Model Answer
M.Sc. (Botany) IV Semester
Paper – LBT 402
Applied Phycology

Section - A

Ans. 1. (i) c – Dinophyta

(ii) d- All of the above

(iii) d- Biodiesel production

(iv) a- Starch and cellulose

(v) c- Raceway pond

(vi) b- Si

(vii) c- Oxygen

(viii) b- Rhodophyta

(ix) a- Watanabe et al.

(x) Cyanophage

Section – B

Ans. 2. Causal factors of the freshwater and marine algal blooms:

The freshwater and marine blooms are initiated and exacerbated by

1. Excessive nutrient (N and P) loading

2. High surface water temperature (> 20° C)

3. Persistent water column stratification (vertical and horizontal water column stability)

4. Long water residence time

5. Organic matter enrichment

6. Low total and dissolved N:P ratio

7. Close metabolic coupling between sediments and water column

8. High irradiance, long daylength.

9. Low turbulence and non-mixing of water

10. Iron and Zinc availability (in marine and freshwater habitat respectively)

11. Algal bacterial synergism

12. Lack of macrograzer

Dynamics of freshwater and marine algal bloom:

Bloom dynamics are controlled internally through behavioural adaptation. Adaptive strategies such as mobility behavior (phototaxis, vertical migration, swimming patterns and aggregation) and life cycle strategies which include temporary phases and resting cysts interact with the surrounding environment in development and maintenance of blooms. The dynamics of algal blooms varies from place to place, not depending alone of the hydrographic and topographical conditions but also on ecological and biological characteristics of the causative organisms.

In marine ecosystem evidences show that the inter-annual variability of some algal bloom events are a result of the interaction between local and global forcing like climatic events such as North Atlantic Oscillation or El-Nino respectively. Each species has a specific life history adapted to the surrounding environment and that gives them advantage over the rest of the community. For this reason knowledge of growth the phases of the life cycle, the mortality rates and their relationships with the local circulation and the biological environment is basic. The expansion of the geographical distribution of a species could be due to an anthropogenic introduction a natural phenomenon or it could be a ‘hidden flora’ species; the species was already in the area but in undetectable concentration.

In both freshwater and marine ecosystem vertical migration of algae is a mechanism to seek nutrients, but is also a determining factor in the spatial distribution of the population as well as adaptation to minimize population loss. The key role of summer breeze forcing and its coupling with organism behavior in determining the occurrence and persistence of the high biomass blooms that occurs recurrently during summer in some marine beaches. A low water renewal favors the nutrient availability and maintenance of certain position for species with swimming ability, enabling them to avoid being washed away. Nutrient input is a necessary but not sufficient condition.

The resting cyst stage of bloom forming genera is commonly found in sediments of many marine and freshwater habitats. They are readily transported vertically and horizontally and can survive many years in benthic and planktonic stages. These cysts emerge readily on advent of the favourable environmental condition and onset the bloom phenomenon. The conditions favouring germination of these cysts include presence of stable overlying water mass, increase in temperature, influx of allochthonous organic matter and inorganic nutrients, upwelling, bringing nutrient rich deep water in to the eutrophic zone, accumulation of ammonia and presence and active growth of associated microorganisms that produce and excrete chemical factors favoring their germination.

The bacterial-algal interactions do play a role in dynamism of algal blooms. There are sufficient evidences which suggest that phycosphere-associated bacateria act as mutualistic companion during algal bloom development and proliferation. Bacteria were suspected of recycling essential CO_2 and Phosphate supplies which may provide host with a competitive nutrient cycling and productivity advantage during period of dense algal bloom formation.

Ans. 3.

Different methods for controlling algal blooms:-

Physical control:

It involves removal of algal bloom by skimming the surface water to remove algae. It consisted of generation of fine bubbles attaching to the algae, which were then collected by skimmer. Ultrasonic waves can also be used which causes the algae to aggregate at node; but it works only on top 50 cm of the water. Another physical method to control the freshwater algal bloom is the application of barley straw. Upon decomposition of barley straw in water under aerobic conditions, phenolic compounds such as ligin and other oxidized phenolic compounds slowly leached into surrounding water and suppress algal growth.

