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MICROALGAE APPLICATIONS IN AQUACULTURE

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Abstract

Microalgae are at the base of the aquatic food chain, even of the life on our planet, and able to accumulate unique molecules for all livings. These microorganisms, which are mostly photosynthetic, are rich source of essential and functional nutrients such as amino acids, polysaccharides, polyunsaturated fatty acids (PUFAs), minerals, vitamins etc. In the last decades, the use of the whole microalgae biomass and microalgae-derived compounds as food supplements has increased. However, the role of microalgae in aquatic ecosystems, especially in aquaculture, is crucial and irreplaceable since they are the essential part of the system and the primary producers. This article aimed to review the favourable effects of using microalgae in aquaculture.

Keywords: Microalgae, aquaculture, feed, concentrate, carbon dioxide

Introduction

Microalgae are the primary producers in aquatic ecosystems and the higher trophic levels depend on the energy produced in this level of aquatic food chain. Although microalgae are largely photosynthetic, few species can also grow non-photosynthetically (Castillo et al., 2021). Photosynthetic microalgae are microscopic organisms that convert inorganic carbon (carbon dioxide) to organic carbon using sunlight through photosynthesis (Koh et al., 2020), while organic carbon sources are used in heterotrophic microalgae growth. These tiny organisms have a wide range of uses especially in health food and nutrition fields since they are rich in protein, carbohydrates, fatty acids, minerals, pigments, vitamins and many other nutritive and bioactive molecules (Göksan et al., 2007). However, above all, microalgae have a special importance in aquatic ecosystems since they are the natural part of the system and at the base of the food chain. As a result, microalgae in the natural aquatic environment have a vital importance both in the early larval stages of various aquatic animals and throughout the life of bivalve mollusks. The feeding on microalgae might be directly or indirectly via zooplankton. The feeding strategy





of the aquatic animals in the nature is mimicked under the controlled conditions in hatcheries and microalgae play again leading role in aquaculture, especially in the culture of marine animals due to the nutritious properties.

The cultures of microalgae require a lot of space, labor, time and expertise. In addition, one of the major problems with the cultures is the sudden collapse, which may be due to the lack of the monitoring of the main culture parameters, e.g., cell density, pH, temperature, macro nutrients, or the biological contamination. As such, aquaculturists and industry have been looking for alternative products that can replace microalgae and meet the requirements of cultured species. Various microalgal products have been developed for this purpose and have largely eliminated the difficulties of on-site microalgae productions. In this study, the use of the microalgae in aquaculture was reviewed, and the measures to reduce the production costs were evaluated since the costs are the main limiting factor for the widespread use of microalgae.

Common Species Used in Aquaculture

In aquaculture industry, microalgae are mostly used in the cultures of marine organisms. The species commonly used in the hatcheries are *Nannochloropsis* sp., *Isochrysis* sp., *Tetraselmis* sp., *Phaeodactylum* sp., *Pavlova* sp., *Chaetoceros* sp., *Thalassiosira* sp., and *Skeletonema* sp. (Saucedo et al., 2013). Interestingly, although *Chlorella vulgaris* is a freshwater species, it is higly demanded in zooplankton culture and green water technique in marine fish hatcheries.

In marine fish larvae culture, especially in green water technique, the ease and simplicity of the microalga culture is more important rather than the biochemical composition. With this respect, fast growing species like *Chlorella* sp. and *Nannochloropsis* sp. are preferred by the producers. However, when it comes to the feeding of the shrimps, bivalve molluscs and to the enrichment of zooplankton such as rotifers, artemia and copepods, the priority shifts to biochemical composition, especially to polyunsaturated fatty acid (PUFA) content, rather than fast growth. Marine fish larvae and bivalve molluscs can not synthesize the PUFAs, so microalgae species rich in EPA and DHA essential fatty acids are of vital importance (Pettersen et al., 2010). In addition, total lipid amount of the microalgae positively affects the feeding behavior of bivalve mollusks (Li et al., 2014). The strains from Bacillariophyta (diatoms, yellow-brown) and Haptophyta (golden-brown), shown in Table 1, are the mostly used microalgae rich in PUFAs for bivalves. Consequently, the combination of the microalgae species for feeding will increase the survival, support the growth and be complementary.

