

Technological Review of Algae-based Proteins for Alternative Protein Applications

An overview of technology development across the microalgae and seaweed value chain and scope of algal protein industry for alternative protein applications in India

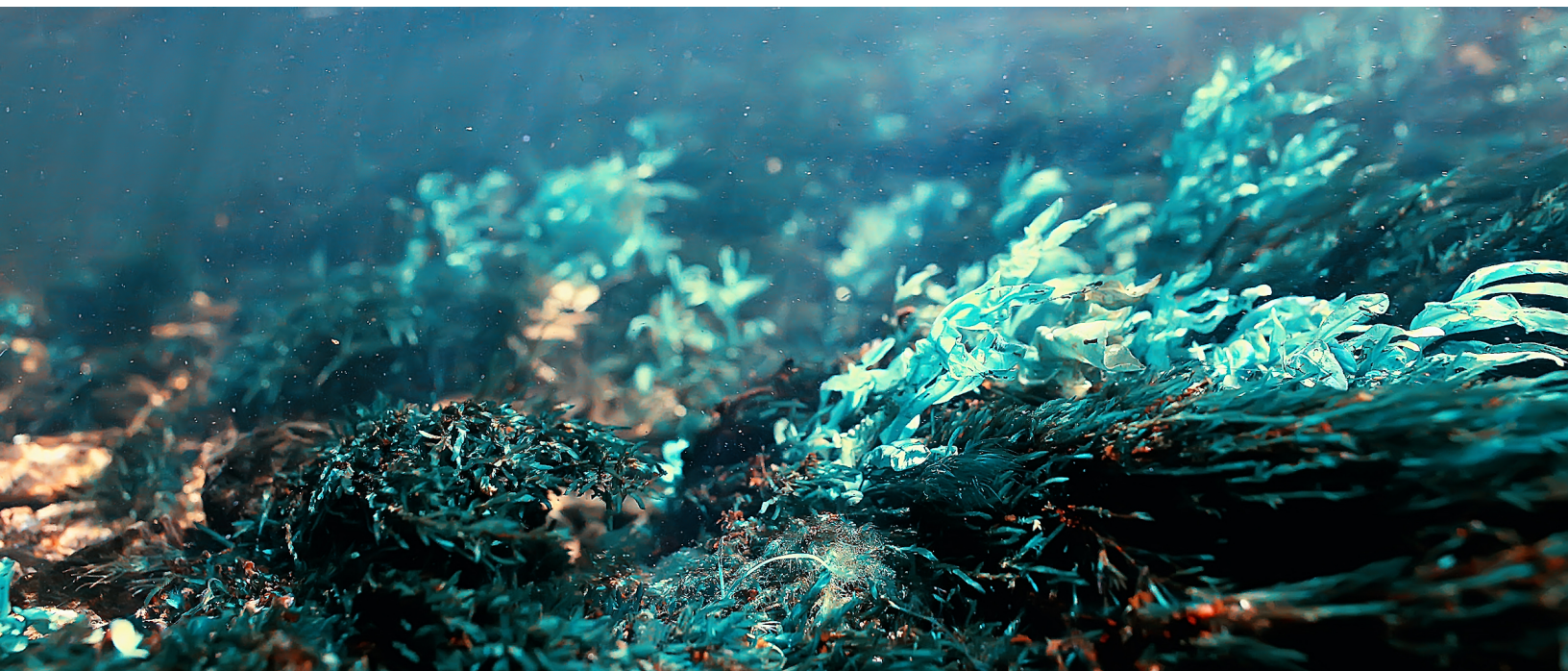


Table of contents

Introduction	4
What are algae?	4
Algae - a protein source	5
Strain selection	6
Microalgae strain selection	7
Isolation methods	7
Screening criteria	8
Strain optimization and selection in India	10
Seaweed strain selection	11
Strain optimization and selection in India	12
Algae cultivation	12
Microalgae cultivation	12
Photo-autotrophic cultivation	13
Heterotrophic cultivation	14
Mixotrophic cultivation	14
Microalgae cultivation in India	15
Seaweed cultivation	16
Seaweed cultivation in India	17
Harvesting and dewatering	18
Harvesting and dewatering microalgae	18
Microalgae harvesting and dewatering in India	20
Harvesting seaweed	21
Seaweed harvesting in India	21
Drying of algal biomass	22
Extraction of proteins	23
Cell disruption	24
Cell disruption in India	26
Protein separation	26
Protein concentrates and isolates	27
Protein concentrates and isolates in India	28
Algal protein functionality and quality	29
Cost of algal proteins	31
Safety and regulatory considerations	33
Safety and regulatory landscape in India	34
Current Policy Landscape	37
Major government bodies governing microalgae and seaweed	37
FDI, Import- Export, National Taxes and Duties	37

Policy Initiatives	38
Talent pool in India and opportunities	39
University courses	40
Research Programmes	42
Developing a talent pipeline for alternative protein	44
Market developments in alternative protein	45
Potential applications of microalgae and seaweed in alternative seafood products	46
Next steps : Maturity of the algal protein industry in India	47
Conclusion	49
Acknowledgements	49
References	50

Introduction

The problems of industrialized animal agriculture are well known, with many well-documented examples of negative environmental, nutritional, public health, and animal welfare outcomes. Animal agriculture is a major contributor to many global issues, including climate change, deforestation, pollution, biodiversity loss, soil erosion and degradation, antibiotic resistance, water overuse, and zoonotic diseases. Alternative proteins sourced from plants, microbial fermentation, and animal cell culture have the potential to create food products that are healthier and more ecologically sustainable.

While the alternative protein industry has made impressive technological advances and demonstrated product-market fit through the rapid commercialization of plant-based meat, egg, and dairy products, diversifying our food supply to be unreliant on animal proteins is no small feat. Feeding billions of humans and tens of billions of farmed animals is among the world's greatest logistical challenges. The first generation of shifts to a sustainable food system have been led by plant-based protein sources but a lot of work is ongoing currently to supplement the momentum from fermentation-derived ingredients. .

In this context, algae as a source of food complementing land-based agricultural food sources can contribute to the growing demand for food, and creation of alternative meat, egg, dairy products. Algal systems can grow both in freshwater and saline water, act as carbon dioxide sinks, be cultivated on non-arable land, and produce higher protein per unit area per unit time than traditional crops. The protein yield from microalgae has been reported to range from 4 to 15 tons/ha/year compared to protein productivity of 0.6 to 1.2 tons/ha/year from soybean (Bleakley and Hayes 2017). Algae-based food products can be nutritionally superior due to the presence of a high concentration of proteins, vitamins, minerals, and bioactive compounds.

In the coming years, India is expected to surpass the population of China, making India the most populous country in the world. In addition, rising income levels in India are leading to higher consumption of animal-based products. Thus, food demands need to be addressed sustainably. The first generation of shifts led by plant-based, cultivated, and fermentation-derived alternatives of animal protein can be complemented with development of the algal protein industry in India. India already has cultivators producing microalgae at small scale and supplying to nutraceutical industries. While wild harvesting of seaweed in India contributes to less than 1% of global seaweed production, India has the potential to become a leader in seaweed production with its 8100 km long coastline holding rich biodiversity of seaweeds.

What are algae?

Algae are a diverse group of aquatic organisms that have the ability to conduct photosynthesis. Seaweeds are the most commonly known algae because of their application in food in form of Nori in sushi while microalgae are commonly attributed as pond scum and the algal blooms in lakes. However algae encompasses a variety of species. Algae can be broadly classified as microalgae and macroalgae (seaweed). Microalgae can be divided into *cyanophyta* (blue-green algae), *pyrrophyta* (dinoflagellates), *chrysophyta* (golden algae), *bacillariophyta* (diatoms) and *chlorophyta* (green algae) (Bleakley and Hayes 2017). *Cyanophyta* are prokaryotic cyanobacteria, while *chlorophyta* are eukaryotic unicellular microalgae. Seaweed are typically classified into three groups based on the presence of specific pigments - brown algae (*Phaeophyceae*) due to the presence of fucoxanthin, red algae (*Rhodophyceae*)

due to the presence of phycoerythrin and phycocyanin, and green algae (*Chlorophyceae*) due to the presence of chlorophyll a and chlorophyll b (Åšcieszka and Klewicka 2019). The reported diversity of algae species ranges from 30,000 to 1 million, although according to Guiry (2012), the estimated number is over 350 million. Even the sizes range from a few micrometers to several hundred meters (Grossmann et al. 2019).

Algae is more commonly known for its use as a gelling, thickening, and stabilizing agent in food products. Seaweeds are cultivated all over the world as a source of carrageenan, agar, and alginates. In countries like Korea, Japan, and China, seaweeds are directly consumed in the form of salads and soups and sometimes eaten fresh. Recently seaweeds powders and extracts have been used to fortify products like noodles, pasta, milk, bread, and other animal-based food products. Microalgae, on the other hand, has been associated with biofuel production as an alternative sustainable energy source. The ability to capture CO₂ from the atmosphere and use sunlight as an energy source made microalgae an attractive option for renewable fuel production. However, depending on the species, microalgae can be rich sources of carbohydrate, protein, lipids, and other bioactive compounds. Nutraceutical products like beta carotene, astaxanthin, antioxidants, sterols, and polyunsaturated fatty acids have been derived from microalgae, and demand high selling prices.

Algae are sources of high-value products like polyunsaturated fatty acids, omega-3 fatty acids, polyphenols, sterols, and pigments in addition to proteins, lipids, carbohydrates, polysaccharides, vitamins, and minerals like calcium, sodium, magnesium, phosphorus, potassium, zinc, iodine, etc. Some seaweeds can contain up to 100 times more vitamins and minerals per unit dry matter than plant or animal-based foods. Several studies demonstrate that algae can be exploited for their antioxidants, antibacterial, antiviral, antidiabetic, antifungal, anti-inflammatory, immunomodulating, neuroprotective, anticoagulant, prebiotic and anticancer properties (Åšcieszka and Klewicka 2019). The scope to exploit metabolic properties from different species is immense, given the diversity of algae species.

Production of microalgae such *Arthrospira* spp. for phycocyanin, vitamin B12, and whole biomass protein, *Chlorella* spp. for carbohydrate extract and whole biomass protein, *Dunaliella salina* for beta carotene, *Haematococcus pluvialis* for astaxanthin, and *Odontella aurita*, *Schizochytrium* sp, *Cryptocodinium cohnii* and *Nannochloropsis* sp for eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is being done commercially globally (Mobin and Alam 2017). Similarly, the cultivation of seaweeds such as *Eucheuma* spp. and *Kappaphycus alvarezii* for phycocolloids, *Saccharina japonica* for alginates, *Gelidium* spp. and *Gracilaria* spp. for agar, *Undaria pinnatifida* known as wakame and *Porphyra* known as nori for food purposes, is carried out on a large scale in countries with a coastline (Patarra et al 2011).

Algae - a protein source

The production of protein extracts, concentrates, or isolates from algal sources is still at its infancy. The demand for microalgae whole biomass with high protein has been majorly led by the popularity of spirulina. Spirulina is the common name of a filamentous Cyanobacterium, *Arthrospira platensis*, which has a protein content of up to 70% (Amorim et al. 2020). Spirulina in the form of ready to mix powders and tablets and enriched beverages, cereals, sauces and dressings, snacks, bakery, and bars has become common over the past decade. Food supplements with *Chlorella vulgaris*, with protein content up to 60%, have also entered the market. While several species of microalgae can be found which have protein content more than 50%, the highest protein content in seaweed species, found in *Porphyra*, is

only up to 47%(Harnedy and FitzGerald 2011). This doesn't mean that seaweeds cannot be considered as an alternative protein source. In fact, the advantage of high protein content in particular microalgae species is offset by the low density of microalgae during cultivation and the cost associated with the concentration process which is not the case with seaweeds where a higher biomass is obtained during harvesting. Currently, algal proteins are not cost-competitive with crop-based proteins as further optimization of strains, cost-efficient engineering systems, and development of large scale manufacturing capability are required. In this report, we will deep dive into each process leading up to the isolation of protein from algae. The value chain for algal protein extraction includes strains selection, cultivation, harvesting and dewatering, cell disruption, protein separation, extraction, and drying. Each of these processes requires optimization specific to the choice of strain used. Each of these steps requires research and development to reach commercial viability in the future.

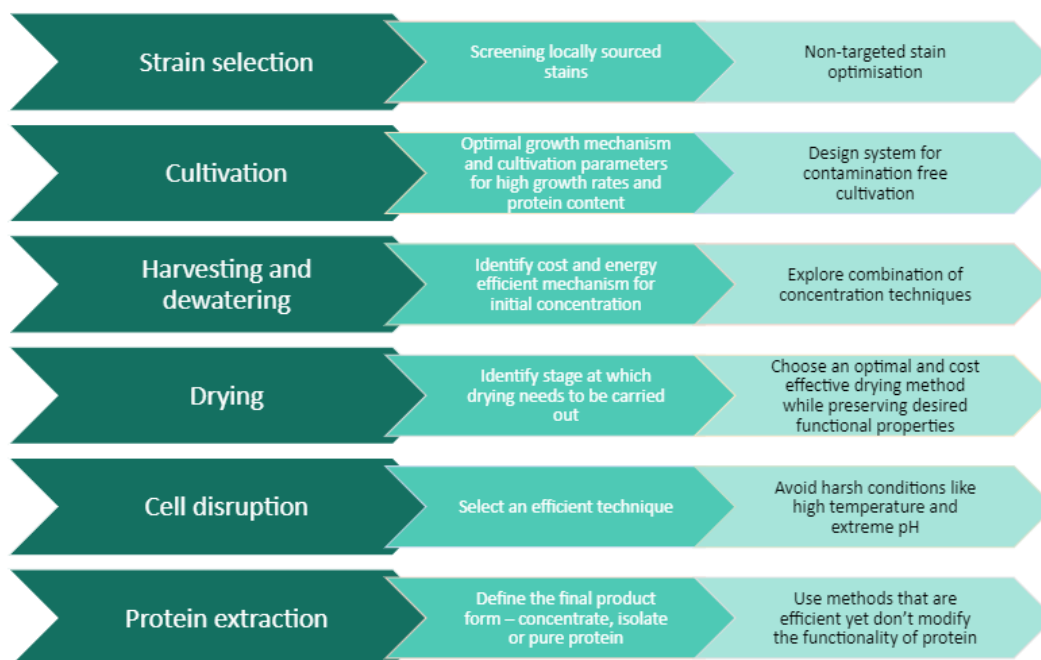


Fig 1 Steps for setting up algae protein production systems

Strain selection

Algal species are sensitive to external environments like climate, geography, and growth media. The design of cultivation technique, cultivation media, and downstream operations specific to each species is critical to obtain maximum yield from the entire process. Algae collections such as ones at the University of Coimbra (Portugal) Göttingen University (Germany), University of Texas (U.S.A), and the National Institute for Environmental and Studies (Japan) act as repositories of thousands of local microalgae strains and species(Duong et al. 2012). In India, the National Repository of Freshwater Microalgae and Cyanobacteria at Bharathidasan University (Tamil Nadu), the botany department from the University of Madras (Tamil Nadu), and The National Collection of Type Cultures at Biochemistry Division of the National Chemical Laboratory, Pune (Maharashtra) have microalgae collections while the National Centre for Seaweed Herbarium at Marine Algal Research Station (MARS) (the largest collection of seaweed species in India), Botanical Survey of India at Kolkata, National Institute of Oceanography at Dona Paula, Goa and CMFRI Mandapam have seaweed collections.

The success of large scale cultivation of both microalgae and seaweed species depends on how well the algal species adapt to the climatic and geographical conditions at the site of production. Hence having local algae strains available in local conditions is extremely critical for strain screening and optimization activities.

Microalgae strain selection

Despite the availability of varieties of collections as mentioned above, only a few hundred strains have been chemically examined and even fewer cultivated for industrial production of biofuels, pharmaceuticals, food, and nutraceuticals. Microalgae grow on soil, rocks, water, and even in and on other organisms, but their main habitats are freshwater, marine water, brackish water, and volcanic waters. The choice of the source should be such that the environment is suitable for outdoor cultivation. An environment with harsh and fluctuating conditions such as tidal rock pools, estuaries, and rivers naturally select for microalgae that are fast-growing and have survival mechanisms to adapt to changing conditions (Duong et al. 2012). For outdoor axenic cultivation of microalgae, collect species that are resistant to foreign growth by choosing a local area that can mimic climatic and ecological conditions of the production site. For the selection of high protein producing microalgae that can be used in alternative meat, egg, dairy products, it is desirable to screen not only a multitude of strains from specific environments, but also strains whose proteins have the desired nutritional composition, functional properties like water holding capacity, gelation, etc. and neutral organoleptic properties. Unlike selection of microalgae for biofuels, where systematic investigation of lipid-rich strains can be performed over a wide variety of taxonomic groups present in various ecological and geographic locations, selection of microalgae for food applications might be constrained to a few strains due to legal and regulatory challenges to approve a new strain without a history of safe use.

The US Food and Drug Administration classifies algal biomass as 'other dietary supplement'. *Spirulina*, *Schizochytrium*, *Chlorella*, *Haematococcus*, *C. cohnii*, *Dunaliella*, and *P. cruentum* fall under the Generally Recognised as Safe (GRAS) category. Microalgae derived nutraceutical products like oils are classified as GRAS for species like *Schizochytrium* and *Ulkenia* (Garcia et al. 2017).

Nevertheless, the selection and isolation of microalgae strain beyond these few strains should be attempted as strain properties impact the cost of downstream operations, hence the commercial viability of algae-based proteins and their use in alternative meat, egg, dairy products. One such species is *Chlamydomonas reinhardtii*, which received GRAS status from the US FDA in 2019 and has been used to commercially produce biomass that can be used as a source of protein (Adams 2019). More information on GRAS status and food safety status of various strains is available in the 'Safety and regulatory considerations' section of this report.

Isolation methods

After the collection of microalgae culture from a desirable location, the next step is isolation of pure species followed by identifying growth rates and protein content. Commonly used isolation techniques include micromanipulation using a micropipette and serial dilution followed by cultivation in liquid media or agar plates (Lim and Schenk 2017). Though these techniques are time-consuming and require sterile cultivation equipment and media, they yield pure cultures. An automatic single-cell isolation technique, flow cytometry (FCM), has been widely used for microalgae cell sorting based on fluorescence properties

of microalgae to differentiate between species like diatoms, dinoflagellates, or prokaryotic phytoplankton. But FCM is relatively expensive over traditional isolation methods(Davey and Kell 1996; Reckermann 2000).

Screening criteria

The selection of microalgal strains for commercial production of proteins needs to account for growth rate, protein content and composition, ease of maintaining an axenic environment during cultivation, ease of cell disruption, and absence of toxins. Staining with Nile red reagent prior to cell sorting has been commonly used to help isolate cells with high lipid content(Lim and Schenk 2017). An alternative method to identify proteins coupled with FCM should be explored for the high-throughput selection of protein-rich species.

Microalgae species	Protein content (% w/w)	Source
Anabaena sp	43-56	Sousa et al. 2008
Arthrospira Maxima	60-71	Amorim et al. 2020; Chronakis et al 2011; Sousa et al. 2008
Chlamydomonas reinhardtii	48	Sousa et al. 2008
Chlorella Vulgaris	51-58	Amorim et al. 2020; Chronakis et al 2011; Sousa et al. 2008; Sidari et al. 2019
Dunaliella salina	26-61	Amorim et al. 2020; Chronakis et al 2011; Sousa et al. 2008
Euglena gracilis	39-61	Sidari et al. 2019
Haematococcus pluvialis	48	Chronakis et al 2011; Sousa et al. 2008
Isochrysis galbana	40-56	Amorim et al. 2020; Sousa et al. 2008
Nannochloropsis sp.	47.7	Chronakis et al 2011; Sousa et al. 2008
Scenedesmus obliquus	50-63	Sidari et al. 2019
Spirulina platensis	46-63	Sidari et al. 2019
Synechococcus sp.	50-63	Sidari et al. 2019

Table 1 Compilation of protein content in microalgae, as reported in the literature

Seaweed species	Protein content (% w/w) dry weight	Sources
Phaeophyta		
Undaria pinnatifida	11-24	Ruperez and Saura-Calixto 2001
Laminaria digitata	8-16	Marshall et al.(2007)
Laminaria saccharina	6-11	Morrissey et al. 2001
Fucus vesiculosus	5-10	Ruperez and Saura-Calixto 2001
Fucus serratus	17	Marshall et al. 2007; Munda 1977
Ascophyllum nodosum	3-15	Morrissey et al. 200; Fleurence 1999b, 2004
Alaria esculenta	9-10	Morrissey et al. 2001
Himanthalia elongata	6-11	Morrissey et al. 200)
Rhodophyta		
Porphyra sp.	24-47	Sanchez-Machado et al. 2004
Chondrus crispus	11-20	Ruperez and Saura-Calixto 2001
Palmaria palmata	12-21	Marshall et al. 2007; Morgan et al. 1980

Chlorophyta		
Ulva species	15-30	Fleurence 2004
Enteromorpha intestinalis	10-18	Morrissey et al. 2001

Table 2 Compilation of protein content in different seaweed species as reported in the literature, adapted from Harnedy and FitzGerald 2011.

