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Nanoparticles in Microalgae Biorefineries: Insights from a Bibliometric Analysis

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Nanomaterials have the potential to revolutionize the microalgae industry by addressing challenges faced by microalgae biorefinery and enabling sustainable production of biomass, biofuels, and high-value compounds. There is a growing emphasis on harnessing nanotechnology to accelerate the progress and development of microalgae biomass as a viable and eco-friendly feedstock for producing biofuels and other valuable products. This study presents a comprehensive bibliometric analysis of global research trends on microalgae–nanoparticle studies from 2010 to 30th October 2025. Data were retrieved from the Web of Science (WoS). A total of 2,330 relevant documents were identified and analyzed using the WoS, VOSviewer and Microsoft Excel. Results revealed that research articles were the most dominant publication type (83.4%), followed by review papers (10.8%). Publication output increased steadily over the years, reflecting rising scientific interest in nanotechnology-enhanced microalgae research. The global distribution of publications revealed Asia as the leading contributor, with China (498) and India (476) ranking highest, followed by the USA (314). In Africa, Egypt (128) contributes the most, followed by South Africa (24), Nigeria (15), and Ethiopia (14). The keyword co-occurrence analysis revealed four major thematic research clusters: nanoparticle toxicity and environmental impacts, nanoparticle-enhanced microalgae biorefinery for biofuel production, green synthesis of nanoparticles using microalgae, and metal oxide nanoparticle interactions with freshwater microalgae. Overall, this bibliometric analysis highlights growing interdisciplinary focus and provides valuable insight for guiding future research directions in microalgae–nanotechnology applications.

Keywords: Nanomaterials, Microalgae, Biomass, Cultivation and Harvesting

1. Introduction

Using fossil fuels has played a significant role in causing air, water, and soil pollution, adversely affecting public health (Fawzy et al., 2020; Saravanan et al., 2023). With a substantial impact on the environment, fossil fuels are accountable for producing approximately 2 billion tonnes of CO₂ in 2021 and roughly 300 million tonnes of CO₂ in 2022 (Olabi et al., 2022). Despite developing alternative and new energy resources, fossil fuels remain the world's leading energy resource (Gao et al., 2020). Soon, a biofuel made from living biomass is expected to gain widespread acceptance as a sustainable and eco-friendly source of energy (Mujtaba et al., 2023; Saleem, 2022). Algae-based biofuels are less harmful to the environment due to their minimal dependence on land and freshwater resources, adequate and fast growth rate, enabling short harvesting cycles (within 1-10 days), and their cultivation does not require arable land or freshwater, thus minimising competition with food production and the use of resources (Hasnain et al., 2023). Still, algal fuels have not touched commercial

production yet due to limitations in biomass harvesting.

Nanotechnology has become a worldwide focus of researchers to enhance microalgae growth by improving biomass production, biofuels, and high-value products (Vasistha et al., 2021; de Moraes et al., 2021). Therefore, utilising nanomaterials in microalgae biorefinery has a high likelihood of enhancing the economic viability of microalgae biorefinery (Khanra et al., 2020). Despite the benefits of nanoparticles, examining their characteristics, concentrations, and potential interactions with microalgae is imperative. Because nanoparticles can potentially cause harm to microalgae due to their toxic effects (Hazeem, 2022; Abbasi et al., 2023). Thus, assessing microalgae type, size, surface charges, and nanoparticle concentrations is essential. Such assessments are necessary to properly apply nanoparticles in microalgae-based food and pharmaceutical industries and microalgae growth and development (Alishah Aratboni et al., 2023). Interestingly,

nanoparticles exhibit antimicrobial properties that hinder the growth of undesirable bacteria, fungi, and other microbes that may compete with microalgae for nutrients during the cultivation phase (Wan Mahari et al., 2022).

Nanomaterials are promising for growing, harvesting, lipid extraction, and biofuel quality (Hossain et al., 2019). Employing nanoparticles during microalgae cultivation can promote growth, but an excessive amount can be detrimental. Hence, optimising nanoparticle concentration is critical for successful microalgae cultivation (Wan Mahari et al., 2022). This approach can enhance the biomass of algae and improve the efficiency of biofuel production. Nanoparticles can stimulate the production of primary metabolites and enhance lipid sources, which can increase biofuel production (Agarwal et al., 2022; Hasnain et al., 2023). Overall, utilising nanoparticles in biofuel production appears to be a viable approach. However, continuing research is essential to fully understand nanomaterials' potential benefits and drawbacks throughout biorefinery processes.

The emergence of nanomaterials in microalgae biorefinery is a rapidly growing area of research that has attracted significant attention in recent years. This area of research involves the application of nanomaterials to improve the efficiency and effectiveness of microalgae-based biorefineries. The use of nanomaterials in microalgae biorefinery has been shown to enhance various processes, such as growth and biomass production (Varaprasad et al., 2022; Shrasti Vasistha et al., 2021a; Wang et al., 2021), lipid production (Rana et al., 2020), and biomass harvesting (Gerulová et al., 2022; Mohamed et al., 2022), which are crucial for the successful operation of a biorefinery. However, despite the increasing interest in this field, there is a lack of systematic analysis of the research trends and patterns in this area. Therefore, the present study brings a novel perspective to nanomaterials in microalgae biorefinery by conducting a comprehensive bibliometric analysis of research trends. This review paper fills the literature gap by systematically analyzing nanomaterials use in microalgae biorefinery and identifying research hotspots. The findings of this study provide valuable insights into the emerging field of microalgae technology and pave the way for future research and advancements in sustainable biotechnology.

2. Bibliometric review methodology

2.1. Data Source and Search Strategy

All data used in this study were retrieved from the Web of Science Core Collection (WoSCC) on 31 October 2025. The literature search was restricted to publications from 2010 to 2025 using the following keyword query: ("Microalgae" OR "Micro-algae" OR "Alga*") AND ("Nanoparticle" OR "NP" OR "NPs"). The 2010–2025 period was chosen because it represents the most active phase of research

connecting nanoparticles with microalgae. Publications before 2010 were too limited for reliable trend analysis. Extending coverage to 30 October 2025 allows a complete fifteen-year overview of the field's development and emerging research directions. Only research published in English language were considered for inclusion. Furthermore, under the advanced search settings, the query was refined to the following subject areas: Energy, Environmental Science, Chemical Engineering, Biochemistry, Agricultural and Biological Sciences, Genetics and Molecular Biology, Chemistry, Immunology and Microbiology, and Materials Science. To ensure consistency, all records were downloaded on the same day to avoid variations resulting from database updates. A total of 2,330 relevant publications were identified and included in this bibliometric analysis (Fig. 1).

