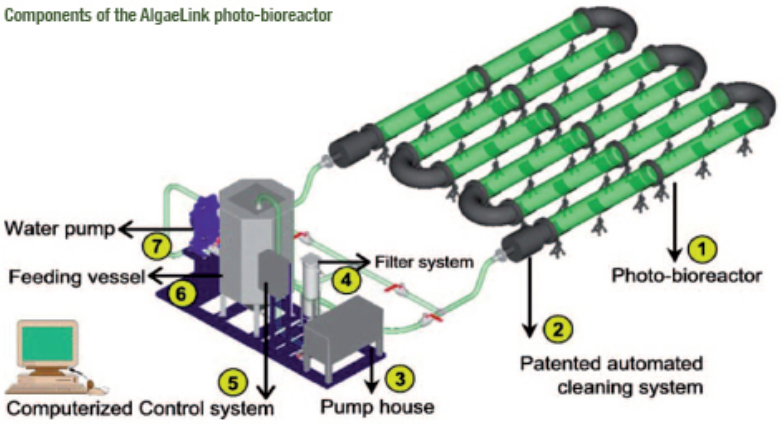


Figure 36. A schematic of a photobioreactor.[64]

Figure 37. A photobioreactor in use.[64]

5.0 Project Goals

The main purpose of this project was to design a system that could mitigate the effects of eutrophication. Authorities believe that the most feasible way to do so is to control the nutrient load received by aquatic systems and to do so before water quality deteriorates. The system designed in this project took a different approach in several aspects. First, the designed system would likely be more effective in the presence of already high nutrient concentrations. Second, the proposed system utilizes microalgae to mitigate the problem of excessive nutrient loading instead of directly reducing microalgae populations. However, it is hoped that in reducing nutrient loads, microalgae populations will consequently diminish.

5.1 Proposed Mitigation System

The system proposed to mitigate excessive nutrient loading and, in turn, eutrophication involves submerging a pouch like container of microalgae into affected water systems. The pouch walls will consist of a thin polymer membrane. **Figure 38** shows a schematic of this system.

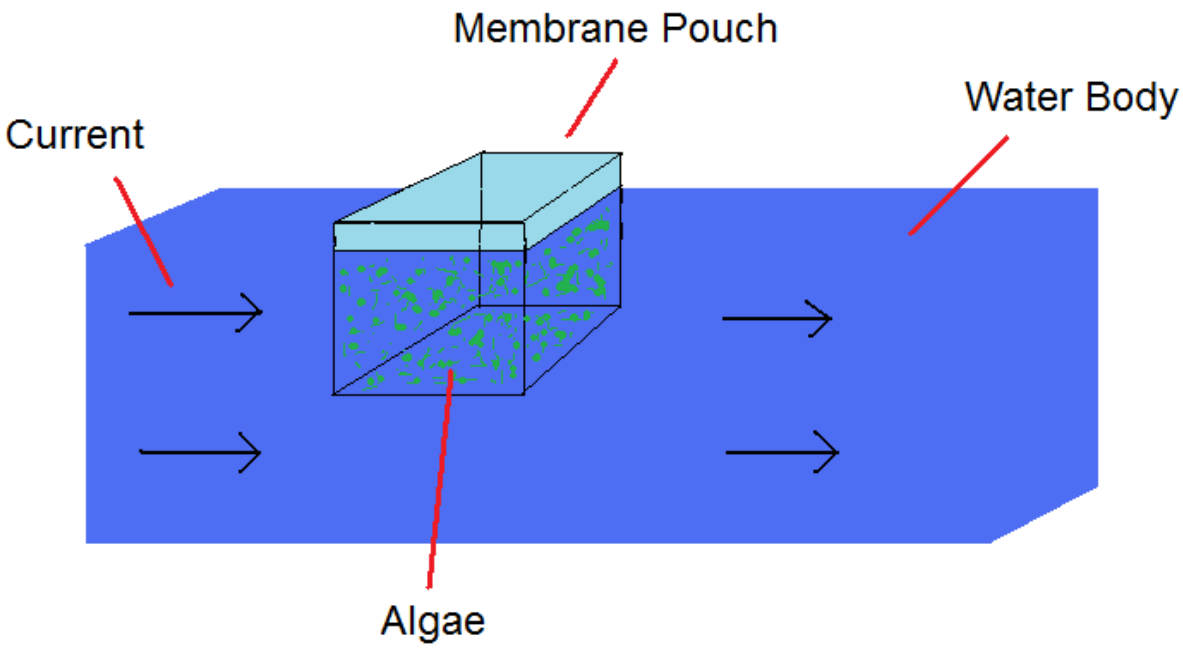


Figure 38. A schematic of the purposed mitigation system tested in this project.

It is hoped that by tailoring the pore size of the membrane, the pouch may be made to prevent microalgae cells from passing through it while allowing for much smaller water and nutrient molecules to pass through it. If nutrients are allowed to interact with the contained microalgae cells, it follows that they would be absorbed. However, any resulting microalgae growth would be contained within the pouch and could be removed from the system when desired. As discussed in **Section 3.4**, an appropriate amount of microalgae could be removed from the pouch before the cells become over populated. Additionally, microalgae grown in the pouch could be used as fertilizer, food supplements or a bio fuel crop.

5.2 Experimental Objectives

In testing the functionality of this proposed system, several design goals were considered. These goals formed the basis of the experiments conducted in this project and are listed as follows:

1) Determine if the membrane used disallows the passage of microalgae cells

2) Determine if the membrane used allows for the passage of nutrients

3) Determine whether the microalgae cells can adapt and grow within this environment

4) Determine the relative proportions of nutrients absorbed by microalgae within the pouch

II. METHODS AND MATERIALS

1.0 Experimental Model

In order to simulate the purposed system, an experimental model was designed (**Figure 39**). As shown, a tank with two partitions, splitting the container into three equal sections, was envisioned. The purpose of this was to allow for the center section to simulate the region enclosed by the microalgae pouch. The two end sections represent the water body outside of the pouch. This basic design was chosen over others in part because it simplifies the process of modelling diffusion rates throughout the tank. Additionally, measuring the volume of each section and nutrient concentrations may prove less challenging with this simple configuration.

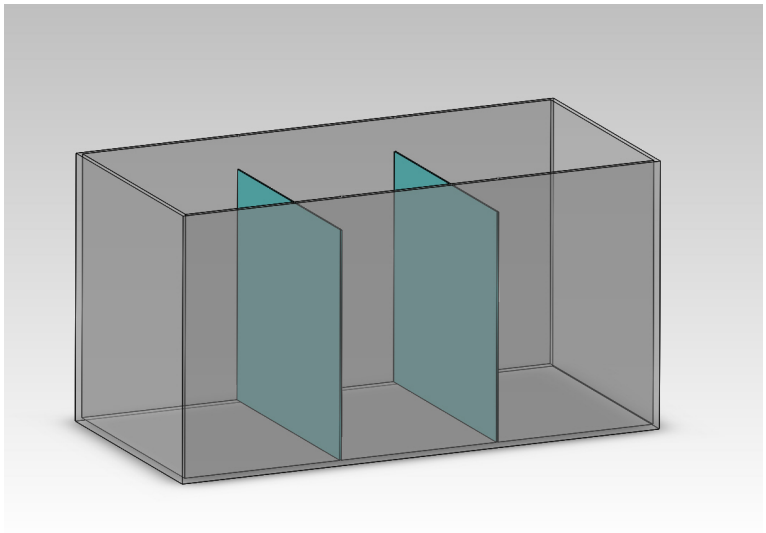


Figure 39. A schematic of the proposed experimental model.

1.1 Model Materials and Construction

Several materials were required to construct this experimental model. The grey container shown in **Figure 39** represents a 10 gallon aquarium with a height of 12 inches, length of 20 inches and a depth 10 inches. The blue partitions shown represent polymer membranes used to simulate the walls of the pouch. For this material, a microporous polycarbonate sheet membrane (**Figure 40**) with a pore diameter of 1 micrometer was obtained. Two pieces of the membrane were cut with dimensions of approximately 11 by 11 inches. These sheets were then adhered to the inner walls of the aquarium using an all-purpose silicon adhesive similar to those used to adhere the glass walls of aquariums. The length of the tank was divided into thirds and marked with tape strips. Lines of silicon adhesive were applied along the tape strips, first along the bottom of the tank and then along the vertical tank walls, one wall at a time. Each polycarbonate sheet was then layed ontop of the line of silicon adhesive so that about a half inch of membrane lay flush against each aquarium surface to which they were applied. A textbook was then used to apply pressure to the membrane and silicon adhesive for five minutes on each side applied to the aquarium walls. After adhering both membrane sheets, the adhesive was allowed to cure for 48 hours. The resulting height of the membranes from the floor of the tank was roughly 10 inches. It was originally intended for the membrane to be stretched tight along the width of the tank.

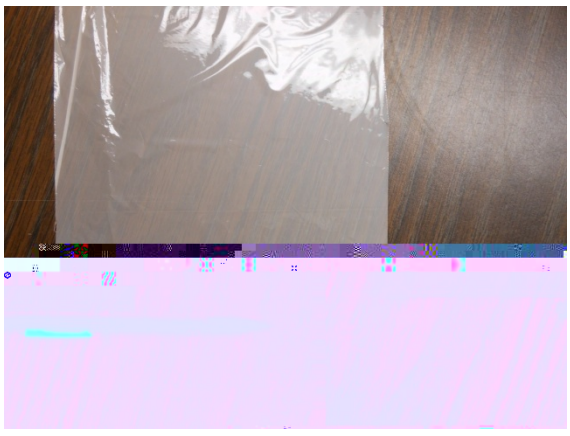


Figure 40. A piece of the microporous polycarbonate membrane used.

However, the material tears easily. Because the aquarium was to be filled with solution during testing, it was thought best to leave slack within the membrane so that it could better respond to any applied stresses. Though, this varied the intended volume of each section slightly as a result. **Figure 41** shows the completed test model. Yellow tape running above the tank was additionally attached to the top of the membrane sheets during experimental trials to help support them after the tank was filled.

1.2 Experimental Methodology

The main experimental focus of this project involved filling each section of the aquarium with a solution of deionized water and micronutrients essential for microalgae growth. However, phosphates and nitrates were added to one of the the end sections of the tank alone. In this manner, a concentration gradient of these nutrients would be formed between the nutrient-loaded section and the remaining two sections. The laws of diffusion hold that these nutrients will then migrate from regions of high concentration to regions of low concentration. Through water quality testing, it could then be determined if nitrates and phosphates were allowed to diffuse through the polycarbonate membrane sheets. To do so, water samples were to be drawn periodically from the non nutrient-loaded sections of the tank. A Hach DR 3800 spectrophotometer was to be used to detect the concentrations of phosphates and nitrates within the water samples. To do so, water samples were mixed into phosphate and nitrate TNT test vials. These vials contain reagents that react to these two compounds. In doing so, the contents of the vial change color (blue in phosphate test vials and red in nitrate test vials). These test vials could then be placed into the spectrophotometer for analysis. Decreased light transmittance throught these vials indicates an increased concentration of nutrients within the water sample. Thus, drawing periodic samples from sections of the tank could determine if nutrients are allowed to permeate the membrane, and, if so, at what rate this process occurs.

