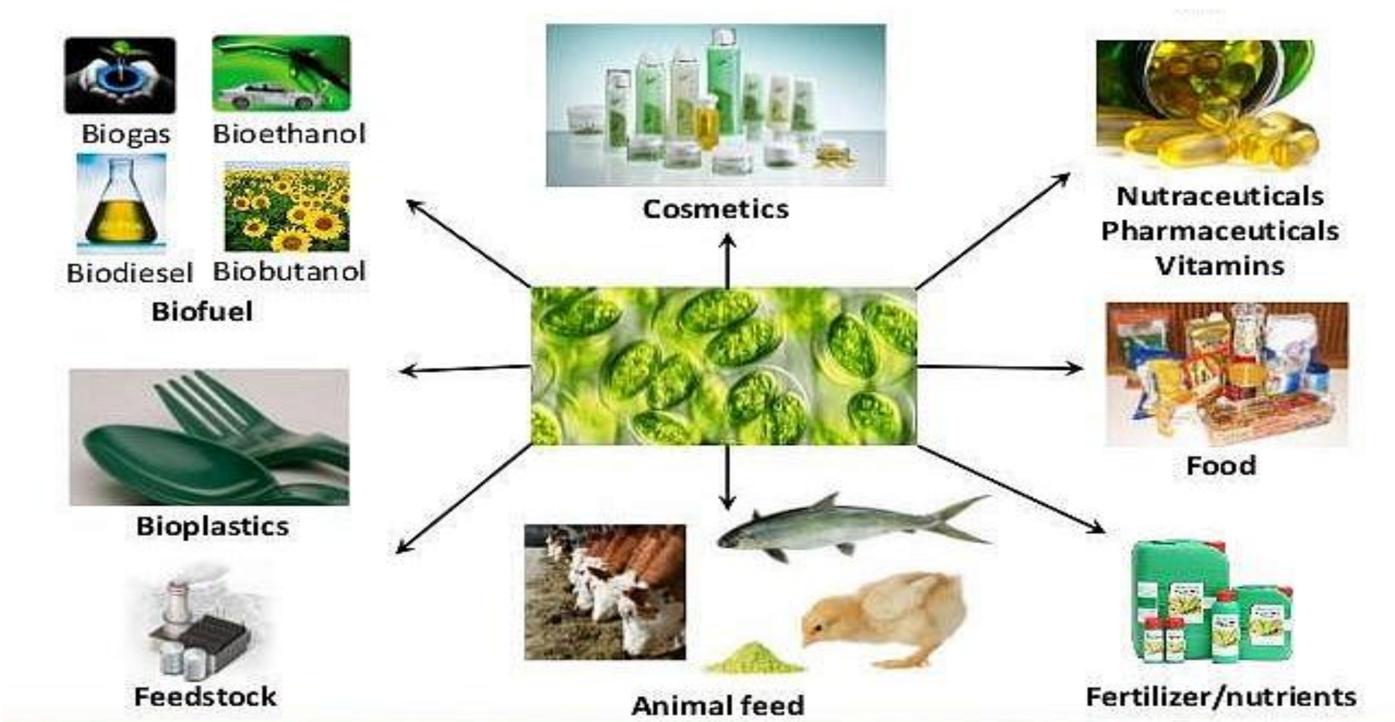
Special Course

For Applied Microbiology Diploma Students

Faculty of Science

Department of Botany & Microbiology



BY

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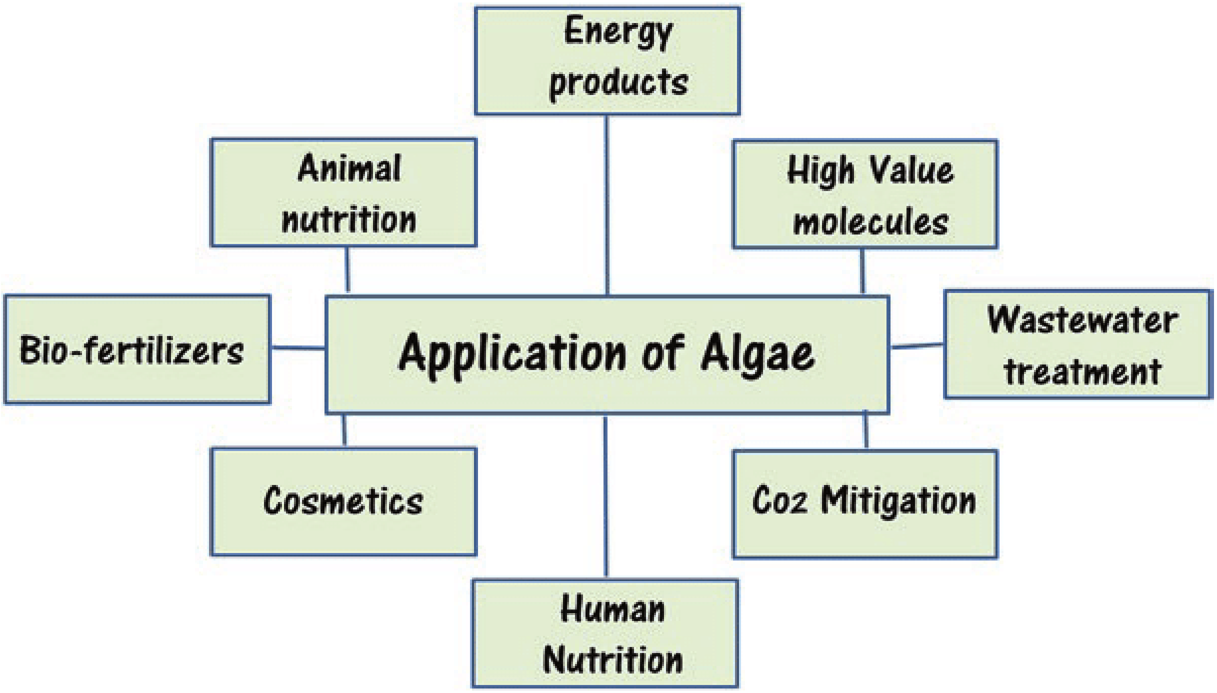
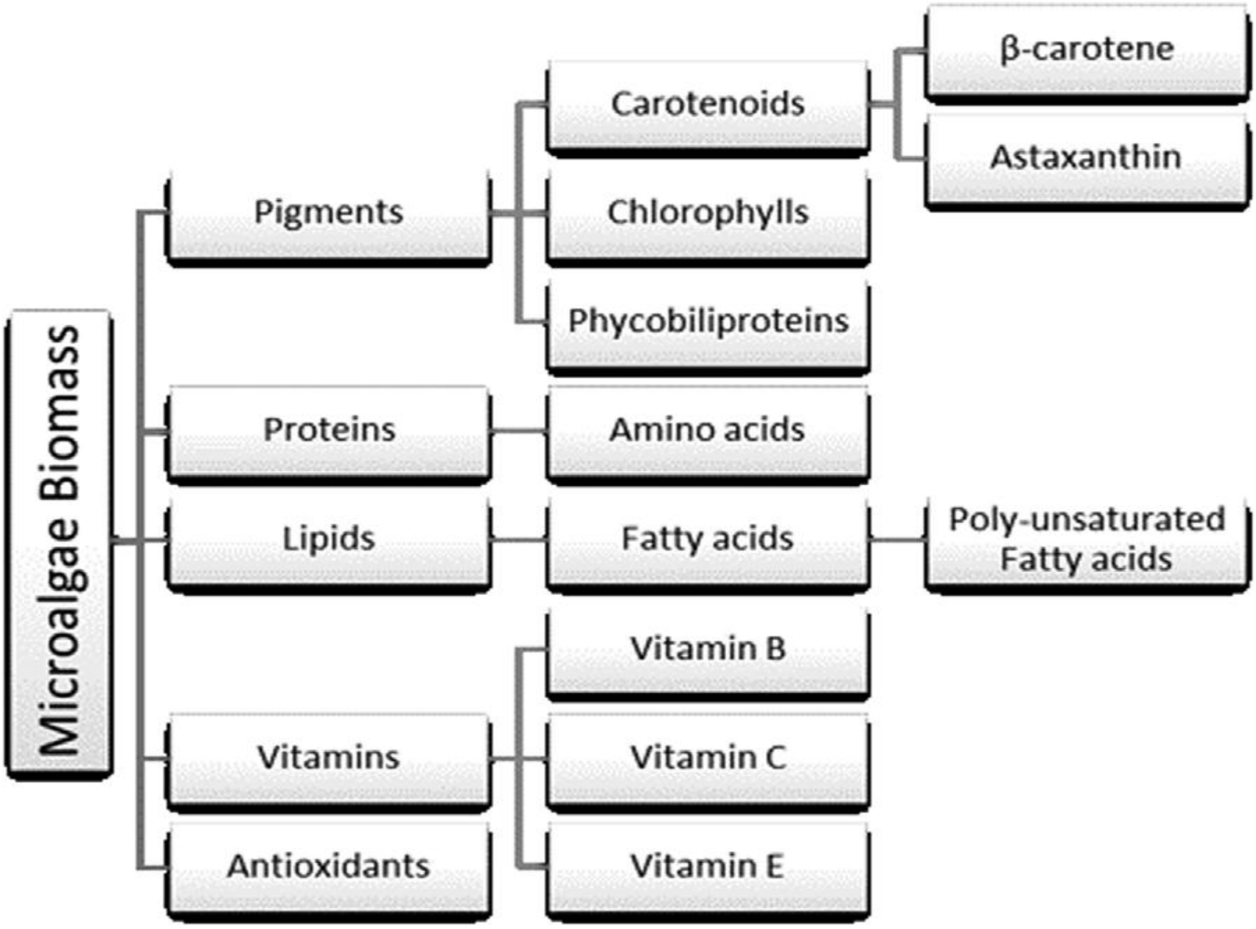
Applications of Algae





I. INTRODUCTION

Living system on earth comprises of plants and animals. Plants are everywhere. They are primary producers which is a source of many nutrients. Algae are a class of photosynthetic organisms found in both marine and freshwaters habitats. Algae are a very large and diverse group of **photoautotrophic organisms** that can prepare their own food and they produce oxygen during photosynthesis.

Based on photosynthetic pigments, the nature of storage products, cell wall components, flagella, and cell structure، algae are divided into 11 classes: **Cyanophyceae**, **Chlorophyceae** (green algae), **Euglenophyceae**, **Xanthophyceae** (yellow green algae،(**Chrysophyceae** (golden algae), **Bacillariophyceae** (diatoms،(**Phaeophyceae** (brown algae), **Pyrrophyceaes**, **Cryptophyceae**، **Rhodophyceae** (red algae), and **Chloromonadineae**.

Algae are divided into macroalgae (macroscopic algae) and microalgae (microscopic algae). A special group of microalgae are blue green algae, also called cyanobacteria because of their prokaryotic cell type, identified as one of the most promising group of organisms for the isolation of novel and biochemically active natural products.

Algae range from unicellular to multicellular forms with high protein content. At the same time, algae are important producers of vitamins, minerals and fatty acids also. Many species of algae find their applications in food, dairy, pharmaceutical, cosmetics and industry. Certain beverages are prepared from sea algae. Mainly marine algae have been used as food and medicine for many centuries. Edible algae are recognized as complete foods which provide correct balance of proteins, carbohydrates, vitamins, and minerals. The importance of investigating new options offered by algae cultivation is motivated by the fact that algae are very efficient at converting light, water and carbon dioxide (CO_2) into biomass in a system that does not necessarily require agricultural land. Therapeutic properties of algae are used for promotion of health. Algae are used as one of important medical source due to its antioxidant, anticancer, antiviral properties. Scientists are looking for biologic drugs which are cheaper than the existing chemical drugs.

The biologic drugs manufactured in mammalian cell culture or by bacteria or yeasts for treating diseases like diabetes, multiple sclerosis and cancer cost too much. The alternate is green algae, which is abundant, resilient, cheap to grow, and efficient at folding complex proteins. Various industrial products are made up from macro algae also. They can act as antibiotics, antihypertensive agents, and cholesterol reducers in blood, dilatory agents, anticoagulants, insecticides, and anti-tumorigenic agents. In cosmetics, the role of algae is as water-binding agents, thickening agents, and antioxidants. Some algae are also potential skin irritants. Most algae do not have lignin associated to the cellulose of the plant cell wall, so the cellulose extraction is easier, less expensive and produces microcrystalline cellulose with chemical properties that can optimize the use of traditional cellulose in some applications as paper production, cosmetics, medicines and membrane filtration.

Biomass as a renewable resource is known to be almost carbon neutral over the life cycle of its use. Algal CO_2 biofixation is one of the successful alternatives to eliminate CO_2 from the atmosphere. Microalgae can capture CO_2 from three diverse origins: CO_2 from soluble carbonates, CO_2 from the atmosphere, and CO_2 present in stack and discharge gases from industries. About 10.4% of world's total energy refers to biomass energy. On other hand, approximately 77.4% of the world's renewable energy comes from biomass energy sources.

II. Advantages of using algae

Algae is proving itself to be one of the most promising and long term source of food, animal feed, medicines, cosmetics other co-products and most importantly oil for fuel. They are found in a large number and have a wide variety of benefits associated with them which makes them so attractive. Algae have evolved over billions of years to produce and store energy in the form of oil. It is done more efficiently than any other process, be it natural or engineered.

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

a. Algae grow fast

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

b. Algae can have high biofuel yields

Algae store energy in the form of oils and carbohydrates, which, 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.

c. Algae Consume CO_2

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

d. 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 traditional agricultural processes.

e. Algae can purify wastewaters

Algae can be grown in nutrient-rich waters like municipal waste waters (sewage), animal wastes and some industrial effluents, while purifying these wastes at the same time and producing a biomass suitable for biofuel productions.

f. Algal biomass used as energy source

After oil extraction, the remaining algal biomass can be dried and "pelletized" and used as fuel that is burned in industrial boilers and other power generation sources.

g. Algae can be used to produces many useful products

Algae can be cultivated to produce a variety of products for large to small markets: plastics, chemical feedstocks, lubricants, fertilizers, and even cosmetics.

h. Biomass used as feed and food also

Algae in addition to fuel can be used as an animal feed and the biomass remaining can be also used as a food supplement.

i. Job creating Industry

Algae can be grown in a variety of ways using a variety of methods, which will create a large number of jobs ranging from research to engineering, construction to farming, from marketing to financial services.

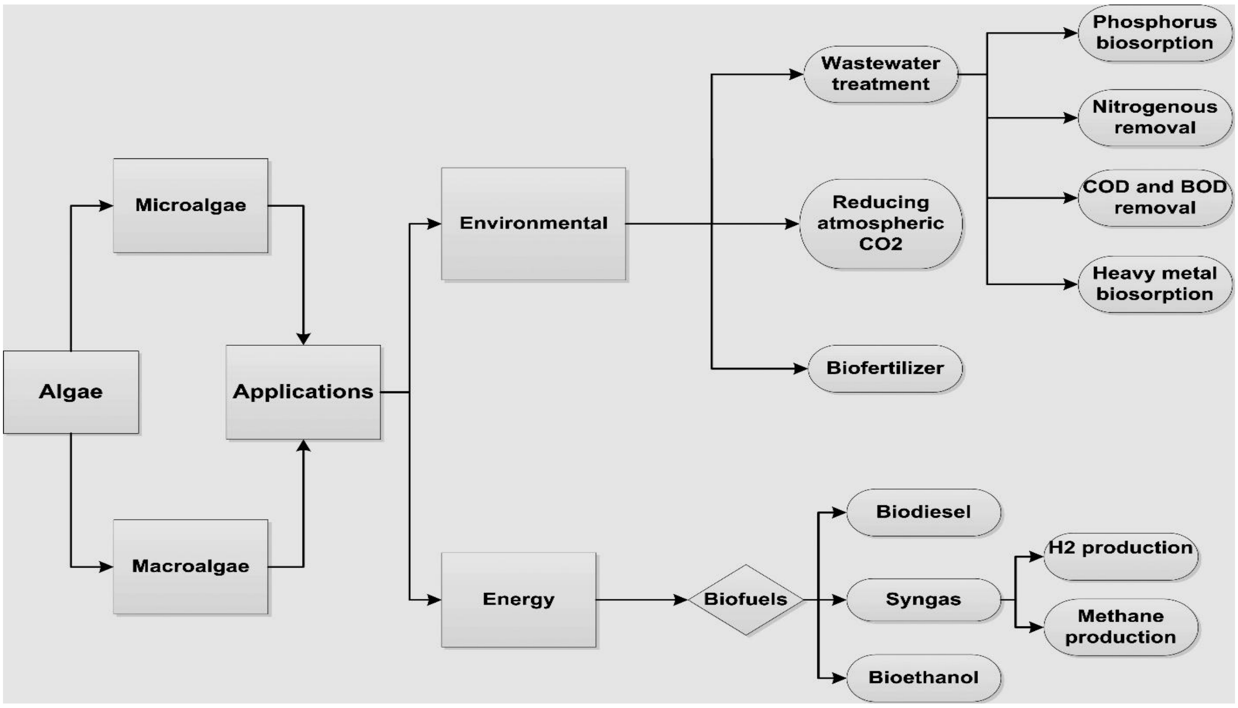
III. Disadvantages of using algae

a- Costly

b- Difficulty in finding cheap source of sterile CO_2.

c- Some species cause fish kills and contamination.

Industrial and Biotechnological Applications of Algae

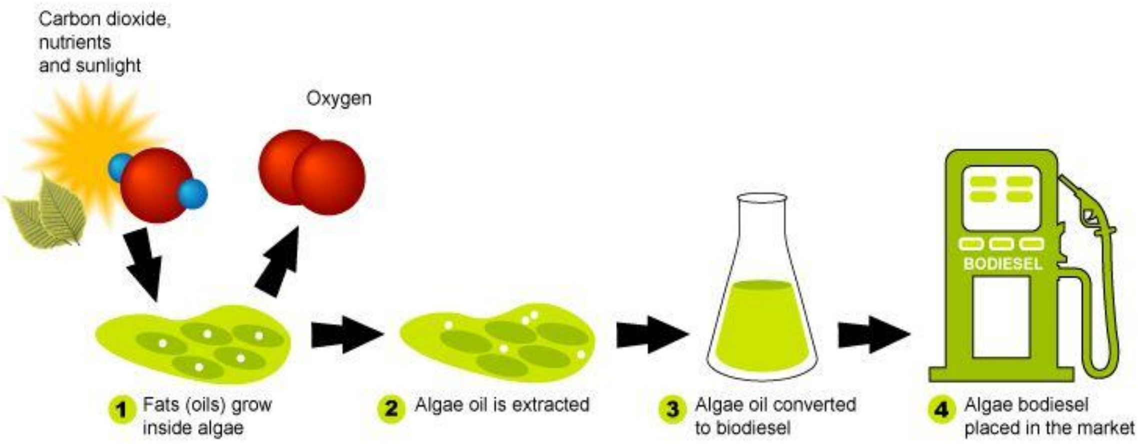


Algae are a class of photosynthetic organisms found in both marine and freshwaters habitats. As these organisms have a short doubling time, they are considered among fastest growing creatures. They have different pathways to fix atmospheric carbon dioxide and to efficiently utilize the nutrients to convert it into biomass. In few years, a focus has been shifted towards these organisms due to their food and fuel production capability. In fuel industry algae biofuels have been emerged as a clean, nature friendly, cost effective solution to other fuels. Algae fuels are categorized into **bio-ethanol, biogas, bio-hydrogen, biodiesel and bio-oil**. Algae as a food have been explored for different applications as in production of single cell proteins, pigments, bioactive substances, pharmaceuticals and cosmetics.

