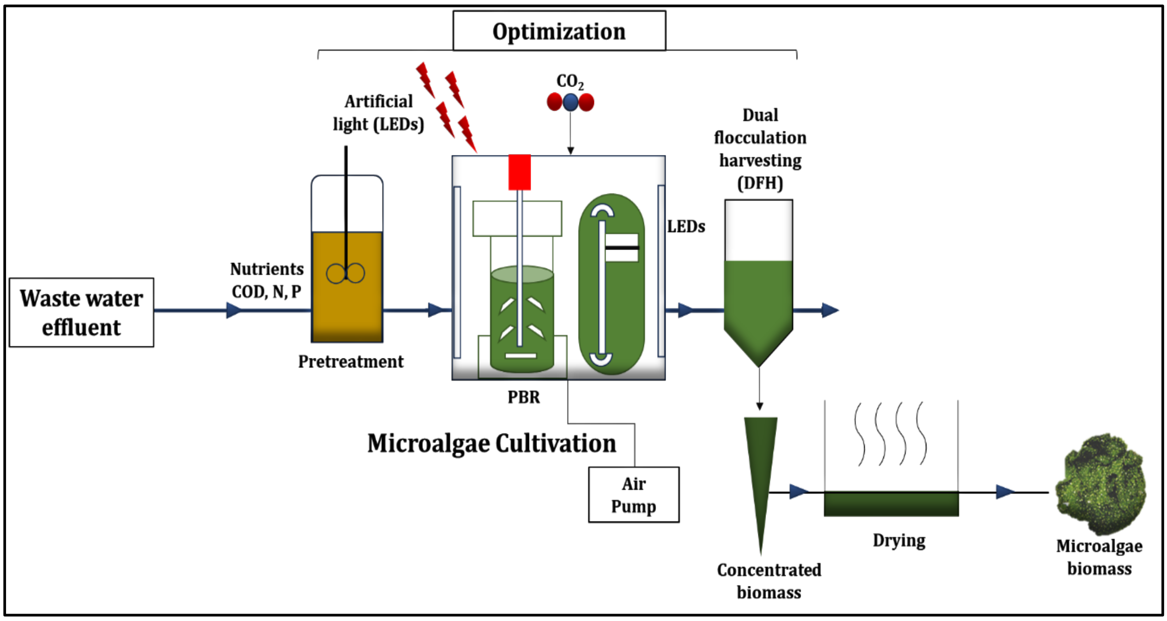
Chapter 1

Introduction



1. Introduction

In recent decades, there has been an increase in the global discharge of greenhouse gases and wastewater as a result of rapid industrialization, urbanisation, and globalisation. Many nations are ignoring these issues in their quest to boost their economies [1]. Around the world, 380 billion m^3 of wastewater are produced annually by human activity. Wastewater generation might rise by 24% in 2030 and 51% in 2050 at the current rate [2]. 80% of the wastewater produced worldwide is discharged into bodies of water without being properly treated [3]. Only 8% of the wastewater produced in low-income nations is treated; in lower-middle-income countries, 28%; in upper-middle-income countries, 38%; and in high-income countries, more than 70% of the wastewater produced is treated [3]. The natural environment may be in danger if there is an excessive amount of wastewater discharged into water bodies, which raises the nutrient level and causes eutrophication [4]. Additionally, the effects of eutrophication on fishing and real estate operations pose a threat to the economy. Eutrophication causes an estimated 2 billion dollars in losses per year [5]. Currently, wastewater is treated using conventional activated sludge (CAS) treatment. Organic waste from wastewater can be effectively treated by CAS, while inorganic nutrient remediation necessitates a separate treatment step. For instance, phosphorus is treated via chemical precipitation, while nitrogen is treated using the anammox process [6], [7]. However, CAS with extra procedures is expensive and uses a lot of energy, accounting for 3% of all power produced globally. The amount of electricity used increases with the amount of wastewater produced [8]. Additionally, wastewater treatment facilities produce a significant quantity of greenhouse gases, which represent 1.3% of all global greenhouse gas emissions [9]. According to the 2019 IEA Fuel Combustion Report, India, United States, and China each released 2.2, 4.8, and 9.8 metric tonnes of CO_2 into the atmosphere, respectively [10]. The Intergovernmental Panel on Climate Change (IPCC, 2018) reports that there has been an increase in global temperature that is between 0.8 and 1.2 ºC above the target threshold for industry [11]. At the Paris Climate Change Agreement, it was resolved that nations should step up their efforts to cut greenhouse gas emissions and keep global warming to less than 1.5 °C [11]. However, the United Nations Environment Programme's (UNEP) analysis indicates that current efforts will only result in a 0.01 ºC drop in global temperature by 2050. As a result, by the end of the century, the temperature will have increased by 3.2 °C, causing global warming and an increase in ocean water levels [12]. According to other forecasts, there is only a 50% possibility that the world's temperature will rise by 1.5 °C [13], [14]. Therefore, it is essential to create environmentally friendly technology that can treat wastewater while also capturing greenhouse gases and recovering resources.

