REVIEW



Greening up the fight against emerging contaminants: algae-based nanoparticles for water remediation

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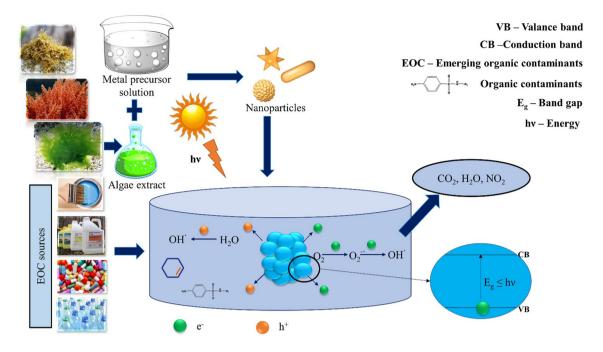
Abstract

Nanoparticles are commonly used for different purposes, including as photocatalysts, biosensors, antibacterial, antifungal, and anticancer agents. Recently, the synthesis of nanoparticles via biological techniques has become popular due to cost efficiency, sustainability, and the least secondary pollutants generation. Plants, algae, and microorganisms are primarily used to synthesize bio-nanoparticles. Algae-based nanoparticles have gained more attention due to their catalytic activity against emerging organic contaminants such as dyes, phenols, and organosulfur compounds. Nevertheless, a systemic evaluation of the potential of algae-based nanoparticles in environmental remediation is yet to be conducted. This paper reviews recent progress in the biosynthesis of algae-based nanoparticles and the potential use of algae-based nanoparticles in environmental remediation. Furthermore, the review examines the factors that affect the properties and behaviors of algae-based nanoparticles. Additionally, the review briefly discusses other medical and industrial applications as well as advantages over physically and chemically synthesized nanoparticles. Challenges associated with the production process and usage of algae-based nanoparticles are also discussed, including the difficulty of predicting the properties of nanoparticles and adapting to large-scale processes. Overall, algae-based nanoparticles have several advantages, including their high stability and surface activity due to the presence of surface functional groups from algae species used for the synthesis of algae-based nanoparticles. However, further research is required to address the knowledge gaps and potential key research areas.

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Graphical Abstract



Keywords Microalgae · Nanoparticle · Photocatalytic degradation · Emerging organic contaminant · Biosynthesis · Nanoflower

Introduction

Modern lifestyles are heavily dependent on synthetic organic compounds that are now being detected in the environment. These, known as emerging organic contaminants (EOCs), were previously not detected or thought to be insignificant (Mukhopadhyay et al. 2022). The main types of EOCs are pharmaceuticals and personal care products (PPCPs), organic dyes, plasticizers, pesticides, and endocrine-disrupting chemicals (Rigoletto et al. 2022). The EOCs enter the surface waters mainly through haphazard disposal and the effluents discharged from the wastewater treatment plants (WWTPs). In natural waters, EOC concentrations range from ng/L to μ g/L. Unfortunately, existing water treatment techniques are not efficient against EOCs (Parida et al. 2021). Therefore, there is an urgent need to develop novel materials for water treatment to remove EOCs.

Nanoparticles have gained attention as a promising approach to removing pollutants present in water (Manikandan et al. 2021). They can be engineered to have specific properties that could be beneficial for water treatment (Karthigadevi et al. 2021). Novel nanoparticles, such as absorbents and catalysts have been developed using biological materials due to their cost-effectiveness, abundance, and renewability (Saleem and Zaidi 2020; Singh et al. 2016). Plants, algae, fungi, bacteria, and cyanobacteria are some of the biological agents used to synthesize bio-nanoparticles (Bandeira et al. 2020). They are considered to be safe and non-toxic allowing them to be used in a wider range of industries (Çalışkan et al. 2020). Biomolecules in these biological materials contain hydroxyl, carboxyl, and amine functional groups, they have the potential to reduce metal ions and cap the nanoparticles (Shafey 2020). Nanoflowers are a type of nanoparticle that has a flower-shaped structure and a size range of 100-500 nm. Compared to regular nanoparticles, the multiple petal layers of nanoflowers greatly enhance their surface area (Tran and Kim 2018). Nanoflowers have been used as adsorbents and photocatalysts to adsorb and photocatalytic degradation of various contaminants (Ahmadpoor et al. 2021). A range of natural sources was used to synthesize nanoflowers, for example, MgO nanoflowers were produced using an aqueous extract of Rosmery fresh flowers (Abdallah et al. 2019). The ZnO nanoflowers synthesized using Chlamydomonas reinhardtii were able to degrade more the 90% of methyl orange (MO) due to their high surface area and unique morphological properties (Rao and Gautam 2016). Also, ZnO nanoflowers were synthesized using leaf extract of Bridelia retusa and seed extract of Withania coagulans (Hasan et al. 2021; Vinayagam et al. 2021).

Algae are widely used to manufacture valuable commercial products, remediate water, and produce biofuel and bioenergy (Lee et al. 2015). They are capable of surviving in trace metal-contaminated water, hence, algae are used to biosynthesize commercially important metallic nanoparticles (Jacob et al. 2019). Hydroxyl and carboxylic functional groups of microalgae help them act as excellent stabilizing and capping agents (Lau et al. 2022; Pugazhendhi et al. 2019). During the past decade, algaebased nanoparticle synthesis has gained interest due to its enhanced removal efficiency and shorter production time (Agarwal et al. 2019). Recently, reviews focusing on the usage of microalgae (Abdelfattah et al. 2023) and algae-based nanomaterials (Agarwal et al. 2019; Chan et al. 2022; Khan et al. 2022) for environmental remediations were published. Abdelfattah et al. (2023) reviewed the contribution of microalgae to the removal of heavy metals and organic pollutants. The main objective of the review published by (Chan et al. 2022) was an elaboration of the synthesis mechanism of algae-based nanoparticles. Despite the review by Khan et al. (2022) providing information on the synthesis process of algae-based nanoparticles and their photocatalytic activity due to its broad scope, it has given lower priority to the photocatalytic degradation of EOCs. Hence, this is the first review which directly aimed at the photocatalytic degradation of EOCs. Furthermore, the present review critically discusses the factors influencing the photocatalytic degradation of EOCs which were not covered by any other reviews published previously although research conducted using algae-based nanoparticles has provided data related to factors influencing photocatalytic degradation. Additionally, the review briefly explains reusability, and biomedical applications

of algae-based nanoparticles, discusses challenges faced during the manufacture and usage of algae-based nanoparticles, and highlights aspects requiring future attention.

