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Enhanced lipid productivity approaches in microalgae as an alternate for fossil fuels – A review



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ABSTRACT

Renewable energy sources especially biofuel is the focus in energy field. Microalgae have rapid biomass production and high oil content which is a promising oil producing alternative for fossil fuels and oil based crops. Changes in algal lipid production and composition have been attributed to variations in environmental and culture conditions. Stressful environmental conditions change the use of carbon uptake by algae for proliferation to energy storage in the form of oil. Fatty acids and triacylglycerols are energy storage compounds in microalgae accumulated under nutrient deprivation and stress conditions. The balance between maximum biomass and lipid production against the culturing conditions will be the ultimate goal of biofuel production from algae. Identification of suitable approaches for triggering algal lipid biosynthesis and lipid accumulation opens the door for enhanced lipid production with absolute quality and quantity of algae based fuels. This review lays the foundation for lipid induction strategies in microalgae to accelerate the application of algal biofuels as an alternate of fossil fuels for sustainable environment. Of the various approaches discussed, the one most often considered is that of nutrient limitation. Given the limitations of nutrient influenced lipid production, identifying other feasible approaches like CO₂ influence, temperature, salinity and heavy metal stress will reduce the negative impacts of existing approaches thereby to obtain maximum microalgal lipid production.

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1. Introduction

Biofuel has received considerable attention in recent years, as it is a biodegradable, renewable and non-toxic fuel. It contributes no net carbon dioxide or sulphur to the atmosphere and emits less gaseous pollutants than fossil fuel [1–3]. Oil based crops such as soybean and oil palm that are currently being used to create biodiesel usually have an oil concentration of less than 5% of dry biomass [4] whereas it is quite common for algae to have lipid concentrations ranging from 20 to 50% of dry mass [5,6]. The interest in the production of microalgae as biofuels is increasing due to their high oil content, rapid biomass production and small foot print which is a promising alternative for oil production [7]. However, the production cost of fuel from microalgae is as high as fossil fuel-derived diesel. To solve this problem, many researchers are trying to lower production costs by screening microalgae that have high lipid content and increase the lipid productivity through various environmental conditions. The ability of microalgae to survive or proliferate over a wide range of environmental conditions results in the production of an array of many secondary metabolites, which are of considerable value in biotechnology fields including aquaculture, health and food industries [8]. Algal oils are usually accumulated as membrane components, storage products, metabolites and sources of energy under some special production conditions [9]. Under suitable environmental conditions, microalgae synthesize fatty acids mainly for the production of membrane glycerolipids, such as glycolipids and phospholipids. However, under unfavourable growth conditions, many microalgae change their lipid biosynthetic pathways to produce large amounts of neutral lipids mostly in the form of triacylglycerol (TAG), which are mainly stored in cytosolic lipid bodies. Despite of the historical and renewed research in algae based fuels, induction of fatty acids remains incomplete as most of the current approaches exhibits varying lipid composition and regulation.

Lipids act as a secondary metabolite in microalgae, maintaining specific membrane functions and cell signalling pathways while responding to the environmental changes. The quantity and quality of oils produced by algal cells are directly proportional to the stimulus

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received from the surroundings [10–12]. Stressful environmental conditions change the use of carbon uptake by algae for proliferation to energy storage in the form of oil. The lipid content, composition and the proportions of various fatty acids of microalgae vary according to the environmental or culturing variables such as light intensity, growth phase, photoperiod, temperature, salinity, CO₂ concentration, nitrogen and phosphorous concentration [13–15]. Microalgae are rich in lipids like tri- and diglycerides, phospho- and glycolipids, hydrocarbons and others. The total content of lipids in the microalgae can range from 1 to 90% of the dry weight, depending on the species and culturing conditions [16,5]. Berglund et al. [17], (2001) reported that both the quantity and quality of lipids produced will vary with the identity of the algal species. Further, fatty acid contents of microalgae are influenced by the environmental and cultural conditions selected for its growth [18].