Rapid growth has been observed in the amount of research being done on technology that can reduce carbon emissions and recover resources from wastewater treatment facilities. Several methods have made progress, including the creation of biochar, microbial electrolytic carbon capture, microbial electrosynthesis, built wetlands, and microalgae-based wastewater treatment technology [15]. A sustainable method for treating wastewater and recovering nutrients in the form of microalgae biomass is biological wastewater treatment mediated by microalgae. They have a great potential for fixing CO_2 through photosynthesis and can absorb nutrients from wastewater more quickly [1]. Some of them could even grow heterotrophically in the dark by using the organic carbon in effluents. Microalgae from wastewater absorb some of the necessary elements, such as carbon, nitrogen, and phosphorus, for the synthesis of useful biomass [16], [17]. When used for wastewater treatment, microalgae have the following benefits: (i) the microbial biomass generated can be used to make biofuel, animal feed, and protein supplements (single cell protein); (ii) fertilisers can be made from microbial biomass; and (iii) oxygen is produced during photosynthesis [18], [19].

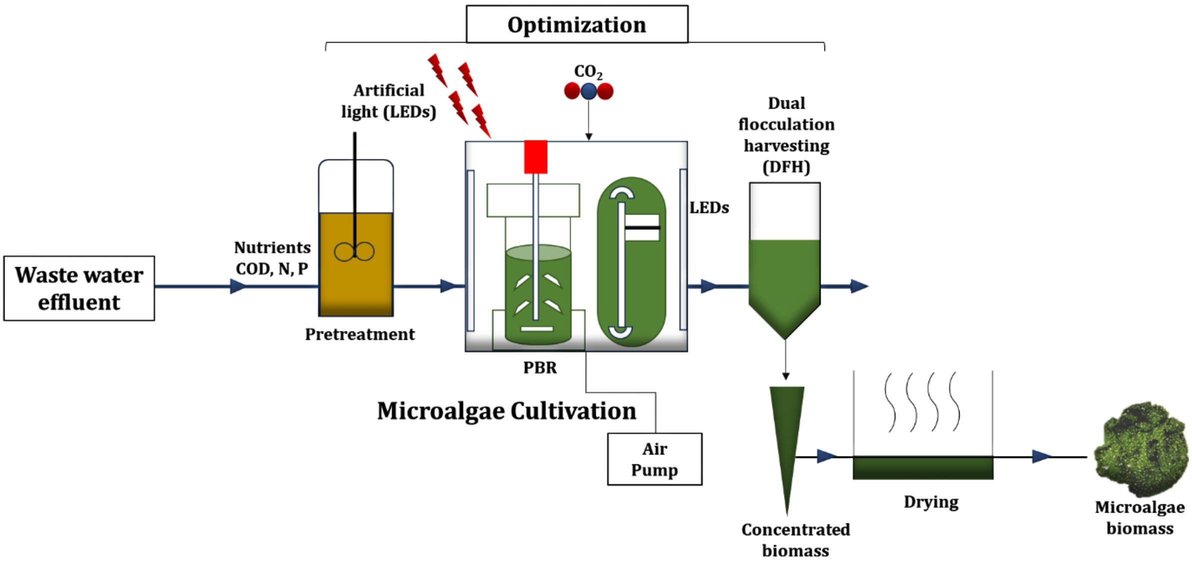


Figure 1.1. Process flow diagram depicting wastewater treatment by microalgae and simultaneous biomass production.

An integrated microalgae-based wastewater treatment process is shown in **Figure 1.1.** It shows how CO_2 released during industrial combustion or respiration and nutrients from wastewater are used to develop microalgae. Through biosorption, biodegradation, and bioaccumulation processes, a wide range of microalgae species have been employed to treat various types of wastewater (WW) originating from home, municipal, industrial, agricultural, and livestock sources [20]. The temperature, initial inoculum level, pH, light intensity, photoperiod, nutrient concentration in wastewater (BOD, COD and N/P Ratio) and other factors all have a significant impact on microalgae growth (**Figure 1.1**). These elements interact with one another in a mutual way and work together to influence the course of treatment [21]. It becomes crucial to optimise these variables and offer the proper mix in order to increase the treatment capacity and biomass output of microalgae. However, testing every possible combination of variables in a single study is essentially impossible [22]. Using conventional statistical methods like the response surface approach, numerous studies have been published in the literature that have sought to optimise predictor factors for increasing biomass productivity and wastewater treatment capabilities (RSM). [23]–[31] The models, however, only function for a limited set of input parameters, and model building from RSM necessitates multiple experimental attempts. In the Web of Science core collection group, 2705 publications have reported microalgae biomass production in wastewater, generating a large dataset (**Figure 1.2**).