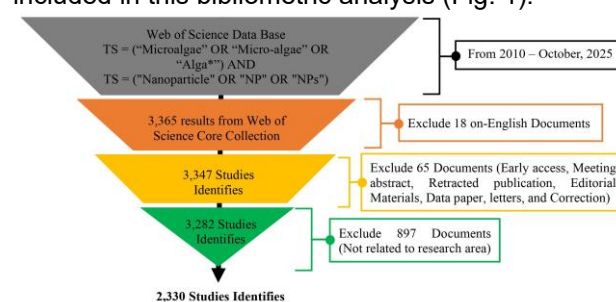


Figure 1: Search strategy employed for the bibliometric analysis. using the following keywords: ("Microalgae" OR "Micro-algae" OR "Alga*") AND ("Nanoparticle" OR "NP" OR "NPs").

2.2. Bibliometric Analysis

To examine bibliometric trends, data were retrieved from the Web of Science database and exported in CSV format. The WoS "Analyze Results" feature was initially employed to extract publication trends, subject areas, and countries contributions. For bibliometric mapping and visualization, VOSviewer version 1.6.19 (developed by Leiden University, Netherlands) was used. This software enabled the construction of keyword co-occurrence maps through its robust clustering and visualization capabilities. Additionally, Microsoft Excel 365 was utilized to support further quantitative analyses and create descriptive visual representations of the data. This analysis provides comprehensive results on global research trends within the field of microalgae biorefineries, therefore providing valuable guidance for the determination of future research directions.

3. Results and discussion

3.1. Document types

The analysis of document types in the WoS database reveals that research articles dominate, comprising 83.4% of the indexed publications, highlighting their role as the primary medium for disseminating original research (Figure 2). Review articles account for 10.8%, playing a crucial role in synthesizing existing knowledge and guiding future research. Conference papers represent 5%,

emphasizing their significance in presenting emerging studies and fostering academic discussions, though their classification in bibliometric databases can vary (Tang & Hu, 2020). Book chapters, making up only 0.8%, contribute to in-depth scholarly discussions but are less commonly indexed. This distribution reflects conventional academic publishing trends, with journals being the preferred platform for knowledge dissemination, while other formats supplement specific scholarly needs. The substantial increase in article numbers demonstrates increased interest in this research field.

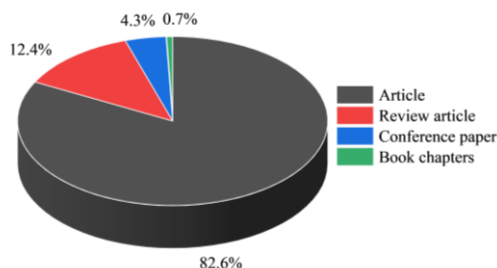


Figure 2: Types of documents in Web of Science database

3.2. Trend in Publications

The publication trend in the Dimensions database from 2010 to 2025 demonstrates a steady and significant increase in academic interest in microalgae research, reflecting its growing relevance in addressing global challenges (Figure 3). Beginning with 33 publications in 2010, the field experienced gradual growth until 2018, followed by a sharp surge from 2019 onward, peaking at 274 publications in 2024. This upward trajectory aligns with advancements in open-access publishing, interdisciplinary collaborations, and increased funding initiatives (Huang *et al.*, 2022). The slight decline observed in 2023 may be attributed to external factors such as funding fluctuations or publication backlogs, which are common in scientific research. Overall, this trend underscores the expanding scholarly focus on microalgae research and its sustained momentum over the years.

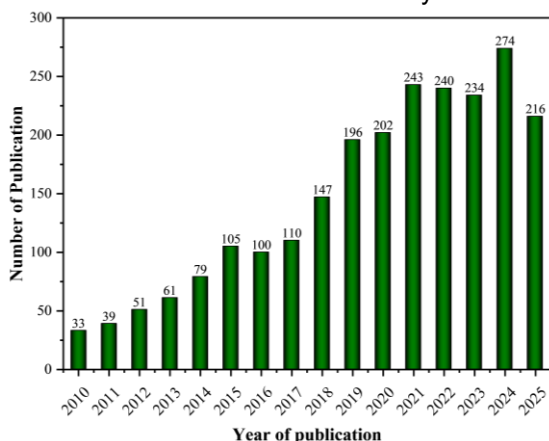


Figure 3: Annual number of publications from 2010 to 31st October 2025 (Source: WoS 2025)

The distribution of publications across subject areas, as shown in Figure 4, highlights the interdisciplinary nature of research involving microalgae and nanoparticles. Environmental Sciences and Ecology is the most dominant field, reflecting strong scientific interest in microalgae for bioremediation, carbon capture, and wastewater treatment. Substantial contributions from Chemistry and Science & Technology emphasize research on biomass composition, metabolite extraction, and nanotechnology applications for enhancing microalgal productivity and value-added products. Engineering demonstrates its key role in optimizing cultivation systems, photobioreactor design, downstream processing, and scaling up technologies for industrial applications. Biotechnology and Applied Microbiology contributes to genetic and metabolic improvements aimed at increasing biomass yield, lipid accumulation, and stress tolerance. Research in Marine & Freshwater Biology, Toxicology, and Materials Science focuses on strain adaptability to environmental conditions, nanoparticle toxicity assessments, and microalgae-derived biomaterials. Although fields such as Energy & Fuels, Plant Sciences, and Microbiology contribute smaller percentages, they support advancements in microalgae-based biofuels, biofertilizers, pharmaceuticals, and biodegradable materials. Overall, the diverse subject areas highlights a strong collaborative approach that integrates environmental, biological, chemical, and engineering innovations.

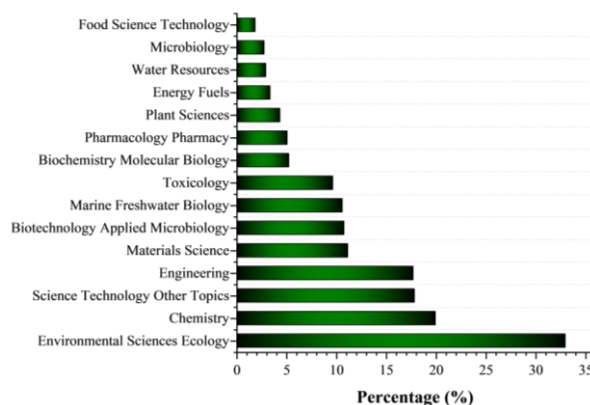


Figure 4: Major Research Area of microalgae research in biorefineries

The global distribution of research on microalgae-nanoparticle studies show clear regional distribution, with output concentrated mainly in Asia, Europe, and North America (Figure 5). China (498) and India (476) lead globally, highlighting Asia's strong dominance, followed by the USA (314). Europe is another major hub, with notable contributions from Spain, Italy, Germany, France, and the UK. In the Middle East, Saudi Arabia and Iran show growing involvement, while Brazil (112) leads South America and Australia (81) leads Oceania. In Africa, Egypt (128) contributes the most, followed by South Africa

(24), Nigeria (15), and Ethiopia (14). Overall, Asia remains the most productive region.

