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Review Article

Potential of Microalgae as Feed Supplements for Sustainable Aquaculture

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ABSTRACT

Aquaculture is a fast-growing industry mainly depends on the key feedstuffs, fishmeal (FM) and fish oil (FO) that will be limited with the passage of time due to the insubstantial resources available for wild fish harvesting. Therefore, other sources of feedstuffs need to be investigated to substitute FM and FO in aquafeeds. Terrestrial crops can be used to substitutes a portion of the FM however; they can result in changes in the nutritional quality of the fish produced. Microalgae can be considered as a favorable alternative that can substitute FM and FO ensuring the principles of sustainability in aquaculture. Microalgae are reasonably rich in proteins, lipids, carbohydrates, vitamins, minerals, pigments, etc., which are essential for not only sustaining fish health but also its unique array of bioactive compounds can improve coloration and quality of fillet. The aim of this review is to provide an update of the current knowledge of microalgae as a supplement or feed additive to substitute FM and FO in aquafeeds. This review will provide a platform to highlight the potential of microalgae-based aquafeeds for a sustainable aquaculture industry.

INTRODUCTION

Aquaculture is an important and fast-growing food sector in the world playing a vital role in human's life. It has undoubtedly contributed significantly to improve the nutritional value of human's diet in the form of seafood and improved the standard of living due to financial gain from the rapidly developing aquaculture industry. Since 2016, worldwide fishery and aquaculture foods have shown tremendous growth of ~ 171 million tons that costs 194.78 billion euros through aquaculture to give 54.5% of the overall production. Statics shown that 19.3 million people have obtained employment in the fisheries and aquaculture sector that accounts 30% of the total jobs, playing a significant role in uprising their socio-economic status. The entire fish production without marine floras is likely to upsurge ~ 204 million tons in 2030 that accounts an overall increase of 15 % over 2018 (FAO, 2018, 2020).

Aquaculture is a vital source of animal protein, which is nearly half of the total production that need outward feed contributions to overcome the food consumption demand by the growing human population. Aquafeeds normally contain fishmeal and oil take out from trivial pelagic forage fish, for instance sardines, herrings and anchovies, and little amount after fish embellishments and castoffs. Fish meal (FM) and fish oil (FO) mainly used to fulfil the protein and fatty acid requirements of farmed aquatic species as palatable and inexpensive feed components. Aquafeeds generally increases fish productivity, but an alternative to FM and FO must be find out for sustainable fish farming (FAO, 2016; Turchini *et al*., 2020). Many fodder fisheries are fully or over exploited, and reports have portrayed the existing trend of fishmeal and oil utilization as a big challenge for marine biodiversity and human food safety (Froehlich *et al*., 2018). The current fishmeal and oil are very costly, expected to upswing than that of plant oils and protein meals over the following decade. Keeping in mind all issues alternative aquafeed resources are required with high digestibility and nutritional cost analogous to FM and FO that needs to be developed through eco-friendly

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strategies as extraordinary and economical feedstuffs (Cottrell *et al*., 2020).

Marine-based FO contain high quantity of omega-3 (n-3) long-chain (≥C20) polyunsaturated fatty acids (LC-PUFAs), namely eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). Biological conversion of the PUFA, alpha-linolenic acid (ALA; 18:3n-3), normally found in terrestrial oil kernels of canola used in marketable salmonid nourishments, to EPA and DHA in fish and humans is not sufficient to fulfil their nutritional rations, therefore it is necessary to take in the n-3 LC-PUFA in food (Saini and Keum, 2018; Bou *et al*., 2017). DHA play an essential role in cell signaling as well as the structure, function, and fluidity of cell membranes while EPA triggers anti-inflammatory response via eicosanoid production. Addition of EPA and DHA in salmonid feed not only safeguard ample growth and development of the fish, but also a carrier to deliver these EFAs to humans, which have significant health benefits in preventing rheumatoid arthritis, cardiovascular, and neurological ailments (Hart *et al*., 2021; Siscovick *et al*., 2017; Laye *et al*., 2018).

The current aquaculture is over relied on terrestrial crops and animal-based materials like soybean meal, canola oil, poultry fat, and blood meal, which involves worries about deviation of crops and animals from human feeding towards aquafeed (Colombo, 2020). As crops growing sector appearances a universal task to nourish nearly a billion of hungry folks, and risks turning the rapidly expanding aquaculture sector into an environmentally unsustainable agrarian practice for the world's grains and oils consumption. Their use in aquafeed has many shortcomings e.g., unbalanced essential amino acid, high levels of antinutritional elements and insufficient level of EAA and EFA cannot fulfil the requirements of fish and human health (Fry *et al*., 2016; Sprague *et al*., 2016).

Microalgae are eukaryotic photosynthetic microorganisms that use solar energy, nutrients, and carbon dioxide (CO_2) to produce proteins, carbohydrates, lipids, and other valuable organic compounds. Recently an increasing attention focused all over the world on commercial-scale production of microalgae for aquaculture feeds due to their better fatty acid profiles. Compared to terrestrial plant proteins and oils, microalgae have reasonable quantity of DHA and EPA (Acquah *et al*., 2020). They can propagate under different conditions (autotrophic, heterotrophic and mixotrophic) by assimilating simple nutrients and accumulate useful metabolites like n-3 LC-PUFA and carotenoids (Hardwood, 2019).

