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Microalgae in the Mediterranean area: A geographical survey outlining the diversity and technological potential

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ABSTRACT

Microalgal diversity enables the possibility to employ them in technological applications, as widely shown by the modern literature. While there exists an extensive body of literature concerning the technological applications of microalgae, the scientific knowledge of microalgal species remains relatively limited. Therefore, there is still potential for unlocking new opportunities through the study of the microalgal biodiversity, particularly in the Mediterranean region, which is unique because of its sub regional diversity. While some studies have assessed microalgae distribution in the Mediterranean area, and others have focused on specific aspects of their technical exploitation, this review seeks to offer a comprehensive overview of isolated microalgal species and their technological applications. Microalgae from the Mediterranean area share common characteristics, such as low half-saturation constants and acclimation to high light intensity, making them ideal for specific technological applications. While the search for new microalgae for technological purposes can help in biodiversity conservation, numerous species still remain underexplored, offering potential for innovative applications. However, the key finding from the critical analysis of the literature is that the diversity of microalgae in the Mediterranean region is its true richness, allowing for their versatile applications across various processes. The work focuses on the Mediterranean area, i.e., having coastlines along the Mediterranean Sea and on aquatic microalgae, coming from water with different salinity levels. This review offers an intrinsic ecological and technological perspective and provides a fresh outlook on the microalgal sector, promoting its expansion in the Mediterranean area and the development of sustainable bio-industries.

1. Introduction

Microalgae constitute a large group of microorganisms within the phytoplankton category and are characterized by a notable biodiversity resulting from the number of species encompassed within this group. Most studied microalgae are unicellular and grow in aquatic environments all around the planet, including in the most extreme conditions, comprising hundreds of thousands of different species [1]. These microorganisms are also characterized by their impressive degree of adaptability to their environments, enabling them to survive in a wide variety of situations and conditions. Originating from primary, secondary, and sometimes tertiary endosymbiosis events, microalgae possess characteristics typical of photosynthetic eukaryotes and prokaryotes, granting them unique and highly adaptable traits and abilities. For example, they may grow in a very large salinity range, from fresh waters with very low salt concentrations, to salt saturation. The concept of species is not well-defined when talking about microalgae. Several criteria may be used to define microalgal diversity, such as morphology, physiological differences and reproductive barriers. However, small subunit (SSU) ribosomal RNA (16S/18S rRNA) homology is the most used criterion nowadays [2]. Despite the employed criterion, a very low number of microalgae species is nowadays scientifically known and studied (about 44,000), and a small fraction of them (about 3000) are kept in culture for laboratory research [3]. Following the Mata et al. definition [4], microalgae may fall in two large family of organisms with different cellular structure: the prokaryotic and the eukaryotic. The formers are represented, above all, by the class of *Cyanophyceae*, and the latter by the classes of *Chlorophyceae* and *Bacilariophyceae* (in particular

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diatoms). About the latter classes, it is possible to state that are the most important from a technological point of view [5]. From a biochemical perspective, algae contain all the major groups of macromolecules exhibiting remarkable diversity. They contain carbohydrates, such as starch or starch-like products [6]; lipids, occurring in a variety of different compounds, with fatty acid composition varying among microalgae species and environmental conditions [7], and proteins. As well known, microalgae respond to environmental stress by increasing lipid production [8,9]. Some algae are a good source of high-nutritional value proteins and may reach a protein content of 40 to 70 % of the dry weight; algal genera such as *Chlorella* or *Tetraselmis* have a percentage of 50 % on average [10]. Moreover, algae possess photosynthetic pigments, including chlorophylls, carotenoids and phycobilins. All these molecules are of interest from a technological perspective as they have market value and microalgae cultivation is often aimed at producing them.

Besides oxygen producers, microalgae represent the bottom of the animal food chain and are the principal source of carotenoids for animals [11–13]. However, they also represent a good resource to produce biomaterials, biofuels, anti-inflammatory compounds, food supplements and various biochemical and consequently, in the last decades, they attracted the interest of many research groups for a multitude of applications. Wastewater treatment is another attractive microalgal application since their ability to assimilate nutrients such as NO_3^-, PO_4^{3-}, NH_4^+ and heavy metals [14,15], even present in urban wastewaters, as well as industrial wastes. For these wide application fields, nowadays, industrial plants for microalgae culturing have been opening worldwide [16].

Microalgae are a hot topic, as evidenced by the sharp rise in the number of publications in recent years, achieving 106,906 Scopus-indexed works about microalgae or cyanobacteria by 2023.

The present study provides a critical perspective on the microalgal applications in the Mediterranean Sea basin. The Mediterranean Sea is a unique environment characterized by several distinct features: it is a quasi-enclosed sea, oligotrophic with a widespread phosphorous deficit, and experiences irradiance levels 20 % greater than those of similar latitudes in the Atlantic Ocean. Additionally, it boasts a very high diversity of habitats, resulting in high species richness but low abundance [17]. Apart from ecological reasons, the diversity of the Mediterranean can be attributed to its historical significance, with a tradition of study longer than almost any other sea, and to its paleogeographic context. The geological history over the last 5 million years has led to distinct biogeographic categories, further contributing to its unique biodiversity [18]. These factors create the ideal conditions for the proliferation of various species of microalgae and cyanobacteria.

However, Mediterranean biodiversity is currently threatened by the combined pressure of both global change and human impact and despite recent efforts to protect it, further actions are needed. Microalgae already have a role in the protection of biodiversity, as they are currently employed as bioindicators to assess the health of the environment. These microorganisms, in fact, have been reported as potentially useful in monitoring the quality of water bodies, with several advantages as they are a potentially viable economic alternative to conventional sophisticated methods [19].

In our perspective, furthermore, the search for new microalgae aimed at technological applications may contribute to biodiversity conservation efforts, as reported in Fig. 1.

Microalgae adapted to the Mediterranean region exhibit intrinsically adaptive characteristics suited to their environment, reflecting the diverse conditions therein and showing a broad spectrum of taxonomic diversity. Consequently, the technological applications of such microalgae are expected to be equally diverse.

The aim of this review is to critically explore the microalgal biodiversity of the Mediterranean area. The work focuses on the Mediterranean area, i.e., having coastlines along the Mediterranean Sea and on aquatic microalgae, coming from water with different salinity levels. We aim also to study the Mediterranean species mentioned in the literature along with their technological applications, considering the processes

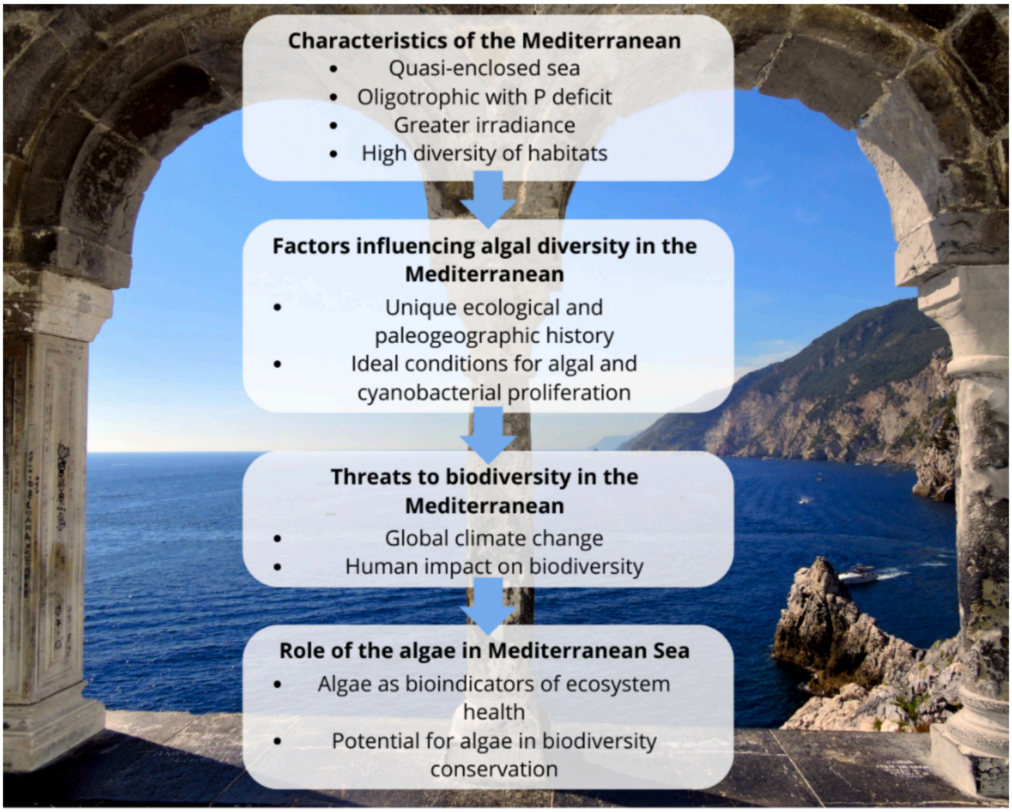

Fig. 1. Role of microalgae in Mediterranean Marine ecosystem.

sustainability as well, in order to highlight potential application gaps. To facilitate the reading, a reader's guide is provided in Fig. 2.

Firstly, a summary of the technological processes involving microalgae is provided, emphasizing tools for economic and environmental assessments. Subsequently, we have examined the ecological characteristics of Mediterranean microalgae and the applications that best suit them, along with an analysis of the isolated microalgae from Mediterranean area with technological applications. In the final section, a bibliometric analysis of microalgal research in Mediterranean countries is provided, together with the diverse technological applications across different regions. With its innovative character given by its intrinsic ecological and technological perspective, this review offers a new point of view on the microalgal sector, promoting its growth in the Mediterranean area.

2. Microalgal bioprocessing routes

Microalgae-based engineering processes can be categorized into two main sections: upstream processes, mainly involving the cultivation of microalgae, and downstream processes, focusing on the processing of biomass to obtain the final product. The upstream section of microalgae-based engineering is focused on cultivation strategies that optimize the growth and productivity of live microalgae biomass. The main efforts are directed towards cultivation techniques, growth medium composition, and bioreactor design to achieve high biomass yields and desired characteristics. Once the biomass has been harvested, the downstream section involves extracting and refining valuable products. This stage aims to isolate specific compounds, such as lipids, pigments, or bioactive molecules. In this case, the key challenge is the separation and purification methods employed to obtain high-quality products. The final product, biomass or extracted compounds, finds applications in various sectors including food, feed, health, and cosmetics. The price of microalgae biomass in these applications varies from 5 to 500 €/kg, with a market size reaching up to 100 kt/year. Furthermore, emerging applications such as biofuels, biofertilizers, wastewater treatment, and chemicals have significantly lower biomass prices (<5 €/kg) but boast substantial market sizes [20]. To better understand the different process steps involved in microalgae biomass production, it would be beneficial to first focus on the upstream processes followed by the downstream processes as reported in Fig. 3.

2.1. Upstream processes

As depicted in Fig. 3, several parameters must be defined before cultivation starts, depending on the intended application, including the selection of the microalgal strain, the choice of reactor and operational mode, and the composition of the culture medium. Genetic engineering may be considered to optimize strains for specific applications, and it is also common to work with consortia microalgae-microalgae or bacteria-microalgae, e.g. in wastewater treatment.

The choice of the photobioreactors is also of fundamental importance: photobioreactors for microalgae cultivation are broadly categorized into open-air systems and closed systems [21] where the latter are typically used for axenic cultures. Tubular photobioreactors and open raceway ponds, typically mixed by paddle wheels with water depths of 15–20 cm [22,23], are the most used among the two reactor classes, especially for their suitability in large-scale applications. Closed photobioreactors offer several advantages, including the regulation and control of crucial biotechnological parameters. Their fundamental benefits include a decreased risk of contamination, prevention of CO_2 losses, the ability to maintain reproducible cultivation conditions, control over hydrodynamics and temperature, and a flexible technical design [22,24,25]. Among closed photobioreactors, the main categories are stirred tank reactors, tubular configurations, airlifts, bubble columns, flat panels [26–28].

It is also necessary to choose if working in via autotrophic, heterotrophic or mixotrophic nutritional metabolism mode. Autotrophic microalgae synthesize organic molecules from inorganic substances, primarily utilizing light through photosynthesis. In contrast, heterotrophic microorganisms derive energy from external organic compounds d between the previous metabolic states.

