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Lipid induction in microalgae

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ABSTRACT

Microalgae are now the center of research due to their potentiality of high growth rate and high amount of lipid accumulation. Lipids, in the shape of triacylglycerides, classically provide a storage function in the cell that enables microalgae to tolerate unfavorable environmental conditions. Essentially algal biomass and triacylglycerides fight for photosynthetic assimilate and a reprogramming of physiological pathways is required to excite lipid biosynthesis. There has been a wide range of studies carried out to identify and develop efficient lipid induction techniques in microalgae such as nutrients and osmotic stress. In counting, numerous genetic strategies for amplifying triacylglycerides production and inducibility are currently being established. In this review, we discuss microalgae and its various uses with special focus on polyunsaturated fatty acids increment by using various stress conditions.

Keywords: Microalgae, polyunsaturated fatty acid, nutrient stress, osmotic stress.

INTRODUCTION

Algae are recognized as one of the oldest forms of living organisms on earth. They are primitive chlorophyll bearing plant showing no differentiation into true tissues. They lack true roots, stems, leaves, embryo, vascular system (Brennan & Owend, 2009) and so they are called thallus. Algae consist of both prokaryotic as well as eukaryotic cells with nuclei and organelles. According to their size, algae can be classified into microalgae and macroalgae (Ibanez *et al*., 2012). Marine macroalgae are called seaweeds. Algae can be heterotrophic or autotrophic on the basic of nutrient. If autotrophic, they need only carbon dioxide, salts and light energy for growth. If heterotrophic, they require nutrient as energy source. Some photosynthetic algae are also called mixotroph i.e. they have ability for photosynthesis as well as acquire nutrients (Brennan & Owend, 2009).

Microalgae are single unicellular microscopic (2-200µm) cell (Mutanda *et al*., 2010) that exist in different ecological habitats such as fresh water, brakish water, marine, hypersaline, dams, river and coastal areas (Brennan & Owend, 2009).They include blue green algae, green algae which may be unicellular (*Chlamydomonas*), colonial (*Volvox*) or filamentous (*Spirogyra*), red algae (*Polysiphonia*), brown algae (*Macrocystis, Sargassum)*. Microalgal classification can be grouped into *Chlorophyceae* (green algae), *Phaeophyceae* (brown algae), *Rhodophyceae* (red algae), *Pyrrophyceae* (dinoflagellates) and *Chrysophyceae* (yellow-green algae).

Member of the Chlorophyceae (green algae) are presently targetted as a potential candidate for production of biofuels (Adarme-Vega *et al*., 2012), bioremediation of polluted water (Christenson and Sims, 2011; Osundeko, 2013), source for a wide variety of nutraceuticals such as omega -3 fatty acids (Adarme-Vega *et al.* , 2012) and also as a food for human and animal consumption (Spolaore *et al.,*2006).

Here are 10 reasons why algae are a promising new source of fuel and other products:

1) Algae Grow Fast

Algae can double their numbers every few hours, can be harvested daily, and have the impending to manufacture a volume of biomass and biofuel many times greater than that of our most fruitful crops.

2) Algae Can Have High Biofuel Yields

Algae store energy in the form of oils and carbohydrates, which when combined with their high productivity, means they can produce from 2,000 to as many as 5,000 gallons of biofuels per acre per year (Chisti *et al.,* 2007, Haag *et al.,* 2007, John and Mayfield, 2012)).

3) Algae Consume CO_2

Like any other plant, algae, when grown using sunlight, devour (or absorb) carbon dioxide (CO_2) as they grow, release oxygen (O_2) for the rest of us to breathe. For high yield, algae need more CO_2, which can be supplied by emission sources such as power plants, ethanol facilities, and other sources.

4) Algae Do Not Compete With Agriculture

Algae cultivation uses both land, that in many cases is unsuitable for traditional agriculture, as well as water sources, that are not useable for other crops, such as sea-, brackish- and wastewater. As such, algae-based fuels complement biofuels made from tradition agricultural processes.

5) Microalgal Biomass Can Be Used for Fuel, Feed and Food

Microalgae can be cultivated to have a high protein and oil content, for example, which can be used to produce either biofuels or animal feeds, or both. In addition, microalgal biomass, which is rich in micronutrients, is being used for dietary supplements to advance human health.

6) Macroalgae Can Be Grown in the Sea

Macroalgae (seaweeds) are grown in the sea, or even on land with seawater, and their sugars can be converted into biofuels and chemicals.

