

CHAPTER 2

Literature Review

2. Literature reviews

The introduction chapter sheds light on the third- and fourth-generation biofuels derived from microalgae feedstock having the potential to replace petroleum. However, due to issues in commercial-scale production of microalgae biomass, microalgae-based biofuels are not yet able to replace with petroleum and be commercially successful. To tackle these issues, the current research aims to design, develop, and optimize a microalgae cultivation system capable of commercial-scale microalgae production and to study the parameters of the microalgae biomass produced in the developed system.

A thorough systematic study based on literature reviews was carried out to conduct the research. A literature review was conducted to study the available microalgae culture systems and their pros and cons, followed by factors affecting the design of the microalgae culture. Subsequently, studies on various optimization methodologies to optimize the parameters of the developed system were carried out. Detailed literature reviews are presented in the following sections.

2.1 Commercial microalgae culture systems

In the natural environment, multiple microalgae species with different physiochemical and biological characteristics coexist; thus, it is difficult to be utilized for any specific application. This necessitates the isolation and monoculture of microalgae species for specific usage. The first successful mono-species microalgae culture was reported to have been done by Beijerinck in 1890 with *Chlorella vulgaris* microalgae species [1]. However, research focusing on the mass culture of microalgae was started much later in the late 1940s in the USA, Germany, and Japan [1]. The first commercial microalgae culture of *Chlorella* microalgae was done in Japan in 1960 [2], followed by the culture of *Spirulina* microalgae in 1970 in Mexico [3]. By 1980, around 46 commercial microalgae facilities produced around 1000 Kg of microalgae biomass per month in Asia [1]. In just 30 years from the beginning of its first commercial production, i.e., by 1996, Japan alone traded 2000 tons of *Chlorella* [1]. The microalgae market has been growing and diversifying ever since. Amongst the various significant factors affecting the success of large-scale commercial microalgae culture, developing a cost-effective commercial-scale culture system is of prime importance. Table 2.1 summarizes the prominent microalgae culture systems currently being

deployed for large-scale microalgae culture, with their advantages and disadvantages, highlighting their strengths and weaknesses for better understanding.

Table 2.1: Prominent large scale microalgae culture systems, their advantages and disadvantages.

Culture system	Advantages	Disadvantages	Reference
Open Systems			
Raceway ponds	<ul style="list-style-type: none"> • Low capital and operational costs. • Ease of operation and maintenance. • No issue of oxygen built up. 	<ul style="list-style-type: none"> • Low productivity • High contamination • Difficulty in monoculture • High rate of evaporation • Need a large open area • Low surface to volume ratio 	[4-8]
Stirred circular ponds	<ul style="list-style-type: none"> • Low capital and operational costs. • Ease of operation and maintenance. • No issue of oxygen built up. 	<ul style="list-style-type: none"> • Low productivity • High contamination • Difficulty in monoculture • High rate of evaporation • Need a large open area • Low surface to volume ratio 	[1, 9-11]
Closed culture systems			
Horizontal tubular photobioreactor	<ul style="list-style-type: none"> • High production efficiency • Lesser cost of harvesting • Highly contamination resistant • Higher control of the operation • Higher stability of culture 	<ul style="list-style-type: none"> • High capital cost • High cost of temperature control • Culture damage due to oxygen inhibition • Biofouling 	[12-15]
Helical tube photobioreactor	<ul style="list-style-type: none"> • High productivity • High efficiency • High illumination surface area • Contamination resistant • High CO₂ dissociation rate 	<ul style="list-style-type: none"> • High capital and operational cost • High energy consumption in mixing • High hydrodynamic stress on cells • Biofouling 	[16-20]
Vertical column photobioreactor	<ul style="list-style-type: none"> • Efficient gas/liquid transfer • High CO₂ dissociation rate • Efficient discharge of dissolved oxygen • Low cost of mixing • Low hydrodynamic stress 	<ul style="list-style-type: none"> • High capital and operating costs • Biofouling • Challenging to clean up • Low economic viability 	[21-25]