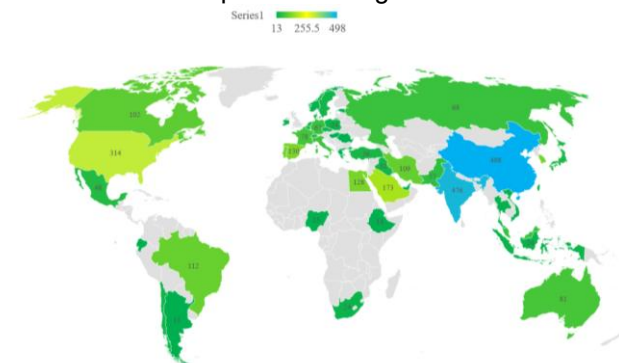


Figure 5: Global distribution of research publications on microalgae–nanoparticle studies

3.3. Author keywords

The author keyword through co-occurrence analysis generated by VOS viewer is depicted in Fig. 5. The minimum number of a keyword occurrence was set to 3 and 44 keywords were deemed significant enough to meet the threshold and were included in the keyword co-occurrence network. The analysis of keywords highlighted four distinct clusters represented by different colors in the visualization map of the network. The clusters have been classified based on their respective colors and sizes, where larger circles represent keywords of greater significance and frequency. The strength of the relationship between two keywords is indicated by the strength of the link and the distance between them, with a closer distance indicating a closer relationship.

Cluster 1 (Red) which include the keywords such as toxicity, ecotoxicity, engineered nanoparticles, oxidative stress, adsorption, aggregation, marine microalgae and growth inhibition. This cluster emphasizes the environmental and biological impacts of engineered nanoparticles on microalgae, particularly focusing on toxicity mechanisms, cellular stress responses, and ecological risks. Studies in this cluster investigate how nanoparticles interact with microalgal cells through processes such as adsorption and aggregation, leading to oxidative stress and growth inhibition. The recurring focus on ecotoxicity and marine microalgae highlights growing concerns regarding environmental safety, biodegradation, and regulatory implications associated with nanoparticle release into aquatic systems.

Cluster 2 (Green) contains major keywords including microalgae, *Chlorella vulgaris*, biomass, lipid production, biodiesel, harvesting, flocculation, extraction, nutrient removal and algae. This cluster centers on the biotechnological application of nanoparticles to enhance microalgae-based biorefineries, especially for biofuel production and biomass valorization. Key topics include improving microalgal biomass accumulation, lipid productivity, and biodiesel yield, while also addressing efficient

harvesting, flocculation, and extraction processes. The frequent occurrence of “*Chlorella vulgaris*” reflects its role as a model organism in microalgal bioprocessing. Overall, this cluster highlights the potential of nanoparticles to optimize biorefinery efficiency and support sustainable bioenergy generation.

Cluster 3 (Blue) contains keywords such green synthesis, biosynthesis, silver nanoparticles, gold nanoparticles, microalgae and green chemistry. This cluster highlights the eco-friendly synthesis of nanoparticles using microalgae and biological systems, aligning with green chemistry and sustainable nanotechnology principles. Research in this cluster focuses on the biological production (biosynthesis) of metal nanoparticles such as silver and gold, using microalgae as reducing and stabilizing agents. The prominence of “green synthesis” reflects a sector shift away from chemical and toxic routes, emphasizing environmentally safe nanoparticle generation for industrial and biomedical applications.

Cluster 4 (Yellow) contain major keywords: ZnO, cytotoxicity, freshwater, nanoparticles, oxidative stress. This cluster concentrates on the interaction between metal oxide nanoparticles especially zinc oxide (ZnO) and freshwater microalgae, with emphasis on cytotoxic and physiological responses. Research here examines the biochemical and cellular effects resulting from nanoparticle exposure, including cytotoxicity and oxidative stress pathways. The cluster highlights ZnO as a widely studied nanoparticle due to its extensive use, environmental release, and potential risks, reflecting the scientific community’s interest in assessing nanoparticle behavior in freshwater ecosystems.

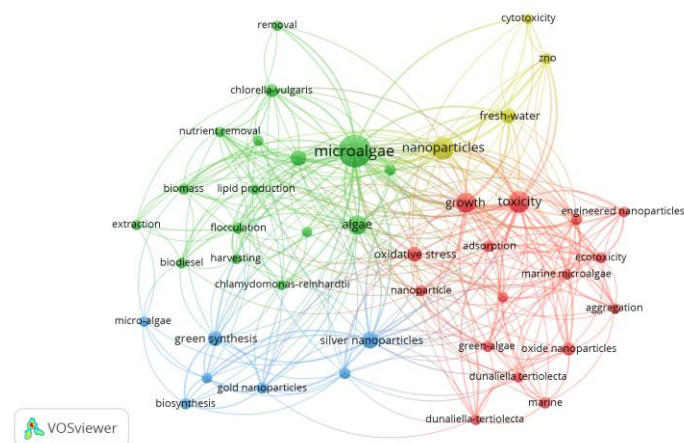


Figure 5: Co-occurrence network visualization of the author keyword.

3.4. Discussion of the major research field

In this bibliometric analysis, five (5) prominent themes were identified through content analysis and are discussed in the following subsections:

3.4.1. Nanoparticles used in Microalgae Cultivation

Improvements in growth, lipid accumulation, biomass production could be achieved by using

nanoparticles in microalgae cultivation (Dey et al., 2023; Alishah et al., 2023). Metallic and hybrid nanoparticles are becoming increasingly common in microalgae cultivation. Numerous studies have investigated the use of diverse nanomaterials to enhance microalgae cultivation, improving growth and increase biomass yield (He et al., 2017; Rana et al., 2020; Nada et al., 2022; Makhi et al., 2022). During microalgae growth, nanoparticles can serve as a nutritional supplement to enhance growth and improve lipid composition (Wan Mahari et al., 2022). It was reported that when the concentration of nanomaterials is low, it promotes growth, but as the concentration increases, it leads to growth inhibition (Lau et al., 2022). Some previous studies suggest nanoparticles can help address the lack of minerals and nutrients required for microalgal growth (Radzun et al., 2015).

