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Perspective

Large Scale Microalgae Biofuel Technology—Development Perspectives in Light of the Barriers and Limitations

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Abstract: Microalgal biomass can be used to derive many different types of biofuels. In order to widely commercialize this technology, its limitations and weaknesses must be eliminated. Many technical and economic issues also need to be clarified and unknowns answered. Microalgae-based technologies have been shown to be versatile, efficient and suitable for practical and commercial use. However, the current technological readiness level (TRL) of most microalgae-based bioenergy production systems precludes their quick and widespread implementation. Their development is limited by a combination of factors that must be precisely identified, after which their negative impact on scale-up prospects can be eliminated or minimized. The present study identifies the main barriers to the development of industrial microalgae-production and microalgae-to-biofuel systems. In addition, it proposes measures and efforts necessary to achieve a higher TRL, which will provide investors with sought-after performance and cost-efficiency data for the given project. The main barriers to the development of microalgae cultivation and processing systems have been identified to include: the complex nature of the cultivation process with multiple variables involved; lack of sufficient data from pilot-scale and near-full-scale plants, which often precludes reliable life cycle assessment (LCA); and insufficient legal assistance, advisory assistance, subsidies and funding for innovative projects. Potential ways of improving performance and competitiveness of microalgae-based systems include: process optimization techniques, genetic engineering, yield improvement through advanced process control and monitoring, use of waste as feedstock and dedicated support programs. The presented summary of the current stage of microalgal biofuel production technology development indicates the directions for further research and implementation work, which are necessary for the final verification of the application potential of these solutions.

Keywords: microalgae; biofuels; technology development; large scale; technological readiness level (TRL); life cycle assessment (LCA); molecular engineering; sustainable development

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1. Introduction and background

Microalgae are single-celled organisms that convert solar radiation energy into chemical energy via photosynthesis [1]. Their cells can accumulate a broad array of value-added bioactive substances, such as proteins, lipids, carbohydrates, fatty acids, pigments and vitamins [2]. The fact that microalgae are often grown on waste materials is often cited as a feature with great potential for application in general environmental protection and engineering [3]. However, it seems that microalgal biomass may find its greatest contribution in bioenergy production [4].

Research to date has demonstrated that microalgae can be harnessed to produce liquid fuels (such as biodiesel and bioethanol), gaseous energy carriers (including biomethane and biohydrogen), as well as pyrgas and syngas [5,6]. It has also been shown to produce energy from direct combustion [7,8]. Many studies have also pointed to the numerous advantages and strengths of microalgal biomass production processes, and technologies for converting such biomass into value-added products and energy carriers [9]. The wide range of biofuels and other value-added products obtainable from microalgal biomass is presented in Figure 1.

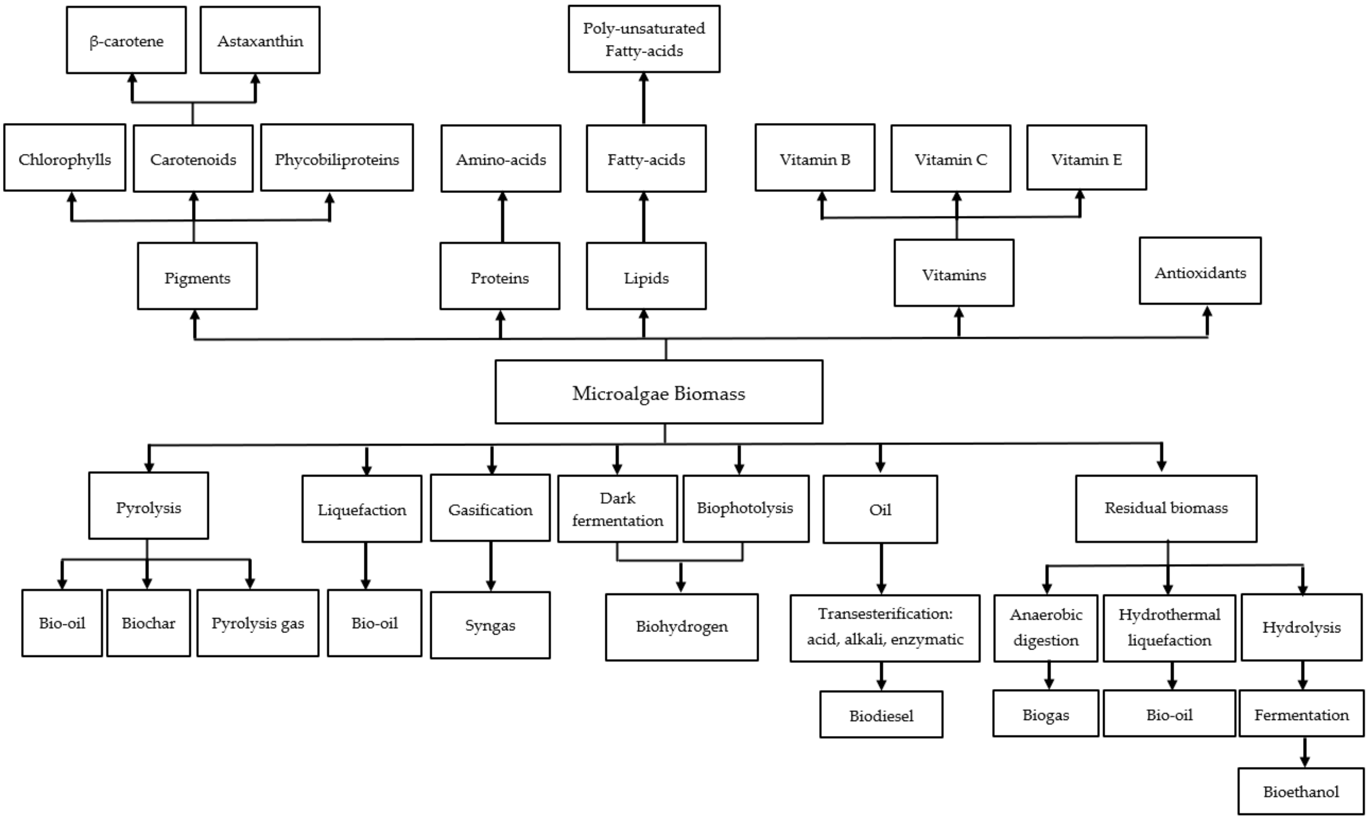

Figure 1. Primary processes used for converting microalgal biomass into biofuels, together with the major value-added products harvestable from microalgae.

The ability to incorporate various types of waste into the culture medium is considered an advantage of microalgal cultivation systems, as it can reduce operating costs and specific costs of biomass production [10]. This approach also reduces the associated environmental pollution [11,12]. Attempts have been made to pair microalgae photobioreactors with processes for sewage and leachate treatment, waste/sludge management, biogas upgrading, carbon dioxide biosequestration or flue gas treatment [13,14]. Indeed, there have been reports on photobioreactors successfully processing waste from industrial and domestic sources, thus, opening the door to practical, commercial systems of microalgal biomass production being established on land unsuitable for agricultural use. Furthermore, there have been proposals to site photobioreactors near agricultural biogas plants, waste-to-biogas plants, wastewater treatment plants, heating/cogeneration plants, landfills and other industrial objects that generate CO_2 and biogenic compounds [15]. Waste streams compatible with microalgal biomass production systems are listed in Figure 2.

