Light Configuration in Algal Photobioreactor for Wastewater Treatment

Margot Amitrano [redacted]
Elizabeth Filine [redacted]

McGill University
Fall 2018

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Abstract

A photobioreactor design for lab-scale algal growth and wastewater treatment is presented. The aim of the project is to provide researchers with an easy-to-use algal photobioreactor which efficiently removes nutrients found in tertiary streams of wastewater. It is expected that the design can be used for biomass production, harvest and post-production research, and wastewater treatment studies. Lighting is currently one of the major drawbacks of bioreactor efficiency; therefore, this system strives to improve the lighting configuration of the photobioreactor to enhance biomass productivity. The conceptual system was tested using a series of computational models to understand the reactor's parameters and their effects on reactor efficiency. In comparison with existing research, this design is predicted to be efficient at nutrient removal, but its biomass production is limited and does not reach that of existing designs. It is also expected to be a more affordable system, when compared to other lab-scale equipment, especially when considering manufacturing costs. A preliminary analysis of environmental impacts of the design was conducted to determine impact categories for the life of the system. A discussion of recommendations is included to specify improvements, limitations and implementation of the proposed system.

Introduction

The Macdonald campus of McGill University is home to the faculty of Agricultural and Environmental Sciences, where undergraduate and graduate-level degree programs are offered in fields of study relating to agricultural, nutritional, environmental and sustainable sciences. The department of Bioresource Engineering is located on this campus and encompasses an array of engineering disciplines. Bhalamurugan Loganathan is a graduate student pursuing his Ph.D. in Bioresource Engineering with a focus on the valorization of wastewater. Our team approached Mr. Loganathan with the idea of working on a design project focusing on the decontamination of wastewater through the use of algal photobioreactors. Mr. Loganathan found the idea of a capstone project, which focuses on similar work as his graduate project, appealing, and he became involved as the project's representative client.

As our team's client, Mr. Loganathan provided us with information to help guide this project from the start. He also provided us with some technical information required for this project. He explained that his research work involved the algal strain *Scenedesmus obliquus,* and that most research laboratories typically work with this strain since there is already a strong knowledge base for its properties in the literature. He also pointed out the need, in the photobioreactor industry, for a system which can homogenize the algal growth solution so that the cells receive an even distribution of light. Lack of sufficient lighting and photoinhibition are currently part of the main issues with photobioreactors because the microalgae tend to grow closer to the light source. This can form a biofilm, which then leads to the death of the cells inside that are no longer receiving light. This can lead to clogging of the instrument, as the algae grow only in the area of the light source.

With Mr. Loganathan's help, our team was able to clearly define a need, and from it, a project proposal that aims to improve the light configuration efficiency in photobioreactors.

Currently, 80% of wastewater is globally being released into the environment without receiving any prior treatment (UN Water, 2018; UN Water, 2017). In high income countries, 30% of the wastewater is not treated before discharge (UN Water, 2017). Still, the treatment this wastewater receives can sometimes be insufficient. This leads to harmful contaminants entering ecosystems, which can be fatal to the present wildlife. One of the extreme consequences of contaminated water being released to an ecosystem is eutrophication. Eutrophication is the over-fertilization of the water, which promotes excessive plant growth and habitat imbalances, resulting in the deaths of certain species. It is mainly caused by nutrients, especially nitrogen and phosphorus (Government of Canada, 2014).

In order to deal with the increasing impact of wastewater on ecosystems, several solutions have been developed to better control its release. Wastewater treatment plants have been the main solution to this issue for several decades. Decontamination is usually carried out in three different processes. Primary treatment is the settling of solids by sedimentation. Secondary treatment is the removal of organic and suspended solid through the use of microorganisms in aerobic conditions. Tertiary treatment is the removal of specific wastewater compounds that could not be removed in the previous steps, which can be done through filtration, flocculation, and disinfection through the use of ozone gas, chlorine or ultraviolet light (FAO, 1992).

However, the government of Canada admits that wastewater treatment plants have not been entirely successful in treating contaminated water. It has been shown that the insufficient wastewater treatment has had both environmental and health consequences, especially harming wildlife populations (Government of Canada, 2014). With the increasing population and increasing barriers to accessing clean water, there is a stronger need for wastewater decontamination, and this has put a greater stress on the industry. Microbial indicators are commonly detected in treated wastewater effluent. It is estimated that 88% of diarrheal diseases as well as 1.7 million deaths worldwide are the direct results of unsafe water (Naidoo & Olaniran, 2013). It is clear that existing methods have not been sufficient at properly treating wastewater, and it is becoming crucial to develop more advanced methods.

Our design team has been striving to develop a system that would benefit the wastewater treatment industry. There is research currently being done on the use of algae to remove nutrients from wastewater. Algal bioreactors are usually used for tertiary treatment, and appear to have a potential to improve current techniques. At this tertiary stage, the large majority of substances contaminating the wastewater have been removed and all that remains should be nutrients, heavy metals and microorganisms. The use of algae for nutrient uptake has long been studied and implemented at this stage. Our team has been working on improving an existing photobioreactor by focusing on light configuration to improve algal efficiency. The main design is centered on a lab-scale photobioreactor, although the team has been considering ways of scaling up the design so it could be implemented in the industry as well.

1.1 Vision Statement

The vision of the team is to optimize photobioreactor conditions and parameters to increase nutrient removal efficiency on a small-scale wastewater setting using microorganisms for biomass and breakdown of contaminants.

2.0. Customer Needs Assessment

As Mr. Loganathan mentioned to us early on in the project, he was not thinking of using a photobioreactor for his work in the future. However, since his work focuses on the optimization of algal biomass conversion to secondary products, he was still interested in working with us on this project as it is directly related to the step prior to his work. He also let us know that he believed it was an interesting concept with a lot of potential that still requires many improvements. Since he was not hoping to use our product, he did not provide very specific guidelines as to how our team should approach this problem. However, he did provide a few recommendations to help our research run more efficiently along the way, which have been combined into Fig. 1.

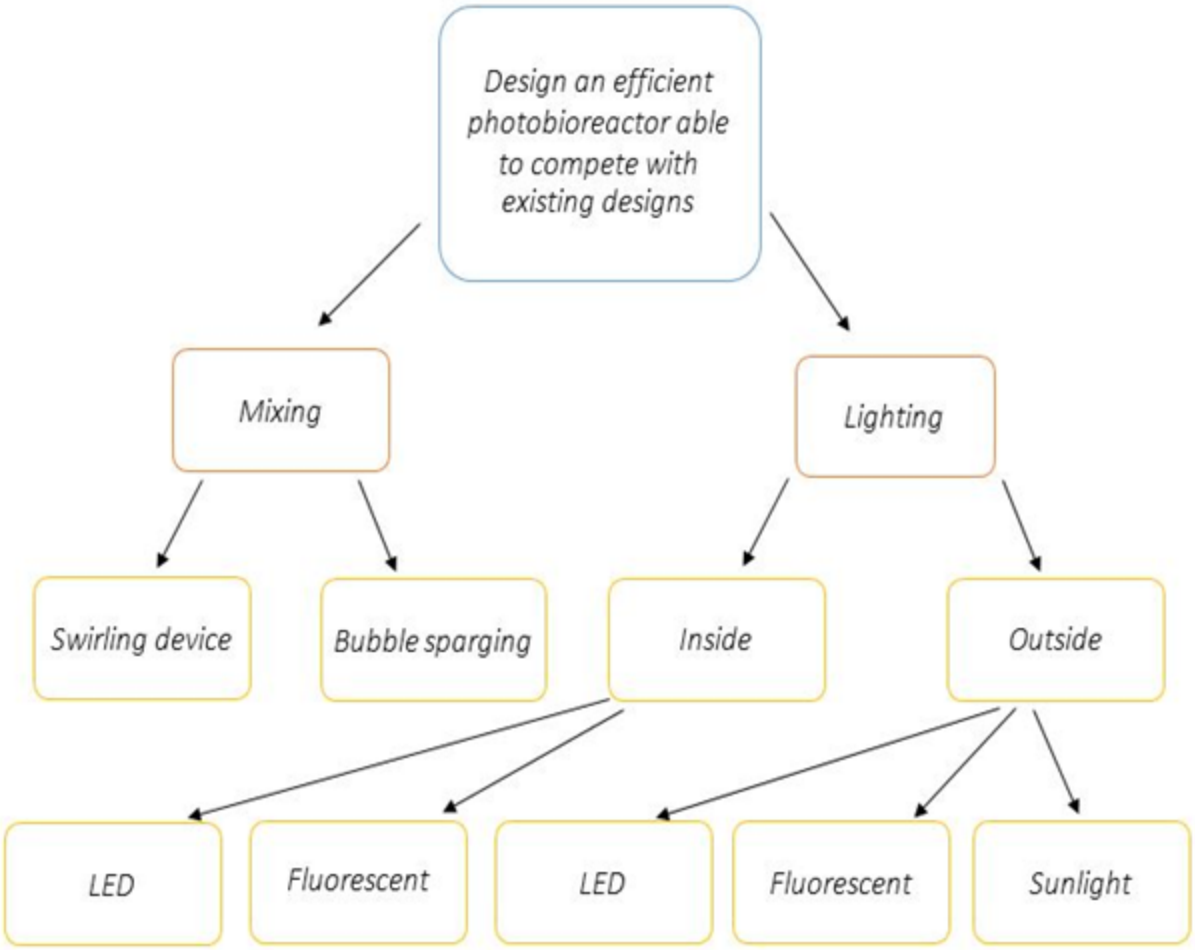

Figure 1. Decision flow chart based on client recommendations. Legend: Blue contour – Goal; Red contour – Criteria; Yellow contour – Alternatives.

Our client was very specific in letting us know that the most important components of the photobioreactor that need improvements are the mixing and lighting systems. From his advice, we decided to focus on these two main aspects of the design. We later conducted a thorough literature review to analyze the different alternatives of each component, see section 5.0 of the report for more details. Another important consideration our client pointed out for us was that we should focus on studying the algal strain *Scenedesmus obliquus*. This strain is widely studied in the scientific community, including by our client himself, which would allow us to gather required data efficiently. This strain is also very efficient at biomass assimilation (Shen et al., 2015), making it a strong candidate for biomass production in our proposed photobioreactor design. In fact, *S. obliquus* has been determined to have a photosynthetic efficiency of up to 5% in CO2 supplemented environments (de Marchin et al., 2015), which is much higher than the approximated 2% for terrestrial plants (Anyanwu et al., 2018). Additionally, the client suggested that we do not focus on the light-dark cycle lighting systems, as those were not as efficient for biomass production for short-term batch growth. From these recommendations by Mr. Loganathan, we conducted our own research to propose a design that would satisfy his needs and potentially the industry. His preferences were taken into account throughout the design consideration, and allowed us to work more efficiently.

A variety of bioreactor designs already exist on the market, each with different benefits and advantages. Selecting the appropriate design often depends on the purpose and the scale it will be used for. A difficulty also associated with bioreactors is the wide range of requirements that varies with the strain or species being grown. This aspect makes it more difficult to be able to find a standard design to work with.

However, one of the biggest challenges that the industry is facing is the unviable cost associated with bioreactors. This cost comes from both the manufacturing side as well as the operating part of the bioreactor since they are very energy intensive, and often do not produce enough biomass to justify this cost. While benchtop photobioreactors for research purposes have a small market, many companies have been unsuccessful at using bioreactors for commercial profit in the biomass industry (Gendy & El-Temtamy, 2013). In fact, Mr. Loganathan guided us to Dr. Lefsrud who has previously worked on designing and building a bioreactor, along with Dr. Lyew. They were able to provide us with the data they had collected for the cost of manufacturing their design and the final estimation was around 14,000 CAD for a 1.5L bioreactor.

We decided that since we were mainly focusing on designing a photobioreactor for laboratory and research purposes, we were going to try to reduce the costs of our design as much as possible to make it accessible to more researchers. The data that Dr. Lyew provided was later used to compare our own data to determine the cost efficiency and the cost benefits of our design. We also realized that most of the operating costs for bioreactors were high due to the energy required to power them, so we focused on making the bioreactor more energy efficient in our

design process. Doing so would be beneficial both for the economic and environmental aspects of our design.

3.0 Revised Needs Statement and Target Specifications

3.1 Client Recommendations

Mr. Loganathan has expressed his willingness to oversee the project in a way which allows for flexibility in design and constraints. As a PhD candidate, he cannot rely on designs from a year-long design process, nor is he able to directly use the product in his own research due to his own tight project time constraints and needs. However, having been in the algal research field for several years, our client has shared limitations in the field, and current challenges with algal growth conditions on the lab scale. According to Mr. Loganathan, most lab-scale experiments utilize previously-established optimized conditions for algae growth, but experiments are often conducted on shaker tables in flasks. While this is suitable for small-scale experiments, if a large volume of biomass must be obtained, a photobioreactor with a controlled environment can be advantageous. This is especially true for researchers who study biomass processing and harvesting, which includes our client.

The original criteria were established at the beginning of the project for the design of the photobioreactor for algal growth.

- **Affordability***:* First, Mr. Loganathan expressed that lab equipment, including photobioreactors, tends to be expensive and unaffordable for laboratories that do not specialize solely on biomass harvesting or post-processing. He established that a successful design would result in consistent, reliable productivities while keeping the costs of manufacture, maintenance and operation at a minimum.
- **Minimization of Clogging*:*** Second, Mr. Loganathan explained that because of algae's tendency to aggregate near light sources, one challenge to solve would be to minimize aggregation by establishing mixing methods, as well as proper light distribution throughout the reactor. A related challenge is the relatively quick decline in productivity from a decrease in light intensity due to an initial increase in turbidity from growth. This can be addressed through gentle mixing and proper light distribution.
- **Nutrient Removal***:* Third, the reactor must be effective at decreasing typical nutrient concentration profiles in a typical, lab-standard solution representing the average chemical makeup of fresh tertiary stage wastewater.
- **Ergonomic***:* Fourth*,* the reactor must be easy to use in the lab without excessive energy or time inputs. It must be easy to sterilize, safe to use, and easy to clean off algal and nutrient residues.

In addition to these original four client specifications, the design must adhere to standards for bioreactor design, considering safety factors, as well as performance indicators which must be addressed and optimized to ensure a successful design.

3.2 Minimum Design Standards

Although the design is not associated with high biohazard risk due to the non-toxic nature of the algae being cultivated, there are several standards of quality and safety which must be considered and observed to ensure the design's compliance with minimum design standards for laboratory equipment. The design must not pose high risk of electrocution, contamination, physical injury or other damage; additionally, in case of major damage, there must be failure systems in place to ensure minimization of damage. The World Health Organization (WHO) has summarized the minimum design standards of the International Standard Organization (ISO) in its Laboratory Quality Standards and their Implementation report (WHO, 2011).

For laboratory equipment WHO has set the following relevant technical guidelines which are relevant to the the design and operation of a photobioreactor:

(1) The equipment must have access to uninterrupted power supply, and have control systems for temperature, etc. Additionally, decommissioning instructions must be included for all systems.
(2) The design must not interfere with any existing lab safety protocols. In this system, for instance, the reactor cannot be positioned in any way which impedes escape routes, can cause spillage, or can cause any harm to lab users or technicians. Furthermore, the use of CO_2 for the sparge system requires handling high pressure gas in the lab setting. Therefore, the setup must not interfere with existing protocols, including the securement of the gas tanks with an appropriate belt at all times, as well as safety features in the design which minimize the risk of the sparge system's failure.
(3) The reactor system must be properly labelled, and come with an operation and maintenance manual. All lab protocols for the recording of use, maintenance dates and problems must be observed with the same rigor as other laboratory equipment. The system must also be adequate for general lab inspections for overall safety conditions in the workspace.

3.3 Performance Indicators

Basic performance indicators that can be monitored throughout operation to ensure proper functioning of the reactor include passing laboratory safety inspections, and fulfilling basic criteria which are required for optimal growth conditions. All research facilities and institutions including McGill University have Environment, Health and Safety (EHS) divisions which conduct routine laboratory inspections to ensure all practices and equipment meet safety guidelines set by that institution. These guidelines are mostly based off other standards and guidelines such as those set

by ISO and the World Health Organization. Examples of passing criteria for the reactor then, include safety measures against electrocution and pressure failure, as well as placement and operation within the lab which does not interfere with other lab safety requirements (placement, operation, power input, etc). (McGill EHS, 2017).

Measurement of effectiveness of the design can be validated with experimental tests, modelling, and the assurance of parameters which are required for all optimized growth settings. These parameters pertain mostly to general needs for photobioreactors. The purpose of this design is to serve as a photobioreactor on a small scale. However, since all lab-scale technology usually have industrial applications for larger-scale settings, scale-up considerations are briefly considered, though not thoroughly analyzed.

(1). Contained Environment. Most algal wastewater treatment approaches involve only High Rate Algal Pond (HRAP) systems or raceway ponds because of the relatively small energy input and ability to store large volumes of water (Hadiyanto et al., 2013). However, there are several drawbacks to these systems. First, contamination from bacteria, yeasts, protozoa, and other microalgae organisms results in a competitive environment, which can reduce the consistency and quantity of the microalgae harvest (Kligerman et al., 2015). Although some researchers have found that using a bacteria-algae consortium can be helpful for nutrient balance, post-processing steps as well as sanitation methods of the water after treatment can prove challenging as well (Wang et al., 2016). By controlling the system for pH levels, carbon sources, temperature and light frequency and intensity, these challenges can be avoided. Additionally, since HRAP's are only useful and effective in regions of the world where there are consistent light/dark cycles and temperatures around 25-30°C, an open pond system would not be feasible in Canada or many other regions of the world (Grönlund & Fröling, 2014).

(2). Light Distribution. The nature of the system must maximize light distribution within the reactor in order to decrease the growth plateau often seen in populations experiencing rapid growth. The limiting factor for growth in a wastewater treatment setting must be the nutrients, not the other conditions, since the aim of wastewater treatment is to remove potentially harmful contaminants that can cause ecological or health risks. Therefore, the system must, to as great an extent as possible, provide equal light intensities to each part of the total volume. This can be limited by practical factors, including the ease of cleaning light fixtures, wiring and material costs and volumes. Thus, light distribution is an optimization problem which must be solved in an innovative way by considering ergonomics, economics and algal growth. Additionally, ideal wavelengths of light for optimal growth, reducing overheating from lighting elements, and other specifications should be considered to save inputs and maximize productivity.