The industrial potential of microalgae remains to be met, largely due to the incomplete knowledgebase surrounding the approaches governing the induction of algal lipid production. A recognized approach of enhancing the lipid production is varying growth conditions. This review have set out to examine the various approaches and understanding the initiatives to mass culture microalgae with enhanced lipid production for bio-energy applications.

2. Approaches for enhanced lipid production

2.1. Nutrient concentration

Both lipid content and biomass production rate are dependent on lipid productivity and hence it is of particular importance in large-scale microalgal lipid production processes. Appropriate medium composition is of importance to achieve the best lipid production performance of microalgae species [19]. Change in macronutrients in the environment will result in the change of cellular macromolecular composition. The growth and lipid accumulation of microalgae are affected by nutrient concentration of the growth medium [20–23]. Cellular lipid levels of microalgae will increase under nutrient stress with triacylglycerols as the dominant proportions [24]. Phosphate limitation caused significant changes in the fatty acid and lipid composition of *Monodus subterraneus*. The proportion of phospholipids was significantly reduced from 8.3% to 1.4% of total lipids, and the proportion of triacylglycerols (TAG) increased from 6.5% up to 39.3% of total lipids [21]. Enhanced lipid production can be achieved by nitrogen limitation or starvation [25] as the excess carbon from photosynthesis is channelled into storage molecules such as triglyceride or starch [26]. A linear relationship between the nitrogen source concentration and the lipid content was observed [27]. Further, changing microalgal cells from normal nutrient to nitrogen depleted media will gradually change the lipid composition from free fatty acid-rich lipid to mostly triglyceride-containing lipid [28].

The deprivation of nitrogen enhances the lipid production in microalgae [29–31] and produces more favourable triacylglycerols by inducing changes in fatty acid chain length and saturation for biofuel conversion. The lipid contents of *Chlorella zofingiensis* were higher in media deficient of nitrogen and phosphate in which highest lipid productivity of 87.1 mg l⁻¹ d⁻¹ was obtained from nitrogen deficient media indicating that nitrogen deficiency was more effective than phosphate deficiency [32]. In another study, 18 mg l⁻¹ d⁻¹ was obtained using *S. quadricauda* under nitrogen starvation [33]. A maximum lipid content of 23% was observed with nitrogen starvation in *Desmodesmus* sp by Rios et al. [34], whereas 54.26% was obtained in nitrogen starved *Nannochloropsis oculata* [35]. A 22.84% of lipid content in *Chlorella minutissima* was observed under nitrogen starvation [36]. However combination of nitrogen deficiency and higher iron level was found to increase lipid accumulation in *Botryococcus* spp [37,38].

2.2. CO₂ influence

Among the atmospheric pollutants, CO₂ is recognized as most important one that contributes to the greenhouse effect. Reduction in the build-up of atmospheric CO₂ can be accomplished by utilizing photosynthetic organism which has ability to use CO₂ for their growth. Microalgae are photosynthetic microorganisms able to use solar energy to combine water with carbon dioxide to create biomass.

Microalgae are efficient biological factories capable of taking zero-energy form of carbon and synthesizing it into a high density liquid form of energy (natural oil) and are capable of storing carbon in the form of natural oils or as a polymer of carbohydrates [39]. CO₂ is known to influence the lipid content of algae [40–42]. Alterations in the composition of the fatty acids are dependent on the CO₂ concentration during the algal growth [43,44]. Enhanced lipid production in cell at various CO₂ concentrations was also reported [45,46]. *Chlorella vulgaris* has produced maximum lipid productivity of 29.5 mg l⁻¹ day⁻¹ at 8% (v/v) CO₂ in which higher saturated fatty acids were obtained [47]. In another study, highest lipid productivity of 40 mg l⁻¹ day⁻¹ was obtained under cultivating conditions at 1.0 mM KNO₃, 1.0% CO₂ and 60 μmol photons m⁻² s⁻¹ at 25 °C [48]. The growth of *C. vulgaris* was enhanced with increased CO₂ concentration and highest lipid productivity was obtained by Widjaja et al., [49]. Further, increasing CO₂ concentration of up to 1% of air will increase lipid produced by algae [50]. However under high concentrations of CO₂, the algal growth was affected which was due to that as CO₂ increases, unutilized CO₂ will be converted to H₂CO₃ thereby reducing the pH of the medium. By contrast, when the CO₂ level is low, algal growth will be inhibited by the low carbon source [51]. Hence optimum CO₂ levels are required to obtain maximum biomass and enhanced lipid production.

