SCALABLE ALGAE PRODUCTION SYSTEMS FOR WIDE APPLICATIONS

Dr. Simrat Kaur, Fatema Diwan, Brad Reddersen

Climate Survival Solutions

Executive Summary

As non-renewable energy resources became depleted and use of fossil fuels was discouraged, nations around the globe began to explore renewable alternatives.

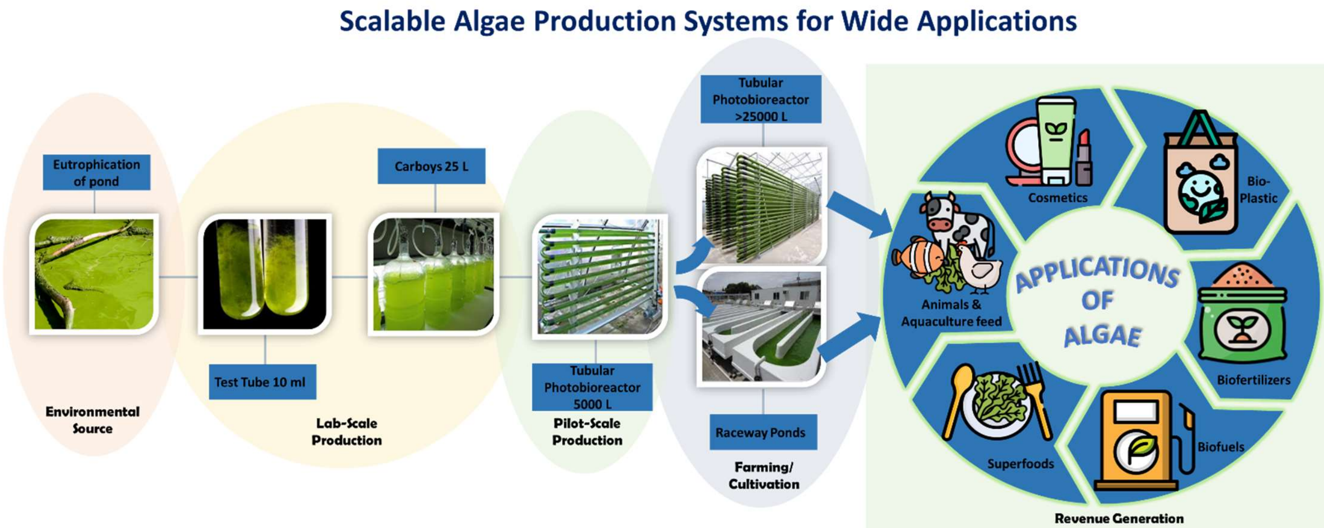
With that came the discovery of fossilized algae in organic remains of oil shales and petroleum source rocks, which in turn triggered advanced research and development efforts on how to obtain biofuels from microalgae. Among the discoveries which followed was that the hydrocarbon oils derived from the microalga *Botryococcus braunii* are now understand to be a major component of crude oil.

Algae are primitive photosynthetic organisms which had transformed Earth's reducing environment to an oxidized state. This evolutionary phenomenon is the most important contribution of algae which principally led to the origins of biodiversity on our planet. The combined photosynthetic CO_2 sequestration and release of oxygen by algae is equally revolutionary, as today's planet has become a greenhouse of toxic and thermogenic gases. The contributions of algae are enormous and most relevant in context of our rapidly degrading biosphere.

In addition to fossil fuels, we are diminishing and polluting many other precious resources of life such as freshwater, nutrient reserves (phosphorous), biodiversity, fresh air, soil and land. The urgency to restore all these resources is evident as we are witnessing global scale water-food shortages, toxic air pollution, loss of fertile soils and nutrient run-offs in the oceans. Algae provide sustainable solutions to mitigate the effects of these environmental crises at different scales. Wastewater is converted to various resources such as bio-fertilizers, biofuels and CO_2 capturing units, as well as greywater capable of recycling, using different types of microalgae. The biodiversity loss in the marine habitats due to ocean acidification can be tackled by direct CO_2 capture using algae in open race way ponds in coastal regions.

Algal diversity is an incredible bio-wealth which in future needs to be tapped to greater extent for securing our needs for food, clean water, fuel and life enriching biosphere. This white paper is intended to offer technical recommendations on various cultivation practices to successfully produce valuable microalgae biomass.

Graphical summary



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Introduction: The Past, Present and Future of Algae

Algae are the progenitors of the photosynthetic life forms which represent group of micro and macro organisms. These primitive photosynthetic organisms have played an important evolutionary role of creating oxidizing environment on planet Earth. In the presence of sunlight, CO_2 and water, algae use their specialized organelle called chloroplasts which provide surface area and cellular machinery that allow the breakdown of the water molecules to release oxygen into the environment.

The German botanist Theodor Wilhelm Engelmann performed a classic experiment on filamentous algae with spiral chloroplasts to demonstrate that photosynthetic organisms including plants do not like all kinds (wavelength) of light. High oxygen evolution from the chloroplasts takes place in blue and red ends of the spectrum where the aerobic bacteria tend to stick to the algae filament (Figure 1). The spectral range of photon absorption is a function of various accessory chlorophyll pigments such as Chls b, c, d and f; the latter two are only present in cyanobacteria which allow absorption of near-infra-red radiations. Cyanobacteria are also among the large group of prokaryotes which display ancestral adaptations to life on land such as tolerance to desiccation, high photosynthetic active radiation (PAR), high ultra violet (UV) radiations, and high salinity (Larkum, Ritchie, & Raven, 2018).

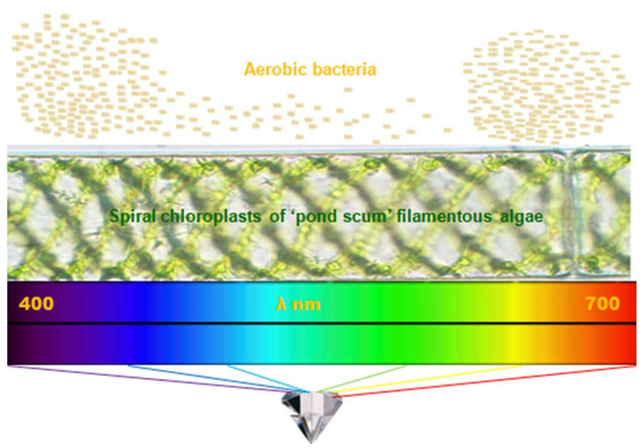

Figure 1. Photosynthetic oxygen evolution demonstrated in a classic experiment.

Oxygen is a potent electron acceptor which led to evolution of aerobic organisms and an enhanced productivity of the primitive oceans. On other hand, the reactive oxygen species promoted cellular compartmentalization and evolution of more complex life forms or in other words the enrichment of cellular biodiversity (De Clerck, Bogaert, & Leliaert, 2012). The origin of multicellular life forms is considered as major event in the eukaryotic (organisms in which the genetic material is present inside the nucleus) evolution. Algae possess remarkable cellular complexities in terms of their size, shape, and cell wall compositions. In fact, some of the largest and most complex single cell organism on our planet belongs to green microalgae, for example *Acetabularia* (Umen, 2014). The conquest of terrestrial ecosphere by oldest freshwater green algae led to the evolution of all multicellular land plants on Earth.

Microalgae are evolutionary significant groups of photosynthetic organisms because they have inherited genes from varied life forms such as photosynthetic organisms, heterotrophic eukaryotes and bacteria. This heterogeneity in their genetic framework allow algae to thrive in environments with varied conditions such as high turbulent coastal sites, freezing temperatures of polar ice, hot springs, high and low nutrient loadings, open oceans with high irradiance and desiccation.

Algae can thrive in habitats ranging from terrestrial, aerial, snow, hot, fresh and marine waters. An extant colonial microalga *Botryococcus braunii* found in fresh and brackish waters synthesize characteristic long-chain (C18-C40) liquid hydrocarbons which are the source of oils in Paleozoic oil-bearing rocks, boghead coals and oil shales. It indicated the noticeable contribution of *Botryococcus* to fossil fuel generation. The hydrocarbon oil content of 75% (dry weight) which is mostly cited in the path finding scientific articles on algae feedstock for biofuels is obtained from the *Botryococcus braunii* (Hillen, Pollard, Wake, & White, 1982) (Chisti, 2007). The discovery sparked interests in deriving fossil fuel alternatives from microalgae.

Other distinct groups of algae such as diatoms and coccolithophores possess unique cell walls made of silica and calcium carbonate respectively. The cell wall of diatoms which comes from silica dioxide (also called frustules) has survived for thousands of years as diatomaceous earth or diatomite. In addition to photosynthesis, the processes of calcification and silicification shown by these two groups allowed the precipitation of organic carbon and the hard materials to ocean interior. These two nutrient processes have huge geologic significance in the history of our planet. Due to large precipitation of calcium carbonate or 'coccoliths' and diatomite, the atmospheric CO_2 was captured into deep oceans followed by the formation of limestones, cherts, oil and gas reserves (Benoiston, et al., 2017).

Together with geo-climatic changes on Earth, the biological sequestration of CO_2 to deep oceans in the form of organic carbon and calcium carbonate by marine phytoplanktons had most likely contributed to the transformation of warm Mesozoic era to ice age in the Cenozoic (Benoiston, et al., 2017).