(3). Gentle Mixing. Like lighting, the mixing system must provide a way to circulate nutrients and algae to provide an even distribution of algae, as well as to reduce clogging effects caused by algae's natural tendency to aggregate near the light source (Ugwu et al., 2008). However, it is important to consider that highly turbulent fluid conditions would also inhibit algal growth, and

possibly even cause cell death (Molina et al., 2002). In natural settings, algae are most commonly found in stagnant, warm waters with high nutrient levels and light exposure (Paerl et al., 2001). While a photobioreactor has distinctively different properties than a natural pond ecosystem, the necessary requirements for life and reproduction of the cell must be met.

(4). Scalability. Although the design is aimed to fulfill laboratory needs, it is crucial to consider the effects that scale-up would have on the efficiency and impact of the design. For this reason, when selecting materials, some decisions may have to be made that would not necessarily have the smallest impact financially and environmentally in a laboratory, but would be required upon scalability. For instance, while using some recycled, post-consumer products such as water jugs may be appealing on a lab scale due to the small financial investment and repurposing capability, using recycled or repurposed plastic would not be feasible financially or environmentally on an industrial scale.

4.0 External Search

4.1. Literature Review

4.1.2 Photobioreactor Designs

With algae's potential for biofuel production and its ability to be transformed into other useful products, extensive research has already been performed on this topic. Bioreactors are closed system vessels with controlled environment used to grow living organisms, usually for the production of a derived product (Bhatia and Bera, 2015). Being able to better control the medium allows the user to manage the system's parameters, such as temperature, pH, oxygen and carbon dioxide. These parameters have effects on the growth kinetics and the biomass productivity of the microorganisms in the system (Najafpour, 2007). Photobioreactors have been extensively studied since the 1950s, and there are a variety of different designs already available on the market; however, these designs have limitations regarding productivity. The three main characteristics of photobioreactors that affect their efficiencies are light distribution, mixing, and pH (or dissolved CO_2 availability) (Huang et al., 2017). Existing designs have strived to improve each of these factors for an optimal biomass productivity.

In comparison to bioreactors, open pond systems are also used for treatment and algal biomass production, but the systems are difficult to control because of their open-system nature. This can make it difficult to obtain a desired organism growth rate or productivity. Still, open ponds currently remain the most industrially applied system because of their low cost (Costa and de Morais, 2014). This highlights the high and inefficient cost of bioreactors. The volumetric productivity in photobioreactors is larger than that in open pond systems (Posten, 2009), but their theoretical maximum productivity is never achieved. Additionally, their high capital costs and

operational costs caused by their energy consumption have made competition with open pond systems difficult. Photobioreactors have a strong potential, however, as they are able to reach a volumetric productivity that is on average 13 times higher than that of open ponds. and only require about two thirds of the space open ponds need to occupy (Chisti, 2007). They have a good potential for the biomass industry, which has led to an increase in research for the optimization of their output to input ratios, especially regarding the cost and energy requirements.

As more photobioreactor research is being performed, various designs have surfaced, each with their own specifications and performance rates. One of the most prominent photobioreactor is the bubble column design. In this model, the lighting is at the exterior of a transparent column made of glass or plastic. An aeration device is inserted into the bottom of the system to provide adequate mixing by pushing air bubbles through the medium. This aeration mechanism is also a way to provide carbon dioxide required for microorganism photosynthesis. This design is advantageous due to its relatively low cost and its large surface area, which results in high lighting efficiency. However, it is limited by its space requirements and photoinhibition effects, caused by excess light which damages the microorganisms' photosystem II and reduces photosynthetic rates (Patyna and Witczak, 2016). A strong lighting control for this design is therefore required.

The airlift photobioreactor is a recent design that has gained attention for its productivity potential. It is composed of two different sections: one with the gas inlet receiving the mixing, and the other with no mixing occurring (Patyna and Witczak, 2016). This design is growing in popularity because of its good mixing rates and photosynthetic efficiency. However, this is only true at low biomass concentrations; the mixing and growth becomes inefficient once the cell density is too high (Al-Mashhadani et al., 2015). In this case, the lighting system is also on the outside of the bioreactor because of the interior sectioning and mixing requirements. Research for scaling up this design is being conducted because of its productivity at a small-scale, but it is experiencing difficulties being implemented for large-scale applications.

Panel photobioreactors' defining characteristic is that they are able to minimize the light path, or light penetration. Since they are very thin, the light can reach the entire system, which means photosynthesis and light use tend towards the system's theoretical efficiency. However, to attain a significant volume of biomass, this requires more space. Scaling up this design is difficult and the cost evolves linearly, as it requires multiple identical units. This design reaches optimal mixing using bubble or gas sparging at the bottom of the panel (Patyna and Witczak, 2016). This system also enables efficient excess gas removal to prevent oxygen accumulation. It also appears to be relatively inexpensive to manufacture and easy to maintain, which should also lower running costs (Ugwu et al., 2008).

The shape of tubular and spiral photobioreactors is also unique, with the aim of increasing photosynthetic efficiency. They consist of multiple loop- or spiral-shaped pipes, so that they can

be placed directionally towards the light source, allowing the microorganisms to efficiently use the light they receive. This design also uses a gas exchange system for carbon dioxide input and oxygen removal. The issue with this design is mainly its high energy consumption, and therefore running costs (Patyna and Witczak, 2016). It also has fouling effects, and could be prone to biofilm formation on its wall surfaces, which would reduce lighting and photosynthetic efficiency (Ugwu et al., 2008).

Tank photobioreactors are often used in wastewater treatment. This is mostly because of the conventional design with typical mechanical mixing at the bottom of the tank and light penetrating the system from the outside. The gas sparging is efficient, as the bubbles can enter from under the agitation device, so they will be uniformly distributed inside the bioreactor. This ensures both carbon dioxide assimilation and adequate mixing. Although the shape allows for efficient mixing, it also reduces the light distribution in the system, as it is usually too deep for the light to reach the microorganisms in the center of the tank (Patyna and Witczak, 2016; Luangpipat, 2013).

Photobioreactors have developed over the past few decades, and improvements have been made to optimize their parameters. Even though their biomass productivity is quite high, at around 1.5 kg/m^3 per day (Chisti, 2007), they are expensive and difficult to use on an industrial scale. The average biomass productivity can also vary depending on the system, its parameters, and even the microorganisms used (Majid et al., 2014). The most important characteristic of a photobioreactor is its light distribution, since it determines the photosynthetic efficiency of the system. The mixing of the microorganisms is usually a good way to efficiently expose them to light, provided an adequate light path exists. The gas inlet and outlet are also major components of the design, as carbon dioxide is required for photosynthesis, and the presence of oxygen could eventually cause photooxidation. Thus, there is still crucial work that remains for photobioreactor development. It is necessary for systems to incorporate each of these parameters effectively to increase biomass yield and decrease running costs. Photobioreactors have a potential for efficient biomass and biofuel production, and could compete with fossil fuel production in the future.

4.1.2 Lighting Characteristics and Requirements

The lighting system used in photobioreactors must be closely monitored to satisfy light requirements for optimal growth. While photosynthesis requires a minimum light penetration, photosynthetic efficiency may also be limited by excess light because of photo-inhibiting effects (de Mooij et al., 2016). There are many aspects of the light system that affect efficiency, and it is necessary to look in-depth at each of these as this is currently one of the limiting factors to reach theoretical efficiency.

One of the reasons algae has such a high potential for biofuel production and why it is regarded as a good fuel alternative is because it can reach up to 80% of its theoretical maximum

photosynthetic efficiency, even in low light intensities (de Mooij et al., 2016). To further enhance productivity, light wavelengths should be examined for lighting optimization. Most algal strains used in photobioreactors have similar requirements. In general, algae absorbs light photons through green pigments, or chlorophylls located inside the chloroplasts of a cell. The pigment has an optimal absorption rate for photons of wavelengths of 450-475 nm and 630-675 nm.

However, in a light color absorption optimization study for algal photobioreactor efficiency, de Mooij et al. (2016) found that the color yellow (570-590 nm), is optimal for algae absorption and assimilation. Other studies show that using red lights (650-700 nm) for photosynthesis results in a more efficient growth rate because chlorophylls absorb those wavelengths better (Carvalho et al., 2011).

The system's irradiance is also a major factor because it directly affects the quantity of photons received by the chlorophylls of the cell, and therefore the rate of photosynthesis. Irradiance is more difficult to control in the lighting system because it depends on cell density, the light path and the algal strain. The benefit of using algae in photobioreactors is their photosynthetic efficiency, which is around 5-11% compared to 1-2% for terrestrial plants. This photosynthetic efficiency for algae could theoretically reach over 25% if all the conditions were optimal for the strain (Gutierrez-Wing et al., 2014). Irradiance is also affected by the light path, or the depth of the medium (usually water), that the light has to reach. The irradiance does not decrease linearly with depth, however, as there is a stronger light attenuation in the first few centimeters of the light reaching the algal culture. Various models have been created to study the effect of the light path on the irradiance, as well as the effect of irradiance on photosynthetic efficiency. These can be useful in the optimization of a photobioreactor design for enhanced photosynthesis and algal proliferation.

Similar to light path, cell density has an impact on the irradiance available along that path. A high irradiance will provide sufficient photon availability for continued algal growth when there is already a high cell density, but would potentially lead to photoinhibition during the initial phases of the system. This leads to the possibility of evolving the light intensity with time as cell density increases. Another possibility would be to expose the algal cells to the light intensity for a shorter period of time. Pulsating lighting systems, creating short light/dark cycles, have been previously researched, with various frequencies addressing light intensity issues, although their efficiencies can also be counter-productive for cell proliferation. Models also exist to characterize the increase of irradiance needs with the increase in cell density or turbidity, but these can be difficult to generalize, since irradiance requirements may vary with the algal strain (Gutierrez-Wing et al., 2014).

Since light requirements differ with the strain of algae used, it is necessary here to look further into the optimization of light for *Scenedesmus obliquus*. A study by Sforza et al. (2014)

looks in depth at the irradiance requirements of this strain and provides an overview of how to meet its demands using photobioreactors. It appears that the optimal irradiance for *Scenedesmus obliquus* is around 150 $\mu mol.m^{-2}.s^{-1}$, or approximately 32 $W.m^{-2}$. This light intensity provides the most efficient results for the specific growth rate of algae. This value is only accurate for batch photobioreactor systems, whereas continuous photobioreactors would require about twice this light intensity. The lipid content is not highest for this irradiance, but still remains at 42% of dry weight. This study also explores the effect of pulsed light on this algal strain, and it appears to have led to a retarded algal growth (Sforza et al., 2014). It would therefore be best for this strain to monitor the cell density increase with time and potentially adjust the light intensity with it.

Since the purpose of photobioreactors is to grow microorganisms for a future purpose, it is possible to adjust the parameters inside the photobioreactor to meet the demands of the industry for which the algae is grown. For biofuel production, the industry requires a high oil content, which can be obtained from the lipid or fatty acids content of the microalgae. Various studies have shown the evolution of lipid content with light intensity; however, lipid content may depend on other factors in the photobioreactor environment such as pH (or CO_2 availability) and temperature. It has been shown that the combination of a low light intensity system with a high pH could risk having a low lipid content in the output biomass (Cuellar-Bermudez et al., 2015). Different strains and species of microalgae behave differently under light intensities, as they will have different requirements and possibly different methods of using light energy. It appears that for *Scenedesmus spp*., a gradual increase in light intensity results in a similar increase in biomass productivity. However, it is important to note at which light intensity the strain could potentially induce photoinhibition, since this would decrease biomass productivity, and therefore the lipid content. It is crucial to keep in mind the optimal light intensity range for biomass productivity, as this increases the overall components in the cell. It is also interesting to look deeper in that range, given that the lipid content increases with increasing optimal light intensity. It has also been reported that high irradiance towards the end of the process can increase lipid content inside the cell (Diffusa et al., 2015). These various techniques could be beneficial for the biofuel industry.

4.2 Benchmarking

4.2.1 Commercial Designs

The design of the photobioreactor for the client must be comparable in performance parameters and productivities in order to be considered as research-grade equipment. Table 1 summarizes select specifications of five different commercial models, including reactor type, efficiency, lighting, mixing and others. Several existing models include a variety of sensor systems, as well as tunable settings. While this makes the system more flexible for a variety of applications, the addition of many different sensors, timers and other instrumentation features can make each reactor expensive. Since the objective for the client's needs is to create an inexpensive alternative, some of the features included in commercial products will be omitted, while

maintaining some of the control and tunability for optimization purposes. One of the most common lab-scale algae photobioreactors is known as the Elara Bench top reactor, which is based on the flat panel design, but can also be incorporated in a vessel-type reactor. Flat panel reactors, while efficient on small scales, can be limited for long-term large scale due to maintenance and breakage issues (Yang, 2007). Additionally, flat panel reactors have large space requirements, and require constant outer lighting systems, which are two conditions that are not acceptable for this design context (Sledgers et al., 2011). Other reactors generally have vessel type conformations with a variety of mixing and lighting systems, including bubble gas sparging, air lift, and internal and external lighting, respectively. Generally, designs of photobioreactors are diverse, and therefore, can be designed for the needs specified. The objective for this design is to optimize the light distribution in a similar way to a flat panel reactor, while maintaining a large volume relative to the material, in a vessel-style reactor. Therefore, an examination of commercially successful designs can help in the selection and design criteria of our own design.

Table 1. Comparison of specifications for five commercially available laboratory photobioreactors

System	Category	Size	Efficiency	Lighting	Mixing	Other	Source
Bodega Alga, LLC	Vessel	N/A	N/A	Internal solar lighting	Flue gas sparging	Modular/ stackable	Bodega Algae (n.d)
Subitec	Flat panel air lift	1.8x1.6x0.4 m 928 L capacity	Up to 3 g/L; 500 g/m2/day	High pressure sodium vapor lamp	Gassing membrane; pressure differential recirculation	Continuous or batch reaction	Subitec (2017).
Commercial Algae reactor	Vessel	100L	125- fold increase in in 7-10 days	N/A	N/A	Operating cost $46/batch	Commercial Algae (2015).
Elara Bench top reactor	Vessel or Flat Panel	4 L or 1.6 L	N/A	Dimmable LED system from 0-3000 umol photon/m2	CO2 addition:manual or pH based	N/A	Solaris (2018).
Qubit Systems	Controlled flow bubble	0.5x0.25x0.35 m (400 ml unit)	N.A	LED system: intensity and spectrum control	Controlled gas bubbling	Chlorophyll fluorescence monitor, 90-240 V	Quibit (2014).

5.0 Concept Generation and Selection

5.1. Design Options and Selection

Since the commercialization of photobioreactors in the 1950s, a variety of designs have reached the market. Each possibility has its own specific emphasis on parameters the inventor considered most important. Most designs focus on improving the light distribution and mixing efficiency, and they tend to approach the possibility of scaling up the system. Hybrids of these designs also exist, to benefit from both designs' strengths. The following Pugh chart was used to

analyze and weigh the benefits of existing designs, with a score of 1 to 5 being assigned for each system component (1 being very low and 5 being very high).

Table 2. A Pugh decision matrix was used for the determination of reactor category for the project. According to the scoring criteria, the bubble column and internal illumination have the most desirable traits for the design problem.

		Types of photobioreactors								
	Weight	Column	**Bubble column**	Airlift	Flat panel	Tubular horizontal	Spiral	Tank	**Internal illumination**	In series
Energy consumption	-4	1	1	1	1	3	1	1	1	5
Surface/Volume ratio	5	5	5	5	5	5	5	1	5	5
Mixing efficiency	4	5	5	5	5	5	5	5	5	5
Overall efficiency	4	1	5	5	5	5	5	5	5	N/A
Space required	-1	1	1	1	5	5	1	5	1	5
Ease of variable control (T, pH, O2, etc)	2	5	2	5	1	1	1	5	5	5
Cost	-3	1	1	5	2	2	1	1	2	5
Maintenance	-2	1	1	3	1	2	3	1	3	5
Scalability	1	5	5	1	1	5	5	5	5	5
Total Score		54	**64**	50	51	45	58	46	**63**	N/A

The lighting system of the design is mostly affected by the light path and light distribution. This can interpreted by the surface area to volume ratio of the bioreactor. A higher ratio generally corresponds to a better light distribution and shorter light path, and most existing designs aim to maintain a high surface area to volume ratio. The tank design, however, has a large cubic-like volume that does not allow for efficient penetration of light.

The mixing system of the design is another important factor to consider, since it is the only factor that ensures proper algal suspension in the solution. It is necessary for the nutrients to be homogenous within the bioreactor so that all microorganism cells have the same capacity to grow. Also, mixing is the principal factor that moves the microalgae around inside the bioreactor (cells have some autonomous mobility, but is considered negligible for this design). This allows them to receive equal amounts of light and nutrients. It also ensures that if the lighting system is not

optimal, each algal cell would still receive the same amount, and no cell would constantly be in the dark, or in low light intensity. This is also used to prevent the formation of biofilms, as that would completely prevent the light from penetrating the bioreactor. The usual mixing used is either mechanical mixing or air bubble sparging.

Factors such as space required, ease of variable control, maintenance and cost are important for the researcher using the design. These factors represent the investment the user would need to make, in manufacturing and labor costs. They also include the risks associated with the design and the ease of use for the workers. These considerations are important for both the company and its employees, as they indirectly affect the efficiency of the design. Designs with unique shapes, such as the tubular, spiral and panel photobioreactors, appear to have a more complicated use and would require more expertise for handling.

Using the Pugh matrix analysis, a hybrid design combining the two most efficient designs was chosen. The hybrid photobioreactor would then combine the benefits of both designs. This design should obtain a high mixing efficiency from the bubble column design, as well as a strong potential for scalability and an ease of variable control from the internal illumination system. The relatively simple parameter control inside the system could prove useful for the researchers using the design, as they would be able to vary those parameters according to their needs. Also, since the system has a high mixing efficiency, the biomass output should be quite high, as long as the lighting system is efficient.

