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Review

A Critical Review on the Status and Progress of Microalgae Cultivation in Outdoor Photobioreactors Conducted over 35 Years (1986–2021)

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Abstract: Microalgae, a renewable bio-resource, are considered a potential value-added commodity and a tool to combat climate change. Microalgal research has received worldwide attention recently. Different perspectives have been explored, but cultivation in outdoor photobioreactors (PBRs) is still a less explored field. This review summarizes the studies conducted on the microalgae cultivated in outdoor PBRs only. The locations, algal strains, PBRs, and cultivation media used in these studies were identified and tabulated. Different aspects of outdoor algal cultivation in PBRs, such as temperature control, light intensity control, photosynthetic efficiency (PE), the outdoor adaptation of strains, PBR designs, and algal growth and biochemical composition variation from the weather, were studied and reviewed. A brief review of downstream processes and environmental and economic impacts was also conducted. This review summarizes what has been carried out in this field so far and will help researchers to determine what further work needs to be conducted and in which direction to proceed.

Keywords: microalgae; outdoor cultivation; photobioreactor (PBR); cultivation strategy; temperature control; growth factors; environmental impact

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1. Introduction

Global warming has changed our perception of how we should view our lifestyles. It has driven us to re-evaluate our individual, social, national, and regional spheres, as well as our commercial and industrial practices. Policies, regulations, and practices to reduce greenhouse gas (GHG) build up in the atmosphere are becoming increasingly common around the world. Various strategies have been adopted to reduce GHG emissions and decrease their concentration in the atmosphere. While increasing forest cover has a crucial role in reducing CO_2 in the atmosphere and halting deforestation is important, other options should also be considered and explored. More than two-thirds of the Earth's surface is covered by oceans, which house numerous micro-organisms that have the potential to contribute to utilizing CO_2 if their biomass can be utilized before decomposition. Commercial cultivation of microalgae has the potential to utilize atmospheric and flue gas CO_2 as the produced algal biomass can be used for various applications [1,2]. Microalgae can play a crucial role in combating global warming and reducing the consumption of chemicals used in traditional gas upgrading plants [3,4]. However, microalgae are not currently being considered to play a significant role in addressing global warming.

Among all ocean organisms, microalgae are an essential component. They are unicellular plants that grow using carbon, water, and other nutrients through the process of photosynthesis. Microalgae cells are free of roots, stems, or leaves, and they uptake nutrients more efficiently and grow faster than terrestrial plants. Microscopic organisms, phytoplanktons, including microalgae, are essential for maintaining biodiversity in oceans, and with their high multiplying capability, they contribute to around 50% of the planet's primary production [5]. Algal biomass is a source of protein, lipids, and carbohydrates,

and depending on the species, they can be used to produce food, feed, fuel, bioplastics, cosmetics, and other value-added products [6,7].

Microalgae can be produced in properly designed cultivation units built on non-fertile or semi-fertile substrates where growing traditional plants and crops is challenging. Microalgae can grow in an environment where only a few nutrients are available. Although optimized algal growth cannot be achieved, microalgae accumulate different components in a nutrient-starved medium. For example, *Rhodomonas* sp. is shown to produce enhanced lipids in a nitrogen-starved medium [8]. By manipulating the nutrient ratios, the biochemical composition of microalgae can be altered. They can be grown commercially in indoor environments, as well as outdoors, such as on a roof. EnerGaia, a start-up from Bangkok, is an example of this. They grow Spirulina in 15 L to 20 L cylindrical photobioreactors (PBRs) on rooftops [9,10]. Another example is Algalif, an algal production facility in Iceland that produces natural astaxanthin products from microalgae and cultivates microalgae inside indoor tubular PBR systems [11].

Microalgae can be cultivated commercially using open ponds or closed PBRs. Open ponds are commonly used due to their lower cost and ease of maintenance. However, they lack the ability to facilitate complex operations, which are necessary for providing additional benefits. PBRs have the potential to fill this void, but their higher construction, operation, and maintenance costs pose a challenge for commercial use. Most laboratory-scale experiments on PBRs have been conducted indoors, where optimum control can be achieved but at a higher cost. Algal productivity in outdoor environments is typically lower than laboratory yields, as outdoor temperatures and light intensities are more variable and extreme. These environmental conditions are often not replicated when selecting strains indoors [12]. According to Dahlin et al. (2018), microalgae that are grown in outdoor environments have to cope with natural fluctuations in temperature and light conditions, making it challenging to identify strains that can be cultivated successfully in a controlled laboratory setting [13]. It is worth mentioning that most large-scale commercial microalgae production farms, including both open ponds and closed photobioreactors, are operated outdoors (personal communication). Despite this, few studies have been conducted on outdoor PBRs.

In this review, we conducted a thorough search for research articles on microalgae cultivation in outdoor PBRs from major scientific publishers such as Elsevier, Black & Wiley, Taylor & Francis, Springer Nature, and MDPI. We also consulted experts from academia and industry through personal communication. In this review study, we considered PBRs located outdoors, inside greenhouses, or those associated with roofs as outdoor PBRs. To date, review articles on microalgae cultivation in outdoor PBRs are scarce, if any exist. In this review, we provide a comprehensive review of upstream and downstream processes, simulations, models, and different types of impacts and comparisons. While some downstream processes and impacts may be similar to other algal review articles, the upstream process is a unique contribution of this study.

2. Drivers of Algae Research

The algae industry has demonstrated significant commercial potential in producing food, feed, biofuel, and biomaterials, which presents a great opportunity for achieving sustainability and decarbonization [14]. These advantages have garnered attention from policymakers worldwide. For instance, a study conducted on several experts and stakeholders stated that European Union policymakers are adapting regulations in favour of the algae market in Europe [14]. Additionally, consumers are opting for sustainable and environmentally friendly products more frequently, which has led to a greater demand for algae [14]. Consequently, the Blue Economy Forum is investigating gaps in financial support, lack of support due to proper policies and regulations, and market challenges [14]. In Europe, there are hardly any socio-cultural barriers to algae products and applications, except for genetically modified microalgae [15]. A new study revealed the potential of algae-based protein food as a viable alternative [16]. The study identified several important

drivers for consumer acceptance, including health and nutritional benefits, taste, natural and fresh characteristics, organic or sustainable certification, and less environmental impact [16]. These drivers are encouraging the algae industry to expand further. For instance, the demand for algae products, such as *Spirulina* and *Chlorella*, is on the rise in Europe [15]. A study analysed data from multiple studies conducted in different regions and found that consumer perceptions varied across regions [16]. For instance, Australian respondents did not exhibit significant concerns about food safety and responsibility [17]. Food neophobia, lack of trust in the promoters, and perceived high cost were identified as major consumer concerns [16]. In order to address the concerns and meet the requirements of specific consumer groups, as well as attract new groups, further research is necessary.

Microalgae are gaining practical considerations in national and regional policies of North America, the Asia Pacific, and Australia, as well as being used as a tool to combat climate change and promote renewable resources of food, feed, and fuel [18–20]. Microalgae are already present in the North American and Australian markets, and attempts are being made to diversify their uses. In the Asia Pacific region, markets are being assessed, feasibility studies are being conducted, and even pilot-scale experiments collaborating with the private sector have been carried out [19,20]. However, algal research faces barriers in all regions, particularly in developing countries. The predictability of the yield of microalgae, as well as the overall cost and energy efficiency of production, are still questionable. The uncertainties, followed by the immature status of the current algal market, have played a significant role in deterring investors from funding new microalgal projects. This is one of the dominant factors hindering the growth of the algal market in developing countries [19].

The majority of research activities related to the microalgae market applications have been dedicated to six major market opportunities: pharmaceuticals, biofertilizers, biofuels, nutraceuticals, bioplastics, and cosmetics [21]. European experts believe that these four sectors will play a major role in the commercialization of microalgae in the near future. These sectors include food (such as protein and polysaccharide production), bioremediation and biofertilizers, the invention and commercialization of antibiotics and antimicrobial compounds, and the production of vaccines and recombinant proteins [15]. A recent study on the European Atlantic algal market identified key obstacles to industrializing microalgae, including the need to strengthen existing growth and production systems for effective technology transfer from R&D platforms to the industrial sector, the absence of information on reproducible levels of bulk production, and the challenge of ensuring sustainable long-term production. Addressing these obstacles was deemed crucial to promoting the industrialization of microalgae [15]. Other major barriers include the lack of a financially efficient commercial metabolite extraction and purification system, energy and cost-efficient biomass harvesting techniques, and innovative and energy-efficient production processes [15]. Other areas, such as biomass production and harvesting, extraction and purification, PBR design, regulation, and certification, and scale-up of production systems, were identified as medium-speed evolving areas [15].

3. Trends and Concerns of Outdoor Algal Cultivation Methods

3.1. Ideal vs. Non-Ideal Conditions

Ideal PBRs are characterized by optimal algal productivity, which cannot be surpassed by any practical technology. The concept of an ideal PBR postulates that its productivity is impervious to any modification or adaptation concerning radiation, as ideal PBRs do not consider productivity to be a function of geographical location but rather depend on the highest solar radiation available on Earth [22]. However, solar intensity fluctuations and deviations from ideal conditions result in lower performance for a given location. The location of interest is also impacted by varying meteorological conditions, induced shading by other PBRs, differing lengths of daytime, and light intensity fluctuations at different periods of the day [22]. Furthermore, the efficiency of radiation utilization by microalgal cells, which is influenced by biological kinetics, is directly affected by temperature, pH,

harvesting strategies, and other experimental factors [22]. A non-ideal or real PBR has to contend with these factors daily.

3.2. Challenges and Limitations of Outdoor Experimentations

Using naturally available resources to cultivate microalgae can reduce costs. However, unpredictable outdoor weather makes algal growth uncertain, which is a concern for commercial production. Outdoor cultivation requires the selected algal strain to not only be a competitive feedstock for value-added chemicals but also to adapt to diurnal changes. The majority of current studies have been conducted indoors because exposure to complete outdoor conditions increases the risk of contamination with viruses, bacteria, fungi, insect pupae, rotifers, protozoa, or other unwanted algae [23,24]. The simple body structure and fast reproduction capability of rotifers and protozoa allow them to rapidly colonize an ecosystem [24]. Especially when investigating new species, close-to-ideal experimental conditions are necessary to determine their optimal cultivation parameters. Properly controlled indoor experiments better serve this purpose. Indoor cultivations provide information about the optimized culture conditions of a strain, but outdoor cultivation is necessary to overcome the challenge of commercial applications.

3.3. Batch Culture

The structural simplicity, flexibility in uses, and low construction and operation costs make batch microalgae culture systems a popular choice among researchers worldwide [25]. Batch production techniques are widely used for algal cultivation due to their ability to accommodate different types of experiments, such as nutrient limitation experiments, more conveniently than continuous mode. Each batch cycle requires starting from a pre-stock culture and typically has a shorter cycle period that allows for lower sanitary demands [25]. The shorter cycle time does not allow predatory or competing organisms to achieve high concentrations [26]. Although scaling-up benchtop PBRs to an industrial scale remains a challenge, research institutes prefer batch culture. As a relatively new field of research, many research institutes and universities have recently initiated algal research. Like all other new research initiatives, they face the challenge of limited resources to conduct research and train new researchers. Batch experiments play an important role in exploring new knowledge of microalgae within limited resources.

3.4. Online Monitoring Tool

Improving the commercial cultivation of microalgae requires significant effort, including better process control and the acquisition and study of real-time data. One way to achieve this is by developing online monitoring tools. In the laboratory, all analyses and observations are based on physical procedures, where samples are collected from the production units and later analysed. Some of these analyses are laborious and time-consuming. Offline analysis systems do not provide many facilities, such as knowing when the highest biomass or any particular metabolite yield has been achieved. Among the spectrophotometric technologies, fluorescence spectroscopy has been recommended as an effective real-time monitoring tool for simultaneously and noninvasively monitoring several compounds in microalgae production [27–32].

