

## Bibliometric analysis of biomass production of chlorella vulgaris through the integration of scientific databases and vosviewer

### Análisis bibliométrico de la producción de biomasa de chlorella vulgaris en bases de datos científicas y vosviewer

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## Abstract

**Introduction:** Microalgae are unicellular photosynthetic organisms with a high capacity for CO<sub>2</sub> fixation and adaptation to diverse cultivation conditions. Among them, Chlorella vulgaris stands out due to its nutritional and biotechnological value. Its cultivation enables the production of lipids, proteins, and pigments useful for biofuels, food, and bioremediation. Despite their ecological advantages, microalgae face economic challenges due to high production costs. Their metabolic versatility makes them key components for sustainable biorefineries. Bibliometric analysis allows the identification of research trends to improve their industrial utilization.

**Objective:** This study reviews the biomass production of Chlorella vulgaris through the integration of scientific databases and the use of VOSviewer to analyze bibliometric networks, aiming to determine research trends related to productivity, culture media, systems, production scales, and key nutrients.

**Methods:** Studies were collected from the Scopus database using five search equations. The most relevant documents were selected and analyzed through co-occurrence analysis using VOSviewer.

**Results:** The bibliometric analysis revealed a growth in studies on Chlorella vulgaris between 2014 and 2024, focused on technological and sustainable alternatives. The searches yielded 4,354 documents, predominantly scientific articles. The main subject areas were environmental sciences, chemical engineering, energy, and agricultural sciences.

**Conclusions:** Alternative nutrient sources are essential to reduce production costs. The use of wastewater can reduce nutrient costs by approximately 50% by providing nitrogen, phosphorus, and organic compounds, becoming a high-quality culture medium.

**Keywords:** Chlorella vulgaris, Bibliometric analysis, Nutrients, Productivity, Scale-up, VOSviewer, Bioremediation, Biofuel

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Spanish version

## Resumen

**Introducción:** las microalgas son organismos unicelulares fotosintéticos con gran capacidad para fijar CO<sub>2</sub> y adaptarse a diversas condiciones de cultivo. Entre ellas, Chlorella vulgaris destaca por su valor nutricional y biotecnológico. Su cultivo permite obtener lípidos, proteínas y pigmentos útiles en biocombustibles, alimentación y biorremediación. Aunque presentan ventajas ecológicas, enfrentan retos económicos por los altos costos de producción. Su versatilidad metabólica las convierte en clave para biorrefinerías sostenibles. El análisis bibliométrico permite identificar tendencias para mejorar su aprovechamiento industrial.

**Objetivo:** este estudio revisa la producción de biomasa de Chlorella vulgaris mediante la integración de bases de datos científicas y el uso de VOSviewer para analizar redes bibliométricas, con el fin de determinar tendencias de investigación sobre productividad, medios de cultivo, sistemas, escalas de producción y nutrientes clave.

**Métodos:** se recopilaron estudios en Scopus usando cinco ecuaciones de búsqueda. Los documentos más relevantes fueron seleccionados y analizados mediante coocurrencia en VOSviewer.

Resultados: el análisis bibliométrico mostró un crecimiento de estudios sobre Chlorella vulgaris entre 2014 y 2024, enfocados en alternativas tecnológicas y sostenibles. Las búsquedas arrojaron 4.354 documentos, predominando artículos científicos. Las áreas principales fueron ciencias ambientales, ingeniería química, energías y ciencias agrícolas.

**Conclusiones:** las fuentes alternativas de nutrientes son clave para reducir los costos de producción. El uso de aguas residuales puede disminuir aproximadamente un 50 % los costos de nutrientes al aportar nitrógeno, fósforo y compuestos orgánicos, convirtiéndose en un medio de excelente calidad.

**Palabras clave:** Chlorella vulgaris, Bibliométrico, Nutrientes, Productividad, Escalamiento, VOSviewer, Biorremediación, Biocombustible.



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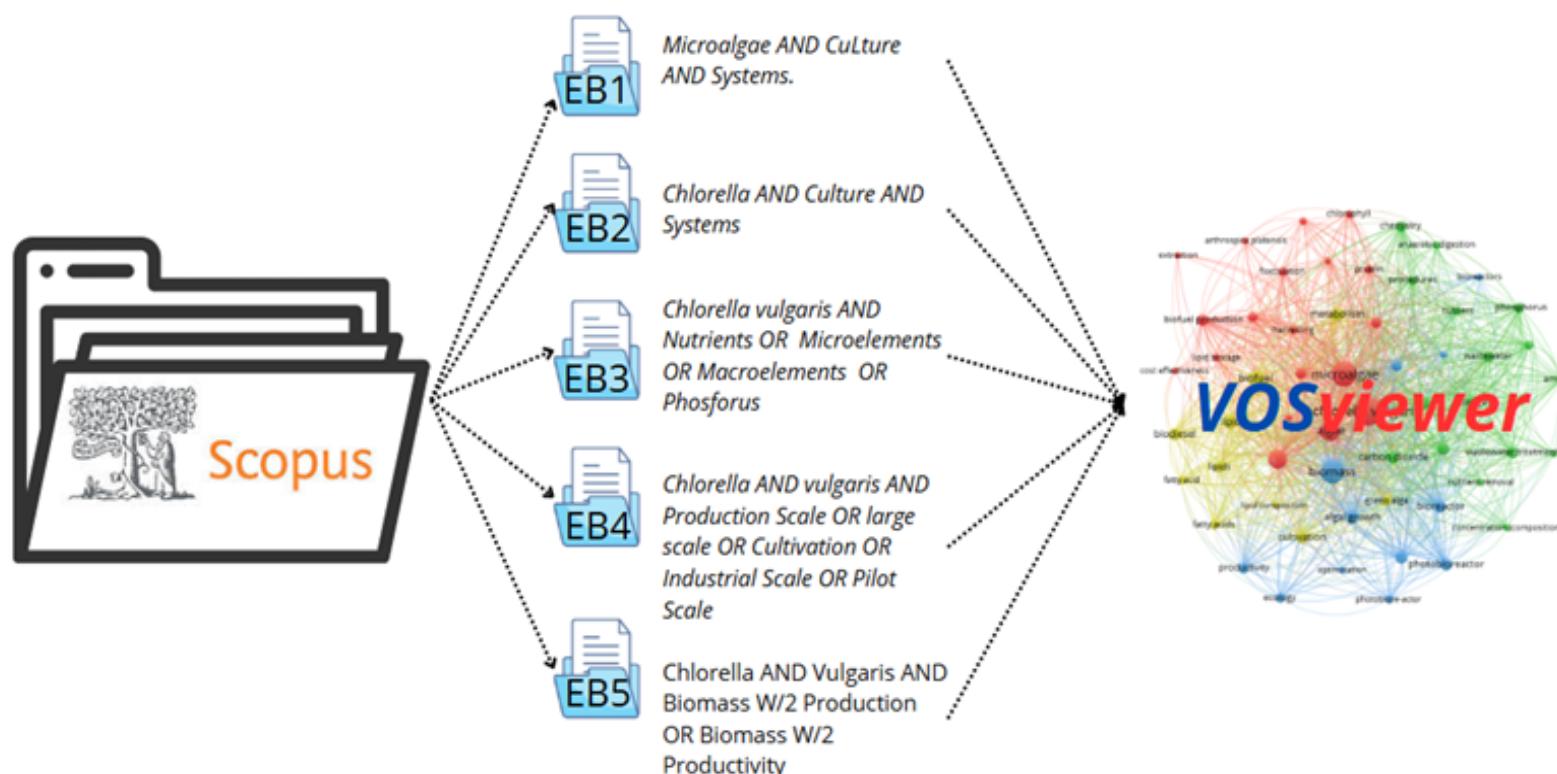