For commercial purposes, it is desirable to screen microalgae with at least 50% protein content. It reduces the downstream extraction costs and opens the possibility of direct usage of cell biomass without cell disruption leading to creation of whole foods as well as ingredients with high protein content. Growth rates or biomass productivity influence the operating costs incurred during cultivation processes. Microalgae that can grow in extreme conditions like high pH and high salinity make outdoor cultivation viable. As outdoor cultivation like raceway ponds can lead to contamination, extremophile microalgae can grow easily due to high selectivity to its environmental conditions (Prokop et al. 2015). This eliminates dependence on photobioreactors for maintaining axenic cultures, hence reduces overall production cost. The harvesting and dewatering process of algae is largely dependent on the density of algae in the water. Species like spirulina can easily sediment hence require no chemical interventions for concentrating cells during the harvesting process. However, harvesting of species like Nannochloropsis requires a flocculation technique. Flocculation requires the addition of chemical or biochemical flocculants to precipitate microalgae. Hence, adding to costs for chemical removal in the end product to prevent contamination. Since proteins need to be extracted from algal biomass, especially for use as functional ingredients in plant-based food products, the cell wall needs to be broken for proteins to be released. The energy consumption during cell disruption adds to operating costs. Hence, selecting strains and species with less rigid cell walls would be beneficial from a cost perspective.

Target	Desirable traits	Microalgae	Seaweed
Meets basic criteria for commercial viability and safety	High growth rate	Applicable	
	High protein content		
	No toxin/allergen productions		
Ease of Cultivation	Tolerance to fluctuating climatic conditions		Applicable
	Locally sourced species/strains		
	Adapted to local conditions		
	Resistant to grazers and predators (desirable for outdoor cultivation)		
	Ability to grow heterotrophically to achieve high growth rates		Not applicable
Ease of Harvesting	Cell size, shape and properties suitable for low cost harvesting methods like flocculation and sedimentation		
Ease of Extraction	Easy to disintegrate cell wall		
Extra market value	Presence of high value compounds like beta carotene, astaxanthin etc. which can make biorefinery approach feasible		Applicable

Table 3. Screening checklist for algal strain selection

It is likely that no one species can demonstrate all properties desirable for energy-efficient and cost-efficient production. But knowing the target traits will help in the intelligent screening process, saving subsequent production costs. Targeted selection, metabolic engineering, and breeding for the domestication of microalgae are still at the early stages. However, unlike traditional agricultural crops, microalgae have several advantages like short life cycles (in order of days), unicellular nature, and small size leading to lower development time and cost for breeding (Lim and Schenk 2017).

Non targeted genome editing by means of UV light and chemical mutagens followed by automated high throughput screening methods can help select mutated strains of microalgae with desirable traits like high growth rates and high protein content. From a regulatory perspective, these are considered non-GMO methods and require no knowledge of genome sequences and biochemistry of microalgal strains.

Predictive modeling using bioinformatics can lead to the discovery of new microalgae strains with high protein content, and their phylogenetic group can indicate species with similar traits or properties. Phylogenetic analysis can be performed using primer design, DNA/RNA extraction, PCR amplification, denaturing gradient gel electrophoresis, and sequencing (Duong et al. 2012). Though there is limited data on microalgae genome sequences, this data is critical for metabolic engineering and designing microalgae properties with high specificity. The key challenge with genetic engineering microalgae for protein products is the concern of the use of genetically modified organisms (GMOs) for human consumption.

In the Indian context, GMO based food products are not acceptable as per regulatory norms. Thus, strain selection should rely on high volume screening from natural sources or collections followed by mutagenesis to develop the desired characteristics.

Screening for optimal traits at the lab scale is not enough. The species need to demonstrate similar productivity, protein content, and stability at a medium or large scale that is representative of the final industrial production facility.

Strain optimization and selection in India

Currently, all research on strain optimization and selection is conducted in house by companies who manufacture microalgae. This makes strain selection a highly research intensive, capital intensive and time-consuming process. Hence there is a huge scope of intervention for research & development in academic and research institutions as well as commercial laboratories -

1. Outsource strain selection and optimization to third party labs and research institutions
2. Create accessible local strain collections and repositories
3. Access to strains without IP restriction and on a fee payment basis
4. Open access research on high throughput screening of algae for high protein content
5. Explore non-targeted gene modification

The stakeholders at the roundtable discussion on 'potential of microalgae for the alternative protein sector' agreed that strain selection is one of the most crucial steps which impacts all downstream operations. Hence, emphasis on high throughput screening of microalgal strains and procuring local strains play a big role in the successful production of microalgae. Preliminary screening with a focus on

strains and species which are safe for human use, with high protein content and quality is desirable especially for food applications like in alternative meat, egg, seafood, and dairy products.

Seaweed strain selection

The diversity in seaweed species is much lesser compared to microalgae. Few thousand species of green, red and brown seaweeds are found in nature. Of these 221 species are of commercial value while about 110 species are used for phycocolloid production (Bhaskar 2018). Like microalgae, only selected species of seaweed are edible or have a history of safe use. About 145 seaweed species have some kind of food applications. Cultivation of seaweed species as sources of food-grade agar, alginates, and carrageenan is much more prominent than for direct consumption or as a source of protein. Thus the clarity on the species which can be grown for direct consumption or protein extraction purposes, especially in India, is missing. Since many species can only be cultivated in specific climatic and geographical regions, the focus for selection of seaweed species for protein production should be first on using approved seaweed species and on improving the quality of the desired species. Seaweeds can be wild harvested or cultivated using established cultivation techniques. Vegetative propagation is commonly used as a low-cost method and small scale farming method. It is an energy-intensive process involving selecting, cutting, and attaching vegetative propagules or seedlings to ropes or nets. The biomass required as a seedling for production in a hectare is in order of several tons accounting to one-fourth of the harvest. This means that seaweed farmers require separate seedling production, which is ready when the season for cultivation arrives (Radulovich et al. 2018). Apart from the disadvantage of labor-intensive and time-consuming process of growing seedlings, the continuous use of the same genetic materials leads to a decline in the quality of biomass and genetic diversity (Gupta et al. 2015). Hence effective breeding for selecting strains of seaweeds with desirable properties like high growth rates, high protein content, resistance to pathogens, and tolerance for environmental variability becomes crucial for obtaining good quality strains of target species. Identification of phenotypes of seaweed strains using morphological features is a time-consuming process, and complexity in morphological characteristics have, in some cases, led to the misidentification of species. Molecular and genomic data to identify markers for germplasms can be used for identification and subsequent selective breeding. Techniques for reproduction using germplasms to create spores and other propagation techniques are well reported and documented. Several handbooks and comparative studies can be referred to for this purpose (Radulovich et al. 2015; Valderrama et al. 2015; Reddy et al. 2008; Yarish et al. 2012; San 2012).

Selective breeding, as described above, has been limited to a few species like *Porphyra* and *Laminaria*. Similar to the domestication of agricultural crops, which have been improved over thousands of years, there is a huge scope of improving seaweeds genetically by using traditional domestication techniques such as crossbreeding, mutagenesis, protoplast fusion, polyploidy, transgenesis, and genome editing. Species produced by transgenesis will be classified as genetically modified organisms; hence should be avoided from the food applications perspective. Mutagenesis and the use of mutant strains should be carefully evaluated as these can impact the genetic makeup of natural populations, especially if the cultivation is carried out offshore.

The key criteria for selecting species for seaweeds should be based on improving cultivation yield and quality of biomass. Hence the desirable properties include high growth rates, high protein content, tolerance to environmental stressors, resistance to pathogens, and adaptability to the local environment where actual cultivation will take place.

Strain optimization and selection in India

With 844 species of seaweeds spread across the 8100 km coastline, India relies on wild harvesting of very few species of seaweeds, including *Kappaphycus alvarezii*, *Hypnea musciformis*, *Sarconema filiforme*, *Gelidiella acerosa*, and several *Gracilaria* spp. (Ganesan et al 2019). This has led to depletion of naturally growing seaweed along the coastline. India's seaweed cultivation is limited to few coastal districts where vegetative propagation is the main technique. There are studies indicating the use of protoplast breeding for improving the quality of seaweed species. However, work needs to be done on the validation of these species in either a natural setup or on a commercial scale. Indigenous species with high protein content and adaptability to the local environment should be explored while selecting for species with desirable traits. The key recommendations for improving the seaweed strains for commercial purposes are listed below:

1. Explore breeding techniques to generate high quality strains
2. Establish database for available species, seedbank and genebank for seaweed species

The stakeholders at the roundtable discussion on 'potential of seaweeds for the alternative protein sector' commented on the need to screen species not just strains for alternative protein applications. Exploring improved strains adapted to the local conditions through breeding techniques in addition to creating seed banks will benefit the indigenous cultivation of seaweeds in India. For alternative protein applications, choosing species and strains which are safe for human use, with high protein content and quality are desirable especially for food applications like in alternative meat, egg, seafood, and dairy products.

Algae cultivation

Algae cultivation systems and conditions determine the yield and quality of protein obtained in downstream processing. This is especially important in food applications like alternative meat, egg, dairy as proteins are the bulk and functional ingredients in these products. Hence it is important to understand the impact of different cultivation techniques, and optimize the processing conditions accordingly. Microalgae and seaweed have radically different cultivation methods. This is simply because microalgae is a unicellular organism, while seaweed is a multicellular organism.

Microalgae cultivation

Naturally found algae utilize sunlight for photosynthesis, CO₂ from the atmosphere, and minerals in water as nutrients converting them into proteins, lipids, and carbohydrates. Photosynthesis involves a light reaction in which solar energy is transformed into chemical energy and a dark reaction in which reduced sugar molecules are produced from CO₂ (Grossmal et al. 2019). This route of growth using solar energy with CO₂ as the only carbon source is known as photo-autotrophic or autotrophic growth. However, certain species of algae can also grow in the absence of sunlight using sugars and O₂ as feedstock. This mode of growth is known as heterotrophic growth. Combining the benefits of the above two growth mechanisms, mixotrophic growth utilizes both sunlight and organic carbon as energy sources simultaneously.

Maximum growth rates either in autotrophic or heterotrophic environments can be achieved with optimization of process conditions like the intensity of light, photoperiod, mixing rates, salinity, pH and

temperature, and nutrient concentrations like CO₂, O₂, phosphates, nitrates, and organic carbon-based feedstocks like sugars. Though all microalgae can grow autotrophically, only limited species can grow heterotrophically. Inability to grow heterotrophically can be attributed to the lack of suitable transporters and incomplete metabolic pathways, preventing the consumption of reduced carbon as an energy source. The species which can grow heterotrophically include *Amphora*, *Nannochloropsis*, *Chlamydomonas*, *Ochromonas*, *Chlorella*, *Tetraselmis*, *Chlorococcum*, *Ankistrodesmus*, *Cryptocodinium*, *Dunaliella*, *Euglena*, *Cyclotella* and *Nitzschia*(Prokop et al. 2015).

Photo-autotrophic cultivation

Microalgae can be grown in open systems like shallow ponds, circular ponds, and open raceway ponds (ORP) with a depth of about 0.2m to 5m(Gorssman et al. 2019). Paddlewheels or air pumps are used to maintain water circulation to make the algae and nutrient dispersions homogeneous and allow penetration of sunlight and exposure to CO₂ from the atmosphere at the surface by preventing settling. Wastelands can be utilized for creating these open systems reducing productive land dependence for algae cultivation. Due to the ease of construction and operation, open systems are the cheapest to manufacture and operate compared to other methods of cultivation. However, due to the open nature of these systems, the key challenges are controlling parameters like temperature, the concentration of CO₂, water loss due to evaporation, and light intensity and preventing contamination with predators and eukaryotic grazers. Due to the lack of control of cultivation conditions, the yield from open systems is lower than closer system cultivation in photobioreactors. Typical biomass growth rates from open raceway ponds range from 0.06–0.1 g/L/day, while growth rates in photobioreactors (PBRs) can reach up to 0.36 g/L/day in closed photobioreactors (Perez Gracia et al. 2015).

Closed autotrophic cultivation can be carried out in different types of photobioreactors such as horizontal tubular system, helical tubular system, flat-panel standing bag system, and flat-panel hanging bags. The material of construction can be glass or plastic tubes with a diameter in the order of 0.1 m spread over a length of up to 500 km in different shapes and configurations. Research groups working on microalgae cultivation and companies working on commercial production of algae for biofuels and nutraceutical applications have built in house variants of photobioreactors adding to the diversity of the commonly known tubular systems. The advantages of closed systems, including high surface area to volume ratio, low risk of contamination and control of culture environment, gas transfer, temperature, and water loss leading to higher productivity, make photobioreactors an attractive option. However, the manufacturing cost of PBRs is much higher than raceways ponds. In cases where it is not possible to get axenic cultures in open systems, PBR can compete as a viable alternative. However, in cases where axenic cultures can be cultivated in open systems, the improved yield in PBR doesn't justify the fixed as well as operating costs. In addition, oxygen removal from PBR systems remains a challenge. National Renewable Energy Laboratory, U.S.A. (NREL) has conducted an in-depth techno-economic analysis comparing various PBR systems with open raceway ponds. For different autotrophic cultivation systems, the study accounted for the cost of inoculum, support structure, piping system, valves & fittings, field equipment, and electrical equipment required for setting up the manufacturing facility corresponding to each system. The horizontal tubular system was the closest, in terms of the fixed cost, to open pond systems, while other systems cost 3-10 times the fixed cost of open pond systems. Similarly, power requirements for mixing, pumping, CO₂ delivery, aeration, and cooling for each system were compared, and open ponds were found to be most economical while helical tubular systems were most expensive, costing nine times the operating cost for open pond systems(Clippinger and Davis et al. 2019). The

scale-up of PBR systems is challenging and expensive, the operating and fixed costs for PBRs need to be lower to make them viable for commercial production of low value compounds like proteins.

Heterotrophic cultivation

In heterotrophic growth, the productivity of biomass, as well as target compounds like lipids and proteins, can increase multifold compared to photo-autotrophic growth under the same conditions. Biomass productivity in a heterotrophic environment can range from 4 to 20 g/L/day of microalgae, almost 10-50 times more than typical autotrophic growth rates (Perez Gracia et al. 2015). High productivity of microalgae in heterotrophic systems is a consequence of the high energy density of carbon source in form of organic carbon and no limitations of varying photo intensity over the growth cycle. In addition to the above, heterotrophic cultivation benefits from the closed nature of bioreactor, allowing for control of nutrient feed rate, oxygen supply, and agitation.

Since bioreactors or fermenters are already commercially used for generating pharmaceutical and nutraceutical products from bacteria, fungi, and yeast, sterile cultivation of axenic cultures of microalgae is not an issue. However, microalgae growth in fermenters competes with other microorganisms like bacteria that can feed on organic carbon, which adds to energy requirements for sterilization and hence operating costs. Though the direct use of land utilization drops dramatically for heterotrophic cultivation as growth occurs in bioreactor vessels, this method uses cultivated land indirectly using organic carbon as a feedstock (Prokop et al. 2015).

The key challenges with heterotrophic cultivation include limited microalgal species that grow heterotrophically, the requirement of oxygen-enriched air, and high feedstock costs due to the addition of organic carbon such as glucose, acetate, crop flours, wastewater, wastes from the food industry, and lignocellulosic materials derived from plant-based biomass. The cost of feedstock can be optimized by utilizing side streams from industrial food processing units, such as molasses from the sugar industry. Scale-up of the fermentation process has been established for other organisms like yeast, bacteria, fungi etc. However, there is a scope to further optimize for cost due to high operating costs associated with sterilization, agitation, and aeration and fixed cost of equipment. It may appear that the sustainability benefit of using photosynthetic algae is lost in heterotrophic cultivation due to dependence on high-value organic carbon derived from crops. However, the energy requirements for harvesting and dewatering operation in photo-autotrophic cultivation needs to be accounted for. A life cycle assessment modelling by Smetana et al. compared the impact of using ORP, PBR and heterotrophic fermenters as cultivation systems for protein production (Smetana et al. 2017). For the case of *Chlorella vulgaris*, heterotrophic cultivation used for production of dried defatted protein meal demonstrated the least environmental impact. Such detailed techno-economic and life cycle assessments of comparing existing cultivation techniques need to be carried out to evaluate the sustainability and efficiency of each of these methods.

Mixotrophic cultivation

Mixotrophic cultivation as a growth method is still in its infancy. Although it has been studied at a laboratory scale, the commercial application of mixotrophic growth mode has not been demonstrated yet. Mixotrophic cultivation combines the advantages of photo-autotrophic cultivation and heterotrophic cultivation. Due to simultaneous dark respiration, mixotrophic growth is not limited by low illumination, photo-inhibition, and oxygen accumulation. The amount of organic carbon required for mixotrophic growth is lower than heterotrophic growth, reducing the media costs. It can demonstrate higher growth

rates, short growth cycle, and higher lipid and protein productivity. Though mixotrophic cultivation is commonly carried out in PBRs with both inorganic and organic carbon sources, it can also be carried out in a fed-batch reactor improving the yield (Perez Gracia et al. 2015). Several lab-based studies of mixotrophic growth of microalgae have demonstrated superior yield compared to photo-autotrophic cultivation and, in some cases, higher than heterotrophic cultivation (Li et al. 2012; Perez Gracia et al. 2015; Smetana et al. 2017, Cheirsilp et al. 2012).

Parameters	Photo-autotrophic		Heterotrophic
	Open raceway ponds	Photobioreactors	Fermenter
Fixed cost	Low	High	Low-Medium
Operating cost	Low	High	Low
Maintenance overheads	Low	High	High
Contamination potential	Medium	Low	Low
Yield	Low	Medium	High
Raw Material costs	Low	Low	High
Ease of harvesting operations	Low	Medium	High
Use in India	High	Nil	Nil
Potential to explore	Low	Medium	High
Manufacturing capability in India	Medium	Low	High

Table 4. Comparison of various cultivation techniques based on internal evaluation

Microalgae cultivation in India

India is one of the largest cultivators of spirulina (*Arthrospira platensis*) and exports more than 95% of biomass produced. Photo-autotrophic cultivation in raceway ponds is most commonly used for cultivating species like spirulina, chlorella, dundelina, haematococcus, and nannochloropsis. As a tropical country, India has the climatic conditions that are favorable for photo-autotrophic cultivation due to exposure to the sun throughout the year. Due to the limited availability of risk capital, cultivation practices are limited to the cheapest available option, i.e. open raceways ponds. Many companies have built their raceway ponds in house while others have been supported by technology consulting firms specializing in the design and installation of microalgae production systems. Despite the availability of manufacturing equipment for fermentation technologies, heterotrophic cultivation has not yet been evaluated for commercial purposes. The main reason is the lack of clarity on the commercial viability of indigenous heterotrophic cultivation systems. Hence cost and energy comparison of heterotrophic cultivation (at lab and pilot scale) with photo-autotrophic cultivation for species of interest should be conducted. As mentioned before, mixotrophic cultivation is still at an R&D stage, and further validations

need to be made before conducting pilot-scale trials. The following whitespace opportunities exist in the Indian market for cultivation:

1. Academic research:
 - a. Explore heterotrophic and mixotrophic cultivation
 - b. Develop optimization rules for the composition of growth media
2. Pilot-scale validation
 - a. Develop scale-up principles for going from lab scale to manufacturing scale for open raceway ponds
3. Manufacturing capability
 - a. Explore heterotrophic cultivation using the fermentation process for relevant species like chlorella
 - b. Establish photo-bioreactor design firms and technology consultancies
4. Investment
 - a. Invest in contract farming for cultivation and harvesting of algae
 - b. Lease infrastructure and equipment for the cultivation of microalgae

For photo-autotrophic cultivation, the stakeholders at the roundtable discussion emphasized the need to bring down production costs through innovation in engineering systems for reducing the overall cost of biomass and subsequent costs of algal proteins. Site selection was also identified as an important decision point along with the choice of species which determined the success of scaling up production. Another opportunity as pointed out by one of the stakeholders is to develop a growth medium with lower costs and potential benefits of preventing contamination.