3.5. Virtual Laboratory

A study introduced the concept of a virtual laboratory designed for outdoor algal cultivation, which was researched by the Department of Chemical Engineering at the University of Almeria, Spain [33]. The virtual laboratory can serve both teaching and research purposes regarding PBR systems. It is intended to aid in comprehending the interrelationship between various parameters of a PBR. Given that hydrodynamic and geometric parameters are critical decision makers for the algal productivity of a PBR, this interactive tool can analyse the performance of a particular PBR design [33]. Moreover, the virtual laboratory can help to identify and address complications during microalgal

production and optimize PBR design. Multiple controls over the growth environment of the algal cultivation unit can be manipulated, resulting in a compelling way of achieving the highest productivity [33].

According to the study, the interactive virtual laboratory is capable of (a) "accurately reproducing the structure of a real plant", (b) "simulating a generic tubular PBR by changing the PBR geometry", (c) "simulating the effects of different operating parameters, such as culture conditions (pH, biomass concentration, dissolved O_2, injected CO_2, etc.)", (d) "simulating the PBR in its environmental context, by changing the geographic location of the system or the solar irradiation profile", (e) "applying different control strategies to adjust various variables such as CO_2 injection, culture circulation rate, or culture temperature to maximize biomass production", and (f) "simulating the harvesting process". The simulator was developed using "Easy Java Simulations", a tool specifically designed for interactive dynamic simulations [33].

4. Design Question of Outdoor PBR Systems

The productivity of outdoor microalgae cultivation in PBRs depends on various factors, including location, climatic conditions, sunlight intensity during cultivation, types of algal strains, the concentration of the seeding culture, PBR geometry, and wall orientation, as well as the wall's reflection and refraction [25,34].

When selecting algal strains or designing cultivation systems, several factors need to be considered. Some factors are technology-related, such as chemical composition and extraction technologies, while others are market-related, such as price and demand for algae-derived products [35]. By enhancing bioprospecting efforts in a particular region, commercially valuable new strains with higher resilience to light and temperature can be identified. To achieve sustainable long-term outdoor cultivation, high photosynthetic efficiencies, strain selection, bioreactor design, and outdoor weather, such as light and temperature, need to be actively considered [36].

While maximum productivity is a crucial factor in commercial cultivation in PBRs, optimizing the cost of PBR development and lowering operation and maintenance costs and energy requirements should also be given high priority. Factors that influence cost reduction are related to the types of algal species, PBRs, and microalgae production mechanisms selected. Algal biomass production costs can be significantly reduced by lowering the construction and maintenance costs of PBRs [37]. Several questions need to be addressed when designing a PBR, such as the efficiency of light distribution, effective mass transfer, and scalability. To date, no PBR fulfils all these requirements [33].

Annual areal productivity is a measure of how much biomass can be produced on a specific land area. Higher annual areal productivity requires less land to produce a given amount of algal biomass. A compact design of piping systems and infrastructure can reduce costs for plants with high areal biomass output. Alternatively, microalgae yield can be related to the number of reactor panels, and capital costs can be significantly reduced by lowering the number of PBRs. Fewer PBRs with higher panel-specific yields can achieve a certain amount of biomass productivity [38].

Scaling up outdoor PBR conditions is challenging for several reasons, including predicting solar radiation. The discrete solar radiation components that penetrate a PBR depend on geography, weather, and PBR design. Therefore, optimizing the layout and spacing between PBRs in farms should be given equal priority to optimizing the geometry of individual PBRs [39].

5. Current Scenario of Outdoor Algal Cultivation

5.1. Chronological and Geographical Progress of Algal Research in Outdoor PBRs

This study considers research published over the last 35 and a half years, from January 1986 to June 2021. During this time, a total of 96 documents, including research articles and patent applications, were identified. To the best of our knowledge, no article on algae cultivation in outdoor PBRs was published before 1986. Figure 1 presents a timeline

chart of published documents, including geographic information. Researchers from 27 countries across 5 continents (Figure 2) contributed to the research and publication of these 96 documents. The frequency of publication gradually increased over the years. Europe is in the leading position both in terms of participating countries and in the number of studies published, accounting for two-thirds of the total. In the early years of this period, most studies were carried out in Italy, which held the leading position until 2021, followed by Spain, the USA, the Netherlands, Portugal, China, and Japan. The latter five countries contributed only during the last two decades.

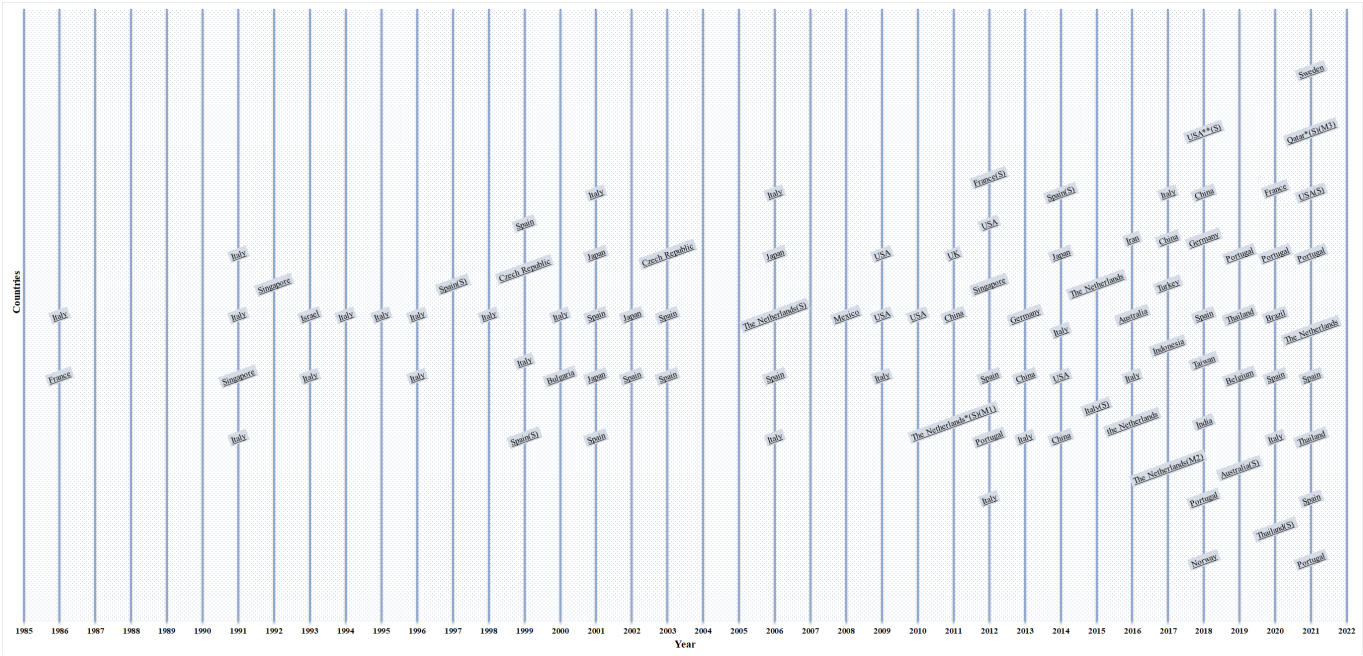

Figure 1. Timeline chart of published articles. (M indicates that this study was conducted for multiple countries; M1—the Netherlands, France, and Algeria; M2—The Netherlands, Norway, Brazil, and Spain; M3—Qatar, Kuwait, Oman, Saudi Arabia, and UAE; * Represents the country where the study was conducted; ** Indicates that this study was conducted for the USA in Germany by German researchers).

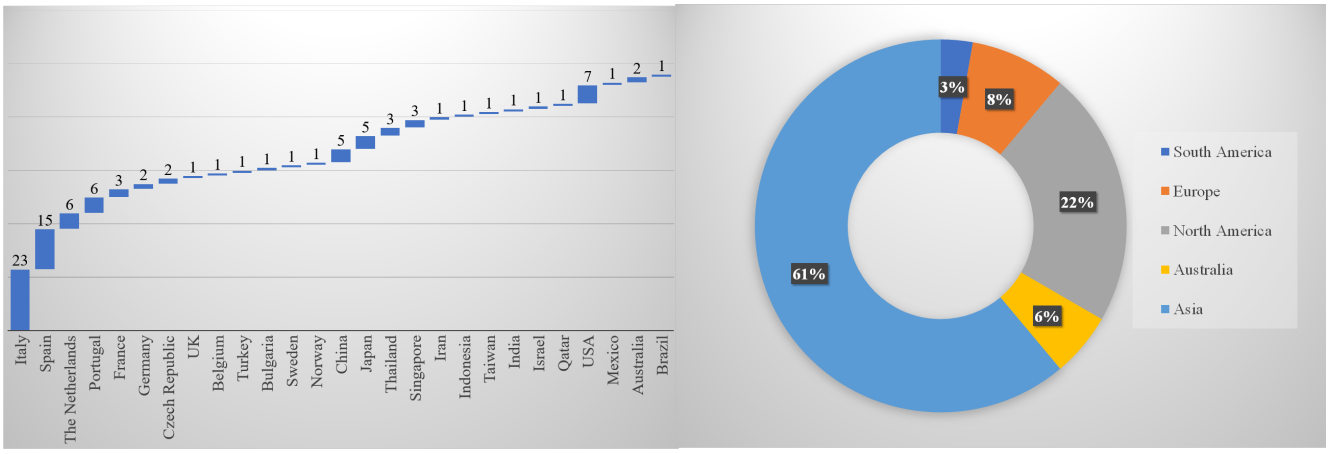

Figure 2. (**A**) Country-wise number of published documents. (**B**) Contribution of the continents to the research publications.

It is difficult to estimate the exact motivations that drove researchers to conduct studies on outdoor algal PBRs. In the 1950s, several start-ups were established in Germany

and the USA. In the early 1960s, the Chlorella industry for healthy food and nutritional supplements was established in Japan and Taiwan, and the market expanded to China and other Asian countries in the 1970s [40]. Spirulina production was first reported in Mexico City, Mexico, in the early 1970s, and in the USA in the early 1980s [40,41]. Production facilities of *Dunaliella* salina, a source of β-carotene, were first reported in the early to mid-1980s in Israel, the USA, and Australia [40]. All these algae production facilities were open pond systems. There is a knowledge gap about the beginning of the algae industry in Europe, but at least 10 algae companies were registered before 1980 [21]. In Europe, 75 microalgae and 222 Spirulina-producing companies were found, with 71% of microalgae-producing and 17% of Spirulina-producing companies adopting PBR technologies as their producing technology [21]. France, Ireland, and Spain are the top three countries with macroalgae cultivation facilities, while Germany, Spain, and Italy are the top three for microalgae. France, Italy, Germany, and Spain are the top four for Spirulina [21].

No study was found that points directly to any reason that motivated researchers from those particular countries to carry out research on algal cultivation in outdoor PBRs. Although most top algae-producing countries from Europe also appeared in Figure 2B and most documents from Asia came from Japan and China, the pioneers of the algae industry in Asia, without hard evidence, it cannot be speculated that the national capacity of algae production and research activities on algal cultivation in outdoor PBRs were directly linked, especially in the early years. It should be noted that at that time, algae were produced in open ponds. PBRs have drawn commercial attention only recently. The only thing that can explain this is that a few researchers started working on PBRs because of personal interest and to unravel the unknown. Some of them had outdoor PBR laboratory facilities. At first, PBRs became well recognized, and eventually, outdoor PBRs started receiving attention because many companies are showing interest in outdoor PBRs. In European countries, which are leading in publication, researchers affiliated with one or two universities frequently appeared as authors in the published articles. This implies that these universities have the resources and facilities to study microalgae cultivation in outdoor PBRs.