Flocculation:

By using a flocculant, algal cells are concentrated and clustered together, becoming heavier and eventually sink. Flocculants such as aluminium sulphate and ferrates have been used extensively. The flocculant with greatest potential is clay. Modifications to clay, using polyaluminum chloride, sophorolipid and chiton-modified sepiolite have demonstrated increased removal efficiency of algae.

Chemical Control:

The first chemical used to control the algal blooms was copper sulphate. Though, it provides immediate effect but the effect is not long lasting and it may cause great harm to the other aquatic organisms.

Nutrient limitation in to water bodies particularly the phosphate and nitrate represents the bottom up control. Phosphate can be removed by precipitation with ferric sulphate and by causing the water source to flow over limestone chips.

The lanthanum modified clay products like 'Phosloc' is capable of efficiently removing the soluble reactive phosphates as it precipitates a number of different phosphates and descends through a water column. Its optimum performance pH range is 6-8. But lanthanum is slightly toxic to mammals and highly to *Daphnia* sp.

Aponin is a sterol surfactant that lowers the surface tension is suggested as control agent for algal blooms. Terbutryn kills the most submerged vegetation and is effective in controlling the bloom. Dichlobenil is used to control the bloom of *Chara* but other filamentous algae are resistant to it.

Alum can also be used to remove phosphate. In the water, alum forms $Al(OH)_3$ which removes soluble phosphate as it descends through the water column. But $Al(OH)_3$ is toxic chemical and there is decrease in pH during alum treatment.

Biological control:

A natural biological limitation for phytoplankton is grazing by zooplankton which may be important in population dynamics. Biological techniques use top down grazing and bottom up bacterial and viral infections observed in population and examine possiblilities to imply such methods for control of algal blooms.

The biomanipulation, (top down control) involves more fundamental alterations to lentic ecosystem. This method was first introduced by Shapiro (1975). In this method the organisms which cause the bloom forming genera mortality, like cyanophages, bacteria, parasites and predators are introduced in water bodies. It involves the substantial reduction of plankivorous and bentivorous fish in shallow lake. This reduction cause large filter-feeders like bivalves and zooplankton to extent a higher grazing pressure on bloom forming plankton. Among all zooplankton, it is the population of *Daphnia* sp. that frequently has the greatest impact, which consumes its own biomass per day.

This method has many advantages over other methods but it is highly specific to target organism, limited to survival of agent and has problem with large scale production.

Ans. 4.

Continued use of fossil fuels is now widely recognized as unsustainable, because of depleting supplies and the contribution of these fuels to the accumulation of carbon dioxide in the environment. Renewable, carbon neutral, transport fuels are necessary for environmental and economic sustainability. Biodiesel, derived from oil crops like palm oil, *Jatropha* oil, ethanol derived from corn and sugarcane is a potential renewable and carbon neutral alternative to petroleum fuels. Unfortunately, biodiesel from oil crops, waste cooking oil and animal fat cannot realistically satisfy even a small fraction of the existing demand for transport fuels. The problem with using plant matter is that they will require a large land area for production as compared to algae production which are able to grow in submerged area and also in seawater.

Algae appear to be the only source of renewable biodiesel that is capable of meeting the global demand for transport fuels. Like plants, algae use sunlight to produce hydrocarbon but they do so more efficiently than crop plants. Many species of algae accumulate large amount of oils that to a large extent are made up of triacylglycerol. The non or mono-unsaturated fatty acids of 16 or

18 carbon length are preferable sources to use for the production of biodiesel. Further, the content of lipids and oils in algae can be increased by various stress induced methods like salinity stress. The algal oil can be converted in to biodiesel by trans-esterification process. Oil productivity of many microalgae greatly exceeds the oil productivity of the best producing oil crops. Algal cultivation is not only able to provide biofuel, but also provides green house gas saving as it utilized large amount of CO_2 during cultivation.

