Microalgae: A source of Bio-colours

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Abstract

Since past many years, manufacturing of artificial pigments has been immensely developing because of its growing use in lots of fields like food-stuff, diary, cosmetics, prescription drugs, paints etc. But their intake through numerous manners can be reason of unsafe results and numerous poisonous illness like cancer, allergic reactions, chronic diseases etc. Due to this, severe organic manner had been investigated which incorporates the extraction of bio-colours from microalgae, bacteria, fungal etc. Microalgae is taken into consideration as a giant supply for the manufacturing of bio-colours that not only presents big benefits in numerous fields but also, additionally produces some of pigments which can be applied in numerous industries. This paper emphasizes upon the different phylum of microalgae and the pigments produced from them. Extraction process of different pigments from six phylum has also been discussed in brief. At the end, the emphasis has been given upon the importance and benefits of bio colours in different industries. It has been concluded in this paper that different bio-colours from microalgae have high utility yet its usage is still in its earliest stages however in not so distant future and can be taken as an entryway for development of sustainable and green industry.

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INTRODUCTION

Colour is the main thing to enhance the final appetizing value and customer popularity closer to components and beverages. In current scenario, use of artificial colours is more than bio colours in order to gain benefits of different properties like advanced appearance, low price, colouration stability, mild colouration depth and uniformity (Dilrukshi et al., 2019). But, considering the fact that since many years, artificial colours were proved disadvantageous because of its tendency to increase toxicity, pollutants etc., while such cases are not shown with bio colours. Due to this reason, use of bio-colours are growing now-a-days. Environment friendly strategies have turn out to be critical in manufacturing of artificial colours and dyes. Natural Pigments are especially acquired from flora and microorganisms like bacteria, fungi, algae etc (raju and Radha, 2015).

In order to enhance the nutritional value of conventional foods, microalgae have become an interesting source to develop new food products as they have valuable biochemical composition. In current study, we are discussing different phylum of microalgae, their pigments and related usage along with their future scope.

Literature Review

In recent years, many researches have been performed related to bio-colours out of which few are discussed below.
(Cardozo et al., 2007) discussed the metabolites of algae and their economic impacts. Focus was maintained on the substances that are biosynthesized by algae and their potential economic impact in food science, pharmaceutical industry and public health. 

(Sharmila et al., 2017), used macro algae for microbial fermentation to extract dye. Different micro algae were collected like Chaetomorphaantennina, Gracilariacorticota and Ulva fasciata from Koyalam, Mahabalipuram and Tamil Nadu. Micro Algae was used as a substrate for fermentation and Aspergillus Niger was used as an inoculum for fermentation. SSF was carried out for 10 days and dye was extracted. 

(Sharma and Sharma, 2017) discussed the industrial and biotechnological applications of algae. Focus was made on applications of algae as food and fuel. Along with this, different algal products which are available commercially were also discussed.

Since past many years, microalgae, including diatoms and cyanobacteria have been studied. They have been under investigation due to their photosynthetic production of natural pigments and biomedical applications (Fu et al., 2019). Microalgae are photosynthetic microorganisms that are found in earth ecosystems and feature the capacity to provide useful merchandise. They are especially developed in aquatic environments and use light and CO$_2$ to create biomass. Their membrane components, storage merchandise and sources of energies are lipids and fatty acids (Kumar and Viswanath, 2019). Their fast increase is fantastic to many better plants, whereas, their metabolic capacities assist in manufacturing of numerous molecules. They are an important source of pigments since they produce large varieties of molecules like chlorophylls (green), carotenoids (red, orange and yellow) and phycobiliproteins (red and blue) etc. The use of algae is more because of their faster growth rate, higher biomass productivity, capacity for sus-
Table 1: List of Phylum and Colour Extracted from them

| Phylum            | Common Name          | Pigments Produced                         | Colour Extracted                  |
|-------------------|----------------------|-------------------------------------------|------------------------------------|
| Chlorophyta       | Green Microalgae     | Chlorophyll, phospholipid, phycobiliprotein | Green, blue, yellow, orange, red, purple |
| Diatomophyceae:   | Diatoms              | Chlorophyll a and c, furcobiliprotein      | Blue-Green, Yellow-Orange          |
| Cryptophytes      | Cryptomonads         | Chlorophyll a and c, phycobiliprotein      | Red and Blue, Blue-Green, Yellow-Orange |
| Cyanobacteria     | Blue - Green Algae   | Phycocyanin, carotenoids                  | Blue Colour, Yellow-Orange, Red, Purple |
| Euglenophyta      | Euglenoids           | Chlorophyll a and b, phycobiliprotein      | Green, Blue, Yellow, Orange        |
| Dinophyta         | Dinoflagellates      | Chlorophyll a and c, carotenoid (beta-carotene) | Blue-Green, Yellow-Orange |

