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PHYTOREMEDIATION OF AQUACULTURE WASTEWATER: A REVIEW OF MICROALGAE BIOREMEDIATION

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ABSTRACT

Current aquaculture practices have a detrimental impact on the environment, in particular due to the release of high concentration of nitrogen and phosphorus that can induce eutrophication. To avoid these harmful impacts, phytoremediation could be employed. The phytoremediation of aquaculture wastewater with microalgae has great potential due to its high nutrient removal efficiency and low cost. In microalgae-based bioremediation, algae fix carbon dioxide and release oxygen by photosynthesis and increase biological oxygen demand in contaminated water. It is the use of microalgae to remove pollutants from the environment or to convert them into harmless form. Furthermore, aquatic animals require protein in large quantity, in which microalgae are excellent requirement to solve this need. They are required for larval nutrition either for direct consumption as used for mollusks and shrimp or indirect consumption as in case of live prey fed to small fish larvae. The aim of this review is to converse the role of microalgae in aquaculture and bioremediation of aquaculture wastewater.

Keywords: Microalgae, Aquaculture, Feeds, Fish, Nutrition

INTRODUCTION

Aquaculture referred to as aqua-farming, it is the farming of fish, mollusks, crustaceans, plants, algae and other aquatic organisms. It involves cultivating freshwater and saltwater populations under controlled conditions (Guldhe *et al*., 2017). Aquaculture is one of the fastest-growing food producing sectors in the world, providing almost about 50% of all fish for human consumption; within 2030, this share is projected to rise to 62% (FAO, 2004). On the other hand, aquaculture represents one of the major contributors to the increasing levels of dissolved and particulate nutrients in the aquatic ecosystems (Lamprianidou *et al*., 2015). A high nutrient loading into the aquatic environment, in particular nitrogen and phosphorus may cause eutrophication, oxygen depletion and siltation (Burford *et al*., 2003).The increase in nutrients in aquaculture waste and their adverse environmental impacts has led to interest in adopting more efficient, cost-effective wastewater treatment methods as alternatives to conventional processes. The phytoremediation of aquaculture wastewater with microalgae has great potential due to its high nutrient removal efficiency and low cost (Nie *et al*., 2020).

Micro-algae are microscopic photosynthetic organisms that are found in both marine and freshwater environments. As described by Priyadarshani *et al*. (2011), microalgae refers to the aquatic microscopic plants (organisms with chlorophyll a and a thallus not differentiated into root, stem and leaf), and the oxygenic photosynthetic bacteria, that is, the cyanobacteria, formerly known as Cyanophyceae. In microalgae-based bioremediation, algae fix carbon dioxide and release oxygen by photosynthesis and increase biological oxygen demand in contaminated water (Singhal *et al*., 2021). It is the use of algae to remove pollutants from the environment or to convert them into harmless form.
Algae are widely acknowledged to be the ultimate source of both cellular carbon and chemical energy needed by other organisms (Fuhrmann, 2021). They are also categorized as macroalgae (sea weeds). Light, carbon dioxide and nutrients are the necessary requirement for the overall development of microalgae. Various industries such as pharmaceutical and aquaculture industries make use of microalgae either as biofilters to remove nutrients from wastewater or for production of useful compounds (Sirakov *et al*., 2015).

Aquaculture is among the widely recognized sectors worldwide. Various species of microalgae used in aquaculture include *Nannochloropsis* spp., *Chlorella* spp., *Tetraselmis, Scenedesmus* spp., *Skeletonema* spp., *Pavlova* spp., *Phaeodactylum* spp., and *Thalassiosira* spp., (Sirakov *et al*., 2015; Ansari *et al*., 2017). Microalgae nutrient compositions play an important role in aquatic food chain, and are known for their nutrient composition and they do not have toxins (Roy and Pal, 2015). The use of microalgae in aquaculture feed is gradually increasing due to their high nutritional value (Viegas *et al*., 2015). Microalgae are mainly used in aquaculture for larval fish, mollusks and crustaceans. Live microalgae are used in the removal of excess dissolved nutrients from aquaculture effluents (Velichkova *et al*., 2014). There is evidence of a cost-effective microalgae-based tilapia feed that enhances growth metrics and the nutritional quality of farmed fish, according to a study, which suggests that microalgae may likely reduce dependency on fishmeal and fish oil (Sarker *et al*., 2020). The aim of this review is to converse the role of microalgae in aquaculture and bioremediation of aquaculture wastewater.

