EVALUATION OF MICROBIAL GROWTH PARAMETERS AND ENVIRONMENTAL IMPACTS OF MICROALGAL BIO-FACADE MODULAR SYSTEMS

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ETHICAL DECLARATION

I hereby declare that I am the sole author of this thesis and that I have conducted my work in accordance with academic rules and ethical behavior at every stage from the planning of the thesis to its defense. I confirm that I have cited all ideas, information and findings that are not specific to my study, as required by the code of ethical behavior, and that all statements not cited are my own.

Zehranur TEKIN

16.01.2024

ABSTRACT

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Master's Program in Bioengineering

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This Master's thesis investigates the potential of microalgal bio-facade modular systems in bioengineering and environmental sustainability. The study focuses on evaluating the challenges and opportunities associated with optimizing photobioreactor systems for microalgae cultivation in building facades. It emphasizes the unique growth characteristics of microalgae, including their rapid growth rate, adaptability to diverse environments, high lipid content, and their role in capturing carbon dioxide and producing oxygen. The research assesses the environmental and economic benefits of integrating microalgae into building facades, highlighting their potential to contribute to the visual and environmental appeal of urban buildings. The thesis also proposes design guidelines for photobioreactor systems to maximize growth and productivity while minimizing environmental impacts. Overall, this research aims to contribute to the development of sustainable and environmentally friendly solutions in bioengineering, aligning with the goals of environmental sustainability and decarbonization within the European Green Deal objectives.

Keywords: Microalgae, *C. vulgaris*, Bio-façade systems, Photobioreactor systems, Environmental sustainability.

ÖZET

MİKROALGAL BİYO-CEPHE MODÜLER SİSTEMLERİNDE MİKROBİYAL BÜYÜME PARAMETRELERİNİN VE ÇEVRESEL ETKİLERİNİN DEĞERLENDİRİLMESİ

Tekin, Zehranur

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Ocak, 2024

Bu yüksek lisans tezi, mikroalgal biyo-cephe modüler sistemlerinin biyomühendislik ve çevresel sürdürülebilirlikteki potansiyelini araştırmaktadır. Çalışma, bina cephelerinde mikroalg yetiştiriciliği için fotobiyoreaktör sistemlerinin optimize edilmesiyle ilgili zorlukların ve fırsatların değerlendirilmesine odaklanmaktadır. Hızlı büyüme oranları, farklı ortamlara uyum sağlama, yüksek lipit içeriği ve karbondioksit yakalama ve oksijen üretmedeki rolleri de dahil olmak üzere mikroalglerin benzersiz büyüme özellikleri vurgulanmaktadır. Araştırma, mikroalglerin bina cephelerine entegre edilmesinin çevresel ve ekonomik faydalarını değerlendirip, bunların kentsel binaların görsel ve çevresel çekiciliğine katkıda bulunma potansiyellerini açıklamaktadır. Tez ayrıca çevresel etkileri en aza indirirken büyümeyi ve üretkenliği en üst düzeye çıkarmak için fotobiyoreaktör sistemleri için tasarım yönergeleri de önermektedir. Genel olarak bu araştırma, Avrupa Yeşil Anlaşması hedefleri kapsamında çevresel sürdürülebilirlik ve karbondan arındırma hedefleriyle uyumlu

olarak biyomühendislikte sürdürülebilir ve çevre dostu çözümlerin geliştirilmesine katkıda bulunmayı amaçlamaktadır.

Anahtar Kelimeler: Mikroalgler, *C.vulgaris,* Biyo-cephe sistemleri, Fotobiyoreaktör sistemleri, Çevresel sürdürülebilirlik.

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CHAPTER 1: INTRODUCTION

In the past few decades, microalgae have emerged as a promising way for sustainable bioenergy, food production, and high-value bioactive compounds (Koyande et al., 2021). Their rapid growth rate, adaptability to diverse environments, and high lipid content make them a valuable bioresource (Udayan et al., 2023). Furthermore, microalgae play a crucial role in creating a habitable environment by capturing carbon dioxide and producing oxygen, highlighting their significance in the context of environmental sustainability (Chew et al., 2021).

One innovative application of microalgae lies in their integration into building facades (Elrayies et al., 2018), where they can serve as a renewable energy source and contribute to the visual and environmental appeal of urban landscapes (Carcassi et al., 2021). However, the successful design and analysis of photobioreactor systems for microalgae cultivation in building facades present multifaceted challenges (Elrayies et al., 2018). These challenges encompass the optimization of light distribution, carbon dioxide and nutrient supply, temperature control, mixing, and oxygen transfer within the photobioreactor systems (Ahmad et al., 2021).

This master's thesis aims to explore challenges related to the cultivation of microalgae in building facades using photobioreactor systems. The study will include a comprehensive review of current knowledge on microalgae cultivation and photobioreactor design, focusing on the unique growth characteristics that make microalgae valuable bioresources. Additionally, the thesis will assess potential environmental and economic benefits of integrating microalgae into building facades and propose design guidelines for photobioreactor systems to maximize growth and productivity while minimizing environmental impacts. The ultimate goal is to contribute to the development of sustainable and efficient microalgae cultivation systems with customized facade designs, addressing pressing issues related to climate change and energy security.

CHAPTER 2: LITERATURE REVIEW

2.1. Microalgae

Approximately 3.5 billion years in the past, prokaryotic microalgae initiated the process of shaping the atmosphere (Vincent, 2009). And 1.5 billion years ago, the endosymbiosis theory was proposed, suggesting the merging of a eukaryote with a prokaryote to give rise to another eukaryote (Finazzi et al., 2010; Cooper and Smith, 2015). As photosynthesis evolved, the biogeochemistry of the Earth underwent changes, transforming it into a conducive environment for the emergence of multicellular organisms within an oxygen-rich atmosphere (Finazzi et al., 2010). Throughout this phase, photosynthesis played a crucial role in creating a habitable environment for all organisms, with the evolution of algae proving to be significantly influential (Xiong et al., 2009; Hohmann-Marriott and Blakenship, 2011). Microalgae, particularly, garnered attention in this oxygenated atmosphere due to their unique growth characteristics and their relevance in both biotechnological applications and innovative, forward-looking design concepts (Talebi et al., 2022). According to a widely accepted definition, microalgae are tiny, colonial, or free-living organisms that can be found in soil and fresh, brackish, or marine water (Sasso et al., 2012; Safi et al., 2014).

Microalgae are a valuable source of bioresources since they are microscopic photosynthetic organisms that grow in aquatic conditions (Randrianarison and Ashraf, 2017). Photosynthetic microorganisms known as microalgae are crucial components of biological and ecological systems (Tandon et al., 2017). Utilizing carbon dioxide and solar energy via photosynthesis, microalgae generate organic substances, playing a crucial role in the carbon cycle and the production of oxygen in the atmosphere (Prasad et al., 2021). Simultaneously, microalgae are gaining recognition as promising reservoirs for applications in biotechnology (Thore et al., 2023). Understanding the microbial growth parameters within microalgal bio-façade modular systems is crucial for optimizing performance. Parameters such as nutrient availability, light intensity, temperature, and pH play pivotal roles in shaping microbial dynamics (Hasnan et al., 2020). Their rapid growth rates and diverse biochemical composition make them

promising candidates for various applications, ranging from biofuel production to wastewater treatment (Salama et al., 2017). The utilization of microalgae within bio-façade systems capitalizes on their ability to efficiently convert solar energy into valuable biomass, contributing to the sustainable production of bioactive compounds (Sedighi et al., 2023).

2.2. Chlorella vulgaris and its Applications

Chlorella vulgaris, as a representative of green algae, is a single-celled organism of broad biological and industrial importance in the world of microalgae (Deviram, 2020). This microalgae species is widely found in freshwater ecosystems and is noted for its ability to rapidly multiply (Nava et al., 2021). Morphologically defined by its spherical and cross-shaped cells, *C. vulgaris* has the ability to biologically convert carbon dioxide and sunlight by producing energy through photosynthesis (Barsanti et al., 2006).

Chlorella vulgaris, a unicellular green microalga, has gained prominence in various scientific and industrial applications due to its rapid growth rate, high lipid content, and adaptability to diverse environmental conditions (Ru et al., 2020). The successful cultivation of *C. vulgaris* requires careful consideration of several key growth parameters and environmental factors (Blair et al., 2014). *Chlorella vulgaris* is a photosynthetic organism highly sensitive to light conditions (Daliry et al., 2017). Optimal growth is achieved under moderate to high light intensities, typically ranging from 100 to 300 μmol photons m^{-2} s^{-1} (Metsoviti et al., 2019). The photoperiod, or the duration of light exposure, also plays a crucial role (Amini et al., 2012). Most studies suggest a 12:12 light-dark cycle, ensuring a balance between photosynthesis and cellular respiration (Daliry et al., 2017).

Temperature profoundly influences the metabolic activity and growth rate of *C. vulgaris* (Serra-Maia et al., 2016). The optimal temperature range for cultivation is generally between 20°C and 30°C (Bamba et al., 2015). While *C. vulgaris* can tolerate a broad spectrum of temperatures, maintaining a consistent and suitable temperature is essential for sustained growth (Serra-Maia et al., 2016).

The pH of the growth medium significantly impacts nutrient availability and enzymatic activities within *C. vulgaris* (Pandit et al., 2017). The recommended pH range for cultivation is typically between 6.5 and 8.5 (Qiu et al., 2017). Maintaining a slightly alkaline pH creates favorable conditions for nutrient uptake and biochemical processes essential for growth (Cheng et al., 2022).

Chlorella vulgaris requires a balanced nutrient composition for robust growth (Mandalam et al., 1998). Key nutrients include nitrogen, phosphorus, potassium, trace elements (iron, manganese, zinc), and vitamins (Fox et al, 2018). Nitrogen, often supplied as nitrates or urea, is particularly crucial for protein synthesis, while phosphorus is essential for energy transfer processes (Hulatt et al., 2012).

As a photosynthetic organism, *Chlorella vulgaris* relies on carbon dioxide for photosynthesis (Clément-Larosière et al., 2014). 1 kg of microalgal biomass would fix 1.8 kg of atmospheric CO_2 (Masoumi and Dalai, 2021). Adequate CO_2 availability is essential for maximizing growth rates (Kumari et al., 2021). In closed systems, such as photobioreactors, the regulation of CO_2 concentrations is critical to ensure optimal carbon assimilation (Eloka-Eboka et al., 2017).

Effective aeration and mixing of the cultivation medium are essential for preventing sedimentation, promoting nutrient distribution, and facilitating gas exchange (Chang et al., 2017). Proper mixing helps maintain uniform conditions throughout the culture, preventing the formation of gradients that could negatively impact growth (Yen et al. 2019).

Chlorella vulgaris can be cultivated in various systems, including open ponds, closed photobioreactors, and bio-facade modular systems (Inam et al., 2021). The choice of system depends on factors such as scalability, resource efficiency, and the specific goals of cultivation.

The biochemical composition of *C. vulgaris* is important, especially as it contains high amounts of protein, carbohydrates, vitamins, minerals, and fatty acids (Ru et al., 2020). These properties make *C. vulgaris* attractive for use not only in biological research but also in various industrial applications such as food supplements, animal feed, and biofuel production (Chisti et al., 2007). In addition, the ability of this microalgae species to adapt to various stress conditions and its special

metabolic properties at the cellular level increase its potential in terms of industrial biotechnology and environmental technologies (Dasgupta et al., 2017).

Today, the sustainability and energy efficiency of urban areas are at the forefront of the architectural design and construction industry (Choe et al., 2022). In this context, innovative solutions for green energy sources have become a fundamental element of building design (Belussi et al., 2019). It has been observed that microalgae, especially *chlorella vulgaris*, have a significant potential in terms of energy production and environmental sustainability by integrating into building facade systems (Metsoviti et al., 2019).

By accelerating carbon dioxide absorption and oxygen production with its photosynthetic activity (Diaconu et al., 2020), *C. vulgaris* not only contributes to bioenergy production (Cardozo et al., 2007), but also provides an aesthetic touch and a green environmental contribution to building design (Arianti et al., 2020). Microalgae integrated into building facade systems produce bioactive components by capturing solar energy and thus have the potential to meet the energy needs of buildings (Rezazadeh et al., 2021).

2.2.1. Cultivation of Chlorella vulgaris under Sterile and Non-Sterile Conditions for Industrial Applications

Chlorella vulgaris, a versatile microalga with immense industrial potential, can be cultivated under both sterile and non-sterile conditions (open and closed systems) (Ray et al., 2022), each presenting unique advantages and considerations in the context of large-scale production (Borowitzka et al., 2017).

Sterile Cultivation

Sterile cultivation involves maintaining a completely aseptic environment to eliminate contaminants and ensure the exclusive growth of *C. vulgaris* (Pinto et al., 2021). This method is often employed in applications where product purity and

consistency are paramount, such as pharmaceuticals, nutraceuticals, and high-value biochemical (Popa et al., 2018).

Aseptic Techniques: Sterile techniques are imperative throughout the cultivation process, from media preparation to inoculation and harvesting (Wang et al., 2019). This involves the use of autoclaved equipment, filtered air, and rigorous sanitation practices (Carvalho et al., 2014).

Closed Systems: Photobioreactors or closed fermentation systems provide a controlled and isolated environment, minimizing the risk of external contamination (Yen et al., 2019).

Sterile Nutrient Supply: All nutrients and growth media must be sterilized before introduction to the culture, ensuring a contaminant-free environment (Coimbra et al., 2019).

Advantages

- **Purity of Product:** Sterile conditions minimize the risk of contamination, resulting in purer biomass suitable for applications requiring high product quality (Prosenc et al., 2021).
- **Precise Control:** Parameters such as temperature, pH, and nutrient concentrations can be tightly controlled, optimizing growth conditions (Darvehei et al., 2018).

Disadvantages

- **Energy Consumption:** The energy-intensive nature of sterilization methods, such as autoclaving, contributes to elevated energy consumption (Coimbra et a., 2019). This not only impacts operational costs but also runs counter to the principles of sustainable and energy-efficient cultivation practices (Choudhary et al., 2022).
- **Limitation on Cultivation Systems:** Sterile cultivation is often more suited to closed systems, such as photobioreactors, which offer better control over environmental conditions (Yen et al., 2019). However, this limits the

scalability and adaptability of cultivation systems, potentially hindering the feasibility of large-scale production (Debowski et al., 2022).

Non-Sterile Cultivation

Non-sterile cultivation offers a more cost-effective approach suitable for applications where absolute purity is not a primary concern, such as biofuel production, wastewater treatment, and certain agricultural uses (Olabi et al., 2023).

Open Systems: Open pond systems are commonly used for non-sterile cultivation due to their simplicity and lower operational costs (Katarzyna et al., 2015). However, this makes the culture susceptible to contamination (Molina et al., 2019).

Natural Nutrient Sources: Non-sterile systems often utilize natural nutrient sources, such as organic fertilizers or wastewater, reducing the need for sterilization of the growth medium (Agustin et al., 2022).