The homogeneity of nutrient concentration, salinity, temperature, pH has a key role in microalgae growth and may be controlled by tuning the hydrodynamics of the system. Among various factors, the

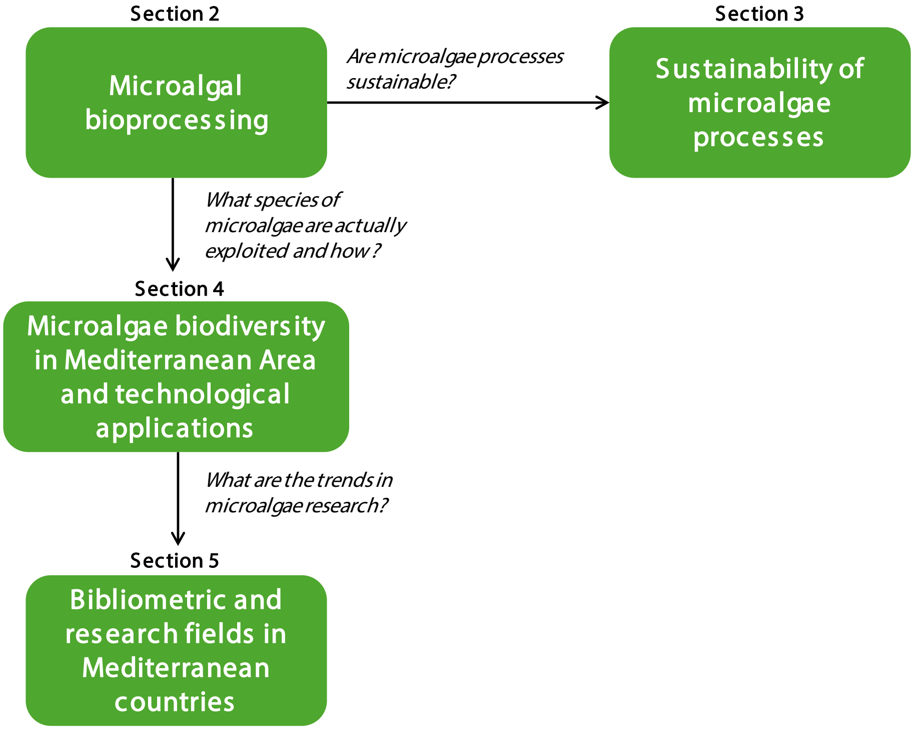

Fig. 2. Reader's guidelines for the review.

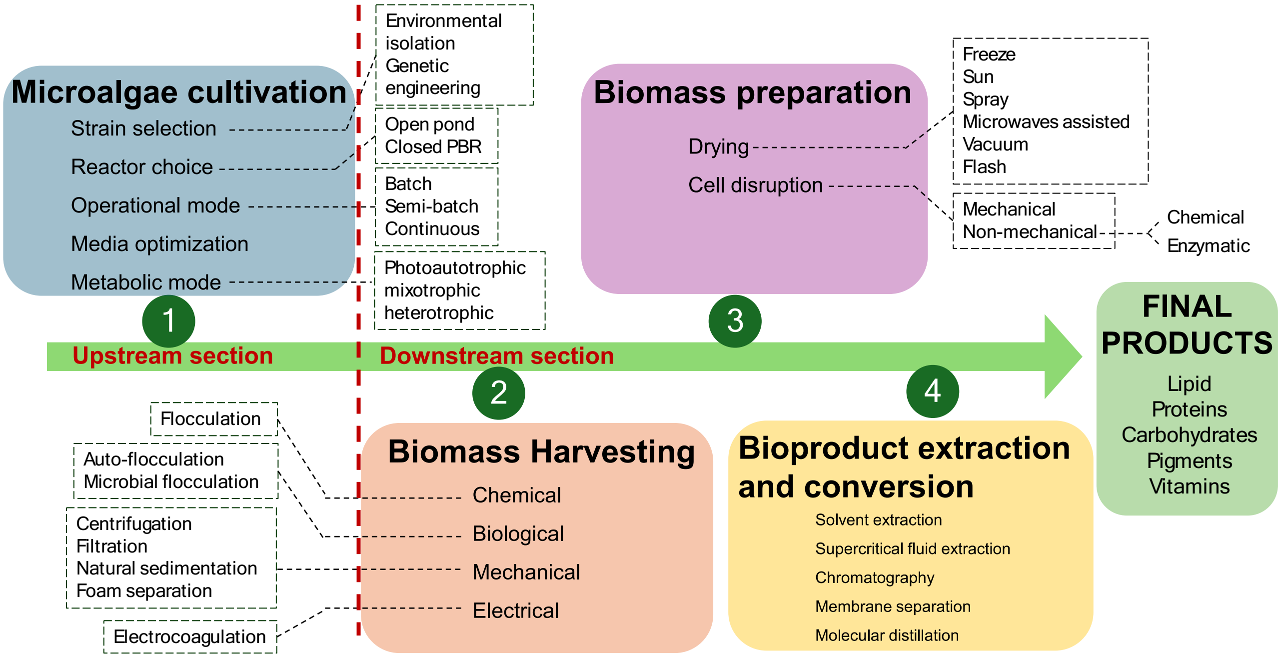

Fig. 3. The main steps of the processes related to the extraction of bioproducts from microalgae and subdivision in the upstream section and downstream section.

distribution of light and its spectrum is considered the most critical [26] considering that the efficiency of photosynthesis is directly connected to the amount of light absorbed by microalgae [29].

The growth temperature also significantly impacts microalgae growth, as it influences enzyme activity and can lead to alterations in composition, particularly in lipids [30]. Temperature control is crucial for evaluating the feasibility of large-scale algae production, with no photosynthetic microorganisms identified with an optimal growth temperature above 75 °C [31,32].

2.2. Downstream processes

Downstream processes focus on extracting valuable products from microalgal biomass. Various technologies and process chains can be designed focusing on the desired final products. As depicted in Fig. 3, the downstream section involves biomass harvesting, biomass preparation (drying and cell disruption), and bioproduct extraction and conversion.

For what concerns biomass harvesting, an efficient recovery of microalgal biomass is crucial for large-scale production of bioproducts. Harvesting operation can be continuous (described by a dilution rate, for a chemostat operation) or discontinuous (in a batch growth system) [33]. Because of its low density in cultures, the biomass harvesting and dewatering is a complex and often expensive operation [34], being the main bottleneck of the entire process. Selecting an optimal harvesting method involves considerations on the microalgal cell characteristics (size, charge, and morphology) and equipment costs. Various methodologies, including chemical (primarily flocculation), biological (auto-flocculation and microbial flocculation), mechanical (centrifugation, filtration, natural sedimentation, flotation, and foam separation), and electrical (electrocoagulation) methods, can be employed [35,36].

Biomass drying represents an optional but essential step in the chain of production of bioproducts from microalgae and is sometimes considered to be an economic bottleneck because of its high energy demand [37,38]. The heat transfer and water diffusion are key mechanisms that must be optimized in order to reduce the water content in the harvested microalgae [39]. Freeze drying, sun drying, and spray drying are the most common drying processes [37].

To make the biocompounds available for extraction, cell disruption is often required to break down the rigid and resistant cell wall. Several methods were proposed for cell disruption, divided into mechanical and physical methods and non-mechanical methods. In the first category, for example, there is high-speed homogenization, pulsed electric fields (PEF), and microwave irradiation [40]. Among the physical cell disruption methods [65–67], the thermal methods, such as the thermolysis, autoclaving, and steam explosion [65–67], are characterized by their simplicity. These processes are however characterized by low efficiency and high energy consumption. Furthermore, the generation of substantial amounts of undesirable cell debris and the thermal resistance of the target product to be extracted limits their application field [40]. Non-mechanical methods include chemical methods, enzymatic techniques and osmotic shock [40–45].

Various extraction techniques, either applied directly to the cell or combined with cell disruption methods, can be employed to assist the recovery of the bioproducts. Extraction with solvents can exploit organic solvents, ionic liquids, deep eutectic solvents, supercritical fluids and other kinds of solvents. Organic solvents are extensively utilized for extracting biomolecules from microalgae, often chosen based on the cell disruption method [46]. Traditional methods such as the Folch [47], Bligh and Dyer [48], and Soxhlet techniques [49] are commonly employed for lipid extraction, albeit with varying efficiency and environmental concerns [50]. To address these issues, there has been a shift towards utilizing green solvents, including supercritical carbon dioxide, ionic liquids, deep eutectic solvents, switchable water, and biosolvents [51,52].

The latter, derived from natural sources, provide eco-friendly alternatives to fossil resources [53], classified as green solvents due to their environmental friendliness and harmlessness to humans [50,54–56].

2.3. Wastewater treatment

An emerging new alternative for growing microalgae involves the use of wastewaters as growth medium, bioremediating the effluent and, simultaneously, producing biomass.

After the traditional treatment process, very often, the streams do not meet the criteria for the discharge due to the high concentration of nitrogen and phosphorous, and they are recycled in the plant for further treatments, increasing the operational costs [57–59]. As shown in Fig. 4, introducing a microalgae-based system as a tertiary step in wastewater

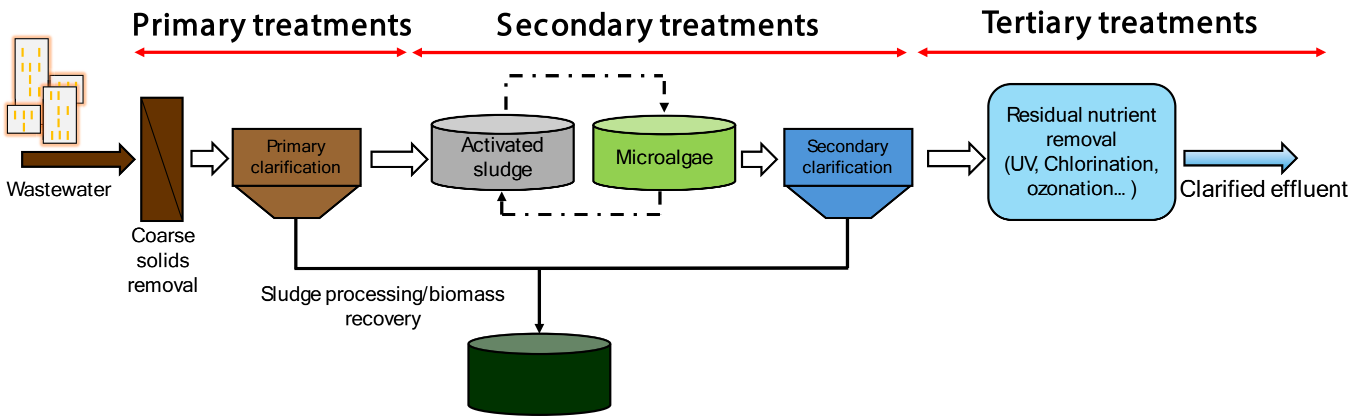

Fig. 4. Flow of the traditional wastewater treatment crafts.

treatment plants is considered an economically and environmentally friendly alternative for nutrient removal from wastewater [59–61]. Furthermore, microalgae exhibit the ability to eliminate micropollutants like heavy metals and persistent organic contaminants, including chlorinated hydrocarbons, dyes from textile industries, and herbicides, [62–65]. Aside from the phytoremediation benefits, utilizing microalgae for wastewater treatment leads to the generation of algal biomass with applications in energy, nutraceuticals, agriculture, and animal feed sectors [66,67]. This dual-purpose approach enhances the economic viability of both processes, decreasing the treatment costs [68]. The selection of microalgal strains represents a key factor for the development of efficient nutrient abatement systems and often, microalgal strains naturally grown in wastewaters are isolated and cultivated for this purpose, because of their high adaptability, resilience to fluctuations in composition and tolerance to other microorganisms [59,69,70].

Microalgae-bacteria consortia were also proposed as a promising and economical solution for wastewater treatment [71], since the photosynthetic activity, operated by microalgae, lead to a self-oxygenation of the culture, eliminating or significantly reducing the need for mechanical aeration [72]. Simultaneously, the CO_2 generated from heterotrophic metabolisms serves as a carbon source for microalgae and nitrifying bacteria [73,74].

3. Sustainability of microalgal bioindustries

Despite the large number of studies and efforts to optimize the various process, as see in Section 3, the commercialisation of microalgal biomass is still limited by several bottlenecks [75], including crucial economic [76] and environmental [77] obstacles. Bio-industries are characterized by uncertainty in the marketability (i.e., profitability) and the generation of potential environmental burdens (impacts on ecosystems, human health, and climate).

3.1. *Main tools for economic and environmental assessments*

The primary tools for the evaluation of the environmental and economic dimensions of sustainability are the life cycle assessment (LCA) and the techno-economic analysis (TEA), respectively. They can facilitate the design and decision-making actions in the implementation of sustainable solutions.

The LCA methodology, in particular, is recognized as a standard reference tool to define the environmental profile of goods and services by evaluating the environmental burdens in terms of resource consumption, waste generation, and environmental impacts related to the inputs and outputs of materials and energy involved in the full life cycle of the product. The LCA implementation is internationally standardised by the framework of the ISO 14040:2006 and 14044:2006 norms [78,79]. According to them, the LCA encompasses four stages of analysis, briefly described in the following. (1) In the goal and scope definition, the aims of the LCA study are defined, and the main methodological features are chosen (e.g., functional unit and system boundary). (2) The life cycle inventory (LCI) analysis includes the collection, calculation, and analysis of input /output flows of materials and energy to/from the product system, typically with the help of a flow diagram with several unit processes. The foreground inventory is built by gathered data describing the system under study, while the background inventory is built through international industrial data (the Ecoinvent database is the most used). (3) In the life cycle impact assessment (LCIA) phase, different methods (e.g., ReCiPe, CML, and Cumulative Energy Demand) can be used to classify flows into impact categories and characterize (i.e., calculate) the environmental impacts. Optionally, the results can be normalized to a reference unit, grouped into one or more sets and weighted to reflect the relative importance of the impact categories. (4) The interpretation step summarizes and discusses LCI and LCIA results, drawing conclusions and recommendations.