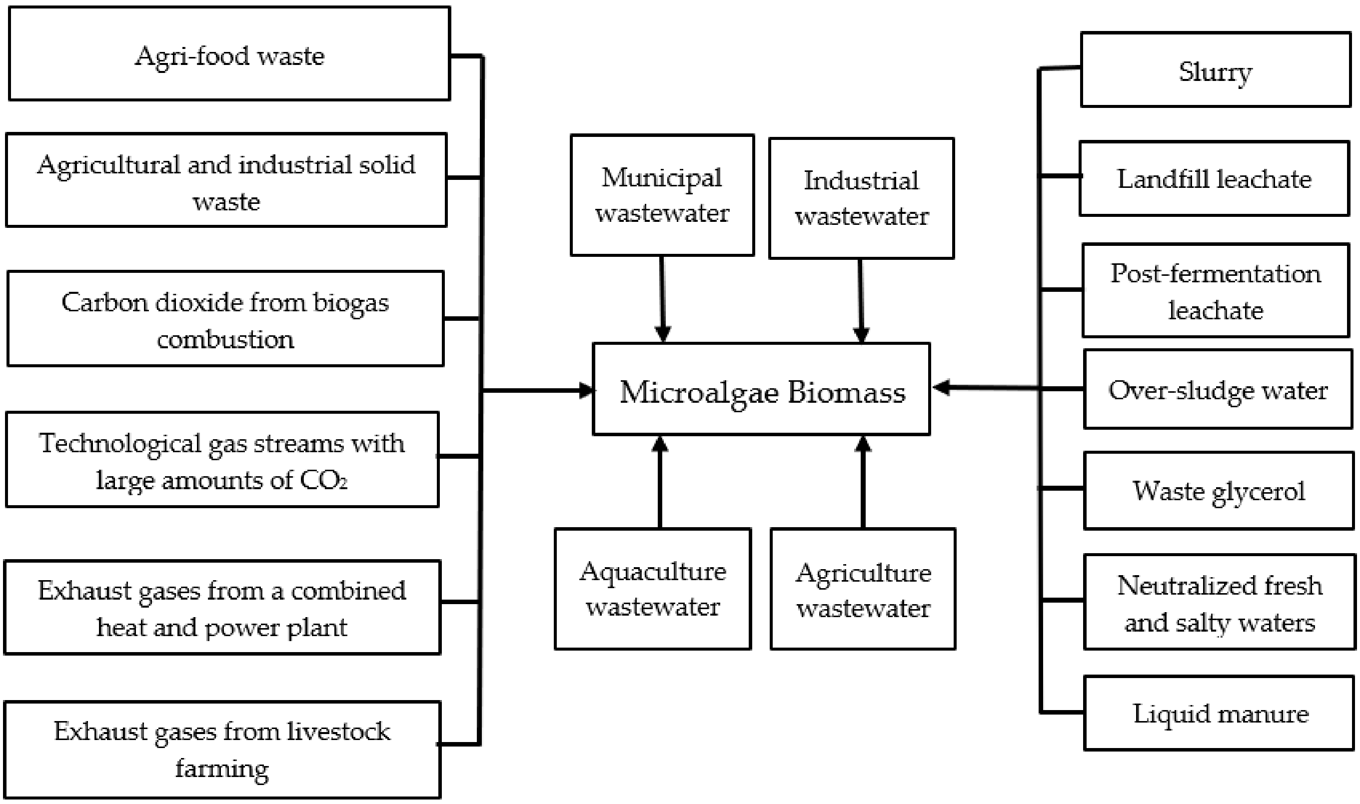

Figure 2. Primary types of waste used for microalgal biomass production.

Many authors have rightfully concluded that the yields from microalgal biomass cultivation systems far surpass those obtained from traditional land-based energy crops, due to the high photosynthetic efficiency of microalgae [16,17]. The results of the analysis of major research databases are a testament to the growing interest in microalgae-to-bioenergy technologies. The 2010–2021 statistics for the search terms "algae biofuels", "bioenergy from algae" and "algae cultivation", shown in Figure 3, suggest that the topic is drawing ever-growing interest from research institutions worldwide.

The promising results of research work have prompted institutions to include microalgae-to-biofuel technology in their draft plans and implement energy policies [18]. One such policy is the European Union (EU) bioeconomy development framework, a major component of which consists of microalgae and their applications in environmental protection technologies, bioenergy, and the production of valuable nutrients for humans and animals [19]. The microalgae sector is expected to gain an increasing foothold in the EU's blue bioeconomy, especially in coastal areas [20]. According to the 2019 EU Blue Economy Report, the EU algae sector boasts an annual turnover of 1.5 billion EUR from direct activities, with an additional 240 million EUR tied to indirect activities, such as R&D [21].

A perusal of the many available reports from studies on microalgal cultivation methods shows them to be very promising and sustainable technologies [22,23]. The overwhelming majority of such studies highlight the competitive advantages and strengths of these solutions and indicate that they are a viable and cost-effective means of biofuel production [24–26]. By now, we have accumulated a great deal of knowledge and repeatedly verified experimental data on the subject. This then begs the question—why has the large-scale implementation and deployment of microalgae-based technologies been relatively underwhelming? What has prevented their widespread take-up? It seems that for the technology to progress, it must first be determined which aspects of development need to be improved and which shortcomings need to be eliminated or reduced, so that the strengths indicated by so many authors can finally be harnessed in large-scale plants.

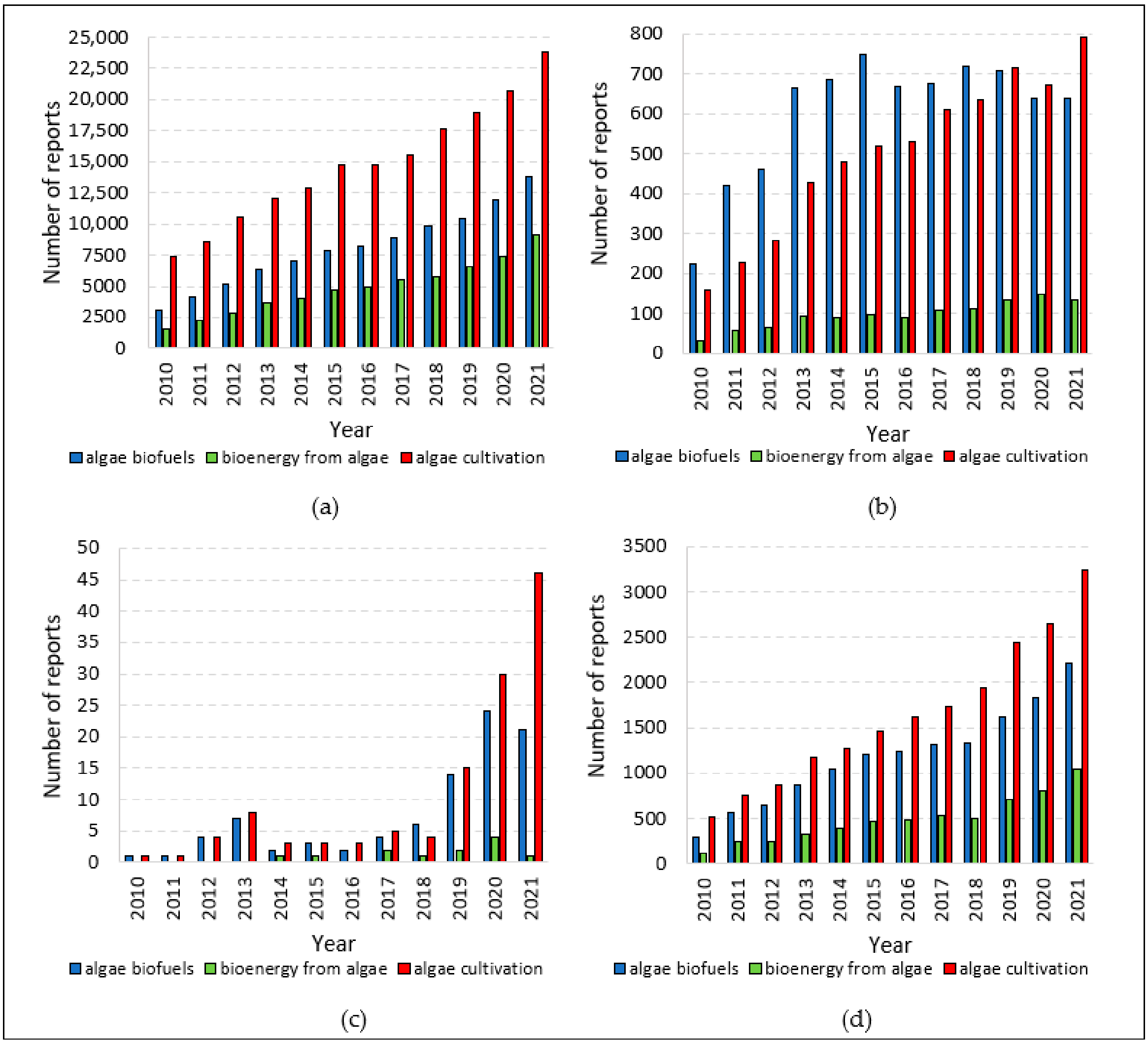

Figure 3. Article results in (**a**) Google Scholar, (**b**) Scopus, (**c**) MDPI and (**d**) Science Direct between 2010 and 2021 for the keywords "algae biofuels", "bioenergy from algae" and "algae cultivation". Accessed 24 October 2022.

