Macrophyte biotechnology: a brief review of production methods and industrial application of seaweed products.

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Introduction

The intensive culture of aquatic plants represents a major proportion of global aquacultured production, value and trade. Total yields of seaweeds in 1983, through controlled culture, were estimated as 2.4 million tons (Pillay, 1993; see Fig. 1), with more recent indications suggesting that cultured seaweeds now contribute between 5-7 million mt annually (Anon., 1994; FAO, 1997). Over 50% of global seaweed production is used for human consumption (*cf.* Tables 1 and 2); and the value of food macrophytes is some six times greater, on a raw materials used basis, than that employed by industrial concerns (Jensen, 1993; *cf.* Tables 1 and 2). Nevertheless, even though the above noted production increases in seaweed culture are astonishing when compared against other forms of aquaculture, it should be remembered that wild harvests of seaweeds by far outweigh that produced by aquaculture. This should not come as a surprise, since it has been calculated that the yearly global production of wild seaweeds approaches 350,000,000 tons (Jensen, 1993).

Table 1. Global industrial application of cultivated seaweeds (from Indergaard and Jensen, 1991).

product	Value US$	Production (T/yr)	Fresh wt used (t)
Alginate	230 m	27000	500000
Agar	160 m	11000	180000
Carrageenan	100 m	15500	250000
Seaweed meals	5 m	10000	50000
Manures	10 m	510000	550000
Liquid fertilizers	5 m	1000	10000

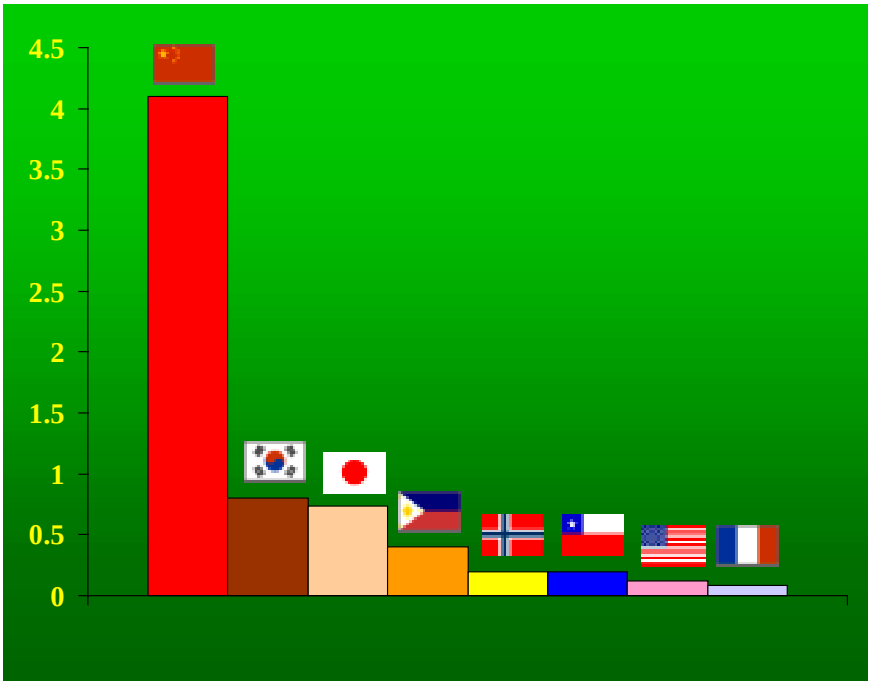
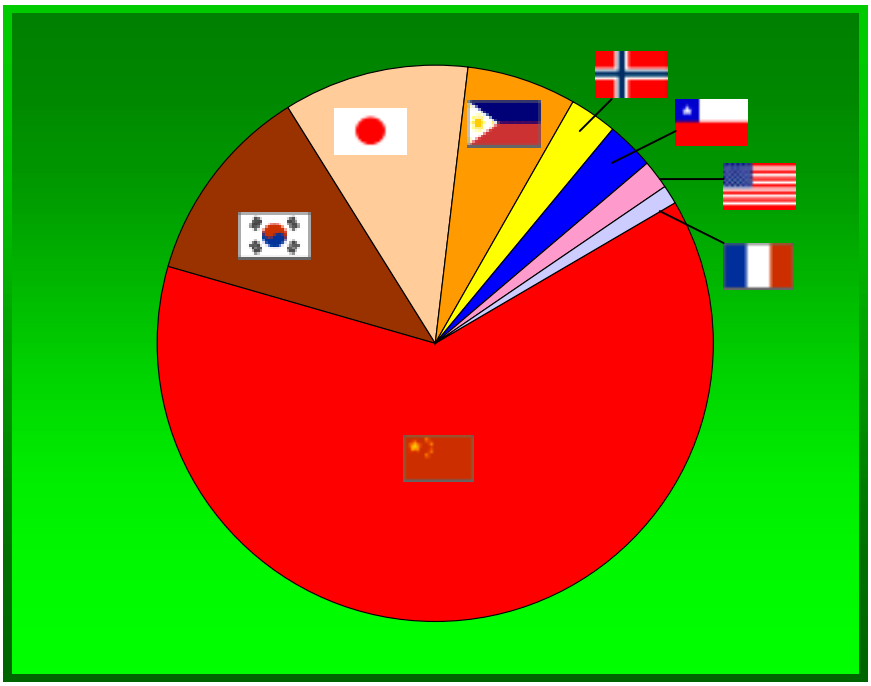


Figure 1. Most important producers of cultured seaweed by nation, in million tons fresh weight (upper) and global percentage contribution to production (lower).