Since this design includes internal illumination, there are few optimal systems available. The usual set-up is composed of light sticks, parallel to the length of the reactor. For this project, the team decided to opt for a unique shape for the design: a helical lighting system. This design will be mounted on rods to obtain the desired shape and to ensure ease of removal, for cleaning or maintenance. This shape will require more material, but it is also expected that the light distribution would be more efficient and that it would cover more volume inside the reactor.

5.2. Lighting System

The use of various lighting amounts to about 20% of the total electrical consumption in developed countries. This stresses the fact that light is very energy consuming. For a photobioreactor this will have cost implications, and it is therefore necessary to obtain the most cost-efficient and optimal lighting system for the algae. There are a variety of lighting options available for bioreactors: incandescent lighting, compact fluorescent lighting (CFL), light emitting diodes (LEDs) and natural sunlight.

The different lighting options vary significantly in efficiency, so it is important to understand the implications of each system. The light received by the algal chloroplasts can be measured by a physical unit called the lumen. The lumen is expressed in terms of sensitivity of the human eye, where peak sensitivity is achieved at a wavelength of 550 nm. The ideal efficiency of

a lighting system is reached when 683 lm/W is produced at 555 nm with no losses, and the lowest lighting efficiency would be 0 lm/W, for ultraviolet- or infrared-emitting systems.

The lighting systems currently available have relatively low lighting efficiencies. The incandescent light bulb is only able to reach an efficiency of 15 lm/W, while CFL efficiencies can reach 80 lm/W. Additionally, only about 5% of the electrical power consumed can be converted to visible light by incandescent lighting systems, and this is even less for emitting specific colors. The rest of the power consumed is emitted as heat. This could be an issue for photobioreactors, as it would either result in a loss of energy, or, if placed inside the medium, in an increase in temperature above the threshold required for optimal microorganism growth (Gayral, 2017). By comparison, the conversion of electrical power to visible light by CFLs is around 27%, significantly higher than that of incandescent light (Mills, 2004). LED systems are also known to be more efficient, which is one of the reasons they have grown in popularity over the past few decades.

Besides lighting efficiency, it is important to look at relative costs of each lighting system. In fact, LEDs are much more expensive than CFL which is also more expensive than incandescent, at the initial purchase. However, incandescent lights have a life-expectancy of about 2,000 hours, whereas CFL can last for 8,000 hours and LEDs can last for up to 25,000 hours. These variations in life-expectancies will have a significant impact on the running costs of the photobioreactor. Moreover, incandescent light bulbs would require 100 W of electrical power and CFL would require 25 W, whereas LEDs would only require about 13.5 W. Even though electrical power is not very expensive in Quebec, this would still impact the running costs of the photobioreactor. In the end, the LED system would be the most cost-efficient for a photobioreactor. Although LEDs' predicted life expectancies are generally over their actual life-expectancies, if the LED system only lasts for a fifth of the expected life expectancy, it would have about the same cost-efficiency of a CFL system (Gayral, 2017).

The impact of the lighting system on the environment is also a major concern when deciding which one to use, so it can prove useful to look at each life assessment. An important factor to take into account is the energy consumption, and it has been observed that incandescent lighting systems are very inefficient for this reason (Sangwan et al., 2014). In fact, incandescent light bulbs have even been banned in some countries, including those in the European Union, in order to promote energy savings (European Commission, 2008). LED systems are not much more environmentally-friendly than CFL systems. However, the main difference between both systems is that CFL bulbs contain mercury, a hazardous material if the bulbs are broken, and this requires proper recycling (Gayral, 2017). Thus, LED systems appear to be the better lighting system when taking into account environmental considerations.

A final lighting option is sunlight. A significant number of photobioreactors use the sun as their source of light. The main reason for this is to drive the overall costs down, both for manufacturing and running costs. This system would also be very environmentally friendly since there is no material or energy use. However, the use of sunlight for photobioreactor lighting also

has many disadvantages. Firstly, this would require the photobioreactor to be placed outside, which makes it prone to climate conditions. Also, there would be no photosynthesis and therefore biomass growth during the night. This is a major productivity loss for the system. Finally, it implies that there would be no control on the lighting system, which is risky since lighting is one of the main factors affecting photobioreactor efficiency and biomass productivity. These major drawbacks have very strong implications, and the benefits that would come from using sunlight were considered to be insufficient for the design.

LEDs appear to be the most efficient lighting system overall, and they have many other advantages that would benefit the photobioreactor greatly. In fact, using LEDs allows for good color control; the system can have almost a single wavelength output. This would increase the efficiency of the photobioreactor since algal strains have optimal rates of photosynthesis at certain ranges of wavelengths. It is also possible to change the brightness level of the system easily, which is not the case for CFLs. This would be useful for our design, since there will need to be an increase in light intensity with increasing cell density. LEDs are a cold light source, and will not emit heat, so they should not affect the temperature inside the bioreactor. They are also said to have a better light distribution efficiency; this will also increase productivity (Mills, 2004). Finally, they have the best power conversion efficiency and life-expectancy. So, they will reduce the overall cost of the system while increasing the biomass productivity, which is why they were chosen for the light source of this design.

5.3. Outer Structure

The outer structure of the design represents the bulk of the material used in this system. It was therefore necessary to choose a strong, sustainable and cost-efficient material. Having a transparent material for the outer structure is the common choice for most photobioreactors since the lighting system is often on the outside of the design. Synthesized polymers, or plastic, and glass are the main options for the transparent material to build a structure. The variety of plastic available on the market allows for a diverse set of material properties which makes it convenient for designs including photobioreactors. However, as the popularity of plastic has increased, plastic waste has been accumulating at an increasing rate. This is partly because of the successive degradation of the material's quality after each recycling. Even for plastics that could be recycled to other usable products, only about 9% of the plastic being produced is recycled (Gross, 2017). Plastic is the most popular option for photobioreactors due to its variable characteristics but also because of its very inexpensive cost. Compared to plastic materials, glass has a higher recyclability such that 33% is being recycled (US EPA, 2015). However, it is a much more expensive material, which then becomes extra costs that are not affordable for a photobioreactor design. As a comparison, the cost of plastic, more specifically polyethylene or polyvinyl chloride, ranges between 1.00-1.50 USD/kg; whereas the cost of glass can go up to 3.90 USD/kg for laminates (Ortiz, 2003).

Another material option for the outer structure of the photobioreactor design is a metallic body, such as SAE 304 stainless steel. This material seemed like the better option when compared to the previous ones since it is being recycled at a rate of 73% of material produced (US EPA, 2015). Stainless steel has a higher durability and overall life expectancy than the other materials considered. It is also a relatively inexpensive material, especially compared to glass, though still higher than plastic, at a cost of 2.70 USD/kg (Ortiz, 2003). These characteristics of stainless steel were also part of the decision of having internal illumination for this design.

5.4. System Dimensions

Like the shape, the dimensions of the photobioreactor have a direct impact on the efficiency of the system, it is crucial to study the structure of the bioreactor for optimal performance. The size and shape of the bioreactor has a major effect on the light distribution and penetration, which in turn will affect the photosynthetic rate. They will also have an effect on the mixing, as stronger mixing will be required for larger volumes. Since the chosen design for the bioreactor is a column structure with bubble sparging, it is necessary to determine the most efficient length and diameter for this column.

Fernandes et al. (2010) studied the optimal light regime for bubble column photobioreactors thoroughly in their research and it was possible to model the productivity of a photobioreactor according to its dimensions using their results. It is clear that microalgae require a certain amount of light intensity to reach them so that they receive sufficient photons for their photosystems to be activated and therefore for photosynthesis to occur. Light of wavelength between 400-450 nm and 650-680 nm will dissipate at an even faster rate than for light of wavelength around 550 nm, this is because the microalgae in the photobioreactor will absorb the red and blue light whereas the green light will be left mostly untouched. The data provided for light of wavelength 540 nm was used to model the evolution of light intensity with increasing penetration, since the overall trend followed would remain similar to the preferred wavelengths. By plotting their results, for a cell density of 0.95 kg/m^3, the following decreasing exponential model was obtained:

$$Light\ Intensity = 1.15 \times \exp(-0.59 \times Distance)$$

This equation was obtained with a coefficient of determination of 0.9856. It was possible to obtain penetration depth for the 50% irradiance value, which was about 7 mm for a cell density of 0.95 kg/m^3. Similarly, it was possible to plot the results for light intensity with respect to cell density and the following polynomial function was obtained, for a depth of 5 mm:

$$Light\ Intensity = -0.0675 \times CD^2 + 0.1205 \times CD + 0.8825$$

This equation was obtained with a coefficient of determination of 0.9814. The cell density receiving only 50% of the actual light irradiance would then be of about 1.25 kg/m^3.

From these results, the dimensions for our photobioreactor design were determined. It was decided that the helical lighting structure would contain two separate helixes with a separation of 4 cm between each lighting strip. This allowed for more volume to be covered so that when the cell density becomes higher towards the end of the process (retention time of 2.3 days) most of the microalgae will still receive a strong percentage of the light intensity. Also, at the beginning of the process when the penetration is deep, the microalgae would not receive too much light in order to prevent photoinhibition. The diameter for the photobioreactor was also obtained from these results. It was decided that a diameter of 20 cm would be optimal for the growth of the microalgae. This way, the lighting structure could be placed 5 cm from the sides of the structure.

The dimensions for the photobioreactor's outer structure also depends on the mixing rate. In fact, mixing is crucial inside the bioreactor to ensure proper suspension of the microalgae, and also to ensure they receive a sufficient amount of CO_2 since it will be pumped in by the bubbles. The efficiency of mixing, and therefore the biomass productivity, is affected by the column height, and it appears to decrease linearly when this column exceeds a 1 m height, for a 20 cm diameter (Sanchez Miron et al., 1999). Since the efficiency remains relatively constant until this point, it was decided to opt for a 1 m high column since this will allow for the highest volume with the best overall efficiency. Thus, our photobioreactor design would have a length of 1 m and a diameter of 20 cm with the lighting structure 5 cm from the sides and two helixes separated by 4 cm (see Appendix 1.2 for schematics). This will provide a total volume of 31.4 L of wastewater being treated per batch. From the design and the lighting system, it is expected that an output biomass concentration of up to 4 kg/m^3 could be obtained.

5.5. Carbon Dioxide Delivery

Carbon dioxide sparging is an important process for the optimization of algal growth through pH control. In addition, the bicarbonates formed by the dissolution of carbon dioxide in water can be used as a carbon source for biomass growth (Prathima Devi & Venkata Mohan, 2012). Microalgae tend to preferentially use bicarbonate rather than CO_2 as a carbon source (Kumar et al., 2011). Therefore, because of the cells' affinity for the dissolved carbon, an algal reactor with sufficient column length should theoretically minimize CO_2 leaving the system through exhaust. Additionally, since the formation of bicarbonate lowers a solution's pH, the method of sparging can reduce the alkalizing effects of algal metabolism, maintaining favorable conditions (Pegallapati & Nirmalakhandan, 2012). A quick uptake of bicarbonate also prevents the solution from becoming too acidic. Therefore, with careful control, the system should result in a balanced pH and adequate carbon availability.

The method of CO_2 sparging has also been suggested as a method for carbon sequestration to reduce effects of global warming and the rise of greenhouse gas emissions (Kumar et al., 2011). Previous designs of biofuel technologies involving CO_2 gas sequestration from waste emissions

exist both on the industrial and lab scales. For instance, GreenFuel Technologies (Massachusetts, USA) has used industrial smokestack emissions for the cultivation of *Chlamydomonas* algae (Rosenberg et al., 2008). Typically, depending on the fuel plant, emissions can range from 4-14%; other industries may have different emissions depending on their respective processes (Kumar et al., 2011). For *Scenedesmus obliquus*, a few studies found that 15% CO_2 concentration was optimal for maximum biomass when sparged at a rate of 600 mL/min (Kaewkannetra et al., 2012; Singh & Singh, 2014). On an industrial scale, therefore, a photobioreactor could be directly coupled with the exhaust outlet of a factory or processing plant for biomass, water treatment and exhaust treatment.

It is important to note that most studies in the literature consider optimized biomass or bio-lipid productivity for their optimization studies. While this project has some interest in the biomass productivity, the main goal is to create an effective wastewater treatment system. Usually, a higher biomass productivity corresponds to a proliferating algal population, which corresponds to higher nutrient removal rates. Therefore, most optimization assumptions made in the design lie on the assumption that optimized biomass productivity means more nutrient removal.

In a lab setting, however, CO_2 sparging must be conducted using pressurized gas cylinders for CO_2 enhancement. To obtain the ideal ratios, the near-pure CO_2 must be mixed with sterile ambient air to make a 15% CO_2 concentration. While the study by Kaewkannetra et al. (2012) was conducted with continuous sparging, some studies including the one by Prathima Devi and Venkata Mohan (2012) found that sparging must be intervaled to prevent acidification. They found that the optimal sparging interval for highest biomass was four hours. Because even similar experiments produce a variety of results depending on volume, temperature, and other factors, for our design, we recommend tracking pH, temperature and turbidity between experiments to see how much the parameters change between replications. In the case of a wide variety of values for pH and other factors, the design will incorporate a control system using a pH and turbidity sensor for real-time tracking of conditions. The control system will either notify the researcher of a need for adjustment of flow rate, light configuration, or other parameters via a smartphone app, or will control a valve for the CO_2 sparger, as well as a switch for the lighting setup.

In order to promote proper fluid flow in a bubble column reactor, a few main parameters must be considered. First, the development of the fluid profile must allow for an ideal liquid-gas interface for good mass transfer and dissolution characteristics (Zahradnik et al., 1997). The kinetics of adsorption of CO_2 into the surrounding medium involves reaction of CO_2 into bicarbonate ion, diffusion and convection. All these processes happen at the same time, so modelling or understanding the flow of bubbles upon contact would require consideration of all these processes (Chen, 2012).

Designing a photobioreactor for ideal flow regimes should result in better carbon dissolution, more effective carbon uptake, and less waste of influent CO_2 exiting as effluent. It is crucial that both the geometries and pressures account for the desired characteristics. For the proper gas flow regime to form, a flat plate is often placed at the bottom of a reactor to break up a large gas bubble, resulting in uniform, distributed gas bubbles with relatively high surface area to volume ratio (Singh and Sharma, 2012; Kantarci et al., 2005). Additionally, relatively high heat and mass transfer coefficients of bubble column reactors make this design ideal both for introduction of carbon dioxide, and for potential heating purposes (Kantarci et al., 2005). Because of the complexity of the hydrodynamic flows, diameter and height dimensions also contribute an important role to ideal bubble distribution (Zahradnik et al., 1997). An aspect ratio of 5 has been shown to be significant for the effect of gas hold-up, while designs with radial gas holdup profiles have been modeled to be the most reliable for scaling up and for consistent heat and mass transfer properties (Kantarci et al., 2005; Krishna and Ellenberger, 1996). A flow velocity of above 4 $cm.s^{-1}$ in a reactor with a diameter larger than 10 centimeters results in small bubbles because of the churn-turbulent regime; however, in the presence of slurry (a combination of solid and liquid), the gas bubbles tend to become larger (Kantarci et al., 2005).

An important consideration for the optimization of the bubble sparging design is the concept of weeping, which is a phenomenon that occurs when there is a pressure drop of the sparge gas as it passes through the perforated plates. Such a pressure drop can cause the liquid to leak through the perforations, which has design implications in terms of mixing efficiency, potential liquid-related damage, and overall decreased effectiveness of the system (Thorat et al., 2001). To prevent this, a study on critical weep velocities ($V_{o,\,crit}$) is needed. While this consideration has not been thoroughly explored in this report, a system which resembles our own (a bubble sparger with an aspect ratio of 5), proposed by Kharbanda & Chu (1970), gave an empirical correlation for weep point, given by

$$V_{O,\,min} = 46.4[5.37 * d_O^{\,2.16} * \rho_L - \sigma * d_O]$$

Where d_o refers to the hole diameter, ρ_L is the density of the reactor liquid and $\sigma$is the surface tension of water. According to Guo et al. (2017), the surface tension of water is 72.75 mN m^{-1}. The proposed hole diameter is 5 mm, and the density of water at 20° C is assumed to be approximately 1000 kg/m^3. Therefore, calculated minimum sparge velocity is 2.67 m/s as an instantaneous velocity through the sparge hole; corresponding to approximately an initial velocity within the reactor of $6*10^{-3}$ m/s (See Appendix 2.7 for further details). For our design, we propose an initial bubble velocity (within the reactor) of 5 cm/s, as suggested by Deckwer et al., (1980). This is larger than the minimum critical velocity, so weeping is assumed to be negligible.

5.6. Gas Exhaust

Although an ideal photobioreactor would minimize waste gases of effluent, it is crucial for safety purposes that an outlet for waste gases from the reactor be capable of removing excess

particulate matter, and trapping potentially harmful by-product gases and vapours to prevent these from being released into indoor or outdoor environments. An outlet system is necessary for a semi-continuous bubble reactor, since the buildup of pressure in the reactor is neither safe nor helpful for the growth of a biological organism (Eibl & Eibl, 2008). Most often, reactors are equipped with scrubbers or filters which are sensitive to the emission of such by-products. Using an efficient fibrous filter such as a High Efficiency Particulate Air (HEPA) filter can be useful for retaining particulate matter and some gases. However, because pressure drops across the filters increase with the increase in trapped particles, filters quickly lose efficiency, and therefore must be replaced. As a result, though more expensive, wet scrubbers and bubble chambers may be more reasonable for long-term operation (Braddock et al., 2015; Peillon et al., 2017).

In a wet scrubber system, air pollution can be mitigated using a 'scrubbing' liquid, which is often water. In a more controlled system, the gases are stored in the liquid as dissolved gases or particulates (Braddock et al., 2015). For a lab-scale project, placement of the reactor with an outlet hose leading into a fume hood should be sufficient. However, for a larger-scale project, filtration and scrubbing must be considered in the life cycle analysis, economics and implementation of the design. It may be useful to model particulates from a typical wastewater profile in order to understand the scale of the issue of exhaust, and how much of a mitigation strategy must be undertaken as a result. However, exhaust is not thoroughly examined beyond these recommendations, as it is considered out of the scope of this lab-scale project.