The purpose of this study was to identify the barriers and factors that have impeded the growth of large-scale microalgae-production and microalgae-to-biofuel systems. It drew on a review of the available literature, as well as the long-standing experience of manuscript authors, to delineate the elements and efforts that need to be focused on before the well-established and proven potential of microalgae-based technologies can be implemented and actualized.

2. Technological Complexity

Microalgae-cultivation and microalgae-to-biofuel systems are highly intricate, complex and susceptible to numerous factors and variables, a fact that limits and complicates their commercial applicability [27]. Their final technological and commercial performance is predicated on successfully designing and building the right combination of intertwined process line components, environmental and technological parameters, and individual process steps [28]. Bioenergy systems for microalgal production and conversion are highly complex, consisting of dozens of subprocesses and steps, with the final configuration chosen from the many variants available and described in the literature. Such variables include: the choice of species; type of culture (autotrophic vs. heterotrophic); reactor type and design; origin and composition of the growth medium; type and source of lighting; type of nutrients and mineral elements; carbon dioxide source and feeding mechanism; feedstock supply mechanism; type of stirring or otherwise generating flow; the separation, concentration and harvesting processes; dewatering and desiccation; destruction of microalgae; oil extraction process; biodiesel conversion technology; and the manner of converting deoiled biomass into other energy carriers, which necessitates the development of a secondary process and the selection of individual process steps, technological parameters, and specific equipment [29]. Figure 4 illustrates the variety of available process steps, which makes it difficult to choose and implement a specific system for large-scale deployments.

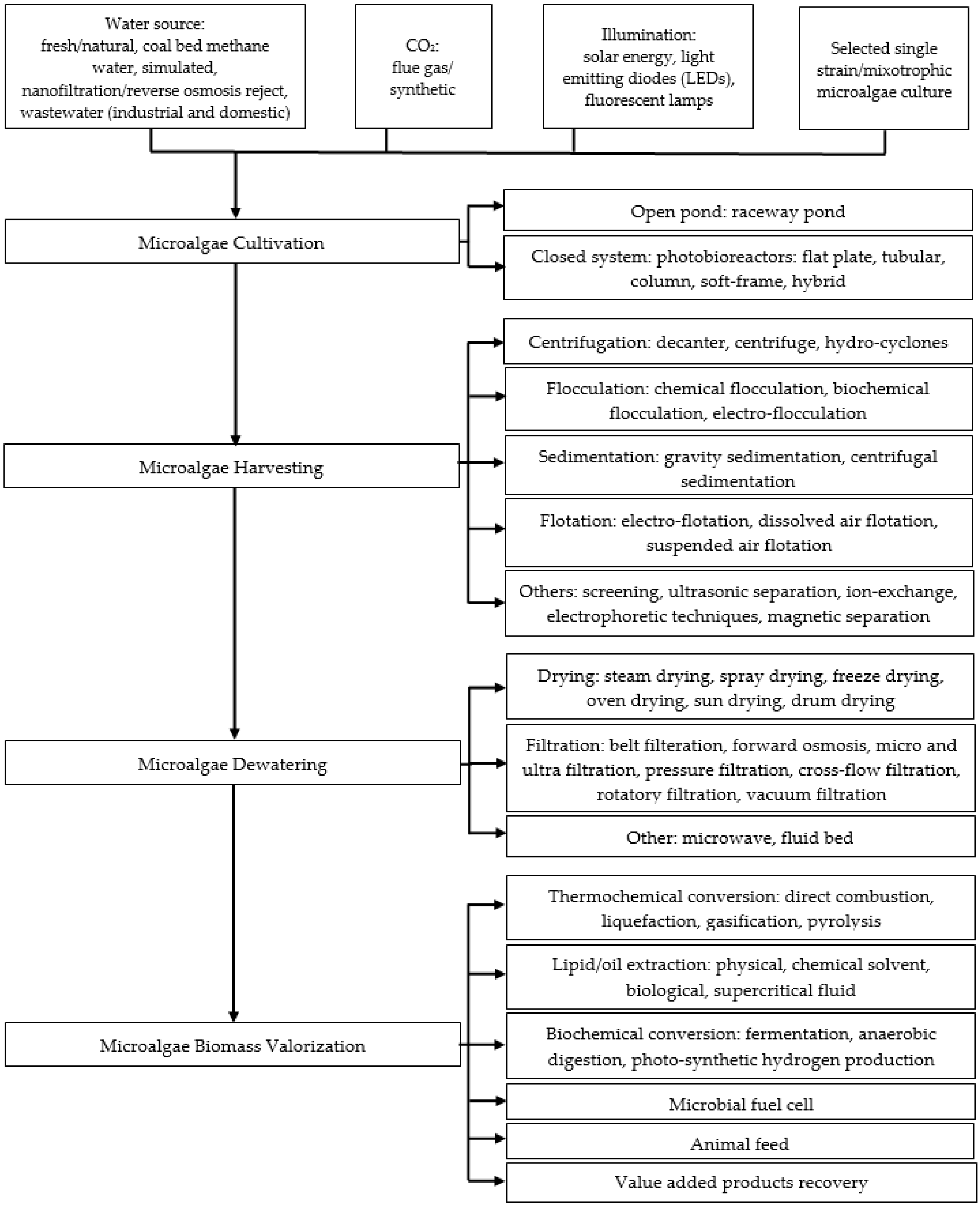

Figure 4. The many available process steps in microalgal biomass cultivation and microalgae-to-biofuel conversion.

It should be noted that technologies for microalgal production and use are much more complex and elaborate compared to other waste treatment and waste-to-energy bioprocesses. For the activated sludge process, commonly used for wastewater treatment, the design guidelines for large-scale installations are extrapolated from just a few key process parameters [30]. These include: target concentration of bacterial microflora in the bioreactor, the specific organic load rate of the activated sludge, hydraulic load and oxygen levels in the bioreactor [31]. Anaerobic treatment systems are even simpler and easier to design, build and operate, relying on only two variables: organic load rate and hydraulic retention time [32]. The significantly simpler and more streamlined nature of such processes is a major advantage and a point in their favor, contributing to their widespread implementation and use in many full-scale installations [33]. It should be noted that these solutions are somewhat competitive with those based on microalgae since they generate surplus sludge, which can be further converted into fuel [34].

The above findings raise the question: which of the myriad available experimental data on microalgae cultivation and microalgae-to-fuel processing will be appropriate to the given specific initial conditions? The fact that most experiments on the subject are small-scale (mainly laboratory-scale) is a well-established barrier to widespread implementation of microalgae-based technologies [35]. Of course, small-scale studies do provide highly valuable data, a basic understanding of a process. They can help explain and describe the biochemical processes at play and can be used to quickly and inexpensively test a multitude of variants. However, experimental efforts often fail to provide real-world data crucial for practical deployment, i.e., those pertaining to the balance of costs and inputs, real-world performance, emergent operational hurdles, the impact of external factors, material and energy flow analysis, actual energy balance, carbon footprint and many others. As a general rule, data from micro-scale studies cannot be used for design or investment projects due to economies of scale. Indeed, deployment cannot realistically be initiated without substantiated and reliable data. The numerous laboratory-scale studies found in the literature demonstrate the potential of microalgae and give a general idea of the scale and versatility of microalgae-based systems, but they do not provide specific data necessary for design, performance assessment, investment outlay estimation, operational cost estimation or environmental impact assessment.