Flat plate photobioreactor	<ul style="list-style-type: none"> • High illumination surface area • Low contamination • Easy removal of dissolved oxygen • High surface to volume ratio • Lesser energy consumption 	<ul style="list-style-type: none"> • Difficult in temperature control • Large system needs significant support material • High capital cost • Biofouling • Difficult to maintain cell suspension 	[26-29]
Plastic bag photobioreactor	<ul style="list-style-type: none"> • Low capital cost • Contamination resistant • Simple design 	<ul style="list-style-type: none"> • Biofouling • Prone to leakage • Difficulty in mixing • Short lifespan • Difficult to clean up 	[30-34]

Following a detailed study of the large-scale microalgae culture systems, their pros and cons, the different factors affecting microalgae culture system designs were studied.

2.2. Microalgae culture system design parameters

Microalgae photobioreactors (PBRs) are systems designed to cultivate microalgae under controlled conditions, utilizing light, carbon dioxide, and nutrients. The design of a photobioreactor significantly influences its efficiency and productivity. Designing a microalgae photobioreactor involves a proper balance of multiple parameters to obtain optimum productivity and efficiency. The primary factors influencing microalgae photobioreactors are light, mixing and hydrodynamics, gas exchange, temperature control, material selection, nutrient supply, and efficient control of the culture parameters [35-37]. Understanding and optimizing these parameters is the key to harness the full potential of the developed system.

2.2.1 Light

Light is one of the most important parameters to be considered while designing a photobioreactor [38]. Light is the primary source of energy utilized by microalgae in the autotrophic mode of cultivation for its photosynthesis [39]. The characteristics of light, like intensity, wavelength, and photoperiod, greatly influence the metabolism of a microalgae species and, thereby, its final composition [40]. The influence of these parameters is discussed in detail in the following section.

2.2.1.1 Light Intensity

Light intensity plays a significant role in the photosynthesis of microalgae, and the availability of adequate light intensity is essential for its metabolism [41]. Low light intensity decreases photosynthesis rate, thereby decreasing productivity [42]. However, increasing light increases photosynthesis and productivity to a certain level known as the photoinhibition threshold, beyond which, if the light intensity is further increased, it causes damage to the microalgae and, thereby, reduces productivity and may lead to the complete collapse of the culture [42]. The photoinhibition threshold varies depending on the microalgae culture and other culture parameters like light wavelength, temperature, pH, and nutrient availability [43, 44]. Thus, maintaining optimal light intensity per the cultured condition and microalgae species is of prime importance.

2.2.1.2 Light wavelength

Light wavelength is another significant parameter of microalgae culture. The wavelength of light used for microalgae culture affects the rate of photosynthesis because wavelengths of light in the blue and red spectrum are more effective in photosynthesis [45, 46]. Researchers have reported blue illumination enhances cell growth and increases lipid accumulation in microalgae species, whereas red illumination enhances cell division [47]. Thus, starting the microalgae culture using red light until the desired cell count is achieved and then switching the light to blue is an efficient strategy for higher productivity [48]. Yuan et al. [49] reported enhanced lipid accumulation using blue illumination and enhanced carbohydrate accumulation using red illumination. LEDs, having a narrow spectrum and highly efficient in electroluminescence conversion, can provide light at specific wavelengths and illuminance to the microalgae culture, thus gaining more research interest as a light source for microalgae cultivation [50-52].