USES

Agar

Agar, a gelatinous substance consequent from red algae, has a number of commercial uses (Lewis *et al*., 1988). It is a good medium on which bacteria and fungi can grow without utilizing them. The gelling agent in agar is an unbranched polysaccharide obtained from the cell walls of some species of red algae, primarily from the genera *Gelidium* and *Gracilaria*. For commercial purposes, it is derived primarily from *Gelidium amansii*. Agar was obtained from the marine algae *Gelidium corneum* by Anselme Payen in 1859 (Payen *et al.,*1859).

Alginates

Alginic acid, or alginate, is extracted from brown algae. Its uses range from gelling agents in food to medical dressings. Alginic acid also has been used in the field of biotechnology as a biocompatible medium for cell encapsulation and cell immobilization. Between 100,000 and 170,000 wet tons of *Macrocystis* are harvested annually in New Mexico for alginate extraction and abalone feed (Macrocystis *et al.,* 2008).

Biofertiliser

Microalgae such as Cyanobacteria are used in agriculture as a biofertiliser (Silva *et al*., 2007). In India, considerable progress has been made in the development of Cyanobacteria based biofertilizer technology and this technology can be a powerful means of enriching the soil fertility and improving rice crop yields (Mishra and Pabbi, 2004).

Bioremediation

Microalgae are also used as a bioremediation to solve environmental problem such as green house effect and industrial air pollution (Chisti, 2007). Micralgae used for waste water treatment for removal of ammonium, nitrate, phosphate, etc by utilizing water contaminants as nutrients (Christenson and Sims, 2011).

Biofuels:

Biofuels are usually classified as follows:

1. First-generation biofuels are directly related to a biomass that is generally edible.

2. Second-generation biofuels are defined as fuels produced from a wide array of different feedstock, ranging from lignocellulosic feedstocks to municipal solid wastes.

3. Third-generation biofuels are, at this point, related to algal biomass but could to a certain extent be linked to utilization of CO_2 as feedstock.

Most recently, research efforts have been aimed at identifying suitable biomass species which can provide high-energy outputs to replace conventional fossil fuels (Miao *et al*., 2004). However, few attempts have been made to produce biodiesel from non-edible sources, like used frying oil, greases, tallow, lard, jatropha, and mahua oils. Nevertheless, the cost of biodiesel production is still a major obstacle for large-scale commercial exploitation, mainly due to the high feed cost of vegetable oils (Lang *et al.,* 2001).

Microalgae represent an exceptionally diverse but highly specialized group of microorganisms adapted to various ecological habitats. Many algae species produce significant biomass as lipids that can be converted to biodiesel in addition to sugars that can be converted to ethanol or hydrogen without utilization of food crops or the conversion difficulties of woody or other lignocellulosic biomass (Jhones and Mayfield, 2012).

Microalgae as nutraceutical source:

Microalgae chiefly are made up of protein, carbohydrates and lipids. They are able to enhance the nutrient content of conventional food. The high protein content of microalgae make it a potential unconventional source of protein (Spolaore *et al*., 2006). Carbohydrate in microalgae can be found in the form of starch, glucose, sugar and other polysaccharides.

Microalgae are source of essential vitamins like A, B1, B2, B6, B12, C,E, nicotinate, biotin, folic acid and pantothenic acid (Spolaore *et al*., 2006). Microalgae are source of pigments like chlorophyll (0.5%-1%), carotenoid (0.1% to 0.2% of dry weight on average and up to 14% of dry weight for β- carotene of Dunaliella) and phycobiliproteins. Moreover, microalgae are good source of polyunsaturated fatty acid.

Algal lipid are composed of glycerol and saturated or unsaturated fatty acids (7 to 22 carbon atoms) present among which ω3 and ω6 families are of particular interest such as eicosapentanoic acid(EPA), arachidonic acid (AA) and docosahexaenoic acid (DHA) (Spolaore *et al*., 2006).

Polyunsaturated fatty acids (PUFAs) are indispensable for human growth and physiology. Among other factors, Polyunsaturated fatty acid (PUFAs) have been proven to reduce the risk of cardiovascular diseases (Brennan and Owend, 2009). Currently, fish and fish oil are the common sources of long chain PUFAs but safety issues raised because of the possible accumulation of toxins in fishes (Spolaore *et al*., 2006) as well as unpleasant fishy smell and poor oxidative stability (Brennan and Owend, 2009; Spolaore *et al*., 2006). Currently, Docosahexanoic acid is the only algal PUFA that is commercially available and important for correct brain and eye development in infants (Spolaore *et al*., 2006) which is present in breastmilk and not in cow milk.

