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Experiments on the Preliminary Curing of Irish Moss, 1944

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Due principally to wartime conditions, the scarcity of agar has created a need for a substitute material. A possible substitute is the mucilage or gelose obtained from an alga, *Chondrus crispus*. This grows in large quantities in the waters of the east coast of Canada particularly around Prince Edward Island. It is harvested by raking and then dried in the sun. Moss once dried is known as black moss or unbleached moss. On soaking in water and leaving the moss exposed to the sun, bleaching occurs and a light brown or cream colour is produced. This is the product principally desired for commercial use.

Bleaching and drying is carried out near the point of collection by the harvester, or sometimes, after being once dried, it is sold to one of the larger dealers who bleach it themselves before export. Some is sold as black moss.

A variety of methods are used for bleaching and drying. The main differences between them are in forms of apparatus for suspension, frequency of wetting and type of moistening solution.

Scope of Investigation

In this investigation, variation in (1) apparatus for suspension, (2) condition of moisture, (3) moistening solution and (4) temperature, light and weather conditions were studied to determine the optimum conditions for natural drying and bleaching. Any effect on the gelose was assumed to be indicated by viscosity measurements. Moss was also bleached under glass and in the laboratory by means of ultra violet light and a General Electric drying lamp of 250 watts, using a thick suspension stirred with a mechanical stirrer or by bubbling air through it. For natural drying and bleaching, board, cement and wire trays were used for suspension. Salt solutions used, other than sea water, were 3% solutions of NaCl, CaCl₂, KCl and fresh water. The moss was wet by soaking for various lengths of time or by sprinkling. While bleaching or drying a record of temperature, wind, extent of sunlight and humidity was kept, and an attempt was made to correlate this with the time necessary for bleaching or drying. Bleaching and drying had to be considered separately since it was essential that the moss be wet in order to bleach. The resultant samples were ground and the viscosity of their gels studied.

Material

Most of the moss used was raked moss obtained from Egmont Bay, P.E.I. Three other lots were gathered (1) at Tignish, P.E.I. (2) Phee's Shore, P.E.I. (3) Yarmouth County, N.S. These last three were compared as regards viscosity of gel. No great difference was found. The drying and bleaching were carried out at Ellerslie, P.E.I. within 14 hours of collection. The Yarmouth County moss was a commercially bleached product.

The Effect of Surface on the rapidity of drying and bleaching.

The surfaces chosen were (1) wire netting of $\frac{1}{2}$ -inch mesh (2) board surface unpainted (3) board surface painted black (4) cement. The trays used were two feet square and had 2" x 2" sides. They were mounted on two rails about 3 feet from the ground. Various depths of moss were used from a single layer to 3 inches. In the drying experiments the material was turned and mixed once each hour. Both bleached and unbleached moss was used. The fresh Chondrus had a moisture content of about 91%. In the bleached Chondrus the moisture had been reduced in bleaching to 40 to 60%.

A preliminary experiment showed overwhelming superiority of the board or cement trays over wire for bleaching. Wire was therefore not used further except for drying.

For drying, specimens of 1000 gm. were placed on wire, board and cement trays. At the end of a given period 20-25 gms. were removed, cut finely with scissors and mixed, and 4-5 gms. used to determine the moisture content. The following results were obtained in unbleached material.

Table 1.

Effect of surface on rapidity of drying

Weather			'Time' 'Hrs.'	Wire	Board	Cement			
Air Temp. °F	Humidity %	Wind	'moisture' 'content	'loss in' 'moisture'	'moisture' 'content	'loss in' 'moisture'			
			%	%	%	%			
60	70	very light	8	24.1	67.4	33.2	58.3	32.2	59.2
			11	13.5	78.1	24.1	67.4		
68	20 min. shower moss covered	moder- ate	15	19.9	71.5	19.1	72.4	15.1	76.4
			18	13.4	78.0	14.9	76.6		
70	50	moder- ate	25	9.7	81.7	8.5	82.9	9.0	82.5
60	63	light	3	36.8	54.7	20.2	72.3	21.0	70.5
			6.5	17.5	75.2	13.5	78.0		
			8.5	13.5	78.0			13.8	77.7

The actual loss in weight in these surfaces was also determined. 200-gm. samples were used. They were dried for a period of 8 hours and then removed and weighed. The moss was carefully turned over each hour so as to dry uniformly. (Table 2). About one quarter of the sample was used in the determination of dried weight.

Table 2.

Effect of surface on loss in weight, initial weight 200 grams.

Day	Type of tray	Final weight	Loss in weight	Dry weight	Difference between trays
		gms.	%		
1	Wire	60.2	69.9	48.3	6.3
	Board	76.1	61.9	61.0	
2	Wire	66.9	62.0	52.7	3.5
	Board	76.9	72.1	59.6	
3	Wire	84.2	57.9	56.6	1.2
	Board	92.1	53.4	61.0	
4	Wire	91.9	54.0	62.4	3.6
	Board	96.2	51.9	69.5	
5	Wire	93.0	53.5	59.6	.1
	Board	94.4	52.8	59.8	
6	Wire	90.4	54.8	51.4	.8
	Board	94.3	52.8	53.0	
2	Cement	74.9	63.0	57.8	wire & cement 3.2
1	Cement	71.0	65.0	60.5	4.5

There occurred a difference in moisture content on the various surfaces. This is shown (Table 3) for several days. 200-gram samples were used and dried for 8 hours. Then, as before, moisture determinations were made.

Table 3.

Effect of surface on moisture content of unbleached moss.

Day	Moisture Content		
	Wire	Board	Cement
1	19.6	19.9	22.6
1	21.1	22.5	14.7
2	30.5	33.8	
2	32.1	27.8	
3	35.8	36.4	
3	43.1	43.2	

Bleached moss gave the following figures. Sea water was used as the moistening agent and when bleached the moss had a moisture content of 52.1%.

Table 4.

<u>Effect of surface on moisture content of bleached moss.</u>						
Av. Temp. °F	Humidity %	Wind	Time hrs.	Moisture Content %		
				Wire	Board	
68	50	light	8	33.2	25.7	
			15	14.6	14.9	
			25	9.5	8.5	

It will be seen from these results that there is very little difference between the wire, board, and cement trays. An inspection of Table 1 shows that the initial drying to about 25% is more rapid in the wire trays due probably to a better circulation of air. Excess water would also drain through to the ground. It was noticed that, in drying in board trays with a considerable thickness of moss (2-3 inches), the surface layer would be dry to touch while the layers near the board would be resting in a collection of water. This could largely be prevented by turning and mixing the moss frequently. On the other hand there is likely to be a loss of material through the interstices of the wire. This is small, however, amounting in 200-gram samples (Table 2) to 0.1% to 6.3% more than in board or cement. Cement compares favourably with board trays. Table 1 shows an increase in moisture from 11 to 15 hours. During this time the weather became dull and there was a 20-minute shower.

The variation in dry weight is from 48.3 to 69.5 grams on 200 grams, giving a yield of 30% or 33% assuming a 10% moisture content of the dried moss.

Effect of increased depths on time of drying.

The time of drying is increased with an increased thickness of moss. Lots of 0.5, 2, and 6 kilograms of unbleached moss were placed on board trays. This gave a depth of moss from a single layer to about 3 inches. The material was turned once each hour and, as before, the moisture content determined in a sample of 25 to 30 grams taken at random.

Table 5 **Effect of depth on time of drying**

Type of tray	Temperature	Time hrs.	Moisture Content		
			Single layer	1½" deep	3" deep
Wire	65	6	20.9	36.2	49.7
Board	65	6	18.8	37.1	50.6

Time required to reduce moisture content to 10%

In order to determine the time required to reach less than 10% from an initial moisture content of 91% the samples were allowed to dry. Moisture determinations were made twice a day.

Table 6.

Time required to reach less than 10% moisture

	Time hrs.	Moisture Content %
Single layer	16	8.2
1½" deep	23	9.6
3" deep	36	9.4

The moss remained out from 9.30 A.M. to 5.30 P.M. and was taken inside at night.

It will be seen that a 3-inch layer requires about three times the length of time to dry as a single layer, the 1½" layer being intermediate. This was the same in both board and wire trays.

Black-painted board compared to unpainted

Samples of 500 gm. were left out from 9.30 A.M. to 5.30 P.M. on each type of board. The samples had been partially dried and had an initial moisture content of 65.4%. At the end of the experiment the one on a black-painted board had dropped to 18.6% while that on the unpainted board 17.9%. This would indicate a negligible difference between the two, if any.

Variation in rapidity of drying with time of day.

Specimens were placed on wire trays at various periods throughout the day and moisture content determined at the beginning and end of two to four hours. Partially dried samples were used as well as the wet material. Table 7.