Advantages

- **Cost-Effectiveness:** Non-sterile cultivation is generally more cost-effective due to reduced infrastructure and operational requirements (Wals et al., 2019).
- **Adaptability:** *Chlorella vulgaris* has a robust nature and can tolerate certain contaminants, making non-sterile conditions suitable for less critical applications (Goswami et al., 2022).

Disadvantages

- **Quality Control Challenges:** The presence of contaminants in non-sterile environments makes quality control more challenging (Pleissner et al., 2020). Ensuring consistent product quality becomes difficult, particularly in applications requiring high purity, such as pharmaceuticals or specialized bioproducts (Tang et al., 2020).

- **Limited Applications in Sensitive Industries:** Certain industries, such as pharmaceuticals or high-end biotechnology, may require a level of purity that is challenging to achieve in non-sterile environments (Shah et al., 2016). This limits the potential applications of *C. vulgaris* biomass from non-sterile cultivation (San Agustin et al., 2022).

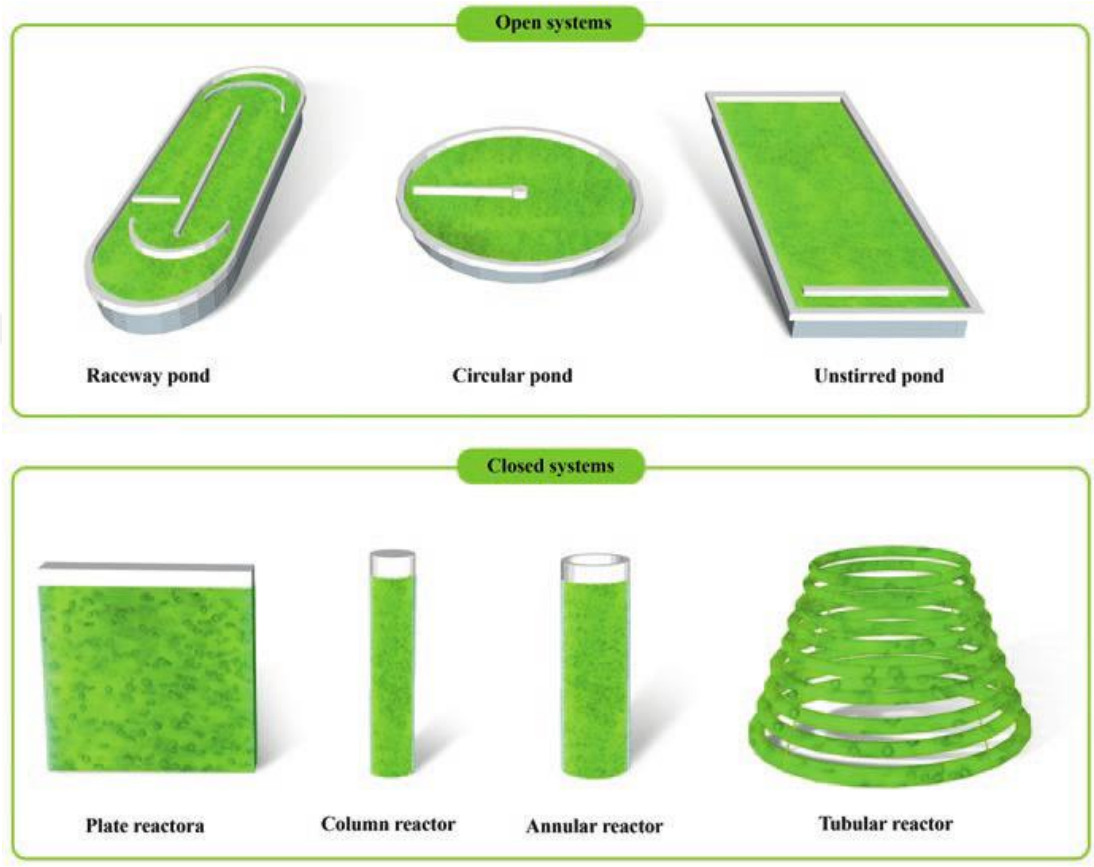

Figure 1**.** Open and closed cultivation systems for microalgae (Source: Zerrouki and Henni et al., 2019)

2.3. Microalgal Façade Systems

With growing awareness of environmental sustainability and energy efficiency, innovative design and building technologies are constantly evolving with the aim of minimizing the environmental impact of our living spaces (Choe et al, 2022). In this context, "Microalgal Façade Systems" offer a groundbreaking approach that has the potential to provide a unique balance between energy production and architectural aesthetics (Öncel et al., 2016).

In recent years, integrating of microalgae into novel bio-façade modular systems has garnered significant attention within the field of bioengineering (Kumar et al., 2023; Singh et al., 2022). These systems, designed for sustainable urban environments, aim to exploit the unique capabilities of microalgae for enhanced microbial growth and positive environmental impacts (Han et al., 2019; Debowski 2020). Microalgae play an important role in bioenergy production thanks to their ability to photosynthesize with solar energy (Heredia-Arroyo et al.,2011). Microalgal facade systems aim to grow microalgae with a special design integrated into the exterior surfaces of buildings and capture solar energy in the process (Choe et al, 2022). These systems contribute to green energy production and reduce energy consumption by increasing the thermal insulation of buildings and taking steps towards a more environmentally sustainable future (Burlew, 1953).

In conclusion, understanding and optimizing these growth parameters and conditions are paramount for achieving high biomass productivity and maintaining the sustainability of *C. vulgaris* cultivation. Researchers and industrial practitioners continue to explore innovative approaches to enhance the efficiency and environmental sustainability of microalgal cultivation for various applications. This study aims to minimize environmental impacts and promote sustainable architectural design by examining in detail the application of LCA to building facade systems. In addition, this study aims to understand the impact of this innovative technology on future building projects by thoroughly examining the advantages microalgal facade systems offer in terms of design, energy efficiency potential and environmental sustainability.

2.4. Photobioreactor (PBR) Design Considerations

A photobioreactor is a type of bioreactor that is used to grow phototrophic organisms, including microalgae, in an enclosed system without allowing material to directly exchange between the culture and its surroundings (Chang et al., 2017). Biotechnological applications of microalgae, especially advances in photobioreactor design (Olivieri et al., 2017), have opened new doors for the sustainability of several industrial processes such as bioenergy production, bioplastic production and water

purification (Talan et al., 2022). Photobioreactors are special systems designed to grow microalgae in a controlled environment and produce energy or bioactive compounds through photosynthesis (Weissman et al., 1988). Effectively designing these systems can increase the efficiency of microalgae cultivation, reduce energy costs, and minimize environmental impacts (Slade et al., 2013).

Photobioreactor design should include several important features (Posten et al., 2009). These features include optimizing light distribution, carbon dioxide and nutrient supply, temperature control, mixing and oxygen transfer (Sathinathan et al., 2023). Success of the design could increase product efficiency by increasing the cellular growth rate of microalgae and minimize undesirable side effects in the process (Singh et al., 2012). Therefore, photobioreactor design is a critical element for sustainable and efficient microalgae cultivation (Suh et al., 2003).

The choice of microalgae, mass, and energy balance for light (shape and volume), and mixing are important design factors for a PBR system (Cañedo et al., 2016). Proper mixing, light route length, and airflow rates are considered for the design parameters in order to improve light penetration and growth (Postern et al., 2009). To avoid high oxygen concentration from building up in the PBR system, an exhaust or degasser can be included in the design, along with a sparger to inject the gas mixture as bubbles. The consistent distribution of nutrients and biomass concentration depends on the medium's lateral mobility as well (Anderson et al., 2014).

The considerations for designing a photobioreactor, as outlined by Tsoglin et al. (1996), can be summarized as follows:

- **Cultivation Capability:** The reactor design should accommodate the cultivation of various microalgal species.
- **Even Illumination and Efficient Mass Transfer:** Design features must ensure uniform illumination of the culture surface and efficient mass transfer of carbon dioxide (CO_2) and oxygen (O_2).
- **Prevention of Fouling:** Microalgal cells tend to adhere strongly, leading to frequent fouling of light-transmitting surfaces. The reactor design should prevent or minimize fouling, especially on these surfaces, to avoid the need for frequent shutdowns for cleaning and sterilization.

- **Elimination of Shear Stress:** High rates of mass transfer, essential for efficient growth, should be achieved without causing harm to cultured cells.
- **Foaming Tolerance:** The photobioreactor must effectively operate under conditions of intense foaming, which is common in reactors with high mass transfer rates.
- **Minimization of Non-Illuminated Areas:** The design should minimize non-illuminated areas within the reactor to optimize overall efficiency.

This thesis aims to open new doors in terms of efficiency, energy efficiency and environmental sustainability in microalgae cultivation by providing an in-depth examination of photobioreactor design features. Current developments in the design of photobioreactors and their potential for microalgae cultivation are important in understanding their role in bioenergy production and industrial applications (Chen et al., 2011). For the purpose of producing algae, several kinds of photobioreactors have been developed (Singh et al., 2012).

2.4.1. Tubular photobioreactor

Tubular Photobioreactor (PBR) systems, typically cylindrical and made of materials such as glass, PVC, or plastic, prioritize optical transparency (Benner et al., 2022). Glass and thermoplastics, chosen for their transparency, maintain sufficient strength within specific height and diameter limits, becoming susceptible to wind if exceeding 4 meters in height and 20 cm in diameter in tubular form (Miron et al., 1999). Despite this vulnerability, they excel in creating a flashing light effect in the PBR due to cyclic bubble recirculation during mixing (Barbosa et al., 2003). Tube diameters range from 10mm to 60mm, while lengths vary from 10-100m (Posten, 2009; Xu, 2007), with smaller diameters chosen to enhance light penetration. When designing a tubular PBR system, ensuring a height greater than twice the diameter is crucial (Singh and Sharma, 2012). This height-diameter ratio promotes effective heat and mass transfer, O_2 gas release, and radial mixing (Kumar et al., 2011).

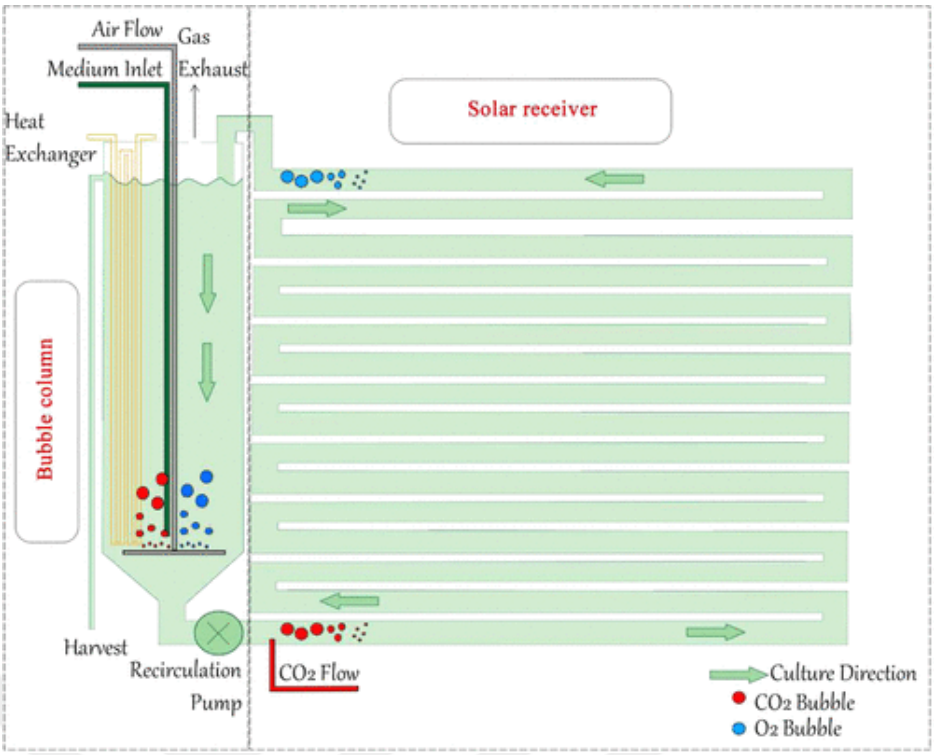

Figure 2. Tubular photobioreactor schematic (Source: Fernandez et al., 2014).

2.4.2. Flat panel photobioreactor

The flat panel reactor, depicted in Figure 3, is characterized by its cubic shape with a minimal light path. It can be constructed using transparent materials such as glass, plexiglass, optical light film, and polycarbonate (Musikant et al., 2020). Featuring a high surface area to volume ratio and an open gas disengagement system, this reactor ensures even light emission from a flat transparent surface screen or overhead lamps (Assunção et al., 2020). Agitation is achieved by either bubbling air through a perforated tube from one side or by mechanical rotation using a motor (Tabernero et al., 2013). Combining several flat panels offers a convenient way to achieve the desired light path in a reactor. However, flat plate systems may encounter challenges related to substantial space requirements, elevated light energy needs, cleaning complexities, and potential inefficiencies in terms of mass production per unit of space (Olivieri et al., 2014). The productivity of these systems relies heavily on the spacing between panels and the areal productivity limitations for outdoor applications (Kumar et al., 2015). For indoor operation, critical factors include the distance of light sources from panels, temperature impacts, illumination on one or both panel sides, and

the light path (Briassoulis et al., 2010). Scaling up flat plate systems might prove challenging due to the rise in hydrostatic pressure with increasing volume (Janssen et al., 2003). Generally, the structural integrity of flat plate systems may not withstand very high pressure (Assunção et al., 2020).

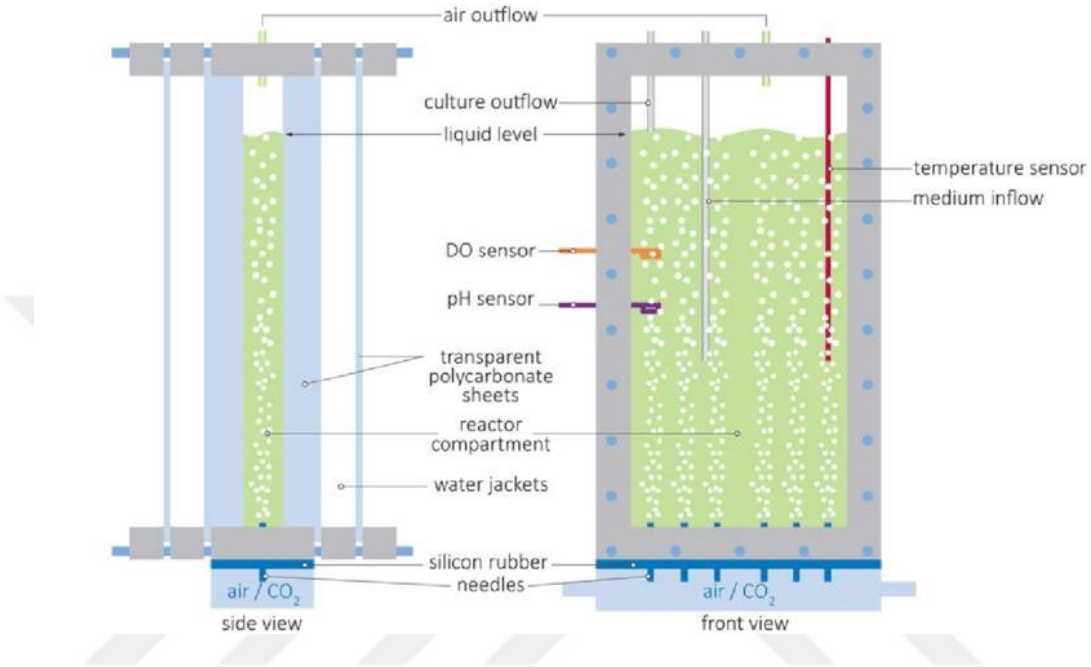

Figure 3. A flat panel photobioreactor schematic (Source: Singh and Sharma, 2012)

2.4.3. Bag or Soft Frame PBR Module

A bag or soft frame photobioreactors typically consists of transparent plastic bags or chambers that are suspended or laid out horizontally to maximize light exposure (Sirohi et al., 2022). The transparency of the material allows for efficient light penetration, essential for photosynthesis (Nwoba et al., 2019). The bags are often equipped with aeration systems to facilitate gas exchange and maintain optimal conditions for growth (Placzek et al., 2017).

