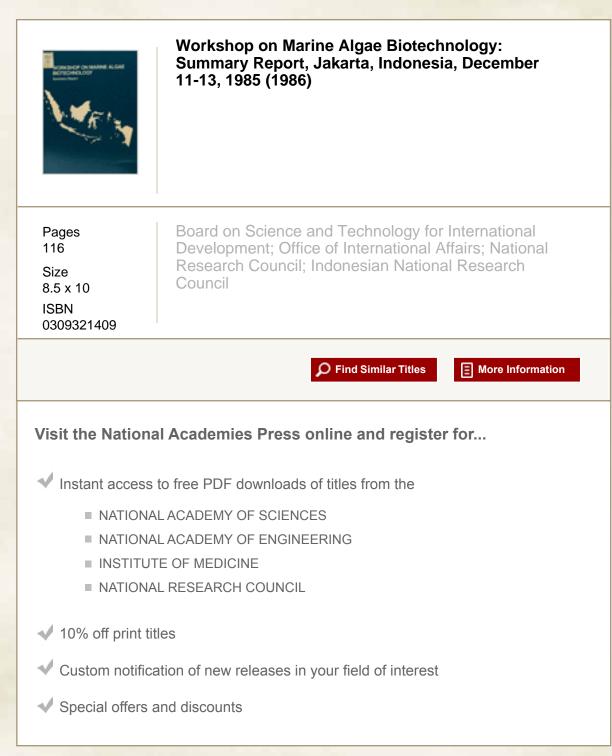
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WORKSHOP ON MARINE ALGAE BIOTECHNOLOGY

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Jakarta, Indonesia December 11-13, 1985

Jointly sponsored by

Indonesian National Research Council Government of the Republic of Indonesia and Board on Science and Technology for International Development Office of International Affairs National Research Council United States of America

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PREFACE

In 1985, the National Research Council of Indonesia (DRN) invited the Board on Science and Technology for International Development (BOSTID) of the U.S. National Research Council (NRC) to join it in sponsoring a workshop on marine algae (seaweed) biotechnology. This workshop was held in Jakarta, Indonesia, December 11-13, 1985. It explored the current and future potential of marine algae as a source of increased income for Indonesians living in coastal areas.

As an island nation, Indonesia is blessed with a wealth of marine resources. One resource, seaweed, is used in the manufacture of food and industrial products. However, this industry is only an evolving one with great potential for further development.

Seaweeds are simple in structure and can be cultivated rather easily. The primary requirements are suitable areas for cultivation and relatively inexpensive labor. As an archipelagic nation, Indonesia has tens of thousands of kilometers of coastal waters in which to establish seaweed farming.

Processed seaweed plays a significant role in many areas of manufacturing as it is used to produce agar, algins, carrageenans, and other substances, which in turn are used in pharmaceuticals and cosmetics as well as for other industrial purposes. Thus, particular attention was given in the workshop to the industrial potential of seaweed products for both the domestic and export markets. Attention was focused not only on the near-term prospects, but also on the longer term which will be affected by the use of biotechnological techniques.

These discussions were one activity in a larger program of cooperation between BOSTID and the Indonesian government. Begun in 1968, this program has featured a series of workshops on food policy, industrial and technological research, natural resources, rural productivity, and manpower planning. BOSTID's participation has been supported in the context of a science and technology loan from the U.S. Agency for International Development (USAID) to the government of Indonesia. The current two-year program with BOSTID calls for a number of activities (panel discussions, workshops, follow-up activities, or small advisory groups) to be organized each year.

ORGANIZATION OF THE WORKSHOP

This workshop was organized by the Seaweed Research Team of the Indonesian Agency for the Assessment and Application of Technology (BPPT) under the sponsorship of the Indonesian National Research Council. Prior to the workshop, site visits to the Bali area where seaweed is being farmed were arranged by the staff of the Seaweed Research Team organizing committee. These included a one-day launch trip around Nusa Ceningan, Nusa Lembongan, and Nusa Penida, followed the next day by visits to Nusa Dua and Pulau Serangan. These visits enabled both the U.S. and Indonesian participants to see firsthand the growing and harvesting methods for marine algae and to inspect the various species of algae in the Bali area. The group also had the opportunity to visit algae warehouses to observe algae in its final state before sales to customers.

Dr. Sediono Tjondronegoro, workshop chairman and secretary of the DRN, and Dra. Rachmaniar Rachmat, chairman of the workshop organizing committee, convened the workshop in plenary session on the morning of December 11. Mr. Richard Howland, deputy chief of mission at the U.S. Embassy, explained at the opening session that this workshop is the first activity under a new collaborative agreement between the U.S. and Indonesian national research councils. This agreement was a product of the U.S.-Indonesia bilateral S&T agreement signed July 1984 in Washington, D.C. (see Appendix A).

Dr. Doddy A. Tisna Amidjaja, vice-chairman of the DRN, officially opened the workshop on behalf of Dr. B. J. Habibie, minister of state for research and technology and chairman of the DRN. He told workshop participants that Indonesia is actively pursuing the production of more nonpetroleum and nongas commodities, especially for export. Thus, resources from the sea such as marine algae merit thorough study (see Appendix B).

A number of papers focusing specifically on marine algae and marine algae biotechnology, and emphasizing industrial applications, were then presented at the workshop and are included as Part I of this report. Subsequently, the participants broke into two working groups that addressed research on marine algae and its development for biotechnological products, respectively.

On December 13, the merged conclusions and recommendations of the two working groups were presented by Dra. Rachmaniar Rachmat. Dr. Dirk Frankenberg, chairman of the U.S. panel and vice-chairman of the workshop, spoke on behalf of his U.S. colleagues about the urgency of undertaking several steps that could enable Indonesia to take advantage of the "window of opportunity" that now exists for supplying quality marine algae to international customers. These steps are included in Part II of this report.

Appendixes to this report describe the Center for Research and Development in Biotechnology at Cibinong (Appendix C), relate closing remarks made at the workshop by Mr. Howland and Dr. Habibie (Appendixes D and E, respectively), and list the workshop agenda and participants (Appendixes F and G, respectively). This workshop report was prepared by Rose Bannigan of the BOSTID staff using papers written by the Indonesian and NRC workshop participants. The papers have been edited to eliminate duplication, but they accurately reflect the discussions. The final draft was reviewed and approved by the members of the NRC panel and the Indonesian organizing committee. Sabra Bissette Ledent, BOSTID consultant, edited the report.

Participants would like to acknowledge the valuable contribution of the workshop's organizing committee to the final arrangements for the workshop as well as the site visits to the islands near Bali. The organizing committee was chaired by Dra. Rachmaniar Rachmat of BPPT, and Drs. Jana Anggadiredja served as vice-chairman. The site visits were arranged by Ir. Sri Istini, Drs. Jana Anggadiredja, and Ir. A. Zatnika.

The participants would also like to thank the members of the workshop secretariat for the excellent organization of the workshop. The secretariat was under the supervision of Drs. Jana Anggadiredja.

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PART I

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Presentations on Marine Algae

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THE POTENTIAL OF MARINE ALGAE FOR BIOTECHNOLOGICAL PRODUCTS IN INDONESIA

Aprilani Soegiarto and Sulistijo National Institute of Oceanology Indonesian Institute of Sciences (LIPI)

INTRODUCTION

The Indonesian archipelago is located between 94° and 141°E and between 6°N and 11°S, or between the continents of Asia and Australia and the Pacific and Indian oceans. It consists of 13,667 islands with more than 81,000 km of coastline. Because water covers two-thirds of the Indonesian territory, it is only natural that Indonesia should have an important role in marine matters and would wish to realize a return from its maritime opportunities. Thus, the Republic of Indonesia is devoting considerable attention and effort to scientific surveys and research on its marine environment.

Modern marine research stems from the Siboga Expedition of 1899-1900, which focused on marine flora and fauna and its biogeography (Tydeman, 1903). It was only through the untiring efforts of Weber van Bosse during and after the Siboga Expedition that the field of phycology became better known in Indonesia. According to van Bosse (1928), 555 species of marine algae (seaweed) were collected from Indonesian waters during the Expedition. Of these, only about 55 species have been utilized by Indonesians as food and for some medicinal treatments (Zaneveld, 1955).

Unfortunately, it is only recently that the economic value of seaweed has been appreciated. Commercial seaweeds from Indonesia are mostly agarophytes (<u>Gracilaria</u>, <u>Gelidium</u>, <u>Gelidiella</u>, and <u>Gelidiopsis</u>) and carrageenophytes (<u>Eucheuma</u> and <u>Hypnea</u>). Some of the chemicals derived from seaweed have particular properties that give them special value for industrial or medical purposes. Seaweed can therefore be considered an important marine resource.

Indonesia is one of the important suppliers of seaweed in Asia. The average annual production of seaweed in Indonesia during the period 1979-1983 was about 7,600 tons. Before 1975, the average annual production was less than 3,000 tons. Because almost all production was harvested from natural stock, it was thus unreliable and the value of Indonesian seaweed was lower than that from other countries. To improve seaweed production and its value, Indonesia has begun to cultivate carrageenophytes in several areas in the Indonesian archipelago. It is hoped that through this workshop on marine algae biotechnology more modern technology--in particular, biotechnological

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research and development--will play a role in Indonesian efforts to enhance seaweed production.

SEAWEED PRODUCTION

The results of a number of surveys carried out in Indonesia show that the distribution and density of marine algae in a region vary according to the type of bottom, season, hydrographic conditions, and species composition at a given time. The great variations in standing crops from one region to another and from one species to another are described for several areas of Indonesian waters in Table 1. Figures 1-3 show, respectively, the distribution of commercial seaweeds in Indonesia as summarized by Soegiarto et al. (1978), the distribution of Gelidium spp. as reported by Atmadja and Sulistijo (1983a), and the distribution of Eucheuma spp. as also reported by Atmadja and Sulistijo (1983b). Table 2 shows the kinds of commercial seaweed, most of which comes from natural stocks, produced in 15 provinces in Indonesia. During the period 1974-1983, seaweed production increased (Table 3), and more than 70 percent of this production came from the Maluku region. Eucheuma spinosum* cultivated in Bali accounted for about 2.5 percent of 1983 production.

Unfortunately, a great quantity of seaweed is still neglected. Even if this seaweed were harvested and used in manufacturing or exported, it would still not be used up to its full potential. For example, seaweed production in 1983 was 9,607 tons (valued at Rp. 515 million), but only 3,405 tons with a value of Rp. 347 million were exported. The unexported products could not be utilized because most consisted of <u>Eucheuma</u> spp. At the same time, Indonesia imported 350,182 kg of agar to supply domestic demands (valued at US \$551,000).

SEAWEED EXPORTS

Commercial seaweed activities in Indonesia most likely began with seaweed exports to China over a century ago. Before World War II, seaweed was exported at the rate of more than 1,000 t/yr, but over the last few years exports have tended to increase, with the best export year (about 5,923 tons) in 1966 (Table 4).

^{*} The terms "Eucheuma spinosum" and "Eucheuma cotlonil" are utilized differently by scientists and those in the trade. To scientists, these terms connote specific species of Eucheuma while those in the trade use these terms in a more generic sense to designate <u>iota</u>-carrageenian and <u>kappa</u>-carragenan, respectively. The latter usage of these terms prevails in this and the following presentations.

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The most important market and shipping center for seaweed is Ujung Pandang, South Sulawesi. At present, seaweed is exported mainly to Singapore, Denmark, Hong Kong, Japan, and France. Singapore and Hong Kong are important as in transit ports for the European markets, the United States, and Japan. About 90 percent of seaweed exported from Indonesia consists of Eucheuma which is used as a source of carrageenan. Gelidium and Gracilaria are usually exported to Japan.

Ten years ago exports of Eucheuma from Indonesia dominated the world market. Since its successful cultivation in the Philippines, however, the Eucheuma market is dominated by Philippine products. In 1983, about 26,000 tons of Eucheuma were cultivated in the Philippines, mostly for export (Porse, 1985).

Location	Species	Standing Crop (t/ha)	Source
Waworada Bay, West Nusa Tenggara	Eucheuma spinosum	4.0-18.0ª	Soegiarto, 1966
Maluku and East Nusa Tenggara	E. spinosum	0.6 3.4 ^b	Mubarak, 1974
Seribu Island, Java	E. spinosum	0.11 ^a	Sulistijo & Atmadja, 1977
Tanjung Benoa, Bali	E. serra Gracilaria lichenoides Hypnea spp Ulva spp	0.46 ^a 0.96 ^a 1.52 ^a 1.63 ^a	Sulistijo & Atmadja, 1976
Southeast Maluku	Eucheuma spinosum	2.27 ^a	Sulistijo & Yusuf, 1977
Central Maluku	E. edule Gracilaria spp	5.02 ^a 2.13 ^a	Sulistijo & Kurnaen, 1977

TABLE 1 Variation in the Standing Seaweed Crop in Indonesia

^aWet weight. ^bDry weight.

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DOMESTIC UTILIZATION OF SEAWEED

Seaweed As Food

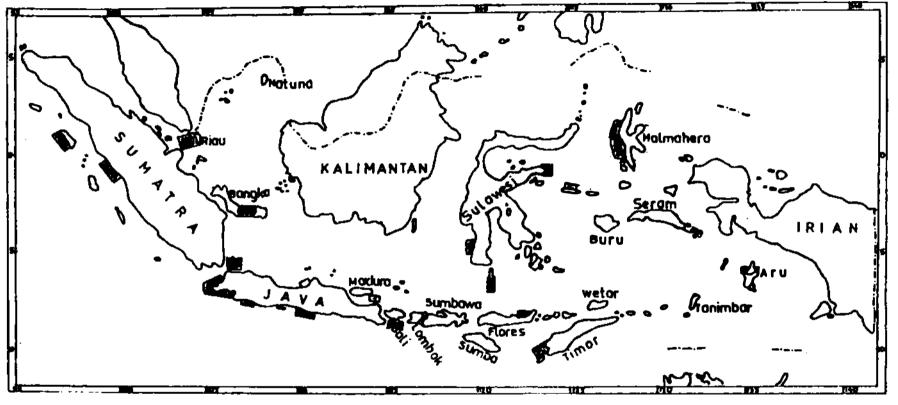
Indonesians have collected marine algae for centuries to use as a food supplement, especially as a vegetable; however, consumption is small. Zaneveld (1955) mentions some 55 useful species of seaweed found in Indonesia, including the economic algae of the Southeast Asian waters.

Seaweed is consumed in various forms--for example, raw as salad, boiled as a vegetable, pickled, and cooked with coconut milk. It is also used for thickening soups, puddings, and sweetened jellies. In the eastern part of Java, puddings and sweetened jellies are made from <u>Hypnea</u> spp. The organic composition, including vitamins and minerals, of six species of seaweed found in Indonesia is listed in Tables 5 and 6.

The nutritional value of seaweed varies from one division to another, from one species to another, and even from one part of a plant to another. Protein is generally found only in small quantities, but very little of that can be assimilated by humans. Carbohydrates are present in large amounts, chiefly as cell wall components and as intracellular storage matter, but they are not of great value as a source of energy. The carrageenan coefficient of digestibility is estimated to be only 6 percent in humans, 33 percent in dogs, and 50 percent in laboratory rats. That for agar is estimated to be about 28 percent in laboratory rats (Walford, 1958). It has been suggested that people who eat seaweed from childhood may acquire certain bacterial flora in their intestines that help them digest these foods.

Seaweed as a Raw Material for Agar Manufacture

In spite of the fact that "agar" is an Indonesian word, an agar industry was only recently developed in Indonesia. In 1910, the first literature appeared mentioning an attempt to manufacture agar commercially, although, no doubt, many unrecorded attempts had been made before. The first large-scale agar factory in Indonesia was established in Kudus, Central Java in 1930. Immediately thereafter other factories were started in other cities. Unfortunately, the Indonesian agar factories suffered from of the competition with imported agar, a lack of a continuous supply of good-quality raw material, and a lack of technologies. Table 7 depicts the situation of agar factories in Indonesia between 1954 and 1964, while Table 8 depicts that of agar industries in Java. Because agar factories in Indonesia are mostly home industries, these factories will have a hard time surviving competition with imported agar without government protection.



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FIGURE 1 The distribution of commercial seaweeds in Indonesia. (Source: Soegiarto et al., 1978)

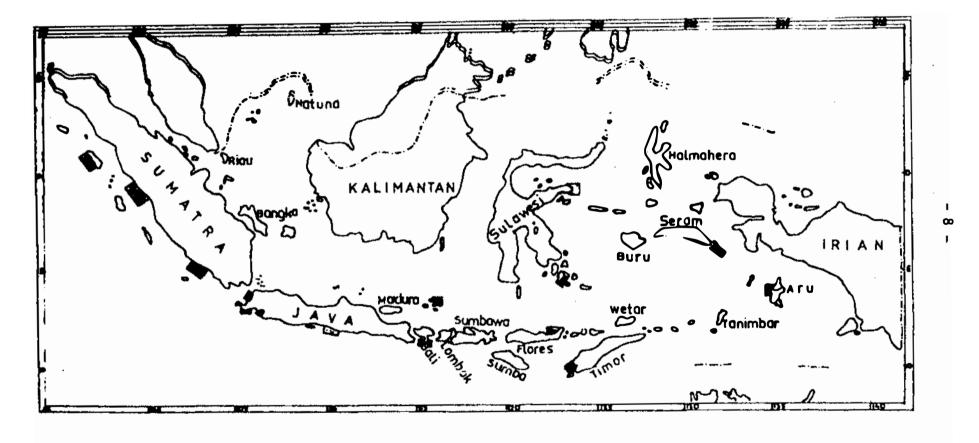


FIGURE 2 The distribution of Gelidium spp. in Indonesia. (Source: Atmadja and Sulistijo, 1983a)

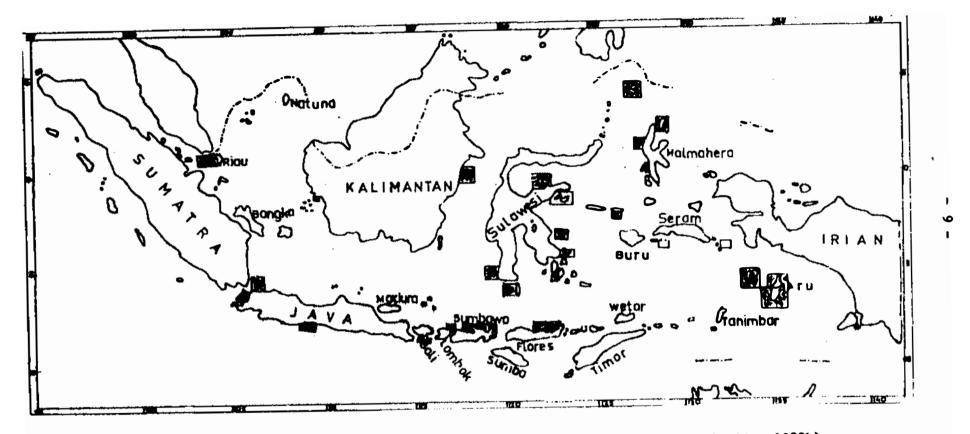


FIGURE 3 The distribution of <u>Eucheuma</u> spp. in Indonesia. (Source: Atmadja and Sulistijo, 1983b)

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Province	Species
Bengkulu	Gelidium
Lampung	Gelidium
Riau	<u>Gelidium,</u> <u>Gracilaria</u> , <u>Eucheuma</u>
West Java	<u>Gelidium, Gracilaria, Hypnea</u>
Central Java	<u>Gelidium</u> , <u>Gracilaria</u>
Yogy akarta	Gelidium
East Java	<u>Gelidium, Gracilaria, Hypnea</u>
Bali	Gelidium, Gelidiella, <u>Gracilaria</u> , <u>Eucheuma, Hypnea</u>
West Nusa Tenggara	<u>Gelidium, Gracilaria</u>
East Nusa Tenggara	<u>Gelidium, Gelidiella, Gracilaria,</u> Eucheuma
Central Sul awes i	<u>Gelidium, Eucheuma</u>
Southeast Sulawesi	<u>Gelidium, Eucheuma</u>
South Sulawesi	<u>Eucheuma, Gracilaria</u>
Maluku	<u>Eucheuma, Gelidium, Gracilaria,</u> Hypnea
Irian Jay a	Eucheuma

TABLE 2 Commercial Seaweed Production in 15 Provinces in Indonesia

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Year	Production (tons)	Value (Rp. 1,000)
lear		(kp. 1,000)
1974	4,232	——————————————————————————————————————
1975	8,325	
1976	3,750	95,897
1977	4,098	202,760
1978	5,621	742,486
1979	5,945	333, 354
1980	7,848	421,272
1981	7,251	362,000
1982	7,479	398,000
1983	9,607	515,000

TABLE 3 Indonesian Seaweed Production and Value, 1974-1983

SOURCE: Central Bureau of Statistics, Jakarta.