There are around 19500 existing species of algae, representing approximately 90% of all marine plants. The major algal groups were initially separated based on color. Thus, "red algae" (containing the pigment phycoerythrin) include the Rhodophyta, while "green algae" or Chlorophyta, contain pigments similar to higher plants (chlorophylls a and b and β-carotene). In contrast, “brown algae” or Phaeophyta, gain their color due to the presence of xanthophyll pigments. Although the simple use of thallus, blade or frond color remains in use by modern taxonomists, it is now pigment type that is used as the major delineator of seaweed groups.

Table 2. Food industry consumption of macrophytes (from Indergaard and Jensen, 1991).

product	Value US$	Production (T/yr)	Fresh wt used (t)
Nori	18000 m	40000	400000
Wakame	600 m	20000	300000
Kombu	600 m	300000	1300000

Because algae are photosynthetic, they are necessarily confined to illuminated areas of the sea - the photic zone; the depth of which varies geographically (averaging 100 m). And, since algal growth is dependent upon exposure to photosynthetically active radiation, which may be absorbed and scattered during passage through the water column, this further limits algal depth distribution, particularly in coastal waters near to population centers, since turbidity and the presence of particulates are generally higher. Seaweed survival may be influenced by prevailing temperature and salinity (Lüning, 1990) and a variety of other biotic and abiotic factors (*e.g.*, desiccation stress, N_2, O_2, CO_2, wave action etc.). It is of note that some crustaceous red algae have been collected at depths greater than 250 m - 0.001% of surface light, but these species have extremely slow rates of growth (Table 3).

Macrophyte reproduction

Algae express both sexual and asexual reproductive strategies. Many species possess a variety of morphological phases and most are able to propagate vegetatively following fragmentation of the thallus. As with higher plants, marine algae alternate between a haploid gamete-producing phase termed the gametophyte, and a diploid spore-producing phase called the sporophyte. These two phases are morphologically and cytochemically distinct. Indeed, the variation in form and coloration between phases (heteromorphism) posed difficulties to early taxonomists who often allocated two or more specific scientific names to the same species. The heteromorphic life history is common

to all cultured macrophytes. In both brown and green algae isomorphic life history phases are expressed, where the two phases in a life cycle have identical morphologies but distinct cytologies, with one phase being haploid (1n) and the other diploid (2n), but with both producing different types of reproductive body. Where two 2n phases occur, the algae are termed the carposporophyte and the tetrasporophyte. The 2n carposporophyte develops on the female gametophyte.

Table 3. Comparison of maximal growth rates of various species of seaweed maintained under optimal conditions. (modified from Lünning, 1990).

species	Cm/day	Cm/yr
Macrocystis pyrifer	30	
Nereocystis luetkeana (blade)	14	
Laminaria angustata (blade)	7-13	
Sargassum muticum	3-4	
Fucus spiralis	0.1	
Tropical crustose coralline algae		1-2
Cold temperature crustose algae		0.3

The initial phase in the development of independent stages of algae rely upon the attachment of a germinated spore or zygote to a substrate. In temperate regions (zoo)spore release occurs during late Autumn and winter months. As the zoospore settles, its cells divide to form a gametophyte that over-winters (due to decreased light intensity) before undergoing a rapid vegetative growth stage in early spring. Of considerable significance during the settlement phase, due to the inhospitable environment (wave action, etc.), is the speed at which the zoospores are able to attach to a suitable substrate. The zoospore attaches to its substrate following secretion of polysaccharide adhesives. After settlement, a rhizoid develops which secures adherence.

Sexual reproduction in all species of marine macrophyte appears to be light dependent, as gamete release fails to occur in the absence of a circadian rhythm. The method and timing of gamete release is species dependent. Gamete release may follow lunar periodicity or may be synchronized to occur as soon as the light cycle commences. In *Laminaria* and other species, the release of eggs is accompanied by co-production and release of pheromones, which stimulate male thalli to release spermatozoids, which in turn are attracted to the egg by pheromone presence. Differences in pheromone chemical structure may provide a means of determining phylogenetic relationships between seaweeds. Figure 4 outlines the life cycle, and provides nomenclature for the various stages for *Laminaria japonica.*

Culture techniques

One of the major limitations to the artificial propagation of marine macrophytes is the availability of suitable settling and on-growing substrates and sites. The simplest technique of encouraging seaweed growth in a specific area is to place large rocks or stones on a sandy or muddy bed, at an appropriate depth for the species of interest and then to provide the area with cuttings. Indeed this technique has been used extensively in Japan for *Gracilaria* and *Gelidium*, and farmers in Eire used this approach to encourage seaweed growth in sandy and muddy protected bays until quite recently. In various parts of South-east Asia and South America this technique still continues.

