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## Green Technology Strategy for Bioremediation of Dairy Effluent for Production of Lipid Byproducts

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### Abstract

Considerable research endeavors are currently in progress exploring Using different types of microalgae for purifying dairy wastewater, aiming to contribute to the waste-to-bioproducts economy. Numerous studies have primarily concentrated on removing essential nutrients such as phosphorus and nitrogen from dairy wastewater through microalgae-based treatment methods. Microalgae exhibit a dual functionality, serving both as effective bioremediation and prolific producers of valuable components including lipids, proteins, pigments, antioxidants, and vitamins. Among these compounds, microalgal lipids are versatile in applications across human health, nutraceuticals, cosmetics, pharmaceuticals, and biofuels. This current review hunts into the pivotal role of microalgae in offering a promising solution for dairy effluent treatment, while also serving as a nutrient reservoir for production of biomass and the accumulation of lipids, such as polyunsaturated fatty acids (PUFAs), carotenoids (pigments), and oxylipins (oxygenated PUFAs). These accumulated compounds hold substantial potential for the production of biodiesel and other commercially viable products. The advancement of technologies related to biohydrogen production, systems biology, and algal trans genomics is advocated for a comprehensive approach towards sustainable development in this domain.

**Keywords:** Dairy effluent; lipid by-products, microalgae, Phytoremediation

## INTRODUCTION

Water stands as the paramount natural resource crucial for the ongoing survival of every species. Water that is clean and free of toxins is essential for a prosperous life. Our dairy industry's growth contributes significantly to water contamination and adversely impacts water bodies by releasing significant amounts of pollutants into the water. (Kolev salalov 2017; Qadir et al.2020; Gramegna et al. 2020; Ummalyma et al. 2021). By introducing toxins into the food chain, untreated wastewater further damages the ecology and has a detrimental effect on the survival of living things. Consequently, for a sustainable environment, wastewater management and treatment are essential. One of the major contributors of wastewater is the industry of dairy products. Milk is regarded as the most significant source of nutrients needed by the body and one of the main food elements in the world. Water is the primary ingredient utilised in the dairy industry to produce milk products, which generates large volumes of wastewater, making disposal a difficulty that cannot be disregarded (Swain et al., 2020). According to reports, the dairy sector produces 2 to 10 L of waste water for every litre of milk processed (Ummalyma and Sukumaran, 2014; Ragunath et al.2016; Daneshvar et al. 2019; Pang et al. 2020; Gramegna et al. 2020; Kusmayadi et al., 2022). The loss of dissolved oxygen is one of the major issues brought on by raw dairy wastewater discharged directly into the environment. Because dairy effluents are high in oil and fat molecules, they form a thin layer on the water's surface that prevents oxygen from getting to the water's surface, making it difficult for the local flora and wildlife to survive. (Karadag et al. 2015; Choi 2016; Sharma et al. 2022).

Additionally, the majority of the whey production is discarded without being properly treated, in many cases due to an increasing cost for its treatment and disposal operations which leads to an excessive financial burden (Choi 2016; Gramegna et al.2020).

A stream with significant qualitative and quantitative fluctuations results (Porwal et al. 2015; Choi 2016; Panday, Srivastava & Kumar. 2019; Ummalyma et al.2022). Despite the compositional variations, dairy effluents are characterised by a significantly higher temperature. Dairy wastewaters are treated using mechanical, physicochemical, and biological techniques. To balance variations in both volumetric and mass flow, mechanical treatment is required. Additionally, some of the suspended particles are reduced. While emulsified compound removal can be accomplished with physicochemical procedures

(Kothari et al. 2011; Kolev Slavov, 2017; Swain et al. 2020). The highly biodegradable pollutants make biological wastewater treatment systems the preferred method. Employment of microalgae for remediating the dairy waste water results in decontamination of dairy effluent along with mass cultivation of microalgae (Gani et al. 2016; Choi, Jang & Kan. 2018; Kawamura et al. 2018; Sirohi et al. 2021a, 2021b; Joun et al. 2021; Ummalyma 2021; Ummalyma et al. 2022). Mass cultivated microalgae are harvested for lipids produced which are utilized in diverse industrial applications (Nguyan et al. 2022). This review focuses on various environmentally-friendly green technologies to recover lipids from microalgae using dairy wastewater as growth medium.

## **DAIRY INDUSTRY**

The dairy sector holds significant importance in the Indian economy, offering substantial employment opportunities, and continues to expand steadily. Despite being a major source of wastewater, dairy industries contribute positively to the economic growth of the nation. India, as per FAO (2020), holds the title of the world's leading milk producer, contributing 22% to global milk production, followed by the USA and Pakistan. However, dairy operations globally leave a considerable environmental footprint, consuming substantial amounts of water, estimated at approximately three liters per liter of milk produced (Porwal et al., 2016). The production and consumption of dairy products, particularly milk, have been steadily increasing, with a projected global consumption rise of 13.7% by 2023 (Choi 2016).