Stakeholders commented on the benefits as well as limitations of heterotrophic and mixotrophic cultivation. The key advantage of heterotrophic and mixotrophic cultivation which was highlighted was high productivities pertaining to the use of the fermentation process to generate microalgal biomass. Also, the ability to shift from green color to yellow color for microalgal biomass in case of heterotrophic cultivation enables the use of microalgae in food applications with a lower impact on the color profile of the final product. An opportunity area in this context which was reiterated was to explore the heterotrophic and mixotrophic routes in India and develop bioreactors for purposes of microalgal cultivation.

Seaweed cultivation

Most of the seaweed cultivation is done on shallow coastal waters. Seaweeds require sunlight to grow, and shallow waters allow sufficient penetration of light while being attached to the seafloor's base through their holdfast. Unlike the roots of terrestrial plants, holdfast doesn't provide nutrients and water but the only anchor for the growth of the seaweed body. Some species like *Sargassum natans* and *Sargassum fluitans* float freely in water due to the presence of air bladders. Seaweed farming using floating structures to provide a base for seaweed attachments is the most commonly used offshore cultivation technique. There are several configurations of floating structures based on which offshore cultivation techniques can be classified further(Radulovich et al. 2015). These include net method, bottom monoline method and floating monoline method. The choice of offshore farming technique depends on the farm environment, availability of seeding and harvesting technology and regulatory requirements to be fulfilled. Farming methods need to be optimised and tested during the growing season to minimize costs and maximise yield(Flavin et al. 2013). Due to seasonal variations, offshore cultivation produces variable nutrient profile, quality and quantity of seaweeds. Although land-based

seaweed cultivation may have space constraints, they can allow for year round production and harvesting, and support species which can't grow using line methods(Cunningham 2018). Inland cultivation technique, also known as tumble culture, utilizes tanks or closed environments to cultivate seaweed. The key advantages of tumble culture are the ability to standardize growth conditions such as nutrients, light penetration, water quality preventing heavy metal build-up and water circulation. The contamination by industrial effluents and sewage discharges can be avoided and there is a possibility of automation in the future. The disadvantages of tumble cultivation include higher costs incurred for set up cultivation systems inland and regulatory hurdles for seawater use and spent medium discharge. Inland cultivation is currently used by select few companies and still at a development stage (Askew 2020)

The key parameters that determine seaweed growth include nutrient quality, penetration of sunlight, temperature, salinity, water circulation, and oxygen and carbon dioxide concentration. The growth of seaweeds is highly specific to the climatic and geographical conditions. For example- species that grow easily in temperate zones can't grow in tropical zones. Biotic factors also impact the growth of seaweed. These include pathogenic microorganisms, organisms like cyanobacteria attaching themselves to seaweeds' surface, organisms like fishes, crabs, and snails feeding on seaweed(Smetana et al. 2017). Hence site selection plays a crucial role in determining the efficiency of cultivation.

Seaweed cultivation in India

Cultivation and farming of seaweeds is not practiced widely in India. The first commercial cultivation of seaweed was carried out by Pepsi Food Ltd. on a contract farming basis, in Madapam in Tamil Nadu, with support from MarineAlgal Research Center, CSMCRI, Mandapam. While seaweed cultivation is taken up commercially by companies like AquaAgri Processing, Tata Chemicals, Coromandel Fertilizers, and Mars Petcare Company, most seaweed production is community-level activity relying on wild harvests from the sea(Bhaskar 2018), This contrasts with global cultivation accounting for 95% seaweed production and wild harvesting accounting for the rest 5%. A feasibility study was conducted recently by CSIR demonstrating that commercial farming of *Gracilaria dura* in Gujarat by seaweed farmers is an economically viable option(Mantri et al. 2020).

Hence, there is a tremendous need and scope to implement commercial cultivation systems in India for seaweed production for protein extraction and other industries. Below mentioned are interventions to aid this development:

1. Pilot-scale optimization
 - a. Establish pilot-scale setups for evaluating and optimizing various cultivation methods
 - b. Establish pilot-scale studies to explore inland cultivation of seaweeds
2. Manufacturing capability
 - a. Promote seaweed cultivation by making training programmes and tools accessible for offshore cultivation for existing and potential seaweed farmers
3. Investment
 - a. Invest in contract farming for cultivation and harvesting of seaweed

Key stakeholders involved in seaweed farming or development methods for seaweed cultivation emphasized the need to scale up seaweed farming in India. Stakeholders also provided a holistic perspective on inland cultivation. For inland cultivation, quality control and the ability to scale-up seaweed cultivation on non-arable land were identified as key advantages however costs involved in

setting-up infrastructure and nutrition supplementation were identified as key cost barriers for commercialization of this cultivation route.

Harvesting and dewatering

With a low biomass concentration, both microalgae and seaweed need to be concentrated before further processing for downstream operations. However, there is a vast difference in the order of magnitude of concentration between microalgae and seaweed. Microalgal concentration can be as low as 0.05% in the medium of growth, and dry matter concentration in seaweed tissue is close to 10%. While microalgae require multistep concentration, seaweeds require an additional step before concentration, which is a literal harvest, like crops, from the sea. The various harvesting techniques are discussed in detail in the below sections.

Harvesting and dewatering microalgae

Harvesting and dewatering can contribute to 10-30% of algae biomass production costs due to the processing of a high volume of water, especially in open systems (Barros et al. 2015; Uduman et al. 2010; Christenso et al. 2011). The cultivated microalgae need to be separated from the aqueous growth media and concentrated for downstream processing. Harvesting of microalgae is a challenging process due to the low concentration of algae in the aqueous environment and its small size in the order of a few micrometers. The biomass concentrations are as low as 0.5g /L in open systems and 5g/L in PBRs.[9] The biomass can be concentrated either in single-step harvesting and dewatering or a multistep process with the first step as harvesting followed by single or multiple dewatering steps. Typically when using combination methods, three stages are involved. The primary concentration or harvesting step increases the biomass concentration from 0.05% to up to 1%. In the next step, further concentration or thickening of primary concentrate leads to slurry-like consistency with about 5-10% dry matter. The final dewatering step concentrates the slurry to a wet paste with up to 15-25% dry matter (Pahl et al. 2013). In cases where the biomass needs to be converted into powder format, it is dried to get 95% dry matter using spraying drying, tray drying, freeze-drying, or drum drying. The maximum concentration achieved by different available methods such as flocculation, flotation, sedimentation, centrifugation, and filtration determines the choice of method or combination required to obtain a wet paste with up to 25% dry matter. The choice of microalgae influences specific properties like particle shape, size, weight, and charge, which also impacts the choice of harvesting and dewatering technique. Since microalgae grown in heterotrophic conditions are likely to have much higher concentrations, multi-step harvesting and dewatering may not be required. Also, the techniques for concentration and extracting intracellular components from the typical fermentation process are well established and rely on the principles of techniques described in this section (Kishore et al. 2011).

Sedimentation

Sedimentation is commonly used for microalgae with high density and can settle. It is typically used in combination with flocculation. Spirulina is known for its ability to settle. After sedimentation, it is separated either by micro sieving or using cloths as a filter to collect algae. Sedimentation is a time-consuming process as settling can take several hours even for species with a natural tendency to settle. The yield from sedimentation is as low as 2-3% dry matter.

Coagulation and flocculation

Coagulation and flocculation can be induced by using organic and inorganic compounds, biochemicals, ultrasound, and electrocoagulation. Commonly used chemical methods involve adding chemicals such as metallic salts, polyelectrolytes, and polyacrylamide polymers or organic materials such as chitosan and cationic starch to coagulate the algae biomass from water followed by aggregation and precipitation of the flocs. Flocculants work on the principle of neutralization of negative charge on the microalgae's surface, allowing them to form aggregates and coalesce into larger flocs (Pahl et al. 2013). The final separation takes place when the floc sediments. The key challenge with flocculation techniques is the difficulty of separating the added compounds from algae, which can act as contaminants in the final product. This is especially important for algae used for food purposes. It is therefore recommended to use food-grade flocculants like chitosan to maintain the edibility of the final product. The energy requirement for flocculation is the lowest after sedimentation, primarily driven by mixing required to disperse flocculants uniformly in the microalgae containing fluid. However, using non-chemical or biochemical compounds for coagulation can add to additional material-related costs.

Centrifugation

Centrifugation utilizes the density difference between microalgae biomass and aqueous medium to separate them. Types of centrifuges include disc stack centrifuges, perforated basket centrifuges, imperforated basket centrifuges, decanters, and hydrocyclones. Lewis et al. have evaluated different types of centrifugation technologies (Pahl et al. 2013). Based on their analysis, disc stack centrifuge has moderate energy requirements. It has a concentration capacity up to 10% dry matter making it a better choice than decanters with high energy requirements and hydrocyclones with low concentration capacity. Also, according to Boxtel et al., disc stack centrifuge can separate microalgae in the size range of 5 to 10 micrometers and has advantages due to low manual interference (Fasaei et al. 2018). Despite readily available centrifugation technologies, the capital cost and operating cost are usually the highest compared to other harvesting and dewatering technologies.

Filtration

Filtration involves the use of membranes or a porous medium that allows only particles with a size smaller than the pore size of the membrane to pass through it and larger-sized particles retained. Hence filtration systems can be custom designed based on the desired particle size of algae to be separated. Depending on the particle size to be separated, membrane separation can be classified as particle filtration, microfiltration, ultrafiltration, nanofiltration, and reverse osmosis to decrease pore size hence increasing power requirements. The energy requirements for membrane filtration are driven by pumping costs and pressure or vacuum required to pass the microalgae containing fluid through the membrane. Microfiltration and pressure and vacuum filtration are used to separate microalgae and operate at a pressure between 1 to 2 bar. Although filtration can be performed as continuous operation, the microfiltration membrane performance drops over time due to fouling and concentration polarisation hence limiting the maximum achievable concentration for microalgae to 5% dry matter (Fasaei et al. 2018).

Flotation

Flotation, commonly used in the coal and mineral industry for ore separation, involves creating a solid-liquid suspension using bubbles of air or particular gas that stick to microalgae and bring them to the surface from where they can be collected. This method can concentrate the biomass up to only 5% dry matter. Flotation involves low energy consumption and has a short operating period compared to centrifugation; however, it needs to be combined with flocculation or sedimentation to obtain the concentrated biomass (Lopes et al. 2019).

Other less commonly used methods include spiral plate technology, magnetic separation, microwave, osmotic shock, freeze press, and supercritical fluid extraction. These techniques have not been discussed in this section owing to the limited usage of these techniques and challenges with applications at commercial-scale (Pahl et al. 2013; Fasaei et al. 2018; Lopes et al. 2019; Branyikova et al. 2018).

Although much literature is available comparing the energy requirements and efficiency of each of these processes, few papers discuss the techno-economic analysis comparing the combination of techniques required to arrive at 25-30% dry matter. For example, flocculation can't be directly compared to membrane filtration. To obtain 25% dry matter, flocculation is followed by sedimentation and filtration or centrifugation. Similarly membrane filtration is followed by centrifugation. Combination of steps leading up to the same concentration of dry matter must be evaluated. Based on literature review, table 4 indicates the comparison between individual harvesting and dewatering techniques.

	Sedimentation	Flocculation	Flotation	Pressure Filtration	Vacuum filtration	Membrane filtration	Centrifugation
Recovery of biomass	10-90%	80-98%	>90%	98%	98%	99%	95-99%
Concentration potential	0.5-3%	3-8%	<5%	22-27%	18-22%	1.5-10%	10-20%
Energy requirement	Low	Low	Medium	Medium	High	High	Medium
Fixed cost	Low	High	High	Medium	High	Low	High
Operating cost	Low	Low-medium	High	Low	High	High	High

Table 5. Comparison of various harvesting and dewatering techniques

Fasaei et al. (2018) have made a detailed comparison between using permutations and combinations of harvesting and dewatering techniques. Taking into account energy and processing costs, the findings suggest that flocculation with chitosan followed by pressure or vacuum filtration is the most economical method while using spiral plate technology followed by centrifugation was the costliest.

Microalgae harvesting and dewatering in India

In India, a majority of microalgae cultivators concentrate microalgal biomass by flocculation using food grade materials followed by sedimentation and sieving. The choice is primarily driven by cost. Key recommendations for the Indian market:

1. Research and development
 - a. Create a database of food-grade flocculants and information on flocculant cocktails specific to strains that can be used to precipitate microalgae
 - a. Develop a techno-economic model for comparing harvesting and dewatering techniques suitable for both heterotrophic and autotrophic cultivation
 - b. Map selectivity of each technique to microalgal species
2. Pilot-scale validation
 - a. Determine scale-up parameters when going from the lab to manufacturing scale

Stakeholders at the roundtable echoed the need for developing low-cost harvesting and dewatering systems at a manufacturing scale to tackle overall production costs. The challenge of creating a highly concentrated microalgal solution from extremely low concentrations is further heightened by the

selectivity of harvesting methods to the species or strains of interest. Hence innovation in scaling up harvesting and dewatering technologies should enable the acceleration of low-cost microalgal production systems.

Harvesting seaweed

The harvesting and post-harvesting operation of seaweeds can add high costs in the form of operating costs and labor costs. Harvesting can be done manually, yielding high-quality materials while sea debris and contaminants are removed in the process. On the other hand, mechanical harvesting using winches and cranes mounted on large boats makes the harvesting process time-efficient but requires additional cleaning. The choice between manual and mechanical harvesting depends on the scale of operation, available technology, and weather conditions. Depending on the type of seaweed cultivated and stage of growth, total or partial harvesting techniques could be used. Total harvesting involves the removal of complete seaweed while partial harvesting involves removal of partial seaweed tissue allowing the residual seaweed, attached to the nets, to grow again. Partial harvesting is suitable for obtaining multiple harvests, as in the case of *Porphyra*, *Gracilaria*, and *Sargassum*. This reduces farming costs as the frequency of replantation goes down. Total harvesting is preferred when seaweed has reached complete maturity. Species like *Eucheuma* and *Kappaphycus* accumulate the highest carrageenan content upon maturity hence undergo total harvesting (Radulovich et al. 2015).

Post harvesting, the seaweed needs to be cleaned, preserved, and dried for downstream operations. Cleaning involves washing with seawater, followed by final washing with fresh water. This step is critical from a safety perspective, especially when seaweeds or seaweed extracts are used for food applications. Microbial and chemical contamination should be removed at this stage to avoid downstream contamination. After washing, seaweeds can be cut or minced depending on end-product usage. Further preservation and drying may also be performed. Solar drying is commonly used to bring down the moisture content from 90% to 30-35%. This is a slow process and labor-intensive as it requires turning seaweeds for even drying. Despite its low cost, solar drying in the open can expose seaweeds to contaminants and induce variability in moisture content due to unexpected weather conditions.

Seaweed harvesting in India

Since most seaweed is wild-harvested in India, and harvesting is done at the community level, harvesting is a manual and labor-intensive process. Cleaning after harvesting is also done manually, and the dry matter is obtained by solar drying. There is an opportunity to introduce automation and carry out commercial-scale operations for harvesting seaweeds in India. This should happen in conjunction with commercial-scale cultivation. For a commercial-scale operation to become commonplace, seaweed farmers should be provided with capital to procure materials and harvesting machines, just like in agriculture, for seaweed cultivation and harvesting. The key recommendation for harvesting and dewatering practices in India is :

1. Manufacturing capability:
 - a. Development of harvest methodologies, protocols and automation tools for seaweed harvesting and drying

Drying of algal biomass

Depending on the end-use of the algae, drying operations can be performed before or after downstream operations. For selling whole biomass algae, drying is performed after the dewatering step to obtain 95% dry matter biomass. Drying after harvesting also reduces handling, storage, and transportation costs while also extending the shelf life of the biomass. However, the impact of drying on protein functionality and structure should be evaluated as the harsh conditions can lead to protein denaturation. The type of method used to dry also depends on the corresponding shelf life desired for the final food product.

The choice of drying methods can also influence protein extraction efficiencies. Ansari et al.(2017) have studied the impact of sun drying, freeze-drying, and oven drying on protein extraction from *Scenedesmus obliquus*. Higher protein extraction was observed for oven-dried biomass(50-60%) compared to sun-dried(40-50%) and freeze-dried biomass(45-55%). Due to the application of temperatures below 60o C, oven-dried cells have better protein extractability, dispersibility, and digestibility. Similarly, Neoh et al.(2016) studied the impact of freeze-drying, oven drying, sun drying, and vacuum drying on the composition, phytochemicals, and antioxidant activity of the edible red seaweed, *Kappaphycus alvarezii*. It was found that oven drying retained antioxidant activity in dried biomass while the highest total phenolic content was preserved in vacuum dried biomass. Species-specific studies need to be conducted to evaluate the best choice of drying technique. The cost of drying algae can also dictate the order of unit operation for obtaining protein as an end product. Intermediate drying after harvesting and dewatering requires additional solubilization of dried cell material to extract value-added components in downstream operations. Thus, increasing the cost of the final product due to additional operating costs.

While several conventional drying techniques can be applied to microalgae, most of the techniques are either energy-intensive or capital intensive. The most commonly used techniques are briefly described in this section.

Spray drying is a highly efficient, rapid, and energy-intensive method. It is commonly used in the food industry for the production of tea, coffee, soup, and whey proteins. However, since the biomass is exposed to high temperatures for a short period, it is critical to control operating conditions to prevent protein degradation.

Rotary drying or drum drying is an energy-intensive method leading to both sterilization and cell disruption. Drum drying involves heating of algae paste covering a rotating drum, which is heated by steam. Dryers demand higher maintenance and capital cost due to lower capacity as compared to spray dryers, although operating costs are similar. The temperature in the drum surface can rise significantly due to the use of steam and can cause denaturation of proteins during the drying process.

Solar drying doesn't require an external supply of energy and is an inexpensive method, but quality cannot be controlled due to variation in weather conditions. It can be utilized in community-level cultivation setups, where access to capital intensive technologies is limited. However, dried biomass quality needs to be monitored because if the drying process is not rapid, there are chances of microbial contamination.

Freeze-drying can also be employed to remove water via sublimation at low temperature and pressure. Although freeze-drying can improve protein stability and retain protein activity, it can lead to a higher concentration of salts in biomass or protein extract, which can result in the denaturation of proteins. (Amorim et al. 2020)

Other methods like oven drying, tray drying, vacuum drying, and incinerator drying have been reported in the literature. The choice of method used will depend on capital investment, operating costs, end-product application, downstream processes, the impact of the drying method on functional properties of biomass, and shelf life desired for the dried product.

Extraction of proteins

As described earlier, algae are rich sources of proteins, lipids, carbohydrates, minerals, polyunsaturated fatty acids, and other high-value compounds. The wide range of applications of microalgae extracts ranges from biofuels to polymers to nutraceutical ingredients. Depending on the end use of the algae, drying operations can be performed before or after downstream operations. For selling whole biomass algae, drying is performed after the dewatering step to obtain 95% dry matter biomass. Drying after harvesting also serves the purpose of reducing handling, storage and transportation costs while also extending the shelf life of the biomass (Soto-Sierra et al. 2018). However the impact of drying on protein functionality and structure should be evaluated as harsh conditions during drying can lead to protein denaturation and loss of functionality. Harsh conditions during drying can also lead to creation of off flavors which may not be preferred in food products. Intermediate drying also adds to significant processing costs.

The significant physical difference between microalgal and seaweed biomass form, obtained after harvesting, require different sets of downstream operations. Seaweed is multicellular and consists of intercellular matrices with high viscosity and anionic cell wall polysaccharides that may hinder the release of intracellular components. Hence, seaweeds may require pre-treatment such as chopping, milling or mincing to aid cell disruption (Bleakley and Hayes 2017). The techniques for the extraction of protein from both microalgae and seaweed biomass will follow the same principles. The choice of technique will, however, be specific to the properties of the species selected. Although extraction of proteins from seaweeds has not been studied as extensively as the extraction of proteins from microalgae, cell disruption, protein concentration, and separation techniques, discussed in this section, should apply to seaweeds as well.