Biosynthesis of nanoparticles

Green synthesis of nanoparticles using plants and microorganisms has become popular owing to its cost-effectiveness, simplicity, and environmental friendliness (Singh et al. 2018). The most commonly used biological sources are bacteria (Fang et al. 2019), fungi (Shamsuzzaman et al. 2017), plants (Bao et al. 2021), algae, and photosynthetic organisms (Jeffryes et al. 2015) (Fig. 1). The whole plant or extracts from plant leaves, seeds, flowers, and fruits were used for the synthesis of nanoparticles. These biologically synthesized nanoparticles were effectively used to remove EOCs from water. For example, Iron-based nanoparticles synthesized using green tea extract removed approximately 96% of malachite green (MG), and the removal of MG fitted well with the pseudo first order model (Weng et al. 2013). Xiao et al. (2020) synthesized iron nanoparticles using polyphenols extracted from green tea extract which exhibited high cationic dye (MG, rhodamine, and methylene blue) removal efficiency, and kinetic data were fitted to pseudo first order model. Recently, algae and their extracts widely used to synthesize nanoparticles because they contain biologically active compounds and secondary metabolites that assist in forming metal and metal oxide nanoparticles.

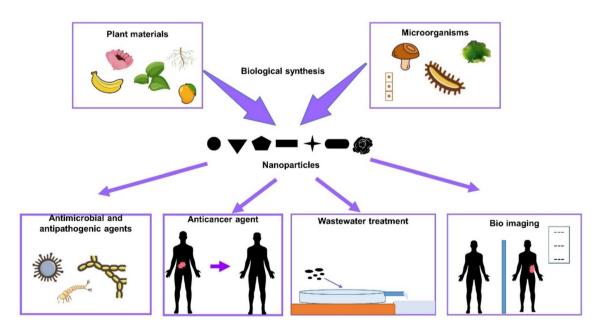


Fig. 1 Biological synthesized nanoparticles and their environmental and biomedical applications

Biological synthesis of nanoparticles using algae

Both living and dead algae can be used to synthesize nanoparticles (Omar et al. 2017). Polypeptides and polysaccharides present inside and outside of the algal cells can capture metals, and the carboxylic groups present on the cell surfaces catch metal ions via electrostatic interactions, which leads to the synthesis of metallic nanoparticles. During the synthesis of bio-nanoparticles, alkaloids, terpenoids, phenolics, and polyphenols act as reducing and capping agents (Negi and Singh 2018), that prevent further reaction and aggregation of nanoparticles and increase their stability (Makarov et al. 2014). Alginic acid, ascorbic acid, protein, carbohydrates, tannins, flavonoids, mannitol, and lipids in Sargassum myricocystum acted as both reducing and stabilizing agents during the synthesis of nanoparticles. Proteins available in algae membranes contribute to the stabilization of nanoparticles and act as templating agents thus, improving their possible usage as nanomedicines (Sharma et al. 2019). Galaxaura elongate, a marine alga is rich in bioactive compounds such as halogenated terpenes, fucoxanthins, flavonoids, C-phycoerythrin, and polyphenols, which contribute to the formation of Ag nanoparticles (AbdEl-Mongy et al. 2017).

The synthesis process of algae-based nanoparticles is comprised of three steps, the first step is the preparation of algal extract in water or an organic solvent by heating or boiling for a certain time duration. Secondly, the preparation of molar solutions of ionic metallic compounds, and finally, the incubation of the mixture of algal solution and metallic ion solution under controlled conditions for a specific time period, with or without stirring (Rajaboopathi and Thambidurai 2017). For example, to synthesize Au particles, microalgal biomass was lyophilized and subjected to reverse-phase high-performance liquid chromatography (RP-HPLC) until the isolation of gold shape-directing protein (GSP), which is an isolated protein type accountable for directing the shape of nanoparticles. Then, this protein was mixed with HAuCl₄ aqueous solution to produce Au nanoparticles (Agarwal et al. 2019). There are three primary techniques for algaebased nanoparticle synthesis, which are intracellular synthesis, extracellular synthesis, and pigment-based synthesis (Fig. 2) (Khan et al. 2022).

Intracellular synthesis

The production of nanoparticles using algae occurs within the cells and is regulated by various biological factors (Mukherjee et al. 2021). These factors include respiration, photosynthesis, and nitrogen fixation which produce reducing agents that aid in the reduction of ions within the cell membrane, cytoplasm, and thylakoid (Sharma et al. 2016). The two main pathways used to produce algae-based nanoparticles via intracellular synthesis are; (i). During the logarithm phase, the cellular extract is isolated through centrifugation, and then biomass is washed and mixed with a precursor solution (Othman et al. 2019), (ii). Individual microorganisms are incubated with an aqueous solution of

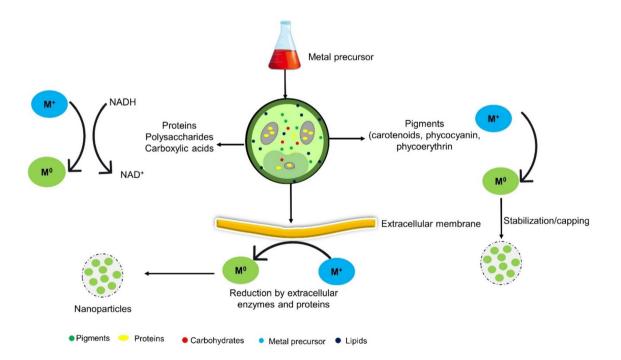


Fig. 2 Algae-based nanoparticles synthesis via. a Intracellular synthesis, b extracellular synthesis, and c pigment-based synthesis

bulk material under optimum conditions for approximately a fortnight (Merin et al. 2010).

Extracellular synthesis

During the extracellular synthesis, metal ions attached to the algal cell surfaces are reduced by exudate metabolites including proteins, lipids, ions, pigments, antioxidants, non-protein DNA, RNA, and enzymes. These metabolites function as reducing agents and capping agents. The biomass was eliminated via centrifugation, then, the filtrate was mixed with a metal precursor solution (Vijayan et al. 2014). The size and structure of nanoparticles are highly reliant on medium pH, temperature, and concentrations of substrate and metal precursor solution (Nadeem et al. 2017). Although, the synthesis of nanoparticles via extracellular synthesis easy cleaning and blending are essential prerequisites (Dahoumane et al. 2016).

Pigment-based synthesis

Various types of cellular pigments have been used to synthesize nanoparticles, the most prominently used ones are phycocyanin, phycoerythrin, and carotenoids (Venil et al. 2021). Phycocyanin is linked with the binding of heavy metals (Gelagutashvili 2013), for example, phycocyanin extracted from cyanobacteria (*Limnothrix* and *Spirulina sp.*) used to synthesize Ag nanoparticles of various sizes and shapes, these differences were because of the purity and molecular weight difference of phycocyanin extracted from both strains. Therefore, the quality and quantity of the produced pigments are important when nanoparticles are synthesized using pigments. To align with the commercial requirement, enhancement of pigment production is necessary, which could be accomplished by manipulation of abiotic stress or via genetic modifications (Khan et al. 2022).