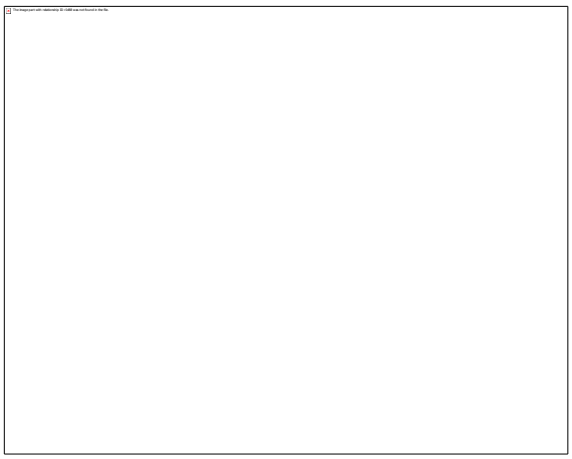


Figure 41. The finished test model during an experimental trial.

Barring the results of this initial experiment, it was then determined that two different experimental trials would be conducted, with and without microalgae present within the center section of the tank. In comparing nutrient concentrations in the end section opposite that initially loaded with phosphates and nitrates between the two trials, it could be determined whether the cells were absorbing nutrients. Additionally, water samples were to be periodically taken from the center section and placed in vials for spectrometer analysis. By reading absorbance values of these samples corresponding to the wavelengths at which chlorophyll exhibits peak absorbance, changes in microalgae cell population could be monitored. Increases in absorbance at these wavelengths would correspond to increases in the concentration of chlorophyll within the sample. Additionally, water samples could be drawn from the two end sections not holding microalgae to establish whether cells are allowed to pass through the membrane. To establish the feasibility of these experiments, it was necessary to perform several preliminary tests.

1.3 Preliminary Tests

Several simple preliminary tests were performed to establish constraints for the main experiments conducted in this project. The first of which was to determine whether the silicone adhesive had formed a proper seal between the membrane and tank walls. To do so, each section was filled with approximately two inches of water, one section at a time. The rate at which water accumulated on the opposite side of each membrane was observed for inconsistencies. The tank was rotated to test each side of the adhered membranes. It was discovered that one of the original membrane sheets had a tear near the bottom of the sheet. It was subsequently removed and replaced.

The next test performed was to determine whether the polycarbonate membranes would become damaged with exposure to a bleach solution. It was researched that high concentrations of bleach can degrade this polymer. However, bleach solution was needed to rinse the tank between experimental trials, as this chemical kills microalgae cells. A solution of 1ml / L of bleach in deionized water was prepared in an erlenmeyer flask. Two squares of polycarbonate were then cut, and one was submerged in the bleach solution for 5 hours. It was then removed, rinsed with deionized water and dried. The two squares were then taped over the top of separate erlenmeyer flasks. 10 mL of deionized water was then poured on top of the squares. The two squares showed no difference in the rate at which the water permeated the material, and it was concluded that the bleach solution would not affect this aspect of the experiments.

After establishing these results it was necessary to determine how long experimental trials involving nutrient diffusion throughout the tank would need to be conducted. Shorter trial times would potentially allow for more trials to be conducted. However, for the Hach spectrophotometer, the water samples tested must contain phosphate and nitrate concentrations within a target range. If a tested sample's nutrient concentration fell outside of this range, the spectrophotometer would still return a concentration measurement, but the accuracy of this value would be affected. The further outside of the acceptable range a sample concentration is, the higher the degree of inaccuracy in the measurement. The measurable ranges for nitrate and phosphate concentration within water samples were 1-60 mg/L and 0.15-4.5 mg/L, respectively. Because of this, enough time would have to be given to allow for nutrients to diffuse into other sections of the tank in suffiently large amounts.

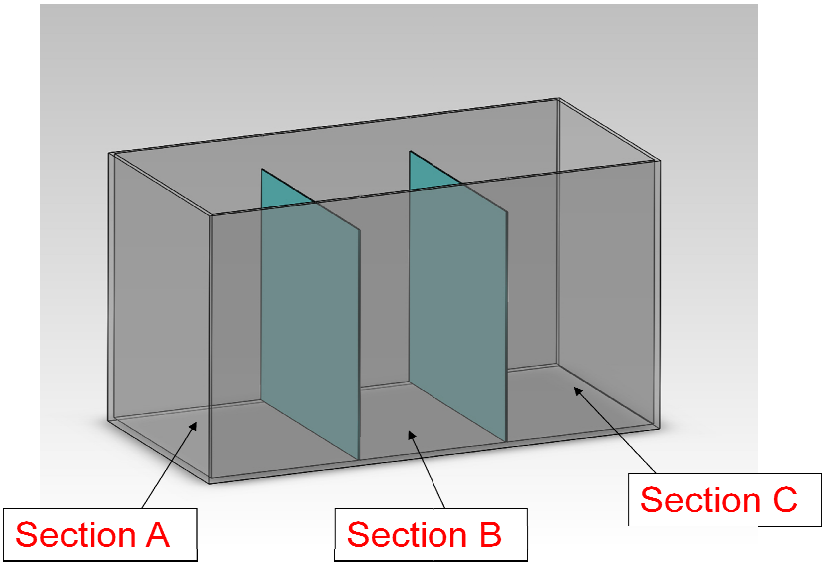


Figure 42. A schematic of the test model used with Sections A, B and C labeled. This same labeling method will be kept consistent throughout the remainder of this report.

In order to develop a rough model for the diffusion rates of these nutrients, three large buckets were obtained. To each bucket was added a 10 liter solution of deionized water and microalgae nutrients. The tank sections were labeled, moving left to right, Section A, Section B and Section C, as shown in **Figure 42**. This same labeling method will be used throughout the remainder of this project report. The solutions were prepared following a recipe for microalgae culturing called Bristol medium developed at the University of Texas at

Austin. **Table X** shows the nutrients and their relative amounts used in Bristol medium. A 10 L volume of this solution was prepared and added to each of the 3 buckets. However, because a phosphate and nitrate concentration gradient was to be established across the length of the tank, nutrients from the Bristol medium containing these compounds were include in the Section A solution, but were excluded from the Section B and Section C solutions. Deionized water was added in the proper volume to replace the presence of these nutrients in the solutions for Section B and Section C. **Table XI** lists the contents of the solution prepared for each section of the aquarium. As shown, 300 mL of deionized water was added to Section B and Section C in place of 100 mL each of $NaNO_3$, K_2HPO_4 and KH_2PO_4.

After mixing each of these solutions thoroughly in the buckets, 3 cups were used (one for each solution) to pour the solutions into the corresponding sections of the tank. Care was taken to alternate sections with each cup poured. This provided for a relatively even water level througout the tank and prevented unequal pressure from being exerted on the delicate membranes. The solutions were poured to a height of approximately 9 inches within the tank. This nearly emptied each bucket (perhaps 100 mL would be left in one bucket each trial due to slightly unequal volume of the tank section and shifting of the membrane due to slack).

Table X. Bristol Medium Recipe [65]

Nutrient	Amount	Stock Solution Concentration	Final Concentration
$NaNO_3$	10 mL / L	10 g / 400 mL dH_2O	2.94 mM
$CaCl_2$, $2H_2O$	10 mL / L	1 g / 400 mL dH_2O	0.17 mM
$MgSO_4$, $7H_2O$	10 mL / L	3 g / 400 mL dH_2O	0.3 mM
K_2HPO_4	10 mL / L	3 g / 400 mL dH_2O	0.43 mM
KH_2PO_4	10 mL / L	7 g / 400 mL dH_2O	1.29 mM
NaCl	10 mL / L	1 g / 400 mL dH_2O	0.43 mM

After each section was filled and it was verified that the solutions were at equal levels within each section, the experiment time began. An initial sample was drawn from each section by dipping Whirl-Pak bags (**Figure 43**) into each section and obtaining around 30 mL of sample. Subsequent samples were pulled from each section and the elapsed time at which this occured was recorded. For the main experimental trials, pipets were used to extract 15 mL water samples from the direct center of each section. This was a more exacting approach than simply dunking the bags into each section, as the pipets allowed for the sample to be obtained from a specific point as well as for a more precise sample volume to be obtained. Because this trial involved diffusion in stagnant solutions, it was predicted that an appreciable amount of time would be required to see a measurable rise in phosphate and nitrate concentrations in Section B and especially Section C of the tank. All told, 8 sets of samples were drawn over the course of 6 days and 20 hours of elapsed time.

Figure 43. A Whirl-Pak bag used to collect water samples during the experiment.

After analyzing samples pulled from Section B and Section C, it was determined that, at an elapsed time of approximately 4 days, a sufficient amount of the two nutrients had diffused into Section B and Section C of the tank. However, it was desired that this experimental time be reduced, as it proved difficult to pull samples at consistent time intervals over so long a time frame. See **Appendix A** for these test results.

Table XI. Experimental Solution Constituents

Nutrient	Section A (mL)	Section B (mL)	Section C (mL)
Deionized Water (dH_2O)	9400	9700	9700
$NaNO_3$	100	0	0
$CaCl_2$, $2H_2O$	100	100	100
$MgSO_4$, $7H_2O$	100	100	100
K_2HPO_4	100	0	0
KH_2PO_4	100	0	0
NaCl	100	100	100
Total Solution Volume	10,000	10,000	10,000

To facilitate the process of nitrate and phosphate diffusion throughout the tank, an aquarium water pump was attached to the tank wall in Section A, as shown in **Figure 41**. The previous experiment was then repeated with two changes. When the solutions had been fully poured into each section in this trial, the pump was turned on and water samples were immediately drawn from each section, marking t = 0 for the trial. The second change was that the experiment was run for 2.5 hours, and water samples were drawn from each section every 30 minutes. Samples pulled at 2.5 hours of elapsed time were analyzed. It was discovered that this amount of time had allowed for a sufficient amount of phosphate to diffuse into Section C of the tank. However, the nitrate concentration for Section C at this elapsed time was still lower than the minimum readable value of the spectrophotometer. However, this method allowed for samples to be drawn at more uniform time intervals. Thus, this method was used in the main experimental trials of this project. It was predicted that increasing the total elapsed time to 4.5 hours would allow for sufficient levels of both nutrients to diffuse into Section C of the tank. See **Appendix A** for these test results.