Table 7.

Variation in rapidity of drying with time of day

Air Temperature		Period of Day	Time hrs.	Initial	Final	Loss in Moisture %
Max.	Min.			Moisture Content %	Moisture Content %	
Unbleached Moss						
42	54	9.30 A.M. to 12.30 P.M.	3	91.5	34.3	57.2
47	61	12.30 P.M. to 3.30 P.M.	3	91.5	20.9	70.6
46	66	3.30 P.M. to 5.30 P.M.	2	91.5	20.9	70.6
42	64	9.30 A.M. to 12.30 P.M.	3	49.7	18.6	34.1
45	62	12.30 P.M. to 4.30 P.M.	4	31.8	17.7	14.1
46	59	9.00 A.M. to 12.00 P.M.	3	19.9	13.4	6.5
48	70	12.00 P.M. to 3.30 P.M.	3	24.1	10.0	14.1
73	64	10.00 A.M. to 12.00 P.M.	2	14.6	12.0	2.4
Bleached Moss						
62	54	10.00 A.M. to 12.00 P.M.	2	23.1	11.52	11.6
		12.00 P.M. to 2.30 P.M.	2.5	11.52	8.35	3.1
		12.00 P.M. to 2.30 P.M.	2.5	16.4	9.31	7.1
75	62	2.30 P.M. to 4.00 P.M.	1.5	9.3	10.2	0.9
		2.30 P.M. to 4.00 P.M.	1.5	9.15	10.9	1.7

It is noteworthy that the last figures were obtained on a day when it became dull at 3.30 P.M. and there was a shower at 4.30 P.M. The sun appeared again at 8.00 P.M.

Table 8.

Increase in moisture content between 5.30 and 10.00 P.M.

No.	Initial Moisture Content %	Final Moisture Content %	Increase in Moisture %	Time hrs.
1	9.8	22.1	12.5	4.5
2	13.1	31.8	18.7	4.5
3	13.7	28.8	15.1	4.5
4	16.8	28.2	11.4	4.5
5	6.35	18.5	12.1	4.5

(No. 2 was sea-water washed but from North Shore of Island, a much bushier plant.)

It will be seen from Table 7 that an initial drop in moisture from 91.5% to 20 to 30% can occur in three hours. A decrease below this value requires a longer time. The greatest decrease occurred in the afternoon - a decrease of 70.6% from 12.30 A.M. to 3.30 P.M. in comparison to 57.2% from 9.30 A.M. to 12.30 P.M. Material with an initial moisture content of about 20% to 24% showed the same giving a decrease of 6.5% in the morning to 14.1% in the afternoon. Conditions for drying were good the humidity was low and there was a light breeze.

The last two figures in the table show an absorption of moisture rather than a decrease. This was undoubtedly due to absorption from the atmosphere during the rain. The same phenomena occurred at night (Table 8) an increase of 11.4% to 18.7% showing in 4½ hours.

Effect of moistening agent on drying of bleached moss.

No uniformity of results was obtained on varying the concentration of salts in the water. (Table 9). Drying was, however, slightly more rapid after using an acid moistening agent (Table 10).

Table 9.

Weather				Initial	Final			Type of
Temperature				Moisture	Moisture	Loss in	Time	Moistening
Max.	Min.	'Sunlight'	Wind	Content	Content	Moisture	'hrs.'	Agent
'F	'F			%	%	%		
78	60	Part cloudy	'Strong winds'	25.9	16.9	9.0	8.5	Sea water
68	63			24.8	18.9	5.9	8.5	Sea water
				18.65	17.3	1.6	8.5	2/3 sea water
		50% Humidity		21.5	18.9	4.6	8.5	2/3 sea water
				20.4	13.95	6.5	8.5	Fresh water
				17.2	15.2	2.0	8.5	Fresh water

Table 10.

Rapidly of drying after bleaching at pH 2 using HCl + CaCl ₂ and HCl + NaCl.								
Weather				Initial	Final	Loss in	Time	Type of
Temperature	Min.	Sunlight	Wind	Moisture	Moisture			
Max.	°F			Content	Content	%		Agent
				%	%	%		
69	50	Bright 59% Humidity	Strong winds	52.1	39.9	12.2	5	Sea water
				49.6	34.6	15.0	5	HCl CaCl ₂ 3%
				50.1	41.2	8.9	5	HCl NaCl 3%
68	53	Part Cloudy 52% Humidity	Moder- ate wind	39.9	25.4	14.5	7	Sea water
				34.6	24.6	10.0	7	HCl CaCl ₂ 3%
				41.2	23.2	18.0	7	HCl NaCl 3%
68	53	Overcast	Light	25.4	13.1	12.3	15	Sea water
75	62	Bright 48% Humidity		24.6	13.7	10.9	15	HCl CaCl ₂ 3%
				23.2	15.8	6.6	15	HCl NaCl 3%

Effect of moisture content on mould.

It is of interest that several samples with a moisture content of 29% and over developed mould in the laboratory. Those below 15% did not.

Effect of moistening agent on gelose content after drying.

There was no uniform variation in gelose content with any of the variously dried specimens.

Table 11.

Gel content after drying	
Moistening Solution	% Gelose
Sea water	50.0
Fresh water	52.3
Sea water	50.4
Fresh water	50.7
2% CaCl ₂	50.4
2% CaCl ₂	47.0
2% KCl	54.2
3% NaCl	47.0

The gelose content was determined after the manner of Hass and Russell-Wells (Analyst 52, 265-9). Other samples showed the same range of 45% to 60% gelose. In the preparation of the gelose, however, by precipitation with alcohol only a 30% - 40% yield was obtained.

In bleaching with fresh water as moistening agent the moss was soaked repeatedly in the same wash water. The results (table 11) would indicate that fresh water unless in considerable excess or on prolonged contact with the moss does not remove appreciable gelose.

Experiments on conditions for bleaching.

Conditions of moisture and moistening solutions were changed, and, as in the drying experiments, a record of the weather was kept.

An attempt was first made to measure extent of bleaching in a definite time. The colors of the solutions prepared by dissolving fresh moss, partially-bleached moss (commercial "one-half bleached") and bleached moss did not differ enough to form a basis for comparison with a colorimeter. The fresh moss gave a green solution, the other two were brown and differed somewhat but gradations between the two could not have been read. The gel itself gave a better indication of extent of bleach and gradations might possibly have been measured with a tintometer. The time of extraction and concentration of the final gel would need to be rigorously controlled.

The possibility that the color could be extracted by some solvent was not overlooked and the following were tried: Ethyl and methyl alcohol, ether (ethyl) butyl and isopropyl alcohol, acetone, benzene, xylol, petrol, diethylene, ethylene glycol dioxane, chloroform, cyclohexane, tetralin, decalin, ethyl butyrate, and pyridine. The coloring material in bleached or dried moss was insoluble in all the reagents. Ethyl alcohol however extracted the color from green moss leaving it a creamy white. 50% alcohol also extracted some of the gel. In 95% alcohol the moss became very brittle probably due to dehydration. Phenol when added to a solution of the moss separated into a layer containing all the pigment leaving a colorless aqueous layer.

Since from a commercial standpoint the fully-bleached moss was the required product, bleaching was judged on the time required to reach a completely bleached stage. The color varied somewhat and was never white after drying but shades of brown. Green and all shades of purple, however, had to disappear before the material was considered bleached. Bleached moss before drying had a much whiter shade.

The salt used in the moistening solution affected the whiteness of the resulting moss and so, to some extent, did the rapidity of bleaching. Judged solely by the unaided eye the whiteness of the resulting bleached moss while wet was in the order 2% CaCl₂, 2/3 sea water, fresh water, 2% NaCl, sea water. On drying any difference disappeared. It will be shown that the moistening solution had considerable effect in the viscosity of the resulting gelose.

Optimum moisture content for bleaching

20-gm. samples of moss from which the stems had been removed were soaked in sea water for from 2 to 35 minutes. These samples had previously been dried at 70°C to 12.1% moisture. On being removed they were superficially dried and divided in two parts. The first of about 5 grams was used for the determination of moisture content. The larger portion of about 15 grams was placed in a 500 cc. Erlenmeyer flask which was stoppered and had neck and cork coated with paraffin wax. The flasks were then inverted in the sun. Moisture condensed in the sides of the flasks in all but No. 1.

Table 12.

Optimum moisture content for bleaching		
Moisture Content %	Time required for bleaching	Time of soaking mins.
23.2	slightly after 7 hours	2
30.2	ends bleached after 7 hours	4
43.1	5.5 hours	10
44.4	5.5 hours	15
60.2	5.5 hours	30
69.0	5.5 hours	35
90.5	green after 7 hours	remained in sea water

The time required for the moss to bleach varied with the amount of moisture in the specimens. Those immersed in sea water did not bleach except when very near the surface. Neither did specimens with a moisture content less than 20%. The quickest bleaching occurred in samples with over 40% moisture and remained about the same short of complete immersion, with further increase in moisture content.

