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Algal biotechnology industries and research activities in China

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Abstract

In old China there were very few people engaged in the study of the algae, but in new China, freshwater and marine algae are studied by over one hundred old and new phycologists. There is now an algal biotechnology industry consisting of an aquaculture industry, producing large amounts of the seaweeds *Laminaria*, *Porphyra*, *Undaria*, *Gracilaria*, eucheumoids, and the microalgae *Dunaliella* and *Spirulina*. There is also a phycocolloid industry, producing algin, agar and carrageenan; an industry producing chemicals and drugs, such as iodine, mannitol, phycocyanin, β-carotene, PSS (propylene glycol alginate sulfate) and FPS (fucose-containing sulfated polysaccharides) and an industry producing food, feed and fertilizer. The *Laminaria* cultivation industry produces about 900,000 t dry *Laminaria*, probably the largest producer in the world and 13,000 t algin, undoubtedly one of the largest algin producer in the world.

Introduction

China has long used a few algae for food and other purposes, notably *Porphyra*, *Laminaria* and *Nostoc* for food, *Gloiopeltis* for colloidal substances and *Sargassum* for fertilizers. A few hundred years ago, the Chinese, especially those of Fujian province, began to cultivate *Gloiopeltis* and *Porphyra* by rock cleaning methods. The algal industry started with the formation of New China in the 1950s. There are now sizable industries in aquaculture, phycocolloids, chemicals and drugs and food, feed and fertilizer, involving many individual enterprises. Several hundred thousand people in China are now engaged in the production and trade of algae and their products.

Aquaculture indusry

Traditional methods for the production of algae for food and other purpose in China were initiated several hundred years ago, but scientific methods for algal aquaculture started in the 1950s with the culture of *Laminaria japonica*, a species endemic to the Sea of Japan. However, *Laminaria* has been used in China as a food for more than 1000 years. Long ago Chinese physicians applied the name ‘Kunbu’ to an East China Sea seaweed, *Ecklonia kurome* (Tseng & Chang, 1961), which was used as an anti-goiter drug. Subsequently the Chinese found *Laminaria japonica* in Korea, which proved to have a similar function, and then found that it was produced in even larger quantities in Japan, a discovery that occurred over one thousands years ago. From then on, China imported large quantities of *Laminaria* from Korea and Japan, reaching as much as 46,000 t dried *Laminaria* in 1929.

In 1927 when the City of Dalian (Dairen) was under occupation by the Japanese, large quantities of logs were imported from Hokkaido and northern Honshu in Japan, on which were sporelings and young plants of *L. japonica*. With these sporelings and young plants as the basis, the Japanese started *Laminaria* cultivation in Dalian using the traditional Japanese method by throwing stones to the sea. Tseng et al. (1955) devised the summer sporeling method, in which the *Laminaria* zoospores are collected in early summer. The young gametophytes and juvenile sporelings are frst cultivated in enriched seawater in a cool room maintained at about 10 °C and moved to the sea when seawater temperature drops to about 20 °C in the autumn. This 'summer sporeling method' was accepted by the industry in the 1950s and has become the standard method for large-scale *Laminaria* cultivation industry in the country today. The industry produces about 900,000 t dry *Laminaria* (>4 million t wet *Laminaria*) per year, by the raft method as devised by Li Hongji and his colleagues (Li, 1990) of the Shandong Aquaculture Institute in the early 1950s. Studies are underway to improve the *Laminaria* protein gene (Qin et al., 1994).