Classification of Microalgae

Microalgae are large group of eukaryotic photoautotrophic protists and prokaryotic cyanobacteria which represent part of group called phytoplankton. Microalgae are classified more than twelve (12) groups, based on pigment composition, storage profile and ultrastructural features (Singh and Saxena, 2015). The major six (6) groups of algae are the Chlorophyta (green algae), Rhodophyta (red), Phaeophyta (brown algae), Chrysophyta (golden algae), Bacillariophyta (diatoms) and Cyanophyta (blue green-algae) (Baweja and Sood, 2015)(Figure1).

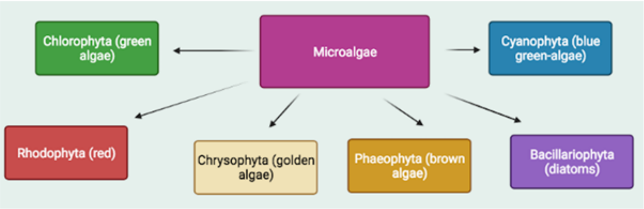

Figure1: Classification of microalgae

Chlorophyta (green algae)

It refers to a highly paraphyletic group that includes all green algae within the family of green plants (Viridiplantae), which is primarily composed of aquatic photosynthetic eukaryotic organisms. The Chlorophyta and Streptophyta are two of the most significant lineages of green algae, which are among the most numerous and diverse types of algae in river systems all over the world (with the latter including the land plants) (Sherwood, 2016).

Rhodophyta (red)

Rhodophyta (Red Algae) is a phyla of macroalgae that makes up a distinct group and is distinguished by having eukaryotic cells devoid of centrioles and flagella, chloroplasts that lack external endoplasmic reticulum and contain unstacked (stroma) thylakoids, and the use of phycobiliproteins as accessory pigments, which give them their red color (Cole and Sheath, 1990). The majority of Rhodophyta members are marine seaweeds, however there are also several taxa that live in freshwater and on land and few taxa from several orders have moved to freshwater habitats, although the majority of their members are marine. Bostrychia and Caloglossain Ceramiales species, for instance, are found in estuarine settings but have also been described from exclusively freshwater aquatic habitats (Szinte *et al*., 2020).

Phaeophyta (brown algae)

The class Phaeophyceae, or brown algae, is a sizable group of multicellular algae that includes several seaweeds that are found in cooler waters in the Northern Hemisphere (Bringloe *et al*., 2020). The predominant seaweeds in the polar and temperate zones are brown algae. In all cooler parts of the planet, they are the dominant species on rocky coasts. (Song *et al*., 2015).
According to Eze *et al*. (2018), the Phaeophyta (brown algae) can be identified by five main characteristics:

1) The photosynthetic pigments include chlorophyll-a and chlorophyll-c, carotene, fucoxanthin, violaxanthin, diatoxanthin, and other xanthophylls; fucoxanthin is present in enough amount to cover the green color of chlorophylls and to lend its own brown color to these algae;
2) Some whitish granules termed fucosan vesicles are typically present in the cell;
3) extra photosynthate is typically retained as laminarin and mannitol, seldom as fat droplets;
4) Cellulose, fucinic acid, and alginic acid make up the cell wall and
5) The flagellated structures have two unequal flagella that are laterally inserted, with the bigger flagella being anterior and pantonematic and the smaller flagella being posterior and acronematic.