Soft frame photobioreactors are highly scalable, allowing for easy adjustment to different production scales, from laboratory settings to large industrial operations (Sirohi et al., 2022). The simplicity of the design and use of lightweight materials contribute to cost-effectiveness, making soft frame photobioreactors an attractive option for large-scale microalgal cultivation (Bhatia et al., 2021). The setup of bag

photobioreactors is straightforward, and their flexibility allows for easy adaptation to various locations (Zittelli et al., 2013). Maintenance is generally simpler compared to more complex photobioreactor designs (Chang et al., 2017). Ensuring uniform light distribution within the bag is crucial to promote consistent growth throughout the microalgal culture (Sivakaminathan et al., 2020). Uneven illumination can lead to variations in biomass productivity (Cho et al., 2019). Effective temperature control is essential to provide an optimal environment for microalgal growth (Gatamaneni et al., 2018). The plastic material of the bags may influence temperature, requiring careful monitoring and control (Carvalho et al., 2006). Proper mixing of nutrients is vital for homogenous nutrient distribution. In bag photobioreactors, achieving uniform nutrient mixing can be challenging and may require innovative solutions (Benner et al., 2022).

2.4.3. Sheet PBR Module

There aren't any sheet Photobioreactor (PBR) systems available right now. The bag PBRs design, which makes use of plastic bags, is the most similar to sheet PBR. To keep the algal biomass from sedimenting, these bag PBRs require a supporting frame with an aeration system (Ting et al., 2017). Nevertheless, they have disadvantages as well, such as the have to change bags on a regular basis and an adverse effect on the environment because of the significant amount of plastic garbage they produce (Wang et al., 2012). Other difficulties with bag systems include poor mixing, restricted light exposure, and leakage possibilities (Huang et al., 2017). Design parameters for bag systems include frame structure, aeration techniques, material selection, and size. Bag PBRs have a low capital cost advantage despite their disadvantages (Huang et al., 2017). Bag PBRs have been used in experiments for a variety of species in volumes ranging from 5 L to 250 L (Chen et al., 2013; Sierra et al., 2008).

2.5. Environmental Analysis and Life Cycle Assessment (LCA)

Life Cycle Analysis (LCA) is a methodology that systematically evaluates and provides understanding of the environmental impacts of a product, a process, or a service (Kjaer et al., 2016). LCA covers the environmental impacts that occur at various stages throughout the entire life cycle of a material, a product, or a process (Chang et al., 2014). These stages are generally; material extraction, production, distribution, use and waste disposal (Joshi et al., 199). LCA provides a holistic view, considering not only the effects during the direct production phase of a product, but also the effects during the material extraction, transportation, and disposal of the final product (Azapagic et al., 1999).

This method of analysis is used in sustainability assessments, improving environmental performance and reducing environmental impacts in product design (Hens et al., 2018). LCA allows objectively assessing the environmental sustainability of a product or process by evaluating energy consumption, greenhouse gas emissions, water consumption and other environmental indicators (Chang et al., 2014).

2.6. Life Cycle Assessments of Different Façade Systems

In the evolution of architectural design, building façade systems play an important role not only in aesthetics but also in terms of energy efficiency and environmental sustainability (Kovacic et al., 2016). In this context, the assessment of environmental impacts throughout the entire life cycle of building facade systems, from design to construction, from use to recycling, has become a critically important issue for decision makers for a sustainable future (Gilani et al., 2022; Ingrao et al., 2018). At this point, life cycle analysis (LCA) stands out as a powerful tool to objectively evaluate the environmental performance of building facade systems (Ottele et al., 2011).

LCA aims to systematically examine the environmental impacts that occur at every stage of building facade systems, from material selection to production and assembly processes, from energy consumption to waste management (Kim et

al.,2011). This analysis includes various environmental indicators such as energy use, greenhouse gas emissions, water consumption, and this data provides important guidance on how building façade systems can be optimized in terms of sustainability and energy efficiency (Soares et al., 2017). This study aims to contribute to minimizing environmental impacts and promoting sustainable architectural design by examining in detail the application of LCA to building facade systems.

CHAPTER 3: MATERIAL AND METHODS

3.1. Preparation of Microalgae Cultures

Different microalgae species require specific conditions for growth and reproduction. Model microalgae provided from the algae culture collection at Ege University, with preference given to those that are easier to culture and adapt quickly. Sampling and inoculations will be conducted under sterile conditions in a laminar flow cabinet. Daily microscopic checks will ensure axenic conditions in flask cultures, with microbiological cultivation methods applied when necessary. Cultures of *Chlorella vulgaris* (SAG 211-12) will be maintained in liquid BG-11 culture medium with slight modifications to organic carbon and nitrogen sources for photomixotrophic cultivation. The culture medium (table 1), excluding organic substrates, will undergo sterilization in an autoclave at 121°C for 20 minutes. The liquid cultures will be cultivated and maintained under continuous illumination of 12.2 $W.m^{-2}$ in a rotary shaker at 120 rpm to ensure effective mixing and homogeneous dispersion of light. Subsequently, the cultures upscaled to 250-mL flasks containing 100 mL BG-11 medium with a 15% (v/v) inoculum or 500-mL flask containing 250 mL BG-11 medium with a 15% (v/v) inoculum. The cultures were inoculated with an initial cell concentration of 1×10 g/L during the mid-logarithmic growth phase. The initial pH was adjusted to 8-8.5. Cultures were achieved by maintaining an 8:16 dark: light cycle. No intervention was made on process parameters during the production, as reported by Isleten Hosoglu et al. (2012), Kose et al. (2017), and Metsoviti et al. (2019).

Table 1. Chemicals that make up the BG-11 nutrient medium and Trace elements.

BG-11 MEDIUM		
Component	**Medium Amount (g/L)**	**Final concentration (mM)**
Sodium Nitrate (MA:84.99 g/mol)	1.50	17.6
Dipotassium Hydrogen Phosphate K_2HPO_4 (MA: 174.2 g/mol)	0.040	0.23
Magnesium Sulfate Heptahydrate $MgSO_4 \cdot 7H2O$ (MA: 246.48g/mol)	0.074	0.3
$CaCl_2 \cdot 2H_2O$ (MA: 147.01 g/mol)	0.0353	0.24
Citric Acid$\cdot H_2O$ (MA: 210.14 g/mol)	0.0065	0.031
Ferric Ammonium Citrate (MA: 261.98 g/mol)	0.0055	0.021
Sodium Carbonate Na_2CO_3 (MA: 105.99)	0.0201	0.19
Ethylenediaminetetraacetic acid disodium salt dihydrate $Na_2EDTA \cdot 2H_2O$ (MA: 372.24 g/mol)	0.00101	0.0027
Sodium Thiosulfate Pentahydrate (agar media only,sterile) (MA: 248.17 g/mol)	0.248	1
Trace Elements:		
Boric Acid H_3BO_3 (MA: 61.83 g/mol)	2.86	46
Manganase (II) Chloride $MnCl_2 \cdot 4H_2O$	1.81	9
Zinc Sulfate Heptahydrate $ZnSO_4 \cdot 7H_2O$	0.22	0.77
Sodium Molybdate $Na_2MoO_4 \cdot 2H_2O$ g	0.39	1.6
Copper Sulfate Pentahydrate $CuSO_4 \cdot 5H_2O$ (MA:249.68 g/mol)	0.079	0.3
Cobalt (II) Nitrate $Co(NO_3)_2 \cdot 6H_2O$ (MA:292 g/mol)	0.0496	0.17

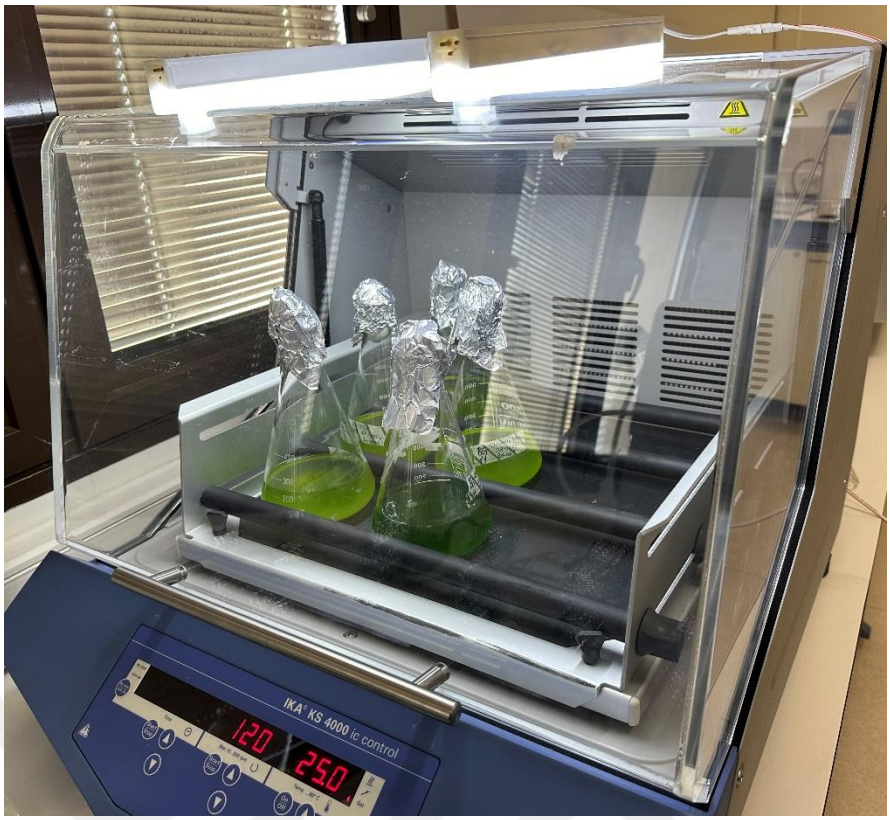

Figure 4. *C. vulgaris* cultivation in an orbital shaker

Since there is a possibility of damage to PBR designs when trying to sterilize them with an autoclave device and such sterilization methods cannot be used in large systems, experiments were first carried out to observe growth in non-sterile conditions. Growth of *C. vulgaris* was observed with 15% inoculation in BG-11 nutrient medium prepared with pure water and ultrapure water under non-sterile conditions. In this study, while the light intensity remained the same, a pump providing 1.75 L air flow per minute was used instead of shaking motion.

Figure 5. Nonsterile media with distilled and ultrapure water

3.2. Design of PBR System

It was determined to work with *Chlorella vulgaris*, which has a high value-added metabolite content and can be easily grown under laboratory circumstances or in controlled indoor spaces, to produce a modular PBR system design that would serve as a proof of concept on building facades. Our primary goal is to see how well such a system works with the outside and inside of already-existing structures. The project views the potential presented by the United Nations, the Sustainable Development Goals, and the lack of advancements in sustainable building technologies. The objective is to construct several photobioreactors in order to verify design concepts and maintain ideal environmental conditions for the growth of microalgae and sustainable building systems. The first step is to examine past projects to understand how the design was applied in similar processes. Next, we've looked into sustainability aspects in design to identify key points relevant to this project. During the literature review, we've modified existing designs (plate and tubular) and plan to incorporate any needed adjustments for the conceptualization of an extra design idea (Sheet) and the prototyping processes.

3.2.1. Learning from Past Projects: Insights for Designing

Microalgae can thrive in photobioreactors through heterotrophic, autotrophic, or a combination of both conditions. In heterotrophic settings, some organisms rely on organic carbons for energy, allowing them to grow in complete darkness. Conversely, in autotrophic conditions, microorganisms utilize light as their energy source. This study aims to assess the impact of natural and artificial light sources on growth performance within closed systems of various designs, such as tubular, plate, and sheet configurations, placed in diverse locations.

Various factors were considered in the design phase, including cost, constraints related to dark zones (either solar or artificial light), limits on the thickness of the culture medium to enhance volumetric productivity, ease of scaling, and energy efficiency. Another crucial factor is the well-stirred reactor, which allows for a uniform mixture, incorporating biomass. However, achieving uniform light distribution throughout the entire reactor volume is challenging due to light absorption by microalgae causing light attenuation.

The design aspects of spargers, including their geometry, diameter, spacing, orifice size, and the number of orifices, play a crucial role in the design process. When designing spargers, it is important to consider the prevention of weeping and, to a lesser extent, non-uniformity (Joshi and Kulkarni, 2011). Weeping occurs when the gas pressure injected into the sparger is lower than the overall pressure of the growth medium, causing the growth medium to enter the sparger through the holes instead of proper bubbling. Factors influencing weeping include pressure drop along the sparger length, liquid height, and the surface tension of the liquid. Uneven gas transfer along the sparger length leads to high non-uniformity, resulting in increased pressure drop and a higher risk of hole clogging in the sparger. It is essential to select a sparger with a bubble diameter ranging from 3mm to 7mm, and the flow rate should be chosen to keep microalgae suspended, while a superficial gas velocity providing homogeneous flow is preferable for scale-up. Various commercially used sparger types include sieve plate, radial, porous, spider, and ring types (Kulkarni et al., 2007).

In this project, we employ the bubbling method, where gas is introduced through the sparger, initiating bubble formation upon contact with the liquid. This

method is preferred for larger reactors due to its ability to create a homogeneous flow. To achieve such uniformity, large bubbles and higher superficial gas velocities are necessary (Veera and Joshi, 1999; Joshi et al., 2002).

The sparger type, geometry, gas velocities, and bubble hydrodynamics significantly influence the design of the photobioreactor (PBR). These factors are intricate and interconnected. The quantity of gas transferred to the photobioreactor plays a crucial role in shaping the flow pattern. Both bubble diameter and flow pattern are key considerations in designing the sparger and optimizing photobioreactor performance. Sparger design involves three types of bubbles: small bubbles, with a volume-equivalent diameter less than 0.1 mm, spherical in shape; intermediate bubbles, which are ellipsoidal; and larger bubbles, with diameters exceeding 18 mm, typically cap-shaped with a volume greater than 3 cm3 (Xu, 2007).