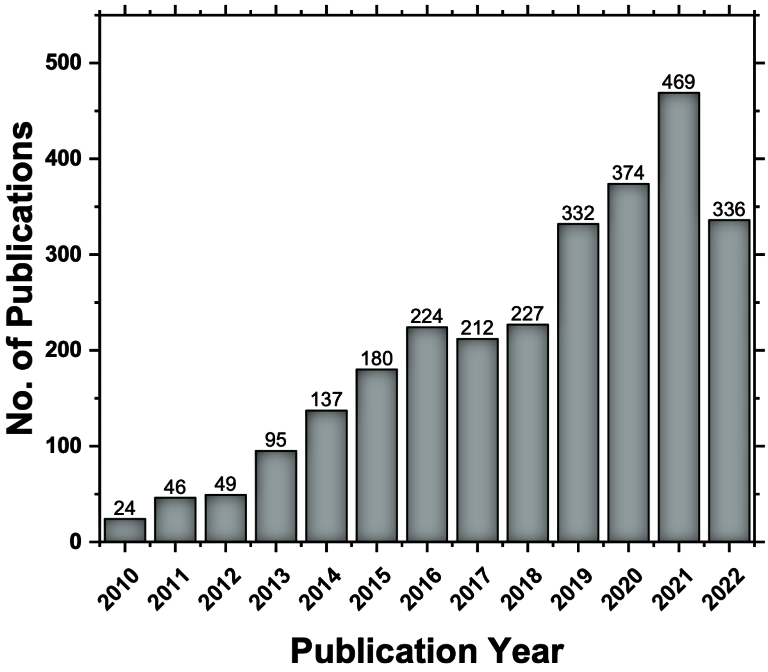


Figure 1.2. Chart representing the number of publications and years that appeared in search of “Web of Science Core Collection” for the keyword “microalgae,” “wastewater” and “biomass” [32].

Artificial Neural Networks (ANN) in combination with Genetic Algorithms (GA) has been recognized as a suitable and powerful tool for the optimization of these input parameters. This review aims to highlight the advantages and applications of ANN-GA in microalgal bioremediation to optimize input parameters such as CO_2 in the inlet air, temperature, initial inoculum level, pH, light intensity, photoperiod, and nutrient concentration in wastewater. Microalgal bioremediation systems involve intricate and non-linear relationships between input parameters and output responses [33]. ANN-GA offers a data-driven, non-parametric approach that can capture the complex interactions among multiple input variables, leading to accurate predictions and optimized parameter settings. ANN is known for its ability to learn from data and model complex relationships [34]. But the computational time and resources required by the ANN models for prediction are more than that required by RSM models [35]. When combined with GA, which efficiently searches for the optimal parameter values, ANN-GA exhibits high prediction accuracy and generalization capability. This ensures robust and reliable optimization results in microalgal bioremediation processes. Traditional optimization methods often require a significant number of time-consuming and resource-intensive experimental trials to determine the optimal input parameters [36]. ANN-GA significantly reduces the experimental efforts by iteratively searching the parameter space, converging towards the global optimum with fewer trials. In real-world applications, experimental data can be noisy or incomplete. ANN-GA is capable of dealing with such noisy data and can still produce meaningful results [37]. It also enables the incorporation of prior knowledge and expert inputs, enhancing the optimization process. In microalgal bioremediation, multiple input parameters need to be optimized simultaneously to achieve the best results [38]. ANN-GA enables multivariate optimization, considering the interactions among various input variables, resulting in a more comprehensive and efficient optimization process [36]. Microalgal bioremediation systems often operate under dynamic conditions with varying environmental factors. ANN-GA's real-time adaptability allows the system to adjust and optimize input parameters in response to changing conditions, ensuring continuous and efficient pollutant removal. The trained ANN-GA model can be easily transferred to different microalgal bioremediation systems with similar characteristics. This scalability and transferability make ANN-GA an attractive tool for various microalgal bioremediation applications, saving time and resources in parameter optimization. By achieving optimal input parameters with ANN-GA, microalgal bioremediation processes can be designed more sustainably. Efficient pollutant removal, along with the production of valuable biomass, contributes to a circular and eco-friendly approach to waste effluent treatment. In conclusion, the integration of ANN-GA as a suitable tool for optimizing input parameters in microalgal bioremediation has shown great promise [39]. Its ability to handle complex and non-linear relationships, high prediction accuracy, and capability to minimize experimental efforts make it an attractive and efficient approach for achieving optimal performance in microalgal bioremediation processes. By harnessing the power of ANN-GA, we can pave the way for more effective and sustainable waste effluent treatment solutions using microalgae. Further research and application of ANN-GA in this field will undoubtedly contribute to the advancement of eco-friendly bioremediation technologies.