Factors influencing the biological synthesis of nanoparticles

The properties of algae-based nanoparticles, including size and structure influenced by different factors including the type and concentration of metal precursor and biomolecule used for the production, reaction time, medium pH, and temperature (Subramani et al. 2021) (Fig. 3).

Algae type and concentration

Nanoparticles synthesized using alginic acid were smaller compared to the nanoparticles produced using starch and agar extracted from Gracilaria gracilis (Francavilla et al. 2014), which is compatible with the previous research (Luque et al. 2013). A shapeshift was observed with the increase of leaf broth concentration, for example, hexagonal, triangular, rod, and radial nanoparticles were generated when Sargassum myriocystum extract reacted with Zn(NO₃)₂ when the concentration of Sargassum myriocystum decreased the shape of nanoparticles transformed to spherical shape (Nagarajan and Kuppusamy 2013). Similar shape and size shifts were observed in biosynthesized ZnO nanoparticles when the concentration of Calotropis procera extract changed (Singh et al. 2011). The nanoparticle formation rate increased with the increase in the concentration of algae extract (Edison et al. 2016). The size of

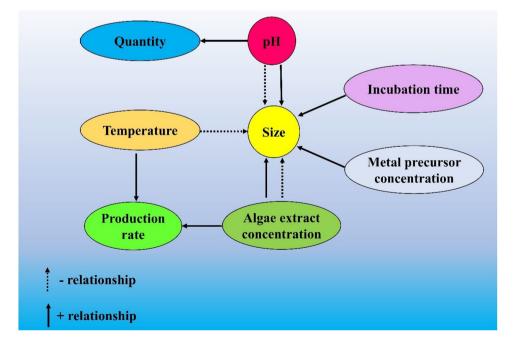


Fig. 3 Factors influencing size, production rate, and quantity of algae-based nanoparticles

the ZnO nanoparticles was increased with the decrease in leaf broth concentration (Edison et al. 2016). The intensity of the surface plasmon resonance (SPR) band amplified resulting in a blue shift toward 435 nm during the production of Ag nanoparticles, demonstrating a reduction in the size of Ag nanoparticles as the Caulerpa serrulata extract concentration increased from 5 to 20% (Aboelfetoh et al. 2017). The SPR peaks of Ag nanoparticles for 1, 3, and 5 mL of algal extract were formed at 436, 438, and 441 nm respectively, indicating a slight increase in the size of Ag nanoparticles at high algal extract concentrations. The yield and the size of Au nanoparticles produced using Chlorella vulgaris as feedstock varied with the GSP concentration; as the concentration of GSP increased both the yield and size of Au nanoparticles decreased (Xie et al. 2007).

Metal precursor concentration

The size of the algae-based nanoparticles is affected by the concentration of metal precursor solution. For example, the increase of HAuCl₄ concentration resulted in a slight shift in λ_{max} toward lower wavelengths (González-Ballesteros et al. 2019a), while at low AgNO₃ concentrations, the surface plasmon resonance (SPR) band was broad and less intense, however, with the increase of AgNO3 concentration small shift toward λ_{max} was observed which is associated with the size variation of Ag nanoparticles. However, both Ag and Au nanoparticles formed at high HAuCl₄ and AgNO₃ concentrations were less stable, with a high tendency toward precipitation and aggregation. As the concentration of metal precursor solution decreased, smaller nanoparticles were synthesized rapidly owing to the availability of more functional groups (Punjabi et al. 2015).

Medium pH

The pH also influenced the shape and size of nanoparticles, with variations observed at different pH values. For example, at pH 5 spherical nanoparticles of 5-20 nm diameter, 15-18 nm nano-triangles, 34 nm nano-hexagons, and 100×51.5 nm rod-shaped nanoparticles were produced, while at pH 7, spherical nanoparticles of 13-22 nm were formed, and at pH 9, monodispersed nanoparticles containing nanospheres of 16 nm diameter were produced (Parial and Pal 2015). At high pH values, small Ag nanoparticles were formed in large quantities (Aboelfetoh et al. 2017), while lower pH favored the aggregation of small Ag nanoparticles to form larger nanoparticles over nucleation (Nagarajan and Kuppusamy 2013).

Temperature and incubation time

The temperature has influenced the morphology and size of nanoparticles, for instance, high temperatures can cause agglomeration of nanoparticles, leading to an enhancement in size, size of Ag nanoparticles synthesized using Cystophora moniliformis increased with the increase of the temperature (Prasad et al. 2013). However, in some cases, a higher temperature can result in smaller nanoparticles, for example, a considerable decrease in the size of Ag nanoparticles was observed as the reaction temperature reached 90 °C (Nagarajan and Kuppusamy 2013). The stability of nanoparticles is affected by the zeta potential, nanoparticles having a higher negative zeta potential are more stable due to the strong electrostatic repulsion between nanoparticles which prevents agglomeration (Pugazhendhi et al. 2019). Additionally, the incubation time can affect the properties of nanoparticles, with longer incubation time leading to aggregation and forming bulk particles, for example, Ag nanoparticles aggregated as incubation continued for 48 h (Kannan et al. 2013). The shape of Au nanoparticles synthesized at the beginning of the reaction (6-18 h incubation) was spherical and undefined and after 24 h incubation, bulk particles were formed due to the aggregation of small particles (Rajeshkumar et al. 2013).

Nanoparticles synthesized from algae

Metallic nanoparticles are the most prominent type of nanoparticles produced using algae, the most prominent metallic nanoparticles synthesized are MgO, ZnO, Au, Ag, Pd, and CuO. Transition metals are the most suitable for the production of metal/metal oxide nanoparticles because their partly filled d orbitals make them more redox-active, which facilitates nanoparticle aggregation (Maize et al. 2021). Distinctive electronic, magnetic, catalytic, and optical properties of metallic nanoparticles are different from those of bulk metals. The production of metallic nanoparticles utilizing algae is a simplistic room-temperature process in which aqueous algae extract is mixed with a metal salt solution of known concentration (Fawcett et al. 2017).

Various types of metallic nanoparticles were produced using a range of macro and microalgae species (Table 1). Pugazhendhi et al. (2018). Çalışkan et al. (2020)used *Galdieria* sp. to biosynthesize Ag, Fe, and Zn nanoparticles; Ag nanoparticles were synthesized using *Laurencia papillosa* (Omar et al. 2017); ZnO nanoparticles produced using *Chlorella*, a green microalgae species (Khalafi et al. 2019); *Hypnea musciformis* a red macroalgae species used to synthesize Ag nanoparticles (Vadlapudi and Amanchy 2017). Aboelfetoh et al. (2017) synthesized Ag nanoparticles using Greening up the fight against emerging contaminants: algae-based nanoparticles for water...