Depending upon the specific strain, algae produce variety of other biomolecules such as proteins, carbohydrates and fatty acids that have found its way into the humans, animals and aquaculture food and feed. The two algae *Spirulina* (blue green algae) and *Chlorella* (microalga) have become the well known brand names for alternative proteins. Both these species grow fast and are competitive. The *Spirulina* can easily be grown in warm alkaline waters. Due to this *Spirulina* is easy specie to cultivate in the open ponds as compared to other microalga such as *Haematococcus pluvialis* (rich and potent source of natural astaxanthin) which is highly prone to predation by zooplanktons and fungi. An excessive R & D on pest management and expertise to handle the delicate *Haematococcus pluvialis* is mandatory to make the open pond cultivation feasible and economical for commercial production of the astaxanthin.

We offer an effective pest management strategy to tackle different types of biological contaminants that impact microalgae productivities in production systems.

The Algae beyond 'pond-scum'

The negative connotation is often assigned to the algae such as pond-scum and sea-'weeds'. Common people look upon these as nuisance and toxic forming blooms in their lakes. Although the algal blooms in fresh and marine water bodies should be an acceptable natural response to our derogatory actions on the environment. Most of these bloom forming algae belong to cyanobacteria which should be thanked for three of the major deliverables to livable planet viz. production of oxygen, CO_2 sequestration and fixation of the molecular nitrogen.

So, next time when we observe algae blooming in our water bodies we should first curse our unsustainable methods of crop production, livestock farming, industrial and municipal discharges which provide 'loose' nutrients in the form of nitrogen, phosphate, and heavy metals. Next, we thank algae for showing mankind the path to mitigate the nutrient pollution in water bodies. Instead of letting undesired species to control these polluted aquatic habitats, the useful and selected members of algae can be employed to recycle, recover or restore all the nutrients. In fact, the sinking of vast algal blooms in the oceans after the nutrient sequestration highlights their importance in cycling of the inorganic and organic products (Hopes & Mock, 2015).

After decades of progress in the production systems for cultivating microalgae, we have now started realizing the dream of 'Algae Farming'. Compared to conventional farming, algae do not have obligatory requirements for fertile lands and freshwaters. Soil degradation is one of the most pressing issues of our times which is not just threatening our food production but is also cause of biodiversity loss. One of the reasons is mono-culturing of food and biofuel crops such as sugarcane and palm. Our fertile soils and habitats rich in biodiversity have become the victims of crony capitalism which is destroying natural productive systems in favor of their own interests in making 'unsustainable' and 'green washed' biofuels.

Algae come as powerful players in harnessing the energetic potential of wastewaters that allow us to reduce our water and energy footprints. Algae have remarkable capabilities to absorb nutrients such as nitrogen and phosphorous from the wastewaters and sequester the ever-increasing atmospheric carbon dioxide. As explained in the opening paragraph of this introduction, algae are potent emitters of molecular oxygen to attract aerobic bacteria which lead to increased efficiency of the aerobic wastewater treatment process. This property of algae to provide aeration to the bacterial co-symbiont has been found useful for reducing the capital costs associated with oxygen inputs to the facultative sewage treatment process. The symbiotic association between algae and aerobic bacteria also provides a basis for the future commercial success of the microbial fuel cell technology.

Wide variety of applications

The 'extinctions' of taxonomists represent a stumbling block to get a fair estimation about "how many species of algae are there?" (Guiry, 2012)*. While only around 44,000 algal species have been documented to date, the estimated number of species includes more than a million species. Irrespective of these figures, only handful of algae names is known in the commercial world. Imagining beyond the famous CHLORELLA, SCENEDESMUS, HAEMATOCOCCUS, SPIRULINA, PORPYRIDIUM, DUNALIEALLA, NOSTOC, diatoms, NANNOCHLOROPSIS, ISOCHRYIS would open up an avenue of countless applications. These would be far from the three F's-fuel, feed, and food.*

Environmental applications

The vivid habitat and species diversity of microalgae (Figure 2) play noteworthy roles in assuring ecosystem stability and achieving environmental sustainability. The word "environmental sustainability" refers to a responsible interaction with the environment by the present generation avoiding the depletion or degradation of the natural resources to maintain the long-term environmental quality.

Figure 2. Microalgae (*Euglena*) blooms in the wetlands receiving agriculture run-off in Assam, India. Reproduced with the permission of the author.

Sustainable practices ensure that the needs of the present generation are fulfilled without comprising the ability of the future generation to meet their own needs. The diverse developments in the algal sectors have stimulated the assurance of obtaining sustainability. The ever-increasing global population and rapid civilization have pushed mankind to exhaustively utilize environmental services and its vibrant resources. The un-exercised, urbanization, industrialization, and deforestation have drastically altered the balance of nature, leading to a devastating climate crisis.

Developments and advancements in the field of phycology and other applied fields have opened new doors for the green conversion and achieving environmental sustainability. Algae has proved to be the most promising candidate for being employed in development activities, as it reverts/replaces various needs and deeds of humans. A wide spectrum of algae is utilized in several applications to ensure a

suitable and sustainable environment for our succeeding generation. Few of the environmentally valuable algae are illustrated (
Table 1).

Carbon sequestration			Wastewater treatment			Phycoremediation		
Algae	**CO_2 (%)**	**CO_2 assimilation rate (g/L/d)**	**Algae**	**Wastewater type**	**% Removal of Ratio-nutrients**	**Algae**	**Phenomena Utilized**	**Hydrocarbon Substrate**
Chlorella vulgaris	10	0.25 (Eduardo Bittencourt, et al., 2010)	*Chlamydomonas reinhardtii*	Industrial Effluent (undiluted)	83% TKN 14.45% P (Kong, Ling, Martinez, Chen, & Ruan, 2010)	*Selenastrum capricornutum*	Bioaccumulation or biotransformation	BTEX, chlorobenzene, 1,2-dichlorobenzene, nitrobenzene, etc. (Semple, Cain, & Schmidt, 1999)
Scenedes-mus obliquus	10	0.55 (Shih-Hsin Ho, Chun-Yen Chen, Kuei-Ling Yeh, Wen-Ming Chen, Chiu-Yue Lin, & Jo-Shu Chang, 2010)	*Chroococcus sp.*	Greywater	89-98% TP9 8% NO_3-N 100% NH_3-N (Sanjeev Kumar, Kaushik, Malik, & Vijay, 2013)	*Prototheca zopii*	Biodegradation	Total Petroleum Hydrocarbon (TPH). (Walker, Colwell, & Petrakis, 1975)
Spirullina platensis	15	0.92 (Kumar, Yuan, Sahu, Dewulf, Ergas, & Langenhove, 2010)	*Chlorella* sp.	Effluent after secondary treatment (Domestic WW)	92% TN 86% TP (Min, et al., 2011)	*Agmenellum quadruplicatum*	Biotransformation	Naphthalene (Cerniglia, Gibson, & Van Baalen, 1979)
Botrycoccus braunii	10	0.64 (Rodas-Zuluaga, et al., 2020)	*Spirulina platensis*	Sago starchy wastewater (anaerobic digestion)	98.0% COD 99.9% NH3N 99.4% phosphate (Phang, Miah, Yeoh, & Hashmi, 2000)	*Chlorella sorokiniana*	Bio-removal	Phenanthrene (Raul, Guieysse, & Mattiasson, 2003)

Table 1. Some of the environmentally valuable algae

Alternatives to fossil fuels

Like several plants such as corn, soybeans, sugarcane, wood, and other plants, algae use photosynthesis to convert solar energy into chemical energy. They store the energy in the form of oils, proteins, and carbohydrates. The stored algal oil (lipids) can be efficiently converted into environmentally safe and clean biodiesel (Figure 3). The oleaginous microalgae suitable for biodiesel are listed in (Table 2).

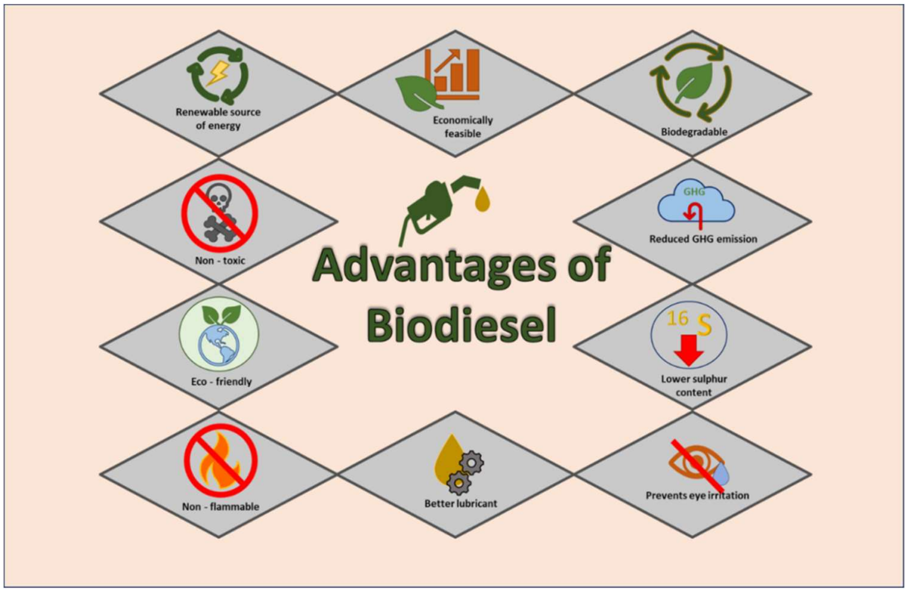

Figure 3. Several advantages of biodiesel.