Algae have a potential to fix atmospheric carbon rich gases, due to which they act as quenchers of carbon dioxide and nitrogen oxides released from different sources. About 1 kg of algae biomass is capable to fix approximately 1.8 kg of carbon dioxide. Algae can use wastewater containing high amount of nitrogen and phosphorus for their growth with benefits of providing biofuels and also helping to get rid from excess nitrogen and phosphorus.

Algae fuel

Biofuel from algae- Is it a viable alternative?



Different Algae based Fuels:

In modern era, fossil fuel depletion and global warming has led to the world's eyes on production of bioenergy from algal biomass. Therefore the key plans to reduce poverty are increased access and energy security.

Biofuel is considered as a solution to substitute petroleum fuel and to reduce the carbon dioxide emissions contributing to global warming.

Currently, the only alternative to replace the fossil fuel consumption and dependency is the production of biofuels from algal biomass.

The most known biofuels used are biodiesel, bioethanol, and biohydrogen extracted from biomass. For a long time, these biofuels were produced from edible crops that compete with the food of humans and animals. Therefore, research is focused worldwide on alternative biomass sources. Scientists are looking for innovative biofuel feedstocks such as agricultural wastes, algae, and jatropha. The first generation of biodiesel mainly depended on vegetable oils for conversion to methyl esters and glycerol.

Biofuel is a type of fuel whose energy is derived from biological carbon fixation. Biofuels include fuels derived from biomass conversion, as well as solid biomass, liquid fuels and various biogases. Although fossil fuels have their origin in ancient carbon fixation, they are not considered biofuels by the generally accepted definition because they contain carbon that has been "out" of the carbon cycle for a very long time. Biofuels are gaining increased public and scientific attention, driven by factors such as oil price hikes, the need for increased energy security, concern over greenhouse gas emissions from fossil fuels, and support from government subsidies. Biofuel is considered carbon neutral, as the biomass absorbs roughly the same amount of carbon dioxide during growth, as when burnt.

The classification of biofuels is shown in Fig. 1. These classifications are:

a) Natural biofuels, **b)** Primary biofuels, and **c)** Secondary biofuels.

Natural biofuels are generally derived from organic sources and include **vegetable, animal waste and landfill gas**. On the other hand, primary biofuels are fuel-woods used mainly for **cooking**, **heating**, **brick kiln** or **electricity production**. The secondary biofuels are **bioethanol** and

biodiesel produced by processing biomass and are used in **transport sectors**. The secondary biofuels are sub classified into three so called generations, namely, **a**) First generation biofuels, **b**) Second generation biofuels, and **c)** Third generation biofuels based on their different features such types of processing technology, feedstock and or their development levels.

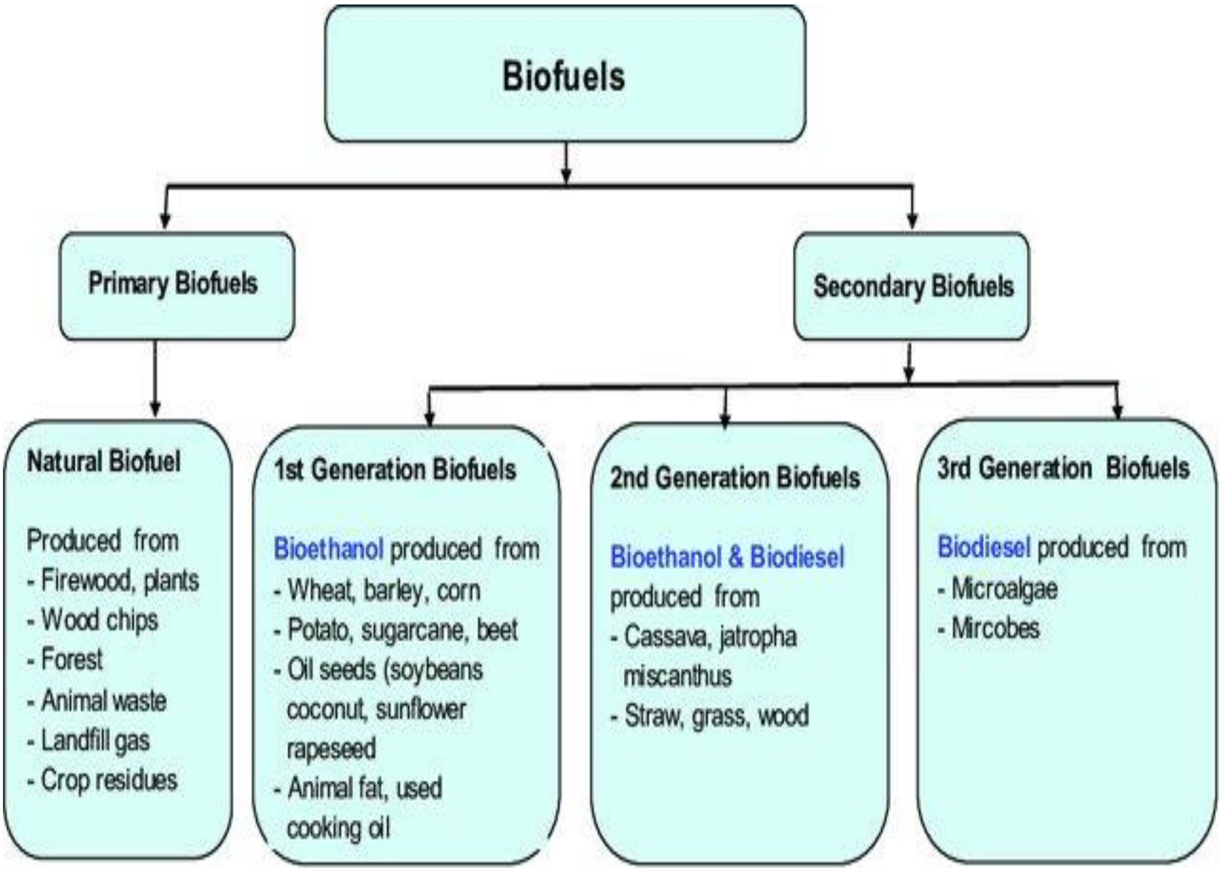


Fig.1: Biofuel production sources (biomasses)

Different sources of biofuel

Here are **4 biofuel sources**, with some of their application in developmental stages, some actually implemented:

1. Algae

Algae come from stagnant ponds in the natural world, and more recently in algae farms, which produce the plant for the specific purpose of creating biofuel. Advantage of algae focude on the followings: No CO_2 back into the air, self-generating biomass, Algae can produce up to 300 times more oil per acre than conventional crops. Among other uses, algae have been used experimentally as a new form of green jet fuel designed for commercial travel. At the moment, the upfront costs of producing biofuel from algae on a mass scale are in process, but are not yet commercially viable.

2. Carbohydrate (sugars) rich biomaterial

It comes from the fermentation of starches derived from agricultural products like corn, sugar cane, wheat, beets, and other existing food crops, or from inedible cellulose from the same.

Produced from existing crops, can be used in an existing gasoline engine, making it a logical transition from petroleum. It used in Auto industry, heating buildings ("flueless fireplaces")

At present, the transportation costs required to transport grains from harvesting to processing, and then out to vendors results in a very small net gain in the sustainability stakes.

3. Oils rich biomaterial

It comes from existing food crops like rapeseed (aka Canola), sunflower, corn, and others, after

it has been used for other purposes, i.e food preparation ("waste vegetable oil", or WVO), or even in first use form ("straight vegetable oil", or SVO). Not susceptible to microbial degradation, high availability, re-used material. It is used in the creation of biodiesel fuel for automobiles, home heating, and experimentally as a pure fuel itself. At present, WVO or SVO is not recognized as a mainstream fuel for automobiles. Also, WVO and SVO are susceptible to low temperatures, making them unusable in colder climates.

4. Agriculture wastes (organic and inorganic sources)

It comes from agricultural waste which is concentrated into charcoal-like biomass by heating it. Very little processing required, low-tech, naturally holds CO_2 rather than releasing it into the air. Primarily, biochar has been used as a means to enrich soil by keeping CO_2 in it, and not into the air. As fuel, the off-gasses have been used in home heating. There is controversy surrounding the amount of acreage it would take to make fuel production based on biochar viable on a meaningful scale. Furthermore, use of agriculture wastes which rich with inorganic elements (NPK----) as compost (fertilizer) in agriculture.

Growing Algae for Biodiesel

Algae require nutrients, light, water and a carbon source, most often CO2, for efficient growth. The major nutrients required by most algae include phosphorous, nitrogen, iron and sulfur. algae are very efficient at sequestering these nutrients when present in their environment.

Most of algal species are phototrophs and therefore require light for their growth. The phototropic microalgae are commonly grown in open ponds and photobioreactors. Open pond systems are shallow ponds in which algae are also cultivated. The open pond cultures are economically more favorable, but raise the issues of land cost, water availability, and appropriate climatic conditions. Photobioreactors are different types of tanks or closed systems in which algae are cultivated. It is possible to identify three primary ways to grow algae, and biodiesel manufacturers have worked hard to tweak these processes to customize and perfect the growing process.

Fig: Open raceway ponds for algal cultivation, A. Raceway pond with thick dividing section (Source-SARDI, South Australia), B. Open raceway pond (Source-Arban Infrastructure Pvt. Ltd., Biotech division), C. Laboratory scale high rate algal pond (SourceNew Mexico State University), D. Schematic of the horizontal pond or bioreactor (Source-Brown et al., 2015).

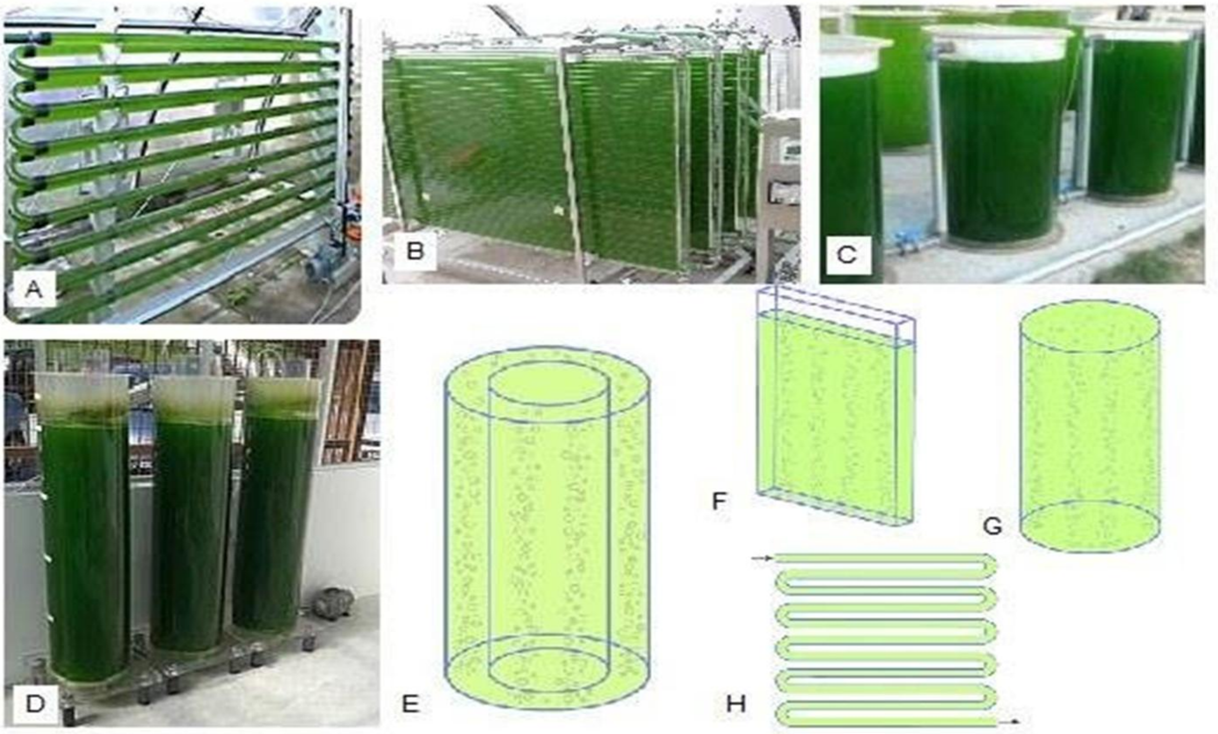


Fig 2. Showing different type of Photobioreactors for algal cultivation, A. Tubular (Source-Chempure Technologies Pvt. Ltd.), B. Plate (Source-IGV Biotech, Wikipedia), C. Column (Source-Oilgae), D. Annular (Source-Tan, Gallery), E-H. Diagrammatic representation of photobioreactors (Source-Hallmann, 2015), E. Annular, F. Plate, G. Column, H. Tubular.

Algal biofuel

Algae fuel, **algal biofuel**, or **algal oil** is an alternative to liquid fossil fuels that uses algae as its source of energy-rich oils. Also, algae fuels are an alternative to commonly known biofuel sources, such as corn and sugarcane. Several companies and government agencies are funding efforts to reduce capital and operating costs and make algae fuel production commercially viable. Like fossil fuel, algae fuel releases CO_2 when burnt, but unlike fossil fuel, algae fuel and other biofuels only release CO_2 recently removed from the atmosphere via photosynthesis as the algae or plant grew. In fuel industry algae biofuels have been emerged as a clean, nature friendly, cost effective solution to other fuels. Because of the high oil content and rapid biomass production, Algae has been recognised as a potentially good source of biofuel production.

Algae can be converted into various types of fuels, depending on the technique and the part of the cells used. The lipid, or oily part of the algae biomass can be extracted and converted into biodiesel through a process similar to that used for any other vegetable oil, or converted in a refinery into "drop-in" replacements for petroleum-based fuels. Alternatively or following lipid extraction, the carbohydrate content of algae can be fermented into bioethanol or butanol fuel.

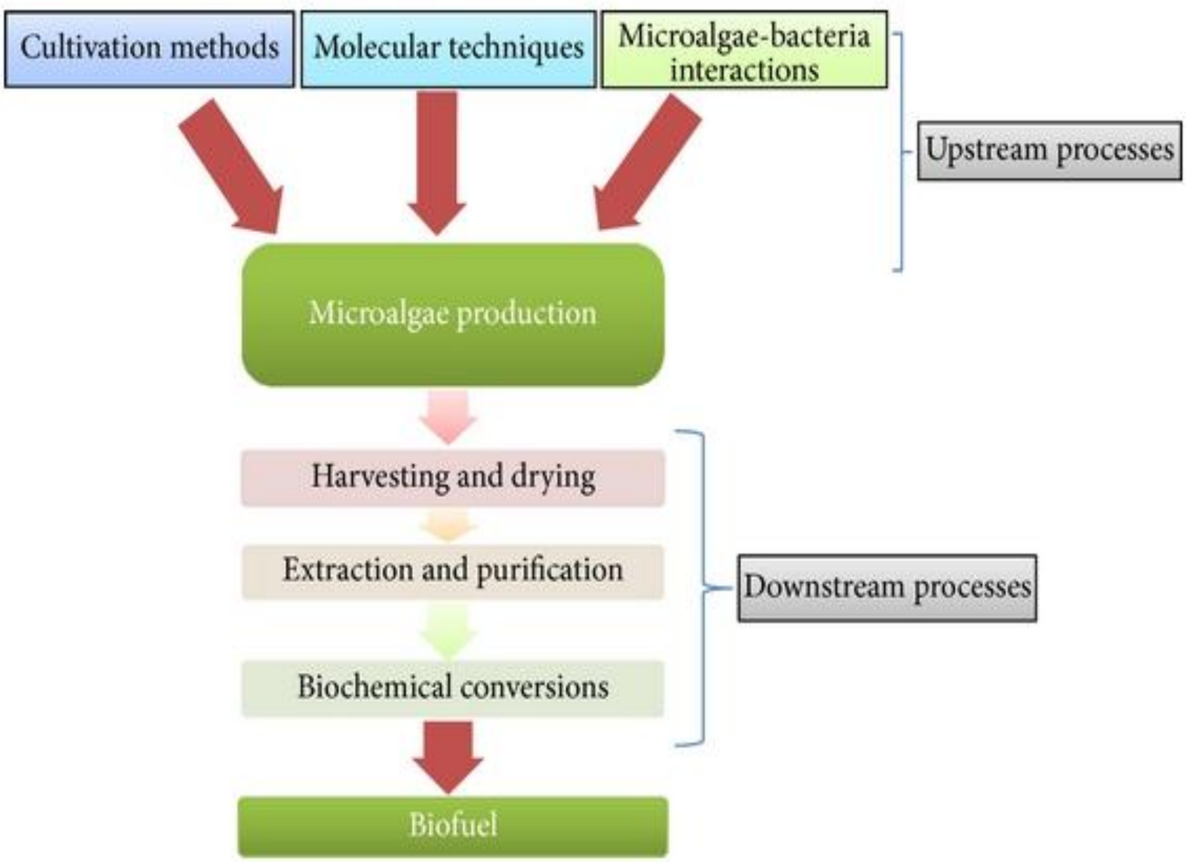


Fig.1: Different strategies involved in microalgae biomass and biofuel production.