### **Types of waste generated during the processing of dairy products**

The wastewater discharged from dairy operations poses a substantial environmental hazard. These effluents typically contain suspended solids and organic matter, along with heightened levels of nitrogen and phosphorus. Additionally, they often contain oil, fat, and grease compounds. Furthermore, residues from the cleaning products utilized in utensil and equipment washing can also be present, (Kushwaha, Srivastava & Mall. 2011; Ahmad. 2014; Kolev Slavov. 2017; Chandra et al. 2021). According to their origin and composition, dairy effluents can be categorized into the following three types; a) processing water, b) cleaning wastewater and c) sanitary wastewater.

**Processing water:** Condensates resulting from milk or whey evaporation, along with the cooling process in specialized coolers and condensers, yield processing water. While evaporators may introduce milk or whey droplets, the vapor produced during milk and whey drying, upon condensation, generates the purest effluent. Generally, processing waters exhibit minimal contaminants and can be recycled or discharged alongside stormwater following basic pre-treatment measures. (Kolev Slavov. 2017).

**Cleaning wastewater:** This effluent typically originates from washing machinery that comes into touch with milk or other dairy products, such as tanker washing, refrigeration points, floor washing, boiler houses, etc. spills of milk and whey, mistakes in operation, and broken equipment are all included. More than 90% of the organic solids found in effluents consist of cheese particles, whey, cream, separated water, starter cultures, yogurt, fruit concentrates, and stabilizers. These effluents must undergo additional treatment because they are produced in large amounts and are highly contaminated (Carvalho, Prazeres & Rivas. 2013; Karadag et al. 2015).

**Sanitary wastewater:** The sanitary wastewater is produced in restrooms, showers, etc. And is typically piped directly to sewage works and shares a similar composition to municipal wastewater. Prior to further aerobic treatment and can be employed as a source of nitrogen for imbalanced effluents of dairy. Dairy product processing results in a huge amount of chemically modified liquid waste which needs proper treatment before discharge (Slavov 2017; These waste materials can be efficiently repurposed as raw materials for manufacturing other industrial goods or for generating energy. (Tan et al. 2018; Chandra et al., 2021).

### **Components of dairy effluent**

The dairy industry primarily produces liquid effluent during the processing of different useful products from whole milk like buttermilk, condensed cream, sweet whey, curd, cheese, butter, ice cream, etc. The distinguishing features of dairy wastewater might be varying according to the formation of final products. Generally, dairy effluents contain high concentrations of lipids such as triacylglycerides, phospholipids, saturated and unsaturated

fatty acids, carbohydrates such as; lactose, glucose, galactose, etc. The dairy waste water also contains two major soluble proteins casein and whey protein etc., resulting in high BOD (biological oxygen demand) and COD (chemical oxygen demand) levels (Mehrotra et al., 2016; Patel et al., 2020). Its high BOD depletes the dissolved oxygen content and creates anaerobic conditions in the aquatic system. Moreover, dairy effluent contains various inorganic and organic compounds of nitrogen and phosphorus, present in higher concentration and responsible for the alkalinity of the dairy waste other than it also contain a trace amount of some mono-valent and bi-valent cations like Na, Mg, Ca, Fe, K, Ni, Mn, etc. Use of detergents and chlorine in the dairy industry further deteriorates the quality of effluent (Kushwaha, Srivastava & Mall. 2011; Ahmed 2014; Patel et al., 2020; Gramegna et al. 2020; Vidya et al.2021).

Dairy effluents are biologically active because of the presence of a high organic and inorganic load and when it is discharged into water streams either untreated or semi-treated leads to water pollution. Hence, the proper treatment before the disposal of dairy waste effluent has become a major problem for the vitality of the dairy industry and the environment. Various attempts have been already made to solve this problem by reusing the nutrients rich dairy waste with diverse microorganisms to metabolize organic matter.

### **Dairy effluents treatment approaches**

Various technologies can be used for the processing of dairy waste prior to release into the natural habitat or surroundings. Dairy waste treatment is done in two ways:

***Physico-chemical treatment:*** Several Physico-chemical methods are used for the treatment of dairy wastewater, such as chemical precipitation, coagulation, coagulation/flocculation, adsorption, electrocoagulation, electroflocculation, membrane bioreactor, combined electrode system, etc. There are various demerits regarding the use of physicochemical treatments such as its higher cost, and generating secondary pollutants, because chemical reagents were used and they only perform partial treatment (Kushwaha, Srivastava & Mall. 2011; Shete and Shinkar 2013; Slavov 2017; Patel et al., 2020; Gramegna et al. 2020; Vidya et al. 2021).

**Biological treatment:** In order to compensate for the negative aspects of physico-chemical approach, biological methods are a suitable alternative for the treatment of dairy wastes. These are eco-friendly, cost-effective, and highly efficient to remove the contaminants from dairy effluents (Kolev Slavov 2017; Gramegna et al. 2020). Microorganisms have been employed in biological treatment methods. Among these, the microalgae are well-known bioremediator because of their ability to grow photoautotrophically and mixotrophically (Choi,2016; Chokshi et al. 2016; Guldhe et al. 2017; Ahmad et al. 2019; Gramegna et al. 2020; Goswami, Agrawal & Verma 2022; Encarnacao et al. 2022; Xing et al. 2022). Dairy wastewater contains high contents of phosphorus, nitrogen, and organic matter thus, the nutrient-rich dairy waste effluent proved to be a suitable medium for the growth of microalgae (Gonçalves et al. 2017; Gramegna et al. 2020; Vidya et al. 2021).