1.4 Experimental Procedures

After conducting the preliminary tests, the procedures for the main experimental trials were established. Two trials were conducted using the experimental method just described. One trial would be run without microalgae in the tank, and the second trial would contain microalgae in Section B of the tank. However, one detail pertaining to the microalgae's growth patterns complicated the experimental proceedings. As shown in **Figure 29**, microalgae experiences a lag phase upon being introduced into a new environment. In this phase, the cell population demonstrates negligible growth in cell population. Thus, it did not make sense to conduct the experiment with the cells in this growth phase; even if they were able to absorb diffusing nutrients, these results would only be evident in growing microalgae populations.

The amount of time that a microalgae culture takes to acclimate to a new environment and move past the lag phase is difficult to determine without empirical study. Among other factors, it may be a function of temperature, salinity, pH, availability of nutrients, photoperiod and the particular species of microalgae. However, an estimate of between 12 and 24 hours was suggested for the duration of the lag period upon making this transfer. Because the conditions provided by the tank were not considered to be particularly stressful to the microalgae used, a 16 hour period was allotted for the cells to adjust. Thus, in the two main experimental trials conducted, the solutions were poured into each section of the tank as previously described. However, on completion of this task, the tank was allowed to remain stagnant for a 16 hour period. At the end of this period, the pump was activated. The pump was then allowed to run for 4.5 hours, and samples were drawn from each section every 30 minutes. Additionally, a growth light was placed adjacent to the tank (**Figure 44**) and switched on during the 16 hour period. It was hoped that these adjustments might overcome the issue of the cells' slow initial growth. To conclude the

experimental procedures, the microalgae used in this experiment, as well as the water testing equipment will now be discussed.

1.5 Microalgae Test Specie

Chlorella Vulgaris was the microalgae of choice in this project. Several other microalgae species are cultured on campus by Dr. Hampson, including Dunaliella. However, Chlorella Vulgaris was selected among them for its high growth rates, adaptability and relative ease with which it may be cultured. In fact, this species is considered as the "guinea pig" in many current microalgae studies.

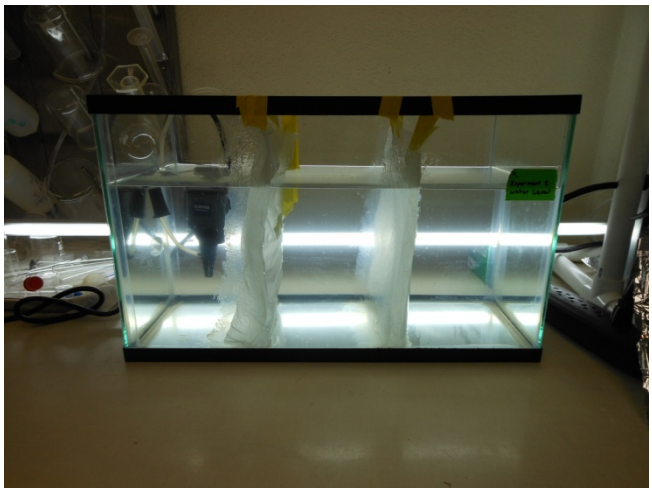


Figure 44. The tank with a growth light placed adjacent to it to stimulate microalgae growth (this photo was not taken during the actual experiment but depicts the arrangement of this equipment during the tests).

Another important constraint on the type of microalgae used was cell size. Because the membrane obtained had pore diameters of 1 micron, the success of the tested system relied in large part on the use of a microalgae with cells of larger dimensions. As shown in **Figure 45**, chlorella vulgaris cells are spherical and roughly 2 to 10 microns in diameter.

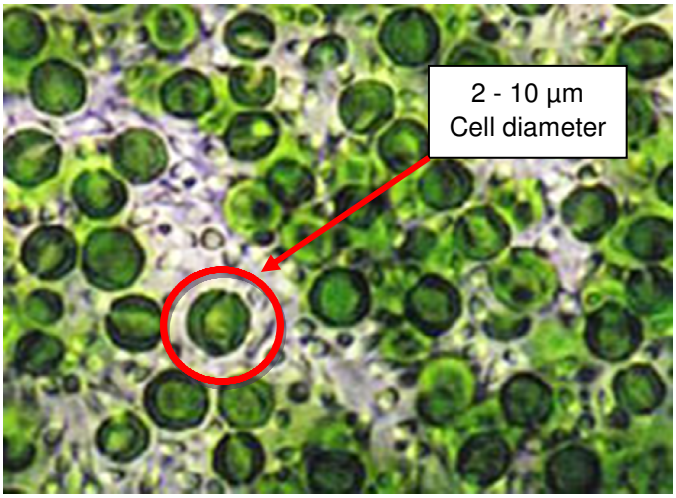


Figure 45. Chlorella Vulgaris microalgae cells.[66]

As previously discussed, one of the experimental trials excluded microalgae from the tank while the other trial included it. In this latter trial, 1 liter of the contents of a chlorella vulgaris culture was obtained. This was added to the solution prepared for Section B of the tank, replacing 1 liter of deionized water within this solution. Afterward, the three solutions were poured into the tank as previously described.

1.6 Sample Analysis Procedure and Equipment

After running each experimental trial, the obtained water samples were analyzed for nitrate and phosphate concentration using the spectrophotometer shown in **Figure 46**. To do so, samples were prepared using Hach TNT test kits (**Figure 47**). When testing for nitrate concentration, 1 mL of sample and 0.2 mL of a solution of isopropanol and water were slowly added to test vials. The vial cap was replaced and the vial shaken several times. Each nitrate test vial was then allowed to sit for 15 minutes. During this time, nitrate within the vial reacted with sulfuric and phosphoric acid originally present within the test vials. This produced a pink solution. When testing for phosphate concentration, 2mL of sample was added to test vials. The vial cap was then replaced, and the vial shaken several times. Next, the

vials were heated at 100 ^{o}C for 1 hour in a Hach DRB 200 reactor (**Figure 48**). The vials were allowed to cool to 20 ^{o}C before 0.2 mL of a sulfuric acid, ammonium molybdate and water solution was added. The vials were then inverted several times and allowed to sit for ten minutes. A resulting reaction produced a solution of blue-grey color within the phosphate test vials.

After the contents of these vials were allowed to react fully, the vials were placed in the spectrophotometer for analysis. The greater the presence of phosphates or nitrates within the test vials, the darker the color of the solution. Spectrophotometers analyze this color change through transmittance or reflectance properties and convert the readings into a concentration value.

It was previously discussed that sufficiently high concentrations of nitrate and phosphate are necessary to obtain an accurate spectrophotometer reading. This was considered with regard to Section C, as this section inherently had the lowest concentrations throughout the experiments. However, excessive nitrate and phosphate concentrations would likewise produce inaccurate readings. This was of concern in analyzing Section A samples, which inherently had the highest concentrations throughout the experiment. To reduce these concentrations to acceptable levels, Section A samples were diluted for both nitrate and phosphate test vials. Initial nitrate and phosphate concentrations for the Section A solution were approximately 166 and 164 mg/L, respectively. Dilution factors of 3 for nitrate and 50 for phosphate were used for Section A samples and were calculated through the following equation:

$$Dilution\ Factor = \frac{Aliquot\ Volume + Diluent\ Volume}{Aliqout\ Volume}$$

Here, the aliquot volume was the amount of water sample added to the test vials, and the diluent volume was an amount of deionized water used to dilute the sample concentrations.

Figure 46. The Hach DR 3800 spectrophotometer used to analysis water samples for nutrient concentration.

Figure 47. Phosphate and Nitrate TNT test kits used to determine concentrations of these compounds within water samples. Shown are the included test vials, added solutions and a processed vial of each compound.

Figure 48. The Hach DRB 200 reactor used to heat TNT test vials.

It was noticed that, when the spectrophotometer read the same vial multiple times, the concentration reading often varied by several hundredths of a mg/L. Thus, each test vial was measured 3 times and the average of those values was recorded. After all samples from a given section and trial had been analyzed, excel graphs were generated to view any resulting trends.

Although Section B samples were not analyzed for their nutrient concentrations, these samples were collected in order to analyze the Chlorella cell population throughout the experiment. An Ocean Optics Red Tide USB 650 spectrometer was used to take absorbance values of these samples at wavelengths of chlorophyll peak absorbance, as previously discussed. To do so, an absorbance versus wavelength graph was first generated by placing a vial of deionized water into the spectrometer. The wavelength of 660 nanometers was marked. Because the absorbance values rapidly fluctuate, a box car width of 8 was used to smooth the absorbance curve. The spectrometer collector was paused 5 times and the resulting absorbance values at the marked wavelength were recorded. These values were then averaged. The same procedure was employed with Section B samples instead of deionized water in the vial. These averaged values were graphed to note any trend that might exist throughout the trial.

One negative aspect of measuring absorbance values to characterize microalgae growth is that small rises in cell population might not be detectable. This was important with concern to the permeability of Chlorella cells through the polycarbonate membrane. If small quantities of cells were allowed to pass through the membrane, this would be a significant shortcoming in the system's design. However, small quantities of escaped microalgae cells would be difficult to detect in Section A and Section C. To cope with this aspect, the pump in Section A was left running for about a week after the experiment with Chlorella in Section B had been completed. Periodic observations of the tank were made to determine if microalgae growth was ever visible outside of Section B.

1.7 Diffusion Modeling

Techniques have herein been discussed to graphically represent the general trends in nutrient flow throughout the tank. However, diffusion plays a vital role in the proposed mitigation system and deserves a closer analysis. In general, there are several different techniques to model diffusion. When choosing a model for a particular phenomenon, it should first be considered whether a mass transfer or a diffusion model would be most appropriate. Mass transfer models involve the use of a mass transfer coefficient and a concentration gradient to calculate the flux of a species. This is shown in the equation:

$$Flux = k\,(C2 - C1)$$

Here, k is the mass transfer coefficient and C_2 and C_1 represent species concentrations at two points of interest. Thus, the flux, or amount of species moving through a given area in a given amount of time, from one point to another is directly proportional to the concentration difference between those two points. Because of this, k must be assumed to be constant between the two points of interest. However, it is shown experimentally that k often changes over time and with position.

In contrast, diffusion models are better adapted to account for changes in flux of a species along its travelled path. These models often use variables of distance of diffusing species into a system, concentration of diffusing species at those distances, diffusion coefficients and time. Diffusion is often a nonlinear phenomenon due to continuous changes in concentration gradient over time. The error function is incorporated into several diffusion equations to account for the dynamic trends in nutrient concentration over time and distance of a species moving through a system. The simplest diffusion

models make use of Fick's first law. The equation associated with this model is quite similar to that shown for mass transfer and is as follows:

$$J = -D\frac{\delta C}{\delta x}$$

J is the species flux while D is the diffusion coefficient or diffusivity (analogous to the mass transfer coefficient). δC and δx represent changes in concentration and distance, respectively. This model assumes steady state conditions. Situations involving varying concentration gradients with time call for slightly more complex models.