Types of nano- particles	Algae species and quantity	Precursor and quan- tity	Reaction condi- tions	Size range/ Avg nm	Shape	Functions	References
ZnO	Chlorella vulgaris	Zinc acetate	80 °C, 1 h pH-8.0, 800 rpm	35-56	Sheet structure	_	Zhang et al. (2019)
ZnO	<i>Chlorella</i> 0.5% W/V, 20 mL	Zn(CH ₃ COO) ₂ ·2H ₂ O 80 mL	58 °C, 1 h, 150 rpm, pH-8.0	19.44	Hexagonal	Photocatalyst	Khalafi et al. (2019)
ZnO	Ulva fasciata 1.0% W/V, 2 mL	Zn(CH ₃ CO ₂) ₂ ·2H ₂ O 10 mM	72 °C, 3-4 h Calcina- tion-455 °C, 4 h	53-96	Spherical	-	Alsaggaf et al. (2021)
CdO–ZnO	Padina gym- nospora 0.5 g, 50 mL	$ \begin{array}{l} ZnCl_{2}.6.H_{2}O\ 0.25\ M\\ CdCl_{2}.H_{2}O\ 0.1\ M \end{array} $	RT, 5 h		Distorted hexagonal		Rajaboopathi and Thambi- durai (2017)
MgO	Sargassum wightii	$Mg(NO_3)_2 \cdot 6H_2O$	90 °C, 6 h	68.02		Catalyst, anti- microbial, and antican- cer agents	Pugazhendhi et al. (2019)
Ag, Fe (II), Zn	Galdieria sulphuraria	Silver, iron (II), and zinc metal solutions 1-15 mM	RT, 24 h, 200 rpm	Ag-1134 Fe (II)-340 Zn-390	-	Antimicrobial agent	Çalışkan et al. (2020)
Ag	<i>Chlorella pyr- enoidosa</i> 100 mL	AgNO ₃ 2 mM, 250 mL	RT, 6 h, 120 rpm	-	Spherical, cuboidal	-	Kusumanin- grum et al. (2018)
Ag	<i>Gelidium</i> amansii 2 mg	AgNO ₃ 1, 3 and 5 mM	Below 40 °C, 150 rpm	27-54	Spherical	Antimicrobial agent	Pugazhendhi et al. (2018)
Ag	Caulerpa ser- rulate 1% W/V, 5–25 mL	AgNO ₃ 1 mM, 95-75 mL	RT, 24 h	10	Spherical and ellipsoidal	Catalyst, antimicrobial agent	Aboelfetoh et al. (2017)
Ag	Chlorella ellipsoidea 1 g	AgNO ₃ 1 mM, 100 mL	RT, 12 h	220.8	Spherical	Catalyst, antimicrobial agent	Borah et al. (2020)
Pd	<i>Chlorella vulgaris</i> 1.0% W/V, 10 mL	PdCl ₂ 1 mM, 50 mL	60 °C	5-20	Spherical	_	Arsiya et al. (2017)
Au	<i>Cystoseira</i> <i>baccata</i> 1 g/mL, 1 mL	HauCl ₄ 0.01 M, 75 μL	RT, 24 h	8.4	Spherical	Anticancer agent	González-Bal- lesteros et al (2017)
Ag	Hypnea musci- formis 5% W/V, 10 mL	AgNO ₃ 1 mM, 190 mL	Exposed to direct sunlight	16-42	Triangles, hexagonals, pentagons, and spherical	Antibacterial agent	Vadlapudi and Amanchy (2017)
Au	Galaxaura elongate 1 g	HAuCl ₄ 0.001 M, 100 mL	-	3.85-77.13	Spherical, rod, triangular, truncated tri- angular, and hexagonal	Antibacterial agent	Abdel-Raouf et al. (2017)
Fe ₃ O ₄	Sargassum vulgare, Ulva fas- ciata, and Jania ruben 1% W/V	FeCl ₃ ·6H ₂ O 0.1 M	RT, 3 h, 800 rpm	17.05-34.09 22.73-39.77 22.22-33.33	-	Antibiofilm agents	Salem et al. (2020)
Au	Ulva armori- cana	HAuCl ₄ 1 mM	RT, 90 min	200	Spherical, triangular, rod	Reducing agent	Mukhoro et al (2018)

Table 1 Size, shape, and functions of biosynthesized nanoparticles using various macro and microalgal species

Table 1 (continued)

Types of nano- particles	Algae species and quantity	Precursor and quan- tity	Reaction condi- tions	Size range/ Avg nm	Shape	Functions	References
Au Ag	Ulva lactuca 1 g/mL 0.5 g/mL	HAuCl ₄ 0.5 mM AgNO ₃ 0.16 mM	RT, 24 h Reflux, 1 h	Au-7.9 Ag-31		Anticancer agents	González-Bal- lesteros et al. (2019b)

RT room temperature

Caulerpa serrulata—spherical-shaped Ag nanoparticles synthesized from *Chlorella ellipsoidea* (Borah et al. 2020).

Applications of algae-based nanoparticles

Algae-based nanoparticles have various environmental and biochemical applications (antibacterial, antifungal, anticancer, and wound healing) (LewisOscar et al. 2016). Nanoparticles synthesized using algae were used for the remediation of EOCs present in aqueous media. Furthermore, they have been used as antibiofilm agents, biosensors, and drug delivery agents.

Remediation of emerging organic contaminants in water and wastewater

Photocatalytic degradation is an ecologically friendly and sustainable wastewater treatment practice that can help to decrease the impact of anthropogenic activities on the environment (Koe et al. 2020). During photocatalytic degradation, photocatalysts are used to break down organic compounds in the presence of light. The process includes the transfer of electrons from the photocatalyst to the reaction molecule, which then undergoes a sequence of chemical reactions that results in degradation. The photocatalytic degradation process can be divided into numerous steps (Fig. 4). The first step is the adsorption of light by the photocatalyst, once the energy of photons exceeds the band gap (energy difference between the conduction band and valence band), electrons in the valence band excite to the conduction band generating electron-hole pair $(e^{-}h^{+})^{-}$ (Ejsmont et al. 2022; Viswanathan 2017). These photo-induced electrons and holes reacted with O2 and H2O and produced reactive oxygen species (ROS) including hydroxyl radicals (OH·) and superoxide anion (O_2^-) radicals that have a high oxidation potential (Koe et al. 2020). The following step is the adsorption of pollutants onto the photocatalysts' surface. Once adsorbed, electron from the photocatalyst is relocated to the reaction molecules, which then undergoes a series of reaction, leading to their degradation into smaller, less toxic compounds like CO₂ and H₂O (Viswanathan 2017).