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are long chain polyunsaturated fatty acids (LC-PUFA) belonging to the omega-3 fatty acids group, which have established to be nutritionally important for the expansion, cardiovascular and brain function of higher eukaryotes (Knapp *et al*., 1989, Romieu *et al*., 2005, Von Schacky *et al.*, 2007, Von Schacky *et al.*, 2008). Microalgae are the chief source of LC-PUFAs in the marine ecosystem; therefore study has bowed towards marketable land-based fostering of algae with lofty nutritional content.

In the marine food web, long-chain polyunsaturated fatty acids (≥20 carbon atoms and >3 double bonds) are chiefly shaped by phytoplankton and pass onto herbivorous zooplankton, thus moving food class for organisms at higher trophic levels (Brett *et al.*, 1997).

Various auto- and heterotrophic marine species from diverse module create Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA), whereas, Arachidonic acid (AA) is regularly initiated in restricted amounts (Thompson *et al*., 1996, Bigogno *et al.*, 2002). Bacillariophyceae (diatoms) and Chrysophyceae species may be rich sources of Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA). Cryptophyceae, Prasinophyceae, Rhodophyceae, Xanthophyceae, Glaucophyceae and Eustigmatophyceae can symbolize attractive sources as well. Docosahexaenoic acid (DHA) is found in major amounts frequently in Dinophyceae, Prymnesiophyceae, and Euglenophyceae (Hu *et al*., 2008, Lang *et al*., 2011). Interestingly, many species producing long-chain polyunsaturated fatty acids (LC-PUFA) include low levels of C18 fatty acid precursors (Khozin-Goldberg *et al.* 2011). R ecently several species is encountered who have high lipid contents 30%–70% dcw (Ward *et al.* 2005). The sector has progressed substantially in recent years and product diversity, in terms of growing processes and subsequent fatty acid profiles, is estimated to enlarge, particularly by developing ingredients with higher (Eicosapentaenoic acid) EPA and (docosahexaenoic acid) DHA concentration in the near future (Grebow *et al.* 2012).

Strategies to improve stress based lipid enhancement in microalgae:

The microalgae are able to grow in diverse and extreme condition with variable pattern of cellular lipid (Sato et al.,2000). Under unfavorable environmental or stress conditions many microalgae alter their lipid biosynthetic pathways towards the formation and accumulation of neutral lipids (20–50% dcw), enabling microalgae to endure these adverse conditions.

Nutrient Starvation

Nutrient accessibility has a major bang on growth and propagation of microalgae and extensive effects on their lipid and fatty acid (FA) composition. Environmental stress condition habitually cause a gradually decline in cell division rate. However, active biosynthesis of fatty acids is maintained in some algae species under such conditions, provided there is sufficient light and CO_2 accessible for photosynthesis (Thompson *et al.,*1996). When algal growth slows down and there is no requirement for synthesis of new membrane compounds, the cells divert and deposit fatty acids into triacylglyceroids (TAG). In this situation, TAG construction might serve as a defensive mechanism. In normal growth conditions, ATP and NADPH formed by photosynthesis are consumed by generating biomass, with ADP and $NADP^+$ eventually being available again as acceptor molecules in photosynthesis. When cell growth and proliferation is impaired due to lack of nutrients, the pool of the major electron acceptor for photosynthesis, $NADP^+$, can become depleted. Since photosynthesis is mainly controlled by light, and cannot be shut down completely, this can lead to a potentially dangerous situation for the cell, damaging cell components. NADPH is consumed in FA biosynthesis; therefore, increased FAs production (which in turn are stored in TAGs) replenishes the pool of NADP+ under growth-limiting conditions (Thompson *et al*.,1996, Hu *et al*., 2008).

Nutrient starvation is one of the most broadly used and functional lipid orientation techniques in microalgal TAG fabrication and has been reported for many species (Table1). For example, when the diatom *Stephanodiscus minutulus* was grown in silicon, nitrogen or phosphorus limitation, an enhancement in TAG accumulation and a decline of polar lipids (as percentage of total lipids) was noticed in all of the nutrient-limited cultures (Lynn *et al.,*2000). In the green alga *Chlamydomonas moewusii*, nutrient limitation resulted in decreased PUFA C16:3, C16:4, and C18:3 contents, whereas, overall levels of C16:1 and C18:1 FA were increased (Arisz *et al.,* 2000).