The case of nutrients moving from Section A to Section C of the tank do not represent steady state conditions. Nutrients are not being replenished into Section A and are not allowed to exit once accumulated in Section C. Thus, the concentration gradient between these two sections will change over time. To represent these concentration changes, several diffusion equations could potentially be used. One involves the assumption of an "infinite source" of diffusing species starting at a distance into the system of zero. In this model, the concentration of species diffusing into the system does not change with time. The equation associated with this model is as follows:

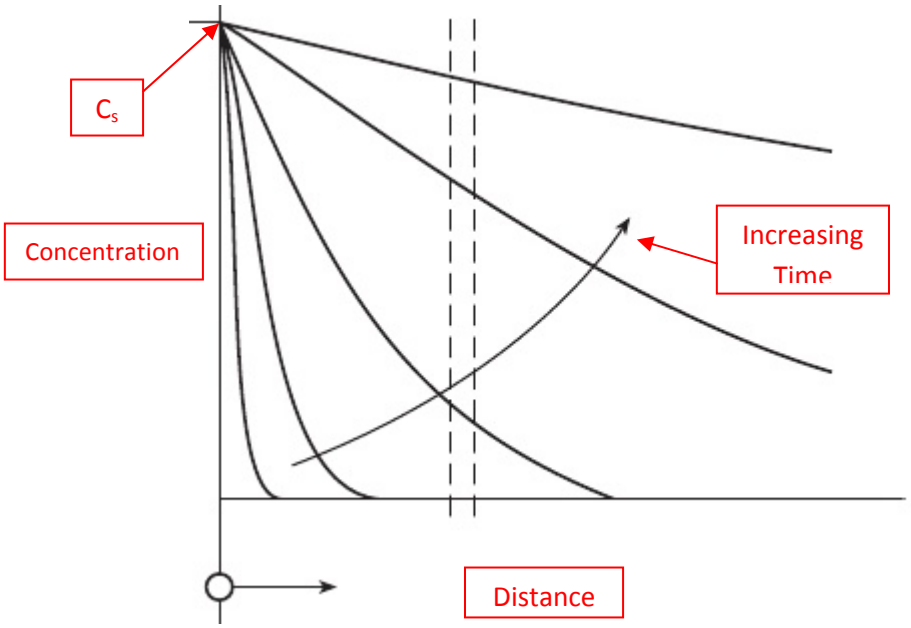


Figure 49. A graph representing a non steady-state "infinite source" diffusion model. Various concentration profiles are plotted to show the effects of increasing time. In accordance with this model, the concentration of species at a distance of zero into the system remains the same. [67]

$$\frac{\mathrm{Cx-Co}}{\mathrm{Cs-\ Co}} = 1 - erf\left(\frac{X}{2\sqrt{Dt}}\right)$$

Here, C_s represents the concentration of diffusing species initially present within the system. C_o represents a constant supplied concentration of this species at a distance into the system of zero. C_x represents the concentration of diffusing species at some distance into the system. Erf refers to the error function. The variables X, D and t represent distance into the system, diffusivity and time, respectively. Although the concentration of nutrients in Section A would not remain constant as required by this model, it may still prove an effective representation over short periods of time. **Figure 49** shows a concentration profile associated with this model. Different plots are shown on the graph to represent how the concentration of diffusing species changes throughout the system over time.

Already the circumstances of the experimental model have somewhat increased the complexity of characterizing the diffusion of nitrates and phosphates. However, there are several additional factors that will further increase the difficulty in modeling this phenomenon. To begin with, two membranes exist within the system. Because of the slower diffusion rates that are a characteristic of such membranes, the

concentration profiles for these nutrients will most likely see interruptions at these points within the system. **Figure 50** shows a representation of this aspect. According to the concentration profile in the

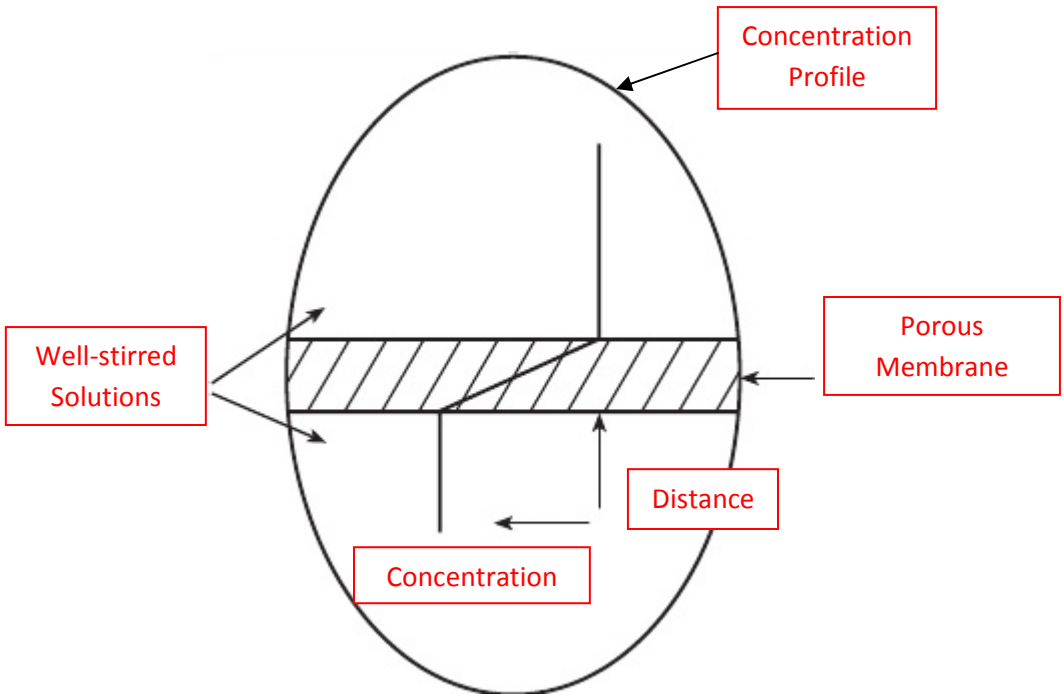


Figure 50. A depiction of the sharp concentration drop in diffusing species across porous membranes.[67]

figure, there is an abrupt decrease in diffusing species concentration across the membrane. Thus, in addition the trends of decreasing nutrient concentration with increasing distance away from Section A of the tank, the concentration differences will further decrease over the polycarbonate membranes. **Figure 51** shows a rough graph of the trends expected in nutrient concentration throughout the tank with increasing elapsed time. As shown, concentration profiles moving from red to blue indicate increases in elapsed time.

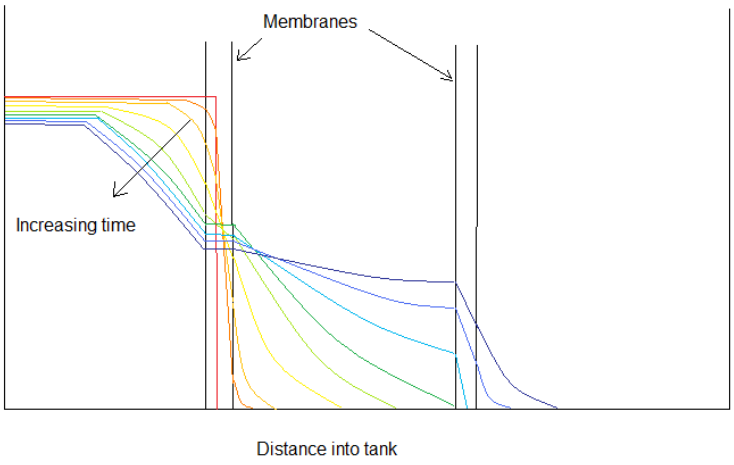


Figure 51. A representation of the changes in nutrient concentration predicted to occur in the experiment. Concentration profile lines are color coded according to amount of elasped time, progressing from red to blue.

III. RESULTS

1.0 Qualitative Results

The results acquired from the experimental trials conducted included both qualitative and quantitative information. The qualitative results included observations made with regard to the functionality of the test system as well as observations concerning the health and growth of Chlorella within the system. Additionally, the ease with which this experimental model was constructed was considered.

1.1 Polycarbonate Membrane Functionality

Several aspects of the polycarbonate membrane were investigated in this project. These were as follows:

1) Ability of membrane to be adhered to tank wall

2) Resistance of the membrane to bleach cleaning solution

3) Ability of membrane to block passage of Chlorella cells

4) Ability of membrane to allow passage of nutrients

5) General handling characteristics of the membrane

Through experimentation, it was shown that the membrane was able to be adhered to the tank wall to form a water tight seal. As previously discussed, when water was poured into one section of the tank alone, the rate at which water permeated the membrane showed no increase near the sealed edges of the membrane, so long as the membrane was completely sealed with adhesive. Again, one of the original polycarbonate sheets was torn slightly during installation and had to be replaced. Another instance occurred, in which it was observed that water placed in Section A of the tank flowed into Section B through a small gap near the bottom of the sheet. It was found that there was a break in the line of applied sealant, and that this was allowing for water to bypass the membrane barrier. After reapplying sealant over this area, the membrane functioned normally. As discussed, an applied bleach solution did not damage the polycarbonate membrane, at least not to an extent that produced observable differences in the permeability of water through the material.

Positive qualitative results were found with regard to the capacity of chlorella to permeate the polycarbonate membrane. After allowing chlorella cells to remain in Section B of the tank for approximately a week, microalgae growth was observable within this section. However, growth was not found in Section A or Section C, with the exception of a small amount of chlorella particulates. The location of these particulates is shown in **Figure 52**, and the reasons behind their presence in Section C will be later discussed. In addition to blocking most of the microalgae cells, the membranes easily allowed for the passage of nitrates and phosphates into Sections B and C of the tank. The quantitative results of this nutrient diffusion will be discussed shortly.

From a manufacturing standpoint, the overall ease with which the polycarbonate membranes were able to be incorporated into the tank were considered. These membranes were difficult to work with for several reasons. First, the electrostatic properties of this material and its great flexibility caused it to cling to nearby surfaces. This created great difficulty when installing these sheets, especially after the silicon adhesive had rubbed onto several unintended surfaces of the tank. Additionally, the thinness of the membranes allowed them to become easily torn. This made moving the tank and filling its sections with solution a slow and delicate process.

