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NPTEL ONLINE
CERTIFICATION COURSE

WASTE TO ENERGY CONVERSION

CULTIVATION OF ALGAL BIOMASS AND TREATMENT OF WASTE WATER-1

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Contents

- Why algal cultivation?
- Algal metabolism and synthesis of fat and protein
- Classification of algae
- Lipid content in algal cell
- Algal lipids and bio oil quality
- Conditions influencing algal lipid quality and quantity
- Enhanced lipid accumulation
- Cultivation/ growth of microalgae as an important step for algae to oil
- Reactor systems for cultivation/ growth of microalgae
- Harvesting of algal biomass
- Treatment of wastewater



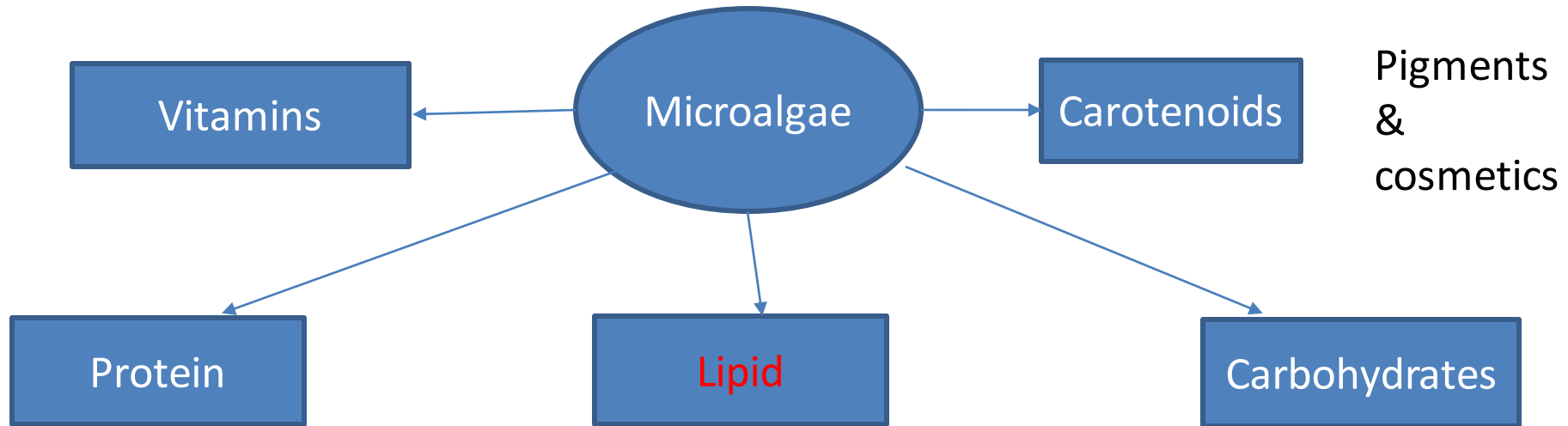
Why algal biomass?

Important metabolites

- Photosynthetic aquatic organism

$\text{CO}_2 + \text{water} + \text{light} \rightarrow \text{Biomass}$

- Help treating waste water



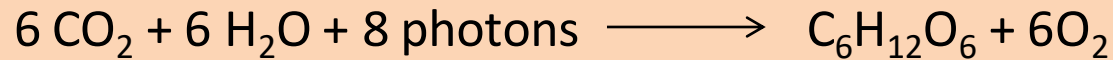
Pigments
&
cosmetics

Application in health, food and feed additives, cosmetics and for **energy production**

- Rapid growth
- Grow in saline or brackish water
- Some species produce high yields of lipids
- Use all nutrients, minimize eutrophication
- Requires CO₂ for growth therefore fuel is carbon neutral
- It can be used as fodder, manure
- Diverse species, microscopic to giant kelp, varied composition
- Untapped resource with high potential

Algal metabolism and synthesis of fat and protein

- Micro algae grow like plants through photosynthesis process, during which it captures carbon-dioxide and photons.
- Photonic energy, water and carbon dioxide are converted to sugars; then sugars are converted to macromolecules such as lipids, carbohydrates, protein etc.