## MICROALGAE

Microalgae are unicellular, eukaryotic microorganisms which include algae, dinoflagellates, diatoms, etc. They can exist independently as a single colony or in a consortium as a group. Microalgae are a broad group of aquatic organisms that have the efficiency and ability to remove pollutants and produce lipids with high biomass production (Kalra, Gaur & Goel, 2021; Calijuri et al. 2022; Bhatt et al. 2022; Devi et al. 2022 Jiang et al 2022). Table 1 shows the different microalgae, their photosynthetic pigments, and reserve food material with examples (Heimann and Huerlimann, 2015; Enamala et al., 2018; Kalra, Gaur & Goel, 2021; Calijuri et al. 2022; Bhatt et al. 2022; Devi et al. 2022 Jiang et al 2022).

**Table: 1. Types of microalgae based on their pigment and reserve food**

<b>Class</b>	<b>Photosynthetic pigments</b>	<b>Reserve food material</b>	<b>Representative Genera</b>
Chlorophyceae (Green algae)	Chlorophyll a, b, Carotene	Starch	<i>Chlorella</i> sp., <i>Heamatococcuspluvialis</i> , <i>Dunaliella salina</i>

Xanthophyceae (Heterokont or Yellow green algae)	Chlorophyll a, c, b-Carotene, Xanthophylls	Fats, leucosin	<i>Chlorochromonas, Chlorosuccus, Vaucheria, Tribonema, Botrydium</i>
Chrysophyceae (Yellow-brown algae)	Chlorophyll a, b, Carotenoids	Oils and leucosin	<i>Ochromonas, Dinobryon, Mallormonas, Synura, Rhizochrysis</i>
Bacillariophyceae (Diatoms)	Chlorophyll a, c, Carotenes	Leucosin and Fats	<i>Cyclotella, Rutelaria, Corethron, Navicula, Bacillaria</i>
Cryptophyceae	Chlorophyll a, b-Carotenes, Xanthophylls	Starch	<i>Falcomonas, Rhinomonas, Plagioselis, Chilomonas, Cryptomonas</i>
Dinophyceae (Dinoflagellates)	Chlorophyll a, c, Carotenoids, Xanthophylls	Starch and oil	<i>Dinophysis, Polykrikos, Gonyaulax, Gymnodinium</i>
Chloromonodiae (Raphidophytes)	Chlorophyll a, b, Carotenes, Xanthophylls	Oils	<i>Vacuolaria</i>
Euglenophyceae (Phototrophic-euglenids)	Chlorophyll a, b	Fats and paramylon	<i>Euglena</i>
Phaeophyceae (Brown algae)	Chlorophyll a, Xanthophylls	Laminarin, mannitol and fats	<i>Pelagophycus, Laminaria, Pelvetia, Sargassum</i>
Rhodophyceae (Red algae)	Chlorophyll a, phycoerythrin	Floridian starch	<i>Vacuolaria, Gomyostomum, Chattonella, Psammomonas</i>
Myxophyceae (Blue-green algae)	Chlorophyll a, b-carotene, c-Phycocyanin and phycoerythrin	Glycoproteins, droplets of oil	<i>Chlorococcus, Darmocarpa, Pleurocaspa, Oscillatoria, Stigonema</i>

### **Dual role of microalgae**

Microalgae present a hopeful biological solution for dairy wastewater treatment owing to their adaptable metabolism, capable of engaging in photoautotrophic, mixotrophic, or heterotrophic activities. Their capacity to utilize inorganic nitrogen and phosphorus renders microalgae an efficient option for tertiary and quaternary treatments of dairy waste. (Choi et al., 2018). This type of bioremediation/ phycoremediation coupled with the production of potentially valuable biomass, which can be used for several purposes. Earlier researches suggested that cultivation of microalgae for the production of lipid components from dairy waste, had provide better results than any other wastewater used as microalgae cultivation medium (Chandrabose et al., 2013; Hu et al., 2018; Panday, Srivastava & Kumar. 2019; Swain et al. 2020; Pang et al. 2020; Gramegna et al. 2020; Biswas et al. 2021).

Many microalgae cultures displayed high growth on the diluted effluents from dairy. Moreover, the microalgae have the ability to use solar energy for its photosynthetic activity and combine water with carbon dioxide in order to make their food autotrophically, further; it converts the environmental carbon di oxide to oxygen. Due to this ability of microalgae, they have been used to remediate the dairy waste as the nutrient sources for their growth, and to reduce the concentrations of nitrogen, phosphorus and other inorganic substances. It has been reported that the microalgae are the better source for manufacturing of bio-fuel due to its elevated photorespiration activity, and rapidly growing biomass activity Panday, Srivastava & Kumar. 2019; Gramegna et al. 2020).