Before any technology can be deployed and propelled from the laboratory to the commercial scale, it requires not only research and performance assessment, but also optimization, process modeling, process design, estimation of economic, social and environmental costs, lobbying and extensive promotion/marketing [36]. An important step in the investment decision-making process for the commercialization of innovative products is to assess their current "technological readiness level" (TRL). With TRL, the maturity of a technology can be expressed on a spectrum from the concept stage (TRL 1) to the maturity stage (TRL 9), the latter relating to concepts that have been developed into marketable and deployable technologies through R&D and preimplementation analyses [37]. TRL allows investors to quantify the level of work on the evolution of a new technologies, prospects for further progress, the investment outlays necessary to increase the TRL, and the innovative risk [38]. As such, it serves as a universal metric for assessing how far a technology has progressed and whether it is ready for commercial deployment. The assorted TRLs are described in Figure 5.

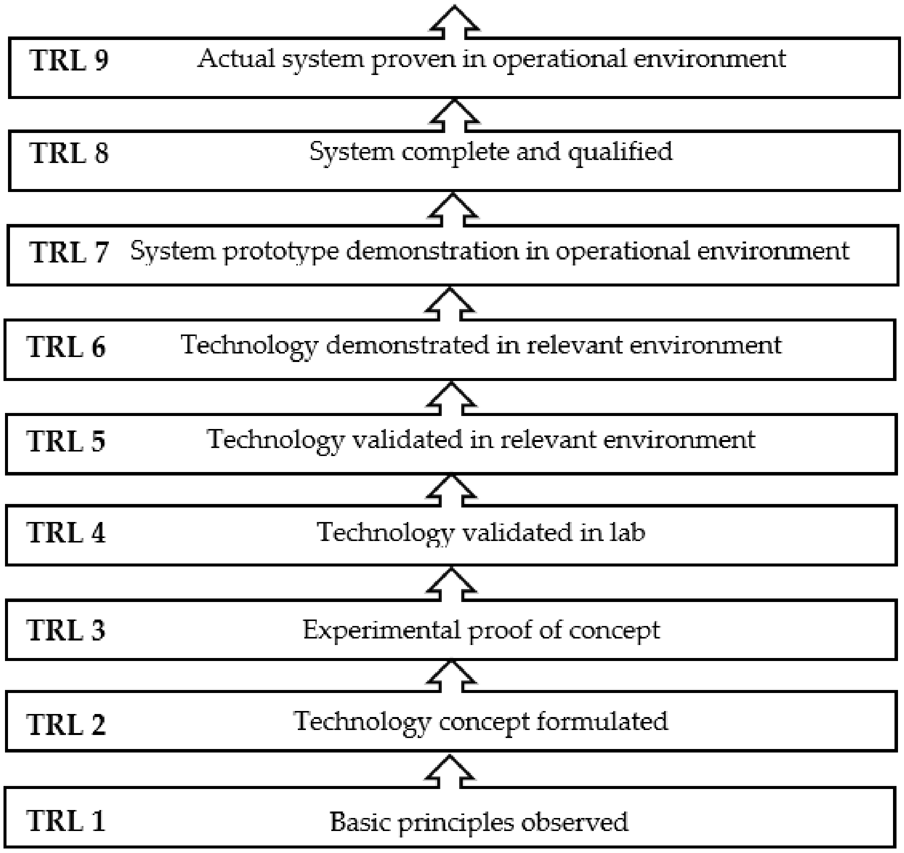

Figure 5. Technical readiness levels.

Brutyan (2017) [39] confirms that the current information on TRL allows all interested parties to know the current state of development of the production of microalgae alternative fuels. The evolution of these technologies by reaching successive levels of TRL reduces the risks associated with their implementation on a large scale. It is an important and practical tool for the successful development and commercialization of microalgae biofuel. It was found that currently the production of biofuels from microalgae is at the 6–7 TRL level. Level 8 can only be achieved if successful commercialization is supported by an effective biofuel production business model based on signed sales contracts and an assessment of the production plant's environmental impact using an independent, internationally approved methodology. Severo et al. (2021) [40], based on the analysis of patented technologies based on the integration of photobioreactors with fuel combustion systems, concluded that due to the low TRL level, they could be implemented on a commercial scale. Low productivity and unsatisfactory system efficiency, high investment and operating costs, engineering problems related to CO_2 capture and conversion, difficulties in maintaining long-term, continuous and stable production of metabolites were indicated. These criteria need to be carefully considered before they can be designed to operate in a free market [41]. In terms of TRL, only a few of the patented technologies are tested in commercial installations, reaching level 9, referred to as true "flight-proven" systems. The vast majority remained at the proof-of-concept stage with a low TRL of 3–5 [40]. In turn, Rumin et al. (2020) [42] claim that, after several decades of research, it can be considered that the production of biofuels from microalgae has reached the TRL of 6. However, it is indicated that the improvement of competitiveness in microalgae growth techniques, technology acquisition and genetic engineering are still needed to make it commercially viable on a large scale [43].

3. Process Optimization

Appropriate procedures, optimization tools, and statistical tests must be chosen and applied at all levels of industrial R&D endeavors to achieve sufficient TRL for deployment [44]. This extends to the very earliest stages of experimental design and subsequent data analysis, especially for technologies whose final performance is significantly and directly determined by multiple interrelated variables. As we elaborated above, microalgal biomass production and its subsequent conversion to energy carriers is one such technology. Appropriate experimental design, i.e., the manner of modeling and optimizing technological parameters, processes and final products, involves the development of a mathematical model using the experimental data [45]. Such models are used to predict or determine the optimal parameters for the product or process against the target values. These algorithms can also be used to forecast how changes in lighting parameters, growth medium composition, stirring parameters, carbon dioxide/oxygen levels, biomass concentrations and other physicochemical parameters will impact the output of desired microalgal metabolites [46]. Such studies often draw on dynamic flux balance analysis (DFBA). The DFBA integrates genome-scale metabolic models with the law of conservation of mass, as applied to the extracellular environment [47]. The accuracy of the model's predictions is verified by collecting independent experimental data, which are controlled by another optimization model [28]. Other popular techniques for design and optimization of experimental data on new microalgae-based technologies are: Plackett-Burman design, response surface methodology, central composite design, and empirical equations derived from multiple stepwise regression [48,49]. If the optimization procedures and statistical tools are applied correctly, the model can produce reliable results and data with low risk of error. Verified data guarantees a reasonable level of assurance for potential investors interested in implementing microalgal biofuel production technology. Providing such data falls within the purview of the research institutes that develop and improve solutions with potential for application.

4. Research Scale-up and Reliable LCA

The next step towards achieving sufficient TRL is to transition the research from the laboratory scale to the semi-industrial scale, the pilot scale and finally, and full scale [50]. This is necessary to avoid scale-up problems later on [51]. The size of the plant is crucial for reliably identifying the right feedstock type, materials, equipment, process steps and technological parameters. Care must be taken to ensure that estimates based on pilot-scale operational data do not create problems after further scale-up [52]. This is a critical consideration for variables, such as process design, type of facility, thermal energy exchange estimates, stirring efficiency estimates, fittings, auxiliary devices and control/measurement equipment. It also enables reliable identification of target plant performance and realistic estimates of investment and operating costs. Such data can be a deciding factor in determining whether or not to move forward with an investment and bring it to completion. Relatively few reports in scientific and technical journals present technical/technological designs and operational data for large-scale microalgal systems [53]. As it stands, this area of research needs to be expanded and developed much further. In other words, there is a legitimate need for establishing and operating pilot-scale and full-scale installations to apply and test the findings of laboratory studies. Currently, LCA analyzes concern the evaluation of the possibility of using various waste streams, including sewage and leachates, as well as waste gases, as elements stimulating the economic and environmental effects of microalgae biofuel production technologies [54]. LCA analysis provides an environmentally sustainable view of the proposed strategy. The results of LCA analysis have great significance for the improvement of the microalgae culture medium, the optimization of the technological process of biofuel production, and the strategy of environmental control in the whole process [55]. The scale of the gap between all published research works and full-scale studies on microalgal biomass production is clearly illustrated in Figure 6.