6.0 Design Modelling and Performance Predictions

To verify the viability of the design proposed, the design team modelled the algal growth in the reactor to find an approximation for the biomass accumulation over the 2.3 days. Biological modelling can have many limitations and uncertainties, however, both due to natural variability of biological systems, and due to the complexity of conditions associated with 'optimized conditions.' Modern day modelling methods and scientific understanding, though rapidly increasing, are limited in capacity and power. Despite uncertainties in modelling capabilities, using computational simulations of engineering systems is a powerful way to perform preliminary analyses of the effectiveness of systems, as well as to compare systems using controlled, user-specified conditions. It can also serve as a way to immediately address system inefficiencies before capital and time expenditure on real system prototypes. Finally, simplifications of real systems can help elucidate relationships between various components by isolating phenomena and studying their effects on the system without the interference of other variables (Gerlee & Lundh, 2016).

For the proposed system, several models and simulations were used as an assessment of the effectiveness of the design. The principal model was a two dimensional simulation of algal growth in a cross section of a circular photobioreactor with a light ring at the center, representing the helical lighting configuration. This was used in order to approximate the final biomass output and to compare varying placements of lighting systems. The modelling environment used for this

was NetLogo, which is an open-source, agent-based program specialized in the simulation of natural and social behaviors. Although there exist different programming languages that are agent-based, NetLogo was an appropriate tool for this design since it is a very user-friendly approach to simulation. Python is another example of an agent-based programming language and could have been used as well; however, because of NetLogo's user-friendly interface and preloaded GUI, using NetLogo helped the team setup and accelerate the design modelling process. The representations consist of turtles, or mobile agents (representing floating algae cells in this simulation) and patches, or stationary agents (representing light distribution and the shape of the reactor) (NetLogo, 2018). An image of the graphical user interface is shown in Appendix 1.1, as well as a sample output. Results, which were exported in CSV format, were subsequently analyzed for the calculation of biomass output.

6.1 Design Assumptions

The following assumptions were made in creating this model. It is important to note that these assumptions simplify the system but add uncertainty to the output.

(1). Algal movement: The model assumes a uniform suspension of algae due to the sparge-induced mixing. The first model assumed a circular movement of the algae, which showed a phenomena known as clogging, or fouling of the light source. An algal mass (shown in Appendix 1.1-B) accumulated near the light source due to energy advantages. However, this was incongruous with the speculating mixing pattern provided by the CO_2 delivery system. Therefore, a random Brownian motion model of the algae cell representations was adopted, since this most likely was the effect of small gas bubbles hitting algae cells in random ways. However, this results in a model limitation; namely, that a uniform suspension of algae is maintained throughout the production time. For a more thorough discussion of the limitations of the modelling procedure, as well as the recommendations for improving the accuracy of the model, see section 6.4.3.
(2). Growth Relationships: All growth relations, coefficients and equations were found in the academic literature and incorporated directly in the models. These are referenced directly in each program script, and are summarized in Appendix 2.
(3). Cell population representation: Due to agent limitations in NetLogo, it is not tractable to monitor and count individual algae cells in the simulation. Therefore, representative model turtles were made to represent a programmer-defined number (in this case, 1 turtle was defined arbitrarily as 6.3*10^6 cells to account for the initial concentration of 10^5 cells/mL, as reported in accepted literature methods). Concentration calculations for turtle definition were calculated assuming that the two-dimensional model represented a 2 cm thick horizontal cross section of the reactor. The thickness observed was considered to be 2 cm since that is the height for which the helix makes half of a revolution, therefore a full revolution is complete since the system uses a double helix, hence the circular representation of light in the model. The biological implication of this assumption is that "colonies" of cells are formed and are partitioned as cells multiply. While *S.*

obliquus has been shown to display colony action (Lewis & McCourt, 2004), colonies of 6.3*10^6 cells would not exist in reality; a typical colony size for *S. obliquus* is under ten cells (Verschoor et al., 2009). However, this was a necessary simplification due to computational power limits. We predict that this is a conservative assumption, since less overall dispersion implies less light delivery to individual cells, and therefore we do not adjust for this in the model.

(4). Constant mixing distributions along length of reactor: For final biomass approximations, each horizontal cross section of the bioreactor was assumed to be representative of the average cross section. Therefore, a profile depending on height, differences in sparge speed, settling rates, etc. was not predicted. This is a simplification, because in reality, despite algal buoyancy, natural settling rates should occur (Samil et al., 2012). Additionally, since CO_2 from the sparge bubbles will dissolve into the growth medium, sparge velocity decreases lengthwise as it rises through liquid, which should increase settling rates, and potentially decrease photosynthetic rates due to carbon availability problems. However, a study by Sanchez Miron et al. (1999) observed the evolution of mixing efficiency by bubble sparging with the height of a column bioreactor. As is explained in section 5.4, the height of the cylinder was restricted to 1 m as this was the maximum height before a significant drop in efficiency could be observed. Therefore, it is reasonable to assume that the mixing will remain constant throughout the length of the reactor and the modeled cross section can be considered representative of the entire cylinder.

(5). Wastewater and nutrients: The model presented does not directly address the role of nutrients in the growing medium on the rate of photosynthesis. Although the principal objective of this project is to serve as a nutrient removal mechanism, for the modelling of the algae growth, an ideal mixture of nutrients is assumed, and therefore, nutrient dependence is neglected. This has obvious limitations, including the omission of the nutrients' roles in reaching a realistic carrying capacity. To adjust for this, however, the data is subsequently compared to experiments in real wastewater settings, both to estimate total removal of nutrients, and to judge the level of uncertainty presented by this assumption. Furthermore, the role of CO_2 in photosynthetic growth is also omitted from the model. This is considered an acceptable omission, however, because the sparge system is designed to maintain a constant pH, the carbon availability can be assumed to be sufficient for the required photosynthetic demands of the growing algae populations.

(6). Absorptivity limited to chlorophyll content: Using the Beer-Lambert law for absorption of light requires using molarity coefficients for the substances being studied. In the case of algal cells, a molarity coefficient could not be determined as the dimensions of the cell exceed the molar realm. A study by Rinanti (2016) observed that the maximum chlorophyll content for *S. obliquus* was of 0.25 mg/L for a cell concentration of $0.37*10^9$ cells/L. Adding on, the molar extinction coefficient for chlorophyll had been studied by Griffiths (1978), and this coefficient had already been used in the case of *S. obliquus* in previous research (Urbig et al., 1995). Therefore, the molar extinction coefficient used in our Beer-Lambert calculations was that of chlorophyll, so our algal concentration was also multiplied by the chlorophyll concentration. Such an assumption is a very clear underestimation of turbidity effects of cell concentrations, since there are many cell structures and other suspended solids which affect light absorption. Therefore, two additional coefficients

were added to the chlorophyll attenuation coefficient, in the model representing the effects of nutrient solution (only phosphorus in water considered) and the effects of the cells themselves. The calculations for these coefficients are summarized in Appendix 2.

(7). Photosynthetic efficiency: Although algae absorbs a considerable quantity of photons, not all of it is converted to energy or biomass. Photosynthetic efficiency is largely dependent on the conditions of the environment the algae is growing in. A study by de Marchin et al. (2015) measured photosynthetic efficiency of *S. obliquus* in environments differing with regards to carbon dioxide availability. A photosynthetic efficiency of 5% was observed under CO_2 conditions. This value was therefore used in our modified Beer-Lambert law to represent more accurate growth rates in NetLogo by accounting for the fact that not all light absorbed would serve as energy for the algae. The photosynthetic efficiency differs significantly between algae and terrestrial plants and can be used as a reason to focus on algae for biomass production. In fact, terrestrial plants' efficiency remains around 2% whereas algal strains can have efficiencies up to 8% (Anyanwu et al., 2018).

6.2. Description of Model

6.2.1. Algal Reproduction and Growth Dynamics

Algae reproduction in the NetLogo model did not adhere to traditional population dynamics models such as Monod or Lotka-Volterra models because of the nature of the agent-based programming. In order to reproduce in this program, the turtles, representing algae, accumulate energy at each time step, referred to here as "ticks." In each iterative cycle, the turtles are associated to an energy level depending on the light intensity of the patch at each turtle's location. An arbitrary number of energy "points" were selected (in this case, 90 points), to indicate the energy level at which the algal cell could divide. Since ticks are also an arbitrary time unit, all these parameters were adjusted later to growth characteristics in similar conditions and environments. A discussion of the limitations and uncertainties are discussed further in Section 6.4.3. However, despite the uncertainties associated with this modelling process, the user control should account for some inaccuracies presented by this method. First, the method of fitting the data to calculate the number of ticks needed to solve for a real-life doubling time (DT), see section 6.2.2 for more details.

Second, the ratio of reproduction-to-death rate has been explored in the literature to ensure that growth dynamics are accounted for in the model. According to Lurling (2003), a mother cell will undergo n = 3 or 4 successive division cycles, yielding 2-16 daughter cells. Therefore, in our model, it is assumed that the reproduction rate is 4 times that of the death rate. Regardless of the true lifespan of an algal cell, the overall effect is assumed to be 4 cells reproduced to one dying cell. This is a simplistic approach, since it also does not consider other causes for slowing reproduction rates including nutrient limitations and genetic mutations. Additionally, it assumes

that reproduction occurs in a very short time period directly prior to death. In reality, the interphase in the cell's reproductive cycle could be much longer proportionally than what was assumed in the model, since cell reproduction is not only dependent on the amount of photosynthetic energy it accumulates. Therefore, while this is an approach which can give a simplistic overview of the cell reproduction relationships in real algae, there are clear limitations to the approach. The uncertainties and inaccuracies of these calculations can only be explored through further research and real experimentation. Thus, biomass productivities, while in a reasonable range according to existing experiments, are not conclusive and must be further investigated to indicate the viability and true efficiency of the project. In future sections, these limitations and uncertainties will be mentioned and discussed, but all further calculations, assumptions, etc. will be based off the output of the model for a sample analysis approach and tentative validation for the purpose of this report.

6.2.2 Time Definition

The time variable in NetLogo is a measure of the number of times the code has been run. The unit used is a tick, so a one tick increase implies that the entirety of the code has been run once. The time variable of the model was initially undefined and needed to be related to existing experiments for the model to be representative of our own experiment. Two different techniques were used to create an appropriate relation between biological time and ticks.

Firstly, the doubling rate of the model was to be calculated so that it could then be related to the doubling rate of existing experiments in similar conditions. The doubling rate is highly dependent on experimental conditions so there is some inaccuracy associated with using other experimental data to represent our own. However, multiple papers focused on the doubling rate of *S. obliquus* grown in photobioreactors and carbon dioxide influx, so these papers were used as a reference for the expected doubling time of *S. obliquus*. A study by de Morais and Costa (2007) studied more in-depth the maximum specific growth rate of this strain but also provided the equation in Appendix 2.3 for doubling rate dependent on maximum specific growth rate. From this paper, an expected doubling time of 15.825 hours was determined. Adding on, a paper by Celekli et al. (2008) took a similar approach by studying the maximum specific growth rate as well and providing the same equation. The determined doubling time from this paper was of 16.32 hours. Based on the results of these studies, a doubling time of 16 hours was used for the remainder of the time calculations. So, over the duration of the experiment, a total of 2.3 days or 55.2 hours, the algal concentration should double approximately 3.45 times.

To obtain the doubling time from the model, in ticks, the equation for generation time was used, as it is defined in microbiology as the time for a cell concentration to double, it can be found in Appendix 2.4. This equation could then be used for every data point at each tick. However, as the ticks increased the doubling time changed as well since algal growth is not linear. This creates uncertainty in the doubling time, as it depends on when the simulation is stopped. For the five replicates studied, it was decided that the simulation would stop once the growth curve began

taking its linear shape, which it then maintained until saturation. This occurred at around 90 ticks. This is an arbitrary decision; if it had been decided that we were to study the growth rate until it plateaus, the results for doubling time would have been much higher and therefore, the output concentration would have increased as well. The average doubling time observed for the five replicates was then 10.5 ticks.

Therefore, since the algal concentration would double 3.45 times throughout the experiment and one doubling time took 10.5 ticks to complete, the experiment would last a total of 36.25 ticks, so a total of 37 ticks were used to complete the experiment in the simulation. The relation between time and ticks was as follows: 0.6566 ticks = 1 hour.

To verify that the definition of time for our model was appropriate, the curve obtained, with the x-axis now defined, was matched to *S. obliquus* growth curves from existing experiments in the literature. These are analyzed and shown in greater detail in Section 6.3. Although the curve for our design seemed to have a steeper slope than that of actual experiments, the concentration within the timeframe was reasonable. The curves were therefore not a very accurate match, but it could be concluded from the graphs that the time associated with our growth curve was appropriate. This helped verify the relation between ticks and time for our design, as mentioned above. Also, it helps support the assumption that stopping the simulation as soon as the curve begins increasing linearly was not unreasonable to make for the design.

6.2.3. Light Evolution

Our model encompasses important structural designs as well as light diffusivity and fluid flow considerations. The main goal that this design strives to achieve is the optimization of light diffusivity in an algal photobioreactor. This means that the lighting structure should be able to provide the necessary amount of light for each algal cell to thrive in its environment, regardless of its distance to the light source. Therefore, the photobioreactor simulation was used to measure the efficiency of our lighting system on algal reproduction. Since nutrients were neglected in the simulation, algal reproduction was then only dependent on the availability of light in the system. This allowed us to focus more strongly on the lighting component of the system and its effect on algal cells.

The Beer-Lambert law was implemented in the energy uptake section of the model. Since the energy of a cell determined its ability to duplicate, it was only dependent on the light that the cell could absorb. The Beer-Lambert law consists in measuring the evolution of light intensity with respect to the material located in the light path. The decline of light intensity of a material is an indication of light being absorbed by that material. This law was crucial in the development of the model as the evolution of light and the evolution of algal cells were both dependent on each other. Therefore, in the implementation of this equation in the model, both light intensity and algal concentrations were variable and changed with time in the simulation. The calculations for the

explicit formula used in the simulation can be obtained in Appendix 2.2. The initial light intensity was fixed at 150 μmol/m^2/s, as it was determined to be the optimal irradiance for *S. obliquus*. The path length of light was determined depending on the location of the algal cell and its distance from the light source. The molar attenuation coefficient was determined based off published literature as previously mentioned in Section 6.1.

This equation was used to determine the amount of light that is absorbed by each cell in the model. Since recalculating the light intensity for each patch in the cylinder at every tick would have significantly slowed down the simulation, the equation was not used to change the representation of light in the model. However, implementing the equation at the algal energy level allowed for the energy uptake of algae to act as if the light level was evolving with time. The light intensity therefore varies with the algal concentration at t-1 and the algal concentration varies with light intensity at t. The overall equation was then multiplied by the photosynthetic efficiency coefficient. This allowed for the equation to more accurately represent the actual value of light that would be converted to biomass.

Therefore, the Beer-Lambert law was used to represent the variation of light intensity in the model due to algal concentration. Since light was the only component affecting algal growth, this law was used to measure the exact amount of light being absorbed that could then be converted to energy and biomass.

6.2.4. Preliminary Mixing Study

A secondary, physical study was conducted in the COMSOL Multiphysics program to verify if the assumption 4 in Section 6.1 (that mixing is uniform across the length of the reactor) is a reasonable assumption. An array of sparge holes on the bottom of a reactor was represented by a linear array along a 2D axisymmetric geometry. An inlet velocity of 5 cm/s was used in a water tank. Reaction of species was neglected, as was interference from the light geometry (See Section 5.4). The steady state velocity profile is shown in Appendix 5.1. The study showed that the velocity of mixing was not entirely uniform over the length of the reactor. However, this may not have significant effects on true photosynthetic efficiencies of the algae. To verify this, more studies may be conducted which consider diffusion of the bicarbonate ions within the reactor, and by assessing buoyancy of *S. obliquus* and the effects of the existing velocity profile on settling rates. Furthermore, a sweep of inlet velocities and sparge plate distribution can also be implemented to determine if a higher sparge velocity can be implemented without causing turbulent flow.

6.3 Design Model Results

6.3.1 Simulation Output

The simulated experiments were run five times. The average values of these five experiments are plotted in Figure 2 shown. Error bars on the graph were added based on the standard deviation between experiments (Note that this is not related to true experimental variation). Since the experiments were run with set initial values, but there was stochastic variability in spatial distribution of the colonies, the error bars increase, as expected. This reflects uncertainty in all systems subject to random phenomena and movement, and cannot be controlled in simulation or reality. The resultant curve had significantly more linear behavior than expected; however, it is important to note that due to software limitations, death counts are not included in the output. Therefore, true biomass as determined by the model (not experimental) is higher than in the reported output.

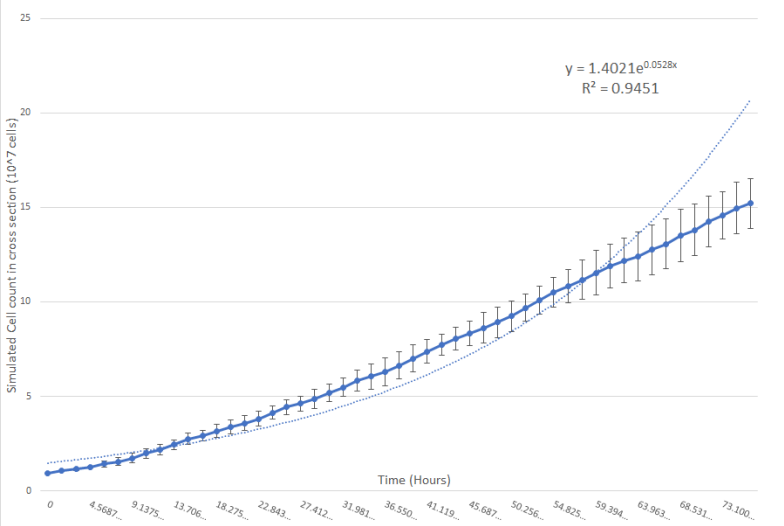

Fig. 2. Average simulated output of cells over 72 hours. Note that the proposed system would run for 55.2 hours but a higher time was added for reference.