2.3. Temperature influence

Changes in culture conditions divert the biosynthetic metabolism to lipid synthesis, thus obtaining fats as the main constituent instead of proteins. Among the varying culture conditions, temperature affects the lipid accumulation in microalgal cells. Enhancement of lipid production in cell under temperature stress conditions is studied long ago [52,53]. It has been shown that many algae display increasing growth and total lipids with increasing temperature [54–56]. Highest total lipid was observed at 13 °C in fresh water microalgae [57]. Microalgae respond to decreased growth temperature by increasing the ratio of unsaturated to saturated fatty acids. Total fatty acid content increased under low temperature (17 °C) in *Nannochloropsis salina* [58]. An increase of the cultivation temperature from 20 °C to 25 °C has increased the lipid content of *N. oculata* from 7.9% to 14.9% [59]. Whereas, triacylglycerols content were decreased with the decrease of temperature in *Nitzschia laevis* reported by Chen et al., [60]. Higher temperature has increased the lipid content of *Ettlia oleoabundans* at a concentration of 10.37 mg l⁻¹ independent of nitrate depletion [61].

An optimum temperature of 20 °C was found for lipid production in *C. minutissima* [36]. The effect of varying temperatures was studied in *Tetraselmis subcordiformis* and *N. oculata* where highest total lipid was yielded at 20 °C and 30 °C. Increased temperature resulted in decreased neutral lipid and polyunsaturated fatty acids while saturated and monounsaturated fatty acids were increased [62]. In *Mono-raphidium*, highest lipid content (32.9%) was obtained at 25 °C, whereas the highest biomass concentration and lipid productivity of 29.2 mg l⁻¹ d⁻¹ was achieved at 30 °C [63]. Improvement in total and neutral lipid content of 24.5% and 10.2% were observed at 30 °C during microalgal cultivation by Subhash et al., [64]. High growth temperature has been related to significant decrease in protein content together with increases in lipids and carbohydrates. This indicates that the response of microalgal chemical composition to high and low growth temperatures varies from species to species.

2.4. Salinity stress

Algae produce some metabolites to protect from salt injury as salinity alters the surroundings' osmotic [65]. Salinity influences physiological and biochemical mechanisms of microalgae and can lead to increment in the lipid content due to changes in the fatty acid metabolism [66]. Algae have gained significance in salt tolerance studies as they are inhabitants of varying salinities. Restoration of turgor pressure, regulation of the uptake and export of ions through the cell membrane, and accumulation of osmo-protecting solutes and stress proteins gets activated when cells are exposed to salinity. This leads to stress generation inside the algal cells causing increased total lipid accumulation which act as a reserve energy material until favourable conditions arise [67,68]. Further, increase in unsaturated fatty acids proportion was observed under salt stress on the other hand higher levels of saturated fatty acids has also been reported under high salt conditions [69].