The TEA methodology is crucial to assess the economic performance and (potential) feasibility of a project or technology throughout its lifetime. TEAs can draw baselines, characterize and compare different scenarios, identify process bottlenecks, guide system operation and design, assist process scaleup, and drive research and development towards profitable investments [80]. There is not a standard methodology for TEA. However, the steps of a complete TEA can be represented similarly to those of LCA [81]. (1) The goal and scope definition phase identifies the aim of the TEA study and the system analyzed, as well as the criteria (parameters like profitability) and indicators (measures of criteria like net present value) of the economic evaluation. (2) The inventory phase comprises the collection of technical data, such as energy and material flows and balances, conversion factors, and operating conditions, and of economic data, which define the price of flows [82]. (3) In the economic assessment step, calculation methods are selected and calculations are executed. Model equations provide economic indicators for the production cost through capital expenditure (CapEx) and operating expenditure (OpEx) [82]. By estimating revenues and selecting an adequate discount rate, profitability indicators (such as Net Present Value, Return On Investment, and Minimum Selling Price) can be calculated. An example of an estimation of the various cost items and economic indicators for microalgal biorefineries is reported in ref. [83]. (4) The interpretation step is conducted in parallel to all TEA phases, checking the quality, consistency, completeness, and reliability of model inputs (inventory) and outputs (economic indicators). Based on the indicators, the project economics is judged in a final evaluation.

3.2. *State-of-the-art and perspective*

In the last decade, hundreds of studies have assessed the economic or environmental sustainability of microalgae-based applications. To provide an overview of the current status and prospects, Table 1 summarizes the highlights of recent review articles, including some information related to economic and/or environmental issues. Overall, Table 1 reveals that data on biomass cultivation are scattered over several orders of magnitude (e.g., production cost from ~1 to 100 $/kg, and greenhouse gas (GHG) emissions from ~0.1 to 1000 kg $CO_{2,eq}$/kg biomass), depending on several features such as algal species, bioreactor configuration, climatic conditions, and dewatering technique. There is generally a consensus on the fact that microalgal biofuels are uncompetitive compared to conventional fuels. Similarly, microalgal technologies are not mature enough for industrialization to produce biochemicals. However, their high market value can push the research towards rapid developments.

Energy requirements and the use of chemical fertilizers are critical issues. On the other hand, the deployment of renewable energy and waste nutrient sources has been demonstrated as a valid approach to significantly improve the sustainability of microalgal systems. Indeed, incorporating valorization strategies of bioremediation for wastewater treatment and carbon capture from flue gas can yield economic and environmental benefits. Multi-product biorefineries with the zero-waste approach could be effective in implementing profitable production systems with mitigated environmental burdens. However, the scale-up from lab- or pilot-scale studies, which is essential to demonstrate the feasibility of field applications, requires more research efforts. Beyond that, methodological heterogeneities and different model assumptions encountered in the literature led to significantly different outcomes. Therefore, homogenization within a common framework is a key point for future assessments.

In a more holistic approach, TEA and LCA tools can be combined to conduct multi-criteria optimization studies based on suitable modelling approaches, such as superstructure methods [84]. The complexity of the problem should not be overlooked, especially considering that TEA and LCA assessments, as well as other process system engineering tools, are based on multi-scale spatial and temporal input data [85]. Useful insights on the sustainability of microalgal processes can be drawn from exergoeconomic and exergoenvironmental assessments [86]. From a broader perspective, the social dimension of sustainability of microalgae-based products should be evaluated, including the evaluation of social impacts [84] and consumers' acceptability [87–89], which can act as either drivers or barriers. In this regard, SWOT (Strengths-Weaknesses-Opportunities-Threats) analysis can evaluate the current status and devise a successful strategy for methodological tools including data and/or some discussion (among others) on economic and/or environmental aspects (TEA and/or LCA in most cases) of microalgae applications.

Table 1
Overview of some recent review articles, including data and/or some discussion (among others) on economic and/or environmental aspects (TEA and/or LCA in most cases) of microalgae applications.

Highlights and main data	Reference
• The conversion of microalgae biomass into high-value biochemicals was characterized by high costs for cultivation in the range from 0.5 to 6 $/kg, with the expenditure dominated by infrastructure, maintenance, and labor. • ORP systems were more economical than closed PBR configurations. However, closed bioreactors offered more controllability with enhanced bioactive compound content (e.g., astaxanthin in *H. pluvialis* was at 1.5–3 % from ORP, while it was over 4 % from PBRs. • Astaxanthin extraction was a considerably costly and inefficient process (7000 $/kg vs. 1000 $/kg in chemical synthesis). • Multi-product schemes in the more general biorefinery approach could significantly enhance the economic viability. For example, the payback period of 6.38 years in protein production was reduced to 2.62 years when considering 3 products (protein, fatty acids, and pigments). • Other figures of merit: Return on Investment (ROI) of 38.22 % for the abovementioned multi-product process; ROI and payback time of 1.87 % and 11 years, respectively, in another TEA of protein production; price of *β*-carotene of either 1370 or 920 €/kg.	[90]
• Green methods (supercritical fluid, pressurized liquid, ultrasound, microwave, pulsed electric field) for the extraction of metabolites (phycocyanin, carotenoids…) from microalgae could produce lower environmental impacts compared to conventional technologies. • LCIA results on GHG emissions for different FUs included 1.86 t $CO_{2,eq}$/800 g astaxanthin, 0.33 kg $CO_{2,eq}$/MJ biodiesel, 0.6 kg $CO_{2,eq}$/kg biodiesel, ~500 kg $CO_{2,eq}$/kg β-carotene, ~1000–2000 kg $CO_{2,eq}$/kg phycocyanin (ultrasound extraction), 9.09 kg $CO_{2,eq}$/kg *Schizochytrium* oil, ~140 kg $CO_{2,eq}$/kg phycocyanin (pulsed electric field extraction). • Energy demand was ~36 % and 56 % for cultivation and lipid extraction, respectively. Pure CO_2 usage increased GHG emissions and energy requirements by 25–30 % compared with waste CO_2 utilization. • Data from laboratory-scale studies introduce inaccuracies in the LCA results, but data from large-scale plants were insufficient.	[91]
• The cost of lipid extraction from microalgae was still too high, accounting for up to 50 % of the total cost of biodiesel. The minimum sale price of microalgal biocrude and biodiesel was 4.85 $/l and 5.57 $/l, respectively, which were uncompetitive while the diesel cost (1.59 $/l). • The main operating cost of extraction methods using solvents was due to chemicals. Hundreds of kilograms of solvents were required for the extraction of 1 kg lipids, involving a cost of ~1000 $/kg lipid or much more in the case of green solvents. The cost associated with energy consumption for lipid extraction was between ~10–40 $/kg, with the lowest value exhibited by supercritical CO_2 extraction.	[92]
• The cost of microalgae bio-oil fluctuated in a wide range, e.g., 0.44–8.76 $/l, thus being not competitive against that of conventional fuels. • Only a small number of pilot projects have been realized, while several obstacles, resulting in high operation and capital costs, still hinder the scaling up and commercialization of microalgal biofuels. • Compared with conventional fuel crops, microalgae grown in wastewater could improve the sustainability of biofuel production. Cultivation in wastewater could save up to 90 % of freshwater and minimize the need for chemical nutrients. • Future technological advances have the potential to lower fossil fuel consumption and overall carbon footprints.	[93]
• Microalgal biofilm achieved higher biomass yield and productivity compared to conventional suspended cultivations. The energy requirement for harvesting was reduced by up to 83 % compared with that of suspended biomass centrifugation. • The biofilm-based algae cultivation was not practised on a large/commercial scale due to several technical limitations. A preliminary TEA was conducted by using small-scale outdoor data, showing that the biomass production costs by biofilm-based cultivation were 8–10 times lower (e.g., ~1 vs. 10 $/kg) than that of suspension-based cultivation, due to benefits from higher biomass productivity, lower water and power consumption and labor cost.	[94]
• Scientific research is driving the microalgae industrialization. However, techno-economic limitations related to the high cost of	[89]

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4. Exploring microalgal diversity and applications in the Mediterranean region

The Mediterranean Sea is a large marine ecosystem with unique characteristics as it is a hot-spot of biodiversity, being significantly impacted by resource exploitation, maritime traffic, and coastal urbanization, driven by a high-density population. Furthermore, subregional seas and coastal areas display high variability due to their hydrographical and climatological regimes [101]. Considering that, according to the Baas-Becking view of microbiology, "everything is everywhere, but environment selects" [102], the diverse geographical features found in the Mediterranean region are mirrored in the multitude of distinct species that have been identified in this area.

Allocating microalgal species to a specific environment is challenging, as there is still debate over whether microalgae are cosmopolitan or endemic [2]. There are two perspectives regarding microorganisms in general, which also apply to microalgae. The first establishes they are generally ubiquitous, meaning they may be found everywhere. The other claims that they are endemic to certain areas and environments. Consequently, identifying the indicative species of an

Table 1 (*continued*)