Microalgae can deliver many vitamins specially vitamins D and K produced in little quantity in the land-dwelling plants. This insufficiency can be fulfilled by adding microalgae in the aqafeed that can also provide other vitamins (A, B, C, D, and E) (Del Mondo *et al*., 2020; Kiran and Mohan, 2021). In a study, *Arthrospira platensis* and *Chlorella vulgaris* were used in the aquafeed to replace fishmeal given to post-larvae of freshwater prawn (*Macrobrachium rosenbergii*) to investigate its influence on vitamin C and E, antioxidant potential, catalase, and lipid peroxidation activities. After 3 months, a 50% substitute of the fishmeal with *A. platensis* has significantly enhanced the growth of *M. rosenbergii* (Radhakrishnan *et al*., 2016). Recently the total folate content was determined in different species of marine microalgae. The marine microalgae *Picochlorum* sp. showed the highest folate content (6,470 ± 167 µg/100 g dry biomass), followed by *Chlorella vulgaris* (3,460 ± 134 µg/100 g dry biomass), and other tested strains (Woortman *et al*., 2020). In another study among seven microalgae species cyanobacterium (*Anabaena cylindrica*l) was find out as a rich source of vitamin K1 producing 200 µg g^{-1} on a dry-weight basis, which is about six-fold greater than its rich dietary sources (spinach and parsley) and can be further increased by optimizing the growth conditions (Tarento *et al*., 2018).

Microalgae strains display a tremendous variation in the inorganic content (ash) or mineral composition due to their existence in diverse habitats, wide-ranging environmental factors, and different genetic composition. The minerals content in microalgae varies from 20-40% that play a significant role in the structural, physiological, catalytic, and regulatory functions of the aquatic organisms (Fox and Zimba, 2018). In a study, the micro and macro minerals of five maritime microalgae strains showed capricious ranges in calcium, phosphorus, magnesium, potassium, sodium, and sulfur as 0.26-2.99, 0.73-1.46, 0.26-0.71, 0.67-2.39, 0.81-2.66, and 0.41-1.38%, respectively. The chlorophyte (*Tetraselmis chuii*) showed the highest level of calcium and phosphorus as 2.99 and 1.46%, respectively. Similarly, the bacillariophyte (*Phaeodactylum tricornutum*) showed highest level of magnesium, potassium, sodium, and sulfur content respectively. Recently the mineral conformation was investigated in maritime microalgae comprised 26 chemical elements (Al, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Sn, Sr, Ti, Tl, V, and Zn). The tested strains showed a high inorganic content ranges from 12.9-36.3% mass of the examined elements per dry biomass (Tibbetts *et al*., 2015; Silva *et al*. 2015). It is also useful for a sustainable aquaculture industry through blue revolution by the minimum use of water, farming land, nutrient recycling, CO_2 conversion, and remediation of wastewater (Tibbetts, 2018; Yarnold *et al*., 2019). Consequently, microalgae-aquaculture association is an emerging paradigm for sustainable aquaculture production that will ultimately shift the aquaculture industry into an ecofriendly circular bioeconomy.

NUTRITIONAL POTENTIAL OF MICROALGAE IN AQUACULTURE

Microalgae are very diverse group of eukaryotic photosynthetic organisms usually existed in marine and freshwater environments (Daneshvar et al., 2018). They can propagate in different forms such as single cells or in chains or in the form of trivial clusters (Postma et al., 2016), and play a vital role in marine environment by utilizing the sunlight and CO_2 for the synthesis of different biomolecules like proteins, polysaccharides, lipids, vitamins, or pigments (Ibrahim et al., 2020; Moha-Leon *et al*., 2018). Consequently, they play an important role in nourishing the trophic chains in the aquatic environments and world widely distributed with >7000 species in diverse environments (Bellou *et al*., 2014; Shah *et al*., 2016).

Microalgae are the principal food source by providing necessary nutrients to the zooplankton and lower to higher trophic fish in the food chain (Yarnold *et al*., 2019). Different microalgae species comprise up to 60% protein, 60% carbohydrates or 70% oils based on the specificity of the strain and respective growth conditions (Draaisma *et al*., 2013) and contain valued pigments, growth hormones and secondary metabolites with substantial antimicrobial, antioxidant, anti-inflammatory and immunostimulant characteristics that are very beneficial for aquatic organisms (Garcıa-Chavarrıa and Lara-Flores, 2013; Shah *et al*., 2017). Consequently, microalgae can be incorporated in aqafeeds to nourish the fish larvae, mollusks, and crustaceans. It can be also used as live food to feed the zooplanktonic organisms like rotifers and micro-crustaceans (*Copepod*, *Cladocera* and *Artemia* sp.) that are live prey of maritime and crustacean larvae (Conceicao *et al*., 2010; Hemaiswarya *et al*., 2011; Perez-Legaspi *et al*., 2018; Yarnold *et al*., 2019). Microalgae is a balanced feed source of protein, lipid, and carbohydrate suitable to protect fish health. Table I shows the nutritional content of microalgae compared with other alternative feed ingredients.

Table I. Nutritional content of alternate feeds.

Feed ingredient	Protein (%)	Lipid (%)	Carbohydrate (%)	Reference
Wheat meal	12.2	2.9	69	Sørensen *et al*., 2011
Soybean meal	44	2.2	39	El-Sayed, 1994
Corn-gluten meal	62	5	18.5	Liu *et al*., 2020
Fish meal	63	11	-	Hodar *et al*., 2020
Saccharomyces cerevisiae	50.1	1.8	4.6	Blomqvist *et al*., 2018
Hermetia illucens	30.87	23.07	37.93	Varelas, 2019
Hydrolyzed feather meal	84.2	10.4	-	Yu *et al*., 2020
Isochrysis	41	17.72	14.46	Madeira *et al*., 2017
Haematococcus	30.87	23.07	37.93	Madeira *et al*., 2017
Botryococcus braunii	39.9	34.4	18.5	Tavakoli *et al*., 2021
Dunaliella sp.	40.46	15.51	20.44	Madeira *et al*., 2017
Spirulina maxima	60-71	6-7	13-16	Madeira *et al*., 2017
Spirulina platensis	55.8	14.2	22.2	Madeira *et al*., 2017
Schizochytrium	12.5	40.2	38.9	Samuelsen *et al*., 2018
Porphyridium aerugineum	31.6	13.7	45.8	Madeira *et al*., 2017
Phaeodactylum tricornutum	39.6	18.2	25.2	Sørensen *et al*., 2016
Chlorella vulgaris	37.5	14.4	26.6	Viegas *et al*. 2021
Chlorella sorokiniana	29.46	26	29.74	Guldhe *et al*. 2017
Anisancylus obliquus	40.56	15.34	16.97	Ansari *et al*. 2021
Scenedemus obliquus	21	22.6	48	Viegas *et al*. 2021 NA
Pavlova sp.	24-29	9-14	6-9	Madeira *et al*., 2017
Nannochloropsis granulata	33.5	23.6	36.2	Tibbetts *et al*., 2017
Isochrysis galbana	23.2	36.6	34.5	He *et al*., 2018