Nitrogen is the most important nutrient affecting lipid metabolism in algae. A common fashion toward gathering of lipids, particularly TAG, in response to nitrogen deficiency has been observed in numerous species or strains of various microalgae (Yeh *et al.,* 2011, Praveenkumar *et al*., 2012, Hsieh *et al*., 2009). Hu *et al.,*2006 conducted a study on nitrogen stress responses of several green microalgae, diatoms and cyanobacteria and all tested species showed a momentous increase in lipid production. A complete 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 Rodolfi *et al.,* 2009. In the diatom *Cyclotella cryptica,* higher levels of neutral

lipids (primarily TAG) and higher proportions of saturated and mono-unsaturated FAs were produced due to silicon deficiency (Miao *et al*., 2006). However, only a small increase in TAG levels (from 69 to 75% from total lipids) together with phospholipids (from 6 to 8%) was reported for the microalga *Phaeodactylum tricornutum* as a result of reduced nitrogen concentrations (Alonso *et al*., 2000). *Scenedesmus* sp. subjected to nitrogen or phosphorus limitation showed an increase in lipids as high as 30% and 53%, respectively (Xin *et al.,* 2010). Lipid content of freshwater green alga *Chlorella vulgaris* could be notably increased by 40% by culturing in low nitrogen-containing medium (Illman *et al*., 2000).

With manipulated culture conditions of 1 mM KNO_3, 1.0 % CO_2, 60 µmol photon m^{-2} s^{-1} and 25 °C, lipid production of *C. vulgaris* was amplified by 2.5-fold (Lv&Zhang *et al*., 2010). In addition, lipid stimulation in *Chlorella* was also achieved *via*. silicon deficiency (Griffiths *et al.,* 2009) and iron supplementation (Liu *et al*., 2008). Moreover, it was found for *C. vulgaris* that changing from normal nutrient to nitrogen depletion media gradually changed the lipid composition from free FA-rich lipids to lipid mostly containing TAG (Widjaja *et al*., 2009).

Nitrogen starvation in microalgae not only affects the fatty acid metabolism, but also affects pigment composition. For *Parietochloris incise* grown in nitrogen-replete medium, an extensive increase in the ratio of carotenoid and chlorophyll contents was recorded (Solovchenko *et al.,* 2008).

Phosphorus limitation resulted in increased lipid content, mainly as TAG, in *P. tricornutum*, *Chaetoceros* sp., *Isochrysis galbana* and *Pavlova lutheri*, but decreased lipid content in *Nannochloris atomus* and *Tetraselmis* sp. (Reitan *et al*., 1994). Due to phosphorus deprivation, production of C16:0 and C18:1 increased, while production of C18:4ω3, C20:5ω3 and C22:6ω3 decreased (Reitan *et al.,* 1994) .

In contrast, for phosphorus-starved cells of the green alga *Chlorella kessleri*, a superior level of unsaturated fatty acids containing all identified individual lipids, namely phosphatidylcholine (PC), phosphatidylglycerol (PG), digalactosyldiacylglycerol (DGDG), monogalactosyldiacylglycerol (MGDG), and sulfoquinovosyl diacylglycerols (SQDG) were found (El-Sheek et al., 1995). Phosphorus limitation was also found to increase the overall TAG production from 6.5% up to 39.3% with a regular decrease in eicosapentaenoic acid (EPA) concentration. The cellular total lipid content increased, mainly due to TAG accumulation in *Monodus subterraneus* (Khozin-Goldberg *et al*., 2006). A study by Sato *et al.,* 2000 on sulphur and phosphorus depletion in green alga *C. reinhardtii* showed that sulphur depletion leads to decrease in SQDG, but on the other hand, PG was increased by 2-fold, representing a compensatory mechanism, where lipids containing sulphur are substituted by lipids containing phosphate. When *C. reinhardtii* was grown in media with limited phosphorus, it showed a 40% decrease in PG and also stimulated increase in the SQDG content. Thus, mechanisms that keep the total sum of SQDG and PG concentrations constant under both phosphorus and sulphur-limiting conditions appear to occur (Sato *et al.,* 2000) . Other studies have also shown that sulfur deprivation led to increased total lipid content in the green algae *Chlorella* sp. and *C. reinhardtii* (Matthew *et al*., 2009).