Highlights and main data	Reference
cultivation facilities, nutrient supply, and licenses to market microalgae-related products are providing grand challenges. Biomass production costs of closed PBRs and open ponds of 12.4 and 1.6 \$/kg, respectively, were reported, with this gap being caused by the significant difference in the facility investment (2000 \$/m^3 for PBRs vs. 50 \$/m^3 for ORPs). • Biorefining strategy could maximize the economic value of microalgae biomass and offset the production cost of various microalgae products. The co-production of biodiesel and high-protein food led to a cost of 0.43 \$/kg food with an additional revenue of 0.97 \$/kg food. By changing the order of biorefining and isolating the protein before the lipid extraction brought a revenue of 6.23 \$/kg protein. In contrast, the single-purpose production of algal protein had a cost higher than 10 \$/kg protein. • The valorization of industrial waste CO_2 produced a cost of 0.20 \$/kg biomass for low-value biofuel. • GHG emissions estimated by different LCA studies covered several orders of magnitude (up to almost 1000 kg $CO_{2,eq}$/kg biomass) and were affected by various factors, including the cultivation technologies, process conditions, culture scales, process inputs (flue gas and nutrients), harvesting methods, and algae strains. • Energy consumption varied between 4 and 800 MJ/kg biomass, and fossil-based electricity use was identified as the major contributor to the global warming impact category. • The use of renewable energy and waste nutrient sources (wastewater, waste gas, or food waste) was a valid approach to significantly mitigate the environmental burdens and even achieve negative carbon emissions.	
• Several TEAs were reviewed, showing great variability in the production cost of microalgal biofuels, which was compared with the commercial price: 0.77 or 0.8–3.5 \$/l vs. 1.15 \$/l for biodiesel, 19.45 or 1.3 \$/gal vs. 2.72 \$/gal for bioethanol, 0.57–13.53 \$/kg vs. 2–8 \$/kg for biohydrogen, 0.55 or 0.3 \$/m^3 vs. 0.25–2.7 \$/m^3 biomethane, 2.2 or 0.7 \$/l vs. 0.48–0.53 \$/l for biocrude, 1.48–1.8 or 0.58 \$/l vs. 0.71 \$/l for pyrolysis oil, 5.89 or 8.45 \$/l vs. 0.9 \$/l for biojet fuel. • The incidence of CapEx on the total production cost was between 57 % and 84 % for most biofuels, while it reduced to 42 % for biocrude and 30 % for bioethanol. • To obtain realistic outcomes from TEAs, the need for data collection at a large scale (e.g., pre-commercial scale) was claimed. • Values of net energy ratio (NER, defined as total energy produced over processing energy consumed) < 1 were reported for microalgal bioethanol, biodiesel, biomethane, and biocrude. A value of ~1.3 was also reported for biocrude oil produced from a large-scale HTL system and biohydrogen production with supercritical water, while the highest NER of ~2.2 was achieved by bio-oil production via pyrolysis. • GHG emissions in the range ~1–7.5 kg $CO_{2,eq}$/kg biofuel were reported, with the lowest and highest values for bioethanol and biohydrogen, respectively. • The values of NER and GHG emissions could promote HTL and pyrolysis as potentially sustainable technologies. The overall practical feasibility of microalgal biofuels will be determined by strain selection, harvesting techniques optimization, and wet biomass processing development.	[95]
• In wastewater treatment, HRAPs could reduce costs and environmental impacts compared to traditional systems based on activated sludge (0.18 €/m^3 vs. 0.26 €/m^3, global warming of 0.146 vs. 0.458 kg $CO_{2,eq}$/m^3, and eutrophication of 126×10^{-6} vs. 158×10^{-6} kg $PO_{4,eq}^{3-}$/m^3). Moreover, HRAPs required only 22 % of the electricity demand of activated sludge. However, the net environmental benefit of an activated sludge-based sequencing batch reactor was slightly larger than HRAPs because of the removal rate of nutrients. • Co-pyrolysis of sewage sludge and wastewater-grown microalgae for biofuel production produced the largest net profit (9 % higher than sewage sludge) when considering a 1:1 mixture. However, the sewage sludge alone scenario had better environmental performance. The high moisture content resulted in a drying step being the most energy-intensive operation (69–88 % of total used energy). • The treatment of food-processing wastewater with unialgal culture had lesser environmental burdens than the treatment with mixed cultures. Bioproduct recovery from microalgae wastewater treatment systems can minimize environmental impacts by up to five times compared to a conventional system using a standard growth medium. • Ideas proposed to overcome present economic challenges included the addition of organic substrates, the adoption of microalgae and bacteria consortia, and the development of low-cost pre- and post-treatments.	[96]
• Bioplastics could reduce GHG emissions thanks to the use of raw materials from renewable resources and the elimination of toxic production processes. For example, PLA bottles decreased GHG emissions by 20 % compared to PET bottles, while saving two-thirds of energy. Regarding the end-of-life, incineration, landfilling and recycling were not considered suitable for bioplastics, while biodegradation was considered the best option.	[97]
• The environmental performance of microalgae cultivation depends on location, season, scale, algal species, and nutrient source. Phototrophic cultivation is less productive than heterotrophic cultivation, thus requiring larger cultivation volumes. • More than 70 % of total energy (335 or 250 kWh/kg$_{DW}$, depending on the algal species) was consumed for centrifugal harvesting and spray-drying after biomass cultivation in ORPs. In contrast, 80 % of the total energy (686 kWh/kg$_{DW}$) was required for cultivation in closed PBRs. The GHG emissions of *C. vulgaris* cultivation in closed PBRs and ORPs were 220 and 141 kg $CO_{2,eq}$/kg$_{DW}$. • Nutrient supply could be critical for the process sustainability. For example, GHG emissions associated with nutrients were reduced by 80 % and 20 % by using slurry and wastewater, respectively. • Based on a weight basis, the GHG emissions and non-renewable energy impact categories could be much higher for microalgae than for beef and other plant raw materials.	[98]
• 16 LCA studies carried out with primary data from pilot to industrial scale. Only a few LCAs with data from near-full-scale plants (cultivation volume in the order of 10 m^3 or higher) were found. • Electricity (especially for artificially illuminated systems) and infrastructure were the major environmental hotspots in microalgae cultivation. • Microalgal biofuels were not competitive with conventional (bio) fuels in energy and environmental performances. However, multi-product scenarios were promising. • Systems for producing high-value biochemicals (e.g., antioxidants and biostimulants) had small and poorly impactful downstream sections. Valorization strategies of co-products (residual biomass) and waste streams (flue gas and wastewater) enhanced the environmental performance. • The results covered several orders of magnitude. For example, GHG emissions spanned in the range 0.33–4256 kg $CO_{2,eq}$/kg$_{DW}$ biomass and 378–6119 kg $CO_{2,eq}$/kg astaxanthin. Behind the results' variability, technical and methodological reasons were identified. Homogenization and clarity were claimed as essential requirements for future assessments, along with the availability of data from large-scale plants.	[99]
• Due to the lack of standardization in the methods, comparisons among different LCAs were difficult and mean trends were uncertain. GHG emissions were in the range from −75 to 534 g $CO_{2,eq}$/MJ biofuel, with HTL tending to be better than other biomass conversion methods due to a higher energy efficiency. Integrating wastewater treatment with algae cultivation led even to negative GHG emissions. • The NER (input over output energy) ranged from ~0.34 to 1.25, while the minimum selling price was from 2.1 to 10.4 \$/GGE. The Energy Return on Investment (EROI) was <1, thus requiring further efforts for the development of competitive systems.	[100]

environment, especially a large one, is a complex challenge. Nevertheless, some microorganisms can only thrive under specific environmental conditions, reflecting the importance of environmental features, particularly in the Mediterranean basin, which has unique ones.

Although oceanic microalgae are subject to constant mixing by the oceans and are therefore more likely to be ubiquitous, the same cannot be said for algae belonging to the Mediterranean Sea, which is a closed sea with physical barriers. It's worth noting that the presence of physical containment is one of the factors that promote speciation. Under dispersal limitation and environmental heterogeneity, microalgal diversity is higher due to local environmental factors promoting endemic species [2]. Environmental specialization can lead to extremely high diversity, especially if several trophic interactions are simultaneously relevant. Population density, dispersal ability, and body size also affect the ability of microalgae to colonize an environment. Consequently, in the Mediterranean, there is a high degree of horizontal diversity due to habitat differences. In general, microalgae inhabiting oligotrophic seas like the Mediterranean cope with low levels of nutrients and high irradiances. As an evolutionary mechanism, they often have low half-saturation constants for nutrients such as nitrogen and phosphorus [103]. This can be well applied, for example, to the bioremediation of wastewaters involving microalgae, as they can effectively decrease even very low concentrations of nutrients, as recently shown [104]. On the other hand, microalgae in this area need to cope with high irradiance levels, resulting in low chlorophyll levels and in the production of high amounts of pigments for photoprotection. Therefore, they may be easily employed in producing high-value compounds such as pigments and antioxidants.

While some studies have assessed microalgae distribution in the Mediterranean area [105,106], this section aims to provide a comprehensive overview of isolated microalgal species with technological applications. Pathogens are intentionally excluded from this discussion. The main species isolated from the Mediterranean area with technological applications are indicated in the supplementary material (Table S1). The table also includes the location of isolation and the GPS coordinates. In a separate column, additional applications, different from those mentioned in the isolation articles, are listed along with the relevant citations. Here, we reported the same species gathered in classes for brevity (Table 2). Among them, the main species with technological applications are from the class *Bacilariophyceae, Chlorophyceae* and *Cyanophyceae.* The first two classes are mainly employed for extracting high-value compounds for pharmaceutics or nutraceuticals and as an alternative for bioremediation purposes, while the third is for the production of nanoparticles or for biocompounds for pharmaceutics. In the following section, a thorough examination of the species present in the Mediterranean area is provided.

Regarding the coastal presence of microalgae, numerous species have been documented across various studies. Quijano-Scheggia et al. documented the presence of species in the genus *Pseudo-nitzschia* spread in NE Spanish coast across 2005–2006 [107]. This marine diatom is of the pennate genus and it is known mainly for being toxic due to the production of domoic acid [108], even if it may be applied for some technological applications. The authors found several strains, including *Pseudo-nitzschia multistriata*, which was also found in the Gulf of Naples in Italy, and it was reported to be a good producer of intracellular domoic acid [109]. Quijano-Scheggia et al., more recently, observed that this genus is widely spread across all the Mediterranean Sea, describing its distribution [110]. It has been found, for example, in Italian and Greek costs. Another study by El Aroussi et al. [111] identified several species of microalgae from Moroccan coasts and assessed their availability for biodiesel production. By assessing the 57 isolated species, authors concluded that *Nannochloropsis* sp., *Dunaliella tertiolecta*, *Isochrysis* sp. and *Tetraselmis* sp. are promising species as biodiesel feedstock based on parameters such as growth, lipids quantity and quality, fatty acid profile but also robustness and ease of culture [122]. In another study conducted along the Mediterranean coast of Morocco, researchers identified several microalgae species including *Nannochloropsis gaditana*, *Nannochloris* sp., *Phaeodactylum tricornutum*, and *Tetraselmis suecica*. Analysis of the lipid fraction revealed promising potential for these microalgae in lipid production, suggesting their suitability for use as supplements in aquatic and animal feed enriched with polyunsaturated fatty acids (PUFA), as well as in the production of other food products with higher omega-3 fatty acid content. Notably, *Nannochloris* sp. exhibited the highest lipid productivity at 15.93 mg/l/day, indicating its potential utility as a source for dietary supplements or biofuels feedstock [112]. In another study on microalgae isolated from Moroccan seawater, *Nitzschia* sp., *Nannochloropsis* sp., and *Tetraselmis* sp. were assessed as possible sources of biodiesel, and *Nannochloropsis* sp. was identified as the best candidate due to its high lipid content [113]. *Nitzchia* sp. is a species investigated for several purposes; for example, it has been used for purifying sea cucumber aquaculture wastewater [114] and metabolite production, antibacterial activity, and slow-release biofertilizer [115]. On the other hand, *Tetraselmis* is a very deeply studied microalga with many applications; for example, it may be applied in lipid production and bioremediation and as source of biomolecules and antioxidants [116]. Among the other identified strains, it was reported that *Nannochloris* may be applied in for landfill leachate biotreatment and lipids production [117]. In a different work assessing microalgae from Sicilian littoral, three strains were identified as *Chlorella* sp. Barcarello, *Chlorella* sp. Pozzillo and *Dunaliella viridis* [118]. Biochemical analyses were performed and *Dunaliella* resulted ideal for its antioxidant content, while in all the strains there is an interesting content of poly unsaturated fatty acids (PUFAs). These characteristics may indicate applications in nutraceutics. In a conceptually similar work, five microalgal strains were isolated from the Adriatic coast in Croatia and identified as *Nitzschia* sp. S5, *Nanofrustulum shiloi* D1, *Picochlorum* sp. D3, *Tetraselmis* sp. Z3 and *Tetraselmis* sp. C6, and the cyanobacterium *Euhalothece* sp. The strains were characterized for the content in macromolecules, pigments, antioxidant activity. Authors concluded that *Nanofrustulum shiloi* D1 is a potential feedstock for biodiesel production due to its high content in lipids. *Tetraselmis* sp. Z3 can be used as a fish oil replacement due to its PUFAs content. All selected microalgae are a good source of proteins and pigments that may be applied in nutraceutics and all microalgal extracts strongly inhibited the growth of Gram-negative *E. coli* and *S. typhimurium* and Gram-positive *S. aureus*. [119]. Another study reported that *Picochlorum* may be used also for phycoremediation of nitrogen and phosphate [120]. A research was carried out on several microalgal strains isolated from the Aegean

Table 2

A resume of microalgae reported as classes isolated from the Mediterranean area and their primary application.

Class/family	Application	Isolation location	Reference
Bacilariophyceae	Source of lipids, aquaculture, biodiesel production, source of bioactives	Italy, Turkey, Tunisia, Croatia, Morocco, Spain, Egypt	[107,109,112,113,119,121,122,144,146,168,172,173,179]
Chlorophyceae	Source of beta carotene, nutraceutics, biomass production	Italy, Turkey, Tunisia, Morocco, Spain, Greece, Egypt	[111,112,118,134,142,146,161,163,165,168,171,172,174,177,178]
Cyanophyceae	Production of FAMEs, synthesis of nanoparticles, bioremediation, pharmaceutics, nutraceutics	Italy, Croatia, Tunisia, Malta, Egypt	[119,123,146,168,172]
Chlorodendrophyceae	Biodiesel feedstock, fish oil replacement, bioremediation	Morocco, Croatia, Greece	[111–113,119,165]
Eustigmatophyceae	Biodiesel feedstock, nutraceutics	Italy, Morocco	[111–113,146]
Dinophyceae	Nutritional, cosmetic, and drug additives	Turkey	[122]
Prymnesiophyceae	Nutritional, cosmetic, biodiesel feedstock	Turkey, Morocco	[111,122]
Trebouxiophyceae	Nutraceutics/phycoremediation of nitrogen	Croatia	[119]
Fragilariophyceae	Biodiesel feedstock	Croatia	[119]
Hymenomonadaceae	Pharmaceutics	Turkey	[121]

Sea, in Turkey. Among the several isolated strains authors found *Amphora* cf *capitellata* and *Nitzschia communis*, with antibacterial activities, *Nitzschia thermalis*, with anticancer activity, *Ochrosphaera* sp. with antifouling activity. The isolated strains may be applied therefore in marine pharmacology [121]. In another study, *Nitzschia Navis-varingica*, *Heterocapsa pygmaea* and *Chrysochromulina alifera* were isolated from the surface water of Mersin and Erdemli coast, in Turkey. These strains were tested for their anticancer proprieties, and authors concluded that microalgal extracts may be used in nutritional, cosmetic and drug additives for cell growth as well as wound healing [122]. Numerous strains were isolated and characterized from the central Mediterranean region, specifically along the coastline of Malta. These strains encompass a variety of cyanobacteria, including *Leptolyngbya*, *Phormidesmis*, *Nodosilinea*, *Phormidium*, and *Lyngbya*, as well as heterocytous *Calothrix* species. Additionally, coccal cyanobacteria such as *Aphanocapsa* and *Chroococcus* were identified, along with coccal microalgae from genera *Chlorella*, *Chlamydomonas*, and *Coelastrella*. The community also included diatoms belonging to *Navicula* species [123]. Among the aforementioned strains, almost all have at least one technological application. For example, the phycobiliproteins of *Leptolyngbya* may be employed for natural illuminated colourant beverages [124]; *Phormidesmis* may be considered source of immunomodulators and antioxidants with possible application in dietetics and medicine [125]; *Nodosilinea* is rich in carotenoids, and may be used in skin care formulations [126]; *Phormidium* may be used for deriving copper oxide nanoparticles for biomedical and environmental applications [127]; *Lyngbya* phycochemicals and biosynthesized nanoparticles may be used as antimicrobial and anti-cancer agents [128]; methanolic extracts of *Calothrix* have bioactive potential and could employed as pharmaceutical sources [129], they may be also employed in the synthesis of gold nanoparticles [130]. *Aphanocapsa* was investigated as biofertilizer [131] and *Chroococcus* may be employed for the decontamination of polluted soils [132].