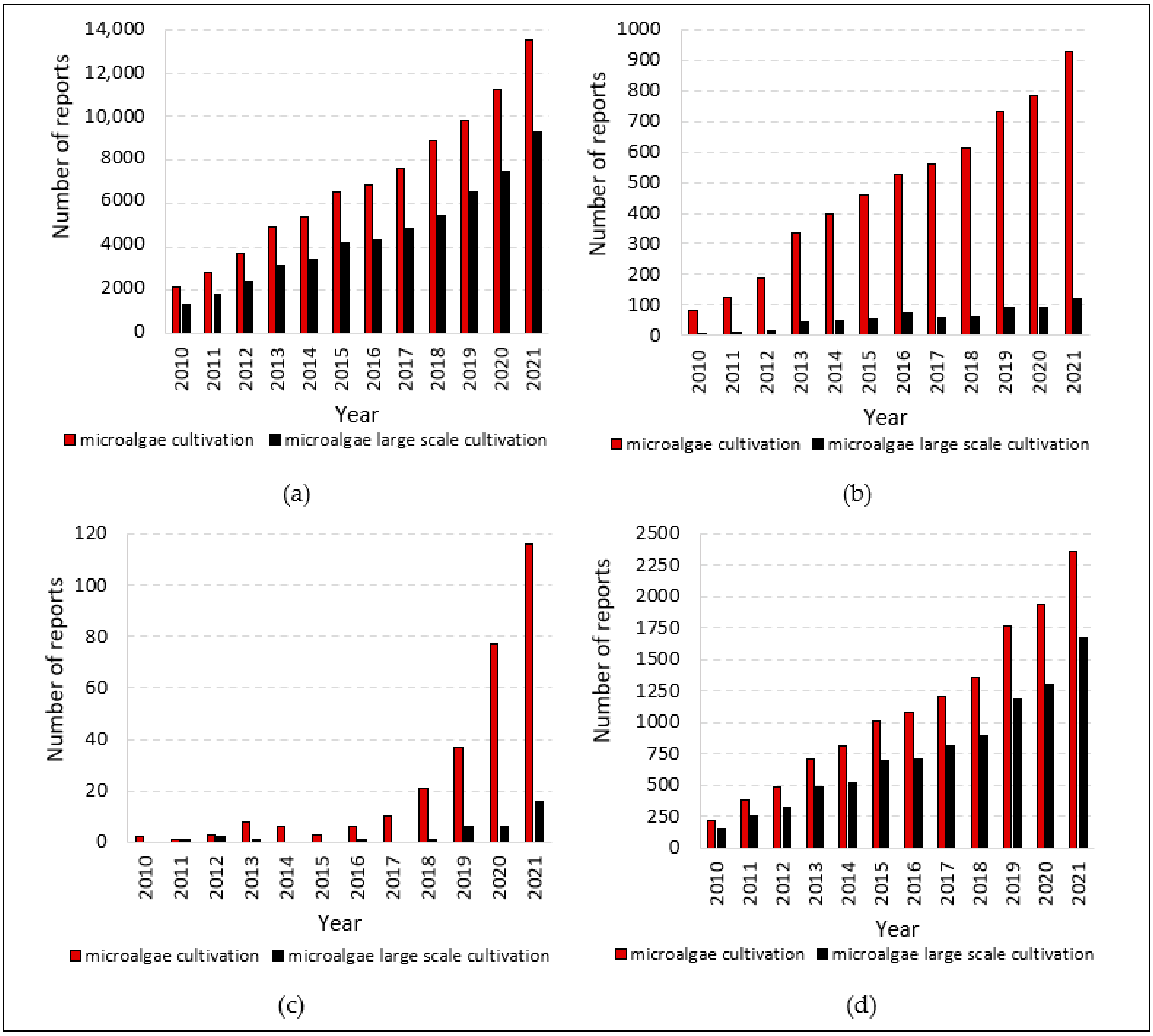

Figure 6. Article results in (**a**) Google Scholar, (**b**) Scopus, (**c**) MDPI and (**d**) Science Direct between 2010 and 2021 for the keywords "microalgae cultivation" and "microalgae large scale cultivation". Accessed 24 October 2022.

A major and indispensable part of all preimplementation work is a life cycle assessment (LCA), which should be applied to every promising new technology with high potential for practical application [56]. LCAs are necessary for preparing materials and compiling documentation, including environmental documentation required to obtain the relevant permits to launch the investment process [57]. Microalgae-production and microalgae-to-bioenergy technologies are no exception. A well-conducted LCA based on reliable and verified data can also provide great insight into the material and energy requirements of the plants, help estimate associated costs and predict emissions/waste generation [58].

Thus, a robust life cycle assessment (LCA) for microalgae-to-biofuel technologies is required to obtain a realistic balance, performance assessment and cost-effectiveness analysis. Different literature studies can often provide conflicting results due to different LCA variables and assumptions, and/or due to disregarding certain factors, which can severely alter the final results of the analysis. LCAs are often based on theoretical analyses and estimates adopted for calculation purposes, which were never actually verified [59]. The authors of LCAs often base their findings on laboratory-scale studies, which is a blatant mistake and produces completely unreliable estimates [60]. Any realistic LCA of a microalgae-production and microalgae-to-biofuel technology must include consideration of the following: siting; microalgae species; full process infrastructure (including facility specifics, fixtures/fittings, auxiliary equipment and control/measurement systems); type and size of transport involved; power sourcing and cost; availability of water or other growth media; nutrient sourcing and profile; by-product disposal, recycling and neutralization; functional and temporal units; waste management; and many others [61]. These factors should be considered on the basis of long-term operational data from near-full-scale installations ("long-term" meaning "from at least one full growing season" for facilities situated in temperate areas). Only then can results on energy consumption, materials, maintenance/repair costs and process efficiency be obtained.

As was stated multiple times, technologies for the production and use of microalgal biomass have their performance shaped by an extensive set of variables, with a very wide range of potential process combinations [62]. Each envisioned solution has different energy/material requirements and produces a different array of by-products, which may be waste materials or may have valuable uses for material or energy. This process variety is a source of considerable controversy and discrepancies in technical, economic and environmental results among LCAs for different microalgae-to-biofuel processes [63].

LCA must be approached as a reliable tool for lifetime environmental impact assessments for microalgae-derived biofuels—from feedstock sourcing and supply to production processes, final disposal and waste recycling [64]. This integrated approach can provide information on environmental footprints throughout the entire process chain, which should then be used as a decision-making tool to achieve a better, more efficient process with minimum impact on the environment [65]. The LCA must define the environmental impact of a process throughout its whole life cycle, including estimates for net energy and material inputs/outputs [66]. Unfortunately, data from large-scale plants are often not available, as is the case with microalgae-to-bioenergy technologies [67]. Instead, energy and balances for full-scale processes are obtained via mathematical modeling and simulations [68]. We consider this a major and common misstep, as such simulations and optimization procedures are only suitable for small-scale processes with low TRLs. Such measures can be used to reduce the number of laboratory-scale experiments and studies, but their results should always be verified and confirmed on a pilot scale. Only then can they become a source of input and output data for the assorted process steps and form the basis of the LCA [69]. Otherwise, the data obtained via LCA becomes wildly erratic, which greatly reduces the investment and deployment value of the technology.

5. Monitoring, Measurement and Control Systems

As already elaborated above, implementing efficient and cost-effective microalgae production and microalgae-to-bioenergy conversion technologies is fraught with hurdles and limitations [70]. They stem from the massive number of interrelated variables that together determine the final performance of a system. Scientists, technologists and operators of such plants face the challenges of designing microalgal cultivation protocols, choosing methods for separation, thickening, dewatering and desiccating of biomass and creating technologies for harvesting value-added products [71]. Another key issue is identifying, screening and effectively monitoring factors that promote microalgal biomass growth and increase the accumulation of desired substances, including chemical and physical properties of the medium [72].