Material

Nearly all types of photobioreactors are constructed using transparent materials to enable the passage of natural or artificial light, ensuring the required light intensity within the reactor (Wang, Lan, and Horsman, 2012) (Tables 2 and 3). Commonly used materials for constructing photobioreactors include glass, polyethylene (PE), polycarbonate (PC), polyvinyl chloride (PVC), acrylic (Plexiglass, PMMA), silicate, and fiberglass (Posten, 2012). It is essential that the chosen materials are non-toxic, possess high strength, are chemically stable, and are easy to clean, addressing concerns of contamination and accommodating the high growth rate and biomass yield of algae.

Table 2. Physical properties of PBR construction materials (Source: Wang, Lan, and Horsman, 2012)

Materials	Energy Content (MJ/Kg	Modulus of Elasticity (psi)	Poisson's ratio	Material Density (Kg/m^3)	Melting Point (^{o}C)	Shear Strength (psi)	Material's life (yrs)	Tensile Strength (psi)
Glass	25	9137377	0.20	2230	-	-	20	-
Polyvinyl Chloride	74	420000	0.10 (@ 73 ^{o}F)	1400	60	-	-	7450
Polyethylene (PE)	78	530000	-	920	136	10500	3	6240
Polycarbonate (PC)			-					
Plexiglass, PMMA	131	425000	-	1180	140	9000	20	9600
Fiber glass	11	-	-	-	-	-	-	-

Table 3. Optical properties of materials used for PBR (Source: Wang, Lan, and Horsman, 2012)

Materials	Light transmission (%)	Critical Angle	Refractive Index	Industry
Glass		43^o	1.52 (1.473)	SCHOTT tubular PBR
Polyvinyl chloride (PVC)	75		1.5	+GF +
Polyethylene (PE)	92 (1/8 inch)	46^o	1.51	
Polycarbonate (PC)			1.60	
Plexiglas, PMMA	95	42.16^o-45^o	1.49	
Fiber Glass	90	-	-	Solar Components Corporation

Since *C. vulgaris* growth was successful in non-sterile conditions, microalgal growth was wanted to be observed with PBR prototypes in the laboratory environment in the next experiment. For this purpose, two sheet reactors made of polyethylene were used. One was cleaned with 5% bleach solution, exposed to UV light, and set up with

sterile BG-11 nutrient medium under aseptic conditions. The other one was cleaned with only 70% alcohol and installed in non-sterile BG-11 nutrient medium.

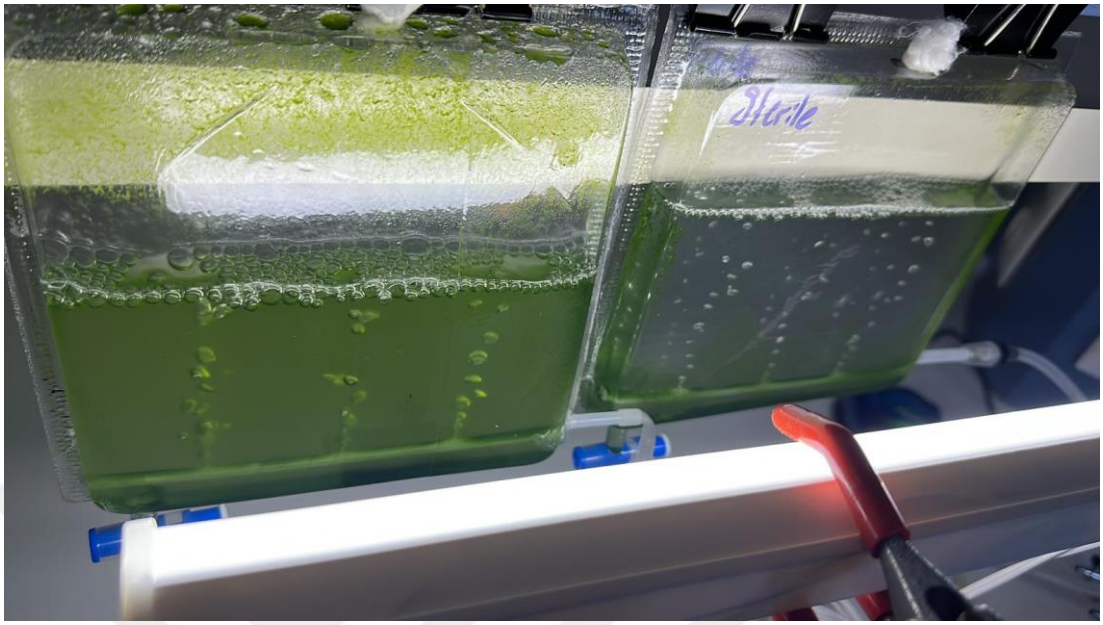

Figure 6. Non-sterile and sterile polyethylene PBR sheet trials at laboratory (prototype 1 at figure 24)

For this research, we have supplied Polyethylene sheets to build prototypes of sheet photobioreactors, given the material's ease of application and cost efficiency.

When aerating the liquid medium, it is crucial to take into account the size of air bubbles. Previous studies have identified four ways in which bubbles can impact cells: (1) when bubbles are at the diffuser's surface, (2) during the ascent of bubbles, (3) when bubbles burst in the medium, and (4) when bubbles burst at the air-medium interface. Surface events and bubble ruptures in the medium are found to be non-damaging to cells, but research indicates that bubbles breaking at the air-medium interface, especially small bubbles, can cause cell damage. Although some data suggest that damage from rising bubbles can be disregarded, the turbulence generated behind rising bubbles can be detrimental to cells, particularly with small bubbles. Larger bubbles, moving faster and interacting with fewer cells, rupture less frequently, making them more suitable for cell cultivation (Yang et al., 2018).

Recognizing the crucial role of air bubbles in algae cultivation, the design approach will experiment with various geometries, textures, and motion patterns to better explore possibilities for optimizing a commercial sparger to deliver an optimal airflow. By directing air bubbles through a nonlinear ascent, the goal is to generate bubbles with dimensions suitable for mixing without harming algae cells and, simultaneously, small enough to create a consistently homogeneous flow in the mixture.

Prototype Trials

In the ongoing studies, firstly, prototypes of 5 different designs created from acrylic material on a single facade (south-west) were worked on. *C. vulgaris* grown on BG-11 nutrient medium with only pump and sunlight grew successfully in all designs. These prototypes, which grew successfully, were subsequently reinstalled on two different building facades, the south-west and north-east building facades.

Design adjustments have also been made to these designs to facilitate the growth and harvest of microalgae. The tubular design system with added spiral polymer material is designed to have a positive effect on the harvesting process. It is planned that the leaf pieces in the tank will have a positive effect on the growth parameters of *C. vlugaris* by increasing movement in the nutrient medium.

These prototypes, which grew successfully, were subsequently reinstalled on two different building facades, the south-west and north-east building facades in figure 9, figure 10 and figure 11.

Figure 7. Designed prototypes: 1) donut, 2) spiral, 3) tank with leaf pieces, 4) tank only, 5) tank with “S” shaped PP sheet (prototype 2 at figure 24).

Figure 8. Tubular PBR design spiral sheet added at 3rd floor, south-west façade (prototype 2 at figure 24)

3.2.2. Site Selection based on Case Studies' Analysis

Taking these considerations into account, we have chosen the Design School building at IEU as the venue for our case study. The suitability of this building for a photobioreactor (PBR) system was thoroughly assessed by the project team to identify suitable locations. The first selected location is on the 4th floor, north-east façade offering ample indoor space and an outdoor terrace for testing our PBR concepts. Both areas receive natural light from the east, south, and west directions, ensuring consistent lighting conditions indoors and outdoors that will support the algae growth.

The second location is the south-west façade on the 3rd floor, also in D block. It was intended to observe the effect of two different facades on the same building on growth in terms of sunlight.

Figure 9. Prototypes at 4th floor, north-east façade (Tank only, spiral, donut, sheet on window, prototype 3 at figure 24)

Figure 10. Tank only PBR system at 3rd floor, south -west façade (prototype 3 at figure 24)

Figure 11. Prototypes at 3rd floor, south -west façade (donut, spiral, sheet on window, prototype 3 at figure 24)

3.3. Life Cycle Assessment of PBRs

Life Cycle Analysis (LCA) is an important methodology used to evaluate and optimize various industrial processes, products, or services in terms of environmental impacts (Aksoy et al., 2023). In this context, the online tool "CCalc2" is a software tool designed to make the process of performing complex LCA calculations and analyzing environmental impacts more accessible and effective (Ozcakir et al., 2023).

CCalc2 allows its users to evaluate important parameters such as energy consumption, greenhouse gas emissions, water use and other environmental indicators that occur at various stages throughout a product's life cycle (Adsal et al., 20202). This can help users strengthen their sustainability efforts, optimize their environmental performance and create a better environmental impact profile (Aksoy et al., 2023).

3.3.1. Life-cycle Assessment, Goal, and Scope of the Study

A useful method for analyzing the quantitative assessment of the environmental effects of a specific process or product of interest is life-cycle assessment (LCA). This assessment included and assessed the process's associated emissions, energy use, and resource consumption. The four steps of LCA in this study are as follows: (i) goal and scope; (ii) inventory analysis; (iii) effect assessment; and (iv) outcomes interpretation. The "cradle to gate" LCA technique is taken into consideration in this case study, along with the culture of *Chlorella vulgaris* in both lab scale photobioreactor and PBR design (Spiral), which produces biomass. The aim of the study was to evaluate the cultivation of microalgae *C. vulgaris* on the basis of environmental impacts through life cycle analysis. The functional unit (F.U.) was assumed as 0.87 g of microalgal biomass.

System Boundaries

Setting up system boundaries is the first and most important stage in the Life Cycle Assessment process. Figure 12 discussed the system boundaries that the "cradle to gate" study explained. The case study involved the comparison of microalgae cultivation processes in two different cultivation areas (in a laboratory environment using a sterile light source and on an office front with non-sterile natural light). The microalgal cultivation process in this study included the following unit operations: i) microalgal cultivation using lamps in sterile photobioreactors, ii) harvesting, iii) drying of the harvested biomass. Similarly, the unit operations mentioned in the other scenario are: i) microalgal cultivation using natural sunlight in non-sterile bioreactors, ii) harvesting, iii) drying of the harvested biomass. The system boundaries of both processes are water and electricity consumption. The input data of the inventory are assumed based on experiments carried out on a laboratory scale.

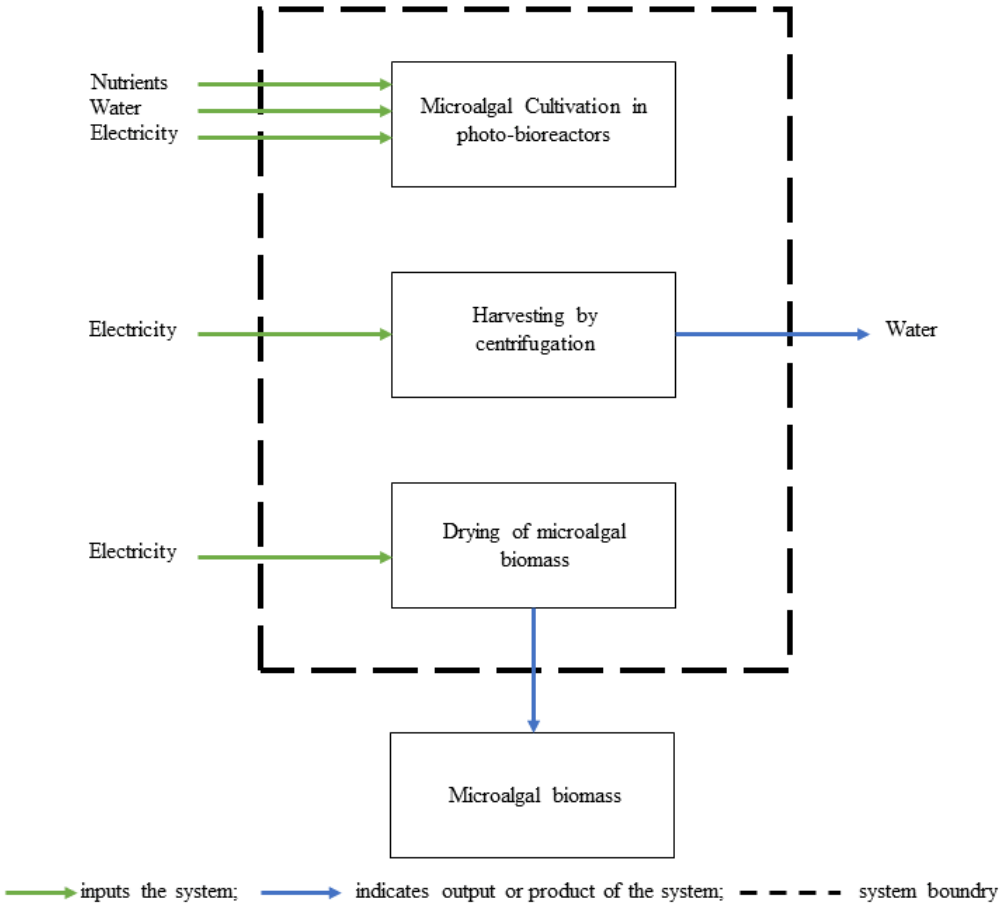

Figure 12. System boundaries of LCA

Inventory

According to laboratory-scale experiments, the biomass produced from BG-11 growth medium was 0.84 g/L in 12 days. In the Spiral PBR, where the highest growth was observed among photobioreactors, the biomass obtained in BG-11 growth medium was 1.78 g/L in 12 days. Since the functional unit is 0.84 g *C. vulgaris*, calculations for the prototype were made over 9 days.

BG-11 nutrient medium chemicals defined in the table as Chemical inorganic plant are as follows: sodium nitrate, dipotassium hydrogen phosphate, ferric ammonium citrate. These chemicals are not in the database of the CCalc2 program. Therefore, the total number of grams of these chemicals needed to produce 1 liter of BG-11 nutrient medium was calculated in grams. Trace element chemicals are neglected. Table 4 and table 5 provide information about system inventory.

Table 4. Inventory data of laboratory scale microalgal cultivation for 0.84 g *C. vulgaris* biomass

Stage	Inputs	Amount	Eco invent Dataset
Microalgal Cultivation in Photobioreactors	Calcium Chloride $CACl_2$	0.035 g	Calcium Chloride, $CaCl_2$, at plant
	Chemical inorganic, at plant	3.05 g	Chemical inorganic, at plant
	Citric acid	0.0065 g	Citric acid from corn - 1
	EDTA	0.001 g	Ethylenediaminetetraacetic acid, plant
	Magnesium Sulphate	0.740 g	Magnesium Sulphate, at plant
	Sodium Carbonate	0.201 g	Sodium carbonate from ammonium chloride production, at plant
	Deionized water	1000 g	Deionized water – from ground water
Use	Electricity (Pump + Centrifuge + Freeze dryer)	197 MJ	Manually Defined (*)

(*) The amount of energy (in MJ) required for 216 hours (9 days) of use has been calculated based on Turkish electricity.