Coastal lagoons of the Mediterranean, whether hypersaline or freshwater, exhibit a nutrient content significantly influenced by factors such as increased evaporation, resulting in higher salinity and deposition of salts like calcium carbonate. Additionally, these lagoons are often affected by human activities, leading to the accumulation of various wastes. Consequently, the organisms inhabiting these lagoons can differ greatly from those found in nearby marine environments [133]. In studies focusing on freshwater lagoons, diverse microalgae have been documented. For example, 19 microalgal species were identified from Sarıyar Dam reservoir, in Turkey [134]. The most interesting among them are *Dictyosphaerium pulchellum*, which may be employed in genetic transformation [135], and *Scenedesmus acuminatus* which showed high potential in the simultaneous production of biomass and carbon fixation [136] and may be grown in liquid digestates from anaerobic digestion [137]. These and other microalgae are studied [138] and preserved in a Turkish collection [139]. Lortou et al. identified several strains from different locations, such as freshwater lakes and a lagoon. The authors found several microorganisms and different new taxa. Among the found species it is possible to observe several species of the genus *Desmodesmus* and *Chlorella*, together with *Asterarcys quadricellulare*, which may be employed for wastewater bioremediation and biomass production as biodiesel feedstock [140], and *Monoraphidium* sp. which may be used as lipid feedstock, [141–143]. Several strains were also isolated from different Karst springs distributed in various places in Italy [144]. Among the most interesting strains there are: *Nitzschia frustulum*, which may be used for producing photoluminescent nanocomb structures from seawater through natural evaporation. Despite their man-made origins, this kind of system is very important because of the high habitat heterogeneity and biodiversity. In salted ponds, extremophile microorganisms can proliferate, showing a high ability to tolerate extreme conditions such as very high salinities and temperatures. These microorganisms are of particular interest due to their potential biotechnological and industrial applications because of the bioactive compounds they produce, often as a survival mechanism [145]. In one of the earliest works addressing microalgae isolated in these systems, from 1990, authors described 111 species in the salt works of Tarquinia, located on the Tyrrhenian coast of central Italy, noting that diatoms prevailed up to 110 ‰ salinity. They were replaced by Cyanophyta at higher salinities [146]. There are several isolated strains with technological applications divided into Cyanophyta, Euglena, Bacillariophyta and Chlorophyta. From the first phylum: *Aphanothece halophytica* may be employed for an enhanced production of fatty acid methyl esters (FAMEs) [147]; aqueous extracts of *Chroococcus minutus* may be used for the synthesis of silver nanoparticles with antibacterial activity [148]; *Chroococcus turgidus* may be employed in bioremediation of municipal wastewaters with bioproduct applications of its biomass [149]; *Spirulina subsalsa* is a source of phycocyanin [150]; *Oscillatoria limnetica* and *Oscillatoria princeps*, *Lyngbya* sp. and *Phormidium* sp. may be applied in the production of nanoparticles of various types [127,128,151,152]. Conversely, *Euglena* sp. has several immune and antiviral effects and is a potential of value-added metabolites [153,154]. In the class of bacillariophyta, *Navicula cincta* may be used as source of triacylglycerols for biodiesel and exopolysaccharides [155], while *Navicula salinarum* has been investigated as source of only exopolysaccharides [156]; *Amphora coffeaeformis* accumulates lipids which may be applied as aquaculture feed [157] while *Amphiprora* sp. may be applied as a source of lipids [158]. In the chlorophyta phylum, *Dunaliella salina* is a well-known microalga cultivated for its content in beta-carotene [159] while *Cladophora* sp. may be applied for example, in the generation of electricity as biocathode [160]. More recently, in 2018, from the same ponds a halo-tolerant strain of *Dunaliella* sp. (genus Chlorophyceae) was isolated [161]. As highlighted by the authors, this strain produces high levels of lutein, an important carotenoid with many functions related to health protection [162]. In another study from the same authors, a strain of *Dunaliella salina* was isolated from the same saltworks [163]. Authors suggest that this strain may have applications such as feed production, nutritional reinforcement as a vitamin A, precursor and production of pharmaceuticals and fine chemicals (mainly carotenoids such as beta-carotene). In another region of Italy, in the south, several strains were isolated from the saltern ponds of Trapani, Sicily [164]. In this work, *Dunaliella viridis* (genus Chlorophyceae), *Dactylococcopsis salina* (cyanobacteria) and *Navicula* sp. (diatom) were isolated, cultivated, and their bioactive effects were assessed. The authors concluded that the biomass cultured in high-salinity conditions might be applied in cosmeceutical/nutraceutical applications due to the production of a characteristic pool of carotenoids (e.g. lutein, fucoxanthin, neoxanthin). The cyanobacterium *Dactylococcopsis* showed a high cell repair activity, while *Brevibacterium* sp. showed anti-proliferative activity on cancer cell lines; they may be therefore applied in pharmaceutics. In another saltworks, in Messolonghi, Greece, a survey about the plankton biota was conducted in 2015. Authors found several categories, namely Cyanobacteria, Chlorophytes, Diatoms. Several microalgal species were identified, such as *Asteromonas gracilis*, *Tetraselmis marina*, *Dunaliella* sp. [165]. *Asteromonas gracilis* showed to be a promising feedstock for biodiesel production [166], while *Tetraselmis marina*, may be employed in bioremediation and as a potential source of compounds of interest and as feed for aquaculture [167]. *Dunaliella* sp., instead, is a well-established strain for microalgal biotechnology [159]. Saltworks in Egypt were also assessed, and, in particular, the solar saltern of Port Fouad. Researchers found several species belonging to the categories of cyanobacteria, diatoms, dinoflagellates, *Euglenophyceae* and *Chlorophyceae* [168]. Among *Cyanobacteria*, as already mentioned, *Chroococcus turgidus* may be applied in bioremediation of municipal wastewaters [149], while *Leptolyngbya fragilis* for bioremediation of soil contaminated with dodecane [169]. As already observed, *Spirulina subsalsa* is a source of phycocyanin [150]; *Synechococcus elongatus* secretes extracellular vesicles which promote angiogenesis [170]; *Synechocystis salina* may produce polyhydroxyalkanoates and is investigated under a biotechnological point of view. In another work from Chtourou et al., a strain of *Dunaliella* sp. was isolated from the Sfax-Tunisia Solar

Evaporating Salt-Ponds and identified. Authors concluded that the strain is usable for biodiesel production due to its content in fatty acids [171]. From the same saltern pond, more recently, three strains were isolated and identified as *Dunaliella salina* (*Chlorophyceae*), *Phormidium versicolor* (*Cyanophyceae*), and *Cylindrotheca closterium* (*Bacillariophyceae*). The photosynthetic and antioxidant activities of the species under light and salinity stress conditions were assessed, revealing that high irradiance and high salinity stimulated carotenoid synthesis and that the strains could provide promising sources of extremolyte for several purposes [172]. In another study assessing the same saltern pond of Sfax, an interesting strain of the genus Amphora was isolated and characterized for the first time. According to authors' conclusions, the strain resulted ideal as biodiesel feedstock due to the high content of lipids and the composition of them, mainly saturated [173]. In another work on three different saline sites in the northern region of Tunisia (North Lake Lagoon, Sebkha of Sijoumi, and Sebkha of Sahline), *Chlorella sorokiniana* ES3 and *Neochloris* sp. AM2 were isolated and characterized. Examination of their fatty acid profile and biodiesel parameters indicated that *Chlorella sorokiniana* shows promise as a viable candidate for the production of high-quality biodiesel [174].

It is worth separately mentioning microalgae isolated from contaminated sites, as their potential use can be linked to bioremediation strategies. Specifically, regarding heavy metal pollution, microalgae have shown promise as biosorbents for heavy metal ions in wastewater. This is due to their advantageous characteristics, including low cost, ready availability, relatively large specific surface area, and binding solid ability [175]. In this context, several researchers investigated on microalgal bioabsorption ability. For example, *Desmodesmus* sp. was isolated from freshwater in Turkey and studied for its Ag/TiO_2 removal ability [176]. A microalga belonging to the genera *Coccomyxa* was isolated from Tinto River, a river flowing through a mining area in the south of Spain. Although this alga may well-grow in acidic environments contaminated with Iron, Copper, Manganese, Nickel, Aluminium, authors proposed that it might have potential for xanthophyll production [177]. Similarly, a strain from the same genera was isolated from a polluted river in Sardinia, Italy, and the authors propose its employment as a source of carotenoid [178].

As demonstrated by this literature review, numerous species of microalgae (including cyanobacteria) isolated in the Mediterranean region are currently being exploited under a technological point of view. In particular, referring to the information in Table S1, we gathered the following number of species for each class and reported them in Table 2: Bacilariophyceae: 22; Chlorophyceae: 20; Cyanophyceae: 25; Chlorodendrophyceae: 5; Eustigmatophyceae: 3; Dinophyceae: 1; Prymnesiophyceae: 2; Hymenomonadaceae: 1; Trebouxiophyceae: 1; Fragilariophyceae: 1.

However, there is still a significant number of them that are currently unutilized, mainly found in freshwaters. These could represent an exciting resource when looking into new applications. At the same time, it is evident that several of the above-discussed applications still require further research to refine the technologies to make them ready for the industry, especially for what concerns energy/biomaterial applications. On the other hand, exploring novel compounds from microalgae is an active and promising research area, yielding significant results in recent years. Similarly, the production of nanoparticles starting from cyanobacteria is an expanding topic with room for innovation. In these fields, other microalgal and cyanobacteria may still be assessed to uncover new bioactive compounds and different kinds of nanomaterials. Mediterranean microalgae present similarities due to the common evolutionary selective pressure, such as owing low half-saturation constants for nutrients and acclimation to high irradiance levels [103]. However, this review primarily highlights how the greater richness of Mediterranean microalgae lies in their diversity, enabling them to have applications across a wide range of fields, as elucidated in the next section. Fig. 5 represents the key findings of the current section.

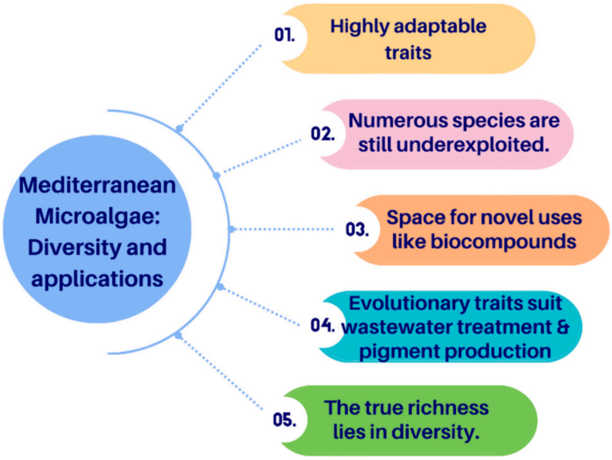

Fig. 5. Main factors connected to Mediterranean microalgal diversity.

5. Technological application of microalgae in the Mediterranean area

5.1. Research criteria and bibliometrics

A bibliometric research was conducted to provide an in-depth analysis of the technological applications of microalgae in the Mediterranean area, utilizing the electronic database SCOPUS. 41,428 titles were found using the keyword "microalgae" until 2023. By restricting the research, 8347 are the titles relative to countries belonging to the Mediterranean area, i.e., having coastlines along the Mediterranean Sea. Starting in 2020, a remarkable increase of interest in microalgae technologies has taken root across the Mediterranean region. This new interest can be attributed to several factors, such as growing environmental concerns, heightened focus on sustainable practices, and the exploration of innovative avenues for resource utilization. Due to this heightened interest, there has been a noteworthy escalation in the number of scientific publications. The transition from 2019 to 2020 witnessed a substantial increase of approximately 17 % in articles dedicated to this subject (Fig. 6). This increase in scientific output underscores the growing recognition of microalgae's potential across various sectors. Among all the works published from 2020 to 2023, the vast majority is relative to the two subject areas of Agricultural and Biological Science and Environmental Science (almost 40 %). The other large subject area is related to the Engineering issue (Chemical Engineering and Engineering), with almost 1000 titles, followed by Biochemistry, Genetics and Molecular Biology, with nearly 430 titles. This demonstrates how the technologies related to microalgae have been a crucial research issue in recent years.