Based on the literature reviewed, it is clear that amongst all nutrient starvation approaches, nitrogen starvation technique is most widely applied and studied in almost all the microalgae species that can be considered for the production of biofuel (Table 1). Nitrogen is the most growth-limiting factor for eukaryotic microalgae and would be one of the first nutrients to be depleted during algae cultivation. It is relatively easy to apply controlled nitrogen stress on microalgae by subtracting the nitrogen source in the growth media. Moreover, all the microalgae species studied so far (Table 1), seem to increase TAG production under nitrogen stress. Hence, nitrogen starvation is the most victorious lipid inducing technique at present. However, high lipid production due to nitrogen stress may take 2–5 days and is complemented with slow growth rates and low cell counts and thus, finally effecting the total biomass and lipid productivity as detailed by Widjaja *et al.* 2009.

Jubilee *et al*, 2017 cultured *Chlorella ellipsoidea* in BG11 medium and reinoculated in modified WC medium under nitrogen stress condition, which resulted in increament of total fatty acid from 18.83 to 24.12% dcw.

Table 1. Examples of different types of nutrient starvation stress which have been studied to induce lipids in microalgae.

Microalgae species or Strain	Nutrient stress	Changes in lipid profile after induction	Reference
Chlamydomonas reinhardtii, Scenedesmus Subspicatus	Nitrogen limitation	Increase in total lipids (lipid: amide ratio)	Dean *et al*., 2010
Nannochloropsis Oculata	Nitrogen limitation	Total lipid increased by 15.31%	Converti *et al.*, 2009
Chlorella vulgaris	Nitrogen limitation	Total lipid increased by 16.41%	Converti *et al.,* 2009
Chlorella vulgaris	Nitrogen limitation	Lipid productivity of 78 mg/L d	Yeh e*t al.*, 2011
Chlorella sp.	Nitrogen limitation	Lipid productivity of 53.96 ± 0.63 mg/L d	Praveenkumar *et al.,* 2012
Phaeodactylum Tricornutum	Nitrogen limitation	TAG levels increased from 69 to 75%	Alonso *et al*., 2000
Dunaliella tertiolecta	Nitrogen limitation	Five times increase in lipid fluorescence	Chen*et al*., 2011
Chlorella vulgaris	Nitrogen medium	Lipids increased by 40%	Illman *et al*., 2000
Chlorella vulgaris	Nitrogen limitation	Increase in TAG	Widja *et al.,* 2009
Chlorella sp.	Nutrient-deprived conditions (nitrogen, phosphate-potassium, iron, and all three combined)	Total lipid production of 49.16 ± 1.36 mg/L d	Praveenkumar *et al*., 2012
Chlorella sp.	Urea limitation	Total lipid productivity of	Hsieh *et al*., 2009

		0.124 g/ L d	
Neochloris Oleoabundans	Ammonium nitrate	Lipid productivity of 0.133 g /L d	Li *et al*., 2008
Scenedesmus sp., *Coelastrella* sp.	Combined effect of P and N-limitation	Increase in TAG	Gardner *et al.,* 2011
Phaeodactylum tricornutum , Chaetoceros sp., *Isochrysis galbana*	Phosphorus limitation	Increase in total lipids with higher relative content of 16:0 and 18:1	Reitan *et al*.,1994
Monodus subterraneus	Phosphorus limitation	Increase in TAG	Khozin-Goldberg *et al*., 2006
Scenedesmus sp	Nitrogen and phosphorus starvation	Lipids increased 30% and 53%, respectively	Xin *et al*., 2010
Chlorella sp.	Silicon deficiency	Lipids increased 30% and 53%, respectively	Griffiths et al., 2009
Chlorella kessleri	Phosphorus limitation	Increase in unsaturated FAs	El-Sheek *et al*.1995
Chlamydomonas Reinhardtii	Sulphur limitation	PG was increased by 2-fold	Sato *et al*., 2000
Chlamydomonas Reinhardtii	Sulphur limition	Increase in TAG	Matthew *et al*., 2009
Cyclotella cryptic	Silicon starvation	Increased in total lipids from 27.6% to 54.1%	Roessler *et al.*,1988

Salinity induced lipid production

Dunaliella species tolerate high salt concentrations. The capability of *Dunaliella* species to multiply over the saturation range of salinities makes them one of the favorite candidates to study salinity effects on microalgae (Azachi *et al*., 2002, Takagi *et al*., 2006, Xu *et al*., 1997). In a study, cells of *D. salina* were transferred from 0.5 to 3.5 M (29 to 205 g/L) NaCl, and there was a notably higher ratio of C18 (mostly unsaturated) to C16 (mostly saturated) FAs in the cells grown in 3.5 M (205 g/L) NaCl compared with those grown at 0.5 M (29 g/L) NaCl (Azachi *et al.* 2002). 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 a rise of intracellular lipid content and a higher percentage of TAG (Takagi *et al*., 2006).