Another advantage of cultivating microalgae under high alkaline salt conditions is to limit contaminants and competing microorganisms. Salinity acts as growth limiting factor within natural biotic communities in open pond systems by inhibiting invasive organisms. Microalgal growth is influenced by salinity [70,71] and many microalgae can accumulate high amount of lipids under environmental stress such as nutrient deficiency or salt stress [72]. Higher lipid contents were observed in *Scenedesmus* species that were subjected to salt stress [51,73,74]. Duan et al. [75], has reported a 21.1% increase of lipid yield in *C. vulgaris* under salt induced osmotic stress. Increased salt concentrations resulted in higher intracellular lipid content to 70% in *Dunaliella* cells [76]. Salinity stress triggered both biomass growth and lipid synthesis in microalgae significantly with total and neutral lipid content of 23.4 and 9.2% along with higher amounts of saturated fatty acid methyl esters [77]. However, higher salt conditions inhibited the cell growth at the same time. Rao et al. [78], reported increase in the relative proportions of palmitic acid and oleic acid in *Botryococcus braunii* at different levels of salinity. Similar results were obtained by Ruangsombon [79] (2012) along with varying light and nutrient conditions. Lipid accumulation of *N. salina* under salinity stress has significantly increased at a concentration of 36%. Elevated salinity conditions leads to increased non-polar lipid content and decrease in membrane lipid content [80]. Various sodium salts have been used to induce lipid accumulation and NaCl was found to increase the lipid productivity of 67.08 mg l⁻¹ d⁻¹ in *Desmodesmus abundans* [81]. Increased salinity is a simple approach to manipulate environmental factors and simultaneously promote selected algal species growth for higher lipid production and limit competitor or predator growth.

2.5. Metal influence

Metal ions influence the algal biomass and lipid production. The total lipid content and lipid productivity of *Scenedesmus* increased 28.2% and 29.7% in the presence of iron, magnesium and calcium with the addition of EDTA during cultivation [82]. Liu et al. [37], reported the effect of iron on *C. vulgaris* and the total lipid content was raised up to 56.6%. Heavy metals like cadmium, copper and zinc are known to increase the total lipid content of *Euglena gracilis* [83,84]. The lipid metabolism of algal lichen photobionts were altered by copper and lead [85]. Lipid accumulation in *Chlorella protothecoides* by copper stressed lipid biosynthesis was studied by Li et al. [86], (2013) where optimized biomass and lipid yield were achieved by 6.47 g l⁻¹ and 5.78 g l⁻¹. Copper stress has influenced the lipid production in *Chlorella* at qualitative and quantitative manner. Higher concentrations of fatty acids were observed in *C. vulgaris*, *C. protothecoides* and *Chlorella pyrenoidosa* at copper levels of 4 mg l⁻¹ [87]. The effect of hexavalent chromium on fatty acid composition and lipid peroxidation was studied in *E. gracilis* by Rocchetta et al., [88].

2.6. Oxidative stress

Environmental stresses trigger the excessive formation and accumulation of intracellular reactive oxygen species (ROS) in algae which cause damage through the oxidation of cellular components. However, algal cells are able to mediate anti-oxidative defence and under oxidative stress, the lipid profile of many microalgae is reported to be altered. Kang et al. [89], has used oxidative stress to induce lipid production in *Chlorella vulgaris*. Yilanciooglu et al. [90], has determined the oxidative stress mediated increased cellular lipid content up to 44% by application of exogenous H₂O₂. Nitrogen depletion results in the co-occurrence of reactive oxygen species and lipid accumulation in diatoms [91]. Association of increased reactive oxygen species levels and cellular lipid accumulation under different environmental stress conditions was also shown in green microalgae [92]. Osundeko et al. [93], has reported that lipid content of *Chlorella luteoviridis* and *Parachlorella hussii* were increased under oxidative stress which could be used for biofuel feedstock production. However, a mechanistic understanding of the connection between oxidative stress and increased algal lipid accumulation requires further investigation [94].

3. Conclusion

Oleaginous microalgae are capable of producing higher quantities of fatty acids and triacylglycerols hence they are promising feedstock for the production of biofuels and bioproducts. This review emphasizes how environmental factors play an important role for enhanced production of microalgal lipids. Biomass and lipid production costs will very much depend on the systems considered-at present, there are many approaches to enhance lipid productivity. Of these approaches, the one most often considered is that of nutrient limitation. Given the limitations of nutrient influence lipid production, identifying other feasible approaches like CO₂ influence, temperature and heavy

metal stress will reduce the negative impacts of existing approaches. At its simplest, following new methods with a fine balance of biomass yield, lipid content and composition gains enhance lipid productivity in microalgae for biodiesel and high value end products. Further, these approaches could improve the rate and total accumulation of microalgal lipids which promotes the commercialization of algal biofuels.

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