5.2. Topics Covered by the Studies

The 96 documents (presented in Table S1 of the Supplementary Materials) cover a diverse range of topics listed in Table 1. Among these, a total of five patent applications were found in four documents, as listed in Table S2 of the Supplementary Materials. Most of the articles focused on growth factors, such as PE, light intensity, optical path, and temperature. Several studies also addressed challenging matters that are of great importance to outdoor cultivation, such as outdoor temperature control and outdoor adaptation of algal species. Furthermore, various studies attempted to explore novel or modified designs of PBRs for outdoor conditions. Other important cultivation parameters, such as O_2 accumulation and fluid velocity, were also discussed. Downstream processing for the extraction of components with commercial potential was also investigated, with lipids and fatty acids being the most frequently studied components. Although several documents discussed topics with present and future importance, such as environmental impact, climate issues (CO_2 sequestration), algae growth medium, bioenergy potential, and economic potential, these topics were not the main focus of most studies. In addition, a handful of documents covered uncommon topics, such as bacteria and the pore size of trays.

5.3. Simulation Studies

The use of computer-based operations is advantageous in inquiry-based studies, as they offer flexibility, interactivity, and dynamism, which apply not only to algal research but to various fields of study [42,43]. Simulations have become a prevalent tool in scientific and technological studies due to their ability to present vast amounts of information in a visual manner. This includes information on material and energy flow, as well as the production of products, by-products, and waste streams. Anyone can run trial and error

tests to verify and/or adjust any particular outcome, and simulations allow one to see how one parameter affects others. Computer-based simulations are particularly useful when investigating critical conditions within a short period [44,45]. However, only 13% of all articles were simulation studies, with the majority being conducted during the last two decades. Simulations can be conducted for multiple places, but this advantage was not fully utilized, as only one study was conducted in the USA by German researchers. Furthermore, only four articles were found where simulations were conducted for multiple cities/countries.

Conducting simulations is challenging, and the quality of a simulation study depends on the accuracy of the models and the quality of the input data, as well as the skill sets of the people involved in modelling and data collection [44,45]. Until now, outdoor research activities have been limited in multiple ways, leading to a lack of data, empirical formulas, and simulation tools. These limitations may explain why fewer simulation studies have been conducted. Although simulations offer many opportunities, these limitations have prevented them from covering a wide range of topics. For example, experimental studies covered 36 topics, and all patents were related to experiments, whereas only 15 topics were studied in simulation studies, mostly related to upstream processes and technical and economic potential. Among these topics, the investigation of novel or modified designs of outdoor PBRs was the most common. The comparison between multiple cities within the same country was the only topic investigated by simulation studies but not by experimental studies.

5.4. Algae Species, Growth Media, and PBRs Used in the Studies

Table 2 presents a list of algae species, growth media, and PBRs used in the studies reviewed. A total of 48 species were investigated across all the articles, with *Spirulina platensis* (in 15 documents) and *Phaeodactylum tricornutum* (in 14 documents) being the most commonly studied microalgae. *Chlorella vulgaris* (in six documents), *Chlorella pyrenoidosa*, *Nannochloropsis oceanica*, and *Nannochloropsis* sp. (in four documents each), and *Chlorella sorokiniana* (in three documents) appeared in multiple studies. All other algae species were used only once or twice.

The main barriers to the commercialization of algae are economic and technological factors. While outdoor PBRs are energized by natural sunlight and affected by diurnal changes, they require intensive installation and maintenance costs. Therefore, extensive research is needed to optimize algal behaviour and growth conditions for outdoor PBRs to achieve maximum growth. Although 48 known species were tested, only 2 species were investigated in more than 10 studies (Tables 1 and 2, and Table S1). Moreover, only a few algae species are used as sources of particular end products. For instance, *Dunaliella* is a major source of β-carotene supplements, *Haematococcus* is used for Astaxanthin supplements, *Spirulina* and *Chlorella* are used for whole-cell nutraceuticals, *Tetraselmis* and *Isochrysis* are used for animal feed (Aquaculture), *Botryococcus, Scenedesmus,* and *Neochloris* are used for energy (fuel cells), and *Symploca* is used as a source of pharmaceuticals [46]. Based on market demand, the number of species used for mass production is even more limited. *Spirulina, Chlorella* spp., *Nannochloropsis* spp., and *Haematococcus pluvialis* are the most cultivated algae in Europe [21], while *Chlorella* and *Spirulina* dominate the algae market in Asia and North America [46]. To expand the algae market beyond the traditional food and feed market to emerging biofuel, biofertilizer, and bioplastic markets, more research on the species listed in Table 2 is needed. Furthermore, species tested in other forms of cultivation experiments and wild species should be investigated in outdoor PBRs to gain knowledge about their behaviour in such conditions. Only one article has investigated unknown wild species to date [24]. Interpolation of research data for algal growth must be carried out carefully. Most European countries and more than a quarter of Asian countries in the figure experience cold weather. Thus, research output such as data and information related to cultivation and harvesting from cold countries may not be directly applicable

in hot countries. Algae should be selected based on region-specific weather conditions to create a multidimensional algal market.

A total of 5 studies were based on continuous operations and 26 studies were on semi-continuous operations, with the rest of the articles using batch mode. Mostly, column and flat-panel PBRs of various shapes, structures, and arrangements were used in all the experiments as a means of cultivation. Modified designs of tubular or flat-panel PBRs were used no more than once. Only one article reported custom-made industrial PBRs (AGS 3 and AGS 4). PBR technology is still in its early stages of development, leaving room for innovative design and process mechanisms. Research is especially needed to determine how to achieve the best performance from an outdoor PBR under varying light intensity and temperature.

Table 1. List of algae species, growth medium, and types of PBRs used in the studies. (The sources of presented data in the table are listed in the Supplementary Materials, Tables S1 and S2).

Algae Species	Algae Growth Medium	PBRs
Alexandrium minutum [47], *Anabaena azollae* [48], *Anabaena siamensis* [49], *Arthrospira platensis* [22,50,51], *Chaetoceros calcitrans* [52], *Chlamydomonas reinhardtii* [25,53], *Chlorella ellipsoidea* [54], *Chlorella pyrenoidosa* [55–57], *Chlorella sorokiniana* [39,58,59], *Chlorella* sp. [60], *Chlorella vulgaris* [10,35,54,61–63], *Chlorella zofingiensis* [64,65], *Chlorococcum littorale* [52,66,67], *Coccomyxa onubensis* [68], *Desmodesmus* sp. [69], *Fistulifera* sp. (formerly *Navicula* sp.) [23], *Galdieria sulphuraria* [70], *Haematococcus pluvialis* [71], *Heterosigma akashiwo* [47], *Karlodinium veneficum* [47,72], *Monoraphidium* sp. [73], *Monodus subterraneus* [74], *Muriellopsis* sp. [75], *Nannochloris atomus* [76], *Nannochloropsis oceanica* [77–80], *Nannochloropsis oculate* [81,82], *Nannochloropsis salina* [81], *Nannochloropsis granulata* [83], *Nannochloropsis* sp. [84–87], *Neochloris oleoabundans* [67], *Phaeodactylum tricornutum* [34,88–101], *Porphyridium cruentum* [102], *Pseudanabaena limnetica* [37], *Rhodobacter capsulatus* [103], *Rhodobacter sphearoides* [53], *Rhodopseudomonas palustris* [104,105], *Scenedesmus obliquus* [62,106], *Scenedesmus obtusus* [69], *Skeletonema marinoi* [83], *Spirulina maxima* [107], *Spirulina platensis* [48,49,107–115], *Synechocystis aquatilis* [116], *Tetradesmus obliquus* [98], *Tetraselmis suecica* [117,118], *Tetraselmis* sp. [119,120], *Thalassiosira pseudonana* [34], *Thalassiosira* sp. [121], *Tisochrysis lutea* [93,94], Unknown wild species [24]	**Lab-made chemical solutions:** f/2 salts solution [101], Guillard's f/2 medium [23,80], Jaworski formulation nutrients [106], Modified Zarouk's medium [49], BG-11 medium [24,37,98,122], Mann and Myers' medium [88,90,92], Modified SOT inorganic medium [123], Modified Ukeles medium [99], Modified van Niel medium (vN-B) [105], Norsker's solution (new solution) [67], 1/2 SWES [98] **Fertilizer solutions:** Agricultural fertilizer-based media such as NPK-fertilizer solution, urea, and triple superphosphate [36,55,64,68,121], Flory Basic Fertilizer 1 (Euflor, Germany) with KNO_3 and NH_4NO_3 [62] **Others:** Deep-sea water-based medium [77], Local mountain spring water [64], Enriched seawater [121], UV-treated natural seawater [85], Artificial seawater medium [66], Wastewater [10,35,54] **Hybrid solution:** Fresh f/2 (−Si) medium in seawater [63,120], Basal medium supplemented with f/2 salts [100], Groundwater supplemented with commercial NaCl [95], Modified seawater BG11 medium [37], Seawater supplemented with NutriBloom® plus [82,93]	Bubble column [23,47,71,72,74,99], Vertical column [23,68,117], Air lift [50,91,99,121], Flat panel [34,36,37,51,53,57,65,72,78,80,84,119], Vertical flat panel [38,70,82,116,123], Tubular (all the articles except where conical flasks were used and the articles cited in this column), Tubular loop [56], Vertical tubular [24,64,84], Horizontal tubular [36,66,84,86,87,106,119], Curved tubular [112], Helical tubular [90,124], Air-lift tubular [49,89], Air-lift bag column [69], Plastic bag type [77], Vertical cylindrical [63], Vertical alveolar panel [48], Dome-shaped reactor [52], Ring shaped [125], Floating horizontal [76], Underwater tubular [104,105], Penthouse-roof PBR (tubular) [108], Green Wall Panel (flat panel) [85,93,96,97,118,122], AGS 3 and AGS 4 (industrial outdoor PBRs) [81]

Table 2 lists the growth media used in the studies, which included traditional lab-made solutions, fertilizers, natural water from various sources, and hybrid media. Although traditional lab-made solutions are ideal for investigating the cultivation parameters of a new species, they are not commercially feasible due to their high cost. In contrast, fertilizers are practical for commercial use because they are affordable and widely available. However, using seawater, mountain water, or wastewater as a growth medium requires satisfying several factors, including site suitability, transportation of the liquid medium, permission from authorities, and environmental safety. The economic feasibility of hybrid solutions is difficult to predict and depends on cost and market availability. The choice of algal growth medium can have a significant impact on operation costs. While only seven articles have studied the impact of growth medium on algal growth performance and two have examined the potential for water conservation, no article has explored the economic importance of algal growth medium.

Table 2. Topics covered in the studies on algal cultivation in outdoor PBR.

