

SEAWEED RESOURCES AND EXTRACTION OF ALGINATE AND AGAR

B. N. DESAI

International Biological Programme, Ernakulam

SINCE the dawn of civilization man has utilized seaweeds as food, fertilizer and also as herbs for making medicines. In countries like China, Japan, Russia and America, people have made use of algae as food in raw, dried or cooked form. It is well known that the seaweeds are important sources of chemicals like sodium carbonate, iodine, potassium and valuable polymers like agar, algin, mannitol, carragenin, laminarian, fucoidin, etc. Many authors have described the uses of algae in different regions of the world. Brooks (1949), Chapman (1940), Tresseler and Lemon (1951) and Fretson (1953) have described the utilization of algae in different forms.

Seaweed resources of India, though not large, are very important. Many varieties which are available in the Gulf of Kutch and the Gulf of Mannar are highly valuable. The brown and red algae found in these regions are valuable sources of commodities like agar and alginate. In Japan and USA, a great deal of attention has been paid to produce these commodities on a large scale and many industrial plants have been in operation for a long time. Unfortunately in India no attempt has so far been made to produce any of these commodities on a commercial scale.

The Central Marine Fisheries Research Institute, Central Salt & Marine Chemicals Research Institute and some universities in South India have initiated research on the two products, namely agar and sodium alginate, and have established methods of their preparation. Both agar and alginate are extensively used in pharmaceutical and chemical industries and the latter in textile industry, and this has necessitated a large-scale import of these two commodities. About 60 tons of alginate and 30 tons of agar are imported every year. As our demands for these products increase and more restriction or total ban on the imports levied, it seems desirable to give an up-to-date picture of our resources of the raw material and describe revised methods of manufacturing these two chemicals on a commercial scale.

Resources

Chidambaram and Unni (1947) have estimated earlier the quantity of seaweeds washed ashore. In certain areas of Tanjore and Tirunelveli districts the estimated production of weeds is about 120 tons. Thivy (1952) estimated the annual harvestable quantity of agarophytes in the Pamban area to be about 100 tons. Pillay and Chacko (1955) have estimated that about 75 tons of brown algae are washed ashore around Krusadi and Rameshwaram Islands. Chacko and Pillay (1958) have indicated that about 6000 tons of wet red algae and 60,000 tons of wet brown algae can be harvested in a year from Point Calimere to Cape Comorin.

All the above estimates have been based on the quantity of washed algae. No detailed and systematic estimates on harvestable quantities have so far been made in the Gulf of Kutch, nor in the Gulf of Mannar.

Survey and estimate of seaweeds in the Gulf of Kutch

The survey of the extent of growth and quantity of brown and red algae in the Gulf of Kutch was initiated in 1962 and the studies were continued till June 1965. Three successive seasons of algal growth were observed. The survey was conducted at Sikka near Jamnagar and various points from Okha to Veraval.

The brown algae were found growing in almost all the places where the wave action is comparatively less and the area is sheltered from the heavy surf. The weed substrata usually were sandy with pebbles and boulders. It was observed that the brown algae do not thrive in muddy substratum. The growth was profuse near Sikka, Okha, Porbandar and Veraval, but the area near Sikka was found to be the richest for brown algae. Kappanna *et al.* (1962) have described some 14 species of brown algae around the Gulf of Kutch. Of these *Sargassum tenerrimum*, *S. cinereum*, *Sargassum* (unidentified species) and *Cystophyllum muricatum* were found in large quantities near Sikka. No brown algae were found growing near Jamnagar.

The estimate of the total quantity of algae was made from results obtained by random sampling as well as transect sampling. For the quantitative survey of total brown algae, four stations were set up along the coast at Sikka. Each station was harvested by a team of fishermen with eight boats. Collection per hour and the area covered were noted. Station No. 1 was 500 sq yds in area situated near Pearl Oyster Park at Sikka. Stations No. 2