### Why was this study conducted?

Conducting a bibliometric analysis on Chlorella vulgaris production systems is relevant because this approach allows the quantification and visualization of scientific output in the field, identifying the most studied topics, collaborations among researchers, and leading institutions. This analysis reveals temporal trends in research, such as increasing interest in more efficient cultivation technologies or the use of agro-industrial residues as alternative substrates for microalgal growth. Addressing these gaps promotes technology transfer from laboratory to industry, fostering the adoption of more sustainable and cost-effective microalgae production systems.

### What were the most significant results? What do these results contribute?

The results show significant growth in the production of microalgal products, particularly nutrients and energy-related compounds. To address the challenges associated with nutritional requirements, it is recommended to integrate microalgal biomass production systems with wastewater treatment systems, industrial effluents, and process residues. By utilizing compounds present in these effluents, microalgae directly contribute to bioremediation while obtaining the nutrients required to generate biomass with high nutritional and energetic value at lower and more sustainable production costs.



## Introduction

Microalgae are a group of autotrophic microorganisms that inhabit marine, freshwater, and saline ecosystems and produce organic substances through photosynthesis. They are unicellular eukaryotic photosynthetic organisms (2–200  $\mu\text{m}$ ) capable of growing under autotrophic or heterotrophic conditions (1). In general, they are highly efficient in  $\text{CO}_2$  fixation. Due to their high metabolic flexibility, adaptability to diverse cultivation conditions, and rapid growth potential, research on their use as sources of biologically valuable products has increased rapidly. Currently, integrated technologies for microalgae cultivation are being explored to isolate various biologically active compounds from biomass, thereby increasing production profitability (2).

Microalgae require relatively simple nutrients such as carbon, nitrogen, and phosphorus (3), enabling the generation of biomass useful for lipid and protein production, biofuel oils, pigment synthesis, and wastewater bioremediation systems, among other applications. However, production costs remain relatively high compared to other plant- or animal-based protein sources commonly used in aquaculture feed production. Several factors influence microalgal biomass production costs, including growth media and light sources (4).

The production of microalgae, particularly *Chlorella vulgaris*, a green unicellular alga with a spherical shape measuring 2.5–10  $\mu\text{m}$  in diameter (5), has gained relevance in recent decades due to its biotechnological potential (7). Its green color originates from chloroplasts responsible for photosynthesis, a characteristic that gives the genus *Chlorella* its name, meaning "small" (6). Despite technological and scientific advances, large-scale implementation of *Chlorella vulgaris* production systems face significant challenges limiting economic viability and environmental sustainability (8). These challenges include high biomass production costs, dependence on expensive and complex culture media, and technical difficulties in efficiently scaling cultivation processes.

Microalgae have gained significant importance in the biofuel sector. *Chlorella vulgaris* is particularly rich in proteins (61.6%), lipids (12.5%), and carbohydrates (13.7%), and contains vitamins, minerals, and pigments (9), making it a highly complete nutritional source. It is also used in wastewater treatment and  $\text{CO}_2$  capture, highlighting its potential in energy, food, and health applications (10). In recent decades, biorefinery design and construction have increased due to the need to obtain biofuels and bioproducts from renewable sources (11).

Microalgae exhibit different nutritional modes: autotrophic, using solar energy and inorganic carbon such as  $\text{CO}_2$  (12); heterotrophic, obtaining energy from organic compounds such as sugars, glycerol, acetates, or glutamates (13); and mixotrophic, combining both processes by simultaneously assimilating  $\text{CO}_2$  and organic carbon (14). This metabolic versatility makes microalgae a promising feedstock for biorefineries using chemical, biochemical, or thermochemical technologies. These biorefineries must integrate sustainability, product diversification, and economic profitability (8).

Open systems are the most widespread for microalgae production worldwide, accounting for more than 90% of global production. These systems consist of shallow ponds that facilitate light penetration and biomass productivity, with paddle wheels maintaining culture circulation (3).