2.2.1.3 Light duration

Light duration or light/dark cycle is the time the microalgae culture is exposed to illumination in the 24-hour cycle of a day. Along with the light wavelength and light intensity, light duration plays a vital role in the performance of microalgae photosynthesis. Velez-Landa et al. [53] found a 12:12 light-dark cycle to perform best for *Verrucodesmus verrucosus* microalgae species to perform best, while Wahidin et al. [54] found a 16:8 light-dark cycle to perform best for *Nannochloropsis* sp. microalgae. On the other hand, Zhang et al. [55] reported photoperiod of 3:3 s and 5:5 s light-dark

cycle resulted in higher biomass and lipid productivity of *Nannochloris oculata*, *Chlorella* sp., and *Chlorella pyrenoidosa* compared to 30:30 min, 12:12 h light/dark and 24 h light cycle. Thus, it can be concluded that photoperiod significantly affects photosynthesis in a microalgae species, and thus, it needs to be considered and optimized for the optimum performance of a photobioreactor.

2.2.1.4 Light distribution

Uniform light distribution within a photobioreactor to achieve equal illumination in all the regions of the photobioreactor is essential for maximizing the performance of the photobioreactor [56, 57]. Uniform light distribution ensures that each microalgae cell receives optimum light energy for its photosynthesis reaction. Light uniformity can be achieved by internal illumination [56, 58, 59], transparent materials [60-62], and reflective surfaces [63]. Other strategies to enhance uniform light distribution are the use of light guides [64, 65], optical fibers [66, 67], or strategically placed LEDs [68, 69]. Additionally, moving the culture through different light zones within the reactor can help achieve uniform exposure. While designing a photobioreactor, it is essential to consider uniform light distribution to enhance productivity and efficient light utilization.

2.2.2 Mixing and hydrodynamics

Proper mixing of the culture media is essential for keeping the microalgae afloat and preventing it from settling to the bottom, allowing adequate exposure to light and nutrients [70]. Additionally, mixing also helps in the proper gas exchange required for efficient operation of the microalgae culture system [71]. However, excessive mixing can cause CO₂ outgassing, essential for microalgae growth. It can cause stress to microalgae cells and damage them, as well as lead to energy loss due to excessive power consumption by the mixing system [71].

Mixing the culture media induces different flow patterns inside the culture system. Laminar flow minimizes energy consumption, while turbulent flow ensures better mixing. The choice depends on the reactor design and the microalgae species. Laminar flow reduces shear stress but may not provide adequate mixing. Turbulent flow enhances mixing but at the cost of higher energy consumption and potential damage to delicate microalgae cells.

Different agitation methods can be implemented for mixing the microalgae culture, like mechanical stirring [72, 73], air bubbling [74-77], and the use of paddle wheels [78, 79], depending on the culture system parameters. Each method has advantages and limitations and can potentially damage microalgae cells if the mixing is too intense. Mechanical stirring can provide adequate mixing but may also introduce shear stress. Air bubbling is gentle but may not be as effective in larger reactors. Paddle wheels offer a balance but require careful design to avoid dead zones. Thus, the cautious selection, design, and optimization of the implemented mixing method are crucial for the success of the microalgae culture system.

2.2.3 Gas exchange

One primary requirement of a microalgae culture system is to facilitate proper gas exchange. Microalgae need CO₂ as a nutrient source for their growth and produce oxygen during photosynthesis [36, 80]. A limited supply of CO₂ can cause a decrease in photosynthesis, thereby reducing productivity. On the other hand, excessive CO₂ supply can make the culture acidic, leading to a drop in the pH level, harming the microalgae species, or even damaging the microalgae culture [81-83]. The rate and supply of CO₂ depend on various factors like microalgae species being cultured, the concentration of microalgae in the culture, nutrients, and illumination conditions, culture system design and configuration, temperature, etc. Controlled CO₂ supply ensures optimal pH levels and supports photosynthesis. Diffusers [84] and spargers [85] are often used to introduce CO₂ into the microalgae culture. CO₂ must be supplied at rates matching the microalgae consumption, avoiding deficiencies and excess that can lead to suboptimal growth conditions. Thus, properly optimizing the CO₂ supply to the culture system is essential.