MICROALGAE AS A POTENTIAL PROTEIN SOURCE IN AQUAFEED

The microalgae biomass contain protein as an important component and its yield is dependent on many aspects e.g. type of specie, growth circumstances (pH, light, and temperature), nutritive value and environmental conditions. Nitrogen is an essential element to increase the protein yield of microalgae. A higher quantity of protein has been reported in microalgae when grown in high nitrogen concentration. The aquaculture industry uses ~70% of high-protein aquafeeds for the enhanced growth of aquatic organisms (Ansari and Gupta, 2019; Hua *et al*., 2019). The aquafeeds usually contain high quantity of protein with all the required amino acids however, majority of the current aquafeeds based on terrestrial plant protein are missing in some of the essential amino acids for instance lysine, methionine, threonine etc. It has been reported that microalgae contain virtually all the required amino acids therefore instead of terrestrial plants inclusion of microalgae in the aqafeed can produce more nutritious aquatic organisms that will be beneficial for human health (Chrapusta *et al*., 2017).

According to the recommendations of WHO/FAO/UNU vis-a-vis humans body need for essential amino acids the microalgae species (*Chlorella* and *Arthrospira*) contain high quality proteins and their amino acids profiles are almost similar to the protein sources (eggs and soybean) (Chronakis and Madsen, 2011). Microalgae can be added as feed or feed additives in the prospective aquafeed formulation of fish, shrimp, crab, shellfish, sea cucumber and other aquatic organisms. Microalgae was grown in an outside raceway reactor provided with digestate that was partially substituted (10% of the diet) in aquafeed of the *Acipenser baerii*. The outcomes of the experiments have confirmed the practicability to grow microalgae on digestate shown higher yield (6.2 gDM m^{-2} d^{-1}) with enhanced nutrient removal and reducing the chemical oxygen demand. The feeding test of the experiment compared with control groups ($p > 0.05$) shown better growth recital, somatic directories, fillet nutritional configuration and celiac function point out the significance of microalgae as protein source could be used in Siberian sturgeon aquafeed (Bongiorno *et al*., 2020).

MICROALGAE AS A NATURAL SOURCE OF POLYUNSATURATED FATTY ACIDS

Microalgae are a rich source of lipids that represents 74% of microalgae's total biomass depends on the species (Bernaerts *et al*., 2019). The lipids are made of fatty acids by 12-24 carbon atoms that comprise polyunsaturated fatty acids of n-3 PUFAs and n-6 PUFAs families, respectively (Patras *et al*., 2019). Microalgae produces eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that are vital for the growth, reproduction, immunity, and nutritional value of aquafeed (Remize *et al*.2021). Many researchers are currently investigating the effectiveness of microalgae that can be developed as non-FM or -FO diets. In one of such study fish feed and oil was substituted by algae feed (*Schizochytrium* sp. powder) and plant proteins. The results imply the potential of microalgae as an alternative source of Omega-3 fatty acid proceeding marine fish (Oliver *et al*., 2020). Currently the nutritional digestibility of a maritime microalga (*Schizochytrium* spp.) enriched in DHA and LC-PUFA was determined as an alternative lipid source fed to rainbow trout (*Oncorhynchus mykiss*). The results demonstrated the ADCs of the nutrients, energy, DHA and other fatty acids profiles of *Schizochytrium* spp. as an excellent alternative of fish oil replacement that may be provided with extra LC-PUFA in fish feed with vegetable oils (Bélanger *et al*., 2021).

Microalgae embody an auspicious prospect of Omega-3 PUFA yield, and several species have the capability to naturally synthesize EPA in high quantity such as *Nannochloropsis*, *Phaeodactylum tricornutum*, *Odontella aurita*, *Monodus subterraneus*, and *Pophyridium cruentum* (Bernaerts *et al*., 2018). The EPA levels (12.74 ± 1.84% and 10.93 ± 1.84%) have been reported in *Nannochloropsis salina* CCMP1176 and *Nannochloropsis oceanica* CCMP537 proceeding total fatty acids (Ma *et al*., 2014). Similarly, in another study, EPA levels (22.4 - 31.4 ± 1.7%) was reported in *Phaeodactylum tricornutum* (Ryckebosch *et al*., 2014). At present, numerous enterprises are producing EPA-rich microalgae biomass under photoautotrophic conditions such as Shenzhen Qianhai Xiaozao Technology Co., Ltd. of China harvests EPA rich lipid extract from *Nannochloropsis salina*. Additional companies are Arizona Algae Products, LLC (US) or Simris Alg AB (Sweden) (Oliver *et al*., 2020). Currently microalgae (*Aurantiochytrium* sp.) which as key source of n-3 PUFAs has been investigated to determine its potential on the growth performance and immune response of *Trachinotus ovatus*, supplied with different microalgae content (1.00-11.00%) for 8 weeks in its diets. The results revealed that adding *Aurantiochytrium* sp., in the diet has a beneficial paraphernalia on the fish survival, weight gain, and explicit growing rate improved by 1.02, 1.16, and 1.08 times, respectively (Li *et al*., 2021).