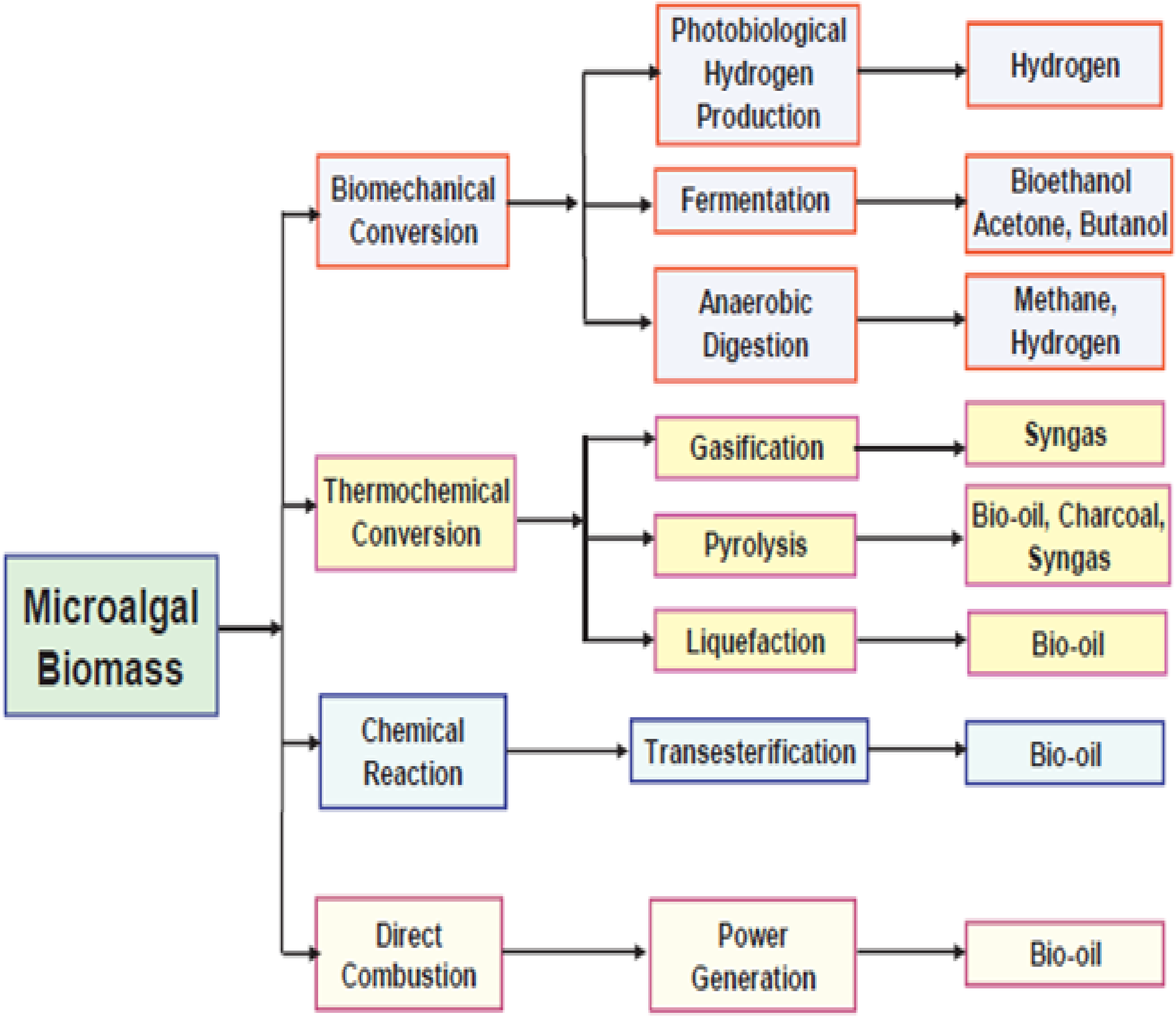


Fig2. Biofuel production processes from microalgae biomass.

Several species of microalgae can have oil contents up to 80% of their dry body weight. As mentioned earlier, some microalgae can double their biomasses within 24 hours and the shortest doubling time during their growth is around 3.5 hours which makes microalgae an ideal renewable source for biofuel production.

Table . Oil contents of microalgae grown in fresh water.

Name of microalgae		(% dry wt)
Fresh Water Algae	*Botryococcus braunii*	25 -75
	Chlorella sp.	28 -32
	Chaetoceros muelleri	34
	Nitzschia sp.	45- 47
	Botryococcus sp.	25 - 75
	Chlorella vulgaris	5 - 58
	Chlorella pyrenoidosa	2
	Scenedesmus obliquus	11 - 55
	Scenedesmus quadricauda	2 - 19
	Scenedesmus sp.	20 - 21

Table . Oil contents of microalgae grown in marine (salt) water.

Name of microalgae		(% dry wt)
Marine Water Algae	*Dunaliella salina*	6 - 25
	Dunaliella primolecta	23
	Dunaliella tertiolecta	18 -71
	Spirulina platensis	4- 17
	Nannochloropsis oculata	23- 30
	Nannochloropsis sp.	12 -53
	Pavlova salina	31
	Pavlova lutheri	36

Biofuels Production Processes from Microalgae

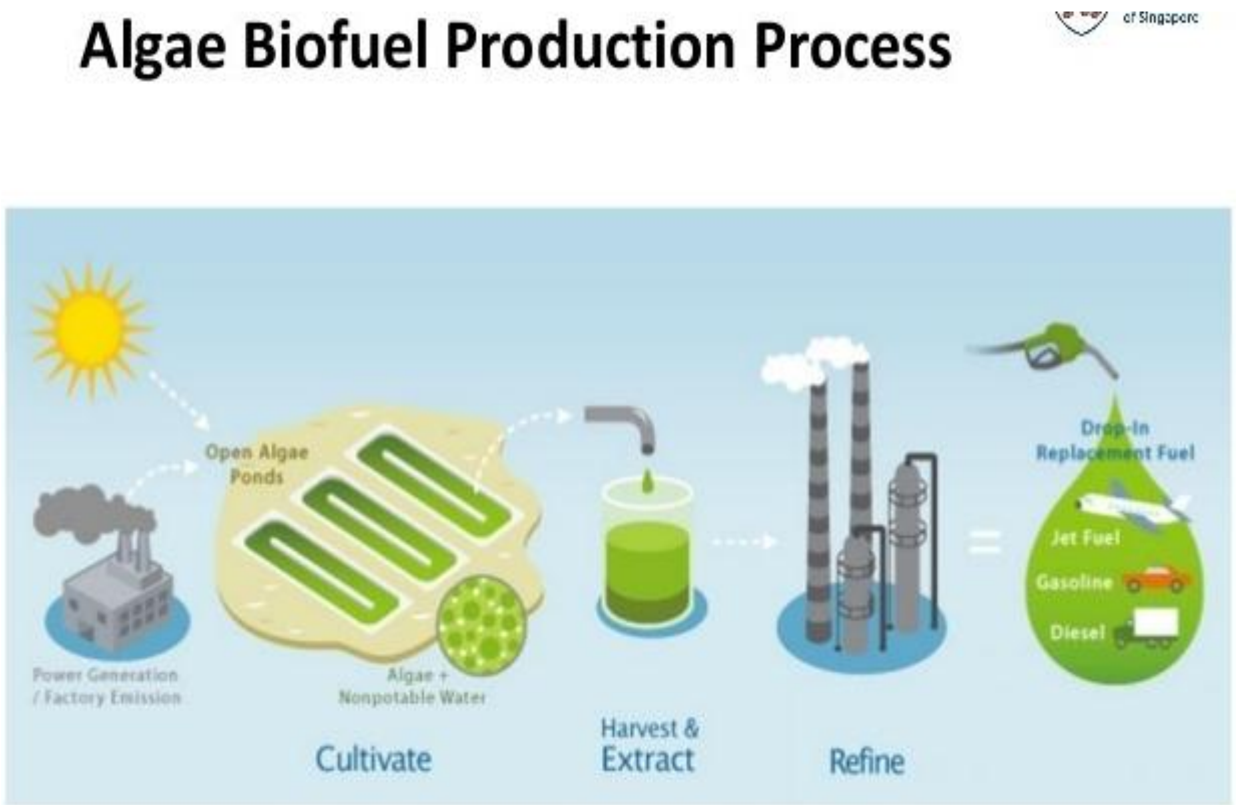
Algae have oil contents with different compositions depending on the specie types. Some species were identified that they have good fatty acid values. In the same way, some algae have more components of fatty acids by their dry masses. Micro algae can grow in different conditions even in availability of fewer nutrients. They are best to be chosen for cultivation.

The collection of sample needs care so that the whole biofuel contents could be obtained through careful handling of the instruments. The growth is also affected by different environmental factors which are not specifically known for every region, so the process needs careful attention accordingly. The simple method of fatty acids extraction and separation of biodiesel is the blending method on small or experimental scale. This process consists of several steps which have been shown in Figure 1. The production of microalgae biomass for extraction of biofuels is generally more expensive and technologically challenging than growing crops.

Photosynthetic growth of microalgae requires light, CO_2, water and inorganic salts. The temperature regime needs to be controlled strictly. For most microalgae growth, the temperature generally remains within 20°C to 30°C. In order to reduce the cost, the biodiesel production must rely on freely available sunlight, despite daily and seasonal variations in natural light level. A number of ways the microalgae biomass can be converted into energy sources which includes: a) biochemical conversion, b) chemical reaction, c) direct combustion, and d) thermochemical conversion (Fig.2). As mentioned previously, microalgae provide significant advantages over plants and seeds as they: i) synthesize and accumulate large quantities of neutral lipids (20 50 % dry weight of biomass) and grow at high rates; ii) are capable of all year round production, therefore, oil yield per area of microalgae cultures could greatly exceed the yield of best oilseed crops; iii) need less water than terrestrial crops therefore reducing the load on freshwater sources; iv) cultivation does not require herbicides or pesticides application; v) sequester CO_2 from flue gases emitted from fossil fuel-fired power plants and other sources, thereby reducing emission of greenhouse gas (1 kg of dry algal biomass utilise about 1.83 kg of CO_2).

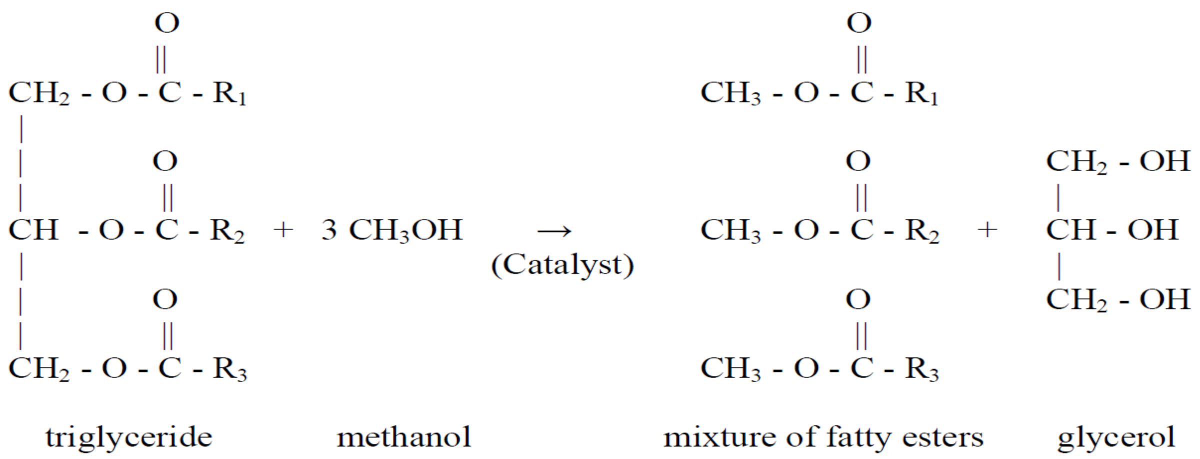
There are different ways microalgae can be cultivated. However, two widely used cultivation systems are the open air system and photobioreactor system. The photoreactor system can be sub-classified as a) tabular photoreactor, b) flat photoreactor, and c) column photoreactor. Each system has relative advantages and disadvantages. More details about these cultivation systems can be found in.

The production of biofuel is a complex process. The process consists of following stages: a) stage 1 - microalgae cultivation, b) stage 2 harvesting, drying & cell disruption (cells separation from the growth medium), c) stage3- lipid extraction for biodiesel production through transesterification and d) stage 4 starch hydrolysis, fermentation & distillation for bioethanol production. However, these processes are complex, technologically challenges and economically expensive. A significant challenge lies ahead for devising a viable biofuel production process.



Transesterification to biodiesel

The transesterification process is a reversible reaction and carried out by mixing the reactants – fatty acids, alcohol and catalyst. A strong base or a strong acid can be used as a catalyst. At the industrial scale, mostly sodium or potassium methanolate is used. The end products of the transesterification process are raw biodiesel and raw glycerol. In a further process these raw products undergo a cleaning step. In case of using methanol as alcohol FAME (fatty acid methyl ester) biodiesel is produced. The purified glycerol can be used in the food and cosmetic industries, as well as in the oleochemical industry. The glycerol can also be used as a substrate for anaerobic digestion.



Reaction equation of the transesterification process

Algae fuels are categorized into bio-ethanol, biogas, bio-hydrogen, biodiesel and bio-oil. There are a no. of methods for the production of biofuel using algae.

Biodiesel

Biodiesel as one from important biofuel types is made from vegetable oils and animal fats. Biodiesel can be used as a fuel for vehicles in its pure form, but it is usually used as a diesel additive to reduce levels of particulates, carbon monoxide, and hydrocarbons from diesel powered vehicles. Biodiesel is produced from oils or fats using transesterification and is the most common biofuel in Europe.

Studies have shown that some species of algae can produce 60% or more of their dry weight in the form of oil. Because the cells grow in aqueous suspension, where they have more efficient access to water, CO_2 and dissolved nutrients, microalgae are capable of producing large amounts of biomass and usable oil in either high rate algal ponds or photobioreactors. This oil can then be turned into biodiesel which could be sold for use in automobiles. Regional production of microalgae and processing into biofuels will provide economic benefits to rural communities.

Microalgae can have faster growth rates than terrestrial crops. Also, they can convert a much higher fraction of their biomass to oil than conventional crops, e.g. 60% versus 2-3% for soybeans. The per unit area yield of oil from algae is estimated to be from 58,700 to 136,900 L/ha/year, depending on lipid content, which is 10 to 23 times as high as the next highest yielding crop, oil palm, at 5,950 L/ha/yea.

Algae can potentially contain over 80% total lipids. However, if the production is under normal conditions then the lipid concentration is low (less than 40%) and high oil content is always associated with very low yields. Under stress conditions like insufficient nitrogen availability, the production of various lipids can be stimulated. But due to this the non-lipid part of the biomass is reduced which can be used as a source for other co-products.

Steps to produce Biodiesel

1. Microalgae cells are specially grown and selected for their oil content. They contain oil inside their cells.
2. The oil is taken out of the microalgae by the use of chemicals. The oil may also be squeezed out of the microalgae cells.
3. The oil is then changed chemically so it can be used as biodiesel.

Advantages of biodiesel

Biodiesel has several advantages as compared with other fuel sources. Since the fuel is made from biomass,

- It does not contribute to CO_2 emissions into the atmosphere.
- Biodiesel produces fewer emissions than petroleum diesel.
- When biodiesel is used instead of petroleum diesel, emissions of soot, sulphur, unburned hydrocarbons, and polycyclic aromatic hydrocarbons are significantly reduced. The infrastructure required for biodiesel production is already in place.
- Biodiesel can be blended with petroleum diesel up to 20% in existing diesel engines or run unblended in engines with simple changes.
- Biodiesel's lubricating qualities can extend engine life because it has twice the viscosity of petroleum diesel.
- Biodiesel has low toxicity and is biodegradable.
- Biodiesel has a cleaner burn and complete combustion.

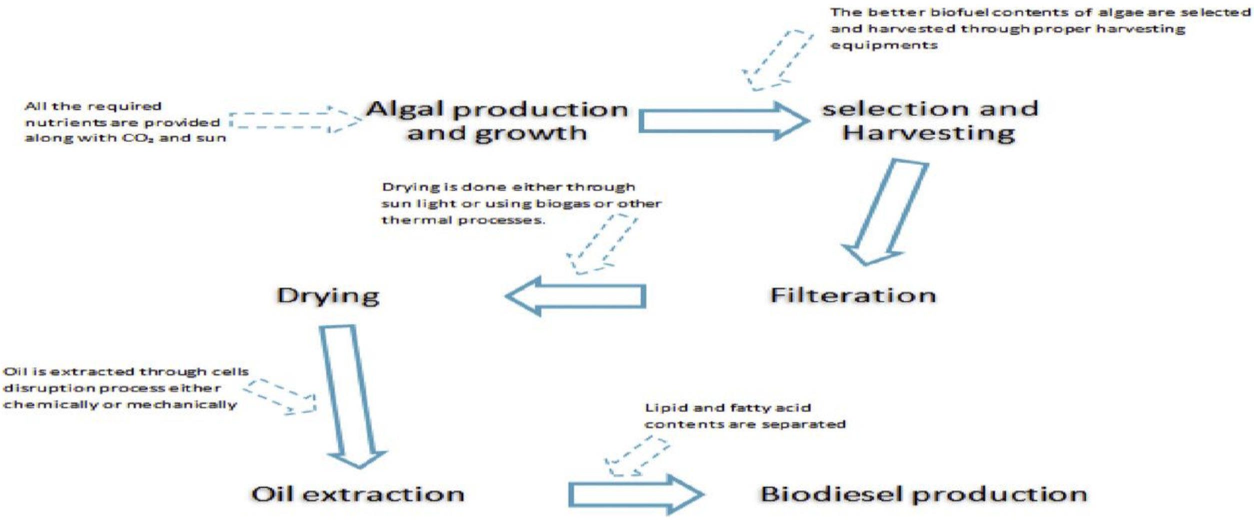


Figure. This figure illustrates the general process of biodiesel production from algae on small scale and for experimental purpose. This method could be used to compare different algal species for the oil contents. Dotted arrow indicates the addition to specific steps which have been highlighted with bold letters and full lined arrows.

Bioethanol

Bioethanol is an alternative energy source for fossil fuels. Bioethanol is an alcohol made by fermentation, mostly from carbohydrates produced in sugar or starch crops such as corn or sugarcane. Cellulosic biomass, derived from non-food sources such as trees and grasses, is also being developed as a feedstock for ethanol production. Ethanol can be used as a fuel for vehicles in its pure form, but it is usually used as a gasoline additive to increase octane and improve vehicle emissions. Bioethanol is widely used in the USA and in Brazil.

The production of bioethanol from macroalgae has been proposed as a sustainable, renewable, and environmentally friendly choice of biofuel.

Macroalgae can be considered a substrate for sustainable production of bioethanol as it does not compete with food crops, for cultivated land and fresh water. Algal bioethanol is produced from biological conversion of biomass by fermentation. The fermentation process can be conducted in presence or absence of oxygen. Algal biomass needs additional pretreatment to start fermentation.

The carbohydrates present in macroalgae can be converted to bioethanol with appropriate enzymes, bacteria, or yeast. Bioethanol produced in studies using different species of macroalgae and different options for **pretreatment**, **hydrolysis**, and **fermentation**.

Pretreatment of Macroalgae

The cell wall of macroalgae is composed of external and internal layers, also known as primary and secondary layers, respectively. Pretreatment of macroalgae is a vital step to breakdown the outer layer of the cell wall for releasing biomolecules such as cellulose and other sugar polymers (e.g., galactan and glucan), so they can be converted to bioethanol. The internal or secondary cell wall is made up of pectin, hemicellulose, and sulphated sugar polymers, while external or primary cell wall comprises more or less rigid structures. Algal cell walls consist of cellulose which is derived from b-D-glucopyranose units with crystalline structure. This crystalline cell wall structure needs to be disrupted by a pretreatment and further enzymatic hydrolysis of cellulose is applied to form simple monomeric sugars.

The range of pretreatments includes physical, chemical, biological, or thermal methods. The efficiency of pretreatment processes can be influenced by three factors: hemicellulose fraction, cellulose crystallinity, and the surface area available for enzymes to hydrolyse.

Mechanical Pretreatment

Mechanical pretreatment consists of three steps, washing, drying, and milling. After collection, macroalgae is washed to remove salts, sand, dust, and adhering and epiphyte material. It is then dried in sunlight or a hot oven to remove the moisture content. The moisture content of the biomass is a vital parameter in bioprocessing. Finally, the dry macroalgae are milled into granules and sieved for desired particle size. Smaller particles are the most efficient for bioethanol production due to higher surface area.