Nanomaterials can affect microalgae growth both directly and indirectly. Nanomaterials can influence microalgae growth through both direct and indirect mechanisms. Directly, nanoparticles may penetrate the cell membrane, leading to the release of intracellular contents. Indirectly, their attachment to the cell surface can interfere with membrane function by blocking active sites or hindering nutrient uptake (Smith et al., 2019). However, high metal concentrations such as Fe can influence the accumulation of lipids in microalgae and potentially compete with the uptake of nutrients (Khoo et al., 2020). Also, adding nanoparticles to microalgal cultures can enhance photosynthesis and promote algae growth by improving CO₂ adsorption and light conversion efficiency. On the other hand, the adverse effects of nanomaterials on algal growth depend on several factors, including their concentration, physicochemical properties (such as particle size, crystal structure, and oxidation state), the composition of the culture medium, and the specific algal species involved (Salehi and Wang, 2022).

Table 1: Different types of nanoparticles used for microalgae cultivation.

Microalgae species	Cultivation medium + NPs	Biomass yield (g/L)	Lipid (%)	Period (days)	Observations	References
<i>C. pyrenoidosa</i>	BG-11 medium + Fe ₂ O ₃ NPs (20 mg/L)	2.14	13.77 wt%	15	Iron oxide NP enhanced both microalgae growth and lipid.	(Rana et al., 2020)
<i>Scenedesmus</i> sp.	BG-11 medium + SiC NPs (150 mg/L)	3.18	40.26	7	SiC NP enhanced substrate use, stress, and lipid biosynthesis efficiency.	(Ren et al., 2020)
<i>Chlorella</i> sp. UJ-3	BG-11 medium + Fe ₃ O ₄ NPs (20 mg/L)	1.2	42.9	12	Fe ₃ O ₄ NP boosted biomass and increased lipid production.	(Wang et al., 2021)
<i>Chlorella vulgaris</i>	BG-11 medium + ZnO NPs (50 mg/L)	2.56	17.58	25	ZnO NP enhanced microalgal biomass and lipids.	(Nada et al., 2022)
<i>Scenedesmus</i> sp.	Bold Basal Medium + CdS NPs (15 mg/L)	2.0	-	10	Low cadmium levels safe; higher concentrations reduce growth and delay.	(Jena et al., 2015)
<i>C. vulgaris</i>	BG-11 medium + SiO ₂ -CH ₃ NPs (0.2 wt%)	1.49	-	10	SiO ₂ -CH ₃ nanoparticles enhanced <i>C. vulgaris</i> growth	(Jeon et al., 2017)

Interestingly, apart from nanoparticles boosting biomass and lipid accumulation by microalgae, their role in enhancing CO₂ biofixation is also significant. Given the critical importance of CO₂ as a carbon source in green algae cultivation, improving its fixation not only enhances algal productivity but also contributes to the mitigation of atmospheric CO₂ (de Morais et al., 2021). The incorporation of nanoparticles into microalgal cultivation systems has been shown to markedly increase the absorption of atmospheric CO₂, thereby elevating biomass yield (Vargas-Estrada et al., 2020). This enhancement is primarily due to the small size of nanoparticles, which provides a larger specific surface area and more adsorption sites, enabling more effective CO₂ capture and prolonged retention during cultivation (Comitre et al., 2021). Remarkably, the addition of nanomaterials to *Chlorella* cultures has led to a 94% improvement in carbon utilization efficiency compared to cultures without nanofibers (Comitre et al., 2021).

Previous studies have demonstrated that certain nanoparticles can enhance microalgae growth and productivity. For instance, low concentrations of TiO₂ nanoparticles were found to stimulate photosynthesis by increasing antioxidant capacity as reported by Xia et al. (2015). Iron-based nanomaterials, valued for their biocompatibility and low toxicity, are also essential for cellular functions in microalgae (Sajjadi et al., 2018). Some nanoparticles, like SiC and g-C₃N₄, promoted biomass and lipid accumulation in *Scenedesmus* sp., while others like TiO₂ and TiC had inhibitory effects. Additionally, gold nanoparticles showed no adverse effect on the photosynthetic system of *C. zofingiensis*. Overall, nanoparticles hold potential to improve microalgae cultivation by enhancing light absorption, CO₂ fixation, and nutrient availability, as summarized in Table 1.

<i>Chlorella vulgaris</i>	BG-11 medium + α -Fe ₂ O ₃ NPs (50 mg/L)	Reduced growth by 41.2%	39.7	12 – 16	Low α -Fe ₂ O ₃ nanoparticle doses maintained similar growth and lipid levels.	(Bibi et al., 2021)
<i>Amphiprora</i> sp.	F2 medium + SiO ₂ NPs (30 mg)	0.2567	81.47	29	SiO ₂ nanoparticles enhanced oil yield; 30 g gave optimal results.	(Jayakumar et al., 2021)
<i>S. obliquus</i>	B.B.M. + Al ₂ O ₃ NP (10 mg/L)	0.55	NR	14	The impact of Al ₂ O ₃ NP on <i>S. obliquus</i> was minimal and only noticeable at high concentrations	(Ahn et al., 2022)

NPs = Nanoparticles, BBM = Bold basal medium

3.4.2. Nanoparticle-Assisted Microalgae Biomass Harvesting

Harvesting processes separates microalgae biomass from the growth culture medium, which presents a significant challenges in producing microalgae biomass (Hadiyanto et al., 2022; Matter et al., 2019). Nanoparticles have demonstrated their usefulness in harvesting microalgae in both their singular form and hybrid form with different compounds. Typically, harvesting microalgal biomass contributes approximately 20–30% of the total production cost; however, this expense could be reduced through the application of nanoparticles (Duman et al., 2019). Nanoparticles can collect biomass from microalgae through flocculation (Lee et al., 2015), and various factors, such as microalgae size, shape, concentration, environmental conditions, and surface charge, can influence flocculation. The flocculation process is initiated by the electrostatic attraction between positively charged nanoparticles and the surface of microalgal cells, and this interaction is closely influenced by the zeta potential (Khanra et al., 2020; Singh et al., 2022). A higher zeta potential value indicates a stronger attraction between nanoparticles and the negatively charged microalgal surface, promoting stable aggregate formation and efficient flocculation (Mamdouh et al., 2022).