and No. 3 were towards the south-east of Pearl Park. Station No. 4 was near the salt works at Sikka. All types of brown algae were harvested from these four stations in successive seasons. From each station about 200 wet tons of algae were harvested. The ratio of wet to dry algae worked out to 10:1, i.e. about 20 tons of dry brown algae were collected from each station. In the mixed collection, *Sargassum tenerrimum* and *Sargassum cinereum* were in greater proportion. It was observed that brown algae grow abundantly from the area west of Jamnagar to Path Finder Inlet near Salaya. This area of algal growth is approximately 90 sq miles. From the results of harvesting in three seasons it is estimated that about 50 tons of dry algae can be collected every year from 1 sq mile area. Therefore, approximate yield of brown algae for the 90 sq mile area will be about 4500 dry tons per annum. It was further observed that the growth of algae was more profuse in the second and third seasons than in the first. It is estimated that another 5000 tons of dry brown algae can be harvested from Okha, Veraval and Porbandar area including the islands. Both random and transect sampling methods were employed for the total estimation of brown algae in the Gulf of Kutch and, therefore, an estimate of 10,000 tons of dry harvestable algae per year seems to give a more correct picture than the previous estimates.

Red algae

A similar survey was also undertaken for the red algae. Only two varieties, *Gracilaria* and *Gelidiella*, were found growing in some quantities in the gulf. The former has a wider range of growth all along the coast where a hard or sandy substratum exists. *Gelidiella* is restricted to rock pools near Okha, Porbandar and Veraval. From the random sampling it is estimated that not more than 5 tons of wet *Gelidiella* and about 20 tons of dry *Gracilaria* can be harvested from the Gulf of Kutch.

Survey of algae in the Gulf of Mannar

The growth of brown algae has been estimated by a number of workers for the Gulf of Mannar region, and it was found that the results were identical with those obtained by the present investigation. It was, therefore, decided that a detailed survey of the red algae only may be conducted. Thivy (1952) has estimated that

about 100 tons of wet red algae can be harvested per year from the Pamban area. A 10-mile survey in the Gulf of Mannar was undertaken in the Rameshwaram area north-east of the temple and a 20-mile area off Kilakarai in Ramnad district. The growth of red algae was found to be profuse in a 10-mile area near the temple. It extended from the littoral zone to about 5 fathoms in the inshore region.

Three varieties of *Gracilaria* and a single variety of *Gelidium* were collected. *Gracilaria* was also found in abundance in the Pamban bridge area. A number of underwater observations were undertaken in the Pamban bridge area, but no trace of *Gelidium* was observed. A number of divers for underwater observation were employed in a 20-mile area north and south off Kilakarai. The richest ground for *Gelidium* in India was the Kilakarai shore in Ramnad district. A profuse growth of *Gelidium* was observed in a continuous half a mile wide strip running parallel to the shore all along the 20-mile coast at Kilakarai. It is estimated that nearly 2000 tons of wet *Gelidium* can be collected from this area. More than 100 tons of dry *Gelidium* were exported to USA and Japan by a private firm as a result of this investigation. The quantity of *Gracilaria* was found to be ten times more than that of *Gelidium*. Therefore, even from the most conservative estimates dry *Gelidium* and *Gracilaria* would amount to 300 and 3000 tons respectively per annum.

From the above data it becomes clear that resources of brown and red algae are ample for the production of sodium alginate and agar on a commercial scale.

Estimation of alginic acid in *Sargassum*

Chacko and Pillay (1958) have estimated algin content in 14 species of brown algae, collected from the south coast of India. Their figures vary from 15 to 29 per cent. Kappanna *et al.* (1962) made a similar estimate for the algae collected from the Gulf of Kutch. Their results vary from 5 to 29 per cent for different species. These estimates for algin content were made on dry weight basis and on laboratory scale. Two species of *Sargassum* and *Cystophyllum* from the Gulf of Kutch area and five species from the Gulf of Mannar were studied for the present investigation using a 50 kg sample of each alga. The results obtained differed from those observed by previous authors. The yield of algin in the algae from the Gulf of Kutch was only 7-9 per cent, while that from the

Gulf of Mannar varied from 10 to 18 per cent. The quality of alginate produced from South Indian algae was found to be higher.

Estimation of agar in red algae

Thivy (1952) has described a method for extraction of agar. She has recorded about 30 per cent yield for *Gracilaria* and 43 per cent for *Gelidium*. Experiments on large-scale production have also shown similar yields. Yield of agar from *Gelidium* is about 48 per cent and from *Gracilaria* 35 per cent.

Revised method for commercial grades of sodium alginate

Stanford in 1883 discovered the presence of alginic acid in the cell walls of brown algae. Several other methods were then devised for the extraction of alginate. Two industrial processes are those of Kelco Co. and Algin Corporation. Cameron *et al.* (1948) have described the laboratory and plant scale methods for the manufacture of sodium alginate. Chacko and Pillay (1958) and Kappanna *et al.* (1952) have also described laboratory methods for the extraction of alginate.