TABLE 4 Indonesian Seaweed Exports (Dry Weight, Tons), 1961-1983

Year	Export	Year	Export	Year	Export
1961	921.6	1969	2,183.3	1979	1,836.1
1962	417.9	1970	3,007.4	1980	596.6
1963	158.0	1971	3,733.5	1981	690.2
1964	285.0	1972	3,721.9	1982	2,110.7
1965	1,132.0	1973	3,251.2	1983	3,405.1
1966	5,923.0	1974	3,301.3		•
1967	918.2	1975	1,602.6		
1968	2,394.7	1976	1,988.0		

SOURCE: Central Bureau of Statistics, Jakarta.

	1	2	3	4	5	6
Moisture	27.50	16.99	19.01	24.91	12.95	25.15
Protein (6.25 N)	5.40	2.48	4.17	3.14	9.98	1.59
Carbohydrates	33.22	63.19	42.59	37.52	54.43	32.25
Fats	8.62	4.30	9.54	.52	11.09	5.81
Crude fiber	3.01		10.51	9.14		11.43
Ash	22.25	23.04	14.18	15.77	11.55	23.77

TABLE 5 Organic Composition of Indonesian Seaweeds (Percent of Air-dried Weight)

- 1. Eucheuma spinosum 4. Gracilaria confervoides 2. Eucheuma spp. 5. Gelidiopsis spp. 6. Hypnea spp.
- Gracilaria spp. 3.

SOURCE: Soegiarto (1968).

TABLE 6 Nutritional Value of Eucheuma and Gracilaria

	<u>E. spinosum</u> Bali	<u>E. spinosum</u> S. Sulawesi	<u>E. cottonii</u> Bali	<u>G. gigas</u> Bali
Moisture (%)	12.90	11.80	13.90	12.90
Crude protein (%)	5.12	9.20	2.69	7.30
Fats (%)	0.13	0.16	0.37	0.09
Carbohydrates (%)	13.38	10.64	5.70	4.94
Crude fiber (%)	1.39	1.73	0.95	2.50
Ash (%)	14.21	4.79	17.09	12.54
Minerals, Ca (ppm)	52.820	69.250	22.390	29.920
Fe (ppm)	0.108	0.326	0.121	0.701
Cu (ppm)	0.768	1.869	2.736	3.581
Pb (ppm)		0.015	0.040	0.190
Thiamin (mg/100g)	0.21	0.10	0.14	0.02
Riboflavin (mg/100g)	2.26	8.45	2.70	4.00
Vitamin C (mg/100g)	43.00	41.00	12.00	12.00
Carrageenan (%)	65.75	67.51	61.52	
Agar (%)	- -			47.34

SOURCES: BPPT Seaweed Research Team and Food Technology Development Center, Bogor Agriculture Institute)

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Although the appearance of Indonesian-made agar is almost the same as that produced elsewhere, its gel strength is less. The end products of Indonesian agar factories are mostly agar block and agar paper; only one factory in Surabaya produces agar powder.

Year	Number of Factories	Number of Workers	Raw Materials (dried seaweed, kg)	Annual Production (kg)
1954	7	166		22,901
1955	5	92		13,734
1956	9	163		27,229
1957	13	212		33,083
1958	14	292	514,954	40,225
1959	14	284	588,151	46,055
1960	11	194	422,838	34,045
1961	10	187	498,536	34,157
1962	11	214	490,240	28,114
1963	12	288	398,810	32,934
1964	6			13,934

TABLE 7 The Agar Industry in Indonesia, 1954-1964

SOURCE: Central Bureau of Statistics, Jakarta.

At present, the main raw material used by agar factories in Indonesia is <u>Gracilaria</u>; only a few use <u>Gelidium</u> (Table 8). Factories could probably improve their products by using a good proportion of <u>Gelidium</u> mixed with <u>Gracilaria</u> to give the right properties. Research is badly needed in this field to support increased production and to make a better-quality agar in Indonesia. Establishment of a carrageenan factory is also urgently needed to support the increasing production of carrageenophytes.

Other Uses of Seaweed

For many centuries seaweed has been used as fertilizer to increase crop yields in Japan, China, Great Britain, France, Canada, and other countries that have extensive seacoasts. Until now, there has been no application of seaweed as fertilizer in Indonesia. In 1980, a factory tried to produce fertilizer from seaweed; however, thus far there is no acceptable market in Indonesia. As an agricultural country, Indonesia has a good opportunity to explore the use of seaweed as fertilizer, especially in regions within distance of the coast.

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Name and Location	No. of Wo rkers	Species Used	Raw Material Needed (kg/mo)	Production (kg/mo)
Universal Surabaya	4	Gracilaria spp.	710	42
Sinar Kencana Surabaya	32	<u>Gracilaria</u> spp., <u>Gelidium</u> spp.	13,000	800
Sinar Kencana Wonocolo	15	<u>Gracilaria</u> spp., <u>Gelidium</u> spp.	3,470	208
Sriti Surabaya			250	15
Sari Jaya Surabaya		· 	2,278	166
Oen Brothers Surabaya	7	<u>Gracilaria</u> spp., <u>Gelidium</u> spp.	417	25
Sumber Laut	7	Gracilaria spp., Gelidium spp.	1,000	60
Hasalin Jakarta	60	<u>Gracilaria</u> spp., <u>Gelidium</u> spp.	20,000-30,000	2,000-3,000
Djawa Jakarta	25	<u>Gracilaria</u> spp.	15,000	1,500

TABLE 8 Description of Nine Agar Factories in Java, 1978

SOURCE: Soegiarto et al. (1978).

In the United States and many European countries such as Denmark, Norway, France, and Scotland, seaweed has been used as a feedstock for a long time, and these countries manufacture this product themselves. In Indonesia, there has been no scientific attempt to use seaweed as a feedstock, although inhabitants of Nusa Tenggara believe that sheep that occasionally feed on seaweed tend to have sweeter and better meat.

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USES OF MARINE ALGAE IN BIOTECHNOLOGY AND INDUSTRY

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INTRODUCTION

All biological organisms, including marine macroalgae (seaweeds), contain a variety of chemical compounds--organic, inorganic, and mixed. The commercially important hydrocolloids--algins, carrageenans, and agars (from which different types of agaroses are derived)--are all major components of macroalgae, but not the same ones. These polymeric carbohydrates, or polysaccharides, have unique properties which have led not only to a wide variety of industrial and food applications, but have made them indispensible to biotechnology as well.

Worldwide production of these hydrocolloids is approximately 5,000 tons for agars, 12,000 tons for carrageenans, and 15,000 tons for algins. Most of this production is consumed by food and industrial applications, with the overall growth rate projected to be less than 3 percent per year over the next 10 years. At this rate, the current world manufacturing capacities should be sufficient for this period. Representative prices are \$4.10-7.70/kg for algins, \$6.60-13.20/kg for carrageenans, \$15-20/kg for food- and pharmaceutical-grade agars, \$100-260/kg for bacteriological-grade agars, and \$500-2,000+/kg for purified, standardized agaroses and their derivatives.

Before discussing the origin, properties, and applications of these biopolymers, it should be explained that hydrocolloids are hydrophilic polymers which are soluble or dispersible in water to the extent that the particles cannot be seen using an ordinary light microscope. As "water modifiers," hydrocolloids can be used as thickeners or viscosifiers--for example, nongelling <u>lambda</u>-carrageenan, sodium alginate, carboxymethylcellulose, microcrystalline cellulose, and xanthan. Or, they can be gelling agents--for example, agar, agarose, kappa- and iota-carrageenans, calcium alginate, and gelatin.

The four major classes of seaweed are <u>Rhodophyta</u> (red algae), <u>Phaeophyta</u> (brown algae), <u>Chlorophyta</u> (green algae), and <u>Cyanophyta</u> (blue-green algae). Only the red and brown algae are currently sources of commercial products of significant value. The three types of carrageenan (designated <u>kappa-</u>, <u>lambda-</u>, and <u>iota-</u>) and agar (from which agarose is derived by purification) are obtained from red algae, but not from the same species. Algins are obtained from a number of species of brown algae and are present in all.

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ALGINS

Although algin was discovered in Great Britain in 1880, commercial production was not begun until 1929, by Kelco in California. Alginates, the salts of alginic acid or algin, are composed of D-mannuronic and L-guluronic acid residues. Three kinds of polymeric segments have been discovered in algins, the relative ratios of which depend on the source and method of extraction (see Cottrell and Baird, 1980; Cottrell and Kovacs, 1980; Guiseley, 1968; and Renn, 1984b: all were used as sources for this section). One segment is composed essentially of D-mannuronic acid subunits, another of essentially L-guluronic acid subunits, and the third of alternating D-mannuronic, L-guluronic acid residues (Table 1). The proportion of these components varies, depending on the source of the algin (Table 2).

TABLE 1 Mannuronic Acid and Guluronic Acid Composition of Alginic Acid

Species	Mannuronic Acid (M) wt. %	Guluronic Acid (G) wt. %	M:G R at io	M:G Ratio Range
Macrocystis pyrifera	61	39	1.56	
Ascophyllum nodosum	65	35	1.85	1.40-1.95
Laminaria digitata	59	41	1.45	1.40-1.60
Laminaria hyperborea (stipes)	31	69	0.45	0.40-1.00
Ecklonia cava and Eisenia bicyclis	62	38	1.60	

SOURCE: Cottrell and Kovacs (1980).

TABLE 2 Proportions of Polymannuronan, Polyguluronan, and Alternating Segments in Alginic Acid from Different Sources

Source	Polymannuronan	Polyguluronan	Alternating
Macrocystis pyrifera	40.6	17.7	41.7
Ascophyllum nodosum	38.4	20.7	41.0
Laminaria hyperborea	12.7	60.5	26.8

SOURCE: Cottrell and Kovacs (1980).

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Isolation

Although processing of seaweed polysaccharides is generally proprietary, a typical algin recovery procedure might include the following steps: washing the weed, alkali treatment to dissolve the algin, clarification, calcium chloride precipitation, color removal, ion exchange to the sodium form, drying, and milling.

Properties

Free alginic acid is essentially insoluble in water, but the ammonium and alkali metal salts dissolve readily in cold water to form viscous solutions. Insoluble salts are generally formed with the alkaline earth and group III ions. This property enables the formation of calcium alginate gels. Propyleneglycol alginate is important commercially because this derivative is more acid-stable than native algin and stabilizes water-in-oil emulsions.

Applications

Food

Because of their thickening, gelling, water retention, and suspending properties and nontoxic nature, alginates are important for their use in food. They are used in frozen foods to maintain texture during freeze-thaw cycles, in bakery icings to counteract stickiness and cracking, in syrups to suspend solids, in salad dressings and sauces as emulsifiers, and in beer to enhance the foam. The gelling properties of alginates are important for preparing instant pudding mixes, pastry fillings, dessert gels, and fabricated foods.

Industrial Uses

The same properties that make alginates useful for food applications also make them valuable for industrial uses. Alginates are used in paper coatings and sizings, adhesives, textile printing and dyeing, oil drilling muds, air-freshener gels, explosives, boiler compounds, polishes, ceramics, welding rods, tabletting agents, and cleaners. Alginates also function as excellent media for dental impressions, although this is not really an industrial or food application.

Biotechnological Uses

Biotechnology is defined in <u>Webster's New World Dictionary</u> as "the use of the data and techniques of engineering and technology for the study and solution of problems concerning living organisms." The algal hydrocolloids are playing an increasingly important role in biotechnology, and the alginates are no exception.

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When a solution of sodium alginate is added in drops to a solution containing calcium ions, water-insoluble calcium alginate gel droplets are formed. This procedure can be used to encapsulate microbial, plant, and animal cells, and, as with carrageenan, these caged cells are being evaluated as bioconversion systems. Calcium alginate gels are not thermoreversible, but they will dissolve in the presence of a calcium-sequestering agent such as EDTA.

Kyowa Hakko Kogyo, Ltd. in Japan now has a commercial ethanol process based on algin-immobilized yeast cell, fluidized bed reactors. In addition, in a laboratory test pancreatic cells contained in a calcium alginate sac and implanted subdermally in laboratory animals continued to secrete insulin for approximately one month. Finally, Damon's cell entrapment system for producing monoclonal antibodies from hybridomas is algin reacted with polylysine to decrease water solubility.

Calcium alginate fibers are used in medicine as swabs for physiological sampling and as pads to control hemorrhaging of wounds.

CARRAGEENANS

"Carrageenan" is a generic term for a complex family of sulfated polysaccharides extracted from a number of different red seaweeds (see Guiseley et al., 1980; Guiseley, 1968; and Renn, 1984b). They are all sulfated linear galactans, whose idealized basic structural unit, carrabiose, is an alternating α 1,3-D galactose, β 1,4-3,6 anhydro D-galactose disaccharide. Figure 1.

Residents of County Carragheen on the southern coast of Ireland are reported to have used the seaweed <u>Chrondrus crispus</u> in foods and medicine about 600 years ago. Known as "Irish moss" and containing carrageenans, this seaweed was used primarily in early times to make

B Units:

Found in:

o - Galoctose	λ,θ
p - Galactase 2 - sulfate	λ.ξ.θ
D - Goloctose 4 - sulfate	μ,ν,κ,ι

A Units :

o - Galactose 2 - sulfate	3
o - Galactose 6 - sulfate .	μ
o - Galactose 2,6 - disulfate	λ,υ
3,6 -Anhydro - o - Galactose	R
3,6 - Anhydra - _D - Galactose 2 - sulfate	ι.θ

FIGURE 1 Repeating structure of carrageenans.

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milk puddings or so-called flans. The first process for extracting carrageenans from Irish moss was patented in 1871, but it was not until 1937 that commercial production was started.

Isolation

A typical process would include the following steps: washing the weed, extraction and/or modification using alkaline reagents, removal of weed residues, concentration, coagulation, drying, and milling.

Properties

Three major types of carrageenans, designated <u>kappa-</u>, <u>lambda-</u>, and <u>iota-</u>, are used commercially. These differ in the amounts and position of ester sulfate substituents and content of 3,6 anhydro-galactose. <u>Kappa-carrageenan gels in the presence of potassium ions to form</u> strong, crisp gels, while <u>lamba-carrageenan</u> is nongelling but forms viscous solutions. <u>Iota-carrageenan gels in the presence of calcium</u> ions to form elastic gels. Because of their strongly anionic nature, these carrageenans exhibit a high degree of protein reactivity. The free acids are unstable and rapidly undergo autocatalytic degradation. Commercial products are usually mixtures of sodium, potassium, or calcium salts.

<u>Chondrus crispus</u> is the primary source of the <u>lambda-carrageenan</u>, <u>Eucheuma cottonii</u> of the <u>kappa-carrageenan</u>, and <u>Eucheuma apinosum</u> of the <u>iota-carrageenan</u>. At least 26 different red seaweeds are reportedly used for manufacturing carrageenans, including <u>Gigartina</u> <u>radula</u> which contains <u>kappa-</u> and <u>xi-carrageenans</u>.

Applications

Food

Carrageenans can be used to gel, thicken, suspend, and stabilize; thus, they are valuable in a number of food applications. Products benefiting from or made possible by their availability include chocolate milk, milk puddings, ice cream, infant formulas, evaporated milk, processed cheeses, water dessert gels, low-calorie jellies, and pet foods--to name a few.

Miscellaneous Consumer Items

Important uses for carrageenans which are neither food nor industrial include binders for toothpaste, bodying agents for shampoos and rinses, ingredients in skin creams and lotions, air-freshener gels, and pharmaceutical suspensions.

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Industrial Uses

The suspending and thixotropic properties of <u>iota</u>-carrageenan make it ideal for abrasive, pigment, and agricultural agent suspensions. Because of their strongly anionic character and protein reactivity, the carrageenans are used as processing aids in the fining of beer and wine and as flocculants for protein recovery.

Biotechnological Uses

Although it is well known that <u>lambda-carrageenan</u> is used to produce rat-paw edema and other inflammatory conditions for screening synthetic and natural product candidates for anti-inflammatory agents, only the <u>kappa-</u>, or rigid gel-forming, carrageenan has found significant use in new biotechnological applications. Adueous solutions of <u>kappa-carrageenan</u> form strong, transparent, thermoreversible gels in the presence of potassium salts.

Living or killed, but enzymatically active, cells of yeast or bacteria, for example, can be encapsulated or immobilized in a beaded cage of <u>kappa</u>-carrageenan by introducing the sodium salt into a solution containing potassium ions. The beads can be used directly or insolubilized by subsequent treatment with glutaraldehyde or polycations and then used for bioconversions. Dr. Ichiro Chibata and his associates at Tanabe Seiyaku Co., Ltd. in Osaka, Japan pioneered this technique and developed several commercial processes based on it, including conversion of glucose to ethanol and production of L-aspartic and L-malic acids. A number of other research groups are looking into the immobilization media potential of carrageenan in additional commercial bioconversions, including some systems using plant and animal cells.

AGARS

Agars are mixtures of polysaccharides extracted from certain red seaweeds, particularly <u>Gracilaria</u>, <u>Gelidium</u>, <u>Pterocladia</u>, <u>Acanthopeltis</u>, and <u>Ceramium</u> genera (see Meer, 1980; Renn, 1984a, 1984b; and Guiseley, 1968). They have achieved commercial importance because of their ability to gel aqueous solutions at low concentrations.

In the form of sweetened and sometimes flavored gels, agars have been used as desserts in the Orient for several centuries. They have been known in the dried state since the mid-1600s when the currently used freeze-thaw process for purifying them was accidentally discovered by an innkeeper in Japan, who found the gel structure of his jellies destroyed by this temperature cycling. Agars were introduced into Europe and America from China in the middle of the nineteenth century as gelatin substitutes for making gelled desserts.

Worldwide production of agars is estimated to be about 5,000 t/yr. Chief producers are Japan, Spain, Taiwan, Korea, Morocco, Chile, Portugal, and the United States. About two-thirds of this production is used in foods and medicines and as a component of dental impression - 23 -

materials. Most of the remainder is used for bacteriological media. Less than 1 percent is used as a raw material for agarose production. Agarobiose, the idealized disaccharide repeating subunit of agar, is composed of 1,3 linked β /-D galactopyranose and 1,4 linked 3,6 anhydro α -L-galactopyranose.

Isolation

Agar-bearing seaweeds are frequently pretreated with hot alkali to improve gel strength of the extract by converting ester sulfates in the 6-position of the L-galactose to 3,6 anhydro groups. The treated weed is extracted with acidic, basic, or neutral hot aqueous solutions, the residue is removed, the filtrate is concentrated and allowed to gel, and the gel is frozen and thawed, pressed and washed, then dried and milled.

Properties

An aqueous agar solution at low concentration ($\leq/2$ percent w/v) will form a firm gel when cooled below about 40°C, and will then retain its gel characteristics until the gel melts at about 85°C. This melting point-gelling point difference is a property known as hysteresis. Gel formation at low concentrations, low reactivity with other compounds, degree of hysteresis, resistance to common microorganism degradation, and ability to retain quantities of moisture are among agar's most valued properties for food and biotechnological applications.