Only the articles related to technology (subject area of Agricultural and Biological Sciences, Environmental Science, Chemical Engineering, Engineering, Energy, Chemistry and Material Science) were evaluated in this work, and 2290 titles published in the last three years were considered in the bibliometric analysis. In particular, in Fig. 6 it is possible to observe also the evolution of the time of documents published in the last three years, distributed by the subject areas of interest. It could be interesting to observe the strong increase in published articles in the Environmental Science area, passing from 2020 to 2022 and 2023, confirming the appeal of the green microalgae technology in these last years.

Concerning the affiliations, the Centre National de la Recherche Scientifique (CNRS), located in Paris, France, is the most productive research centre in the Mediterranean area, with 267 published articles about the microalgae issue. It is followed by the Universidad of Almería (142), sited in Spain, and by the Consiglio Nazionale delle Ricerche (83), located in Rome, Italy.

In the subject areas of interest, Algal Research is the journal with

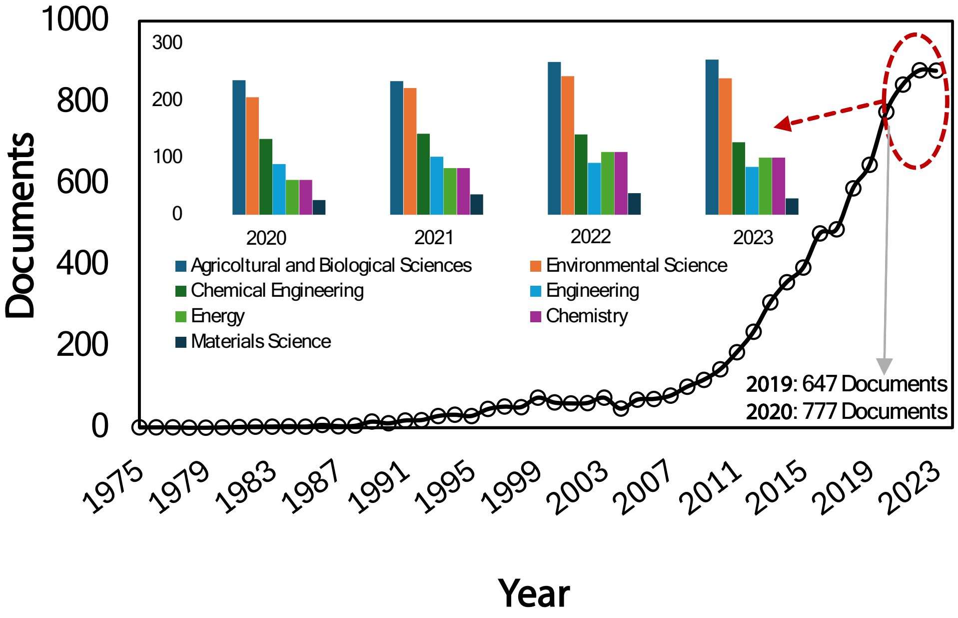

Fig. 6. Documents per year. Research was done on the Scopus database with the criteria presented in the supplemental material (Table S2). The evolution of the published documents from 2020 to 2023 in the seven areas of interest is also shown.

more articles (142), followed by the Journal of Applied Phycology (76) and Science of Total Environment (73). Behind there are Bioresource Technology, Applied Sciences Switzerland and Environmental Science and Pollution Research.

Among over 1600 significant keywords provided by the authors, about 32 % is inherent to pharmaceutical and nutraceutical issues, and 9 % to the use of microalgae biomass as a biostimulant or feed for vegetables or animals. Another large portion of the author keywords is related to wastewater treatment (30 %), comprising the emerging pollutants, micro- and nano-pollutants, metals, microalgae-bacteria consortia and waste valorization. The studies of bioreactors comprise 10 % of the authors' keywords, a percentage that growth to 16 % considering the keywords connected to kinetic, modelling, and computational fluid dynamics works. The last most crucial issue, in terms of published authors' keywords, is related to a biorefinery approach for energy production, with about 11 % of keywords. Other keywords inherent to life cycle assessment and economic analysis, rheology, and machine learning approach are less than the 3 % for each area. In Fig. 7 is represented a histogram with the main addressed issues in the universities of the Mediterranean area and the relative occurrence percentage of the relative significant authors keywords. From this analysis it is possible to understand how the most discussed topics published in article by the Universities in the Mediterranean area are the use of microalgae in wastewater treatments, in pharmaceutical and nutraceutical application, the use as biostimulant and as feed in animal farming, followed by biofuel production.

5.2. Technological diversity in the Mediterranean

In this section of the work, the main articles published in the seven areas of interest over the last four years are analyzed, divided by country. The research was conducted with the same key words described in the previous paragraph restricted with the name of each country. The affiliation of the first author is taken as priority, and the works are selected considering their inherence to engineering issues and their relevance in terms of novelty and citations.

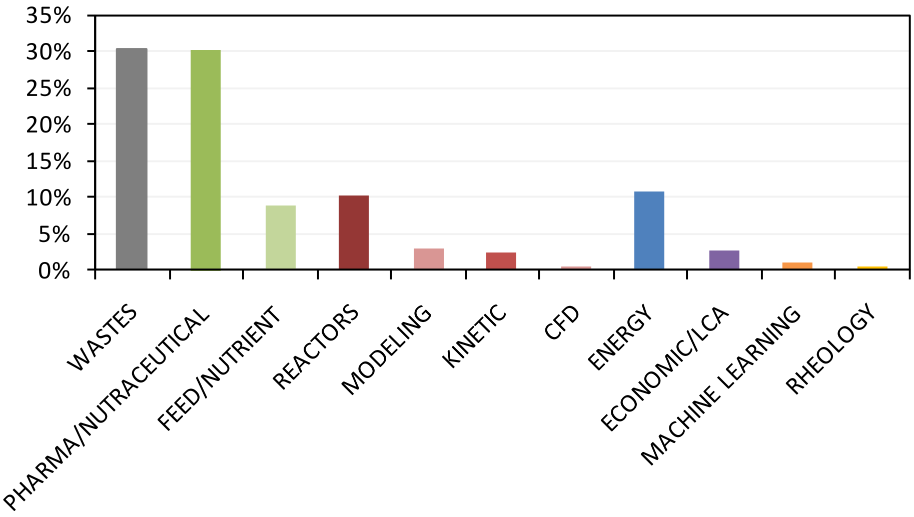

Fig. 7. Authors keywords analysis.

5.3. Spain

Spain sees the University of Almería-CIEMAT as the most active centre in the microalgal research, with 142 published works among 707. The most discussed topics are related to agricultural and biological sciences (with 21.8 % of the entire number of papers) and environmental science (20.3 %), followed by chemical engineering (11.7 %). Recently, many efforts were dedicated to the treatment and exploitation of wastewaters generated from pig farms. Many studies were carried out to explore the exploitation of wastewater [180], containing a high nutrient content generated from piggery, for cultivating algae and producing algal biomass for biostimulant applications [181]. The experimental campaign involved the use of an open thin-layer cascade reactor. Based on the obtained data, an economic analysis was conducted for a plant with a treatment capacity of 37.77 m^2/day, considering four scenarios depending on whether the biostimulant production utilized membranes or a simple centrifugation method for separation. Additionally, the study evaluated the co-production of a biopesticide through solvent extraction. The most cost-effective separation method for biostimulant production turned out to be the membrane system, with a cost of 65.5 €/m^3, and it remained competitive with commercial fertilizer when the crop distance was <300 km. Ciardi et al. [182,183] proposed to use diluted pig slurry to reduce water consumption in the cultivation of *Scenedesmus almeriensis*. After an optimization study [183], they found an optimum dilution of pig slurry for maximum biomass productivity of 5 %, with a productivity rate of 0.68 $g \cdot l^{-1} \cdot day^{-1}$, comparable to that obtained through the standard growth medium. Moreover, they proposed to use a sequence of thin-layer photobioreactors over a 16-month period to reduce the water consumption in microalgae cultivation [182,184]. A nutrient recovery strategy was proposed consisting in cultivating microalgae in brewery wastewater [185] and in nitrified urine, with or without adding supplements [186]. The treatment and reuse of urban wastewater was studied in lab-scale by Gonzalo Ibrahim et al., demonstrating how the concentration of the pathogen agent reached was compatible with the reclaimed water limits despite the absence of ultraviolet light [187]. A circular approach was also proposed [188,189] with the recovery of valuable compound. Zambrano et al. evaluated *Scenedesmus almeriensis* microalgae–bacteria consortia for the removal of veterinary antibiotics, such as tetracycline, ciprofloxacin, and sulfadiazine from the liquid fraction of pig slurry first in a laboratory scale batch system [190] and then in a pilot-scale photobioreactor [191]. The main results showed that microalgae can remove up to 99.9 % of tetracycline, 78.0 % of ciprofloxacin and 78.0 % sulfadiazine in the laboratory scale batch photobioreactor, meanwhile they go down to 77 %, 90 % and 69 % sulfadiazine respectively in the pilot-scale reactor. Another profitable operation on microalgae biomass is the extraction of saponifiable lipids, which may be rich in eicosapentaenoic acid. Jiménez Callejón et al. extracted these compounds from *Nannochloropsis gaditana* biomass [192]. Arashiro et al. explored the cultivation of three microalgae (*Nostoc* sp., *Arthrospira platensis*, and *Porphyridium purpureum*) in industrial wastewater with the goal of producing phycobiliproteins [193]. The three strains demonstrated efficient removal capabilities, achieving up to 98 % removal of COD, 94 % of inorganic nitrogen and 100 % of phosphate. Moreover, phycocyanin, allophycocyanin, and phycoerythrin were successfully extracted from the biomass, reaching concentrations of up to 103, 57, and 30 mg/g dry weight, respectively. In this way, the authors demonstrated the possibility of integrating microalgae for industrial wastewater treatment and the recovery of high-value phycobiliproteins. Serrà et al. presented the synthesis of a hybrid helical Cu@Cu2O@CuO–microalgae photocatalyst for the photodegradation of antibiotics [194]. The synthesis process involved the electroless deposition of copper and its controlled oxidation, using a *Arthrospira platensis* as a biotemplate. This hybrid photocatalyst demonstrated enhanced efficiency in the photocatalytic degradation of tetracycline, particularly in energy consumption, and it may be easily recycled once their effective lifetime is reached, allowing the creation of microalgal pellets. Belachqer El Attar et al. studied the rheology of microalgae concentrates of *Scenedesmus almeriensis* and *Nannochloropsis gaditana* [195,196] aiming to characterize the solutions in different culture media. Villarò et al. [197] in a recent work, proposed to use the microalga strain *Arthrospira platensis* BEA, provided by the Spanish Bank of Algae (Spain), grown in an 80 m^2 raceway as food colourant for macarons. The generated biomass primarily consisted of protein (57.0 % in weight) and contained natural and valuable pigments such as chlorophylls (6.7 %), carotenoids (1.8 %), phycocyanins and allophycocyanins (<1 %). López-Rodríguez et al. studied how to enhance the extraction of carotenoids, fatty acids, and amphidinols from *Amphidiniumcarterae* strains (ACRN03 and Dn241EHU) by optimizing cell disruption and solvent extraction methods [198]. The best carotenoid extraction was obtained at 60 °C without prior cell disruption. Also Morillas-España et al. [199,200] analyzed the production of the microalga *Scenedesmus* sp. in pilot-scale reactors. In a first study [200], along a yearlong experimental campaign, they found that the biomass productivities achieved in thin-layer cascade reactors during the months of increased photosynthetic activity reached 30–35 $g/m^2 \cdot day$. A preliminary economic analysis indicated that using wastewater for microalgae production could cut production costs by about 0.44 € per kilogram. Sánchez-Zurano et al. explored also the microalgae-bacteria consortia in processes for the depuration of municipal effluents [201,202]. The authors have suggested respirometric techniques to identify and calibrate proper kinetic models for the microalgae-bacteria process, to be eventually applied as an optimization tool to improve the efficiency and stability of consortia-based wastewater treatment. Other authors have suggested models for microalgae-bacteria consortia treatment systems. These models can then be used as optimization tools to improve the efficiency and stability of these wastewater treatment processes [203]. Sánchez-Zurano in another paper proposed a photo-respirometric method to evaluate the activity of microalgae, heterotrophic bacteria, and nitrifying bacteria within a microalgae-bacteria consortium [204]. This approach enables the separate determination of the activity of these microbial components. The treatment of marine aquaculture wastewater through microalgae-bacteria consortia was studied by Perales-Pérez et al. [205]. In this work, the authors focused on the biomass separation step, through a preliminary coagulation-flocculation pre concentration process and then comparing the settling and flotation processes to separate the biomass. Morillas-España et al., instead, proposed an ultrafiltration membrane to separate the microalga *Scenedesmus* sp. in consortia with bacteria, within a wastewater treatment raceway reactor [206]. The ultrafiltration membrane, positioned in the reactor sump, separates water from cells and was employed to distinguish the cell residence time, from the hydraulic retention time. Otálora et al. [207,208] proposed a technique based on machine learning to classify microalgae. The two proposed models have the capability to differentiate between *Scenedesmus almeriensis* and *Chlorella vulgaris*. In the most recent work [208], they showed the capability to differentiate among six distinct genera of microalgae. In practice, the model achieved an impressive classification accuracy of up to 97.27 % when analyzing a culture. A significant group of researchers [209–212] investigated the use of microalgal biomass as nutrient source for aquacultured fish. About the extraction process of valuable substances, Navarro-López et al. [213] investigated the effect of different parameters on the extraction of biostimulant molecules from *Scenedesmus almeriensis* microalgal biomass and underscored the biostimulant potential of the microalga. The optimization of the extraction process was determined by assessing the germination index in watercress seed bioassays. Various combinations of solvent extraction ratios, temperatures, and extraction durations were subjected to experimentation and the most effective combination was selected for each solvent. Optimal conditions were achieved using environmentally friendly organic solvents like acetone or ethanol. López Pastor et al. performed a techno-economic analysis on the use of solar thermal energy for microalgae drying [214]. The author proposed a 200 m^2 surface collector operating in recirculation mode and demonstrated that the cost of the drying step reduces to 1.16 € for kg of biomass, compared to a cost of 2.37 €/kg for conventional fossil fuel-based spray dryers. López-Herrada et al., in a recent work [215] carried out a life-cycle assessment of the production process for a microalgae-based fungicide derived from amphidinols. The study was based on a production target set at 22,000 l of fungicide annually. In another study, Inostroza et al. [216,217] optimized the design of 500 m^2 raceway reactors using a Computational fluid dynamics approach. Thanks to simulations the dynamic behavior of the optimal configuration was analyzed. The monophasic analysis, conducted using the Finite Element Method (FEM) in COMSOL Multiphysics™, confirmed that the utilization of deflectors in the baffle partition bend type resulted in superior performance in terms of fluid velocity, reduction of dead zones, shorter residence time, and an appropriate cell Reynolds number. To complement the analysis, a multiphasic analysis was conducted using the Finite Volume Method (FVM) in ANSYS Fluent, considering the geometry and rotation speed of the paddlewheel. An important study about the adhesion behavior of flagellated microalgae has been addressed by García-Abad et al. [218]. Seven different surfaces with varying water adhesion tension properties and two microalgae strains were analyzed: *Chlamydomonas reinhardtii* and *Isochrysis galbana*, which were cultivated under batch and fed-batch conditions. Cells and exopolymeric substance adhesion were measured and a direct correlation between cell and exopolymeric substance adhesion was observed, inversely related to biomass generation in the cultures. These results are particularly interesting in the biofouling context.