MICROALGAE POLYSACCHARIDES AS IMMUNOSTIMULANTS IN AQUACULTURE

Aquaculture is the rapidly growing industry faces the problem of disease outbreaks usually controlled by traditional methods of using antibiotics and chemical disinfectants; however, they have the problems of resistance development and bioaccumulation of toxic residues in aquatic organisms. Vaccines are an effective way of disease control, but its use is time consuming, expensive, and traumatic to the fishes. Therefore, immunostimulants are natural compounds that trigger the host defense system against infections. Currently microalgae polysaccharides are used as immunostimulants to control the diseases in aquatic organisms have been focused due to its less toxicity, bioactivity, and environment friendly nature (Marudhupandi and Inbakandan, 2015). The bioactivities and applications of sulfated polysaccharides from microalgae has been reported as anti-inflammatory, immunomodulatory, antiviral and antioxidant properties in the aquatic organisms (Raposo *et al*., 2013; Amna *et al*., 2018; Mohan *et al*., 2019; Nastasia *et al*., 2020). In a recent report, sulfated polysaccharides have been isolated from *Codium fragile* that have immuno-stimulatory effects on *Olive flounder* and can be utilized as feed additive to heighten the immunity of fish (Yang *et al*., 2019).

Microalgae can be used as nutritional supplements in the aquafeeds for their potent immuno-stimulatory effects in aquatic organisms. Currently it was observed that supplementing *C. vulgaris* at 10% in the meal of *O. niloticus* has protected it beside arsenic-induced immunotoxicity and oxidative stress (Zahran *et al*., 2018). Similarly, adding *C. vulgaris* at 6% in the meal of gigantic freshwater prawn (*M. rosenbergii*) has shown enhanced prophenol oxidase activity with the entire quantity of hematocytes of *M. rosenbergii* post larvae that might improve the larval survival to *Aeromonas hydrophila* infection (Maliwat *et al*., 2017). In another study *C. vulgaris* was added as dietary supplementation of nile tilapia (*O. niloticus*) to protect it against sub-lethal concentrations of penoxsulam herbicide and improve its anti-infective capacity against *Aeromonus sobria* (Galal *et al*., 2018). Similarly, the dietary intake of 5% *Schizochytrium limacinum* has encouraging results in improving the intestinal health and nutrient utilization potential of rainbow trout *O. mykiss* (Lyons *et al*., 2017).

MICROALGAE CAROTENOIDS AS FUNCTIONAL FEED ADDITIVES IN AQUACULTURE

Microalgae produce carotenoids with distinctive antioxidant and coloring characteristics including xanthophylls e.g., zeaxanthin, lutein, antheraxanthin that are found in land-dwelling plants. Moreover, they can also produce other pigments (astaxanthin, fucoxanthin, diatoxanthin, diadinoxanthin) specifically found in algae, cyanobacteria, and some species of yeast (Novoveská *et al*., 2019; Ambati *et al*., 2014, 2019). Numerous carotenoids are used in the aquaculture industry to color farmed fish especially astaxanthin is utilized to augment the pigmentation in farmed salmon. The pigmentation of fish is an important factor that can stimulate the consumer's choice to buy it. Carotenoids are not only important for coloring but also show a significant role in the growth, reproduction, and health care of aquatic organisms (Alfnes *et al*., 2006; Lehnert *et al*., 2019; Costa and Miranda-Filho, 2019).

The aquatic animals are unable to synthesize carotenoids therefore, microalgae can be provided as feed additive in their meal which are their naturally producers. An important carotenoid astaxanthin that is commercially used in the aquaculture industry produced by a microalga (*Haematococcus pluvialis*) at>4 percentage per DW that is a promising yield as compared to other organisms (Butler *et al*., 2018). Spirulina was added as a carotenoid source (0, 2.5, 5, and 10% of fishmeal weight) in the feed of yellow tail cichlid *Pseudotropheus acei*. The data shows a significant increase in total eggs production, percentage of eggs hatching, enhanced growth rate and raised carotenoids level in the skin of experimental one as compared to the control group of fishes (Güroy *et al*., 2012). In a study, four fish meals were supplemented with the carotenoids (astaxanthin, lutein, canthaxanthin and lutein+canthaxanthin) standardized at 50 mg kg^{-1} in the diet of goldfish juveniles compared to control (without carotenoids). The meal with lutein, astaxanthin and canthaxanthin showed a greater persistence values and increased carotenoid pigmentation if the skin of goldfish juveniles as compared to control treatments (Besen *et al*., 2019).

MICROALGAE AS POTENTIAL SUBSTITUTION OF THE CONVENTIONAL CONSTITUENTS IN AQUAFEED

The aquaculture production has tremendously increased during the last decade due to the amassed consumer's demand. Hence, this sector needs massive quantities of aquafeed that depends on FM, FO, and terrestrial plants, problems of low nutrients status, less availability, and expansive. To overcome these issues, microalgae is the best economical and alternative feed ingredient in aquafeed. Currently live microalgae strains as a whole or lipid-extracted algae (LEA) have been tried in aquafeed that have significantly enhanced the growth performance, physiological movement, and nutritional status of the aquatic species (Ansari *et al*., 2021). Numerous studies have been conducted to determine the potential substitution of conventional constituents in aquafeed with microalgae as shown in Table II.

Table II. Studies conducted to determine the potential substitution of conventional constituents in aquafeed with microalgae.