A technological improvement to culture activities was the placement of substrates that could be more easily moved to assist during harvesting operations. In its simplest form, as seen in South-east Asia, it is not uncommon for operators to use bamboo canes or brushwood planted in areas where zoospores may settle naturally. Methods of algal culture still rely heavily upon the production of cuttings and or collection of zoospores from the wild with their subsequent on-growing. A major advance in *Porphyra* cultivation was made following the discovery of its alternate shell-boring *Conchocelis* life history phase (Drew, 1949). This finding had immediate impact upon the reliability of supply from the *Porphyra* industry, since the *Conchocelis* phase could be cultivated in tanks. Today, this controlled system permits the collection of non-motile zoospores that are then used for propagation. As an alternative to wooden structures, a number of other settling substrates are now employed, or have been examined, including calcite granule covered vinyls (Saito, 1979) and nylon ropes with various attached substrates (*e.g.*, Blinova and Trishna, 1990; Berchez *et al.*, 1993). Cultivation ropes may be set in a variety of layouts, including long-lining from the shore, raft-based rope units, floating nets, and so on.

Cultivation of *Porphyra*

In Japan, *Porphyra*, or nori culture, is not only the largest existing mariculture operation, but is also one of the oldest. *Porphyra* culture has been practiced on various Japanese islands since the early 1600s (Tamura, 1966). This genus forms large red bladed seaweeds that are most often encountered as the wrapping around sushi. Japanese *Porphyra* production for 1989 was 403,300 mt (Ikenoue and

Kafuku, 1992) although declining harvests have been witnessed since that time. Commercial cultivation of nori is a 4-stage process and commences with the cultivation of *Conchocelis* filaments. Mature blades (collected Dec-Mar.) are ground into a suspension. Following removal of debris, the resulting suspension of carpospore-bearing fronds is added to a tank. The carpospores are used to seed oyster shells, which are spread over the bottom of concrete tanks, at a seawater depth of 10-15 cm (Fig. 2). The carpospore-bearing shells are then hung in similar tanks outdoors, and left until the filamentous *Conchocelis* phase develops. Optimal temperatures during this phase range between 15-20 ^{o}C. By late September the *Conchocelis* starts to release spores, a process that may be artificially manipulated by lowering temperatures or shortening photoperiod.

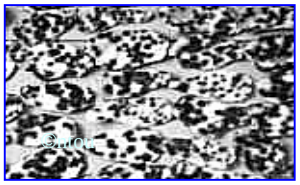


Figure 2. Carpospore-bearing oyster shells.

The second phase of nori production requires seeding conchospores onto a suitable substrate. This may be undertaken either in ocean-settings, where oyster shells bearing the *Conchocelis* are placed in bags attached to the lower side of a nori net. The nets are then seeded naturally upon conchospore release. Alternatively, the concrete tank-based systems used for *Conchocelis* production may be used with conchospores being collected by lying nets directly into the water. The water may be bubbled to assist conchospore dispersal in the water column. Nets are then set in the sea, usually supported by bamboo or wooden poles or rafts. After 3 wk germling *Porphyra* appear and, 4 wk later, these will have grown to 2-3 cm. At this stage, the nets or ropes act as nurseries. These are used for on-growing, which occurs between October and April. The nets are usually placed in regions where there is high nutrient levels and seawater movement. Harvesting commences in November, at which point the thalli achieve approximately 30 cm length. Smaller thalli may be left on the nets for later harvesting. The nori may be processed by the culturist into dried sheets before sale.

Global sales of nori are presently around US$ 2 billion, with Japan representing the largest market (the average Japanese spends US$ 300 per year on nori sheets). The Japanese market supports incredible product differentiation for nori, characterized by a wide range of prices per processed sheet, ranging from 3 ¢ to $20 each. Estimates for growth in nori sheets in the EC and North American markets are around 8-12% per year. Of recent interest in *Porphyra* culture has been the application of fertilizers to stimulate production. Diseases, such as red wasting disease, caused by the fungus *Pythium* together with green spot disease – a *Vibrio* infection, have become problematic to the culture of *Porphyra* in recent years.

Culture of *Gracilaria*

A comprehensive overview of *Gracilaria* culture is provided in Santelices and Doty (1989). The following presents a brief overview of global *Gracilaria* cultivation methods. In Hawaii and Malaysia, intensive culture of *Gracilaria* employs a nursery tank containing various settlement substrates that include clamshells, coral and monofilament line. Fertile *Gracilaria* collected from the wild are placed onto a net that overlies the tank. Upon drying, the thalli release spores into the tanks' water column (0.5 m), which subsequently settle on bottom substrates. The depth employed is not arbitrary; rather, trial and error has demonstrated that 0.5 m is an optimal depth for spore dispersal in the water column. The spores are allowed 1-3 days to attach to the substrate, after which they are outplanted to a farm site.