The oxygen produced during the photosynthesis reaction, if it accumulates, can lead to increased dissolved oxygen concentration in the microalgae culture, inhibiting photosynthesis due to an increase in respiratory activity of the mitochondria [86]. High oxygen levels can lead to oxidative stress, affecting cell health and productivity. The combination of intense light and high oxygen concentration results in photooxidative damage to algal cells. Thus, excessive oxygen built-up eventually leads to a loss in microalgae biomass. Efficient degassing systems, such as airlift designs or membrane contactors, help maintain appropriate oxygen levels [87, 88].

2.2.4. Temperature control

An essential factor in the growth of microalgae is temperature. Although microalgae are adaptive to a specific range of temperature fluctuation, to obtain maximum growth, the temperature must be within the region of favorable growth temperature for the cultured microalgae species [89]. Optimal growth temperature varies depending on microalgae species. Renaud et al. [90] evaluated the ideal temperature for the growing of different algae strains. They found ideal temperatures of 33-35 °C for *Chaetoceros* sp, 27-30 °C for *Chaetoceros* sp., *Cryptomonas* sp., *Isochrysis* sp and *Prymnesiophyte NT19*, and 25-27 °C for *Rhodomonas* sp. Thus, maintaining the appropriate temperature of the culture media to achieve optimal growth of the microalgae strain being cultured is of prime importance.

Large-scale microalgae culture systems might require sophisticated cooling systems for temperature control. The heat generated by light sources and metabolic activity can raise the temperatures of microalgae culture [91]. Without temperature control systems, temperatures in an outdoor closed photobioreactor can rise to as much as 30 °C above the surrounding air temperature [36, 91]. Water jackets, heat exchangers, and evaporative cooling systems are some techniques currently used to regulate the temperature of microalgae culture [92-94]. Cooling systems must be designed to handle the thermal load without causing rapid fluctuations that can stress the culture. Proper insulation minimizes external temperature fluctuations, ensuring a stable internal environment. Insulation materials and designs can help maintain a consistent temperature, reducing the energy required for active cooling or heating.

2.2.5 Material selection

The material used to build the photobioreactor significantly affects the systems' performance, durability, and cost [95, 96]. Materials used in photobioreactor construction must be durable, non-toxic, and compatible with the culture medium [96, 97]. When selecting material for photobioreactor design, specific considerations are transparency, chemical resistance, and mechanical strength. Materials like glass or certain plastics ensure good light transmission. The choice of material affects the reactor's overall efficiency of light utilization. Material used for photobioreactor development must resist corrosion from nutrients and byproducts. Some materials may degrade or leach chemicals into the culture medium, affecting microalgae growth. The

material must certainly ensure structural integrity, especially in large-scale systems. The material must withstand pressure changes, mechanical stresses, and environmental conditions without failure.

2.2.6 Nutrients supply

Microalgae need additional nutrients from those in the photosynthesis reaction for appropriate growth and reproduction. Nitrogen and phosphorus are the major nutrients microalgae require for its growth and metabolism. Additionally, other micronutrients like boron, sulfur, molybdenum, hydrogen, oxygen, potassium, copper, magnesium, sodium, chlorine, iron, calcium, manganese, vanadium, cobalt, nickel, silicon, and selenium are required for their metabolism [89]. The content and concentration of these minerals vary depending on the microalgae species being cultured. Thus, maintaining the proper chemical recipe for the microalgae being cultured is of prime essence. To ensure adequate nutrient availability for the cultured microalgae in a photobioreactor, a nutrient delivery system must be designed to provide a balanced supply of nutrients and avoid limitations or toxicities. The nutrient delivery should ensure a homogeneous distribution of nutrients that prevents localized depletion and promotes uniform growth. Uneven nutrient distribution can lead to poor growth and reduced overall productivity. The optimal concentration of nutrients varies with species and growth phase. Automated dosing systems can help maintain appropriate levels of nutrients in the culture. Excessive nutrients can lead to waste and potential contamination, while deficiencies can limit growth.

