Lipid Accumulation in Microalgae and its Induction under Different Stress Conditions for Biodiesel Production

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Abstract: The ever-increasing energy demand, diminishing fossil fuel reserves, global warming and other environmental as well as economical concerns necessitate alternative forms of energy that are renewable and sustainable. The oils stored in biomass are energy rich and have structures that resemble the fossil fuels and are therefore, important precursors for making biodiesel. Oils can be produced in significant amounts by some microalgae. Microalgae potentially provide several advantages i.e. high biomass productivity, fed on carbon dioxide and thus mitigate greenhouse gases, have ability to grow on non-agricultural land and higher efficiency in converting solar energy into chemical energy. The study of various biosynthetic pathways that lead to the formation and accumulation of triglycerides and understanding the mechanisms that how these pathways can be utilized for commercial production of microalgae feedstock for biodiesel production is required. Microalgae have an ability to produce substantial amount of (20-50% dry cell weight) triglycerides as storage lipids under photo-oxidative stress and other adverse environmental conditions. Efficient lipid induction techniques have been developed in microalgae such as nutrient stresses (nitrogen or phosphorus starvation), osmotic stresses, temperature, radiation, heavy metals and chemicals. A thorough understanding of unique metabolic steps that control the partitioning of carbon between lipids and other storage products is required for metabolic engineering of algal cells for increased production of lipids. The chemical, biochemical and catalytic processes can be employed to convert algal oil into biodiesel. The transesterification process is usually employed to convert microalgal triacylglycerol into fatty acid alky esters i.e. biodiesel. However, there is a need to focus on the development of efficient processes for processing of algal extract into biodiesel for reducing the cost and energy consumption.

Keywords: Lipid induction, Triacylglycerol, Microalgae, Biodiesel

1. INTRODUCTION

The ever-increasing energy demand, diminishing fossil fuel reserves, global warming and other environmental as well as economical concerns necessitate alternative forms of energy that are renewable and sustainable. The fossil fuels contribute to global warming by transferring previously sequestered carbon molecules into the atmosphere as CO_2, a greenhouse gas, a major source of air

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pollution and through other combustion products found in the exhaust [1].The oils stored in biomass are energy rich, and have structures that resemble the fossil fuel and are important precursors for making biodiesel Various sources for commercial biodiesel production are soya beans, canola oil, animal fat, palm oil, waste cooking oil [2] and Jatropha oil [3]. However converting existing agricultural land for biodiesel production has several associated controversial issues such as food versus fuel, environmental and social impact. The oils can be produced in significant amounts by some microalgae. Microalgae grow quickly as compared to terrestrial plants. They double in size every 24 hours. During the peak growth phase, they can double every 3.5 hours [4]. Microalgae potentially provide several advantages including: i) High biomass productivity ii) utilize carbon dioxide and thus, mitigate greenhouse gases iii) have ability to grow on non-agricultural land thus overcoming the food versus fuel issue iv) Much higher efficiency in converting solar energy into chemical energy v) require much less water than plant crops [5].

2. LIPID BIOSYNTHESIS AND ACCUMULATION IN MICROALGAE

The basic pathways of lipid synthesis are conserved in the green plants. In a photosynthetic cell, triacylglycerol synthesis occurs in several sub-cellular compartments involving several enzymatic reactions [6]. The oil biosynthesis and accumulation involve three major steps

1. Fatty acid synthesis in the chloroplast
2. Assembly of glycerolipids in the endoplasmic reticulum (ER)
3. Accumulation of triacylglycerol into the oil bodies

In algae, the *de novo* synthesis of fatty acids occurs primarily in the chloroplast. Microalgae can fix CO_2 into sugars using energy from the sun. The fixed sugars are further processed to produce acetyl-CoA (coenzyme A) and more than one pathway may contribute to maintain the acetyl-CoA pool. Acetyl-CoA provided by photosynthesis serves as the precursor for fatty acid synthesis in the chloroplast [7,8] through series of enzymatic reactions. Fatty acid synthesis requires stoichiometric amount of ATP, acetyl CoA and NADPH for each two carbon added to the growing fatty acyl chain. Photosynthetic reactions thus provide not only a carbon source, but also help in generating reducing power (NADH and NADPH) and energy (ATP) for fatty acid synthesis. In most of algal species, the final acyl chains emerging from the chloroplast are 16- or 18- carbons in length.

The triacylglycerols are formed in the endoplasmic reticulum by the sequential acylation of *sn*-glycerol-3-phosphate (G3P) backbone with three acyl-CoAs catalyzed by a group of enzymes named acyltransferases [9]. After synthesis of triacylglycerols in endoplasmic reticulum, droplets of oil are cut off from the membrane of endoplasmic reticulum thereby forming distinct cell organelles [10]. The dispersion of these oil droplets in the cytoplasm is by a single-layer membrane made of phospholipids with the hydrophilic head groups on the surface. These subcellular compartments are called oil bodies, lipid droplets or oleosomes. Oil bodies are spherical organelles consisting of a neutral lipid core enclosed by a membrane lipid monolayer coated with proteins.