The microalgae strain which contains large number of fatty acids, like polar and non-polar or neutral lipids, and also flourishing in the native domain of dairy waste effluent have yet to be used for bio-fuel production. Micro algal feedstock is one of the best medium for bio-fuel production and as a replacement source of fossil fuel and also proved beneficial to eradicate greenhouse gas emissions (Mwangi et al. 2015; Khan et al. 2018; Shokravi et al. 2019; Debowski et al. 2021).

***Lipid producing microalgal strains:*** Numerous species of microalgae have been scrutinized for their fatty acid compositions, with approximately 20-50% of dry biomass comprising lipid constituents. Such as species of *Nannochloropsis*, *Neochloris*, *Chlorella*, *Dunaliella*, *Cryptocodinium*, *Chlamydomonas*, *Cylindrotheca*, *Isochrysis*, *Nannochloris*, *Nitzschia*,



*Schizochytrium*, *Phaeodactylum*, *Porphyridium*, and *Tetraselmis* have been recognized for containing certain classes of polar and non-polar lipids. Nonpolar or neutral lipids like sterols, free fatty acids acylglycerols, etc. and polar lipids like glycoacylglycerides, phosphoglycerides, and sphingolipids have been reported in microalgae (Mata et al. 2010; Ghosh, Roy & Das. 2017; Chen et al.2018; Panday, Srivastava & Kumar. 2019; Swain et al. 2020).

**Table 2: Some lipid containing micro-algal species**

Sr. No	Microalgae species	Lipid Content (% Dry Weight)	Reference
1.	<i>Botryococcus braunii</i>	25–45%	Lee et al., 2015; Ferreira et al. 2019
2.	<i>Schizochytrium sp.</i>	30–60%	Sajjadi et al. 2018; Menegazzo & Fonseca. 2019
3.	<i>Neochloris oleoabundans</i>	25–44%	Breurer et al., 2012
4.	<i>Nannochloropsis sp.</i>	31–68%	Ma XN et al., 2016; Shokravi et al. 2020
5.	<i>Nitzschia sp.</i>	45–47%	Jiang et al., 2015
6.	<i>Chlorella sp.</i>	35-58%	Ghosh, Roy & Das. 2017; Mirizadeh et al. 2020
7.	<i>Scenedesmus sp.</i>	40-68%	Kumar et al., 2021
8.	<i>Chloccum sp.</i>	40–67%	Mahapatra and Ramachandra., 2013
9.	<i>Cylindrotheca sp.</i>	20 -35%	Demirel et al., 2015
10.	<i>Dunaliella sp.</i>	36-47%	Gharajeh et al.,2020
11.	<i>Chlamydomonas reinhardtii</i>	55- 65%	Xu et al., 2018; Yang et al, 2018; Hang et al. 2020,

12.	<i>Phaeodactylum triconutum</i>	18- 57%	Yang et al. 2017; Desmukh et al. 2019
13.	<i>Chlamydomonas sp.</i>	21-27%	Desmukh et al. 2019
14.	<i>Pavlova salina</i>	12-30%	Desmukh et al. 2019
15.	<i>Phorphyridium</i>	9-14%	Desmukh et al. 2019

### Biosynthesis of lipid in microalgae

The green microalgae generally synthesis two categories of lipids as free fatty acids or neutral fatty acids and triacylglycerols. Neutral fatty acids are synthesized in chloroplast while triacylglycerols are, in smooth endoplasmic reticulum. 14–20 carbon chains fatty acids are utilized in the preparation of biofuel and long carbon chains of 20 or more carbons which employed in food industry (Jacob-Lopes et al. 2015; Ratomski & Hawrot- Paw 2021). Microalgae produce and store large amounts of lipids in the form of triacylglycerides as oil droplets in the various cell organelles. The conversion and accumulation of lipid content, varies according to cultural conditions and microalgal strains (Bellou et al. 2014; Yang et al, 2017; Konget et al. 2019; Nayak et al. 2019; Grijalva et al 2020; Abomohra et al 2020; Udayan et al. 2022; Rawat et al. 2022). Biosynthesis of lipid in microalgae depends upon the production of carbon compounds by the process of photosynthesis. It includes light dependent reactions and light independent reactions also. In light dependent reactions oxygen, ATP and NAD(P)H are produced while in light independent reactions (Calvin cycle), a three-carbon compound (glyceraldehyde-3-phosphate) is produced, which is converted into acetyl CoA via glycolysis. This acetyl CoA is a major substrate for biosynthesis of lipid and precursors for proteins, nucleic acids, carotenoid and carbohydrates etc. The transformation of acetyl CoA to malonyl CoA is the first confide path in biosynthesis of lipids (Lv et al., 2010; Griffiths, Dicks and Richardson. 2011; Goldberg, 2016; Mimouni et al. 2018; Dolganyuk et al. 2020; Chen & Wang 2021). Many factors which are responsible for the accumulation of lipids inside the cells, such as availability of nutrients (mainly nitrogen, phosphorus and silica), temperature, salinity, pH, light, humidity and enzymes are

mainly responsible for metabolic reactions (Han et al. 2012; Bartley et al. 2013; Lin & Wu, 2015; Sun et al. 2017; Chen et al. 2017; Chen & Jiang. 2017; Gao et al. 2021).