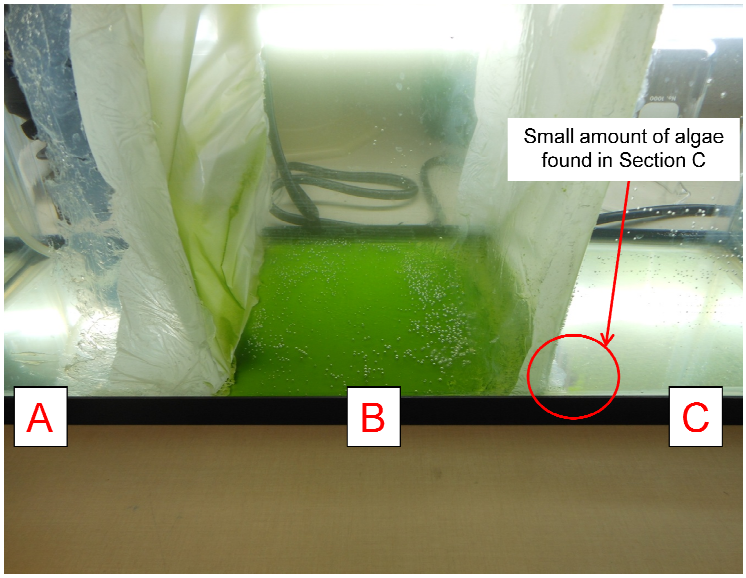


Figure 52. A picture of added chlorella cells present within Section B of the tank. The location of cells found outside of this section is indicated.

1.2 Chlorella Growth Observations

Although some chlorella cells were found outside of Section B, positive observations were also made in regard to microalgae growth. As shown in **Figure 53**, green patches of chlorella growth were observed on the membrane separating Section A and Section B of the tank. However, none were seen on the membrane separating Section B and Section C. Additionally, it was noted that a large portion of chlorella cells had sunken to the bottom of the tank.

It was intended that these qualitative observations of chlorella growth be backed by absorbance measurements from Section B water samples. A plot of 660 nanometer light absorbance versus elapse time was made. The trend of this plot was weak, and it was soon after determined that an improper graph type had been generated on the Spectrasuite program. By the time this error was resolved, the Section B samples were judged no longer able to yield absorbance trends that would represent growth in cell population throughout the experiment because chlorella cells within these bagged samples would still be able to grow in the presence of nutrients within the sample. Additionally, this growth would likely be greater for samples drawn later in the trial than earlier, as the nutrient concentrations would be larger for longer elapsed times. Thus, the data gathered from these spectrometer readings was discarded.

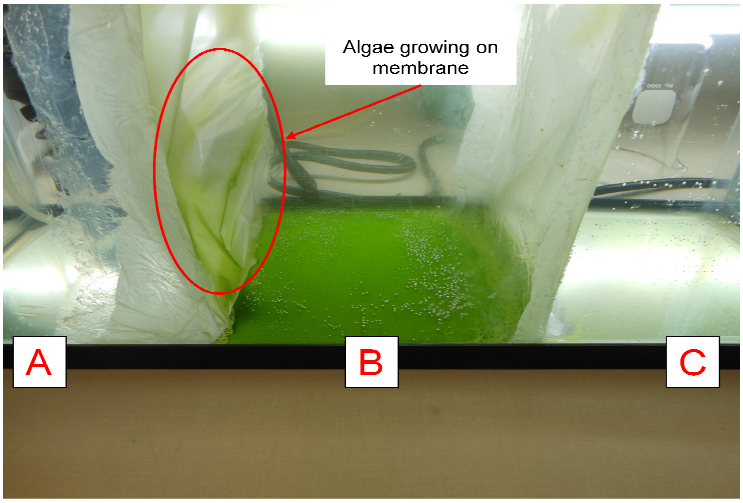


Figure 53. A picture demonstrating the regions of chlorella growth following its introduction into Section B of the tank.

2.0 Quantitative Results

Quantitative results obtained included nitrate and phosphate concentration data. In addition to being separated by nutrient, this data is further divided into that taken from Section A samples and Section C samples and whether chlorella was or was not present in Section B during the trial. In the interest of organization, the results will be first categorized by tank section, secondly by nutrient and lastly by whether chlorella was or was not present in Section B for that trial. Nutrient concentration data was compiled and graphed for each trial. From these graphs, the rate at which nutrient concentration changed, the initial concentrations, and the R^2 values were considered. As will be discussed, linear trend lines were included on these graphs as a result of the unexpectedly high linearity of the plots.

2.1 Section A Water Sample Data

After pouring solutions into each section of the tank, it was predicted that nitrate and phosphate present in Section A would diffuse into the other sections of the tank. This trend was confirmed in the gathered concentration data, signifying that both nitrate and phosphate were capable of permeating the polycarbonate membrane.

In the trial without chlorella present, the initial concentration of phosphate in Section A was 137.5 mg/L. Over the course of 4.5 hours, this concentration diminished by 15.5 mg/L to a final concentration of 122 mg/L. This yielded a rate of 47.6 µg/L/min leaving Section A. A linear trend line was placed on the data plot. This had an R^2 value of 0.9059 and an equation of $y = -0.0476x + 134.57$.

In the trial with chlorella present, the initial concentration of phosphate in Section A was 141.5 mg/L. After 4.5 hours of elapsed time, the concentration had decreased by 22.0 mg/L to a final concentration of 119.5 mg/L. This yielded a rate of 61.3 µg/L/min leaving Section A. The linear trend line showed an R^2 value of 0.8377 and an equation of $y = -0.0613x + 140.83$.

Without chlorella present in the tank, the initial nitrate concentration in Section A was 127.47 mg/L. After 4.5 hours of elapsed time, the concentration of nitrate had decreased by 20.0 mg/L to a final concentration of 107.47 mg/L. This data yielded a rate of 67.3 µg/L/min leaving Section A. A linear trend line of the plot had an R^2 value of 0.9309 and an equation of $y = -0.0673x + 125.46$.

With chlorella present in the tank, the initial concentration of nitrate in Section A was 132 mg/L. By the end of the 4.5 hour trial time, the concentration had decreased by 21.2 mg/L to a final concentration of 110.8 mg/L. The plot of this data resulted in a rate of 74.9 µg/L/min leaving Section A. The linear trend line fitted to the plot had an R^2 value of 0.9382 and an equation of $y = -0.0749x + 131.62$. A summary of these results is listed in **Table XII**. Additionally, the graphs produced from this data are shown in **Figure 54**.

From **Table XII**, several trends may be seen. The observed trends for several of these factors are summarized in **Table XIII**. Although the initial nutrient concentrations were higher for phosphate than nitrate in both trials, the average rate of concentration change was higher for nitrate in each case. Additionally, it was found that initial nutrient concentrations as well as rates of concentration change were higher for both nutrients in the trial with chlorella. Both nitrate data sets exhibited higher R^2 values than were seen for phosphate.

Table XII. Section A nutrient concentration data

Trial Conditions	Initial Concentration (mg/L)	Change in concentration over trial (mg/L)	Rate of concentration change (µg/L/min)	R^2 Value	Linear trend equation
Phosphate Without Chlorella	137.5	- 15.5	-47.6	0.9059	y= -0.0476x + 134.57
Phosphate With Chlorella	141.5	- 22.0	-61.3	0.8377	y = -0.0613x + 140.83
Nitrate Without Chlorella	127.47	- 20.0	-67.3	0.9309	y = -0.0673x + 125.46
Nitrate With Chlorella	132	- 21.2	-74.9	0.9382	y = -0.0749x + 131.62

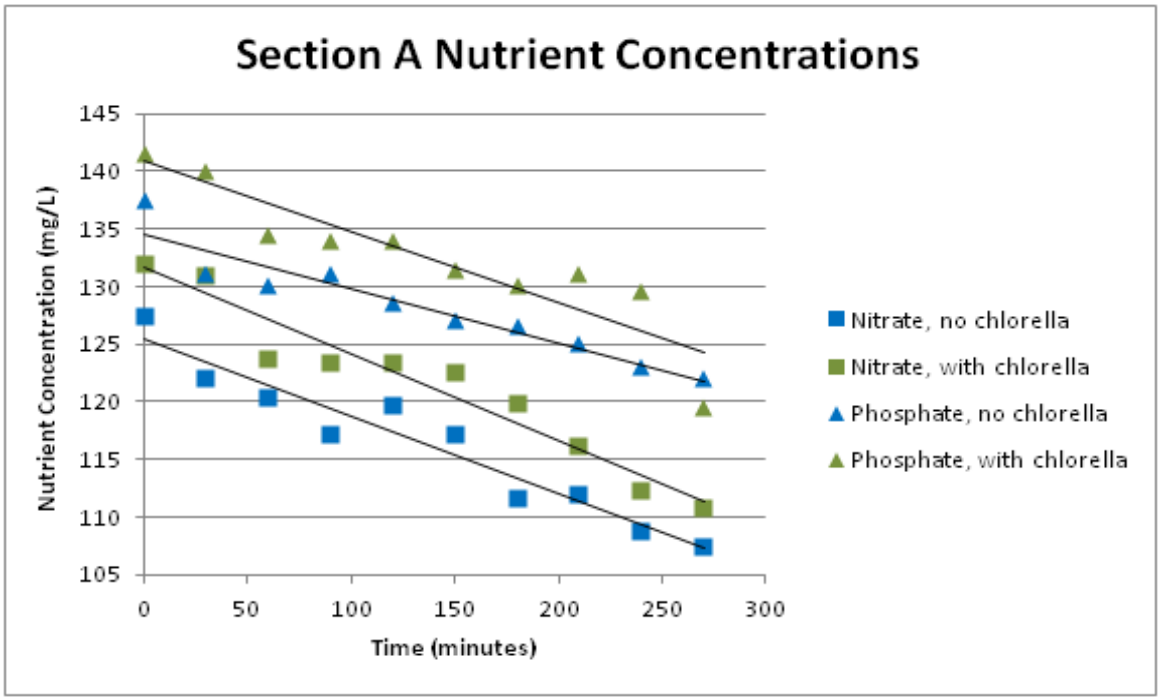


Figure 54. Section A concentrations for nitrate and phosphate from both trials. Nitrate data points are square and those of phosphorus are triangles. Additionally, the data from the trial without chlorella is represented with blue data points, while that from the trial with chlorella is represented with green data points.

Table XIII. Observed trends in Section A concentration data

Factor	Observed trends
Initial nutrient concentration	• higher for phosphate in both trials • higher for both nutrients in the trial with chlorella
Rate of concentration change	• higher for nitrate in both trials • higher for both nutrients in the trial with chlorella
R^2 Value	• higher for nitrate in both trials

2.2 Section C Water Sample Data

Water sample data from Section C was graphed and analyzed using the same methods as used for Section A samples. However, it was predicted that the nutrient concentrations for this section would increase over time, as nutrients diffusing from Section A reached the other end of the tank. This prediction proved correct, as is shown in the following results.