The second algal aquaculture industry to be developed in China was *Porphyra* cultivation. This industry started in China a few hundred years ago in Fujian Province by the traditional rock cleaning method. The rocks that had good growth of *Porphyra* were cleaned some time in autumn to create space for the *Porphyra s*pores to attach and grow. Our ancestors, however, did not know anything about such spores! It was Kathleen Drew (1949) who found that *Porphyra* spores do not germinate to become leafy *Porphyra*, but rather gave rise to the shell-boring microscopic plant, the *Conchocelis*; however, she did not realize that the leafy *Porphyra* grows from the *Conchocelis*. Kurogi (1953a, b) in Japan and Tseng & Chang (1954) in China solved this problem independently. We now know that in nature the *Conchocelis* grows on molluscan shells and its spores, the conchospores, develop to become the leafy *Porphyra*. In the early 1960s the Chinese Ministry of Fisheries conducted a national compaign in Fujian to grow *P. haitanensis* in two different ways, the conchospore and the traditional methods. The success of the conchospore method finally convinced the public that the theory of Tseng & Chang was correct. Numerous experiments by X.G. Fei have shown that preservation of the various strains is best effected by growing free-living *Conchocelis.* Fei has at present 20 species and 120 strains in cultivation from China and other parts of the world (Fei, 1999). The laver industry is next to the *Laminaria* industry in size and is the second largest seaweed cultivation industry in China, producing about 40,000 t dry *Porphyra* or about 400,000 t fresh weight.

The third algal cultivation industry is that for *Undaria*. *U. pinnatifida* is a food alga superior to *L. japonica* in taste and protein content. As with *L. japonica*, *Undaria* was first cultivated in Dalian, when still under Japanese occupation in the 1940s, by the traditional throwing stones method. China has her own *Undaria* in Zhejiang and Fujian Provinces, but the *Undaria* under cultivation in Dalian came from Japan. In the early 1940s, Qingdao also had *Undaria* under cultivation, but the original stock came from Korea. In the early 1960s *Undaria* was cultivated by methods similar to those devised for *Laminaria* cultivation. The gametophytes and young sporelings were cultivated, however, at normal room temperature and no cooling is necessary since it is a warm temperate plant. At present *Undaria* is produced in Liaoning and Shandong provinces. The total annual production is about 50,000 t wet weight, about 90% of which is produced in Dalian, Liaoning Province (Wu, 1993, 1998; Wu et al., 1999).

The fourth algal cultivation industry is that based on eucheumoids for producing carrageenan. This is based on three genera: 1) *Eucheuma denticulatum* (formerly called *E. spinosa* and *E. muricatum*) producing ι-carrageenan, the raw material produced in large quantity in Taiwan and known in China as *Qilincai* (meaning unicorn vegetable), a rather common food alga in China; 2) *Betaphycus gelatinum* (formerly known as *Eucheuma gelatina*), producing β-carrageenan, under cultivation in the Qionghai and Wenchang districts of Hainan Province since 1962; 3) *Kappaphycus alvarezii*, producing κ-carrageenan and introduced by C.Y. Wu from the Philippines in 1985, and cultivated in several places in Hainan Province.

Betaphycus gelaltinum grows naturally in Hainan in the sublittoral region and is most abundant about 1 m below the low tide region. At first divers were sent to insert cuttings on the sublittoral reefs, but since 1974 a new cultivation method has been devised. The living thalli are collected by divers, cut into pieces and fastened to coral branches with rubber rings or threads and thrown into sublittoral reefs where divers rearrange them in order. The annual production has remained at about 300 t dry plant for quite a few years (Tseng, 1981).

Kappaphycus alvarezii was initially cultivated on floating rafts where pieces of the alga were tied to the raft ropes. When Wu introduced this species to Hainan, he cultivated it on rafts and was bothered by schools of small fish eating the seaweed. It is now grown in Qionghai and Lingshui districts on the east and Chengmai district on the west of Hainan Province. The annual production is as yet unknown.

The fifth algal cultivation industry is the *Gracilaria* industry. *Gracilaria*, known as *jiangli* in China, has been appreciated as a food and feed for culturing marine animals and formerly also as a binding matural in the preparation of lime for painting walls. The most important use of *Gracilaria*, however, is the production of agar. Formerly, almost all the agar was made from *Gelidium* spp., especially *G. amansii* in Japan and China, and practically monopolized by Japan. However, with *Gracilaria* now the most important agarophyte, several countries are involved and *Gracilaria* agar constitutes about 60% of the agar of the world (Zhang et al., 1999). At present most of the agar in China comes from *G. lemaneiformis* and other imported *Gracilaria*, while *G. tenuistipitata* var. *liui* is used as a good feed for growing marine animals.