Chrysophyta (golden algae)

The Chrysophyta (golden algae) is a vast family of algae that is primarily found in freshwater (Eze *et al*., 2018). It is also known as chrysophytes, chrysomonads, golden-brown algae, or golden algae. These division Planktonic unicellular and colonial algae found in fresh or salt water are included in the phylum Chrysophyta (chryso = golden and phyton = plant). They are crucial to the functioning of nature's economy since they are both photosynthesizers and the base of numerous food chains. Eukaryotic cells make up chysophytes. The predominant green pigment is chlorophyll a, and surplus food is either stored as the glucose polymer chrysolaminarin (leucosin) or as oils (Levasseur *et al*., 2020). In addition, many chrysophytes have silica in their cell walls and the walls of their resistant spores. Diatoms (Bacillariophyceae) and golden algae (Chrysophyceae) are the two subgroups of the phylum Chrysophyta (Eze *et al*., 2018).

Bacillariophyta (diatoms)

Bacillariophyta (diatoms) are a category of unicellular organisms that are very diverse and made up of many species of microalgae that may be found in the world's oceans, rivers, and soils (Khan *et al*., 2018). A large fraction of the biomass of the planet is made up of living diatoms: A siliceous cell wall (frustule) with two overlapping portions is present in them (valves). Sets of siliceous hoops (girdle bands) that cover and efficiently protect the cell's midsection are also subtended by each valve. A growth constraint is placed on the cell by the siliceous cell wall. Because of this, each mitotic division causes a decrease in cell size. The diatoms eventually grow to the point at which they can begin reproducing sexually (Medlin *et al*., 1993).

Cyanophyta (blue green-algae)

Cyanophyta, also referred to as the Cyanobacteria, is a phylum of Gram-negative bacteria that produces energy by photosynthesis (Heidorn *et al*., 2011). Biologically speaking, bacteria are small, photosynthesizing organisms known as blue-green algae. Since dense growths frequently color water green, blue-green, or brownish-green, they were previously referred to as blue-green algae. All lakes have these algae, which are a typical component of the lake ecosystem.

Pigments and Bioactive Compounds from Microalgae

Algae are very important in the production of pigments and bioactive compounds (Larkum *et al*., 2012). Various algal species have been identified in the production of pigments. The following algal species are very influential in the production of b-carotene, astaxanthin, lutein, canthaxanthin and fucoxanthin: *Dunaliella salina, Haematococcus pluvialis, Chlorella* spp. *Scenedesmus* spp. *Botryococcus braunii, Spirulina platensis*, and diatoms (Zhang *et al*., 2014). Pigments produced by algae differ from each other the reasons for this might be because of the various environmental conditions of the species producing the pigments (da Silva Ferreira and Sant'Anna, 2017). The living conditions of microalgae is very complex, their environmental condition is usually changing. This gives them the ability to adapt quickly to the new environment they found themselves, in order to survive (Chia *et al*., 2017). *Tetraselmis* spp. produces the pigment lutein which proves to be of nutritional benefit to the zooplankton *Artemia* used as fish feed (Lee *et al*., 2021). The assimilated lutein could be converted into vitamin A from halibut larvae when the crustaceans are used as feed (Samat *et al*., 2020). Lutein and astaxanthin produced by

Haematococcus pluvialis, phycocyanin by *Spirulina platensis*, polyunsaturated fatty acids by *Schizochytrium* spp. *Chlorella* spp. biotin and vitamin E from *Euglena gracilis* are among the high quality bio-products produced by microalgae (Vadiveloo *et al*., 2019).

Bioremediation of oil spills and heavy metals biodegradation

Bioremediation is the use of microorganisms in breaking down environmental pollutants or detoxify dangerous pollutants in the environment (Kensa, 2011; Adams *et al*., 2015). Cultivating microalgae requires order of engineering complexity. The main methods of cultivating microalgae in aquaculture includes; Open ponds or tanks, provided with or without aeration/stirring, bubble or airlift columns and closed photobioreactors (Naumann, 2013; Goswami *et al*., 2018).

Oil spill is a big environmental concern. Microalgae are significant in the bioremediation of spilled oil. They have great potential for the removal of excess nitrogen and phosphorus from wastewater including the farm runoff. They can capture carbon dioxide from coal fired power plants thereby reducing greenhouse gas and also producing algal biomass, which can be converted into biofuel. A study conducted by Praepilas and Pakawadee (2011) shows the potential of microalgae in utilizing industrial wastewater as a cheap nutrient for their growth and oil accumulation. *Scenedesmus quadricauda* and *Chlorella* spp. were used in the study. The cultures yielded the highest lipid content at 18.58 % and 42.86% for *S. quadricauda* and *S. obliquus* respectively.