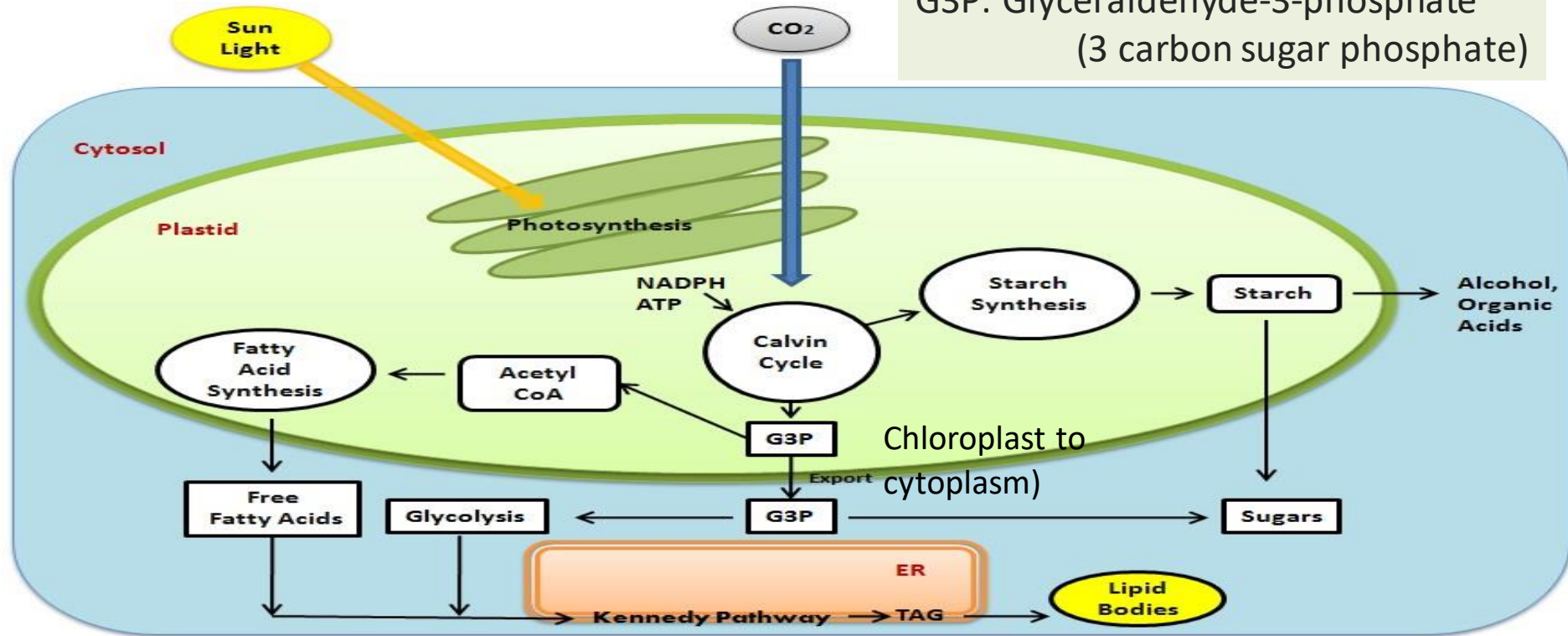


- The lipid content in algae contributes to bio-oil



Metabolic pathways of algae rendered for biofuel production

G3P: Glyceraldehyde-3-phosphate
(3 carbon sugar phosphate)