6.3.2. Biomass Production

The production of biomass from the photobioreactor design was one of the principal components set for this innovative design to increase. In fact, if the device could maximize biomass production, this could then be sold to external companies in various sectors that are interested in converting plant biomass into useful by-products such as biofuel. Although microalgae biomass is relatively cheap on the market, it can still provide a significant new revenue if it can be sold over the long-term. This would not be applicable in the laboratory setting, but if a similar system were to be implemented on an industrial scale, this would be a significant consideration.

From the five replicate curves representative of the design's efficacy, it was possible to determine the final concentration of algae at the end of each batch. From the tick-time relation, it

is possible to determine the final time of the experiment as being 37 ticks. Over the five replicated experiments, the average output at 37 ticks was of 161.6 algal dots, the equivalent of $1.02*10^9$ for the volume of one cross section. This is slightly less than a log 2 increase from the initial concentration, which was of $6.28*10^7$ cells in the volume of one cross section. Therefore, over the course of the experiment, over 2.3 days, approximately $9.57*10^8$ new algal cells are hatched in the 628 mL volume of the cross section. This also takes into consideration the algal cells that have already died, since their biomass could still be used in the conversion to secondary by-products. These results can be computed for the cylinder as a whole. With a height of 1 m and a diameter of 20 cm, the cylindrical bioreactor holds a volume of 31.4 L. Considering the volume of a cross section, there are exactly 50 cross sections in one cylinder. So the total initial concentration of algal cells becomes $3.14*10^9$ and the total final concentration becomes $5.11*10^{10}$ cells.

The overall final weight of biomass produced can be computed, since biomass is sold on a per dry weight basis. The dry weight of *S. obliquus* cells was obtained from a study by Hodaifa et al. (2013) to be 37 pg/cell. So, using our final concentration, we find that the final biomass output of our design would reach 1890 mg, or 1.89 g. The final biomass output can be written as 0.06 g/L. From the calculation in Section 5.4, the expected output for the design to prove successful would have required 4 g/L to be produced, as this was the goal set for this design. This is significantly higher than the obtained output, which indicates that this design does not provide sufficient biomass for it to be more successful than existing designs. Possibilities to improve on this will be discussed more in-depth in later sections, but include increasing the input concentration of algae or increasing the length of the experiments.

6.3.3. Nutrient Removal

One of the major goals of this design was to accomplish successful removal of nutrients in wastewater, which through more academic and industrial research and development, could in the long term provide municipalities with a clean device that would supply cleaner water. However, the model used to simulate the design does not take into account nutrients. This decision was made in order to simplify the model and focus more strongly on algal growth. The final algal concentration could then be simply related back to nutrient removal later.

In Canada, strict governmental and municipal regulations were put in place to oversee that no contaminated water is discharged into natural environments. Most of the wastewater is able to be transported to wastewater treatment plants to undergo thorough decontamination processes before being discharged and reused. The Canadian Environmental Protection Act of 1999 regulates the concentration of toxic substances such as nutrients in wastewater effluent. However, the report by Chambers et al. (1997) provides more descriptive quantities of each component in wastewater. Although this is a report from over two decades ago, it has been archived by the Government of Canada and was last reviewed in March 2019. The concentrations provided were therefore used as references for this design report. From the paper by Chambers et al., the effluent concentration of

phosphorus is limited to 0.5 mg/L, for treatment plants in Quebec. It was decided earlier on in the design process that the nutrient removal would mostly focus on phosphorus, since this is the limiting nutrient for growth, and also the nutrient with the most intensive regulations in Canada. Phosphorus is also usually removed from wastewater with less efficacy than nitrogen, since nitrogen can be removed through a variety of mechanisms including biological sequestration and volatilization as ammonia (Wang et al., 2016).

Our calculations were adapted from the work by Martinez et al. (2000), since their experimental setup was similar to our design in the way that they worked with *S. obliquus* in a cylindrical bioreactor with air bubbling for mixing to treat wastewater. The doubling time that they reported (15.8 hours) was very close to that which we assumed for our model. Martinez et al. provided data about the initial phosphorus concentration in the wastewater used, which was of 135 µmol/L or 12.8 mg/L. This was helpful in the evaluation of our own design. They also provided a conversion rate of phosphorus to biomass to be 1.53 mg-biomass/µmol-P. Therefore, with a final phosphorus concentration requirement of 0.5 mg/L or 5 µmol/L, the experiment should produce 199 mg of algal biomass for the design to be considered successful regarding nutrient removal efficacy. These results are explained by more in-depth calculations in Appendix 2.5.

In fact, the biomass produced can be simply calculated by taking the difference between final and initial algal concentration in the bioreactor and adjusting this value with the dry weight of the cells. The total dry weight of biomass produced is then 1.77 g or 1770 mg, as seen in Appendix 2.6. This is almost nine times more than the necessary dry weight required to filter out the phosphorus to a concentration below that provided in Canadian regulations for Quebec. Therefore, the design can be considered very successful at nutrient removal of phosphorus and it can be assumed with some degree of confidence that the phosphorus levels in effluent water will be well below that of the governmental policies. However, this may also be considered as a limitation for our design, since the algae may run out of phosphorus early on, which would limit its growth before the end of the 2.3 day batch.

6.4. Analysis of Design Output

6.4.1. Evolution of Design Factors

The model of the photobioreactor design focused on two major components: light intensity and cell density. These two factors are related, as light intensity will diminish with increasing cell density, and cell growth will slow and eventually plateau with decreasing light intensity. Since the design's main goal was to maximize final cell density, as this increases biomass for by-products as well as the nutrient removal rate, it was necessary to closely monitor the light level during the simulations.

As previously mentioned, the light intensity evolved following the Beer-Lambert law adjusted to represent our design parameters. Since the experiments only last for a duration of 2.3 days or slightly over 55 hours, it was not expected that the lighting would be entirely blocked out by the increasing algal density. As can be observed in Fig. 3, the light intensity does decrease with time and algal concentration, but this is not extremely significant, especially since this is the light level measured at the edges of the bioreactor. Even if the experiment were to last much longer, cell density would still have a relatively low impact on light intensity. However, it may be possible to explain this result with the assumptions that were made during modeling, for example the fact that the nutrients are not represented or that the algal absorptivity is restricted to chlorophyll content with a few other simplified factors (cell structure and dilute aqueous solution). This result seems to indicate that the cell density would be able to grow without being limited by light, and that the nutrient content may be the limiting factor for algal growth in this system.

Algal growth is the determinant factor of our design, since the reactor should be able to create a hospitable environment for *S. obliquus* cells to proliferate. From Fig. 3 and the results mentioned in previous sections, the algal cells were clearly able to reproduce and multiply at a rapid pace. The lag phase that normally occurs during cell growth is almost nonexistent as they begin growing exponentially almost immediately. The curve used to represent cell growth in Fig. 2 is an exponential model that best fit the actual data, with a coefficient of determination of 0.867. If the experiment were to last longer under the same conditions, the algae would continue reproducing until the turbidity eventually affected lighting. In the simulation, this occurred at approximately 375 ticks. However, as mentioned in section 6.3.4., nutrient uptake appeared to be very efficient and would become a limiting factor before the lighting system. Since the goal of this design focused on improving lighting systems in photobioreactors, these results support the proposed lighting structure.

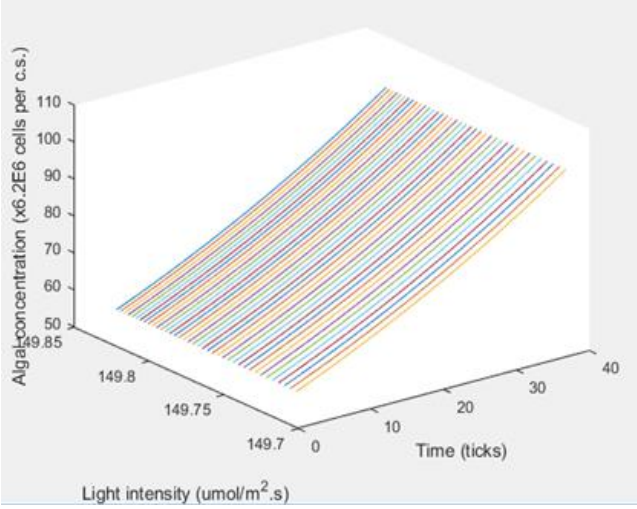

Figure 3. Evolution of algal concentration and light intensity with time in a 1/50th representation of the proposed photobioreactor design

6.4.2. Scale-Up Considerations

The timeframe for the design development unfortunately did not allow for an in-depth study and model of a scaled-up proposed design. Some points were still considered throughout the design process so that this design could potentially benefit not only lab-scale research but also the industry and wastewater treatment facilities.

The two main factors that could be modified in the proposed design are the height and the diameter of the bioreactor. One of the main difficulties in increasing either of those dimensions comes from the mixing system. In fact, the height of the proposed design was limited to 1 m because that was the maximum length that would not decrease growth efficiency. If the height of the cylinder were to be increased, it would be necessary to perform an in-depth fluid motion study in order to quantify the decrease in efficiency and to measure a reasonable margin for the height increase before a significant decrease in efficiency would occur. Another possibility would be to increase height and introduce a second air sparging mechanism at the middle of bioreactor. An additional sieve would then also be required to break down the air into bubbles and spread them out over the bioreactor's diameter.

Expanding the diameter of the bioreactor would appear as a simpler way to increase the volume treated. The difficulty that would arise from increasing the diameter is the need for an adequate repartition of air bubbles throughout. One sieve to break up the sparged air into bubbles would probably not be sufficient to provide adequate mixing over the entire diameter. To account for this, a possibility would be to create multiple air sparging vents at the bottom of the cylinder instead of only one so that the bubbles would cover the entire diameter once broken up by the sieve. Adding on, a diameter increase would also affect the lighting inside the bioreactor since, as mentioned in Section 5.4, a distance of 5 cm is optimal for the lighting to provide energy to the algal cells. Increasing this distance would cause light to become limiting for algal growth. However, this could be solved by installing a second, or multiple, light structures in a similar manner as the one currently used but with a larger diameter. This approach was slightly studied on NetLogo but the software was not powerful enough to simulate two different light sources as the light rings would create interference and the lighting would therefore not be represented accurately.

There are various possibilities and approaches to increase the volume that could be treated in this photobioreactor to meet industry demand. However, more thorough research and testing on the proposed considerations are required to analyze these possibilities. Modeling would be an appropriate tool to perform these tests, but the software required would need to be more powerful than NetLogo to simulate fluid flow and multiple lightings.

6.4.3. Design Limitations

Several limitations to the design have been mentioned throughout the modelling process reporting; these limitations are important to consider in all models, including that of this proposed design because the model cannot be confused with reality. One of the largest limitations to the modelling approach reported, is the lack of validation against real systems. Because of the variability in methodology in experimental reports in academic literature, it is not possible to find relationships between growth parameters that are completely representative of the proposed design. The only true way to verify and validate the design is through controlled experimentation

of the proposed design. Therefore, all reported results and calculations can only be viewed as tentative.

Moreover, the assumptions stated in Section 6.1 are all simplifications which were required for the modelling process because of computational and knowledgebase limitations. All assumptions, therefore, added to uncertainty and inaccuracies in growth projections. For instance, Assumption 1, which states that algal movement represented a uniform suspension, neglects that biological organisms have adaptive strategies which add uncertainty in the behavior of the suspended cells. One of these adaptive behaviors which could affect the efficacy of our system is the cells' adaptation to light. A study conducted at the Max Planck Institute for Dynamics and Self-Organization reported that green algae have the ability to detach their flagella (the appendage which serves as the cell's propulsion mechanism) in the presence of light. This results in of the algal adhesion to a surface closest to the light (Kreis et al., 2018).The proposed system in this case, would not deliver light in the expected way because of light clogging. The client had mentioned this phenomenon, which served as the basis for the mixing system and light distribution proposal. However, this would likely still be a challenge in a real system. Kreis et al. (2018) suggested that specialized lighting systems which suppress this adhesion response could be a solution. Using exclusively red light, for example, reduced adhesion phenomena, but also reduced photosynthetic rates. The research also suggested the use of algae with modified blue light photoreceptors in the presence of blue light; however, this is out of the scope of this design.

Assumptions about the time definition mentioned in 6.2.2, as well as the omission of nutrients in the light and energy model in NetLogo also have the potential for adding substantial error to the projected outputs, since both of these assumptions together imply a system which does not limit cell growth in any way besides with light intensity. This is known to be inaccurate. Factors such as nutrient levels are key to the determination of carrying capacity of a biological system. Additionally, certain other unforeseen phenomena could prove to be detrimental to the system. For instance, it has been shown that nitrates have the tendency to form ammonia in aqueous systems. Ammonia inhibits cells' ability to take up N-compounds, therefore limiting their ability to grow and reproduce. High-ammonia solutions are also more likely to be toxic for most species of algae. This can be controlled by keeping temperatures lower to prevent the nitrate-ammonia reaction from happening at a high rate (Wang et al., 2016). However, the lowering of the temperature could also reduce growth rates of algae (Kligerman et al., 2015).

Limitations to the design itself include those associated with vessel reactors. In general, vessel reactors have low surface area-to-volume ratios, and therefore tend to have less efficient light and mixing distributions. This was a payoff which was deemed acceptable for this design because of the low space requirement, lower manufacturing cost and the specialized lighting design. In addition, since the bubble reactor was designed with maximum dimensions for gas holdup and velocity distribution in mind, increasing dimensions in case of scaleup needs will also

require additional systems to offset inefficiencies and poorer sparge characteristics with increasing volumes. Because of the client specifications, however, the design limitations, though important to consider, were not significant enough for extreme modifications to the proposed design. Further design inefficiencies may be observed upon testing a real prototype. Thus, the design cycle cannot be considered complete in its entirety until it has been fully investigated and optimized in real laboratory settings.

6.5. Refinement of Preliminary Design

Throughout the design process, we kept in mind that although our preliminary analysis of photobioreactor designs had yielded a theoretical advantageous new design, the testing phase would be a crucial step in the validation of this design. The choice of computational simulating over building the prototype came from the short timeframe, the budget and the lack of experience in manufacturing this type of device. Although models are never accurate representations, their appropriate use can prove very useful in creating predictions, all the while keeping in mind their limitations. The benefits of having used simulation methods mainly revolve around the possibility to test out various dimensions and to redesign the device after testing to improve on its efficacy based on observed results.

We were able to simulate multiple designs of photobioreactors, especially looking at the lighting structure and the mixing component. Regarding the mixing aspect of the design, we decided initially to use bubble sparging because it seemed more efficient, as it both mixes the solution and delivers carbon dioxide gas for the algae. In our model, we simulated both swirling and bubble sparging to compare the results. When the swirling method was used, it was clear in the simulation that the algae near the edges of the cylinder were dying off almost immediately and the surviving algae proliferated, causing the algae to agglomerate at the light source. This was to be expected, at least partially, from the design, since this biofilm formation at light sources has been a source of decreased photobioreactor efficiencies. When the bubble sparging method was used, the mixing was much more efficient, and there was a much more homogeneous distribution of the algae around the cylinder, making the use of its space more cost efficient. The use of bubble sparging was therefore an appropriate choice for our photobioreactor's design. Not only does it provide the algae with a more even distribution of light, but it also allows the algae to occupy more space and therefore have access to more nutrients as well. We predict that the implementation of bubble sparging in our photobioreactor will greatly increase its overall efficiency both at the biomass production level and at the wastewater treatment level.

6.5.1 Light Gradient Design

The primary project approach was to optimize light distribution to ensure better energy access to cells throughout the cylindrical volume of the reactor. Thus, it was a crucial step to verify that the placement of the light source was adequate and maximized the amount of energy input for

a uniform light distribution. The lighting system was optimized and verified for the design using two approaches: the physical and the biological approaches.

In the biological approach, the principal NetLogo code was executed for nine different placements of light rings, which was achieved by changing the radius of the light source. This also assumes that the light source has an equal initial light intensity (150 μmol photons/m^2); however, the material used in the reactor is not considered, and therefore, effects of reflection or absorptivity of glass are not considered. The graphs of three configurations of light within the same reactor are shown in Fig. 4, with the curves of real experimental data in existing laboratory photobioreactors form the literature graphed for reference. Since all parameters are kept completely constant for both the biological and physical lighting validation studies, these can serve as a more certain comparison of different systems than simply comparing the model output to experimental data. Despite lack of controlled protocols in the scientific literature, therefore, a relative assessment of different systems can be made with a higher degree of certainty.

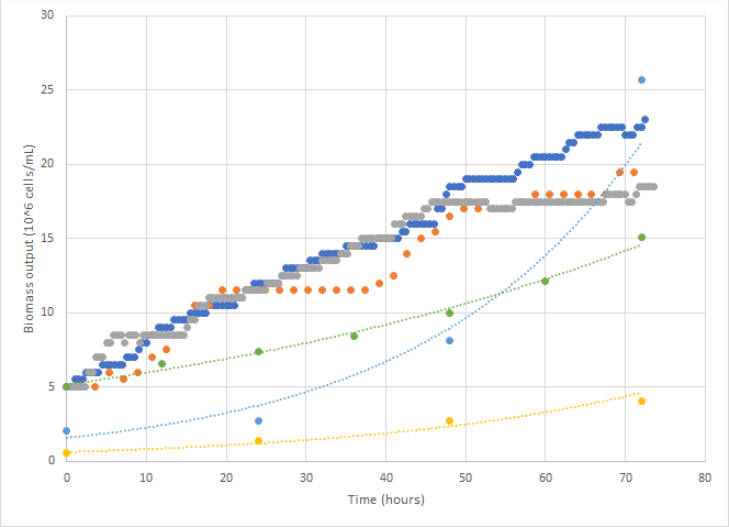

Figure 4. A comparison of three light configurations with existing literature results.
Blue dots: Helical internal lighting (r = 5 cm); Orange dots: External uniform lighting source; Gray dots: Central light rod (r = 1 cm); Yellow curve: Experimental data from Arbib et al. (2013). Light blue curve: Experimental data from Gris et al. (2014); Green curve: Experimental data from Krzeminska et al. (2014).