Co-cultivation of two microalgae involves growing two different microalgal species together in the same culture medium to enhance their growth and productivity. Co-cultivation refers to the simultaneous growth of two or more microbial species (e.g., microalgae-microalgae or microalgae-bacteria) in the same culture system to exploit their synergistic interactions for improved biomass productivity, nutrient removal, or metabolite production. The co-cultivation of two microalgal species holds significant importance in enhanced bioremediation and biomass production [40], [41]. Co-cultivating two microalgal species can create a synergistic effect, where their combined metabolic activities complement each other, leading to enhanced bioremediation capabilities [41]. Each species may have different pollutant removal efficiencies, allowing for a more comprehensive and efficient remediation of diverse pollutants in waste effluents [42]. Different microalgal species have varying nutrient uptake preferences and capabilities. Co-cultivation enables the efficient utilization of available nutrients in the growth medium, minimizing nutrient limitations and promoting sustained microalgal growth and biomass production. The co-cultivation of compatible microalgal species can lead to increased biomass production compared to monocultures [43]. This is because the species may utilize different resources, reducing competition for nutrients and light, resulting in higher overall biomass yields. Co-cultivating different microalgal species enhances the metabolic diversity within the system. This diversity can lead to the synthesis of a wider range of bioactive compounds, such as pigments, lipids, and proteins, which have numerous valuable applications in industries like food, pharmaceuticals, and cosmetics. Co-cultivation allows for the utilization of various waste streams, as different microalgal species may have varied tolerance levels and abilities to assimilate specific pollutants. This approach enables the conversion of waste into valuable biomass while simultaneously reducing the environmental burden. Co-cultures often exhibit higher stability and resilience to changes in environmental conditions compared to monocultures [44]–[46]. This stability ensures a consistent performance in bioremediation and biomass production, even under fluctuating conditions. Co-cultivation can promote positive microbial interactions, such as mutualistic relationships, symbiosis, and cross-feeding. These interactions can improve the overall efficiency of nutrient cycling and utilization, contributing to a more efficient and sustainable microalgal bioremediation system [47], [48]. Co-cultivating microalgae in wastewater treatment systems can effectively remove a wide range of pollutants, including nutrients, heavy metals, and organic compounds [49]. This process can significantly improve the quality of treated effluents, making them suitable for safe discharge or reuse. Microalgal biomass produced through co-cultivation has the potential to sequester carbon dioxide, contributing to climate change mitigation efforts. This biomass can be used for bioenergy production or as a carbon sink, reducing greenhouse gas emissions.