Research to date indicates that the development of solutions that are cost-effective, technologically viable, and environmentally friendly will be the determining factor for the feasibility of cultivating microalgae for industrial purposes [73]. One performance-enhancing measure that tends to be ignored by scientific publications is the optimization of physical, chemical and biological medium conditions [74]. This may be achieved by using advanced and practical systems for monitoring, command and control of microalgal cultivation systems [74]. One effective measure for industrial settings and full-scale plants may be to implement on-line monitoring for full and ongoing process control, which can directly improve cultivation productivity and, thus, increase pollutant removal and the production of value-added substances while minimizing operational costs. Such monitoring requires accurate sensors for ongoing measurements of the external environmental conditions, the physicochemical parameters of the culture medium, and the biological process variables for the photobioreactor interior [75]. Since the end product of microalgae-based technologies is shaped by so many environmental parameters, the ongoing analysis has to draw on numerous metrics. Parameters requiring ongoing control include: physicochemical profile of the bed/medium, lighting intensity, light-dark cycling regime, on-line assessment of microalgal growth rates, biomass concentration and changes in microalgal species composition [76]. The final performance of intensive microalgal biomass production is also mediated by levels of CO_2, O_2, nitrogen, phosphorus, carbon and micronutrients in the medium, pH, temperature, bed turbidity, light transmittance, biomass concentration and chlorophyll *a* levels [77]. Ensuring optimal cultivation conditions and the ongoing monitoring thereof requires the use of multiparameter sensors and analyzers to improve photobioreactor performance, increase microalgal biomass production, and optimize materials/energy use [78]. Innovative control and measurement systems can do much to shape biomass composition and profile, including the accumulation of commercially valuable substances or energy products in the cells. Features to be considered for advanced systems include flow sensors, IR spectroscopy, RPG light sensors, microscopy, in situ, virtual sensors, the internet of things (IoT), artificial intelligence (AI), online monitoring of taxonomic structure, and others [79]. The physicochemical parameters requiring monitoring and control, together with the applicable types of sensors, are listed in Table 1.

Table 1. Factors monitored and analyzed in microalgae cultivation systems.

Monitored Parameters	Sensors	Control Options/ Monitoring Method	Acceptable Range or Off-Line/ On-Line	Comment	Refs.
pH	Optical pH sensor; pH glass electrode	CO_2 injection	7–10	Value out of range: growth rate decrease	[80,81]
Temperature	Thermoelement	Water bath; Water spraying; Heat exchanger; Shading	15–35 °C	High: culture death; Low: slow growth	[80,82]
Light density	Quantum sensor: flat cosine, fiberoptic spherical, PAR dosimeter, integration solarimeter	Culture density; PBR design; mixing	10–250 µM/m^2/s (optimal); 0–2000 µM/m^2/s (actual)	High: PE sinks at PFD > 250, Photoinhibition at >1500; Low: slow growth	[80,83]
Mixing	None	Agitation intensity; Gas addition rate	Re < 6000–10,000	High: mechanical damage to cells; Low CO_2 limitation/ O_2 inhibition	[84,85]
Inorganic nutrients	Colorimetric assays; UV spectroscopy; Ion-selective electrodes	Nutrient addition	Varies with nutrient	Low: growth limitation, lipid or starch accumulation	[86,87]
O_2	Paramagnetic and polarometric analyzer	-	Depends on O_2 and mixing intensity	High: growth rate decrease	[88,89]
CO_2	Mass spectrometer; IR analyzer	CO_2 feed	>0.15%	Low: growth rate decreases	[80,90]

Table 1. *Cont.*

Monitored Parameters	Sensors	Control Options/ Monitoring Method	Acceptable Range or Off-Line/ On-Line	Comment	Refs.
pCO_2 (liquid phase)	IR analyzer + flow meter; pCO_2 electrode	CO_2 injection	>0.1 kPa	Low: growth rate decreases below 0.1 kPa	[80,90]
Cell number concentration; Cell mass concentration; Cell morphology; Population composition	Microscope + CCD	ISM	On-line	Image-analysis software is critical	[91,92]
Cell mass concentration	CCD camera	Color analysis	Off-line	Data analysis software is critical	[93,94]
Cell mass concentration	OD sensor; Turbidity sensor	OD, turbidity	Off-line and on-line	Wavelength choice depends upon pigments	[80,90]
Protein; Lipid; Carbohydrate content	ATR flow system; Fiberoptic probe	IR radiation (MIR, NIR, FTIR)	Off-line and on-line	Data analysis software is critical	[80,90]
Lipid content; Cell size	Flow cytometer	FC	Off-line and on-line	Sample processing necessary for lipids and starch	[80,90]
Pigments; Fatty acids	Spectrophotometer	Absorbance spectrum	Off-line	Fatty acid levels may be estimated by correlation to pigment ratio	[90,95]
Pigments; Lipids; Photosynthetic efficiency; Quantum yield	Pulse amplitude modulated fluorometer	Fluorometry	Off-line and on-line	PAM identifies stress leading to lipid production onset	[90,96]

One interesting and forward-looking solution that can improve the efficiency of microalgae-production, separation and microalgae-to-energy conversion installations is the internet of things (IoT). This concept calls for individually addressable objects (things) that can indirectly or directly store, process and/or exchange data via smart power-line communication or computer networks [97]. This approach has found use in processing industry, urban governance, health services and household appliances [98]. Bringing the IoT to microalgal cultivation can help overcome many of the hurdles currently encountered by the industry through a reduction in operation costs while also enabling operators to monitor microalgal growth and productivity in real time [99]. The term IoT refers to independent federated applications and services that support high-level autonomous data capture, event reporting, interoperability and network interconnectivity. The system integrates mechanical or digital machines, computer equipment or objects, and provides a unique identifier (UID) for network data communication without the need for human-human or human-computer interaction [100]. Cloud technologies and cloud computing allow data to be collected via IoT. The cloud is the best medium for complex processing of data, as it enables quick configuration and integration of new objects and the maintenance of cost-effective deployment. It also allows everything to be connected, traced and managed via dedicated websites and built-in applications. Effective monitoring and control of smart objects through the cloud has been made easier by the availability of fast networks [101].

Employing automation and IoT can reduce human labor and manual work via resource automation, thus, providing an optimal operational model for microalgae-production and microalgae-to-energy conversion installations [102]. The effectiveness and rate of microalgal growth, as well as the increase in the accumulation of substances important for the production of biofuels in their biomass, are influenced by many environmental factors. For this reason, the optimization of these technologies must in the future be associated with the use of multiparameter monitoring of photobioreactors and the implementation of an automatic control system and the modification of process conditions. In large industrial installations, it will be based on automation and online process control using, among other things, IoT tools.

6. Genetic Engineering

Advanced genetic engineering is one of the research avenues that can significantly improve the cost-effectiveness of microalgae-based bio-energy technologies [103]. The features and capabilities of genetically modified microalgal populations are believed to facilitate high biomass productivity and promote the accumulation of commercially valuable substances or energy products in the cells [104]. This approach to microalgae cultivation can be used to produce an improved biomass from molecular biology while limiting energy and substrate input [105]. Most microalgae species make use of cell structures called antennae to efficiently capture sunlight [106]. The effective take-up and conversion of light energy by these complexes is what directly fuels and determines biomass growth. Modification of the DNA responsible for these antennae enables better light penetration through the cells [107]. Transformation methods employed for transferring DNA into microalgal cells include electroporation, artificial transposons, viruses, particle bombardment, agitation of cell suspension in the presence of DNA and glass beads, grobacterium infection, silicon carbide whiskers and the more recent agrobacterium-mediated transformation [108]. Chemical and physical agents can also be used to induce spontaneous mutations [109]. Genetic engineering of microalgae has become increasingly efficient and relatively inexpensive for advanced techniques of DNA restructuring. This is because DNA restructuring (such as enhanced evolution, sequencing, metagenomics and hybridization) could be used to imbue microalgae with tolerance to harsh conditions [110]. New operationally and commercially desired qualities could also be attained via nontransgenic methods, which may produce microalgae that could better withstand a wide range of abiotic and biotic circumstances [111]. However, some have pointed out that such metabolic changes may favor and develop more narrow applications over others—better biofuel yields could draw all the attention at the expense of other food and nonfood applications [112].

Improvement of microalgae-to-bioenergy conversion technologies with genetic engineering could meaningfully reduce the costs of operating photobioreactors, which has been the primary challenge for large-scale plants [113]. Another important issue is the need to introduce suicide genes to prevent dangerous microalgae species from surviving in natural waters in the event of an incidental escape [114]. This is because these so-called dangerous algae strains present a high chance of proliferation and environmental danger [115]. Genetic engineering and molecular techniques to modify the characteristics of microalgae at the DNA level are very promising, but at present they should be approached with great caution due to social concerns and real environmental risks. The development of protocols for obtaining microalgae strains with high potential for bioenergy must be combined with the elimination of their potentially dangerous impact on natural biocenoses. This is a prerequisite for the commercialization of these solutions and their safe use in practice. Figure 7 presents genetic engineering techniques used to predispose microalgae to higher production of biomass and energy products.