In some instances, nutrient-based stress can be induced in the microalgae being cultured to achieve the desired products from the microalgae. Goiris et al. [98] reported tocopherols and ascorbic acid levels were higher in nutrient-limited cultures, particularly under phosphorus limitation in *Phaeodactylum tricornutum*, *Tetraselmis suecica*, and *Chlorella vulgaris* microalgae species. Nitrogen limitation can induce stress on certain microalgae species, leading to increased lipid content; however, nitrogen limitation decreases biomass productivity, thus adversely affecting overall lipid productivity [99]. Therefore, proper optimization of nutrients plays a vital role in the final output of a microalgae culture system.

2.2.7 Efficient control of the culture parameters

As discussed in the previous sections, numerous factors affect the overall performance of microalgae growth. These parameters must be precisely controlled for optimum performance of the microalgae culture system. Automated control systems enhance the precision and efficiency of photobioreactors [100, 101]. They monitor and adjust environmental parameters, including light, temperature, pH, and nutrient levels. Advanced systems can use sensors and feedback loops to optimize conditions continuously. Real-time monitoring of critical parameters allows for immediate adjustments, maintaining optimal growth conditions. Sensors for light intensity, temperature, pH, and nutrient concentrations are essential components. Additionally, automated systems reduce the need for manual intervention, increase reproducibility, and can operate continuously, improving overall productivity [102, 103]. Advanced control systems can also include data logging and analysis capabilities for process optimization [104, 105].

2.3 Optimization techniques

Following the design and development of a system, the input/control parameters of the systems need to be optimized to achieve the most desired outcome. Various statistical and mathematical tools are available that can be used to optimize a developed system systematically and efficiently. This section demonstrates some of the popular optimization tools that can be implemented to achieve this purpose.

2.3.1 Response Surface Methodology

A set of statistical and mathematical methods called Response Surface Methodology (RSM) are used in the development, enhancement, and optimization of processes [106]. It helps engineers and researchers find the best settings for process variables to achieve desired outcomes efficiently. It investigates the connections between one or more answer variables and a number of explanatory variables [107]. RSM determines the optimal combination of factor levels (continuous variables) that maximize or minimize a specific response (e.g., yield, quality, or performance). The main idea is to use a designed experiment sequence to obtain an optimal response. The critical components of RSM are (i) experimental design, (ii) modeling, (iii) optimization, (iv) analysis, and (v) validation.

Experiments can be designed using the Central Composite Design (CCD) [108] or Box-Behnken Design [109] approach. The CCD approach is a commonly used design that includes a factorial or fractional factorial design with center points, augmented with a group of 'star' points that allow curvature estimation [110]. The Box-Behnken Design is a spherical, rotatable design based on three-level incomplete factorial designs without an embedded factorial or fractional factorial design [111].

Modeling the system can be achieved by developing polynomial models followed by regression analysis. The polynomial models are usually second-order (quadratic) polynomial models used to approximate the true response surface. The regression analysis fits the polynomial model to the experimental data to create a response surface.

Optimization is carried out with the help of Contour plots and surface plots, which are visual tools to understand the relationship between variables and responses. The desirability functions combine multiple responses into a single criterion for optimization.

Analysis of variance (ANOVA) assesses the significance of model terms and the fit of the model, and finally, the validation confirms the model's predictions with additional experiments.

2.3.2 Taguchi Method

The Taguchi Method, initially developed by Japanese engineer and statistician Genichi Taguchi, is a statistical approach used to improve the quality of manufactured goods and processes. It is primarily known for its use of Design of Experiments (DOE) principles, which help identify and control variables that influence product quality [112]. The method emphasizes robustness, ensuring that products and processes perform consistently under varying conditions [113].

Taguchi introduced the concept of the quality loss function, which quantifies the loss incurred by society when a product deviates from its target value [114]. This approach shifts the focus from merely meeting specifications to minimizing variation and achieving optimal performance. The Taguchi Method employs orthogonal arrays to design experiments efficiently [115, 116]. These arrays allow for the systematic variation of multiple factors simultaneously, providing comprehensive data with fewer experiments compared to traditional methods [117]. The S/N ratio is a crucial metric in the Taguchi Method, used to measure the robustness of a process or product.