Table 5. Inventory data of prototype (Spiral) microalgal cultivation for 0.84 g *C. vulgaris* biomass

Stage	Inputs	Amount	Eco invent Dataset
Microalgal Cultivation in Photobioreactors	Calcium Chloride $CACl_2$	0.035 g	Calcium Chloride, $CaCl_2$, at plant
	Chemical inorganic, at plant	3.05 g	Chemical inorganic, at plant
	Citric acid	0.0065 g	Citric acid from corn - 1
	EDTA	0.001 g	Ethylenediaminetetraacetic acid, plant
	Magnesium Sulphate	0.740 g	Magnesium Sulphate, at plant
	Sodium Carbonate	0.201 g	Sodium carbonate from ammonium chloride production, at plant
	Deionized water	1000 g	Deionized water – from ground water
Use	Electricity (Orbital incubator + Lamps Centrifuge + Freeze dryer)	475 MJ	Manually Defined (*)

(*) The amount of energy (in MJ) required for 288 hours (12 days) of use has been calculated based on Turkish electricity.

Impact Assessment

In this study, life cycle modeling and the estimation of various midpoint impacts, including acidification potential (AP), eutrophication potential (EP), global warming potential (GWP), human toxicity potential (HTP), ozone layer depletion potential (ODP), and photochemical smog potential (PSP), were conducted using the CCaLC2 Life Cycle Assessment (LCA) software in conjunction with the Ecoinvent2

database, following the CML 2001 method. Figure 13 shows the interface visual of the CCalc2 software.

Figure 13. The interface of CCalc2 Carbon Footprinting LCA Software tool (Source: CCalc2 Carbon Footprinting Tool)

3.4. Analytical Methods

3.4.1. Determination of Optical Density (OD)

There are multiple crucial phases involved in determining the optical density (OD) of microalgae (Nielsen et al., 2019). To attain homogeneity, a representative sample is first taken out of the microalgae cultures and properly mixed (Xu et al., 2009). The microalgae sample's optical density is then determined using a UV-VIS spectrophotometer (Myers et al., 2013). This measurement, which is usually carried out at a certain wavelength, such 650 nm (Liu et al. 2021) or 700 nm (Griffiths et al., 2021), indicates how many microalgal cells are present in the culture. The acquired optical density data is essential for tracking the microalgae culture's density and growth over time, offering important insights into a range of growth parameters

(Myers et al., 2013). All things considered, this methodology provides a basic way to measure the optical density of microalgae cultures, which is an important indicator of the growth and bioproduction potential of these cultures (Wagenen et al, 2014).

In this context, the cell concentration (optical density) was determined by measuring the absorbance of the suspension at 650 nm and 700 nm using a UV–vis spectrophotometer (Perkinelmer, Lambda 750, USA) of the 15% inoculated cultures for 28 days.

3.4.2. Direct Cell Counting with a Microscope

Microscopic counts were conducted using the Neubauer counting slide to assess the daily fluctuations in cell number. Following the homogenization of the culture through pipetting, a cell count was performed on the sample taken on the Neubauer slide. Subsequently, the cell count was quantified as cells/mL by utilizing the calculation specific to the Neubauer counting chamber (Guillard et al., 2005).

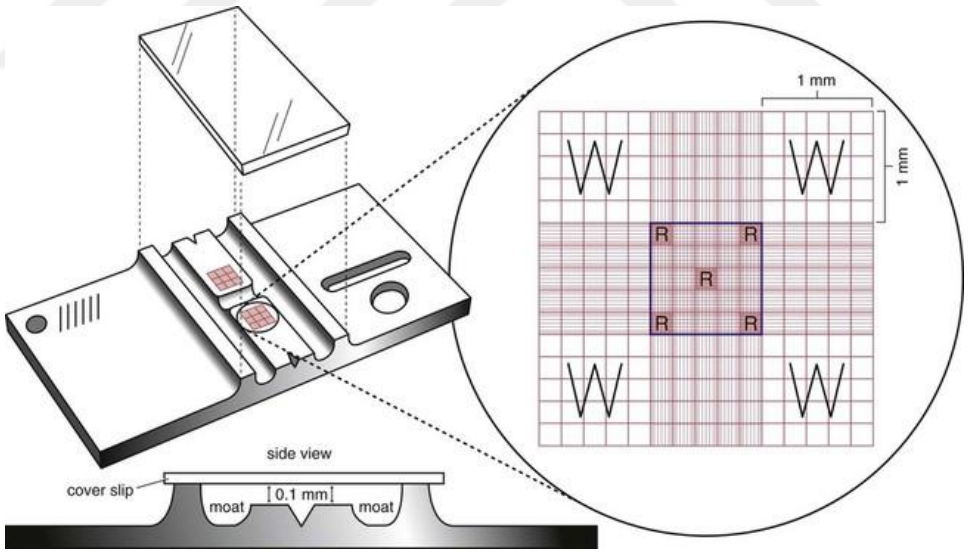

Figure 14. Naubauer chamber (Source: https://www.labtestsguide.com/platelet-count-test-procedure)

Cell number (cell/mL) = A x DF x 10^4

A: Number of cells counted on slide

DF: Dilution factor

3.4.3. Dry Weight Determination

To monitor the increase in microalgae cell concentration, dry weight measurements were also conducted. This measurement involved two stages: filtration and drying. Initially, a specific volume of the sample was taken and passed through a filtration device using pre-weighed filter paper (Sartorius, Germany) (Shekhar et al., 2017).

For dry weight determination, 5 mL samples were taken from each culture. The samples were passed through tared 0.45 μm glass microfiber filters (Sartorius, 0.45 μm, Germany) using a vacuum pump and the biomass was separated from the culture medium (Richardson et al., 1969).

Minerals etc. originating from the nutrient medium on the filter paper. The filter surface was washed with 5 mL of distilled water to remove residues (Richardson et al., 1969). The filter papers were dried in the drying oven at 60 °C overnight and brought to room temperature in the desiccator and then weighed on a precision scale (Ratha et al., 2017).

3.4.4. Total Chlorophyll and Carotenoid Content

Changes in the amount of chlorophyll in microalgae grown phototrophically function as an indicator for biomass growth (Malapascua et al., 2014). For phototrophically grown microalgae, the measurement of chlorophyll a levels will be carried out in tandem with the establishment of growth kinetics. The process described below will be used to determine the levels of carotenoid and chlorophyll (Mhatre et al., 2018; (Tebiani et al., 2015):

In order to measure the amount of carotenoid and chlorophyll, 5 milliliters of culture liquid are first put into a tube, centrifuged for 5 minutes at 4000 rpm, and the supernatant is then extracted. The 5 mL harvested biomass content will be supplemented with 5 mL of 100% methanol. For one to five minutes, the methanol-containing wet biomass will be vortexed, causing mechanical and chemical disruption of the cell. The sample will be vortexed and then heated to 60 °C for thirty minutes.

They will then undergo another vortex and a 10-minute centrifugation at 4100 rpm. The absorbance of the supernatant can be measured using UV-vis spectroscopy at wavelengths of 470, 645, and 662 nm by obtaining a sample of it. The absorbance values in the following equations substituted to determine the amounts of chlorophyll and carotenoid pigments:

chlorophyll a (mg/L) = (11,75 x A662) -(2,35 x A645) (Mhatre et al., 2018)

chlorophyll b (mg/L) = (18,65 x A645) -(3,96 x A662)

Total chlorophyll content (mg/L) = chlorophyll a + chlorophyll b

Total carotenoid content (mg/L) = (1000 x A470) -(2,27 x chlorophyll a) -(81,4 x (chlorophyll b/227))

3.4.5. Environmental Conditions for Microalgal Growth

The light: dark cycle, temperature, and continuous agitation play a crucial role in the growth of microalgae (Allaguvatova et al., 2019; Josephine et al., 2022).

To maintain optimal conditions, the temperature was carefully controlled and set at 23 ± 2°C using a shaker incubator (IKA-KS 4000 ic control, IKA Turkey Laboratuvar ve Proses Teknolojileri A.Ş./İstanbul, IKA, German) as shown in figure 4 LED lights were chosen to simulate sunlight due to their energy-saving benefits and practicality for microalgal growth simulation. Two LED lights with 5 watts each (2800-3200 lux in total) were employed for the light cycle to induce photosynthesis. Light illuminance was monitored using a Digital Lux meter (AS803, China) known for its accuracy and 2% repeatability. Although *C. vulgaris* can thrive in a broad range of illuminance (2500-10,0000 lux) (Febrieni et al., 2020), choosing illuminance above 3000 lux requires higher energy consumption. Therefore, the range of 2800-3200 lux was selected. To convert photometric to radiometric values (lx to $W.m^{-2}$) for light intensity, the following equation (Thimijan and Heins, 1983) was applied.

$$\frac{(lm.m^{-2}lx^{-1})(lx)(mW.lm^{-1})(h\ of\ light)}{(1000\ mW.W^{-1})(24\ h)} = W.m^{-2}$$

Fundamentally, the transformation from lux (lx) to watts per square meter ($W \cdot m^{-2}$) relies on both the light source and the specific wavelength under consideration. For instance, converting the illumination from a 10-kilolux (klx) cool-white fluorescent light over a 10-hour daily period to $W \cdot m^{-2}$ (averaged over 24 hours, spanning 400-700 nm) would involve:

$$\frac{(10{,}000\ lx)(2.93\ mW.lm^{-1})(10\ h)}{(1000\ mW.W-1)(24h)} = 12.2\ W.m^{-2}$$

To maintain the dark cycle, a cover is utilized, and this procedure is manually performed every 16 hours (light) and 8 hours (dark) (Gammanpila et al.,2015). In addition to maintaining a constant temperature and light:dark cycle, the agitation of microalgal cultures is crucial. The shaker incubator's rotation speed was set to 120 rpm to ensure continuous mixing for cells, gas, and nutrients. This mechanical agitation compensates for the lack of air pumping, as introducing air can result in bubble formation, disrupting the culture. However, utilizing air pumping would lead to higher energy consumption, which is why it was not employed for the small-scale (flasks).

CHAPTER 4: RESULTS

4.1. Growth Curve of C. vulgaris

The absorbance value of *C. vulgaris* in the samples taken from the liquid medium was measured almost every day at 650 nm and 700 nm with a UV-VIS spectrophotometer device. The study was carried out on a 3.5 ml sample taken and BG-11 was used as a blank. Measurements were made starting from the second day of subculture until the end of 28 days and the result graph is given in figure 15 and figure 16.

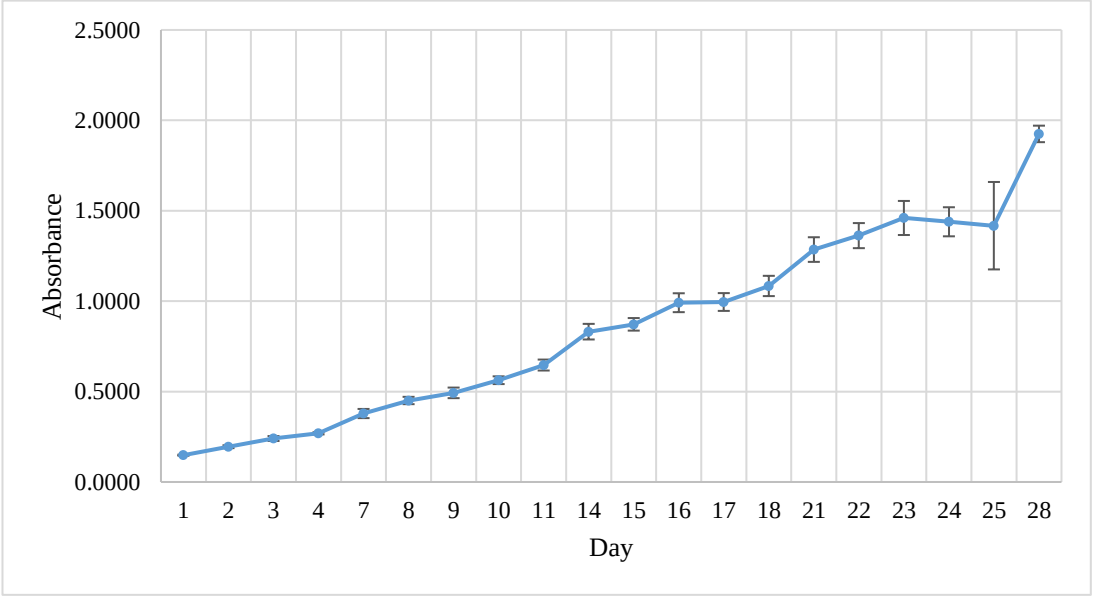

Figure 15. *C. vulgaris* growth curve at 650 nm

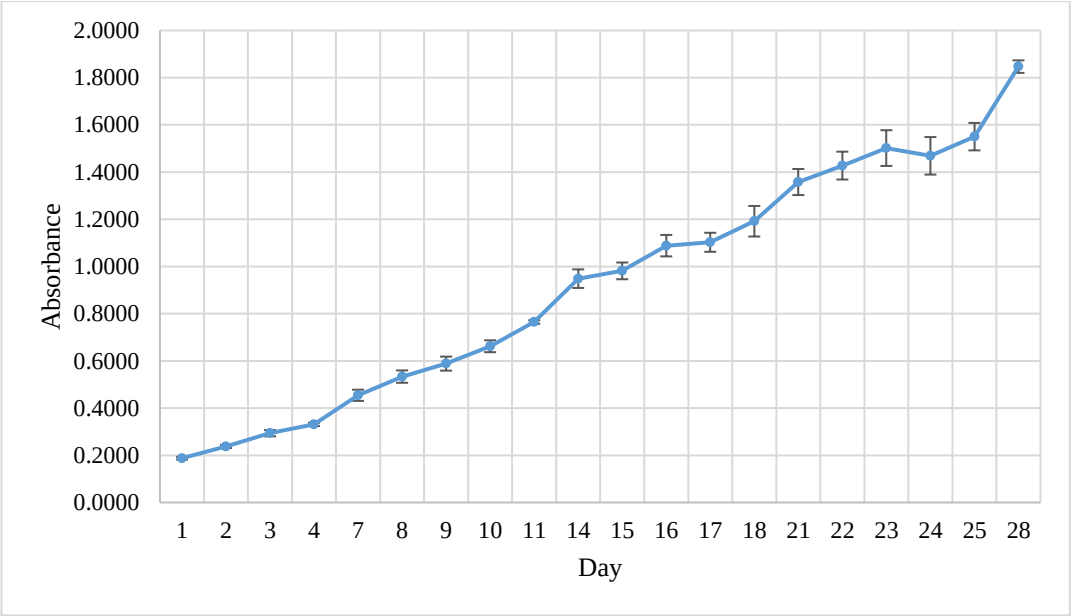

Figure 16. *C. vulgaris* growth curve at 700 nm

The count of *C. vulgaris* in the samples taken from the liquid medium was performed almost every day under a light microscope with a Neubauer slide. Cell counts were performed in the 0.1 ml sample taken. Cell counts were made starting from the second day of subculture until the end of 28 days. Standard curves of *C. vulgaris* cell counting values and the microscope view are given in figure 17 and figure 18 respectively.