Alternative methods of *Gracilaria* farming, which employ nets and ropes, are practiced in Burma, India, Brazil and the West Indies. Here, large cuttings are tied onto nets or ropes which and then set at suitable on-growing sites. Another approach has been to use monofilament line as a spore substrate. The monofilament line can then be used as cultivation sets at appropriate farm sites. In China raft-type propagation is employed, wherein ropes of approximately 70 cm length are hung from a bamboo or wooden floaters.

Because *Gracilaria* does not require either strong water movement or stable salinities, it is also possible to culture these algae in tanks, ditches and ponds (see: Ugarte and Santelices, 1992). Pond farming is practiced in Taiwan and Hawaii, whereas ditch culture has been used in China. Culturists simply chop wild-taken *Gracilaria* to 10 cm lengths and spread the fragments over the pond, tank or ditch surface. The fragments subsequently develop into mature fronds. A critical factor in this type of culture however, relates to water pH, which should be maintained at approximately 8. Water exchange in such systems is usually undertaken every 2-3 days to maintain salinity and mineral levels. Fertilizers may also be added to the systems to enhance algal growth rates. Under tank conditions, it might become necessary to add CO_2 to the water and or to aerate. Mineral addition however, has the effect of enhancing the growth of epiphytes that grow on the algal fronds, decreasing overall seaweed and agar production potential. Using the enclosed methods, *Gracilaria* is harvested using scoop nets or rakes. The yield is sold as a dried product to agar manufacturers.