Sr. No.	Microalgae	Oil Content (wt% of dry basis)
1.	*Chlorella sp.*	28-32
2.	*Botryococcus braunii*	25-75
3.	*Dunaliella primolecta*	23
4.	*Monallanthus salina*	>20
5.	*Nannochloropsis sp.*	31-68
6.	*Neochloris oleoabundans*	35-54
7.	*Schizochytrium sp.*	50-77

Table 2. Some of the high lipid producing microalgae species.

Microalgae biomass can act as a potential source of the fermentable substrate due to high levels of carbon compounds that are directly available for fermentation or after pre-treatment are successfully utilized for bioethanol production. The bioethanol available after refining and purification can be further utilized as motor fuel or as an additive to gasoline, providing an alternative "renewable source" of energy. Biohydrogen is another promising biofuel, obtained from algae. The production of photobiological hydrogen increases exponentially as per the carbon content of the algal biomass. The microalgae can be cultivated to produce hydrogen under optimized conditions in closed systems.

Algae derived bioplastics

Polyhydroxyalkanoates (PHAs) are the natural polyester comprising of the units of hydroxy-alkanoic acids with similar properties to petroleum plastic (Mei-Hui Jau, et al., 2005). These PHAs are bio-accumulated by the microalgae as a reserve source of carbon and energy. Other classes of polymeric compounds such as polyhydroxybutyrate (PHB) and its copolymer polyhydroxybutyrate-co-valerate (PHB-HV) are efficiently synthesized by cyanobacteria under several specific cultivation conditions (Sharma, Singh, Panda, & Mallick, 2007). Owning to physical and chemical properties, PHB can be easily processed in the equipment commonly utilized for polyolefins and synthetic plastics. Some of the algal species and the bioplastics obtained from them are as listed:

- *Chlorella* – Chlorella/PVA blend film. (Sabathini, Windiani, & Gozan, 2018)
- *Spirulina platensis* – bioplastic biofilm. (Dianursanti, Noviasari, Windiani, & Gozan, 2019)
- *Phaeodactylum tricornutum*–bioplastic PHB (Hempel, et al., 2011)
- *Calothrix scytonemicola* –PHA, plastic film (Johnsson & Steuer, 2018)
- *Scenedesmus almeriensis* – bio-based plastic film (Johnsson & Steuer, 2018)

Algal biofertilizers

Nitrogen is one of the major nutrients required by crops for their growth and development. Though, nitrogen is found abundantly in the atmosphere but is not readily absorbed by the crops. This demands the utilization of synthetic nitrogen fertilizers to provide nitrogen to crops. Bio-fertilizers derived from the various blue-green algae (BGA) species namely *Nostoc sp*., *Anabaena sp., Tolypothrix, Aulosira sp*. efficiently fix the atmospheric nitrogen in moist soil, especially in paddy fields. The algae can either be unicellular or filamentous. The filamentous BGA possess specialized cells known as heterocysts (Figure 4) (e.g. Anabaena and Nostoc) (Bhatnagar, 2019) which are the sites for nitrogen fixation.

Biofertilizers assume a crucial function in supplying several nutrients to the soil for efficient uptake of essential elements by the crops. Biofertilizers fix the atmospheric nitrogen in the soil and convert them into a usable form for crops. They substantially convert the insoluble phosphate form to readily absorbable form taken up by plants. Similarly, biofertilizers induce root growth by the production of growth hormones, vitamins, and amino acids (Yeeun, Minjeong, Changki, Suyea, & Seonghoe, 2021). Biofertilizers equally enhance the nutrient cycling in the soil and ameliorate several adverse soil conditions. Additionally, they improve the immunity of plants and prevent several pest and disease outbreaks.

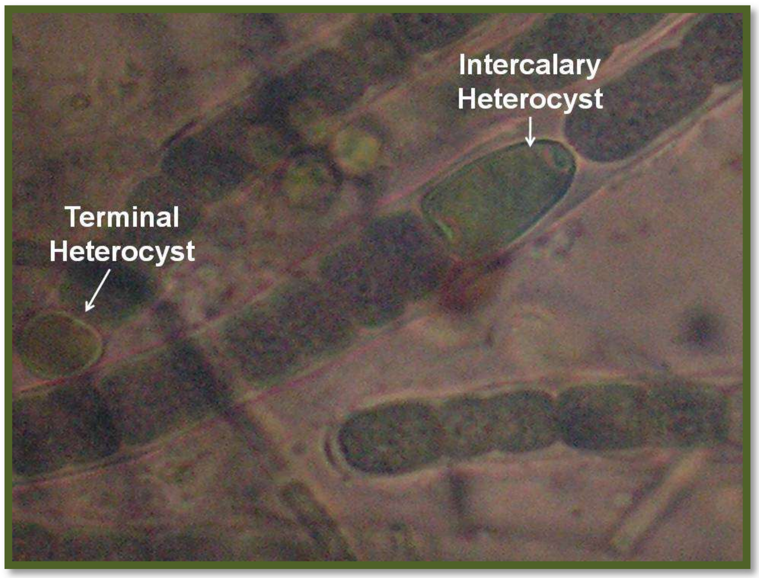

Figure 4. Microscopic image of filamentous BGA showing two types of specialized nitrogen fixation cells called heterocyst. Courtesy by Author.

Aquaculture and animal feed

Microalgae essentially form the base of the aquatic food chain due to their high nutritional value. As a rich source of protein, microalgae promote the growth of aquaculture organisms. For example, the biomass of *Spirulina plantensis* consist of 65– 77 % of dry weight proteins which is rich in several essential amino acids (lysine and threonine) is sustainable replacement for the low protein cereal feed. Spirulina feed could promote growth, enhance digestion and feed uptake, and improve immunity and survival rate of shrimps. The protein content of marine alga *Isochrysis sp.* account for 30.5% on dry weight bases (Bleakley & Hayes, 2021) is a potential meal in aquaculture of marine fishes.

Microalgae which are good source of β-carotene, astaxanthin and lutein aid in improving the organism's color. Their high polysaccharides content is essential for enhancing the immunity of the organisms. Microalgae are also a rich source of polyunsaturated fatty acids (such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) which enhances the nutritive value of the aquatic organisms. *Chlorella* is the major species utilized as the feed for aquaculture around the world. *Chlorella minutissima* (unicellular algae without flagella) demonstrate several characteristics (such as easy cultivation, rapid growth, high amount of amino acids and polyunsaturated fatty acids (PUFAs) making them an ideal choice for aquaculture feed.

Microalgae as feed powder

The utilization of microalgae in a powder form primarily involves the selection of species illustrating the desired nutrients needs. For example, choosing out the microalgae comprising; high protein content (such as *Spirulina platensis*), high unsaturated fatty acids content (such as *Schizochytrium* and *Nannochloropsis oculata*), and rich in pigment source (such as *Spirulina*) (Li, et al., 2015). The selection and screening of algae are followed by the culturing of these algae on large scale to get algal powder. The powder is subsequently added to the feed of different proportions to benefit the organisms and to study the effect of microalgae on the body colour, development, immunity, and meat quality of farmed organisms.

Algae as Animal feed

Algae are a rich source of several nutritive compounds, promoting the production of the value products and enhancing the animal's health. The inclusion of microalgal biomass into feeds provides vitamins, amino acids, polysaccharides, mono- and n-3 and n-6 polyunsaturated fatty acids (MUFA and n-3 and n-6 PUFA, respectively), minerals, and pigments (such as carotenoids and chlorophylls (Priyadarshani & Rath, 2012). Microalgae significantly provide PUFA and pigments that improve meat coloration and their antioxidant properties. *Schizochytrium* sp., *Chlorella* sp., *Arthrospira* sp., *Isochrysis* sp., *Porphyridium* sp., *Pavlova* sp., and *Nannochloropsis* sp., are major microalgae species utilized or supplemented in animal feed (Madeira, et al.).

Poultry produces a huge amount of meat worldwide. The utilization of protein-rich microalgae to substitute the vegetable protein in poultry feed lessens the stress on agriculture. Chickens reared with microalgal feeds portray higher weight gain, higher feed efficiency, and declined food intake. A study illustrates reduced cholesterol, elevated astaxanthin levels, and carotenoid pigmentation in egg yolk by utilization of *Haematococcus pluvialis* as feed (Dineshbabu, Goswami, Kumar, Sinha, & Das, 2019).

Human nutrition- super foods for body and skin

Superfoods are foods having a high nutritional value, with a low number of calories. They comprise a high number of antioxidants (naturally occurring molecules in the food) which neutralize the free radicals formed in the body as the by-product of energy generation in the body. Free radicals in the body can lead to heart disease, stroke, respiratory disease, immune deficiency, arthritis, cancer, emphysema, Parkinson's disease, etc. Superfoods are not cure-all food and are only beneficial along with a balanced diet. Currently, there has been a steep rise in the demand for superfoods (such as microalgae), not only for their nutritive characteristics but also to support sustainable food cultivation techniques.