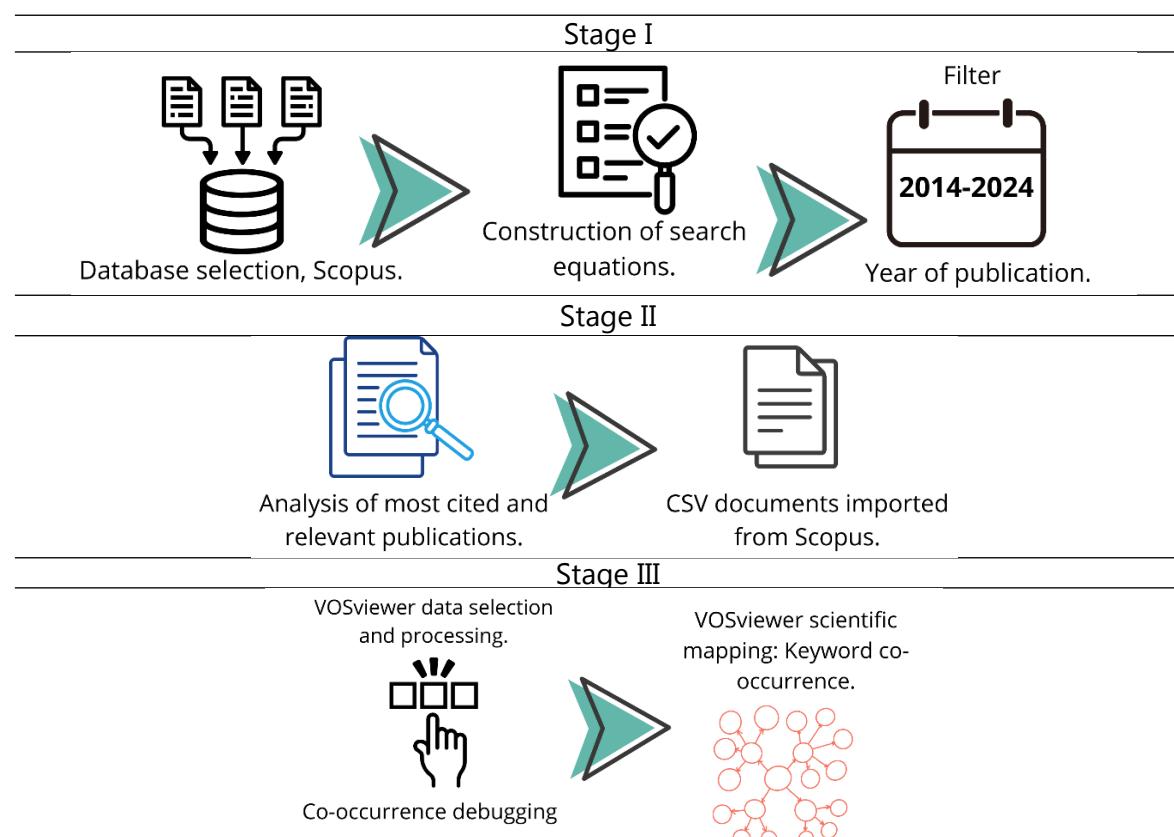
However, they present disadvantages such as easy contamination, limited operational control, evaporation losses, low cell density, and high dependence on climatic conditions (15).

Closed systems such as photobioreactors are used to cultivate microalgal strains that cannot withstand extreme conditions present in open systems. These systems are suitable for producing high-value compounds that justify higher operational costs. Various closed photobioreactor designs have been proposed, including bubble columns, helical systems, and flat panels, with tubular photobioreactors being the most common at commercial scale (3). Closed systems offer advantages such as reduced land requirements, lower contamination risk, and greater production flexibility (15).

Conducting a bibliometric analysis of Chlorella vulgaris production systems is relevant as it quantifies and visualizes scientific output, identifying key research themes, collaborations, and leading institutions. This approach reveals temporal research trends, such as increased interest in efficient cultivation technologies and agro-industrial waste utilization as alternative substrates (11), fostering technology transfer from laboratory to industry and promoting sustainable and cost-effective production systems.

## Methodology

A bibliometric analysis of scientific articles addressing the cultivation and production systems of Chlorella vulgaris was conducted, focusing on nutrients, biomass generation, and production scale-up. The Scopus database was used to identify key researchers, subject areas, and keywords, compiling relevant academic information (Figure 1).



**Figure 1.** Methodological stages

## Construction and analysis of search equations

Search equations were constructed and configured using the parameters and tools permitted by the scientific database Scopus. These equations made it possible to identify and collect information on the scientific production of the most highly recognized academic publishers, including Elsevier, Taylor & Francis, Wiley Blackwell, Springer Nature, Sage, Oxford, among others. The equations were developed considering keywords related to microalgae, biomass production, cultivation systems, Chlorella vulgaris, culture media, and nutrients, combined using the Boolean operators allowed in Scopus: AND, OR, and NOT (see Table 1).

**Table 1.** Formation of search equations

search	equations	number of results
EB1	"Microalgae" AND "Culture AND Systems"	2.465
EB2	"Chlorella AND Vulgaris" AND "Culture AND Systems"	600
EB3	"Chlorella AND Vulgaris" AND "Nutrients OR Microelements OR Macroelements OR Phosphorus"	481
EB4	"Chlorella AND Vulgaris" AND ("Production scale" OR "Large Scale" OR "Cultivation" OR "Industrial Scale" OR "Pilot Scale")	367
EB5	"Chlorella AND Vulgaris" AND ("Biomass W/2 Production" OR "Biomass W/2 Productivity")	451

## Analysis of search equations using VOSviewer software

The information obtained through the search equations was also processed using VOSviewer software version 1.6.14 (open access). Co-occurrence maps were generated to represent and establish nodes, interactions, and connections among the set of keywords obtained as a result of the search equations in Scopus (16,17).

CSV files downloaded from Scopus were used and imported into VOSviewer for processing. Specific filters were applied to ensure high-quality results, such as the minimum number of keyword co-occurrences (EB1: 25, EB2: 20, EB3: 20, EB4: 15, EB5: 15) and the document type (research articles). Using the software, density maps and keyword co-occurrence networks were generated, producing a set of important "clusters" (groups of words) for identifying relevant research topics, emerging thematic areas, and scientific trends related to the cultivation, biomass production, and scale-up processes of Chlorella vulgaris.

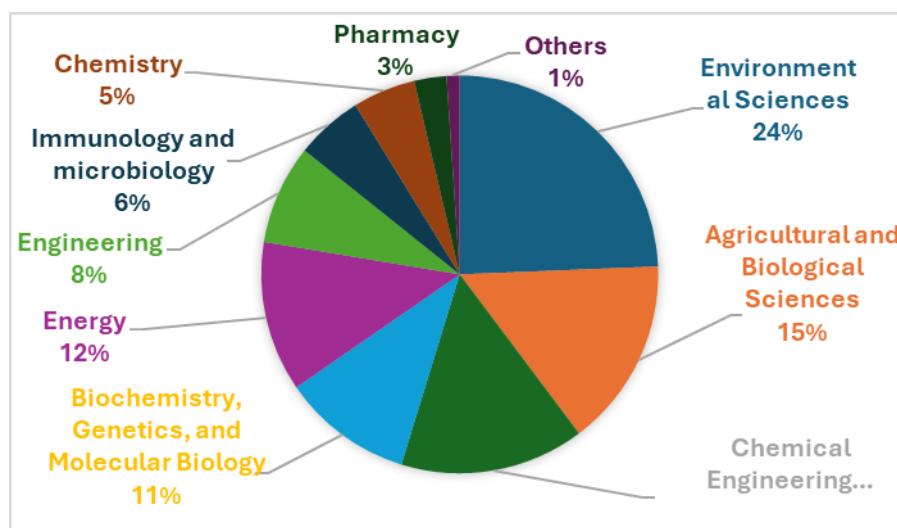
## Results and discussion

The study of biotechnological processes and the utilization of microalgal biomass encompasses various areas of knowledge, ranging from environmental sciences where microalgae play a key role in the removal and remediation of contaminated Waters to specialized cultivation for the production of high-value bioproducts. This reflects a growing interest in improving cultivation

conditions, scale-up strategies, and trends associated with microalgae development, particularly Chlorella vulgaris.

