



## Pilot plant scale extraction of alginate from *Macrocystis pyrifera*. 1. Effect of pre-extraction treatments on yield and quality of alginate

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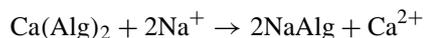
**Key words:** alginate, pilot plant process, pre-extraction, *Macrocystis pyrifera*, acid treatment, formalin treatment

### Abstract

In the extraction of alginate from brown seaweeds, the acid pre-extraction treatment has been considered by many authors as an essential step because it makes the alginate more readily soluble in an alkaline solution. At pilot plant level, extractions were made (i) using formalin treatment prior to the acid pre-extraction treatment (ii) using different acid treatments so the calcium ions exchanged varied from 83% to 4%. The use of formalin treatment gave a product with less color. During the acid pre-extraction treatment, it was possible to reduce the calcium exchanged from 33.4% to almost zero with a maximum reduction in alginate yield of 7%. The degree of acid treatment was positively correlated to calcium exchanged and yield but negatively correlated with alginate viscosity. Using strong acid conditions the viscosity was 168 mPa s, while mild acid conditions produced an alginate with 623 mPa s. The direct extraction from calcium alginate to sodium alginate is possible because strong alkaline conditions were used, pH 10 at 80 °C for two hours and with a low water volume. The best pre-extraction treatment to obtain an alginate with high viscosity is to hydrate the alga with 0.1% formalin overnight, then wash the alga once with hydrochloric acid at pH 4 using a batch system with continuous agitation during 15 min.

### Introduction

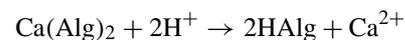
In brown seaweed alginic acid is present mainly as the calcium salt of alginic acid, although magnesium, potassium and sodium salts may also be present. The first aim of the extraction process is to convert the insoluble calcium and magnesium salts into soluble sodium alginate. If the seaweed is treated with alkali, then the process necessary for extraction is an ion exchange (Bashford et al., 1950):



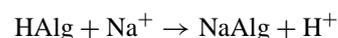
However it has been stated by many authors (Haug, 1964; Secconi, 1967; Myklestad, 1968; Duville et al.,

1974; Hernández-Carmona & Aguirre-Vilchis, 1987) that a more efficient extraction is obtained by first treating the seaweed with dilute mineral acid:

(1) Pre-extraction:



(2) Extraction:



According to several authors, the calcium alginate is converted to alginic acid and this is more readily extracted with alkali than the original calcium alginate.

However, no one had studied how much of the yield is lost if the amount of calcium exchanged is reduced.

In the early patents for the industrial production of alginate, the acid pre-extraction step was described as an essential step to make the alginate more readily soluble in the alkaline solution (Clark & Green, 1936; Bescond, 1948; Secconi, 1967). Green (1936) cited that algae contain 35–40% of ash and proposed the acid leaching to reduce it to 5–15% of the dry weight of the algae. Le Gloahec and Herter (1938) also mention that certain advantages can be gained by first treating the alginous material with dilute acid to dissolve any residual alkaline earth.

None of the above authors gave any chemical explanation for the use of the acid treatment. The first scientific explanation was given by Haug (1964) who assumed that alginate occurs as an insoluble salt with calcium as the main cation, so the extraction of alginate could be regarded as a two step process, a transformation of insoluble alginate into soluble alginate i.e. sodium alginate, followed by a diffusion of the soluble alginate into the solution. He proposed that this transformation could be conveniently carried out by converting the algal alginate into alginic acid, followed by neutralization of the alginic acid with an alkaline sodium salt.

The Russian patents (Lukachyov & Pochkalov, 1965; Baranov et al., 1967) do not follow this principle, they simply treat the algae with boiling alkali. However these patents do not give any information of the yield or quality of the product obtained. The first scientific paper that gave quantitative data comparing the use and non use of acid treatment was published by Shah et al. (1967). Working with *Sargassum* spp. the authors found that after acid treatment the seaweed gave 13.8% alginic acid on extraction with sodium carbonate, while the untreated seaweed gave 13.7% alginic acid, suggesting that acid treatment of the seaweed is not essential for the extraction of sodium alginate. They also proposed that alginic acid is present in the seaweed mostly as free acid and not as calcium salts as reported by other authors.

The studies of Myklestad (1968) demonstrate that alginic acid occurs in the algae as a mixture of salts with calcium as the main ion. He gave a detailed explanation of the Ca/H ion exchange reaction during the pre-extraction treatment. This author demonstrated that the exchange rate depends upon factors such as acid concentration, particle size, agitation and residence time of the acid. The exchange follows a first order reaction and the exchange velocity is propor-

tional to the logarithm of the acid concentration. He showed that this exchange process is governed by a film diffusion mechanism, and the diffusion of calcium across a thin liquid film enveloping the particle is the rate-determining step. However this author did not relate his results either to the percentage of alginate obtained or the quality of the product.