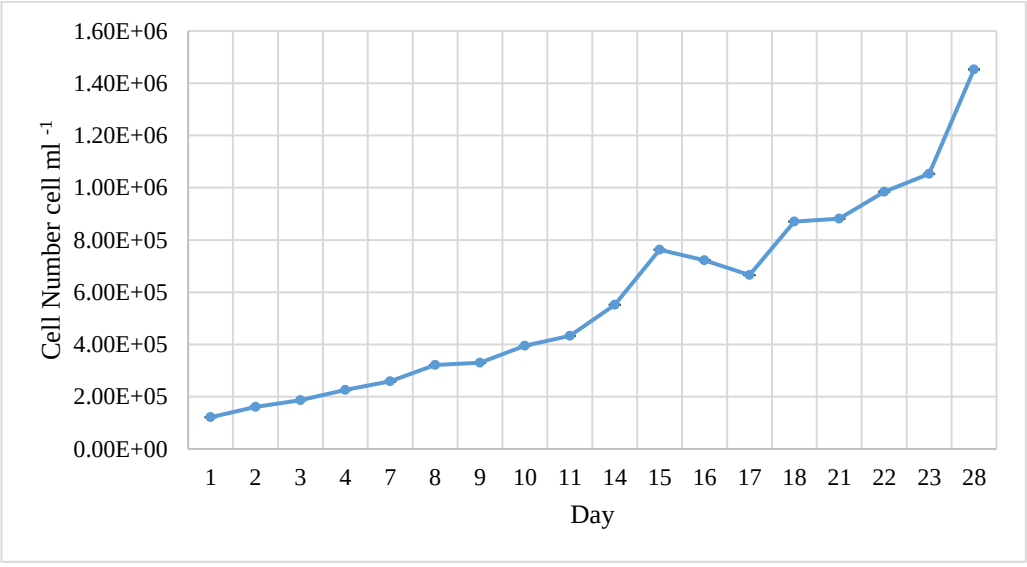

Figure 17. *C. vulgaris* cell number ml^{-1}

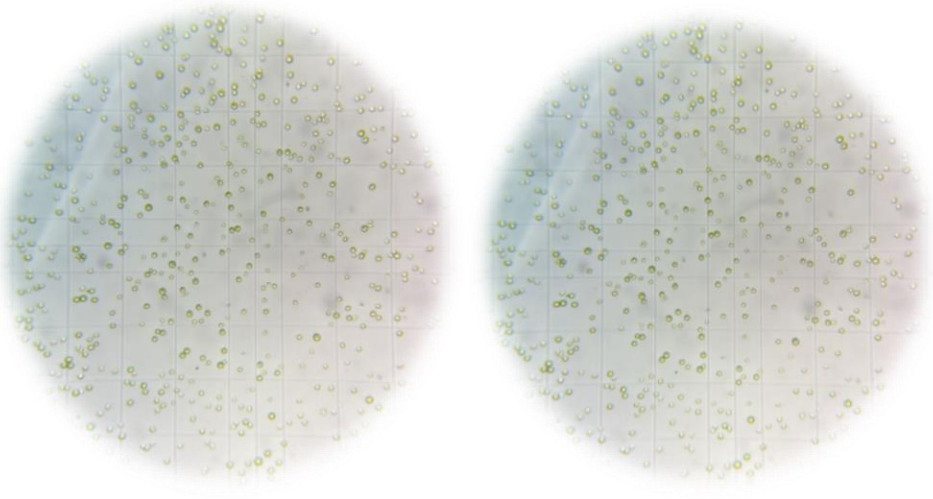

Figure 18. 40x view of C. vulgaris cells on Neubauer slide under microscope.

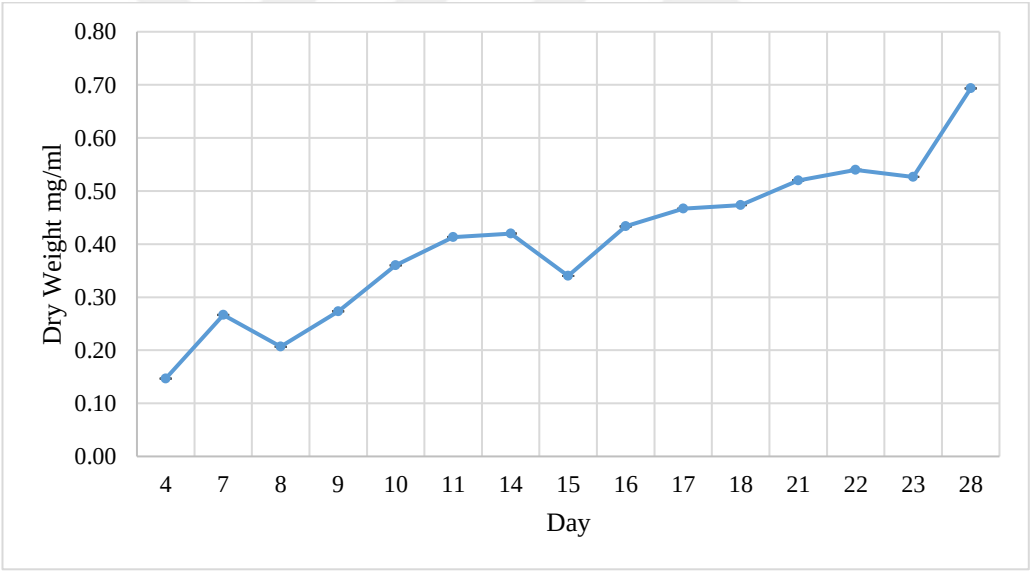

Figure 19. *C. vulgaris* dry weight mg/ml

Chlorophyll was extracted from the cells, which were separated from the nutrient medium by centrifugation at 4000 rpm for 5 minutes, using methanol. The total amount of chlorophyll and the total amount of carotenoids were measured spectrophotometrically using different wavelengths (470, 645 and 662) (Mhatre et al., 2018). Standard curves of *C. vulgaris* optical density values versus chlorophyll amounts are given in figure 20 and figure 21, respectively.

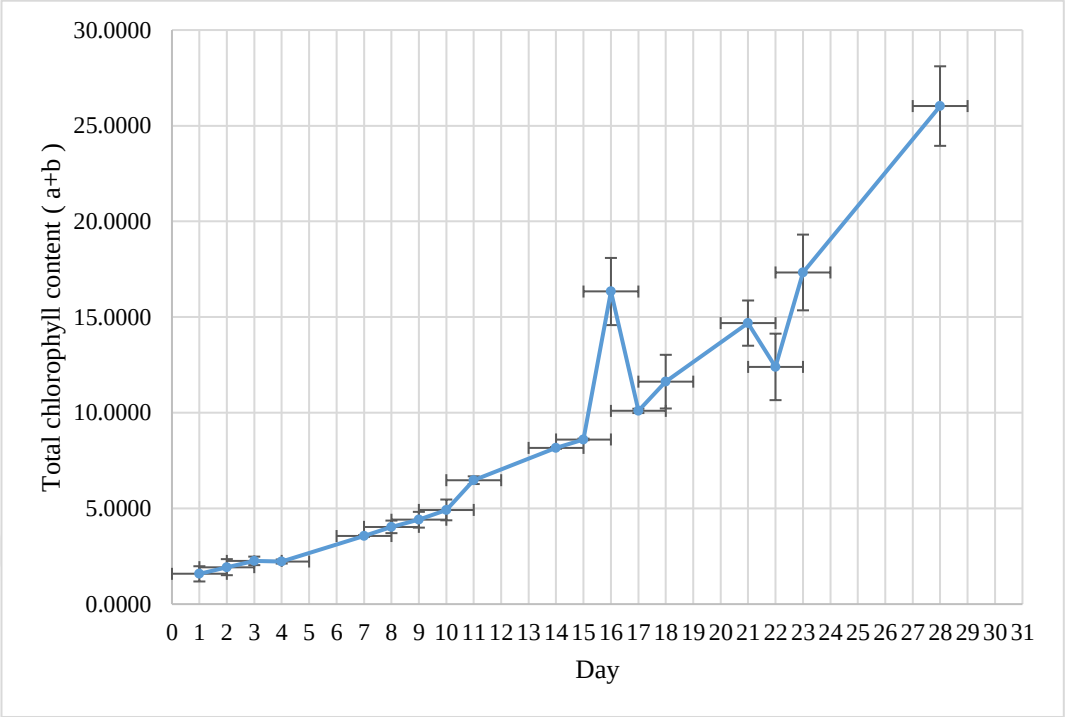

Figure 20. *C. vulgaris* total chlorophyll content

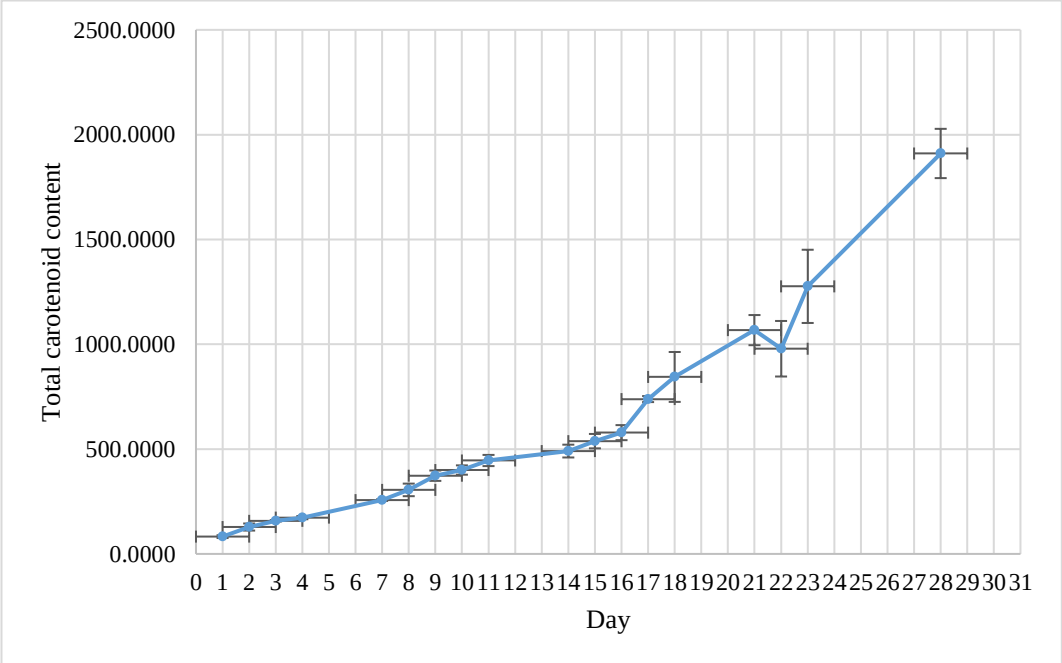

Figure 21. *C. vulgaris* total carotenoid content

4.1.1. Non-sterile Media with Distilled Water and Ultrapure Water Trials

C. vulgaris showed growth characteristics in growth media and materials without any sterilization process. The result is shown in figure 22 and figure 23. It showed similar growth properties to *C. vulgaris* subcultured under aseptic conditions in BG-11.

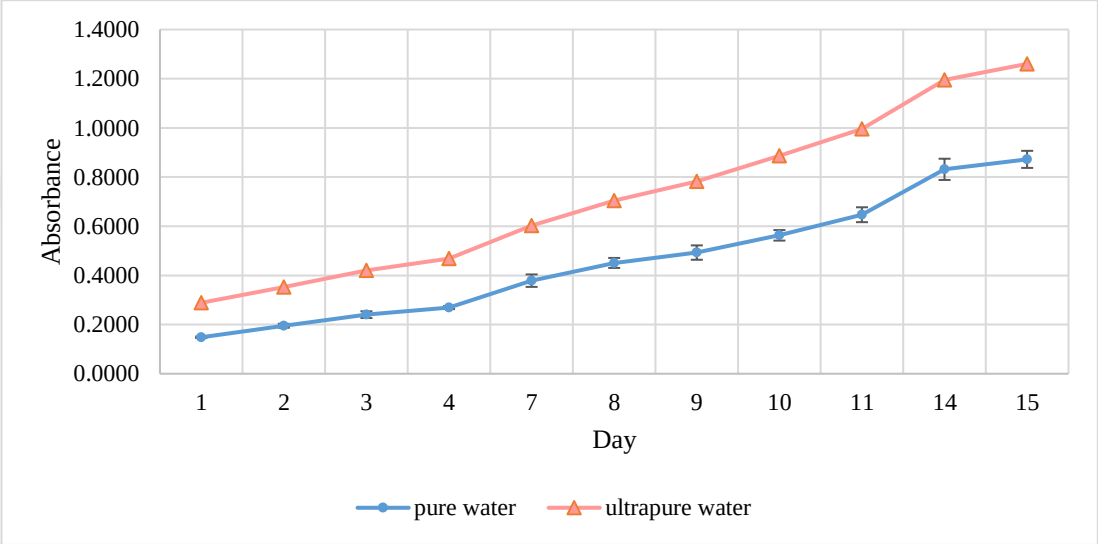

Figure 22. *C. vulgaris* growth curve at 650 nm (non-sterile ultra-pure water BG-11 media)

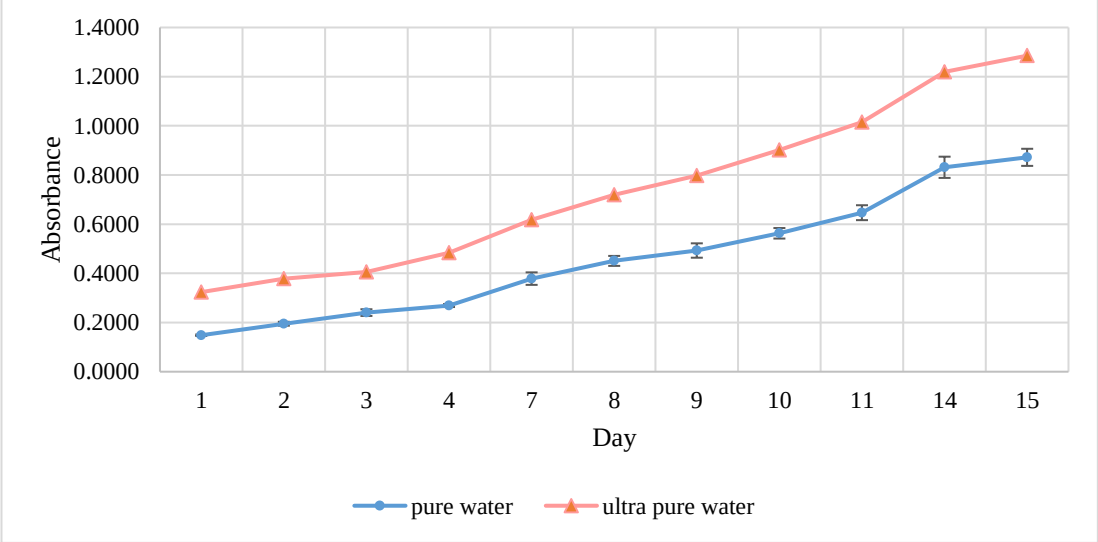

Figure 23. *C. vulgaris* growth curve at 650 nm (non-sterile pure water BG-11 media)

4.1.2. Designed prototypes

In Table 6 and Table 7, the cell number and biomass results of the prototypes used in the first facade trials are given. In line with these results of prototype number 2 (figure 24), a comparison was made in terms of biomass and cell number. Faster growth was detected in the prototypes compared to the growth media in the laboratory. It was observed that the fastest growth was in the tubular prototype to which spiral material was added.