5.4. France

In France the most active centre in the microalgae technology issue is the Centre National de la Recherche Scientifique with 268 titles. Among 436 total papers, the 19.4 % is related to agricultural and biological sciences, and 16.9 % falls in the environmental science issue. Nzayisenga et al. examined the biomass and fatty acid production of four microalgal strains (*Chlorella vulgaris*, *Desmodesmus* sp., *Ettilia pseudoalveolaris*, *Scenedesmus obliquus*), as sources for biodiesel. The biomass was cultivated under three different light intensities (50, 150, and 300 $\mu E\ m^{-2}\ s^{-1}$) [219], and the main results showed that elevated light intensities contributed to an increase in biomass concentration in all four species. A significant rise in fatty acid content was observed in both *Desmodesmus* sp. and *Scenedesmus obliquus*; moreover, the increase in fatty acid content is inversely associated with a decrease in protein content in all cases. Analysis of fatty acid composition demonstrated that higher light intensity resulted in increased oleic acid (18:1) and decreased linolenic acid (18:3). Wils et al. explored natural deep eutectic solvents (called NaDES) for the extraction of bioactive compounds from *Spirulina*, focusing on pigments and free fatty acids [220]. The glycerol/glucose-based solvent exhibited a diverse profile, spanning polar phycobiliproteins to free fatty acids, while a fatty acid mixture-based solvent demonstrated high selectivity for free fatty acids. The intensified extraction process led to the evaluation of six spirulina-NaDES formulations and solvents for their impact on cutaneous inflammation. Zhao et al. explored the effects of charge and corrugated surface on membrane filtration performance and a synergistic approach for cultivating high-density microalgae and achieving cost-effective harvesting using a pH-responsive, charge-switchable, patterned membrane [221,222]. The membrane, consisting of polyethylenimine (PEI)-crosslinked polyvinylidene fluoride (PVDF), exhibits different charges based on pH, influencing the interaction with microalgae. Demir et al. employed force spectroscopy atomic force microscopy (AFM) to examine the molecular-scale interactions between *Chlorella vulgaris* cells and chitosan, aiming to elucidate the flocculation mechanism [223]. This important research identifies distinct mechanisms at different pH levels, emphasizing the complexity of these interactions and providing valuable insights into the flocculation process. Results revealed that, at pH 6, chitosan engages with the *C. vulgaris* cell wall primarily through biological interactions rather than electrostatic forces. Additional AFM experiments demonstrated a different mechanism at higher pH, characterized by chitosan precipitation. Lacroux et al. investigated the mixotrophic growth of five microalgae species (*Acutodesmus obliquus*, *Auxenochlorella protothecoïdes*, two strains of *Chlamydomonas reinhardtii*, and *Chlorella sorokiniana*) in the presence of acetate or butyrate under varying pH conditions [224]. The assimilation of acetate was efficient for all strains, while butyrate uptake varied significantly among strains. Growth rates were affected at pH levels above 8, and values below 5 or 6 inhibited the growth on acetate and butyrate, respectively. These results are very important in a framework of optimizing processes that integrate bacterial fermentation with microalgae cultures. Galès et al. by assessing the CO_2 conversion efficiency of microalgae cultivated in open land-based raceways, found that its highest conversion photosynthetically fixed CO_2 into carbon biomass (40 %) occurred at pH 7 [225]. Moreover, the same author studied cyanobacteria-microalgae consortia for treating urban wastewaters within open ponds in different climes (temperate oceanic and Mediterranean climates) [226]. The results of this work showed how similar ecological successions were observed in the two cases. Together with the microalgae, bacteria participated substantially in the complete consumption of ammonia. The resulting competition for NH_4^+ influenced the removal efficiency levels of disCOD by bacteria and PO_4^{3-} by microalgae. A study of Peyrton et al. focused on the production and characterization of polyols synthesized from microalgae extracted oil [227]. A new foam incorporating 25 wt% biobased polyols was proposed, demonstrating compliance levels comparable to a fossil-based reference foam. Moreover, this study achieved a catalyst-free foam with a density similar to the reference using a biobased triglyceride catalytic polyol. In a comparative study, Morales et al. analyzed the immobilized culture systems in large scale, i.e. systems in which the biomass grow attached on the surface of a support medium. They offer various advantages over suspended counterparts, such as increased biomass productivity and simplified harvesting and concentration processes [228]. A comparative life cycle assessment was conducted, evaluating the environmental impacts, energy consumption, and material requirements of large-scale production of *Tetraselmis suecica* in both conventional Open Raceway Ponds and Rotating Algal Biofilm systems. With identical productivity levels, the environmental impacts were 26 % higher per kilogram of biomass and 24 % higher per protein powder (algae meal). Jimenez et al. in 2020 proposed the production of a slow-release fertilizer using a *Monoraphidium* sp. microalgal strain, grown in a liquid digestate which served as nutrient source [229]. The effect of the microalgal biomass on tomato plants was positive, enhancing the plant growth by 32 %. An interesting work, conducted by Zhang in 2020, proposed a procedure to recovery biomolecules from microalgae through a high voltage [230]. *Nannochloropsis oculata* microalga was used and the process consisted in two steps, involving initial aqueous extraction followed by secondary organic solvent extraction from vacuum-dried microalgae. Moreover, the effects of high-voltage electrical discharges (at 40 kV/cm, 4 ms pulses) were investigated, highlighting how the high voltage treatment improved the kinetic of vacuum drying and significantly influenced the organic solvent extraction of chlorophylls, carotenoids, and lipids. Clavijo Rivera studied a wet biomass processing method, involving harvesting, cell disruption, and fractionation of target compounds, with membrane filtration [231]. The focus of the study was on the lipid recovery from aqueous extracts of *Parachlorella kessleri* using cross-flow filtration eliminating the need for costly drying procedures.

5.5. Italy

In Italy the research trend is equally divided between Agricultural and biological sciences (18.3 %) and Environmental science (17.4 %) and the most active research centre is the *Consiglio Nazionale delle Ricerche*, with 83 published papers among 532. Casagli et al. described the evolution of the algae-bacteria ecosystem in an outdoor raceway for wastewater treatment through a model named ALBA [232]. The model incorporates mass balances of COD, C, N, P, H, and O, taking into account growth and interactions among algae, heterotrophic and nitrifying bacteria, with local climate influencing light and temperature. Calibration and validation were performed using data from a 56 m^2 raceway in the South of France treating synthetic wastewater over >400 days. The ALBA model highlights the impact of paddle wheel regulation on the ecosystem, emphasizing the need for optimal control to balance mixing, aeration, and degassing effects efficiently. As reported by the authors, this model can be used to support advanced control strategies, including smart regulation of the paddle wheel velocity to balance the mixing, aeration and degassing effects more efficiently. An interesting genetic engineering study conducted by Perozeni et al. investigated the possibility to employ synthetic redesign of the ab-carotene ketolase gene to facilitate its constitutive overexpression from the nuclear genome of *Chlamydomonas reinhardtii*, a microalga lacking the inherent ability to synthesize high-value ketocarotenoids [233]. This optimized carotene ketolase overexpression extended the native carotenoid biosynthesis, resulting in a shift in the green algal colour to reddish-brown. Robust overexpression allowed the conversion of up to 50 % of native carotenoids into astaxanthin and over 70 % into other ketocarotenoids. Di Pippo et al., in a water pollution context, and the possible exploitation of microalgae for wastewater treatment, used advanced techniques to examine the tiny ecosystems formed on microplastics (plastisphere) collected from lakes in Italy [234]. The plastic surfaces have their own unique microbial communities, different from the surrounding water. Despite some variations based on location, there was a consistent group of microorganisms living on the plastic. Interestingly, the types of microbes didn't change much with different plastic materials. Moreover, less degraded plastics attracted generalist microbes, while more degraded ones had a more diverse community. Bolognesi et al., in a circular economy view and a wastewater treatment context, studied the possibility of recycling sewage sludge and microalgae mixture for the production of biochar. The proposed method involves a pyrolysis of a mixed sludge/bioalgae matrix under various conditions. This approach recovers a material with versatile potential end uses and eliminates residuals destined for landfills. Moreover, the algae were subjected to preliminary solvent oil extraction and the results indicated a significant increase in biochar production (25–33 %). Lima et al. dedicated studies to the effect of flashing lights on *Nannochloropsis gaditana*, *Koliella antarctica* and *Tetraselmis chui* microalgae [235,236]. In fact, light pulses are known to promoting growth or triggering the production of high-value biocompounds in microalgae. Main results showed that in the presence of flashing light conditions, a rise in lipid content and a reduction in polyunsaturated fatty acids and chlorophyll occurred under nutrient-deficient conditions, whereas contrasting effects are evident under nutrient-abundant condition. Furthermore, subjecting concentrated cultures to low-frequency flashing light for a duration of four days resulted in a three-fold increase in the productivities of eicosapentaenoic acid and specific carotenoids [237]. In another work, Lima et al. assessed the extraction of the sugar content and its conversion into 5-hydroxymethyl furfural (5-HMF), used in small quantitative as alimentary additive, to valorize the *Chlorella* sp. microalgal biomass [238]. In the initial phase of the process, the authors tuned the pretreatment of biomass using a combination of sonication and hydrothermal treatments, complemented by the presence of acetic acid and SiO_2 pellets. This optimization aimed to achieve the maximum release of carbohydrates. The second step was carried out under hydrothermal conditions and focused to the catalytic isomerization/dehydration of monosaccharides derived from previous reaction step. The process utilized two commercially available niobium-based catalysts and led to 21 % yield to 5-HMF on the total sugar, and 29 % in a reactive extraction process. Regard the wastewater treatment with microalgae-bacteria consortia, many studies were carried out using autochthonous microalgae [237,239]. In particular, the *Chlorella* sp., was proven as one of the most effective autochthonous microalgae, in synergy with activated sludges, leading to a total nitrogen removal of about the 77 % and total phosphorus removal above the 60 %. Moreover, an increase in saturated fatty acid production was monitored in presence of bacteria and the residual microalgae biomass contained a high quantitative of carbohydrates that could be used in other applications.