Fig. 3. Seaweed harvesting

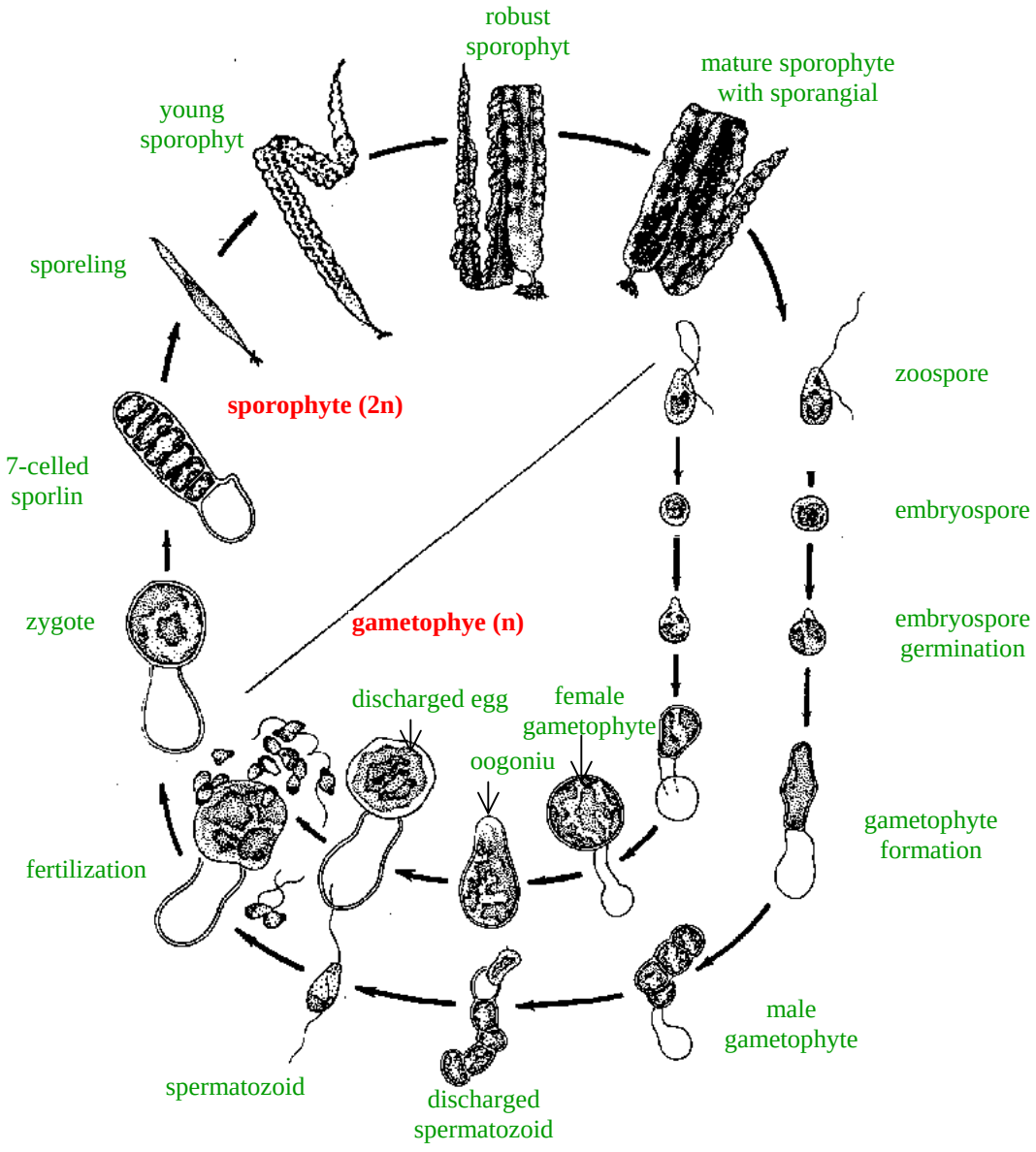


Figure 4. Diagrammatic representation of the haploid (n) and diploid (2n) life cycle of *Laminaria japonica*. Slightly modified from Tseng (1987).

Outlines of other types of production technology for various species of edible and industrial algae and freshwater plants such as cress, water spinach, sagitarria and wild rice may be found in Bardach *et al.* (1972), Pillay (1993), among a host of other textbooks. It should be noted that recent production figures for Taiwan indicate that macrophyte cultivation may be an unstable business. For example, in the period 1981-1990, Taiwanese production of *Gracilaria* remained fairly secure. However, the value of harvest declined from approximately US$1.2 m in 1981 to US$120,000 in 1990 (Ajisaka and Chiang, 1993). In contrast to this position however, Indonesian production of seaweeds, which remained fairly steady between 1987-1990, was matched by a rise in total harvest value (Luxton, 1993).

Industrial uses of macrophytes

There have been two phases in the application of seaweeds for industrial utilization. Since before 1800, seaweeds were burnt and sodium and potassium salts, together with iodine, extracted from the resultant ash. Seaweed ash was also the major source of soda, which was used in the production of glass and soaps pre-1800. The production of potash and iodine from kelps was continued through to the 1930s. During the Great War (1914-18), kelp components, notably potash and acetone, were used in the production of cordite, a smokeless gunpowder (Neushul, 1987). However, the costs incurred in the harvesting and processing of seaweeds for iodine and potassium salts, when compared against chemical and other industrial processes, eventually eliminated the industry; forcing it to adapt to new processes and products. Today, there are three major groups of extracts from seaweeds, which are commercially important.

As noted in Table 1, it is the alginates that predominate in terms of macrophyte product value. Alginates, which are polysaccharides containing D-mannuronic and L-guluronic acids, are used in numerous processes (Table 4), but are particularly useful in providing viscosity to aqueous solutions (Clare, 1993; Renn, 1997). Alginates are commonly employed as emulsions and dispersions for an array of products such as ice cream, shoe polish and oil drilling mud (Jensen, 1993). Alginate gels are formed with the addition of calcium to heated material. The calcium ions work by linking adjacent chains of polysaccharides, forming what has been termed an "egg-box" structure. Alginates are present at levels of between 18-40% in the Phaeophyceae, with many of the *Laminaria sp.* providing yields of between 25-38%. These seaweed products have also been employed as a tobacco substitute and to improve the quality of paper texture.

Table 4. Example industrial applications of alginates.

Industry	Application
Food	Emulsifier and suspension agent in various dressings and stabilizer for ice creams and thickener for gravies etc.
Textiles	As a thickening agent for printing and improved material absorptivity.
Pharmaceuticals	Employed in syrups as emulsifier, smoothing agent for creams and other topical preparations etc.
Brewing	Provides creamier froths and smaller bubbling.
Cosmetics	Used as a smoothing agent for creams and gelling product for consistency of facial masks.

In addition to alginates, the red algal-derived phycocolloids, agar and carrageenan, are also gel-forming polymers. *Gelidium* and *Gracilaria* are particularly important species in phycocolloid production, and both are cultured. Agar content of seaweeds varies considerably but in *Gelidium cartilagineum* this may reach up to 30%, provided the weed is grown in cooler, more turbulent waters. The most important producers of agar seaweeds include Japan with 2000 t production in 1990, China, with a harvest of approximately 1000 t, Chile and Spain (600 t), Portugal and Argentina (400 t). Denmark, together with the United States, New Zealand and France each produced around 100 t in 1990. Prices for agar in 1991 were US$ 26/kg for food grade gums, US$ 55/kg for microbiological supplies and up to US$ 100/kg as agarose (Selby and Whistler, 1993). Agars are graded in various countries based on physical and sensory measurements. In Japan a sub-grading system is also used. Agar may be divided into two components: agarose and agaropectin, and it is the relative proportions of these two components that determine the structural characteristics of the formed gels and their value on the international marketplace. The applications of agars are diverse. Their use in microbiology requires little introduction. Similar to bacterial culture use, agar has been employed in meristem culture operations. Application of agars to pharmaceutical products has been increasing for a number of years, especially in separation and purification steps (gel electrophoresis, gel-bead chromatography, size-exclusion chromatography) for fine chemicals, hormones, enzymes, and vaccines. They have been used as laxative agents and as a means of suspending barium meals for x-ray of the gastrointestinal tract. Sustained-release devices, suppositories and many other surgical lubricants have an agar base. Agars are also applied in the production of intricate casts used in dentistry, criminology and dye making. While non-digestible, agars are commonly used as ingredients for food in South-east Asia and elsewhere (Fleurence, 1999). Seaweeds used in the production of agars may be stored in baled form for in excess of 4 years, provided that moisture content is maintained below 20%.