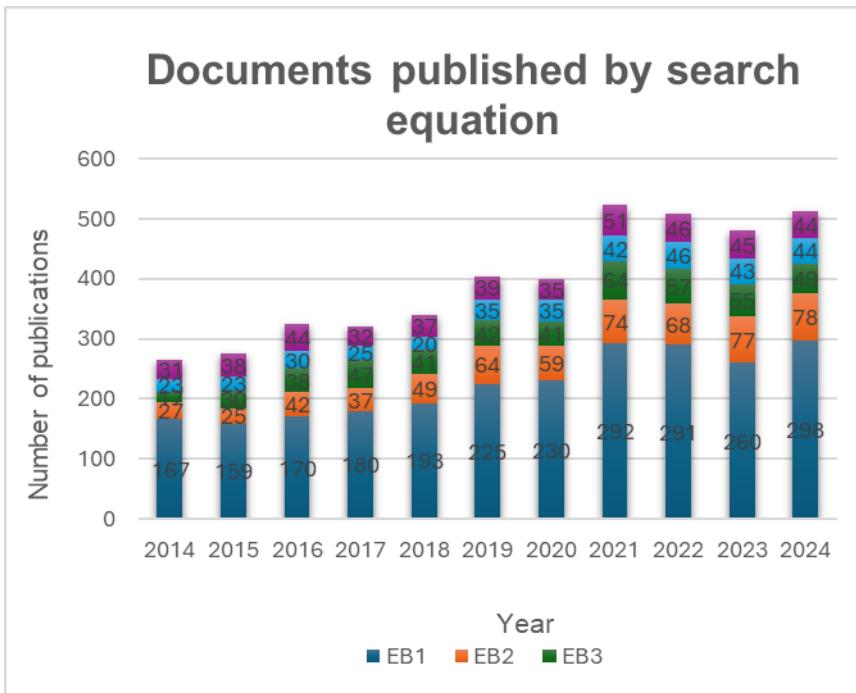
#### According to the number of publications

A total of 4,354 documents were identified as a result of the five search equations applied in the Scopus database for the period 2014–2024. Specifically, EB1 recorded 2,465 documents, EB2 yielded a total of 600, EB3 identified 481, EB4 produced 366, and EB5 yielded 442 documents. The identified research subject areas (see Figure 2) show that 24% of the publications correspond to environmental sciences, highlighting applications in the remediation of effluents from different industries. Additionally, 15% of the publications are concentrated in agricultural and biological sciences, emphasizing the use of Chlorella vulgaris in animal supplementation, biofertilizers, and biomass production. Furthermore, chemical engineering accounts for 15% and the energy sector for 12%, reflecting interest in crop optimization, the development of value-added products, and biofuel production as innovative and sustainable alternatives to address current energy and environmental challenges.



**Figure 2.** Thematic areas according to publications from the search equations (EBs)

A 78.44% growth in the number of publications was observed between 2014 and 2024 for EB1 (see Figure 3), indicating that processes related to microalgae are expected to remain a growing research trend in the coming years. This trend is driven by the increasing consideration of microalgal biomass as a potential feedstock for the production of chemicals and biofuels (biodiesel, biogas), combining wastewater treatment with symbiotic systems involving microalgae and/or bacteria to generate a sustainable and efficient business model that is key to overcoming the current energy and environmental crisis ([16,17](#)).



**Figure 3.** Documents published 2014-2024 for each search equation

The publication trend for documents published between 2014 and 2024 corresponding to EB2 shows a growth of 188.89%. Figure 4 allows the inference of a positive trend in the increase of publications and research associated with the search for new sustainable energy sources, with Chlorella vulgaris being an important feedstock in the development of bioenergy. Chlorella vulgaris exhibits an excellent capacity to achieve high productivities in different production systems, depending on cultivation conditions. The amount of biomass that can be obtained from this microalga ranges from 0.5–2.8 g/L in open systems to 3.48–30 g/L in mixotrophic systems, with a lipid content of approximately 19%–50%. This enables the biomass to be projected for applications in biodiesel and bio lubricant production. These values correlate with Monod kinetics, in which the growth rate depends directly on nutrient concentration. In open systems, light limitations and microbial competition explain the lower productivities observed (18–22).

A significant growth of 188.24% in documentary production was observed between 2014 and 2024 for EB3 (Figure 4), reflecting strong interest in the search for strategies to minimize nutrient production costs. In 2021, a notable peak in growth was observed; in the context of the COVID-19 pandemic, research on supplementation and nutraceutical products intensified to address this issue from a preventive perspective. For example, studies focusing on mineral supplementation in different products aimed at increasing immune system resilience against the virus have been reported (23–27).

Large-scale microalgae cultivation requires substantial amounts of water and nutrients, which increases production costs. For this reason, research has focused on the study of alternative nutrient sources. Various wastewaters have been identified that meet the nutritional profile required for the growth of Chlorella vulgaris, such as effluents generated during tofu production and

livestock-related processes such as aquaculture. These sources have demonstrated high biomass productivities and nutrient removal efficiencies ranging from 42.2% to 100% (28,29).

Figure 4 also shows a 91.30% growth between 2014 and 2024 for EB4, highlighting the importance of research on scaling up cultivation to large volumes and/or industrial systems capable of producing biomass in large quantities. The ability to accurately predict algal productivity across a wide range of environmental conditions, reactor geometries, and operating regimes is crucial for assessing the feasibility of large-scale algal production. Consequently, research is exploring mathematical models and simulators incorporating cultivation parameters such as temperature, pH, operational regime, and light. This modeling and simulation approach addresses the main challenges associated with accurately predicting algal productivity at real scale (30).

Additionally, a 41.94% growth in publications was observed between 2014 and 2024 for EB5. This trend reflects the focus of research on addressing challenges related to commercial application, with one of the main issues being the achievement and maintenance of high production levels. To this end, it is essential to provide microalgae with appropriate growth conditions (temperature, photoperiod, and pH). The optimal cultivation conditions for *Chlorella vulgaris* are within a temperature range of 25–30 °C and a pH between 6.5 and 8.0. Under these conditions, it is possible to obtain biomass with a maximum biomass concentration productivity of 2.37 g·L<sup>-1</sup>, as well as lipid accumulation ranging from 0.36 g·L<sup>-1</sup>·d<sup>-1</sup> to 0.47 g·L<sup>-1</sup>·d<sup>-1</sup> of dry biomass, depending on cultivation time. This range corresponds to the stability of photosynthetic enzymes and the maximum activity of Rubisco, whose thermal optimum lies between 28–30 °C. At higher pH values, the availability of dissolved CO<sub>2</sub> decreases, affecting photosynthetic efficiency (25–27).