The idea of using microalgae as a source of fuel is not new, but it is now being taken seriously because of the escalating price of petroleum and, more significantly, the emerging concern about global warming that is associated with burning fossil fuels. Algae can provide several different types of renewable biofuels. These include methane produced by anaerobic digestion of the algal biomass, biodiesel derived from microalgal oil, ethanol, and photobiologically produced bio-hydrogen. Hydrogen can be produced by algae in two different ways: by direct photolysis, and by indirect photolysis due to presence of enzymes like hydrogenase and nitrogenase in blue-green algae (Cyanobacteria).

Limitations associated with use of algae as biofuel:

The use of algae for the production of biofuel has a promising future. But, still there are certain limitations associated with the practical application of the same which must be removed to make it commercially feasible.

The major current problem for the commercial viability of biofuel derived from the algae is the low selling price of biofuel. The cost of production of biodiesel from the algae is still high in comparison to the existing market price of fossil fuel. With regard to biodiesel production, the productivity should be about 100 grams of algae per m^2 per day are needed to achieve commercial viability. Economics of producing algal biodiesel need to improve substantially to make it competitive with fossil fuel.

The bio-oil derived from the algae has high oxygen content of some 11% compared to fossil oil with 0.05 to 1.5%. Key to improving the bio-oil quality is increase in lipid content in biomass. But the methods which increase the lipid content in biomass are responsible for decreasing in productivity of the biomass.

The major factors affecting the cost of hydrogen production by algae are the cost of huge photo-bioreactors, cost of hydrogen storage facilities and requirement of adequate light through the night or during cloudy periods of day.

Ans. 5.

Toxins produced by Cyanobacteria:

Cyanobacterial toxins may be divided into three groups according to their effects on mammals as Neurotoxins, Hepatotoxins and Irritants.

Neurotoxins compose a group of toxins with a diverse chemical composition, although they produce similar symptoms eg. Anatoxin-a, Saxitoxins and neosaxitoxins. Anatoxin-a is the only naturally produced organophosphate known to date. Saxitoxins produce blockage of sodium ion channels while Anatoxin-a mimics acetylcholine and it binds to acetylcholine receptors inducing contraction in muscles. These toxins damage the central and peripheral nervous system.

Hepatotoxins may be divided in three groups: microcystin, nodularin and cylindrospermopsins according to their chemical composition. The first two are peptides while the last one is alkaloid. Microcystins and nodularins are mainly produced by *Microcystis* spp. and *Nodularia* spp. respectively. These two toxins disrupt the cytoskeleton and inhibit eukaryotic protein phosphatases 1 and 2A. Cylindrospermopsins are produced by *Cylindrospermopsis*, *Umezakia* and *Aphanizomenon*. Besides damaging the liver, this toxin induces pathological changes in kidneys, spleen, thymus and heart by inhibiting glutathione synthesis and protein synthesis.

Irritants are the toxins that may be considered least harmful to human beings. These toxins are produced mainly by *Lybgbya majuscule* and cause severe dermatitis and are tumor promoters.

Toxins produced by Dianoflagellates:

Dianoflagellate toxins are structurally and functionally diverse, and many present unique biological activities. Based upon the syndromes produced by the toxins these have been groups in to five major seafood poisoning syndromes.

1. Paralytic shellfish poisoning (PSP): In this group the main toxins are Saxitoxins and Gonyautoxin. Which are produced by *Alexandrium* spp. and *Gymnodinium* spp. respectively. These toxins block the voltage-gated sodium channel 1.

2. Neurotoxic shellfish poisoning (NSP): These symptoms are produced by Brevetoxin and Yessotoxins. The main toxin producing genera are *Kerenia brevis* and *Lingulodinium* respectively. These toxins affect the voltage-gated sodium channel 5 or voltage gated calcium or sodium channel.

3. Ciguatera Fish Poisoning (CFP): This syndrome is produced by Ciguatoxins which is the most commonly reported marine toxin in the world. It is produced by *Gambierdiscus toxicus*. It blocks the voltage gated sodium channel 5. Another toxin in this category is Maitotoxin which is also produced by *G. toxicus*. It blocks the voltage-gated calcium channel.