In the trial without chlorella, the initial phosphate concentration was 2.22 mg/L. Over the course of the trial, this concentration increased by 1.7 mg/L, yielding a final concentration of 3.92 mg/L. This change gave an overall rate of phosphate concentration change of 6.5 µg/L/min. After fitting a linear trend line to the data plot, it was found to have an R^2 value of 0.9619 and an equation of y = 0.0065x + 2.1602.

In the trial with chlorella, the initial phosphate concentration was 2.72 mg/L. After the 4.5 hours of elapsed test time, this concentration increased by 1.4 mg/L to give a final concentration of 4.12 mg/L. This yielded an overall rate of phosphate concentration change of 5.1 µg/L/min. A linear trend line fitted to this data set had an R^2 value of 0.985 and an equation of y = 0.0051x + 2.6309.

In the trial without chlorella, the initial nitrate concentration was 2.69 mg/L. After 4.5 hours, this concentration increased by 3.54 mg/L to give a final nitrate level of 6.23 mg/L. The overall rate of change in nitrate concentration was 15.9 µg/L/min. An R^2 value of 0.9435 and an equation of y = 0.0159x + 2.4556 were found for this data set.

In the trial with chlorella, the initial nitrate concentration was 5.74 mg/L. By the end of the trial, this concentration had increased by 1.6 mg/L to a final concentration of 7.34 mg/L. The rate of change in nitrate concentration was calculated to be 4.9 µg/L/min. A linear trend line was fitted to this data set. The R^2 value of the trend was 0.3469 and its equation was y = 0.0049x + 5.4376. **Table XIV** lists a summary of these results. Additionally, graphs produced from this data are shown in **Figure 55**.

Table XIV. Section C nutrient concentration data

Trial Conditions	Initial Concentration (mg/L)	Change in concentration over trial (mg/L)	Rate of concentration change (µg/L/min)	R^2 Value	Linear trend equation
Phosphate Without Chlorella	2.22	+ 1.7	+ 6.5	0.9619	y = 0.0065x + 2.1602
Phosphate With Chlorella	2.72	+ 1.4	+ 5.1	0.985	y = 0.0051x + 2.6309
Nitrate Without Chlorella	2.69	+ 3.54	+ 15.9	0.9435	y = 0.0159x + 2.4556
Nitrate With Chlorella	5.74	+ 1.6	+ 4.9	0.3469	y = 0.0049x + 5.4376

From the data in **Table XIV**, several trends were observed. First, initial concentrations for both nutrients were higher in the trial with chlorella. Also, the initial concentrations of nitrate were higher than of phosphate in both trials. The changes in nutrient concentration were higher without chlorella in the tank

than with it present. In turn, the rates at which both nitrate and phosphate concentrations rose were greater when chlorella was not added to Section B. A trend of higher R^2 values was seen for phosphates. However, it should be noted that a peculiarly low R^2 value was obtained for the nitrate data set with chlorella present. Thus, it is difficult to draw any definite trend from these values. These findings are summarized in **Table XV** for several factors.

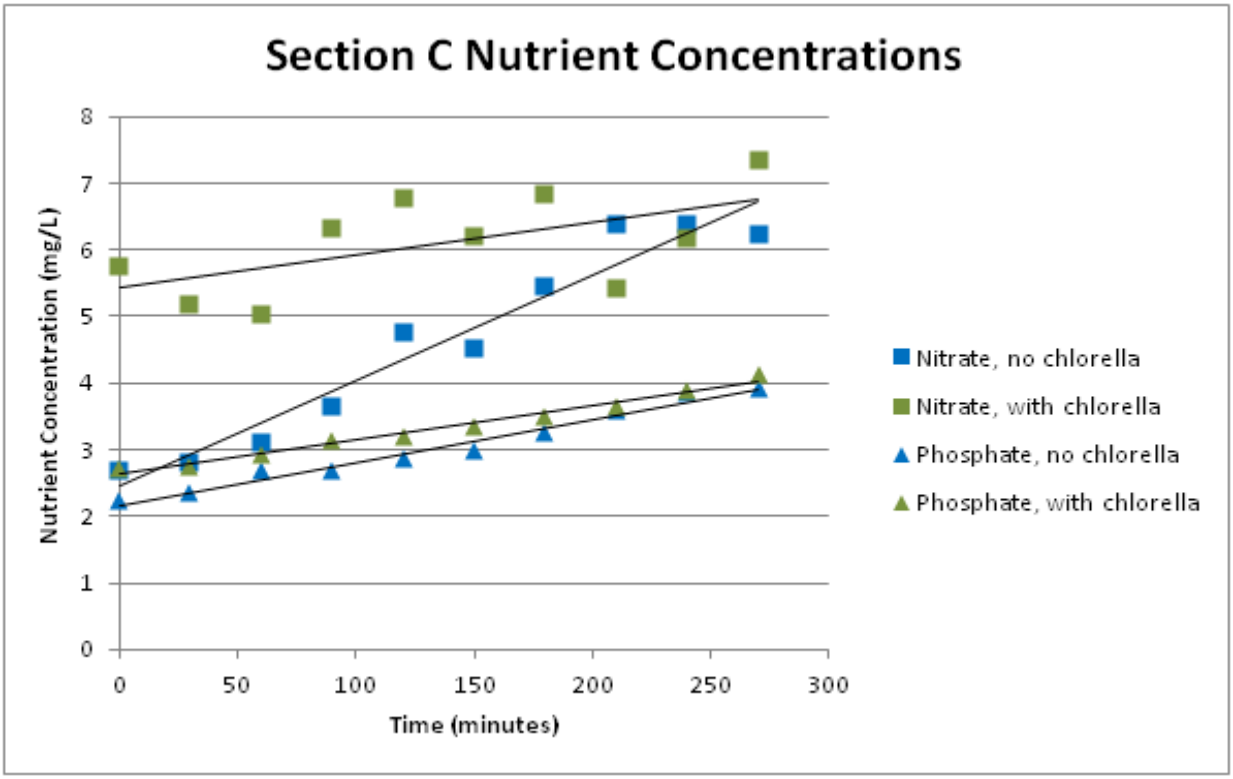


Figure 55. Section C concentrations for nitrate and phosphate from both trials. Nitrate data points are square and those of phosphorus are triangles. Additionally, the data from the trial without chlorella is represented with blue data points, while that from the trial with chlorella is represented with green data points.

Table XV. Observed trends in Section C concentration data

Factor	Observed trends
Initial nutrient concentration	• higher for nitrate in both trials • higher for both nutrients in the trial with chlorella
Rate of concentration change	• higher for both nutrients in trial without chlorella
R^2 Value	• higher for phosphate in both trials (however, this trend may not be valid)

IV. DISCUSSION

1.0 Analysis of Results Overview

After considering the obtained results, an assessment was made of the experimental model. An analysis of the model's functional features was fairly straight forward. However, interpreting the data compiled from Section A and Section C water samples proved more complex. This data was helpful in determining whether nutrients could permeate the polycarbonate membrane and gaining a general idea of nitrate and phosphate diffusion rates throughout the tank. Trends with this data help to establish the effect of adding chlorella vulgaris cells to Section B of the tank. However, there are several factors that may have influenced these trends. Ultimately, it was clear that both the number and validity of conclusions drawn from the experiment was hindered by the quantity of data points and the number of trials conducted.

1.1 Discussion of Quantitative Results

The polycarbonate membrane selected demonstrated both positive and negative qualities with regard to its role in the proposed microalgae pouch. Not only did it properly house chlorella vulgaris cells within a contained region (so long as it was properly sealed), but it also allowed for the permeation of water and nutrients. These material qualities are crucial for this application. Additionally, the membrane showed no adverse reactions with exposure to a dilute bleach solution, allowing for the membrane to be cleaned if necessary. The silicone adhesive used was able to form a water tight seal between the glass tank walls and the membrane. The thinness of the polycarbonate membrane provided great flexibility and allowed for it to move in response to applied water pressure within the tank. This quality proved valuable in preventing damage to the material once the tank had been filled with solution. However, the exceptional thinness of the membrane made it more prone to tearing during installation. As previously mentioned, this did in fact occur. Using a thicker membrane would be a good solution to this problem. The electrostatic behavior of this material made handling and installation difficult, as it would readily cling to nearby surfaces. Improving this quality would speed up the process of constructing the proposed microalgae pouches. Microporous polymer membranes are not cheap. Thus, durability and associated cost would play an important role in manufacturing and using these systems in an actual eutrophic water body.

In this project, the quantitative analysis of microalgae growth was not successful. However, several observations were made after adding chlorella cells into Section B. Referring to **Figure 53**, microalgae growth was visible on the membrane dividing Section A and Section B. This shows that at least a portion of the cells were able to adapt to the environment of the tank. The fact that this growth was not seen on the membrane separating Section B and Section C indicates that the cells sought the more nutrient rich water arriving from Section A. This was achieved despite the current generated by the pump in Section A that pushed the water in the opposite direction. Thus, the proposed system might not be hindered by modest currents within a water body. Despite these positive results, **Figure 53** also shows a large amount of chlorella cells accumulated on the bottom of the tank. While this does not necessarily indicate that these cells had died, growing microalgae cells tend to create gas which makes them more buoyant. As a result, growing microalgae populations tend to accumulate near the surface of water bodies. Available light may also play a factor in this observation. Light intensity diminishes with the amount of water it passes through. This also may explain why microalgae grow more densely near the water's surface. The growth light placed adjacent to the tank may have directed light of higher intensity towards

the bottom of the tank. However, it does not seem as likely that this was the cause of chlorella accumulation on the floor of the tank as the possibility that these cells had simply died in the environmental transition.

2.0 Discussion of Qualitative Results

The compiled nutrient concentration data demonstrated some positive results, as well as some that were not predicted. Ultimately, the trends shown from these datasets beg further investigation. Thus, this analysis aims to interpret what the concentration data suggests as well as to identify factors that may, for better or worse, have influenced these results. Additionally, measures that may be taken in order to improve the quality of these results and expand upon them will be discussed shortly.

2.1 Analysis of Water Sample Data Trends

Perhaps the most important trend noted from the nutrient concentration results involved the rates at which phosphate and nitrate diffused into Section C between the two experimental trials. Because the microalgae was intended to absorb nutrients before they reached Section C, it was hoped that the flux of these nutrients into Section C would be lower in the trial including chlorella than when this microalgae was not present in the tank. Likewise, it was hoped that the Section C nutrient concentrations would be lower in the trial with chlorella than in the trial without it.