Studies and research associated with EB1 (see Table 2) are oriented toward advances in microalgae cultivation, focusing on the consideration of different culture media types (freshwater, saline water, and wastewater) and the most efficient cultivation systems. Although the use of wastewater is promising from a sustainability perspective, a limitation is the relatively low biomass yields obtained (0.01–0.5 g/L) and the high harvesting costs, which represent between 20% and 50% of total production costs. Mathematical models are also analyzed as tools to perform simulations aimed at process optimization, along with co-cultivation systems involving other microorganisms that can improve yields and reduce costs. In addition, topics such as polyculture strategies to enhance production and the industrialization of volatile organic compounds (VOCs) produced by microalgae are also reviewed (31–39).

**Table 2.** Most cited documents EB1

Title	Author # Citations
A review on the use of microalgae consortia for wastewater treatment	<a href="#">(31)</a> 598
Heterotrophic cultivation of microalgae for pigment production: a review	<a href="#">(32)</a> 334
Potential industrial applications and commercialization of microalgae in the food and functional feed industries.	<a href="#">(33)</a> 302
Kinetic growth models for microalgae cultivation: a review	<a href="#">(34)</a> 289
A mini review: photobioreactors for large-scale algae cultivation	<a href="#">(35)</a> 248
Cultivation of heterotrophic microalgae for sintering biodiesel production with waste remediation: progress and prospects	<a href="#">(36)</a> 235
Use of marine microalgae as a chassis for the degradation of polyethylene terephthalate (PET)	<a href="#">(37)</a> 233
Micropollutant removal in an algae treatment system fed by source-separated wastewater streams	<a href="#">(38)</a> 229
Effects of C/N ratio on biofloc development, water quality, and performance of juvenile <i>Litopenaeus vannamei</i> in a biofloc-based, high-density, zero-exchange outdoor tank system	<a href="#">(39)</a> 227

The studies analysed under EB2 (see Table 3) highlight advances in the cultivation of Chlorella vulgaris and its performance in wastewater treatment, biomass production, and bioproduct generation. Sustainable cultivation systems are evaluated, such as consortia with bacteria and activated sludge, which improve nutrient removal, reduce sludge production, and enhance carbon capture. Additionally, autotrophic, heterotrophic, and mixotrophic cultivation conditions are optimized, and innovative technologies such as microbial fuel cells incorporating microalgae are developed. These studies demonstrate the potential for integrating bioenergy, bioremediation, and waste valorization [\(40–44\)](#).

**Table 3.** Most cited documents EB2

Title	Author # Citations
Interaction between Chlorella vulgaris and nitrifying-enriched activated sludge in the treatment of wastewater with low C/N ratio	<a href="#">(40)</a> 524
Microalgae for high-value products for human health and nutrition	<a href="#">(41)</a> 464
The interactions of the algae-bacteria symbiotic system and their effects on nutrient removal from synthetic wastewater	<a href="#">(42)</a> 243
Centrate wastewater treatment with Chlorella vulgaris: Simultaneous enhancement of nutrient removal, biomass and lipid Production	<a href="#">(43)</a> 148
The effect of different light intensities and light/dark regimes on the performance of photosynthetic microalgal microbial fuel cells	<a href="#">(44)</a> 130

The microalga Chlorella vulgaris has demonstrated excellent capabilities for wastewater treatment, CO<sub>2</sub> capture, and bioenergy production, among other applications. Research has focused on its ability to remove nutrients (N and P), heavy metals, and organic compounds, both in pure cultures and in microbial consortia (see Table 4). Although different cultivation modes (autotrophic, heterotrophic, and mixotrophic), cellular configurations (free and immobilized), and operational modes (batch and semicontinuous) have been explored, certain cultivation strategies—such as nutritional stress—have been implemented to enhance lipid and carbohydrate accumulation. Chlorella vulgaris integrates a sustainable production model, as the incorporation of biotechnology into cultivation systems and the use of industrial effluent treatment as a nutrient source for the production of high-value products represent an important link in the search for new sources of green products (45–49).

**Table 4.** Most cited documents EB3

Title	Author # Citations
Growth and nutrient removal of free-living and immobilized green algae in batch and semi-continuous cultures treating real wastewater (45)	571
Potential carbon dioxide fixation by industrially important microalgae (46)	464
A comparison of product yields and inorganic content in process streams after thermal hydrolysis and hydrothermal processing of microalgae, manure, and digestate. (47)	211
Bioethanol production from the microalga Chlorella vulgaris induced by nutritional stress by enzymatic hydrolysis and fermentation with immobilized yeast (48)	210
Heavy metal removal (copper and zinc) in secondary effluent from wastewater treatment plants by microalgae (49)	137

In Table 5, the studies mainly address the barriers in Chlorella vulgaris production related to cultivation scale-up, since increasing system volume reduces the efficiency of light and gas transfer, thereby limiting the attainment of higher biomass yields. This phenomenon can be explained by the Lambert–Beer law, which establishes that light intensity decreases exponentially with depth and cell density in the medium, directly affecting the photosynthetic rate and productivity. For this reason, pilot-scale configurations have been developed, such as 150 L photobioreactors with microalgal consortia, which optimize light distribution and achieve nitrogen and phosphorus removal efficiencies above 90%, improving energy conversion and nutrient utilization (50–51). When scaling up to industrial levels, adequate facilities and large quantities of nutrients are required; therefore, wastewater pretreatment methods and strategies to optimize lipid and carbohydrate accumulation in these systems have been explored. Finally, techno-economic models have demonstrated the feasibility of producing bioethanol from microalgae in tropical contexts, consolidating their role as a sustainable and cost-effective solution for environmental treatment and bioproduct generation (50–53).