An even stronger increase in salinity from 0.4 to 4 M (23 to 234 g/L) in *Dunaliella* sp. raise the proportion of total saturated fatty and monounsaturated fatty acids, but the proportion of PUFA was reduced (Xu *et al.,* 1997).

Higher amounts of EPA were produced by diatom *Nitzschia laevis* when grown under osmotic stress (Chen *et al*., 2008). In higher salt concentrations, the degree of FA unsaturation of both neutral and polar lipid fractions increased sharply when salt concentrations raised from 10 to 20 g/L, but decreased at salt concentrations of 30 g/L (Chen *et al.,* 2008). Highest contents of total fatty acids, EPA and polar lipids were obtained at NaCl concentration of 20 g/L, under which 71.3% of total EPA existed in polar lipid fractions (Chen *et al.,* 2008). The quantity of total free sterols was also improved with a boost in salt concentration. In three marine heterotrophic microalgae strains, *Crythecodinium cohnii* ATCC 30556, ATCC 50051 and RJH grown at different salinities, the FA composition was affected (Jiang *et al.,*1999). At 9 g/L NaCl, *C. cohnii* ATCC 30556 had the highest total FA content and DHA (C22:6) proportion. In contrast, *C. cohnii* ATCC 50051 and *C. cohnii* RJH had the highest DHA content at 5 g/L NaCl and *C. cohnii* ATCC 30556 and ATCC 50051 had the highest DHA yield (132 and 68 mg/L respectively) at 9 g/L NaCl, while, *C. cohnii* RJH had the highest DHA yield (129 mg/L) at 5 g/L NaCl ((Jiang *et al.,* 1999). Growth, lipid content and FA composition of heterotrophic microalga *Schizochytrium limacinum* OUC88 at different temperatures (16 °C, 23 °C, 30 °C and 37 °C) and salinities (0, 9, 18, 27 and 36 g/L) were analyzed. Highest lipid content was obtained at salinities of 9–36 g/L at a temperature range of 16–30 °C and the content of saturated fatty acids C15:0 and C17:0 was increased greatly. In addition, the ratio of DHA to DPA changed at different temperatures and salinities (Zhu *et al.,* 2007). Moreover, all the microalgae species studied so far (Table 2), seem to increase total lipid content under osmotic stress.

Table2: Showing microalgal lipid stress due to osmotic stress

Microalgae sp	Salinity change	Changes in Lipid profile after induction	Reference
Dunaliella salina	Transferred from 29 to 205 g/L NaCl	Increased concentration of C18 FA	Azachi *et al*.,2002
Dunaliella tertiolecta	Transferred from 29 g/L to 58 g/L NaCl	Lipid content and TAG increased	Takagi *et al*., 2006
Dunaliella sp.	Raised salinity from 23 to 234 g/L NaCl	Boost in total FA and monounsaturated FA	Xu *et al.*, 1997
Nitzschia laevis	NaCl concentration raised from 10 to 20 g/L	unsaturated FA amount raised	Chen *et al.,*2008
Crythecodinium. cohnii ATCC 30556	At 9 g/L NaCl	Raising of total FA content and DHA	Jiang *et al*., 1999
Schizochytrium Limacinum	Salinity at 9–36 g/L at temperature range of 16–30 °C	Saturated FA C15:0 and C17:0 was greatly increased	Zhu *et al.*, 2007

Conclusion and future directions

In this review, we focused on production and induction of microalgal lipid. Here studies have been done on different lipid induction techniques that can be used to stimulate lipid biosynthesis in microalgae, in particular triacylglycerol (TAG). It is clear that different induction technique have different effects on different microalgal species and thus on their lipid composition and amount.

Now the main question before an algal technologist is which technique must be used ?

The answer is that we should consider a particular technique by taking into consideration the environmental conditions and the microalgal species.The most popular method we can imply to understand environmental effect on lipid metabolisms of microalgal species is to study them in pilot scale in the laboratory conditions. From this review we can observe that nitrogen is an important factor in altering lipid metabolisms of microalgal species. Change in nutrient stress and salinity can also induce lipids effectively, but may be difficult to regulate on a large-scale cultivation system. The lipid accumulation phase may be further varied by introducing a combination of different induction types

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