Table 6. *C. vulgaris* cell count ml^{-1}

Day	Donut	Tank with leaf pieces	Spiral	Tank Only	Tank with S shaped pp sheet
1	72	52	19	24	54
5	141	65	183	88	38
7	106	108	162	11	48
12	145	76	408	179	124

Table 7. *C. vulgaris* dry weight in g/ml

Day	Donut	Tank with leaf pieces	Spiral	Tank Only	Tank with S shaped pp sheet
12	0.0010	0.0006	0.0018	0.0012	0,008

4.2. Trials with Photobioreactors in Different Façade Designs

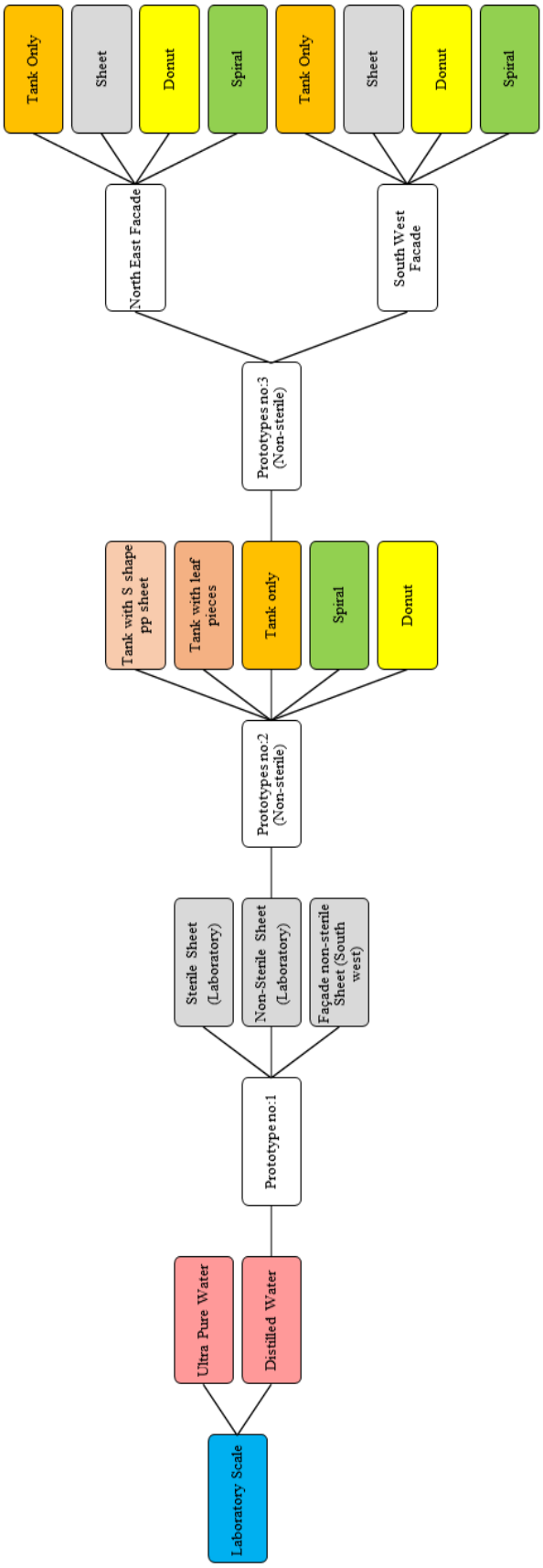

Figure 24.Trials with prototypes in different façade designs

4.2.1. Site Selection based on Case Studies' Results

Table 8. Absorbance value at 650 nm of prototypes at 4th floor, north-east façade

Day	Donut	Spiral	Sheet on Window	Tank with suspended S PP Sheet
1	0.2259	0.2441	0.1938	0.0888
4	0.5960	0.7424	0.2289	0.3591
5	0.8277	0.8595	0.3531	0.3620
6	0.8346	0.8941	0.2814	0.4809
7	0.8367	0.9106	0.2548	0.7020
8	0.9155	1.0154	0.2463	0.6540
11	1.0640	0.9977	0.2028	0.5615
12	1.1023	1.0076	0.2392	0.7591
13	1.1342	1.0845	0.2277	0.7736
14	1.1661	1.1614	0.2162	0.7881
15	1.1980	1.2383	0.2047	0.8026

During the work, there was an unforeseen leak in the sheet prototype on the north-west front, so the culture there quickly became extinct. Since the experiment could not be reconstructed due to limited time, some of the results on one front were incomplete. Therefore, there was missing data in table 9 and table 11.

When compared in terms of absorbance values, growth was observed in all prototypes. When compared in terms of absorbance values, growth was observed in all prototypes. Since direct sunlight is used as the light source in the prototypes, there may be differences in growth rates depending on the days they are installed. While there was no difference in the growth of *C. vulgaris* microalgae grown on two different fronts (prototypes number 3, figure 24), growth was faster in prototypes number 2, which were established in sunny weather.

Table 9. Absorbance value at 650 nm of prototypes at 3rd floor, south-west façade

Day	Donut	Spiral	Sheet on Window	Tank Only
1	0.2529	0.2526	0.1905	0.1984
4	0.7729	0.7098	0.9302	0.2862
5	0.8766	0.9424	1.1512	0.3619
6	0.8934	0.9948	1.1661	0.4617
7	0.9150	1.0741	0.9174	0.4946
8	0.9660	1.1430	0.8175	0.5609
11	1.0529	1.3240	-	0.5092
12	1.4698	1.3460	-	0.5154
13	1.5269	1.3712	-	0.4734
14	1.5840	1.3964	-	0.4314
15	1.6411	1.4216	-	0.3894

Table 10. Absorbance value at 700 nm of prototypes at 4th floor, north-east façade

Day	Donut	Spiral	Sheet on Window	Tank with suspended S PP Sheet
1	0.0258	0.2705	0.2173	0.0975
4	0.6388	0.7751	0.2316	0.4010
5	0.8640	0.8873	0.3600	0.4024
6	0.8241	0.9030	0.2768	0.4980
7	0.8537	0.9158	0.2587	0.7085
8	0.9170	1.0151	0.2467	0.6809
11	1.0703	0.9977	0.2082	0.5818
12	1.1076	1.0066	0.2442	0.7680
13	1.1368	1.0845	0.2273	0.7996
14	1.1660	1.1624	0.2104	0.8312
15	1.1952	1.2403	0.1935	0.8628

Table 11. Absorbance value at 700 nm of prototypes at 3rd floor, south-west façade

Day	Donut	Spiral	Sheet on Window	Tank Only
1	0.2733	0.2423	0.1752	0.2101
4	0.7997	0.7406	0.9396	0.2943
5	0.8881	0.9492	1.1553	0.3833
6	0.9001	0.9988	1.1591	0.4816
7	0.9177	1.0684	0.9170	0.5098
8	0.9635	1.1404	0.8205	0.5609
11	1.0532	1.3058	-	0.5175
12	1.4473	1.3284	-	0.5296
13	1.5103	1.3627	-	0.4808
14	1.5733	1.3970	-	0.4320
15	1.6363	1.4313		0.3832

4.3. Carbon Footprint Results

The environmental impact of the prototype and laboratory methods designed to produce 0.84 grams of *C. vulgaris* was calculated. Carbon footprint values are as in table 12 below.

The purpose of this LCA study is not to measure the total environmental impact of a process or to calculate the shares of the stages contributing to that environmental impact, but only to compare two different methods for the same production process. It is a common approach to not take the same inputs (raw materials) into account in the method. Therefore, in this thesis, we adopted this approach and modeled only the inputs that were different. This is the amount of electricity consumed during production. Therefore, there is no value for raw materials and other processes in this thesis. As seen in Tables 4 and 5, exactly the same 1 liter of nutrient medium was prepared in both growth environments.

Table 12. Total carbon footprint of two microalgae growth method

Product	**Total Carbon Footprint/Functional Unit ($kgCO_2$ eq/fu)**
Designed reactor (spiral)	45.39
Laboratory methods	79.29

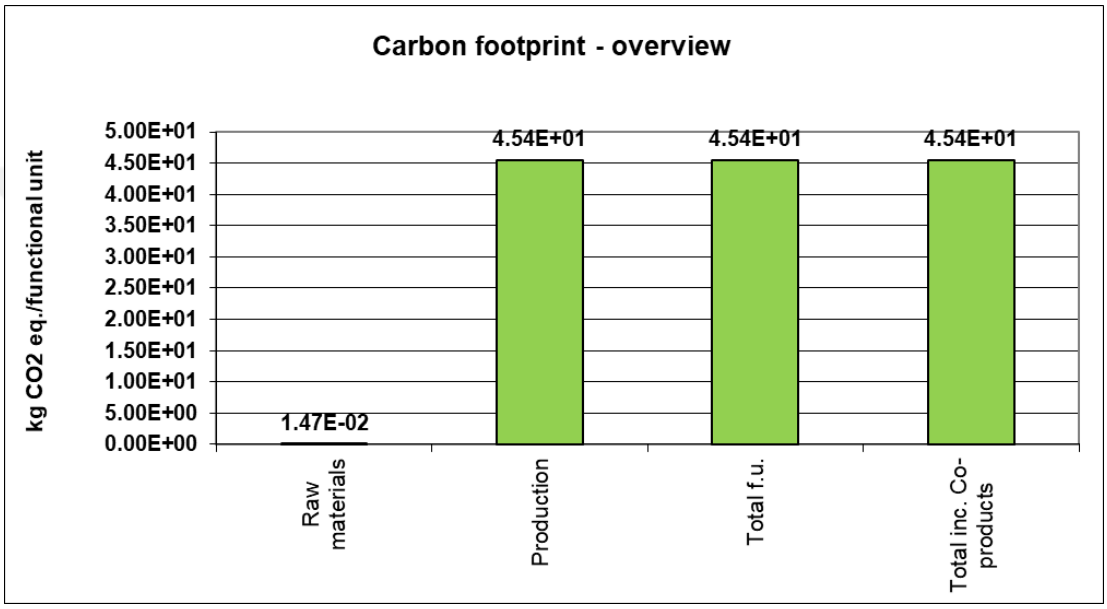

Figure 25. The carbon footprint ratios of designed reactor (spiral-prototype 2 at figure 24)

According to the results of this study, the same amount of *C. vulgaris* produced in a laboratory setting has a higher carbon footprint compared to the prototype method. While it takes 12 days to produce 0.84 g of algae in a laboratory environment, this amount was reached in 9 days in the spiral prototype.

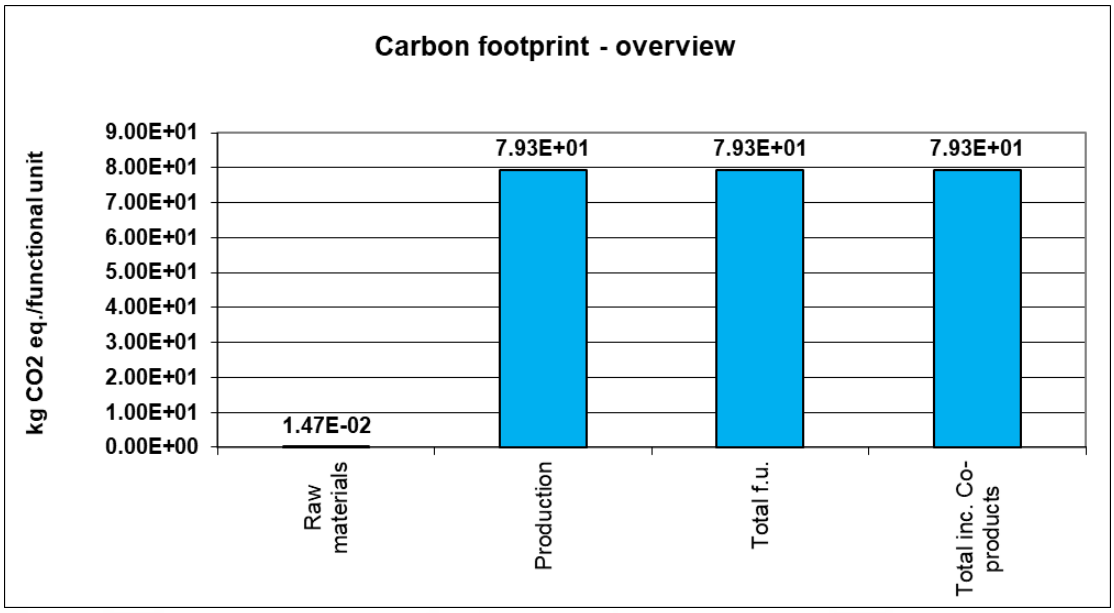

Figure 26. The carbon footprint ratios of laboratory methods (laboratory scale)

As seen in Figure 27, the material with the highest carbon footprint among raw materials is deionized pure water. As it is known, microalgae and *C. vulgaris* can be grown in various water sources. The water source to be used can be changed to reduce the carbon footprint of raw materials.

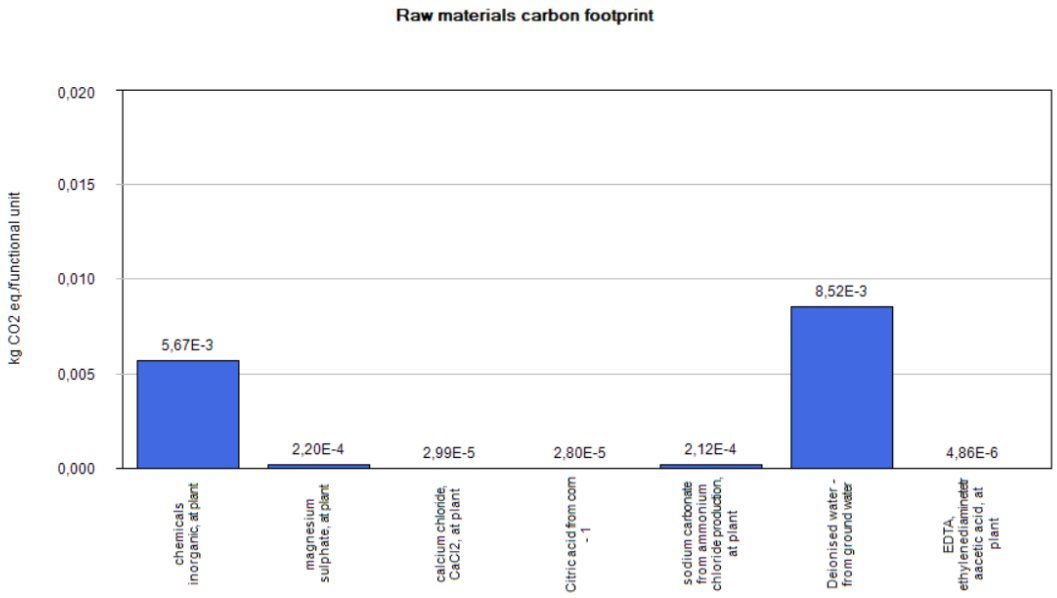

Figure 27. Impact of raw materials on total carbon footprint score for spiral and laboratory scale microalgal growth

The raw materials used being exactly the same, while the carbon footprint data influenced by them remains the same, the carbon footprint caused to produce the same amount of *C. vulgaris* in a laboratory environment is nearly 1.75 times that of the carbon footprint caused to produce *C. vulgaris* with the spiral prototype.