Applications

Food

The major applications for agars continue to be in foods. Although some are still used for jelly-type desserts, ices, and canned meats, fish, and poultry (including pet foods), most are used by bakers and confectioners. Bakers use agars in icings, where they serve as a moisture barrier to prevent drying of unwrapped goods such as doughnuts and sweet rolls and running of icing on goods enclosed in tightly sealed packages. Confectioners use agars to prepare jelly candies and specialty products such as marshmallows and sugared fruit slices.

Miscellaneous Consumer Items

In the pharmaceutical field agars are used as laxatives and in dental impressions and other casting media, as well as for suppositories and carriers for topical medications.

Industrial Uses

No large-scale industrial applications are reported for agars, although they have been used for certain casting procedures requiring high precision.

Biotechnological Uses

In 1882, Dr. Robert Koch formally announced the use of agar as a new solid culture medium for microorganisms following his now-famous experiments on tuberculosis bacteria. Thus, it can be said that agar was first used in a biotechnological application in the 1880s. Amazingly, this the most significant use of nonfood and pharmaceutical agar has not changed in 100 years; it is still the medium of choice for general microbiological growth and identification. With the advent of recombinant DNA and cell fusion techniques, much of the selection, cloning, and propagation of modified bacteria and yeasts are being done on agar.

AGAROSES

Agar has its limitations. Thus, it cannot be used for a number of biotechnological applications because of the presence of varying and ill-defined ionic moieties (see Guiseley and Renn, 1977; Womer, 1982; and Renn, 1984a, 1984b).

The concept that agar is composed of neutral "agarose" and ionic "agaropectin" is an oversimplification which persists throughout much of the current literature. Most of the components of agar do have the agarobiose backbone or a precursor. Although not always present concurrently, sulfate ester, methoxyl, ketal pyruvate, and carboxyl groups can appear on the agarobiose backbone in an almost infinite number of combinations. The conditions used for separation determine in which fraction specific molecules appear. Duckworth and Yaphe (1971), based on their comprehensive chromatographic and enzymatic studies, recommended the following as a practical definition of agarose: "... that mixture of agar molecules with the lowest charge content and, therefore, the greatest gelling ability, fractionated from a whole complex of molecules called agar, all differing in the extent of masking with charged groups."

Isolation

Most commercial processes for isolating agaroses from agars are based on differences in solubilities or chemical reactivity associated with the anionic character of the "agaropectins." The most important of these commercial processes include selective removal of the agaropectins using quaternary ammonium salts or other specific precipitants and fractional precipitation of the agaroses using reagents such as polyethylene glycol.

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The physical and chemical properties of each agarose preparation reflect the seaweed source, including location and stage of growth cycle, agar recovery procedure, and process used to isolate the agarose. To assure lot-to-lot agarose equivalence and thus permit reproducibility for a given assay, a number of characterization parameters have been explored and methods developed for their quantification. The most important of these are gel strength, gelling and melting temperatures, and electrical properties of the gel.

Properties

Agarose is at present the only commercially available, thermoreversible, ion-independent gelling agent. Although an idealized agarose preparation that contains anionic substituents has not yet been reported, some types of agarose are sufficiently devoid of charged residues that they can be said to be essentially neutral and to exhibit virtually no nonspecific protein reactivity. Because agarose gels water at 1.0 percent or less, mechanically stable gels with large relatively uniform pores are easily formed. The exclusion limits of spherical proteins vary with the concentration of agarose in the gel.

Like those of agars, the gelling and melting temperatures of any agarose preparation are not identical as a result of hysteresis. The temperature at which an agarose solution gels under given conditions has been found to be directly related to the methoxyl content, with very few exceptions. Thus, agarose of <u>Gelidium</u> origin, having inherently fewer methoxyl substituents, gels in the range of $34^{\circ}-38^{\circ}$ C, whereas that from <u>Gracilaria</u> gels at $40^{\circ}-52^{\circ}$ C. These measurements were obtained using dynamic conditions, cooling at the rate of 0.5° C/min. Gelation occurs at somewhat higher temperatures when considerably slower cooling rates are used.

Electroendosmosis (EEO) is an important factor where applications involve using agarose gels in an electric field. Although predominantly neutral, the agarose matrix contains some anionic residues--sulfate and pyruvate. Associated with these residues are hydrated counterions. When an electric potential is applied across an agarose gel, the counterions migrate toward the cathode carrying their water of hydration and any neutral sample molecules with them. Thus, there is a net flow of water in the gel toward the cathode, while the fixed anionic groups in the matrix are unable to move. This liquid flow is termed "electroendosmosis," and some important applications depend on this property.

Applications

Almost all reported applications of agaroses can be characterized as biotechnology oriented. They fall into five main categories: electrophoresis, immunology, microorganism culture, chromatography, and immobilized systems technology. Because applications in each category are extensive, this presentation highlights only a few.

Electrophoresis

Agarose gels containing the appropriate buffers provide excellent media for separation of polyelectrolytes, particularly proteins and nucleic acids and their derivatives, by charge or mass, using an electric potential. Separations by charge are based on differential rates of migration of charged particles toward the oppositely charged electrode when an electric potential is applied across the gel. Electrophoretic separation by mass or molecular size depends on the relative ability of particles to migrate through the pores of the gel matrix. The smaller the molecule, the less is the restriction, and, therefore, the faster is the movement. This type of electrophoretic sorting is frequently termed "molecular sieving electrophoresis." Different proteins contain different charge:mass ratios. At times, chemicals such as the detergent sodium dodecyl sulfate (SDS) are used to minimize or completely mask the charge effect. Thus, separation proceeds on size alone.

Since serum components reflect various charge:mass ratios, agarose gel electrophoresis is routinely used in clinical laboratories to identify protein abnormalities, including enzyme variations in serum and plasma as well as in other biological fluids.

Two critical procedures in recombinant DNA or genetic engineering techniques rely on agarose gel electrophoresis: (1) separation and isolation of desired gene DNA fragments, and (2) gene mapping. Because charge densities are essentially equal in DNA and restriction enzyme cleaved fragments, all migrate according to size in electric fields stabilized by agarose. Using 0.1-2.5 percent agarose gels, resolution of about 0.1-900 kilobase pairs is possible. One can separate particles such as phages, viruses, and capsids using even lower concentrations of agarose (0.035 percent). With the use of NuSieveTM agarose, a recently introduced FMC product, separation of gene fragments of 10-1,000 base pairs can be resolved. This product could replace polyacrylamide for this purpose.

Frequently, a scientist wishes to recover separated nucleic acids or fragments from electrophoretic separations. The two low-gelling, low-melting temperature hydroxyethyl agarose derivatives SeaPlaqueTM and SeaPrepTM agaroses (FMC Corporation) offer the flexibility of thermal disruption of the agarose matrix at temperatures below the denaturation point of the polynucleotides.

Cross-linked polyacrylamide gel electrophoresis has been used frequently for molecular sieving of smaller molecules. This material is produced by polymerization in situ and can be difficult to form reproducibly. In contrast to agarose gels, it is also difficult to dry a run film one wishes to save without cracking it. A recent discovery was that a nongelling olefinic agarose derivative, known as AcrylAideTM cross-linker, can be substituted for N,N'-methylenebis-acrylamide to cross-link polyacrylamide gels while maintaining a resolving capacity. These gels can be readily oven dried to a coherent, undistorted film even at 15 percent acrylamide-1 percent AcrylAide cross-linker concentrations, particularly on FMC's - 27 -

GelBondTM PAG film. This simplifies both autoradiographic and fluorographic procedures for identifying gene products.

A special form of electrophoresis known as isoelectric focusing (IEF) takes advantage of the varying isoelectric points of amphoteric biopolymers. Done traditionally on polyacrylamide gels, it has now been adapted to specially designed, negligibly charged agarose media. FMC's Marine Colloids Division produces one such product, IsoGelTM agarose, which is a mixture of a very low EEO agarose and a galactomannan which further suppresses charge. Pharmacia has designed a derivatized IEF agarose which has balanced anodal and cathodal EEO and can therefore be used for isoelectric focusing.

Recently, the use of agarose for two-dimensional electrophoresis has been reported, utilizing IsoGel agarose for isoelectric focusing in the first dimension and a SeaPlaque, SeaPrep agarose gradient in the second. This development should allow examination of mixtures of large molecules which are totally excluded by polyacrylamide gels.

Immunology

Applications of agarose in immunology for the detection and study of various antigenic materials, particularly disease indicators, and their specific antibodies are so numerous that this presentation can barely scratch the surface. "Antigens" are any substance not recognized as "self" that give rise to an immunological response. "Antibodies" (immunoglobulins) are specific proteins formed by specialized animal cells. They are synthesized in response to an antigenic stimulus and will combine specifically with that antigen to neutralize its threat to the animal. Many of these antigen-antibody complexes are insoluble; thus, if an antigen and the antibody specific to it diffuse separately through an agarose gel, the position where they come together will be marked by a cloudy or white so-called precipitin band marker. Because of the macroporosity of the agarose gel matrix (antibodies are large molecules), its relative chemical neutrality, and its high clarity, agarose is an ideal medium for immunological reactions. A number of immunological techniques, all of which employ agarose and rely on visualized antigen-antibody interactions, have been developed. These include gel diffusion, radial immunodiffusion, immunoelectrophoresis, electroimmunodiffusion, and counterelectrophoresis.

Some of the immunobiological assays, such as plaquing to detect single cell antibody producers, hemolysis-in-gel, and migration inhibition factor assays, have benefited from development of low-gelling, low-melting temperature agarose products in which cells can be incorporated at temperatures at which they remain viable. Furthermore, agarose particles, to which antigens, antibodies, enzymes, or enzyme substrates are attached, have enabled highly sensitive specific molecule or microorganism assays to be developed.

Microorganism and Cell Culture

Agar has long been the standard medium for microorganism and cell culture. However, even the bacteriological-grade agars vary considerably from lot to lot and contain varying proportions of unknown entities, some of which are reported to be toxic to microorganisms and plant and animal cells. This leads to slower or no growth of sensitive cells and microorganisms. Agarose, because of its higher degree of purity and consistency, is being used increasingly by scientists for critical cultures. Antibody-producing hybridomas, after formation by fusion, have been found to reproduce more consistently on agaroses.

One of the problems with agar, including standard agarose preparations, is the relatively high gelling temperature. With the availability of lower gelling- and melting-temperature hydroxyethyl agarose derivatives, such as SeaPlaque and SeaPrep agaroses, cells and other heat-labile substances can be incorporated into gelling media at considerably lower temperatures than before. In addition, these hydroxyethyl agarose derivatives seem to encourage cell growth.

Chromatography

Columns of beaded agarose get particles, sold under such trademarks as Sepharose (Pharmacia) and Bio-Gel A (Bio-Rad Laboratories), serve as media for molecular-size separations because of the uniform effective pore size related to a particular concentration of agarose in the gel. Agarose is the preferred chromatographic medium for separations of molecules greater than 250,000 daltons where minimal nonspecific binding to the medium and retention of biological activity of the molecules being separated are important. Affinity chromatography is a rapidly growing extension of agarose gel chromatography. In this technique, an antigen, antibody, enzyme, co-enzyme, or substrate is bound physically or chemically to the agarose gel particle. These bound ligands interact specifically with molecules having a particular physical or chemical conformation, and thus remove them selectively from complex mixtures containing them. The specific molecules can subsequently be eluted by changing the composition, pH, or ionic strength of the eluant, thus effecting purification in a single step with high yields.

Agarose may also be derivatized so that functional hydrophobic group ligands are available. Work by a number of investigators in the field of agarose-based hydrophobic chromatography indicates promise for this versatile technique.

More restricted pore media for chromatography have been developed by combining agarose with polyacrylamide. Such products are available commercially under the trademarks Ultrogel (LKB Instruments) and Sephacryl (Pharmacia).

Finally, a number of ionic agarose derivatives useful for chromatography have been developed. These and the aforementioned media have found numerous applications in the field of biotechnology, assuring a strong future for agarose beads and granules and their derivatives in separation and purification science.

Immobilized Systems

Agarose-immobilized cells and enzymes are important bioconverters. There have been many reports in the literature regarding the use of agarose gel films, particles, or beads to attach enzymes or encage cells and subsequently utilizing them as bioconverters to transform one chemical to another. The interested investigator is urged to consult the literature for further specific information on this technique.

One specific application recently described, which may have interesting consequences, is the use of low-gelling temperature SeaPrep agarose to encapsulate insulin-producing pancreatic Islet of Langerhan cells for transplantation.

Finally, the use of agarose to encapsulate activated charcoal and ion-exchange resins in agarose beads for hemoperfusion to detoxify individuals suffering from a drug overdose looks promising.

Many other as yet minor niches in the area of biotechnology have been found for agarose, but space does not permit describing these. New biotechnological uses are continually being discovered for agarose and its derivatives.

CONCLUSION

The unique properties associated with algins, carrageenans, agars, and agaroses--polysaccharides from seaweeds or marine algae--have been the foundation of a number of successful commercial ventures, ranging from seaweed cultivation, harvesting, and processing to specialty applications. Although biotechnology does not at present require large volumes of these algal polysaccharides, they have enabled many pioneering advances to be made in this rapidly evolving field.

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BIOTECHNOLOGICAL AND ECONOMIC APPROACHES TO INDUSTRIAL DEVELOPMENT BASED ON MARINE ALGAE IN INDONESIA

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INTRODUCTION

High-technology industrial developments based on marine algae (seaweed) show promise for Indonesia, a country with a long record of exporting marine algae. This paper addresses the biotechnological and economic aspects of producing seaweed as the raw material for such industrialization.

Seaweed production is the practical side of seaweed physiological ecology. Very little reliable information is available on the latter such as would be obtained from field experimentation. Yet, as a result of a growing world population and changing material desires, the market demands for substances such as the algal hydrocolloids are expanding.

Farming of seaweeds has become a necessity in Indonesia as the wild crops are neither adequate in volume and reliability nor competitive in cost. Commercial interests in Indonesia have begun to farm one type of seaweed, the red alga <u>Eucheuma</u>, but its market development has paused near the 600 t/yr level, apparently because of the quality of the product.

All seaweed-producing countries need technical information to perfect their seaweed production in relation to their national objectives. This information is beginning to appear for temperate-zone seaweed. Yet adequate traditional or scientifically reliable biotechnological information from which to draw agronomic or management guidance for the quite different tropical seaweeds and their widely varying tropical environments is not available. Fortunately, attainment of the current \$8-10 million annual world export value returned for farming Eucheuma has provided some experience that may be useful in determining the causes behind the Indonesian market situation and obtaining relief from it. Relief would mean access to the growing world market.

The carrageenan stored in seaweed competes with other hydrocolloids on the market beyond its special value to the dairy industry. To receive an acceptable return on its capital investment, no company producing this gel can afford to pay much more than about \$1.85/kg plus shipping for carrageenan as it is received in seaweed form at the extraction plant.

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In addition, the seaweed must be, on average, <u>standard</u> in both carrageenan yield and quality. In this presentation, the striking difference between the carrageenans derived from the two commercial species of <u>Eucheuma</u> known in the trade as "spinosum" and "cottonii" is disregarded for the sake of simplicity. Similarly, quality standardization will be applied only to gel strength. In reality, there are other qualities to be considered, and usages of these gels usually require that they be chemically modified to fit the final product's requirements.

Extracting companies sell special blends of materials. At present, the seaweed produced by farms in Indonesia is of "low quality" based on indexing of value. A figure for indexing may be obtained for a given lot of exported Eucheuma by linear factoring, an example of which is shown in Table 1. In this table, five factors are taken into account. Their arithmetic product multiplied by 100 yields an evaluation (Col. 6) for extraction of a low grade seaweed in comparison with a standard grade seaweed. While the individual percentage values given in Table 1 are characteristic, they refer to no particular lot or source of seaweed. To make a final product of higher quality (e.g., standard gel strength), a company would have to either blend the low-grade carrageenan with stock having unusually high gel strength or add more carrageenan. Either procedure would be more costly than buying seaweed with standard carrageenan in it.

Seaweed Quality	Dry Weight (1)	CAY (2)	Gel Strength (3)	Ship- ping (4)	Pro- cessing (5)	Value Index (6)
Standard	0.70	0.40	1.00	1.00	1.00	280
Low grade	0.60	0.30	0.60	1.00	1.10	119

TABLE 1 The Value Indexes of Standard and Low-Grade Seaweed

Note: Dry weight = ratio of anhydrous dry weight to weight recived; CAY = clean anhydrous carrageenan yield. Cols. (1)-(3) are standard terms to which a cost of processing factor (5) is added. The value 1.00 is 100 percent of the standard quality.

A company using <u>Eucheuma</u> only from the present Indonesian farms could soon be bankrupt from buying and processing, as per Table 1, over twice (280/119) as much low value-index seaweed as a company using the standard quality. Thus, expanding farm production of seaweed with a low value index--that is, substandard or low-quality seaweed--will hardly be encouraged by its market. A decision to ignore this situation or to encourage the farming without first determining whether or not quality and yield can be improved to standard would prove to be akin to premature investment.

Fortunately, the farming of <u>Eucheuma</u> in Indonesia has a bright side, even though the seaweed now being produced is low grade. The introduction of seaweed farming and its spread have provided a significantly high-volume process that is working successfully, despite the possible sacrifice of some quality and yield. Furthermore, seaweed farming has been spread over a desirable range of ecological situations. These features and the participation of government have created an excellent opportunity to carry out an experimental program leading to control of product quality and improvement of product value from industry's point of view. With success, the market is certain to expand desirably and quickly.

An appropriate experimental program used for these purposes by production engineers is known as the Experimental Development of Process (EDOP). The EDOP method is used to obtain a rational change in a working method that has been developed to operational success without the input of the philosophical niceties of the scientific approach. It is also used in cases, such as the farming of <u>Eucheuma</u> in Indonesia, where management is attempting to obtain "control" of a process to increase profitability or eliminate problems. EDOP must be applied at an appropriately higher operational level than that at the introduction of the process.

The experimental development program suggested below is designed to cope with Indonesia's specific <u>Eucheuma</u> seaweed industrialization problems, but generally it would be appropriate in any other country seeking seaweed industrialization. Such a program can hardly be undertaken, however, unless the requisite biological, hydrocolloid, environmental, and statistical techniques are available and rigorously applied operationally. Unfortunately, it seems at present that the appropriate combination of personnel and facilities for such a wide scale project are not available in Southeast Asia. Indonesia could well develop such a combination.

In view of the bright future for new seaweed processes and products, Indonesia's sights should well be set beyond solving just the problems concerned with the current opportunities in <u>Eucheuma</u> and carrageenan or <u>Gracilaria</u> and agar. At the same time, Indonesia cannot afford to risk awaiting the development of adequate facilities and personnel before coping with the immediate needs for higher-quality farmed <u>Eucheuma</u>. To do so would risk in turn delaying or eliminating Indonesia's opportunity to take advantage of a nearly ideal situation for using <u>Eucheuma</u> as a stepping-stone to industrialization of its seaweed industry. While solving current problems is essential, in the long run establishment of an ongoing academic and technical university-level training program in marine agronomy may be even more important.

Indonesia could attain these objectives most efficiently by employing a combination of foreign expertise and training opportunities. Such a step could deal with the present problem and, at the same time, provide training so that Indonesians can cope with future problems. It would also leave Indonesian institutions of higher learning equipped to produce future generations of phycological scientists. Such a step would be desirable in any geographic area. In Indonesia, however, it is critical to attaining seaweed industrialization goals that the process be initiated now.

Because seaweed is one of Indonesia's significant readily exploitable but renewable national resources, it is hoped that Indonesians will choose to obtain such foreign assistance, acquire the appropriate laboratory facilities, create the necessary job positions, and, obtaining the requisite personnel, provide the incentives that will yield the desired biotechnological and industrial development goals. In reaching for these goals, it is recommended that specific efforts be directed toward the areas of agronomics, genetics, and economics, which are described in the following sections.

AGRONOMIC PRACTICES

Site Planting and Harvesting Routines

As observed on Indonesian farms, planting and harvesting routines may be faulty in a number of respects aside from mere inefficiency. Use of the EDOP approach should, with thought, result in an improvement in quality of farmed Eucheuma. Thus, this work is among the most important of the immediate objectives.