The utilization of nanoparticles has been recognized as a practical approach for enhancing the efficiency of microalgae harvesting, owing to their ability to offer advantages such as reduced energy consumption, improved harvesting performance, recyclability, and faster processing time (Lee et al., 2015). Nanoparticles for microalgae harvesting have recently gained attention, and it has been suggested that their usage may be restricted to biofuel production. The investigation of the potential use of nanoparticles in harvesting microalgae biomass for food and feed production is under scrutiny (Singh et al., 2022). Although, it was reported that applying nanomaterials in food packaging can increase thermal resistance and provide potent antimicrobial

activity (Góral et al., 2023). The incorporation of nanoparticles in the microalgae harvesting process has yielded impressive outcomes, as the harvesting efficiencies of different microalgae species exceeded 90% (shown in Table 2). The table provides a comprehensive overview of various nanoparticle types, their concentration, microalgae species, and the harvesting efficiency achieved. The nanoparticles mentioned in the table include magnetite, iron oxide, chitosan, silica, aluminium, and calcium. Among the various nanoparticles, magnetite-based nanoparticles (Fe₃O₄ NPs) are among the most commonly used nanoparticles for microalgae harvesting. Some of the characteristics of Fe₃O₄ NPs include high surface area, which allows them to interact effectively with microalgae and form stable aggregates, resulting in efficient flocculation. Interestingly, Fe₃O₄ nanoparticles have a superparamagnetic property, which enables them to be easily separated from the microalgae biomass using a magnetic field. This unique characteristic has significant advantages over other harvesting techniques. It allows for rapid, low-energy separation of microalgal biomass from the harvesting media, resulting in higher harvesting efficiency and cost reduction (Kim et al., 2018; Patel et al., 2022). Other metal-based nanoparticles such as CaO, Ti, NiO, and ZrO₂ are promising for microalgae harvesting. These nanoparticles exerted their action by adsorbing onto the surface of the microalgae cells and then aggregating the cells into larger flocs that can be easily separated from the growth medium. This mechanism is based on electrostatic interactions between the positively charged nanoparticles and the negatively charged microalgae cells, allowing efficient flocculation (Khanra et al., 2020; Mohamed et al., 2022). It is vital to ensure that the use of metallic nanoparticles in microalgae harvesting does not cause harm to the environment, the microalgal biomass, or any subsequent applications of the harvested biomass. (Hidalgo et al., 2023).

Table 2: Application of Different Nanoparticles for harvesting microalgae biomass.

Microalgae species	Nanoparticles (Concentration)	Flocculation Conditions	F.E (%)	Time (min.)	References
<i>Synechocystis</i> sp. PCC 6803	CaO NPs (250 mg/L)	pH 9.8, 45 °C	99.3	5	Mohamed et al., 2022
<i>Chlorococcum</i> sp.	Ti NPs (15 ppm)	NR	82.46	45	(Khanra et al., 2020a)
<i>C. vulgaris</i>	NiO NPs (5 mg/L)	pH 7	98.75	1	(Huang and Kim, 2016)
<i>Chlorococcum</i> sp.	ZrO ₂ NPs (15 mg/L)	pH 10	99.59	4	(Khanra et al., 2020b)
<i>Scenedesmus</i> sp.	Fe ₃ O ₄ NPs (0.14 g/L)	pH 7.38	95	8	(Abo Markeb et al., 2019)
<i>C. sorokiniana</i>	Fe ₃ O ₄ NPs (200 mg/L)	pH 3, 450 rpm	>99	5	(Patel et al., 2022)
<i>S. obliquus</i>	MgAC- Fe ₃ O ₄ NPs (4.19–4.92 mg/L)	pH 4.0	> 80	10	(Kim et al., 2018)
<i>C. minutissima</i>	Chitosan-TiO ₂ NPs (90 mg/mL)	pH 4–8	> 98	2	(Almomani and Bhosale, 2021)
<i>C. sorokiniana</i>	MnFe ₂ O ₄ NPs (0.15 g/g)	pH 2–12	90	5	(Lapeñas et al., 2022)
<i>Tetraselmis indica</i>	LS NPs (140 mg/L)	pH 7, 50°C, 150 rpm.	86	90	(Kumar et al., 2022)
<i>A. protothecoides</i>	PEI coated Fe ₃ O ₄ NPs (0.268 mg/L)	pH 4	99	1.5	(Kucmanov et al., 2022)
<i>C. minutissima</i>	Chitosan-TiO ₂ NPs (0.09 g/g)	pH 7 ± 0.2	> 98	2	(Dineshkumar et al., 2017)
Mixed algae	MgAC- Fe ₃ O ₄ NPs (4.19–4.72 g/L)	pH 4	> 80	10	(Kim et al., 2018)
<i>C. vulgaris</i>	Cationic starch NPs (7.1 mg/L)	pH 11.84	92	NR	(Bayat Tork et al., 2017)

NR = Not Reported, LS = Lime Sludge nanoparticles, PEI = Polyethyleneimine, and MgAC = Magnesium Aminoclay

3.4.3. Nanoparticles for Microalgae Biodiesel Production

Several categories of nanomaterials, such as nanofibers, nanotubes, and nanometal, have been utilized to assess their effect on the generation and growth of biofuels and biodiesels (Gardy et al., 2019; Arya et al., 2021). The characteristics of nanoparticles such as their high surface-to-volume ratio, strong dispersibility, high reactivity, and selectivity, have been shown to enhance the production of biofuels from microalgae biomass. It was shown that applying nanoparticles in pretreating biomass from microalgae can enhance substrate digestibility and increase biodiesel production yield (Ahmed et al., 2023). During the lipid extraction stage, nanoparticles have shown great promise by breaking down the rigid cell walls of microalgae, thereby facilitating the efficient release of intracellular lipids. This improved cell disruption enhances the digestibility of the substrate, ultimately leading to higher biofuel yields during the production process (Ahmed et al., 2023). Compared to traditional solvents used in extraction processes, nanoparticles are less harmful, more stable, and reusable (Nguyen et al., 2019). According to Duraiarasan et al. (2016), magnetic nanoparticles were utilized to break down the polysaccharide cell walls of microalgae for lipid extraction, and the addition of these nanoparticles to immobilized cellulase enhanced cell wall disruption, resulting in a significant 93.56% improvement in biodiesel

production. Similarly, Vasistha et al. (2021) reported that the application of titanium (Ti) nanoparticles at a concentration of 15 mg/L resulted in a high lipid extraction efficiency of 74.29% in *Chlorococcum* sp., further demonstrating the potential of nanoparticles in improving biofuel recovery from microalgae. The efficiency of lipid extraction is influenced by the concentration of nanoparticles used, as the optimal dosage can enhance cell wall disruption and maximize lipid release. Razack et al. (2016) evaluated the impact of silver nanoparticles on the intracellular oil extraction of *Chlorella vulgaris* and reported that increasing the nanoparticle concentration from 50 to 150 µg/g led to a notable rise in oil extraction efficiency from 8.44% to 17.68%, highlighting the importance of dose optimization in nanoparticle-assisted lipid extraction processes.