In the 1950s the author studied spore germination in *G. vermiculophyllis* (formerly variously called *G. asiatica*, *G. confervoides* and *G. verrucosa*); this gives rise to disc-like thalli and requires several months to give growth of erect branches (Tseng & Chen, 1959). It is evidently not practical to take such a long pre-cultivation time to grow an annual plant. The idea of employing spores in mariculture of *Gracilaria* was therefore abandoned and we tried to use cut branches for propagation. In the case of*G. lemaneiformis*, cut branches grew in Qingdao to about 20 times in its best growing season with water temperatures of 12–22 °C for about 4 months a year. Recently X.G. Fei cultivated the Qingdao plant in Zhangjiang City, Guangdong Province, and obtained an increase of 100 times in a growing season. From these plants he selected a strain which can endure higher temperatures and grew it in Nanao, Shantou City, Guangdong Province, where several kinds of marine animals were cultivated. He obtained a growth of a thousand times the original weight in a growing season of about 4 months (X.G. Fei, pers. comm.). This strain is excellent for growing in a warm temperate region which is highly polluted.

In Hainan, *G. tenuistipitata* var. *liui* is cultivated in ponds just by scattering the cut plants at random. In Taiwan, cultivation of the *Gracilaria* was initiated in 1967. By 1977 *Gracilaria* ponds occupied 2.21 ha, with production reaching 6804 t. The production areas are located mostly in the Pingdong (Pingtung) and Tainan districts in south Taiwan. Cuttings of the *Gracilaria* are uniformly planted in the bottom of the ponds tied to bamboo sticks or covered with old fishing nets to avoid drifting (Tseng, 1981). About 4000–6000 kg fresh *Gracilaria* are planted in one hectare.

The sixth algal cultivation industry concerns microalgae. Two kinds are cultivated on a large scale. *Spirulina* was introduced to China by Jian-Ren Miao of Jiangxi Academy of Agriculture in 1982 from France and India. In 1985, R.D. Fox , based in France, visited Miao and gave him *S. platensis* and *S. maxima* (Miao, 1999). B.T. Wu of the South China Sea Institute of Oceanology also obtained material of *S. platensis* from Lake Chad and after a few years of selection experiments he obtained the SCS strain adapted to seawater cultivation. He conducted experiments in Sanya, Hainan and pointed out several advantages of cultivating the seawater strain in tropical parts of Hainan, such as higher cultivation temperatures, higher protein contents and higher yield and lower costs than freshwater culture (Wu et al., 1992). In the national research program (Project 75-05-03) on proteinaceous feeds, it was decided to try large-scale cultivation of *Spirulina*. It was concluded that the best places for *Spirulina* experiments should be tropical or subtropical regions, and that seawater culture should be employed since preliminary experiments showed that seawater culture produced an average 12 g m^{-2} day^{-1}, whereas freshwater culture produced less than 7 g m^{-2} day^{-1}. In the subtropical Huilai county, Guangdong Province, cultivation was carried out in 3000-m^2 outdoor raceway ponds and between 14 August and 5 November an average yield of 10.3 g m^{-2} day^{-1} was achieved. It was further pointed out that culturing in seawater medium has an added advantage of utilizing waste land near the beach, rather than valuable farm land. Production in Sanya City has reached as high as 20 g m^{-2} day^{-1} (Wu et al., 1993). An overview of the status of the *Spirulina* industry in China in 1996 was presented by Li (1997).

Mass cultivation of *Dunaliella salina* in China is effected with brine rather then seawater. The brine comes from a solar saltwork and solvents are added. The production plant has 8 rectangular production ponds with an area of 1350 m^2 (Guo, 1991).