Microalgae such as *Spirulina, Chlorella, Dunaliella, Haematococcus, and Schizochytrium* have been categorized as food sources in GRAS (Generally Regarded as Safe) category by the U.S. Food and Drug Administration.

The widely utilized microalga with several desirable characteristics is *Spirulina* which is usually cultivated on the large scale and forms 70% of the global market of dietary supplements for cattle and human consumption (Wikfors & Ohno, 2001). *Spirulina* is considered as super food, owing to its chemical composition. It comprises high protein content (65% - 77%) of its dry weight (Bleakley & Hayes, 2021), a balanced dose of carbohydrates (12-25%), lipids, and essential amino acids (18%), vitamins (Vitamin E, Vitamin B12) and pigments (Carotenoids, Chlorophyll a, Phycocyanin), minerals (such as calcium, magnesium, phosphorus, sodium, potassium, and iron) (Vrenna, Peruccio, Liu, Zhong, & Sun, 2021). The active ingredients of Spirulina also have remarkable anti-ageing, anti-inflammatory, anti-wrinkle and collagen synthesis properties due to which Spirulina has also established a niche in the cosmetic industry.

The nutritive content of *Spirulina* as compared to some of the other foods is as mentioned under:

- Calcium – 180% > whole milk
- Protein – 670% > tofu
- β carotene – 3100% > carrots
- Iron – 5100% > spinach
- More antioxidant and anti-inflammatory activity in 3g of *Spirulina* as compared to 5 servings of fruits and vegetables (Moorhead, Capelli, & Cysewski, 2005)

Several challenges need to be addressed by food scientists and engineers to make microalgae the mainstream food for human consumption. (Table 3)

Sr. No.	Parameter	Challenges
1.	Cost-effective production	• Formulating novel growth media • Energy-efficient systems • Attaining constant biomass productivity
2.	Recovery and Preservation of biomass	• Effective dewatering systems • Designing drying process with minimum damage to desirable biomolecules • To ensure stability of algal compounds
3.	Environmental sustainability	• To develop energy-efficient systems • To reduce freshwater footprints • CO_2 recycling
4.	Safety issues	• To regulate biomass safety • To adhere to clean production protocols • Conducting safety regulations on the algal biomolecules
5.	Food technology & engineering	• Innovative product development • Utilizing new processing technologies • To establish new packaging strategies to preserve the bioactivity of extracted compounds
6.	Consumer acceptance	• To attain good flavour and odour masking properties • Consumer outreach about unconventional microalgae products

Table 3. Challenges associated with adoption of microalgae derived unconventional foods.

Why choose fewer when many could be cultivated?

Despite there being over 40,000 published algae species, only handful of strains have been successfully cultivated. The four widely cultivated microalgae genera worldwide are *Spirulina*, *Chlorella*, *Haematococcus* and *Nannochloropsis*. These microalgae have been cultivated for decades for their biotechnological applications mainly as nutrient supplements and as biodiesel feedstock. Besides these freshwater microalgae, the other widely grown marine microalgae that are commercially grown in different countries of

Europe are *Tetraselmis* sp., Tisocrysis *lutea*, *Porphyridium* sp., and species of diatoms such as *Phaeodactylum tricornutum*, *Thalassiosira* sp., *Chaetoceros muelleri* (Araújo, et al., 2021). The cyanobacterium *Spirulina* is regarded as a "super food" and shares a large number of companies in Europe (Figure 5), most of which are located in France (Araújo, et al., 2021). There are expensive and laborious administrative burdens as any new specie to be exploited commercially must go through the Novel Food Regulation in Europe; therefore, the stake holders resist exploiting the new microalgae (Araújo, et al., 2021).

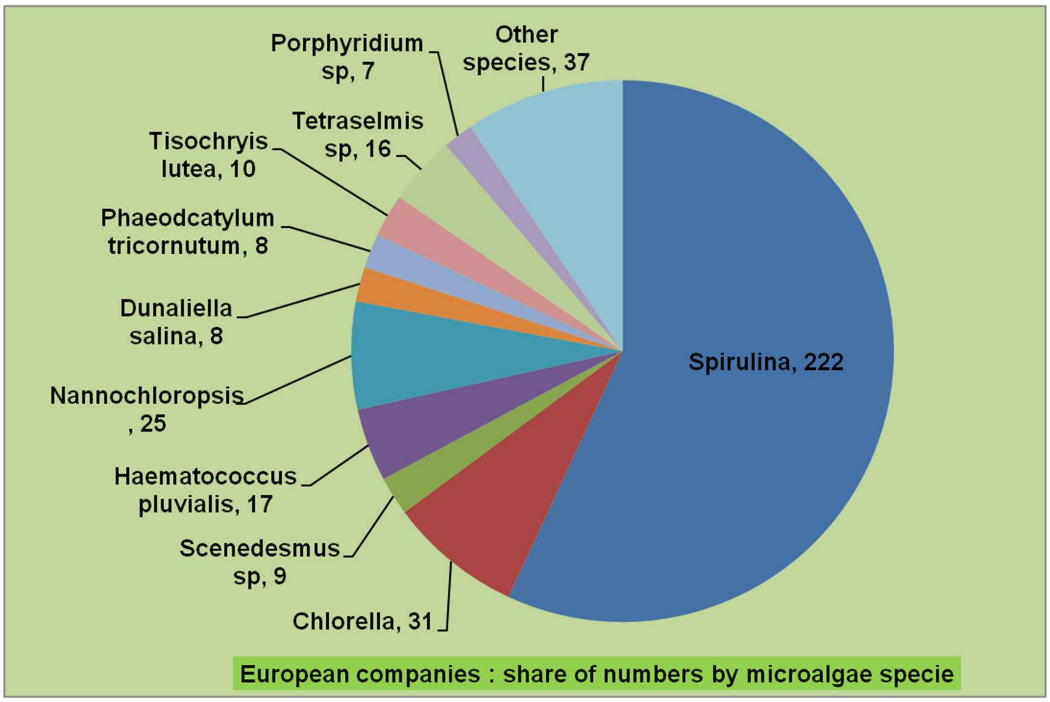

Figure 5. Number of microalgae companies in Europe by species. The 37 other companies grow *Thalassiosira* sp, *Acutodesmus obliqus*, *Chaetoceros muelleri*, *Cyanidium caldarium*, *Euglena gracilis*, and *Odentella aurita*. Data source: (Araújo, et al., 2021).

Ecologically, algae sit at the bottom of the food pyramid as primary producers. Whether in the freshwaters or in marine ecosystems, both small (zooplanktons) (Figure 6) and large (fish) herbivores feed on the algae to obtain their nutrition. In addition, other algal species and the non-photosynthetic microorganisms such as certain bacteria and fungi act as competitors for the inorganic and organic nutrients in their niches. Beside the biotic factors, the abiotic environment is also crucial for the growth of algae. As progenitors of plants and old group of organisms, algae must have witnessed the dynamic environments during the process of evolution on Earth. Perhaps, this is the reason that algae inhabit diverse forms of habitat.

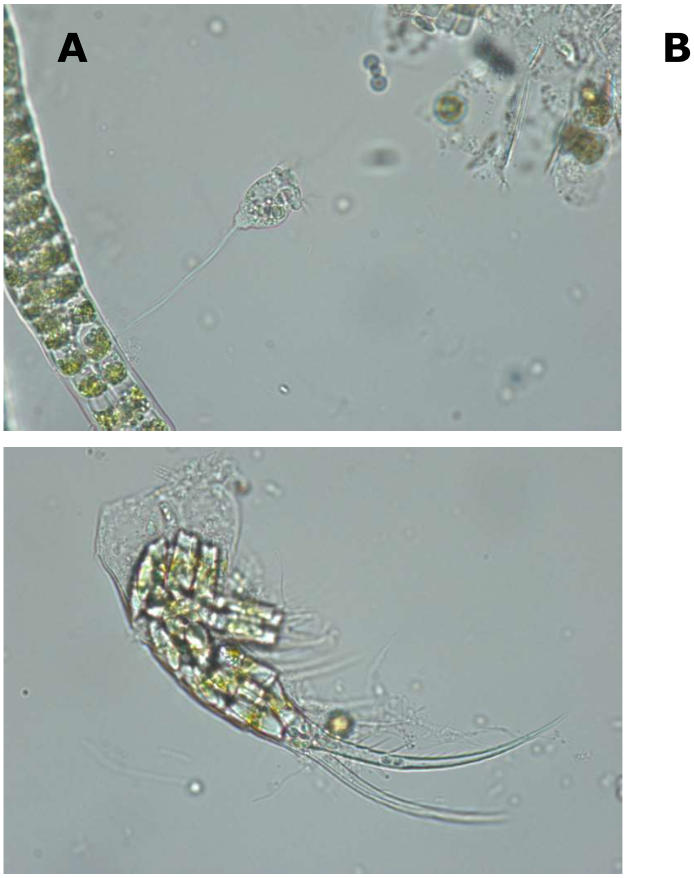

Figure 6. Microscopic images of surface sea water samples showing A) a zooplankton attached to the filamentous microalga B) diatom cells inside a zooplankton. Courtesy by author.