There are various strains of microalgae (*Chlamydomonas*, *Chlorella* sp. etc.) which have been employed as an experimental design to examine its anabolic and catabolic activities and functions (Scaife et al 2015). Genomes of *Chlamydomonas reinhardtii* have been sequenced and its molecular and genomic tools is also available. It can also accumulate considerable amount of triacylglycerols under salt stress or nitrogen deprived conditions (Li et al., 2010; Dean et al., 2010; Siaut et al., 2011; James et al., 2011; Fan et al., 2012; Msanne et al., 2012; Lei et al. 2012; Bartley et al. 2013; Liang et al. 2013; Li et al. 2014; Viegas et al. 2015; Sibi et al.2016; Juppner et al. 2017; Lei et al. 2018; Gifuni et al. 2019; Sulochana & Arumugam 2020; Hang et al 2020; Shokravi et al. 2020). Micro algal species collected from the dairy waste indicated great prospective for low-cost cultivation and producing lipid utilizing dairy waste, without modifying the procedure. It was reported that the *Chlorella* sp. isolated from the dairy waste water showed good biomass production when grown on dairy effluent, reduced its COD, and nutrients (Wang et al. 2010; Choi et al., 2018). This microalga was identified by using 18S rDNA sequencing, and the DNA sequences were analyzed via BLAST representing the high homology with *Chlorella* sp. ArMoo29B (98% homology). Microalgae *Chlorella protothecoides* displayed good growth in a pre-treated whey solution. Since the use of microalgae approach alone was not sufficient for satisfactory removal of pollutants, therefore using hydrolysis and flocculation-struvite methods, the treated whey with 25:75 dilutions exhibited sufficient pollutants removal by designing biochemical approach. Using this approach, approximately up to 99% or 91–100% lessening of inorganic and organic contaminants was attained after nine days of cultivation of microalgae (Patel et al. 2020). In the similar study, reported that freshwater green microalgae *Chlorella* sp., UMACC344 was able to produce higher lipid amount in dairy waste, which could be utilized as potential biofuel.

In another study, *Chlorella* sp. isolated and cultivated in dairy wastewater, resulted in higher algal biomass production along with good amount of lipid production, the microalgae reduced BOD and COD, trace elements and other nutrients, thereby remediated dairy waste water (Choi et al., 2018; Hu et al. 2019). In another study, the dairy effluent supported the growth of *Chlorella vulgaris* and *Anabaena ambigua*, with significant lipid, carbohydrates,

protein productivity. The lipid and carbohydrate content were higher in *Chlorella vulgaris* compared to *Anabaena ambigua*, which exhibited high protein content. A significant decrease in both COD and BOD along with other nutrients was also observed (Ahmad, 2014; Rani et al. 2015; Kolev Slavov, 2017; Ummalyma et al 2022)

*Chlorococcum* sp. RAP13 grown in dairy effluent produced great amount of biological mass and lipids, particularly under heterotrophic growth conditions. Lipid production and biomass increased expressively under heterotrophic cultural conditions. Higher amount of lipid production by these micro algae makes it a suitable candidate for biodiesel production. Moreover, use of dairy effluent as a source of energy and carbon by micro algae remediated the effluent in terms of BOD and COD (Ummalyma & Sukumaran, 2014).

Kothari et al. (2013) cultivated *Chlamydomonas polypyrenoideum* on dairy wastewater, which resulted in considerable lessening of nitrogen (90%) and phosphate (96%) on 10<sup>th</sup> and 15<sup>th</sup> day respectively. Biomass of microalgae after 10<sup>th</sup> day treatment exhibited 42% (w/w) lipid contents, which was analogous to the bio-oil extracted from other natural resources as per FTIR results analysis. These outcomes proposed the possible usage of microalgae for biodiesel production as well as phyco-remediation of the dairy wastewater (Sharma et al 2022).

The following section discusses the role of micro-algae in diverse fields, where lipids produced by microalgae employed in commercial products.

### **Applications of microalgal lipids**

Microalgae are rich in lipids, high density lipoproteins, oxylipins, carotenoids, proteins and carbohydrates etc. (Pohl & Kock. 2014; Santosh, Dhandapani, & Hemalatha. 2016; Bošnjaković and Sinaga 2020; Randhir et al. 2020). Many micro algal strains are able to synthesize the long chain fatty acids, with yields between 25 to 75% of their total lipids. Many fresh water microalgae such as *Chlorella* sp., and *Scenedesmus* sp. etc. and seawater microalgae *Thraustochytriacea* sp. and *Cryptocodiniacea* sp. are currently used for the production of PUFAs (Udayan et al. 2021).

Microalgae strains have incredible adaptability to survive in extreme environmental conditions, and also have the huge potential for producing several advantageous by-products. Nowadays, the micro algal species are considered as lipid biofactories because of producing

various kinds of lipids with remarkable applications in various fields such as in food and feed manufacturing industries, biofuel production, cosmetics, and pharmaceuticals etc. (Nascimento et al. 2013; Matos et al. 2017; Hess et al. 2018; Bhalamurugan et al. 2018; Maltsev & Maltseva 2021; Devadasu & Subramanyam 2021; Liu et al. 2022; Kuravi et al. 2022).