Duville et al. (1974), working with *Macrocystis pyrifera* and *Lessonia fuscescens* found that 67% of alginic acid is combined with calcium and magnesium ions. Without acid pre-treatment they obtained 15.5% alginate in a cold process and 16% in a hot process (50 °C). Using the acid pre-treatment and extraction at 50 °C, the yield increased to 23%. Again, these authors did not give any information on the product quality.

Hernández-Carmona and Aguirre-Vilchis (1987) studied the acid pre-treatment and gave details of the ion exchange process using *Macrocystis pyrifera* as raw material. They found that using the pre-extraction acid treatment as described by Myklestad (1968) the alginate yield was 35%, but using seawater to prepare the acid the yield was 29.3%. This means that with only 65% of the calcium ion exchanged, the difference in yield was only 5.7%. The reduction of calcium ion exchange, on a laboratory scale, was studied by Hernández-Carmona et al. (1992) and Reyes-Tisnado et al. (1992) by using recirculation methods. They found that acid could be recirculated with the consequent reduction of calcium ion exchanged, without affecting the alginate yield. Arvizu-Higuera et al. (1995) demonstrated that acid treatment could be reduced down to 0.006 M (that exchanged only 26% of the calcium ions) without significant difference in the yield (28.6% with 0.1 M HCl and 26.5% with 0.006 M HCl).

During the alkaline extraction, which follows the acid pre-treatment, a brown discoloration develops and this carries through the rest of the process resulting in a dark sodium alginate powder. Decoloration of the alginate was first proposed by Le Gloahec and Herter (1938), by treating the clarified sodium alginate extract with gelatinous aluminum hydroxide. In a later patent Le Gloahec (1939) claimed that, by soaking the algae in a formaldehyde solution prior to extraction, the 'chlorophyllian colored matter' became fixed to the algal tissues and discoloration during alkaline extraction was markedly reduced. Haug (1964) demonstrated that phenolic compounds are responsible for the discoloration. He also showed that when samples of brown algae are extracted with dilute acid,

as in the acid pre-extraction treatment, the extract contains phenolic reducing compounds.

Formalin treatment can therefore fix some of the phenolic compounds to the algal tissue and the acid pre-treatment may remove any remaining soluble phenolic compounds. McHugh (1987) proposed the use of formaldehyde after the acid treatment, stirring the algae with water containing 0.1–0.4% commercial formalin solution during 15–30 min; the acid treatment would remove most of the soluble phenolic compounds allowing a more efficient use of the formaldehyde. None of the above authors have given quantitative data for differences between the use or non use of the formalin treatment.

This study was undertaken on a pilot plant scale to examine the effects on alginate yield and quality, of pre-extraction treatments of *Macrocystis pyrifera* with formalin and acid, or acid alone.

## Materials and methods

### *Determination of the calcium concentration in the alga*

#### *(i) Determinations of Ca/H exchange in a continuous flow system (laboratory level)*

30 g dried and milled alga, *Macrocystis pyrifera* (L.) C. Agardh, were hydrated with 270 mL of 0.1% formalin solution overnight. The residual formalin solution was drained and analyzed for calcium concentration (time 0 min). The continuous flow system designed by Myklestad (1968) was used to determine the rate of calcium/hydrogen exchange and the total amount of calcium. A hydrochloric acid solution (0.2 M) was pumped through the reactor cell containing the hydrated *M. pyrifera* at a speed of 250 mL min<sup>-1</sup> for 12 min with constant stirring at 800 rpm.

The exchanged calcium ions that were carried out in the effluent were collected at one minute intervals, and also the residual solution in the reactor was collected (time 13 min). The calcium concentration was measured by complexometric titration using the method described by Schwarzenbach and Flaschka (1969). The continuous flow system assures that 100% of the calcium ions are exchanged during the reaction and this value can be compared with the exchange resulting from the different experimental treatments.

To correct the values of calcium exchanged, a control was run, hydrating the alga with 270 mL of water overnight (time 0 min), pumping into the reactor distilled water and measuring the calcium extracted. The

value obtained corresponds to the free calcium salts and was subtracted from the total amount of calcium determined.