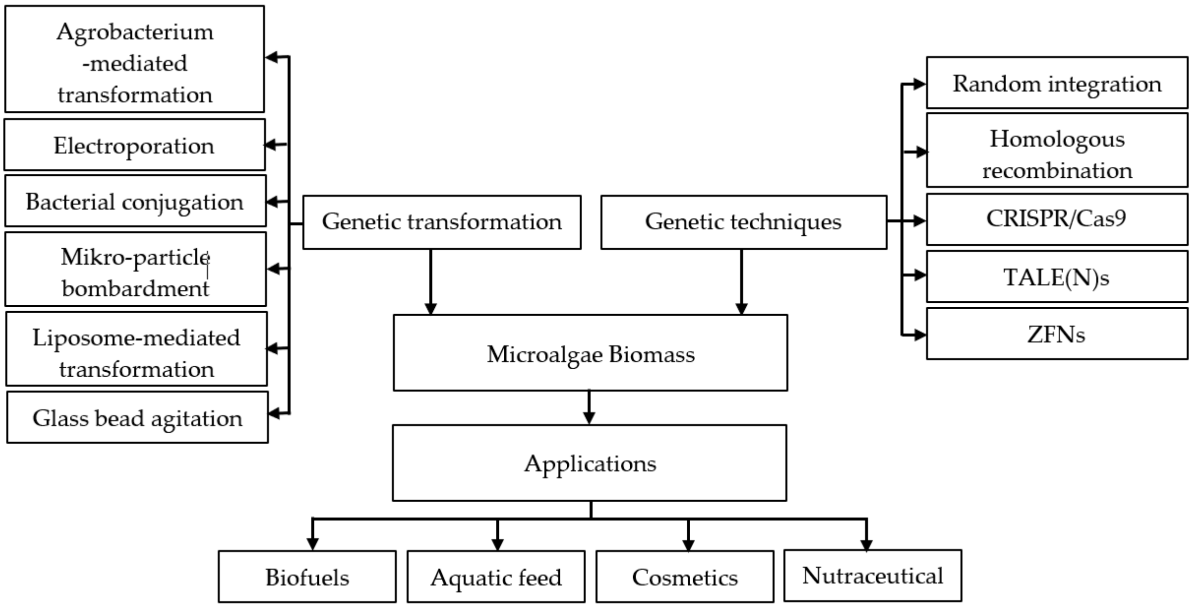

Figure 7. Genetic engineering methods used in microalgae-based technologies.

7. Symbiotic Systems

A new direction in the development of microalgae technologies that may contribute to their more widespread use are systems based on the symbiosis of microalgae-bacterial, microalgae-fungal or microalgae-yeast [116]. Currently, work is underway on the selection of cultivation conditions, the effects of the interaction of autotrophic and heterotrophic organisms and possible technological effects [117]. In symbiotic systems, microalgae intensify the processes of removing nitrogen and phosphorus compounds, which is of great technological and economic importance for wastewater treatment and are responsible for the production of molecular photosynthetic oxygen [118]. The oxygen produced by microalgae supports the metabolism of aerobic bacteria and fungi, which directly improves the technological and economic efficiency of the symbiotic system [119]. On the other hand, the biodegradation of organic pollutants by heterotrophs results in mineralized forms of nitrogen, phosphorus and carbon dioxide, which intensify the development of microalgae [120].

An additional competitive feature of the symbiosis of microalgae with heterotrophic organisms, indicated by the researchers, is the improvement of the sedimentation and separation properties of the produced biomass [121]. It is extremely significant from the point of view of the economics of the process because it is estimated that the techniques of separation and dewatering of microalgal biomass account for almost half of the costs incurred in the technological process of microalgal biomass cultivation [122]. The positive influence of symbiotic systems and their interactions on the quantity and quality of biofuels and other economically valuable products obtained from the obtained biomass was also found [123].

There are many benefits to the co-cultivation strategy, but it can also have many disadvantages. The commonly indicated ones include the secretion of toxic extracellular metabolites, enzymes that degrade cell walls, and lysis of microalgae cells during the culture, which in turn inhibits their growth [124]. Therefore, the industrialization of symbiotic methods very often requires sterilization of the breeding system, which significantly increases the total cost of system exploitation. When it comes to binary breeding, one of the main challenges is the proper selection of the microalgae strain. They should be species with high adaptability to difficult and changing environmental conditions [125]. Moreover, the commercialization of production methods based on autotrophic-heterotrophic symbiosis is limited by the fact that such systems are more dependent on ambient conditions, such as the level of nutrient accessibility, pH and temperature fluctuations, which leads to greater heterogeneity of the breeding medium [126]. Therefore, the development of an appropriate cultivation medium that would accelerate the growth of the strains in binary culture is another important task. Another critical point in the operation of large-scale bioreactors for symbiotic systems is the formation of regions poor in gaseous and mineral components of the culture medium. These kinds of dead zones will result in poor mass transfer and thus generate a lot of cellular stress for the cooperating microorganisms. Fortunately, these depleted areas can be alleviated by synergistic oxygen-carbon dioxide exchange between mixotrophic/autotrophic/heterotrophic strains in non-axenic cultures [127].

Taking the above into account, it is necessary to optimize the rate of aeration, mixing and feeding of nutrients to binary cultures. It mainly boils down to the correct design of reactors with optimized cultivation parameters, which is a prerequisite, but a necessary condition for a successful symbiotic production process. Another key aspect of this co-culture system that requires attention is access to a light source when culturing symbiotic organisms with high cell density. Such a dense population can effectively block the light pathway, inhibiting the growth of microalgae and the productivity of lipids or other value-added products.

8. Incentives, Subsidies, and Law Regulations

Programs aimed at supporting clean energy production systems and reducing environmental emissions could do much to help raise interest in microalgae-production and microalgae-to-energy conversion technologies [128]. Development and implementation of regional or national assistance programs, co-funding schemes, and preferential/attractively-rated loan programs would raise interest in such technologies and promote implementation. Support initiatives may also take the form of grants; subsidies; tax benefits; business plan/environmental documentation assistance; advisory assistance on microalgal biomass production systems and/or biofuel/waste management strategies; co-funding of R&D; and reduction of barriers and import duties [129].

Governments and international organizations may and should leverage policies to encourage biofuel use, including microalgae-derived biodiesel or biogas, to reduce greenhouse gas emissions from fossil fuels and mitigate the fast-progressing climate change [130]. Government and corporate assistance should focus on supporting research and pre-implementation works for plants at near-real-world scale. Engineering (rather than strictly scientific) endeavors should take precedence in terms of identifying, reducing or eliminating all of the main shortcomings and deficiencies of microalgae-based technologies.

There is also the issue of the multilateral conflicts of interest between the agricultural, energy, food, environmental and social sectors—an important socio-economic factor to consider in striving for better technological and commercial prospects for developed microalgae-to-bioenergy conversion processes. Some economists have noted that budget subsidies for the evolution and implementation of alternative energy sources may have negative repercussions for economies, reducing societal welfare and standard of living [131]. The reasons for this are twofold—first, the transfer of government funds to a single sector, and second, the increase in energy prices for individual consumers and businesses [132]. It is often said that double-use biomass sources (those that can be used both for biofuel production and for food/feed production) can also negatively affect societies, especially by reducing the land for growing food and driving up the prices of consumer goods [133]. Although the impact of bioenergy policies on land use has actually been poorly documented, there is nevertheless strong evidence supporting their impact on land demand and deforestation [134]. However, these problems are mostly irrelevant to microalgae, as they do not compete with the food and feed supply chains, require much less land and water, can be used to process wastewater, do not drive deforestation, and are not a threat to biodiversity (to the same extent as other biomass materials) [135].