Chemical Pretreatment

Chemical pretreatment includes different steps such as depolymerization of cellulose, solvation of hemicellulose, and structure modification. Diluted acid or alkali pretreatment with enzyme hydrolysis is an effective pretreatment approach for bioethanol production. The cellulose and hemicellulose content of macroalgae varies between 46 and 86% by dry weight, and can be converted into simpler sugars by chemical pretreatment to improve saccharification efficiency during enzyme hydrolysis. The hydroxyl derivatives of potassium and sodium salts are commonly used for alkali pretreatment. The disadvantage of using alkali pretreatment is the wastage of water for desalting. Diluted sulfuric acid is common for pretreatment for all macroalgae types.

Enzymatic Pretreatment

Enzymatic pretreatment is performed with enzymes such as cellulase, agarase, and amylase to hydrolyse complex macroalgae polysaccharides into reducing sugars. There are several factors affecting pretreatment processes. These include pretreatment time, temperature, pH, and concentrations of enzyme and substrate.

Thermal Pretreatment

Thermal pretreatment is effective for improving extraction of polysaccharides and release of sugar from macroalgae. This pretreatment may result in increased bioethanol yield by increasing eroded surfaces and exposing fibers of macroalgae, which facilitates enzymatic degradation. However, structure of the macroalgae cortex is not affected by thermal pretreatment. Therefore, an appropriate pretreatment method is needed to hydrolyse these components effectively.

Hydrolysis of Macroalgae

Hydrolysis is the process that degrades complex carbohydrates (i.e., mannitol, agar, laminarin, carrageenan, cellulose, ulvan, and alginate) into the simplest forms of sugar (i.e., xylose, glucose, arabinose, galactose, fucose, and mannose), suitable for fermentation to produce ethanol. Prior to hydrolysis, different pretreatment methods are applied as discussed above.

Acid Hydrolysis

Acid hydrolysis is most commonly used method for bioethanol production from macroalgae, due to the shorter reaction time compared to other methods. Hydrochloric and sulfuric acids are commonly used for macroalgae hydrolysis. However, a major limitation of using acids is the formation of by-products that inhibit fermentation (i.e., 5-HMF, levulinic acid, and furfural). The inhibitors are produced due to dehydration of pentose and hexose, caused by long reaction time and low pH.

These inhibitors affect fermenting organisms by damaging DNA and blocking protein and RNA synthesis, thus decreasing the fermentation of reducing sugars. Filtering hydrolysate with activated carbon before fermentation is an effective method to remove inhibitors.

Filtering hydrolysate through activated carbon reduced the concentration of 5-HMF from 30 to only 5 g/L. Acid hydrolysis is comparatively easy and commonly used compared to the other hydrolysis methods, as the concentration of acid used is very low 0.01–0.90 M. A neutralization step is required before fermentation, to provide suitable conditions for yeast, this is commonly achieved with a similarly low concentration of a base.

Alkaline Hydrolysis

Alkaline hydrolysis is the opposite of acid hydrolysis. In this process, a base is used as a catalyst instead of an acid. Hydroxide ions in bases react with hydrocolloids in macroalgae to form gels, which are too viscous

Enzymatic Hydrolysis

The conversion of polysaccharides into reducing sugars through enzymatic hydrolysis is an effective and efficient process that forms fewer toxic compounds than other methods. Cellulases are the most commonly used enzymes for hydrolysis; they are naturally secreted by fungi (such as Penicillium, Schizophyllum, Trichoderma, and Fusarium) and cellulolytic bacterial species (such as Thermomonospora, Streptomyces, , Clostridium, Microbispora, Bacillus, and Bacteroides).Three types of cellulases have been mostly studied for enzymatic hydrolysis of bioethanol feedstock: β-glucosidases, endoglucanases, and exoglucanases. Each cellulase type performs a unique action on a substrate during saccharification.

Fermentation of Macroalgae

The reducing sugars produced by hydrolysis can be converted to ethanol by fermenting microorganisms. Ethanol is the major product of fermentation with some by-products, i.e., water and carbon dioxide. The organism used for fermentation is the major factor affecting ethanol production. Many yeast, bacteria, and fungi have been studied for fermentation. *Saccharomyces cerevisiae* (yeast) has been commonly used in different studies because of its high ethanol yield, fermentation rate, and high selectivity and producing few by-products.

There are two ways to produce sugar by hydrolysis enroute to ethanol production. The Embden-Meyerhof and Leloir pathways are used to produce ethanol from glucose and galactose. The Embden-Meyerhof pathway involves glucose in its two main stages. First, conversion of the reducing sugars to a common intermediate (glucose-6-phosphate), while the second stage involves converting the intermediate into pyruvate. The end-products of this pathway are microorganism-dependent. If yeast is used, it converts pyruvate to ethanol and carbon dioxide by alcoholic fermentation. On the other hand, the Leloir pathway comprises conversion of galactose-1-phosphate to glucose-1-phosphate followed by glucose-6-phosphate. This pathway is more complicated for galactose metabolism compared to glycolysis, which results in slow degradation of galactose compared to glucose during fermentation. It reduces ethanol yield while fermenting mixed sugar hydrolysate with *S. cerevisiae*.

Purification

Bioethanol can be separated from the fermentation broth by techniques such as extractive distillation, heterogeneous distillation, pressure-swing distillation, reactive distillation, adsorption, and membrane separation. Distillation is used to separate the component of a mixture with respect to their volatility and consumes significant amount of energy. A distillation unit commonly comprises of an energy source (usually steam), feed (ethanol), and a condenser, and produces two output streams: overhead and bottom product. Ethanol is in the top product, components with a high volatility, vapor-rich stream while the bottom product comprises components with less volatility in a liquid-rich stream.

Steps to produce Bioethanol

1. Macroalgae is chosen because it has a high sugar content .
2. The macroalgae is cut up and treated. It appears like sludge and the substance is now called feedstock.

3. Micro-organisms called yeast are added to the mixture in a big tank. The feedstock is changed by the yeast to ethanol and other components. This process is called fermentation.
4. The ethanol is seperated from the other components and filtered. This can now be used with petrol.

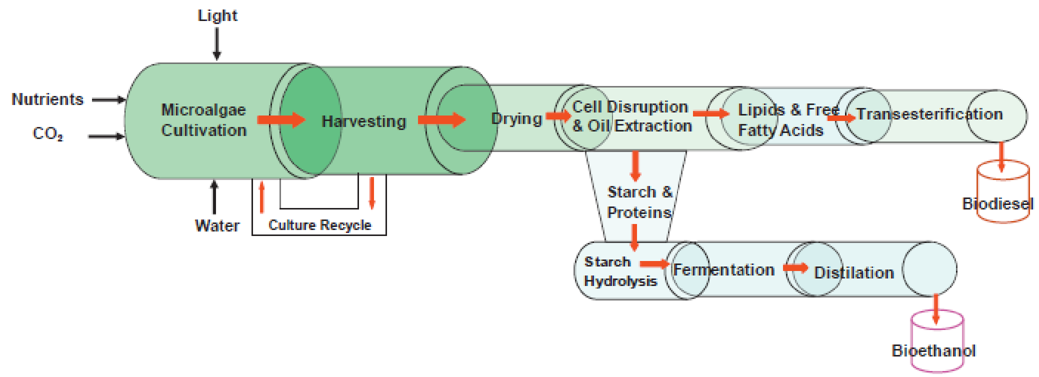


Fig. Biodiesel and Bioethanol production processes from microalgae.

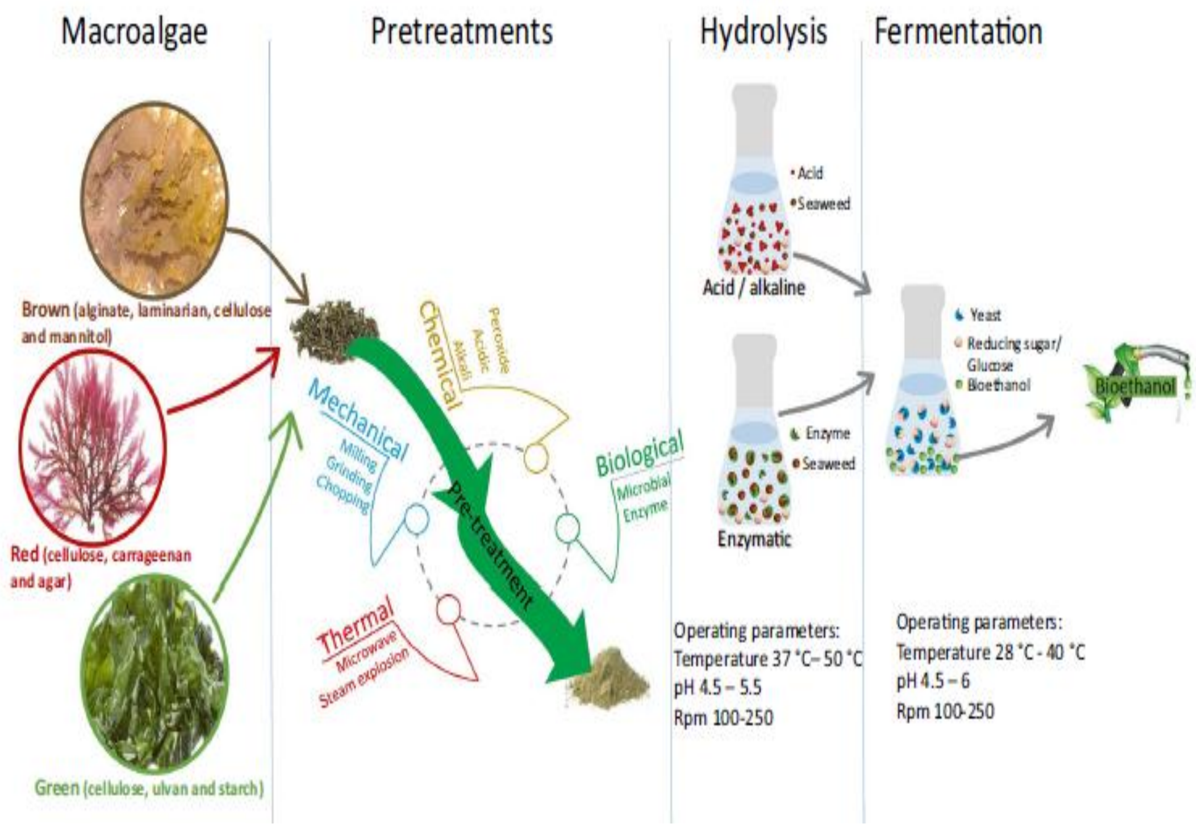


Fig. Steps involved in bioethanol production from macroalgae

Biobutanol

Butanol can be made from algae or diatoms using only a solar powered biorefinery. This fuel has an energy density 10% less than gasoline, and greater than that of either ethanol or methanol. In most gasoline engines, butanol can be used in place of gasoline with no modifications. In several tests, butanol consumption is similar to that of gasoline, and when blended with gasoline, provides better performance and corrosion resistance than that of ethanol or E85.

The green waste left over from the algae oil extraction can be used to produce butanol. In addition, it has been shown that macroalgae (seaweeds) can be fermented by *Clostridia*genus bacteria to butanol and other solvents.

Biogas

When wet biomass undergoes anaerobic digestion than the organic matter is converted to biogas containing 60-70% biomethane and rest CO_2. It has many advantages like, CO_2 produced can be fed back to algae, no need of drying as the process uses wet biomass, nutrients present in digested biomass can be recovered from liquid and solid phase.

Steps to produce Biogas

1. Biogas can be made using macroalgae. The algae chosen must contain a high amount of sugars.
2. The macroalgae is then washed and cut up. Micro-organisms help to decompose the macroalgae. They help to break down the sugars. This makes it easier to produce biogas. The substance is now called feedstock.
3. The next step is called aerobic digestion. It takes place in big tanks. Special micro-organisms are added. They can breakdown the macroalgae at

this stage without oxygen. They need their environment to be warm, acidic and free from oxygen (air). The mixture undergoes a series of reactions thanks to the special micro-organisms called anaerobes. Methane gas is produced at the end of this process.

4. Methane gas is the main ingredient of biogas which can be used as fuel.

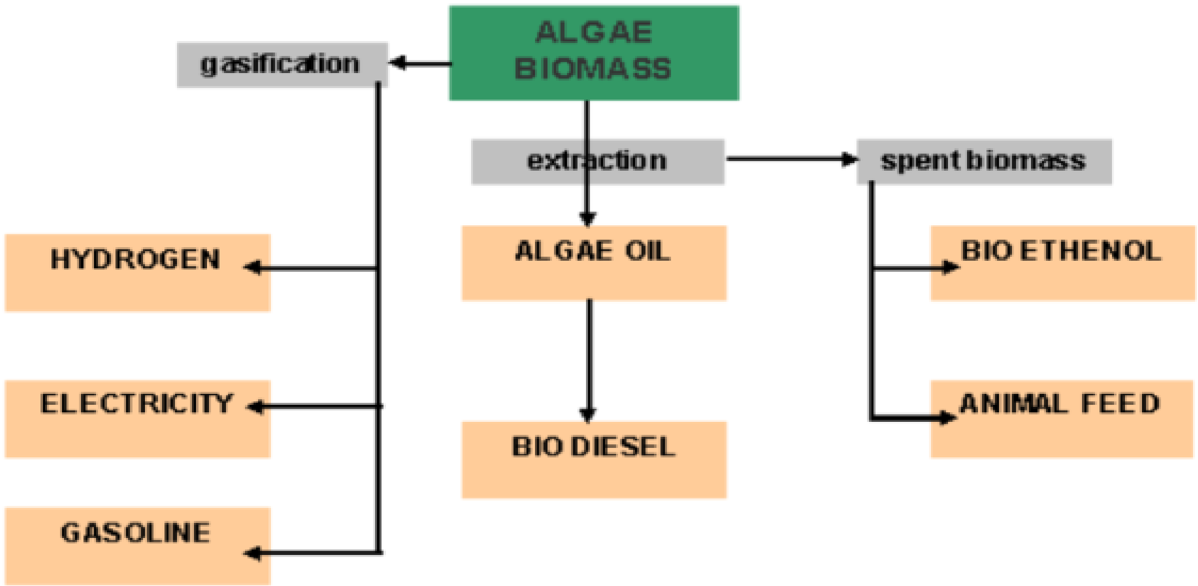
Syngas

Syngas is generated during the biomass gasification process.The gasification process is capable of converting biomass into gaseous mixtures which can be employed to produce hydrogen. Hydrogen as renewable energy is considered a possible source of energy to be used as a fuel feedstock. Syngas or synthesis gas is defined as fuel gas mixture. It mainly consists of hydrogen, carbon monoxide, and carbon dioxide. The name comes from synthetic natural gas (SNG), in addition to methanol or ammonia production. Syngas is mainly used in synthol processes for methanol and ethanol fuel production and generation of electricity. As a fuel, it is combustible and regularly used in internal combustion engines. Gasification is a chemical process which converts carbonaceous substances to a combustible mixture of gas in the presence of oxygen or air at 800–1000°C. Syngas can be produced by catalytic or non-catalytic gasification reaction of biomass with steam, oxygen, or air.

Biohydrogen

Hydrogen gas is produced by some algae by manipulating it. But the yield is low as the cells loses energy during formation of hydrogen, due to which less biomass is produced and hence lesser co-production.

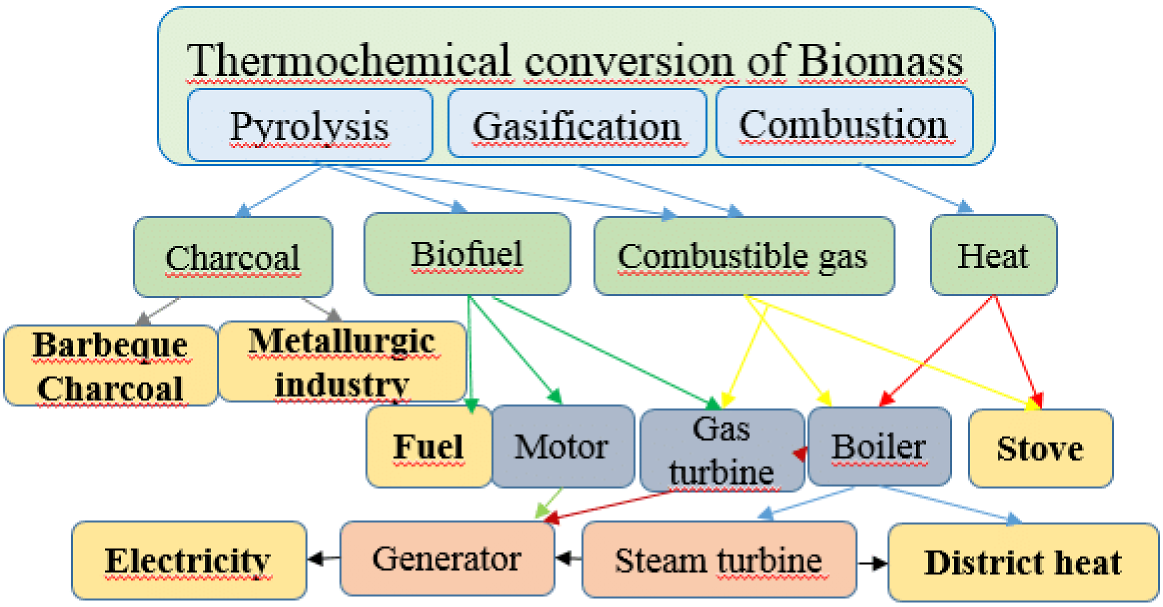
In current years, a much attention has been paid towards the bio-hydrogen production. But still, production of bio-hydrogen on large scale is not feasible due to **costly process** and **low biomass concentration**. In some studies it has been reported that exposing some algae species to various environmental stress like **depriving light in algae**, could trigger appreciable amount of hydrogen gas. But this technology is still in its beginning stage and process can be developed or improved till higher level . The three pathways suggested for the production of hydrogen are: 1. **Direct photolysis** 2. **Indirect photolysis** 3**. ATP driven pathway**. **Direct** photolysis is made feasible only when produced hydrogen and oxygen are continuously removed. In this process, photosynthesis and water splitting are linked, leading to production of hydrogen and oxygen together, which results in bigger security risks, also increasing the cost for separation of hydrogen and oxygen. Furthermore, the hydrogenase enzyme used in process are very oxygen sensitize. Due to these reasons, **indirect** processes are favored mostly. Under anaerobic and sulfur limited environment, starch contained in cell walls of algae converted into hydrogen up to some extent. In most of the studies it has been found that cyanobacteria are main producers of biohydrogen through biological approach, hydrogenase and nitrogenase enzymes act as catalyst in this process.



Thermochemical Treatment

Under very high pressure and temperature, biomass undergoes a chemical conversion. It ends up in a raw gaseous, liquid or solid phase, depending on the content of water and the extreme conditions applied, which can be upgraded to use as a biofuel. But the energy intake in this process is high in comparison to the production of biogas.