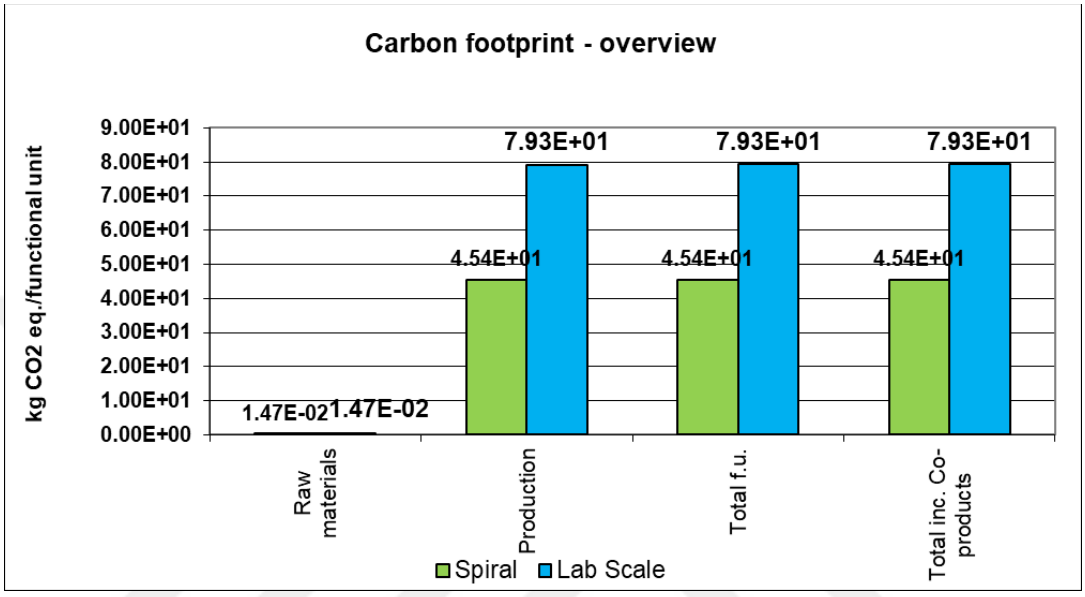

Figure 28. The comparison of carbon footprint scores of both growing system

The comparison chart of the production stages in laboratory scale production and spiral prototype is given in Figure 29. The data is the same because the harvesting and drying methods are the same. The differences applied during the production phase are seen in terms of carbon footprint value. *C. vulgaris*, with the same amount of biomass grown in a laboratory environment, causes 2.4 times more carbon footprint than the microorganism grown in the spiral prototype.

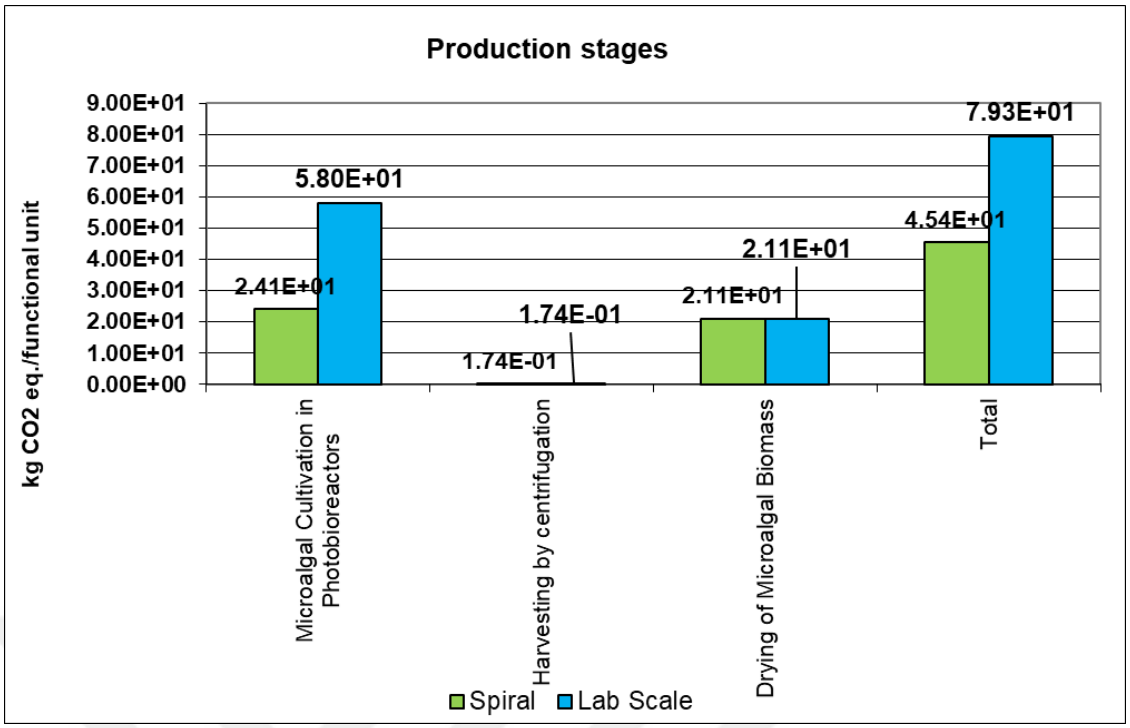

Figure 29. Comparison of carbon footprint of laboratory scale and spiral prototype in terms of production stages

Acidification potential (kg SO_2 eq./f.u.), eutrophication potential (kg PO_4 eq./f.u.), ozone layer depletion potential (kg R11 eq./f.u.), photochemical smog potential (kg C_2H_4 eq./f.u.), and human toxicity potential (kg DCB eq./f.u) values are the same for both production methods. Because these values come from raw materials.

CHAPTER 5: CONCLUSIONS

This thesis provides insights into the potential of microalgae in bioengineering and environmental sustainability. Through a comprehensive exploration of microalgae cultivation and photobioreactor design, the thesis sheds light on the unique growth characteristics that make microalgae valuable bioresources. The study also investigates in depth the challenges and opportunities associated with integrating microalgae into building facades, emphasizing the multifaceted nature of optimizing photobioreactor systems for microalgae cultivation.

Furthermore, the thesis evaluates the environmental and economic benefits of incorporating microalgae into building facades, offering a glimpse into the potential contributions of microalgal bio-facade modular systems to decarbonized built environments within the "European Green Deal" objectives. By addressing the complexities of microbial growth parameters and environmental impacts, this research paves the way for the development of sustainable and environmentally friendly solutions in bioengineering.

In conclusion, this thesis not only enriches our understanding of microalgal bio-facade modular systems but also highlights the potential of bioengineering to address environmental challenges and promote sustainable practices. This research serves as a resource for scholars, practitioners, and policymakers seeking innovative approaches to decarbonize built environments and advance the goals of environmental sustainability.

CHAPTER 6: FUTURE PERSPECTIVES

The thesis discusses challenges and opportunities in improving photobioreactor systems for growing microalgae. It suggests future research directions, including innovative design and engineering for enhanced productivity and reduced environmental impact. The study highlights the potential of microalgal bio-facades in urban buildings, proposing further research on their integration into buildings and public spaces to assess impacts on urban sustainability. Additionally, the thesis evaluates the environmental and economic benefits of using microalgae on building facades, suggesting future research focus on developing sustainable microalgal bio-products for circular economy practices. Lastly, the importance of considering social and cultural implications in integrating these systems into built environments is emphasized, suggesting future research to explore stakeholders' perceptions and attitudes.

To approach it from a design perspective, as seen in Figure 31, the spiral material made of polyethylene creates a surface to which *C. vulgaris* can attach. Thanks to these types of designs, new approaches can be gained to the harvesting methods of microalgae. A similar product is the leaves for the prototype leaf tank shown in figure 32. When these two prototypes were compared in terms of growth rates, they showed a faster growth compared to the growth data in the laboratory environment.

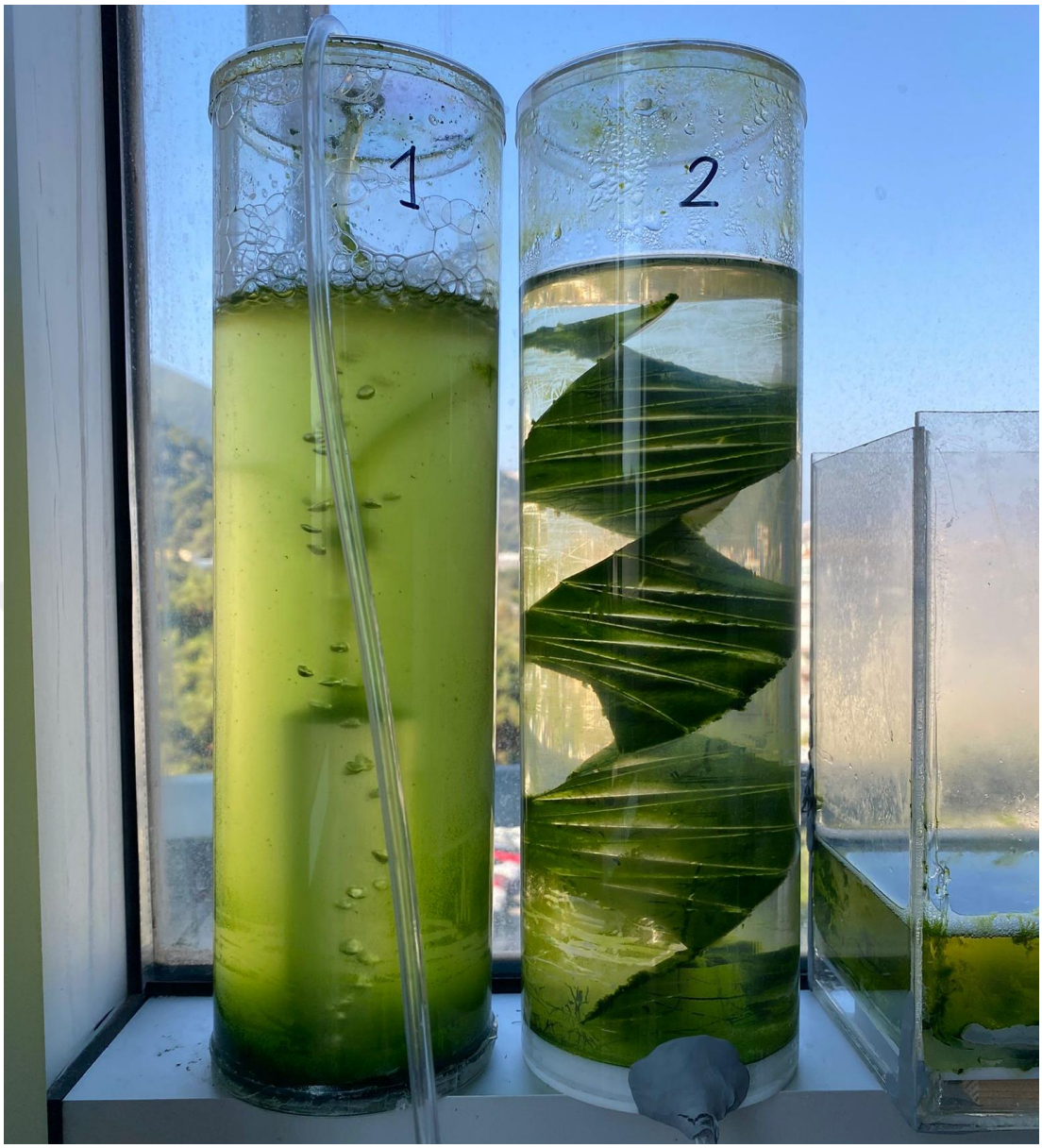

Figure 30. Tubular prototypes 1) donut, 2) spiral (prototype 2 at figure 24)

Figure 31. Spiral from prototype

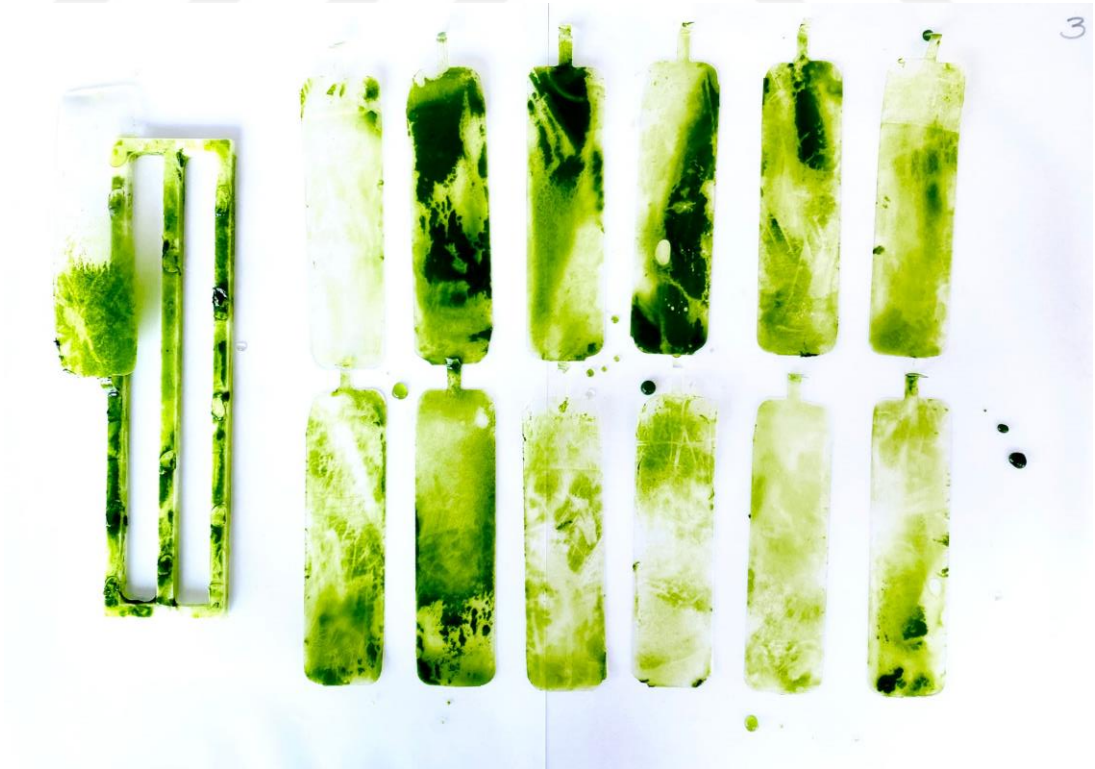

Figure 32. Leaf pieces from prototype (tank with leaf pieces, prototype 2 at Figure 24)

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