Larval feeding of microalgae in tropical countries is done in hatcheries indoors which in turn improves the quality and dependability of production. This implies that culturing of algae outdoors causes lysis of cells due to severe and rapid fluctuations in light and temperature (Nash, 2011). Araya *et al*. (2010) reported 20L capacity polycarbonate carboys containing PVC filaments for culturing of mixed benthic diatom strains, which were successfully administered to post larval *Haliotis rufescens*. The same research group also described a photobioreactor design for diatoms, based on an aerated acrylic cylinder coupled with a bottle brush-like array of plastic bristle (Silva-Aciares and Riquelme, 2008).

Heavy metals are not biodegradable and tend to accumulate in living organisms. In order to find a suitable solution to heavy metals non-biodegradable issues, various methods have been tested (Sun *et al*., 2018). Microalgae, related photosynthetic eukaryotes, and many fungi have preferentially evolved the production of peptides which are capable of binding to heavy metals (Spain *et al*., 2021). These biomolecules, as organometallic complexes, are further partitioned inside the cell vacuoles to facilitate appropriate regulation of the cytoplasmic levels of heavy metal ions, thus neutralizing their potential toxicity (Cobbett and Goldsbrough, 2002). Centrally to this mechanism employed by eukaryotic organisms, prokaryotic cells make use of ATP consuming efflux of heavy metals or enzymatic change of speciation to neutralize toxicants. Microalgae prove to be more significant in remediation processes, this is due to the fact that wide range of toxic and other wastes can be treated with algae and they are non-pathogenic. Microalgae reduce the risk of accidental release of pollutants into the environment. Microalgae use the environmental wastes as nutritional sources and enzymatically degrade the pollutants (Ayse *et al*., 2005).

Microalgae in Water Purification

Microalgae are cultivated in nutrient media to aggregate biomass used for food or biofuel production (Velichkova *et al*., 2012). Wastewater from recirculation system in aquaculture, as they are rich in inorganic and organic substances are used as media for algal culture (Sirakov *et al*., 2013). Various species of algae have a potential for integrated use in purification of waste pond water and biomass production. Such species include, *Chlorella vulgaris*, *Nanochlopsis oculata* and *Tetraselmis chuii,* they exhibit high potential to accumulation in terms of phosphorus and nitrogenous compounds present in the wastewater from aquaculture and could be utilized for their remediation (Sirakov *et al*., 2014).

One of the means of providing efficient nitrogen and phosphorus in wastewater is the cultivation of microalgae (Gismondi *et al*., 2016). The wastewater should be free of other toxic pollutants such as heavy metals, which would be accumulated in the algal cells, and subsequently in the animal tissues, if the algal biomass was targeted as a food or feed ingredient (McHugh *et al*., 2003). Liu and Hu (2013) studied the efficient phytoremediation of primarily treated domestic effluents with *Chlorella* spp. The study reported that phytoremediation can be a feasible approach to reduce the release of both organic and inorganic compounds into natural aquatic ecosystems, and valorize the resulting biomass by converting the nutrients present in wastewater into useful biomolecules such as protein, lipids, and pigments.

Culturing Microalgae on Wastewater

Wastewater decreases the production cost compared to cultivation with commercial growth media. Treatment of effluent (wastewater) makes production sustainable, as it reduces biological oxygen demand (BOD) of the effluent, which can therefore return safely to the environment without costly effluent treatment procedures. Safafar *et al*. (2016) reported that culturing microalgae on industrial wastewater enhances nutrients composition. Safarar *et al*. (2016) cultivated *Chlorella vulgaris* and *Chlorella pyrenoidosa* and in pre-gasified industrial process water with high levels of ammonia representing effluent from a local biogas plant. Both species grew well in industrial process water. The protein content was significantly affected by the growth media and the duration of cultivation. *Chlorella pyrenoidosa* produced the highest amount of protein (65.2% -1.30% DW) while *Chlorella vulgaris* accumulated extremely high levels of the pigments lutein and chlorophylls (7.14 -0.66 mg/g DW and 32.4 - 1.77 mg/g DW respectively).