Beside these products from China's aquaculture industry there are also a few other algae under cultivation, but on a very small scale, such as *Bangia atropurpurea*. Experiments on the cultivation of other economic algae such as the *zhegucai*, *Caloglossa leprieurii*, and the *facai*, *Nostoc flagelliforme* have also been effected. *Facai* is a food plant and is rather common in Chinese dishes. *Facai* in Chinese means 'hair vegetable', signifying the chair-like form of the product, but in Cantonese it also means 'to get rich', a term greatly appreciated by the people of Guangzhou and Hong Kong. This *Nostoc*, which has annual production of several hundred tons, normally grows on arid and semi-arid steppes in northern and western China (Gao, 1998). People gather it by tools, which more or less destroy the vegetation, and recently the Chinese government has prohibited further collection for the sake of environmental protection. A few phycologists in northern and western China were engaged for some years in attempts to cultivate this alga, but all in vain. However, more recently a few scientists at the Institute of Oceanology and the Institute of Hydrobiology, CAS, have been engaged in studies on *N. flagelliforme* aquaculture and have obtained some good results. Hopefully, their work will be successful and there will eventually an aquaculture of the *facai.*

Phycocolloid industry

Three kinds of phycocolloids are involved, algin, carrageenan and agar. For many years, China had a very small agar industry, employing traditional methods of extraction. The first phycocolloid industry of importance was the algin industry. When I was in the USA in the 1940s, I was involved in research on agar raw materials and made a survey of the American seaweed industry. I visited Kelco Co. of San Diego, California, which used *Macrocystis* as the raw material for the manufacture of a unique commercial commodity, algin. Algin was discovered by Stanford in 1883 and the manufacturing process patented by Krefting in 1896, H.C. Green in 1936 and V.C.C. Le Gloahec in 1938, 1959, 1940 and 1941. I was surprised by the widespread uses of algin in various industries, especially in stabilizing ice cream, making dental and other impression materials, and sizing textiles (Tseng, 1945, 1946). When I returned to China I made the decision to help to start an algin industry there. In 1951, we started to extract algin from a common local brown seaweed, *Sargassum confusum*. In 1952, we succeeded in obtaining algin by alkali digestion (Ke Xue Tong Bao, 1953). To make the production of the product an industry, it must have commercial uses. The first thing I tried was as a sizing material in textile industry. As a sizing material, algin is much better than starch in that it fills the cloth more completely, is tougher, and is more elastic; most important of all, the starch has to be made from cereals and China in the early 1950s was facing a serious shortage of food. So our experiment was to employ algin to take the place of starch as a sizing material. We conducted an experiment in the fifth textile factory in Qingdao. We succeded. We reported to the municipal government and in a few year a part of the Qingdao alcohol factory was devoted to algin production, using wild *S. confuscum* as the raw material. The wild *Sargassum* was practically depleted in a few years. Fortunately the cultivation of *L. japonica* was successful and the raw material of the industry has shifted to *Laminaria* ever since. The *Laminaria* is, however, a cultivated plant and more expensive as a raw material. It is important, therefore, to try to find more uses for the *Laminaria*. At last, from a sample of the *Laminaria* we produce, beside the algin, also iodine and mannitol (Ji, 1997; Ji et al., 1963). Both the iodine and the mannitol are thus by-products of the algin industry.

The algin industry in China formally started in the late 1960s with cultivated *L. japonica* as the raw material. At present, beside our own *Laminaria*, we have to import raw materials such as *Ecklonia* from South America. Our maximum annual algin production capacity is 13,000 t, undoubtadly one of the largest algin producers in the world. Uses of our algin have extended to various food industries as stabilizers, as a thickening agent in medical industries, as an impression material in dentistry and as anti-coagulant material in making toothpaste (Ji, 1997).

The second phycocolloid industry was the production of the carrageenan. The raw material is provided the by eucheumoid algae, especially *Betaphycus gelatinum* from Hainan and *Eucheuma* and *Kappaphycus* imported from the Philippines. For a long time, *Betaphycus* was treated as an agarophyte and employed as a minor material together with *Gelidium* in the production of agar. In the 1960s and 1970s, *Betaphycus* was dealt with independently and the product was sold in thread form as ‘agar’. In recent years carrageenan is produced independently and 85% of the raw materials, *Kappaphycus* and *Eucheuma*, are imported from the Philippines; only 15% are local product, the *Betaphycus*. At present, about 2500 t carrageenan are produced in China, mostly in powder form. About 60% of the carrageenan product is exported.