The Stanford process consisted of macerating the algae with ten times the weight of sodium carbonate, and acidifying the extract to obtain alginic acid. It was either mixed with sodium or calcium salts to make sodium or calcium alginate. The Kelco and Algin Corporation processes were further modifications of earlier processes where the algae are first treated with acid or calcium chloride to reduce the salt content. Sodium alginate in crude form extracted by digestion with sodium carbonate is treated with calcium chloride solution to form calcium alginate and then acidified before converting to sodium alginate.

The method described by the present author was very nearly the same as that used by the above companies. It was, however, observed that the sodium alginate obtained was of very low quality. Viscosity of the alginate solution is a very important property for its use in textile and pharmaceutical industries. Viscosities as high as 4000-5000 centipoise are required by the industry whereas those obtained from using the above methods are very low, i.e. about 50 cps. It was, therefore, considered desirable to modify the process so that different grades of alginate can be prepared by modifying the process.

The fresh dry algae were thoroughly cleaned in running water and then washed for 2 hr in hot water at 52°C. This temperature was arrived at by numerous experiments of raising and lowering the temperature. The cell wall collapsed when the temperature was raised to more than 60°C and at temperatures lower than 50°C, very little cleaning effect was observed. Washing at 52°C removed most of the water-soluble salts and brown pigment. It was found advisable to change the water at 1 hr interval. The water was drained out and the algae were immersed in 0.3-0.5N sulphuric acid. Ratio of algae to this solution was 1:3 or 1:5 and the temperature was kept at 42°C. This temperature facilitated easy removal of acid-soluble salts and also rupture of the cell wall, so that maximum extraction could take place in alkaline medium. The acid was washed out and the pH of the algae was brought to neutral. The algae were then digested in 4 per cent solution of sodium carbonate at room temperature and left overnight. The solution was centrifuged and filtered through a filter press to obtain a clear fluid of crude sodium alginate. It was then bleached with sodium hypochlorite till a yellow colour was observed. It was then immediately decolorized by sodium bisulphite. The usual method of precipitation of alginic acid by hydrochloric acid was found to lower the viscosity of sodium alginate. Therefore, the resultant solution was mixed with half the volume of 90 per cent industrial alcohol. This resulted in coagulation and flocculation of sodium alginate and the steps of acidification and mixing with sodium salt were eliminated. The used alcohol could be redistilled for further use and only a small quantity was thus utilized for each batch. Sodium alginate is collected and dried at 70°C in an oven fitted with an alcohol condenser.

This method has been found to give most suitable quality of sodium alginate. Different grades of viscosities can be easily produced by varying the time and temperature of acid wash. If the temperature and time are both increased, the viscosity is lowered and if the temperature is reduced with a slight increase in time, higher viscosity is obtained.

Revised method for agar

Agar is known to mankind for a very long time. The ancient Chinese prepared sweet jellies by boiling the red algae and adding some sweetening agents. Thivy has described the method for extraction of agar from Indian species of *Gracilaria* and *Gelidium*.

In this process the algae are pulverized and then boiled with soft water which is slightly acidified, the boiled mass is filtered and allowed to settle to form the jelly. It is then frozen at 5°C and defrosted further to remove the water, dried and pulverized. This method has been found most suitable for the manufacture of agar. However, a slight modification in the final step was considered necessary. For a large-scale plant for this purpose, costly freezing equipment becomes necessary. In Japan and USA the freezing is done by simple exposure and no costly equipment is involved. For eliminating this cost the filtrate is treated with 90 per cent industrial alcohol so that agar is flocculated.

References

- BROOKS, A. J. 1949. The Seaweeds and Their Uses. *New Biology*: 789-803.
- CAMERON, M. C., ROSS, A. G. & PERCIVAL, E. G. V. 1948. *J. Soc. chem. Ind. (London)*, **67**: 161.
- CHACKO, P. I. & PILLAY, M. 1958. *Rep. Fish. Dept. Mad.*
- CHAPMAN, V. J. 1940. *An Introduction to the Study of Algae*, 1-387. Cambridge University Press, London.
- CHIDAMBARAM, K. & UNNI. 1947. *Madras Agri. J.*
- FRETSON, E. C. 1953. *Proc. 1st international Seaweed Symposium*, 1952: 70-71.
- KAPPANNA, A. N., VISWESWARA RAO, A. & MODY, I. C. 1962. *Curr. Sci.*, **11**: 463-64.
- PILLAY, M. & CHACKO, P. I. 1955. *Rep. Fish. Dept. Mad.*
- THIVY, F. 1952. *Proc. Indo-Pacific Fisheries Council 3rd Meeting*.
- TRESSELER, D. K. & LEMON. 1951. *Marine Products of Commerce*.