Like agar, carrageenan also acts to solidify and emulsify solutions. Approximately half of all carrageenan produced is derived from cultured sources, and particularly from members of the genus *Eucheuma*. The Phillippines represents a major producing country for cultured *Eucheuma* (Doty, 1986), as too does Indonesia (Luxton, 1993); African nations, such as Zanzibar, have recently entered large-scale production (Lirasan and Twide, 1993). Carrageenans are composed of alternating units of modified galactose. Several forms of carrageenan have been identified but the two main groups of carrageenan are assigned as κ- and l-types (De Ruiter and Rudolph, 1997). Carrageenans are generally used by the food industry, and especially by the baking, confectionery and dairy product markets . Carrageenans are used to prevent chocolate milk from creaming and sedimenting and to permit solidification of flans, custards, ice creams and milk puddings. Fillings for tarts, as seen in the gels which overlie mixed berries, relies upon the gelling properties of carrageenan for structural stability, while cooked ham and poultry products have been processed with carrageenans to enhance salting characteristics and to improve sliceability (Therkelsen, 1993). Carrageenans at 0.2-1.0% are used in canned meat and fish products as a means of gelling broths and for stabilizing the product in containers. Beers are clarified using carrageenans to complex and precipitate proteinaceous impurities. Toothpastes and canned and frozen pet foods also incorporate carrageenans during the production process to assist in gel formation and solidification. At present, the industrial demand for carrageenans and agars by far outpaces supply.

Seaweed processing

The initial phase in preparing seaweed extracts for the production of agar is boiling. The extract obtained from the boiled seaweed is then frozen and later thawed. During thawing, water separates from the agar, carrying with it soluble impurities. Carrageenans too are prepared following boiling of the appropriate seaweed. However, instead of being frozen, the product is precipitated from a crude extract with the addition of propanol. For alginates, brown algae are washed, macerated, and then digested with sodium carbonate. The resultant filtrate is then added to sodium or calcium chloride, which forms a fibrous precipitate of calcium or sodium alginate. The alginate may be converted to alginic acid following treatment with dilute hydrochloric acid. After further chemical and purification steps, the alginate is powdered.

Food and agricultural applications

The vast majority of consumed seaweed products are found in the Orient, although in Europe and North America algae is still eaten. In the west, three species of red seaweed are utilized, mainly for the production of breads. In the Far East, seaweeds as food have a long tradition. Nori is the single most valuable product from marine algae (Table 2), being processed into sheets, powders, flakes and confectioneries. Nori, together with wakame and kombu are important components of many Japanese foods. Seaweeds have also been used extensively in European agriculture, particularly as animal fodders and as manures and fertilizers. However, most of the traditional practices of European farmers declined rapidly by the end of the 1800s. Nevertheless, seaweeds are still employed as additives for animal feeds in NW Europe and Canada (Dring, 1982).

Future perspectives

The growth of the macrophyte industry has been steady on a global scale, and it has been predicted that such growth will continue apace with population increases and with the development of novel applications for algal products. Already we have witnessed the production of synthetic prostaglandin-like substances from a variety of species (Gerwick *et al.*, 1993) and significant advances have been made in drug production from marine macrophytes in China (Tseng, 2001). India too has commenced research upon novel biotechnological applications for seaweeds with particular emphasis upon selective breeding for higher agar yields (Kaladharan and Gopinathan, 2001). Alginates have also been employed in the development of sustained-release drug delivery systems, both for use in fish (vaccines, GnRH) and humans. Another form of delivery system derived from alginates is seen in the encapsulation of transplanted islets of Langerhans, which, following injection, are capable of producing and delivering insulin to treated animals (Soon-Shiong *et al.*, 1992). However, the sustainability of algal resources, together with the application of their yields to the development of novel products, will be dependent upon the stabilization of a high quality macrophyte supply (Renn, 1997). This can only be achieved with the continued evolution in efficiency of seaweed farming methods. Genetically engineered Laminaria have already been employed as a means of producing chlormaphenicol and other pharmaceuticals (Qin *et al*., 1999) and this approach to enhancing productivity, quality and use of macrophytes will likely increase. The use of seaweeds as a means of processing polluted and effluent waters has

not gone untried. For example the integrated culture of fish, mollusks and seaweeds has been proposed as a means of minimizing environmental impacts of aquaculture (Shpigel *et al.*, 1993). A considerable number of studies have examined the potential for seaweeds, and particularly kelps as a renewable energy source (Neushul, 1987), and it is likely that such projects will continue into the new millennium. Already, seaweed extracts have been employed in the mass production of ethanol (Horn *et* al., 2000). Proposals have also been made to examine the culture potential for algae in otherwise non-productive areas of the world's oceans. However, much depends upon the economic feasibility of these plans as much as investor confidence.