As further confirmation fronds were used which were soaked as follows with the results stated.

1. 10 minutes each hour required for bleaching 5 hours.
2. 10 minutes each 2 hours required for bleaching 8 hours.
3. 30 minutes each day required for bleaching over two days.
4. 10 minutes each $\frac{1}{2}$ hour required for bleaching 5 hours.
5. 20 minutes each 4 hours required for bleaching 16 hours.

In order to determine approximately the time required by the moss to reach a sufficiently high moisture concentration to bleach, 500 gms. were dried at 60°C for 12 hours and then placed in 4 litres of water. At intervals 5 to 10 gms. were removed and after being dried superficially in paper towels had their moisture content determined. After 6 minutes the material felt "sticky" to touch when soaked in fresh water. In sea water of 3% NaCl solution the moss remained firm.

From the standpoint of rapidity of bleaching it will be seen that little is gained by soaking after 10 minutes (table 12). In the case of very dry moss however the stems do not take up moisture at the same rate as the fronds, being much slower. As a rule, however, after half an hour a moisture content over 40% is reached. After the initial wetting of the dry moss a soaking of 10 minutes each hour served to maintain its moisture content over 40%.

The effect of covering with glass

The same trays were used as in the drying experiments with the addition of board trays covered with glass, and of a water-proof tray kept half full of water and covered by a wire tray in which the moss was placed, the whole being covered with glass.

The board trays covered with glass gave (Table 13) a much more rapid bleach and a higher final moisture content than did uncovered board trays.

Table 13.

Effect of covering with glass			
Type of tray	Time hrs.	Moisture Content %	Degree of bleaching
Board	10	39.8	$\frac{3}{4}$ bleached
Board		34.5	$\frac{3}{4}$ bleached
Board		33.0	$\frac{3}{4}$ bleached
Board glass covered		70	bleached
Board glass covered		88	bleached

The moss was soaked for 20 minutes at the end of five hours. The extent of bleaching was estimated by comparison with commercial grades given as " $\frac{1}{2}$ ", " $\frac{3}{4}$ " and "fully" bleached. The temperature of the glass-covered samples varied between 37 and 43°C.

It seems that ultraviolet light is not necessary for bleaching since this would be absorbed by the glass covering. The maintenance of increased moisture might be responsible in these same trays for the more efficient bleaching as might the higher temperature. A comparison was made between glass-covered trays (A.) and trays made as indicated above with an additional tray containing water under a wire tray containing the moss (B.). Commercial black moss was used which had a moisture content of 17.6% (Table 14).

Table 14.

Comparison of glass-covered trays			
Tray	Moisture Content %	Degree of bleaching	Time hrs.
A	75	90	8
B	86	bleached	8
A	72	90	8
B	82	bleached	8
Board uncovered	25.2	75	8

The extent of bleaching was judged by the eye. For moss to be called bleached, both stems and fronds had to have a uniform amber to cream color with no green or purple. The degree of admixture of the latter, decided what percentage of bleaching was listed. Commercial "50%" and "75%" bleached were also used for comparison. The temperature of the covered specimens rose to 47°C; the uncovered averaged 23°C.

Using 3% CaCl₂ (Table 15) the moss had a slightly lighter shade. A series gave essentially the same results as in Table 14, with the exception of a lower moisture content which could be due to the additional time.

Table 15.

Effect of 3% CaCl₂ on bleaching

Tray	Moisture Content	Extent of bleaching	Time
A	32.9	bleached	10½
B	45.9	bleached	10½
A	40.5	bleached	10½
B	54.0	bleached	10½
uncovered	22.3	bleached	10½
uncovered	28.0	bleached	10½

Effect of immersion in sea water.

A cement tray was used and sufficient sea water was kept in it to about half cover the moss. The moss above the water was not kept moist, however, but would dry out. The material was then turned. Only the wet material uncovered with water appeared to bleach to any extent. Table 16 gives a comparison of the results with those on 'B' and uncovered trays. Commercial black moss was again used. See also Table 19.

Table 16

Tray	Moisture Content %	Extent of bleach %	Time hrs.
cement containing water	53.9	90	8
B	45.9	bleached	8
uncovered	22.4	75	8
B	59.8	bleached	8
uncovered	30.7	75	8

Effect of temperature on bleaching.

The temperature of some black moss after soaking was maintained at 60°C with a burner and another sample at 12°C by running water around a tin tray containing the sample. A tin tray was also used to hold the heated material. At the end of four hours the moss at the higher temperature was bleached. The second sample at the end of eight hours was only about 3/4 bleached. The material was kept wet with frequent soakings. Since wetting solutions other than sea water were not found to be superior, a diluted sea water was tried.

The effects of an initial drying on bleaching.

The effects of an initial drying before bleaching were compared with those of keeping the moss moist from the start. 1000 gms. of moss were spread on each board tray and treated as follows:

1. soaked 10 minutes in sea water each hour.
2. soaked 10 minutes in sea water each hour after drying for 4 hours.
3. soaked in 2/3 sea water 10 minutes each hour.
4. soaked in 2/3 sea water 10 minutes each hour after drying for 4 hours.
5. soaked in fresh water 10 minutes each hour.
6. soaked in fresh water 10 minutes each hour after drying for 4 hours.

The samples were left out from 9.30 A.M. to 5.30 P.M. each day until bleached. The number of hours required for bleaching are given in Table 17. One sample was continuously sprinkled with sea water. The initial drying had no effect.

Table 17.

Comparison of sea water, 2/3 sea water and fresh water as moistening solution. Also effect of initial drying.

Moistening agent	Temperature	Temperature	Time required for bleaching	
	1st 8 hours	2nd 8 hours	(No initial drying) hrs.	(Initial drying) hrs.
Sea water	64°F-88°F	70°F-88°F	11	11
2/3 sea water			10	10
Fresh water	bright sunlight	bright sunlight	9.5	9.5
Sprinkled			20	20

Effects of dilution of sea water on bleaching.

While wet, the moss appeared whiter in the order: fresh water, 2/3 sea water, salt water, but little difference was apparent on drying.

Effects of pH and calcium in moistening agent.

Moss kept moist by solutions with an acid reaction bleached much more rapidly. Beard trays were again used. As usual the moss was soaked 10 minutes each hour. 1000 gm. samples were used and soaked in:

1. sea water.
2. water at a pH of 4 with HCl.
3. water at a pH of 12 with NaOH.
4. water at a pH of 8.
5. continuously sprinkled with sea water.
6. immersed in sea water during bleaching.

Table 18.

Effect of pH on bleaching		
Temperature °F	pH of moistening solution	Time required for bleaching hrs.
53 - 69	sea water	12
bright sun	4	5.5
	12	over 8
	8	10

Table 19.

Comparison. Soaking. Sprinkling and immersing in sea water.		
Method of wetting	Temperature °F	Time required for bleaching hrs.
Soaking	53 - 69	12
Sprinkling	bright sun	over 16
Immersion		over 16

The acid-bleached material was sticky to touch and had obviously lost much gel. It was very difficult to dry and felt soft after two days. Some of the moistening solution was filtered. This gave a precipitate with benzidine chloride.

The pH of sea water was adjusted with HCl to give an acid reaction and used as a moistening agent. 3 litres of solution were used to wet 1000 gms. of moss. Trays were used containing 1000 gms.

1. sea water at pH 2
2. sea water at pH 4
3. sea water at pH 6
4. 2/3 sea water
5. sea water

Table 20.

Effect of sea water with an acid reaction on time of bleaching.			
Temperature 1st day	Temperature 2nd day	pH	Time hrs.
50- 69	62 - 75	2	6
Bright sunlight	Bright sunlight	4	6
		6	6
		2/3 sea water	12
		sea water	18

As before, the moss remained out from 9.30 A.M. to 5.30 P.M. and time over 8 hours means continuation of the bleaching the next day. In adjusting the pH a B.D.H. universal indicator was used.

3% NaCl and 3% CaCl₂ made acid with HCl was tried. The pH was adjusted to 4. The moss in each case remained firm to touch.

Table 21.

<u>Effect of acid in 3% NaCl and CaCl₂</u>			
<u>Temperature</u> °F	<u>Moistening</u> <u>agent</u>	<u>pH</u>	<u>Time</u> hrs.
53 - 69	Sea water		13
	3% NaCl	4	6
	3% CaCl ₂	4	6

Effect of depth of moss on bleaching.