Based on the protein content, protein products from algae can be classified as whole-cell protein, protein concentrates, protein isolates, and purified proteins. Further other forms of proteins like hydrolysates and bioactive peptides can also be obtained from these proteins. Whole-cell protein consists of the whole algae biomass with protein trapped inside the cell. Although edible algae like spirulina with up to 70% protein content can be consumed as a whole biomass power, the protein can be made more bioaccessible if extracted from the algal cells. *Chlorella vulgaris* has been reported to have much lower digestibility compared to plant protein due to the presence of higher content of polysaccharides in cell walls. In general seaweeds and microalgae have low digestibility when consumed in raw and unprocessed form (Bleakley and Hayes 2017).

Thus, to obtain an accessible and digestible protein, which can also act as a functional ingredient to produce alternative meat, seafood, egg, and dairy, it is important to break the algal cell wall and

concentrate proteins to prevent undesirable interaction from other components from the cell. Protein concentration can also impact the taste, odor, and color profile of protein extract. After cell disruption, the proteins can be solubilized, followed by fractionation to water-soluble and water-insoluble proteins. Further purification may be required as an additional step to obtain highly pure protein isolate.

Cell disruption

Cell disruption can be carried by three routes - physical, chemical, or biochemical and mechanical. Physical methods include sonication, microwave, and pulsed electric field. Chemical methods include the use of acids, bases, and enzymes, and mechanical methods include bead milling and homogenization, to break the cell wall. The choice of route is determined by the desired end product/s. For protein extraction purposes, it is critical to maintain mild conditions to prevent denaturation of protein, and loss of techno-functional properties. Conditions like high pH and high temperature can damage sensitive components of the cell, including proteins. Since proteins of interest to us are targeted for human consumption purposes, incorporation of non-food grade chemicals or enzymes should be avoided to prevent contamination and additional downstream costs for separation. The use of a cell disruption technique and the processing parameters associated with it will depend on the choice of algal strains. As mentioned before, cell disruption techniques for microalgae have been extensively studied while a limited number of studies have been carried out for seaweed. Cell disruption using enzymes like cellulase and xylanase have been studied for protein extraction from *Palmaria palmata* (Joubert and Fleurence 2008). Use of homogenisation and osmotic stress to obtain proteins from *Porphyra acanthospora*, *Sargassum vulgare* and *Ulva fasciata* have also been reported in literature (Barbarino and Lauren 2005). Chemical extraction using sodium hydroxide and hydrochloric acid has been demonstrated for *Ascophyllum nodosum* (Jordan and Vilter 1991; Harnedy and FitzGerald 2013; Kadam et al. 2017).

Although lab-scale data comparing a few techniques for specific species is available, a general quantitative comparison between various methods, experimental or simulated, for commercial-scale has not been reported in literature yet. In this section, different techniques are discussed in detail with qualitative comments on their efficiency and energy consumption.

Ultrasonication

Ultrasonication works on the principle of cavitation in which microbubbles grow and collapse to create shock waves that disrupt the microalgal cells permeating through the cell wall and cell membrane (Show et al. 2015). Sonication usually requires high energy inputs that create high local temperature and pressure in regions of cavitation, impacting the functional properties of proteins and other sensitive intracellular materials. The high energy requirements also pose a challenge to cell disruption on a large scale due to high operating costs.

Pulsed electric field

Pulsed electric field (PEF) works by the application of pulsed electric fields of high intensity for a short duration of time in the range of microseconds to milliseconds to disrupt the lipid bilayer of the cell membrane either partially or wholly. Coustels et al. (2015) have established that field intensity for *H. pluvialis*, *C. vulgaris*, and *Nannochloropsis salina* was inversely proportional to cell size since cell size is directly proportional to the transmembrane pressure (Coustels et al. 2015). Hence microalgae with a smaller size such as *Nannochloropsis* will require more energy to break down than larger sized

microalgae like *C. vulgaris*. PEF is a gentle method of extraction (Grossman et al. 2019, Postma et al. 2016; Goettel et al. 2013). However, in some cases, it is not sufficient for complete disruption of the cell wall and the complete release of intracellular protein. Hence PEF should be considered in combination with other cell disruption methods. PEF can also be considered as a method for continuous extraction without killing the microalgal cells due to the formation of reversible or irreversible pores and defects on the cell wall (Luengo et al. 2014; Buchmann et al. 2019).

Bead mill

Bead mill is one of the most commonly used methods for cell disruption. It is an energy-intensive process in which steel, zirconium, glass, or ceramic beads spinning at high speeds collide, causing mechanical disruption of the microalgal cells. The efficiency of disruption is a function of properties of microalgal species like cell density and cell wall strength, flow rate, and bead type and size. Bead mill has been used at both lab and industrial scale. Despite high energy requirements for a large scale bead milling operation, bead milling is considered one of the most efficient and practical cell disruption techniques for large scale operations (Suarez et al. 2018; Grossman et al. 2019)

Homogenization

With high cell disruption efficiency and high energy efficiency, homogenization is one of the most commonly used methods for disruption. Homogenization involves passing microalgae through small holes in a high shear environment with cavitation and turbulence facilitating cell disruption. It is superior to other methods like ultrasonication, PEF, and bead milling. The efficiency of homogenization depends on pressure, flow rate, the strength of the microalgal cell wall, and the number of passes. It has been proven successful in large scale operations but requires high energy inputs.

Enzymatic cell disruption

Enzymatic cell disruption is a mild extraction technique; however, enzymatic cell disruption technique is not sufficient alone to achieve cell lysis. Due to the complexity in the composition of cell walls for algae, the choice of enzymes used, and the combination of enzymes need to be highly specific to the target species' cell wall. Enzymes can also impact the functionality and structure of proteins, which need to be retained for food applications. However, if the desired end product is a protein hydrolysate, then enzymes can act as both cell disruptors as well as hydrolyzing agents. Though enzymatic cell disruption requires low energy and mild operating conditions, the cost of the enzyme cocktails can make the cell disruption process cost-prohibitive (Amorim et al. 2020).

Chemical extraction

Cells can be disrupted using a wide range of chemicals such as antibiotics, chelating agents, chaotropic agents, detergents, solvents, hypochlorites, acids, and alkalis (Amorim et al. 2020). Like enzymatic extraction, the choice of chemical used is highly specific to the target species and its cell wall composition. The use of sodium hydroxide has been reported as a low energy disruption technique. The toxicity of chemicals and the impact of chemical consumption on human health should be carefully evaluated as it will be retained in the disrupted biomass. Chemicals will also increase downstream costs for separation if proteins are extracted for food applications.

Of the above methods, high-pressure homogenization and bead milling have been proven effective at manufacturing scale for cell disruption. In contrast, other methods need to be developed further for commercial applications.

	High pressure homogenisation	Ultrasonication	Chemical disruption	Enzymatic disruption	Bead milling	Pulsed Electric Field
Scalability	High	Low	Medium	Low	High	Medium
Operating cost	High	Medium	Low	High	High	Medium
Efficiency	High	Low	Medium	Medium	High	Low
Energy requirement	High	Medium	Low	Low	High	Medium
Residue	Particulates	Particulates	Chemical residue	Enzymatic residue	Particulates	None
Harsh conditions	High T*	Local high T and P			High T*	
Selectivity	Low	Low	High	High	Low	High

*temperature can be lowered upon modification of traditional equipment with an inbuilt cooling system

Table 6. Comparison of various cell disruption techniques

Cell disruption in India

Very few companies in India have cell disruption facilities. Most of the microalgae producers are selling the whole biomass powder, obtained after spraying drying of dewatered biomass, directly to consumers or supplying to nutraceutical companies who have expertise in extracting high-value components from microalgae. Most of the seaweed producers cater to agar, alginate and carrageenan industry. As per our knowledge, cell disruption for commercial algae protein production either as mainstream or sidestream product is non-existent in India. Hence there is scope for the development of companies specializing in downstream operations. The following opportunities exist in the cell disruption technology domain, bringing India one step closer to commercial algae protein production:

1. Research and development
 - a. Explore and optimize low energy disruption techniques like PEF and ultrasonication
 - b. Conduct academic research on food grade chemical and enzymatic extraction methods
 - c. Map cell disruption techniques to microalgal species
2. Pilot-scale validation
 - a. Determine scale-up rules for going from lab to manufacturing scale
3. Manufacturing capability
 - a. Determine indigenous equipment design and technology for low cell disruption and protein extraction

Stakeholders at the roundtable discussion reiterated the importance of cell disruption particularly for microalgae and its impact on downstream processes. Few stakeholders mentioned using bead milling or ball milling as a mature technology for cell disruption and the need to optimize processing parameters depending on microalgal species and target components to be extracted.

Protein separation

Protein separation is typically based on the principle of solubility of proteins in water. The aqueous phase is enriched with protein, which is then dried or purified. The first step in protein separation is to solubilize protein in water. The solubility of protein depends on the strength of protein-protein interactions and protein-water interactions in the aqueous phase (Amorim et al. 2020). The ionic strength and pH of the aqueous phase impact the protein interactions. The higher the concentration of

ions in water, the lesser will be the solubility of proteins as salts will compete with protein to remain solubilized due to common ion effects. It is well known the proteins precipitate at pH equal to their isoelectric point. Hence it is desirable to maintain pH values that are different from the isoelectric point to facilitate aggregate solubilization and hydration. Thus combining pH adjustments with cell disruption can be considered for protein extraction to improve protein yield. However, harsh conditions like high temperature and pH can also cause protein aggregation, degradation, and functional properties loss which will impact the use of proteins in food applications like alternative meat, egg, dairy products.

Centrifugation is used to concentrate the protein in supernatant while other insoluble components and insoluble proteins accumulate in the solid phase. At the lab scale, centrifugation is feasible. However, high energy requirements for attaining acceleration in order of 10,000g and maintaining low temperature at a large scale are challenging (Amorim et al. 2020). In centrifugation, pigments can adhere to the fine colloidal particles in the aqueous phase, thus imparting color to the protein concentrate. For food applications, this may be undesirable if the protein is used as an ingredient. Hence other techniques like filtration and ultrafiltration can be used in conjunction with centrifugation to obtain cleaner proteins.

Protein concentrates and isolates

Protein concentrations and isolates contain above 70% content on protein. Due to higher concentration and not being trapped inside the cell wall, they have higher protein digestibility corrected amino acid score(PDCAAS) and exhibit functional properties like foaming, emulsification, and stabilization capacity. The harsh conditions during extraction like high temperature or extreme pH can lead to denaturation of protein and undermine the functionality of protein concentrates. Protein concentrates at lab scale have been reported for microalgae such as *Arthrospira sp.*, *Chlorella sp.*, *Scenedesmus sp.* and *Nannochloropsis sp.* and seaweeds such as *Sargassum tenerrimum*, *Hypnea charoides*, *Hypnea japonica* and *Ulva lactuca* have been reported in literature (Lum et al. 2013; Wong and Cheung 2001).

The protein needs to be separated out of supernatant by means of membrane filtration, precipitation at the isoelectric point, ion-exchange chromatography, or dialysis to get concentrated protein. Waghmare et al.(2016) have studied a three-solvent system for protein concentration. However, protein concentration using chemicals such as ammonium sulfate or trichloroacetic acid is undesirable due to the retention of chemicals in the concentrate, making it unsafe for human consumption. Impurities like pigments are removed at this stage.. Ultrafiltration uses a semipermeable membrane through which components with size lower than membrane pore size pass through while higher sized particles get retained in the membrane. The choice of membrane pore size determines the protein yield and purity. Freeze-drying can also be used to remove water by sublimation at low temperature and pressure. This reduces the water content, thus concentrating the proteins(Matejtschuk et al. 2007; Amorim et al 2020). However, salt present in protein extract can increase the ionic strength and pH of the solution leading to salting out and change in protein properties. The optimization and analysis of protein concentration techniques on a large scale need to be done. Protein purity can be increased further by using ion-exchange chromatography to remove polysaccharides and other impurities.

Protein isolates have a protein concentration of up to 90%, hence cost higher. Protein isolates may be used as functional ingredients or as food supplements. Alternative meat, egg, dairy products typically have protein isolates in their formulations as these products typically aim to have a high protein content, and at the same time aim to derive functional properties from proteins. Protein isolation involves the

extraction of protein fractions based on their physical and chemical properties such as density, size, or solubility. The methods of isolation and fractionation work on the principle of differences in solubility of proteins due to different isoelectric points. The precipitation or dissolution of protein at a particular ionic strength and pH of the solution can help to segregate proteins with similar properties and functionality. A mechanical route to separate proteins based on their density can be deployed using gradient centrifugation (Walker 2010). Membrane filtration can be used to fractionate proteins based on their size. The sensitivity of each of the techniques to the differences in properties to each fraction of protein makes the process of isolation an expensive one. Protein purification has not been discussed in this report as high purity (>99%) protein is typically used for niche purposes in the form of functional peptides with specific health benefits or for research purposes to understand the physical, chemical, and biological properties of proteins. Purification is typically carried out using various chromatography techniques. However, the cost of purification is too high, and the process too complex for commercial production of such proteins. Though protein isolates are likely to have less off flavors due to the high purification level, lower purity proteins can demonstrate better functional properties due to presence of polysaccharides and lipids.

Protein concentrates and isolates in India

As indicated earlier, as per our knowledge, protein extraction from algae has not been commercialized in India yet. The following interventions can strengthen the algae extraction supply chain making algal proteins relevant for alternative meat, seafood, egg, and dairy products:

1. Research and development
 - a. Determine protein functionality, organoleptic properties, and nutritional value of algal proteins
2. Protein extraction
 - a. Optimize extraction methods for neutral organoleptic properties
 - b. Map protein functionality desired as per final products usage
 - c. Explore biorefinery approach to valorize all value-added components from algae
3. General
 - a. Drive consumer awareness and education for microalgae-based products in the Indian market

The stakeholders at the roundtable discussion pointed out that the use of microalgae in alternative protein applications requires access to food grade microalgal biomass at a feasible price for universities to conduct research on food applications. Another important opportunity raised by the panel was the need for building consumer acceptance of microalgae-based food products which will pave the way for consumer interest in alternative protein applications using microalgal proteins.

For seaweeds, the biggest challenge highlighted by one of the stakeholders is the availability of seaweed biomass in India which is limited due to the low production volume of cultivated seaweeds. An important opportunity the panel emphasized was the need to valorize all side streams through a biorefinery approach to make proteins from seaweeds a commercially viable product.

The stakeholders at both, microalgae and seaweed, roundtable discussion pointed out the need for more research to be done for not only determining the right techniques for extraction of protein from microalgae and seaweed but also exploring the nutritional value as well as techno-functional and organoleptic properties of algal proteins.

While using algal proteins as the only protein ingredient in alternative meat, seafood, egg, and dairy products is not cost-competitive to soy at this stage, incorporating algal proteins in conjunction with soy or pea protein in plant-based meat, seafood, egg, and dairy products will facilitate market adoption, demand generation and optimization of algal protein properties.

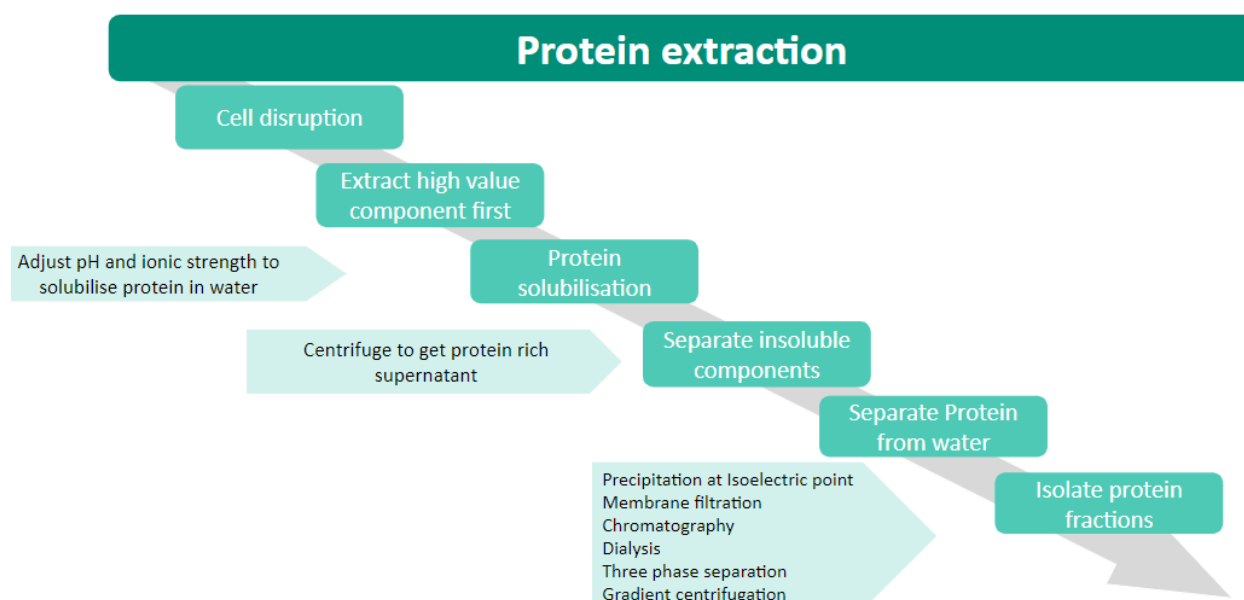


Fig 2. Extraction of protein concentrates and isolates

Algal protein functionality and quality

For algal proteins to compete with existing protein sources, the nutritional profile, digestibility and functionality of these proteins need to be either the same or better than animal or plant-based proteins. A key indicator of protein quality is its amino acid profile. Since not all amino acids are produced in the human body, the essential amino acids need to come from external protein sources as part of the food. The protein quality from algae depends on various intrinsic and extrinsic factors such as choice of algal strain, cultivation conditions, growth media, and nutrients supplied and downstream processing.

The amino acid profiles of various microalgal and seaweed species have been reported in the literature(Chronakis 2000 ,Becker 2013). However intrinsic factors like strain type and extrinsic factors such cultivation systems, growth conditions, climate fluctuations and nutrient supply can lead to variability across studies. In general, most seaweeds contain all the essential amino acids and are a rich source of the acidic residues aspartic and glutamic acid(Fleurence 1999). Microalgae such as *Chlorella vulgaris* and *Arthrospira platensis* have amino acid profiles similar to soybean. Hence algal proteins demonstrate promise as alternative protein due to their composition.

When optimizing production systems for algae, the amino acid profile should be targeted such that it meets the FAO reference standards on the recommendation of essential amino acid content in a protein mix. It would be advantageous to have amino-acid profiles superior to proteins derived from eggs and soy. This can be evaluated using the Essential Amino Acid Index(EAAI)), which relates the content of

each essential amino acid in a protein to the amino acid content in egg protein. Apart from the amino-acid profile, protein bioavailability and digestibility are crucial parameters that indicate the absorption of nutrients in the human body. The Protein Digestibility Corrected Amino Acid Score (PDCAAS) is an index recommended by FAO in 1989. However, Digestible Indispensable Amino Acid Score (DIAAS) was recommended in 2013 as a more precise digestibility index than PDCAAS as DIAAS considers the digestibility of each essential amino acid separately while PDCAAS takes into account protein digestibility in the form of nitrogen. Few studies report the EAAI and PDCAAS scores of algae (Grossman et al. 2019, Amorim et al 2020). This can be attributed to the lack of readily available algal protein for purposes of nutritional studies. Thus, bioavailability, bioaccessibility and bioactivity of proteins need to be studied to evaluate their impact after human consumption. Upcoming studies should also focus on the impact of extrinsic factors on protein quality and evaluate the digestibility of both whole biomass algae as well as protein extracts for a fair comparison with plant-based proteins.