Parameters	Experimental	Simulation and Modelling	Total
Number of articles	84	12	96
Patent application	4		4
PE	18	1	19
Multiple months	17		17
Light intensity and dilution	18	3	21
Types of light and optical path	4	1	5
Temperature control	12	3	15
Outdoor adaptation	9		9
Lipid	14		14
Fatty acid	9		9
EPA	5		5
Carbohydrate	4		4
Protein	6		6
Lutein	2		2
Fucoxanthin	2		2
Astaxanthin and carotenoid	2		2
Biochemical composition	8		8
Novel and/or modified design	18	3	21
Economics	15	3	18
Comparison between different types of outdoor PBRs	13	1	14
Comparison of outdoor PBRs with indoor PBRs	8		8
Comparison of outdoor PBRs with outdoor ponds	8		8
Arrangement/orientation of outdoor PBRs	6	2	8
CO_2 sequestration	6	2	8
Environmental impact	4	1	5
Bioenergy potential	10	1	11
Algae growth medium	7		7
O_2 accumulation	5	1	6
Fluid velocity	8		8
Biomass loss at night/winter	7		7
The initial concentration of pure strain	6		6
Bacteria	2		2
Pore size	1		1
Algal species screening technology	1		1
Comparison of multiple strains	2	1	3
Comparison of different operational modes	1	1	2
Comparison between multiple cities in one country		1	1
Comparison between multiple countries	1	2	3

6. A Review of the Findings on Upstream Processes

Several problems act as major barriers to achieving high biomass productivity, such as maintaining optimal temperature, limited availability of light when algal density is high, cost-intensive biomass harvesting, difficulties in process optimization, and lack of computer-aided methods to utilize solar energy effectively [115].

6.1. Temperature Control

To prevent photo saturation and photoinhibition in the Calvin–Benson cycle, enzyme activities can be lowered through temperature control [74]. Laboratory experiments have demonstrated that active and passive methods can be used to regulate the temperature of outdoor operations during both the summer and winter seasons. Active temperature control techniques include water cooling through methods such as spraying water on the surface [49,107], circulating water through a stainless-steel coil supplied by a water bath [55], and using a floating unit in a water pool regulated by an external chiller [76]. Additional methods include using a heat exchanger [92] and a water basin [113], where the temperature was maintained by controlling the depth of water in the basin. Comparing two cooling techniques, a study found that solar-tracked (a device that orients a payload toward the Sun) PBRs achieved higher biomass concentration and volumetric productivity than using cold water aggregate [62]. Although active methods are effective, they require higher costs and energy utilization. To address this issue, passive temperature control techniques can be employed. These include using greenhouses and polythene insulations, which have been reported as effective [106]. However, greenhouses and polythene may also alter light transmission characteristics and reduce energy efficiency. Other passive techniques include using mesh, which can change the water temperature inside a PBR [124], or using a shade made of plastic sheets painted with a dark colour to reduce the amount of received solar radiation [107]. The latter method requires at least 80% shading of the PBR surface for 5–6 h daily to achieve a higher biomass yield [107]. Finally, PBRs can be installed in such a way that two or three positions overlap to reduce sun exposure [107], although this technique can be difficult and its effectiveness is limited.

To maintain a higher temperature in cold seasons, various passive methods have been employed to control the temperature of PBRs. For example, a greenhouse was used to maintain the temperature of PBRs in winter [49]. In Bulgaria, a PBR similar to a commercial absorber was used to cultivate green microalgae, where the temperature was controlled at 32–35 °C by regulating evaporation and minimizing convective heat loss, while the ambient temperature was 13–15 °C [126]. Waste heat from a block heat and power plant was used to maintain PBR temperature in cold seasons [62]. Simulation studies have shown that in countries such as the Netherlands, temperature control had a minor effect in summer, but it was significant in spring and autumn [74]. Additionally, heating PBRs in the morning resulted in increased productivity in both colder (i.e., The Netherlands) and warmer countries (i.e., Israel) [74]. A study presented a temperature model integrated with light availability to estimate algal biomass production potential in a vertical flat-panel PBR array [38]. Another simulation study predicted that PBR temperature increases with increasing PBR spacing until it reaches the temperature of an isolated PBR [127]. Under sub-tropical conditions in Brisbane, PBRs with large diameters had low and stable temperatures. Alternative design strategies, such as reducing PBR-to-PBR distance and widening reactor diameter, could be used to reduce heat load and culture temperature fluctuations passively [127]. However, the design should be optimized to ensure that the microalgae growth rate is not affected by lower PBR spacing or increased PBR diameter.

Controlling temperature is a major contributor to the high operating costs of PBRs [55,56]. However, the installation of heat exchangers, chillers, pipes, and pumps can negatively impact economic viability. Furthermore, pumping cooling water, particularly in areas where water is scarce, can lead to energy imbalances in the cultivation system and environmental and social risks in the surrounding production site [38]. To address this issue, Ong et al. (2010) suggested cultivating thermotolerant algal species such as *Chlorella* sp., *C. zofingiensis*,

and *C. sorokiniana*, which have shown productivities comparable to, or higher than, those attained in several studies carried out outdoors without temperature control [122,128]. In a study conducted in Italy during a very hot summer, the temperature was maintained 12–15 °C higher than the optimum temperature of *T. suecica* for 3–4 h by circulating room-temperature tap water, which allowed the algal species to tolerate the high-temperature range [117].

6.2. Outdoor Adaption

Temperature and light are the two most critical factors to consider for the outdoor adaptation of algae strains. Before outdoor cultivation of any strain, a fresh culture is prepared and maintained in a controlled environment for a brief period. However, when algae are transferred from a controlled environment to PBRs for outdoor cultivation, they experience a sudden change in environment, and they need to adapt to these new conditions to perform optimally. Different types of algae species respond differently to frequent changes in temperature and light intensity. For example, a study conducted on *Alexandrium minutum*, *Karlodinium veneficum*, and *Heterosigma akashiwo* suggested that the range of temperature variation should not exceed 10 °C [47]. The study also noted that in the summer, attention should be given to light intensity, as it can increase the temperature inside the PBRs despite limiting temperature variation [47]. Studies on *Chlorella vulgaris* and *Scenedesmus obliquus* have recommended operating PBRs in offset mode, allowing algal cells to undergo photoadaptation effectively under natural outdoor light regimes [62].

The adaptation period can vary depending on outdoor conditions, as demonstrated in a study that found *Thalassiosira* sp. took two days longer to adapt during the dry season than in the rainy season, after which it began to grow rapidly [121]. A study showed that the productivity of *Nannochloropsis* sp. in September was less compared with September in the previous year. In between this time, the productivity of *Nannochloropsis* sp. decreased in summer due to the hostile environment and the algae did not recover from that stress completely [86]. Some algae species can never fully adapt. For instance, the outdoor productivity of *C.* onubensis was nearly half that of indoor cultivation in March, even when both maximum and minimum temperatures remained moderate [68]. Therefore, identifying suitable species for commercial production is essential. For example, *Chlorella zofingiensis* is a promising feedstock for biodiesel, as it adapts well to varying weather conditions [65]. Another study reported *Tetraselmis obliquus* as a preference, not only for its higher productivity and CO_2 fixation rate but also for its higher adaptability to diurnal and seasonal changes [98]. Thus, algae species should be screened for commercial viability.

Identification and classification of organisms based on conserved and variable regions of 16S or 18S rDNA and ribosomal spacer sequences, including ITS regions, have been commonly used in the taxonomy field for discriminating genetic variation in green algae [129]. In addition, 18S rRNA genes are widely used in the field of biodiversity [130]. Huo et al. (2017) isolated and identified a wild strain from an outdoor culture of *Chlorella zofingiensis* in a PBR using 18S rRNA molecular technology, which belonged to the Scenedesmus genus and was named *Scenedesmus* sp. FS [24]. This new strain showed good alkali resistance, robust adaptation to the outdoors, and the potential for large-scale cultivation for biodiesel production [24].

6.3. Light Intensity and Dilution

In addition to other factors, the PBR material can also affect the efficiency of light utilization. Sarker and Salam (2019) reported that the light transmission efficiency of polyethylene terephthalate (PET), the PBR material they used, decreased with increasing sunray intensity [10]. Moreover, PBRs can also shade each other, which can have a significant impact on their productivity. For instance, a simulation study revealed that the volumetric productivity of arrayed PBRs was significantly lower than that of a single PBR due to shading effects [127]. The same study also showed that algal culture in PBRs with larger diameters was more exposed to dark zones than that in PBRs with smaller diameters,

and larger diameter PBRs were more shaded by surrounding PBRs than smaller diameter ones [127].

The geographic location is another crucial factor that needs to be considered when designing PBRs to optimize their light utilization. Furthermore, the shape of the PBRs can also affect their exposure to sunlight. Tredici and Zittelli (1998) found that tubular PBRs had a higher surface area than flat-panel PBRs and received significantly higher photosynthetic photon flux density, which contributed to the higher productivity of *Arthrospira platensis* [51]. Similarly, López et al. (2006) reported that tubular PBRs received 10 times more irradiance than bubble-column PBRs, resulting in higher productivity of *Haematococcus pluvialis* [71]. However, in warm regions during summer, tubular PBRs can cause photoinhibition due to their high light distribution capacity. In a higher irradiance zone, bubble column PBRs were recommended over tubular PBRs because they reduced photoinhibition [71]. Nonetheless, PBR design alone may not be sufficient to address photo inhibition. Different techniques can be adapted to dilute the light intensity of a PBR. For example, Gutiérrez et al. (2008) suggested using a simple mesh-type solar shade that is commonly used in agriculture [124]. Rosales et al. (2018) and Huo et al. (2018) recommended using nets to reduce irradiance intensity [64,72]. A study suggested that mesh or net could reduce irradiance by more than 60% [124]. Sarker (2022) conducted experiments in Thailand, a hot country, and suggested using a shade/roof with proper dimensions over the PBRs [54].

Haze or clouds can reduce solar irradiance [69]. Zhang et al. (2001) reported that *Synechocystis aquatilisin* was more productive in sunny weather than in cloudy conditions during all three seasons in Japan [116]. Campo et al. (2001) found that *Muriellopsis* sp. was more productive in May at lower dilution rates than in November when irradiance was lower in Spain [75]. This was likely due to higher cell densities in November experiencing light limitation, known as the self-shading effect caused by high biomass concentration, which led to a decrease in the received light per cell. Although light intensity positively affects biomass growth and productivity, when biomass density is high, it can dilute irradiance inside the culture. Zittelli et al. (2006) found that the highest intensity received by the column surface on a very hot summer day was about 20% lower than that on a horizontal surface during noon/midday, while the opposite occurred in the early morning and evening, with about 30% higher solar intensity on the column than on the horizontal [117]. In countries such as Spain where adequate sun rays are available, photoinhibition was observed at lower algal density [92]. To avoid this situation, the algal culture needs to be inoculated up to a certain biomass density. For example, the authors suggested an inoculation density of 0.5 g/L for *Phaeodactylum tricornutum*, which can be used for outdoor cultivation in PBRs [92].

Androga et al. (2017) proposed a mathematical model for an outdoor tubular PBR that can estimate the path length of each light ray to a specific point inside a culture [103]. The authors claimed that the model is capable of obtaining appropriate growth kinetics in outdoor culture by conducting simulations to assess yearly algal productivity and optimize the PBR diameter based on the chosen species and their behaviour under varying light intensities.

6.4. Types of Light and Optical Path

PBRs receive heat through three solar components—direct, diffused, and reflected solar radiation [103]. All three components should be considered during PBR design because the percentage of received radiation of these three components depends on the culture density and layout of the PBR [39]. Reflected irradiation reaches zones of the PBR where direct and diffused irradiation barely reaches, such as close to the ground. Reflected irradiation contributes to a more homogeneous distribution of light in the PBR and has a significant effect on overall biomass yields, despite its limited contribution to the total energy input [38]. This justifies the importance of considering every component. A smaller optical path in an algal cultivation unit results in higher productivity. De Vree et al. (2015) reported that a flat-panel PBR provided the highest areal productivity by having the smallest optical

path, followed by vertical tubular, horizontal tubular, and raceway pond [84]. Lee (1997) developed a model to calculate direct, diffused, and reflected solar radiation around PBRs and to predict changes in the light regime and temperature simultaneously [46]. Solar radiation with different frequencies may have a different impact on a specific algal strain. For example, UV-A radiation does not affect the growth of *C. vulgaris* but UV-B radiation hurts its growth [61].