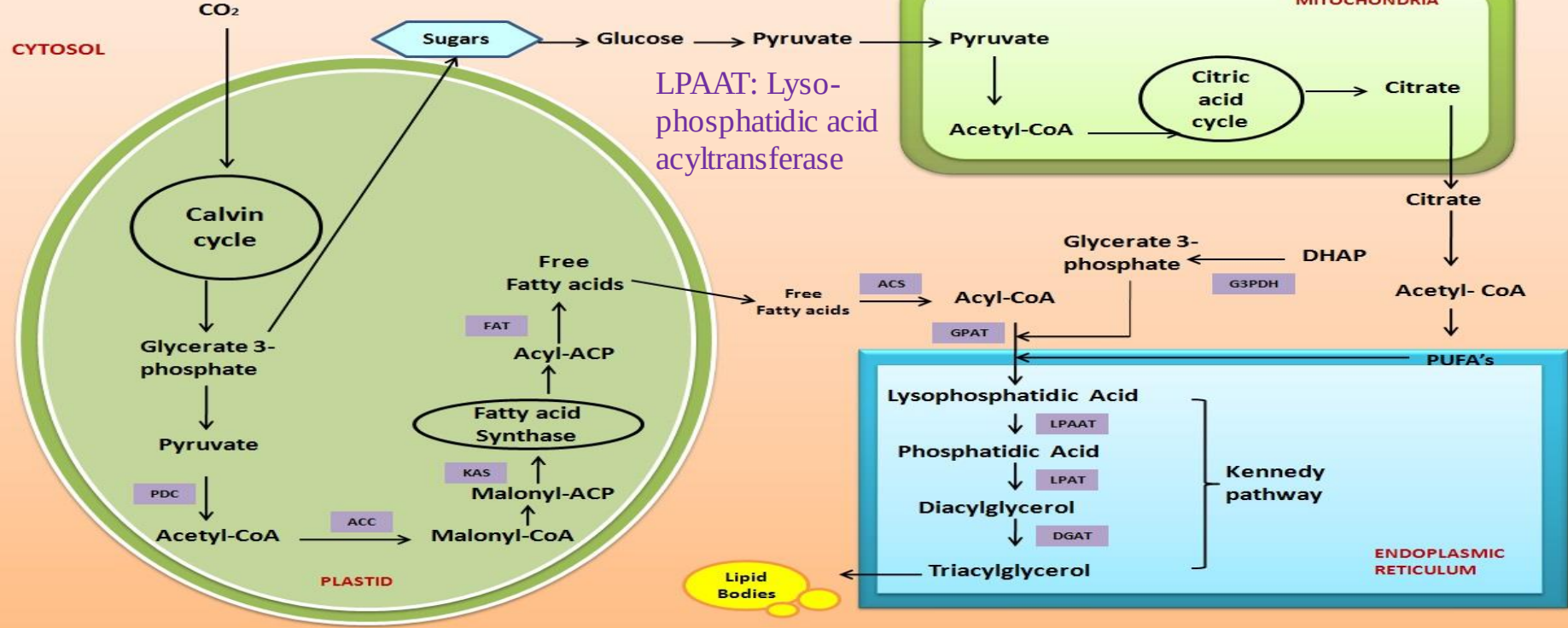


(Modified from Radakovits et al. 2011; Beer et al. 2009).

Simplified scheme showing lipid synthesis in microalgae

ACS: Acyl-CoA synthetase

ACC: Acetyl CoA Carboxylase, KAS: 3-ketoacyl-ACP synthase



PDC: Pyruvate dehydrogenase complex, GPAT: Glycerol-3-phosphate acyltransferase, FAT: Fatty acyl-ACP thioesterase

(Modified from Bellou and Aggelis 2012; Chen and Smith 2012; Radakovits et al. 2011).

Classification of microalgae phyla

(Rodolfi et al., 2009)

Algal Phylum	Description
Green algae or <i>Chlorophyta</i>	Algal cells have green chloroplast that contains chlorophyll a and b. xanthophyll as their accessory pigments These cells have mitochondria. Some species have flagella.
Red algae or Rhodophyta	Cells have chloroplast with chlorophyll a and d, and phycobillins. They have double cell wall, but do not have centrioles and flagella.
Cyanobacteria Blue-green algae	Class of prokaryotic cells that contain chloroplast normally with chlorophyll a. These are bacteria; but they are assimilated to algae due to their growth through photosynthesis process similar to microalgae. Some strains can grow in soil, marine or fresh water.



Classification of microalgae phyla

Algal Phylum	Description
Eustigmatophytes	Class of eukaryotic algae which contain yellow-green chloroplast. They include strains growing in marine, freshwater or solid medium such as soil. Algae in this class have chloroplast containing chlorophyll a.
<i>Brown algae</i> <i>Phaeophyta</i>	Contain chlorophyll a and c. Their accessory pigments are xanthophylls and carotenoids, including fucoxanthin.
Diatomaceae	Diatomaceae cells have chloroplast carrying chlorophyll a and c. They have hard wall due to the presence of silica. Most of these cells can be found in fresh or salted sea. Majority of diatom species live in cold water.