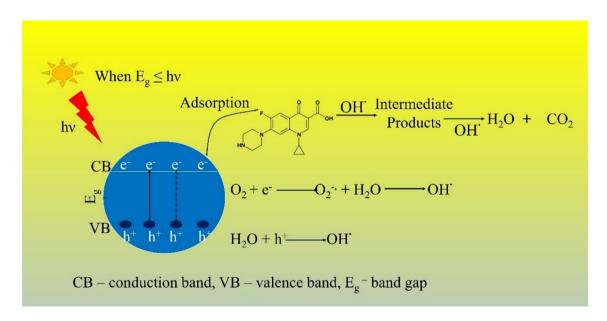


Fig. 4 Photocatalytic degradation mechanism of EOCs

The adsorption process helps to concentrate pollutants onto the surface of the photocatalyst increasing the probability of interaction with the photocatalyst and enhancing the degradation process (Abebe et al. 2018). Finally, the degraded products are desorbed from the surface of the photocatalyst, and the process continues with the adsorption of new molecules. The photocatalytic degradation process is applied to remove toxic and non-biodegradable contaminants and pathogens from contaminated water (Shan et al. 2017). Metal and metal oxide nanoparticles produced via physical and chemical techniques are often used to degrade organic pollutants present in wastewater, for example, TiO₂ was used to degrade sulfamethoxazole (SMX) and trimethoprim (TMP) in water (Abellán et al. 2009). The Ag-TiO₂ nanoparticles exhibited high photocatalytic degradation efficiency of ciprofloxacin (92%) and norfloxacin (94%) under exposure to visible light irradiation for 4 h (Wang et al. 2021).

Nanoparticles synthesized using both macro and microalgae were utilized for the photocatalytic degradation of organic pollutants (Table 2). Ag nanoparticles produced using Caulerpa serrulata at high temperatures showed the highest catalytic activity (Aboelfetoh et al. 2017). Similarly, agar extracted from Gracilaria gracilis was used for ZnO nanoparticle synthesis which acted as a promising photocatalyst and degraded around 52% of phenol under solar radiation. Moreover, Ag and Au nanoparticles produced from Caulerpa serrulata and Caulerpa racemosa extracts showed high photocatalytic degradation efficiency of Congo red (CR) and methylene blue (MB) dyes in water (Aboelfetoh et al. 2017; Edison et al. 2016). Microalgae-based nanoflowers have been used to remediate waters contaminated with organic contaminants, for example, hybrid nanoflowers formed by mixing ZnO and microalgae acted as a photocatalyst and degraded more than 90% of MO within two hours owing to their elevated surface area and exclusive morphology (Rao and Gautam 2016). Malachite green dye solution was exposed to sunlight in the presence of algae-based Ag nanoparticles synthesized from Gracilaria corticata was completely degraded within 6 h (Poornima and Valivittan 2017). The reduction of Rhodamine B (RhB) started spontaneously in the presence of both NaBH₄ and Au nanoparticles synthesized using Turbinaria conoides and Sargassum tenerrimum (Ramakrishna et al. 2015). The photocatalytic degradation efficiency of MB under sunlight in the presence of algae-based MgO nanoparticles was 8.2 times higher than the degradation efficiency in the absence of the catalyst (Pugazhendhi et al. 2019). Dibenzothiophene (DBT) degradation efficiency by ZnO nanoparticles synthesized using Chlorella sp. reached 97% within 2 h (Khalafi et al. 2019).

The photocatalytic degradation efficiency of nanoparticles varies with the size and shape of nanoparticles, and usually, smaller nanoparticles show high degradation efficiency during the photocatalytic degradation process owing to their higher specific surface area (Rajaboopathi and Thambidurai 2017). Degradation efficiencies of RB198 dye by CdO-ZnO nanoparticles produced using *Padina gymnospora* were observed under sunlight, visible light, and UV light irradiation, and the highest degradation efficiency was observed under sunlight (Rajaboopathi and Thambidurai 2017). This may be due to the strong catalytic activity resulting from the sunlight irradiation source which emits both UV and visible light compared to UV and visible light irradiation alone (Brahmia 2016).

The degradation efficiency of organic dyes depends on the medium pH, for example, when pH decreased from 6.0 to 2.0, CR degradation efficiencies of Ni@Fe₂O₃ and CuO nanoparticles increased from 53 to 78% and 50 to 85% respectively whereas CR degradation efficiency dropped when pH increased from 6.0 to 12.0. However, photocatalytic degradation of both MB and RhB increased at high pHs and was the highest at pH 10. This is because pH influences the surface properties of nanoparticles and the ionization of organic compounds. The concentration of contaminant was also found to be affecting the photocatalytic degradation, for example, the degradation of dyes dropped with the increase of contaminant concentration because, at low contaminant concentrations, adsorption capacity is high due to the presence of more active sites (Rao and Gautam 2016).

The rate constant of the reduction reaction of RhB and Sulforhodamine 101 (SF101) by Au nanoparticles produced using *Turbinaria Conoides and Sargassum tenerrimum* increased as the volume of Au nanoparticles added enhanced (Ramakrishna et al. 2015). The time taken to completely reduce RhB and SF101 decreased with the added volume of Au nanoparticles, due to the presence of a high number of active sites (Ramakrishna et al. 2015). The degradation rate of CR increased with the increase of the catalyst dose from 0.1 to 0.3 mL (Aboelfetoh et al. 2017). The temperature of nanoparticles produced also influences their photocatalytic activity, for instance, Ag nanoparticles produced at high temperatures exhibited the highest photocatalytic degradation activity (Aboelfetoh et al. 2017).

Degradation of EOCs can be observed via visual observation as well as UV–Vis spectroscopy. The degradation of dyes was confirmed by the reduction of the intensity of the band appearing at 662 nm and simultaneous formation of the new sharp band at 280 nm and the enhancement of its intensity with time (Borah et al. 2020). In the presence of solar radiation, the peak intensity of the characteristic absorption peak of MO at 460 nm was gradually decreased and the lowest intensity was observed after 12 h of incubation time revealing the degradation of MO (Kumar et al. 2013). Once the reduction of RhB dye started, the color of the dye faded progressively (Ramakrishna et al. 2015).