Microalgae species + Aquatic species	Ingredient substituted	Effects of microalgae specie on the growth performance and feed utilization of aquatic species	Reference
1. Nannochloropsis sp. + *Dicentrarchus labrax*	PR	*Nannochloropsis* sp. partially substitute 10% of the diet has no adverse effects on the growth performance, dietary nutrient consumption, and gut enzymes	Pascon *et al*., 2021
2. Schizochytrium sp. + *Oncorhynchus mykiss*	FO	Microalga can be a better candidate to substitute FO and LC-PUFA in FM due to improved ADCs of the nutrients, energy, DHA and other fatty acids	Bélanger *et al*., 2021
3. *Chlorella vulgaris* + Macrobrachium rosenbergii	FM	Adding 4-8% *chlorella* as a replacement of FM significantly enhanced the explicit growth rate, immune response, and resistance of *M. rosenbergii* postlarvae counter to *Aeromonas hydrophila* pathogen	Maliwat *et al*., 2021
4. Schizochytrium sp. + *Salmo salar*	FO	Microalgae biomass was added as 30% in diets of *S. salar* that specify the Sc biomass as an extremely digestible source of DHA and protein	Hart *et al*., 2020
5. Chlorella sp. + *Cyprinus carpio*	FM	Fresh microalgae that performed well in nutrient assimilation and oxygen production replaced FM, reducing eutrophication and providing O_2 in aquaculture.	Chen *et al*., 2020
6. Schizochytrium sp*.* + *Oreochromis niloticus*	FM	Microalga has modulatory effects on the blood cells and celiac microorganisms, without disturbing the configuration and integrity of intestinal villi	Souza *et al*., 2020
7. Scenedesmus-chroococcus + Acipenser baerii	PR	Microalgae supplemented diet accomplishes the nutrient necessities, confirming appropriate growth, ample fillet quality and a vigorous gastrointestinal tract in fish	Bongiorno *et al*., 2020
8. Nannochloropsis oculata and Schizochytrium sp. + Oreochromis niloticus	FM and FO	FM and FO replaced with two microalgae species in fishmeal has produced highest amount of DHA in the fillet than in those fed conventional feed recommends a cost effective aquafeed for farmed fish	Sarker *et al*., 2020a
9. Nannochloropsis sp., Isochrysis sp., and Schizochytrium sp. + Oncorhynchus mykiss	FM and FO	Microalga showed better results to substitute FM and FO due to improved ADCs of the crude protein, amino acids, lipid, and other fatty acids	Sarker *et al*., 2020b
10. S. obliquus + Oreochromis niloticus	PR	In different microalgae-based FMs, the diet comprising 7.5% of whole and LEA deliver essential nutrients with significant growth performance indicators (FCR 1.36 g/g, PER 1.84 g/g, and HSI 2.01%) in *O. niloticus*	Ansari *et al*., 2020
11. N. oceanica + Anarhichas minor	FM	FM of *A. minor* may be substituted up to 15% of the *N. oceanica* rich in omega 3-fatty level improved in the fish body	Knutsen *et al*., 2019a
12. S. obliquus + Anarhichas minor	FM	Substituting 4% of FM has significant impact on the body weight from 140 to 250 g after 12 weeks with rapid muscle growth, proximate arrangement of muscle, and skin color of fish	Knutsen *et al*., 2019b
13. Haematococcus pluvialis + Perca flavescens	FM	LEA meal mixed by soy protein (10% of the diet) replace 25% of FM in the tested diet has no antagonistic paraphernalia on the growth performance with growth indicators (FCR 1.19 g/g, PER 1.76 g/g, and HSI 2.00%) compared to the control diet	Jiang *et al*., 2019
14. Gracilaria arcuate + Oreochromis niloticus	FM	FM of *O. niloticus* replaced with 20% *G. arcuata* has tremendously increased their body weight from 13.01 to 36.13 g after 12 weeks with the growth indicators (FCR 2.28 g/g, and PER 1.49 g/g)	Younis *et al*., 2018
15. Nannochloropsis oculata + Oreochromis niloticus	PR	Substitution of FM with 33% LEA showed improved growth performance, feed use, and persistence comparable to control diet. After 12 weeks, the body weight increased from 1.98 to 28.06g with the growth indicators (FCR 1.26 g/g, and PER 2.12 g/g)	Sarker *et al*., 2018

Table continued on next page ....

Microalgae species + Aquatic species	Ingredient substituted	Effects of microalgae specie on the growth performance and feed utilization of aquatic species	Reference
16. *Nannochloropsis granulate* + *Litopenaeus vannamei*	PR	DP Protein content of all *N. granulate* meals was adequate and can be potentially added in the meals of *L. vannamei*	Tibbetts *et al*., 2017
17. *Isochrysis* sp. + *Tridacna noae*	LF	Microalgae was used as LF source to determine its consumption and digestion by *T. noae* larvae that was subjective to the type of microalgae and larval age	Southgate *et al*., 2017
18. *Schizochytrium* + *Litopenaeus vannamei*	LF	Addition of 4% *Schizochytrium* in diet showed significantly higher specific growth rate in shrimp larvae without effecting their survival, activities of gut enzymes, and fatty acid profile	Wang *et al*., 2017
19. *Schizochytrium* sp. + *Salmo salar* L	FO	*Schizochytrium* sp. was incorporated as FO in the diet of *S. salar* that showed enhanced growth performance, high fillet quality, nutrient retention, and blood chemistry	Kousoulaki *et al*., 2016
20. *Haematococcus pluvialis* + *Seriola rivoliana*	FM	FM substituted up to 80% without sizable effect on the intestinal effectiveness of *S. rivoliana* with an improved body weight from 2.5 to 74.0 g after 9 weeks, and growth indicators (FCR 0.8 g/g, and HSI 1.1%)	Kissinger *et al*., 2016
21. *Desmodesmus* sp. + *Salmo salar* L	FM	Addition of 20% *Desmodesmus* sp. in FM had no adversative effect on the growth depiction with growth indicators (FCR 0.90 g/g, PER 2.36 g/g, and HSI 1.30%)	Kiron *et al*., 2016
22. *Arthrospira platensis* + *Macrobrachium rosenbergii*	FM	Addition of 50% FM by *A. platensis* considerably improved their growth, feed efficiency, and enhanced amino acids' proteins and oil content	Radhakrishnan *et al*., 2016
23. *Phaeodactylum tricornutum* + *Salmo salar* L	FM	Addition of 6% microalgae biomass has no undesirable effects on the growth, feed conversion or ADCC of protein, lipid, energy, ash, and DM	Sørensen *et al*., 2016
24. *Schizochytrium* sp. + *Oreochromis niloticus*	FO	Microalgae proved a better substitution of FO in the diet of *Oreochromis* sp with better weight gain, feed conversion ratio, protein efficiency ratio without effecting its survival rate	Sarker *et al*., 2016
25. *Pavlova viridis* and *Nannochloropsis* sp. + *Dicentrarchus labrax*	FO	Addition of 50-100% microalgae to replace FO in the diet of *Dicentrarchus* sp has no negative effects on their growth performance and nutrient utilization	Haas *et al*., 2016
26. *Ulva ohnoi* and *Entomoneis* spp. + *Salmo salar* L	FM	Two added algal harvests (2.5 and 5.0%) delivered same fish enactments and feed efficacy (rich in n-3 LC-PUFA) related to the reference intake	Norambuena *et al*., 2015
27. *Chaetoceros muelleri* and *Tisochrysis lutea* + *Panopea generosa*	LF	Compared to the spray-dried, live-microalgae diets showed reasonable protein, carbohydrate, lipid, energy, DHA, n – 6 DPA, and Σn – 3 PUFA content; elevated EPA, AA, and ΣMUFA levels and an elevated Σn – 3/Σn – 6 PUFA ratio	Arney *et al*., 2015
28. *Isochrysis* sp. + *Dicentrarchus labrax* L.	PR and FO	Replacing 20% PR and up to 36% FO by Isochrysis sp. has no adversative effects, feed intake or growth performance as compared to controls	Tibaldi *et al*., 2015
29. *Arthrospira platensis* + *Oncorhynchus mykiss*	FM	Replacing 10% diet of *Oncorhynchus* sp with *Arthrospira* sp has tremendously increased their red and white blood count, hemoglobin, total protein, and albumin levels	Yeganeh *et al*., 2015