The strategy and logistics are routine. Little is required beyond obtaining expertise and a group of collaborators, such as the farmers at Nusa Dua or Nusa Lembongan, and almost immediate laboratory processing of gel, micronutrient, and environmental samples. It is anticipated that the added value to be gained would more than offset any costs to farmers.

A major problem is that the work seems so simple that there is often unjustified confidence in a yet unproven "in-house" capability. Perhaps this accounts for the current lack of useful technical information on the farming of both <u>Eucheuma</u> and <u>Gracilaria</u> and the resulting problems such as the low grade of Indonesian-farmed seaweed.

Use of the phrase "almost immediate" when referring to sample and data analysis merely means available analytic and statistical services of suitably high quality. Samples and data from the field must be given immediate and reliable attention, and quality control must be exercised. This is necessary not only to ensure reliable data; it is also needed to provide the project's interpreters with results quickly so that their interpretations can be used expeditiously in devising and implementing changes or extending or terminating the particular aspect of the work yielding the data.

As for staffing the EDOP field and analytic work as well as the overall direction, part-time students and local farmers can be employed to provide much of the labor for the experimentation. Farmers familiar with the EDOP approach and an operations manager must be hired as well. Field operations costs will be small, but travel, communication, sample-gathering, and analytic costs will be about 50 percent of the - 35 -

total budget. The presence in the field of farmers familiar with EDOP and experienced in experimental seaweed farming is essential and not costly. They will stay at the farms to inspect and adjust the work each day. The operations manager will travel and will be reponsible for measurement, sampling, sample preservation and delivery, analytic follow-through, and record keeping, as well as the professional EDOP design, project direction, data interpretation, liaison between the funding source and the project, and accountability as the project director.

Eucheuma productivity and harvest quality are, apparently, sensitive to many environmental factors. These fall into four major categories: light, temperature, water quality, and water motion. EDOP efforts to develop agronomic methods for improving farmed seaweed quality may be greatly reduced by concentrating initially on seasonal or other independent major variations in each of these categories.

The nature of the bottom materials is important, as is therefore the distance of the thalli from the bottom. Because the thalli are also sensitive to aerial exposure, the elevation at which they are planted with respect to the mean tidal level is important as well.

If the maximum size to which the thalli are allowed to grow before harvesting is held constant and the amount harvested per period is increased, the annual yield is decreased. On the other hand, by decreasing the amount harvested at each harvest period, the annual yield is increased. For example, if 4 grams are harvested from every thallus when it reaches 512, grams the yield would be 1.38 times that obtained when 256 grams are harvested each time. This extreme would, however, require harvesting the thalli three times a day! Increasing the maximum thallus weight from 512 to 2000 grams and harvesting half when the maximum weight is reached would increase the annual yield 3.91 times. Numerous other seemingly small matters that cause significant variations in productivity have been recognized, but very few have been appraised in useful detail. It is not suggested, however, that this project undertake an encyclopedic effort to do so.

A series of EDOP runs should be made for each major farming site and water quality area to determine better combinations of conditions and practices that would lead to obtaining the desired product qualities. Because there is considerable compensation among the four categories of environmental factors just listed, the phrase "better combinations" is used above. Ideally, such sites would be selected before the actual farming begins as this would prevent disappointment were the farming discontinued because control of product quality was not obtained.

Hydrocolloid Gel Quality Control

Different kinds of field treatments that include replication should be undertaken at different water levels to improve understanding of gel qualities and gel strength. Replication is necessary because <u>Eucheuma</u> is a large thallus, and the tendency is to have one thallus per level in each treatment. This is particularly critical as genetically identical clones are not always employed.

The gel toughness of <u>Eucheuma</u> thalli can be likened somewhat to young and old carrots. The gels from young thallus parts that are growing rapidly are low in gel strength and differ in other characteristics from what is found in old or slow-growing parts. Thus, it is not surprising that in some cases the harvesting of old bases rather than young tips provides better gel qualities by at least 10 percent. It is not quite that simple, however. The role of carrageenan precursors and their enzymatic control are quite possibly involved, especially in relation to the role of sulfate radicals.

For Eucheuma it is generally believed that there is a correlation between high growth rate and low gel strength. This should be treated as an hypothesis for testing in the project described here. In the discussion of strain selection in the following section, the disappearance of what were thought to be strains having higher growth rates is said to be possibly related to differences in water quality at different sites. This explanation would not be applicable here, however, since high growth rates and low gel values have been noted for the two years that farming has been under way.

Neishizing to Produce More Nearly Standard Eucheuma

Neishizing is a process in which thalli are physiologically altered by growing them in bright light in water low in inorganic nutrients. Originally applied to Chondrus, this process has not been applied to Eucheuma, but it is well worth trying. It should result in significantly increased gel strength and yield at the expense of growth, and thus the production of significantly improved seaweed.

The possible value of this process as a postharvest treatment needs to be explored with respect to its effects on <u>Eucheuma</u> and the costs of obtaining them. A relatively short-term experimental series would produce the materials for laboratory testing, and a cost analysis would provide the necessary management information.

Fertilization and Ion Balance

Fertilization and ion balance phenomena are anticipated, but very little experimentation on this has been done to date. It will be necessary to determine the levels of various individual inorganic nutrients and the balances between them. Both have been found to be important in terrestrial field and vegetable crop production.

The effects of growth factors and inhibitors are unknown. Application of several of them may have the effect of slowing <u>Eucheuma</u> growth in the field or increasing seaweed quality. As in all agronomic work, the costs and returns of whatever effects are obtained from such experimentation must be appraised.

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Postharvest Techniques

In addition to Neishizing, the other postharvest techniques should be investigated; they are ideal for the EDOP methodology. Such an investigation might well include the introduction of practices to halt the apparent depolymerase or acid hydrolytic actions that lead to lower gel quality. Practices that remove 6-sulfate from the galactose moieties and induce anhydro-linkages could be expected to improve gel quality. Treating harvested <u>Eucheuma</u> with toxic materials that evaporate or degrade quickly in air or seawater could reduce microbiological degradation.

A brief extraction at a high pH level is one way to obtain added value from poor <u>Eucheuma</u> when high gel strength is desirable in its hydrocolloid. Dusting with calcium hydroxide might raise the pH enough to remove significant sulfate.

GENETICS AND GENETIC BIOTECHNOLOGY

Local and Imported Species Comparisons

Available noncommercial species having desirable hydrocolloids should be compared with those now being produced. Industry has traditionally used two species from Indonesia and elsewhere in Southeast Asia: <u>Eucheuma denticulatum</u> and <u>E. alvarezii</u>, prized, respectively, for their <u>iota</u> and <u>kappa</u>-carrageenans. Traditionally, the trade has designated <u>Eucheuma</u> that produces <u>iota</u>-carrageenan as "spinosum" and <u>Eucheuma</u> that produces <u>kappa</u>-carrageenan as "cottonii." It is incorrect to use the trade names as binomial scientific names. For example <u>Eucheuma</u> cottonii is a quite different species which has never been farmed commercially and is not normally seen in commercial shipments. The differences between the scientific names for these seaweeds and trade names causes no trouble if the generic name is not associated with the latter as a binomial.

A number of <u>iota</u>- and <u>kappa</u>-carrageenan-producing species of <u>Eucheuma</u> are not being farmed at present. Some of these are available in the Lombok and Bali straits. These species should be compared as to the suitability of their gels for the market and to their suitability for farming. The effects of the present and other agronomic practices on their gel content and quality should also be appraised. These comparisons, although important ultimately, can be undertaken while growth is taking place in experiments that are more immediately critical.

Selection of Strains of Present Species Using EDOP Methods

The relative desirability of the spinosum and cottonii strains presently found in Indonesia for Indonesian farming conditions should be determined using EDOP procedures. This is an ideal project for participation by outer island institutions or student-trainees, although centralized, qualified direction should be maintained. Apparently, the initial seed stock of the spinosum and cottonii now being farmed in the Lombok and Bali straits areas was selected in the Philippines and brought to Indonesia about two years ago. The Indonesian strains of the same species have provided this country with exports from its wild crops for at least a century. In general, this production has been of good quality except for problems unrelated to the genomes involved. Thus, the testing of Indonesian strains using EDOP procedures could result in local stocks that produce better-quality gel under Indonesian farming conditions. This entails caution, however. The areas now being farmed may not have been known previously as significant Eucheuma-producing areas.

The strains isolated and touted by local farmers usually prove to be either a color variation or fast growing and are soon determined not to be better than the standard stock. The color variations are most often olivaceous brown, green, or pink, and they appear to be genetically stable. Although there is no experimental evidence to support the idea, they are generally thought to follow the same genetic control as color variations in other red algae genera such as <u>Chondrus</u>, Laurencia, and Gracilaria.

Fast-growing strains are usually discovered when wild seed stock is brought from sites remote from the farm where their fast growth was noted. With few exceptions, however, their growth rates (or other peculiarities) dwindle to those of the spinosum or cottonii already growing on the farm, and nothing more is heard of them. It is as though they had accumulated at their original site all the essential materials but one. Perhaps the lacking ingredient was an essential growth factor or a mineral nutrient which, while scarce at the old site, is present in abundance at the new site. The new site, however, may be deficient in yet another essential substance. Thus, a new strain at a new site has a surplus of some material that was in abundance at its original site but which is in short supply at the new site. It will therefore grow spectacularly at the new site for the first few weeks, after which it will grow at the same rate as the strains present earlier.

The fast growth rates in the Lombok and Bali straits are not thought to result from the "fast grower" phenomenon just described, since these rates have been sustained for about two years.

Other Genera and Other Gels

It is highly recommended that <u>Gracilaria</u> species or strains be sought and introduced. Indonesia can then enter the international market and sell agar-producing seaweeds of qualities that are in short supply. To attempt to enter the market and sell food-grade agar would be akin to entering a saturated market. A search for sugar-reactive agars is particularly recommended.

Spore Selection Methods

Spore selection methods have never been studied seriously or developed for either <u>Eucheuma</u> or <u>Gracilaria</u>. In fact, spore growth has not been studied beyond the stages of release, setting, and the first beginnings of disc-like formation. A great deal more is known about <u>Gracilaria</u> as it is common in temperate waters. It grows well in low-water motion conditions, and, being small, the outplanted material is much more apt to "set" artificially on lines or gravel.

Adult Eucheuma can be transplanted widely with, at least in the case of cottonii, little mortality. For <u>Gracilaria</u>, this does not seem to be the case; adult transplants often die. Although almost all viable spores observed in a lot may settle and produce peg-like upward growths that seem vigorous and persist well in the field, the peg-like stages are dormant in most cases. Some dormant stages have been observed to remain healthy for over a year when outplanted to a site other than that from which the spore mother thalli came. When there is success, it is likely to affect only a few sporelings. These few shoot up, assume the mature form for the species in that habitat, and become fertile. At present, this phenomenon is interpreted as being attributable to genetic variability in the spores, with only some genomes suitable for the new outplanting sites.

It is suggested that this approach (through the spores) to obtain new strains could be valuable and should be undertaken immediately, but on a second-priority basis.

Breeding New Genetic Combinations

The breeding of new genetic combinations, as carried out very successfully in Gracilaria, should be introduced into Eucheuma biotechnology. Simply stated, nonfertile tips of female thalli are grown isolated from other thalli until one is convinced that no fertilized carpogonium (fertilized female gamete) is present. Suspected male thalli are likewise cultivated separately. (One might well cultivate the male and female thall1 from the Eucheuma color varieties mentioned above to establish the routines.) A cross is made by merely transferring spermatium-bearing water from the culture of the selected male thallus to the water of the selected female thallus, and agitating the latter. If viable spermatia are then in the water and the female thallus is fertile, the diploid spore-producing structures, the cystocarps, should begin to appear in a few days on the female thallus. The spores from any one such cystocarp are theoretically all genetically identical and diploid. Potentially, these diploid spores would each represent the first cell in the diploid part of the life cycle, the gamete-producing male and female thalli being haploid. The diploid thalli produce a great many spore mother cells, each of which, accompanied by meiotic mitosis, produces four haploid spores which will differ genetically. Theoretically, random segregation could mean that each of the myriad of tetraspores produced is genetically distinct.

Thus, even though they have the ability to grow thalli sufficiently tall to protrude through the boundary layers covering the substratum to which they have attached, only the few tetraspores that eventually produce mature sexual thalli are genetically suited to the new environment. In this way, for a given environment a new strain will have been created by genetic manipulation--a demonstration of the survival of the fittest.

Genetic Biotechnological Engineering

A capability in genetic biotechnological engineering is an important objective for Indonesia's ultimate commercial algae development. This objective would, initially, entail seeking control of the life cycle of as many organisms as feasible through experiments such as the one described above. Subsequently, work would be undertaken on freeing cytoplasts from their cell walls, something little done with red algae to date. Fusions of haploid cytoplasts (artificial fertilization) or "renucleation" of enucleated cytoplasts might be the first advanced cytoengineering techniques to be developed. Use of these techniques would depend on demand at the time they become available.

If such techniques were available now, genetic manipulation to obtain low-sulfate and sugar-reactive agars would be appropriate subjects as well as production of seaweed with desirable farming qualities for each of the carrageenans. (Please note that it is suspected that some of the iota-producing Eucheuma species which are not now farmed may be desirable genetically for their extractability and iota-carrageenan quality. They may be difficult to farm, however, as they require high-energy environments or deep water.) Genetically engineered hybrids may be possible where conventional hybridization proves impossible.

The initial steps described above might well be implemented soon. They require establishment of a shoreside laboratory with unlimited access to "good" seawater in which both <u>Eucheuma</u> and <u>Gracilaria</u> grow well. This laboratory should be designed to encourage the scientific productivity of perhaps three professionals whose results would cover other than the seaweed now available commercially. For example, work is needed on the drug-producing seaweeds found in Indonesia such as Chondria and its relatives.

The requirement for large quantities of seawater and access to wild stocks of seaweed dictate a shoreside site for this work. Convenience to cities and to areas with significant runoff from agricultural developments will result in using water that is rarely of suitable quality.

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Personnel

The above descriptions of six topics suggested for biotechnological research almost totally lack mention of Indonesian personnel. Once facilities are established, however, opportunities and part-time work in developing a methodology would utilize students. Those who excell will be ideal candidates for advanced training.

The initial facilities could be relatively crude when undertaking the first steps in gene engineering as well as the biotechnological work on obtaining control of the life cycles of the organisms. These steps could commence soon, with the final genetic engineering activities being undertaken after establishment of appropriately equipped facilities.

ECONOMIC PRACTICES TO ENCOURAGE BETTER-QUALITY FARMED SEAWEED

Although economic practices to encourage better-quality farmed seaweed are favored by industry, competition usually results in irregular to formless execution of any uniform policy. Consolidator/ exporter practices, such as those indicated below, could be keys to obtaining better-quality seaweed.

Bonuses or higher prices paid by industry for value-added or higher-quality seaweed would provide farmers and consolidators with the incentive to adopt the farming of different seaweeds or agronomic practices resulting from the EDOP experimentation suggested above. There are problems, however, with determining seaweed quality at the time of shipping and paying by letters of credit. Perhaps partial payment could be received when the seaweed is shipped (letter of credit) and final payment received after receipt and assay of the product at the company's extraction plant (sight draft).

Consolidators/exporters could likewise pay higher prices for better material. Again, the problem of objectively determining the quality of the seaweed at the time of purchase makes this difficult. Any desired postharvest treatment of this product can, as a rule, be readily determined by, for example, its color, feel, and general appearance.

<u>On-site post-purchase treatment</u> could also result in better-quality seaweed. After seaweed is purchased from farmers it could be treated on platforms or on barges provided for the purpose at buying stations in the farm areas. Such practices have been implemented successfully at a few locations in the Philippines, where industry has established buying stations in the field with large platforms for washing, treating, and drying the seaweed after its purchase.

Investigation of better <u>drying practices</u> would, likewise, result in better-quality seaweed. Drying seaweed directly on sand or dirt means adding trash (and weight) which reduces the percentage yield. Slow drying or washing by rain (or otherwise) can reduce the quality.

Consolidators, and later the processing industry, could enforce standards for dryness and trash. Eucheuma standards usually call for no more than 30 percent water and 5 percent trash. For <u>Gracilaria</u>, the dryness standard is usually no more than 18 percent water and 5 percent trash.

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<u>Basal diameter standards</u> requiring that a stated percentage of the thalli have basal diameters of over a given size when offered for sale could be set if the EDOP study shows that they would result in higher gel strength in the seaweed shipped. To ensure consolidator compliance with standards other than water and trash, industry could buy standard <u>Eucheuma</u> at a base price and guarantee a bonus based on yield and quality. The base price would necessarily be below the present world price. The bonuses paid would be determined by sample analysis, perhaps only after the seaweed has reached the extraction plant warehouses.

Ensuring a fair partitioning of the export price could contribute to the production of better-quality seaweed. The extracting industry could maintain a skeleton buying organization in each farm area and pay a support price of, say, 70 percent of the export price for the desired quality of seaweed. These support prices would be posted in the field and the company buyers rigorously policed. Paying consolidators the bonus described above would encourage them to treat seaweed in such a way that value would be added.

CONCLUSIONS

With execution of the above suggestions the quality of Indonesian-farmed <u>Eucheuma</u> could be increased from low to standard. A much larger market for a higher grade of seaweed can be expected. The higher annual dollar returns would probably be considerably in excess of the total cost of the EDOP program required to develop and introduce the necessary agronomic methods.

The proximity of the exporting port of Surabuya to seaweed farming means that a middleman first purchaser can easily deliver the product to representatives of the extracting companies. They would then export the product and at the same time control or adjust the quality of the seaweed shipped. If a local exporter is not required, only one middleman level need exist and as much as 75 percent of the export value could go to the farmers at first sale. To the few thousand farmers involved, the socioeconomic benefits would be immense. In the traditional situation the producers often receive 10-40 percent of the export value at first sale leaving many farmers near the poverty level.

Farmers contentment with the returns of farming should introduce a positive stability factor into the raw material supply. This in turn would auger well for a successful national extraction industry. Thus, <u>Eucheuma</u> farming is a rare opportunity for the government to facilitate a break with tradition by favorable support, regulation, taxes, and other actions, thereby promoting both significant socioeconomic advancement and stability in the raw material base for the anticipated industrialization to follow.

In all the projects recommended the costs and returns in terms of socioeconomic value and industrial profitability should be appraised. The availability of this information could provide encouragement for industrialization. Implementation of the more elementary of the recommended projects is an opportunity to plan and initiate the - 43 -

development of personnel and facilities also needed by other industries based on seaweed and various other renewable marine resources.

The approach recommended of utilizing expertise from abroad in a combination work/training project associated with an Indonesian university, preferably located in a seaweed-producing area, could be the most economiclly way of quickly obtaining the higher-grade export seaweed desired. This same approach could, as well, provide Indonesia with training personnel and institutions equipped to cope with the personnel needs and biotechnological problems of seaweed industrialization in the future.

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SCREENING SPECIES OF BROWN AND RED ALGAE COLLECTED FROM THE COAST OF SPERMONDE FOR ACTIVE SUBSTANCES

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INTRODUCTION

Some natural resources have long been a cheap source of raw materials for the manufacture of drugs, dyes, food additives, and other items. In the temperate and subtropical countries, active substances have been found in various kinds of marine algae, and several have been produced commercially. It is hoped that this production will also take place in Indonesia, given its climate and environment. Thus far, no marine chemical industry exists in Indonesia, and little information on the chemical constituents of marine algae grown in Indonesia has been published. For this reason, a series of studies on this topic has been initiated, and several species of marine algae from the coastal area of Spermonde are being screened for active substances.

MATERIALS AND METHODS

The algae studied were collected along the coasts of 10 islands of Spermonde from a depth of between 1 and 5 meters. Collection took place from July to September 1984.

The fresh algae were washed thoroughly with tap water several times to remove some of the salt and epiphytes, and then dried in an oven at 60-70°C. The water content of the dried sample had to be lower than 5 percent. The sample was then ground to a fine powder at about 100 mesh.