The goal is to maximize this ratio, thereby enhancing the performance consistency under various noise conditions (uncontrollable factors) [116]. The control factors are variables that can be controlled during the manufacturing process, while noise factors are external variables that can affect the process but are beyond control. The Taguchi Method focuses on optimizing control factors to minimize the impact of noise factors [116].

The Taguchi Method is implemented through the following steps:

- (i) *Problem Definition*: Clearly define the problem or objective of the optimization process. This includes identifying the key performance characteristics that need improvement.
- (ii) *Selection of Control Factors*: Determine the factors that can be controlled and varied during the experiment. These factors are chosen based on their potential impact on the performance characteristics.
- (iii) *Choice of Orthogonal Array*: Select an appropriate orthogonal array based on the number of factors and levels to be tested. This array will dictate the design of the experiment.
- (iv) *Conducting the Experiment*: Perform the experiments as per the orthogonal array, systematically varying the control factors.
- (v) *Analysis of Results*: Analyze the experimental data to determine the optimal levels of the control factors. This often involves calculating the S/N ratios and using statistical techniques such as ANOVA (Analysis of Variance).
- (vi) *Verification*: Confirmation experiments are conducted to verify that the optimized conditions yield the desired improvements in performance.

2.3.3 Gaussian Process Regression (GPR)

Gaussian Process Regression (GPR), originally known as Kriging, was named after the South African mining engineer Danie Krige and had its roots in geo-statistics [118]. Over time, it has been adapted and expanded into what is now broadly termed Gaussian Process Regression (GPR).

The Gaussian Process Regression (GPR) technique leverages probabilistic models to provide highly accurate predictions and optimization solutions, making it invaluable for complex and data-intensive applications [119]. A Gaussian Process (GP) is a collection of random variables, any

finite number with a joint Gaussian distribution. In GPR, the goal is to infer a function that best describes the underlying data distribution. This is achieved by defining a mean function (often assumed to be zero) and a covariance function (kernel) that encodes assumptions about the function's properties, such as smoothness and periodicity.

The critical components of GPR are [120]:

- (i) *Mean Function*: Represents the expected value of the process at any point. It is often assumed to be zero, simplifying the model without loss of generality.
- (ii) *Covariance Function (Kernel)*: Defines the similarity between different points in the input space. Common kernels include the Radial Basis Function (RBF), Matérn, and Polynomial kernels. The choice of kernel significantly influences the model's performance and its ability to generalize from the training data.
- (iii) *Hyperparameters*: Parameters of the covariance function that are typically learned from the data. These include length scales, which determine the extent of influence of one data point over another, and variance parameters, which scale the output of the kernel.

The GPR process optimization involves refining processes to achieve the best possible performance according to specified criteria, such as minimizing costs, maximizing output, or improving quality [121]. GPR excels in its domain due to several inherent advantages like [122]:

- (i) *Uncertainty Quantification*: One of the standout features of GPR is its ability to provide predictions and a measure of uncertainty. This is crucial in process optimization where decision-making under uncertainty is common.
- (ii) *Handling Sparse and Noisy Data*: GPR is particularly effective when dealing with sparse or noisy data. Its probabilistic nature allows it to make robust predictions even when data is limited or imprecise.
- (iii) *Flexibility and Adaptability*: The choice of kernel function allows GPR to be tailored to various types of data and processes. This flexibility makes it applicable across multiple industries and optimization problems.
- (iv) *Non-Parametric Nature*: As a non-parametric method, GPR does not assume a predefined form for the underlying function, allowing it to naturally model complex and non-linear relationships.

Gaussian Process Regression is a potent tool for process optimization, offering precision, flexibility, and robust uncertainty quantification. Despite challenges related to computational complexity and kernel selection, ongoing advancements in technology and methodology are set to enhance the scalability and accessibility of GPR.

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