Each curve produced a similar growth curve overall, although they did not all reach the same final concentration. However, the curves for each systems did vary slightly: the helical structure provided a sigmoid curve, similar to that of the the central rod lighting. However, its sigmoid plateaued sooner at a lower concentration, and the outer lighting created a step-like function. Additionally, the helical ring system was able to reach a higher final concentration of algae than both other methods tested, being larger than the central rod results and the outer lighting results by 19% and 15% respectively. This is significant result as it highlights the benefits of our own novel lighting structure in comparison to existing lighting systems. By providing a more efficient algal growth, the helical lighting structure allows for a higher biomass production rate and a more efficient nutrient removal and wastewater treatment rate.

In Fig. 5, the final concentrations of the nine experiments are graphed to compare the effectiveness of each configuration. It can be observed that the chosen radius of the internal light source is maximized at the proposed solution of 5 cm. Although there is variation in results and some error (seen especially clearly for r=0.25 and r=1.25 cm), there is an overall trend which indicates that a medium radius tends to have better light distribution, and therefore better algae output.

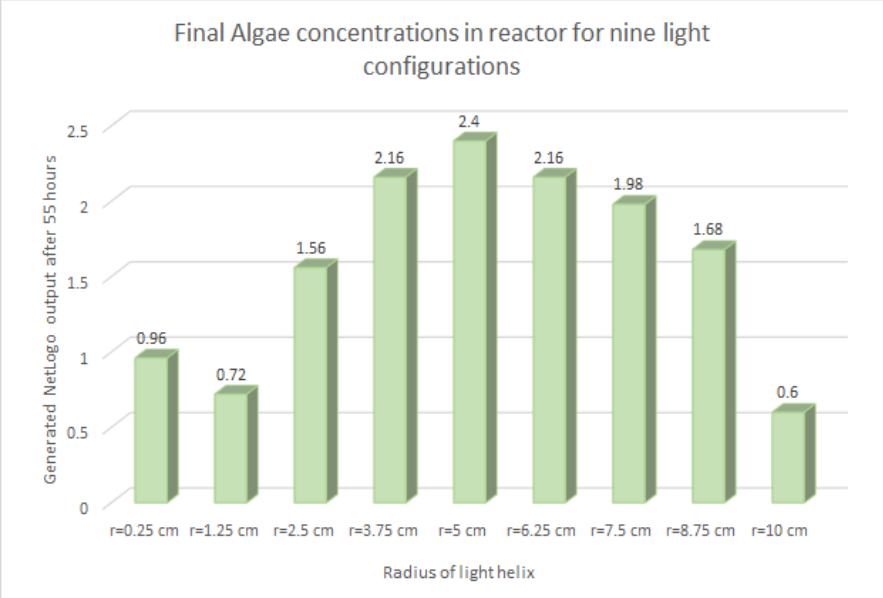

Figure 5. The final output of the nine experiments for different light radii are shown. The numbers recorded or relative, but represent cell count in the cross sectional area discussed, with an initial concentration of $5*10^6$ cells/mL in total. This was chosen as an initial concentration for ease of comparison with experimental data, which frequently start with that initial concentration.

In the physical approach, MATLAB was used to map light distribution in the absence of algae (in water only) in a horizontal cross section of three separate reactor configurations: (1) the reactor is made of a transparent shell (glass or plastic) and is exposed to a lighting source externally. This can either represent a simplistic model of natural light (in reality, the light distribution would be less uniform and subject to change throughout the day), or a more controlled lighting source in a laboratory setting. (2) the reactor is made of stainless steel, as proposed, and is exposed to a central light rod with no geometrical complications. (3) the reactor is made of stainless steel as proposed, and is exposed to light emanating from the helical lighting structure in both directions (as proposed). The coefficients of extinction, reflection factors, and equations were found in the work of Jonasz & Fournier (2007). For a full summary of the calculations and programming approaches, see Appendix 2. The output of distribution is shown in a three dimensional plot in Fig. 6.

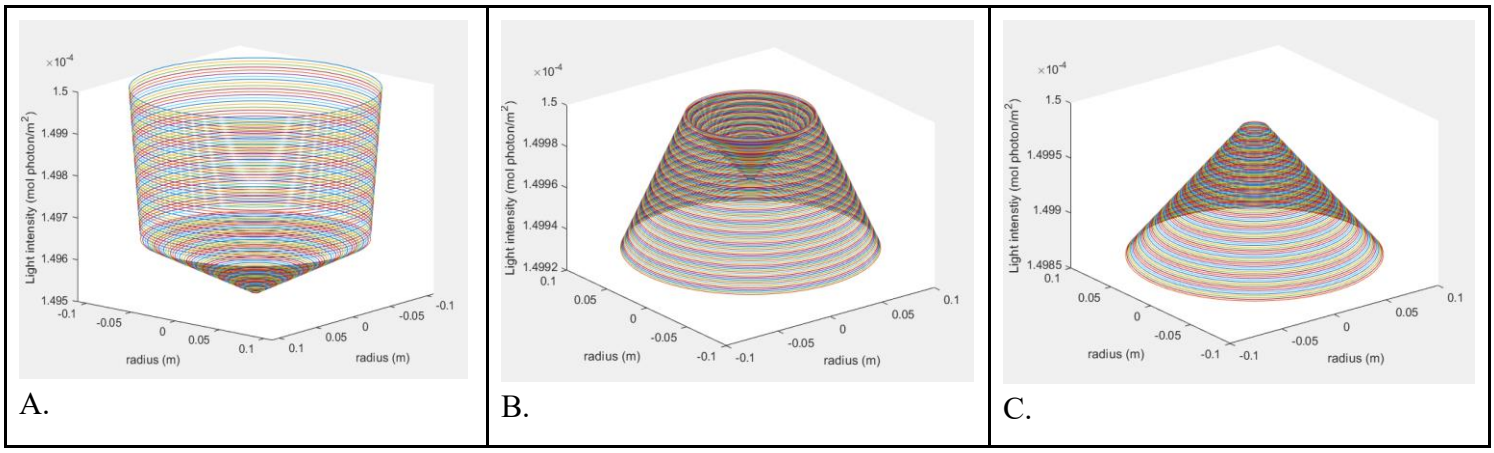

Figure 6. The output of simulated light distribution for three light configurations in water, each exposed to an initial intensity of 150 µmol photons/m^2 . (A) An even distribution of light is applied externally from a transparent glass reactor. (B) The light distribution is provided by the proposed helical lighting system. (C). An internal light rod is placed at the center of the reactor. Though the distribution makes a negligible difference, in water, it can be seen that the distribution is more even and and provides overall higher light intensity across the reactor in configuration B.

6.5.2 Sparge Velocity and Reactor Dimensions

The length of the bioreactor was mainly affected by bubble sparging because the bubbles dissolve with the increasing height of the bioreactor, as observed from previous research by Sanchez Miron et al. (1999). Therefore, the longest possible length at 1 m was chosen. For the diameter of the cylinder, the main component that would restrict its expansion would be the lighting system. From our model, we obtained sufficient data to support the selected diameter of 20 cm. The growth curve and final concentration is appropriate for the volume of water being cleaned and provides a biomass output that nears the goal that our team set out last semester of 4 kg.m-3 per batch in 2.3 days. Expanding the diameter of the cylinder would increase the volume of water that can be filtered per batch but would decrease the final algal concentration and would be considered less cost efficient since the space at the edges would be significantly darker than other areas within the cylinder and the algae would then not be accumulating any biomass in those regions, nor would they be taking up nutrients.

7.0. Final Design

7.1. Manufacturability and Economic Analysis

7.1.1 Functional Description of Reactor Subcomponents

The following is a general overview of key components that are central to the functionality of the reactor. While it lists the most crucial parts, it is not a comprehensive overview of all required components. CAD sketches of some of the subcomponents are provided in Appendix 1.2. It is important to note that this cannot serve as a manufacturing manual, and merely details physical functions within the system.

Reactor body: The body of the reactor is a vessel-type reactor, which consists of a cylindrical central body with a high volume-to-surface area ratio. Vessel-type reactors have advantages and disadvantages which must be considered in the creation of photobioreactors. The main advantages, which were ultimately considered the most important, are the low cost of manufacturing, ease of cleaning, and small space requirement for the output volume. The structure is a simple cylindrical tank with 5 mm thickness, that is covered in an insulating material for thermal conservation. This insulating material is not extremely significant, since the reactor is to run at 20°C. However, a layer of fabric can be used to reduce heat loss. The cylindrical body has a few features, including an bottom plate which is perforated with an array of sparge holes 5 mm in diameter, and 1 cm apart. The perforations are interrupted by an out-jutting groove which secures the lighting system frame (six steel rods, 1 cm thick; 1 m long). Beneath the sparge plate is a funnel-like structure which connects to a flow pipe 5 cm in diameter. The top of the reactor is closed, with a small opening sealed with a sterile filter tube leading to the fume hood of the laboratory. This is a safety precaution, since this prevents pressure buildup within the reactor, and allows exhaust gas to exit the lab space without any risk to the workers in proximity. The entire system can be disassembled, to allow for quick and simple cleaning, sterilization and loading. This is an important consideration, since easy maintenance can improve overall reactor efficiency over time, and can also prevent contamination of sample. In addition, worn components or broken parts can easily be replaced without replacing the entire system.

Lighting structure: The lighting structure is best described as a “double helix” of double-sided, waterproof LED strip lights. The double-helices were chosen for two reasons: (1) a light irradiance can be implemented in two normal directions, rather than for most systems, in which light can only radiate outwards in one normal direction. (2) The helix, though a seemingly complicated geometry, helps to simplify the wiring of the lighting system to an electrical input. This is important for safety reasons and for cost minimization. By mounting the lights on the 6-rod framework mentioned in the reactor body description above, the continuous strip lights can run through the entire reactor

length without interruption. In addition, this system can be easily removed from the reactor body for cleaning and sterilization between batches.

Sparge system: The sparge system must consist of a control valve, which modulates the flow and mixing of concentrated carbon dioxide with sterile ambient air. This control system must use a data acquisition system (DAQ), in which the pH sensor attached to the system provides data for how much CO_2 must be mixed into the gas mixture to maintain a neutral pH. The system should also take safety measures into account as per laboratory protocols. A control valve and the control system requirements have been considered in 7.1.2. However, due to time constraints, this aspect of the project is considered for the most part out of the scope of this project, and thus, a researcher planning on building this sparge system would have to implement the controls using their own design.

7.1.2. Control System Considerations

Many parameters affect the design and its efficiency so it is necessary to monitor them as precisely as possible to maintain optimal functioning of the design. It was decided that two sensors were necessary to monitor appropriately the design parameters, these were a turbidity sensor and a pH/temperature sensor.

The turbidity sensor was crucial for the control of the design since it is able to detect and monitor the level of suspended particles in water. The Gravity analog turbidity sensor in particular was chosen because it specified that it could be used in rivers and streams, which indicates that it is appropriate to use in an environment with fluid motion. It also specified that the threshold level could be changed. So, calculations could be performed using the model to find the duration of the experiment as well as the cell density at which the light level becomes too dim to provide the cells with sufficient energy for growth and reproduction. The sensor could then indicate that the output concentration of algae has been reached and that it is necessary to either increase the light level inside the reactor or to end the experiment, since the efficiency will decrease rapidly.

The pH meter from Extech instruments would be an important addition to the design since it is able to monitor pH and temperature in water. The need to control the pH of the solution comes from the fact that the pH is an indication of the availability of carbon dioxide for algae. As the algal cells take up carbon dioxide, it is necessary to introduce more into the system and this can be done through carbon dioxide-supplemented air sparging. The use of the pH meter will therefore help control and monitor the carbon dioxide levels in the system. As the cell concentration increases, the carbon dioxide requirements will increase as well and this will be observed through a change in pH. It can then be immediately remediated through an increase of the carbon dioxide level in the air being sparged into the system. These two sensors will therefore serve as the main control system for the bioreactor and will help maintain an optimal environment to benefit algal growth.

7.1.3. Economic Analysis of Design Implementation

An economic analysis of the design was necessary to assess its economic viability. The cost of each material was looked into thoroughly in Appendix 4, as well as the potential labor and maintenance costs. A long-term analysis was also carried out to determine the potential monetary yield from this design. The overall costs and revenues associated with the design are described in Table 3.

The economic efficiency of this design was compared to the one currently used in our client's laboratory. Currently, Mr. Loganathan is using Dr. Lefsrud's photobioreactor which is able to hold a volume of 15L per batch, whereas this design could hold a total volume of almost 42L. This increase in volume would allow our client to use larger quantities of wastewater and produce a higher biomass yield. The manufacturing costs of Mr. Loganathan's photobioreactor amounted to a total of 14,000 CAD. From section 5.4.1, this design is expected to cost a total of 1,400 CAD to manufacture. This is a log one reduction in cost and almost a threefold increase in volume, which represents significant savings for the client.

If after further research, results indicate that scaling up the design is a possibility, it would potentially lead to substantial savings for wastewater treatment facilities. In fact, the Canadian government currently offers a variety of funding or grant opportunities for facilities that are making an effort in using clean technologies. Some examples of these opportunities include the 'Clean Growth Program', the 'Emerging Power Renewable Program' and the 'Sustainable Development Technology Fund'. There are additional province-specific programs that aim to fund these facilities as well (Government of Canada, 2018). Adding on, the large-scale design could yield a much higher biomass output, enabling the facility to sell a reasonable amount of algal biomass to industries looking to convert it into a useful product. The cost of algal biomass is relatively high at approximately 650 CAD/ton (Davis et al., 2016). Although it was observed that for lab-scale production selling the biomass did not result in any profit, at an industrial scale there is more potential to profit from the biomass output.

By calculating the net present value and internal rate of return of the design, it was clear that this design would not yield any monetary gain to the user. However, photobioreactors are usually never able to make profit since the design is initially quite expensive and it is a highly energy-consuming product. The main goal of this project was to lower the initial cost of manufacture of the design to make it more affordable to research laboratories. It was observed in the previous section that this goal had been achieved, assuming it all goes accordingly. Although it was very attractive to make this design profitable, no technique allows for photobioreactors to yield profits yet. Still, this design remains economically viable, even if not profitable, due to the significant reduction on investment.

Table 3. Total costs and revenues associated with proposed photobioreactor design (in CAD)

Total Cost of Manufacture	1401.72
Total Cost of Operation and Maintenance *	380.290299
Total Profitability *	0.14926275
Net Present Value (5 years)	-2792.58957
Internal Rate of Return	-175%

* per year

7.2 Design Validation

7.2.1 Final Design Risk Assessment: Usability and Safety

To have a better overview of the risks associated with this design, it was necessary to carry out a thorough risk analysis. This provided insight on the various dangers associated with the design and allowed for a reflection on how these could be efficiently avoided or mitigated. Two major types of risks were observed: risks associated with design failure, and risks associated with worker harm. A design failure may lead to serious environmental or health consequences. Monitoring the system as a whole would be necessary to prevent this from happening. The risk of workers getting harmed could be caused by certain parts and components of the design, either due to improper handling or insufficient maintenance. However, this system was designed for ease of use by all employees. The following risk assessment matrix outlines the potential dangers of our design, by giving them a risk level from one to three corresponding to lowest potential and danger to highest potential and danger respectively.

Table 4. Assessment Matrix Associated with photobioreactor design, including large-scale implications

Risk Factor	Risk Level	Contributing Factors	Mitigation Procedure
Insufficient nutrient removal	3	- Feedstock with higher nutrient concentration - Inefficient nutrient uptake by algae	- Testing output concentration before release - Treat again if insufficient
Electrocution from lighting inside bioreactor	1	- Improper lighting set up or maintenance	- Certified electrician and/or plumber for installation - Worker training - Removable lighting structure for ease of use
Cross contamination	2	- Microbes from surrounding environment - Leak in bioreactor	- Closed system - Regular tests and maintenance
Worker exposure to waste contaminants	1	- Wastewater as feedstock	- Worker training
Acidic pH of media due to CO2 sparging	1	- CO2 input for algae photosynthetic growth	- Monitor pH and remove excess CO2 at output - Possibly add base
Accumulation of photosynthetic oxygen	1	- Oxygen as by-product of photosynthesis	- Output airflow at top of bioreactor
Working with pressurized gas	2	- Improper handling or storage of gas cylinders	- Follow OSHA standards (1910.101) - Worker training
Potential leakage	1	- Equipment failure	- Changing components before the end of their life-expectancy

The highest risk associated with our design would be insufficient nutrient removal. This could only be a problem if the design is implemented in a wastewater treatment facility, if the scaled up design is viable. However, from the results of the lab-scale design, the current parameters

and processes have shown that the algae is able to take up phosphorus efficiently and the effluent concentration should be well below that indicated in regulations, as long as the design is used properly. In the case of the industrial scale photobioreactor design, insufficient nutrient removal could result from a failure of the design or its inappropriate use and would have serious negative health effects on the community. However it can be mitigated by monitoring the system as a whole as well as testing the output water, and possibly reusing the output water as feedstock for another batch if the first treatment was insufficient. Although there are other smaller risks associated with this design, they should be easily avoided through continuous and thorough monitoring as well as careful handling of the system.

7.2.2. Final Design Life Cycle Analysis (LCA) Considerations

To assess the impact of the design manufacturing and long-term use, a preliminary life cycle analysis (LCA) was performed using previously recorded data and studies, as well as assumptions about the quantity of materials required for manufacturing the proposed design. It is recommended to consider the framework and working design when conducting a Life Cycle Analysis, so a modified version of the ISO 14044:2006 (E.) framework was used to guide the design's environmental thinking. A conceptual map of the overall treatment process was also created and is attached in Appendix 3 (a) of this report.

Data collected for the manufacturing of each subcomponent (reactor body, LED light strips, sensors, etc.) was compiled from the literature, as well as from the OpenLCA software database. Approximations of certain parameters, such as potential for global warming, human toxicity, eutrophication, and others (see Table 5 below), were calculated using the United States Environmental Protection Agency's Tool for the Reduction and Assessment of Chemicals and other environmental Impacts (U.S. EPA TRACI 2.1) as well as the Intergovernmental Panel on Climate Change (IPCC)'s 2.10.2 Direct Global Warming Potentials data.