**Table 5.** Most cited documents EB4

Title	Author	# Citations
Microalgal biomass production from wastewater: treatment and costs: scale-up considerations	<a href="#">(50)</a>	215
Cultivation of Chlorella vulgaris in dairy wastewater pretreated with UV irradiation and sodium hypochlorite	<a href="#">(51)</a>	80
Techno-economic and sensitivity analysis of microalgae as a commercial raw material for bioethanol Production	<a href="#">(52)</a>	78
Sustainable microalgal biomass production from food industry wastewater for low-cost biorefinery products: a review	<a href="#">(53)</a>	60

Significant advances have been achieved in the cultivation of microalgae, particularly Chlorella vulgaris, for applications in wastewater treatment and biofuel production (see Table 6). Several studies highlight efficient systems such as photobioreactors, biofilms, and co-cultivation with bacteria, which achieve biomass productivities of up to 0.9 g/L·d and nutrient removal efficiencies above 85%. These improvements are attributed to the optimization of gas and nutrient transfer, as well as to metabolic symbiosis between microalgae and bacteria, which enhances the utilization of carbon, nitrogen, and phosphorus. The application of these technologies has increased profitability and lipid productivity by up to 50%, consolidating them as a key strategy for the development of sustainable biorefineries [\(54–57\)](#).

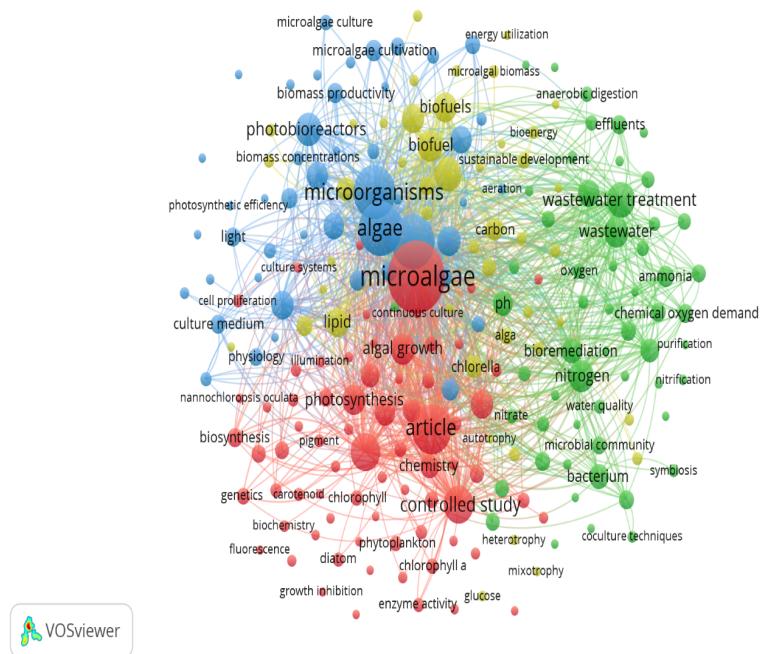
**Table 6.** Most cited documents EB5

Title	Author #Citations
Continuous cultivation of microalgae in aquaculture wastewater using a membrane photobioreactor for biomass production and nutrient removal.	<a href="#">(54)</a> 276
A novel algal biofilm membrane photobioreactor for the growth of attached microalgae and nutrient removal from secondary effluents.	<a href="#">(55)</a> 221
Rapid quantification of microalgal lipids in aqueous media using a simple colorimetric method	<a href="#">(56)</a> 127
Systematic investigation of biomass and lipid productivity by microalgae in photobioreactors for biodiesel application.	<a href="#">(56)</a> 119
Improving microalgal biomass productivity by engineering a microalgal-bacterial community	<a href="#">(57)</a> 98

Based on keyword interactions

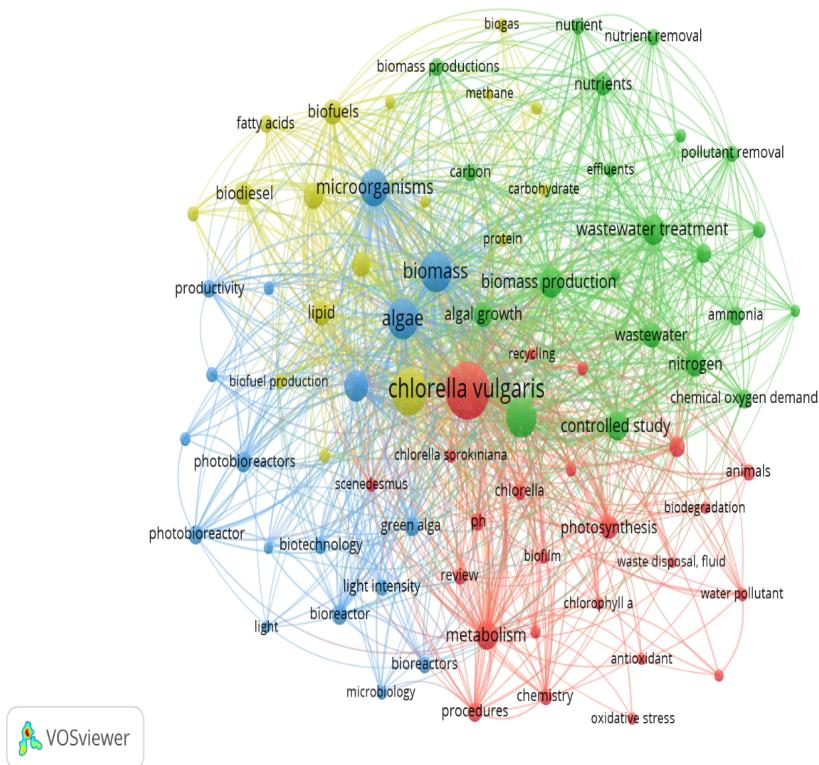
The co-occurrence analysis showed that this is a research topic with a broad spectrum of study areas and a growing trend in scientific production. To obtain more precise results aligned with the objectives of the study, minimum co-occurrence filters were applied to each of the search equations: a threshold of 25 was established for EB1, 20 for EB2 and EB3, and 15 for EB4 and EB5.

Figure 4 presents the classification of the clusters obtained from the co-occurrence analysis. Group I (red) generally address certain microalgae species, biochemical aspects, and some environmental cultivation parameters. Group II (green) includes keywords related to wastewater treatment, biological processes, and environmental variables. Group III (blue) relates keywords associated with cultivation systems, cultivation techniques, biological and industrial processes, and technical production aspects, such as photobioreactors and culture media for biomass production. Group IV (yellow) synthesizes keywords related to the energetic valorization of microalgae, particularly as feedstock for biofuel production and biorefineries.