Certain algae such as diatoms, which have originated through unique evolutionary phenomenon, possess siliceous cell wall structures; owing to which their nutrient requirements differ significantly from other types of algae. Similarly there are other unique members of algae called coccolithophores which live abundantly in upper layers of the ocean and have characteristic $CaCO_3$ miniscule extracellular structures or shell named coccoliths. The coccolithophores play important roles in carbon cycling dynamics over short and geologic time scales (Müller, 2019).

Algae display a diverse range of trophic modes such as autotrophic, heterotrophic and mixotrophic. Recently, a group of researchers have isolated a dark tolerant diatom with facultative heterotrophic function from a depth of 1000 m in the Western Pacific Ocean (Mou, et al., 2022). The metabolic properties of algae that allow them to grow under different trophic modes are advantageous for their utilization in wastewater biological treatment process. Most of us are aware of the harmful algal blooms due to excessive nutrient loadings in the freshwater lakes which pose threats to humans and animals.

However, these provide important opportunities to understand how varying nitrogen and phosphorous levels impact metabolic functions, abundance and dominance of specific species of algae particularly the cyanobacteria. It is crucial to understand the relationship between nutrient dynamics and algal community structures in various ecosystems.

The following sections provide 'toolkit' and 'troubleshooting' guide to overcome various constraints that block the road to cultivate many more algae species. Perhaps the 'un-scientific' factor is the biased selection of only 'commercial' strains to avoid the risks associated with cultivation of new strains.

Nutrient ratio -- an easy tool to control algae

The power of elemental stoichiometry not only describes the biological mechanisms of ocean life but is a potent tool in our hands to control the growth of desired specie of algae. The classic research in early 1930's by prominent marine biologist Alfred Redfield revealed remarkable consistency in N:P ratio across all ocean basins and depths (Editorial, 2014). His results showed a strong linear correlation between dissolved nitrogen and phosphate levels in the oceans. The Redfield ratio is based on the observation that marine life has a fixed C:N:P ratio of 106:16:1; this concept has become a cornerstone in understanding marine carbon and nutrient cycling (Planavsky, 2014).

In this section, we will guide the users on how to utilize the concept of Redfield ratio to optimize the growth of the desired microalgae in their cultivation systems (more relevant in open systems and ponds). Nitrogen and phosphorous are two major elements required for the growth of organisms including algae; however, the amounts of these nutrients required varies with the species. The blue green algae which can fix the atmospheric nitrogen require more phosphate (Table 4), in contrast to green algae which instead require high amounts of nitrogen.

Phosphate PO_4 (mg/l)	Nitrate NO_3 (mg/l)							
	0.01	1	2.5	5	7.5	10	15	20
0.01	1.53	153	382.5	765	1147.5	1530	2295	3060
0.05	0.306	30.6	76.5	153	229.5	306	459	612
0.1	0.153	15.3	38.25	76.5	114.75	153	229.5	306
0.2	0.0765	7.65	19.125	38.25	57.375	76.5	114.75	153
0.3	0.051	5.1	12.75	25.5	38.25	51	76.5	102
0.5	0.0306	3.06	7.65	15.3	22.95	30.6	45.9	61.2
1	0.0153	1.53	3.825	7.65	11.475	15.3	22.95	30.6
1.5	0.0102	1.02	2.55	5.1	7.65	10.2	15.3	20.4
2	0.00765	0.765	1.9125	3.825	5.7375	7.65	11.475	15.3
Favor blue green algae growth			Favor green algae growth			Specie profile does not change		

Table 4. The stoichiometry guide to select the favorable nutrient profile (N:P) for target group of microalgae. Adapted from: https://buddendo.home.xs4all.nl/aquarium/redfield_eng.htm#algen.

The calculated values presented in Table 4 provide a guiding tool to optimize the growth of a particular group of algae in their open production systems. By changing the concentrations of nitrate and phosphate one could easily eliminate the contaminant algae. For example, the contamination of blue green algae (Figure 7) in open ponds can easily be eliminated by manipulating the nitrate and phosphate concentrations. There could be several scenarios in which these manipulations can be tricky and thus require expertise. The intelligent application of this tool save expensive resources by optimizing the use of nutrients and by preventing or overcoming culture crashes due to non-target algae species.

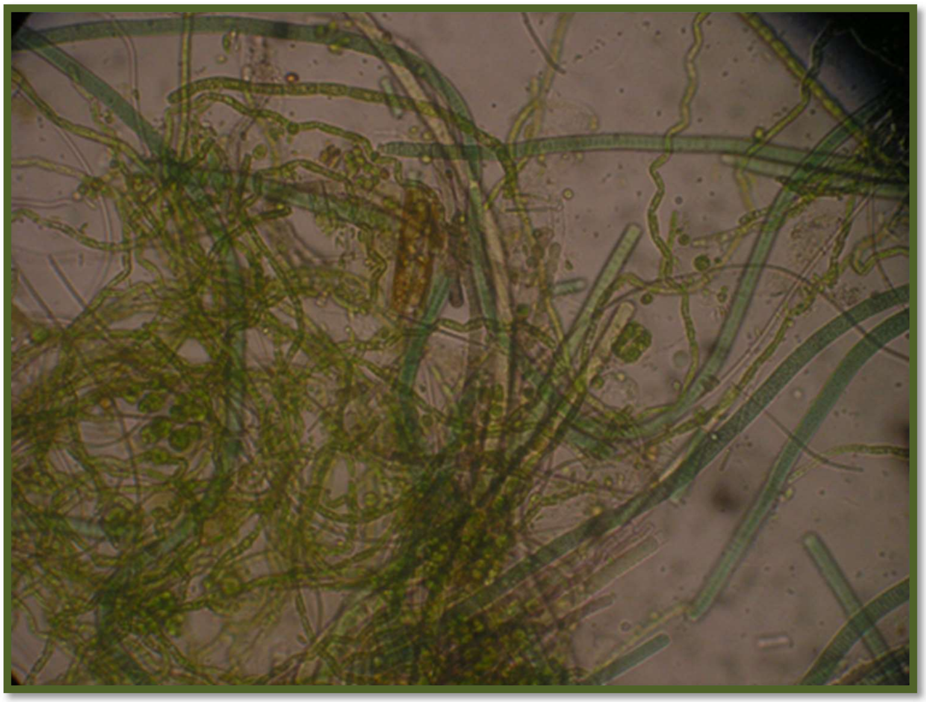

Figure 7. Sample from freshwater habitats of algae showing profuse growth of cyanobacteria as seen under the microscope. Image courtesy of the author.

Wastewater as sustainable substitute for nutrients

Microalgae biomass production has lots of nutrient requirement, particularly for N, P, K and Mg which are expensive and are obtained from the exhaustible resources. Therefore, there is a dire need for economically viable and environmentally sustainable replacements for the nutrients source. Algae can recover nutrients from various types of wastewater sources such as domestic, municipal, agro-livestock (Figure 2), industrial piggery and food processing. Wastewaters such as from the domestic sources not only contain nutrients but variety of other abiotic and biotic elements such as suspended solids, organic carbon compounds and microbes (bacteria, fungi, protozoa, viruses). It is necessary to assess the nutrients, suspended solid particles, turbidity, and biological loads in wastewater to strategically develop models that allow algae mediated nutrient recovery and reclamation of wastewater. For details about the upstream processes involved in phycoremediation of wastewater see our whitepapers (Kaur, Diwan, & M., 2021) (Kaur, Diwan, & Reddersen, 2022).

Light – maintaining a delicate balance

The term photosynthesis literally means synthesis in the presence of light and therefore access to light is a primary requirement for cultivating algae. The reason we have covered light after nutrient is because under natural conditions, it is not an easy to control parameter. Fortunately, algae can easily adapt to the natural variations in light intensity and quality with the aid of their own photosynthetic structures and pigments. Under extreme high light conditions, the accessory pigments of algae such as carotenoids and xanthophylls shield the primary pigments namely chlorophylls a, b. Another way of escaping high light is by moving away from the source through production of motile cells (in some specialist algae such as *Haematococcus*). Light acts as stress in young cultures of algae, both in laboratory and in the production systems.

As light is mandatory requirement when we grow and cultivate algae in autotrophic nutrition mode. The source of light is either natural or artificial using LEDs (efficient and cost-effective). Although controlling the quality and quantity of light when cultivating algae using sunlight is least or not possible; however, as it is the only sustainable and cost-effective approach, we suggest some measures highlighted in Box 1 to attenuate natural light conditions.

Box 1. Measures to control light requirement during algae cultivation

- *Increase inoculums density (cells/ml)*
- *Shade cultures using translucent material*
- *Start inoculation late afternoon or in evening to minimize bright sunlight exposures*
- *Select strain according to geographical locations*

Some of the measures in Box 1 are equally applicable for production systems that rely on the use of artificial light. The light attenuation in photobioreactors (PBRs) that have in-built lighting requires expertise in process engineering. Such PBRs should operate on automated control system that allows users to adjust the illumination.

Light adjustments and growth of cultures run in parallel. The young or newly inoculated cultures are prone to photo inhibitions as more light is available per cell. In contrast, as the cultures become dense and old, the amount of light available to individual cells is lowered due to the phenomenon of self-shading. It is therefore necessary to provide low light during the lag phase when cells are making adjustments to their new environment; and as the culture progresses, gradual increases in the illumination favors optimal growth without photo bleaching the cells. Maximum light should be provided in the old cultures near harvest when light becomes a limiting factor.