4. **Azaspiracid shellfish Poisoning (AZP):** It is comparatively new syndrome which is produced by azaspiracid. This toxin is produced by *Protoperidinium crassipes.* This toxin is significantly different from the other groups of toxins.

5. Palytoxin (PTX): It is very potent toxin in very low concentration. It is produced by *Ostrepsis siamensis*. This toxin blocks the Sodium-Potassium ATPase.

Ans. 6.

Phycoremediation

Phycoremediation, a type of bioremediation, can be defined in a broader sense as the use of macroalgae or microalgae for the removal or biotransformation of pollutants including nutrients and xenobiotics from wastewater and CO_2 from waste air. The metabolism of algae tackles simultaneously more than one problem, a solution not capable by conventional chemical processes for example, problems such as heavy metal removal, pH correction, sludge removal, TDS reduction, BOD and COD removal, etc. can be handled simultaneously by algal treatment (phycoremediation), whereas in conventional methods, separate methods or stages of treatments are used.

Thus, phycoremediation comprises several applications:

(a) nutrient removal from municipal wastewater and effluents rich in organic matter;

(b) nutrient and xenobiotic compounds removal with the aid of algae-based biosorbents;

(c) treatment of acidic and metal wastewaters.

The use of micro algae for the treatment of municipal wastewater has been subject of research and development for several decades. The cell wall of the algae has very high heavy metal binding capacity. Besides, they can synthesize various phytochelators for the intra-extra cellular sequestration of heavy metals. The result of such effort is that some commercial technologies and processes are available in the market such as the Advanced Integrated Wastewater Pond Systems (AIWPS).

Advantages of phycoremediation

We can effectively manage a wide range of industrial effluents to achieve the following:

1. Reduction in COD, BOD

2. Reduction in sludge

3. More than 90% reduction in operation costs in comparison to conventional method

4. Less space requirement and no chemicals are used

5. Ability of algae to tackle more than one problem simultaneously which is not possible by conventional physical and chemical methods.

6. The commercial benefits could be derived from the biomass and other extracellular products.

7. Cost effective as it saves energy and lot of chemicals.

8. Natural oxygenation of environment.

9. Possible co-production of biofertilisers, biogas and biofuel.

Disadvantages.

The disadvantages included along with the practicing of phycoremediation are hygienic aspects, unwanted contaminants and in many countries probably some concern regarding the use of human excretion products to produce consumables.

Ans 7.

Many cyanobacteria are also capable of using atmospheric dinitrogen (N_2) as the source of nitrogen at no cost and the most common method is termed as nitrogen fixation. Like in many other biological systems, nitrogen fixation in cyanobacteria, is brought about by a high molecular weight, oxygen labile, metalloprotein enzyme known as nitogenase. Nitrogenase reduces molecular nitrogen to ammonia in presence of hydrogen. Due to this important characteristic of nitrogen fixation, the cyanobacteria have an ability to enhance the maximum production in agriculture. Many studies have been reported on the use of dried cyanobacteria to inoculate soils as a means of aiding fertility, and the effect of adding cyanobacteria to soil on rice yield. The blue-green algae (e.g. *Aulosira, Anabaena, Cylindrospermum, Nostoc, Tolypothrix, Gloeotrichia, Rivularia, Plectonema, Oscillatoria*) are commonly found in the paddy fields. In water-logging condition, the cyanobacteria multiply, fix atmospheric N_2 and release it into the surroundings in the form of amino acids, proteins and other growth promoting substances. The cyanobacteria contribute about 20-30 kg N/ha/season as well as organic matter to the soil which is quite significant for the economically weak farmers because they are unable to invest purchase costly chemical nitrogen fertilizer.

Algalization

Venkataraman coined the term *'algalization'*. Algalization is the process in which there is an application of blue-green algal culture in a field as biofertilizer. He initiated algalization technology in India. Before algalization, mass cultivation of regional cyanobacterial starter culture is required.