3. LIPID INDUCTION IN MICROALGAE

Under optimal growth conditions, large amounts of algal biomass are produced but with relatively low lipid contents which constitute about 5–20% of their dry cell weight (DCW), including membrane lipids. The occurrence and the extent to which triacylglycerol (TAG) is produced depend upon the species/strain and are ultimately controlled by the genetic make-up of individual organisms [11].Synthesis and accumulation of large amounts of TAG along with changes in fatty acid composition occur in the cell when algae are placed under stress conditions such as chemical or physical environmental stimuli, either acting individually or in combination [12].The major chemical stimuli are nutrient starvation, salinity and growth-medium pH. The major physical stimuli are temperature and light intensity. In addition to chemical and physical factors, growth phase and/or aging of the culture also affects TAG content and fatty acid composition. Under unfavorable environmental or stress conditions many microalgae alter their lipid biosynthetic pathways towards the formation and accumulation of neutral lipids (20–50% DCW), mainly in the form of TAG, enabling microalgae to endure these adverse conditions.

i) Nutrient Deprivation

Nutrient availability has a significant impact on growth and lipid/fatty acid composition of microalgae. Limitation of nutrients invariably causes a steadily decline in rate of cell division. Under such conditions, an active biosynthesis of fatty acids is sustained in some algae species, provided there is enough light and CO_2 available for photosynthesis [13].

When algal growth slows down and there is no requirement for the synthesis of new membrane compounds, the cells instead divert and deposit fatty acids into triglycerides. Under these conditions, TAG production might serve as a protective mechanism. Under normal growth conditions, ATP and NADPH produced by photosynthesis are consumed by growing biomass. ADP and $NADP^+$ thus produced eventually become available again as acceptor molecules in photosynthesis. When cell growth and proliferation is impaired due to the lack of nutrients, the pool of the major electron acceptor for photosynthesis, $NADP^+$, can become depleted. Since photosynthesis is mainly controlled by the abundance of light, and cannot be shut down completely, this can lead to a potentially dangerous situation for the cell, damaging cell components. NADPH is thus consumed in fatty acid biosynthesis and there is increased production of fatty acid, which in turn is diverted to form triglycerides. This replenishes the pool of $NADP^+$ under growth-limiting conditions [7] (Hu *et al*, 2008).

Nutrient starvation is one of the most widely used and applied lipid induction techniques in microalgal TAG production and has been reported for many species. Nitrogen is the single most critical nutrient affecting lipid metabolism in algae. A general trend towards accumulation of lipids, particularly TAG, in response to nitrogen deficiency has been observed in numerous species or strains of various microalgae [14] conducted a study on nitrogen stress responses of several green microalgae, diatoms and cyanobacteria and all tested species showed a significant rise in lipid production. A detailed and large-scale model of lipid induction by nutrient starvation (nitrogen, phosphorus) on several diatoms, green algae, red algae, prymnesiophytes and eustimatophytes is presented in a study carried out by [15]. Phosphorus limitation resulted in increase in lipid content mainly triglyceride in *P.tricornutum*, *Chaetoceros* sp., *Isochrysis galbana*, but decreased lipid content in *Nannochloropsis atomus* and *Tetraselmis* sp. [16].

ii) Temperature Stress

Temperature has profound effect on the fatty acid composition of microalgae. A general trend towards increasing fatty acid (FA) unsaturation with decreasing temperature and increasing saturated FA with increasing temperature has been observed in many microalgae and cyanobacteria [17,18]. The most commonly observed change in membrane lipids following a temperature shift is an alteration in fatty acid unsaturation [19]. The fatty acids with carbon-carbon double bonds cannot be as densely packed as saturated fatty acids, thereby increasing the the fluidity of membranes. Most of microalgae species regardless of their taxonomic status respond to the temperature regime by similar changes in their intracellular fatty acid composition i.e. the relative content of unsaturated fatty acids decreased and saturated fatty acids increased with the increase in temperature. In contrast, no significant change in the lipid content was observed in *Chlorella sorokiniana* grown at various temperatures [20]. As lipid profile changes at different temperatures, the properties of algal-derived biodiesel would also change for different climates and seasons and different algal strains or species can be used for different seasons (e.g., summer or winter strains) for biodiesel production.

iii) Light Irradiation Stress

Light is most important for photosynthesis, without which no autotrophic life can exist. Microalgae grown on various light intensities exhibit remarkable changes in their chemical composition, pigment content and photosynthetic activity [21,22].The different light intensities and wavelengths have been reported to change the lipid metabolism in microalgae altering the lipid profile. High light intensity leads to oxidative damage of polyunsaturated fatty acids and alters the level of this fatty acid in microalgae. The low light intensity induces the formation of polar lipids, particularly the membrane polar lipids associated with the chloroplast, whereas high light intensity decreases total polar lipid content with a simultaneous increase in the amount of neutral storage lipids, mainly triglycerides (TAG) [23,24]. TAG production under high light conditions might serve as a protective mechanism for the cell. The electron acceptors needed by the photosynthetic machinery might be depleted under high light conditions. Increased fatty acid synthesis, which in turn, is stored as triglyceride helps the cell to re-generate its electron acceptor pool.