Apart from their role in lipid extraction, nanomaterials have recently been investigated as potential catalysts for enhancing the efficiency of the esterification process, offering improved reaction rates, higher biodiesel yields, due to their high surface area, catalytic activity, and reusability. One of the most commonly used nanoparticles in this context is calcium oxide, which has been extensively studied for biodiesel production because of its strong basic properties, widespread availability, low environmental impact, long catalyst lifetime, and overall cost-effectiveness (Colombo et al., 2017). Other functionalized nanoparticles, such as methyl-

functionalized silica ($\text{SiO}_2\text{-CH}_3$) and unmodified silica (SiO_2), have been reported to significantly enhance the transesterification process, leading to a maximum fatty acid methyl ester (FAME) yield of 1.00 g/L in *C. vulgaris*. Specifically, the incorporation of 0.2% (wt%) $\text{SiO}_2\text{-CH}_3$ nanoparticles resulted in a remarkable 610% increase in FAME production, demonstrating their effectiveness in boosting biodiesel yield. In summary, nanomaterials have shown promising results for the extraction and esterification of microalgae oil. They effectively increase lipid extraction efficiency, promote higher biodiesel production yields, and provide a sustainable and cost-effective. Figure 6 illustrates nanoparticle roles in the microalgae biorefinery process. The figure shows nanoparticle-assisted microalgae cultivation, biomass harvesting, lipid production, oil extraction, and biodiesel conversion. It also illustrates the potential for recycling nanoparticles within the process.

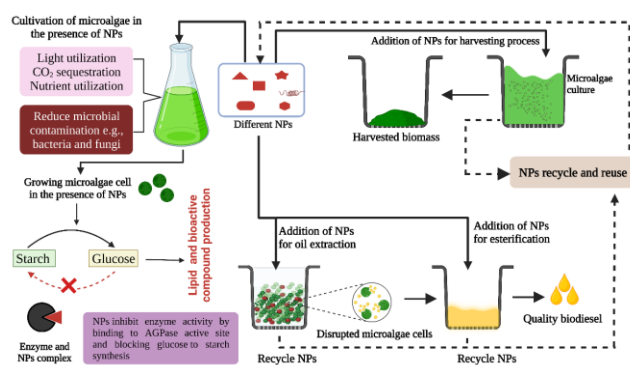


Figure 6: Overview diagram illustrating the multifaceted role of nanoparticles in different phases of microalgae biorefinery

3.4.4. Nanoparticles in Microalgae Bioactive Compounds Production

The production of valuable bio-based products has gained significant interest in scientific and industrial domains owing to its advantageous effects on health and the economy. Bioactive compounds are commonly produced using two methods which are synthetic techniques that involve petrochemical sources or biological pathways present in organisms such as animals, plants, and various microorganisms (Li et al., 2020). Within the group of organisms that produce bioactive compounds naturally, microalgae strains have gained significant interest due to their capability to store significant quantities of bioactive compounds under stressful conditions (Lu et al., 2020; Nasri et al., 2021). Interestingly, integrating nanotechnology in microalgal biorefinery is vital for minimizing costly procedures related to algae harvesting. Furthermore, incorporating nanomaterials in microalgae culture has been shown to stimulate the production of bioactive compounds and other metabolites in algae with pharmaceutical and nutraceutical characteristics (Taghizadeh et al., 2020).

Studies have explored the impact of various types of nanoparticles as stressors on promoting bioactive compound production from microalgae. For instance, Estime et al. (2015) developed a polymer film consisting of silver nanoparticles. They found that this film increased the absorption of blue light in *Chlamydomonas reinhardtii* culture, resulting in a 25% increase in biomass and a 35% increase in chlorophyll and carotenoids by the 10th day of incubation. Hee et al. (2020) conducted an experiment exposing *H. pluvialis* to varying Ag nanoparticle concentrations (10, 100, and 200 ppm). The results indicated that the growth of *H. pluvialis* was initially slow, but it gradually increased as it adapted to the stressful environment. The highest carotenoid production of 0.458 ± 0.002 g/L was achieved at a concentration of 10 ppm of Ag nanoparticles. The researchers observed that carotenoid production was increased in the presence of Ag nanoparticles, regardless of their concentration, with day 14 showing higher production. Li et al. (2020) combined the microalgae *C. zofingiensis* with gold nanoparticles (Au-NPs) to enhance photosynthesis and the production of carotenoids. The findings indicated that *C. zofingiensis* could take up smaller Au nanoparticles (5 nm), which boosted carotenoid production (10.7 ± 1.2 mg/L), a 42.7% increase compared to the control. They also noticed that using Au nanoparticles significantly increased the relative electron transport rate in photosystem II and levels of reactive oxygen species (ROS) in the microalgae. As a result, overall photosynthesis towards carotenoids was enhanced. Table 3 highlights the effectiveness of various nanomaterials in enhancing the production of bioactive compounds across different microalgae species. Notably, nanoparticles such as TiO_2 , ZnO, Ag, Au, and functionalized resins have significantly boosted yields of astaxanthin, carotenoids, β -carotene, TAGs, and phycocyanin. However, the outcomes vary depending on the type and concentration of nanomaterial, microalgal species, and culture conditions, indicating the importance of optimization for maximum productivity.

3.4.5. Synergistic Effect of Nanoparticles and Microalgae for Wastewater Treatment

Microalgae can efficiently remove organic pollutants, heavy metals, and emerging contaminants due to several advantageous properties, such as their high affinity for nutrients, rapid absorption of metals, low energy consumption, environmentally-friendly application, low-cost implementation, and ability to absorb metals in both high and low concentrations (Syafiuddin and Boopathy, 2021). Most conventional wastewater treatment processes are ineffective in removing most emerging contaminants (ECs) from wastewater. The presence of higher levels of organic pollutants, such as nitrogen, phosphorus, and even emerging pollutants, such as antibiotics, in the environment due to inadequate treatment is

causing environmental problems that have adverse effects on both the aquatic ecosystem (eutrophication) and human health (Azzam et al., 2017; Wan Mahari et al., 2022).

Most traditional methods of treating wastewater employ biological methods to remove substances such as organic matter and nutrients through aerobic and anaerobic systems. Recently, research has demonstrated that combining nanomaterials with microalgae is a more efficient method of treating wastewater and has the potential to enhance microalgae growth (Shen et al., 2020;

Shrasti Vasistha et al., 2021; (Kashyap and Kiran, 2021). Interestingly, nanoparticles can address nutrient deficiencies in microalgae cultivation in wastewater or ponds by encouraging rapid absorption of nutrients, lipid accumulation, and antibacterial properties, contributing to rapid microalgae growth. However, to achieve the desired growth of microalgae, nanoparticles need to be used in low concentrations (Chen et al., 2018; Shen et al., 2020).