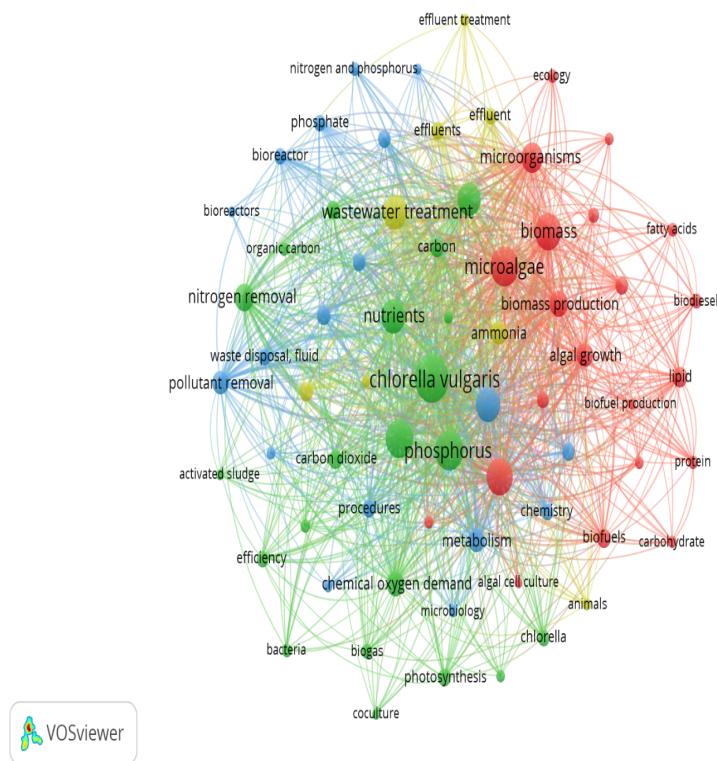
**Figure 4.** Publications co-occurrence network for: "Microalgae" AND "Culture AND Systems" (EB1).

In Figure 5, the classification of the clusters from the co-occurrence analysis is presented across different thematic areas according to research on *Chlorella vulgaris*. Group I (red) broadly address the combination of environmental and biochemical topics and practical applications in industry. Group II (green) focuses directly on wastewater treatment using *Chlorella vulgaris*, highlighting treatment processes and certain contaminants that serve as nutrients in the microalga's metabolism. Group III (blue) relates ecological and technological factors influencing algal biomass productivity, such as production technologies, photobioreactors, and cultivation conditions associated with biofuel production. Group IV (yellow) synthesizes keywords related to biofuel generation from the lipids and proteins of the biomass produced by *Chlorella vulgaris*.



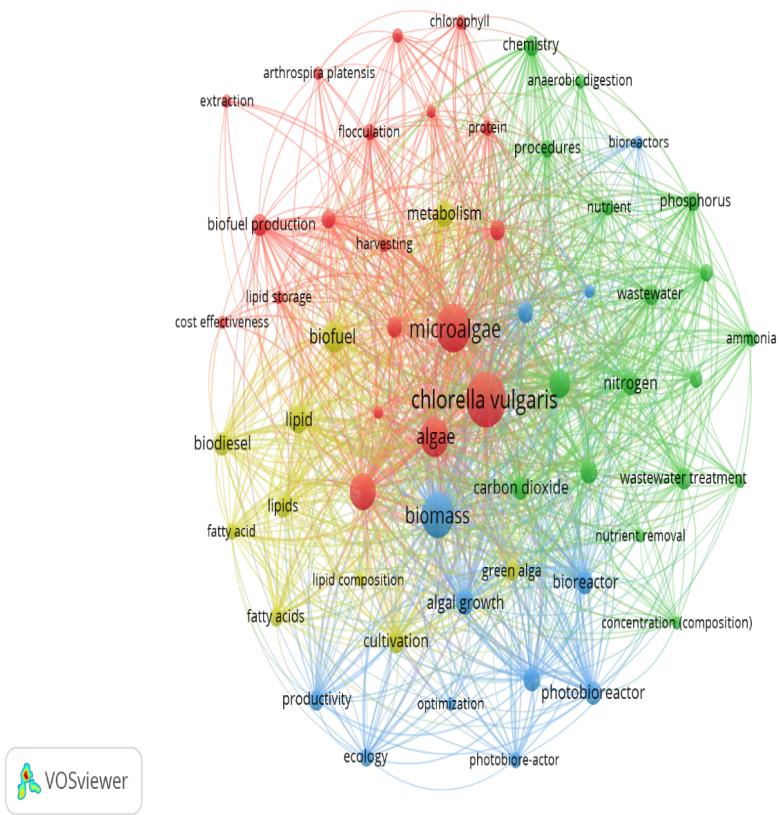
**Figure 5.** Publications co-occurrence network for; "Chlorella AND vulgaris" AND "Culture AND Systems" (EB2).

In Figure 6, the classification of the clusters from the co-occurrence analysis is presented, with a primary focus on environmental sciences, where contaminated effluents are used as culture media for microalgae. Group I (red) broadly address biomass and biofuel production from *Chlorella vulgaris*, covering aspects such as cultivation, growth, and productivity. Group II (green) includes keywords related to *Chlorella vulgaris* in wastewater treatment systems aimed at reducing organic loads while simultaneously producing biomass, with a strong ecological and biotechnological component. Group III (blue) relates keywords associated with biotechnological and microbiological processes applied to the removal of nutrients and contaminants in controlled systems (photobioreactors and bioreactors) for the standardization of cultivation conditions. Group IV (yellow) synthesizes keywords related to the removal of nitrogenous compounds, primarily ammonium.



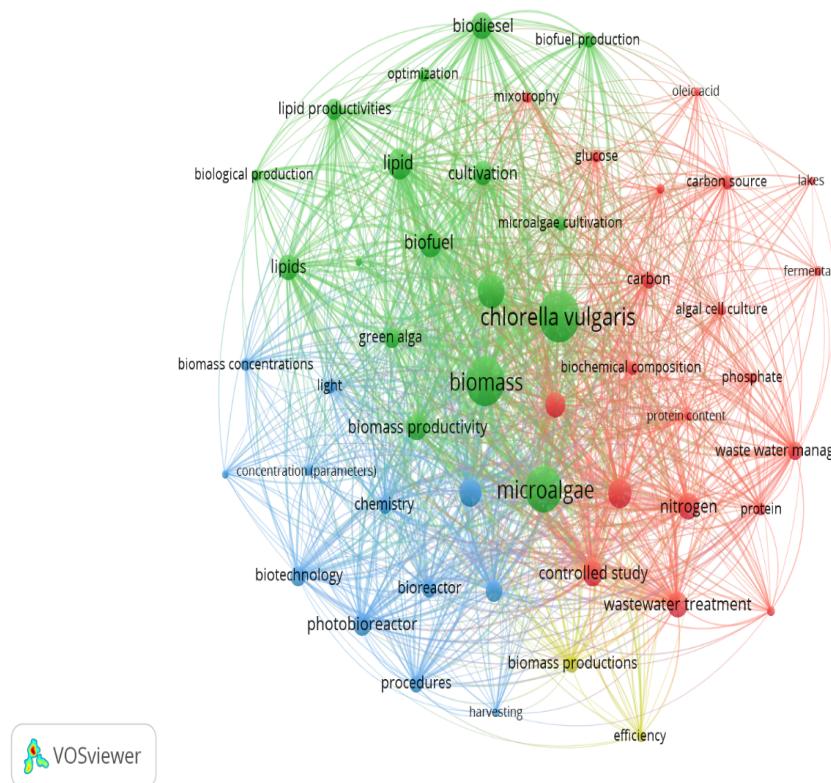
**Figure 6.** Publications co-occurrence network for; "Chlorella AND Vulgaris" AND "Nutrients OR Microelements OR Macroelements OR Phosforus" (EB3)

