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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 CO_2 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, CO_2 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. CO_2 influence

Among the atmospheric pollutants, CO_2 is recognized as most important one that contributes to the greenhouse effect. Reduction in the build-up of atmospheric CO_2 can be accomplished by utilizing photosynthetic organism which has ability to use CO_2 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]. CO_2 is known to influence the lipid content of algae [40–42]. Alterations in the composition of the fatty acids are dependent on the CO_2 concentration during the algal growth [43,44]. Enhanced lipid production in cell at various CO_2 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) CO_2 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 KNO_3, 1.0% CO_2 and 60 μmol photons m^{-2} s^{-1} at 25 °C [48]. The growth of *C. vulgaris* was enhanced with increased CO_2 concentration and highest lipid productivity was obtained by Widjaja et al., [49]. Further, increasing CO_2 concentration of up to 1% of air will increase lipid produced by algae [50]. However under high concentrations of CO_2, the algal growth was affected which was due to that as CO_2 increases, unutilized CO_2 will be converted to H_2CO_3 thereby reducing the pH of the medium. By contrast, when the CO_2 level is low, algal growth will be inhibited by the low carbon source [51]. Hence optimum CO_2 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].

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