6.5. Effect of Varying Outdoor Conditions

Olofsson et al. (2012) experimented on *Nannochloropsis oculata* in a commercial vertical flat-panel PBR at Necton's facility in Olhão, Portugal [82]. The total lipid content was 11% of the dry weight of biomass in winter, 20% in summer, and 30% in autumn, which was attributed to the variation in light and temperature with the season. Pereira et al. (2020) experimented in the same facility with *Tisochrysis lutea* and *Phaeodactylum tricornutum* and found that autumn and winter were suitable for *Phaeodactylum tricornutum*, while spring and summer were suitable for *Tisochrysis lutea* [94]. Simulation results also showed that the productivity and CO_2 fixation rate of *Tetraselmis* sp. in Portugal in horizontal tubular PBR were twice as high in spring compared with autumn [119]. Campo et al. (2001) reported the highest productivity of biomass and lutein of *Muriellopsis* sp. with tubular PBRs in May and July and the lowest in November in Spain, with solar irradiance playing an important role [75]. Specifically, the solar irradiance of September was half of that in May.

Several studies have investigated the effects of varying outdoor conditions on algal cultivation over extended periods. For instance, a study conducted in central Italy over six years demonstrated that the average annual yield of *S. platensis* and *S. maxima* in closed tubular PBRs was nearly twice that of open ponds operated in the same climatic conditions [107]. However, the growth in PBRs was not stable throughout the year, with yields significantly decreasing in autumn compared with July. Additionally, the phycocyanin content in the algal biomass was 40% higher in August than in October [107]. Other studies conducted by the same institute later showed that the productivity of *S. platensis* increased significantly more in September than in July, with the authors suggesting that variations in photosynthetically active radiation and temperature were responsible for this discrepancy in algal yield [109,115].

Another study reported a 25% higher productivity of *Nannochloropsis* sp. in near-horizontal tubular PBRs and its eicosapentaenoic acid (EPA) content in May compared with September of 1997, with 22% lower productivity in September of that year compared with the previous year. The main difference between the two consecutive years' in September was the intensity of solar irradiance, which was lower in September 1997 [86]. Carlozzi and Sacchi (2001) found the highest productivity of *Rhodopseudomonas palustris* in underwater tubular PBRs in July, which was five times higher than in January and November. In this experiment, the highest solar irradiance was recorded in July, and the lowest was recorded in January. The authors also reported a 3.6 times increase in polyhydroxybutyrate (PHB) content and a 2% increase in carbohydrate content of the dry biomass but a 10% decrease in Lowry protein content from January to July [104].

In another study, Rodolfi et al. (2017) reported that the productivity of *Phaeodactylum tricornutum* was consistently lower in spring than in summer. In nitrogen-starved conditions, the EPA content remained stable in spring but decreased in summer [97]. Hulatt and Thomas (2011) experimented on Scenedesmus obliquus in horizontal tubular PBRs in the U.K. and found the highest productivity during the clear skies of June [106].

In Indonesia, a study revealed that the productivity of *Thalassiosira* sp. was 50% higher during the rainy season than in the dry season. However, the biomass had a higher content of saturated fatty acids during the dry season and more unsaturated fatty acids during the rainy season [121]. In the dry season, the culture received twice the solar irradiance compared with the rainy season. Zhang et al. (2001) reported that during the period of three seasons, the biomass density of *Synechocystis* aquatilis was highest in vertical flat-plate PBRs when solar irradiance was higher in Japan [116]. In another study, Sato et al. (2014)

tested *Fistulifera* sp. in vertical column PBRs from spring to autumn and found the highest biomass concentration in summer and the lowest in spring, with the highest oil content in spring and the lowest in summer [23].

Quinn et al. (2012) conducted a three-year experiment on *Nannochloropsis oculata* and *Nannochloropsis salina* in Fort Collins, Colorado (USA), using commercial-grade PBRs designed at Solix Biosystems' facility [81]. They found that both species had almost the same average annual productivity, which they maintained throughout the experiment. Most of the time, both species showed a similar growth pattern. However, in the summer of every year, productivity was recorded as the highest, which was more than twice the average productivity. It should be noted that Fort Collins, Colorado, is located in a region with cold weather. Therefore, it can be concluded that algal density tends to be higher in the summer of cold countries, and in the summer or autumn in hot countries.

Steinrücken et al. (2018) investigated three strains of *Phaeodactylum tricornutum*, namely, Fito, M28, and B58, in Green Wall Panels in Norway [96]. The biomass productivity of each strain was highest in spring, lower in summer, and lowest in autumn. There was no significant difference in the EPA content of strains M28 and B58 in spring and summer, but the EPA content of strain M28 decreased in autumn [96]. In another study, Cheregi et al. (2021) simulated a long winter, spring, and summer using the records for air temperature, irradiance, and sunshine duration for the west coast of Sweden during 2014–2016 to cultivate *Nannochloropsis granulata* (Ng) and *Skeletonema marinoi* in a semi-continuous culture [83]. They suggested a cultivation strategy of using different local strains instead of a single strain, where the selected strains will be cultivated in rotation throughout the year. It is important to note that biomass productivity alone should not be the only parameter for screening algal strains. Biomass composition and energy stored in biomass, specifically energy productivity based on stored energy, should also be considered during the screening process.

6.6. Photosynthetic Efficiency (PE)

The PE of a PBR system represents the ratio of light energy supplied to the algal culture and the energy of the algal biomass produced by photosynthesis [131]. If the light path of a PBR system is longer, the culture may not receive adequate light, resulting in a lower PE of the system [65]. The PE fluctuates as photon flux densities change [84]. A study conducted in Italy reported that the PE trends were oscillatory, except for cultures with low biomass concentrations [105]. For all systems, the average PE was inversely correlated with the intensity of solar irradiance [87]. Low-light-acclimated algal cells were found to have a better capability in utilizing irradiance of lower intensity and had a low maximum light-saturated photosynthetic rate [123]. Lee and Low (1992) found that during the cultivation of *Chlorella pyrenoidosa* in a tubular PBR, the PE was high in the early morning but eventually dropped, resulting in overall lower productivity [55]. Campo et al. (2001) reported that *Muriellopsis* sp. in a continuous tubular PBR had similar PE in May and November, although solar irradiance in November was half of that in May [75]. Maintaining cell density and proper mixing ensured stable PE at two different irradiances. The PE may also change with temperature. A *Spirulina platensis* culture in a tubular PBR demonstrated a lower reduction in PE at 35 °C compared with 25 °C in Italy [111]. However, the authors also claimed that cells could recover their PE when the condition changed to lower light intensity and higher temperature. Hulatt and Thomas (2011) reported that *Scenedesmus obliquus* in a horizontal tubular PBR in the U.K. had a four-fold drop in PE in clear skies compared with cloudy skies [106]. Carlozzi and Sacchi (2001) reported lower mean PE in summer than in winter [104].

A study reported that vertical-plate and tubular PBRs have higher PE than horizontal flat-plate PBRs [51]. During the morning and afternoon, there was no significant variation in PE, but at noon, the PE of horizontal flat-plate PBRs decreased by 30% to 55% compared with the other two PBRs. The inclination of vertical plate PBRs and curved surfaces of tubular PBRs prevented midday orthogonal sun rays from entering the PBRs, resulting

in higher conversion efficiencies of sunlight [51]. Additionally, vertical tube PBRs had higher PE than horizontal tube PBRs [84]. Another study suggested that PBRs with vertical geometry increased PE by reducing the period of time that cultures were exposed to light and the intensity of photoinhibition [39]. Algae in a coil-shaped PBR were exposed less to photosynthetic photon flux density but to a more homogeneous light environment, resulting in higher photo dilution and better PE than horizontal flat-plate PBRs [51]. Especially when sunlight intensity is maximum at noon, light received by the coiled PBR is diluted three times compared with the horizontal flat-plate PBRs. It is worth noting that Vree et al. (2015) reported that open raceway ponds have lower PE than all types of PBRs they experimented with [84]. Quelhas et al. (2015) suggested that in the case of tubular PBRs, PE increased with an increase in the total volume of the tube [95]. PE changes with the weather. Zittelli et al. (2006) investigated PE for several weather conditions and found the highest PE in cloudy summers in Italy [117]. A simulation study found that doubling PE resulted in a cost reduction of 32.7–42.5% [36]. However, higher solar energy harvest does not always refer to higher PE. A study reported that for horizontal flat-plate PBRs, adopting a fixed minimum distance among the PBRs and tilting the PBRs would ensure the harvest of a higher amount of solar energy with a reduced number of plates. However, due to this arrangement, the advantages of light dilution are lost, and PE will decrease [122]. One article reported that the cultivation of *Phaeodactylum tricornutum* with superficial gas velocity inside a helical tubular PBR had 15% PE, which was comparable to 20% PE in outdoor tubular PBRs in Spain [132] or 13% PE in outdoor channels in the USA with the same species [90].

6.7. Novel and Modified Design

Tredici et al. (1988) patented a vertical alveolar panel in which air bubbling was used effectively to remove oxygen from the PBR [48]. This design significantly improved the productivity of *S. platensis*, which reached 9 g/(m^2 day), a remarkable achievement at the time, given the winter conditions in Italy. Furthermore, the PBR exhibited 25% less night-time biomass loss in September than other results.

Richmond et al. (1993) developed a PBR with three main components: an airlift pump, a gas separator, and parallel tubes connected by manifolds [49]. The airlift pump circulated the culture through the transparent tubes, and the gas separator, placed 2.0 m above the tubes, removed dissolved oxygen. Culture circulation also helped remove super-saturated oxygen through the degasser. The authors used water spray to cool the PBR, and the volumetric productivity achieved was a breakthrough.

Doucha and Livansky (1997) patented an inclined PBR where the distribution of the algae suspension under turbulent flow depended on the inclination of the PBR [133]. In this design, the path of the suspension flow was longer than the width, resulting in significant energy savings in pumping. However, to achieve an effective result, circulation was required 24/7, which was energy demanding.

Petkov (2000) cultivated green algae in a PBR that was similar to a transparent plate absorber and used natural sunlight [126]. The temperature was controlled by controlling evaporation and minimizing convective waste heat. Ugwu et al. (2002) also experimented with an inclined PBR [58]. They found that an inclination above 45° showed better mass transfer characteristics, but installing and supporting highly inclined PBRs were technically challenging and expensive.

Acién Fernández et al. (2003) experimented with *P.* tricornutum in a helical-shaped PBR [92]. They noted that, at that time, the major issue was the mass transfer capacity of the PBR, which needed improvement. They recommended increasing the fluid flow velocity to ensure optimum light–dark cycle frequencies. However, these modifications posed a risk of cellular damage to algal cells due to excessive hydrodynamic shear forces induced by conventional pumps. They suggested using two air-lift pumps, one to withdraw liquid from the outlet of the photo stage and another one to force liquid into the inlet of the photo stage, as a potential solution.

Masojídek et al. (2003) experimented with a penthouse-roof PBR that combined the features of indoor and outdoor cultivation units and included solar concentrators [108]. To maintain the liquid temperature of the PBR, a counter-flow heat exchanger was used. Carlozzi et al. (2006) set up an underwater tubular PBR to cultivate *Rhodopseudomonas palustris* and determine its elemental biomass molar composition, combustion heat, and photosynthetic efficiency [105]. They observed that the maximum photosynthetic efficiency of this underwater system in Italy was 11.2%, which was observed at sunset.