LEA, Lipid-extracted microalgae; FM, Fish meal; FO, Fish oil; LF, Larval food; PR, Protein; DP, Digestible protein; ADC, Apparent digestibility coefficients.

The *Nannochloropsis* sp. has been used as living food in aquatic species in a high-rate algal pond (HRAP) system on zootechnical basis considering morphometric parameters, and dietary nutrient digestibility of the celiac system of *Dicentrarchus labrax*. According to the results 10% of terrestrial plant ingredients replaced with microalgae has significantly increased the final body weight of the fish without disturbing its growth performance, dietary nutrient utilization, and gut enzymatic activities (Pascon *et al*., 2021). Microalga (*Schizochytrium* spp.) has been investigated to determine its digestibility, macronutrients availability and individual fatty acids (omega 3-rich) in *Oncorhynchus mykiss*. Thus, the microalgae were found to be a potential ancillary of FO and LC-PUFA in FM (Bélanger *et al*., 2021). Similarly, *Schizochytrium* sp. was included as 30% in the diets of *S. salar* that could reinstate the fillet n-3 LC-PUFA content as well as increase nutritional quality of the product for consumers (Hart *et al*., 2021). In another study addition of *C. vulgaris* (4-8%) in diets of *Macrobrachium rosenbergii* delivered best growth rates and enhanced immunity of the post larvae (Maliwat *et al*., 2021).

The traditional aquaculture is facing with the problems of eutrophication and depletion of oxygen for the aquatic organisms. In this regard, microalgae can be helpful in providing oxygen during its natural photosynthetic process as well as assimilating the nutrients released from the sludge and providing a conducive environment for the better growth and survival of aquatic organisms (Chen *et al*., 2020). The *Schizochytrium* sp. was added in the diet of *O. niloticus* reared in net cages that has beneficial effects on the red blood cells, lymphocytes, and intestinal microbiota without any contrary effects on the structure and integrity of the intestinal villi (Souza *et al*., 2020).

Aquaculture companies have focused to reduce the cost of aquafeed by replacing FM and FO by adding sustainable and cost-effective microalgae to produce fish free feed for *O. niloticus*. Such an effort was carried out by adding two microalgae species to replace FM and FO in the diet of farmed fish, which has beneficial effects such as highest amount of lipid, protein and DHA in the fillet as compared to the conventional feed (Sarker *et al*., 2020a). Similarly, three microalgae species (*Nannochloropsis* sp., *Isochrysis* sp., and *Schizochytrium* sp.) have been added in the diet of *O. niloticus* to substitute FM and FO in their feed. A significant improvement in ADCs of the crude protein, amino acids, lipid, and other fatty acids was observed in the farmed fish (Sarker *et al*., 2020b).

Integrated algae-aquaculture systems provide a suitable platform to develop ecofriendly, economical, and sustainable aquafeeds. Accordingly, different microalgae based FMs, the diet comprising 7.5% of whole and LEA has delivered essential nutrients with significant growth performance indicators (FCR 1.36 g/g, PER 1.84 g/g, and HSI 2.01%) in *O. niloticus* cultivated in a raceway pond (Ansari *et al*., 2020). Spotted wolf fish (*Anarhichas minor*) dominate the cold-water aquaculture. Its FM was substituted up to 15% of the *N. oceanica* that has resulted in high level of omega 3-fatty level in the muscle, liver, and whole body of all treatment sets, replicating the use of ~ 50% plant-based ingredients in the diets (Knutsen *et al*., 2019a). Moreover, *Scenedesumus obliquus* can be used as a substitute and valued feed constituent to partially replace FM to the existence of extraordinary protein content (above 50% of dry matter). Therefore, substituting 4% of FM has tremendously increased the body weight of the fish from 140 to 250 g after 12 weeks with rapid muscle growth and proximate arrangement of muscle suggesting the potential use of microalgae in aquaculture (Knutsen *et al*., 2019b).