A screening procedure consisting of stepwise hot extraction of dried tissue using a Soxhlet extractor has been developed. During the first step of the procedure, the sample was continuously extracted using a relatively nonpolar solvent, and in the second step the residual tissue was extracted using a more polar solvent. The first extract contained lipids, free steroids, color substances, and other soluble components. The second extract contained carboxylic and sulfated carbohydrates, alcohols, glycosides, and other soluble components.

Qualitative analyses of the steroid content were carried out on the crude extract, the hydrolysate, and the crystallization products by means of thin-layer chromatography and color reaction. The total

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steroid content of the plants was estimated using the unsaporifiable fraction of the total crude extract and a spectrophotometric method based on the Liebermann-Burchard reaction. Free and ester steroids were separated from the crude extract by means of silica gel column chromatography.

Carboxylic and sulfated carbohydrates were separated from the second extract by adding sodium hydroxide or sodium carbonate solutions. The two components crystallize after being cooled for four hours. The crystals were filtered and washed with alcohol, and then dissolved in a sodium carbonate solution. The clear solution was subjected to column chromatography to separate the carboxylic and sulfated carbohydrates.

Agar components were precipitated by adding 2N HCL to the filtrate of the second extraction. After washing the precipitate, it was dried and weighed as agar. Alcoholic substances remaining in the solution were analyzed by means of acetylation and crystallization and weighed.

RESULTS

All of the algae studied contained free, ester, and glycosidic steroids, but the quantity varied according to species and the location of the sample collected (see Tables 1-3). Four species had steroid contents that ranged from 0.1 to 0.7 percent. Glycosidic steroids were found in six species in quantities ranging from 0.01 to 0.1 percent. Thus far, the highest ester steroid content found is about 0.09 percent.

Only two species of the algae studied had a sufficently high yield and viscosity in sodium salts of carboxylic carbohydrate. The average content of carboxylic carbohydrate was higher in red algae than in brown algae. Sodium salt of sulfated carbohydrate was found in all red algae studied. Two species contained sodium salt of sulfated carbohydrate in quantities greater than 30 percent (based on dried plants). Brown algae contained a relatively small amount of these salts. A high yield (10-40 percent) of agar was found in all red algae studied, but only a small amount was found in brown algae. Alcoholic components or their acetic derivatives (less than 0.1 percent) were also found in the brown algae.

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	Steroids (%)		Carboxylicteroids (%)Carbohydrate			Sul. Carbo-		
Specie s	Free	Ester	Gly.	Yld.(%)	Vis. (Cps.)	hydrate (%)	Agar (%)	Alc. (%)
L	0.7	0.08	0.03	27	2,510	3	10	0.10
2	0.8	0.09	0.07	29	2,516	1	3	0.08
3	0.3	0.07	0.05	20	760	2	1	0.07
4	0.1	0.03	0.02	15	600	3	2	0.06
5	0.7	0.07	0.06	4	380	32	15	0.03
6	0.6	0.06	0.06	4	150	35	27	0.02
7	0.6	0.05	0.06	7	200	25	25	0.02
8	0.5	0.04	0.06	6	175	20	30	0.01
9	0.2	0.02	0.02	5	200	15	12	0.01
10	0.1	0.05	0.03	3	300	10	12	0.01
1. <u>Sar</u> ;	gassum	ailiquos	յսո		6.	G. eucher	umoid es	
2. Sar	gassum	spp.			7.	Eucheuma	spp.	
	binaria				8.	Gelidium		
	ina spp				9.	Acanthop	nora spe	ecifer
5. Gra	cilaria	crasa			10.	Galauxau		

TABLE 1	Results of	Analyses	of Algae	Species	Collected	in the	Lae-Lae
	and Gusung	Islands					

TABLE 2 Results of Analyses of Algae Species Collected in the Samalona Islands

	Steroids (%)			C arboxylic C arb ohydrate		Sul. Carbo-		
Species	Free	Ester	Gly.	Yld.(Vis. %)(Cps.)	hyd ra te (%)	Agar (%)	Alc. (%)
1	0.6	0.05		30	1,670	5	4	
2	0.7	0.06		29	2,050	3	5	
3	0.4	0.04	0.02	15	1,000	4	3	
4	0.3	0.02	0.02	22	600	1	1	0.03
5	0.4	0.03	0.03	3	550	33	20	0.02
6	0.5	0.02	0.05	4	300	40	30	0.02
7	0.7	0.07	0.02	4	200	27	30	0.02
8	0.6	0.04	0.02	7	175	25	27	0.03
9	0.1	0.05	0.03	5	150	20	15	0.02
10	0.1	0.05	0.04	4	250	11	13	

	Steroi	.d s (%)			xylic hydrate	Sul. C arb o-		
Species	Free	Ester	Gly.	Yld.(Vis. %)(Cps.)	hydrate (%)	Agar (%)	Alc. (%)
1	0.8		0.02	35	1,750	3	4	0.02
2	0.6	0.02	0.02	25	1,500	3	3	
3	0.7	0.03	0.05	20	900	4	2	0.03
4	0.3	0.01	0.02	11	750	3	3	0.02
5	0.6	0.02	0.01	6	700	37	22	0.02
6	0.6	0.03		5	500	32	32	
7	0.5	0.03		7	278	28	30	0.02
8	0.6	0.04		3	350	25	25	0.10
9	0.4		0.03	5	200	21	15	
10	0.2	0.02	0.02	4	150	10	11	+-

TABLE 3 Results of Analyses of Algae Species Collected on Kodemgareng Keke Island

SEAWEED AS A RAW MATERIAL FOR INDUSTRY

Rachmaniar Rachmat, Jana Anggadiredja, Achmad Zatnika, Sri Istini, and Suhaimi BPPT Seaweed Research Team

INTRODUCTION

In seeking national development, Indonesia is looking toward its vast marine resources to provide more diversified earnings. One marine resource that has not been tapped wisely is seaweed. Most of the seaweed now harvested grows naturally along the coasts of Riau, West Sumatra, Bangka Belitung, South Java, South Sulawesi, Central Sulawesi, Southeast Sulawesi, Bali, the Nusa Tenggara Islands (NTT), and the Mollucas (Soegiarto et al., 1978).

The Siboga Expedition of 1899-1900 identified approximately 555 different kinds of seaweed (van Bosse, 1928), of which five are of commercial value: <u>Gracilaria</u>, <u>Gelidium</u>, and <u>Gelidiella</u> for agar and Hypnea and Eucheuma for carrageenan.

Seaweed has long been known as a raw material for food and pharmaceuticals in the United States, Japan, China, and Europe. Based on the present processing technology, alginate, agar, and carrageenan are used in the manufacture of various industrial commodities, most of which are produced by the United States, Denmark, France, Korea, Japan, Taiwan, and Canada. Because the demand for these commodities is increasing, the demand for the raw material, the unprocessed seaweed, is increasing concurrently.

Indonesia is looking forward to this increased demand as it has important marine algae sources. They will provide a new source of income for Indonesians living in coastal areas, reduce pressure on the land, and earn foreign currency.

PRESENT SITUATION

The real potential of seaweed in Indonesia is not well established as a detailed study of the resources has yet to be done. A number of studies indicating the distribution of various species of seaweed have been undertaken, however. In fact, some areas already produce substantial amounts of seaweed, as indicated in Table 1.

Seventy percent of seaweed production comes from the Mollucas, 9 percent from Bali and NTT, and 5 percent from South Java. Table 2 shows a more detailed breakdown of seaweed production by location.

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Year	Volume (tons)	Value (Rp. 1,000)
· 1979	5,945	334,000
1980	7,848	421,000
1981	7,251	362,000
1982	7,479	398,000
1983	9,607	515,000

TABLE 1 Production and Value of Seaweed in Indonesia, 1979-198	TABLE I	Production an	d Value of	Seaweed in	Indonesia,	1979–198
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SOURCE: Directorate General of Fisheries, Jakarta, 1985.

TABLE 2 Seaweed Production (Tons) of Eight Coastal Areas of Indonesia, 1979-1983

Location	1979	1980	1981	1982	1983
West Sumatra	6	4	8	6	62
East Sumatra	39				
Malacca Strait	251	572		7	
North Sul awes i	108	119	105	123	69
South Sulawesi	436	78	1 38	280	501
South Java	191	486	208	407	772
Bali/NTT	496	550	451	315	1,755
Moliucas	4,418	6,039	6,341	6,341	6,448
Total	5,945	7,848	7,251	7,279	9,607

SOURCE: Central Bureau of Statistics, Jakarta.

The amount of seaweed produced in Indonesia, most or all of which comes from uncultivated resources, is very low compared to that produced in the Philippines (Table 3). The production of <u>Eucheuma</u> per hectare in Indonesia is 0.6-3.4 tons dray weight per month. By farming seaweed, production could reach 6-8 t/ha/mo in Southeast Sulawesi and 5-6 t/ha/mo in Bali. At present, the production of seaweed in Bali is 120 t/mo from 22 hectares. The average growth rate of seaweed in Bali for the period December 1984-January 1985 is shown in Table 4. In the Philippines, intensive cultivation has increased <u>Eucheuma</u> production from 398 t/yr in 1970-1973 to 20,000 t/yr in 1978-1984 (Porse, 1985).

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TABLE 3	Production of	Eucheuma	spp.	in	the	Philippines,	1978-1984
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Year	Production (tone)
1978	13,000
1979	14,000
1980	17,000
1981	18,000
1982	26,000
1983	26,000
1984	23,000

SOURCE: Porse (1985).

TABLE 4 Average Growth Rate (Percent per Day) of Seaweed in Bali, December 1984-January 1985

Species 1	lusa Dua	Nusa Lembongan	Serangan
Eucheuma spinosur	n 3.9	5.6	
Eucheuma striatum	3. 5	6.0	5.09
Gracilaria gigas			1.33

Table 5 indicates the production of Eucheuma in 1985 and its estimated products.

TABLE 5:	Eucheuma	Spino s ium	Production	in	1985	and	Product	Estimation
of Euc	cheuma sp	in Bali						

		uma Spinosium ction, 1985	Products Estimation Euchema Sp.		
Location	Area (ha)	Production (tons)	Area (ha)	Production (tons)	
Nusa Lembongan/					
Nusa Penida	38	190	110	550	
Nu sa Ceningan/					
Nusa Dua	2	10	10	50	
Serangan	3	15	100	500	
TOTAL	43	, 215	220	1,100	

Source: The Seaweed Research Team Of BPP Technology, 1985.

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Besides Eucheuma, other possibly useful seaweeds are <u>Hypnea</u>, <u>Gracilaria</u>, <u>Gelidium</u>, and <u>Gelidiella</u>. The production value of these genera is not known as the statistical data are mixed in with those on <u>Eucheuma</u>. The total production of seaweed is only 0.3 percent that of fish, however (Table 6).

TABLE 6 A Comparison of the Product Values (Rp. 1,000) of Seaweed and Fish in Indonesia, 1977-1983

Year	Seaweed	Fish and Other Sea Products	Seaweed/ Fish (%)	
1977	203,000	57,884,000	0.4	
1978	742,000	66,207,000	1.1	
1979	334,000	105,664,000	0.4	
1980	421,000	121, 374,000	0.3	
1981	362,000	136,073,000	0.3	
1982	398,000	132,284,000	0.3	
1983	575,000	179,772,000	0.3	

SOURCE: Directorate General of Fisheries, Jakarta, 1985.

MARKET SITUATION

Most of the seaweed produced in Indonesia is exported (Table 7), but some is used domestically. Within the country, seaweed is largely processed as agar. The demand for <u>Eucheuma</u> is estimated to be 50,000 t/yr, while the current supply is about 44,000 t/yr. It is expected that the demand for alginate will reach 50,000 t/yr in the next 5-10 years. This is equivalent to 1 million tons of fresh seaweed.

According to the Central Bureau of Statistics, the price of seaweed for export is in the range of Rp. 100-200/kg dray weight at the farm gate level. After processing by wholesalers, the price could reach Rp. 450-500/kg FOB.

The following example of the price breakdown of the marketing chain is based on a case in the Mollucas:

- o Seaweed farmers, Rp. 100
- o Local collectors, Rp. 150
- o Middlemen, Rp. 350
- o Exporters, Rp. 450.

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Year	Volume (Kg.)	Value (US \$)	
1979	1,836,076	170,132	
1980	596,629	143,016	
1981	690,291	61,302	
1982	. 2,110,703	166,201	
1983	3,402,139	346,619	
1984	3,061,122	658,842	

TABLE 7 Seaweed Exports, 1979-1984

SOURCE: Central Bureau of Statistics, Jakarta.

SEAWEED PROCESSING INDUSTRIES

The seaweed processing industries in Indonesia are presently limited to processing seaweed into agar. None have the capacity to produce carrageenan and alginate. In 1978, there were 11 factories with a production capacity of 5,816 kg/mo (Soegiarto et al., 1978). They were located in Surabaya (6), Wonocolo (1), Jakarta (3), and Tangerang (1). Of these, only three factories are still in operation: two in East Java and one in Jakarta.

Because the processing of seaweed continues to be done largely in the traditional ways, the products are low in quality and unable to compete with imported products. From 1981 to 1984, Indonesia imported quality agar valued at US \$410,958 per year for industrial uses (Table 8). Indonesia is also importing alginate. Statistics for carrageenan are not available as carrageenan is probably mixed with other chemicals. In comparing the value of imports and exports of agar and alginate alone, Indonesia is spending 18 times more on imports than it receives for exports. It is hoped that this proportion can be reduced to an acceptable economic level.

Year	Agar (kg)	Value (U.S.)	Alginate (kg)	Value (US)
1978	95,518	556,763		
1979	25,872	144,670		
1980	159,349	848,019		_ _
1981	43,372	300,710	4,639,508	5,114,598
1982	261,947	542,193	2,938,303	4,764,968
1983	350,111	526,957	3,717,901	4,848,997
1984	162,885	273,973	3,652,365	5,473,142

TABLE 8 Imports of Agar and Alginate, 1978-1984

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CONCLUSION

Seaweed resources have the potential to contribute to industrialization in Indonesia through better resource identification and seaweed culture. Imports of quality seaweed products could be reduced through the development of more modern factories. Establishment of these factories and the culture of seaweed rather than harvesting from uncultivated natural sources will create jobs, generate income, diversify resource utilization, earn foreign currency, and reduce the pressure on the land.

The Seaweed Research Team has concluded that the following actions are needed to develop a marine algae program in Indonesia:

- o Mapping marine algae by area and estimating production and quality of the current resource
- o A market study
- o Manpower development for the cultivation, postharvest, and processing phases
- o Development of better cultivation techniques
- o Establishment of a research center.

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MARINE ALGAE BIOTECHNOLOGY: POSSIBILITIES AND REALITIES

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INTRODUCTION

Marine algae contain a variety of chemical compounds--organic, inorganic, and mixed. Some of these compounds are now being used commercially, while others may be in the future. They are being applied in the areas of food, biomass energy, medicine, industry, agriculture, and biotechnology.

Algae are living organisms. Thus, they contain chromosomes and other nucleic acid components which are subject to genetic manipulation through conventional crossbreeding or the emerging new methods of biotechnology. Because algae have photosynthetic capabilities, they require only light, air, water, and salts to grow, reproduce, and produce commercially interesting metabolites. Blue-green algae even fix their own nitrogen.

The biotechnological techniques applied to bacteria, fungi, and land plants are increasing rapidly, but genetic manipulations with algae have scarcely been explored. It is hoped therefore that the same techniques applied to bacteria, fungi, and land plants, or modifications of them, will likewise be applicable to algae. Cell culture methods have only recently been applied to algae, and the few reported successes seem to be limited to the unicellular blue-green algae.

Before discussing and putting into proper perspective some of the numerous possibilities for improving various characteristics of algae or using them as foreign gene hosts, it is essential to have an elementary understanding of the techniques involved.

EXPERIMENTAL TECHNIQUES

Cell Culture

The cell culture cycle for land plants is illustrated in Figure 1. Each plant cell is "totipotent," meaning that, theoretically, each and every plant cell is capable of producing a regenerated plant under the appropriate conditions. Each plant cell has its own genetic diversity, and neighboring cells in the same plant may have entirely different

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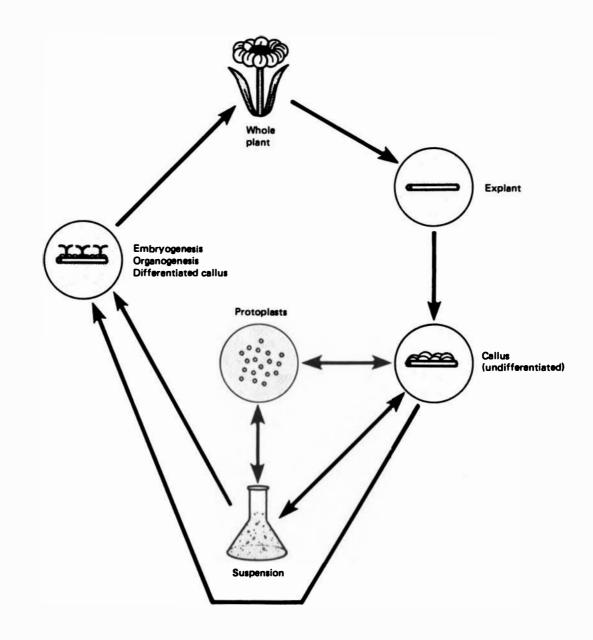


FIGURE 1 Plant cell culture.

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genetic components and yield different strains upon regeneration. This so-called "somaclonal variation" has been demonstrated in a number of land plants. They can be treated to produce single or aggregate cell suspensions and then cultured this way to produce the desired products. Whether any or all of these principles of plant molecular biology apply to marine algae remains to be seen.

Cell Fusion

Cells can be stripped of their outer coats using a variety of enzymes or other reagents. The resulting "naked cells" are called protoplasts. These protoplasts, or their parent cells, can be fused with other cells under the appropriate conditions to form hybrids from which a regenerated plant showing characteristics of both cell lines can be obtained. If this technique is proven applicable to marine algae, new species could be generated.

Cell Transformation

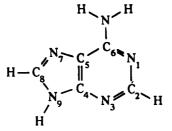
Using microinjection, liposome fusion, or electroporation it has been possible to introduce foreign genes or DNA fragments directly into land plant cells. The transformed cells can be cultured or developed into regenerated plants. The net effect is plant cells or a regenerated plant producing the material coded for by a foreign gene. It is conceivable that algae or their cells could be used as factories for foreign gene products (e.g., increased digestible protein content).

Genetic Engineering

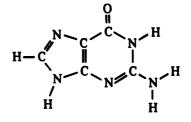
Genetic engineering is generally assumed to refer to applications of recombinant DNA technology, and often when people speak of biotechnology, they are thinking of genetic engineering. It is actually only a small segment of biotechnology, however, and its applications are rather limited. But it is a powerful tool, nevertheless.

Each protein in a living system, whether structural, storage, or reactive enzyme, is not formed directly. Precise information coding for amino acids, peptides, or proteins is stored in genes found in nuclear chromosomes or other specific cellular components such as the plasmids of bacteria. Each normal human cell nucleus has 46 chromosomes, but marine algae have considerably fewer, two or more, and each chromosome contains many gene segments coding for specific proteins and peptides.

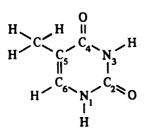
Genes are carried within the nucleus of each cell and are replicated indefinitely. They are composed of deoxyribonucleic acid, or DNA strands, each of which is made up of combinations of nucleotides, consisting of one of the four organic bases--adenine (\underline{A}) , guanine (\underline{G}) , cytosine (\underline{C}) , and thymine (\underline{T}) --with the sugar component,

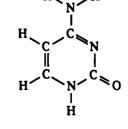


Adenine (6-aminopurine)



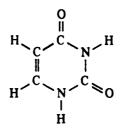
Guanine (2-amino-6-oxypurine)





Thymine (2, 4-dioxy-5-methyl pyrimidine)

Cytosine (2-oxy-4-aminopyrimidine)



Uracil (2, 4-dioxypyrimidine)

FIGURE 2 Purine and pyrimidine bases of DNA and RNA.