DISCUSSION

Dr V. Krishnamurthy: Dr Desai mentions that harvesting did not interfere or reduce the amount of seaweeds available, particularly *Sargassum*; but, on the contrary, it increased the amount present. In this connection, I would like to know the exact method of harvesting that has been used, how much was harvested, and for how many successive periods or years. He also mentions that the viscosity of alginic acid varied in different species. Since alginic acid is a versatile substance, I would like to know the viscosity requirements of different industries.

Dr B. N. Desai: Plants were cut above a certain level so that the basal portion remained. Harvesting was done in three successive years, viz. 1962-63, 1963-64 and 1964-65, at various

centres in the Gulf of Kutch and the Gulf of Mannar, and annually about 200 tons were harvested. Harvesting was done in marked transect areas, and the total quantity harvested was found to increase considerably. Viscosity requirements for the various industries are different. Some require low grades, i.e. 2 per cent alginic acid solution having 70-80 centipoise, in the textile industries for printing with reactive dyes 1200-1500 centipoise, making plates for dental patients 700-800 centipoise, in electronic industries 2500-3000 centipoise. In the preparation of dental moulds very thin viscous solution is required, i.e. 10-15 centipoise, and for the ice-cream industry 15-20 centipoise.

Mr R. Venkataraman suggested that manufacture of agar-agar should be taken up in the country as the cost of production of agar would be far lower than the cost of import of agar.

Dr C. S. P. Rao: It is said that *Sargassum swartzii* and *S. cinereum* have higher percentage of alginic acid with higher viscosity, while *S. tenerrimum*, a more abundant species, produces only a low viscosity product. There are two methods available for improving the viscosity. The technologist is there on one side to devise the process to increase the viscosity and the fundamental biologist is there on the other side who could bring about hybridization of *Sargassum*. An attempt could be made in this direction.

Dr E. R. S. Talpasayi: Dr Desai, would you kindly tell us the method of collection and preservation of algae and what time would elapse from the time of collection till the final analysis and extraction are made? There might be some hydrolysing enzymes which might act on polysaccharides.

Dr B. N. Desai: Collection and preservation of algae are a most important factor. Algae that are collected should not be dried straight away, but have to be washed in fresh water before they are dried. They should be dried to such an extent that the moisture content would be no more than 10-11 per cent. If it is more than 15-16 per cent, we do get putrefaction, and the viscosity of the product goes down. I found it much better to wash with fresh water. Four per cent formalin will preserve much better.

Mr I. C. Mody: Dr Desai mentions that bleaching with reducing or oxidizing agents does not decrease or affect viscosity while it is a general observation that prolonging time with excessive reagents does decrease viscosity of sodium alginate. He also mentions that oxidation or precipitation with acidic pH rather reduces viscosity. Is he precipitating with very strong reagent? At pH 5-6 alginic acid is not precipitated; but

actual precipitation takes place at pH 4-5 or 3-4, and if it is kept at this pH for long, acidic condition may affect viscosity, and not precipitation. He further mentions that coagulation increases the viscosity due to the removal of soluble salts which are coming as impurities if it is precipitated.

Dr B. N. Desai : Point about viscosity and drop in the viscosity is a very big question, and I would like more work to be done in this direction. My own observation is that acidification of alginic acid is found between pH 3 and 4. At this pH viscosity may be lowered. Main point is that changes in pH affect the viscosity of the alginate solution, and not just the precipitation. Just to prevent these industrial alcohol is used.

Mr C. R. Easwaran : Dr B. N. Desai mentions coagulation of sodium alginate by commercial alcohol. To filter crude alginate, we have to dilute it tremendously for aiding it in filtration, so a large amount of alcohol is required. I would like to know whether it is commercially feasible to use alcohol. Will it be cent per cent recoverable ?

Dr B. N. Desai : It is true that the quantity of alcohol required is very great. We require about 50 per cent of industrial alcohol of 95 per cent grade. It is possible to recover about 95 per cent of the alcohol by industrial recovery process. This will not affect the cost of production.