Microalgae in Aquaculture

Microalgae are living cell with high nutritional value and play important role in aquatic food chain. Microalgae used in aquaculture were first reported in 1910 and thereafter have been widely used and recognized in aquaculture (Brown and Robert, 2015). One of the most growing sectors in the world is aquaculture and microalgae. This is because microalgae comprise of nutritional compositions which are of values to aquatic animals and serve as feed to some aquatic animals (Brown and Robert, 2015). Application of microalgae in aquaculture involves the use of green water feeding technique. In this technique, microalgae are added as suspension to the organism environment grown simultaneously in tanks with larvae (Pérez-López et al., 2014).

Microalgae as Valuable Feed Additives in Aquaculture

Increasing need for protein and high cost of fishmeal, has led to search for new alternatives. Microalgae are an excellent alternative to solve this need and requirements in the aquaculture industries. Microalgae are used as live feeds for the growth of various aquatic cultures such as mollusks, crustaceans and some fish species, also microalgae can be fed to zooplankton for aquaculture food chains (Roy and Pal, 2015; Kaparapu, 2018). In the application of microalgae, direct or indirect method can be used in feeding the cultured organisms. *Artemia,* rotifers, and *Daphnia* are the routes of applying indirectly. Also different species of microalgae can be used together instead of just one microalgal species (Guldhe *et al*., 2017).

Microalgae nutritional value is composed of protein, vitamin and polyunsaturated fatty acids. Environmental conditions such as nutrients, light intensity and temperature are among the factors used to improve the content of polyunsaturated fatty acid (PUFA) in microalgae, this give room for the modulation of lipid composition and subsequent optimization of their productivity. The use of microalgae as feed additives in aquaculture have recently received a lot of attention due to their effects on weight gain, increase in triglyceride and muscles protein deposition, decrease in nitrogen output into the environment, improved resistance to disease, increased fish digestibility, carcass quality, physiological activity and starvation tolerance (Fleurence, 2012).

Addition of Microalgae to Fish Larval Rearing Tanks

There are several techniques involved in the addition of microalgae to fish larval rearing. Fish larval rearing using microalgae is common and yield higher survival and growth rates than larval rearing in clear water (Kaparapu, 2018). Green water technique involves microalgae or zooplankton to be stored in ponds or large tanks where fish larvae are also kept (Shields, 2001). For this purpose, cultured microalgal strains can be introduced into fish rearing tanks provided the water used in the system has been pre-treated to eliminate competing microorganisms. Various investigations have been done to understand the mechanisms by which microalgae enhances superior rearing performance of fish larvae and optimization of their delivery in hatcheries (Conceiçao *et al*., 2010). Molina-Cárdenas *et al*. (2014) suggested that processes such as microbial conditioning by microalgae may lead to impeding cell-to-cell signaling (quorum sensing) by pathogenic bacteria. In a laboratory study focused on commonly used microalgal strains in aquaculture, several of the assessed strains interrupted signaling by pathogenic *Vibrio harveyii*, leading to postulation that such microalgae offer potential to be used as biocontrol agents in aquaculture (Vargas-Albores *et al*., 2021).

Nutritional Composition of Microalgae

Microalgae grows rapidly and are one of the most important sources of feed in aquaculture, due to their high nutritional value and ability to synthesize and accumulate high amount of α- 3 - polyunsaturated fatty acid (PUFA) in their cells (Patil *et al.,* 2005). Nutritional analysis of six microalgae showed that they contain 35.4 – 50 % total intracellular protein, 28.3 – 32.3 % carbohydrate and fiber, 5.6 – 15.6 % total lipids (Matos *et al*., 2016). In an investigation, the microalgae *Tetraselmis chuii* cultured in different media enriched with or without wastewater was evaluated for its growth, proximate composition and carotenoid production of the species (Khatoon *et al*., 2018). The findings showed that significantly high growth (4.3 × 105 cells mL^{-1}) and carbohydrate (20% dry weight), protein (56.4% dry weight) and lipid (44% dry weight) contents were found in *T. chuii* when cultured in the medium containing both wastewater and Conway.