Processes for Mass cultivation of cyanobacterial biofertilizers

According to Venkatraman, mixture of 5 or 6 regional acclimatized strains of cyanobacteria are generally used for starter inoculum for outdoor mass cultivation of cyanobacterial biofertilizers. The following four methods are used for mass cultivation :

(i) shallow metal troughs method
(ii) pit method
(iii) field method cemented tank method and
(iv) Nursury –cum-cyanobacterial method.

Shallow metal trough method:

In this method, 2m x3mx 23 cm of tray made of iron sheets are used. 8 to 10 kg of soil is transferred in this tray. Trough is filled with water and it is left to be undisturbed so that the suspended matter settles down. 200 gram superphosphate is added. 5 to 15 cm of water level is maintained in the tray. Soil pH is maintained through adding lime. Carbofuran (3%) is added to avoid insect infection. Once the soil is settled, clean culture is sprinkled in this tray. The unit is exposed to the sun in the open air. Water is added intermittently to make up the loss of water due to evaporation. During summer the inoculated cyanobacteria grow vigorously and form thick mat in 7 to 10 days. Then, watering is stopped so that mats are dried and cracks into the flakes which are collected and stored for field use. The flakes are removed from the tray and the tray is again filled with water and a handful of dried flakes are added as an inoculum for further propagation. The process can be repeated 3 to 4 times. Two harvests of flakes thus produce 3-5 kg of active material sufficient to biofertilize one hectare of rice field.

Pit method:

This is also a method for mass cultivation of cyanobacteria and this method is cheap and easy to do for small farmers in their own farm. Instead of trough, shallow pits are dug in the ground and lined with thick polythene sheet to hold the water. Rest process is same.

Field Method

It is a large scale version of trough or tank method and it is used for generating bulk quantities of cyanobacterial fertilizer. 40 square meters of plot is selected and soil is well puddle. Plots are flooded with water. Superphosphate (12 kg per plot) and furadon (250 gram per plot) are also added in the tank. The cyanobacterial inoculants are sprinkled over the water and the water level is maintained intermittently. In sunny weather and clayey soil, growth becomes optimal within two weeks while in loamy soil, it takes four weeks to achieve such growth. After making floating mat stage, watering is stopped and sundried cyanobacterial flakes are collected and stored in sacks for use at the farmer's field. Again the plots are reflooded and superphosphate as well as insecticides is also added. The cyanobacterial propagation and harvest can be repeated many times without further inoculum. In each harvest it biofertilize 16-30 kg/plot.

Nursery-cum-cyanobacterial method:

Farmers produce cyanobacterial biofertiliser along with rice seedlings. Plot of 40 square meter is used and rest is same as in field method. In this method, 15-20 kg cyanobacteria/plot is used to biofertilise 1.5 hactare of rice field.

Application of cyanobacteria and its benefits as a biofertilizer and in reclamation of usar soil

Application of cyanobacterial biofertilizer improves the physico-chemical properties of the soil by enriching them with carbon, nitrogen, available phosphorus, etc. There is considerable reduction of exchangeable sodium and soil pH (towards neutrality) by using cyanobacteria as a biofertiliser. Cyanobacteria also reduce sodium ion content of the soil by making calcium ions available through solubilisation of calcium carbonate nodules, possibly by releasing various organic acids like, oxalic-, oxaloacetic-, lactic-, succinic acids, etc. Protection against alkaline environment is also provided by the synthesis of certain fatty acids, sugars like sucrose and various osmotic stress-induced proteins. It increases the grain yield in rice by increasing N content of the grain and straw, leaf length, number of ears, number of spikelets per panicle and amount of dry matter. In addition to contributing nitrogen, cyanobacteria benefit crop plants also by producing various growth promoting substances (like gibberellins, auxins like indole-3-acetic acid, indole-3-propionic acid and vitamin B_{12}, free amino acids (like serine, arginine, glycine, aspartic acid, threonine, glutamic acid, etc), extra- and intra-cellular polysaccharides (like xylose, galactose, fructose) etc. Such substances have several beneficial effects like improved soil structure and stimulation of growth of crop plants.