According to the compiled concentration data, both phosphate and nitrate demonstrated lower rates of concentration change in Section C of the tank when chlorella was present in Section B. This suggests that the microalgae is absorbing nutrients from the system and performing as intended. For phosphorus, the average rate of concentration change in Section C was 6.5 µg/L/min with chlorella and 5.1 µg/L/min without chlorella in the tank. For nitrate, the average rate of concentration change in Section C was 15.9 µg/L/min without chlorella and 4.9 µg/L/min with chlorella present in the tank. These results were encouraging, and since a fairly low chlorella cell concentration was first introduced into the system, these rates might be further lowered with the introduction of larger numbers of cells into Section B.

As discussed, it was additionally hoped that the actual nutrient concentrations would be lower in the trial with chlorella than in the trial without it. However, it was observed almost exclusively that both phosphate and nitrate concentrations were higher throughout the trial with chlorella than throughout the trial without it. However, several factors may have affected these results. First, pouring the solutions into each section of the tank was a slow process, taking approximately 20 minutes to unload about 30 Liters of solution. Care was taken to pour the solutions slowly and with as little agitation of the solutions as possible. Though, it is probable that the solutions were agitated to different degrees between trials. This agitation likely increased diffusion rates, if only for a short period of time, throughout the tank. Thus, this could have caused the nutrient concentrations to be higher in Section C for the trial with chlorella.

Another interesting trend with respect to these results was that, in the trial with chlorella, both Section A and Section C demonstrated this phenomenon for both nitrate and phosphate. If the same amount of nitrate and phosphate were initially added to Section A in the two trials, it would seem logical that a higher relative nutrient concentration in Section C of the tank would be accompanied with a lower relative concentration in Section A of the tank. The fact that, throughout the trial with chlorella, higher nutrient concentrations were simultaneously seen in both Section A and Section C of the tank than were seen in the trial without chlorella seems an indication of some experimental error. One potential explanation is

that a larger percentage of the tank's total nutrients were present in Section B throughout the trial without chlorella than in the trial with chlorella, thus accounting for the lower relative nutrient levels in Section A and Section C. Another source of error could have been that slightly higher amounts of nutrients were added into the Section A solution in the trial with chlorella than in the trial without it.

A third source of this trend could have resulted from a procedural error. When chlorella was taken from the culture container for use in the experiment, 1 Liter of the container's contents was extracted. It was indicated that the culture had not received replenished nutrients in an appreciable period of time and that the solution within the container was likely devoid of nitrate and phosphate. Thus, these contents were added straight to the Section B solution without testing for nitrate and phosphate concentrations. It is possible that these nutrients were still present within the 1 Liter of culture water added to Section B during this trial. Since this task was not performed in the trial without chlorella, this could have accounted for the higher nutrient concentrations in the trial with chlorella. However, the trend still stands that, in the trial with chlorella, the concentration of nutrients in Section C rose less rapidly than when chlorella was not present. In fact, **Figure 55** shows that the concentrations of both nutrients in the trial without chlorella have nearly surpassed the nutrient concentrations of the trial with chlorella within 4.5 hours of elapsed test time with the pump activated.

The trend just discussed involved a factor largely representative of the proposed system's functionality. However, other trends within this data were examined as well. As previously discussed, trends existed within the data sets for a single section. For example, data from Section A showed that in both trials the rate of concentration change was higher for nitrate than for phosphate. Given that nitrate has a higher diffusivity than phosphate (1.9×10^{-5} and 1.0×10^{-5} cm^2/sec for nitrate and phosphate, respectively), this trend is logical. Species with larger diffusivities should move more rapidly through a system. However, this trend did not exist within the Section C data sets. This indicates that other factors may have played a role in the diffusion rates of these two nutrients, as will soon be discussed.

Trends within the R^2 values existed within section data sets, but were not visible when all data sets were considered. For example, phosphate data sets exhibited higher R^2 values in Section C for both trials. However, Section A saw higher R^2 values for nitrate data for both trials. Had both sections demonstrated higher R^2 values for a single nutrient between both trials, further inference would have been made. Though, it was observed that R^2 values for each nutrient and each trial were greater in Section C data than in Section A data, with the exception of the Section C nitrate data set for the trial with chlorella. The R^2 value for this particular data set (0.3469) was incredibly low compared to the other R^2 values. Although not nearly as severe, the phosphate data set for the trial with chlorella taken from Section A displayed an R^2 value appreciably lower (0.8377) than the rest of the values for that section. Sources of error in these values could have resulted from a number of different procedural tasks. Although it was intended for water samples to be drawn from the exact center of each section through a pipet, it was difficult to keep the location of the pipet tip completely consistent when drawing samples. If the pipet tip was placed in different positions throughout the trials, this could have affected the concentration of the sample drawn. Additionally, samples were drawn into pipets and transferred into test vials for spectrophotometer analysis. Because only 1 and 2 mL of nitrate and phosphate sample, respectively, were required, small deviations from this amount transferred into the test vials might have had an appreciable impact on measured concentrations. As previously discussed, samples were drawn and transferred into plastic bags where they were then stored for times as long as a week before they could be analyzed. The top of the bags were not sealed but were designed so that the top was rolled several times to close them. Because of this, evaporation of the samples likely occurred, at least in some small amount. This could have affected the nutrient concentration of the samples. Lastly, one error that could have accounted for the low R^2 value seen in the Section C nitrate data set with chlorella was the

accidental switching of test vials. Vials were labeled with the sample set and elapsed time circumstances in which they were drawn. However, improper labeling of this sample set may have placed vials out of order according to the elapsed time at which they were taken and resulted in a lower R^2 value.

An additional observation was made concerning R^2 values; this was that these values were higher for Section C data sets than for Section A data sets (with the exception of the previously discussed Section C nitrate sample with chlorella). This is believed to have been the result of the varying amounts of water agitation created by the water pump. After turning on the pump, it was observed that the membranes reduced the amount of current generated in Section B and Section C. However, ripples were still readily visibly in Section C. The differences in the amount of agitation between Section A and Section C may have played a role in the lower degree of linearity shown in R^2 values of these data sets. However, the linear trends of nutrient concentrations seen in this experiment require further discussion.

2.2 Analysis of Diffusion Linearity

As predicted, the concentration of both phosphates and nitrates decreased in Section A and increased in Section C during both trials. However, the trends of these concentration changes in almost every case were highly linear. This contrasted with the expected trends of nutrient concentration. Because non steady-state diffusion rates change with respect to time and concentration gradient, diffusion profiles tend to be nonlinear. However, **Figure 49** might offer a potential reason for the linear behavior observed. As shown in this graph, diffusion profiles tend to decay, or decrease in concentration, less dramatically over time. The top most profile even appears to be nearly linear. The fact that the solutions were allowed to sit in the tank for a period of 16 hours before the pump was activated might have allowed for subsequent diffusion rates to become more linear.

Another factor that may have influenced the rate of change in nutrient concentrations could have come from the Colligative properties present within the test model, most notably osmotic pressure. These properties, which involve the ratio of solute to solvent particles within solutions, give a new perspective to the proposed system. The proposed microalgae pouch in a way functions similarly to how a living cell does; semi-permeable membranes surround cells and allow for the selective intake of nutrients from their surroundings. In turn, osmotic pressure relates to the relative amounts of ions (or nutrients in this case) on either side of the membrane. If a higher concentration of nutrients is present outside of the cell, water will actually diffuse across the membrane and out of the cell in an attempt to achieve equilibrium between the separated concentrations. This phenomenon will proceed until the gravitational force acting on the system overcomes this tendency.[68] **Figure 56** demonstrates this process. This is analogous to the experimental test model in that water molecules may have been simultaneously diffusing towards Section A while nutrients diffused away from this region. The current generated from

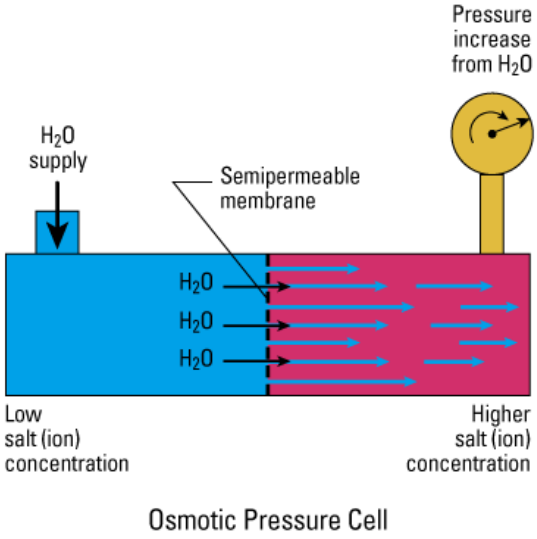


Figure 56. A depiction of how osmosis causes the diffusion of water across membranes in response to ion concentration gradients.[69]

the pump may have discouraged water from diffusing across the membrane into Section A though. Additionally, the polycarbonate membrane used may not have been capable of the semi-permeable characteristics involved in osmosis. However, the net movement of water as an additional diffusing species may have influenced the nutrient concentrations if this phenomenon did indeed occur within the tank.

Membrane potential may have also influenced nutrient diffusion rates in the experiment. As ions diffuse across a membrane, their net charge can create an electric potential between the two separated solutions that depends on the concentration of ions in each. As electrically charged ions diffuse across a membrane, their accumulated charge may discourage further ions from diffusing into the region. This may especially occur with semi-permeable membranes.[70] In addition to this electric potential across the membrane, the charge of dissociated electrolytes in the solutions to either side of the membrane impact the rates of their diffusion. Solutions of dissolved anions and cations, although not without exception, diffuse across membranes at the same rate. This is caused by the need of electroneutrality. Even though smaller dissolved ions may naturally diffuse more rapidly through the system than larger dissolved ions on their own, the principle of electroneutrality holds that the diffusion rate of each electrolyte influences that of the other. These two rates on their own become averaged as dissolved anions and cations both diffuse through a membrane.[71] Again, it is outside the scope of this project to determine the relative role that these phenomena played in the experimental phosphate and nitrate diffusion rates.

It is noted that there are several different ways in which nutrients may move across membranes. Although the pores in the polycarbonate membrane likely facilitated the diffusion of nitrate and phosphate through this barrier, diffusion through the material itself may have also been simultaneously contributing to the movement of these nutrients. Another potential factor with regard to the membrane material that may have affected diffusion rates is membrane charging. Membranes may contain fixed charges within them that act to encourage the passage of oppositely charged ions and hinder the passage of ions with the same sign of charge. For example, fixed negative charges in sulfonated polystyrene easily allow for the permeation of positive ions. However, the diffusion of negatively charged ions is more difficult as the fixed negative charges in the membrane tend to repel the negatively charged ions. With regard to the project experiment, it seems possible that this had a role in the nutrient diffusion trends observed. The polycarbonate exhibited the accumulation of static electricity and clung to nearby surfaces. It seems likely that this material then had some net charge on its surface during the experiment.