Types of nanoparti- cles	Algae species	Contaminant	Kinetic model	Degradation constant/ s ⁻¹	Degradation time/s	Degradation efficiency/%	References
Ag	Caulerpa rac- emosa	MB	Pseudo first order	1.114×10^{-3}	1800	_	Edison et al. (2016)
Ag	Caulerpa ser- rulata	CR	Pseudo first order	$\begin{array}{c} 2.17 \times 10^{-3} (\text{Ag-} \\ 0.10 \text{mL}) \\ 4.33 \text{x} 10^{-3} (\text{Ag-} \\ 0.20 \text{mL}) \\ 5.67 \text{x} 10^{-3} (\text{Ag-} \\ 0.25 \text{mL}) \\ 7.67 \text{x} 10^{-3} (\text{Ag-} \\ 0.30 \text{mL}) \end{array}$	960 720 540 360	96 99 99 99	Aboelfetoh et al. (2017)
ZnO	Chlorella sp.	DBT	-	-	10,800	97	Khalafi et al. (2019)
Ag	Chlorella ellip- soidea	MB	Pseudo first order	7.87×10 ⁻⁴	-	-	Borah et al. (2020)
		МО	Pseudo first order	5.40×10^{-4}	_	-	
MgO	Sargassum wighitii	MB	-	-	9000	100	Pugazhendhi et al. (2019)
Au	Turbinaria conoides	RhB	-	4.49×10^{-3} (Au-10 µl) 29.77 × 10 ⁻³ (Au-25 µl) 109.79 × 10 ⁻³ (Au-50 µl)	600 70 20		Ramakrishna et al. (2015)
		SF101		3.99×10^{-3} (Au-10 µl) 14.49 × 10 ⁻³ (Au-25 µl) 19.91 × 10 ⁻³ (Au-50 µl)	660 225 90		
		4-nitrophenol	Pseudo first order	9.37×10^{-3}	300	100	
		p-nitroaniline	Pseudo first order	18.73×10^{-3}	123	100	
Au	Sargassum tenerrimum	RhB		3.98×10^{-3} (Au-10 µl) 10.51×10^{-3} (Au-25 µl) 68.0×10^{-3} (Au-50 µl)	550 281 45		
		SF101		2.67×10^{-3} (Au-10 µl) 13.45 × 10 ⁻³ (Au-25 µl) 24.89 × 10 ⁻³ (Au-50 µl)	650 146 86	- - -	
		4-nitrophenol	Pseudo first order	10.64×10^{-3}	300	100	
		p-nitroaniline	Pseudo first order	16.07×10^{-3}	140	100	
Ag	Ulva lactuca	МО	-	_	43,200	-	Kumar et al. (2013)
ZnO	Padina gymno- spora	RB-198	-	-	9000	85.09 (SL) 86.09 (UV) 84.7 (Vis)	Rajaboopathi and Thambidurai (2017)
		Phenol		-	9000	59.7 (SL) 53.4 (Vis)	
CdO-ZnO		RB-198	-	-	9000	80.9 (SL) 80.9 (UV) 65.9 (Vis)	
		Phenol	-	_	9000	51.8 (SL) 48.3 (Vis)	

 Table 2
 Catalytic activity of nanoparticles produced using macro and microalgae

Greening up the fight against emerging contaminants: algae-based nanoparticles for water...

Table 2	(continued)
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Types of nanoparti- cles	Algae species	Contaminant	Kinetic model	Degradation constant/ s ⁻¹	Degradation time/s	Degradation efficiency/%	References
ZnO	Chla- mydomonas reinhardtii	МО	_	_	7200	92 (MO-5 mg/L) 90 (MO- 10 mg/L) 78 (MO- 15 mg/L) 95 (ZnO- 75 mg) 90 (ZnO- 50 mg) 76 (ZnO- 25 mg)	Rao and Gautam (2016)
Ag	Sargassum myriocystum	MB	-	-	3600	98	Balaraman et al. (2020)
TiO ₂	Blue-green algae	MB		$\begin{array}{c} 1.35 \times 10^{-4} \ (\text{pH 4}) \\ 2.06 \times 10^{-4} \ (\text{pH10}) \\ 2.25 \times 10^{-4} \ (\text{MB-} \\ 10 \ \text{mg/L}) \\ 1.23 \times 10^{-4} \ (\text{MB-} \\ 20 \ \text{mg/L}) \\ 1.03 \times 10^{-4} \ (\text{MB-} \\ 30 \ \text{mg/L}) \end{array}$	7200	65 (pH 4) 75.80 (pH 6) 83 (pH 10) 83 (MB- 10 mg/L) 62 (MB- 20 mg/L) 51 (MB- 30 mg/L)	Vu Nu et al. (2022)
SnO ₂	Chlorella vul- garis	МО	-	-	5400	94	Al-Enazi et al. (2022)

SL sunlight, *UV* UV light, *Vis* visible light, *MO* methyl orange, *CR* Congo red, *MB* methylene blue, *SF101* sulforhodamine 101, *RhB* rhodamine B, *RB-198* reactive blue 198, *DBT* dibenzothiophene

Biomedical applications of algae-based nanoparticles

Nanoparticles are versatile in their applications and are used in a variety of fields such as biomedical treatments, energy production, and environmental remediation. However, their use in the biomedical field has received significant attention recently due to their potential as an antimicrobial and anticancer agents. Metallic nanoparticles synthesized using microalgae have been used as antimicrobial agents, for example, Ag nanoparticles produced using Galdieria sp. are used as an antimicrobial agent against gram-negative and gram-positive bacteria. Similarly, Ag nanoparticles synthesized from Chlorella ellipsoidea have been tested for their antimicrobial activity against gram-negative (Escherichia coli, Klebsiella pneumonia, and Pseudomonas aeruginosa) and gram-positive bacteria (Staphylococcus aureus) by diskdiffusion method (Borah et al. 2020). Although the thick cell wall of gram-positive bacteria containing peptidoglycan prevents penetration of nanoparticles, Ag was found to inhibit the growth of both types of bacteria. El Ouardy et al. (2023) synthesized Ag nanoparticles using Parachlorella kessleri and they have shown excellent antibacterial activity against Escherichia coli, multidrug-resistant Escherichia coli, Bacillus clausii, Pseudomonas aeruginosa, Staphylococcus aureus, and Salmonella typhi. The antimicrobial activity of nanoparticles was found to be concentration dependent, for example, the maximum inhibition zone (21 mm) for Escherichia coli was when exposed to 75 μ L of Ag nanoparticle, whereas, the minimum inhibitory zone (10 mm) for Salmonella typhi was when exposed to 50 μ L of Ag nanoparticles (Aboelfetoh et al. 2017). The attachment of Ag nanoparticles to negatively charged bacterial cells can disrupt their shape and affect the penetrability of their plasma membrane. Moreover, the Ag nanoparticles synthesized from Sargassum longifolium exhibited concentration-dependent antifungal activity on Aspergillus fumigatus, Candida albicans, and Fusarium sp. (Rajeshkumar et al. 2014).