Microalgae can be incorporated in FM with a particular percentage to progress the growth configurations, stress comebacks, liver functions, physiological events, and disease resistances of numerous fish varieties. For example, Lipid-extracted microlagae (LEA) meal mixed by soy protein (10% of the diet) to replace 25% of FM in the tested diet has shown good growth performance with growth indicators (FCR 1.19 g/g, PER 1.76 g/g, and HSI 2.00%) matched to the control diet in *Perca flavescens* (Jiang *et al*., 2019). According to Younis *et al*. (2018) the FM of *O. niloticus* is substituted by 20% *G. arcuate* that has significantly increased their body weight from 13.01 to 36.13 g after 12 weeks. Furthermore, Sarker *et al*. (2018) investigated that 33% LEA substitution of the FM has significant impact on the growth performance, feed utilization, and persistence comparable to control diet. After 12 weeks, the body weight increased from 1.98 to 28.06g with the growth indicators (FCR 1.26 g/g, and PER 2.12 g/g). Kiron *et al*. (2016) found that increasing the integration of *Desmodesmus* sp. as of 10% to 20% in FM has no adverse effects on the feed consumption and health of *Salmo salar* L. Similarly, Norambuena *et al*. (2015) found that *Ulva ohnoi* and *Entomoneis* spp. with inclusion levels of 2.5% and 5.0% could be added in FM of *Salmo salar* L that shows an enhanced feed efficacy (rich in n-3 LC-PUFA) related to the reference diets.

Digestible protein (DP) content is an important feed ingredient that is necessary for the development of new diet formulations for the acquaculture industry. The Protein degree of hydrolysis (DH) and predicted protein apparent digestibility coefficients (ADCs) of *N. granulata* algal meals can provided key parameters of its incorporation in the meals of *L. vannamei* (Tibbetts *et al*., 2017). In other report microalgae, concentrates have been used as larval feed of *T. noae* to determine its ingestion and digestion efficiency that was subjective to the type of microalgae and larval age (Southgate *et al*., 2017). The dietary potential of *Schizochytrium* as a meal supplement has been accessed in a feeding trial to investigate the survival, growth performance, digestive enzymes, and fatty acid configuration in the larvae of *Litopenaeus vannamei*. It was observed that addition of 4% *Schizochytrium* meal in microdiets of shrimps could progress their growth performance and other essential life activities (Wang *et al*., 2017). According to Kousoulaki *et al*. (2016), adding 5% whole biomass of *Schizochtrum* sp. in the extruded meal of *Salmo salar* L effectively replaced FO deprived of any adversarial effect on their growth performance, preservative ability of nutritional value. According to Kissinger *et al.* (2016) FM replaced by microalgae up to 80% showed no sizable effect on the growth performance or intestinal integrity of *S. rivoliana* with an improved body weight from 2.5 to 74.0 g after 9 weeks. Sørensen *et al*. (2016) scrutinized the whole cell microalgae *Phaeodactylum tricornutum* as an impending feed constituent for *Salmo salar*. A direct decline in apparent digestibility coefficients (ADC) was detected for protein, lipid, and DM to replace FM with *P. tricornutum* biomass from 0-12% in the feed. The algae biomass can substitute 6% of the FM devoid of any contrary impact on nutrient digestibility, growth and feed consumption of the fish (Sørensen *et al*., 2016).

The enhanced digestibility of crude protein and many essential amino acids found in *Spirulina* sp. recommend it as a good contender to be considered as an alternative protein source while *Schizochytrium* sp., contain maximum quantity of lipid and unsaturated fatty acids is a good candidate of FO substitute in tilapia feed. These microalgae species have been investigated for the apparent digestibility of macronutrients, amino acids and fatty acids in the *Nile tilapia* (Sarker *et al*., 2016). Similarly, the potential of *Pavlova viridis* as a PUFA source was assessed by comparing to *Nannochloropsis* sp. in the diets of *Dicentrarchus labrax* L. during 8-week feeding trial. It was observed that 50-100% microalgae could be added to replace FO in the diet of *Dicentrarchus* sp has no adverse effects on their growth performance and nutrient utilization (Haas *et al*., 2016). In another study the nutrient digestibility, growth performance, biometry, dressing out parameters, fillet muscle proximate and fatty acid composition of *Dicentrarchus labrax* L. have been investigated by replacing 20% PR and up to 36% FO with freeze-dried biomass of *Isochrysis* sp. has no adversative effects, feed intake or growth performance as compared to controls (Tibaldi *et al*., 2015). Another study has assessed the effects of diets comprising 0, 2.5, 5, 7.5 and 10% of *Spirulina platensis* on the hematological and serum biochemical factors of *Oncorhynchus mykiss*. It was found that replacing 10% diet with *Arthrospira* sp has significantly increased the red and white blood count, hemoglobin, total protein, and albumin levels in *Oncorhynchus* sp. (Yeganeh *et al*., 2015).

INTEGRATED MICROALGAE-AQUACULTURE SYSTEM, A SUSTAINABLE BIOREFINERY APPROACH

The current aquaculture industry is facing the problems related to the environmental safety and food security. Therefore, researchers have focused to resolve these problems to develop a sustainable aquaculture. The main problems in the customary aquaculture are water deterioration and antibiotics misuse that are not only responsible for resistance development but also polluting the water body (Han *et al*., 2019). The water deterioration generally occurs due to depletion of oxygen, detrimental algal bloom, and eutrophication of the water used in aquaculture. These problems possess serious threat for rearing the aquatic species as well as causes environmental pollution (Lu *et al*., 2019; Liu *et al*., 2014). One of the main causes of water deterioration is due to the excessive use of customary aquaculture feed comprised biomass contain protein and lipid that is not fully utilized by the aquatic animals. The remaining feed is changed to soluble nutrients by specific microorganisms ultimately causes eutrophication in water body (Han *et al*., 2019). Water deterioration also occurs due to the wastes secreted by the aquatic species that in the end can cause diseases in the aquatic animals ultimately their death (Lamb *et al*., 2017; Bhatnagar and Devi, 2013). To overcome these problems a biorefinery approach can be a cost-effective and sustainable paradigm in which microalgae cultivation and aquaculture, integrated for shared benefits (Shaalan *et al*., 2018). Figure 1 shows an integrated microlage-aquaculture system for sustainable aquaculture production.