References cited

Ajisaka, T. and Chiang, Y.M. (1993). Recent status of *Gracilaria* cultivation in taiwan. *Hydrobiologia*, **260/261,** 335-338.

Bardach, J.E., Ryther, H. and Mclarney, W.O. (1972). *Aquaculture*. John Wiley & Sons, New York.

Berchez, F.A.S., Pereira, R.T.L. and Kamiya, N.F. (1993). Culture of *Hypnea musciformis* (Rhodophyta, Gigartinales) on artificial substrates attached to linear ropes. *Hydrobiologia*, **260/261,** 415-420.

Blinova, E.I. and Trishna, O.A. (1990). Cultivation of *Phyllophora nervosa* (DC) Grev. on rope collectors in the Black Sea. *Aquaculture*, **84,** 257-265.

Clare, K. (1993). Algin. pp. 105-143 In: *Industrial gums: polysaccharides and their derivatives.* R.L. Whistler and J.N. BeMiller (eds.). Academic Press, San Diego.

De Ruiter, G.A. and Rudolph, B. (1997). Carrageenan biotechnology. *Trends in Food Science and Technology*, **8,** 389-395.

Doty, M.S. (1986). Estimating farmer returns from producing *Gracilaria* and *Eucheuma* on line farms. *Monog. Biol.*, **4,** 45-62.

Drew, K.M. (1949). *Conchocelis*-phase in the life-history of *Porphyra umbilicalis* (L.) Kütz. *Nature*, **164,** 748-749.

Fleurence, J. (1999). Seaweed proteins: biochemical, nutritional aspects and potential uses. *Trends in Food Science and* Technology, **10,** 25-28.

Freshwater, D.W. and Kapruan, D.F. (1989). Spheroplast isolation and propagation of *Porphyra carolinensis* Coll et Cox for mariculture. *Aquaculture,* **81,** 101-110.

Gerwick, W.H., Proteau, P.J., Nagle, D.G., Wise, M.L., Jiang, Z.D., Bernart, M.W. and Hamberg, M. (1993). Biologically active oxylipins from seaweeds. *Hydrobiologia,* **260/261,** 653-665.

Horn, S.J., Aasen, I.M. and Oestgaard, K. (2000). Ethanol production from seaweed extract. *Journal of Industrial Microbiology & Biotechnology,* **25,** 249-254.

Indergaard, M. and Jensen, A. (1991). Utnyttelse av marin biomasse. *Inst. Bioteknologi, NTH, Trondheim.*

Jensen, A. (1993). Present and future needs for algae and algal products. *Hydrobiologia,* **260/261,** 15-23.

Kaladharan, P. and Gopinathan, C.P. (2001). Prospects of biotechnology in seaweed mariculture in India. *Perspectives in mariculture*, The Marine Biological Association of India, Cochin (India), 2001, pp. 381-388.

Lirasen, T. and Twide, P. (1993). Farming *Eucheuma* in Zanzibar, Tanzania. *Hydrobiologia,* **260/261,** 353-355.

Luxton, D.M. (1993). Aspects of the farming and processing of *Kappaphycus* and *Eucheuma* in Indonesia. *Hydrobiologia,* **260/261,** 365-371.

Neushul, P. (1987). Energy from marine biomass: The historical record, pp. 1-38 In: *Seaweed cultivation for renewable resources*. K.T. Bird and P.H. Benson (eds.). Elsevier, Amsterdam.

Pillay, T.V.R. (1993). *Principles of aquaculture.* Fishing News Books, Oxford.
Qin, S., Sun, G., Jiang, P., Zou, L., Wu, Y. amd Tseng, C. (1999). Review of genetic engineering of *Laminaria japonica* (Laminariales, Phaeophyta) in China. *Hydrobiologia*, **398/399,** 469-472.

Renn, D. (1997). Biotechnology and the red seaweed polysaccharide industry:status, neds and prospects. *Trends in Biotechnology*, **15,** 9-14.

Selby, H.H. and Whistler, R.L. (1993). Agar, pp. 87-103 In: *Industrial gums: polysaccharides and their derivatives.* R.L. Whistler and J.N. BeMiller (eds.). Academic Press, San Diego.

Shpigel, M., Neori, A., Popper, D.M. and Gordin, H. (1993). A proposed model for "environmentally clean" land-based culture of fish, bivalves and seaweeds. *Aquaculture*,**117,** 115-128.

Soon-Shiong, P., Feldman, E., Nelson, R., Komtebedde, J., Smidsød, O., Skåk-Bræk, G., Espevik, T., Heintz, R. and Lee, M. (1992). Intraperitoneal microencapsulated islets successfully reverse spontaneous diabetes in dogs. *Transplantation*, **54,** no. 5.