Tainable growth and lack of competition with other crops (Lone et al., 2013). During the period, their use has been increasing in bio colour formation.

Phylum of Algae

Phylum is a taxonomic rank which lies below kingdom and above class. It is a taxonomic division that mainly involves one or more classes. All the phylum listed below lies under kingdom Protista. Algae are mainly classified into 6 Phylum (Table 1) that produce pigments. They are classified as:

Chlorophyta

These contain approximately 500-16000 species. They are commonly called as green microalgae. Pigments like chlorophyll, beta-carotene, phycobiliproteins and astaxanthin are produced by species under this phylum. These pigments are biologically active and commercially important. They are also crucial for therapies like tumorigenesis, neuronal disorders etc (Khan et al., 2018).

Diatomophyceae

They are also called diatoms. There are more than 200-100000 species and are also called brown algae. They produce pigments like Chlorophyll a and c, b-carotene, fucoxanthin, diadinoxanthin and are mostly found in two types of habitats benthic (moist and submerged) and planktonic (open water) (Round et al., 1990). This group is mainly called species rich group of algae as they are ecologically widespread and participate in carbon and silicon cycles in nature.

Cryptophytes

They are also called cryptomonads and produce pigments like Chlorophyll a and c, carotenoids and phycobiliproteins. Pigment like phycobiliprotein is mainly used as fluorescent agent. This is a small group of unicellular protists named as algae of class cryptophyceae or protozoa of class phytophagophora. They are mostly found in two types of habitats like marine and freshwater (Wastl et al., 2020).

Cyanobacteria

They are mainly called as blue green algae due to the presence of blue pigment phycocyanin which is soluble in water. Phycocyanin along with chlorophyll a and c helps in capturing of light for pho-
### Table 2: List of Pigments Produced and their Applications

| Pigment          | Species                                                                 | Applications                                                                                                                                 |
|------------------|--------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|
| Beta Carotene    | Green and Brown microalgae as well as euglenoids.                        | Use in Butter, fats, oils, soft drinks, fruit juices, ice creams for food colouring.                                                        |
| Astaxanthin      | Haematococcuspluvialis, Chlorella zofingiensis, Chlorococcum, and Phaffiarhodozyma | Mostly used for food colouring in Salmon, Crabs, Shrimps, Chickens and Eggs production.                                                     |
| Phycobiliproteins| Rhodophyta and Chlorophyta                                               | It has properties like hepatoprotective, anti-oxidants, anti-inflammatory and anti-aging activity and hence can be used in food, cosmetics, pharmaceutical and biomedical industries. |
| Chlorophyll      | Chlorophyll b: Green Algae, Diatoms, Dinoflagellates and Brown algae.    | It is mainly used to colour a variety of foods and beverages like pasta and spirits.                                                        |
| Carotenoids      | Cryptophytes                                                             | Colouring properties of carotenoids mainly used in food, pharmaceutical, cosmetics, and animal feed industries.                           |
| Fucoxanthin      | Isochrysisgalbana, Mallomonas sp., Chromophyta (Heterokontophyta or Ochrophyta), including brown seaweeds (Phaeophyceae) and Diatoms (Bacillariophyta), Heterokont and Haptophyte groups of Algae | It is mainly used as antioxidant which provides brown or olive green colour.                                                                  |
| Phycocynin       | Aphanizomenonflos-aquae and Spirulina, Photoautotrophic Cyanobacteria.   | It is mostly used in Food and Beverages Industries and mainly named as ‘Lina Blue’ or ‘Exberry Shade Blue’ and used in sweets and ice creams. |

Pigments extracted from Micro-Algae

Pigments are simply said to be the natural colouring of plant and animal tissues. They are used in various activities like colouring in paint, ink, plastic fabrics, cosmetic food and other materials. To be used industrially for colour production, they should have high tinting strength. Listed are the brief descriptions of a few pigments that are extracted from microalgae. Advantages and species providing the particular pigment are given in Table 2.