Biomass and Lipid productivity of different phyla of microalgae

(Khan et al., 2009;
Rodolfi et al., 2009)

Algal phylum	Strains	Biomass productivity (g/L/day)	Lipid productivity (mg/L/day)	Habitat
Diatoms	<i>P.Tricomutum</i>	0.24	44.8	Marine
	<i>Skeletonoma sp.</i>	0.09	27.3	
Green Algae	<i>Chlorocuccum sp.</i>	0.28	53.7	Fresh water
	<i>Scenedemus sp.</i>	0.26	53.9	
	<i>Chlorella sorokiniana</i>	0.23	44.7	
Prymnesiophytes	<i>Pavlova lutheri</i>	0.14	50.2	Marine
	<i>Isochrysis sp.</i>	0.17	37.7	
Red algae	<i>Porphyridium cruentum</i>	0.37	34.8	Marine
Eustigmatophytes	<i>Nannochlorosis sp.</i>	0.19	53	Marine
	<i>Ellipsoidion sp.</i>	0.17	47.3	



Lipid content in algal cell

Mondal and Soni, 2012

Algal strain	Lipid content in g lipid per 100 g dry algae
<i>Chlorella sp</i>	28-32
<i>Tetraselmis</i>	15-23
<i>Dunaliella primolecta</i>	23
<i>Isochrysis sp</i>	25-33
<i>Thalassiosira Pseudonana</i>	>30
<i>Nannochlorosis sp</i>	31-68
<i>Porphyridium cruentum</i>	>40
<i>Schizochytrium sp</i>	50-77
<i>Phaeodactylum</i>	20-30
<i>Neochloris oleoabundans</i>	35-54
<i>Botryococcus braunii</i>	25-75



More research is required to identify microalgae having high lipid content



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Category	Fatty acid
Saturated fatty acids	Capric (10:0) : $\text{CH}_3-(\text{CH}_2)_8-\text{COOH}$
	Lauric (12:0) : $\text{CH}_3-(\text{CH}_2)_{10}-\text{COOH}$
	Myristic (14:0) : $\text{CH}_3-(\text{CH}_2)_{12}-\text{COOH}$
	Palmitic (16:0) : $\text{CH}_3-(\text{CH}_2)_{14}-\text{COOH}$
	Stearic (18:0) : $\text{CH}_3-(\text{CH}_2)_{16}-\text{COOH}$
	Arachidic (20:0) : $\text{CH}_3-(\text{CH}_2)_{18}-\text{COOH}$
	Behenic (22:0) : $\text{CH}_3-(\text{CH}_2)_{20}-\text{COOH}$
	Lignoceric (24:0) : $\text{CH}_3-(\text{CH}_2)_{22}-\text{COOH}$

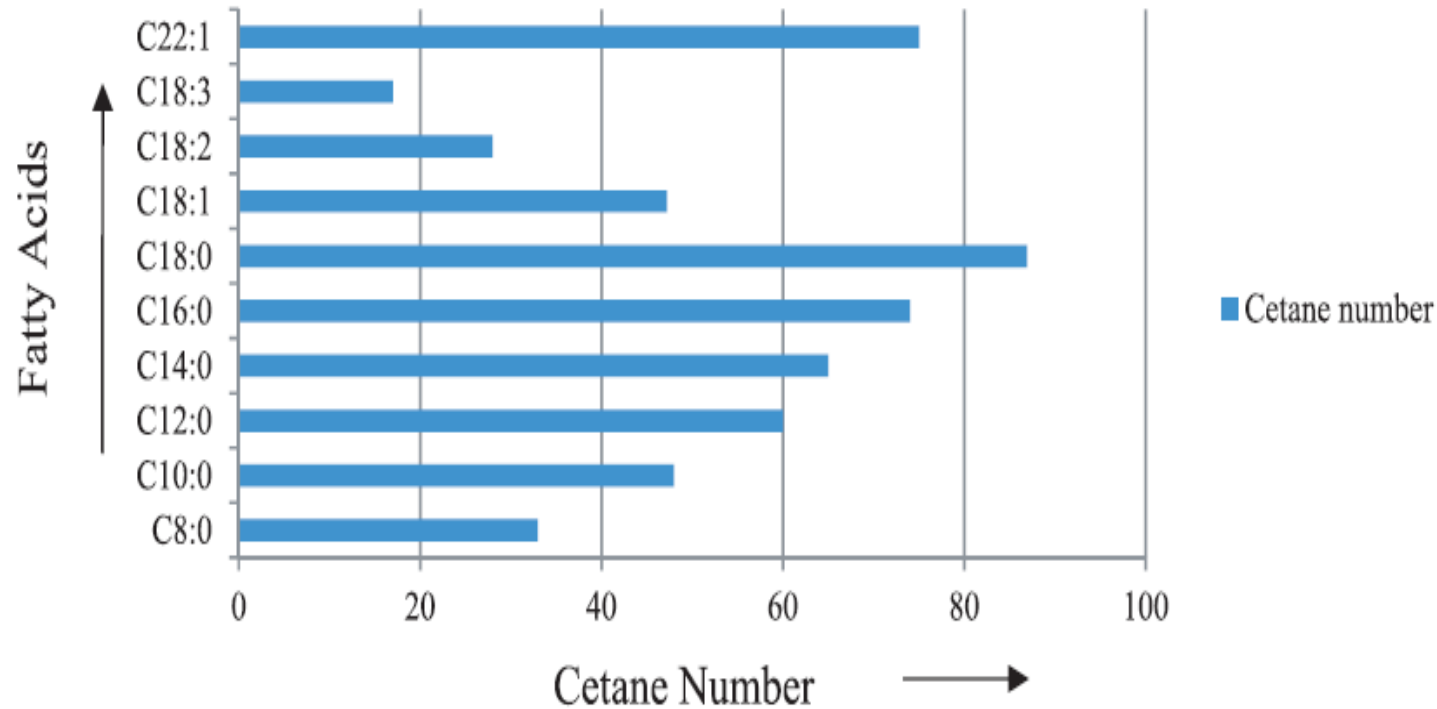
Various types of fatty acids in algal cell