Currently available cancer treatment drugs are often expensive and can cause harmful side effects including neuron damage, skin irritations, and severe pain. Therefore, the invention of nontoxic and cost-effective drugs is crucial (Pugazhendhi et al. 2019). Nanoparticles smaller than 100 nm have the potential to detect and treat cancer due to their strong interactions with proteins, nucleic acids, and lipids in cells. Thus, there is a high possibility of using nanoparticles to detect and treat cancers. At high concentrations, Au nanoparticles synthesized using red seaweed species *Corallina officinalis* showed strong cytotoxic activity against MCF-7 cells resulting in cell necrosis, however, no impact was observed at low concentrations (El-Kassas and El-Sheekh 2014). Similarly, the MgO nanoparticles produced using *Sargassum wighitii* exhibited a concentrationdependent cytotoxic effect on lung cancer cell lines (A549) with the IC₅₀ value of $37.5 \pm 0.34 \mu$ g/mL compared to the IC₅₀ value of cisplatin (Pugazhendhi et al. 2019). Also, Ag nanoparticles synthesized using *Sargassum vulgare* showed toxicity toward cancer cells inducing apoptosis and DNA damage (Govindaraju et al. 2015).

Algae-based nanoparticles have shown great potential in biosensing applications such as monitoring the hormone levels in the human body, and cancer diagnostics due to their excellent optical properties, e.g. Au nanoparticles (Chaudhary et al. 2020). Biofilms which are composed of bacteria, fungi, and algae, can cause considerable health and economic issues, especially, in the medical and industrial sectors (de Carvalho 2018). Pathogenic biofilms can also become resistant to antibiotics due to the abuse of antibiotics. Nanoparticles synthesized using algae serve as effective antibiofilm agents because bacteria can not develop resistance against nanoparticles (LewisOscar et al. 2016). Furthermore, nanoparticles are an eco-friendly alternative to biocides, which can release toxic chemicals. Algae-based nanoparticles have also been used to control malaria pathogens and vectors. For instance, Ag nanoparticles synthesized from Ulva lactuaca showed promising results in reducing the population of Plasmodium falciparum and Anopheles stephensi (Murugan et al. 2015). The LC50 values of Ag nanoparticles for Anopheles stephensi larval stages I, II, III, IV, and pupa were 2.111 mg/L, 3.090 mg/L, 4.629 mg/L, 5.261 mg/L, and 6.860 mg/L respectively. Although some metallic nanoparticles have been used in various industries (Iravani et al. 2014; Singh et al. 2014), algae-based nanoparticles have not been widely utilized. Therefore, further studies are necessary to explore the potential use of algaebased nanoparticles in different industries.

Stability and reusability of algae-based nanoparticles

Stability and reusability, are the most significant factors determining the cost-effective applicability of nanoparticles for the removal of pollutants when they are used on a large scale (Arun et al. 2018). Ag nanoparticles produced using algae were found to be highly stable, for instance, no aggregation of Ag nanoparticles produced using *Calotropis procera* was detected even after one month of production (Edison et al. 2016).

Algae-based nanoparticles exhibited excellent stability and a slight decrease in degradation efficiency after each degradation cycle. The decolorization efficiency of the CdO-ZnO photocatalyst produced using Padina gymnospora was slightly reduced within three reused cycles (99.5, 96.3, and 94.1% respectively) (Rajaboopathi and Thambidurai 2017). The catalytic efficiency of ZnO was slightly decreased after 5 consecutive photodegradation cycles exhibiting high stability and recyclability (Khalafi et al. 2019). SnO₂ nanoparticles synthesized using Chlorella vulgaris have been used for five consecutive MO photocatalytic degradation cycles and it has retained 80% activity (Al-Enazi et al. 2022). Algaebased TiO₂ nanoparticles synthesized via microwave method maintained high photocatalytic degradation efficiency throughout four photocatalytic degradation cycles (Vu Nu et al. 2022). Although the photocatalytic degradation rate maintained during cycle 2 it was decreased slightly during cycles 3 and 4 mainly due to the loss of the photocatalyst during the collection process. However, the cyclic application of algae-based nanoparticles has not been studied adequately, therefore, more attention on this aspect is required in the future.

Advantages and disadvantages of production and application of algae-based nanoparticles

The process of synthesis of the algae-based nanoparticle is an efficient, less time-consuming, and single-step process. Unlike other methods, additional stabilizing and capping agents are not necessary because algae contain compounds that act as stabilizers and capping agents (Makarov et al. 2014). Moreover, the use of toxic chemicals during the synthesis is minimal, resulting in a low release of secondary pollutants into the environment. Since fewer chemicals are used during the biological synthesis of nanoparticles, algaebased nanoparticles are free from toxic byproducts (Singh et al. 2016). Therefore, usage of those for water treatment as well as medical purposes is relatively safe.

- Nanoparticles produced using algae are remarkably stable hence, making them suitable for a wide range of applications (Mukherjee et al. 2021).
- The surface activity of algae-based nanoparticles is high because nanoparticles synthesized using algae usually have surface functional groups that were available in the algae extract used to synthesize nanoparticles (Edison et al. 2016; Ramakrishna et al. 2015)
- Algae-based nanoparticles have exhibited high photocatalytic degradation efficiencies compared to chemically synthesized nanoparticles, for example, the photocatalytic degradation efficiencies of chemically synthesized ZnO, CdO, and TiO₂ are lower than that of ZnO and

ZnO-CdO synthesized using seaweed (Rajaboopathi and Thambidurai 2017).

 Algae-based nanoparticles have shown great potential for treating human breast cancers and lung cancers (El-Kassas and El-Sheekh 2014; Pugazhendhi et al. 2019).

Despite the advantages of algae-based nanoparticle synthesis, some challenges exist in the process.

- The difficulty in producing nanoparticles of uniform size even in the existence of long surfactant molecules which form protective layers around nanoparticle surfaces and prevent aggregation of nanoparticles together.
- Selecting the appropriate algae species for the production of algae-based nanoparticles is challenging since the characteristics of the resulting nanoparticles vary depending on the algae species used.
- Factors such as concentration of reducing and capping agents, temperature, and pH influence the shape and size of nanoparticles (Parial and Pal 2015; Prasad et al. 2013).
- Aggregation of nanoparticles may occur under certain conditions, resulting in a decreased surface area and blocking active sites eventually decreasing pollutant removal efficiency, for instance, larger nanoparticles also have low photocatalytic degradation efficiency due to the low specific surface area of nanoparticles.
- The difficulty in adapting nanoparticle-based wastewater treatment to large-scale processes.

Therefore, it is important to maintain optimal pH and temperature during the synthesis of nanoparticles to produce nanoparticles with high photocatalytic degradation efficiency.

Nanoflowers and cross-linked nanocomposites are more advanced forms of nanoparticles due to their higher efficiency in degrading EOCs. However, the formation process of the aforementioned catalysts is more complex and requires additional steps such as ultrasonication and prolonged incubation. Nanocomposites produced by combining biogenic organic and inorganic compounds exhibited a high degradation efficiency compared to algae-based nanoparticles though, the formation of nanocomposites is timeconsuming. The usage of biomolecules extracted from algae is another advanced step taken to improve the quality of nanoparticles, nevertheless, due to the complexity of extraction and separation processes, it has not been applied widely. Therefore, further studies are necessary to explore the formation of nanocomposites and nanoflowers using algae and their potential use in EOC removal.