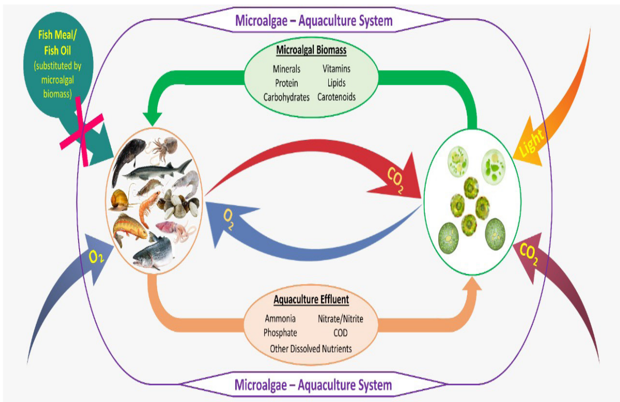

Fig. 1. The integrated microlage-aquaculture system for sustainable aquaculture production.

The integrated system of using microalgae in aquaculture system is an emerging paradigm that can be adopted to develop an ecofriendly and sustainable aquaculture. Aquaculture industry still facing the problems of expansive aquafeeds while its conventional ingredients possess environmental issues. Considering these problems microalgae is the best candidate, which comprise essential nutrients, can replace FM and FO in aquafeeds of the aquatic species to produce cost-effective and high-quality nutritious food for the malnourished population of the developing counters. However, the diversity in microalgae strains, their nutrients composition, environmental factors, and formulation in aquafeeds needs further research. Moreover, an integrated and economically sustainable biorefinery approach can be implemented to cultivate microalgae as aquaculture feed whereas the aquaculture wastewater used as a nutrients source to produce useful biomass. Microalgae has significant environmental benefits of fixing carbon dioxide and wastewater remediation, thus reducing the pollution problem caused by aquaculture wastewater. Therefore, considering the environmental and economic facets, microalgae-assisted aquaculture needs to be developed that will open new avenues in aquaculture and environmental sustainability.

MICROALGAE BASED AQUACULTURE WASTEWATER REMEDIATION

Microalgae have the potential to assimilate nitrate, nitrite, ammonia, phosphate, and organic carbon from aquaculture wastewater for their growth. Aquatic species generate these unwanted compounds in the wastewater body used for rearing. Therefore, microalgae are the best candidate to remove such nutrients and convert into useful biomass that can be used as feed constituent in aquafeeds. Thus, microalgae cultivation in wastewater has exclusive benefits in terms of bio-circular economy e.g., removing nutrients from the aquatic ecosystem and producing cost effective biomass for aquaculture industry. Subsequently biomass harvest, the treated water can be castoff for rearing aquatic organisms or other useful applications to develop a sustainable and ecofriendly environment (Yang *et al*., 2020).

A sustainable biorefinery approach was investigated in aquaculture wastewater of tilapia rearing tanks by growing *Chlorella sorokiniana* heterotrophically to utilize wastewater substrate for dual benefits (nutrients bioremediation and biomass generation). Microalgae has significantly removed phosphate, ammonia, nitrate, and COD (chemical oxygen demand) as 73.35, 75.56, 84.51, and 71.88% from the aquaculture wastewater with biomass productivity comprised proteins, lipids, and carbohydrates as 141.57, 150.19 and 172.91 mg/L/day (Guldhe *et al*., 2017). Similarly, *Scenedesmus obliquus*, *Chlorella sorokiniana* and *Ankistrodesmus falcatus* were cultivated in aquaculture wastewater (AWW) to investigate the biorefinary model to produce biomass with subsequent nutrient removal. *A. falcatus* generated biomass of 198.46 mg $L^{-1}d^{-1}$ with added sodium nitrate (400 mg L^{-1}) while *C. sorokiniana* produced biomass of 157.04 mg $L^{-1}d^{-1}$ with supplemented sodium nitrate (600 mg L^{-1}) in AWW as compared to the BG11 medium. Microalgae grown in AWW showed significant removal of ammonia, nitrate, phosphate, and COD in the range of 86.45-98.21, 75.76-80.85, 98.52-100 and 42-69% respectively (Ansari *et al*., 2017).

The wastewater of the recirculating aquaculture system (RAS) was used as nutrient medium to co-cultivate two different species of microalgae (*C. vulgaris* and *T. obliquus*). Both strains grow vigorously than their monoculture with average removal efficiencies of nitrate (98.73±0.06) and phosphate (99.46±0.04%), respectively (Tejido-Nuñez *et al*., 2020). Numerous strains of microalgae have been grown in aquaculture wastewater for nutrients bioremediation and biomass generation for an ecofriendly and sustainable aquaculture (Peng *et al*., 2020; Nasir *et al*., 2019; Gupta *et al*., 2016). Similarly, consortia of microalgae and associated water-borne bacteria through their extracellular enzymatic activities can potentially remediate the compact wastes of aquatic species. In such a symbiotic relationship, microalgae can effectively assimilate the nutrients from the AWW refining the self-purification capability of aquaculture system by producing high value biomass (Addy *et al*., 2017; Fang *et al*., 2017).

CONCLUSIONS

Aquaculture is a fast-growing sector playing an important role in providing high quality seafood mainly depends on FM and FO in the aquafeeds. Microalgae contain valued source of the essential nutrients required for high quality aquafeeds, comprising omega-3 fatty acids, EPA and DHA, essential amino acids, pigments, and antioxidants. Microalgae is the best source to replace FM and FO in aquafeeds due to their important role in the enhanced growth performance, physiological movement, and nutritional status of the aquatic species. Moreover, the integrated microalgae-aquaculture system provides a sustainable biorefinery approach e.g., removing the wastes of aquatic organisms and converting into cost-effective biomass. Therefore, microalgae-assisted aquaculture is necessary to develop a sustainable circular bioeconomy.

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Statement of conflict of interest

The author has declared no conflict of interest.

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