Mondal and Soni, 2012

Category	Fatty acid
Unsaturated fatty acids	Myristoleic (14:1) : $\text{CH}_3(\text{CH}_2)_3\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$
	Palmitoleic (16:1) : $\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$ (= bond at C7)
	Sapienic (16:1) : $\text{CH}_3(\text{CH}_2)_8\text{CH}=\text{CH}(\text{CH}_2)_4\text{COOH}$ (= bond at C 10)
	Hexadecadienoic (16:2) : $\text{CH}_3(\text{CH}_2)_{10}\text{CH}=\text{CHCH}=\text{CHCOOH}$
	Hexadecatrienoic (16:3) : $\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_2\text{COOH}$
	Oleic (18:1) : $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$
	Linoleic (18:2) : $\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$
	α -Linoleic (18:3) : $\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$



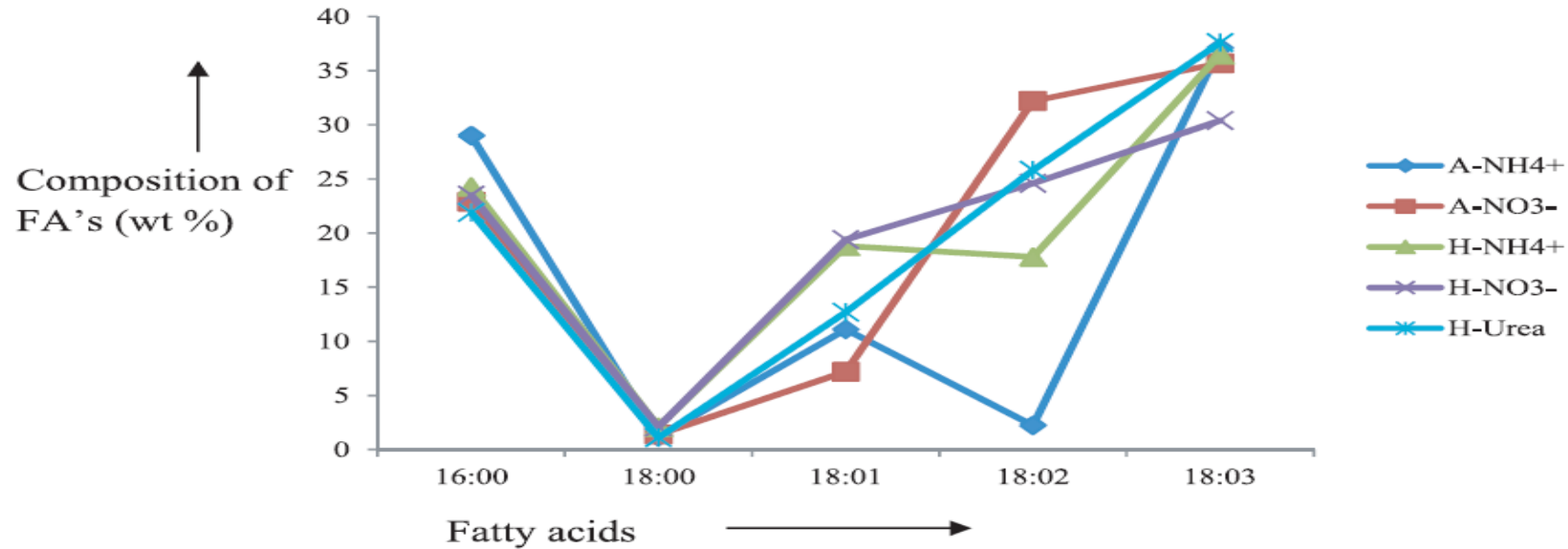
Category	Fatty acid
Unsaturated fatty acids	Octadecatetraenoic (18:4) : $\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}-\text{CH}_2\text{CH}=\text{CH}(\text{CH}_2)_4-\text{COOH}$
	Arachidonic (20:4) : $\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2-\text{CH}=\text{CH}-(\text{CH}_2)_3\text{COOH}$
	Eicosapentaenoic (20:5) : $\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2-\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_3\text{COOH}$
	Erucic (22:1) : $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_{11}\text{COOH}$
	Docosahexaenoic (22:6) : $\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2-\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_2\text{COOH}$
	Docosapentaenoic (22:5) : $\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2-\text{CH}=\text{CHCH}_2-\text{CH}=\text{CH}-(\text{CH}_2)_3\text{COOH}$