Thermochemical conversion aims at converting the entire biomass into gases. Algal biomass, either whole algal cells or extraction residues, are suitable feedstock for thermochemical conversion such as gasification, pyrolysis, and hydrothermal liquefaction and gasification to produce syngas, bio-oil, biopolyols, and biochar.



Bio-Oil

Bio-oils are produced by a process called thermo-chemical conversion which in absence of oxygen converts biomass into oil along with char and gas at very high temperature. Bio-oils are quite similar to petroleum oils due to which they can be used as a substitute. The bio-oil formation process is categorized into **two steps**: pyrolysis and thermo-chemical liquefaction. Pyrolysis is performed at a very high temperature (350-530°C) for production of a liquid, a gaseous and solid part. The liquid part is made up of an aqueous and a non-aqueous phase called bio-oil or tar and biomass is dried. During thermo-chemical liquefaction, wet biomass is hold upon lower temperature and high pressure of about 300°C and 10 MPa respectively.

The bio-oil contains various organic compounds accumulated as lipids, proteins, carbohydrates in algae and as compare to lipids present in algae, and amount of yield is high. A number of microalgae have been investigated to produce bio-oil via pyrolysis or thermal liquefaction for example: formation of different hydrocarbons by pyrolysis of *Dunaliella* sp. biomass. Bio-oil yields from microalgae have been reported up to 41% for *Spirulina* , about 24%–45% for microalgae *Scenedesmus* , about 37% for *Dunaliella* and up to 49% for *Desmodesmus* . Bio-oil yield from macroalgal biomass has been reported up to 23% after liquefaction process while in macrolagal biomass *Laminaria* saccharina it accounts for 63% energy restoration and *Laminaria* saccharina yielded 79% oil after hydrothermal liquefaction, Whereas freshwater macroalgae, Oedogonium and Cladophora yielded only 26% and 20%, respectively.

Advantages of Algae Biofuel

1. It uses large volumes of carbon dioxide. One significant benefit of using algae biofuel is that it will consume carbon dioxide in a massive scale, which means that we will be reducing a main contributor to the problems of climate change. Also, it does not have some negative effects on the global food prices and supply, as it does not require us to take away pieces of land that are currently used in cultivating food crops.

2. Its basic source can reproduce fast.

Naturally, algae grow fast, where the only need sunlight, water and carbon dioxide to thrive. Also, they can be produced using waste and sea water, while not affecting the water sources. Moreover, they are biodegradable and generally has “almost zero” negative impact on the environment, when biomass is being produced from them.

2. It has the potential to produce high energy content.

Biomass production from algae can generate high energy content. According to research, algae have the capability to produce more than 30 times of energy per unit area than other first and second-generation biofuel crops. In fact, the US Department of Energy claimed that algae have the potential to yield 100 times more oil per acre than terrestrial oil-producing crops, such as soybeans.

4-Biofuels reduce air pollution.

5- Algae growth helps us to curb our greenhouse gas emissions.

Greenhouse gas emissions from our transportation needs can make up the majority of the carbon dioxide, methane, and other pollutants that we release each day. When we start using sustainable biomass to create fuels instead of natural gas or oil, then the photosynthesis process of the plants converts the CO2 into oxygen for our atmosphere to enjoy. Algae farms give us the potential to create a neutral environment where the growth cycle absorbs the gases that we create when meeting our everyday needs

Disadvantages of Algae Biofuel

1. Its overhead cost is high.

At present, algae biofuel is still largely in an experimental state, where making investments in them is still very costly, while offering only very little monetary recompense. While its overhead is very expensive, its production facilities require huge amounts of land.

2. It can deplete non-renewable resources.

By producing algae biofuel, we are basically depleting non-renewable resources. For example, a component of harvesting algae is the already scarce and non-renewable element, phosphorus. And if we do not find an alternative to this element in producing such a fuel, the resource may become even more scarce, which can lead to increase in prices.

3. It lacks competition with other sources of fuel.

It is important to note that algae biofuel properties might not be competitive with other sources of fuel. Basically, such a fuel’s composition is substantially different than standard fuels on the market today. As of yet, algae biofuel is still a technology that has been able to provide solid evidence that it can compare to the efficiency of the fuels that are currently used. However, by knowing its essential advantages and disadvantages, we will be able to have a good opinion on what it can do for the world we live today.

Table. Algae strains for various biofuel productions.

Strains Algae	color	Biofuels
Microalgae		
Phormidium Autumnale	Green	Biodiesel
Nannochloropsis	Green	Biodiesel
Phormidium	Green	Biodiesel
Porphyridium cruentum	Red	Bioethanol
Spirogyra sp	Green	Bioethanol
Chlorella vulgaris	Green	Biodiesel
Chlorella sp	Green	Biodiesel/bioethanol
Scenedesmus almeriensis	Green	H2 production
Chlamydomonas reinhardtii	Green	H2 Production/biodiesel
Macroalgae		
Ulva sp.	Green	Methane
Laminaria sp.	Brown	Bioethanol
Gelidium amansii	Red	Bioethanol
Saccharina sp.	Brown	Bioethanol
Kappaphycus alvarezii	Red	Bioethanol
Ulva pertusa	Green	Biodiesel
Alaria crassifolia	Brown	Bioethanol
Enteromorpha sp.	Green	Biodiesel
Gelidium elegans	Red	Bioethanol
Caulerpa peltata	Green	Biodiesel

Bioremediation

Remediate" means to solve a problem, and "bio-remediate" means to use biological organisms to solve an environmental problem such as contaminated soil or groundwater.

Bioremediation is applied to eliminate various contaminants, such as organic, inorganic or other pollutants from the environment.

Bioremediation is a friendly approach for remediation of contaminated water and soil. Biological treatment through use of mixed microbial culture such as bacteria, fungi and algae for the bioremediation of pollutants.

Bioremediation is using of microorganisms and its enzymes to protect the environment from severe pollution. Bioremediation may be employed in order to eliminate specific contaminants, such as chlorinated pesticides or other pollutants from the environment. Microorganisms degrade the different pollutants in a natural environment but some modifications can be done to enhance its degradation efficiency at a faster rate in a limited time frame by using the genetically engineered microorganisms and microalgae.

Using biological materials, it is more effective than the traditional strategies because bioremediation strategies can be used directly at the site of contaminant without the need to transfer contaminant materials.

Why use biological sources in bioremediation?

Microorganisms in general have effective ability to rapidly multiply and increase in huge numbers when be inoculated to decontaminate polluted area, when compared with chemical means. Microorganisms possess efficient enzymatic pathways able to eliminate or modify different pollutants. Moreover, different microorganisms could be added to polluted area (called inoculation) to enhance biodegradation rate. The inoculums may be a mixture of nonindigenous microbes from various polluted sites (specially selected and cultivated for its various pollutant degrading capabilities) or it may be a mixture of microbes selected from the site to depolluted or mass-cultured in the laboratory. Addition of nutrients along with inoculated process shows enhanced results for bioremediation

Environment worldwide is under great stress due to industrialization and human interfering on the limited natural resources. The release of chemicals pollution needs several techniques to treat some of these chemicals, but due to their cost, new technologies should be developing in order to create cost-effective and eco-friendly bioremediation technologies for environmental conversions. Bioremediation is an increasingly popular using microbial and algae strains for degrading waste contaminants.

The major groups of microorganisms commonly used for the bioremediation of metals, which include bacteria, microalgae, fungi, and yeast as well as the mechanisms for bioremediation are summarized in Figure 1.

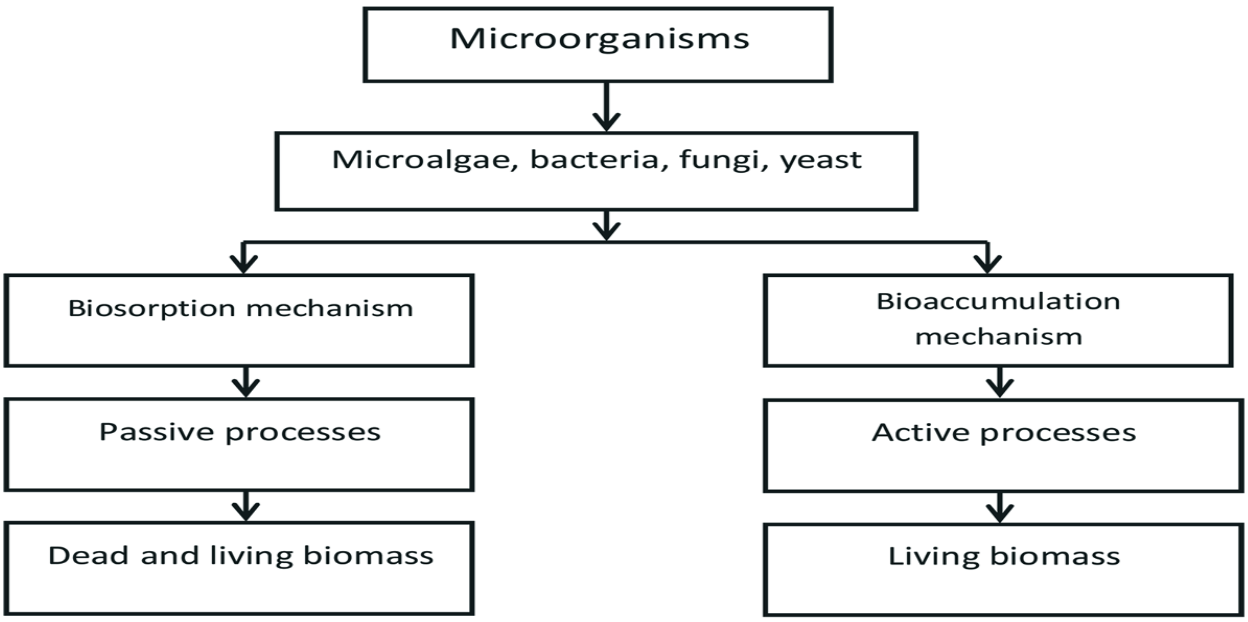
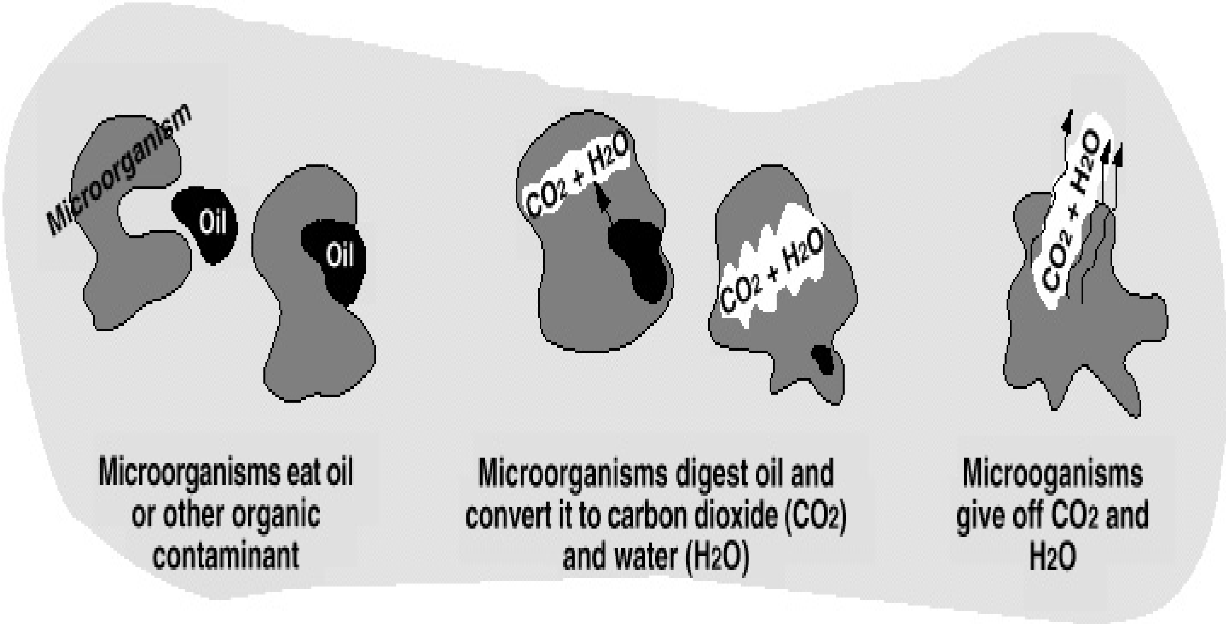


Figure 1. Microorganisms and mechanisms employed in the bioremediation involved in the case of dead and living.

How Does Bioremediation Work?



Uses naturally occurring microorganisms to break down hazardous substances into less toxic or nontoxic substances.

Algal Bioremediation (Phycoremdiation)

Phycoremediation refers to the combination of the words "phyco" meaning "algae" and "remediation" which means "to treat or bring back to the original state".

The process of phycoremediation or algal bioremediation is the use of algae to remove pollutants from the environment or to convert them into harmless form.

The removal or degradation of organic pollutants by the algae or "phycoremediation" is a promising bioremediation technology for decontaminating polluted sites. Phycoremediation in a much broader sense is the use of micro or macroalgae for degrading or biotransformation of different pollutants, including nutrients and xenobiotics from wastewater and CO_2 from air.

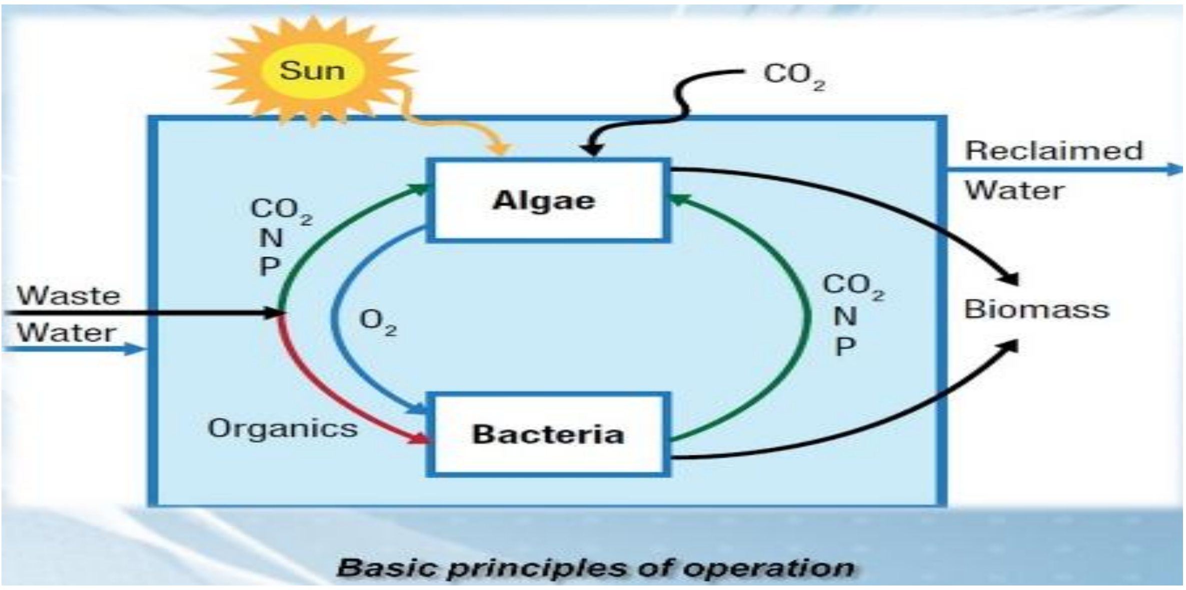
Phycoremediation is a sustainable and environmentally eco-friendly approach for cleaning up polluted areas. Algal degradation of organic pollutants is a natural process which ensures a lower environmental impact compared with mechanical, physical, and chemical removal approaches of organic pollutants.

Algae play a major role in controlling metal concentration of lakes and oceans. It possess the ability to degrade or accumulate toxic heavy metals and organic pollutants such as phenolics, hydrocarbons, pesticides and biphenyls from the environment, resulting in higher concentrations within themselves as compared to surrounding water.

Algal wastewater treatment is effective in the removal of nutrients (C, N and P), coliform bacteria, heavy metals and the reduction of chemical and biological oxygen demand (COD&BOD), removal and/or degradation of xenobiotic compounds and other contaminants.

The advantages of algae-based bioremediation are greater production biomass and high ability to accumulate, detoxify, or degrade xenobiotics and pollutants. In addition, the biomass produced in bioremediation could be economically valorized in the form of bioenergy. A large number of enzymes from algae have been found to be involved in the biodegradation of many organic pollutants.

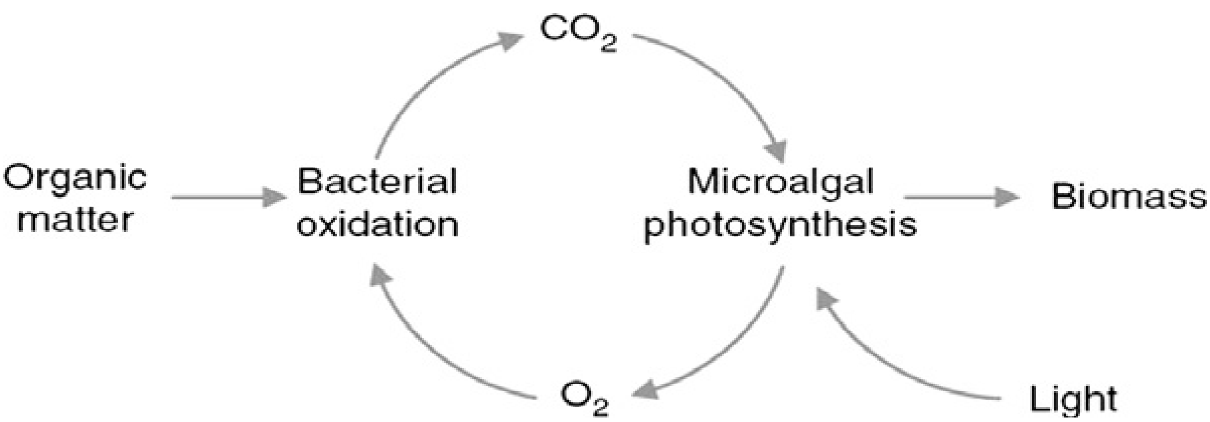
The use of higher plants and bacteria for bioextraction and biodegradation of organic pollutants has been extensively studied. However, the application of macro- and microalgae in absorption and degradation of organic pollutants in aquatic environment has just started. In this chapter, we will discuss the ability of algae to accumulate organic pollutants and the role of different enzymes in degradation processes.



It is applicable to various types of wastewater including:

Human sewage- livestock wastes- agro-industrial wastes- industrial wastes and food processing waste and other agricultural waste substrates.