Protein Source	PDCAAS (%)	Biological value (%)	EAAI (%)
Egg	118	94	100
Milk (bovine)	121	90	92
Meat (bovine)	92	74	86
Soybean	91	74	85
Wheat	42	65	63
Arthrospira sp.	48	51–82	64
Chlorella sp.	N.A.	53–80	85
Scenedesmus sp.	N.A.	60–81	71
Dunaliella sp.	N.A.	N.A.	98

Table 7. Protein quality of microalgae species and common protein sources as reported in literature. Adapted from Becker (2013), Clement et al. (1967), Damodaran et al. (2007), Fabregas and Herrero (1985), Hoffman and Falvo (2004), Kent et al. (2015), Lubitz (1963); Oser (1959), Schaafsma (2000)

The techno-functional properties also determine the quality of protein when used as an ingredient. For ingredient usage, neutral taste, color, and odor profiles are critical for blending into the food product. Spirulina grown autotrophically has a green color. However, the protein extract is deep blue in color due to the presence of phycocyanin. Thus, spirulina protein concentrates require further refining downstream to color removal. Another way to offset the intense colors from microalgae could be by growing them heterotrophically. However, this needs to be validated depending on the species of interest. Some species lose green color in a heterotrophic environment while others don't. In addition to the organoleptic properties, the proteins should also be soluble in water and, depending on applications, display functionalities like water-binding capacity, emulsification, structure building, foaming, and gelation. For alternative meat products, functionalities like water holding capacity, gelation, and emulsification are very important whereas for egg and dairy applications, functionalities like solubility, emulsification and foaming become the key. Further work needs to be done to evaluate the techno-functional properties of protein isolates and protein concentrates from microalgae and seaweed.

The use of microalgae to fortify bakery products and beverages has shown promising results. Several studies where microalgae is added as a functional ingredient in cookies, pasta, and yogurt show that the fortified product has superior nutritional properties due to presence of vitamins, minerals, and antioxidants. The increase in minerals content and improvement in color and texture stabilities for

Chlorella vulgaris and *Arthrospira platensis* has been demonstrated by Wandurraga et al.(2019). Segovia et al. (2017) studied the use of *Isochrysis galbana*, *Nannochloropsis gaditana*, *Scenedesmus almeriensis*, and *T. suecica* as a functional ingredient in wheat bread and didn't find any impact of microalgae addition on the textural properties. Microalgal proteins have been reported to exhibit techno-functional properties such as foaming, emulsification, insensitivity to low pH, and stabilization capacity(Law et al. 2018; Nirmala, Prakash, and Venkataraman 1992; Schwenzfeier et al. 2013; Ursu et al. 2014). Similarly, the use of seaweeds to fortify bread, milk, noodles, and pasta has shown promising results. In a study by Prabhashankar et al. (2010), Wakame was incorporated in pasta up to 10% which improved the amino acid and fatty acid profile of pasta. The sensory properties of pasta were not impacted due to inclusion of seaweed. O'Sullivan et al.(2014) incorporated seawater extracts up to 0.5% in milk and found improvement in milk quality and shelf-life characteristics. Fortifying foods with microalgae and seaweeds, generally speaking, lead to improvement in nutritional characteristics. This paves a way for consumer acceptability and willingness to explore microalgae and seaweed-based food products and eventually algal protein-based meat, seafood, egg, and dairy analogues.

Creating algae-based meat, seafood, egg, and dairy analogues is a real possibility as it has been demonstrated in lab-scale studies. Caporgno and Mathys (2018) combined heterotrophically cultivated *Auxenochlorella protothecoides* with soy concentrates to create fibrillary textured extrudates. The study demonstrated that the light yellow colored microalgae can be obtained from heterotrophic cultivation. This can drive consumer acceptance for use of microalgae as an ingredient. Similarly, studies by Law et al. (2018), Nirmala, Prakash, and Venkataraman (1992), Schwenzfeier et al. (2013) and Ursu et al. 2014 as mentioned earlier have demonstrated algae proteins exhibiting techno-functional properties like emulsification, foaming, pH stability, etc. which can be leveraged to create other alternative protein products like egg and dairy too. More such studies need to be conducted to generate evidence that incorporating algae whole biomass or algae-based proteins in meat analogues is not only feasible but also beneficial from both a nutritional and sustainability standpoint.

Cost of algal proteins

Based on literature review and stakeholder interviews, the cost of microalgal proteins is estimated to significantly higher than other plant-based sources like soy protein isolates. The report 'Sustainable protein technology: an evaluation on the STW Protein programme and an outlook for the future' by Voudouris et al.(2017) presents an overview of various novel protein sources and provides data on costs of commercially available proteins. The cost of microalgal proteins when compared with soy protein isolates indicates a huge gap in costs with soy protein available in the range of 2.2-6.5\$ per kg compared to costs of microalgal biomass in the range of 3-67\$ per kg. Hence the cost of protein isolates from microalgae will be much higher compared to soy protein isolates. The cost of microalgal proteins is a function of the species, the cultivation method, and specific unit operations including harvesting and dewatering, cell disruption, and drying. Food grade biomass of species such as spirulina is available at 3-27\$ per kg while species like chlorella is available at 9-67\$ per kg. Production costs vary with lower costs for photo-autotrophic cultivation if carried out in raceway ponds and higher costs if photobioreactors are used. NREL has done a detailed techno-economic analysis involving both a top-down approach and a bottom-up approach analysing costs involved in microalgae biomass production using various open raceway pond designs(Davis et al. 2016). The analysis is based on determining the costs of setting up a 5000-acre raceway pond facility using various designs obtained from majors players in microalgae production space. Infrastructure costs for the entire facility have been accounted for including costs of production ponds, inoculum ponds, CO₂ delivery system, water delivery,

and circulation system, and dewatering system. Operating costs including the cost of medium, power, water, etc have been estimated. Assuming productivity of 25 gram/m²/day based on existing literature data, the cost price of microalgal biomass concentrated to 20% has been arrived at accounting for all variable costs. The minimum biomass selling price(MBSP) is then calculated based on depreciation for fixed costs, operating costs, and targeted internal rate of return for fixed costs. A discounted cash flow analysis is used to arrive at the minimum biomass selling price required to obtain a zero net present value (NPV) with a 10% internal rate of return (IRR) and plant lifetime of 30 years. It is worth noting that this report was published by NREL in 2016 and used designs available before 2016 in the US. Also, the costs were considered assuming 2011 as the base year. Hence change in fixed costs and subsequently operating costs should be anticipated as a result of innovations that might have come up in the last 4-5 years and also because of the differences in labor, infrastructure, and chemical costs between the US and Indian markets. However, the techno-economic analysis is a useful framework to evaluate the commercial feasibility of a project and define internal targets to bring down costs. Such an analysis to compare photo-autotrophic routes with heterotrophic routes is absent in literature although a separate report by NREL captures economic analysis of different photo-bioreactors systems compared to open raceway ponds(Clippenger and Davis 2019). Availability of data for heterotrophic routes will help guide companies and startups who want to explore microalgae space and have some expertise in fermentation-based bioreactor systems.

The biomass cost for seaweed is relatively low compared to microalgae owing to low resource utilization during the production process. Cultivation of seaweeds in oceans requires capital investment for bamboo rafts for cultivation unlike microalgae or even traditional crops which require nutrition in form of nitrogen, phosphorus, and potassium-based fertilizers for growth. However, the harvesting of seaweed is similar to traditional crops and is a labor-intensive activity. The cost of seaweed biomass depends on the species and procurement route if the species is not available at the site of end utilization. The cost of seaweed species like *Kappaphycus Alvarezii* is 0.6 \$ per kg dry weight in India. Other species used in the hydrocolloid industry like *Gracilaria edulis*, *Gelidiella acerosa*, and *Gracilaria dura* range from 0.65-1.7\$ per kg dry weight.

CSIR-CSMCRI and ICAR-CMFRI have worked closely with seaweed farmers to build techniques for the cultivation of seaweed. Several reports have been published which provide a detailed breakup of costs involved in the cultivation of seaweeds (Johnson et al. 2017, Ganesan et al. 2019). The key fixed costs are related to materials required to create bamboo rafts like ropes, anchors, nets, and bamboo and initial seedling material. The actual yield for each species will differ. For *Kappaphycus alvarezii*, about 40 kg of the wet seedling is required to obtain a yield of 200-240 kg wet weight which requires 500 rafts of 3m by 3m size spread over a hectare for cultivation. The estimated cost of each bamboo raft as per reported data range from Rs 1000 to Rs 1200 while a seaweed farmer from Gujarat reported the cost to be in the range of Rs 2000. From 200 kg wet weight of seaweeds biomass about 20 kg dry weight of seaweed is obtained which is typically sold at Rs 40 per kg. Assuming a 45-day growth cycle (and excluding the monsoon season) leading to 5 rounds of cultivation, the average earning annually for a farmer is Rs 4000 per raft. With each farmer handling 50 rafts, the annual income from seaweed cultivation is Rs 200000 per farmer. An investment ranging from Rs 50,000 to Rs 1,00,000 is required from the farmer's end. However, this can be brought down if the government introduces subsidies for seaweed farming. Automated drying at the current volume of seaweed cultivation is an expensive operation hence solar drying in hygienic conditions is the most commercially viable solution at this stage in India.

Economic analysis of downstream operations including cell disruption, protein extraction, and drying of microalgae and seaweed will depend not only on the choice of technology but also an adaptation of

equipment and process design to suit a particular species of microalgae or seaweed. As in the case of plant-based proteins, the manufacturing equipment designs are different for different raw materials due to variation in composition, similarly, equipment design for protein extraction will differ from one species to another. Also, biomass characterization for protein extraction from microalgal and seaweed species is not as readily available as for soy, pea, or other common plant-based protein sources. Thus, limiting the predictability of equipment design for protein extraction. However, existing technologies for cell disruption of whole biomass and drying of algal proteins can be utilized with the optimization of processing parameters depending on species used. With more academic research and progress in the field of algal proteins, the uncertainty, and variability in the cost of algal proteins are expected to come down.

Safety and regulatory considerations

Toxin producing algae such as cyanobacteria and dinoflagellates have been known to cause harmful algal blooms causing damage to marine organisms and birds. Algae, both microalgae, and seaweed are known to accumulate heavy metals. If these toxins and heavy metals enter the food system due to the consumption of algae, it can be lethal to both humans and other life forms. Hence it is critical to assess the toxicity of algal species and understand the impact of algae consumption on human health. Algal consumption can be toxic due to toxins production, presence of purines from the nucleic acid, use of poor quality water, and the presence of microbial contaminants(Moudrikov et al 2018). Food allergy is another aspect that needs to be evaluated for not only algae without a history of safe use but also new algal strains of species with a history of safe use. It is worth noting that not all species of cyanobacteria and dinoflagellates produce toxins. *Arthrospira platensis* is a cyanobacterium. Although cyanobacteria are known to produce microcystins, some studies have shown *Arthrospira platensis* does not produce microcystins(Polikovsky et al 2019, Carmichael and Boyer 2016 ,van Apeldoorn et al 2007). In contrast, others have shown that it contains levels that are below the limits considered toxic. Nevertheless, *Arthrospira platensis* comes under the GRAS category (Gilroy et al. 2000). Until now, microalgal species used in aquaculture or food supplements have reported no production of toxins. This includes species such as *Isochrysis*, *Chaetoceros gracilis*, *Tetraselmis suecica*, *Pavlova lutheri*, *Skeletonema costatum*, *Dunaliella tertiolecta*, *Nannochloropsis* sp., *Phaeodactylum tricornutum*, and *Chlorella* sp (Erzing et al. 2014).

Though it is known that seaweeds have the potential to accumulate heavy metals and polycyclic aromatic hydrocarbons from seawater and get contaminated (from industrial and sewage discharge), the toxicity and food allergy potential for proteins extracted from seaweed have not been evaluated in detail yet (Chronakis 2011). Algae whole biomass has been used in lower content in the form of supplements or additives in food products like bread, cookies, pasta, etc. Hence, it is essential to evaluate the toxicity and food allergen potency at higher protein levels in concentrates and isolates. This is especially relevant when determining the safety of algae-based meat, seafood, egg, and dairy applications in the future.

Erzing et al.(2012) have reported the following algal species (relevant from a feed or food perspective) have no known toxins: *Navicula* sp., *Synechococcus* sp., *Nitzschia dissipata*, *Tetraselmis* sp, *Phaeodactylum tricornutum*, *Thalassiosira pseudonana*, *Haematococcus pluvialis*, *Odontella aurita*, *Dunaliella* sp., *Skeletonema* sp., *Chlorococcum* sp. ,*Monodus subterraneus*, *Scenedesmus* *Nannochloropsis* sp., *Desmodesmus* sp., *Haptophyta Isochrysis* sp. , *Palmaria palmata*, *Ascophyllum nodosum* *Synechococcus* sp. and *Fucus* sp. Certain species belonging to *Phormidium* sp., *Anabaena* sp.,

Prototheca sp. and *Amphora* sp. have toxin producing and pathogenic characteristics. On the other hand, species such as *Cryptocodinium cohnii*, *Porphyridium cruentum*, *Chlorella* sp., *Arthrospira* sp. *Chlamydomonas reinhardtii*, *Laminaria* sp. and *Undaria pinnatifida* have been given GRAS status by the US FDA (Food and Drug Administration). The following seaweeds species have been given GRAS status for use as flavor enhancers in dried format and categorised under spices, seasonings, and flavorings: *Analipus japonicus*, *Eisenia bicyclis*, *Hizikia fusiforme*, *Kjellmaniella gyrata*, *Laminaria angustata*, *Laminaria longicruris*, *Laminaria Longissima*, *Laminaria ochotensis*, *Laminaria claustronia*, *Laminaria saccharina*, *Laminaria digitata*, *Laminaria japonica*, *Macrocystis pyrifera*, *Petalonia fascia*, *Scytosiphon lome*, *Gloiopeltis furcata*, *Porphyra crispata*, *Porphyra dentata*, *Porphyra perforata*, *Porphyra suborbiculata*, *Porphyra tenera*, *Rhodymenia palmata* (Center for Food Safety 2020).

In India, *Spirulina* from *Arthrospira platensis* is recognised as a nutraceutical ingredient according to FSSAI and it is permitted limits for usage 500 - 3000mg per day. Astaxanthin powder derived from *Haematococcus pluvialis* and kelp or brown seaweed are also listed as nutraceutical ingredients. Processed Eucheuma has been classified as food additive under Schedule VE. Direct reference to any other microalgal species has not been made in FSSAI documentation. In absence of these standards, existing CODEX standards can be followed. FSSAI standards for edible microalgae and seaweeds need to be created. Nutrient profiling, nutrition labelling and protocols to evaluate safety and quality of edible algae need to be developed.

Safety and regulatory landscape in India

Seaweed-based ingredients for food applications

Seaweed-based ingredients are permitted in certain food applications as additives as thickener, stabiliser and gelling agent. Food additive is defined as a substance not consumed as food by itself and which is neither a typical ingredient, but is added for technological purposes (including organoleptic purpose) in the manufacturing, processing, preparation, treatment and packaging steps in the production process. It is regulated by the Food Safety And Standards (Food Products Standards And Food Additives) Regulations, 2011.

Name of Permitted seaweed Additive	Food Applications to be used in	Functional Use	Permitted Usage Limit of the Additive
Agar	Sterilized And UHT Creams, Whipping And Whipped Creams, And Reduced Fat Creams (Plain)	Polysorbates	GMP*
	All types of yoghurts	Stabiliser, Emulsifier	5g/kg max
	Ice cream, Kulfi, Dried ice cream mix, Frozen desserts, Milk ice, Milk lollies and Ice candy	Stabiliser, Emulsifier	10g/kg max
	Liquid Egg, Frozen Egg	Sorbate	GMP
Alginic Acid	Fermented Milks (Plain), Heat Treated After Fermentation	Phosphate	GMP
	Sterilized And Uht Creams, Whipping And Whipped Creams, And Reduced Fat Creams (Plain)	Polysorbates	GMP

	Fish and Fish Products	Sulfite	GMP
Ammonium Alginate	Fermented milks (plain), heat treated after fermentation	Phosphate	GMP
	Sterilized and UHT creams, whipping and whipped creams, and reduced fat creams (plain)	Sorbate	GMP
	Fish Products	Sulfite	GMP
Calcium Alginate	Dairy based drinks, flavoured or fermented(eg:chocolate, milk, eggnog, cocoa) UHT sterilized milk shelf life more than three months	Emulsifying, Stabilising	GMP
	Unripened Cheese	Sorbate	GMP
	Egg Products	Sorbate	GMP
	Fish Products	Sulfite	GMP
Carrageenan	Flavoured/Fermented Dairy Milk, Desert Jelly	Stabiliser, Emulsifier	GMP Value
	All types of Yoghurt	Stabiliser, Emulsifier	5g/kg max
	Evaporated Milk	Stabiliser, Emulsifier	150mg/kg max
	Ice cream, Kulfi, Dried ice cream mix, Frozen desserts, Milk ice, Milk lollies and Ice candy	Stabiliser, Emulsifier	10g/kg max
	Condensed Milk/Evaporated Milk/ Sweet Condensed Milk	-	150 mg/kg
	Sterilised UHT Creams, Whipping Creams and reduced fat creams	Polysorbate	GMP
	Unripened Cheese-only Cream Cheese	Sorbate	5000 mg/kg
	Eggs and Egg Products	Sorbate	GMP
	Fish and Fish products	Sulfite, Phosphates ,Carotenoids	GMP
Potassium Alginate	Sterilized and UHT creams, whipping and whipped creams, and reduced fat creams (plain)	Polysorbates	GMP
	Fermented milks (plain), heat treated after fermentation	Phosphate	GMP
	Fish Products	Sulfite	GMP
Processed Eucheuma Seaweed (PES)	Dairy Products And Analogues Except Fats, Oils And Emulsions (Includes all fat-based products that are derived from vegetable, animal or marine sources, or their mixtures)	Thickener, Stabiliser	GMP

	Fish and Fish Products	Sulfite, Phosphate, Carotenoids, Chlorophylls, And Chlorophyllin Copper Complexes	GMP
	Egg Products	Sorbate	GMP
Propylene Glycol Alginate	Ice cream, Kulfi, Dried ice cream mix, Frozen desserts, Milk ice, Milk lollies and Ice candy	Stabiliser, Emulsifier	10g/kg max
	Unripened Cheese	Sorbate	GMP
	Fat Emulsions	Sorbate	GMP
Sodium Alginate	Dairy based drinks, flavoured or fermented (eg:chocolate, milk, eggnog, cocoa) UHT sterilized milk shelf life more than three months	Emulsifying, Stabilising	GMP
	Unripened Cheese	Sorbate	GMP
	Egg Products	Sorbate	GMP
	Fish Products	Sulfite	GMP

* Good Manufacturing Practice (GMP) refers to usage of a food additive in the lowest possible quantity with the appropriate food grade quality and is handled as any other food ingredient.

Table 8: Permitted use of seaweed-based ingredients in food applications in India

Microalgae-based ingredients in food applications

Microalgae-based ingredients is regulated chiefly by the Food Safety and Standards (Health Supplements, Nutraceuticals, Food for Special Dietary Use, Food for Special Medical Purpose, Functional Food and Novel Food) Regulations, 2016, where the microalgae ingredients are listed as nutraceuticals. To be called a nutraceutical, it must not be presented as a conventional food and therefore it may be formulated in the form of powders, granules, tablets, capsules, liquids, jelly and other dosage forms. Nutritional ingredients listed in the Schedules except Schedule VA to Schedule VF can be used for formulations falling in other categories.

Name of Permitted Microalgae Additive	Permitted Usage Limit per day	Purity Criteria
Phycocyanin from <i>Spirulina platensis</i> Dried powder	50 – 250 mg /day max	Protein: 50-70 %; Carbohydrates: 20-40%; Minerals (Ash): 5.0 – 15%; Moisture: 3.0 – 6.0 %
<i>Spirulina</i> (algae) from <i>Spirulina platensis</i> (<i>Arthrospira platensis</i>)	500 - 3,000 mg / day	(Protein (min.) - 55%, carbohydrates (max.)-30%, fats (lipid) – not more than 10%, minerals (ash)-5-10%, moisture- 3- 6% (also fatty acid profile to be mentioned on the package)
Astaxanthin (from <i>Haematococcus pluvialis</i>), powder or oleoresin	4 mg/day max	Astaxanthin content

Table 9: Permitted use of microalgae-based ingredients in food applications in India

Safety and testing

For food testing and analysis FSSAI recognizes and notifies National Accreditation Board for Testing and Calibration Technology (NABL) accredited food laboratories and also recognises foreign laboratories to reduce the time in clearance of food consignments at ports. FSSAI approved notified laboratories with updated NABL accreditation validity and a list of referral labs in case of appeals can be found [here](#).