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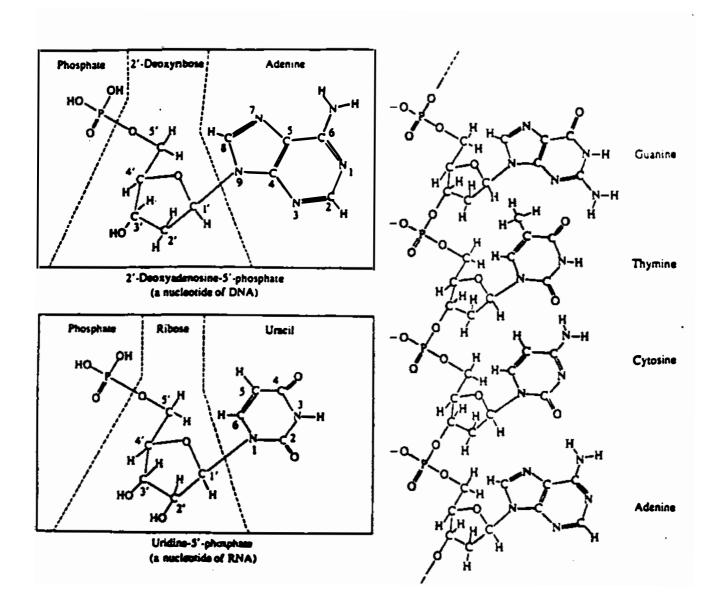
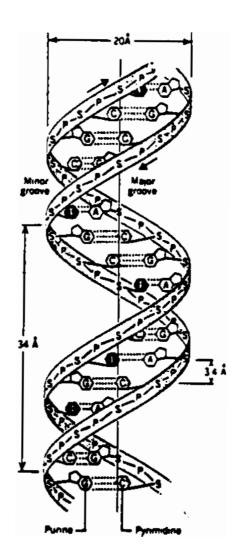


FIGURE 3 Nucleotides.





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in this case deoxyribose, and phosphate residues (Figures 2 and 3). DNA consists then of triplets of these nucleotides joined together in long strands forming double helices (Figure 4). Each strand is matched with a complementary strand so that the <u>A</u>'s match with <u>T</u>'s and <u>C</u>'s with <u>G</u>'s. The complementary strand is used as a template for production of DNA during cell division. Some of the base pairs are initiators, regulators, and terminators, but each specific amino acid is coded for by one or more triplets of nucleotides (Figure 5). Putting these together in appropriate order yields a codon for a particular sequence of amino acids making up peptides or proteins.

It is important to remember that this coding applies only to specific amino acids, polypeptides, and proteins (Figure 6); such molecules are called single-gene products. Nonpeptide materials--such as polysaccharides, pigments, and steroids--require the formation of enzymes or series of enzymes as primary gene products. These enzymes then create the nonpeptide molecules.

How does an organism get from the DNA to the proteins or polypeptides? In the first phase, known as "transcription," particular enzymes initiate the process in which a so-called messenger ribonucleic acid (mRNA) is formed from the coding DNA which, with the help of the cell ribosome, translates the mRNA code into specific amino acids linked together appropriately to form the polypeptides or proteins. RNA is different from DNA in two respects. Uracil, or 5-demethylated thymine, is substituted for thymine, and ribose is substituted for deoxyribose.

How is this system used in modifying proteins by genetic engineering? Enzymes called restriction endonucleases, which have been discovered in nature, cleave DNA between defined base sequences. The DNA sequence coding for a specific protein can be cut from a gene. Agarose gel electrophoresis is used to separate the desired DNA fragment. Concurrently, as a vector or introducing agent, a bacterial plasmid or organelle composed of circular DNA can be isolated and the same or an analogous enzyme used to remove a portion of the plasmid DNA.

The isolated DNA fragment coding for the specific protein is spliced into the plasmid DNA, replacing the portion removed, and is "glued" there by another enzyme called a ligase or DNA polymerase. The altered plasmid is reintroduced into the bacterium and codes for the specific protein desired. By this process, <u>E. coli</u> or other microbial hosts can then rapidly produce, for example, human insulin under fermentation conditions.

For algae, at least three possibilities exist for applying the principles of genetic engineering: (1) use of algae as hosts for foreign genes, such as those coding for digestible storage proteins; (2) transfer of algae genes into bacteria or other microorganisms; and (3) manipulation of algae genes to enhance the productivity of desired products such as polysaccharides by increasing copy numbers of limiting synthesis enzymes, or alteration of photosynthetic partitioning so that less energy goes into cell division and more goes into polysaccharide productivity.

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	Second latter							
I		U U	C	A	5			
	υ		UCU UCC UCA UCG	UAU UAC UAA UAG		J U A U		
le tuer	c					ט א ט כ	Life	
Frat		AUU AUC AUA AUG Met	ACU ACC ACA ACG	AAU AAC AAA AAG	AGU AGC AGC AGC AGC	J U ≪ U	Thed	
	G	GUU GUC GUA GUG	GCU GCC GCA GCG	GAU GAC GAA GAA GAG	GCU GCC GGA GGA GGG	J U ≪ U		

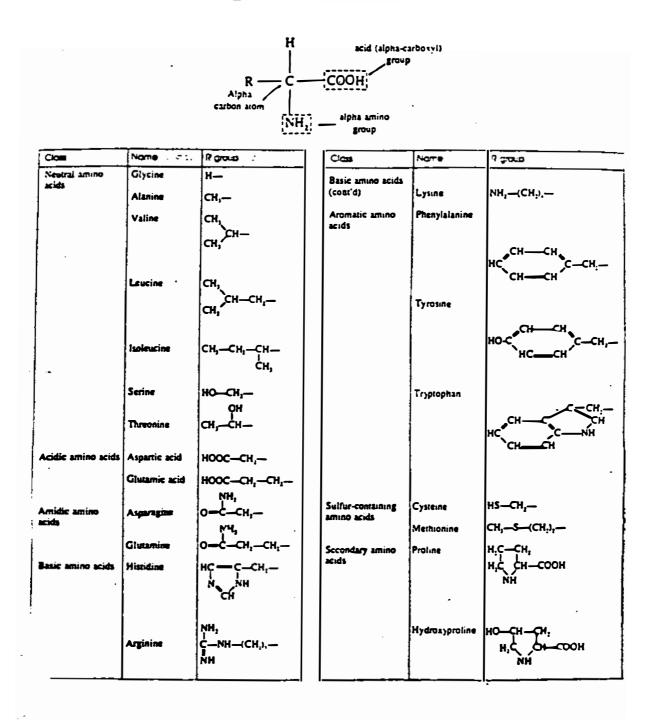
	Three-letter
Amino acid	symbol
alanine	ala
arginine	arg
asparagine	asn
aspartic acid	ąze
asn and/or asp	asz
cysteine	Cys
glutamine	gin
glutamic acid	glu
gin and/or giu	ght
glycine	gly
histidine	his
isoleucine	iteu
leucine	leu
lysine	lys
methionine	met
phenylalanine	phe
proline	pro
serine	SOF
threonine	thr
tryptophan	trp
tyrosine	tyr
valine	val

AUGCGCGCUUCGAUAAAAUGA

- (a) met arg ala ser ile lys met
- (b) ala arg phe asp lys asn l
- (c) | cys | ala | leu | arg |

FIGURE 5 The genetic code. The three triplets UAA, UAG, UGA with no amino acid allocated to them are nonsense codons which lead to the termination of the polypeptide chain.

THE COMMON AMINO ACIDS



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POSSIBILITIES FOR APPLYING BIOTECHNOLOGY TO ALGAE

How can the technology described above be applied to marine algae?

Species Improvement

Selected examples of species improvement are:

- o Faster-growing varieties
- o Higher yields of desired products
- o More desirable products
- o Disease resistance
- o Herbivore resistance
- o Hybrid products
- o Increased protein content
- Secondary metabolite stimulation
- o Creation of new species
- o Environmental adaptability.

Algae as Hosts

Since algae need only air, water, salts, and light to grow and produce metabolites, it is tempting to suggest their use as hosts for foreign gene product production, even for such high value-added products as insulin, human growth hormone, and interferons.

Algae as Chemical Reactors

A number of enzymes have yet to be discovered in algae. It is entirely possible that some of these will be useful for synthesizing various compounds difficult to produce by traditional methods. Whole or homogenized algae, algae cell suspensions, or immobilized systems are all potentially useful as chemical reactors.

REALITIES

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Taking into account how far the biotechnology field has evolved thus far, as well as the limited progress made to date on applying the principles of biotechnology to marine algae, one must apply reason to the aforementioned possibilities in predicting which ones may become realities within given time frames. Genetic manipulation research is expensive and time.consuming, and until now the driving force has been the production of high value.added products. Whether sufficient incentive can be generated to apply biotechnology extensively to marine algae remains to be seen. Nevertheless, the following predictions seem reasonable.

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Short Term (1-5 Years)

Mutant Selection Using appropriate observations of natural species or induced mutation processes together with analytical methods developed to quantify the useful property sought, mutant selection is certainly a short term reality. In fact, this process is already being used to select strains for seaweed farming.

Hybridization Whether this is carried out by sexual crosses (as Dr. John West is doing at the University of California, Berkeley) or asexually by cell or protoplast fusion and then regeneration, or combinations, hybridization should produce selectively superior algae within the short term. Whole plant regeneration from callus has been reported for Agardhiella subulata by P. M. Bradley and D. P. Cheney (1985) of Northeastern University. In the November 1985 issue of Agricell Report, this prediction appears:

Tissue culture procedures may have an even greater potential for rapid breeding and multiplication of desirable seaweed cultivars than for terrestrial crops. In land crops, much of the useful variation has been selected through thousands of years of plant breeding. In contrast, little breeding of marine crops has been done. In vitro culture of multicellular marine algae, however, is still in a very early stage of development. While protoplasts have been isolated from a few seaweed species, very little work has been done on basic tissue culture procedures and none on genetic recombination of these.

Cell Culture, Including Secondary Metabolite Production Techniques are being developed to reduce multicellular marine algae to tissue culture, both callus and suspension. These cells and their protoplasts can be used for hybridization, as described previously, and for production of secondary metabolites (nongene products) via cell culture. The products normally produced by whole algae could possibly be produced by denser cultures of free or aggregate cells.

Cell Culture Manipulations Techniques must be developed for fusions of cells or protoplasts followed by culture or regeneration. Within the next five years, practical methods should evolve.

Development of Foreign Gene Insertion Methods Whether foreign storage protein genes for more nutritious algae, genes for high value-added products, or genes for increasing production of polysaccharides are anticipated, practical methods for these gene insertions will have to be developed. This should be possible within the next five years.

Biopathway Elucidation To increase the productivity of current products, including polysaccharides, by genetic manipulation, it is necessary to understand which enzyme systems are active in biosynthesis of these products. It should be possible to elucidate a number of

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these systems within five years if sufficient effort is applied to the problems.

Enzymatic Bioconversions Certain enzymes existing in marine and other algae may enable production or modification of high value-added products. Free algae or immobilized forms of them should be evaluated, perhaps leading to short term success in their utilization.

Intermediate (5-10 Years)

Foreign Gene Insertions If the development of methods for foreign gene insertion is successful during the next five years, the following five years should see desirable foreign gene insertions and useful expressions of those genes in almost unlimited variations. Even though it is often said that algae would make ideal hosts for foreign gene products because of their requirements for only water, air, inorganic salts, and light, it must be remembered that their doubling times are orders of magnitude greater than those for bacteria and yeasts. Thus, these longer doubling times must be considered, unless they can be reduced by photosynthetic partitioning or some other manipulation.

During the next five years, enough about specific algae genes and their products may be learned to allow us to transplant these genes to more rapidly growing hosts. Perhaps algae polysaccharides will be produced by fermentation techniques, or nitrogen-fixing gene products from blue-green algae will be transferred to and expressed by land-crop plants. A number of these possibilities may become realities.

Biosynthetic Pathway(s) Improvement As stated earlier, the rate of natural production of commercial products such as the polysaccharides may be limited by a particular enzyme or series of enzymes. If the genes producing these enzymes could be manipulated to produce more copies, productivity could be enhanced considerably.

Long Term (Greater than 10 Years)

It is difficult to imagine any biotechnology oriented marine algae project that could not become a reality in the long term if sufficient effort is devoted to it.

CONCLUSION

Considering the number of algae species and the useful products from them (both those known and those yet to be discovered), it is difficult to choose which genetic manipulations on what algae or combinations of algae will have the best chance for economic success. One can only hope that through prudent choices of projects and cooperative efforts by universities, research institutes, government

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agencies, and private industry, at least some of the possibilities discussed will ultimately become, or lead to, profitable realities.

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MARINE ALGAE BIOTECHNOLOGY: ECOLOGICAL CONTEXT AND MANPOWER NEEDS FOR INDONESIA

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INTRODUCTION

Use of Indonesian marine algae for existing commercial purposes and future biotechnological development will be based upon the diversity of marine algal flora in Indonesia and the conscious application of a strategy for developing it. The tropical setting and the extraordinary genetic resources of the Indo-West Pacific biogeographic region are responsible for Indonesia's great diversity of marine algae -- greater, in fact, than that of any other place in the world. This resource is well suited to development through the strategy for industrial transformation articulated by Minister of State for Research and Technology B. J. Habibie. However, application of this strategy will require newly trained scientific manpower. This paper examines the ecological context of Indonesian marine algae resources, describes the potential for commercial development within the Minister's industrial transformation strategy, and outlines some implications that a marine algae development program in Indonesia would have for scientific manpower training.

ECOLOGICAL CONTEXT OF INDONESIAN MARINE ALGAE

Indonesia occupies a 3,000-mile band of ocean between the Pacific and Indian oceans. This area is the heart of the Indo-West Pacific biogeographic region which has the world's most diverse biota. The diversity of Indo-West Pacific Shelf fauna far exceeds that of other tropical regions (Briggs, 1974). For example, this region has an estimated 500 species of reef-building corals, or about 10 times the number found in the Western Atlantic which is the next richest area (Vaughn and Wells, 1943). Similarly, it has more than 1,000 species of bivalve molluscs- the next richest tropical shelf region has fewer than 500 species (Stehli et al., 1967)--and over five times as many conch species (Strombidae) as the next richest area (Abbot, 1960). Briggs (1974) estimates that there are more than 3,000 species of shore fish in the Indo West Pacific, which compares to fewer than 1,000 found in any other tropical shelf region. And algologists believe that Indonesia's algal flora is more diverse than that in other tropical

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areas; 587 of the about 1,500 described species of macroscopic marine algae are found in Indonesia (information furnished by the Seaweed Research Team). It is also said that marine algae found anywhere in the tropical ocean are also found in Indonesia (Max Hommersand, University of North Carolina, personal communication, 1986).

Among Indonesia's algal flora are plants that produce chemicals for which there is a present international market and chemicals that are likely to be valuable in the future. The current Indonesian algae industry is based on Eucheuma strains imported from the Philippines, but it is likely that indigenous species also produce commercially valuable kappa- and iota-carrageenans. The Indonesian species Gratelaupa probably produces lambda-carrageenans, used as a thickener in prepared food products. Similarly, it is almost certain that Indonesian species of Gracillarica, Gelidium, Gelidiopsis, and Gelidiella produce agar, a valuable food product and bacterial growth medium.

Other algae within Indonesia's rich flora are likely to produce chemicals that will be valuable in the future. For example, the extraordinary diversity of the Indo-West Pacific biota includes a wide range of plant-eating herbivores, which are capable of all but eliminating their food plants from the natural ecosystem. Many plants, however, have evolved chemical defenses against herbivores. Over 12,000 biologically active chemicals have been identified from terrestrial plants (Devon and Scott, 1972), including alkaloids, terpenoids, acetogenins, and aromatic compounds known to function as toxins, feeding inhibitors, digestibility reducers, and pathogen inhibitors (Rosenthal and Janzen, 1979).

At least 500 different natural products have been identified from marine algae (Faulkner, 1984), and a large percentage of these appear to be biologically active secondary metabolites (Norris and Fenical, 1982).

Some of these chemicals have already been isolated from Indonesian marine algae (information furnished by Tjodi Harlim, University of Hasanuddin).

COMMERCIAL DEVELOPMENT OF INDONESIAN MARINE ALGAE

Commercial development of natural resources involves several identifiable steps. Minister Habibie has identified four phases of this process in a paper describing a strategy for the industrial transformation of a developing country (Habibie, 1983):

The first, most basic, phase is the use of already existing technologies for added value processes in the assembly and manufacture of products already on the market. This includes both the domestic as well as the international market...

The second phase is the integration of already existing technologies into the design and manufacture of completely new products. In this second phase, technologies are used and developed to create blueprints and designs, thus adding the element of creativity to the first phase... - 71 -

The third phase is the stage of the development of technology. Existing technologies are improved and new ones developed in the effort to design and manufacture the products of the future. If in the second phase one can avail oneself of existing technologies, at this stage there is a need for the creation of completely new ones...

Firms and countries in the third phase of development will very often find that in many cases there exist gaps in theory which need investment in basic research. This may be called the fourth stage in the development of science and technology for technological and industrial transformation.

Commercial development of marine algae products appears to fit nicely into the Minister's model of industrial transformation of a developing country. Indonesia can enter the existing international markets for carrageenan and agar using existing technology; this is being done in the new production facilities at Nusa Dua and Nusa Lembongan. But steps should be taken to improve application of this technology and thereby the quality of algae products. These steps appear minor, however, compared to the strides already made.

The second phase of industrial transformation may begin with analyses of Indonesia's rich algal flora for potential new products, or it may involve linkages between marine algae production and the new Center for Biotechnology in Indonesia. In the previous paper, Dr. Renn outlined some realistic possibilities at the interface of marine algae and biotechnology.

The Minister's third and fourth phases of industrial transformation must build upon the first and second, but a functioning algae products industry with immediate access to the world's most diverse algal flora has an obvious competitive advantage in the development of totally new algae products.

Minister's Habibie has also stated five basic principles that must underlie all phases of industrial transformation through the application of science and technology (Habibie, 1983):

First, education and training in the various sciences and technologies relevant to the nation-building needs of the country must be undertaken. This involves both in-country as well as education and training abroad. This is an essential step It is, however, by itself, not sufficient.

In addition, a clear, realistic, and consistently applied concept of the nature of the society to be developed and the technologies needed for the realization of this future society must also be evolved. These technologies need not necessarily be the most primitive. They may indeed in many cases be the most advanced in the world. The only criterion for the appropriateness of technologies for any particular country, including technologically less developed countries, is their utility in solving actual problems in that particular country. Third and perhaps most important, technologies can only be transferred, adapted, and further developed through their being applied to concrete problems. By their very nature, technologies cannot be learned, let alone be developed, in the abstract. . .

Fourth, and as a corollary to the previous principle, for a country to develop itself technologically it is vital that it solves its own problems by itself. To develop its technology, no country can continue to be a net technological importer indefinitely. At some point it must be able to develop its own technologies.

Fifth, in the very first stages of transforming itself into a technologically advanced nation, every country must protect the growth of its national technological capabilities until its international competitiveness has been established.

Here again, marine algae seem to be a resource well suited to application of these principles. Education and training will be needed during development of Indonesia's marine algae resources, but once these investments in human capital have been made, the newly gained knowledge can be applied immediately to concrete technical problems and to solving Indonesia's problems of foreign exchange generation and import substitution.

MANPOWER NEEDS

The preceeding two sections of this paper have attempted to document two major premises:

- 1. Indonesia's marine algae are a rich and diverse natural resource for industrial development.
- 2. Marine algae provide a raw material well suited to application of science and technology to the industrial transformation of Indonesia.

If these two premises are true, it is useful to examine the role of education and training in the development of marine algae products to further Indonesia's industrial transformation. This is, of course, Minister Habibie's first principle of industrial transformation,

Advanced training will be needed in four areas if marine algae are to be utilized effectively in industrial transformation: algal biology, international market analysis, natural products chemistry, and algal genetics.