It is also believed that, since linear rates of diffusion were seen, this could be the result of limited nutrient permeability of the polycarbonate membranes. Perhaps some maximum rate of nutrient passage through the membranes was met for each set of experimental conditions. This might explain why the diffusion rates showed no acceleration, but only a constant, linear rate. Additionally, the current applied from the water pump may have dominated the diffusion process. The rate at which the current could move nutrients throughout the system is much greater than the rate at which these nutrients would naturally diffuse through stagnant water. As well, the current was not altered during the experiment. Thus, this assisted diffusion rate may have overruled natural diffusion rates in the overall motion of the nutrients, and, because the current was remained constant, kept a steady supply of nutrients flowing out of Section A and into Section C of the tank.

Unfortunately, finding the precise factors that account for the unpredicted linear trend in the nitrate and phosphate diffusion rates is outside the scope of this project. However, it seems that there is a balance within these diffusion systems whereby the rate of nutrient diffusion showed no change over the experimental trials. With the numerous factors that affect the diffusion of ions across membranes, it is easy to see how the rate of these processes may be influenced by the system's characteristics. The process of pulling samples from only 3 distinct points in the tank did not allow for a complete picture of

nutrient movement. However, this proved to be an acceptable method for gaining a relative idea of the concentration changes in phosphate and nitrate in various tank sections over time, especially under constraints of limited time, funds, and TNT test vials with which to conduct this experiment.

3.0 Recommendations for Further Investigation

Well understood behavior of microalgae cells and polymer membranes suggested that their incorporation into the proposed system should be able to create a sort of living filter for excessive nutrients within water bodies. However, no previous research was found on systems that were entirely comparable to this application. Thus, this project was, in many aspects, focused on characterizing the general functionality of the system and the feasibility with which it could be successfully constructed. The yielded results now form a basis upon which the system's functionality may be further investigated. In this regard, it is fortunate that the design of the test model was successful in fulfilling several purposes intended for the proposed microalgae pouch. However, there are many variables and aspects inherent to this system that require further study in order to confirm the experimental results obtained in this project.

In regard to the membrane, other material options could be explored. The polycarbonate membrane performed its required functions quite well. However, its exceptional thinness and electrostatic properties were not entirely desirable. The other main system component, the chlorella cells, likewise performed their intended function, at least from what is suggested in the data. However, many of these cells fell to the bottom of the tank and likely were not in a position to intercept an appreciable quantity of passing nutrients. Thus, greater care should be taken in any further studies to ensure the health of the cells that are transferred into the tank. Agitating the cell population may also have increased the amount of nutrients the added chlorella were able to absorb over the course of the trial.

The concept of the microalgae pouch was simulated by section B of the tank. Because healthy cells tend to accumulate at the water's surface, different configurations of this simulated pouch could be developed that house a larger water surface area for the given volume enclosed within the pouch. Still less complex, an effective experiment to build on the results gained from this project would be to conduct multiple test trials with chlorella in the tank with different cell populations each trial. This would likely give a better representation of the amount of nutrients that could be absorbed within the system for a given concentration of microalgae cells. In addition to experimenting with different tank configurations, it is believed that a membrane pouch may be manufactured by heat welding the edges of two overlaid sheets. This would create a free standing pouch as opposed to that simulated with Section B in this project. The quality of these seals would provide another source of future investigation.

As discussed, many variables could have influenced the diffusion rates of phosphate and nitrate through the system. Running experiments under stagnant water conditions alone would be effective in eliminating some variables that may have obscured test results in this project. However, the expense of TNT test kits and lengthy amount of time required to process a large number of water samples still pose as obstacles to gaining an in depth representation of nutrient diffusion rates throughout the tank. One method to circumvent these challenges may be to use dyes instead of nutrients. The concentration of dye could be tested much more time and cost effectively with a spectrometer. By drawing water samples and using this piece of equipment to measure their light transmittance, the relative concentration of "nutrients" could be determined. Darker samples, of course, would be more "nutrient rich" and show less light transmittance at wavelengths corresponding to the color of the dye. Additionally, these water samples could then be drawn from many points along the length of the tank. This would likely allow for a well developed concentration profile to be made and would yield a better representation of diffusion patterns

throughout the system. The negative side to this approach would be the inability of microalgae cells to uptake the dye. Unless a colored microalgae nutrient could be used, testing the effectiveness of microalgae would not be possible. Though, this method would still be valuable in that more experimental trials could be conducted without added expense and over shorter periods of time. The small number of trials that were able to be performed in this project disallowed for the data to be analyzed through statistical tests. As these tests could help confirm the experimental findings of this project, it is highly encouraged that many trials are run in further experimentation of this system.

In regard to drawing water samples, it was difficult to consistently obtain samples from the same exact point within each section. To overcome this source of experimental error, a device could be constructed above the tank that would hold the pipets in fixed and predetermined positions within the tank. Additionally, water samples would ideally be analyzed directly after they were obtained to rule out sample evaporation as a potential source of error in nutrient concentration measurements.

These proposed topics are in no way a definitive list of future studies that may be performed. The multiple facets involved in this system all have a capacity to be altered and further tested. However, it is believed that the positive results gained from this project justify further study of this system as a potential mitigation device for excessive loading in water bodies.

V. CONCLUSION

Both positive and negative results were gained in this study. In most aspects, the test model functioned as desired. However, some challenges were met in constructing the experimental model. These were primarily relevant to the polycarbonate membrane selected. Additionally, modelling the diffusion of nitrate and phosphate throughout the tank proved cumbersome. Cost and time constraints contributed to these two challenges. These constraints in turn reduced the number of trials that could be feasibly conducted in the project and disallowed the use of statistical tests in interpreting acquired nutrient concentration data. Nonetheless, the overall perfomance of the test model was encouraging. Through this project, the following positive conclusions were drawn:

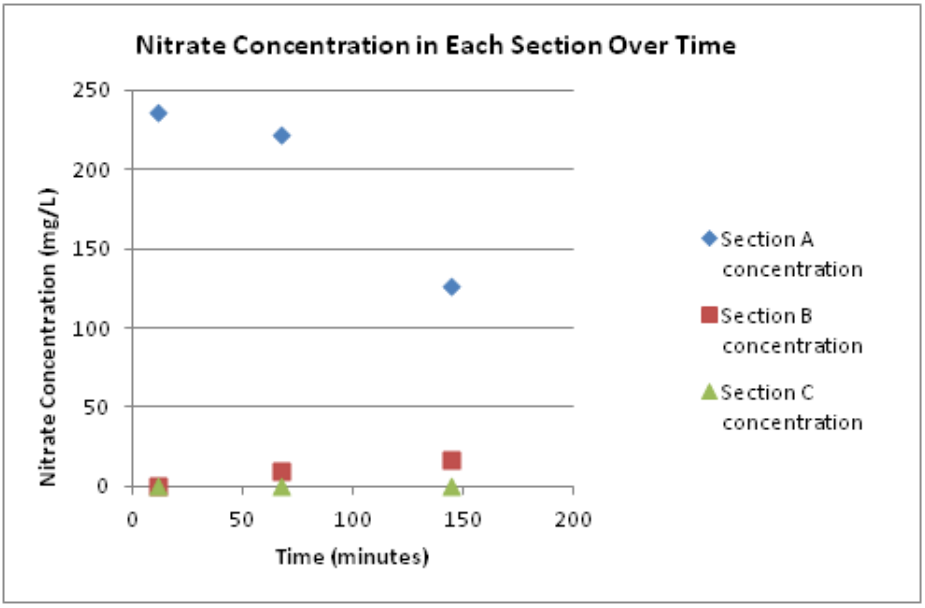
1) the polycarbonate membrane successfully housed chlorella vulgaris cells
2) the polycarbonate menbrane succcessfully allowed for diffusion of nitrate and phosphate
3) added chlorella vulgaris cells were to some degree able to adapt and grow within the tank
4) added chlorella vulgaris cells appeared to absorb some of the nitrate and phosphate diffusing through the tank

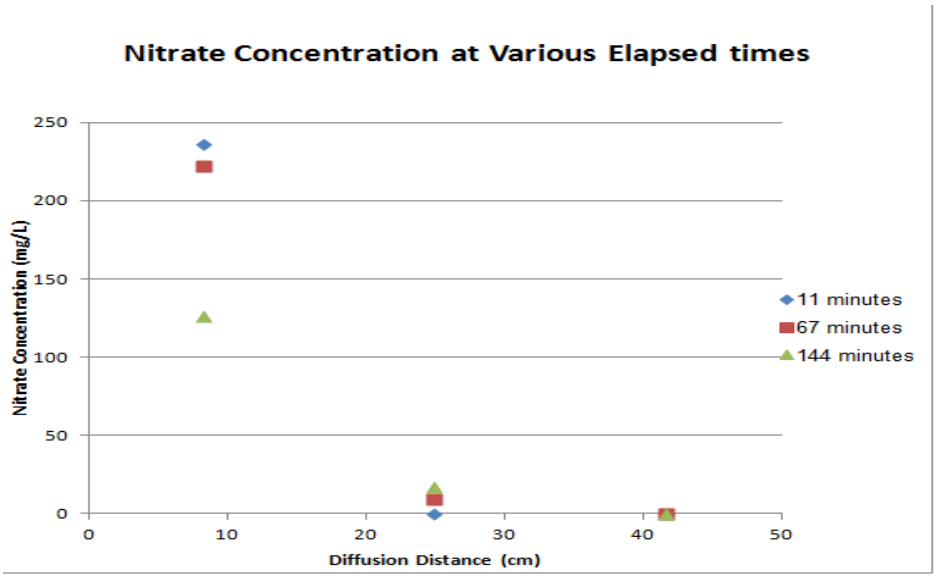
Because of these positive results, it is appears that the introduction of these microalgae pouhces into eutrophic water systems would be able to mitigate excessive levels of nutrients and potentially reduce the ill effects of eutrophication.

APPENDIX A

Preliminary Water Sample Concentration Results

This graph expresses the concentration of nitrate over time. Each data plot represents a different section of the tank. The pump was activated for the entirety of this test, and chlorella was not added to the tank. As shown, an appreciable amount of nitrate was allowed to diffuse into Section B over the elapsed time of this trial. However, a final concentration of 0.115 mg/L was observed in Section C. This was below the readable spectrophotometer range. Thus, it was concluded that a larger elapsed time would be needed in the official experimental trials to yield accurate data. In the following graph, nitrate concentration was plotted againse distance to create a diffusion profile for several different elapsed times. It was noted that these plots demonstrated more nonlinear trends than were shown in the official experimental trials.





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