Current Policy Landscape

Major government bodies governing microalgae and seaweed

The access to biological resources for commercial utilization is regulated by the National Biodiversity Authority (NBA) or the State Authority under the Biological Diversity Act 2002. Indian companies interested in entering the commercial seaweed industry and its export would need to get prior permission from the NBA. The applications for approvals under NBA depends on the purpose of application, and the entity who seeks permission for the access to the biological resources. The forms with the fees can be found [here](#). The Act also mandates that earnings from the commercial activity be shared with the local community as a part of Access Benefit Sharing (ABS).

Under the Ministry of Fisheries, Animal Husbandry & Dairying, the Department of Fisheries formulates policies and schemes for development of inland, coastal and marine fisheries as well as development strategies for the sector and seaweed cultivation is included under it. The department also works closely with academic institutes working to advance research on fisheries through joint projects.

Under Section 14, 'Duties and functions of Coast Guard Act', of Coast Guard Act, the Coast Guard of India is given the duty to protect the fishermen, take measures that are necessary to preserve and protect the maritime environment and to prevent and control marine pollution. The Coast Guard also has powers of levying heavy penalties for the pollution of port waters, example if there is untreated waste water run off from the seaweed cultivation farms. The Coastal Zone Notifications regulates activities on the coastal zone and permits shore activities such as aquaculture, if it does not cross the low tide mark. Additionally the aquaculture farm should be registered under the Coastal Aquaculture Authority. Those seeking to register new aquaculture farms or upgrade existing farms must register with specific details under [Form I](#) issued by the Coastal Aquaculture Authority and the permit will be given for five years.

FDI, Import- Export, National Taxes and Duties

According to the [FDI Policy Circular of 2020](#), given by the Ministry of Commerce and Industry, aquaculture activities can receive up to 100% FDI through the automatic route (without prior government or RBI approval). "Seaweed and other algae" that are fresh, chilled, frozen or dried and are used primarily for human consumption have an IGST rate of 5%.

The general requirements for import and export in India include Registration, License and Import Export Code with details given below:

- The person intending to import or export must be registered as a proprietorship, partnership, private limited company or a public limited company.
- They must have a VAT Registration.
- Importer/Exporter must have an Importer Exporter Code (IEC) and must apply for the same from the [Directorate General of Foreign Trade](#) (DGFT).

Most items are freely exportable unless they fall into the restricted/prohibited list. Under the Agricultural & Processed Food Products Export Development Authority (APEDA), seaweed is categorised as other vegetable products fit for human consumption that are exported. Seaweed and algae with the ITCHS Code of 12129910 can be freely exported. Food Products which are meant for 100% export only, must be prepared as per standard/specification and the labelling requirements of the importing country. No part thereof shall be sold/supplied for domestic consumption. No Objection Certificate (NOC) is imperative to export food, and it will be issued after providing these- company information; IEC that is issued by the DGFT; Food Business License by State/Central Authority ; required food safety tests and labelling compliances as laid down by the importing country for the goods to be imported after which NOC will be issued. Depending on the individual products, other documents may be required. Notices and regulations regarding export of agricultural products can be found [here](#).

An importer must obtain an IEC from the Central Licensing Authority in accordance with the provisions of the [Food Safety and Standards \(Licensing and Registration of Food Businesses\) Regulations, 2011](#) if the importer is importing food items including food ingredients and additives for commercial use. According to India's foreign trade policy, all imported goods shall also be subject to domestic laws, technical specifications, environmental and safety norms as applicable to domestically produced goods. Under the [Food Safety and Standards \(Import\) Regulation 2017](#), guidelines are laid down to streamline the process of imported food clearance. The general requirements to import food and food ingredients to India are to register as an importer and gain the import export code from the Directorate General of Foreign Trade. FSSAI's Import Regulation 2017 also lays down specific rules for shelf life, packaging and labelling requirements, storage of imported food, safety requirements and sampling of imported food. If the Authorising Officer is satisfied there will be a NOC assigned. Certain species of algae are permitted to be imported on the basis of phytosanitary certification provided by the importing nation after inspection by inspecting authorities and after fumigation (if necessary) and other necessary checks as given by the Regulation of Import into India Order 2003 by the Directorate of Plant Protection, under the Ministry of Agriculture & Farmers Welfare. Such specific species are *Ecklonia maxima/ Gelidium/ Gelidiella/Gracilaria/ Pterocladia/ Eucheuma/ Chondrus Kappaphycus* in the form of dried seaweed used for consumption.

*India [Trade Portal](#) contains additional information about duties, trade volume and other import export data for specific items.

Policy Initiatives

Financial and infrastructural support:

- The Finance Minister in her Union Budget 2020 speech announced that cultivation of seaweed and microalgae will be promoted by the setting up of extension services through 3477 SagarMitras and 500 Fish Farmer Producer Organisations. With the help of self help groups and FPOs, the government aims to boost local sales of seaweed, spirulina and other algae.
- As part of the Atmanirbhar Bharat relief package, ₹637 crore has been allocated for seaweed cultivation under the Pradhan Mantri Matsya Sampada Yojana (**PMMSY**) to be spent over the next five years to be available as subsidy support. The following support areas have been outlined under the scheme.
 - Establishment of Seaweed culture rafts including inputs
 - Establishment of Seaweed culture with Monoline/ Tubenet method including inputs
 - Establishment of Brood Banks (including seed banks for seaweeds)

- Under the National Fisheries Policy 2020, the government has called for additional areas for seaweed cultivation to be identified, and technology upgradation for the culture of native species.
- The government has identified seaweed culture as one of the components under the Blue Revolution (also called 'Neel Kranti') Mission and provided 10,510 units of rafts for seaweed culture in its implementation period from 2015-16 to 2019-20.
- The National Mariculture Policy 2019 stipulated designated areas for mariculture activities including seaweed culture, in consultation with local area planning departments, fishermen cooperatives, coastal dwellers and relevant State and Central government departments and other stakeholders. It includes setting up centres for the supply of fresh stock of planting material of seaweed for the southeast and northwest coasts. It additionally includes improvement of culture technologies with regard to control of invasive species, techniques to monitor grazing by fishes and avoiding fertigation usage.
- Under the Ministry of Fisheries, Animal Husbandry & Dairying, the National Fisheries Development Board (NFDB) provides financial assistance for training and demonstration as well as for establishment of seaweed processing units (in the form of equity to the tune of 20% of the investment costs) to Fisheries Institutes, State Fisheries Departments, Fisheries Colleges, NGOs and SHGs. In Ramanathapuram district, 750 fisherwomen beneficiaries have been trained as a part of the first phase of the programme. The NFDB also offers the Fisheries and Aquaculture Infrastructure Development Fund, which is a loan subsidy programme given on a first come first serve basis wherein the eligible people (entrepreneurs, companies, SHG's, collective groups, minority groups) undertaking seaweed farming get 80% of unit cost as loan amount from Scheduled Banks and up to 3% interest subvention of loan on bankable projects. The application requirements as per the guidelines are available on the website [here](#).
- A panel was constituted by the Fisheries Department to study the potential of seaweed farming along the coast of Kerala. The study will cover financial, economic (including employment generation, market linkages) and technical (including suitable species and methods) viability of seaweed farming in the area.

Demand Creation:

- The Karnataka government launched a dietary supplement programme under the Bal Poshna Scheme as one of the initiatives to address the issue of malnutrition in children. As a part of the programme, two grams of *Spirulina* in the form of capsules were given to school children.

Based on the analysis of the current state of Indian policy and regulations with respect to microalgae and seaweed applications in India, several opportunities have been identified across domains of governance, regulations and safety, research funding, livelihood support and security and demand creation. These opportunities have been collated in the opportunities database. Details about the opportunities database have been discussed in the last section.

Talent pool in India and opportunities

Presently the talent needed for developing the algal protein value chain does not exist in India. However, there are ways to extrapolate talent from allied industries and to generate new talent for the alternative protein industry. In this analysis, we will explore spillover benefits to the alternative protein industry from emerging industries, such as innovations in nutraceuticals and biofuels, that creates a large base of suppliers and talent with easily transferable expertise and supply chains to alternative proteins. For the purpose of this study, the microalgal and seaweed value chain for alternative protein solutions has been

categorized by GFI India into the following, strain selection, cultivation, harvesting & dewatering, cell disruption, and protein extraction. In order to access the skill and talent throughout the value chain, it is necessary to access university courses, student groups currently involved, and ongoing research of relevance at academic institutions, startups, and industry. Finally, we will highlight specific interventions needed across the value chain to develop talent needs specifically for accelerating the algal protein sector.

Microalgae and seaweed have been attracting commercial and political interest as a feedstock for bioenergy, including biofuel and biogas, production. Microalgae are being widely researched as a fuel due to their high photosynthetic efficiency and their ability to produce lipids, a biodiesel feedstock. Seaweeds(or macroalgae) do not generally contain lipids and are being considered for the natural sugars and other carbohydrates they contain, which can be fermented to produce either biogas or alcohol-based fuels(Bruton et al. 2019).

India is the world's third-largest consumer of oil and is largely dependent on imports to meet its energy needs (above 80% in 2018). As a response, the Government of India has been actively pursuing a policy to improve oil security. Recently efforts have been made to improve innovation for the development of new sources of bioenergy. The National Policy on Biofuels has set a target of 5% biodiesel oil in diesel by 2030 and 20% blending of ethanol in petrol. Microalgae and seaweed have been extensively researched within this context.

Owing to their superior nutritional properties, due to the presence of vitamins, minerals, and antioxidants, microalgae, and seaweed are increasingly being used in alternative applications such as nutraceuticals, pigments, proteins, functional foods, and other chemical constituents(Wells et al. 2017). Alternative proteins present a promising opportunity. However, the selection of potential strains, low-cost growth nutrients, efficient harvesting techniques, cost-efficient manufacturing, and algal food applications, are all presently under-researched in the context of algae-based alternative protein.

University courses

The alternative protein industry has a significant need for workers and innovators with specialized knowledge spanning multiple traditional disciplines like biotechnology, bioinformatics, biochemical engineering, chemical engineering, mechanical engineering, food science, and industrial engineering. The alternative protein industry needs educational programming that can cover the depth and complexity of knowledge, experience, and skills required within the context of traditional academic institutions. However, few universities offer alternative protein majors or dedicated subject matter. GFI has launched [alternative protein coursework](#) at universities around the globe to help accelerate the alternative protein sector. To ensure a strong talent pipeline it is necessary to evaluate the current state of food science and other enabling sciences that can help propel alternative protein food solutions.

Biosciences (or biotechnology) and biochemical engineering follow a multidisciplinary approach involving electrical, chemical, mechanical, and computer to biology and healthcare. Specializations emerging from this field including tissue engineering, bioinformatics and computational biology, genomics, biomaterials, and bioprocess engineering are especially of relevance within the alternative protein industry. There are presently 279 undergraduate courses and 150 postgraduate and doctorate [biotechnology courses](#) and 11 undergraduate courses and 16 postgraduate [biochemical engineering courses](#) offered in universities across India. Renowned universities including [IIT Guwahati](#), [IIT Kanpur](#), [IIT Bombay](#), [IIT Jodhpur](#), [IIT Delhi](#), [IIT Kharagpur](#), [IIT Madras](#), [Banaras Hindu University \(BHU\)](#), [IISc](#)

[Bangalore](#), and [BITS Pilani](#) offer these programs to batch sizes ranging between 60 -100 students annually.

Bioinformatics is an interdisciplinary field, which harnesses computer science, mathematics, physics, and biology. Through the Department of Biotechnology supported [Biotechnology Information System Network \(BTISNet\)](#) programme 150 bioinformatics centers have been established in various universities and research institutions spread across the country. The centers have been established within institutions with a strong background in biotechnology. Seven Centres of Excellence (COE) in Bioinformatics have been established, including Jawaharlal Nehru University (JNU), New Delhi, Indian Institute of Science (IISc), Bangalore, SuperComputing Facility (IIT), New Delhi.

Food science and technology programs deal with the techniques involved in the production, processing, preservation, packaging, labeling, quality management, and distribution of food products. India's most reputable food technology institutes include [IIT Kharagpur](#), [National Institute of Food Technology Entrepreneurship and Management \(NIFTEM\)](#), [CSIR-Central Food Technological Research Institute](#), and [ICT Mumbai](#). There are presently 153 undergraduate and 58 postgraduate and doctorate [food science and technology programs](#) across India, with batch sizes ranging between 50 - 100 students annually. The Association of Food Scientists and Technologists (India) maintains a directory of graduates and diploma holders in Food Science, Technology and Engineering and those engaged in the profession.

With respect to algal protein development, the following skill-sets are of importance -

1. Strain selection:

- a. Microalgae strain selection:** Sourcing the best microalgal source material for the end-product will depend on open access research on high-throughput screening of algae for high protein content, high growth rates, high adaptability and determination of protein functionality, organoleptic properties, and nutritional value. These high-throughput technologies will generate huge amounts of data and will rely on the field of biotechnology and bioinformatics.
- b. Seaweed strain selection:** Large-scale cultivation of seaweed is highly dependent on how well the algal species adapt to climatic and geographic conditions. Effective breeding for selective strains with desired qualities would require stock improvement and seed production technology to be attempted through biotechnology interventions. To select seaweed species for the large-scale production of algal protein it is essential to understand the genetic composition of the selected strain. The establishment of seaweed seed banks, categorized by its composition, at universities with existing strain banks will enable the promotion of seaweed for alternative uses.

2. Cultivation:

- a. Microalgae cultivation:** Industrial cultivation of microalgae in autotrophic, heterotrophic, or mixotrophic environments rely on the optimization of various process conditions to achieve maximum growth rates. In India, microalgae are most commonly cultivated in open systems like raceway ponds owing ease of construction, operation, and cost-efficiency of such systems. However, the yield remains low. For higher productivity, closed systems cultivation can be carried out in various types of photobioreactors and/or fermentation tanks. Manufacturing and operating such systems would require intervention through a biochemical engineering approach. Repurposing of bioreactors or fermenters already commercially in the pharmaceutical industry would require a similar approach.

-
- b. Seaweed cultivation:** Much of India's seaweed cultivation depends on vegetative propagation. As part of the Government of India's 'Skill India' initiative, CSMCRI conducts training on seaweed cultivation and dissemination of farming along the coast of Tamil Nadu. The Central Marine Fisheries Research Institute (CMFRI), Mandapam, has also been promoting seaweed cultivation from Self Help Groups (SHGs). In countries like Indonesia, China, and Japan, open sea cultivation is carried out at an industrial scale, India doesn't currently possess this type of cultivation technology. Research on different cultivation modes must be explored through mechanical engineering methods. To ensure the quality of feedstock, intervention from a marine biology approach will be essential to monitor various parameters of the ocean to ensure consistent quality of the seaweed.
 - 3. Harvesting and Dewatering:**
 - a. Microalgae harvesting and dewatering:** Typically for microalgae, this involves a harvesting process by concentrating or thickening of the algal biomass into a slurry-like consistency followed by a dewatering process of concentrating the wet slurry into a paste. A variety of mechanical, chemical, and enzymatic methods can be employed here. Designing various mechanical systems - such as centrifugation, filtration, will require a chemical and mechanical engineering approach. Exploration of food-grade flocculants and combinations that can be used to precipitate microalgae will require a food processing intervention.
 - b. Seaweed harvesting:** Harvesting is done either manually or mechanically using winches and cranes. The efficiency of these methods will rely on the scale of cultivation and strong engineering skill sets will make the process cost and yield efficient
 - 4. Protein Extraction:** In order to extract and scale-up protein from microalgae and seaweed, expertise and experience is needed in cell disruption, protein extraction, concentration, and drying technologies This would involve majors like biochemistry, food science and engineering, chemical engineering, mechanical engineering, and industrial engineering
 - 5. Product development and food science:** In order to scale-up a food production process, it is recommended to hire a food scientist. Food scientists can lead the product development process from ideation to launch including working with novel plant protein ingredients, formulation & processing development, and pilot & manufacturing trials. And this may also lead to engagement with external ingredient suppliers, research facilities, pilot plants, and co-manufacturers to effectively deliver the final product with determined specifications and within designated timelines and costs.

Research Programmes

Research is taking place across the algal value chain, however, it remains highly fragmented with researchers, industry, and startups functioning in silos.

1. Microalgae research programmes

National Collection of Industrial Microorganisms (NCIM) - CSIR, one of the largest culture collections in India, consists of around 3700 strains including microalgae. Little information is available on the functionality of proteins extracted from microalgae.

The DBT-Pan IIT Centre for Bioenergy is an initiative with five IITs (IIT Bombay, IIT Kharagpur, IIT Guwahati, IIT Jodhpur, and IIT Roorkee), focusing on the role of microalgae in biofuel production and imparting the necessary skills among over 300 research scholars for the work. Research is currently underway across broad categories through seven research groups. One research group

is focusing exclusively on strain selection and optimization through genetic engineering to produce high amounts of lipids, oil or ethanol. However, it should be noted that GMO in food products is not permitted in India even if the final product does not contain GM proteins or are below a detectable limit. Researchers can explore mutagenesis for the selection of efficient strains for food production. Another group is focusing on process engineering with the objective of designing optimal photobioreactors, to maximize biomass yield, and for cost-effective algal harvesting. Developed strategies will help to improve the overall performance of the technology via combined modifications at the strain (mutagenesis approach) and process level (biochemical engineering approach) which can have direct spillover benefits for the alternative protein value chain. With regard to cell disruption, research is underway on engineering enzymes with desirable properties to improve the efficiency of extraction techniques, this could inform future research on food grade chemical and enzymatic extraction methods.

At CSIR- Central Food Technology Research Institute, Mysore, research is underway for the development of algal metabolites for food applications. Technology has been developed for licensing for the use of high-protein content *Spirulina*. Microalgae are currently underutilized in their application as food ingredients for human consumption due to their inherent organoleptic properties.

Aban Infrastructure Pvt. Ltd, India's largest offshore drilling entity in the private sector, through its biotechnology division, has developed novel commercial-scale technologies for algae cultivation, algae biomass harvesting techniques using catalytic electrodes, and biocrude production from algae feedstocks. Aban is conducting research on algal strain selection and optimization in collaboration with the Centre for Advanced Study in Botany, University of Madras.

Reliance Industries Ltd. and CSIR-IIIM Jammu have partnered on the joint development of therapeutic proteins & formulations with a focus on a formulation of algae-protein with pure algal natural products of therapeutic value.

2. Seaweed research programmes

For seaweed, local strain banks exist at several research institutes in India including National Centre for Seaweed Herbarium at Marine Algal Research Station (MARS), National Institute of Oceanography at Dona Paula, Goa, Central Marine Fisheries Research Institute (CMFRI), Kochi, and CSIR-Central Salt & Marine Chemicals Research Institute (CSMCRI), Bhavnagar. However, the pool of available species is limited and significant additional efforts and resources are required to develop and select seaweed species and strains with specific properties tailored for food, chemical or fuel applications. Advances in understanding seaweed life cycles and efficient breeding techniques will contribute to the improvement of cultivation practices and strain selection.

Mariculture of seaweed has been attempted by The Central Salt & Marine Chemicals Research Institute (CSMCRI), Bhavnagar, Central Marine Fisheries Research Institute (CMFRI), Kochi, and the National Institute of Oceanography (NIO). CSMCRI has been engaged in seaweed-related R&D, including the introduction and acclimatization of species of the red alga, *Kappaphycus alvarezii* on the Diu coast, and further commercialization of the culture technology for commercial seaweed farming in India. CMFRI has been promoting the vegetative propagation of agar yielding varieties of *Gracilaria dura* and *Kappaphycus alvarezii* among Self Help Groups

(SHGs) along the coast of Tamil Nadu and Gujarat. CSMCRI and CMFRI provide both the technology and technical support to the industry for further producing seaweed-derivative products. Industrial-scale farming remains limited by the unavailability of technology and engineering structures for deep-sea propagation.

At the DBT-ICT Centre for Energy Biosciences, research on sustainable seaweed farming technologies together with downstream processes for harnessing the potentials of seaweed resources for energy, food, feed, and chemicals, is presently underway. Currently, most strains cultivated are used for agar, alginate, and carrageenan extraction. A number of technologies have been already licensed to industries for pilot and commercial-scale plants.