Indonesia's marine algal flora is not well known. Although the Snellius Expedition provided a survey and the Snellius II results will expand existing knowledge, even greater knowledge is required to recognize commercially useful species among the diverse array.

An international market analysis is essential if Indonesian products are to fit effectively into the world market. Lambda carrageenans and sugar-reactive agars are high-value products for which a ready market is available. Some algae products such as - 73 -

bacterial agar and purified agaroses are 100 to 1,000 times more valuable than raw carrageenan. A cost-benefit analysis of the production economics of different algae products will be needed in the early stages of applying technology to the Indonesian marine algae industry.

In the later stages of industrial development, advanced knowledge of natural products chemistry will be necessary as algae are examined as a raw material for potential new products.

Finally, because algae produce chemical products through a complex series of steps in intermediary metabolism, knowledge of biochemistry and genetics must be applied to the selection and development of algae strains suited to the environmental conditions and a product mix suited to the Indonesian marine algae industry.

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PART II

Conclusions and Recommendations

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SUMMARY OF CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Participants in the Workshop on Marine Algae Biotechnology reviewed the status of marine algae resources and farming systems in Indonesia and discussed how the application of biotechnology might contribute to the industrial development of these resources. Based on these discussions, the group concluded that:

- o Indonesia has some of the richest, most varied, and potentially most valuable marine algae resources in the world.
- These resources currently produce an array of products for domestic consumption as well as over 9,000 tons of exported material used in the manufacture of food, pharmaceuticals, and scientific products.
- Because Indonesia's marine algae resources offer great potential for further development, the government should strongly encourage capital investment and create a better climate for doing business.
- The international market for marine algae products is becoming more diverse as new uses are developed. Existing markets can be penetrated further, however, through achievable improvements in the quality of Indonesian algae resources.
- Because of the fine efforts displayed thus far by the interministry Seaweed Research Team administered by the Agency for the Assessment and Application of Technology (BPPT), continued development of this team should be encouraged as a mechanism for further developing Indonesia's use of its rich marine algae resources.

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RECOMMENDATIONS

Research and Development

Based on these conclusions, it is recommended that:

- o The current program of marine algae research and development be continued. Although this program should include assessments of the currently underutilized natural crops, it should concentrate initially on developing crops for existing markets. Indonesia has the capacity to grow various types of algae, but the market force should guide program development. For example, the existing export markets for carrageenan products and import substitution for the over \$5 million in agar and alginate products currently imported annually should guide early program development.
- Steps be taken to improve the quality of the algae products now being processed. Further development of cultivated algae could provide substantial employment and income for subsistence level farmers and fishermen. This potential can only be reached, however, through quality production. The program should therefore include establishment of an algae product quality assurance laboratory to be used for both research and industrial purposes.
- Research be undertaken on and an inventory made of the commercially important species of natural crops, including the agar producing Gracilaria and its relatives.
 Herbivore resistant algae should be screened for bioactive chemicals such as toxins, and a profile of the general life history and ecology of these species made to assess the potential uses of algae in aquaculture and agriculture.
- Research on cultivated crops also be conducted, including genetic selection and biotechnological development of algae strains with high growth rates and high levels of valuable chemicals, experimentation to develop optimum spore setting and cultivation techniques, and development of postharvest treatments that preserve or enhance product quality.
- New technologies and products be developed: for example, algal conversion of waste products into materials of value to industry or agriculture: bioactive substances, including steroids for use as pharmaceuticals. and new products from algae using biotechnological research.
- o Market, social, and economic analyses be made, including:
 - -- An analysis of the market potential for <u>lambda-carrageenan</u> production

- -- An assessment of product quality requirements for international market penetration
- -- An evaluation of the product requirements and profit potential of a domestic agar production factory
- -- A continuing assessment of the markets for marine algae to identify new products for which Indonesia might have a comparative advantage.

Manpower and Training

Manpower development, both within Indonesia and abroad, is an integral part of the marine algae R&D program.

Within Indonesia, manpower development should focus on helping solve specific problems such as low product quality. Specific measures might include:

- o Establishment of an algae product analysis laboratory and training appropriate to its needs
- o Short courses on topics of benefit to farmers and researchers
- o High-level workshops to assist with program development.

Abroad, manpower development should focus mainly on training and should include:

- O Short term technical development activities in universities and industry, perhaps joint research, as well as longer-term graduate degree training in institutions with marine algae programs, such as the universities of California at Berkeley and Santa Barbara, Hawaii, Maryland, North Carolina, and Washington
- Rapid attainment of expertise in marine algae systematics, functional ecology, algal agronomy, sol/gel chemistry, and aquacultural economics
- Longer term training in biotechnological areas such as algal genetics, biochemistry, protoplast fusion, and enzyme analysis.

Infrastructure

The participants recognized that the development of marine algae in Indonesia will involve many people, companies, regions of the country, and ministries. Thus, they recommended that several supporting organizations be established as follows:

 A coordinating committee for the marine algae development program under the sponsorship of the BPPT. This committee would provide communication among program components, sponsor

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domestic workshops, oversee information dissemination, and act as a publicly visible focus for the development program.

- A marine algae association to assure coordination and mutual assistance among the large and small precursors.
- o A marine algae information and extension program.

COMMENTS BY CHAIRMAN OF THE U.S. PANEL

Dirk Frankenberg Marine Science Program University of North Carolina at Chapel Hill

It is with pleasure that I offer the appreciation of the U.S. participants for the thoughtful and careful planning that our Indonesian hosts have so obviously lavished on workshop preparations. I also wish to summarize some observations of the U.S. participants on the potential of Indonesia's marine algae industry for development.

The arrangements for the conference have been first-rate in every respect. The U.S. participants have been impressed by the excellence of the field trip logistics and by the significant development of algae production operations at Nusa Lembongan and Nusa Dua. Indonesia can take pride in both the organizing committee for the workshop and the Seaweed Research Team established by the Agency for the Assessment and Application of Technology [BPPT]. We have seen firsthand the good results produced by these scientists.

The U.S participants believe that Indonesia is facing a "window of opportunity" in securing a larger share of the world market for marine algae products. This market is growing, but, more important, concerns about the political stability of one of the major producers of these products is leading manufacturers to search for alternative supplies in other countries. Thus, Indonesia has a significant opportunity to become one of the world's major suppliers of algae products.

Indonesia has a long history as a minor supplier of such products lrom the harvest of wild plants as well as from small-scale aquaculture. The BPPT assisted development of Nusa Lembongan demonstrates that Indonesia is capable of rapidly increasing its production. Unfortunately it appears to the U.S. participants that the quality of the algae products currently processed in Indonesia are below world standards, and thus may not support an increase in, or even retention of, Indonesia's current share of the world market.

Given this fact, coupled with the limited period within which market share adjustments seem likely, the U.S participants in the workshop recommend initiation of a crash program of quality improvement for Indonesian <u>Eucheuma</u> products. Four specific steps should be taken as part of such a crash program:

1. The BPPT Seaweed Research Team should seek immediate technical assistance from expert consultants capable of assessing the production techniques in the Bima Sakti operations in Bali and

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recommending steps to improve product quality. (During the U.S team's brief visit it was noted that it may be possible to improve drying techniques to ensure a cleaner product with a lower moisture content and to harvest older algae which usually yield products with higher gel strengths.) Dr. Maxwell S. Doty of the University of Hawaii is prepared to recommend specific sources of technical assistance.

- 2. The BPPT Seaweed Research Team should formally approach the four major manufacturers of algae products worldwide with a request for technical assistance in improving product quality. The U.S. participants will suggest persons and companies that should be contacted with such requests.
- 3. BPPT should provide funding to establish an algae product quality assurance laboratory capable of assessing the moisture content, cleanliness, and gel strength of Eucheuma. Laboratory analyses should be made available to the researchers and seaweed producers experimenting with techniques to improve the quality of Indonesian algae products. The laboratory should also be used to provide independent analyses of Indonesian products offered for sale on the world market. Technical assistance from private consultants or seaweed product manufacturers may be necessary to establish this laboratory effectively.
- 4. Four to six members of the Seaweed Research Team should be sent abroad in the summer of 1986 for advanced technical training in experimental ecology, algae industry operations, and culture of Eucheuma and agar producing algae. It is suggested that team members arrive in the United States in time to enroll for a five week course in experimental marine ecology at the University of North Carolina Institute of Marine Sciences in Morehead City. After the course the team might travel to the University of Hawaii for a month of directed research training on Eucheuma and agar producing algae. The U.S. participants in this workshop would be pleased to host Seaweed Research Team members during their stay in the United States. We have been told that the funds available through an existing USAID educational exchange program (General Participant Training II Program) can be used to support U.S travel of the team.

The recommended four-step program should lead directly to improved quality of Indonesian algae products. By initiating such a program Indonesia will demonstrate its commitment to effective competition in the world market for marine algae products. This demonstration of commitment is critical at this time when major international purchasers are seeking new sources of supplies.

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The U.S. participants realize that calls to immediate action are difficult to answer, but the opportunity facing Indonesia is too great for us not to recommend immediate, forceful action. If we did not, we would have failed in our responsibilities to the spirit of U.S -Indonesian cooperation demonstrated by this workshop

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APPENDIXES

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APPENDIX A

Opening Remarks

Richard C. Howland Deputy Chief of Mission U.S. Embassy

I have had the good fortune to have been associated with Indonesia for over 20 years. Of the many experiences I have had in Indonesia, the most exciting have been those in which I participated in a new start. This has especially been the case when the new endeavor grew from the seeds of prior cooperative relationships which were mutually beneficial, enlightening, and lasting. This workshop is such a new start, and I am very happy to be here on this occasion to bring you the good wishes of Ambassador Holdridge as well as my own. The ambassador has been a very strong and consistent supporter of cooperation between our countries in the fields of science and technology. Unfortunately, he is not in Jakarta at the moment, but he sends his best wishes for a very successful workshop and project.

Indonesia and the United States have worked together for a long time and have shared interests in the fields of science and technology. We are proud of this cooperation which was recently formalized by Minister Habibie and George Keyworth, President Reagan's science advisor, and which culminated in the signing of an agreement by Minister Habibie and Ambassador Armacost in July 1984.

This agreement calls for cooperation in the fields of "agriculture, health oceanography, natural resources, energy, technology relevant to providing a basis for industrial development" and others. This workshop, under the guidance of the National Academy of Sciences and the Dewan Riset Nasional [DRN], is the first fruit of that agreement.

Both of our nations acknowledge that development depends largely on the members of the scientific and technical communities sharing ideas and experiences. The U.S. Congress established the U.S. National Academy of Sciences to provide the environment within which eminent persons from academia, government, and private enterprise could contribute their ideas, expertise, and experience to a compendium of problems of national significance. Today under the umbrella of the U.S. National Research Council, the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine continue to draw upon the most prominent persons in our country to generate innovative ideas and to resolve major problems not only for our own country but for other countries as well by mutually sharing and discussing ideas with their prominent scientists.

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Earlier this year, President Suharto established a similar national research council for Indonesia, the Dewan Riset Nasional. We are very proud that the chairman of the DRN, Minister Habibie, has invited the U.S. National Research Council to participate in the first steps of establishing this vital enterprise. This first activity has been very ably coordinated by the esteemed secretary of the DRN, Dr. Sediono Tjondronegoro, who is well known for his achievements in sociology and for his keen interest in the problems of achieving effective transfer of technology. We are fortunate that the person chosen by the Academy to coordinate with Dr. Tjondronegoro is Mrs. Rose Bannigan, program development coordinator for the Board on Science and Technology for International Development. Mrs. Bannigan is well known in Indonesia to both Indonesians and Americans as a tireless worker and a first-class organizer. I think that with Dr. Tjondronegoro and Mrs. Bannigan, assuming responsibility for this project, we can look forward to a series of exciting and productive workshops of which marine algae is the first.

This is not a new relationship between the members of the DRN, as individuals, and the National Academy of Sciences. The Academy has been cooperating with prominent Indonesian engineers and scientists for nearly 20 years. The first major workshop on food policy in 1968 resulted from a collaborative program between the Academy and LIPI [Indonesian Insitute of Sciences]. The recommendations of that workshop were incorporated into REPELITA I, which laid the foundations for the very successful program of agricultural research. This work continues to develop through the U.S .. assisted Applied Agricultural Research and Sumatra Agricultural Research projects, both of which have already contributed substantially to Indonesia becoming self sufficient in food. The initial involvement was instrumental in developing jointly funded projects such as the science and technology project, the present program with the National Academy of Sciences, and a current study to provide Indonesia with more support in the field of aquaculture.

As we can see, this collaborative effort has already been very fruitful, and I am very honored to be a participant in the start of a new chapter in what has turned out to be an exciting story with an excellent plot and lasting relationships. We sincerely hope that this will, in turn, lead to exciting new chapters in the future.

Not all projects can catch the attention of the public as much as putting an Indonesian astronaut into space, but all are important to the development of Indonesia and its people. The workshop you are about to engage in today on marine algae biotechnology may not appear to be dramatic, but it is one that has almost limitless benefits, not only for the people of Indonesia but also for the people of the world. Indonesia's position as an island nation in an equatorial area gives it a potential far above that of most other countries. Indonesia can become the leader in the biotechnological development of marine resources. I believe that the subject for this workshop is the right subject for this time and for this place.

I am particularly pleased that at the start of its existence the DRN will have the opportunity to consult with the National Academy of

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Sciences and to use the Academy experience to assist in defining the tasks and the role it has been chartered to play in the life of the Indonesian nation. It is important that the first areas of concentration be not only substantive areas but also those in which Indonesia already has significant experience. Biotechnology and marine resources have unlimited potential for improving the welfare of the Indonesian people, and I congratulate you on choosing as your first subject a very challenging but vitally important area of scientific enterprise. We look forward not only to the reports of this workshop but also to the applications of its deliberations.

Please accept my best wishes for a very successful workshop.

APPENDIX B

Keynote Address

Doddy A. Tisna Amidjaja Vice-Chairman Indonesian National Research Council

On behalf of Professor Habibie, chairman of the Indonesian National Research Council and minister of state for research and technology, it is my honor and pleasure to extend our warmest welcome to you, especially the eminent scientists who came all the way from Hawaii and the mainland of the United States to take part in the Workshop on Marine Algae Biotechnology, which is jointly organized by the U.S. National Academy of Sciences and the Indonesian National Research Council.

Dr. Habibie deeply regrets not being able to welcome you personally and to inaugurate this opening ceremony today. However, he has given us his assurance that he will be present at the last session of the workshop to acquaint himself with the conclusions and recommendations resulting from your deliberations and to convey his remarks and messages in the closing ceremony. He is fully confident that this workshop will be a success, given the outstanding qualifications of the participants and the intrinsic importance of the subject to be discussed.

I am happy to note that the U.S National Academy of Sciences has been helping Indonesia examine several vital problems or topics pertaining to certain sectors of its development process, and come to a scientific understanding in formulating the answers to the problems of concern. In 1968 and 1978, the U.S. National Academy of Sciences, in cooperation with the Indonesian Institute of Sciences [LIPI], held workshops on food, while in October and November 1982, in cooperation with the Team for the Formulation and Evaluation of a National Program for Research and Technology [PEPUNAS RISTEK], workshops were held to discuss the important topics of, respectively, rural productivity and science and technology planning and forecasting.

Once again, today, the U.S. National Academy of Sciences is cooperating with the Indonesian National Research Council -the legal statutory advisory body to the Ministry of State for Research and Technology- on bringing scientists from the United States and Indonesia together to obtain their views and recommendations on a very important topic for Indonesia as well as for the international economy, marine algae biotechnology. The use of algae products in the traditional Indonesian household, especially as vegetables or in delicacy cakes or

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agar cakes, probably dates back to when the ancient Indonesians first invented simple biotechnologies. These included the use of microbes in fermentation techniques to produce tape (fermented cassava or rice) and alcoholic drinks (tuak, brem, etc.) and the use of molds to produce tempe (soybean-base) and oncom (peanut base) cakes. As early as 1292 when the first Europeans sailed the Indonesian seas, they observed that the natives were collecting seaweed to be used as "vegetables "

Scientific taxonomic studies of algae in Indonesia date from 1922 when Heyne mentioned 21 species of useful Indonesian seaweed. Now 587 species of seaweed belonging to 193 genera are known and have been described, and it is certain that still others exist.

Of the known seaweed genera, several are of commercial importance. For example, <u>Gelidium</u>, <u>Gelidiella</u>, and <u>Gracilaria</u> are used as raw material for agar and <u>Eucheuma</u> and <u>Hypnea</u> are used as raw material for carrageenan. The techniques and technologies used to gather, conserve, and pack seaweed are still very simple; these tasks are mostly accomplished by collecting and sun-drying. We have, of course, a long way to go to obtain a product with the highest value added. This will involve a long chain of sequences, beginning with:

- Basic research on seaweed that covers its taxonomy, geographical distribution, physiology and ecology, biochemistry, and identification of the properties of active substances.
- Followed by <u>applied</u> research on farming techniques, conservation of raw material, alternative technologies for the isolation of chemical inclusions (perhaps biotechnology is the order of the day here), and screening techniques for bioactive material, etc.
- Arriving subsequently at the stage of <u>technology</u> assessment, engineering, and <u>pilot</u> projects to be performed with cost-benefit analyses, and the establishment of model industries on a limited production scale which should be demonstrated to interested entrepreneurs.
- o Culminating in <u>upscaling</u> of <u>production</u> to produce an economic commodity with the desired standardized quality and demanded quantity.

This sequence of steps would normally not be carried out by a single institution. Most probably, and indeed desirably it should be a relay race of results from one institution to another. The key to efficient, well-coordinated cooperation is the development of jointly coherent research programs and projects.

In Indonesia's quest to produce more nonpetroleum and nongas commodities for export, resources from the sea should be searched and studied exhaustively. Based on data on the state of the art of its exploitation and economic aspects, marine algae should obviously have high priority for increasing efforts to optimize primary production, either through nurturing natural stocks and applying rational and rotational cropping techniques or through farming technologies It is heartening to note that some experimental seaweed farming efforts, such as Eucheuma in Bali, show optimistic results. However, there is still not sufficient basic information on the biology and ecology of algae species to improve the current satisfactory result in farming techniques. Furthermore, postharvest techniques are needed to obtain good-quality raw material commodities.

It is very disappointing to see that the production of Indonesian seaweed (from harvesting natural stocks) has been declining in both quantity and quality during this decade and is superseded by that of other countries (i.e., Philippines). The lower quality of the Indonesian product is clearly demonstrated by the lower price per ton received for Indonesian Eucheuma. In 1982, this price was US \$100/t, while the Philippines received US \$380/t. It is obvious that to obtain a constant high yield in algae products and to maintain its quality, we must master up-to-date farming systems and postharvest technologies.

Once the farming system technologies are established and the raw material product is controllable in quantity as well as in quality, further efforts should be made to obtain higher value added, not only for exported raw materials, but also for bioactive substances that should be developed into economic commodities.

Before arriving at this stage, however, more information should be gathered and more research and investment should be carried out. It is very important that a well coordinated, cooperative program be established among all institutions and researchers dealing with seaweed research, especially when very sophisticated techniques will be applied. This is where the Indonesian National Research Council can play a role and give guidance and stimulation.

Let me close by once again extending our gratitude to the U.S. Agency for International Development and the U.S National Academy of Sciences for the assistance and cooperation that make this scientific rendezvous between U.S. and Indonesian scientists possible. Our thanks are also due to all the participants in the workshop. We are confident that the deliberations will be fruitful and an invaluable input to the Indonesian National Research Council so that it can formulate suggestions for the Minister of State for Research and Technology, who will in turn formulate policies and programs for research on natural resources.