5.6. *Turkey*

The 21.9 % of the 240 published articles in Turkey are related to Agricultural and biological sciences and 17.2 % to Environmental science, followed by the Energy issue (11.9 %). Among the most interesting works, a research group in 2020 studied the effect of microplastics and metal pollutants on the freshwater microalga *Chlorella vulgaris* [240,241]. The main results show the response in growth and chlorophyll production of the microalga when 0.5 μm-sized polystyrene microplastics at varying concentrations were present in the culture medium. The authors have encountered no significant impact at lower concentrations of microplastics (1 and 5 mg/l), while higher concentrations (50, 100, 1000 mg/l) led to a substantial reduction in the growth and chlorophyll content of cells. Moreover, the combination of microplastics with metals (Cu, Zn, Mn) exhibited greater inhibition of growth and chlorophyll concentration. The results of these studies represent an important step in the possible exploitation of microalgae in the industrial wastewater treatment framework. Tarhan et al. used the process water obtained from waste biomass hydrothermal carbonization for *Chlorella minutissima* and *Botryococcus braunii* microalgae cultivation [242]. An interesting use of *Chlorella vulgaris* biomass was proposed in different studies [243,244] in which the biomass was used for the synthesis of CuB, NiB, FeB catalyst, and sulphur and phosphorus doped carbon catalysts. The resulting metal-free catalysts were utilized for efficient hydrogen (H_2) production from sodium borohydride ($NaBH_4$). Moreover, Saka et al., used *Spirulina platensis* cyanobacteria dried biomass, pretreated with H_3PO_4, for the synthesis of supported-CoB catalysts [245]. Also in this case, hydrogen obtained from $NaBH_4$, demonstrating how the microalgal biomass could be applied for efficient catalytic reactions with a particular attention to the environmental issue. *Chlorella vulgaris* was also studied as supplement in agriculture to alleviate drought stress in broccoli plants subjected to water deficiency [246]. The foliar application of microalgae mitigated the drought stress effects, resulting in improved growth performance. Aghaalipour et al., in a biological remediation point of view, studied the carbon dioxide capture capacity in *Scenedesmus obliquus*, *Monoraphidium contortum*, *Psammothidium* sp., and *Chlorella vulgaris* species [247] and in different conditions. They found that *Chlorella vulgaris* showed the best growth parameters and the vertical column was the best configuration of photobioreactor when fed with 10 % of CO_2 gas.

5.7. *Greece*

The published works in Greece are oriented to Agricultural and Biological Sciences (19.3 %) and Environmental Science (18 %). Metsoviti et al. explored the impact of solar irradiance on *Chlorella vulgaris* cultivated in open bioreactors within greenhouse conditions and investigated the effects of the ratio of light intensity in different wavelength ranges and artificial irradiation provided by red and white LED lamps in a closed flat plate laboratory bioreactor on growth rate and composition [248]. The trends in biomass, lipid, and protein productivities as a function of light intensity were found in greenhouse system laboratory bioreactor. Higher solar irradiance resulted in increased growth, along with elevated lipid content in microalgal biomass. In experiments conducted in the closed bioreactor, an increase in the wavelength ratio correlated with higher specific growth rates and biomass, protein, and lipid productivities. Furthermore, an increase in light intensity using red and white LED lamps led to faster growth rates and higher lipid content (up to 22.2 %). Another important study was carried out by Zarrinmehr et al. [249] focusing into the impact of nitrogen concentrations on the growth rate and biochemical composition of *Isochrysis galbana* microalga. With low nitrogen concentration, cell growth, pigments, and protein content in biomass exhibited a decline, while carbohydrates reached their highest value, 47 %, when total nitrogen was absent. The most interesting aspect was the polyunsaturated fatty acids (PUFAs) increment under sufficient nitrogen concentrations (72 mg/l) compared to nitrogen deprivation. Conversely, the concentration of saturated fatty acids (SFAs) was higher under nitrogen deprivation than in cases of nitrogen sufficiency. Zkeri et al. compared two treatment systems for medium-strength dairy wastewater. The first system consisted of a methanogenic Moving Bed Biofilm Reactor (AnMBBR) and an aerobic MBBR (AeMBBR), while the second system included an AnMBBR and a sequencing batch reactor (SBR) with *Chlorella sorokiniana*. [250]. The microalgae acclimatized to dairy wastewater resulted in enhanced growth, with a protein content of 54.6 %, starch content of 3.4 %, and lipid content of 23.1 %. Pax et al. used exhausted olive pomace for extracting valuable compounds to cultivate the heterotrophic *Crypthecodinium cohnii* microalga [251]. The extraction was carried out through organosolv technology, using water, organic solvents and an acid catalyst to break down the pomace and extract components like sugars, galacturonic acid, and phenolic compounds. The liquid fraction was then used as medium for the biomass growing, suitable for biorefinery processes, in particular for the omega-3 fatty acids extraction. Koutra et al. instead used the digestate of an agro-industrial effluent for growing *Chlorella vulgaris* [252]. The removal of carbon, nitrogen and phosphorus reach up the values of 92 %, 77 % and 94 %, respectively. Moreover, the biomass was used in a biorefinery context to extract antimicrobial compounds, tested with success on *Bacillus subtilis* bacteria.

5.8. *Algeria*

Eighteen articles on microalgae are published in Algeria. One of the most interesting works is proposed by Hasnaoui et al. The authors studied an electrochemical photo-bioreactor to produce H_2 from the *Arthrospira platensis* microalga [253], achieving hydrogen evolution rates of up to 27.49 and 13.37 mol of H_2 d^{-1} m^{-3} for the anode and cathode chambers, respectively, under 0.3 V voltage and ~2.5 mA current, representing a 4-fold increase compared to the production rates without voltage application. Keddar et al. in a study of 2020 used the lyophilized biomass of *Scenedesmus* sp. to extract simultaneously antioxidants with different polarity [254]. Supramolecular solvents with various composition were used, produced with octanoic acid dissolved in ethanol and water at pH of about 3. A yield of 1.04 mg/g of carotenoids was achieved, with a significant part of lutein, and of 10.29 mg/g polyphenols, demonstrating how a green and efficient extraction of bioactives compound is possible. In another important work of 2023, Nouacer et al. studied the biosorption of Nickel in *Auxenochlorella protothecoides* microalga biomass immobilized in sugarcane bagasse [255]. Results showed that microalgae can also be applied in the removal of metals from specific wastes, with a maximum adsorption capacity for nickel of 62.1 mg/g.

5.9. *Tunisia*

Kahla et al. in 2021 [256], in view of wastewater treatment, examined the efficacy of the consortium of the benthic diatom *Nitzschia* sp. and the associated bacteria in the removal of benzo(*a*)pyrene and fluoranthene. The diatom, isolated from a Polycyclic aromatic hydrocarbons-contaminated sediment in the Bizerte Lagoon (Tunisia), was exposed to this pollutant over 7 days in both axenic and non-axenic cultures. The diatom exhibited continuous growth under these conditions and accumulated benzo(*a*)pyrene and fluoranthene with varying efficiencies in axenic and non-axenic cultures. Biodegradation, the primary mechanism for Polycyclic aromatic hydrocarbon elimination, was enhanced in the presence of bacteria, indicating the co-metabolic synergy of microalgae and associated bacteria. Elleuch et al. in 2021 studied the *Dunaliella* sp. biosorption capacity for zinc and other metals in the context of phytoremediation of contaminated wastewaters [257,258]. In particular, the presence of zinc influenced the cell growth and photosynthetic pigment accumulation and reached a maximum zinc removal of 98.95 %. In particular, the presence of zinc influenced the cell growth and photosynthetic pigment accumulation and reached a maximum zinc removal of 98.95 %. Khemiri et al. evaluated the effect of including microalgal biomass in food [259,260]. In the first case, the *Nannochloropsis gaditana* and *Chlamydomonas* sp. biomasses are used as sources of protein in gluten-free bread. The microalgae-enriched bread resulted in higher proteins and lipids content compared to the control bread [259]. In the second case, the authors introduced an innovative ricotta cheese incorporating *Chlorella* sp. as a functional ingredient, maintaining the traditional manufacturing process and preserving acceptable sensory attributes [260]. Karray et al. proposed a novel process for the treatment of the olive mill wastewater, involving an anaerobic co-digestion, an ultra-filtration and a following microalgae treatment [261]. *Scenedesmus* sp. specie was used in the culture with the aim of producing valuable biomass. The maximum productivity in terms of biomass was 0.15 g/l day in a medium consisting of 25 % of filtrated digestated. The maximum nitrogen removal rate was 15.18 mg/l day and the phosphorus and phenolic compounds were almost totally eliminated. Dammak et al. studied stress factors, such as presence of nickel, chromium and cobalt in the growth medium and high irradiance or nitrogen depletion on the lipid production of *Tetraselmis* sp. microalga [262,263]. They assessed this microalga specie as a good heavy metals bio accumulator and at the same time suitable for biodiesel production, due to the high value of produced lipids.

5.10. *Israel*

58 articles are published in Israel about microalgae, 31.6 % of which in the Agricultural and Biological Sciences field. Among the most interesting ones, Harvey & Ben-Amotz outlined a path towards large-scale industrial production of 9-cis beta-carotene through biotechnology utilizing *Dunaliella salina* biomass, enhancing downstream processing efficiency by employing naturally hyper-accumulating carotenogenic strains and leveraging red [264]. Shkolnikov Lozober et al. studied the gelation, i.e. an important techno-functionality for novel protein sources, and characterized the *Spirulina* protein concentrate gel [265]. Moreover, they explored the enhancement of gel properties through high-pressure homogenization prior to thermal gelation. While the high-pressure homogenization increased the protein solubility by 91 %, the gelation occurs at pH 6.5 as inferior limit, due to the insufficient protein solubility. Grossman et al. proposed a treatment system for food processing wastewater, using two local thermotolerant strains of *Coelastrella* sp. and *Chlorella* sp. [266]. The wastewater underwent treatment employing an anaerobic membrane bioreactor, followed by polishing through outdoor photobioreactor cultivation of microalgae, with biomass productivity ranged from 0.25 to 0.8 $g \cdot l^{-1} \cdot day^{-1}$, while surplus sludge underwent treatment via hydrothermal carbonization. The effluent met the standards for irrigation use water and an energy production stem as biogas and hydrochar was also proposed to comply with a near-zero discharge process. Liberman studied the antimicrobial potential of polysaccharides produced by three different red microalgae species [267]: *Porphyridium* sp. (seawater species), *Dixoniella grisea* (brackish water) and *Porphyridium aerugineum* (freshwater). They added zinc and chitosan to increase the antimicrobial effect for wound-dressing materials use, founding that these hydrogels offered a synergistic combination of the antimicrobial and wound-healing benefits of chitosan and zinc, combined with the bioactivities and rheological properties of red microalgal sulphated polysaccharides. In the context of pharmaceutical contaminants in wastewaters, Akao et al. focused the attention on the degradation of iohexol, a contrast

agent used for X-ray imaging technique [268,269]. The study showed how the microalga *Chlorella vulgaris* was able to remove the pollutant: in 27 days 40–50 % of iohexol was eliminated from the medium, with 23–30 % of the agent biodegraded, demonstrating the versatility of this microalgal specie in the reclamation of polluted waters.

Shrestha et al. proposed the *Coelastrella* sp. freshwater microalga biomass as fertilizer for wheat grain [270]. Results showed that, despite the wheat yield was not affected by the use of microalgae-based biofertilizers, the emission of nitrogen oxide from soils was significantly reduced compared to the urea fertilizers. These results open new ways in the use of fertilizers with low impact to environment and human health.

6. Conclusions

This review evaluates the Mediterranean microalgal biodiversity from a technological point of view, focusing on the critical steps of microalgal bioprocessing and the tools for assessing sustainability. Through bibliometric analysis of works on microalgae published between 2020 and 2023 in the Mediterranean area, we observed prevalent keywords related to pharmaceuticals and nutraceuticals, biostimulants or feed, wastewater treatment, bioreactors, kinetics or modelling, and biorefinery. The assessment of microalgal diversity revealed common characteristics among Mediterranean microalgae, such as low half-saturation constants and acclimation to high light intensity, making them well-suited for specific technological applications. While exploring new microalgae for technological applications may contribute to biodiversity conservation efforts, many microalgal species remain underexploited, presenting opportunities for novel applications. However, the main insight which emerges from an accurate analysis of the scientific literature analysis, is that Mediterranean diversity constitutes the true richness that enables various microalgal species to be applied across a wide range of processes. This review serves as a starting point to identify literature gaps and new applications, facilitating the expansion of algal research and industry in the Mediterranean area.

CRediT authorship contribution statement

Alessandro Cosenza: Writing – original draft, Methodology, Investigation, Conceptualization. **Serena Lima:** Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Luigi Gurreri:** Writing – original draft, Visualization, Investigation, Data curation. **Giuseppe Mancini:** Writing – review & editing, Supervision. **Francesca Scargiali:** Writing – review & editing, Visualization, Validation, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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