The effect of different light intensities on algal lipid composition was successfully demonstrated in a detailed study on *Chlorella vulgaris* Berjerinck *and Nitzschiapalea* (Kütz.) Smith by [25]. A culture grown to stationary phase under strong continuous light or under 12:12 h strong light/dark conditions had a higher amount of triglycerides with saturated and monounsaturated fatty acids compared to cultures grown with less light. At the exponential growth phase, however, the proportion of polyunsaturated fatty acid (PUFA) was highest under high light conditions. This demonstrates the important role of growth phase in the accumulation of certain fatty acids. With the onset of stationary phase, algae typically show increased proportions of saturated and monounsaturated fatty acids and decreased amounts of PUFA [23]

iv) Salinity and pH stress

Dunaliella species of microalgae can tolerate high salt concentration and an increase of the initial NaCl concentration from 0.5 M (29 g/L) to 1.0 M (58 g/L) followed by further addition of NaCl to 2.0 M (117 g/L) during cultivation of *Dunaliella tertiolecta* resulted in an increase in intracellular lipid content and a higher percentage of TAG [26]. Changes in the pH of the medium also alter the lipid composition of microalgae. The alkaline pH stress led to TAG accumulation in *Chlorella* CHLOR1 and was not dependent on nitrogen or carbon limitation levels, and led to a decrease in membrane lipids [27].

4. PROCESSING OF ALGAL EXTRACT INTO BIODIESEL

Microalgal cells are suspended in water and hard to settle by natural gravity force due to negative charges. The recovery of algal biomass requires one or more solid-liquid separation techniques and accounts for 20-30% of total cost of production [28]. The harvesting of microalgal cells is dependent upon the characteristics of microalgae such as size and density. The available approaches for micro algal harvesting are flocculation, flotation, centrifugal sedimentation and filtration. Similarly several oil extraction technologies i.e. mechanical methods, solvent extraction methods, supercritical fluid extraction, microwave technology and pulse electric field technology are available. However, most of these technologies are at their early research stage.

The algal oils are primarily composed of various triacylglycerols consisting of fatty acid chains esterified to glycerol. The fatty acyl chains are chemically similar to the hydrocarbons that make up the bulk of the molecules found in diesel. However, most algal/plant oil has a viscosity range that is much higher than that of conventional diesel, thus algal oil cannot be used directly as biofuels. The chemical, biochemical and catalytic processes can be employed to convert algal extract into biodiesel. The transesterification process can be employed to convert microalgal triacylglycerol into fatty acid alky esters usually fatty acid methyl esters (FAME).The process is simply the displacement of an alcohol group from an ester by another alcohol [29]. Transesterification process [30] is a conventional and the most common method for biodiesel production from various plant oils.

Homogeneous (acid/base catalyst) and heterogenous catalyst can be employed for transesterification process. Alkali-catalyzed transesterification proceeds much faster than that catalyzed by an acid and it is the one, which is mostly used commercially [31]. Chemical based process give high conversion of triacylglycerol into biodiesel but have drawbacks such as energy intensive, difficulty in removing the glycerol, removal of catalyst from the product and treatment of alkaline wastewater. Use of biocatalyst such as lipases can address these problems and offer an environmentally more attractive option to the conventional process. The benefits of using enzymes as catalyst over the acid and alkali catalysts are: i) No soap formation, ii) Have ability to esterify both free fatty acids and triglycerides iii) Capitulate a higher quality glycerol iv) waste cooking oils, animal fat and similar waste fractions, with high free fatty acids and water content, can be catalyzed with complete conversion to alkyl ester and vi) Reaction conditions are milder and there is less energy consumptions with lower alcohol to oil ratio than chemical catalyst [32]. Although enzymatic approach have become increasingly attractive option to conventional process but not have been demonstrated at large scale due to high price of lipases, negative effects of methanol and glycerol on lipases.

5. FUTURE PROSPECTS

The metabolic control of flux of photo synthetically fixed carbon and its partitioning into carbohydrates, proteins and lipids is a major area of research. A thorough understanding of unique metabolic steps that control the partitioning of carbon between lipids and other storage compounds is required for increased production of lipids. Fatty acids are the common precursors both for membrane and storage lipids and a complete understanding of distribution of these precursors to distinct destinations or their interconversions need to be elucidated. A thorough understanding of lipid induction stresses helps to achieve optimum lipid productivity in large scale microalgae cultivation system for biodiesel production. There is a need to focus on the development of efficient processes for downstream processing of algal biomass to reduce the cost and energy consumption. Currently, algal-biofuels production is still too expensive to be commercialized. Microalgae contain a large percentage of oil, with the remaining parts consisting of large quantities of proteins, carbohydrates, and other nutrients [33]. This makes the residue after oil extraction attractive for use as animal feed or other value-added products. So in future, an integrated bio refinery approach is required to lower the cost of algal-biofuels production.

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