In another study by Kent *et al*. (2015) to assess the nutritional composition of some Australian microalgae, it was found that *Dunaliella* spp. *Scenedesmus* spp. and *Nannochloropsis* spp. have promising nutritional biochemical profiles. Yusof *et al*. (2011) using culture method, investigated *Chlorella vulgaris* (Cv) for their nutrient's composition. Fatty acid contents and other nutrients were assessed. It was observed that *Chlorella Vulgaris* cultured under 24 hour illumination and 10% CO_2 yielded the best growth rates and contained higher protein, lipid and moisture contents when compared to other culture conditions. There was a positive correlation between the fatty acid of Cv) content and the amount of CO_2. Gas chromatography-mass spectrometry (GC-MS) analysis of the fatty acids profile showed the presence of cis-10-pentadecanoic acid (C15:1), palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), stearic acid (C18:0), oleic acid (C18:1n9c), linoleic acid (C18:2n6c), linolenic acids (C18:3n3) and arachidic acid (C20:0). Polyunsaturated fatty acids such as (linolenic and linoleic acids) were found in abundance compared to other fatty acids in *Chlorella vulgaris*. The concentrations of palmitic, linoleic, oleic, and linolenic acids increased when the amount of carbon dioxide was increased from 1 to 10% under both culture conditions (12 and 24 hour illumination).

Algal Toxins in Aquaculture

Algal toxins are organic biomolecules synthesized by a variety of algae in marine, brackish and fresh water bodies (Carmichael, 2013). Toxin produced by algae is normally associated with algal blooms, or the rapid growth and exceptionally dense accumulation of algae which results to serious water quality deterioration (Wang *et al*., 2011; Weirich and Miller, 2014). Algal toxins when produced in sufficient amounts become a greater problem in aquaculture as it has sufficient potency to kill cultured organisms, decrease feed and growth rates (Fire *et al*., 2011). Some cyanobacteria (blue-green algae), particularly *Microcystis* and *Anabaena* produce toxins referred to as (cyanotoxins) which are poisonous to fish. For example, neurotoxins can attack the nervous system of both invertebrates and vertebrates. Neurotoxins are produced by several genera of cyanobacteria. *Microcystis*, *Aphanizomenon flos-aquae* and *Oscillatoria* blooms have been responsible for animal poisonings in many regions of the world (Svirčev *et al*., 2019; Ndlela *et al*., 2020; Skafi *et al*., 2021). Hepatoxins are produced by many genera of cyanobacteria among them is *Microcystis aeruginosa* which is the most common bloom-forming cyanobacteria species and also causes the deaths of fish, birds, wild animals, livestock and humans around the world (Mecina *et al*.,2019; Esterhuizen-Londt and Pflugmacher, 2020). Baidyanath *et al*. (2006) reported the toxicity of *M. aeruginosa* on the zooplankton *Daphnia magna*, air breathing teleost (*Clarias batrachus* and *Heteropneustes fossilis*) and major carps (*Catla* and *Labeo rohita*). The results revealed that *M. aeruginosa* strains produced a variety of peptide toxins. Eze *et al*. (2018), conducted a study on prevalence of *Microcystis* bloom in fish culture ponds in India and possible control approaches were reported. The population density of *M. aeruginosa* was found to be maximum in Harahi Pond, followed by Dighi Pond and Mahaseh Pond. Bloom-like situation was recorded during summer and was observed only when its population density was greater ($2.5x10^4$ cells/cm^3).

Conclusion

An extensive literature review demonstrated the importance of microalgae for aquaculture wastewater nutrient removal, as microalgae can decrease the N concentration of wastewater, and their biomass can be used for aquacultural feed. They are good bioremediation agents for the treatment and recycling of wastewater in aquaculture ponds.The importance of algae in aquaculture is not surprising as natural food source of these animals. Application of microalgae for aquacultures are associated with nutrition, being used fresh as sole component or as food additive to basic nutrients and for inducing other biological activities.

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