The third phycocolloid industry is the production of agar (Tseng, 1944, 1946; Tseng et al., 1952). Agar is the oldest phycocolloid produced in China, but now the smallest in production. More than 90% of the raw material is from *Gelidium amansii* obtained by diving to about 5 m in the sea in Qingdao. The agar is made simply by cooking the raw materials and freezing the product to get rid of the water. The product is in the form of threads or powder. In recent years, another agarophyte is becoming more and more important. This is *Gracilaria*, which now supplies 60% of the agar raw material in China. Two species of *Gracilaria* are involved, *G. lemaneiformis* and *G. tenuistipitata* var. *liui*. Old thalli of *Porphyra haitenensis* are also employed as raw material (Ji, 1997). Current annual production in China is about 500–600 t.

Agarose is a refined product of agar which contains a very small amount of sulfate (about 0.1–0.5%) and is now increasingly employed for biochemical and medical purposes. To make agarose, *Gelidium* containing only a very small amount of the sulfate is most suitable. At present less than 100 kg agarose is produced in China annually.

Chemical and drug industry

Seaweeds contain many kinds of chemicals and drugs required by humans. One of the best known is iodine, present in large quantities in algae such as *Laminaria*. China is an iodine deficient country and even in new China, almost 40% of the population, more than four hundred million people, suffer from iodine deficiency; there are large annual imports of iodine. The Chinese government declared some years ago that the iodine deficiency problem should be solved in the year 2000. *L. japonica* has almost 5‰ iodine in its thallus and in China it is a by-product of the algin industry. If all the *L. japonica* produced in China could be used in iodine production, more than 2000 t iodine could be produced annually, which would be sufficient for health and industrial use. However only 200 t iodine are currently produced from the *Laminaria*. The Institute of Oceanology has been engaged for many years in iodine studies, and has found that iodine exists in seaweeds in organic and inorganic states. The organic form is readily taken up by humans. In *L. japonica* 12–14% and in some *Sargassum* 38% of the total iodine is in the organic state. The Institute has devised a method of extracting organic state iodine and the method is now used to produce 100 t of seaweed organic iodine tablets. Phycocyanin is extracted from *Spirulina platensis* and β-carotene from *Dunaliella salina*.

Seaweeds also contain substances found to be useful as drugs. *Digenea simplex* from Pratas Island contains the strong anthelminthic, kainic acid, which is also present in *Caloglossa leprieurii* of Fujian and the provinces south of the Changjiang River. In *Chondria armata* and *C. crassicaulis*, a common seaweed also of Fujian Province, another anthelminthic, domoic acid is found. These anthelminthics are recommended for children infected with intestinal worms. FPS is a drug made from fucose-containing sulfated polysaccharide and is effective against uremia. PSS is prophylene glycol alginate sulfate made from algin and is effective in heart and brain disease.

Food, feed and fertilizer industry

Cultivated algae such as *Laminaria*, *Undaria* and *Porphyra* must undergo processing for the food industry. Formerly seaweeds were just dried and the products on sale were often mixed with mud and sand. In contrast, Japan has more than 200 kinds of marketable product from seaweeds. In China there are so far only a few kinds of seaweed food products on the market, such as knob *Laminaria* and sheet *Porphyra*, and artificial jellyfish and sharks fin made from algin. *Spirulina* is now a well known health food and appears on the market in the form of tablets. Undoubtedly the algal food industry will increase and more algal food articles will appear on the market.

Since the initiation of animal aquaculture, unicellnlar algae such as *Tetraselmis*, *Cryptomonas* and *Nitzschia* have been cultured and used as feed for the larvae and also adults. The microalgae are cultivated in culture tanks indoors and in ponds outside and tubular photoreactors are used only in experiments. Macroalgal feeds such as *Gracilaria* for animals such as abalone are collected and fed directly to the animals.

Seaweed has also served as a fertilizer for a very long time. The entire seaweed was used and buried in the soil until rotten or burned and ash obtained; however, this meant that the organic constituents were all lost and only K and a few other inorganic constituents remained. The Institute of Oceanology has cooperated with a firm in the manufacture of seaweed liquid fertilizer and a production of 10,000 t is expected in 1999 (Yan et al., 1999).

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