Startups like Sea6 Energy have developed proprietary bioprocessing methods for identifying various elements within seaweed biomass, isolating, modifying, and commoditizing them into products ranging from agricultural biostimulants, animal feed, bio-polymers, biofuels, and food ingredients. Extraction of protein from seaweed is presently being explored at a lab-scale given the high variability of protein content within seaweed.

Despite there being no specific research ongoing in any part of the value chain for both microalgae and seaweed specific to alternative protein applications, research from allied industries may be extrapolated to a certain degree to assist in building the algal protein value chain. Ongoing research on scaling up of microalgae for biofuel applications at select institutes may directly inform practices for alternative protein stakeholders. Similarly, ongoing industrial research on applications of microalgae in the nutraceutical industry may inform further protein extraction techniques from microalgae.

Similarly, we are seeing growing research on making the cultivation of seaweed more cost-efficient and yield efficient. These efforts assist the industry greatly in reducing upstream costs for downstream food technology applications. Just as research may be extrapolated, similarly talent can also be brought into the alternative protein industry from existing industries.

Developing a talent pipeline for alternative protein

In order to drive talent towards the alternative protein sector, a number of initiatives can be explored -

- a. *Interdisciplinary approaches at existing centers of excellence:* Equipment and expertise hosted within research centers, like the [DBT Pan-ILT Centre for Bioenergy](#), are mostly made available for a fee to both academics and industry. Proactive outreach to these centers studying the value of microalgae and seaweeds from startups and academicians will help to broaden their scope to include aligned research, including that of alternative protein. Extrapolation of existing research will greatly improve the knowledge transfer and timelines for market commercialization of microalgae and seaweeds as alternative protein solutions.
- b. *Grant programs for alternative protein research:* Grant programs and incubation facilities are key elements within the startup ecosystem and play an important role in de-risking venture capital investment. There exist a number of ag-tech programs paying specific attention to promoting deep-science and technology-driven entrepreneurship in the agri-sector, leading to innovation, economic development, and job creation in the agricultural domain. Application to such programs paying specific attention to the development of the algal value chain will play a significant role in developing the overall market for algal products downstream. Top startup accelerators and dedicated accelerators for alternative protein startups, like Big Idea Ventures, Brinc, and Gastrotrope, also have an opportunity to diversify protein sources within the sector

through microalgae and seaweed. You can find an extended list of accelerator and incubator programs [here](#).

- c. *Technology transfer from research laboratories:* Technology transfer within the Indian research ecosystem takes place through dedicated Technology Transfer Offices (TTO). However, currently, there does not exist a uniform technology transfer policy for public funded research across all institutions. Stakeholders interested in learning more about ongoing research projects at specific academic institutions must refer to individual technology transfer policies of universities for guiding any commercial engagement. TTOs determine the incentives to the academic community to engage with industry for the commercialization of ongoing research. Further development of specific technologies may also be possible through the expertise of the institution itself.
- d. *Industry workshops, courses, and training programs:* The lack of educational resources hinders the development of a robust talent and recruitment pipeline since even talented and interested learners do not have a clear educational pathway to enter the alternative protein space. The alternative protein industry needs educational programming that can cover the depth and complexity of knowledge, experience, and skills required within the context of traditional academic institutions as well as post-graduate professional development and training opportunities. Startups and industry interested in the space and looking to develop a talent pipeline may reach out to the list of universities mentioned to facilitate sponsored workshops to drive further engagement.
- e. *Student support groups:* Establishing and supporting university student groups is central to expanding university activities in alternative protein research and innovation. Student groups are also critical to building supportive, on-campus communities around exploring career paths into the alternative protein industry. Student support through research projects will also help drive R&D funding towards the sector to further increase the commercial potential of microalgae and seaweed solutions.

Given the complexity of the challenges in developing the algal protein value chain, talent must be specifically created for this purpose. Stakeholders need to assess skill sets across specific areas of the value chain and perform targeted outreach to academic institutions, research centers, and student groups to draw in expertise that can contribute to the algal protein industry. To foster talent, stakeholders must be willing to dedicate significant resources for knowledge building and research and development if they are looking to address existing bottlenecks to move the industry forward.

Market developments in alternative protein

Algae-derived products like carrageenan, alginates, agar, and oils have been commonly used in food applications for decades. However, in the last few years, several companies have started working on algae-derived proteins as ingredients and using these ingredients as alternative proteins in meat, egg, and dairy replacement. Triton Algae Innovations Ltd., based out of the US, is one such company focussed on producing algae-based ingredients for alternative meat or plant-based products. Using scalable fermentation technology, they are producing *Chlamydomonas reinhardtii* rich in heme and other meat-like compounds. Similarly, Algama, based out of France, is involved in identifying strains to create alternative proteins from algae. Their expertise ranges from commercial extraction process to ingredient development to attain desired organoleptic, physicochemical, and functional properties.

There is interest from big companies like Nestlé and Unilever to enter into the alternative protein space and utilize algae as a sustainable source of protein. Nestlé has entered into a partnership with Corbion

to develop microalgae-based ingredients for use in their portfolio of food products. Unilever has partnered with Algenuity, which specializes in developing microalgae for use in consumer products, to explore food products based on microalgae. Additionally, startups in the alternative protein space innovating to create meat, seafood, egg, and dairy applications derived from microalgae are rising. Some notable startups have been described below.

New Wave Foods is creating sustainable, plant-based shrimp alternatives made from seaweed and other natural ingredients. Odontella, based out of France, has licensed patents relating to the utilization of Odontella and has created a microalgae-based smoked salmon alternative. Kuleana, based out of the US, is creating plant, microalgae, and seaweed-based tuna. Belgium-based company Damhert has a vegan algae burger product in its portfolio already in the market. Netherlands-based company, Seamore, has a wide range of seaweed-based food products, including chips, wraps, pasta, bread, and a bacon replacement. NovaMeat based out of Spain has recently developed a 3D-printed alternative meat product using plant-based and algae-based proteins. VeganEgg, an egg replacement created by Follow Your Heart based out of the UK, was launched in 2017 and was based on algal flour and algal protein. Spira, based out of the US, is working on creating algae-based ingredients to replace animal-derived and petroleum-derived materials. According to their website, the protein isolate as an ingredient for plant-based milk and meat texturizer is under development.

In India, technology consultancy firms like GreenBubble are instrumental in providing engineering support with setup of low-cost cultivation and harvesting and dewatering systems for microalgae. On the other hand companies like SeaGrass Technologies, Parry Nutraceuticals, Shaivaa AlgaeTech and NB Labs are leading the way to expand microalgae production systems. Utilizing the existing extraction capabilities of companies like Synthite Industries will aid algae production companies to go beyond selling algae whole biomass products and venture into downstream processing of algae to create functional protein isolates for use in alternative protein products.

The collaboration of multinational companies with biotechnology startups for developing algae-based food products, and growing number of startups creating algae based meat, seafood, egg, and dairy applications demonstrates that microalgae and seaweeds can be a promising source of novel alternative proteins.

Potential applications of microalgae and seaweed in alternative seafood products

Health benefits associated with seafood consumption are often attributed to the presence of omega-3 fatty acids, high vitamin D and B12 content, and minerals like iron, zinc, iodine, magnesium, and potassium. However fish obtain these nutritional components from the consumption of marine microalgae. Since consumers look for not only taste and convenience but also nutritional benefits from a seafood product, algae become a natural choice for sourcing ingredients that enhance the nutritional value of plant-based seafood products. Microalgae, depending on the species, can be rich sources of beta carotene, astaxanthin, polyunsaturated fatty acids, omega-3 fatty acids, polyphenols, sterols, and antioxidants in addition to minerals like calcium, sodium, magnesium, phosphorus, potassium, zinc, iodine, etc. On the other hand, seaweeds are rich sources of micronutrients. Seaweeds are the best natural sources of iodine. They are also sources of soluble vitamins A, D, E, K, C, B1, B2, B9, B12 and essential minerals like calcium, iron, magnesium, phosphorus, potassium, zinc, copper, manganese, selenium, and fluoride (Mišurcová 2011, Qin 2018). Seaweed-based products like carrageenan, agar,

alginates, and other polysaccharides can also act as structuring agents. Due to the high water holding capacity of seaweed-derived polysaccharides, these can act as gelling agents and binders. Thus, seaweeds can be utilized as sources of texturizing not only alternative seafood products but also alternative meat, egg, and dairy. The characteristic umami flavor found in seafood which is a result of the presence of certain amino acids like aspartic acid and glutamic acid can be achieved in plant-based seafood products by incorporation of microalgae and seaweed as ingredients. However, the type, content, and format of microalgae and seaweed used for such purposes need to be explored.

Next steps : Maturity of the algal protein industry in India

Based on the literature review of the current state of technology development across the algal value chain and insights garnered from stakeholder interviews, the maturity of each stage of the algal value chain in India has been determined. This will help current and potential stakeholders understand the current state of the algal value chain in India. The maturity of each stage of the value chain can be interpreted based on the combination of the technology readiness level and manufacturing readiness level. Based on a scale of 0 to 5, the technology or manufacturing readiness level indicates the level of technology or manufacturing development of a particular segment of the value chain. The technology readiness level ranges from 0, indicating the technology development has not begun in India, to 5, indicating presence of product lines in the market by many companies. Similarly, the manufacturing readiness level ranges from 0, indicating the absence of any infrastructure or manufacturing scale facility where the technology relevant to the value chain segment is implemented, to 5, indicating readily available infrastructure or manufacturing facilities where one or multiple technologies relevant to the value chain segment have been scaled in a cost-effective way. Both the technology readiness level and manufacturing readiness level are determined keeping in mind the Indian market. The table below describes the technology readiness levels and manufacturing readiness levels in detail.

Scale/Type of readiness	0	1	2	3	4	5
Technology Readiness Level	No academic research	Only academic research	Proof of concept validated at lab scale/experimental prototypes/technologies reported by companies/startups	Prototypes/technology demonstrated to be economically viable	Few companies have established product lines for this stage of value chain	Many companies have established product lines for this stage of value chain
Manufacturing Readiness Level	No existing infrastructure capacity to support this activity	No infrastructure capacity to support this activity but infrastructure available for other applications	Infrastructure established by few companies but cost is prohibitive, no scalable solutions exist	Infrastructure established by few companies but cost is prohibitive, scalable solutions demonstrated to bring down costs	Infrastructure established by few companies and cost is no longer prohibitive	Infrastructure is common-place, high scalability of production and processing techniques

Table 10: Technology readiness scale and manufacturing readiness scale

The table below shows the ratings for each stage of the value chain.

Microalgae	Strain selection	Cultivation	Harvesting and dewatering	Cell disruption	Protein extraction	Drying	Algae-based alternative meat, seafood, egg, dairy products
Technological readiness level	1	5	4	4	3	5	2
Manufacturing readiness level	0	3	3	3	1	5	2

Table 11: Maturity ratings for segments of microalgal value chain

Seaweed	Strain selection	Cultivation	Harvesting	Protein extraction	Drying	Algae-based alternative meat, seafood, egg, dairy products
Technological readiness level	1	4	4	3	5	1
Manufacturing readiness levels	0	3	3	1	5	1

Table 12: Maturity ratings for segments of seaweed value chain

As the report demonstrates, based on developments of the algae industry in India as well as outside India, there is significant progress in terms of technological readiness for most value chain segments. However, the exploration of both microalgae and seaweed for not only alternative protein applications but also general food applications in India has been limited to academic research. Manufacturing readiness for most value chain segments has been limited in India given that a limited number of companies have demonstrated technological solutions at scale and also a limited number of companies are working in the microalgae and seaweed sector.

While the maturity rating gives an overview of the microalgae and seaweed industry status in India with respect to alternative protein applications, the key white space opportunities and interventions which will help accelerate the algae industry have been elaborated in detail in our opportunities database. The opportunities database lists out opportunities across value chain segments and categories including R&D, manufacturing, regulatory, investment, end product optimization, and demand generation. The opportunities have also been rated for the extent of their impact on the alternative protein sector, the difficulty of implementation, and the maturity of the intervention in the Indian context. The opportunities database can serve as a guide for various stakeholders working in the algae industry as well as the alternative protein sector to help accelerate the development of alternative protein products using algae-based proteins or algae biomass.

Conclusion

The algal protein industry in India and around the globe is at its development stages, with few companies working globally on commercial production. As the alternative protein sector evolves in India, algal proteins will play a key role in creating a new generation of sustainable proteins to create alternative meat, egg, dairy and seafood products. Given the potential of algae as a sustainable food source, the flexibility to engineer efficient production of proteins and the presence of nutritionally and pharmaceutically valuable compounds from algae, the future of algae-based products is promising. Technological innovation at each stage of the value chain is critical to make algal protein a commercial source of alternative protein. India has a unique advantage as a global bio-manufacturing hub and can be an industry leader in algae-based products with the right balance of academic research and funding from the government, corporate sector, and venture capital firms. The geographical and climatic conditions in India are also favorable for the cultivation of microalgae and seaweed. With the help of existing technical expertise and channeling investments in technological development and academic research across the value chain, India can lead the way to cost-efficient, functional applications of algae in the alternative protein sector, and a new generation of delicious, nutritious, and sustainable meat, egg, dairy and seafood replacements.

Acknowledgements

GFI India would like to thank the various stakeholders and experts who participated in the interviews and roundtable discussions to give their valuable inputs and insights on the microalgae and seaweed industry. Their insights were extremely valuable in identifying the key interventions that need to be focused on to accelerate the algae industry in India especially for alternative protein applications. The participants in our study included - Dr. C.R.K. Reddy, Dr. Greg Mitchell, Harshith Sirigeri, Dr. Stephen Mayfield, Yashraj Jariwala, Sònia Hurtado, Dr. Roberto Bassi, Dr. Kunal Samantaray, Dr. Alexander Mathys, Dr. D Swaminathan, Kushal Aradhya, Jill Kauffman, Dr. Pierre Winsel, Dr. William Chen Wei Ning, Karen Seal, Baruch Dach, Dr. Laurice Poureau, David Hazelback, Dr. S.V. Mohan, Beth Zotter, Marc Geytenbeek, Dr. Rene Wijffels, Dr. Amha Belay, Dr. Jean-Paul Cadoret, Yonatan Golan, Abhiram Seth, Erika George, Lior Roger, Dr. P. Kaladharan, Atul Anjani, Hrishikesh Rajanlankar, Dr. Monica Gajanan Kavale, Dr. Avinash Mishra, Dr. Lutz Grossman, Dr. Reena Pandit, Dr. Vikas Singh Chauhan, Dr. Brijesh Tiwari, Dr. Manish Shukla, Dr. Rajesh Krishnamurthy, Dr. Lukas Böcker, Kevin Parikh, Supriya Srinivasan, Alex Ward, Dr. Raffael Jovine Dr. Carsten Krome, Amod Salgaonkar, Sri Sailaja Nori, and Dr. Arup Ghosh. We are extremely thankful to Dr. Fatma Boukid for reviewing the report and sharing her feedback on the same.

References

- Amorim ML, Soares J, Coimbra JS dos R, Leite M de O, Albino LFT, Martins MA. 2020. Microalgae proteins: production, separation, isolation, quantification, and application in food and feed. *Crit Rev Food Sci Nutr.*:1–27.
- An HSUS Fact Sheet: Greenhouse Gas Emissions from Animal Agriculture [Internet]. 2007. The Humane Society of the United States [Humanesociety.org](https://www.humanesociety.org/sites/default/files/archive/assets/pdfs/farm/hsus-fact-sheet-greenhouse-gas-emissions-from-animal-agriculture.pdf). [accessed 2020e Sep 4].
<https://www.humanesociety.org/sites/default/files/archive/assets/pdfs/farm/hsus-fact-sheet-greenhouse-gas-emissions-from-animal-agriculture.pdf>.
- Ansari FA, Shriwastav A, Gupta SK, Rawat I, Bux F. 2017. Exploration of microalgae biorefinery by optimizing sequential extraction of major metabolites from *Scenedesmus obliquus*. *Ind Eng Chem Res.* 56(12):3407–3412.
- Askew K. 2020 Apr Seakura: We are at the frontier of land-based seaweed cultivation. *Foodnavigator.com*. 2020 Apr 28 [accessed 2020 Sep 4].
<https://www.foodnavigator.com/Article/2020/04/28/Seakura-We-are-at-the-frontier-of-land-based-sea-weed-cultivation>
- Barbarino E, Lourenço SO. 2005. An evaluation of methods for extraction and quantification of protein from marine macro- and microalgae. *J Appl Phycol.* 17(5):447–460.
- Barros AI, Gonçalves AL, Simões M, Pires JCM. 2015. Harvesting techniques applied to microalgae: A review. *Renew Sustain Energy Rev.* 41:1489–1500.
- Becker EW. 2013. Microalgae for Human and Animal Nutrition. In: *Handbook of Microalgal Culture*. Oxford, UK: John Wiley & Sons, Ltd. p. 461–503.
- Bhaskar RN. 2018 Jun 13 COMMENT | Cultivating seaweed could be a massive opportunity for India. *Moneycontrol.com*. [accessed 2020 Sep 3]. <https://www.moneycontrol.com/news/>
- Bleakley S, Hayes M. 2017. Algal proteins: Extraction, application, and challenges concerning production. *Foods.* 6(5):33.
- Branyikova I, Prochazkova G, Potocar T, Jezkova Z, Branyik T. 2018. Harvesting of Microalgae by flocculation. *Fermentation.* 4(4):93.
- Bruton, T. & Lyons, Henry & Lerat, Yannick & Stanley, Michele & Rasmussen, M.B.. (2009). A review of the potential of Marine Algae as a Source of Biofuel in Ireland. *Sustainable Energy*.
- Buchmann L, Brändle I, Haberkorn I, Hiestand M, Mathys A. 2019. Pulsed electric field based cyclic protein extraction of microalgae towards closed-loop biorefinery concepts. *Bioresour Technol.* 291(121870):121870.
- Caporgno MP, Mathys A. 2018. Trends in microalgae incorporation into innovative food products with potential health benefits. *Front Nutr.* 5. doi:10.3389/fnut.2018.00058.
<http://dx.doi.org/10.3389/fnut.2018.00058>.

Carmichael WW, Boyer GL. 2016. Health impacts from cyanobacteria harmful algae blooms: Implications for the North American Great Lakes. *Harmful Algae*. 54:194–212.

Center for Food Safety, Applied Nutrition. 2020. Microorganisms & microbial-derived ingredients used in food. *Fda.gov*. [accessed 2020 Sep 6].
<https://www.fda.gov/food/generally-recognized-safe-gras/microorganisms-microbial-derived-ingredients-used-food-partial-list>.

Cheirsilp B, Torpee S. 2012. Enhanced growth and lipid production of microalgae under mixotrophic culture condition: Effect of light intensity, glucose concentration and fed-batch cultivation. *Bioresour Technol*. 110:510–516.

Christenson L, Sims R. 2011. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. *Biotechnol Adv*. 29(6):686–702.

Chronakis IS, Madsen M. 2011. Algal proteins. In: *Handbook of Food Proteins*. Elsevier. p. 353–394.

Chronakis IS. 2000. Biosolar proteins from aquatic algae. In: Doxastakis G, Kioseoglou V, editors. *Developments in Food Science*. Vol. 41. Elsevier. p. 39–75.

Clément G, Giddey C, Menzi R. 1967. Amino acid composition and nutritive value of the alga *Spirulina maxima*. *J Sci Food Agric*. 18(11):497–501.

Clippinger JN, Davis RE. 2019. Techno-economic analysis for the production of algal biomass via closed photobioreactors: Future cost potential evaluated across a range of cultivation system designs. Office of Scientific and Technical Information (OSTI).

Coustets M, Joubert-Durigneux V, Hérault J, Schoefs B, Blanckaert V, Garnier J-P, Teissié J. 2015. Optimization of protein electroextraction from microalgae by a flow process. *Bioelectrochemistry*. 103:74–81.

Cunningham S. 2018 Nov 5. offshore vs. land-based seaweed farms, and why we went land. — Monterey Bay Seaweeds. *Montereybayseaweeds.com*. [accessed 2020 Sep 4].
<http://www.montereybayseaweeds.com/the-seaweed-source/2018/11/4/offshore-vs-land-based-seaweed-farms-and-why-we-went-land>.