To determine the relative effect of depth of moss on the time required for bleaching, three board trays were used containing 500 gms., 1500 gms., and 5 kilograms. This gave a depth of a single layer in the first and 1½ and 3 inches in the second and third. The material was soaked in sea water 10 minutes each hour and thoroughly mixed. The times required were: for a single layer 12 hours, for a 1½-inch layer 20 hours, and for a 3-inch layer 34 hours.

This as for drying gives roughly three times as long for a three-inch depth as for a single layer.

Slower bleaching of stems than of fronds.

Stems and fronds were separated and bleached. The stems took 12 hours while the fronds only 4 hours.

Effect of salts other than sea water.

Salt solutions other than sea water had no great accelerating effect in bleaching. One series gave: sea water 16 hours, fresh water 14 hours, 3% CaCl₂ 13 hours, 3% KCl₂ 16 hours, 3% NaCl 14 hours. The addition of CaCl₂ 1% to sea water gave a slightly whiter material but the time was the same.

Effect of weather on bleaching.

The Chondrus was found to bleach in fog as well as sunlight. Bright sunlight and high temperature had an accelerating effect however. Fronds cooled to 8°C with running water bleached in 8 hours compared to 3 hours for material heated to 60°C.

To determine the relative effect of natural variations in sunlight and temperature, samples of 800 gms. were used. These were placed on board trays and allowed to bleach. Each hour the specimens were soaked for 10-15 minutes in about two litres of sea water and replaced in the trays. Samples were run on days with varying degrees of sunlight and different temperatures (Table 22). If bleaching was not completed on the first day, it was completed in the same days for all samples. It was assumed that any acceleration in time would be due to the first day. It will be seen (Table 22) that bleaching occurs much more rapidly in bright sunlight and at a higher temperature than in dull weather or at a lower temperature. Bleaching will not occur in darkness even at a high temperature.

Table 22.

Effect of weather on bleaching					
Temperature		Sunlight	Time		Total time hrs.
Max.	Min.		1st day hrs.	2nd & 3rd days hrs.	
A.M. 73	64	Bright	8	3	11
P.M. 88	70				
A.M. 78	66	Bright	8	5	13
P.M. 65	59				
A.M. 78	62	Bright	8	5	13
P.M. 74	68				
A.M. 69	50	Bright	8	8	16
P.M. 68	53				
Day 72	66	Part cloudy	8	8 + 4	20
Day 65	56	Overcast	8	8 + 8	24
A.M. 74	58	Part cloudy	8	8 + 5	21
P.M. 64	60				

Laboratory examination of Irish moss cured in various ways.

Samples of variously bleached and unbleached moss were used. These were ground in a meat grinder and used to prepare both an extract and a purified gelose powder. Viscosity measurements were made on both these products.

In the preparation of the extract 15 gms. of the chopped moss were soaked for 20 minutes in about 500 cc. of distilled water then out quite fine in a Warner blender. This took from 3 to 4 minutes. The material was then diluted to a litre and heated on a steam bath for six hours. Water was added from time to time to maintain a volume of one litre. The material was then centrifuged and filtered. Evaporation was then carried out in a steam bath until the volume was reduced to about 300 cc., 90 cc. were removed and the viscosity determined by means of a Stormer viscometer. The remainder of the solution was poured with stirring into 1 litre of 95% ethyl alcohol and the precipitate further purified and dehydrated by treatment with two 300 cc. portions of absolute alcohol and two 200 cc. of absolute ether for periods of 12 hours each, finally being placed in a vacuum dessicator for three days. Some small samples, of two grams or so, were dried after the initial alcohol precipitation at 40°C by means of a drying lamp. These will be mentioned later. Approximately 2 gms. of the purified gelose was dissolved in 100 cc. of distilled water about 90 cc. of which was used for the determination of the viscosity.

The amount of gelose was measured by precipitation with benzidine chloride and titration with 0.1 N NaOH after the manner of Hass and Russell-Wells (1). The procedure was as follows: About 90 cc. of the solution was placed in the Stormer viscometer and maintained at a constant temperature of 31°C. The remaining solution was used in a determination of pH using a Beckman pH meter. The relative viscosity given was the average for at least five determinations. After the first determination 10 cc. of solution was withdrawn and replaced by distilled water. The solution was used to determine the gelose content. The replacement by distilled water was continued until the concentration fell below 0.2%. At the end of each fourth determination the gelose content was determined and compared with the calculated value made on the basis of the first and fifth measured values. A comparison is shown in Table 23.

Table 23.

Comparison between observed and calculated concentration

Calculated concentration	Observed concentration
%	%
1.70	1.70
1.12	1.12
0.73	0.72
0.47	0.46
0.34	0.34
0.29	0.27

An attempt was made to measure exactly 90 cc. into the cup and calculate the resulting concentration. With higher concentrations of gelose particularly, however, it was found impossible to measure any definite volume with any degree of accuracy. Approximately 90 cc. were therefore used, the calculation being made on the basis of two measurements and then checked as shown above.

In the measurement of viscosity the 50-gm. weight was used and 100 revolutions timed. In some cases, however, when the time required for this weight was as high as four to six minutes a 100- or 200-gram weight was substituted. It was found that the relative viscosity varied depending on the weight. (Table 24). In these cases the relative viscosity given was that obtained using the weight which took a time corresponding to the 50-gram weight at the lower concentrations.

Table 24.

Variation in viscosity with weight
used in Stormer viscosimeter.

Conc. %	Relative Viscosity		
	50 gm. weight	100 gm. weight	200 gm. weight
1.78	27.30	22.73	19.15
1.41	8.07	6.05	5.46
1.25	5.76	4.75	4.64
1.11	4.63	3.92	3.92
0.88	3.22	3.00	3.00
0.78	2.88	2.68	
0.70	2.55	2.55	
0.62	2.40	2.40	
0.55	2.28	2.29	

Weights above 50 grams were not compared directly with water but with the weight below it. The factor was calculated which would give identical viscosities with the smaller weight at its upper limit. In Table 24 at a concentration of 0.88% and 1.11% weights of 100 grams and 200 grams give the same readings. Below 0.55% weights of 50 grams and 100 grams give the same.

Effect of moistening agent on viscosity of product.

An examination of fig. 1 shows that the use of salt water as a moistening agent gives a gelose with a higher viscosity than a diluted sea water or fresh water. Increasing the salt content by the addition of NaCl does not increase it farther. This is true for both the simple extract and that purified by precipitation with alcohol. The addition of potassium salts did not, as will be shown later, increase the viscosity of the lower viscosity gelose, although gelation could be increased in those with KCl at a lower concentration than without. It did, however, increase the firmness (2,3) in accordance with the observations of E. J. Rodman (3).

The viscosity was increased by alcohol precipitation in sea-water-moistened material. Fresh-water-bleached material showed very little change until a concentration of 1.1% was reached when the viscosity of the simple extract became slightly higher. Actual figures show approximately 8.5% difference in precipitated and unprecipitated extracts. In concentrations above 1.0% the differences due to moistening solutions are more marked. (Table 25).

Table 25.

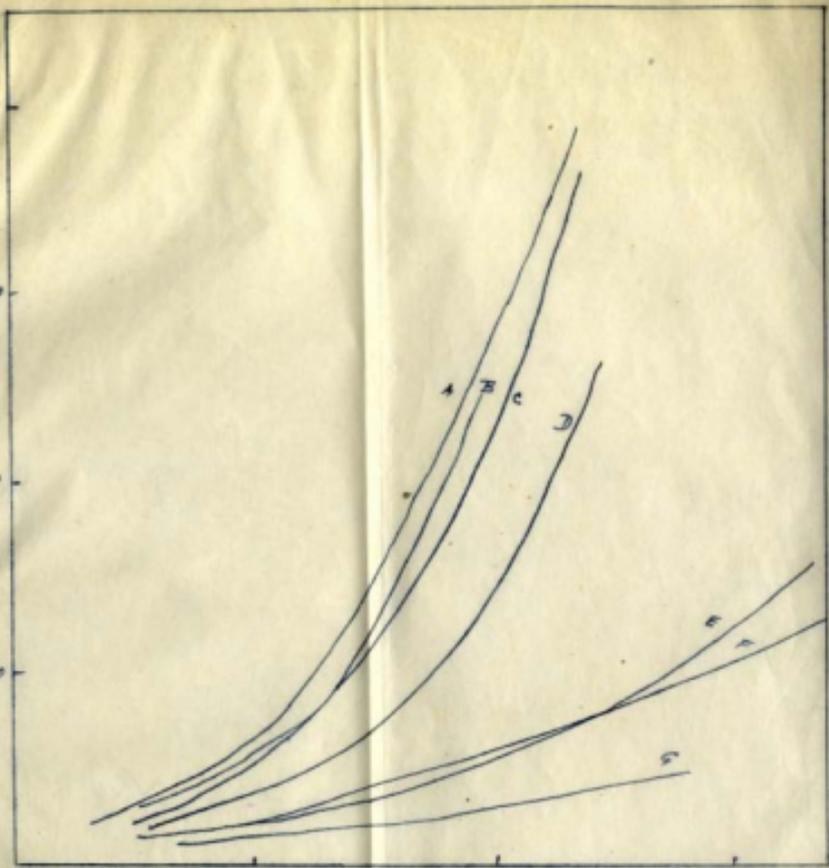
Concentration	Effect of moistening agent on viscosity	
	Relative Viscosity	
	Moistened with sea water	Moistened with 2/3 sea water
1.0	6.30	5.40
1.1	7.50	6.80
1.2	9.20	8.90