Microbial harvesting is a critical step in many biotechnological processes as it directly impacts the efficiency and cost-effectiveness of the overall operation. Proper selection and optimization of the harvesting method are essential to achieve high yields of viable and functional microbial biomass for various applications in biotechnology and environmental remediation [50]. Advances in harvesting technologies continue to contribute to the advancement of microbial-based industries and the sustainable utilization of microorganisms in various fields. The dual harvesting approach, combining one inorganic and one organic flocculant, holds significant importance in enhanced harvesting of microalgal biomass during waste effluent bioremediation for several key reasons [51]. The use of both inorganic and organic flocculants in combination can significantly enhance the harvesting efficiency of microalgal biomass. Inorganic flocculants, such as iron and aluminum salts, have strong binding abilities, facilitating the aggregation of microalgal cells. Organic flocculants, like chitosan, act as bridging agents, further promoting the formation of larger and denser flocs, leading to improved biomass recovery from the culture medium. The dual harvesting approach creates a synergistic effect, where the strengths of both inorganic and organic flocculants complement each other [52]. This combination can overcome the limitations of using a single flocculant and optimize the flocculation process, resulting in a higher overall harvesting efficiency. Inorganic flocculants are generally less expensive than organic flocculants. By using a combination of the two, it is possible to reduce the overall cost of the flocculation process while achieving improved harvesting efficiency [53], [54]. This cost-effectiveness is particularly beneficial for large-scale microalgal biomass production in waste effluent bioremediation. Organic flocculants, like chitosan, are often considered more environmentally friendly and biodegradable compared to synthetic chemical flocculants [55]. By incorporating an organic flocculant into the dual harvesting approach, it is possible to reduce the environmental impact of the harvesting process while maintaining high efficiency. Waste effluents from various industries may contain a complex mixture of pollutants and suspended solids. The dual harvesting approach provides flexibility in handling diverse effluent compositions, ensuring effective biomass recovery in different waste remediation scenarios [56]. Dual harvesting can lead to improved biomass quality. The combined flocculation approach may result in the removal of a higher percentage of impurities and unwanted substances, making the harvested biomass more suitable for further processing and utilization in various applications, such as biofuels, animal feed, and value-added bioproducts. By using a combination of inorganic and organic flocculants, it is possible to minimize the residual flocculant remaining in the harvested biomass [51]. This reduced presence of flocculant in the biomass enhances its potential for applications in food, pharmaceuticals, and other sensitive industries. ANN-GA can be applied in the optimization of input parameters for the dual harvesting of microalgal biomass, where the combination of Artificial Neural Networks (ANN) and Genetic Algorithms (GA) proves to be a powerful tool. The ANN component learns from the data and models the complex relationships between input parameters and biomass yield. Meanwhile, GA efficiently searches the parameter space to find the optimal combination of input variables, such as co-cultivation ratios, nutrient concentrations, and harvesting time, for maximizing biomass productivity [54]. This approach minimizes experimental efforts, provides accurate predictions, and enhances the efficiency of the dual harvesting process, contributing to sustainable microalgal biomass production for various applications in biotechnology and environmental solutions.

The current study evaluated the potential of a newly isolated strain, from the inlet of a sewage treatment plant, *Diplosphaera mucosa VSPA*, belonging to the class Trebouxiophyceae, for treating carpet and textile effluent. Previous literature lacked any publications related to the application of *Diplosphaera mucosa VSPA* for such treatment. Biomass production and treatment efficiency of *Diplosphaera mucosa VSPA* were compared with a well-known strain of the same class, *Chlorella pyrenoidosa*, to assess the feasibility of its application in wastewater treatment. The growth pattern and substrate removal in the photobioreactor were described using the Photobiotreatment Model (PhBT) and Gompertz Model (GP). Additionally, the study explored the impact of wastewater's N/P ratio on biomass generation, which could be crucial for reactor operation. Next, the focus shifted to optimizing different input parameters influencing the growth of *Diplosphaera mucosa VSPA* in textile and carpet effluent. Five critical parameters, including temperature, pH, light intensity, wastewater composition, and Nitrogen/Phosphorus (N/P) ratio, were optimized to increase biomass productivity and nutrient removal efficiency. The optimization process involved generating different models using RSM and ANN, which were then solved using GA to determine the optimal values of the input parameters, leading to high biomass production and nutrient removal efficiency. In next study, treatment efficiency of IWE (petroleum refinery waste effluent) by *Diplosphaera mucosa VSPA* were evaluated and, compared it with *C. pyrenoidosa*. The treatment process was carried out in a lab-scale open raceway bioreactor, and the value addition of the treatment process was determined in terms of microalgae biomass and lipid productivity. Experimental results were simulated and validated using both three-parameter and four-parameter growth models. In another investigation, a co-cultivation of a newly isolated strain and S. obliquus was performed to treat TE (textile and carpet effluent) using a 10-liter bubble column photobioreactor. A photobioreactor is a closed cultivation system designed to grow photosynthetic microorganisms (such as microalgae or cyanobacteria) under controlled environmental conditions. It allows precise regulation of light, temperature, CO_2, and nutrient supply, minimizing contamination and maximizing productivity. The co-cultivation mode was evaluated by comparing biomass production and nutrient removal with the mono-cultivation mode using heterotrophic and mixotrophic cultivation modes. The change in the population of both strains during co-cultivation was analyzed using microscopy and flow cytometry. Final study aimed to optimize the dual harvesting of Diplosphaera mucosa VSPA in bioremediation by optimizing key input parameters, such as pH, chitosan dosage, alum dosage, mixing time, and settling time, crucial for enhancing flocculation efficiency. The optimization process utilized a combination of Response Surface Methodology (RSM) and Artificial Neural Networks (ANN) models for prediction, along with Genetic Algorithms (GA) to determine the optimal values of these parameters, maximizing flocculation efficiency through the optimization process, utilizing the predictive capabilities of RSM, ANN, and the optimization capabilities of GA.