Sato et al. (2006) attempted to optimize mixing in a novel PBR featuring aeration in Japan [52]. They used computational fluid dynamics (CFD) to examine mixing performance for different shapes and selected an optimized chamber for light reception capacity. After several modifications, *C. littorale* showed 80% more productivity compared with existing PBRs, and *C. calcitrans* showed even twice the productivity. Dogaris et al. (2015) claimed to develop a cost-effective semi-continuous horizontal PBR that combined the benefits of open ponds and PBRs [76]. They also stated that their PBR could be used both on water and land, depending on the needs.

Eckerle et al. (2009) filed a patent application for a PBR that used light more efficiently [134]. Tredici and Rodolfi patented a green wall panel PBR with a 110 L culture volume in 2004 [101]. Compressed air was injected in the bubbled form to agitate the culture, CO_2 was provided to regulate pH and as a source of carbon, and the temperature was controlled by spraying water. Narala et al. (2016) developed a hybrid two-stage cultivation system [120]. In the first stage, algae were grown in an airlift PBR until they reached their exponential phase. In the next stage, this biomass was transferred to a nutrient-depleted open raceway pond, where the algae biomass became lipid rich. Biomass grown in this system had more lipid content than other results found in open pond studies. Nutrients were injected into the PBRs only, while open ponds had turnovers of only a few days, reducing the issue of microalgal grazers.

Sebök et al. (2019) experimented with *U. lactuca* in a ring-shaped PBR but found it to be less effective than conventional PBRs [125]. Gao et al. (2021) developed prediction models for biomass and fucoxanthin productivities for outdoor PBR-based algal production systems by experimenting on *T. lutea* and *P. tricornutum* [93]. The authors claimed that industrial process control could be increased by manipulating and monitoring biomass and fucoxanthin using their models.

Rubio et al. (1999) developed prediction models for axial concentration profiles of dissolved oxygen and carbon dioxide in continuous tubular PBRs [102]. The presented model is simple but has the potential to be used as a scale-up tool and can be adapted to other types of tubular PBRs and photoautotrophic strains. Endres et al. (2018) developed a temperature model combined with an elaborate simulation of light availability to evaluate algal growth in an arrayed set-up of vertical flat-panel PBRs [38]. Sarker and Salam (2020) presented a design protocol for outdoor PBRs that considered temperature, light, algae species, pH, the material of construction, and the size and shape of the PBR [35].

6.8. Arrangement/Orientation of Outdoor PBRs

Several studies conducted in Italy, Japan, and the Netherlands have reported that placing outdoor PBRs in an east–west (facing south) orientation resulted in higher volumetric productivity than a north–south orientation [33,34,85,116,123], due to increased solar energy. However, the difference in productivity varied depending on location. Rodolfi et al. (2009) argued that orientation is more critical when installing multiple PBRs in several rows. Their optimized arrangement received reduced diffused and reflected radiation on the reactors' surface, but the direct illumination remained almost the same as a single PBR [85], resulting in unnoticeable effects on productivity. Proper orientation helps PBRs located in the back rows during low solar radiation months to avoid shading from those located in the front. A simulation study conducted in different regions of the USA indicated that north–south orientation provides better productivity [38], but this study does not contradict the previous studies. The simulations conducted in this study were in regions

with high average temperatures and light availability. North–south-facing PBRs received less radiation and experienced less photoinhibition [38]. Thus, in places where sunlight intensity was high, north–south-facing PBRs showed significantly better productivity due to less photoinhibition.

Typically, outdoor PBR panels are arranged in 2D arrays in commercial facilities, where they shade each other as their height increases and distance decreases [127]. To optimize these systems, complex modelling of the temporal and spatial distribution of both light and temperature of the arrayed PBRs is necessary, rather than just modelling individual PBR performance. Shading or light availability can be changed by adjusting PBR height or distance or both. PBR height is a design parameter that depends on other factors, while the distance between PBRs is a matter of arrangement plan. Endres et al. (2018) recommended panel spacings of 0.4–0.75 m for commercial applications in warm regions [38]. Sunlight exposure for each PBR was found to have a positive relationship with panel distances. Thus, higher panel distances had a positive impact on productivity, but this impact was lost when the panel distance was too high. Beyond a panel distance of approximately 2 m, productivity gains were often negligible. Large panel distances may be responsible for the lack of shading, which could lead to high temperatures at the PBRs, particularly in hot geographic locations. If the temperature rises too high, it will decrease productivity. Therefore, maximum productivities per panel were not found at the highest panel distance examined (such as 5 m), where irradiation was strongest for an individual panel [38]. In a simulation study, it was found that volumetric productivity increased inversely to reactor height at low length (L)/height (H) ratios. For larger PBRs, the total culture volume was reduced when L/H increased. The areal productivity was found to be higher for all cases when the L/H ratio was the same at low PBR height [127].

6.9. Fluid Velocity

Studies have shown that increasing the fluid velocity from laminar to turbulent can enhance the productivity of Spirulina [112]. However, there is a threshold level of turbulence beyond which productivity remains unchanged. Turbulence helps in distributing the available nutrients evenly throughout the culture [133] and ensures better light distribution inside the culture [50]. It also keeps algal cells in motion, enabling them to move to both illuminated and darker areas of the PBR. This prevents the cells from being deprived of light for extended periods and shifts algal atoms from the illuminated peripheral zone to the darker interior, thus reducing photoinhibition [89]. Furthermore, it has been found that short intervals of darkness (one second) can enhance the sunlight conversion capacity of algal cells [135,136] by improving the dark catalytic reactions of photosynthesis. These reactions restore the photocatalytic apparatus to full efficiency for the next light period.

The study by Hall et al. (2002) found that increasing the superficial gas velocity had a positive effect on algal production in helical reactors for continuous *Phaeodactylum tricornutum* cultivation [90]. This is because the increased gas flow moves the algal cells within the culture, which enhances mass transfer and fluid dynamics. However, the relationship between dissolved O_2 and airflow velocity was not found to be linear in continuous mode [90]. The fluid dynamics also contribute to the light/dark cycle frequency and exposure to light. Therefore, it is important to determine the optimized air flow rate for each PBR based on the inoculum volume and other factors through experiments rather than interpolating based on the values of one PBR alone [37]. Another study by Zittelli et al. (2006) reported that the mass transfer coefficient for oxygen and the gas hold-up in the annular column affects the O_2 concentration in the culture fluid, which is dependent on gas velocity [117].

6.10. O_2 Accumulation

The accumulation of O_2 in an algal culture in a continuous tubular PBR is dependent on the length of the tubular circuit [107]. The concentration of O_2 in the culture increases with algal photosynthetic activity, which is influenced by temperature and light inten-

sity. Additionally, as the culture moves forward in the tubes, the O_2 concentration also increases. Upon removal from the tubes, a significant amount of O_2 is released into the atmosphere, emphasizing the importance of the circulation pump in degassing the O_2 [107]. Fernández et al. (2001) discovered that O_2 concentration is also dependent on the liquid velocity [89], which determines the residence time of fluid inside the PBR. They observed minimal diurnal variation in high algal density at the highest liquid velocity [89]. The O_2 concentration in an outdoor culture changes over time due to the varying received light intensity and temperature, which affect photosynthetic activity. Additionally, dissolved O_2 due to photosynthesis also changes with the condition of the sky [106]. Another study found that dissolved O_2 concentration, along with temperature, were the dominant factors leading to culture collapse in outdoor PBRs [92]. However, higher O_2 concentration did not cause the collapse of the culture but rather decreased productivity [114]. Rubio et al. (1999) developed a model to predict axial concentration profiles of dissolved O_2 and CO_2 due to photosynthesis in a tubular PBR. The model was validated through experiments with continuous production of *Porphoridium cruentum* in a 100 m tubular PBR with a working volume of 200 L [102]. The advantage of the proposed model is its ability to provide quantitative predictions of specific profile changes. Inside the solar tube, the model can predict the induced liquid velocity for any specified aeration rate in the riser, the consequent changes in the gas hold-up, and the gas–liquid mass transfer characteristics of the various zones [102]. According to the model, during the daylight period, the dissolved O_2 concentration reached very high levels. This led to higher O_2 content at the exit of the solar loop than in the exhaust gas of the PBR. This suggests that a strategy for dissolved O_2 control should be based on measurements at the end of the solar loop where the concentration is the highest [102]. Dissolved O_2 measurements in the degasser zone, which is a frequent practice used in this work, are less satisfactory as they do not reflect the dangerously high levels that occur in the system [102].

6.11. Bacteria

The physiological and metabolic processes of algae and bacteria are mutually influenced, even though bacteria are commonly viewed as a mere contaminant in algae cultures [137]. Several bacterial characteristics, such as motility, chemotaxis, type IV secretion systems, quorum sensing systems, and production of growth-promoting compounds, are potentially significant for their interaction with microalgae and may affect their growth [138]. Several bacteria parasitize algae by lysing algal cells through the activity of enzymes such as glucosidases, chitinases, and cellulases [139,140]. Once the algal cell is lysed, the bacteria can utilize intracellular algal compounds as nutrients. However, competition for existing nutrients between algae and bacteria can result in slower growth rates of algae, leading to lower algal biomass and algal-derived product yields [141]. Sarker (2021) found that if primary wastewater with high turbidity was used, bacterial activity occurred in the culture [54]. This is because bacteria tend to attach to solid particles and compete with algal cells for nutrients. Bacteria have a better ability to process suspended particulates than microalgae. Aeration-induced agitation led to high aerosol formation above the water surface in the PBRs, creating an environment for the bacteria to flourish. Continuous moistening, aerosol-containing nutrients, and the presence of light facilitated the production of a green algal film by the bacteria near or at the water's surface [54]. However, using filtered wastewater resulted in a different scenario because the removal of particulates also removed most of the bacteria, and thus there were inadequate bacteria present to compete with microalgae [54]. Androga et al. (2017) found that PBRs containing bacteria had higher temperatures [103]. The purple non-sulfur (PNS) bacteria, such as *Rhodobacter capsulatus* YO3 (hup−), contain pigments, namely, bacteriochlorophyll-a and carotenoids, that are capable of absorbing light at certain wavelengths. Bacteria use the energy from light for growth, and the excess energy is dissipated as heat, contributing to the increase in temperature within the PBR [142]. Non-axenic batch cultures of microalgae with low or no microbial control may have lower algal cell density compared with pure algal

cultures, and the purification of accumulated target molecules may become more expensive and technically complicated due to lower concentrations of the specific product in cell extracts. Therefore, controlling algae-bacteria interactions is crucial to avoid decreased yields of algal biomass and algal-derived products [137].

6.12. Biomass Loss at Night/Winter

The composition of biomass in an outdoor PBR is directly influenced by the temperature and solar intensity it receives. When exposed to high light irradiance at low biomass concentrations, algal biomass tends to have an increased carbohydrate content [110]. However, the protein synthesis process at night only utilizes a fraction of the excess carbohydrates synthesized during the day. As such, the amount of biomass lost during the night is dependent on the temperature and light irradiance at which the cultures were grown [110]. Experiments conducted by an Italian laboratory found that the night biomass loss of *Spirulina* was higher in the culture grown at 25 °C than at 35 °C [109,110]. Other studies, also conducted in Italy, found that night biomass loss was higher in months with higher average solar intensity, even if the nights were longer [48,115]. Benavides et al. (2013) suggested that outdoor PBRs had higher biomass growth than outdoor ponds but also experienced higher night biomass loss [100]. Similarly, Cabanelas et al. (2017) found that, despite lower algal biomass concentrations in outdoor PBRs than in indoor PBRs, outdoor PBRs still exhibited higher biomass loss. Their experiments conducted in cold regions suggested that starch degradation was responsible for biomass loss, while carbohydrates, triacylglycerols (TAGs), and polar lipids did not show any degradation [66]. Finally, Carneiro et al. (2020) reported that the pattern of night biomass loss followed changes in cell number, which were maximal at the end of the dark period [79].