The fresh-water-bleached material formed a very white powder when dry and was easily ground. The other two formed a brownish colored powder when dry which was fibrous in contrast to the fresh-water-bleached material which had a granular appearance. None gave a test for reducing sugars with Benedict's reagent. The first two formed a reasonably firm gel at a concentration of 1.5% while the last required a concentration of 2.5% and was not as firm. The hydrogen ion concentration of the three were different being: sea-water-bleached pH 6.6; do. alcohol-precipitated pH 6.5; 2/3 sea-water-bleached pH 5.5; do. alcohol-precipitated pH 6.2; fresh-water-bleached pH 4.56; do. alcohol-precipitated pH 6.12.

An addition of KCl was made to the sea-water-bleached extract. Sufficient was added to increase any KCl content by 0.05%. The solution then had a pH of 4.7. A considerable decrease in viscosity occurred (fig. 2). A solution of this same material was dialysed against distilled water for six days. No test for chlorides with $AgNO_3$ in the concentrated dialysate after the first day. A test

Relative
viscosity

500
400
300
200



0.5

1.0

1.5

Percent Galose

Legend

- A. Sea-water-bleached, Alcohol-precipitated extract.
- B. Sea-water-bleached Simple extract.
- C. $\frac{2}{3}$ sea-water-bleached, Alcohol-precipitated extract.
- D. $\frac{1}{3}$ sea-water-bleached Simple extract.
- E. Fresh-water-bleached Simple extract.
- F. Fresh-water-bleached, Alcohol-precipitated extract.
- G. Washed green moss.

Fig. 1.

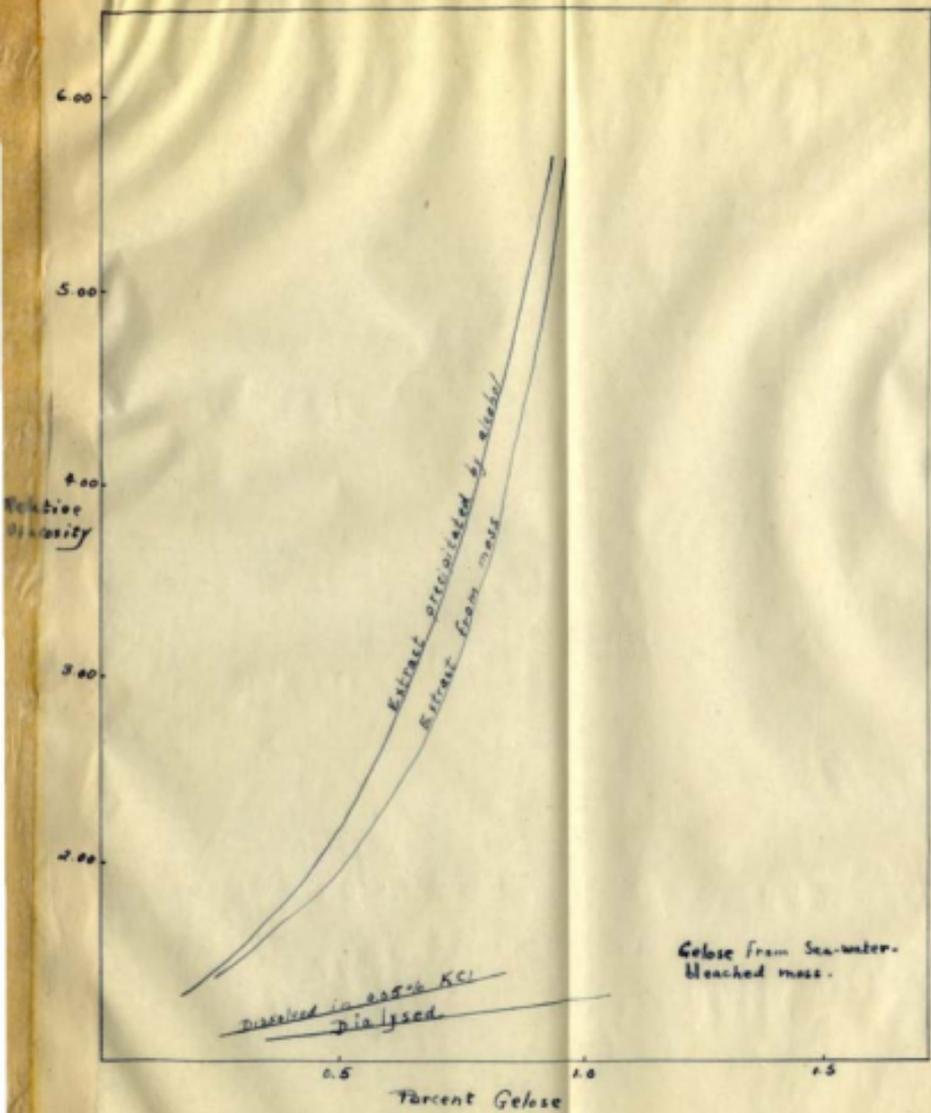


Fig. 2.

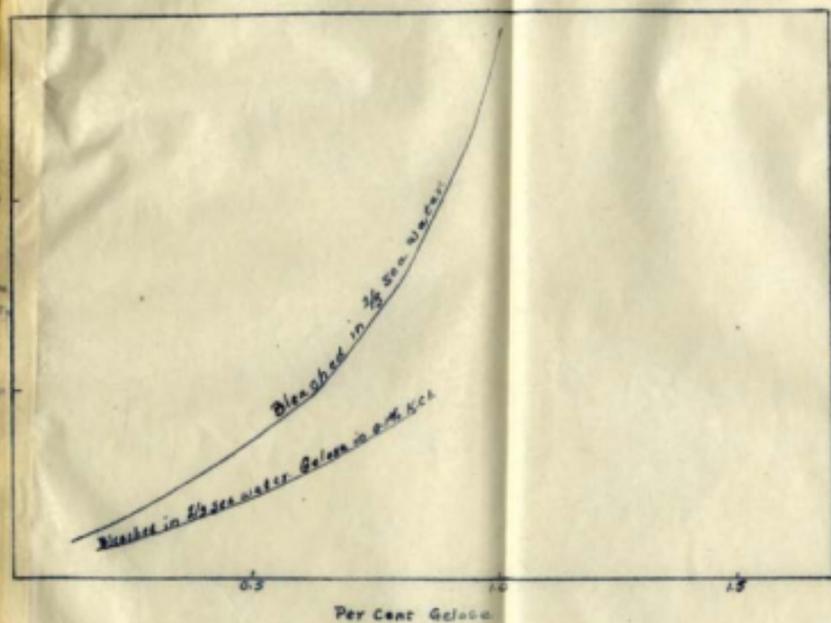


Fig. 3

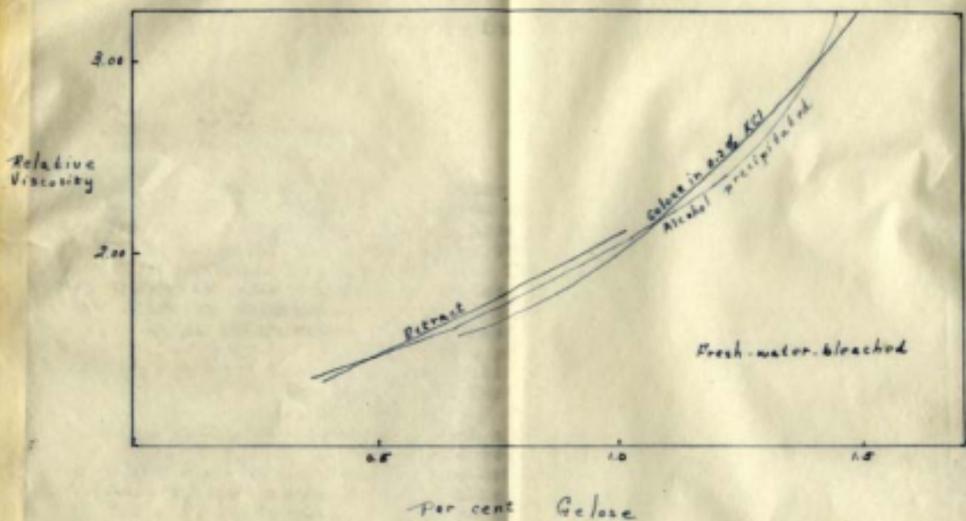


Fig. 4.

for sulphates was obtained with $BaCl_2$ until the sixth day, however. The viscosity of the solution was found to be decreased (fig. 2) and was not increased by the addition of KCl. The gelose before dialysis, dissolved in 0.05% KCl, also showed a decrease in viscosity (fig. 2). A similar change occurred in moss bleached in 2/3 sea water. The gelose in 0.1% KCl showing a decrease in viscosity (fig. 3). The pH changed from 5.49 to 4.66. KCl added to fresh water bleached moss showed the smallest change (fig. 4). The pH increased from 4.56 to 5.28. Gelose from washed green moss was also dialysed. In comparison with that from bleached moss the viscosity from 0.6% to 0.7% was 1.25 in contrast to 1.20.