Table 3: Nanoparticle-Enriched Cultivation Media on Bioactive Metabolite Production from Different Microalgae species

Medium incorporated with nanoparticles	Microalgae Cultivated	Compound Produced	Yield	References
NIES-C + MgAC NP (1.0 g/L)	<i>Haematococcus pluvialis</i>	Astaxanthin	10.3 ± 0.4 mg/mL	(Kim et al., 2020)
BG-11 + ZnO NP (50 mg/L)	<i>Chlorella</i> sp.	Carotenoids	1.23-fold	(Kaliaturthi et al., 2019)
Glucose, meat peptone and 5 g/L NaCl + TiO ₂ NP (0.005 g/L)	<i>Dunaliella salina</i>	b-carotene	25.23 mg/g	(Gonabadi et al., 2022)
F/2 + Gd ₂ O ₃ NPs (100 µg/mL)	<i>Dunaliella salina</i>	Carotenoid	150 % (1.5-fold)	(Makhi et al., 2022)
TAP medium + engineered resin NPs (160 mg/L)	<i>Chlamydomonas reinhardtii</i>	Triacylglycerols (TAG)	520% (5.2-fold)	(Lu et al., 2023)
Carbon nanodots (1.0 mg/mL)	<i>Haematococcus Pluvialis</i>	Astaxanthin	10.0 (1.8-fold)	(Choi et al., 2020)
BBM + Ag NPs (10 ppm)	<i>Haematococcus pluvialis</i>	Carotenoid	0.458 mg/mL	(Bakhtiari et al., 2020)
BBM + Fe NPs (2.49 mg/L) and Zn NPs (4.41 mg/L)	<i>Haematococcus pluvialis</i>	Astaxanthin	7.29 mg/mL	(Rastar et al., 2018)
Zarrouks + tungsten oxide NPs (0.001 g/L)	<i>Spirulina platensis</i>	Phycocyanin	90%	(Padash et al., 2023)
OHM + ZnO NP (200 µg/mL)	<i>H. pluvialis</i>	Astaxanthin	~ 20 mg/g	(Nasri et al., 2021)
BG-11 + TiO ₂ NP (0.1 g/L)	<i>Coelastrella</i> sp.	Carotenoid	13.15 mg/g	(Pushpalatha et al., 2021)
BG11 + 0.5% glucose + AuNP (40 ppm)	<i>C. zofingiensis</i>	Carotenoids	10.7 mg/mL	(Li et al., 2020)

MgAC = Magnesium aminoclay nanoparticles, TAP = Tris–acetate phosphate medium, BBM = Bold Basal Medium, OHM = Optimal haematococcus medium

Interestingly, metallic nanoparticles present in wastewater can serve as supplementary nutrients in microalgal growth media, offering a sustainable and cost-effective strategy to boost lipid accumulation, cellular metabolism, and overall biomass productivity (Nguyen, 2019; Abideen et al., 2022; Alishah et al., 2023). In this context, the conjugation of nanoparticles with microalgae for wastewater treatment has emerged as a novel and promising research direction. Several studies have demonstrated that nanoparticles, particularly iron oxide, can enhance the nutrient removal efficiency of microalgae when treating wastewater. For example, ZnO nanoparticles have garnered significant attention due to their strong pollutant adsorption capacity, high surface reactivity, and ability to absorb visible light (Sadegh et al., 2017). Their wide bandgap (3.37 eV) and high exciton binding energy (60 meV) contribute to enhanced surface adhesion and photocatalytic activity (Li et al., 2019), making them effective in removing contaminants. When

conjugated with microalgae, ZnO nanoparticles have been shown to further augment the removal of nutrients and pollutants from wastewater (Govarthanan et al., 2020; Hesni et al., 2020). In summary, integrating nanomaterials with microalgae offers a promising pathway for advanced wastewater treatment, combining pollutant removal with biomass generation (Table 4). Nevertheless, further research is essential to fully understand the potential risks of nanoparticle use, optimize operational parameters such as nanoparticle type, concentration, and size, and tailor approaches to different wastewater compositions and microalgal species.

3.4.6. Toxicity of Nanomaterials on Microalgae

The toxicity of nanoparticles is influenced by several factors, including their characteristics, such as particle size, oxidation state, crystal structure, the concentration of nanoparticles, the cultivation media used, and the specific species of microalgae being studied (Hazeem, 2022). The nanoparticles toxicity

on microalgae is attributed to the oxidative stress induced by generating reactive oxygen species (ROS) (Chang et al., 2012). ROS are highly reactive molecules that can cause oxidative stress and damage to microalgae cells (Fazelian et al., 2019). The inhibition of growth in microalgae may occur

due to altering their photosynthetic activities because photosynthesis is responsible for light harvesting, energy transfer, and light-energy conversion, which are crucial for the growth and survival of microalgae (Xiong et al., 2023).

Table 4: Microalgae-Nanoparticle Conjugate Systems in Pollutant Removal and Biomass Production

Microalgae sp.	Nanoparticle Conc.	Wastewater	Concentration after treatment (mg/L)	Biomass yield (g/L)	Period (days)	References
<i>Chlorosarcinopsis</i> sp. MAS04	ZnO NPs (10 mg/L)	Primary Treated Wastewater	COD (42±1.0), TN (8.7±0.2), TP (21±0.04) and TOC (11.2±0.05)	3.43	20	(Shrasti Vasistha et al., 2021)
<i>C. vulgaris</i>	Fe ₃ O ₄ NPs (5 g/L)	Local wastewater treatment plant	TN (85%) TP (91.0%)	NR	3 h	(Govarthanan et al., 2020)
<i>C. vulgaris</i>	Fe ₃ O ₄ (5 ppm)	Fish farm effluent	NH ₄ (93.67 %), NO ₃ (92.23 %), NO ₂ (89.3 %), PO ₄ (89.25 %)	NR	10	(Hesni et al., 2020)
<i>C. vulgaris</i>	ZnO NPs (20 mg/L) and Fe ₂ O ₃ NPs (50 mg/L)	Synthetic saline wastewater	TN (55.1%) TP (111%)	2.08	40	(Xia et al., 2021)
<i>C. vulgaris</i>	MWCNTs NPs (1.0 mg/L)	Biogas slurry	COD (90.43±5.15%) TN (77.07±4.12%) TP (78.12±4.33%)	0.81 ± 0.008	9	(Sun et al., 2022)
<i>C. sorokiniana</i> PA.91	MgAC NPs (0.2 g/L)	Municipal wastewater	COD (91.1%)	0.2	15	(Panbehkar and Amini, 2023)

COD = Chemical Oxygen Demand, TN = Total Nitrogen, TP = Total Phosphorus, TOC = Total Organic Carbon, MWCNTs = Multi-walled carbon nanotubes, MgAC-NPs = Magnesium amino clay nanoparticles, PTWW = Primary Treated Wastewater, STWW = Secondary Treated Wastewater

Assessing the toxicity of residual nanoparticles is challenging since it depends not only on the characteristics of the nanoparticles, including their particle size but also on other factors, such as the presence of dissolved organic substances in the water (Zhang et al., 2019). Kang et al. (2014) observed that a high level of nanoparticles could produce a shading impact that diminishes the quantity of light that infiltrates the water, which eventually causes a decline in photosynthetic efficiency. The reduction in the quantity of light accessible to the algal cells results in a reduction in chlorophyll levels and cell development (Fazelian et al., 2019). A study by Saied et al. (2021) reported that the growth of algae was reduced by 22.7%, 35.4%, and 41.1% on day 7, respectively, when exposed to MgO nanoparticles at varying concentrations of 0.8, 8, and 40 mg/L. This reduction in growth was attributed to the formation of agglomerates from nanoparticles, which hindered light penetration and severely impeded algal growth. Recently, Hasnain et al. (2023) examined the effect of Ag nanoparticles on some algal species and reported that the effect of Ag nanoparticles reduced the growth of algae from 5 g initial biomass concentration to 1.8 g in *Oedogonium* sp., 2.3 g in

Ulothrix sp., 1.5 g in *Cladophora* sp., and 1.3 g in *Spirogyra* sp. under a concentration of 0.08 mg/L at day 7. Johari et al. (2018) reported that exposure to Ag nanoparticles slowed the growth of *Dunaliella salina*, with toxicity peaking at 1.0 mg/L.