Another reason used to support the next round of research on microalgae-to-biofuel conversion relates to the regulations on greenhouse gas reduction and the share of components in the traditional fuel blend. This necessitates the implementation of new technologies for advanced biofuel production that support efficient recovery of bio-energy, as well as the deployment of effective carbon dioxide sequestration techniques. Many strategies, including EU policy, aim to increase the share of alternative energy, which is a big effort for most countries. The ILUC (Indirect Land Use Change Impacts) Reports commissioned by the European Commission undermine the ecological sense of the production and use of biofuels produced from biomass that can be a source of food and fodder. There is therefore a need to look for other sources of biomass for biofuel purposes. These goals are to be achieved through the production of advanced biofuels made from non-food biomass and waste. The documents indicate such raw materials, whose energy value is repeatedly counted for the purpose of determining the content of biofuels in energy production. Algae ranks first on this list.

The biorefinery concept is an interesting concept for improving the cost-effectiveness of microalgae-derived bioenergy production, widely recognized as a valid one [136]. This approach calls for expanding the production process to extract additional commercially valuable substances, thus adding value to the entire system [137]. Non-fuel products that can be derived from microalgal biomass include food, diet supplements, animal feed, fertilizers, and medicines [138]. A comprehensive biorefinery approach to microalgal biomass processing is presented in the diagram below (Figure 8).

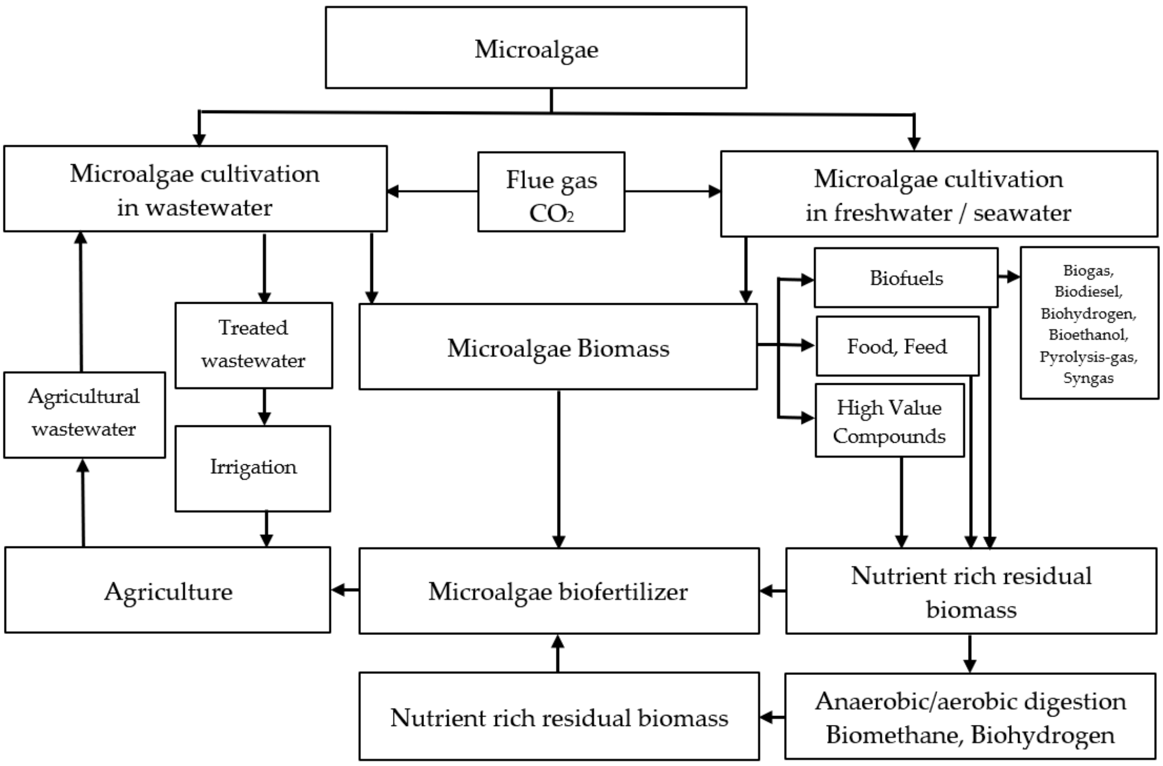

Figure 8. Microalgae biomass in a comprehensive biorefinery concept.

Interest and funding from the private sector can serve as a major driver of microalgae-to-bioenergy conversion technology growth and proliferation. The importance of microalgae-to-biofuel conversion technologies has been recognized and appreciated by large energy corporations. One example is PKN Orlen SA, a leading East-Central European fuel company, which pursued an R&D project to find renewable sources of next-generation biocomponents [139]. A mobile pilot research station was built at the Płock (Poland) Production Plant to grow algae on CO_2 and process wastewater from the refinery units. The innovative R&D endeavor forms part of PKN ORLEN SA's policy of concern for the environment, while also aiming to help reach biofuel use targets mandated by the Renewable Energy Directive (RED) [140,141]. The main purpose of the project was to develop a technology to produce components from oil-producing green microalgae and diatoms in a real-world refinery setting. The experimental oil obtained from microalgae is being examined to assess its applicability for the synthesis of esters. Microalgae residues were also tested for gasification and anaerobic digestion potential, while organic residue from the diatom biomass was examined to see if it could be used to produce polymers. Judging by its performance at the Płock refinery, the technology can be commercialized and used to convert microalgae into biofuels in the future. It can also be implemented in CO_2-generating facilities of the PKN Orlen SA international capital group, and is sure to reduce on-site carbon dioxide emissions [142].

9. Conclusions

Harnessing microalgal biomass for commercial purposes is not a novel idea—in fact, it has been consistently developed for years. Although the overwhelming majority of published publications and reports indicate that culturing and harnessing microalgal biomass under controlled conditions is a viable endeavor, relatively few large-scale plants have actually been established. Algal biomass for use is still predominantly sourced from natural water bodies, but this approach is not without its limitations and controversies. The intensive exploitation of natural algae resources has led to an increasingly evident reduction in populations and the disappearance of commercially relevant species in the EU. This is mainly attributable to aquatic pollution, greenhouse gas emissions (and the resultant global warming), overexpansion and harvesting of biomass and the displacement of endemic algae species by rapidly spreading non-native eurybiontic species. This means that large-scale algae cultivation plants will have to be constructed and operated at some point. However, investment has been slow and inadequate, especially given the significance attributed to microalgae-production and microalgae-to-energy conversion technologies.

As demonstrated in this paper, this slow implementation can be attributed to the fact that large-scale deployment of microalgae cultivation and the use of technologies are fraught with many economic, technological and legal difficulties. In the temperate zone, unsuitable conditions (low temperatures and variable sunlight levels throughout the growing season) are an additional impediment to intensive and efficient production of microalgal biomass. Thus, facilities have to be equipped with solutions that ensure the optimal conditions required for microalgal cultivation. However, such upgrades greatly escalate the investment and operating costs for the technology, limiting the performance and cost-effectiveness of the entire endeavor.

Despite the fact that many available publications and process design, economic, legal, and environmental studies have pointed to the huge potential and bright prospects for microalgae-derived bioenergy production, adoption has been slow. There is no denying that microalgae-to-biofuel conversion technologies, as they are now, cannot compete with traditional energy carriers. Of importance in this regard is the immense instability of fossil fuel prices and the resulting difficulties in forecasting the return on investment and rate of return for innovative microalgae-based systems.

Multiple authors have looked to waste-as-feedstock solutions as a potential way to improve economic returns from microalgae-production and microalgae-to-biofuel plants. Wastewater, leachate, waste gases and flue gases can be used as sources of nutrients in microalgae processing systems, but this can reduce operating costs only in certain cases. Waste streams almost always have to be subjected to difficult pre-treatment before being fed into the medium, whether via pasteurization, UV disinfection, purification, dedusting or additional nutrient supply. The physical and chemical properties of such media (turbidity, high levels of organics and sulfur gas) further inhibit microalgal growth, serving as breeding grounds for competitive microbes (especially bacteria), which compete for nutrients and block the light needed for photosynthesis.

In the authors' opinion, the main barriers to microalgae-based technology development lie in the complex nature of the cultivation process with multiple variables involved; the lack of sufficient data from pilot-scale and near-full-scale plants (which often precludes reliable life cycle assessment); and the insufficient legal assistance, subsidies and funding for innovative projects. Potential ways of improving performance include: process optimization techniques, genetic engineering and yield improvement through advanced process control and monitoring.

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