Aeration -- as a means for resource mixing, reduction in fouling and further attenuation of light

Although raceways represent the closest form of natural habitats for algae; the specialized production systems called photobioreactors (PBRs) have come along through a series of developments to provide optimal habitat for enriched growth of microalgae. The PBRs are equipped with specific control systems to administer optimal pH, aeration (CO_2-O_2 gas exchange), mixing, temperature and lighting. The major variations in the design of PBRs are geometry or shape (tubular, flat panel, helical, cylindrical or tank), illumination source, mixers and aeration apparatus.

In closed PBRs, the mass transfer or gas exchange between substrate (CO_2) and product (O_2) is crucial to ensure maximum photosynthetic efficiency of the system. It is important to maintain the state of homogeneity within the microalgae cultures. In other words, there should be uniform distribution of light, nutrient, CO_2 and microalgae (no aggregates or cell clumping) which is achieved through design engineering of the PBRs. In accordance to the Henry's law which states that "at a constant temperature, the amount of a given gas that dissolves in a given type and volume of liquid is directly proportional to the partial pressure of that gas in equilibrium with that liquid", maintaining a constant temperature is therefore crucial to have uniform dissolution of CO_2 in culture medium.

Mixing of the cultures inside PBRs is therefore crucial for attaining higher photosynthetic efficiencies and to maximize biomass productivity. Mixing allow uniform distribution of nutrients, light and gas exchange. In addition, mixing is important to avoid biofouling at the bottom or on the surface walls of the PBR which would obstruct light penetration; thereby decreasing the biomass productivity. The liquid velocities due to mixing should be below 50 cm/s as microalgae cells are susceptible to shear stress and cell damage above 100 cm/s due to the production of micro eddies (Osama, Hosney, & Moussa, 2021).

Based on geometry, there are several forms of PBRs namely tubular, air-lift, flat panel, stirred tanks and hybrids which are mainly constructed using glass, PVC, acrylic or stainless steel. Depending upon the design, the mixing is done in three different ways- i) mechanical agitation using an impeller, ii) by sparging air-CO_2 bubbles, and iii) combination of aeration and mechanical agitation (Osama, Hosney, & Moussa, 2021). Besides the gas-exchange mixing affects the distribution and utilization of light within the PBRs.

There are two important biological properties of microalgae cells that need consideration while designing the mixing or aeration systems in the PBRs:- 1) microalgae cells tend to move towards the source of light, and 2) become less buoyant when the cells are either get clumped or become bigger in size (depending upon the specie) during cultivation period. Therefore, the drawbacks of air-lifts systems, where mixing is attained by using compressed air, such as that in the column based PBRs is biofouling on the surfaces and biomass settlements at the bottom of the column or tube (Figure 8). However, the columns PBRs have low operating costs and can be easily constructed by any local contractor. Due to these reasons column PBRs have high applicability for pilot scale studies or for household applications.

Hybrid mixing involving mechanical agitation and aeration allow further 'fine-tuning' or light attenuation based on the phenomena of flashing light effect on microalgae cultures. The perpetual movement of microalgae cells from saturated light at the surface or in vicinity of light source to darker zones i.e towards the center of the PBR or at the bottom of ORPs creates flashing light effects which enhance the photosynthetic efficiency of the cultures (Osama, Hosney, & Moussa, 2021).

Figure 8. Air-lift column PBRs showing drawbacks of fouling and biomass settling.

Strain specific selection of cultivation system

There are numerous types of microalgae cultivation systems which are both open and closed. The two most important aspects of any of these cultivation systems are the availability of sufficient amounts of light and aeration. Of the various open production systems such as ponds and tanks; the shallow raceway ponds (30-40 cm depth) offer most reliable yields due to better penetration of sunlight. The open raceway pond cultivation has now been well adopted for certain specialist algae such as the cyanobacterium *Spirulina* which require warm alkaline waters for its growth. This minimizes the issues of contamination by common algae and other microorganisms. The open raceway ponds (ORPs) are the most economical and comparatively low maintaining cultivation systems in many parts of the world, particularly near tropics, in deserts and in regions where there is vast availability of wastelands.

To obtain best yields from ORPs, the selection of microalgae is crucial factor. Apart from highly competitive species including *Chlorella* and specialist strain like *Spirulina*; the other commercially grown microalga which is preferably grown in ORPs is *Haematococcus pluvialis* which has two phase cultivation process due to its unique biological growth cycle (Figure 9). During the green vegetative phase, the cultures of *H. pluvialis* are either grown in closed raceway ponds or in glass tubular photobioreactors mainly to avoid contaminations and photoinhibition (**Error! Reference source not found.**). To avoid photo bleaching in newly inoculated young cultures of *H. pluvialis*, it is mandatory to follow all the rules provided in (Box 1).

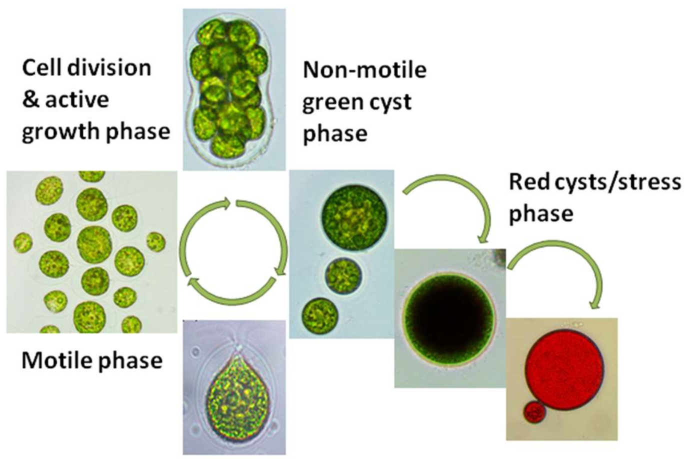

Figure 9. Life cycle of astaxanthin hyper accumulating microalga *Haematococcus pluvialis* showing the green vegetative growth phase and the red astaxanthin accumulating phase.

The retention time or the number of days algal culture stays in the ponds depend upon multiple factors such as geographical location, duration of sunlight, growth rate of algae, seasonal variations and the main objective for cultivation. During summers when ample amounts of sunlight is available the retention time is considerably reduced; however in winter both quality and quantity of light along with sub-optimal temperatures can prolong the cultivation time and can delay the harvest. The retention time also depend on the biomass utilization. When algae are cultivated for proteins such as in case of Spirulina, the cultures are harvested prior to any nutrient limitation. In contrast, when algae such as *Chlorella*, *Scendesmus*, *Nannochloropsis* and *H. pluvialis* are used, the harvest is mainly done after the cultures have attained nutrient limitations which is necessary to trigger the lipid production. The retention time for *H. pluvialis* in ORPs (Figure 10) include 5-7 days of growth during green phase and is variable for the red phase. The bright and long duration of sunlight is necessary for stressing the nutrient starved cells to form green cyst that start synthesizing astaxanthin. The astaxanthin accumulation in red cysts (Figure 9) can be obtained within 10 days during summer months but it can take upto 30 days during cold winter months.