Some of the gelose was ashed. The percentages for four samples were: gelose from

fresh moss washed	18.8%
sea-water-bleached	16.1%
2/3 sea-water-bleached	17.1%
fresh-water-bleached	18.7%

Green moss which had been washed in running water for 7 days as well as black moss which had been washed free from chlorides before extraction did not show a high viscosity. These formed a weak gel at a 2% concentration which was hardened by the addition of KCl (3) in weaker concentrations of less than 1%. The addition of KCl caused the separation of some gel from solution without increasing the viscosity of the solution. Fig. 5 shows the normal viscosity curve for the washed green moss. This formed a brown gel at a concentration of 1.23% in 0.04N. KOH with a pH of 12.6. On cooling to 45°C, when diluted to give a concentration of 0.5%, the pH became 6.3 and the viscosity was about as before. The green moss itself had a pH of 5.48. 1 gram in 100°C of 0.5 KCl formed a colorless gel. On being precipitated with 95% ethyl alcohol washed twice with absolute alcohol and once with ether, and redissolved, the same viscosity measurements were obtained as in the original gelose. Tests with Benedict's reagent were negative. The black moss which had been washed until the wash water gave no test for chloride when evaporated to one tenth had also a low viscosity. Considerable gelose was removed in the washing. Again a gel-like precipitate formed which settled out from the solution. The viscosity of the solution was not increased by the addition of KCl. Table 27 shows this over a series of concentrations. The pH was unchanged, being 5.48 before the addition of KCl and 5.5 after the concentration of KCl was 0.25%.

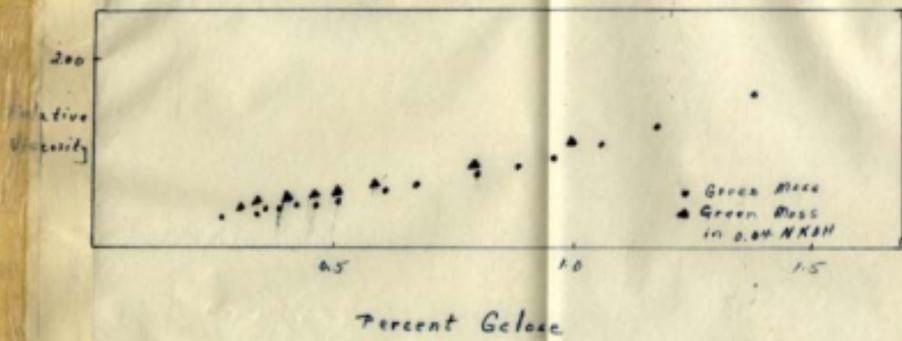


Fig. 5.

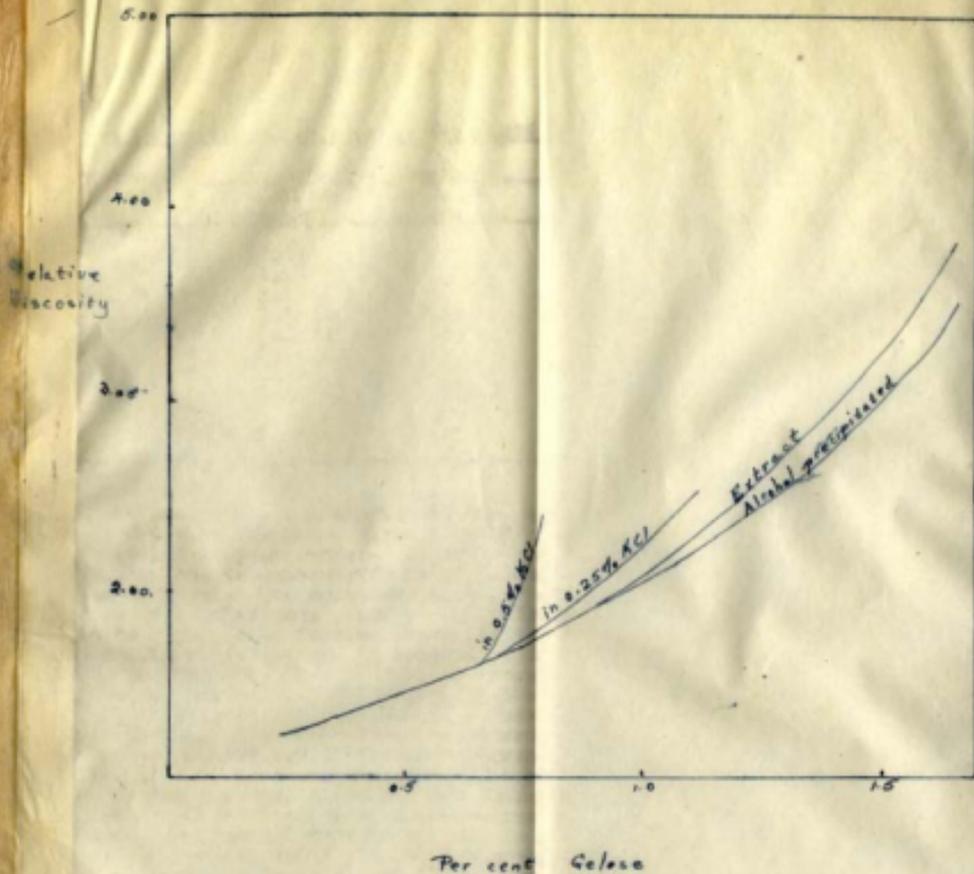


Fig. 6

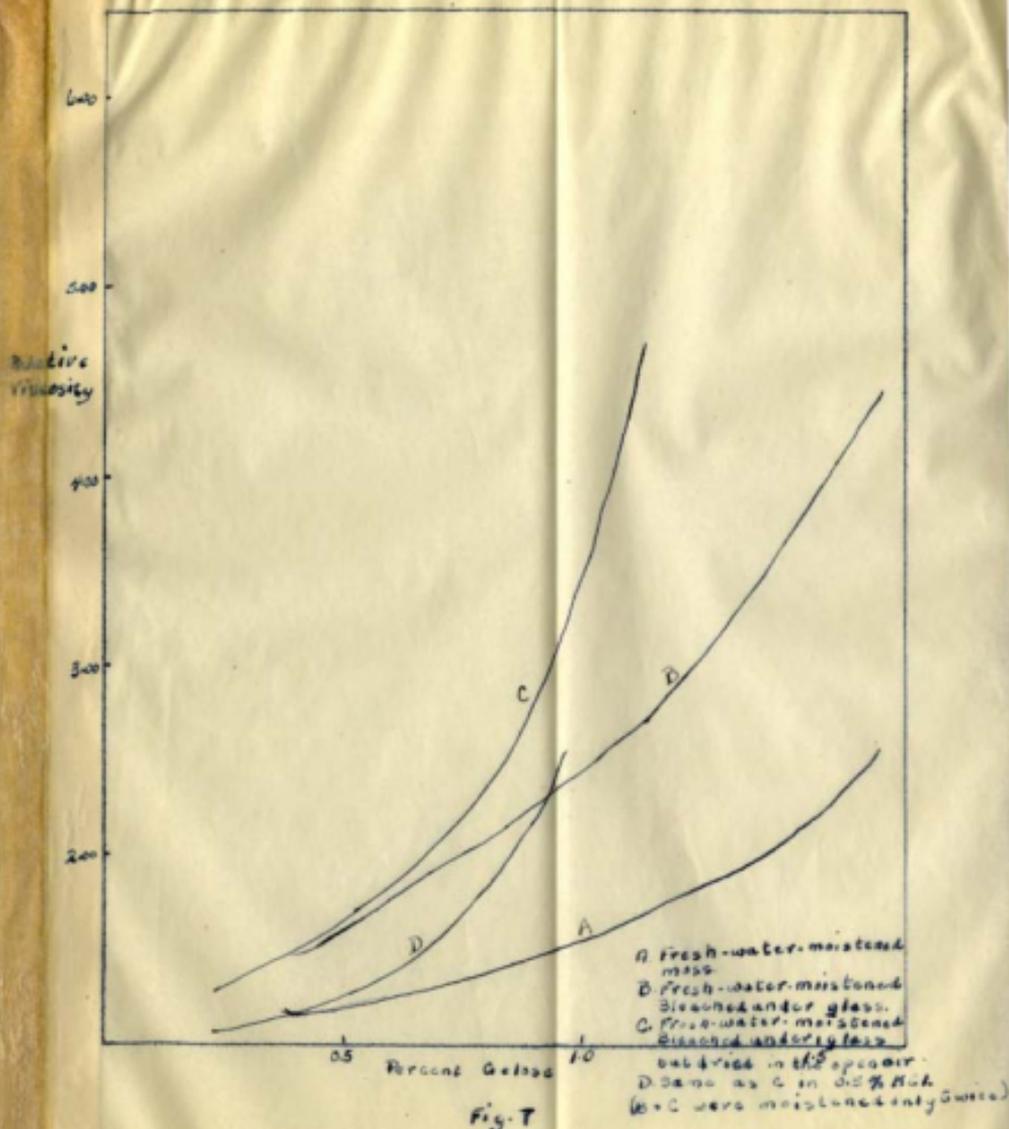
Table 27.