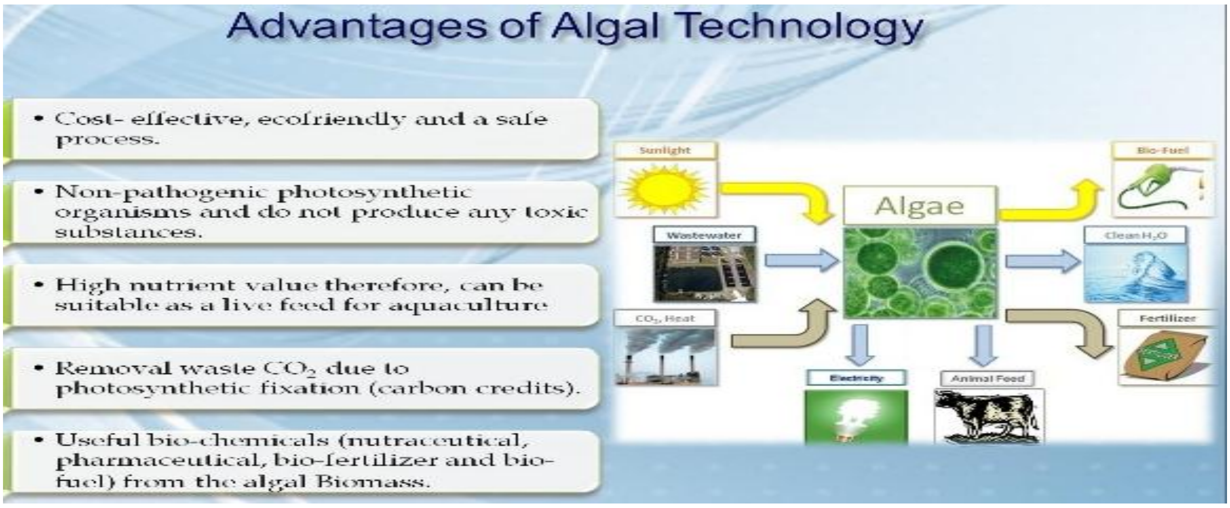
The effective disposal of sewage requires oxygen and this oxygen is brought about by the algal members present in the sewage like *Chlamydomonas*, *Chlorella* etc. The oxygenation by algae also helps to avoid unpleasant odour coming out of sewage bodies.



The phycoremediation shows advantage over other chemical methods as the removal of algal mass from the treated effluents is easy and economic. Phytoremediation has emerged as the most desirable technology which uses plants for removal of environmental pollutants or detoxification to make them harmless. There are several plants used in the phytoremediation has a considerable capacity of metal absorption, its accumulation and reducing the time of decontamination of an ecosystem. Phytoremediation is a green emerging technology used to remove pollutants from environment components.

The following algal species such as *Chlorella vulgaris, Rhizoclonium hieroglyphicum* and mixed algae culture (*Microspora* sp., *Navicula* sp., *Lyngbya* sp., *Cladophora* sp., *Spirogyra* sp. and *Rhizoclonium* sp.) have proved efficiency in waste waters treatment. Furthermore, various species of algae such as *Anabaena inaequalis, Chlorella* sp., *Stigeoclonium tenue*, *Synechococcus* sp. and *Westiellopsis prolifica* developed resistant to heavy metals and thus have been used for the removal of heavy metals. The study revealed that *spirulina maxim* a have good capability for removal of nitrogen and phosphorus from wastewater.

The main two mechanisms of bioremediation by microorganisms are biosorption and bioaccumulation. Biosorption is a passive adsorption mechanism which is reversible and fast. Biosorption needs inexpensive cost because the biomass can be obtained from industrial waste, and it can be regenerated and reused in many cycles. In biosorption, either the living or dead biomass can be used in bioremediation process, however, bioaccumulation involves only living biomass which cannot be reused and therefore it needs cost expensive. Other technology used the metabolic potential of microorganisms to decompose contaminants into simple no toxic compounds as compared with other physicochemical methodologies. Microorganisms can also survive in contaminated habitats because they are metabolically able to exploit contaminants as potential energy sources. In all techniques mentioned above either employing one type of organism or a consortium of microorganisms, high toxic chemicals are converted into less toxic chemicals by biological means. Biosorption of pollutants especially toxic metals can be affected by several environmental conditions such as pH, ionic strength, biomass concentration, temperature, particle size, and presence of other ions in the solution.



Phycoremediation of heavy metals

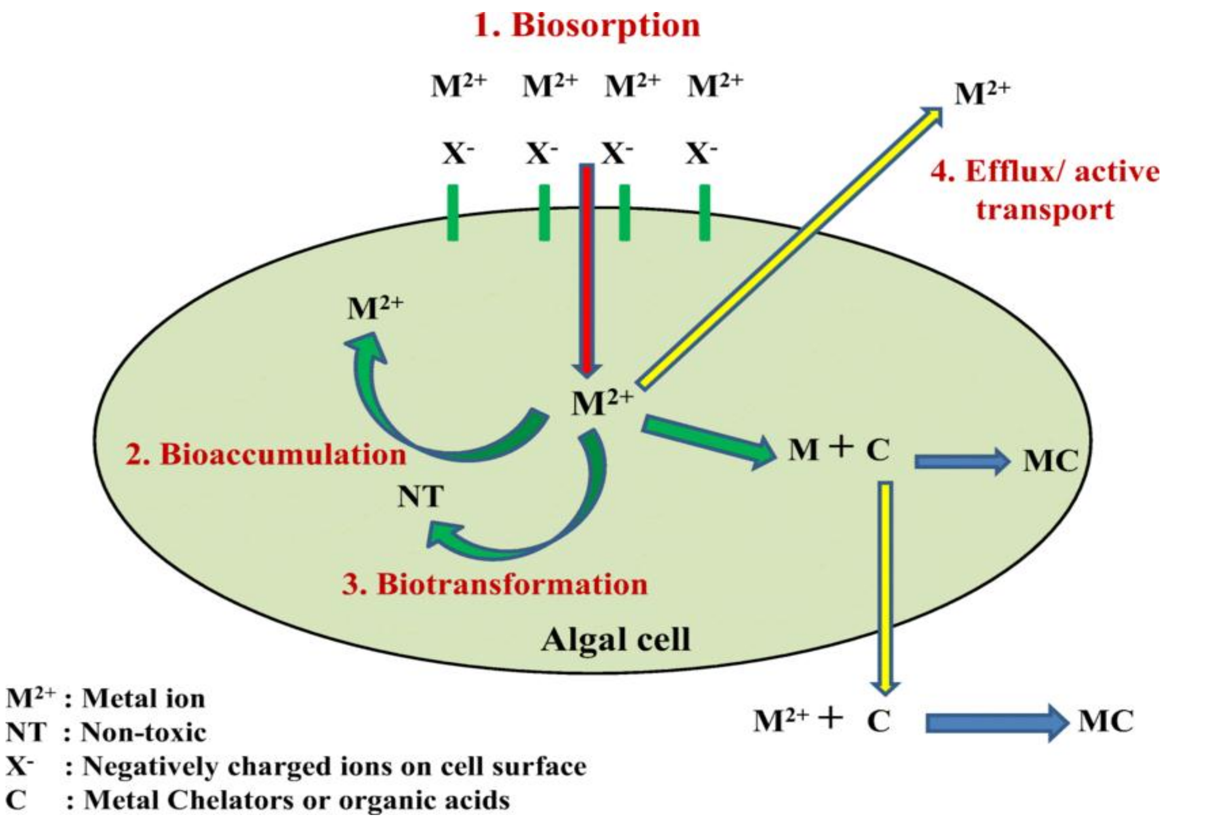
Various algal strains can absorb a significant amount of hazardous pollutants like heavy metals, pesticides and other organic pollutants as their nutrients from wastewater. The process in which algae absorb the heavy metals directly through the cell surface is known as physical adsorption. The process in which the pollutants enter into cytoplasm and the degraded by enzymes to convert them into nutrients is known as Chemisorption.

Algae are being most successfully used micro-organism to remove the heavy metals from the wastewater because algal strains can survive in high concentrations of heavy metals and other toxic pollutants. Algae have large surface area to adsorb much amount of pollutants from wastewater, have ability to grow autographically and heterotrophically. They also have potential to genetic manipulation.

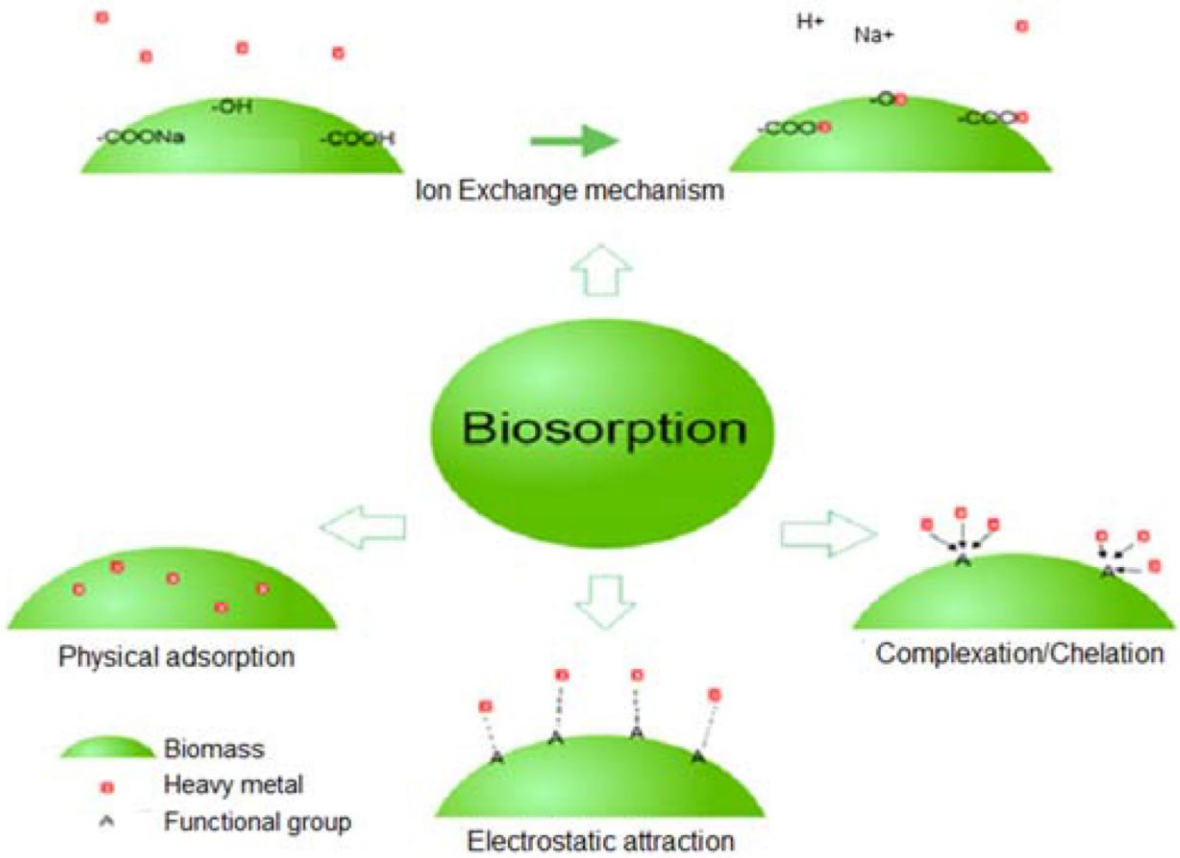
Mechanism of algae for decontamination of heavy metals

Algae can eliminate heavy metals by two processes: **bioaccumulation** and **biosorption**. **Biosorption** is a term that describes the removal of heavy metals by the passive binding to nonliving biomass from an aqueous solution; however, the **bioaccumulation** describes an active process whereby removal of metals requires the metabolic activity of a living.

Algae have peptides which bind with heavy metals. The peptide chains in algae bind with heavy metals and form organometallic complex which enter into vacuoles for controlling the cytoplasmic concentration of heavy metals. In this way algal cells check the toxic effects of heavy metals. The peptide chains are known as Phytochelatins and metallothioneins. The MTs are gene-encoded polypeptides and PCs are enzymatically synthesized peptides. Phytochelatins are also known as class-III metallothioneins. In algae class-II and class-III metallothioneins are found. Class-I metallothioneins are not found in algae. Synthesis of Mt III can be induced by some heavy metals like Cd^{2+}, Ag^{+}, Zn^{2+}, Hg^{2+}, Au^{2+}, Pb^{2+} and Bi^{3+}. Mt III are very important peptide molecules in algae because presences of these molecules make them capable to survive in high concentration of heavy metals. Biosynthesis of MtIII is directly proportional to degree of pollution.



The different defense mechanism employed by algae to combat metal toxicity



Algae for Monitoring and Degradation of Organic Pollutants

Monitoring

Biomonitoring is the specific application of biological response for the evaluation of environmental change for the purpose of using this information in quality control program.

In biomonitoring many organisms are used as bioindicators for environmental assessment. The bioindicators including algae, macrophyte, zooplankton, insect, bivalve mollusks, gastropod, fish, amphibian, and others are enumerated and compared for their advantages and disadvantages in practical biomonitoring of aquatic metal pollution. The potential applications of biomonitoring are proposed to mainly include evaluation of actual aquatic metal pollution, bioremediation, toxicology prediction, and researches on toxicological mechanism.

Algae are an ecologically important group in most aquatic ecosystems and have been an important component of biological monitoring programs.

a marine macroalgae, and knowing the corresponding concentration factors, the mean metal contents in seawater can be estimated. Recently, there has been a growing interest in using algae for assessment and biomonitoring of aquatic pollution by heavy metals and organic pollutants.

The seaweeds are used as bioindicators because of their distribution, size, longevity, presence at pollution sites, ability to accumulate metals to a satisfactory degree, and ease of identification. Algae are ideally suited for water quality assessment because they have rapid reproduction rates and very short life cycles, making them valuable indicators of short-term impacts. Macro algal species can be good biomonitors of contaminants that tend to reside in the dissolved phases (like heavy metals) compared to those contaminants that are lipophilic (like organochlorines). These lipophilic contaminants will not be readily taken up by macroalgal species, due to their low lipid content. Cyanobacteria and microalgae have the capabilities to degrade organic pollutants and are used in monitoring organic pollutant degradation.

Bioremediation of organic pollutants

Algae can eliminate organic pollutants by two processes: **bioaccumulation** and **biodegradation** (Tables 1 and 2). Bioaccumulation is a process that allows living organisms to take up and store certain organic and inorganic substances inside the cell, whereas biodegradation is a natural process by which organic substances are decomposed by algae or other organisms into simpler substances such as carbon dioxide and water. Organic pollutants can be transformed and degraded mainly by biological processes, with degradation resulting sometimes to mineralization.

Many algae have to utilize hydrocarbons as their carbon and source of energy in their metabolism. Algae and protozoa are the important members of the microbial community in both aquatic and terrestrial ecosystems; reports are scanty regarding their involvement in hydrocarbon biodegradation.

The decontamination of polluted areas by living organisms gives much hope on the restoration of polluted mangrove swamps and is being utilized for the degradation of crude oil in soil matrix by using microorganisms, to transform the petroleum hydrocarbons into less toxic compounds. This is achieved by the help of bacteria, fungi, and algae that produce enzymes capable of degrading harmful organic compounds.

The biodegradation of crude oil by Chlorella vulgaris and Scenedesmus obliquus, both algae grow under heterotrophic conditions using crude oil as sole carbon source and could degrade oil effectively when incubated with low concentrations of oil.

Table2. Efficient algae strains for wastewater treatment.

	Efficiency [%]	Applications
Macroalgae		
Cladophora glomerata	89–97	Metal ion removal
Spirogyra sp.	50–99	Nitrogen and phosphorus removal
Oedogonium	–	Metal ion removal
Chaetomorpha linum	87–92	Nitrogen and phosphorus removal
Enteromorpha	–	Controlling water nutrient level
Cladophora	–	Controlling water nutrient level
Sphacelaria	–	Controlling water nutrient level
Ectocarpus	–	Controlling water nutrient level
Ceramium	–	Controlling water nutrient level
Polysiphonia	–	Controlling water nutrient -level
Herposiphonia	–	Controlling water nutrient level
Oscillatoria	–	Controlling water nutrient level

Strains	Efficiency [%]	Applications
Microalgae		
Chlorella PY-ZU1	94	Organic materials uptake
Mixed culture (*Euglena* gracilis (CCAP 1224/5Z) and *Selenastrum* (SCCAP K-1877))	45–99	COD, organic and inorganic materials uptake
Scenedesmus sp.	72–74	BOD, COD removal
Chlamydomonas	–	BOD removal
Euglena	100–400 $kg_{BOD}day^{-1}$	BOD removal
Chlorella sp.	90	COD and BOD removal
Chlorella vulgaris	70–80	Nitrate and phosphorous removal
Scenedesmus obliquus	–	BOD, COD, and nitrate removal
Dunaliella	–	Organic and inorganic materials uptake
Oscillatoria	–	Nitrate and phosphorous removal
Micractinium	–	Nitrate and phosphorous removal
Phormidiumlaminosum	90	Nitrate removal
Golenkinia	96–99	Nitrate and phosphorous removal
Spirullina	80–90	Nitrate and phosphorous removal

Table 2 Bioaccumulation and biodegradation of some organic pollutants by microalg

Pollutants	Species	Type of bioremediation
Atrazine	*Selenastrum capricornutum*	Biodegradation
Benzene	*Selenastrum capricornutum*	Bioaccumulation
Benzopyrène	*Selenastrum capricornutum* *Scenedesmus acutus*	Biodegradation Biodegradation
Benzo[a]pyrene	*Selenastrum capricornutum*	Biodegradation
Bisphenol A	*Monoraphidium braunii* *Desmodesmus* sp.	Bioaccumulation Biodegradation
Chlordimeform	*Chlorella* spp.	Biodegradation
Chlorobenzene and 1,2-dichlorobenzene	*Selenastrum capricornutum*	Bioaccumulation
Diazinon	*Chlorella vulgaris*	Biodegradation
Dimethomorph	*Scenedesmus quadricauda*	Bioaccumulation
2,6-Dinitrotoluene	*Selenastrum capricornutum*	Bioaccumulation
DDT	*Cylindrotheca* sp. *Dunaliella* sp. *Euglena gracilis* *Scenedesmus obliquus*	Bioaccumulation Biodegradation Bioaccumulation Bioaccumulation
α-Endosulfan	*Chlorococcum* sp. *Scenedesmus* sp.	Biodegradation Biodegradation
Fluoranthene	*Nitzschia* sp.	Biodegradation
Lindane	*Chlamydomonas* spp. *Chlorella* spp.	Biodegradation Biodegradation