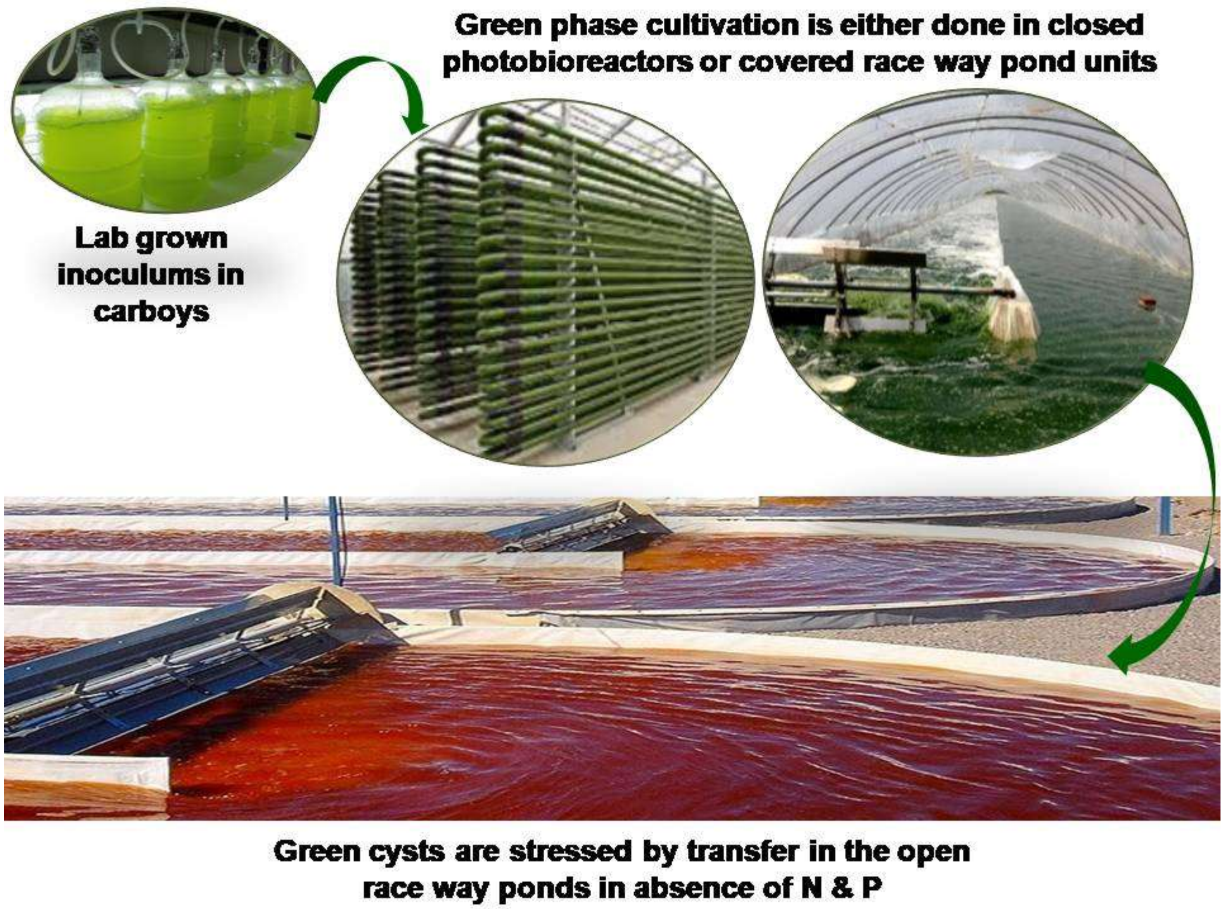

Figure 10. Different operations involved in the open race way pond farming of *Haematococcus pluvialis* for commercial production of astaxanthin.

During shady days when the duration and the amount of sunlight received by cells are lowered, photosynthetic yields is decreased. One of the ways to counteract is by using OPRs which are equipped with LEDs to supplement illumination during shorter days **(**Figure 11**).** ***In the following section we have unique recommendations to address season dependent loss of algal productivities.***

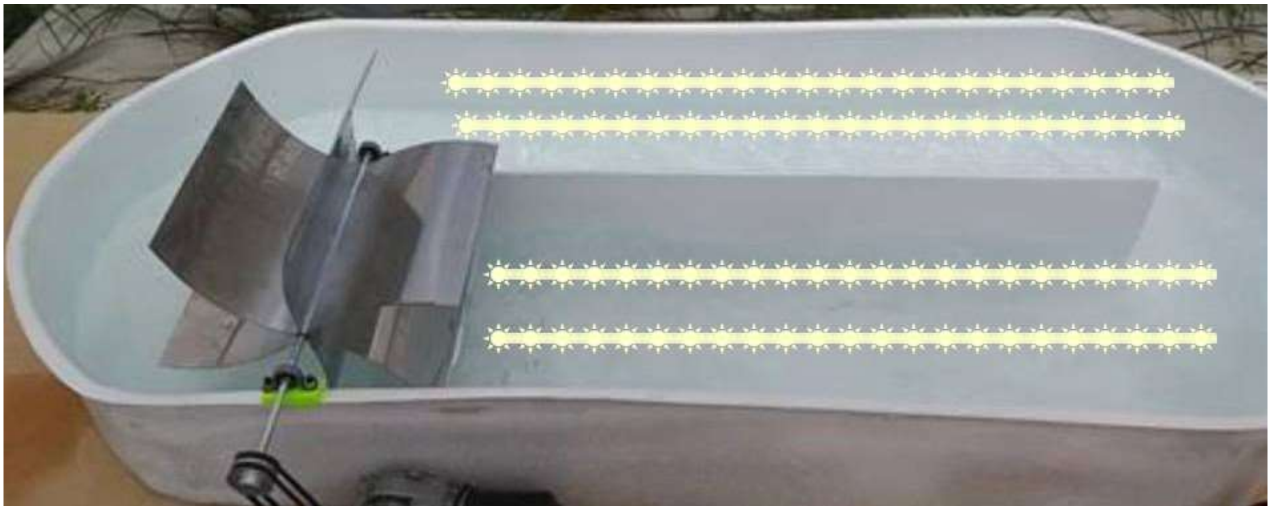

Figure 11. A model of pilot scale race way pond with internal LED illumination to support growth during shady days.

Besides nutraceutical applications, when algae are cultivated for wastewater remediation process, the retention time relates with highest nutrient removal capabilities of the cultivated algal specie. The fast-growing algae efficiently remove N and P within short period of time. An important consideration in selecting efficient nutrient removal species is to look at the N:P as discussed in previous sections. Wastewater containing high amounts of P should be treated with bluegreen algae instead of green microalgae. Another potential strategy for high removals of both N and P is by using mixed cultures of bluegreen and green microalgae.

The major limitations affecting biomass yields in open raceway ponds are evaporative losses, overflow during rainy periods, CO_2 diffusion and photo-inhibition during bright sunny days. To overcome these limitations closed photobioreactors have been designed to optimize yields under sunlight. In addition, the closed photobioreactors are more feasible options when cultivating less hardy and slow growing species. The slow growing species are not favorable for open cultivations due to high risks of contamination and subsequent culture crashes leading to enormous economic losses. The closed systems are also suitable when cultivating algae for high value products.

Concept of 'crop rotation': to revolutionize algae production

Temperature is the most important physical parameter which affects the growing season for a particular specie of microalgae. Few locations such as Chennai (India) and the

Canary Islands where the temperature does not show wide monthly variations (Figure 12) are suitable for outdoor microalgae 'farming' using the best adapted strain.

In contrast, cities like Dallas (USA), Marseille (France) and Madrid (Spain) experience wide variations in monthly average temperatures (Figure 12). The 'hill top' portion of the temperature curves in the above three locations coincides with the most productive months of the year when highest microalgae biomass productivities could be achieved. During the colder months of January, February, March, November and/or December when the average temperature drops below 10°C attaining consistent growth of microalgae become challenging which subsequently affect the yearly yields of high quality biomass. Under such temperature scenarios, it is difficult to select a single microalgae strain which can withstand wide variations in monthly temperatures.

We are introducing a new concept by which the loss in yearly production at any microalgae production can be overcome. Locations where the difference in summer and winter temperatures is beyond the optimal range of the microalga grown at the facility, multiple strains (adapted to low °C and dim light) can be selected and cultivated using the same production systems. In case of some cosmopolitan microalgae, strains or 'geo-variants' from different climatic zones should be isolated and used for cultivation in different seasons of the year. Possibly, rotating the cultivation of green microalgae with blue green algae or the cyanobacteria would minimize the use of nitrogenous nutrients. The use of spent water (culture medium after harvesting green algae) could be used to cultivate species with lower demands for nutrients.

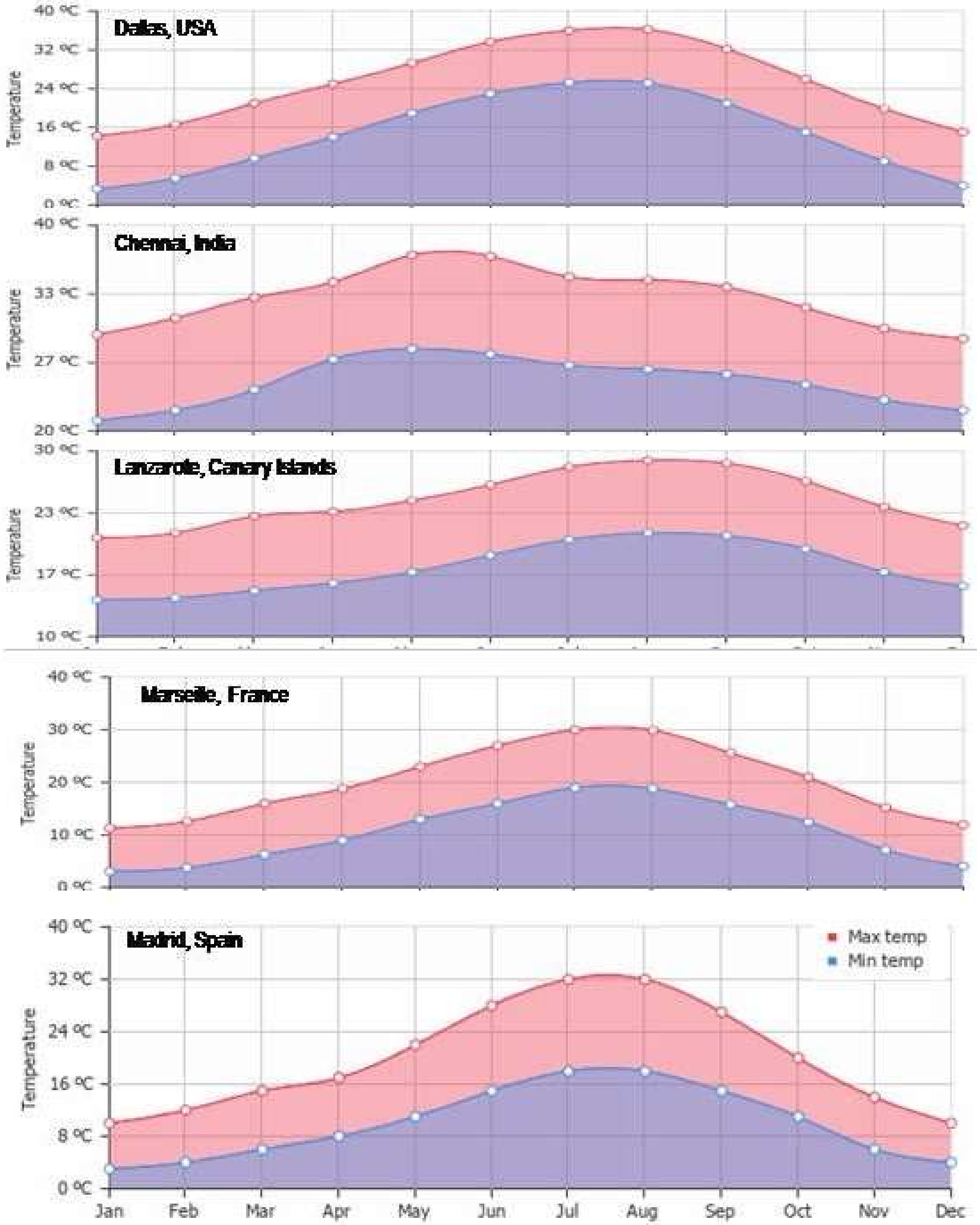

Figure 12. Monthly average temperatures in different regions of the world. Source: weather and climate.com