Over the past few decades, researchers have explored the use of various types of nanoparticles for photocatalytic degradation to remove toxic contaminants from water. However, there is a growing concern regarding the potential toxicity of nanoparticles to both humans and animals. Recent studies have tested the lethal effects of nanoparticles in various organisms due to the risk of penetration of tiny nanoparticles through human and animal tissues. For example, the growth of *Lemna minor* and *Lolium multiflorum* seedlings was inhibited by Ag nanoparticles, and the level of inhibition increased with prolonged exposure (Gubbins et al. 2011; Yin et al. 2011).

Future perspective

Selecting an appropriate algae species for the production of algae-based nanoparticles can be challenging due to the variation of their characteristics with the algae species used for the synthesis. Most of the algae-derived nanoparticles have been produced in laboratory settings, and therefore, the challenges associated with large-scale commercial production are not well understood. The cellular mechanism involved in the biosynthesis of metallic nanoparticles is not fully comprehended, and comprehensive studies that investigate the developmental mechanisms on both cellular and molecular levels are essential. Especially, although there are reports in the literature on the identification of bioactive moieties, identifying the biomolecules involved in the reduction of metals and which molecules are acting as capping agents are important to improve the synthesis efficiency. It is necessary to understand how active compounds from microalgae combine with nanoparticles to improve surface properties and stability.

Most of the studies that have evaluated the photocatalytic degradation efficiency using nanoparticles have been conducted in laboratories, therefore, field application studies are essential to accurately assess the actual efficiency, as chemical and physical parameters fluctuate rapidly in the natural environment influencing photocatalytic degradation. Moreover, the use of fresh algae is not beneficial, given the difficulties and costs associated with culturing, therefore, the usage of algae residue, which is discarded after bio-diesel production also can be used to produce nanoparticles. It is important to develop simple and effective techniques to synthesize algae-based nanoparticles, and asses their efficiency under different operation conditions. While metallic nanoparticles are frequently used for photocatalytic degradation, it is crucial to shift attention toward carbonaceous nanoparticles, as they possess a high specific surface area and display electrical, optical, thermal, and chemical activity.

Although nanoparticles have proven effective in the photocatalytic degradation of various organic compounds a comprehensive investigation of the underlying mechanisms involved in the process has not been conducted. Additionally, many unexplored microalga species could potentially be utilized for synthesizing nanoparticles. Nanoparticles fabricated utilizing algae could also be used for the photocatalytic degradation of antibiotics, plasticizers, volatile organic compounds, and endocrine-disrupting chemicals. However, most of the algae-based nanoparticles have been tested for photocatalytic degradation of organic dyes, organic sulfur contaminants, and phenols. Therefore, the usability of algaebased nanoparticles to remediate other EOC categories such as antibiotics, polyaromatic hydrocarbons, phthalates, and polychlorinated biphenyls needs to be evaluated. The reuse and recovery of algae-based nanoparticles used to remediate EOCs remediation have not received adequate attention, however, it is significant to determine the efficiency and cost-effectiveness of algae-based nanomaterial usage on a large scale. Thus, future studies should focus on determining the reusability and recovery of nanoparticles more often.

New approaches for producing nanoparticles involve combining organic and inorganic compounds to form biopolymer-based nanocomposites, as well as synthesizing nanoparticles using biomolecules extracted from biological sources. These materials have been effectively used for the degradation of EOCs, however, there has been limited research on using compounds extracted from algae to produce the above types of nanomaterials (Ramadhani and Helmiyati 2020). Therefore, in-depth studies focusing on the formation of nanocomposites using algae are necessary. Additionally, although nanoflowers have been synthesized from enzymes, oxidase, and proteins used for environmental remediation, algae-based nanoflowers have not been extensively studied. Hence, further studies are required to compare and contrast the photocatalytic ability of nanoflowers and nanoparticles.

The aim of blending carbonaceous materials is to enhance porosity and surface area, thereby improving adsorption capabilities. Microalgae biomass may serve as a potential oxidizing agent or act as a natural binding agent between carbonaceous materials and photocatalysts. Further investigation is required to fully explore the potential of this novel reactive adsorbent.

Though metallic nanoparticles have been used in microcosms due to their potential for photocatalytic degradation and antibacterial activity (Bessa da Silva et al. 2016), algae-based nanoparticles are yet to be used in either lab-based microcosm studies or the field. However, the usage of algae-based nanoparticles in a microcosm can be a novel approach to studying environmental and biological phenomena because algae-based nanoparticles can be successfully used due to their potential benefits. Algae species have the ability to adsorb metal ions present in water, thus, when processed to nanoparticles this potential could be amplified, allowing efficient removal of contaminants present in microcosm. Additionally, these nanoparticles can be used to monitor the pH changes and targeted pollutants available in microcosms due to their sensitivity to environmental changes. Some algae species increase the growth of plants by providing essential nutrients or promoting favorable microbial activity in the soil, hence, algae-based nanoparticles can be used as potential biofertilizers. Microalgae-based nanoparticles can be incorporated into biofilter and bioretention systems to enhance the removal of pollutants from surface runoff from metropolitan areas. Furthermore, the nutrients captured by nanoparticles can enhance the growth of vegetation and bioretention systems.

Conclusions

Recently, algae-based nanoparticles have been widely used for the remediation of water contaminated with EOCs. The synthesis can be done via three main pathways; intracellular, extracellular, and pigment-based synthesis. Biomass concentration, algae species, metal precursor solution used for the synthesis, medium pH, temperature, and contact time are found to be influential to the properties of algaebased nanoparticles. Therefore, it is important to maintain the optimum synthesis conditions to generate nanoparticles with high photocatalytic degradation efficiency. Algae-based nanoparticles were synthesized using different species of algae and metal precursors, and these nanoparticles have been used for the photocatalytic degradation of azo dyes, phenols, and pharmaceuticals. The degradation efficiency was evaluated under varying operating conditions such as initial concentration of pollutant, pH, and photocatalyst dose under SL, VL, and UV light. The photocatalytic degradation of dyes was found to decrease with the increase of initial dye concentration (MB, MO) (Vu Nu et al. 2022). On the other hand, the increase in photocatalyst dose enhanced the photocatalytic degradation efficiency and degradation rate (Ramakrishna et al. 2015; Rao and Gautam 2016). Although the stability and reusability of algae-based nanoparticles have not been widely studied, the existing results indicated that the reusability of these nanoparticles is high (Khalafi et al. 2019). However, in some instances, the actual reason behind the loss during the latter cycles was due to the loss of photocatalyst during the post-collection process (Vu Nu et al. 2022).

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Declarations

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