Application in Aquaculture	Microalgae Strains					
Formulated Feeds	Arthrospira platensis, C. vulgaris, C. minutissima, C. virginica					
	Dunaliella tertiolecta, D. salina, Haematococcus pluvialis					
Feed for bivalve molluses	Thalassiosira pseudonana, Pavlova lutheri, Isochrysis galbana.					
	C. minutissima, Gomphonema sp., Nitzschia sp., Phaeodactylum					
	tricornutum, Chaetoceros calcitrans, C. gracilis, Skeletonema					
	costatum, Tetraselmis subcordiformis, T. suecica, T.chui					
Rotifer and Artemia Live Prey	Crypthecodinium cohnii, Schizochytrium sp., Ulkenia sp.,					
	Chlorella sp., Chlamydomonas sp., Nannochloris oculata,					
	Tetraselmis tetrathele, T. chuii					
Feed for Crustacean Larvae	Tetraselmis suecica, T.chui, Chaetoceros calcitrans, C. gracilis,					
(Shrimps, lobsters)	Skeletonema costatum, Thalassiosira pseudonana					
Feed for Gastropod Molluscs and	Nitzschia sp., Navicula sp., Amphora sp.					
Sea Urchins						
Green Water	Nannochloropsis oculata, Isochrysis galbana					

Table 1.	Commonly	used microa	lgae in ac	uaculture a	pplications (Kaparapu	1. 2018)
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The both Arthrospira and Chlorella strains are rich in vitamins, minerals, amino acids, antioxidants and many other growth promoters. They contain more than 50% crude protein. They can be directly used in powder and flake forms or added in the feeds at low amounts ranging 1-10 ppm (Alagawany et al., 2021). Dunaliella salina and Haematococcus pluvialis are the strains rich in the carotenoids β -carotene and astaxanthin, respectively. They have been reported as the highest biological sources of the carotenoids (Rammuni et al., 2019; Torzillo et al., 2003). Microalgae are the main source of astaxanthin in aquatic environments. Astaxanthin is important ketocarotenoid pigment and widely used especially in salmonid feeds. Although natural astaxanthin can be used in the feeds, more than 95% of the feeds use synthetic astaxanthin in order to keep the costs lower (Lim et al., 2018). However, natural astaxanthin is more than a coloring agent in aquatic animals. Dietary astaxanthin is absorbed and deposited in aquatic animals such as krill, shrimp, lobsters, crayfish and various fish such as salmons and ornamental fish. Most of the aquatic animals mentioned above require 100-200 ppm astaxanthin in the feeds for a healthy growth. The use of natural astaxanthin improves growth performance, stress tolerance and disease resistance when compared to the use of the synthetic one (Lim et al., 2018).

Green Water Technique

Green water is a technique commonly used in aquaculture and a simple practice in which live microalgae are present in a certain concentration in the culture medium. This technique is called "green water" because many photosynthetic microalgae species are green in color due to the dominance of the chlorophyll molecules. However, the name of the application remains the same "green" even when the species in yellow, brown or any other color such as *Isochrysis* sp. and *Chaetoceros* sp. are used. Another term "pseudo-green water" can also be used in some studies, if microalgae are regularly added into the rearing tanks to keep the microalgae concentration at a certain level.

The presence of microalgae in culture media is not only due to the nutritional content, but also serves many different purposes. The use of green water technique was found to have positive effects due to the essential nutrients such as amino acids, polysaccharides and polyunsaturated fatty acids (PUFAs), on survival and growth of larval fish, shrimp, crab (Palmer et al., 2007; Dash et al., 2017; Basford et al., 2021) and especially shellfish (Pronker at al., 2008). One of the main purposes of green water technique is to serve as a food source for some aquatic organisms. The target organism may be fed directly on microalgae or indirectly on the zooplankton species such as rotifer, artemia and copepods enriched with n-3 PUFA, minerals, vitamins, etc. by the microalgae. The advantages of the green water technique in marine fish culture have partly been shown in the study of Papandroulakis et al. (2002). The technique applied to the larval rearing tank, increased survival, growth and food conversion index parameters when compared to the tank in clear water condition (Papandroulakis et al., 2002).