In Figure 7, the classification of the clusters from the co-occurrence analysis is presented, primarily focusing on cultivation scale-up. Group I (red) broadly address biofuel production, including process stages from harvesting and extraction to the storage of lipids and biomolecules, as well as efficiency and cost analyses. Group II (green) approaches biomass production from anaerobic processes, with an emphasis on remediation and the valorization of organic residues to reduce scale-up costs. Group III (blue) relates keywords associated with the optimization of microalgae cultivation systems in bioreactors, particularly the scaling of processes from laboratory to industrial plant. Group IV (yellow) focuses on biofuel production from a cellular perspective, emphasizing the accumulation of fatty acids during cultivation.



**Figure 7.** Publications co-occurrence network for; "Chlorella AND vulgaris" AND "Production Scale OR Large Scale OR Cultivation OR Industrial Scale OR Pilot Scale" (EB4)

In Figure 8, the classification of the clusters from the co-occurrence analysis is presented, primarily focusing on algal biomass productivity within cultivation systems. Group I (red) address algal biomass production under mixotrophic conditions in controlled environments, considering nutrients and varied cultivation settings. Group II (green) focuses on the production of Chlorella vulgaris through the optimization of cultivation, production processes, and yields. Group III (blue) highlights technical and biotechnological aspects of algal cultivation under controlled conditions, mainly using bioreactors with varied operating parameters, aimed at determining biomass productivity. Group IV (yellow) directly encompasses efficient biomass production, whether in terms of yields, costs, or resource utilization (light, carbon, or nutrients).



**Figure 8.** Publications co-occurrence network for “Chlorella AND vulgaris” AND “Biomass W/2 Production OR Biomass W/2 Productivity” (EB5)

## Conclusions

The bibliometric study evidenced a notable growth in research on microalgae, particularly *Chlorella vulgaris*, during the period 2014–2024, oriented toward technological and sustainable alternatives. The five applied search equations yielded a total of more than 4,354 documents, predominantly scientific articles. Specifically, search equation 1 recorded 2,465 documents, equation 2 yielded 600, equation 3 identified 481, equation 4 produced 366, and equation 5 yielded 442 documents. The proportion of articles ranged between 68% and 92%, with a smaller percentage corresponding to reviews and other publication types. These results reflect a thematic and methodological expansion in the field, positioning microalgae as a strategic resource in diverse sustainable industrial applications. Overall, the findings provide a clear overview of current trends in microalgal biotechnology and their relevance for future research.

According to the analysed publications, several important multidisciplinary fields are identified within the research area. Of the total documents, 25% belong to environmental sciences, highlighting nutrient recovery in the remediation of effluents from different industries. In addition, 14.4% of the publications are concentrated in agricultural and biological sciences, emphasizing the use of *Chlorella vulgaris* in animal supplementation, biofertilizers, and biomass production. Furthermore, chemical engineering (15.6%) and the energy sector (13.6%) reflect strong interest

in cultivation optimization, the development of value-added products, and biofuel production as innovative and sustainable alternatives to address current energy and environmental challenges.

Alternative nutrient sources for microalgae cultivation represent one of the main pillars for overcoming the barriers associated with production costs during scale-up. The use of wastewater as a nutrient source reduces approximately 50% of the costs associated with nutrients. In addition to its economic impact, this approach aligns with the principles of circular economy and mass balance, where the recovery of nitrogen ( $\text{NH}_4^+$ ) and phosphorus ( $\text{PO}_4^{3-}$ ) closes biogeochemical cycles, contributing to process sustainability. Under these conditions, an average lipid productivity of 25% (138.79 mg/L) is achieved, together with effective bioremediation of nitrogen between 80% and 90%, phosphorus between 75% and 90%, and a reduction in chemical oxygen demand (COD) of up to 100%, contributing to the prevention of eutrophication.

The choice of cultivation system directly influences the biochemical composition of *Chlorella vulgaris* biomass. In mixotrophic systems, the simultaneous utilization of  $\text{CO}_2$  and organic carbon sources allows greater lipid accumulation (19–56%), resulting from excess available carbon and the diversion of metabolism toward fatty acid synthesis, making these systems suitable for biodiesel production. Under autotrophic conditions, the exclusive fixation of inorganic carbon under light favors protein synthesis (38–48%) by prioritizing photosynthetic anabolic pathways, although with a risk of contamination in open systems. In contrast, heterotrophic cultures, based on the assimilation of organic carbon under controlled light conditions, promote the formation of pigments, bio compounds, and structural polysaccharides, constituting an effective biotechnological alternative for the production of high value-added metabolites.

#### CrediT authorship contribution statement

**Conceptualization - Ideas:** JSebastian Guaza Lasso, Jorge Luis Sánchez Ortega. **Data curation:** Sebastian Guaza Lasso. **Formal analysis:** Sebastian Guaza Lasso. **Investigation:** Sebastian Guaza Lasso. **Methodology:** Jorge Luis Sánchez Ortega. **Project Management:** Jorge Luis Sánchez Ortega. **Resources:** Jorge Luis Sánchez Ortega. **Software:** Sebastian Guaza Lasso, Jorge Luis Sánchez Ortega. **Supervision:** Jorge Luis Sánchez Ortega. **Validation:** Sebastian Guaza Lasso. **Writing - original draft - Preparation:** Sebastian Guaza Lasso. **Writing - revision and editing -Preparation:** Sebastian Guaza Lasso, Jorge Luis Sánchez Ortega.

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