Davey HM, Kell DB. 1996. Flow cytometry and cell sorting of heterogeneous microbial populations: the importance of single-cell analyses. *Microbiol Rev*. 60(4):641–696.

Davis R, Markham J, Kinchin C, Grundl N, Tan ECD, Humbird D. 2016. Process design and economics for the production of algal biomass: Algal biomass production in open pond systems and processing through dewatering for downstream conversion. Office of Scientific and Technical Information (OSTI).

Duong VT, Li Y, Nowak E, Schenk PM. 2012. Microalgae isolation and selection for prospective biodiesel production. *Energies*. 5(6):1835–1849.

Elsevier. *Novel Macromolecules in Food Systems*, Volume 41 - 1st Edition. Elsevier.com. [accessed 2020 Sep 4].
<https://www.elsevier.com/books/novel-macromolecules-in-food-systems/doxastakis/978-0-444-82932-0>.

Elsevier. 2018. Bioactive seaweeds for food applications: Natural ingredients for healthy diets. 1st ed. Qin Y, editor. San Diego, CA: Academic Press.

Enzing C, Nooijen A, Eggink G, Springer J, Wijffels R. Algae and genetic modification. 2012 May. Cogem.net. [accessed 2020 Sep 6].
<https://cogem.net/app/uploads/2019/07/CGM-2012-05-Algae-and-genetic-modification-research-production-and-risks1-1.pdf>.

Enzing C, Ploeg M, Barbosa M, Sijtsma L. 2014 Apr 1. Microalgae-based products for the food and feed sector: an outlook for Europe - EU Science Hub - European Commission. Europa.eu. [accessed 2020 Sep 6].
<https://ec.europa.eu/jrc/en/publication/eur-scientific-and-technical-research-reports/microalgae-based-products-food-and-feed-sector-outlook-europe>.

Fabregas J, Herrero C. 1985. Marine microalgae as a potential source of single cell protein (SCP). Appl Microbiol Biotechnol. 23(2):110–113.

Fasaei F, Bitter JH, Slegers PM, van Boxtel AJB. 2018. Techno-economic evaluation of microalgae harvesting and dewatering systems. Algal Res. 31:347–362.

Flavin K, Flavin N, Flahive B. 2013 Kelp Farming Manual. Squarespace.com. [accessed 2020 Sep 4]
https://static1.squarespace.com/static/52f23e95e4b0a96c7b53ad7c/t/52f78b0de4b0374e6a0a4da8/1391954701750/OceanApproved_KelpManualLowRez.pdf

Fleurence J, Massiani L, Guyader O, Mabeau S. 1995. Use of enzymatic cell wall degradation for improvement of protein extraction from *Chondrus crispus*, *Gracilaria verrucosa* and *Palmaria palmata*. J Appl Phycol. 7(4):393–397.

Fleurence J. 2004. Seaweed proteins. In Yada, R. Y. [Ed.] Proteins in Food Processing. Woodhead Publishing Limited, Cambridge, UK, p. 197–213.

Fleurence J, Chenard E, Lucon M. 1999. Determination of the nutritional value of proteins obtained from *Ulva armoricana*. J. Appl. Phycol. 11:231–9.

Fleurence J. 1999. Seaweed proteins. Trends Food Sci Technol. 10(1):25–28.

Ganesan M, Trivedi N, Gupta V, Madhav SV, Radhakrishna Reddy C, Levine IA. 2019. Seaweed resources in India – current status of diversity and cultivation: prospects and challenges. Botanica Marina. 62(5):463–482.

García JL, de Vicente M, Galán B. 2017. Microalgae, old sustainable food and fashion nutraceuticals. Microb Biotechnol. 10(5):1017–1024.

Gilroy DJ, Kauffman KW, Hall RA, Huang X, Chu FS. 2000. Assessing potential health risks from microcystin toxins in blue-green algae dietary supplements. Environ Health Perspect. 108(5):435–439.

Goettel M, Eing C, Gusbeth C, Stroessner R, Frey W. 2013. Pulsed electric field assisted extraction of intracellular valuables from microalgae. Algal Res. 2(4):401–408.

-
- Grossmann L, Hinrichs J, Weiss J. 2019. Cultivation and downstream processing of microalgae and cyanobacteria to generate protein-based techno functional food ingredients. *Crit Rev Food Sci Nutr.*1–29.
- Guiry MD. 2012. How many species of algae are there? *J Phycol.* 48(5):1057–1063.
- Gupta V, Kumari P, Reddy CRK. 2015. Development and Characterization of Somatic Hybrids of *Ulva reticulata* Forssk *Monostroma oxyspermum* (Kütz.)Doty. *Front Plant Sci.* 6. doi:10.3389/fpls.2015.00003.
- Harnedy PA, FitzGerald RJ. 2011. Bioactive proteins, peptides, and amino acids from macroalgae. Macroalgae: Bioactive agent source. *J Phycol.* 47(2):218–232.
- Harnedy PA, FitzGerald RJ. 2013. Extraction of protein from the macroalga *Palmaria palmata*. *Lebenson Wiss Technol.* 51(1):375–382.
- Hoffman JR, Falvo MJ. 2004. Protein - which is best? *J Sports Sci Med.* 3(3):118–130.
- Johnson B, Narayanakumar R, Abdul Nazar AK, Kaladharan P, Gopakumar G. 2017. Economic analysis of farming and wild collection of seaweeds in Ramanathapuram District, Tamil Nadu. *Indian J Fish.* 64(4).
- Jordan P, Vilter H. 1991. Extraction of proteins from material rich in anionic mucilages: Partition and fractionation of vanadate-dependent bromo peroxidases from the brown algae *Laminaria digitata* and *L. saccharina* in aqueous polymer two-phase systems. *Biochim Biophys Acta Gen Subj.* 1073(1):98–106.
- Joubert Y, Fleurence J. 2008. Simultaneous extraction of proteins and DNA by an enzymatic treatment of the cell wall of *Palmaria palmata* (Rhodophyta). *J Appl Phycol.* 20(1):55–61.
- K. Y. Lim D, Algae Biotechnology Laboratory, School of Agriculture and Food Sciences, University of Queensland, Brisbane, Queensland 4072, Australia, M. Schenk P. 2017. Microalgae selection and improvement as oil crops: GM vs non-GM strain engineering. *AIMS Bioeng.* 4(1):151–161.
- Kadam SU, Álvarez C, Tiwari BK, O'Donnell CP. 2017. Extraction and characterization of protein from Irish brown seaweed *Ascophyllum nodosum*. *Food Res Int.* 99:1021–1027.
- Kadam SU, Prabhasankar P. 2010. Marine foods as functional ingredients in bakery and pasta products. *Food Res Int.* 43(8):1975–1980.
- Keeney DR. 1970. Protein and amino acid composition of maize grain as influenced by variety and fertility. *J Sci Food Agric.* 21(4):182–184.
- Kent M, Welladsen HM, Mangott A, Li Y. 2015. Nutritional evaluation of Australian microalgae as potential human health supplements. *PLoS One.* 10(2):e0118985.
- Kishor P B K, Rao M, Kumar S. *Pharmaceutical Biotechnology: Himalaya Publishing House Pvt. Ltd* [accessed 2020 Sep 4]. <http://www.himpub.com/documents/Chapter941.pdf>
- Koyande AK, Chew KW, Rambabu K, Tao Y, Chu D-T, Show P-L. 2019. Microalgae: A potential alternative to health supplementation for humans. *Food Sci Hum Wellness.* 8(1):16–24.

-
- Law SQK, Mettu S, Ashokkumar M, Scales PJ, Martin GJO. 2018. Emulsifying properties of ruptured microalgae cells: Barriers to lipid extraction or promising biosurfactants? *Colloids Surf B Biointerfaces*. 170:438–446.
- Li Y, Zhou W, Hu B, Min M, Chen P, Ruan RR. 2012. Effect of light intensity on algal biomass accumulation and biodiesel production for mixotrophic strains *Chlorella kessleri* and *Chlorella protothecoides* cultivated in highly concentrated municipal wastewater. *Biotechnol Bioeng*. 109(9):2222–2229.
- Lopes da Silva T, Moniz P, Silva C, Reis A. 2019. The dark side of microalgae biotechnology: A heterotrophic biorefinery platform directed to ω -3 rich lipid production. *Microorganisms*. 7(12):670.
- Luengo E, Condón-Abanto S, Álvarez I, Raso J. 2014. Effect of pulsed electric field treatments on permeabilization and extraction of pigments from *Chlorella vulgaris*. *J Membr Biol*. 247(12):1269–1277.
- Lubitz JA. 1963. The protein quality, digestibility, and composition of algae, *Chlorella* 71105. *J Food Sci*. 28(2):229–232.
- Lum KK, Kim J, Lei X. 2013. Dual potential of microalgae as a sustainable biofuel feedstock and animal feed. *J Anim Sci Biotechnol*. 4(1):53.
- Mantri VA, Shah Y, Thirupathi S. 2020. Feasibility of farming the agarose-yielding red alga *Gracilaria dura* using tube-net cultivation in the open sea along the Gujarat coast of NW India. *Applied Phycology*. 1(1):12–19.
- Marsham S, Scott GW, Tobin ML. 2007. Comparison of nutritive chemistry of a range of temperate seaweeds. *Food Chem*. 100(4):1331–1336.
- Matejtschuk P. 2007. Lyophilization of Proteins. In: *Cryopreservation and Freeze-Drying Protocols*. Totowa, NJ: Humana Press. p. 59–72.
- Mekonnen MM, Hoekstra AY. 2012. A global assessment of the water footprint of farm animal products. *Ecosystems*. 15(3):401–415.
- Mišurcová L. 2011. Chemical composition of seaweeds. In: *Handbook of Marine Macroalgae*. Chichester, UK: John Wiley & Sons, Ltd. p. 171–192.
- Mobin S, Alam F. 2017. Some promising microalgal species for commercial applications: A review. *Energy Procedia*. 110:510–517.
- Moomaw W, Berzin I, Tzachor A. 2017. Cutting out the middle fish: Marine microalgae as the next sustainable omega-3 fatty acids and protein source. *Ind Biotechnol (New Rochelle N Y)*. 13(5):234–243.
- Morgan K. C, Wright J. L. C, Simpson F. J. 1980. Review of chemical constituents of the red alga *Palmaria palmata* (dulse). *Mar. Biol*. 34:27–50
- Morrissey J, Kraan S, Guiry M. D. 2001. *A Guide to Commercially Important Seaweeds on the Irish Coast*. Bord Iascaigh Mhara/Irish Sea Fisheries Board, Dublin, Ireland, 67 p

-
- Moudříková Š, Nedbal L, Solovchenko A, Mojzeš P. 2017. Raman microscopy shows that nitrogen-rich cellular inclusions in microalgae are microcrystalline guanine. *Algal Res.* 23:216–222.
- Munda I. M. 1977. Differences in amino acid composition of estuarine and marine fucoids. *Aquat. Bot.* 3:273–80.
- Murbach TS. U.S. FOOD & DRUG. [Fda.gov](https://www.fda.gov/fda.gov). [accessed 2020 Sep 3].
<https://www.fda.gov/media/121982/download>.
- Neoh YY, Matanjun P, Lee JS. 2016. Comparative study of drying methods on chemical constituents of Malaysian red seaweed. *Dry technol.* 34(14):1745–1751.
- Nirmala C, Prakash V, Venkataraman LV. 1992. Physico-chemical and functional properties of proteins from spray dried algae (*Spirulina platensis*). *Nahrung.* 36(6):569–577.
- O’Sullivan AM, O’Callaghan YC, O’Grady MN, Waldron DS, Smyth TJ, O’Brien NM, Kerry JP. 2014. An examination of the potential of seaweed extracts as functional ingredients in milk. *Int J Dairy Technol.* 67(2):182–193.
- Oser BL. 1959. An integrated essential amino acid index for predicting the biological value of proteins. In: *Protein and Amino Acid Nutrition*. Elsevier. p. 281–295.
- Pahl SL, Lee AK, Kalaitzidis T, Ashman PJ, Sathe S, Lewis DM. 2013. Harvesting, thickening and dewatering microalgae biomass. In: *Algae for Biofuels and Energy*. Dordrecht: Springer Netherlands. p. 165–185.
- Patarra RF, Paiva L, Neto AI, Lima E, Baptista J. 2011. Nutritional value of selected macroalgae. *J Appl Phycol.* 23(2):205–208.
- Perez-Garcia O, Bashan Y. 2015. Microalgal heterotrophic and mixotrophic culturing for bio-refining: From metabolic routes to techno-economics. In: *Algal Biorefineries*. Cham: Springer International Publishing. p. 61–131.
- Polikovskiy M, Fernand F, Sack M, Frey W, Müller G, Golberg A. 2019. In silico food allergenic risk evaluation of proteins extracted from macroalgae *Ulva* sp. with pulsed electric fields. *Food Chem.* 276:735–744.
- Postma PR, Pataro G, Capitoli M, Barbosa MJ, Wijffels RH, Eppink MHM, Olivieri G, Ferrari G. 2016. Selective extraction of intracellular components from the microalga *Chlorella vulgaris* by combined pulsed electric field–temperature treatment. *Bioresour Technol.* 203:80–88.
- Prokop A, Bajpai RK, Zappi ME, editors. 2015. *Algal Biorefineries*. Cham: Springer International Publishing.
- Radulovich R, Neori A, Valderrama D, Reddy CRK, Cronin H, Forster J. 2015. Farming of seaweeds. In: *Seaweed Sustainability*. Elsevier. p. 27–59.
- Radulovich R, Umanson S, Cabrera R, Mata R. 2015. Tropical seaweeds for human food, their cultivation and its effect on biodiversity enrichment. *Aquaculture.* 436:40–46.

Ranganathan, J. et al. (2016). Shifting Diets for a Sustainable Food Future. Working Paper, Installment 11 of Creating a Sustainable Food Future. Washington, DC: World Resources Institute. World Resources Report. Org. 2018 Dec 4 [accessed 2020 Sep 4]. <http://www.worldresourcesreport.org>.

Reckermann M. 2000. Flow sorting in aquatic ecology. *Sci Mar.* 64(2):235–246.

Reddy CRK, Jha B, Fujita Y, Ohno M. 2008. Seaweed micropropagation techniques and their potentials: an overview. *J Appl Phycol.* 20(5):609–617.

Rupe ´ rez P, Saura-Calixto F. 2001. Dietary fibre and physico- chemical properties of edible Spanish seaweeds. *Eur. Food Res. Technol.* 212:349–54.

San, M. 2012 Mar. The farming of seaweeds. *Fao.org*. [accessed 2020 Sep 3]. <http://www.fao.org/3/a-bl759e.pdf>

Schaafsma G. 2000. The protein digestibility-corrected amino acid score. *J Nutr.* 130(7):1865S–7S.

Schwenzfeier A, Helbig A, Wierenga PA, Gruppen H. 2013. Emulsion properties of algae soluble protein isolate from *Tetraselmis* sp. *Food Hydrocoll.* 30(1):258–263.

Ścieszka S, Klewicka E. 2019. Algae in food: a general review. *Crit Rev Food Sci Nutr.* 59(21):3538–3547.

Show K-Y, Lee D-J, Tay J-H, Lee T-M, Chang J-S. 2015. Microalgal drying and cell disruption – Recent advances. *Bioresour Technol.* 184:258–266.

Sikorski ZE. 2018. Fennema’s Food Chemistry (Fifth Edition) Edited by Srinivasan Damodaran Kirk L. Parkin CRC Press, Boca Raton, Florida, 2017. 1107 pp. ISBN 9781482208122. *J Food Biochem.* 42(2):e12483.

Sidari R, Tofalo R. 2019. A comprehensive overview on microalgal-fortified/based food and beverages. *Food Reviews International*, 35(8), 778–805.

Smetana S, Sandmann M, Rohn S, Pleissner D, Heinz V. 2017. Autotrophic and heterotrophic microalgae and cyanobacteria cultivation for food and feed: life cycle assessment. *Bioresour Technol.* 245:162–170.

Soto-Sierra L, Stoykova P, Nikolov ZL. 2018. Extraction and fractionation of microalgae-based protein products. *Algal Res.* 36:175–192.

Sousa I, Gouveia L, Batista A, Raymundo A, Bandarra N. 2008. Microalgae in novel food products. *Algae: Nutrition, Pollution Control and Energy Sources*.

Suarez Garcia E, van Leeuwen J, Safi C, Sijtsma L, Eppink MHM, Wijffels RH, van den Berg C. 2018. Selective and energy efficient extraction of functional proteins from microalgae for food applications. *Bioresour Technol.* 268:197–203.

Thornton P, Herrero M, Ericksen P. Livestock and climate change. *Cgiar.org*. [accessed 2020 Sep 4]. <https://cgspace.cgiar.org/bitstream/handle/10568/10601/IssueBrief3.pdf>.

Uduman N, Qi Y, Danquah MK, Forde GM, Hoadley A. 2010. Dewatering of microalgal cultures: A major bottleneck to algae-based fuels. *J Renew Sustain Energy.* 2(1):012701.

-
- Uribe-Wandurraga ZN, Igual M, García-Segovia P, Martínez-Monzó J. 2019. Effect of microalgae addition on mineral content, colour and mechanical properties of breadsticks. *Food Funct.* 10(8):4685–4692.
- Ursu A-V, Marcati A, Sayd T, Sante-Lhoutellier V, Djelveh G, Michaud P. 2014. Extraction, fractionation and functional properties of proteins from the microalgae *Chlorella vulgaris*. *Bioresour Technol.* 157:134–139.
- Valderrama D, Cai J, Hishamunda N, Ridler N, Neish IC, Hurtado AQ, Msuya FE, Krishnan M, Narayana Kumar R, Kronen M, et al. 2015. The economics of *Kappaphycus* Seaweed cultivation in developing countries: A comparative analysis of farming systems. *Aquacult Econ Manage.* 19(2):251–277.
- van Apeldoorn ME, van Egmond HP, Speijers GJA, Bakker GJL. 2007. Toxins of cyanobacteria. *Mol Nutr Food Res.* 51(1):7–60.
- Voudouris P, Tenorio AT, Lesschen JP, Kyriakopoulou K, Sanders JPM, van der Goot AJ, Bruins ME. 2017. Sustainable protein technology : an evaluation on the STW Protein programme and an outlook for the future. Wageningen: Wageningen Food & Biobased Research.
- Waghmare AG, Salve MK, LeBlanc JG, Arya SS. 2016. Concentration and characterization of microalgae proteins from *Chlorella pyrenoidosa*. *Bioresour Bioprocess.* 3(1). doi:10.1186/s40643-016-0094-8. <http://dx.doi.org/10.1186/s40643-016-0094-8>.
- Walker J. 2010. Protein structure, purification, characterisation and function analysis. In: *Principles and Techniques of Biochemistry and Molecular Biology*. Cambridge, TAS, Australia: Cambridge University Press. p. 300–351.
- Wells ML, Potin P, Craigie JS, Raven JA, Merchant SS, Helliwell KE, Smith AG, Camire ME, Brawley SH. 2017. Algae as nutritional and functional food sources: revisiting our understanding. *J Appl Phycol.* 29(2):949–982.
- Westhoek H, Lesschen JP, Rood T, Wagner S, De Marco A, Murphy-Bokern D, Leip A, van Grinsven H, Sutton MA, Oenema O. 2014. Food choices, health and environment: Effects of cutting Europe's meat and dairy intake. *Glob Environ Change.* 26:196–205.
- What is Agrobiodiversity? Fao.org. [accessed 2020b Sep 4]. <http://www.fao.org/3/y5609e/y5609e02.htm>.
- Wong KH, Cheung PCK. 2001. Nutritional evaluation of some subtropical red and green seaweeds Part II. In vitro protein digestibility and amino acid profiles of protein concentrates. *Food Chem.* 72(1):11–17.
- Wong K. H, Cheung, P. C. K. 2000. Nutritional evaluation of some subtropical red and green seaweeds: part I—proximate composition, amino acid profiles and some physico-chemical properties. *Food Chem.* 71:475–82.
- Yarish C, Redmond S, Kim JK. 2012. *Gracilaria culture handbook for New England*. [accessed 2020 Sep 3]. <https://opencommons.uconn.edu/wracklines/72>.