Harvesting and downstream processing of cultivated biomass

Once the biomass is cultivated in the production system (open or closed), it need to be separated from the liquid medium or water by pumping the cultures into the settling towers to allow gravity sedimentation. The separation of microalgae cells from the water can also be done within the vertical type PBRs such as columns or stainless steel tanks. In open ponds, the paddle wheels or the blades used for the circulation of water are stopped overnight for the next day harvest. This is called primary harvesting. The energy expenditure is nil in case of vertical PBRs where electrical energy is utilized for operating pumps to transfer cultures from ponds to the settling towers.

In the secondary step, the dewatering or thickening of the microalgae cultures is an energy intensive process which require high power centrifuges to concentrate the biomass into slurry or paste (Figure 13).

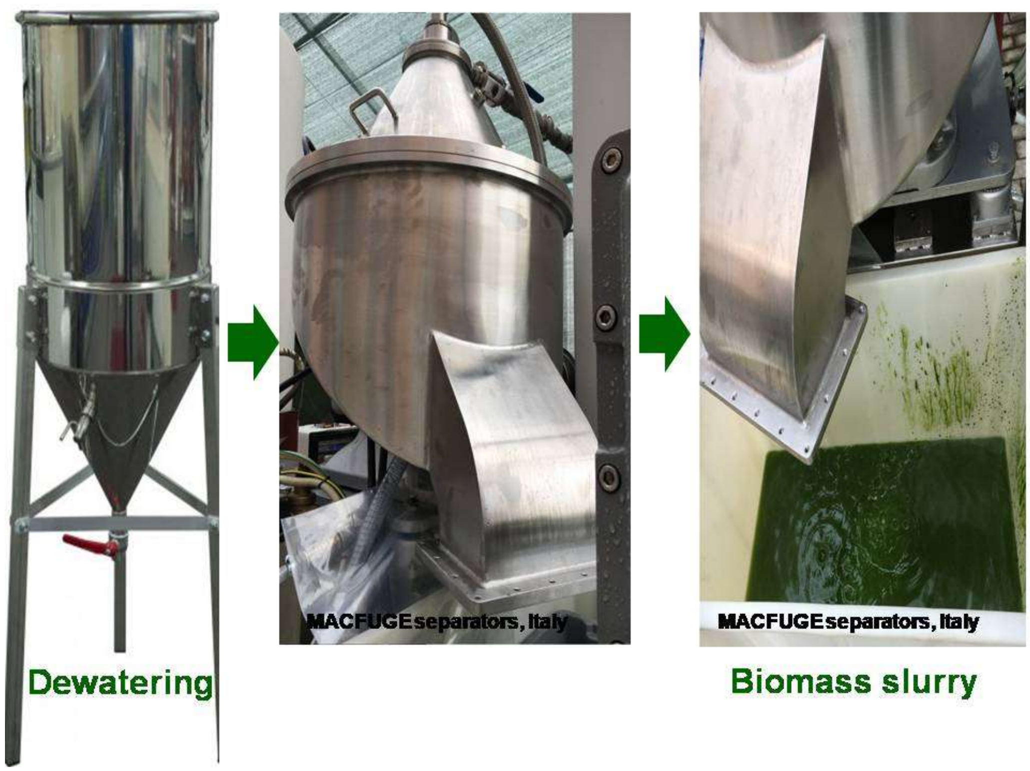

Figure 13. Harvesting process for microalgae cultures grown in open ponds or in PBRs.

Electro Water separation (EWS)

The electro water separation (ESW) is a non-mechanical and non-chemical method to harvest intact algal cells with reduced bacterial loads and with minimum usage of energy. The algal cells are harvested out of the growth medium through electro-catalytic process. It operates in different stages: i) stage one is electro-coagulation which forces algae out of suspension and concentrate the cells, ii) stage two is electro-floatation where bubbles lift concentrated cells to the surface and the concentrated algae is pushed out. The clean water is recovered which could be re-used in aquaculture or to grow the algae again. This method is suitable for harvesting algae and pelletizing it for aquaculture feed. The commercial algae biomass harvesting system from a USA based company OriginClear is based on electro water separation method (Figure 14).

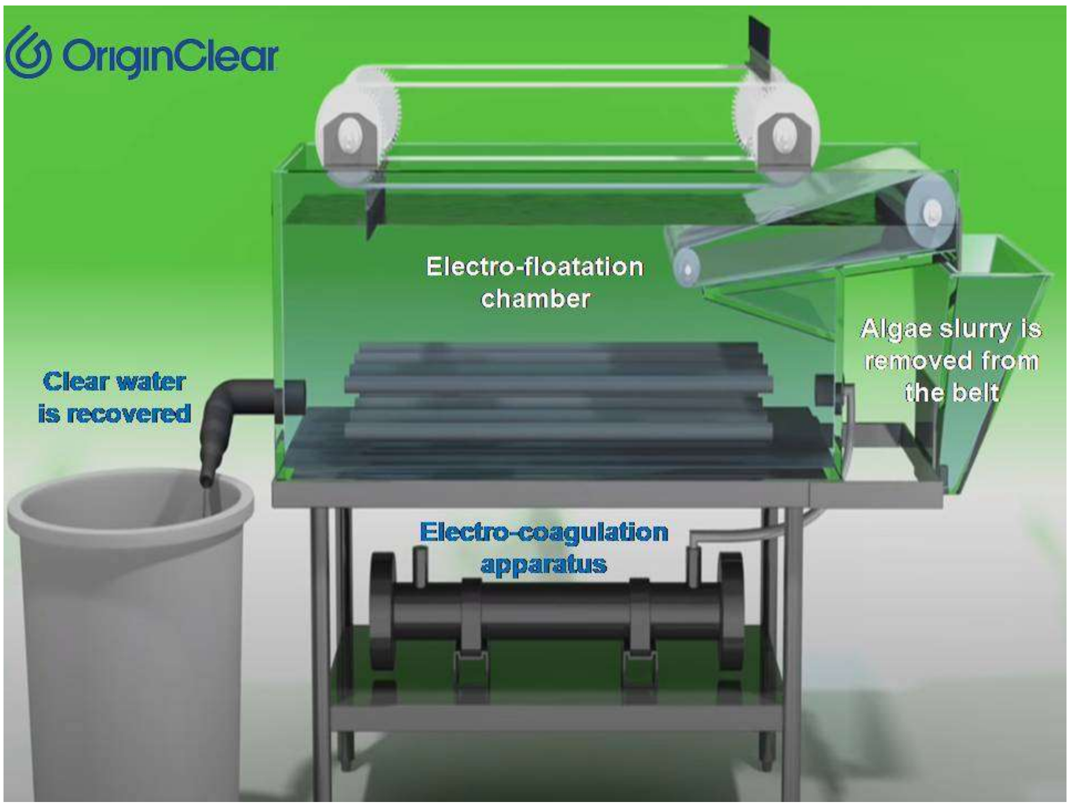

Figure 14. An electro water separation system for harvesting microalgae biomass. Source-OriginClear.

Conclusions

The pressing issues on our planet today are acute water shortages, massive burnings of fossil fuels, increasing atmospheric CO_2 concentrations, and air, water and soil pollution. These issues prevail on a planetary scale and have massive geologic impacts. Perturbations in the physical environment have large scale ripple effects on the biological components of the planet. Increasing CO_2 concentrations and pollution of all three components of our biosphere are deteriorating crop yields and impacting animal health. A ray of hope comes from the most primitive living organisms on the Earth which have witnessed massive evolutionary changes throughout their existence. These primitive organisms are the photosynthetic algae which have the tremendous potential of reversing the unbalance caused to the biosphere due to anthropogenic malpractices. Algae are present in huge numbers all around us; however, we have only explored a handful of the species for applications like biofuels, environmentally friendly bio-materials, animal and aquaculture feed and in human nutrition. The four basic requirements for growing algae are sunlight and CO_2 which are abundantly present and the water and nutrients which are depleting day by day. To overcome the scarcity of freshwater and nutrient resources, algae must be grown on sea water, wastewater, secondary treated water and reclaimed water. Algae farming pose several challenges as algae are being acclimatized to man-made habitats and production systems. Biological expertise is required to isolate microalgae from their vivid habitats, whereas advancements in bio-process engineering allow us to farm naturally growing algae into man-made production systems.

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