6.13. Initial Concentration of Pure Strain and Harvest Rate

Zhang et al. (2001) found that in a semi-batch culture, the initial algal concentration at the start of the culture had a significant impact on biomass productivity. The productivity of *Synechocystis aquatilis* increased with the initial concentration, but when the initial concentration of the pure strain exceeded a threshold concentration (such as 1–2 g L^{-1}), the initial concentration did not influence productivity [123]. Another study conducted in semi-continuous culture in Italy showed that when the initial concentration increased by 3.5 to 6.4 g L^{-1}, productivity was reduced by 23% [115]. Recently published studies on different microalgae conducted in the Netherlands supported both findings [87,93]. Benavides et al. (2013) found that the initial concentration had an impact on the protein fraction too [100]. Torzillo et al. (2012) also studied the effect of the initial concentration and concluded that if the initial concentration was too low, the culture experienced high light stress, and a very high concentration would lead to the shading effect [101]. Light stress caused rapid changes in both chlorophyll fluorescence and photosynthesis parameters, resulting in variations in productivity [101]. The shading effect was responsible for less light, which caused less growth [87]. As the initial concentration impacted light availability, its degree of impact changed with weather and sky conditions [93,101,123] and had an impact on the overall biomass composition as well. During batch laboratory experiments, the culture is started from scratch, but for commercial continuous and semi-continuous cultivation, having an optimized initial concentration is important. Zittelli et al. (2006) experimented with the influence of the harvest rate on productivity [117]. In semi-continuous culture, the highest volumetric productivity was found at a 50% harvest rate, and volumetric productivity decreased with the harvest rate. The increase in the harvest rate from 30 to 50% resulted in a halving of the doubling time and a 25% reduction in the cell weight, although the biochemical composition was less affected [117].

7. Downstream Processes

If the goal of algal cultivation is to produce a specific product, strains selected for outdoor cultivation should not only be chosen for their fast growth but also for their

ability to produce that particular product. For example, *K. veneficum* has been shown to have higher carbon storage (e.g., lipids) due to a high biovolume of cells in outdoor conditions compared with indoor conditions [47]. Although productivity in outdoor PBRs fluctuated, while it remained stable in indoor PBRs, the overall lipid productivity of *K.* veneficum made it a suitable organism for outdoor cultivation. Under the same conditions, the lipid productivity of *N. salina* was almost twice that of *N. oculate* [81]. *S. obtusus* is considered a potential raw material for commercial biodiesel because of its high lipid productivity in outdoor PBRs [69]. Lipid content in algal biomass is directly influenced by outdoor conditions. For instance, one study reported a 50% higher lipid productivity from *Nannochloropsis* sp. at mid-summer compared with the end of summer [85]. A different study reported three times more lipid productivity from *N.* oculata in autumn compared with winter [82]. Another study suggested that *N. oculate* and *N. salina* produce peak amounts of lipids during the summer solstice, which is three times the average lipid productivity [81]. Olofsson et al. (2012) found that the high lipid content of *N. oculata* coincided with high light and high temperature, and vice versa [82]. This was supported by another study that reported that lipid content in the biomass harvested in the evening was higher than that harvested in the morning [79,100]. It can be concluded that to maintain the energy demand required to support the cell division process at night, the cells consume a portion of their lipid reserves [79]. Therefore, algae harvesting may need to occur before the end of daylight.

Overall, the biochemical composition of algal biomass observed in outdoor PBRs and open ponds did not vary significantly [101]. For example, one experiment conducted in Italy on *P. tricornutum* showed biomass grown in outdoor PBRs and open ponds had a protein percentage above 50%, a lipid percentage of about 25%, and a carbohydrate percentage of around 17% [101]. The only difference found was that myristic and stearic acids were higher in algal biomass grown in ponds. A study conducted in Indonesia showed that Thalassiosira sp. grown during rainy and dry seasons had different biochemical compositions [121]. Carlozzi and Sacchi (2001) observed a mean Lowry protein content of *Rhodopseudomonas palustris* that decreased by around 2% in July compared with January, and crude protein always remained 10% higher than Lowry protein content [104]. A study showed that three *Phaeodactylum tricornutum* strains under western Norwegian climate conditions had different EPA and fatty acid contents and compositions, although they showed a similar pattern when cultivated indoors [96]. This is because the three strains responded differently to changing outdoor weather. Fatty acid and EPA contents were found to be very low after re-inoculation, then increased to high values gradually [96]. For *Nannochloropsis* sp., polyunsaturated fatty acid contents decreased considerably in the evening, but monounsaturated fatty acids remained stable under all conditions during semi-continuous operation [79].

Tredici et al. (1991) proposed three different growth strategies based on diurnal changes in carbohydrate and protein synthesis [48]. In sunny weather, carbohydrate synthesis occurred at a rate 2–3 times faster than protein synthesis. On the following night, protein synthesis continued using the additional carbohydrates. Both carbohydrates and proteins were synthesized at almost the same lower rate on cloudy days and were respired at night [48]. However, a sunny sky after four continuous cloudy days led to an increase in carbohydrate content but caused a halt to protein synthesis [48]. In Italy, the carbohydrate content of *Rhodopseudomonas palustris* increased from January to July [104].

Pereira et al. (2021) experimented to cultivate algae for the whole year to produce fucoxanthin, which can be used for industrial production [94]. They cultivated *P. tricornutum* during autumn and winter and *T. lutea* during spring and summer. On average, fucoxanthin content was almost the same in both organisms. This experiment demonstrated a way to produce fucoxanthin from algal biomass to supply the industry with steady production throughout the year [94].

8. Comparisons

8.1. Comparison of Outdoor PBRs with Indoor PBRs

Fuentes-Grünewald et al. (2013) found that three microalgae species exhibited similar growth rates in indoor PBRs but significantly different growth rates in outdoor PBRs [47]. This suggests that outdoor experiments are essential to determine the growth patterns of different species under varying weather conditions. For instance, Buono et al. (2016) conducted experiments on *Phaeodactylum tricornutum* and *Tetradesmus obliquus* in southern Italy using indoor PBRs with more controls and outdoor PBRs with fewer controls [98]. They observed significantly higher growth rates, biomass and lipid productivity, and CO_2 fixation in indoor PBRs. However, Cabanelas et al. (2017) reported lower biomass growth and higher night biomass loss in outdoor PBRs [66,77], despite suggesting outdoor PBRs as a preferable option due to higher maintenance costs. In addition, Steinrücken et al. (2018) reported different fatty acid compositions in outdoor and indoor PBRs for three different *P.* tricornutum strains [96]. For example, the productivity of the highest EPA-producing strain was almost twice that of the lowest EPA-producing strain. Moreover, Sarker and Salam (2019) conducted experiments on *Chlorella vulgaris* in a tropical country (Thailand) and found that outdoor PBRs are preferable in tropical environments because indoor PBRs required significantly higher operation and maintenance costs [10].

8.2. Comparison of Outdoor PBRs with Outdoor Ponds

After six years of experimentation, Torzillo et al. (1986) reported that outdoor PBRs could produce twice the amount of *Spirulina* annually compared with outdoor ponds in Italy [107], a finding supported by other studies conducted on different species in various regions [63,87,100]. In 2012, a study from the same laboratory found that the protein, lipid, and carbohydrate contents of biomass grown in outdoor ponds and PBRs were similar, but open ponds resulted in a higher ratio of saturated to unsaturated fatty acids [101]. Narala et al. (2016) demonstrated that both outdoor PBRs and hybrid systems (outdoor PBR + pond) provided better productivity than open ponds [120]. Ortega and Roux (1986) found that the productivity of *Chlorella* in temperature-regulated PBRs located in less favourable weather was similar to that of open ponds in more favourable weather conditions [57]. Furthermore, a study conducted in Singapore demonstrated that a well-designed PBR can reduce photoinhibition compared with outdoor ponds [39]. De Vree et al. (2015) also found that PE was higher in different types of PBRs than in open ponds [84].

8.3. Comparison between Different Types of Outdoor PBRs

PBRs with different inclinations can have a significant impact on cultivation performance. Lee and Low (1991) conducted extensive outdoor experimentation on PBR inclinations [56]. They found that horizontal PBRs (concerning the ground) received more solar irradiance in the morning, while vertical PBRs received more solar irradiance in the evening. However, if the PBR inclination is parallel to the sun's rays, the PBRs receive less irradiance [56]. It is important to note that the angle of the sunray changes with time. Lee and Low (1991) observed that a PBR inclined at 45° received almost constant solar irradiance throughout the day. They also found that an 80° inclined PBR (concerning horizontal) delivered more than 6 times the productivity per land area than a horizontal PBR [56]. Although algal productivity per culture volume and bioreactor surface did not vary significantly based on inclination, it should be noted that this experiment was conducted a long time ago. However, their study on light-receiving capability based on inclination is still relevant because light availability and intensity play a vital role in algal productivity, especially in tropical countries.

Prakash et al. (1995) set 2 tubes at different planes where the 2-plane PBR had a 2.5 times surface-to-volume ratio and delivered 3 times more volumetric productivity [113]. Carlozzi and Torzillo (1995) found that a curved tubular PBR was more productive than a straight tubular PBR [112]. Tredici and Zlttelli (1998) showed that a near-horizontal straight tubular PBR performed better than a near-horizontal flat-panel PBR in summer

(in Italy) [51], both in terms of productivity and growth rate, though the biochemical composition did not vary. Camacho et al. (1999) concluded that their vertical concentric-tube airlift PBR was a better performer than a horizontal-loop tubular PBR because of a better combination of mixing and light distribution in the riser and the downcomer of the airlift PBR [91]. They also found that the tubular PBR had higher average irradiance inside the culture during summer and spring, while the airlift PBR had higher average irradiance during autumn and winter in Spain [91]. The authors suggested that the vertically oriented concentric-tube airlift PBR offers a reliable option to replace horizontal tubular PBRs for cultivating algae at latitudes close to 45° [91]. The vertical arrangement provided a more appropriate combination of local irradiance distribution, mixing, and ordered flow to ensure a suitable modulation of the light–dark periods. This technology avoided high irradiances in the summer and spring and at noonday all through the year [91].

Mirón et al. (2003) concluded that the vertical orientation of relatively large-diameter column PBRs did not let the algal culture experience photoinhibition even when the algal density was very low [99]. López et al. (2006) argued that reactor shape can be responsible for the varying biochemical composition of algal biomass [71]. They also concluded that tubular PBRs are preferable to bubble columns for the outdoor production of astaxanthin [71]. Because of the variation in arrangement and tube diameter, their tubular PBRs received 10 times more irradiance than bubble columns. De-Vree et al. (2015) conducted experiments on horizontal tubular PBRs, vertical tubular PBRs, and flat-panel PBRs simultaneously and found different ground areal productivities and average PE for each PBR [84]. A simulation conducted by Schipper et al. in 2021 demonstrated that various types of PBR systems in the Middle East offer different large-scale productivity rates [28]. López-Rosales et al. (2018) found that, in Spain, bubble column PBRs were a more favourable option than flat-panel PBRs [72]. Additionally, studies have reported that PBRs of the same type but with differing volumes can yield varying levels of algal productivity, CO_2 sequestration, and production costs [37,95].