Ans 8.

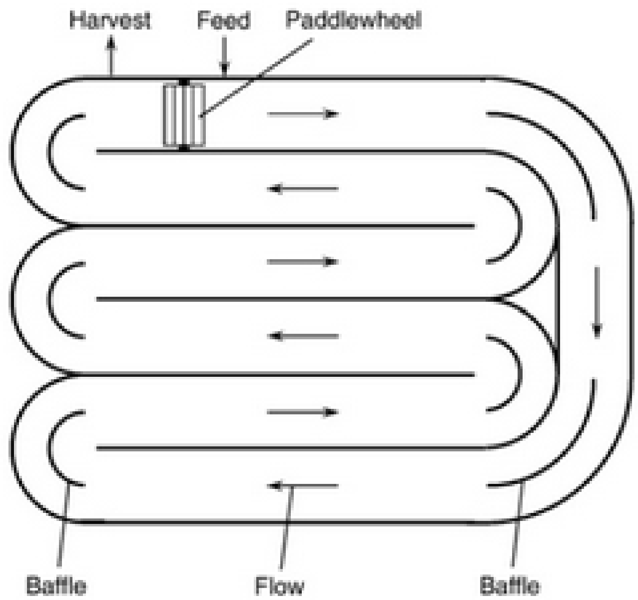
Methods for mass cultivation of microalgae

There are three main methods for mass cultivation of microalgae.

1. Open culture method 2. Hybrid method 3. Photobioreactor method

1. Open culture method: It is the oldest system which is still being used today at industrial level. The algae are cultured in their natural habitats (lake, pond or open sea, lagoons) or cultured in artificially constructed ponds. One of the major advantages of open ponds is that they are easier to construct and operate than most closed systems. However, major limitations in open ponds include poor light utilization by the cells, evaporative losses, diffusion of CO_2 to the atmosphere, and requirement of large areas of land. Furthermore, contamination by predators and other fast growing heterotrophs have restricted the commercial production of algae in open culture systems to only those organisms that can grow under extreme conditions. Also, due to inefficient stirring mechanisms in open cultivation systems, their mass transfer rates are very poor resulting to low biomass productivity.

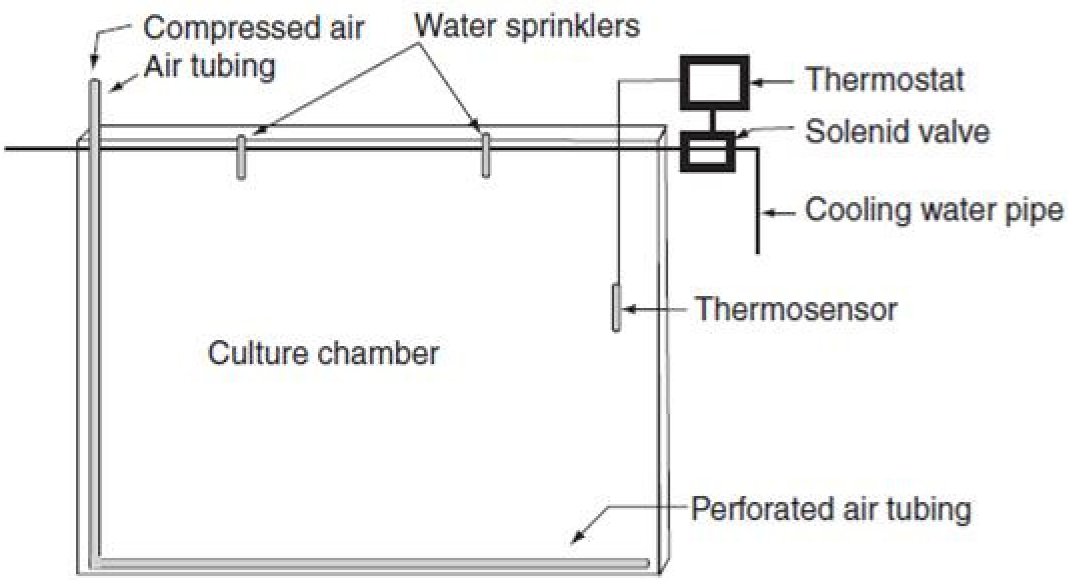
The biggest advantage of these open ponds is their simplicity, resulting in low production costs and low operating costs. While this is indeed the simplest of all the growing techniques, it has some drawbacks owing to the fact that the environment in and around the pond is not completely under control. Bad weather can stunt algae growth. Contamination from strains of bacteria or other outside organisms often results in undesirable species taking over the desired algae growing in the pond. The water in which the algae grow also has to be kept at a certain temperature, which can be difficult to maintain. Another drawback is the uneven light intensity and distribution within the pond.