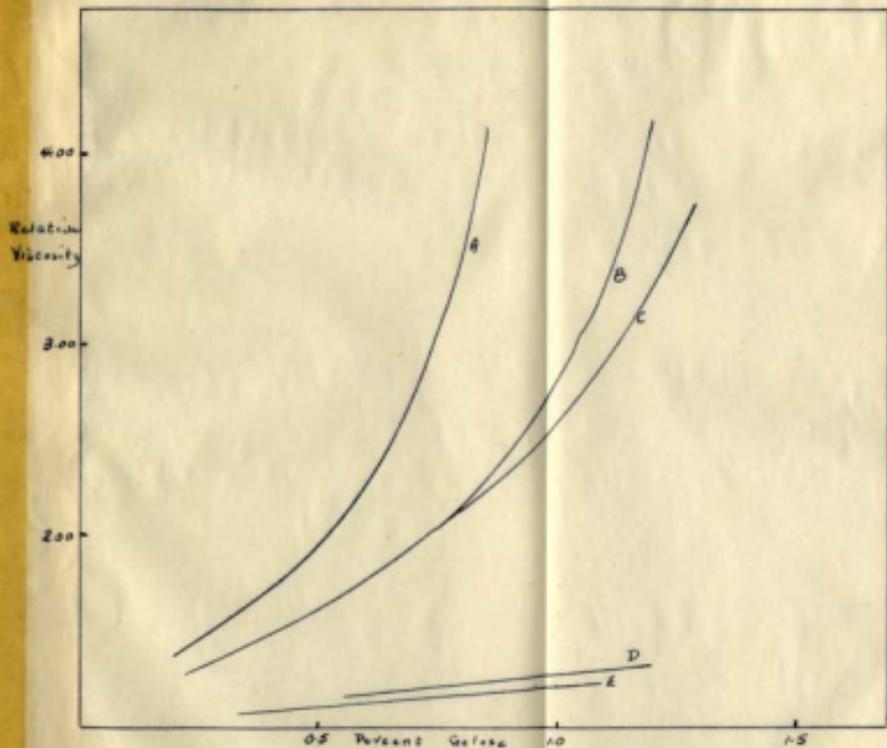
Viscosity of washed black moss in water and 0.25% KCl

Concentration %	Relative viscosity	
	In distilled water	In 0.25% KCl
1.67	2.24	
1.48	1.95	
1.32	1.83	
1.23	1.74	1.64
1.10	1.66	1.54
1.00	1.55	1.45
.90	1.49	1.37
.80	1.46	1.30
.70	1.36	1.25
.60	1.33	1.19

In no case, therefore, was the viscosity of the solution increased by the addition of KCl which might possibly have been leached out by the method of preparation. In every case, however, the addition of KCl causes, in concentrations of gelose of 1% or over, the formation of a gel. The additions to lower concentrations causes the formation of a gelatinous precipitate. Alcohol precipitation which might be expected to further remove salts does not appreciably decrease the viscosity and in some cases increases it. The addition of KCl in quantities of 0.5% will increase the viscosity above a concentration of 0.7% leading to the formation of a gel. (fig. 6). Material that had been bleached under glass had also a lower viscosity than salt-water-bleached material (fig. 7). For comparison a curve is shown for fresh-water-bleached material. The gelose in 0.5% KCl at a concentration of 0.88% was gel-like in appearance and the figure here and above may not indicate a true viscosity. On allowing it to stand at a concentration of 0.88% the apparent viscosity increased from 2.95 to 3.33 in 3 minutes. B and C in fig. 7 were bleached under glass after being washed in fresh water. When bleached while still wet C was removed and dried in the sun. B was bleached under glass and remained there until dry to touch when the glass was removed and drying continued. Only two washings were necessary.

Material kept wet by soaking in a solution where pH was adjusted to 2 bleached much more rapidly. The same was true at a pH of 4 and to about the same extent. Moss bleached at a pH of 2, made so by the addition of HCl and adjusted using a B.D.H. universal indicator, became very gelatinous and did not dry to a crisp product. The material was very white while wet but turned a dark brown on drying. The extract in 2% solution had about the same viscosity as water (1.11) and on precipitation with alcohol did not form a gel-like mass but formed a fine white precipitate which could be either filtered or centrifuged. This, precipitated again, turned brown in dehydrating and drying. A solution made from the purified product gave essentially the same low viscosity in 2% solution (1.12). No

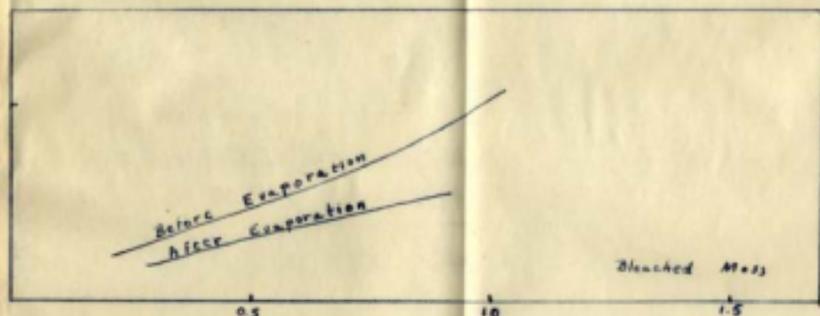




Bleached Mrs.
Moistening Agent

- A. 5% Meal Extract.
- B. 3% Meal Gelose alcohol precipitated
- C. 3% Meal Bleached by temp
- D. 3% CaCl₂ Gelose alcohol precipitated
- E. 3% CaCl₂ Extract.

Fig 8.



Relative Viscosity

Percent Gelose

Solution evaporated with Drying lamp Stirred by air

gel was formed on addition of KCl.

Moss bleached in 2% NaCl and CaCl₂ adjusted to pH 2 with HCl as well as sea water at pH 2 and 4 with HCl was used bleaching was much more rapid. (Fig. 8). It will be seen that in 3% NaCl a relatively high viscosity was reached. 3% CaCl₂ giving a viscosity only slightly higher than fresh water. With the water-bleached material at pH 2 a slight test was given with Benedict's solution. The pH of the resulting gels varied and a slight precipitate occurred in '2' on the addition of 2% CaCl₂.

	<u>pH of extract</u>	<u>pH of Alcohol precipitate</u>
1. 3% NaCl	6.05	6.43
2. 3% CaCl ₂	4.22	5.79

Number 1 did not precipitate as a fine precipitate but as a thin gel which on dehydration became firmer. Hence again the addition of KCl had little effect on the viscosity. The same was true of pH changes. Number 2 extract was adjusted with HCl to pH 3.8 and the pH increased by the addition of KOH. The change in pH had no measurable effect on the viscosity. This as will be shown is not true of all material.

Table 28.