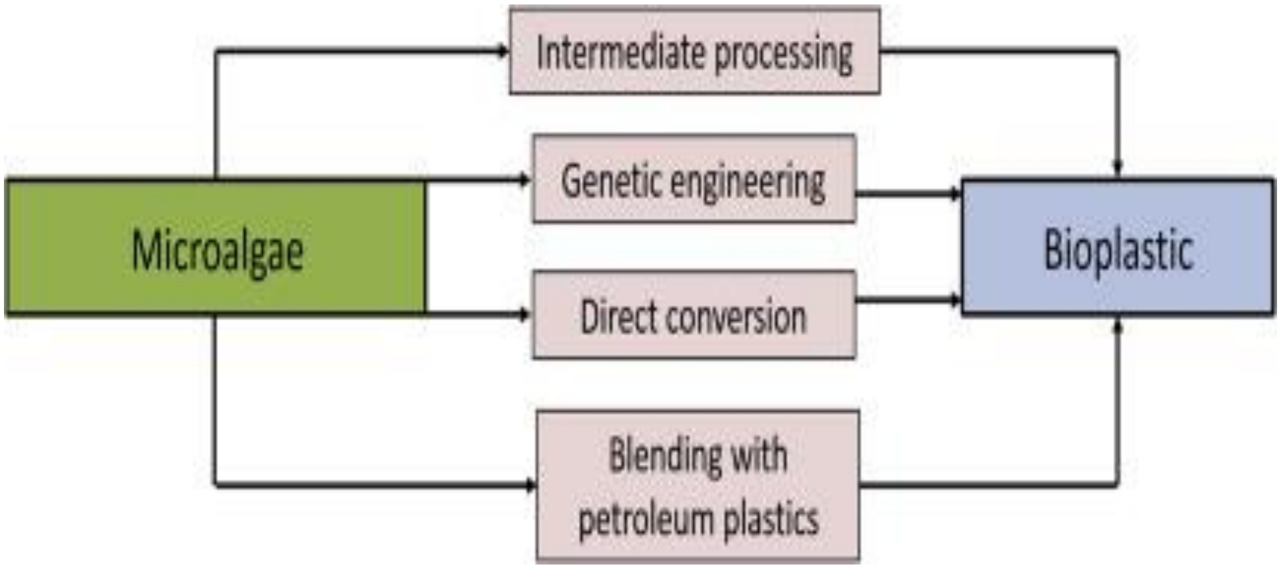
Microalgae as a source of bioplastics

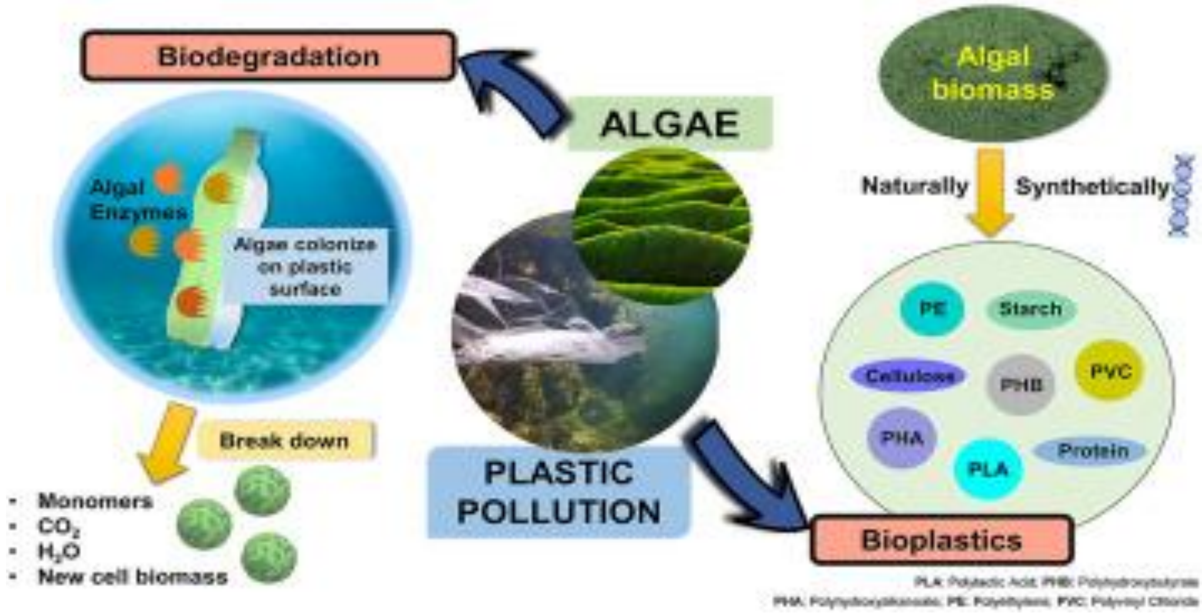
The demand of plastic and plastic-based products have grown significantly in last few decades, which has placed a major strain on the remaining petrochemical resources of our planet. The increasing production of these petrochemical-based plastics has also generated concern regarding plastic pollution worldwide, mostly in marine ecosystems due to their persistence in environment as non-biodegradable materials. Therefore, alternatives to petrochemical-based plastics sources are in high demand, as they would make plastic production sustainable while mitigating the issue of plastic pollution. Algae have the potential to be an economically viable feedstock for bioplastics production, as the biomass can be sold at US$ 970/tonne, which is within the current standard range for other sources of bioplastics (US$ 800 – 1200/tonne.

Microalgal biomass components such as **starch**, **carbohydrates**, and **lipids** can be converted into **plastics**. There are currently three main approaches to produce bioplastics from microalgae, including:

(i) direct use of microalgae as bioplastics, **(ii)** blending of microalgae with existing petroleum-based plastics or bioplastics, and (**iii**) genetic engineering of microalgae to produce bioplastic polymer precursors.

In the first approach, Zeller et al. (2013) have reported production of bioplastics and thermoplastic blends directly from *Sprulina platensis* and *Chlorella vulgaris*, However, the most common approach to making microalgae-based bioplastics is to blend the biomass with existing petrochemical-based plastics, such as polyethylene, polypropylene, polyvinyl chloride. *Chlorella* sp. biomass was blended with polyethylene and polypropylene and was found to possess good thermoplastics processability because of the presence of natural cellulosic type materials.





Algae as biofertilizers

From ancient time, Algae has been used as a fertilizer in many parts of the world. Biofertilizers offer a new eco-friendly technology which would overcome short comings of the conventional chemical based farming. Biofertilizers showed positive influence on both soil sustainability and plant growth. Biofertilizers are not only the alternative to chemical fertilizers but also tend to increase the soil fertility and plant productivity.

Algae have a high mineral content and have a property to help increase the water binding capacity of soil. It is capable of fixing atmospheric nitrogen and thus can be used to make biofertilizers. Algae can be grown simultaneously with the other crops and it will provide the crops with the most basic and important element to grow, that is, nitrogen. Also, plants fertilized with algae resists diseases and attacks by insects.

Algae can the production and secretion of bioactive compounds such as phytohormones, which stimulate the growth of agricultural crops.

Blue green algae belonging to genera Nostoc, Anabaena, Tolypothrix and Aulosira fix atmospheric nitrogen. *Chara* is used to overcome calcium deficiency in the fields. *Fucus* is used as manure.

Seaweed as a biofertilizer

Seaweed plays an important and vital role in the marine ecosystem and growing in large amount in the sea. Seaweed can be regarded as a potential source of bio-fertilizer in of dried or fresh form; it helps to enhance biochemical constituents like carbohydrates, lipids, proteins, fibers, ash, phenol, dietary fiber

The seaweed extract is available as fertilizer in different forms such as SLF (Seaweed Liquid Fertilizers), LF (Liquid Fertilizers) and powder form of seaweed manure have been used as a biofertilizer. In market, seaweed extracts available for several years as fertilizer additives and beneficial results from their use. Seaweed extract is effective for improves the quality of produce and soil conditioner.

Due to presence of P, K, Ca, and some trace elements, the seaweeds are used as fertilizers in many countries, such as Japan, France, United States, England and South India.

The components of seaweed such as macro and micro- element nutrients, amino acids, vitamins, cytokinin, auxins, and abscisic acid (ABA)- like growth substances affect cellular metabolism in treated plants to enhance growth and crop yield.

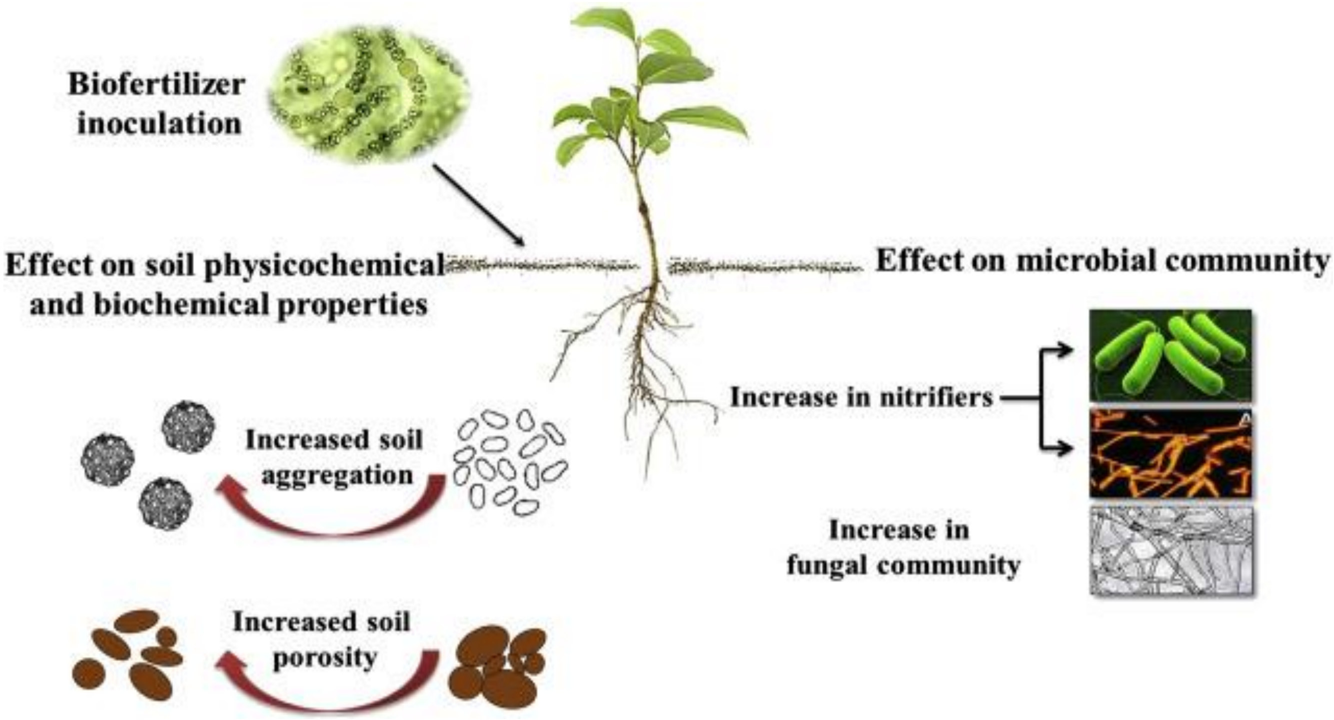


Fig. Effects of biofertilizers on physiological and biochemical properties of soil.

The use of natural seaweed as fertilizer has allowed for partial substitution of prevailing synthetic fertilizer. Some studies have reported a wide range of beneficial effects of seaweed extract applications (like soil drench, foliar spray, soil drench +foliar spray) on plants, such as improving moisture-(water holding capacity) and by promoting growth of beneficial soil microbes enhanced soil health, which are conditioning substances of secretion of soil and which are promote root growth and development, improve nutrient uptake by roots, promote rooting of cutting, early flowering and increase fruit set & yield, elicit abiotic stress tolerance in plants, nematodes, enhance defense against pests and diseases, bacterial and fungal pathogens. Seaweed manure seems to increase resistance to disease. Most of the nutrients including nitrogen compounds are in ionic form and a quick absorption by crops takes place and relatively little is left to be broken down by soil microflora, thus preventing acid conditions of the soil arising from the fermentation. In general, the minerals diffuse out from the sea weed thallus rapidly. Yet another, feature is that sea weed manure holds water and air at the same time and improves the soil in both respects. Like other manures seaweeds have a similar role but also contribute the required potassium, sulphur, phosphorus and calcium. The treatment of seaweed extract increased the seed germination, seedling growth and yield of crop.

Table: Algae Used as Biofertilizer in Different Parts of the World

Major Class of	Algal Biofertilizer Species	Name Contribution
Brown macroalgae	*Laminaria digitata* (Oarweed), *Saccharina latissima* (Sugar Kelp), *Fucus vesiculosus* (Bladder wrack), Ascophyllum nodosum (Knotted wrack),*Ecklonia maxima, Stoechospermum marginatum*	1.Rich in nitrogen, potassium, and phosphorus 2. Carbohydrates (improve aeration and soil structure, especially in clay soils and have good moisture retention properties) 3. Used as source of naturally occurring plant growth regulators. 4. Enhance plant growth, freezing, drought and salt tolerance; photosynthetic activity; and resistance to fungi, bacteria, and virus
Red macroalgae	*Phymatolithon calcareum* (Maerla), *Lithothamnion corallioides* (Maerla)	Trace elements
Blue-green algae	*Nostoc*, *Anabaena*, *Aulosira*, *Tolypothrix*, *Nodularia*, *Cylindrospermum, Scytonema, Aphanothece, Calothrix, Anabaenopsis, Mastigocladus, Fischerella, Stigonema, Haplosiphon, Chlorogloeopsis, Camptylonema, Gloeotrichia, Nostochopsis, Rivularia, Schytonematopsis, Westiella, Westiellopsis, Wollea, Plectonema and Chlorogloea*	**3.** Fix 18e45 kg N/ha in submerged rice field 2. Produce growth-promoting substances
Anabaena Azolla Association	Anabaena azollae	1.Fixes 40e80 kg N/ha 2. Used as green manure because of large biomass

Nutrient content and Bio-chemical parameter of Different seaweeds:-

Table- The comparison of different seaweeds macro- nutrient content

Name of seaweed	Type	N mg/g	P mg/g	K mg/g
Sargassum wightii	B	174.02	45.56	72.83
Sargassum crassifolium	B	0.4	0.009	1.520
Padina pavonica	B	0.01090	0.00926	0.16013
Dictyota dichotoma	B	175.02	44.56	71.84
Laurencia obtuse	R	3.9	3.8	2
Jania rubens	R	4	3.5	1.6
Padina pavonica	B	0.07985	0.00069	0.00278
Ulva linza	G	0.05716	0.00120	0.01265
Ulva lactuca	G	0.12609	0.00300	0.01634
Ulva lactuca	G	174.02	45.56	75.83

Whereas, G= Green Seaweed, B= Brown seaweed, R= Red Seaweed, N-Nitrogen content, P-Phosphorus content and K-Potassium content.

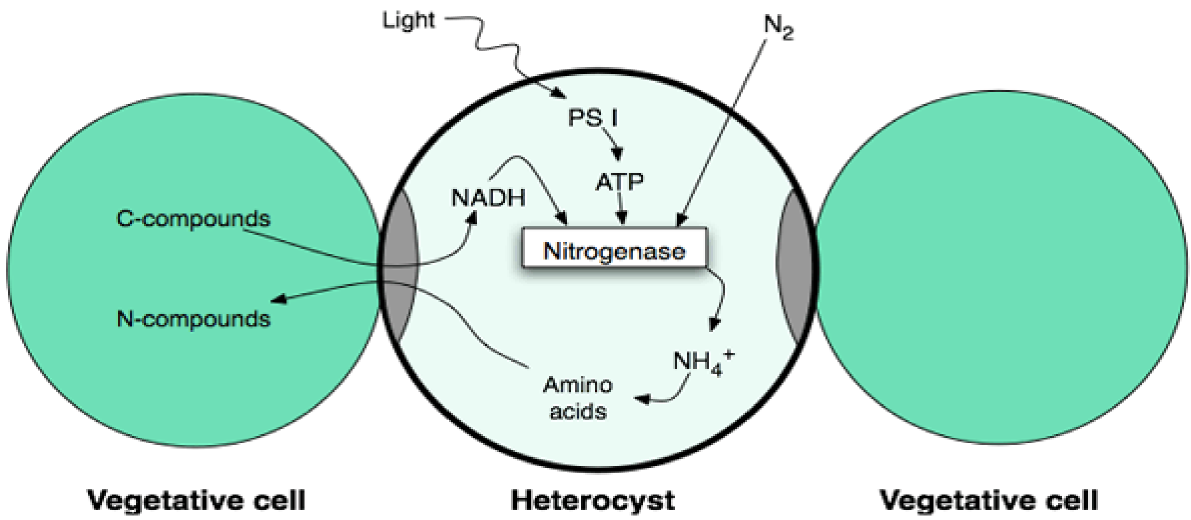
Table - The comparison of different seaweed bio-chemical parameters

Name of seaweed	Type	P %	C %	Ph%	L%	F%	A%
Chaetomorpha crassa	G	25.48	26.94	-	1.50	34.29	26.94
Gracillaria fisheri	R	26.71	47.47	-	0.62	11.78	47.47
Caulerpa racemosa	G	23.42	48.10	-	0.67	6.68	48.10
Caulerpa branchypus	G	26.34	54.38	-	1.42	6.04	54.38
Caulerpa lentilifera	G	12.68	27.19	-	1.09	4.83	27.19
Ulva rigida	G	13.32	67.84	-	0.15	5.69	67.84
Cladophora sp.	G	0.12	0.73	0.066	--	---	----
Padina sp.	B	1.84	0.62	0.380			
Sargassum cinerrum	B	0.13	0.63	0.277			
Caulerpa indica	B	0.14	0.92	0.27			
Jania rubens	R	13.82	374.02	0.053	-		
Laurencia obtusa	R	142.94	199.69	0.529	-	-	20.25

Whereas, P= Protein content, C= Carbohydrate content, L= Lipid content, F= Fiber content, A= Ash content, Ph= Phenol content, TDF= Total Dietary Fiber, G= Green Seaweed, B= Brown seaweed, R= Red Seaweed

Nitrogen fixation by blue green algae (Cyanobacteria)

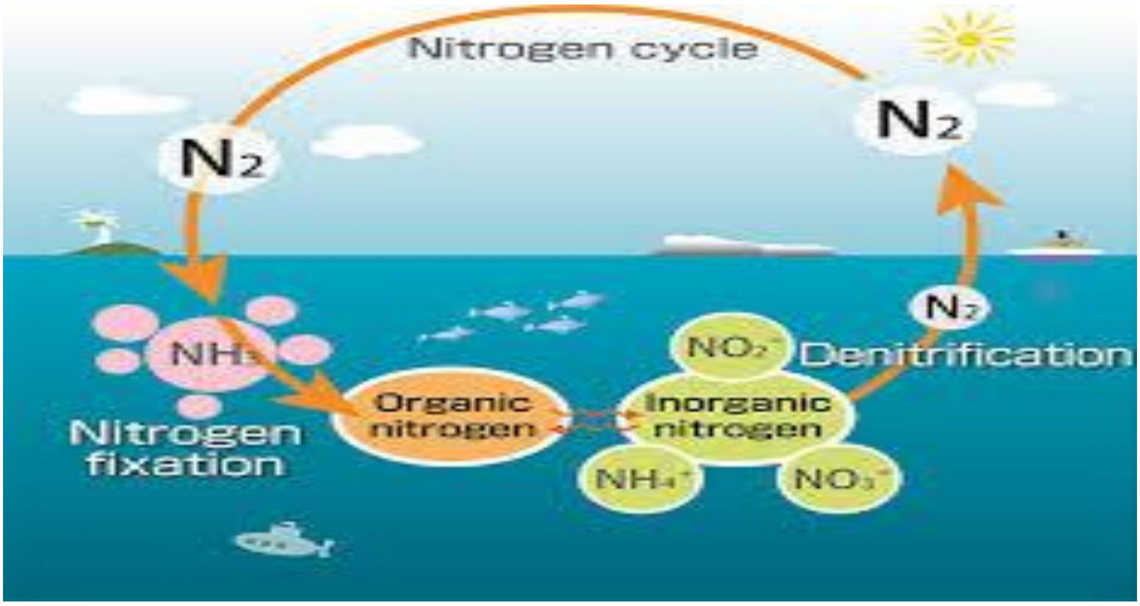
Cyanobacteria are the blue-green algae which have the ability of nitrogen fixation in soil. The nitrogenase enzyme is present in the heterocyst of this organism. The heterocysts are the specialised cells other than the vegetative cells. The nitrogenase enzyme helps in the nitrogen fixation. Soil is a living mass and apart from soil particles there are in it a number of bacteria, fungi, algae and protozoa. According to Russel, algae occupy a volume three times that of the bacteria. For a long time, it was felt that these are the bacteria in the sheath of blue-green algae which fix nitrogen. Blue-green algae (now called cyanobacteria) are the main agents for nitrogen fixation in rice fields, and the part played by other bacteria is relatively unimportant.