Raceway Pond

2. Hybrid systems: An alternative to open ponds are closed ponds where the control over the environment is much better than that for the open ponds such systems are called Hybrid systems. Hybrid systems cost more than the open ponds, and considerably less than photobioreactors for similar areas of operation.

As a variation of the open pond system, the idea behind the closed pond is to close it off, to cover a pond or pool with a greenhouse. While this usually results in a smaller system, it does take care of many of the problems associated with an open system. It allows more species to be grown, it allows the species that are being grown to stay dominant, and it extends the growing season, only slightly if unheated, and if heated it can produce year round. It is also possible to increase the amount of carbon-di-oxide in these quasi-closed systems, thus again increasing the rate of growth of algae.



Hybrid system

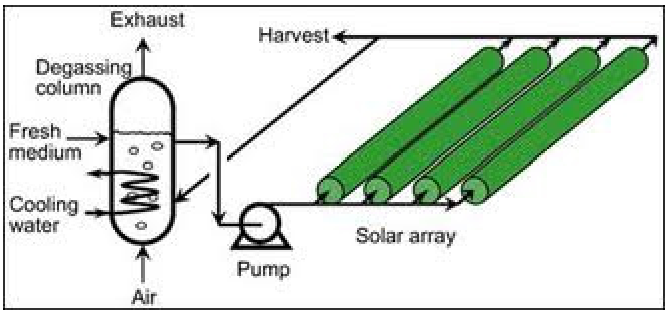
3. Photobioreactor method: A photobioreactor is closed equipment which provides a controlled environment and enables high productivity of algae. As it is a closed system, all growth requirements of algae are introduced into the system and controlled according to the requirements. PBRs facilitate better control of culture environment such as carbon dioxide supply, water supply, optimal temperature, efficient exposure to light, culture density, pH levels, gas supply rate, mixing regime, etc.

The photobioreactor itself is used to promote biological growth by controlling environmental parameters including light. The tubes are made of acrylic and are designed to have light and dark intervals to enhance the growth rate. The photobioreactor has a built-in cleaning system that internally cleans the tubes without stopping the production.

After the algae have completed the flow through the photo-bioreactor, it passes back to the feeding vessel. As it progresses through the hoses, the oxygen sensors determine how much oxygen has built up in the plant and this oxygen is released in the feeding vessel itself. It is also at this stage that the optical Cell Density sensor determines the harvesting rate. When the algae are ready for harvesting, they pass through the connected filtering system. This filter collects the algae that are ready for processing, while the remaining algae passes back to the feeding vessel.

Covering ponds does offer some of the benefits that are offered by photobioreactors, but enclosed systems will still provide better control of temperature, light intensity, better control of gas transfer, and larger surface area-to-volume ratio. An enclosed PBR design will enhance commercial algal biomass production by keeping algae genetics pure and reducing the possibility of parasite infestation.

Capital cost is very high. This is one of the most important bottlenecks that is hindering the progress of algal fuel industry. Despite higher biomass concentration and better control of culture parameters, data accumulated in the last two decades have shown that the productivity and production cost in some enclosed photobioreactor systems are not much better than those achievable in open-pond cultures. The technical difficulty in sterilizing these photobioreactors has hindered their application for algae culture for specific end-products such as high value pharmaceutical products.



Photobioreactor