According to the International Standard for Environmental management -- Life Cycle Assessment guideline (ISO 14044, 2006), a full inventory of Life Cycle can only be achieved with data and monitoring. While some data is available through the literature, future tests of inputs, outputs and other components which are not yet materialized must be conducted in order to complete a full life cycle analysis. The simulation of the design was not sufficient to provide data for a complete LCA and building a prototype would be strongly recommended to study the system's environmental impact.

The calculations to approximate impact are represented using estimated amounts of subcomponents of the product. Note that only the reactor body has been considered, though influents and effluents, as well as accessories to the system will add complexity and increase the impact factors to the system. Impact factors considered were only the ones with the most

significant values according to the approximations and examples of other Life Cycle Analyses in the industry.

LED light approximations.

Using the U.S. Department of Energy's Life Cycle Assessment of Energy and Environmental Impacts of LED Lighting Products, the material content in an LED light was assumed to be a Mid-Power LED, with encapsulated packaging, and that the entire system would have a lifetime of approximately 13.7 years (U.S. Department of Energy, 2012). The strip LED was assumed to be made up of a square sapphire wafer substrate with side dimensions of 0.5 cm with gallium arsenide phosphide (corresponding to emission of red light) grown on top (U.S. Department of Energy, 2012). The process was simplified into a three-step process; namely, the substrate production, LED die fabrication, and packaging. The subprocesses are specified in Appendix 3 (b) of this report. Inputs are scaled from the Life Cycle Analysis according to our specifications as compared to the analysis referenced.

Stainless Steel body approximations.

Values for emissions and involved chemical components were taken from calculations reported by Itsubo & Inaba (2003). Fewer assumptions were made than for the LED lighting component due to fewer variations in the manufacturing processes. The sub-processes and calculations are specified in Appendix 3 (c) of this report.

Table 5. Summary of Life Cycle Analysis key parameters for manufacturing of photobioreactor

Impact Category	**Methodology**	**Approximate Value per Bioreactor Unit per year**
Subcomponent-Lighting component	*Avg. Life: 13.7 years	
Cumulative Energy demand (kWh/year)	U.S. Dept. of Energy	13.98 (includes use per year and manufacturing)
Ecotoxicity (CTU eco/comp, Emissions Fresh water C, freshwater)	TRACI 2.1; IPCC 2.10.2	326
HH Particulate Air (PM 2.5 eq/ comp)	TRACI 2.1; IPCC 2.10.2	$8.29*10^{-2}$
Global Warming	TRACI 2.1; IPCC 2.10.2	Not enough information
Human Health (CTU noncancer/comp, emission to urban air, non-cancerous)	TRACI 2.1; IPCC 2.10.2	$7.6*10^{-4}$
		*End of Life: Non-recyclable (80%)
Subcomponent: Stainless Steel components (Reactor tank, piping)	*Avg. Life: 30 years	
Cumulative Energy demand (kJ/year)	Dusart et al., 2011	0.011
Ecotoxicity (CTU eco/comp, Emission fresh Water C, freshwater)	TRACI 2.1	$3.00*10^{-5}$
Eutrophication	TRACI 2.1	$9.87*10^{-4}$
Global Warming	TRACI 2.1	2.07
Human Health (CTU noncancer/comp, emission to urban air, non-cancerous)	TRACI 2.1	$3.40*10^{-11}$
Acidification air	TRACI 2.1	$7.53*10^{-3}$
		*End of Life: Recyclable

Limitations and additional notes.
As previously mentioned, other subcomponents were not included in this analysis. Some impact factors were ignored for simplicity's sake. Additionally, transport and flows during operation were not used. Calculations were placed in a per-year basis; this is not a standard protocol. However, since the system would function as separable components, and thus have distinctively different life spans, the components were placed in a per year basis for ease of comparison.

8.0 Conclusion

The design's success would depend on three major factors: the biomass output, the nutrient concentration in the effluent and the cost of the design. From the results of the simulation, the design appears to be creating an appropriate environment to benefit algal growth. Over the course of the experiment, where one batch would last 2.3 days, it was expected that the design would be able to treat wastewater so that effluent concentrations would be below nutrient concentrations specified in Canadian regulations. During that time, it was also expected to be able to grow algae to a final concentration of 4 kg/m^3. By thoroughly testing the model multiple times, it was determined that the design was successful at removing nutrients and that effluent concentrations would be well below the recommended level after 2.3 days of treatment. However, the biomass production over that period of time did not meet the expected concentration since the biomass output only reached 0.06 kg/m^3. The design would therefore be successful in a wastewater treatment setting but the level of biomass produced does not allow it to be competitive with other existing bioreactor designs. Regarding the cost of the device, for lab-scale use, it appears to be significantly less expensive than current bioreactors, especially in our client's case. This bioreactor was designed to be more affordable for research purposes and to have a lower environmental impact than existing designs.

While the principal objective of this design was solely for research purposes, bigger-picture thinking is required in justifying the usefulness of such research to the larger society. Therefore, potential for public and commercial services must be considered. Despite the high energetic and maintenance costs of photobioreactors, it is important to note that the use of photobioreactors could still be useful on an industrial scale to offset costs of wastewater treatment through purified, uncontaminated biomass production. The current status of wastewater treatment in most urban settings is that the public cost for treatment of waste effluent is astronomical. Given the expanding market for biofuels and other bio-based markets, some experts estimate that by selling the biomass product, municipalities and industries could offset current treatment and sanitation costs by 10% , bringing costs from 0.25-0.50 EUR/m^3 to 0.23-0.45 EUR/m^3 (Kligerman et al., 2015; Vulsteke et al., 2017). In a large-scale municipal or industrial scale operation, these reductions could be significant. These estimates most likely refer to open-pond systems, which are significantly less expensive than bioreactor systems. However, open pond systems are significantly less efficient, and produce contaminated biomass because of the lack of control available. Therefore, the

implementation of bioreactor systems on an industrial scale may still be justified. A more thorough economic analysis must be performed to make an assessment, especially since these figures are likely dependent on location and thus, are variable. An economic incentive as well as the existing social and environmental one could also drive up standards of sanitation and ecological regulations.

For bioreactor systems involving algae treatment to function, governmental policies must be implemented to encourage such research and development. Bioreactors have already been implemented in various forms in existing wastewater treatment settings. These usually use bacteria and anaerobic microorganisms for removal ammonia and phosphorus (Alberta Capital Region Wastewater Commission, n.d.). Such systems, however, do not provide any source of revenue, and are an enormous public cost. By incorporating autotrophic organisms such as algae, governments and communities can gain many benefits, including carbon sequestration, biomass for a variety of bio-based products, and clean processed water. Biological Nutrient Removal (BNR) has been implemented before, and therefore, implementing a new variety of BNR systems is feasible if the government shows sufficient interest for further research and development. Additionally, encouraging algal treatment of water could drive markets such as algal biofuel industries, further adding to long-term environmental, sustainability, social and economic benefits for the community.

Further work on this design would be necessary prior to implementation. Models are very useful tools to represent systems but they are not accurate. Manufacturing the bioreactor and performing actual tests would provide crucial data to support the conclusions of this report. The scaled-up version of this design was not thoroughly reviewed in this report. If a scaled-up design were to be implemented, fluid flow and light path models would be necessary to perform more in-depth testing and measure the proposed solution's capacity in an industrial setting. Optimizing the biomass output would also be interesting to continue studying. It may be possible to extend the duration of the experiments to allow the algae to reproduce longer. If this path were pursued, nutrient concentration may become limiting and it could be interesting to study a continuous-system for the bioreactor, where feedstock would be added to the device continuously. This could allow for more wastewater to be treated and for increased biomass output.

9.0 References

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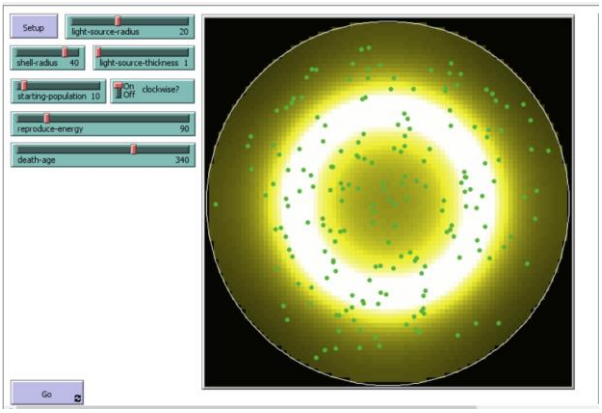
Appendices

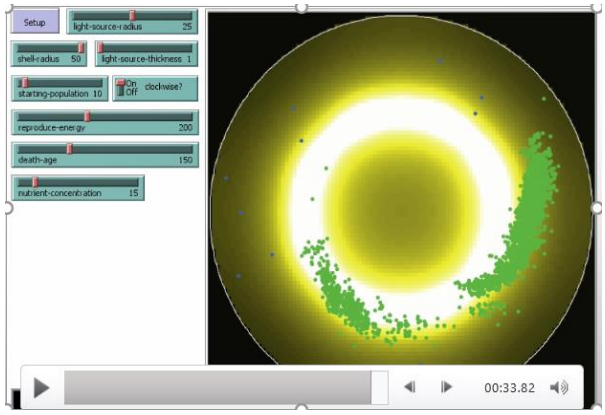
<u>Note:</u> All program files used to report the modelling process can be accessed in the Google Drive Folder here.

Appendix 1. Schematics and Drawings

Appendix 1.1. Example of NetLogo graphical User Interface.

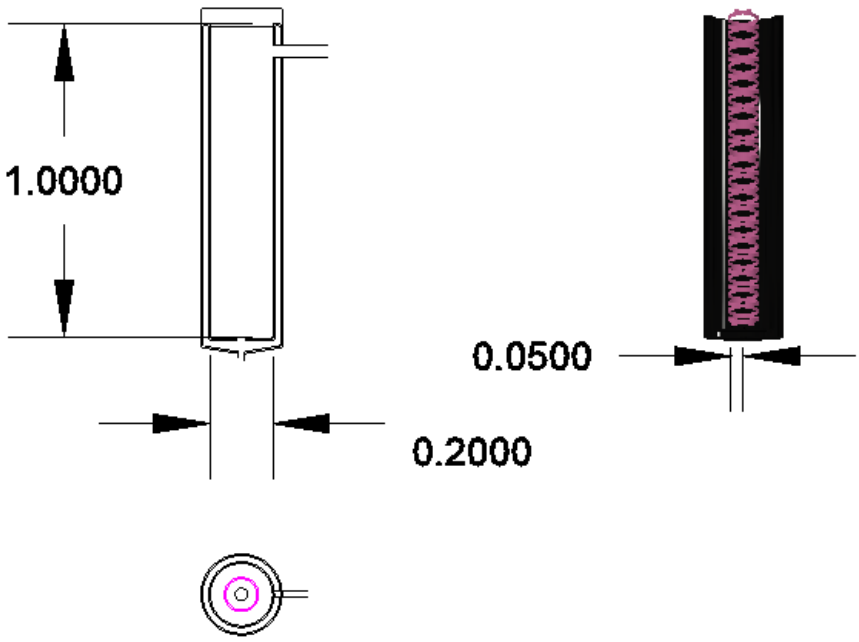


(A) Simulation of reactor with "Brownian motion" of algae agents



(B) Simulation of reactor with swirl mixing of algae agents

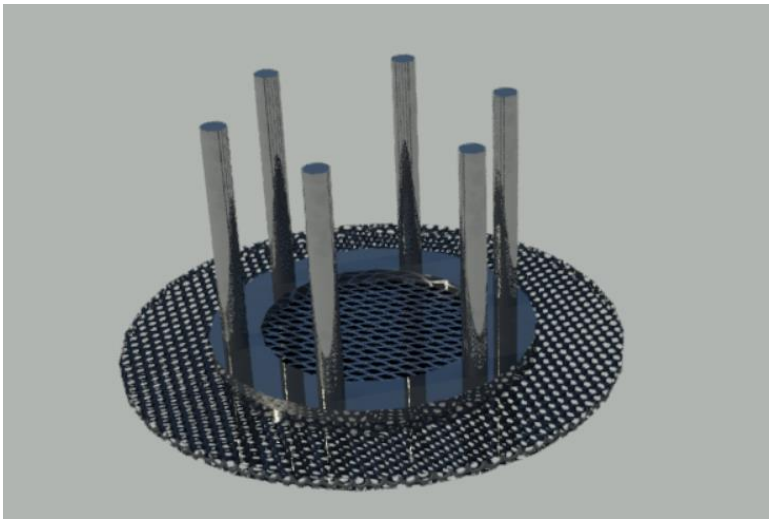
Appendix 1.2. Design Drawings



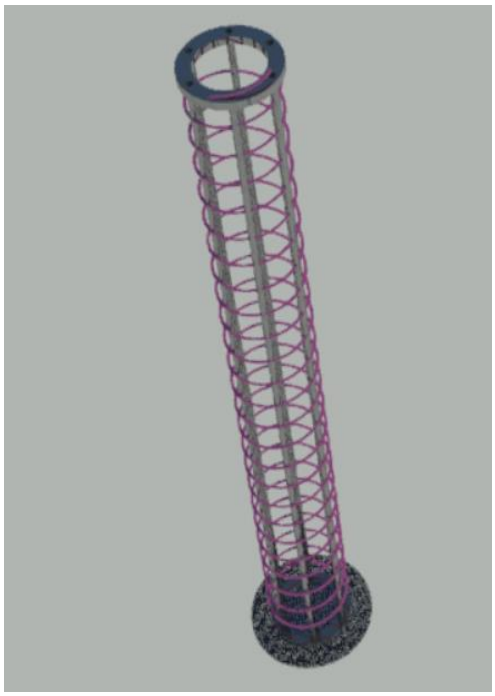
A general schematic representing the proposed design: *Top left*: cross sectional view of design reactor body, including sparging inflow, exhaust and proposed dimensions. *Bottom Left*: Top view of cylindrical photobioreactor including spiral-formed lighting. *Top right*: cross sectional schematic of assembled lighting and design body.

A 3D AutoCAD rendering of the conceptual design is shown here for clearer viewing:

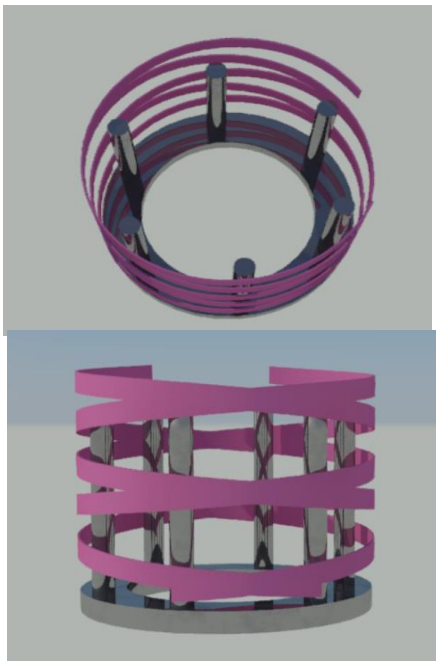
Sparge Plate: 3D AutoCAD rendering of sparge plate design with portion of light rod frame inserted in the light securing system shown for reference. The steel rods with the light helix are one unit which are completely removable from the reactor plate. The mesh represents the sparge plate, which in reality would contain holes 5 mm in diameter, arranged in an array with spacing of 1 cm.



Lighting Structure:



A.



B.

(A). The helical structure mounted on the six-rod frame is shown above. The helix is represented as a thinner structure than the proposed design of 1 cm - thick LED strip lights. This is to help with visibility of the configuration.

(B). Two close-up views of the mounted double-helix structure is shown at the base of the mounting structure. The pink ribbon represents the LED strip light, which consists of a series of small LED bulbs on one continuous light tape.

Appendix 2. Calculations

Appendix 2.1. Algal Concentration Calculations.

Volume modeled by cross section: $V = \pi \times r^2 \times h = \pi \times 0.1^2 \times 0.02 = 6.283 \times 10^{-4}\ m^3 = 628.3\ mL$

Initial concentration (input): $Ci = 10^5\ cells/mL$

Relation between modeled and actual concentrations:

$Nb\ cells\ modeled = Ci \times V = 10^5 \frac{cells}{mL} \times 628..3\ mL = 6.28 \times 10^7\ cells$ (in cross section)

$Starting\ population\ modeled = 10\ dots$

So: $1\ dot\ cell = 6.28 \times 10^6\ real\ cells$

Initial mass concentration: $Ci = 10^5 \frac{cells}{mL} \times 37 \frac{fg}{cell} = 3.7 \times 10^{-6}\ g/mL = 3.7\ microg/mL$

$C = nb\ cells \times 6.28 \times 10^6 \div (628.3\ mL \times 0.001) = 1 \times 10^7 \times nb\ cells\ \ cells/L$

Appendix 2.2. Beer-Lambert Law.

With A: absorbance, ϵ: molar extinction coefficient, l: light path length, C: algal concentration,

I: light intensity, I_0: initial light intensity

So, our Beer-Lambert equation: $A = \epsilon \times l \times C = 9.12 \times 10^6 \times 0.25 \times C = 2.28 \times 10^6 \times C$

So: $I = I_0/10^A = 150/10^{2.28\times10^6\times C}$

Appendix 2.2.1. Light Intensity Calculations.

$$I = I_0/10^A = 150/10^{2.28\times10^6\times1.66\times10^{-20}\times nb\ cells} = 150/10^{3.7848\times10^{-14}\times nb\ cells}\ umol/m^2.s$$

Borrego et al. (1999) have approximated the molar extinction of the BChl bacteria in a solvent solution to 48.9 $mM^{-1}\ cm^{-1} = 4.89*10^6\ M\ m^{-1}$. This factor was later implemented in the NetLogo model to adjust for absorbance values of the cells and solution themselves.