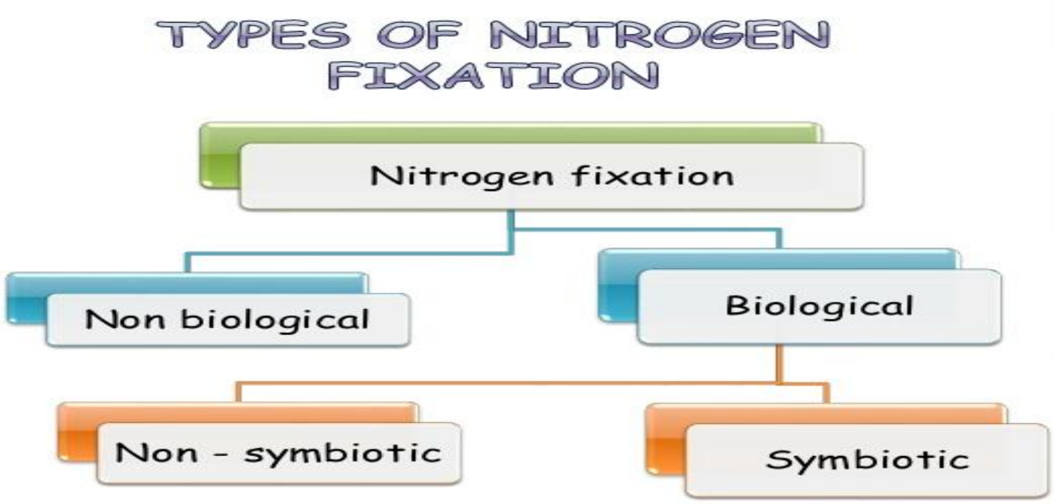


Tolyporthrix tenuis is a strong nitrogen fixer, it could fix as much as 780 lbs. of nitrogen per acre per year. *Anabaena cylindrica* fixes 2,900 lb. of nitrogen per acre per year.

The dominance of the blue green algae, possessing the ability for nitrogen fixation particularly in the tropics, suggests that these organisms are of considerable importance in maintaining soil fertility. By increasing and strengthening the local blue green algal flora of a given habit, it may be possible to increase the fertility of these soils.

As nitrogen content is an important and necessary element for plant growth, adding algae dried biomass to soil may increase agricultural soil quality. On the other hand, some blue green algae such as Anabaena, Nostoc, Aulosira, and Tolypothrix can fix atmospheric nitrogen and utilize nitrogen for plants growth besides CO_2 fixation.





Biological N_2 Fixation: Biological nitrogen fixation (BNF) occurs when atmospheric nitrogen is converted to ammonia by an enzyme called nitrogenase. The reaction for BNF is:

$$N_2 + 8\ H^+ + 8\ e^- \rightarrow 2\ NH_3 + H_2$$

Reclamation of alkaline user soils by blue-green algae (cyanobacteria)

It has been found that some blue green algae form a thick stratum on the surface of saline usar soils during the rainy season when other plants including crop fail to grow. Various species of Nostoc, Scytonema, Anabaena, etc., have been found to be plentifully growing on usar soils during the rains. According to Dr. R.N. Singh (1950), these algae (cynobacteria) can be of use in the reclamation of usar lands.

There is successive growth of the algal crop on such soils in a water-logged condition. After a year of such reclamation, it has been possible to grow transplanted paddy crop with a yield of as much as 1576-2000 lb. per acre. It has been found that the pH of the soil experimented upon in this matter fell from 9.7 to 7.6. Moreover, there was an improvement in the tilth and exchangeable calcium by about 20 to 33 percent. The water holding capacity increased by about 40 percent.

Various potentially other useful effects have been shown for the cyanobacteria, such as antifungal substances. Pre-soaking rice seed with cyanobacterial cultures or extracts has been reported to enhance germination and growth.

Algal plant growth regulators (PGR)

In various algal taxa, essentially all known phytohormones were detected in concentrations comparable with their content in higher plants.

algal hormones such as **auxin**, **abscisic** acid (ABA), gibberellin-like substances (GLS), and cytokinins in certain species of green, brown and red marine algae-macrophyte.

Auxins

The phytohormone auxin is one of the main directors of plant growth and development. Auxins and their inactive analogs were present in brown (Macrocystis and Laminaria), red (Botryocladia), and green (Enteromorpha, Chlorella, and Cladophora) algae and also in cyanobacteria (Oscillatoria) for example. the presence of auxins (IAA in particular) in green and characean algae and in the extracts from brown algae (Fucus and Ascophyllum). In zygotes and adult plants of Fucus distichus and F. vesiculosus, IAA was present at the concentrations only slightly lower than in higher plant tissues (2–9 mg/g fr wt. The content of auxin in the algal thalli varied in dependence on the season and developmental stage. The highest concentration was observed in summer in vegetative tissues.

Cytokinins

It was repeatedly shown that the extracts from the marine phytoplankton exhibited cytokinin activity. These hormones were found in the extracts of fucoid algae. Euglena gracilis chloroplasts contain tRNA with cytokinin activity and a wide spectrum of cytokinins, such as IPA, 2-methylisopentenyladenine, and 2-methylisopentenyladenosine. IPA was found in the cyanobacterium Arthronema africanum, IPA, isopentenyladenosine, zeatin, zeatin riboside, and aromatic cytokinins, topolin conjugates, were identified in characean and green micro- and macroalgae. Basic cytokinins in green microalgae (Protococcus, Chlorella, and Scenedesmus) are free IPA, zeatin (cis-isomer prevails), and riboside and ribotide conjugates. A great diversity of cytokinin conjugates is evidently rather a property of higher plants but not algae. Trace amounts of O-glucosides were detected in some microalgae and brown macrophytes (Sargassum heterophyllum, Macrocystis pyrifera).

Abscisic and lunularic acids

In various groups of algae, some compounds were repeatedly detected, which suppressed plant growth in bioassays. At least one component of this inhibitory complex was isolated from the green alga Enteromorpha compressa and studied. This component was identified as dihydrostilben, lunularic acid. The identification of this compound confirmed a theory of Pryce, who supposed that, as distinct from higher plants, algae and liverworts do not contain ABA and its functions are fulfilled by lunularic acid. However, the increasing body of information appears about the presence of ABA in the algae of various groups. This hormone was found in green microalgae (Chlorella sp., Dunaliella salina, and Haematococcus pluvialis) and also in the thalli of brown macrophytes from the genus Ascophyllum and some species of Laminaria. It might be that the growth-inhibiting complex found in various algal groups comprises several components, including lunularic acid, ABA, and some other so far unidentified biologically active compounds. One of the possible candidates for this role is salicylic acid.

Algae as air pollution control

Numerous research and development projects on CO_2 reduction have been performed in recent years. There were chemical, physical and biological methods in the procedures studied. The biological method is more reliable than other methods. Using microalgae photosynthesis is trusted to be an efficient approach for CO_2 biofixation.

Microalgae are mostly used due to their photosynthetic performance in CO_2 biofixation, high lipid accumulation, high biomass productivity, and valuable nonfuel co-products. The autotrophic or photosynthetic microalgae have numerous abilities for CO_2 fixation and are 10–50 times more effective than terrestrial plants. Only 3–6% of the global CO_2 emissions are diminished by terrestrial plants. In comparison to nitrogen and phosphorus, carbon is the most important element for microalgae growth. CO_2 can be used to cultivate microalgae by injection of flue gas from power plants into the microalgae culture medium or by cultivating it in the raceway pond to minimize atmospheric CO_2.

As CO_2 is converted into organic carbon-like lipids and hydrocarbons in this process, this illustrates the definition of "CO2 fixation process" (Fig. 2).

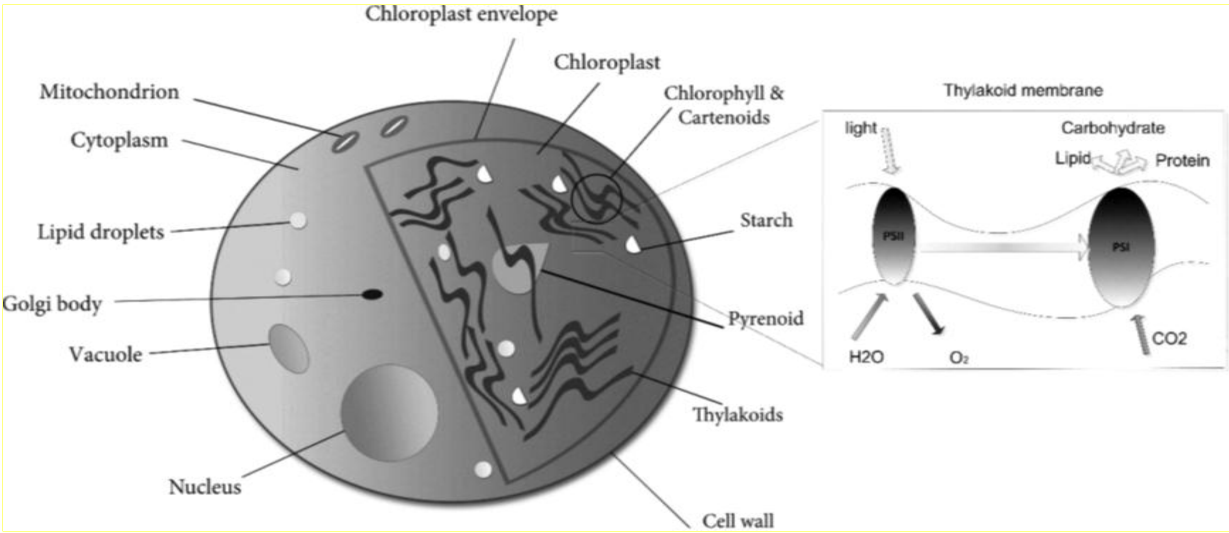


Figure2. CO_2 fixation in photosynthetic process.

Table. Microalgae strains and their efficiency in CO_2 biofixation.

Strains	CO_2 removal efficiency [%]	CO_2 concentration [%]	
		Best growth	Maximum tolerance
Scenedesmus obliquus PF3	87	5	10
Aphanothece microscopica	92	15	–
Euglena gracilis	–	5	45
Chlorella sp.	46–85	10	40
Scenedesmus sp.	40–62	10-20	80
*Chlorococcum littorale**	–	60	60
Dunaliella tertiolecta	–	5	15
Botryococcus braunii	–	20	–
Dunaliella tertiolecta	–	10	24
Chlorella vulgaris	92	5	80
Nannochloropsis oculata	11–47	2–5	15

***Chlorococcum littorale can grow under 60% CO_2 by sequential steps.**

Cosmetics

There is an increasing trend in the usage of photosynthetic microorganisms including macro- and micro-algae in the field of Cosmeceuticals by incorporating the bulk products extracted from its biomass into cosmetic formulations.

Algal products have been used in the cosmetic industry as antioxidants, sunscreens, thickening agents, skin sensitizers, moisturizing agents to enhance the competence of skin against abrasions, tanning, etc.

Algae have natural anti-cellulite and anti-ageing properties. It helps in increasing the elasticity and suppleness of the skins and also stimulates the renewal of damaged skin cells. It can detoxify and cleanse and tone the skin. It increases the lusture of hair, has a moisturizing and softening effect on hair. It forms a gel on reacting with proteins, which has a moisturizing effect on the skin and softens skin and can produce soothing face packs and masks. It has been known long for its anti-inflammatory and tissue renewal properties which can have a positive effect on problems such as facial wrinkles. It has hydrating properties and forms a protective layer on the skin thus acts as a moisturiser and reduces loss of skin moisture through evaporation.

There are many scientific corroboration that proves the competence of algae but of course, it depends on specific type of extract, how it is processed and its application. Due to the growing economic aspect of the cosmetic industry, the need for harmless and efficient natural raw ingredients has become an utmost necessity. According to certain research reports, algal products used in cosmeceuticals have been known to be suitable alternatives with constructive effect even after prolonged usage.

Table. Macro algal species used in cosmetics

Algal species	Type	Pigment	Fatty acids/Metabolites	Applications/ Products
Irish moss	Red algae	Phycoerythrin	Omega-3 fatty acids, Omega-6 fatty acids	Emollient, moisturizing, sheaths damaged or dry hair, nutritive, Skin soothing, anti-inflammatory [41]
Sea Lettuce *(Ulvalactuca)*	Green algae	Chlorophyll-a, Chlorophyll-b, β-Carotene	Oleic acid, Linoleic, and Linolenic acid	Antioxidant, anti-inflanrnmtoiy, skin elasticity, collagen synthesis, anti-wrinkle,emollient, moisturizing [42]
Sea Palm *(Postelsiapal maeformis)*	Brown algae	Chlorophyll-c, Fucoxanthin		Skin softening, anti-wrinkle, nourishing, moisturizing, anti-inflammatory [43]
Fucus vesiculosus	Brown algae	Chlorophyll-c, Fucoxanthin		Tightening effect and stimulates metabolism [44]
Porphyra umbilicalis	Red algae	Phycoerythrin	a-Linolenic acid,	Skin-conditioning agent [45]
Ascophyllum nodosum	Brown algae	Chlorophyll-c	Fucoxanthin, Alginates	Anti-aging agent, anti-wrinkle agent, smoothing agent [46]

Table: Micro algae species used in cosmetics

Algal species	Type	Pigment	Fatty acids/ Metabolites	Applications/ Products
Spirulina	Blue green algae / cyanobacteria	Phycocyanin	Gamma-Linolenic acid, Phycocyanobilin, Phycoerythrobilin	Anti aging, anti-wrinkle, collagen synthesis, anti-inflammatory, nourishing, antioxidant [47]
Isochrysis	Brown algae	Cantaxanthin, Fucoxanthin	Mistiric Acid, Oleic Acid	Antioxidant, suncare, soothing agent, anti-irritant [48]
Dunaliella salina	Green algae	Chlorophyll-a, Chlorophyll-b, β-Carotene	Palmitic acid, Linolenic acid, β-Cryptoxanthin	Antioxidants, smoothing agent, Anti-inflammatory [49]

As a source of vitamins

The marine algae are the richest source of vitamins. The vitamins A, B and E are found abundantly in sea weeds. The vitamin B essentially required for the development of human body is found in great abundance in almost all Phaeophyceae. The cod liver oil is the rich source of vitamin A, which is acquired from sea weeds. Vitamin E is equally important for human beings which are found in many marine algae.

In the sea weeds of Sweden maximum amount of vitamin B12 and folic acid are found in spring and summer and niacin and folic acid in winter. Vitamin B12 content and also that of B1 are higher in green and red algae than in brown algae and that the niacin and vitamin C content appear to be about the same in the above three groups of marine algae. Several vitamins except ascorbic acid have been reported from *Chlorella*. The vitamins found in *Chlorella* are thiamin, riboflavin, niacin, pyridoxine, pantothenic acid, chlorine, biotin, vitamin B12 and lipoic acid.

There are substantial amounts of vitamin B12 in Spirulina algae tablets. The bioavailability of vitamin B12-compounds from spirulina was evaluated using vitamin B12-deficient rats. The results suggested that algal vitamin B12 is a bioavailable source for mammals. However, pseudovitamin B12 (an inactive corrinoid) which predominated in the spirulina tablets are not suitable for use as a vitamin B12 source **Manufacture of soaps and alums**: By burning seaweeds on the sea coast, the alkalies are prepared from seaweed ashes. These alkalies are employed in the manufacture of soaps and alums.

Manufacture of potash: Species of *Macrocystis* and *Nereocystis* (Phaeophyceae) possess 30% of potash in their dry weight.

Manufacture of light weight buildings: Germany has discovered a process, in which the sea weeds are mixed with cement to make buildings light in weight and good heat resistant.

Safety aspects of algal products

Food safety at international level is governed by Codex Alimentarius Commission (CAC), which was created by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO).

Food safety is important for the food products made from various microbial sources. Food products using whole cell algal biomass or extracts from algae are following food safety regulations. An algal product must pass a nutritional physiological and toxicological Safety test before it can be put on the market as food or feed. International recommendations exist for these tests. Special safety aspects apply to the use of algae biomass from wastewater for animal nutrition.

Algae such as *Spirulina* (Arthrospira), *C. vulgaris*, *D. salina*, *P. tricornutum*, and *P. cruentum*, among others that are commercially used in aquaculture and food supplements do not produce toxin. Even within the same species, large differences exist between toxic and non-toxic algae. For example, dinoflagellates and diatoms are best known for their production of toxins that can affect humans, but for instance the dinoflagellate strain *C. cohnii* has a GRAS (Generally Recognized as Safe) status by FDA for ω-3 DHA human food consumption (Table 5). As such, it is very important to know the safety of algae at strain level that will be eventually applied for food or feed application.

Table5. Safety aspects of relevant microalgae for food application source.

Organism	Species	Safety aspect	Organism	Species	Safety aspect
Cyanobacteria	*Spirulina / Arthrospira sp.*	GRAS	**Heterokon-tophyta**	*Navicula sp.*	NT
	Synechococcus sp.	NT		*Nitzschia dissipata*	NT
Chlorophyta	*Tetraselmis sp*	NT		*Phaeodactylum tricornutum*	NT
	Chlamydomonas reinhardtii	NT		*Thalassiosira pseudonana*	NT
	Haematococcus pluvialis	NT		*Odontella aurita*	NT
	Dunaliella sp.	NT		*Skeletonema sp.*	NT
	Chlorococcum sp.	NT		*Monodus subterraneus*	NT
	Scenedesmus	NT		*Nannochloropsis sp.*	NT
	Desmodesmus sp	NT	**Haptophyta**	*Isochrysis sp.*	NT
	Chlorella sp	GRAS		*Pavlova sp3*	NT
	Parietochloris incisa	NT	**Dinophyta**	*Crypthecodinium cohnii*	GRAS
Rhodophyta	*Porphyridium cruentum*	GRAS			

NT = no toxins known, GRAS = Generally Recognised as Safe by the Food Drug Administration (FDA)

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WITH MY BEST WISHES

DR. ABLA AM. FARGHL