**Food and nutrition field:** Polyunsaturated fatty acids (PUFAs) have been known for its contribution in the field of human health benefits. Earlier, the major sources of polyunsaturated fatty acids were fish oil. But in the current scenario, the commercial production of PUFA from fish sources, are declined due to some limitations (fish population and diversity threatening and environmental hazards). Recently microalgae investigated for the production of oils or fatty acids which contain high amount of polyunsaturated fatty acids (EPA and DHA) (Winwood. 2013). Now days these are employed in the food industry, on a large scale. Various strains of microalgae are able to amalgamate various series of the PUFAs like 1-6 series, which contains linoleic acid (LA), and arachidonic acid (ARA), and as well as of the PUFAs 1-3 series, containing linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (Minhas et al., 2016; García, Vicente and Galan 2017; Camacho, Macedo & Malcata 2019; Koyande et al. 2019; Vieira et al. 2020). These fatty acids are having one or more double bond in their carbon skeleton. They have important ingredients for food and feed supplements, with many healthful benefits. The most common species of microalgae which are exploited in lipids production are *Schizochytrium sp.*, *Scenedesmus sp.*, *Arthrospira platensis*, *Ulkenia*, *Haematococcus pluvialis*, *Isochrysis galbana*, *Chlorella vulgaris*, *Chlorella pyrenoidosa*, *Chlorella ellipsoidea*, *Tetraselmis sp* *Dunaliella salina* and *Cryptothecodinium*. (Xia et al. 2013; Park et al. 2018; Enamala et al. 2018; Costa et al. 2019; Gramegna et al. 2020; Mirizadeh et al. 2021).

**In cosmetic industry:** Microalgal PUFAs have been considered very useful and safe in the formulation of various cosmetic products due to having antioxidant and anti-inflammatory properties. Fatty acyls including fatty acids, fatty alcohols, esters, carotenoids, and eicosanoids etc. are the needed oily raw materials used as emulsifiers in cosmetics. In the cosmetic field, the fatty acyls used as the major bio-based oil surfactants (Xu & Qian 2014; Khan, Shin & Kim. 2018; Jeyakodi, Krishnakumar, & Chellappan. 2018; Ambati et al. 2019; Luca et al. 2021).

The microalgal lipids which are used as biosurfactants possess amphiphilic nature due to the presence of both hydrophilic and hydrophobic components within the chain. The main function of these biosurfactants, are reducing the interfacial tension which make easier solubilization of hydrophobic molecules in water and its lower micelle concentrations, allowing it to work at a very small concentration when compared with any synthetically prepared surfactant (Guillerme, Couteau & Coiffard, 2017; Cezare- Gomes et al. 2019; Couteau & Coiffard 2020; Shi et al. 2020; Puchkova et al. 2020; Cikos et al. 2022). They are also having antibacterial, antifungal, anti-tumor bioactivities and very less eco-toxicity. (Mondal et al. 2017; Ahmad & Ahsan. 2020;). Many fatty acids such as palmitic acid, stearic acid, lauric acid, and myristic acid, are playing an important role in the maintenance of normal skin barrier functions and making the skin smooth, soft and brighter (Mukherjee et al. 2011; Bishop, 2012; Thomas, and Kim, 2013; Christaki et al. 2013; Wang et al. 2015; Ariede et al., 2017; Yang, Zhou, & Song, 2020; Kuravi et al. 2022).

**Pharmaceuticals:** Many micro algal species produce high-value lipids which are utilized for pharmaceutical purposes. Production of these high value lipids or fats compounds (bioactive compounds) from microalgae has been considered as an emerging area. (Levine & Fleurence. 2018; Basheer et al 2020). The microalgae strains exploited in production of lipid bioactive compounds (carotenoids), include *Chlamydomonas* sp., *Spirulina* sp., *Chlorella* sp., *Dunaliella*, *Nannochloropsis*, *Nostoc*, *Cryptocodinium* and *Haematococcus* etc. Microalgal produced various kind of carotenoids such as astaxanthin, lutein, zeaxanthin, etc. the green microalgae commonly contain beta carotene which can derived other forms of it by the action of various enzymes like hydroxylase and ketolase. (Novoveska et al., 2019).