This, the final light intensity equation implemented was

$$I = I_0/10^A = 150/10^{2.28\times10^6\times1.66\times10^{-20}\times nb\ cells} = 150/10^{(3.7848\times10^{-14}+4.89\times10^{-6})\times nb\ cells}\ umol/m^2.s$$

Appendix 2.3. Doubling Time Equation. (Celekli et al., 2008; De Morais and Costa, 2007)

$DT = ln(2) \div \mu_{max}$

with DT: doubling time, and μ_{max}: maximum specific growth rate

Appendix 2.4. Generation Time Equation. (Madigan et al., 2015)

$$N = N_0 * 2^{t/g} \equiv g = ln(2) * t \div ln(\frac{N}{N_0})$$

With N: number of cells, N_0 : initial number of cells, t: time, g: generation time

Appendix 2.5. Biomass Production Requirements for Phosphorus Removal.

$$MM(PO_4^{3-}) = 30.97 + 16 * 4 = 95 \frac{g}{mol}$$
$$C_i = 135\,\mu mol/L = 135 * 10^{-6}\,mol * 95 \frac{g}{mol} = 12.8\,mg/L$$
$$C_f = 0.5 \frac{mg}{L} = \frac{0.5}{95} = 5\,\mu mol/L$$
$$So: \Delta C = 130\,\mu mol$$
$$Y_P = 1.53\ \frac{mg - biomass}{\mu mol - P}$$
$$So: Biomass\ Produced = \Delta C * Y_P = 130 * 1.53 = 199\,mg$$

Appendix 2.6. Total Biomass Produced.

$$C_i = 3.14 * 10^9\ cells/batch\ \ and\ C_f = 5.11 * 10^{10}\ cells/batch$$
$$\Delta C = 4.8 * 10^{10}\ cells/batch$$
$$DW = 37\,pg = 37 * 10^{-12}\,g$$
$$So: Biomass\ Produced = 4.8 * 10^{10} * 37 * 10^{-12} = 1.77\,g$$

Appendix 2.7. Calculation of initial sparge velocity within reactor

Mass flow rate of the bubble is constant before, during and after passing through the sparge plate. This can be represented by the following equation:

$$\hat{m} = \rho v_1 * A_1 = \rho v_2 * A_2$$

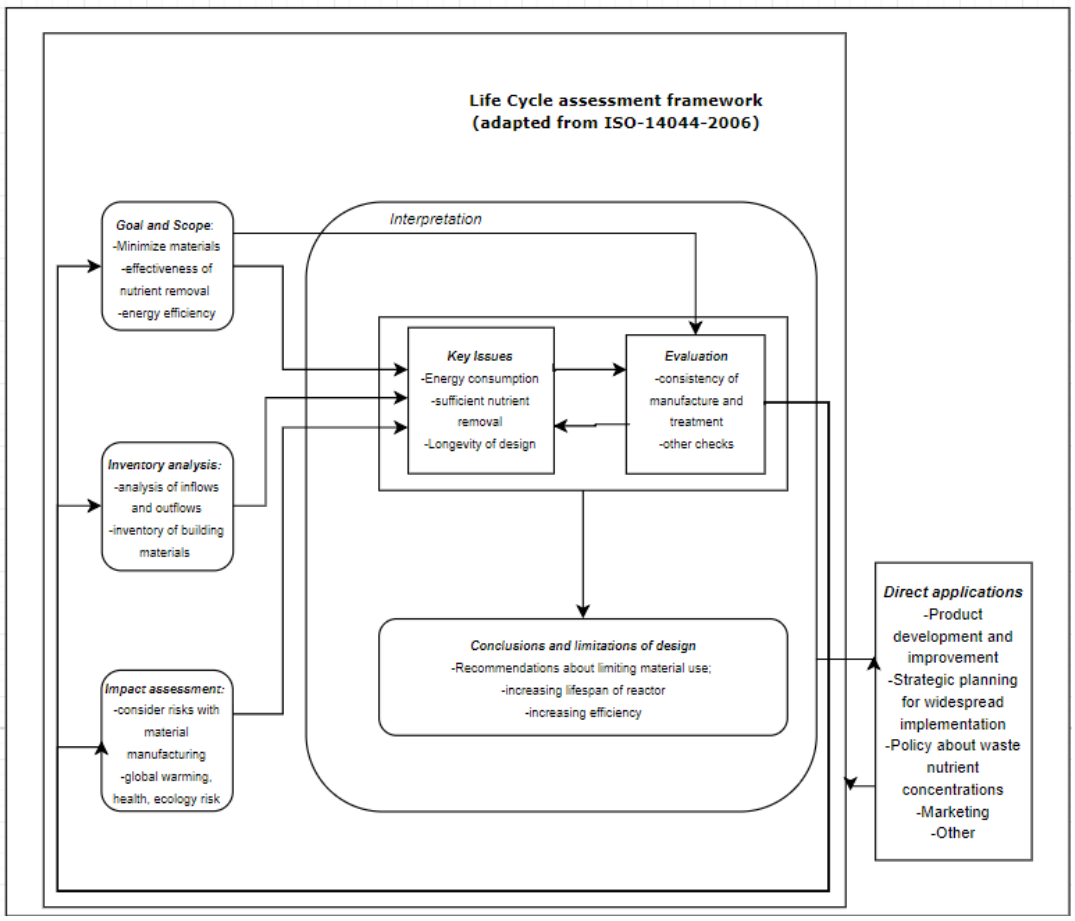
To determine the minimum bubble velocity within the reactor after passing through the sparger, therefore, density is assumed to be constant, and thus

$$2.67 * \pi * 0.005^2 = v_2 * \pi * 0.1^2$$
$$v_2 = 6 * 10^{-3}\ m/s$$

Note that this assumes incompressible flow, and neglects the process of bubble formation. Therefore, this approximation is limited, and further experimental analysis is required.

Appendix 3. LCA

Appendix 3(a) ISO-14044-2006 (E) framework, modified for particulars of proposed bioreactor system.



Appendix 3 (b). Calculations and data for determination of LCA of LED subcomponent.
Step 1: Gather Data for subcomponents and processes in manufacture process (*Note: "original" denotes the values from the US Department of Energy document (2012), while the "scaled" values are approximated values assuming a proportional relationship in manufacture, and using a factor of 3.15 (calculated by approximating the length of LED strip needed for design as compared to manufacture of LED bulb))*.

Sapphire Wafer										
Step	Energy Inputs (kWh/wafer-from Energy.gov table)	*Scaled energy inputs	Alumina inputs (g-original)	*Scaled alumina inputs	Water inputs (L/wafer-original)	*scaled water inputs	Cleaner inputs (original-L/wafer)	*Scaled cleaner inputs		
Boule growth	15.51	48.8503937	16.61	52.31496063	100	314.9606299	0	0		
Core fabrication	1.35	4.251968504	0	0	0	0	0	0		
Wafer slicing	1.24	3.905511811	0	0	2	6.299212598	0	0		
Lapping and beveling	0.09	0.283464567	0	0	0.67	2.11023622	0	0		
polishing and chemical-mechanical planarization	0	0	0	0	0	0	0	0		
geometry and optical inspection	0.001	0.003149606	0	0	2	6.299212598	3.5	11.02362205		
Totals		**57.29448819**		**52.31496063**		**329.6692913**		**11.02362205**		

Die Fabrication										
Step	Energy, original (kWh/wafer)	Energy. Scaled	H2, original (m3)	H2, scaled	NH3, original (m3)	NH3, scaled	TMGa, original (g)	TMGa, scaled	N2, original (m3)	N2, scaled
Bake	8.75	27.55905512	0.06	0.188976378	0.02	0.062992126	0	0	0	0
Nucleation Layer	4.74	14.92913386	0	0	0	0	0	0	0	0
Temp ramp	1.46	4.598425197	0	0	0.02	0.062992126	0	0	0	0
Buffer+ N layer	9.77	30.77165354	1.54	4.850393701	0	0	1.38	4.346456693	1.54	4.850393701
Active layer MQW	4.74	14.92913386	0.01	0.031496063	0.01	0.031496063	0.03	0.094488189	0.01	0.031496063
P layer	3.28	10.33070866	0.06	0.188976378	0.02	0.062992126	0.06	0.188976378	0.06	0.188976378
Totals		**103.1181102**		**5.25984252**		**0.220472441**		**4.62992126**		**5.070866142**

Post-epitaxy steps	Energy, scaled	Target Ag (mm4/wafer, scaled)	Ti scaled (mm4/wafer)	W, scaled (mm4/wafer)	UPW (L/wafer)	N2, scaled (m3)	Acetone, scaled (L/wafer)	developer, scaled (mL/wafer)	etchant Ag, scaled (mL/wafer)	Etchant Metal, scaled (mL/wafer)	GaN Etchant, scaled (mL/wafer)	Photoresist, scaled (mL/wafer(	SF6, scaled (l/wafer)	Target Ni (mm4/wafer)
Wafer Inspection	0.0944882	0	0	0	0	0	0	0	0	0	0	0	0	0
P contact	3.7480315	1.385826772	1.480314961	9.732283465	188.976378	2.204724409	0	0	0	0	0	0	0	0
N contact opening	7.2440945	94.48818898	0	0	188.976378	2.204724409	0.62992126	157.480315	125.984252	188.976378	0.598425197	25.19685039	0	0
GaN pattern	8.5354331	0	0	0	188.976378	2.204724409	0.31496063	78.74015748	0	0	0	12.5984252	0.31496063	0
N contact	3.5590551	0	0	0	188.976378	2.204724409	0.31496063	125.984252	0	0	0	15.7480315	0	1.322834646
Other	7.7795276	0	0	0	0	0	0	0	0	0	0	0	0	0
Totals	**30.96063**	**95.87401575**	**1.480314961**	**9.732283465**	**755.9055118**	**8.818897638**	**1.25984252**	**362.2047244**	**125.984252**	**188.976378**	**0.598425197**	**53.54330709**	**0.31496063**	**1.322834646**

Packaged LED Assembly	Ceramic substrate-2 layer alumina (g)	Energy	ESD diode (silicon) (g)	Gold (g)	Underfill (g)
Totals	**0.0425197**	**0.094488189**	**0.173228346**	**0.000188976**	**0.061732283**

Step 2: Using TRACI and IPCC methods, calculate impact factor sums (per year of life span), by multiplying impact factor by kg of material (*Note: scaling and conversions are included in calculations shown below*).

mass in system	UUID	Formatted CAS #	Substance Name	HH Particulate Air (PM2.5 eq / kg substance)	Ecotox. CF [CTUeco/kg], Em.airC, freshwater	Human health CF [CTUcancer/kg], Emission to cont. rural air, cancer
52.35748031	39148248	39148-24-8	FOSETYL-ALU	0.00E+00	1.33E+00	2.68E-10
11.02362205	68439509	68439-50-9	ALCOHOLS, C	0.00E+00	3.65E-04	n/a
0.220472441	7664417	7664-41-7	AMMONIA	1.88E+00	n/a	n/a
13.88976378	17778880	17778-88-0	NITROGEN	0.00E+00	n/a	n/a
1.322834646	x	x	NICKEL(II)	0.00E+00	8.05E+00	4.11E-09
410.8346457	x	x	SILVER(I)	0.00E+00	2.40E+03	1.04E-02
0.31496063	2551624	2551-62-4	SULFUR HEXA	0.00E+00	n/a	n/a
415.8097638	130154	130-15-4	1,4-NAPHTHO(	0.00E+00	2.04E+03	n/a
412.1574803			COPPER(II)	0.00E+00	4.04E+00	2.32E-09
52.35748031	x	x	ARSENIC(III)	0.00E+00	5.58E-01	1.49E-06
400	101906	101-90-6	OXIRANE, 2,2'-	0.00E+00	n/a	n/a
400	79107	79-10-7	ACRYLIC ACID	0.00E+00	1.45E+00	9.71E-06
1.25984252	67641	67-64-1	ACETONE	0.00E+00	9.99E-05	1.22E-11
Totals per year				8.29E-03	3.26E+02	7.60E-04

Appendix 3 (c.) Calculations and data for determination of LCA of LED subcomponent.
Step 1: Gather Data for subcomponents and processes in manufacture process (*Note: All values are taken from the table below, from Itsubo & Inaba (2003)'s research)*.

Table 2 Inventory table for manufacturing 1 kg of stainless steel sheet and carbon steel sheet.

	Carbon steel sheet (1 kg)	Stainless steel sheet (1 kg)
CH_4	5.50×10^{-6}	1.71×10^{-5}
CO_2	1.98	4.14
N_2O	6.11×10^{-5}	3.86×10^{-4}
NO_x	1.00×10^{-3}	6.40×10^{-3}
SO_2	1.60×10^{-3}	1.03×10^{-2}
SPM	2.56×10^{-5}	1.10×10^{-4}
As	1.13×10^{-9}	3.53×10^{-9}
Cd	5.67×10^{-11}	1.76×10^{-10}
CO	3.11×10^{-4}	1.70×10^{-3}
Cr	1.13×10^{-9}	3.53×10^{-9}

Source: Isubo & Inaba (2003)

Step 2: Using TRACI and IPCC methods, calculate impact factor sums (per year of life span), by multiplying impact factor by kg of material (*Note: scaling and conversions are included in calculations shown below: the assumed mass was 15 kg of steel, and the scaling factor is 15* the inventory values above*).

CAS #	Formatted CAS #	Substance Name	Global Warming Air (kg CO2 eq / kg substance)	Acidification Air (kg SO2 eq / kg substance)	Eutrophication Water (kg N eq / kg substance)	Ecotox. CF [CTUecol/kg], Em.airU, freshwater	Ecotox. CF [CTUecol/kg], Em.fr.waterC, freshwater	Human health CF [CTUnoncancer/kg], Emission to urban air, non-canc.
74828	74-82-8	METHANE	6.41E-03	0.00E+00	0.00E+00	n/a	n/a	n/a
10102440	#######	NITROGE	0.00E+00	4.05E-03	1.69E-03	n/a	n/a	n/a
x	x	NITROGE	0.00E+00	6.72E-02	2.79E-02	n/a	n/a	n/a
124389	124-38-9	CARBON	6.21E+01	0.00E+00	0.00E+00	n/a	n/a	n/a
7446095	#######	SULFUR [	0.00E+00	1.55E-01	0.00E+00	n/a	n/a	n/a
x	x	ARSENIC	0.00E+00	0.00E+00	0.00E+00	3.37E-04	8.06E-04	9.04E-10
x	x	CADMIUM	0.00E+00	0.00E+00	0.00E+00	1.04E-05	2.56E-05	1.17E-10
x	x	CHROMIU	0.00E+00	0.00E+00	0.00E+00	2.74E-05	6.85E-05	1.89E-16
630080	630-08-0	CARBON	0.00E+00	0.00E+00	0.00E+00	n/a	n/a	n/a
Totals			6.21E+01	2.26E-01	2.96E-02	3.75E-04	9.00E-04	1.02E-09
Totals per year			2.07E+00	7.53E-03	9.87E-04	1.25E-05	3.00E-05	3.40E-11

Appendix 4. Economic Analysis: Cost Worksheet

Unit	Quantity	Total Cost (CAD)	Working Period (years)
304 Stainless steel pipe [1]	1	25.00	30
304 Stainless steel sheet [2]	1	53.72	30
LED strips 32 W/m^2	6.3	252.00	15
Pump 12V 1.2L/min	2	44.90	5
Brass valve	2	13.20	10
PVC Plastic Tubing	1	5.28	50
CO2 bone dry gas	1	50.00	0.583
Steel rods [3]	3	39.60	30
Feedstock (Wastewater)	31.4 L	0	-
Scenedesmus obliquus culture	1	165.10	-
Turbidity sensor	1	22.10	1
Waterproof pH meter	1	47.99	1
Labor costs for manufacture	-	500.00	-
Subtotal	-	**1218.89**	-
Safety factor	15%	182.8335	-
Total	-	**1401.72**	-

[1] Pipe with dimensions of 1m length and 20 cm diameter for outer structure of bioreactor

[2] Sheet with dimensions of 50cm x 75cm for top and bottom lids of bioreactor and bubble break for CO2 sparging

[3] Rods of 1m length to hold up lighting structure

Appendix 4.2. Net Present Value Equation.

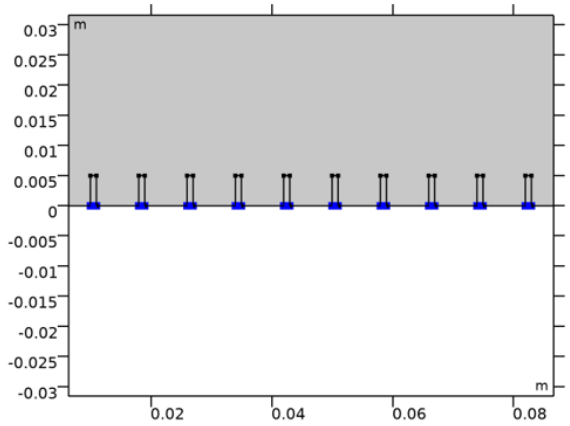
$$\mathrm{NPV} = \sum_{k=0}^{n} \frac{Profit_k - Cost_k}{(1+i)^k}$$

Appendix 5. Secondary physics-based models.

Appendix 5.1 COMSOL Velocity Profile.

A preliminary study of the fluid flow was implemented in COMSOL. This model has limitations, because it did not solve for two phase reacting flow, but rather as a laminar flow reaction. This therefore does not treat the bubble sparge as individual bubbles which slowly release CO_2 into the surrounding liquid using Henry's Law, as a more comprehensive model would. The simplification was made due to the lack of access to required modules and due to time limitations.

The sparge plate was modelled using inlet flows, shown in the screenshot below:



Inlet 1

Select Laminar Flow Equations are built into the software, and are reported here:

$$\rho\frac{\partial \mathbf{u}}{\partial t} + \rho(\mathbf{u}\cdot\nabla)\mathbf{u} = \nabla\cdot[-p\mathbf{I} + \mathbf{K}] + \mathbf{F}$$
$$\rho\nabla\cdot(\mathbf{u}) = 0$$
$$\mathbf{K} = \mu\left(\nabla\mathbf{u} + (\nabla\mathbf{u})^{T}\right)$$

The velocity profile of the sparge system at a steady state, neglecting effects of lighting framework are shown in the screenshot of the time-dependent study shown below. The model was implemented as a two dimensional axisymmetric system, neglecting the effects of the lighting structures on the fluid flow. This results in another obvious limitation to the study. A more comprehensive study of the fluid flow is suggested, using more accurate physical models (two-

phase reaction), and more accurate representations of the geometric interference. Furthermore, this study could examine the effect of scale up on the velocity profile and overall mixing of the system.