Cetane number of various fatty acids present in algal oil

Monitoring of favourable fatty acid content in algal cell

Effect of nitrogen source



- A-NH₄⁺ represents effect of ammonium ion on fatty acid composition when grown autotrophically
- H represents heterotrophic condition for algae growth.

Mondal and Soni, 2012

Strategies for enhanced lipid accumulation in microalgae

Approaches for enhancing lipid accumulation in microalgae

Physiochemical Methods

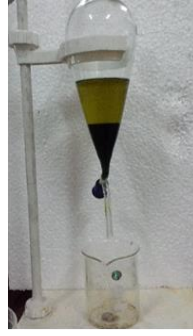
- Nutrient Stress
- Optimization of culture conditions (i.e. light, temperature, salinity etc.)
- Combined stresses (multiple nutrient stress with different culture conditions)
- Co-culturing with bacteria and/or fungi
- Use of phytohormones
- Mixotrophic growth conditions (use of different carbon substrates)

Molecular Strategies

- Gene silencing
- RNAi technology (knockdown of lipid catabolic genes)
- Transformation of microalgal cells
- Overexpression of genes directly linked to lipid biosynthetic pathway
- Overexpression of transcription factors
- Chloroplast engineering
- Gene expression analysis
- Metabolic engineering (pathway engineering via alteration in expression of multiple gene)



Cultivation/ growth of microalgae as an important step for algae to oil



Algae cultivated
in the glass flask

Wet biomass
Obtained

Dry biomass
after oven
drying for 24 hr
@ 60 deg. C

Ultrasonicated by
adding
chloroform:
methanol in 2:1
ratio

Separating the
phases

Vacuum
Evaporation for
separating
chloroform and
algal oil

Algal oil

Algal oil
transesterification
with methanol
and 10% Conc.
Sulphuric Acid

Cultivation/ growth of microalgae as an important step for algae to oil

Biomass and lipid measurement

$$\text{Biomass Productivity} = \frac{X_2 - X_1}{t_2 - t_1}$$

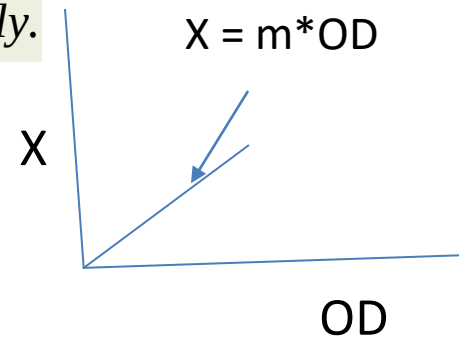
X : Dry cell weight in g/l

OD : Absorbance of algal cells at 682 nm.

where X_2 and X_1 represent the biomass concentration at t_2 and t_1 respectively.

$$\text{Lipid content (\% dcw)} = \frac{\text{weight of extracted lipids}}{\text{dry algae biomass}}$$

$$\text{Lipid Productivity} = \text{Biomass Productivity} * \text{Lipid content (\% dcw)}$$



Lipid content: Bligh and Dyer (1959) method was used to extract lipid.

1 g of dried algal biomass is mixed with 1 ml of chloroform and 2 ml of methanol and then kept for 18 hr at 25°C. After that the mixture is shaken tightly and again 1 ml of chloroform is added and again the mixture is shaken vigorously for 1 min. To the mixture, 1ml of distilled water is added and mixed in a whirlpool again for 2 min. Separation of layer and determination of lipid.

Thanks...