<u>Effect of pH on viscosity of acid-bleached moss</u>						
Concentration	Relative viscosity at pH					
	3.8	4.22	6.99	9.8	10.4	10.9
1.03	1.19	1.19	1.20			
.80		1.17	1.18		1.17	
.07		1.13	1.12	1.14		1.13
.65	1.11	1.11		1.11		
.57		1.08				1.08
.50		1.07	1.07			

For comparison table 29 gives the relative viscosity of acid-bleached moss at pH 4.22 compared to sea-water-bleached moss.

Table 29.

<u>Comparison of acid to sea water as bleaching agent</u>		
Concentration	pH 2 in 3% CaCl ₂	Sea water bleached
1.03	1.19	5.95
.80	1.17	2.95
.70	1.13	2.60
.65	1.11	2.40
.57	1.08	2.31
.50	1.07	2.31

The addition of KCl did not increase the viscosity measurably. .005 N KOH, .01 N KOH, .05% KCl and .5% KCl in concentrations of gelose form .5% to 1.0% had the same viscosity. Some of the gelose from '1' was evaporated with a General Electric drying lamp (250 watts) and stirred by bubbling air through the solution (fig. 8). The viscosity was slightly decreased.

Fresh moss gelose which had been precipitated with alcohol was dissolved in several concentrations of KCl. The viscosity was decreased (Table 30) until a concentration of 0.2% was reached when a thick gel formed. The pH was about 5.3. An 0.88% solution of gelose was used. The initial gelose viscosity at this concentration was 1.58.

Table 30.

<u>Effect of KCl on fresh moss</u>	
<u>Cone. KCl %</u>	<u>Rel. viscosity</u>
0.01	1.51
0.05	1.45
0.10	1.43
1.15	1.41
0.2	1.71

Green moss was bleached with 3% H₂O₂ and 30% H₂O₂. The gel was extracted at the same time. This was filtered and used. (Table 31).

Table 31.

<u>Effect of H₂O₂ on viscosity</u>			
<u>Cone.</u>	<u>Viscosity</u>		
	<u>H₂O₂ 3%</u>	<u>H₂O₂ 30%</u>	<u>Unbleached</u>
0.34	1.48	1.00	1.55
0.31	1.40	1.01	1.45
0.27	1.24	1.01	1.40
0.24	1.19	1.00	1.33
0.22	1.17	1.00	

There was also a change in H ion concentration.

3% H ₂ O ₂ bleached	pH 5.5
unbleached	6.7 and 6.8
30% H ₂ O ₂ bleached	3.96

The last gave a precipitate with CaCl₂. A similar change occurred with sun-bleached material. The solution was stirred by bubbling air through it and evaporated with a drying lamp (fig. 9).

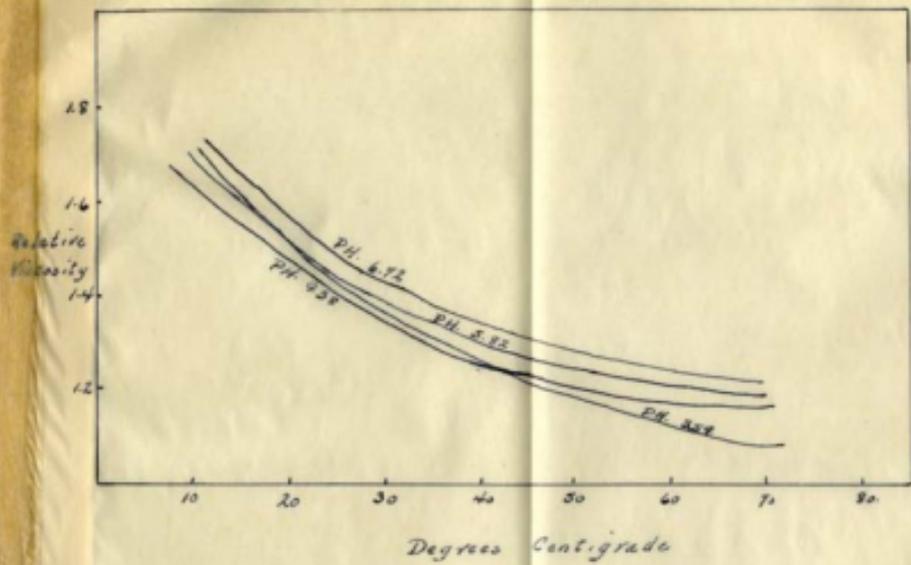
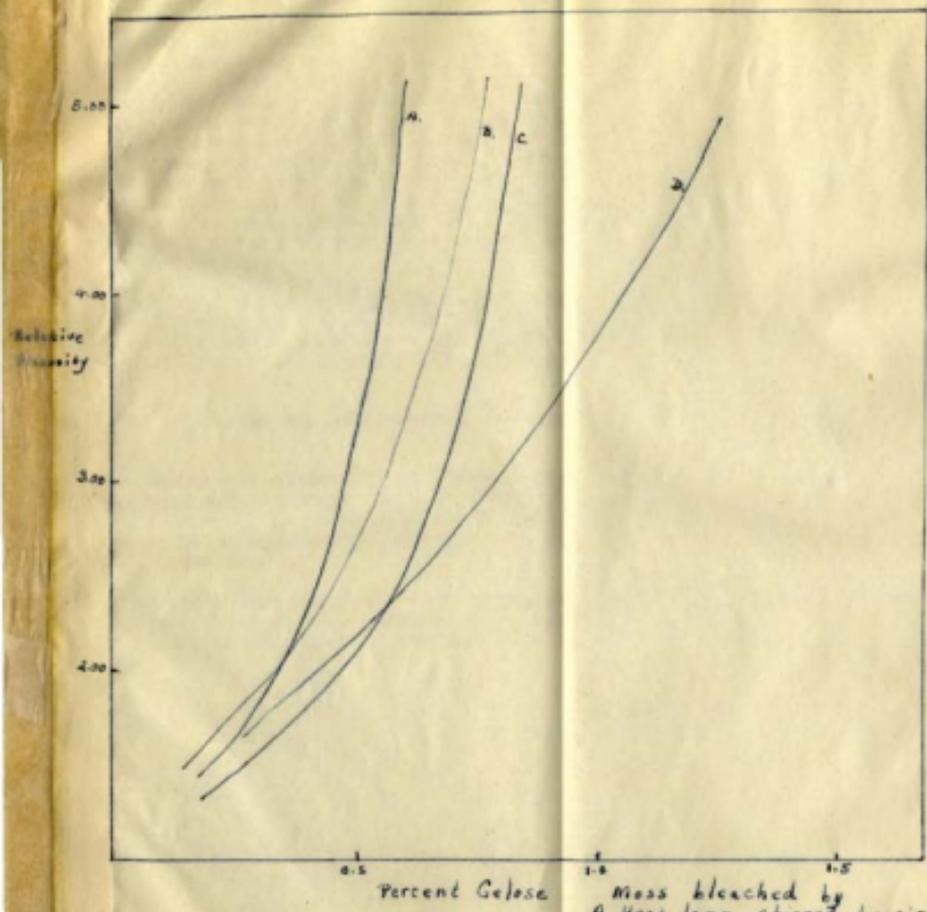


Fig. 10.



- Mass bleached by
- A. Heat lamp, stirred by air
 - B. Ultra violet lamp, Gelose alcohol precipitated.
 - C. Ultra violet lamp, Gelose extract.
 - D. Heat lamp, stirred mechanically.

ing on the temperature, humidity, and wind, with an average of 24 hours. The lowest moisture content reached was somewhat over 8%. This is probably the lowest value obtainable with natural drying. Increasing the depth of moss to over a single layer lengthens the time and necessitates considerably more handling. Moisture is absorbed from the atmosphere by partially dry specimens.

The time required for bleaching depends on the temperature, intensity of sunlight and moisture. The optimum moisture content is over 40%, short of complete immersion. Below this the time required increases. Very little bleaching occurs in specimens with less than 20%. As a general rule the moss should always be wet to touch. Sea water or isotonic salt water are the best moistening agents. Moss can be bleached readily under glass as well as in the open air and in a somewhat shorter time. Samples of 20 grams were also bleached by artificial light in less than two hours. The gelose compared favourably with natural-bleached material as regards viscosity. Comparatively high temperature and white light is necessary.

The viscosity of the gelose was increased by bleaching and was affected by the moistening solution used. For a high-viscosity gelose salt water was the best moistening agent. Small amounts of fresh water had no effect on the product except after a prolonged exposure.

The quality of moss from various regions did not differ significantly.

Bleaching increases the viscosity of the gelose; and while the addition of potassium salts increases the firmness of any gel, its inclusion does not increase the viscosity of an originally low-viscosity material. It will, however, decrease the concentration necessary for gel formation.

Soaking in acid solution shortens the time necessary for bleaching. The product however was poorer. This could be partially prevented by the addition of sodium salts to the solution.