Euglenophyta

They are commonly called as Euglenoids and are a group of protists which consists of phototrophic species and colourless phagotrophic and osmotrophic species (Brosnan et al., 2003). They provide colour range between yellow-orange and green blue to green yellow since they contain chlorophyll a, b and beta carotene.

Dinophyta

They are commonly called dinoflagellates and produce pigments like Chlorophyll a, c, carotenoid (beta-carotene) and peridinin. Out of the produced pigments from this phylum, carotenoids are bio-compounds which have a major role in food, feed cosmetics and biopharma. Pigments produced by dinophytes provide important biological activities and are of great commercial interest (Khan et al., 2018).

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**Astaxanthin**

It is mainly produced by the green microalgae. Astaxanthin is a type of carotenoid which is used
in salmonid and crustacean aquaculture which provides pink colour to species. Its scientific name is 3,3′-dihydroxy-β, β′-carotene-4,4′-dione which is a xanthophyll carotenoid that is normally found in Haematococcuspluvialis, Chlorella zofingiensis, Chlorococcum, and Phaffiarhodozyma (Higuera-Ciapara et al., 2006).

**Extraction of Pigment**

Astaxanthin (Figure 1) synthesis includes the formation of an inflexible molecular wall that impedes direct astaxanthin extraction right into a solvent. During the following downstream processing, the algal broth is mainly harvested with the aid of using centrifugation which is dried and automatically disrupted (Bauer and Minevca, 2019).

**Phycobiliproteins**

They are produced by the algae belonging to Rhodophyta and Chlorophyta phylum. Being water soluble it provides coloured accessory light-harvesting macromolecules in cyanobacteria, red algae and cryptomonads. PBPs also provide unique characteristics like strong absorbance, fluorescence, proteinaceous nature and other properties like hepato-protective, anti-oxidants, anti-inflammatory and anti-aging activity. They have high benefits in diseases like Alzheimer and cancer (T and Zakrys, 2015).

**Extraction of Pigment**

Biomass is mainly harvested by self-sedimentation from the cyanobacteria which is then filtered by a glass fibre filter paper. It is then washed and filtered again. This biomass is called wet biomass which was kept at 20°C to extract phycobiliproteins (Figure 2) Hussein et al. (2017).

**Chlorophyll**

It is one of the important bioactive compounds which can be extracted from the microalgal biomass. They are mainly used as natural food colouring agents which consist of antioxidant and antimutagenic properties (Aris). Two types of chlorophyll are mainly present i.e. a and b in green algae and terrestrial plants. The ratio of chlorophyll a (Figure 3 (i)) to chlorophyll b (Figure 3 (ii)) in higher plants is approximately 3:1. Chlorophyll has a quality to absorb light in red (650 – 700 nm) and the blue-violet (400 – 500 nm) regions of the visible spectrum. Chlorophyll a possesses green-blue colour, and chlorophyll b possesses green-yellow colour (Rajalakshmi and Banu, 2013)

**Extraction of Pigment**

This extraction begins with dewatering and desalting the dilute microalgal culture. This pigment is then extracted by using organic solvent extraction or using supercritical fluid extraction. This process is mainly called as fractionation step in order to separate the chlorophyll and its related pigments (Aris et al., 2010).

**Carotenoids**

These are tetraterpene pigments, which exhibit yellow, orange, red and purple colours and are mostly widely distributed pigments in nature and present in photosynthetic bacteria, some species of archaea and fungi, algae, plants and animals. Two classes of carotenoids are found in nature: (a) carotenes such as beta-carotene, consist of linear hydrocarbon and can be cyclized at one end or both ends of the molecule, and (b) oxygenated derivatives of carotene like lutein, violaxanthin, neoxanthin, and zeaxanthin, called as xanthophylls (Natalia et al.). Carotenoids have colouring properties they are used in the food, pharmaceutical, cosmetic, and animal feed industry.