Therkelsen, G.H. (1993) Carrageenan. pp. 145-180 In: *Industrial gums: polysaccharides and their derivatives.* R.L. Whistler and J.N. BeMiller (eds.). Academic Press, San Diego.

Tseng, C.K. (1987). Laminaria mariculture in China. In: Doty, M.S., Caddy, J.F. and Santelices, B. (editors) *Case studies of seven commercial seaweed species*. FAO Technical Paper 281.

Tseng C. (2001). Algal biotechnology industries and research activities in China. *Journal of Applied Phycology*, **13,** 375-380.

Ugarte, R. and Santelices, B. (1992). Experimental tank cultivation of *Gracilaria chilensis* in central Chile. *Aquaculture*, **101,** 7-16

Additional reference sources and further reading

There have been seventeen *International Seaweed Symposia* for which proceedings are available. These symposia included a mixture of papers which covered a diverse range of fields, including; culture techniques; purification, properties and application of various algal enzymes, and industrial and food processing and application of seaweeds and seaweed products. An example of one of these symposia was published as a multi-edited version (A.R.O. Chapman, M.T. Brown and M. Lahaye) of *Hydrobiologia* (**260/261**) in 1993. Some papers from the same symposia were also included in the *Journal of Applied Phycology* **5,** (2), 1993. A number of FAO/ADB workshops have also been held on seaweed culture *(e.g., ADB Agric. Dep. Rep. Series* #1). The journals *Aquaculture* and *Journal of Applied Phycology* often include articles on various aspects of macrophyte culture and use. The periodicals *Botanica Marina* and *Aquatic Botany* also have regular articles on macrophyte culture and the

propagation of edible and ornamental freshwater plants. The following list includes a number of review articles which may ease preliminary acquisition of information on macrophyte biology and culture.

K.T. Bird and P.H. Benson (eds.)(1987). *Seaweed cultivation for renewable resources*. Elsevier, Amsterdam. 377pp.

Briand, X. (1988). Exploitation of seaweeds in Europe. pp. 53-66, In: *Aquatic primary biomass (marine macroalgae): biomass conversion, removal and use of nutrients.* P. Morand and E.H. Schulte (eds.) COST 48, European Community, Brussels.

Chapman, V.J. (1970). *Seaweeds and their uses.*Methuen, London.

Espejo de la Fuente, M. (1993). Posibilidades del cultivo extensivo de macrofitos. pp. 35-47 In: Seminar on culture and industrial use of seaweed. *Cuad. Area Cienc. Mar. Semin. Estud. Galegos*, **5**.

Dring, M.J. (1982). *The biology of marine plants.* Edward Arnold, London.

Evans, L.V. and Butler, B.M. (1989). Seaweed biotechnology: current status and future prospects. *Biochem. Algae Cyanobact.*, **28,** 335-350.

Guiry, M.D. and Blunden, G. (eds.)(1991). *Seaweed resources in Europe: uses and potential.* John Wiley & Sons, Chichester.

Lobban, C.E. and Wynne, M.J. (eds.)(1981). *The biology of seaweeds.* Blackwell, Oxford.

Lüning, K. (1990). *Seaweeds. Their environment, biogeography, and ecophysiology*. John Wiley & Sons, Inc. New York. 527pp.

Miura, A. (1975). *Porphyra* cultivation in Japan. p. 304-320, In: *Advance of phycology in Japan*. J. Tokida and H. Hirose (eds.). Fischer, Jena.

Neish, I.C. (1979). Developments in the culture of algae and seaweeds and the future of the industry. pp. 395-402, In: *Advances in aquaculture*. T.V.R. Pillay and W.A. Dill (eds.). Fishing News Books, Oxford.

NERAC Inc. (1992) Seaweed culture. Database collection series. NERAC Inc., USA.

Perez, R. (ed.)(1992). *La culture des algues marines dans le monde*. IFREMER, Plauzane, France.

Saito, Y. (1979). Seaweed culture in the North-west Pacific. pp. 402-410, In: *Advances in aquaculture*. T.V.R. Pillay and W.A. Dill (eds.). Fishing News Books, Oxford.

Santelices, B. and Doty, M.S. (1989). A review of *Gracilaria* farming. *Aquaculture*, **78,** 95-133.

Stadler, T., Mollion, J., Verdus, M.-C., Karamanos, Y., Morvan, H. and Christiaen, D. (1988). *Algal biotechnology*. Elsevier, Amsterdam. 521pp.

Stewart, W.D.P. (1974). *Algal physiology and biochemistry*. Blackwell, Oxford.

Tamura, T. (1966). *Marine aquaculture*. 2nd ed. (Translated from the Japanese by M. I. Watanabe); U.S. Dep. Commer., Natl. Tech Inf. Serv., Springfield, VA.

Watson, A.S. (1979). *Aquaculture and algae culture*. Noyes Data Corp., Park Ridge, N.J., USA.