9. Impacts

9.1. Economics

Careful consideration should be given to any modifications that aim to improve cultivation performance as they may result in increased costs. For instance, the use of Fresnel lenses and optical fibres has been shown to increase the PE of PBRs [51], while vertical plates can reduce irradiance to achieve high PE [51]. However, these modifications come at a high capital investment cost, and scaling them up for commercial operations can be challenging. In their study, Tredici and Zlttelli (1998) suggested near-horizontal tubular PBRs for commercial operations due to their satisfactory volumetric productivity and PE, ease of scalability, and ability to provide a homogeneous and stable environment for cells [51]. Similarly, Sánchez Mirón et al. (2003) recommended vertical column PBRs for potential commercial operations, citing their compactness, low cost, and easy monoseptic operation [78].

Dogaris et al. (2015) conducted a study on laboratory prototypes and found that the average manufacturing cost of conventional PBRs was four to nine times higher than that of open ponds, regardless of whether the design was tubular or flat panel [76]. However, the cost of their horizontal photobioreactors (HBRs) was comparable to that of open ponds. This was achieved by using cheap thin plastic film instead of thick plastic tubes or panels to construct the HBRs, which contributed most to the cost reduction. The authors projected that the capital cost of HBRs per kg of algal biomass was two to eight times lower than that of conventional PBRs and comparable to that of open ponds. Moreover, culture contamination or crashing, commonly found in open ponds, was not found over a long period of time in the HBRs. However, it should be noted that this technology was conducted at a small scale in the laboratory, and only considered materials, labour, and overhead costs for manufacturing [76].

The implementation of auto-flocculation (pH-induced flocculation) has been shown to significantly reduce the energy cost of harvesting [85]. Hulatt and Thomas (2011) proposed mounting the photo stage horizontally to reduce the static head and optimize the water flow rate, as well as rearranging the tube layout to minimize pressure drop, as a means of reducing energy consumption [106]. Norsker et al. (2021) also recommended reducing fluid velocity to decrease energy consumption [67]. However, they found that the energy required for an outdoor PBR system could be up to twice the theoretically calculated values. Quinn et al. (2012) conducted large-scale outdoor cultivations in PBRs with *N.* oculata and *N.* salina and reported that energy requirements for mixing and gas transfer in PBRs could be similar to open raceway ponds without affecting productivity [81]. Guccione et al. (2014) suggested that using low-cost PBRs to reduce cooling needs would be an effective way to minimize operational costs for commercial operations [122]. Kusumaningtyas et al. (2017) recommended using low-cost agricultural fertilizers to reduce operational costs [121]. Schipper et al. (2021) conducted a simulation analysis for Qatar, which revealed that doubling the PE could reduce costs by over 40%. They also showed that maintaining a certain temperature could eliminate the need for temperature control and reduce individual costs by 25%. According to their analysis, using flat-panel PBRs with these two features could reduce algae production costs to EUR 1.46 kg^{-1}. Tredici (2010) reported that large regions in the Middle East and North Africa have the potential for large-scale algal biomass production in outdoor PBRs, although this potential has not yet been confirmed by long-term cultivation [143]. Productivities can be improved with applied cultivation systems [84,144,145], but such systems come with higher CAPEX and OPEX footprints [84,144,145]. Moreover, the advantages of outdoor PBRs are not the same in every region. For example, PBRs in deserts and tropical climates will require temperature control systems for smooth operation. The trade-off between increased capital and operation costs of such systems and higher productivity does not always provide a positive outcome [146,147]. "The Arabian Peninsula," and specifically "the Gulf Cooperation Council countries," offer suitable weather conditions for year-long operation, availability of non-arable land, direct local access to seawater, and a high number of CO_2-rich flue gas point sources [36].

Water recycling can reduce the water footprint of a process, but for a PBR operation, it can lead to significant energy usage when water is recycled at higher Reynolds numbers. Carlozzi and Torzillo (1996) showed that the energy required for water recycling at a Reynolds number (this number represents the velocity and associated turbulence of liquid flow) of 20,000 was 50 to 60 times higher than at a Reynolds number of 5000 [112].

For any type of process facility, the per unit cost typically decreases as the capacity of the facility increases [148]. Similar results have been observed for outdoor PBR farms, where the size of the cultivation facility directly affects production costs. One study suggested that scaling up from a 1 ha farm to a 10 ha farm reduced costs by up to 67%, with an additional 13% reduction observed when the facility size was increased to 100 ha [36]. The highest cost reduction was observed in operation costs. In a 1 ha facility, labour costs alone accounted for almost half of the microalgae production cost. For a 10 ha farm size, labour costs ranged from 15% to 24%, with the variation mainly due to the different shapes of PBRs. For a 100 ha farm size, labour costs dropped to as low as 3.45% for tubular PBRs [36]. These findings suggest that multiple smaller production facilities spread out over a wider area can be more attractive than fewer larger production facilities, due to lower costs and less risk [36].

9.2. Environmental Impact

Guerra et al. (2012) demonstrated that the water requirements for cultivating microalgae in PBRs varied depending on the mode of operation. Specifically, semi-continuous and continuous modes required almost the same amount of freshwater, which was 2.5 times more than what was needed for batch mode [80]. The findings of the study suggest that developing a commercial batch operation could serve as a feasible alternative in regions facing

water scarcity challenges. However, further research is necessary to establish a definitive conclusion on this matter [80]. Another option is to replace freshwater with wastewater, which can be reused or recycled for microalgae cultivation. Sarker (2021) successfully cultivated *C. vulgaris* and *C. ellipsoidea* using wastewater in Thailand [54]. This study showed that PBRs provide additional benefits for microalgae cultivation with wastewater, such as the prevention of odour spreading, less human contact with wastewater, and better light distribution. While wastewater is less transparent than freshwater, this disadvantage can be effectively offset by better light distribution in PBRs. For instance, Zhang et al. (2001) found that in a vertical plate PBR, *Synechocystis aquatilis* had a CO_2 fixation rate of 50 g m^{-2} day^{-1}, using CO_2 collected from the flue gas of a liquefied natural gas (LNG) electric power plant [116]. Another study on *Phaeodactylum tricornutum* showed that the CO_2 fixation rate in outdoor PBRs was half that of indoor PBRs [98]. Pereira et al. (2018) demonstrated that in a semi-continuous culture, *Tetraselmis* sp. was capable of fixing 75% of CO_2, with the highest biofixation occurring in spring and the lowest (half) in autumn [119]. Quelhas et al. (2019) found that *Phaeodactylum tricornutum* was capable of fixing 2.5 g of CO_2 per gram of algal biomass formed, with CO_2 fixation efficiencies up to 60% [95]. The improved PE performance of this study with larger PBR volumes strongly suggests that industrial-scale algal production has the potential for CO_2 mitigation. However, the biosynthetic pathway of extracellular metabolites should also be considered to improve CO_2 sequestration, as microalgae are known to release extracellular compounds (e.g., sugars) under specific culture conditions [149,150]. If this is not possible, the highest CO_2 capture is limited to increasing macromolecules with higher carbon contents, such as lipids. Rubio et al. (1999) achieved a 70% biofixation of CO_2 in the continuous outdoor operation of *Porphyridium cruentum* cultivation in a 200 L PBR with a 100 m solar receiver tube [102]. Other studies have suggested that other types of algal species, such as *Alexandrium minutum*, *Karlodinium veneficum*, and *Heterosigma akashiwo*, are also capable of CO_2 biofixation [47,125]. Although these studies suggest that outdoor PBRs could be a potential technology for CO_2 sequestration, more research is needed to address outdoor challenges before considering their use for commercial CO_2 sequestration.

9.3. Bioenergy Potential

To assess the potential of an energy plant, one useful metric is the net energy return (NER). This measures the ratio of energy output to net energy input, taking into account the energy gain over the lifetime of the plant, as well as the embodied energy of all materials used and the energy required for operations. To improve the NER of a microalgae production plant, careful selection of materials and design can reduce net energy input. Additionally, reducing the energy required for mixing and harvesting can further lower net energy input. For example, Rodolfi et al. (2009) found that green wall panels (GWPs) had lower embodied energy than other types of PBRs, and second-generation GWPs have even lower embodied energy [85]. However, the NER of algae cultivation with even second-generation GWPs did not meet commercial requirements, primarily due to the high costs associated with mixing and harvesting [85].

Tredici et al. (2015) conducted a simulation to assess the production of *Tetraselmis suecica* using GWP technology in Tuscany, Italy [118]. The study reported annual productivity of 36 tons (dry weight) ha^{-1} $year^{-1}$, with a net energy ratio (NER) of 0.6. The input energy was composed of the embodied energy of the PBRs (30%), direct energy consumption for mixing the culture (40%), fertilizers production (11%), and harvesting (10%). However, in locations with more favourable growth conditions for microalgae (such as North Africa), twice the productivity can be achieved, but this would only increase the NER by 40% and the overall gain would still be negative [118]. The integration of photovoltaics (PV) with GWP-II technology can increase the NER to 1.7, making GWP more sustainable. Achieving high algal productivity and protein yields (up to 30 times higher than traditional crops such as soybeans) can be possible in a suitable location with algal production carried out in a GWP plant [118].

Microalgae present an opportunity to convert solar energy into hydrogen energy. However, monocultures of *C. reinhardtii* have been reported to have very low solar energy conversion efficiency (0.05–0.06%) [53]. Mix cultures of microalgal species have been found to have higher energy conversion efficiencies, reaching up to 25% [133], but the overall efficiency remains low. Nevertheless, several studies suggest that different microalgal species cultivated in outdoor PBRs have bioenergy potential, including *Chlorella minutissima*, *C. zofingiensis*, *Nannochloropsis*, *S. obtusus*, *Thalassiosira* sp., *Fistulifera* sp., *Chlorella* sp., and *C. sorokiniana* [59,65,69,81,121]. These studies were conducted in various types of facilities, including PBRs in laboratories, pilot scales, and industrial scales, and in diverse weather conditions such as tropical regions, cold places, and during summer, winter, and rainy seasons. This proves that the commercialization of algal biofuel is possible, and the challenge is to identify a suitable species for a particular location. Rodolfi et al. (2017) suggested that algae grown in nitrogen starvation conditions in outdoor PBRs were more suitable for biodiesel production, similar to indoor conditions [97]. Moreover, the biodiesel potential of *Chlorella* sp. increased with the increase in salt in the cultivation medium [60]. Xia et al. (2014) reported the potential for commercial biodiesel production from *S. obtusus* in North China, which is a relatively cold region [69]. Another study showed that algal oil productivity was five times higher than palm oil in the rainy season [121].

10. Conclusions and Outlook

Research on outdoor PBRs for microalgae cultivation is still in its immature stage, and both long-term and midterm considerations must be taken into account when planning research projects in this field. Midterm considerations include finding suitable microalgae strains for specific weather conditions, developing effective cultivation strategies, exploring wild species and analysing their biochemical characteristics, and developing process parameters for commercial production. Long-term considerations include designing complex but efficient PBRs, optimizing processes for outdoor cultivation, and formulating algal growth models for commercial cultivation. Models can be important tools to predict algal growth and biomass composition, but they should be validated for the species and specific weather conditions. In this study, we discussed several topics based on only a limited number of articles due to the scarcity of available studies. These topics include bacterial interactions with microalgae and the initial concentration of pure strains. Additionally, the number of studies conducted on downstream processes and their economic and environmental impact is not adequate, and more work should be conducted in this field to close this gap. While biomass harvesting from outdoor PBRs has been studied by a few research works, feasibility studies and life cycle analyses for outdoor PBRs are not available. Extensive research should be carried out on the mentioned topics, along with upstream and downstream process optimization and process cost optimization, to expand the knowledge sphere and present this field as an attractive area of interest to curious researchers, policymakers, and potential investors.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/en16073105/s1, Table S1: List of documents published on algal cultivation in outdoor PBRs; Table S2. List of documents published on algal cultivation in outdoor PBRs. References [10,22–26,33–39,47,48,48–127,133,133,134,151–153] are cited in the supplementary materials.

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