**Extraction of Pigment**

Conventional (Figure 4) extraction of algae intracellular merchandise is usually carried out from dry biomass and is primarily based totally on maceration and thermal extraction the use of natural or aqueous solvents, relying at the polarity of the goal compounds to be extracted. Carotenoids show off various polarities, solubilities and chemical stabilities (Natalia et al., 2015).

**Fucoxanthin**

Fucoxanthin, a major carotenoid present in the chloroplasts of brown seaweeds, contributes to more than 10% of the estimated total production of carotenoids in nature. A number of studies have examined the metabolism, safety, and bioactivities of fucoxanthin, including its anti-cancer, anti-obesity. Fucoxanthin is an orange-coloured pigment, along with chlorophylls a and c and β-carotene, present in Chromophyta (Heterokontophyta or Ochrophyta), including brown seaweeds (Phaeophyceae) and diatoms (Bacillariophyta) (Peng et al., 2011)

**Extraction of Pigment**

Extraction of fucoxanthin (Figure 5) from moist brown seaweed Undaria pinnatifida (water content material turned into 93.2%) became accomplished with a easy approach with the usage of liquefied dimethyl ether (DME) as an extractant in semi-non-stop flow-kind system. The extraction temperature and absolute strain had been 25°C and 0.59 MPa, respectively. The liquefied DME turned into handed via the extractor that crammed with the aid of using U. Pinnatifida at special time intervals (Kanda et al., 2015).
Phycocyanin

Cultivation of Spirulina sp. uses majority of microalgae, that are cultivated in an open system and controlled system. It uses the phycocyanin as its main component which is present 15-25% of its biomass dry weight. This pigment is mainly used as anti-inflammation, anti-tumour, antioxidant, antiviral, anti-atherosclerosis, hepatoprotective, lipase inhibitor, lipid reducing agent, and inhibitor of environment pollutant into the body (Alassali and Cybulska, 2015).

Extraction of Pigment

The phycocyanin (Figure 6) extraction was carried out by the use of water on the one of a kind biomass-to-solvent ratio and shaking rates. The phycocyanin awareness and purity (A615/A280) acquired have been 1.2 g/l and 0.3. These values are 40% and 20% decrease than the cost acquired from the algae produced by the usage of the artificial chemicals. Further purification produced the extract purity required for meals grade (Taufiqur-rahmi et al., 2017).

Other Applications of Bio-colour

Microalgae are used in bio colour formation to a large extent. Along with this, their usage has increased in various industries from past few years. Below is the brief discussion of a few industries where bio-colours are used.

1. Bio colours are mainly used for protecting the food from microbial spoilage since they have antagonistic activity for certain bacteria, fungi and viruses.

2. Natural food grade like carotenoids used as a sunscreen to maintain the food quality and as a vitamin supplement.

3. Anthocyanin also used as an indicator to evaluate the food quality.

4. Riboflavin is a natural food bio colourant which is essential vitamin source and usually available in milk and leafy vegetables (Abedin and Taha, 2008)

5. Bio colourants are also used in maintaining human health as they contain biological compounds which have strong pharmacological properties.

6. They are also used in paint industry to impart natural properties in huge amount.

7. According to certain studies, it has been showed that bio colourants derived from chlorophylls decreases the risk of colon cancer (Cardozo et al., 2007)

8. Xanthophylls present in bio colours act as a protective antioxidant in macular region of human retina (Kanda et al., 2014)

9. Similarly, betacyanin present in the bio colours contain radical scavenging properties which helps in providing help against stress related disorders.

CONCLUSION

In the field of algae growth, there is a need to help the different innovative work exercises to beat different mechanical boundaries, as the green growth can possibly give novel synthetic substances and bioactive mixes. As discussed above, different bio-colours from microalgae have high worth yet its commercialization is still in its earliest stages however in not so distant future, can be viewed as an entryway to sustainable and green industry. In this paper it is reasoned that microalgae incorporate various species which gives pigments like carotenoids, carotene and so on that help in arrangement of bio-colours. Bio Colours which are extracted from microalgae have different applications in fields of pharmaceuticals, materials, biotechnology and so on which should be examined more.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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