The uptake of nanoparticles by microalgae cells could potentially result in severe damage to organelles, impede respiratory and photosynthesis functions, and even induce cell death (Chen et al., 2019). The toxicity of nanoparticles can differ based on their varying types and characteristics (Parveen et al., 2023). Fazelian et al. (2019) reported that CuO nanoparticles can cause damage to the cell membrane of microalgae when observed under scanning electron microscopy (SEM). Therefore, damage to the cell membrane caused by nanoparticles can result in the leakage of essential cell components and the loss of critical cellular functions, leading to decreased cell viability and growth. Moreover, it can also facilitate the entry of toxic substances into the cell, exacerbating the harmful effects of nanoparticles on microbial cells (Djearmane et al., 2019; Kumar et al., 2023). The growth of *Phaeodactylum tricoratum* was significantly suppressed by titanium dioxide nanoparticles (TiO₂). The study revealed that the

half-maximum effective concentration (EC 50) of TiO₂ nanoparticles was 167.7 mg/L, and the lowest observed effective concentration (LOEC) was 20 mg/L. The diverse morphology of the TiO₂ nanoparticles, which included amorphous, crystalline, and flocculent forms, caused entrapment of the algal cells and direct damage to their cell walls (Wang *et al.*, 2016). Similarly, Widyaningrum *et al.* (2019) revealed that the engineered poly (isobutylcyanoacrylate) resin nanoparticles (iBCA-NPs) with a size of 25 nm could cause sudden cell death in the microalgae *Chlamydomonas reinhardtii*. The toxicity of nanoparticles on microalgal cells is a growing area of concern. Studies have shown that the uptake of nanoparticles by microalgal cells can generate reactive oxygen species (ROS), causing damage to organelles, impairment of respiratory and photosynthetic functions, and even cell death. The toxicity of nanoparticles can vary based on their diverse types and characteristics, and their effects on microalgal growth and photosynthetic productivity can be significant. Factors such as particle size, concentration, and surface area can also influence the toxicity of nanoparticles.

3.4.7. Fate and ecotoxicity of nanomaterials

In recent years, researchers have begun to focus their attention on the fate of nanomaterials in marine ecology, which is an important area of research because nanomaterials are increasingly being used for a variety of purposes; as a result, they are being released into the environment, including water. The fate of nanomaterials, which refers to their behaviour and impacts on the environment, depends on several factors, such as their physical and chemical properties, the conditions of their release, and the characteristics of the environment they are released into (Giri *et al.*, 2022; Singh *et al.*, 2023). Nanomaterials may present risk in terms of toxicity, whereas effectual recovery and reusability remain essential topics of investigation for securing the sustainability of the proposed technology and potential contamination of algal biomass (Reetu *et al.*, 2023).

Research in the laboratory is usually applied to one or two organisms and lasts for hours to days, with very few studies covering a period of months or years; nevertheless, Ag nanoparticles persist from months to years in realistic environments and affect the whole ecosystem (Zhang *et al.*, 2019). Two fundamental factors determine the behaviour and motion of nanoparticles in the environment. These factors include nanoparticles physicochemical properties (such as particle size and shape, aggregation/agglomeration, surface chemistry (e.g., functionalisation), chemical composition, and dissolution), and the surrounding environmental conditions (e.g., oxygen level, natural organic matter, ionic strength and temperature) (Gottschalk *et al.*, 2013; Hazeem, 2022). Therefore, the fate of nanomaterials cannot be determined by a single

factor, as their interactions with the environment and the resulting impacts are influenced by a variety of factors working in combination. Understanding these factors is crucial to managing and minimising any potential adverse effects of nanomaterials on the environment and human health (Zhang *et al.*, 2019). The high ionic strength presence in marine waters will likely cause rapid nanoparticle aggregation. The strong ionic environment reduces the electrostatic repulsive forces between particles, leading to their tendency to come together and form aggregates. Understanding how nanoparticles behave in the marine environment requires investigating their interactions with naturally occurring components of seawater, incredibly natural organic matter (NOM). These interactions play a crucial role in determining the ultimate fate of nanoparticles in aquatic environments (Navarro *et al.*, 2008; Vukosav *et al.*, 2023). According to the research by Zhang *et al.* (2019), the fate and toxicity of silver nanoparticles in realistic environments are influenced by the combined effects of various physicochemical properties, which are cross-correlated. The laboratory studies focus on one or more characteristics which offer only limited insights into the fate and toxicity of nanoparticles in aquatic ecosystems. Therefore, It is essential to consider the interplay between the critical physicochemical properties of nanoparticles when assessing their complete fate and toxicity in a realistic environment (Zhang *et al.*, 2019). Thus, by examining the relationships among the different physicochemical properties, a more comprehensive understanding can be obtained regarding how nanoparticles behave and impact ecosystems.

4. Conclusion

This study provides an overview of global research trends on microalgae–nanoparticle studies from 2010 to 2025, showing the rapid growth of interest in combining nanotechnology with microalgae biorefineries. Research output has steadily increased, with Asia particularly China and India leading, followed by the USA, Europe, and selected countries in Africa, the Middle East, and Oceania. The analysis highlights the interdisciplinary nature of the field including environmental sciences, chemistry, biotechnology, engineering, and materials science, reflecting efforts to improve microalgal biomass production and the recovery of valuable compounds. Keyword analysis revealed four main themes such as nanoparticle-assisted biorefineries, green synthesis of nanoparticles, and interactions between metal oxide nanoparticles and freshwater microalgae and nanoparticle toxicity and environmental effects. Overall, the findings show that nanoparticles can play a significant role in enhancing microalgae biorefinery processes. Future research should focus on ensuring environmental safety, and developing scalable approaches to make these systems practical and sustainable.

Conflict of Interest

The authors declare no conflict of interest.

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