Microalgae represent a good source of natural carotenoids. These are also called isoprenoids or terpenoids organic compounds, which containing 5 carbon atoms units (2 or more). Because of having anti cancerous, antioxidental properties, these are highly used in food, cosmetics, nutraceuticals and also in pharmaceutical industries (Zaid, Hammad & Sharaf. 2015; Lauritano et al. 2016; Yan et al. 2016; Huang et al. 2017; Deniz, Vaquero & Imamoglu. 2017; Hussain & Abdullah. 2020; Vieira et al. 2020; Rahman et al. 2020; Khavari et al. 2021; Togarcheti & Padamati 2021). *Nostoc* sp. has the capability to produce polyunsaturated fatty acids and essential fatty acids (omega 3 and 6 fatty acids) and

*Chlamydomonas reinhardtii* is able to produce glycerol, when grown in sulphur deprived medium, which is widely utilized in pharmaceutical industries (Singh et al. 2014; Yang et al. 2015; Scranton et al. 2015). *Chlorella* species containing high amount of  $\alpha$ -tocopherol (vitamin E) and this can help in preventing macular degeneration and the occurrence of certain cancers (Noguchi, Maruyama & Yamada. 2014; Jayshree, Jayshree & Thangaraju. 2016; Bitto et al. 2020; Ferdous & Yosof. 2021). *Nannochloropsis gaditana* known to produce good amount of eicosapentanoic acid (EPA) and *Cryptocodinium cohnii* for producing docosahexaenoic acid (DHA) (Adarme-Vega et al. 2012; Oliver et al. 2022). Long chain fatty acids EPA and DHA are the most valuable ones as having positive effects in the treatment and prevention of various diseases like; inflammation, hypertension, atherosclerosis, thrombosis, arthritis, and risk of chronic disease (Adarme-Vega et al. 2012; Toumi et al. 2022). DHA promotes eye health by enhancing the performance of retina and it also contribute in the brain functioning (Zang et al. 2020). EPA is showing the significant hypolipidemic activity. Hence it is very useful in preventing cardiovascular disease, and reducing the symptoms of depression (Mozaffarian & Wu. 2011; Kashiwagi & Huang. 2012). It also helps in the maintenance of normal growth and development of bone and hair, relaxation and contraction of muscles (Herrero et al.,2013; Yan et al.,2016; Blasio & Balzano 2021).

PUFAs also serve as precursors of eicosanoids and docosanoids which regulate gene expressions and biosynthesis of steroidal hormones that can maintain the good health of reproductive system. These are utilized in promoting the vasoconstrictive and aggregative activity of platelets in the process of coagulation. PUFAs are able to make some changes in their composition in the plasma membrane so that they can affect the fluidity and signaling process of the cell membrane. Omega-3 fatty acids have inhibitory effects on antibody production, lymphocyte proliferation and it also suppress to the activation of programmed cell death. Due to having above mentioned characteristic features, all these lipids are very important for pharmaceutical formulation (Ekka et al 2022).

**Biofuel:** The microalgal biomass has the ability to produce various kind of biofuel such as biodiesel by transesterification of the fatty acids, biogas by gasification or anaerobic digestion, bioethanol by direct fermentation of carbohydrate fraction of microalgae, methane

gas by anaerobic fermentation of microalgal biomass. Microalgal lipids are the alternate resources which can be used to decrease the consumption of fossil fuels (Laurens. 2017). Microalgae cultivation gives much higher yields than any other conventional bioenergy crops. Some microalgal species eg. *Scenedesmus sp.*, *Schizochytrium sp.*, *Nitzschia sp.*, and *Botryococcus braunii* which contain higher amount of lipid approximately 40 to 60 %, therefore, are an excellent source of biofuel (Mata, Martins, & Caetano. 2010; Chinnasamy et al. 2010; Gong et al., 2011; Gouveia. 2011; Shah, Yadav, & Tiwari. 2011; Shenbaga – Devi et al. 2012; Mata et al. 2013; Sreekumar et al. 2016; Patel, Patel, & Krishnamurthy. 2016; Chye et al. 2018; Sharma et al. 2018; Hossain, Mahlia & Saidur. 2019; Culaba et al. 2020; Patnayak & Mallick. 2021; Moshood, Nawanir, & Mahmud. 2021; Mathushika & Gomes. 2021; Ebhodaghe, Imanah, & Ndibe. 2022; Mofijur et al. 2022).

**Biodiesel:** Nowadays, microalgae are being used as a potential source for production of biodiesel to overcome the ever exhausting and often increasing price of fossil fuels. They can produce high oil content (approx 50 to 80% of their dry weight) than any other traditional crops (Schlagermann et al. 2012; Eman & DM. 2013; Shin et al. 2015; Valdez-Ojeda et al. 2015; Sun et al. 2018; Aratboni et al. 2019; Hawrot- Paw 2021; Udayan et al. 2022).

Microalgal biomass contain good amount of triglycerols which is the main constituent of the biodiesel (Kim et al. 2014; Raheem et al. 2015; Hossain .2019; Lee. 2019; Kumar et al. 2017; Osman. 2021; Ebhodaghe, Imanah, & Ndibe. 2022; Kandasamy et al. 2022). Among them, transesterification is widely used process for the conversion of microalgal lipids into biodiesel. The microalgae biodiesel contains high caloric value and lower density and viscosity compared to the biodiesel obtained from other feedstock sources (Unpapran, Tipnee, & Ramaraj 2015). Microalgae biodiesel also help to reduce the level of carbon monoxide and other pollutants suspension in the atmosphere, as it does not contain aromatics hydrocarbons and sulfates. (Mondal et al. 2017; Bhalamurugan et al. 2018). Moreover, exploitation of microalgae for biodiesel is better option compared to usage of traditional food crops (oleaginous crops) and animal fats for the production of biofuel, since food supply could be affected severely.