All the marine fish hatchery protocols include zooplanktonic live feeds, e.g., rotifers and artemia, in the culture tanks, and nutritional enrichment of these filter feeding organisms just before the feeding of the larvae is necessary. The nutritional value of the rotifer and artemia in the tanks is enhanced by the green water technique and high quality feed for the larvae is guaranteed (Wang et al., 2019). Green water also increases visual contrast since most of the feeds are in red and brown color, helping the reared organism to detect and consume the prey or feed more easily. In addition, increased turbidity has beneficial effects on larvae, lowering stress level and preventing the larvae from rising to the water surface (Naas et al., 1992; Cobcroft et al., 2012). Green water has many other health benefits on the larvae such as the boosting of the immune system and functioning as a probiotic. Live microalgal cells have a





suppressing effect on harmful bacteria in the tank water and larval gut (Ma et al., 2020). Besides, microalgae have an important role in improving the water quality in the culture tanks, absorbing mainly nitrogenous compounds and phosphorus (Taelman et al., 2013).

Apart from these, green water technique originally can be applied to the fresh water fish, i.e., mostly in carps and shrimp cultures in natural ways. The ponds or culture fields are fertilized and microalgal density is increased. Thus, aquatic animals grow much faster and healthier compared to the ones fed with just industrial feeds.

Microalgae Concentrates (Pastes)

Microalgal concentrates or pastes are highly concentrated forms of the algal cells and considered the best alternative to live microalgae. These products have aimed to replace live microalgae required in the farms, and largely eliminated the difficulties and risks of on-site microalgae productions, especially at peak production periods. The storage and usage are simple, and algal concentrates can replace with the live microalgae partly or totally depending on the reared animal and its developmental stage. They are ready to use and can be used any time needed.

The commercial products, in general, can be purchased in two forms; refrigerated form below 4 °C or frozen form at minimum -18 °C. The frozen forms are limited to few species such as Chlorella, Nannochloropsis and Tetraselmis. If an improper method is used to concentrate the microalgae cells, the organic content will leak out of the cell and cause pathogenic bacterial contamination in the rearing tanks. The products can be found at various biomass concentrations, ranging approximately from 10 to 30%, accordingly, the product can be in liquid or cake form. The cells are generally concentrated by high speed disc-stack centrifuges, membrane filter systems or flocculation methods. The lost of motility in flagellates, and/or nutrients especially in diatoms (Knucky et al., 2006) are the significant problems for bivalve cultures. The strains like Isochyrysis sp. and Pavlova sp. are very sensitive to mechanical stress, so, filtration and flocculation methods are preferred in these types of cells, and the dry biomass concentration of the end product is low around 10%. However, Chlorella and Nannochloropsis cells, which are also resistant to freezing, can be concentrated directly by centrifuges and the dry biomass concentration can reach 30% and above. The frozen products can be stored about 2 years, while the storage of the refrigerated products is limited to only about 3 months. Algae pastes may also have some disadvantages, i.e., the cells may be damaged by the concentration process or freezing and thawing, tend to precipitate faster, and have a lower nutrient content compared to live microalgae. Additionally, these cells cannot function like living cells in terms of water quality. Specifically, they are unable to absorb nitrogen compounds or produce oxygen (Rajaa et al., 2018). In cultures where the aforementioned disadvantages are critical—especially in bivalve cultures-the use of live microalgae becomes essential.

Conclusion

In bivalve hatcheries, one of the main problems is the high operational cost of the microalgae production, which may reach at the half of the total hatchery operating cost (Coutteau & Sorgeloos, 1992; Borowitzka, 1997). With this respect, the measures to reduce the microalgae production costs are of vital importance. The production of microalgae at larger scales using efficient culture systems (photobioreactors) is one of the measures. The expertise of the personnel responsible for microalgae production is as important as the use of efficient culture systems.





In microalgae culture, the cost of carbon dioxide (CO_2) is getting more and more important since carbon constitute approximately half of the dry biomass, and in theory, approximately, 1.8 kg CO₂ is needed for the production of 1 kg dry biomass (Chisti, 2007). Today, carbon taxes are very high in order to reduce the emition of CO₂, which is one of the major greenhouse gases. The prices of pure CO₂ tubes used in microalgae cultures are also increasing with the same rate. After heavy metal analysis, microalgae cultures integrated with the flue gases of various industries can be another solution to reduce microalgae production costs.

Ethical approval

Not applicable

Informed consent

Not available

Data availability statement

The authors declare that data can be provided by corresponding author upon reasonable request.

Conflicts of interest

The authors confirm that there is no conflict of interests for publishing this study.

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Contribution of authors

Tolga Göksan: Investigation, Writing original draft, Review, Editing. The author has read and agreed to the published version of the manuscript.

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