APPENDIX C

The Center for Research and Development in Biotechnology at Cibinong

Susono Saono Professor National Biological Institute Indonesian Institute of Sciences Didin S. Sastrapradja Assistant Minister of State for Research and Technology

INTRODUCTION

There is a general consensus that biotechnology will play an important role in the advancement of human welfare in the near future. This results largely from its flexibility and commercial potential in various industrial sectors such as pharmaceuticals, health care, chemicals, energy, agriculture, animal health and nutrition, food, and waste treatment. Because biotechnology is adaptable to diverse conditions, it can be applied to the traditional, simple food and beverage fermentations as well as to modern pharmaceutical manufacturing.

The state of biotechnology in a particular country depends, among other things on its industrial base, the availability of skilled and trained manpower, financial resources, supporting facilities, and the market. In many developing countries where these resources are limited, biotechnology is largely simple and traditional in nature, yielding nigh-volume/low-value products. In contrast, the dominant feature of biotechnology in the developed countries is its sophistication: thus it yields low-volume/high-value products.

Based on its GNP, Indonesia is a developing country. However, because of its rich natural resources, large population (a potential manpower resource and market), and sound economy, there is a real need to introduce the so-called "new" biotechnology. Such an endeavor has a strategic importance because it presents the opportunity to produce a larger variety of high value-added products which will not only save a substantial amount of funds for import substitutions, but could also serve as an additional source of income for the country. As a spin-off, the introduction of the "new" biotechnology is also expected to induce an improvement in science teaching in the schools and universities, especially in basic sciences related to biotechnology such as microbiology, biochemistry, genetics, and molecular biology.

Because development of the "new" biotechnology requires a large capital investment and substantial well qualified manpower, it is more economical with the present situation in Indonesia to establish a new, well equipped and well funded research and development center for

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biotechnology (the Center for Research and Development in Biotechnology at Cibinong) than support the existing research and development institutes under various government organizations.

OBJECTIVES OF THE CENTER

Based on the above considerations, the objectives of the Center for Research and Development in Biotechnology at Cibinong are to develop the national capability to conduct research and development in the "new" biotechnology and genetic engineering and to provide the sophisticated facilities needed for this undertaking. In the long run, it is expected that the biotechnological approach can be utilized to increase and promote the economic value of natural resources for food, feed, medicines, chemicals, and energy.

GUIDING POLICY

In developing the Center, the following guiding principles will be observed:

- The activities of the Center will be cross sectoral in character.
- o The program of the Center will become the focal point of a national network in biotechnology.
- o The products of the Center's activities will be information and products (prototypes) that are low volume/high value in character, with extensive market potential and usable for scaling up studies.

Scope of Activities

Projects

To realize its objectives, the Center will formulate selected research and development projects that support national programs in the fields of family planning, health care, food production, animal production, and the manufacture of pharmaceuticals and other biologically active compounds. For example, projects would likely include the production of steroidal compounds as raw materials for oral contraceptives and drugs, vaccines for human and animal health care, and important pharmaceuticals and other biologically active compounds. Other projects might include cattle breeding by embryo transplantation and economic plant propagation by tissue and cell culture.

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Manpower

So that the Center can function immediately, it is planned that scientists now conducting biotechnological research in various research institutions will be appointed to the Center staff. They will be given support to carry out research on Center projects in their respective institutes before the laboratories and other facilities of the Center have been completed. Simultaneously, new university graduates will be recruited and sent to established universities abroad for advanced degree training in the disciplines underlying biotechnology such as microbiology, biochemistry, genetics, molecular biology, biochemical engineering, and enzyme technology. It is anticipated that by the end of the second five-year phase, the Center will have a staff of at least 20 Ph.D.s, supported by about 40 M.Sc. and other graduate research assistants. The latter will consist mainly of capable technicians such as process development engineers, chemical engineers, microbiological analysts, chemical analysts, mechanical engineers, electrotechnicians, and laboratory technicians.

Facilities

The Center will be located on 200 hectares of government land at Cibinong situated between Jakarta and Bogor. Pharmaceutical and food processing factories, related research laboratories (agriculture, biology, health, animal husbandry), as well as universities are located nearby. It is planned that within the first five year phase the following facilities will be constructed:

- A two-storey research and development laboratory (2,000 m²) for applied microbiology and tissue culture
- A two-storey research and development laboratory (2,000 m²) for biochemistry and molecular biology
- o A general purpose processing laboratory (500 m²)
- Supporting facilities consisting of an emergency power house, a water pump house, a small wastewater treatment facility, a workshop, and a number of greenhouses
- o Staff housing.

During the current fiscal year (1985-1986), preparation will begin for construction of the laboratory for applied microbiology and tissue culture.

MANAGEMENT OF THE CENTER

Since the Center is expected to become the focal point of a national biotechnology network, it will have an advisory board drawn from officials of the various research and development agencies, universities, and the private sector. The forerunner of such an - 96 -

advisory board was set up by the Minister of State for Research and Technology, and it consisted of representatives of various research and development agencies and universities.

The day-to-day activities of the Center will be run by an administration comprising a director, laboratory and administrative supervisors, and a supporting staff.

COOPERATION

It is envisaged that the Center will establish close cooperation with related research and development institutions as well as universities through collaborative research and exchange of staff and information to achieve common goals.

Regional and international cooperation are much needed to develop the Center. It is expected that the developed countries will make a significant contribution during the developmental phase of the Center in the form of equipment and other research supporting facilities, as well as manpower training. The latter is particularly important because almost all the scientific disciplines needed are not offered by Indonesian universities as advanced degree training programs In service training in the established research institutions abroad, especially on the specific techniques of biotechnology, will also be needed to retrain the existing manpower to deal with new developments in this field.

It is also envisaged that joint research and development ventures in selected fields will be established between the Center and biotechnological research institutions abroad.

LONG-TERM PLANNING

As a research and development center as well as a knot in the national biotechnology network, the Center should eventually be able to:

- Become a partner of the university in manpower training in the field of biotechnology and related disciplines
- Provide basic information on and findings in biotechnology to various research and development institutions
- Serve as a link between the national biotechnology network and regional and international institutions or organizations dealing with biotechnology
- o Serve as a clearinghouse for the national biotechnology network.

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FUND ING

During the first and second five-year development phases, the Center will be funded by the government of Indonesia and foreign assistance. Starting with the third five-year development phase, however, the Center is expected to be gradually able to finance its activities through contract research and the provision of facilities as well as consultants in the field of biotechnology. It is expected that this ability will increase with time.

APPENDIX D

Closing Remarks

Richard C. Howland Deputy Chief of Mission U.S. Embassy

During the past three days you have heard about and discussed the limitless conceptual possibilities of scientific development in the study of algae and the near-term product development possibilities from mutant selection, hybridization, etc. You have also heard that in the limited time span of only two years, a small group of Indonesian scientists has made major headway in research and has successfully developed 750 Indonesian families into algae farmers.

As a layman I find the technical developments now being discussed almost beyond imagination. As an informed observer I find the work of the Indonesian scientists and of the algae farmers clear evidence that Indonesia is ready for a major advance in biotechnological development. The big questions are what follows now and how does Indonesia go about achieving it.

In his paper discussing the biotechnological research center in Cibinong, Dr. Didin Sastrapadja made a very telling point that the most important factor in any development is an adequate supply of trained and experienced people. Without such people no progress can be made, and if the numbers available are too few progress can only be slow and constrained to faltering steps. The key to development is adequate numbers of people, well trained and with practical experience.

Minister Habibie, this has been a recurring theme in your speeches and discussions over the last several years. It is a theme that we in the U.S. Embassy agree with wholeheartedly and to the support of which substantial sums have been dedicated. Minister Habibie and distinguished scientists, I congratulate you on a successful workshop. On behalf of Ambassador Holdridge and myself, I wish you a very successful future in this important field, and I thank you again for the great honor of sharing this subject and this time with you.

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APPENDIX E

Closing Address and New Directions

B. J. Habibie Minister of State for Research and Technology Chairman, Indonesian National Research Council (DRN)

In both my capacities as minister of state for research and technology and as chairman of the Indonesian National Research Council, I would like to add a personal word--not only a somewhat belated welcome, but at this point, and perhaps more appropriately, also a word of thanks for your presence, for your participation, and for the valuable contributions that you have made to this Workshop on Marine Algae Biotechnology.

Frankly speaking I have been looking forward to this first in a series of joint DRN National Academy of Sciences workshops since I signed the agreement on mutual cooperation with Professor Keyworth in Washington, D.C. in the early summer of 1984. Therefore, I was delighted to learn two months ago from the secretary of the Indonesian National Research Council that the present workshop was to take place in early December, after the USAID-NAS agreement was signed on October 2, 1985.

Allow me now to dwell on an explanation which I address to all present here concerning the direction in which I would like to see DRN-NAS cooperation and their deliberations develop.

As you may be aware, the first phase of the agreement between DRN and the U.S. National Academy of Sciences covers the period 1985-1987. Within this period four major subjects have been selected thus far for a series of workshops: marine algae biotechnology, oceanology and marine resources, biotechnology, and development of a science and technology information system. A possible fifth topic to be considered is technology transfer to technologically backward ethnic groups.

Each has been selected with a view toward the problems that my country faces in its progress toward a take-off stage in less than 15 years from now. Two of the most relevant and urgent problems in great need of relatively rapid solutions are scientific manpower development and the acquisition of indispensible research facilities. It is my deepest conviction that in a world of rapid technological progress, Indonesia, as a big developing country bestowed with a wealth of natural as well as human resources, cannot afford to remain ignorant of the most sophisticated technological advancements.

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Indeed, I am also aware of my country's potential, and it is precisely because of my awareness of existing backlogs and shortcomings that I deem it necessary to maintain good relationships with technologically advanced countries to enable a continuing dialogue. In that scenario, it is my contention that the North South dialogue on problems of research and technology will benefit both counterparts. It is not only the technologically advanced North that can transfer technology and science. The South offers a tremendous amount of varied conditions in light of which traditional and patterned solutions may have to be revised and improved. It is these new challenges that we as scientists seem to be facing. It is therefore important to establish and maintain an effective communication network through which consultations and continuous exchange of thought will be possible.

With regard to the topic of this workshop, I would like to say that the choice has been guided by the fact that among our many natural resources, our marine resources have been relatively unexplored and unexploited, except perhaps for fisheries. Participants in this workshop have been made aware of our programs to develop biotechnological research relevant to marine resources.

Having listened carefully to the findings and recommendations of this workshop, I am most confident that at least part of the recommendations can be implemented within the foreseeable future It is this spirit of bringing the world more practical policymaking that I have my greatest appreciation for, and I would like to see the forthcoming workshops in the DRN-NAS series conducted in this spirit. I would like to reiterate what I have said on many occasions. It is in the service of development in general, and of human prosperity in particular, that the blend of scientific guidance and the wisdom of policymaking should be put to its best use.

Finally, I am looking forward to the printed proceedings of the discussions that took place in this workshop which would preferably be circulated in two languages--Indonesian and English-for wider distribution among interested scientists as well as policymakers. I thank you again for your contributions to this workshop, and I would like to extend, more particularly to our American friends, my early good wishes for Christmas and the New Year:

The sequence of activities that has already been scheduled by DRN and NAS for 1986 assures me that I can close this fruitful workshop by saying: Sampai bertemu lagi! (See you again!)

APPENDIX F

Workshop Agenda

Wednesday, December 11

Morning **Opening Ceremony** Report by the Seaweed Research Team Organizing Committee Dra. Rachmaniar Rachmat, Chairperson **Opening Remarks** Mr. Richard C. Howland Deputy Chief of Mission U.S Embassy Keynote Address Dr. Doddy A. Tisna Amidjaja Vice-Chairman Indonesian National Research Council Coffee Session I: Dr. A. M. Satari, Chairman; Hasan Mubarak and Untung Suwahyono, Rapporteurs The Center for Research and Development in Biotechnology at Cibinong Dr. Didin S. Sastrapradja, Assistant Minister of State for Research and Technology The Potential of Marine Algae for Biotechnological Products in Indonesia Dr. Aprilani Soegiarto, Director, National Institute of Oceanology Uses of Marine Algae in Biotechnology and by Industry Dr. Donald W. Renn, Senior Research Fellow, FMC Corporation - 101 -

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Lunch

Afternoon	Session II: Dr. Aprilani Soegiarto, Chairman; Mr. Jana Anggadiredja, Rapporteur
	Biological and Economic Approaches to Industrial Development Based on Marine Algae in Indonesia Dr. Maxwell S Doty Professor of Botany, University of Hawaii
	Screening Species of Brown and Red Algae Collected from the Coast of Spermonde for Active Substances Tjodi Harlim, Lecturer, University of Hasanuddin
Coffee	Adjournment
Thursday, December 12	
Morning	Session III: Dr. Indrawati Gandjar, Chairperson; Daja Pamudji, Rapporteur Seaweed as a Raw Material for Industry
	Mr. Jana Anggadiredja on behalf of the BPPT Seaweed Research Team
	Marine Algae Biotechnology: Possibilities and Realities Dr. Donald W. Renn, Senior Research Fellow, FMC Corporation

Coffee

Marine Algae Biotechnology: Ecological Context and Manpower Needs for Indonesia Dr. Dirk Frankenberg, Director, Marine Science Program, University of North Carolina at Chapel Hill

Lunch

Afternoon Group Discussions

Research Group: Dr. Aprilani Soegiarto, Chairman; Mr. Wardana Ismail, Rapporteur

Development Group: Dr. A. M. Satari, Chairman; Mr. Hasan Mubarak, Rapporteur

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Friday, December 13

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Morning	Plenary Session: Dr. Sediono Tjondronegoro, Chairman; Dra. Rachmaniar Rachmat and Achmad Zatnika, Rapporteurs
Lunch	
Afternoon	Summary of Conclusions and Recommendations Dra. Rachmaniar Rachmat
	Comments by Chairman of the U.S. Panel Dr. Dirk Frankenberg
	Closing Remarks Mr. Richard C. Howland
	Closing Address and New Directions Dr. B. J. Habibie, Minister of State for Research and Technology, and Chairman, Indonesian National Research Council

APPENDIX G

Workshop Participants

Steering Committee

- Dr. Sediono Tjondronegoro, Secretary, Indonesian National Research Council (Chairman and Workshop Chairman)
- Dr. Didin S. Sastrapradja, Assistant Minister for Research and Technology
- Dr. A. M. Satari, Deputy Chairman for Basic and Applied Sciences, Agency for the Assessment and Application of Technology, (BPPT)
- Dr. Aprilani Soegiarto, Director, National Institute Of Oceanology, Indonesian Institute of Sciences, (LIPI)

Organizing Committee

Dra. Rachmaniar Rachmat, BPPT (Chairman)

Drs. Jana Anggadiredja, Ministry of State for Research and Technology

- Ir. Achmad Zatnika
- Ir. Sulistijo, National Institute of Oceanology, LIPI
- Ir. Sri Istini, BPPT
- Ir. Dadang Permadi, Ministry of State for Research and Technology
- Ir. Suhaimi, BPPT

Ir. Hasan Mubarak, Agency of Agricultural Research and Development Drs. Tito Kustim, LIPI

Mr. Sutarjo, Ministry of State for Research and Technology

Abdul Firman, Ministry of State for Research and Technology

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Suki Julu Salasikin, LIPI

Budi Minerva, Ministry of State for Research and Technology

Indang Wahyurini, Ministry of State for Research and Technology

Ms. Yessy, Ministry of State for Research and Technology

Ms. Asti Suryani, Ministry of State for Research and Technology

Prapti Mulyani, BPPT

Mr. Dodi, BPPT

U.S. NRC Panel Members

- Dr. Dirk Frankenberg, Director, Marine Sciences Program, University Of North Carolina of Chapel Hill (Chairman and Workshop Vice-Chairman)
- Dr. Donald W. Renn, Senior Research Fellow, FMC Corporation
- Dr. Maxwell S. Doty, Professor. Department Of Botany, University of Hawaii
- Mrs. Rose Bannigan, Program Development Coordinator, Board on Science and Technology for International Development, National Research Council

Other Participants

Mr. Daja Pamudji, BPPT

- Mr. Soemarsono, BPPT
- Mr. Haryanto Dhanutirto, BPPT
- Mrs. Sri Sugati, Department of Health
- Mrs. Lilik Purwaningsih, Department of Industries
- Mr. R. W. Wahyu W., Department of Industries
- Mr. Isroil Samihardjo, Department of Defense and Security
- Mrs. Ida Ikram, Bogor Agricultural University
- Mr. Sumpeno Putro, Fish Research and Development Center
- Mr. A. Basrah Enie, Department of Industries

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Mr. Wanda S. Atmadja, National Institute of Oceanology Mr. Wibowo Mangunwardoyo, University of Indonesia Mr. Edi Setianto, U.S Agency for International Development (USAID) Mr. Bogie, Bosowa Mining Mr. S Matsumoto, Bosowa Mining Mr. M. Syarif Hitam, Bogor Agricultural University Mrs. Indrawati Gandjar, University of Indonesia Mr. Djiwa Darmadja, University of Udayana, Bali Mr. Indriyarso, BPPT Mr. Mochtar Machful, Ministry of State for Research and Technology Mr. Waluyo Subani, Fish Research and Development Center Mr. Ben Budiman, Sinar Mutiara Indonesia Ltd. Mr. Tjodi Harlim, University of Hassanudin, Ujung Pandang Mr. Hariadi Adnan, The Copenhagen P.F. Ltd. Mr. I. M. P. Suardhana, Regional Planning Board of Bali Province Mr. M. Alwi, Regional Planning Board of West Nusatenggara Province Mr. Tuntomo, Dinamika Sulawesi Ltd. Mr. Yoesman, Regional Planning Board of West Nusatenggara Province Mrs. Irma, Amazon Yudha Ltd. Mr. Nico P. M., Amazon Yudha Ltd. Mr. Bambang Tjiptorahardi, Bima Sakti Ltd. Mr. R. Sunaryo Mr. Asmaun Tan, Dunia Walet, Ltd. Mr. Trisna Wijaya, Dunia Walet, Ltd.

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Other Attendees

- Dr. D. A. Tisna Amidjaja, Chairman, LIPI
- Mr. J.H. Hutasoit, Junior Minister of Fish and Husbandry, Department of of Agriculture
- Mr. Jacob Jennes, Neraca News
- Ms. Diah Asri Erowati, BPPT
- Mr. Ron Redman, USAID
- Mr. Bardis Winta, BPPT
- Mr. Endro Kardona, BPPT
- Mr. Desmond O'Riordan, USAID
- Mr. Richard C. Howland, U.S. Embassy, Jakarta
- Ms. Saraswati, BPPT
- Mr. Andri, BPPT
- Mr. K. Algamar, BPPT
- Mr. Todo Tambunan, Science and Technology Research Center
- Mr. Joko P. S., BPPT
- Mr. Effendi, Dunia Walet, Ltd.
- Mr. Hanuna Mappa, Regional Planning Board of South Sulawesi Province
- Mr. William P. Fuller, USAID
- Mr. Rozik B. S., BPPT
- Mr. Maman Rochamar, BPPT
- Mr. Sabana Kartasasmita, Ministry of State for Research and Technology
- Mr. Susono Saono, National Biological Research Center
- Mr. R. Sihombing, Antara News
- Mr. Rusmin, U.S. Information Service
- Mr. B. S. Daniel, Sinar Harapan News
- Mr. Jusdy Achmad, Ministry of State for Research and Technology

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Working Groups

Research Group

Sediono Tjondronegoro

Aprilani Soegiarto Donald W. Renn Ida Ikram Sunaryo Wanda S. Atmadja M. Syarif Hitam Sri Sugati Waluyo Subani Isroil Samihardjo Wibowo Mangunwardoyo Djiwa Darmadja Achmad Zatnika Sri Istini Suhaimi Husan Mubarak Development Group Maxwell Doty S. Matsumoto Yoesman Dirk Frankenberg M. Alwi Sumarsoo A. Basrah Enie Lilik Purwaningsih R. W. Wahyu W. Irma Ben Budiman Nico P.M. Tuntomo Mochtar Machful Rose Bannigan I. M. P. Suardhana Hariadi Adnan Bogie Trisna Wijaya Asmaun Tan Bambang Tjiptorahardi A.M. Satari Sumpeno Putro Sulistyo

Jana Anggadiredja