Therefore, microalgae are proposed to be a better choice for producing biodiesel in a sustainable way because they possess capacity to grow on diverse industrial waste effluent



and they can adapt themselves according to adverse environmental conditions. Many studies have been carried out by using *Chlorella sorokiniana* and *Scenedesmus* sp., for increasing the target number of fatty acids under a variety of culture conditions that could be used as a feedstock for producing good quality of biodiesel, under a variety of stress conditions (Jena et al. 2012; Ekka et al 2022; Khan et al. 2022).

**Biohydrogen:** The first green microalgae, *Scenedesmus obliquus* which was noted to produce hydrogen. After that, many microalgal strains such as *Chlorella sorokiniana*, *Chlamydomonas reinhardtii*, *Chlorococcum littorale*, *Platymonas subcordiformis* and *Chlorella fusca* etc. were used to produce hydrogen. Microalgae produce biohydrogen by employing various processes like photosynthesis, direct and indirect biophotolysis and fermentation (Kothari et al. 2015; Singh & Das. 2018; Hossain & Mahila. 2019; Limongi et al. 2021; Ordonez et al. 2021; Mondal & Khan. 2021). It was reported that microalgae produce low amount of hydrogen in dark but high amount of hydrogen produced in presence of sunlight. The hydrogen production in microalgae depend on the expression of hydrogenase enzyme and as well as the availability of sulphur containing components in the growing media (Amaro et al.,2013; Hariskos and Posten 2014; Eilenberg et al 2016;). It was reported that *Chlamydomonas reinhardtii* has high amount of hydrogenase enzyme around 10 to 100-fold, compared to other microalgal strains (Torzillo et al. 2016) But the commercial production of biohydrogen from this strain could not outstretch due to some technical issues (Batyrova & Hallenbeck. 2017). The studies on marine alga, *Platymonas subcordiformis* and on the fresh water green microalga *Chlorella sorokiniana* & *Chlamydomonas reinhardtii* demonstrated that they produced a high amount of biohydrogen when cultivated in sulphur deprived medium (Skjanes, Rebours, & Lindblad. 2013; Nomanbhav, Purvunathan & Yin. 2017; Vargas, Zaiat & Calijuri. 2021).

*Chlorella vulgaris* TISTR 8680 did not produce hydrogen (Ali, Rakshit & Kanhayuwa 2011; Kumari, Nasrand & Kumar. 2017). To attain profitable achievement in this field, genetically modified strains should be used which can be survive under adverse environmental conditions like sulfur- improvised state (Chader, et al., 2011; Shaishav, Singh, & Satyendra. 2013; Saifuddin, & Priatharsini. 2016; Feng et al. 2022; Ali et al. (2011)

## **CHALLENGES IN THE FIELD OF MICROALGAL BIOMASS PRODUCTIVITY**

Current research on microalgal biomass productivity has been facing many challenges. Some of these are mentioned here as follows:

- To overcome maximum organic and inorganic load from effluent and recycling of dairy waste-water
- To enhance the outdoor microalgae productivity
- To develop efficient photo reactor for enhancing the microalgal growth
- Prevention of contamination from microalgal culture
- To improve the supply of light and CO<sub>2</sub> into dense cultures
- To develop low-cost methods for harvesting process and energy efficient strategies for extraction of lipids and other additional co-products
- To develop economic method for production of hydrogen
- To develop algal transgenomics technology

Converting solar energy into lipid and other organic molecules is the main objective of microalgal culture. The selection of well-characterized strains through genetic engineering and screening for new species are two strategies to overcome these obstacles and achieve optimal lipid productivity in microalgal cells. A variety of methods are employed to increase the amount of light available, such as adjusting the reactor's architecture, utilizing optics to direct light towards the reactor's center, creating flashes, and employing mixed species to use a range of light wavelengths that increase photosynthetic efficiency by trapping carbon. (Schneidermann et al., 2012; Scott and others, 2010). To increase the rate of productivity, the genes and key enzymes that control the metabolic pathways leading to lipid synthesis are presently being studied and altered. On the other hand, altering the nutrition supply can enhance the content of lipid cells (Bhowmick, Koduru & Sen. 2015; Sun et al. 2019; Shahid et al. (2020); Banerjee et al. (2020)).

## **CONCLUSION**

Microalgal cultivation holds significant potential for environmental bioremediation and the production of valuable by-products such as polyunsaturated fatty acids, omega-3 fatty acids, and B-carotenoids. Moreover, microalgae can be genetically engineered to produce alternative fuels like biohydrogen, bioethanol, and biobutanol. These bio-products find extensive applications in various industries including biofuel production, pharmaceuticals, nutraceuticals, food and nutrition, cosmetics, as well as aqua and animal feed. Microalgae biomass could be modified energy containing fuels through thermal processes. In future with continued progress being made in the field of systems biology metabolomics will play an important role as a holistic approach in generation of bio-fuels which produce lower emissions, for green energy production and thereby maintaining environmental sustainability.

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