#### *(ii) Determination of calcium exchange at pilot plant level (batch system)*

Ten kg of dried and milled alga, *Macrocystis pyrifera*, were placed in a tank to hydrate with 90 L of 0.1% formalin solution overnight. The residual solution was drained off and the alga was washed with 100 L of hydrochloric acid with constant agitation during 15 min. The acid conditions were varied as follows:

1 acid washing with 0.1 M HCl

2 acid washings with 0.1 M HCl

no acid washing

1 acid washing with HCl at pH 4

2 acid washings with HCl at pH 4

3 acid washings with HCl at pH 4

no formalin hydration overnight, 3 acid washings with HCl at pH 4

The residual solutions from the hydration and acid washings were drained off, the volumes were measured and sampled to measure the calcium concentration using the method described above.

To correct the values obtained in each treatment the same procedure was carried out but washing the alga with water instead of formalin and acid; the amount of calcium obtained was subtracted from the corresponding treatment with formalin/acid, the result being the amount of calcium obtained by ion exchange.

#### *(iii) Determination of calcium exchange at laboratory level*

In order to make some comparisons of pilot plant and laboratory results, 10 g samples of alga were hydrated with 90 mL of formalin solution (0.1%). The residual solution was drained off and in separate experiments the alga was treated with acid as follows:

1 acid washing with 0.1 M HCl

3 acid washings with HCl at pH 4

To calculate the calcium obtained by ion exchange, controls were run as before, using water to replace the formalin and acid.

### *Determination of yield and quality*

#### *(i) Laboratory level*

After the pre-extraction treatment in both the continuous and batch systems, the extraction was carried out by placing the samples in 25 parts of water to 1 of alga, and adjusting the pH to 10 with 10% sodium carbonate

Table 1. Effect on the alginate yield and viscosity of the percentage of calcium ions exchanged during the acid pre-extraction treatment.  $\text{mg g}^{-1}$  = milligrams of calcium per gram of dry algae. Viscosity values correspond to 1% alginate solution at 22 °C.

Treatment	Total Ca Removed ( $\text{mg g}^{-1}$ )	Total Ca/H exchanged (%)	Yield (%)	Viscosity (mPa s)	Color (% trans.)
Laboratory level (continuous flow system)					
Washing with water	18.0	0.0	–	–	–
Washing with acid 0.2 M	60.7	100.0	22.2	406	88.9
Laboratory level (Batch system)					
Formaldehyde hydration, 3 water washings	20.5	0.0	–	–	–
Formaldehyde hydration, 3 acid washings, pH 4	36.7	37.9	18.6	1016	92.6
Formaldehyde hydration, 1 water washing	21.0	0.0	–	–	–
Formaldehyde hydration, 1 acid washing (0.1 M)	49.0	65.7	17.9	288	90.1
Pilot plant level (Batch system)					
Controls :					
No hydration, 3 water washings	20.7	0.0	–	–	–
Water hydration, 3 water washings	20.3	0.0	–	–	–
Water hydration, 2 water washings	19.7	0.0	–	–	–
Water hydration, 1 water washing	18.7	0.0	–	–	–
Water hydration, 0 water washing	14.1	0.0	–	–	–
Experimental treatments:					
1. Formaldehyde hydration, 2 acid washings (0.1 M)	55.2	83.2	23.4	168	90.2
2. Formaldehyde hydration, 1 acid washing (0.1 M)	35.4	39.3	21.2	342	95.1
3. No hydration, 3 acid washings, pH 4	28.4	18.1	17.9	488	77.4
4. Formaldehyde hydration, 3 acid washings, pH 4	34.6	33.5	17.0	518	90.2
5. Formaldehyde hydration, 2 acid washings, pH 4	29.2	22.2	16.9	491	93.9
6. Formaldehyde hydration, 1 acid washing, pH 4	24.4	13.5	16.2	623	90.1
7. Formaldehyde hydration, 0 acid washing	15.9	4.2	15.7	579	91.3

solution. The sample was heated at 80 °C in a water bath with constant stirring of 800 rpm for 2 h.

The solution was diluted with hot water and filtered in a vacuum filter prepared with filter paper and diatomaceous earth. The clarified solution was treated with 10% calcium chloride solution. The resulting calcium alginate fibers were placed in 20 parts of water to 1 part of alga (by volume) and the pH was adjusted to 2 with hydrochloric acid (1 M) and stirred for 15 min. The sample was filtered and the acid washing was repeated two more times, adjusting the pH to 1.8.

The alginic acid fibers were placed in ethanol-water (1:1), using 15 mL per gram of alga. Sodium carbonate solution (10%) was added until a pH of 8 was obtained. The sample was stirred for 1 h. The sodium alginate was filtered, pressed, and the fibers separated and dried to constant weight in an oven at 50 °C.

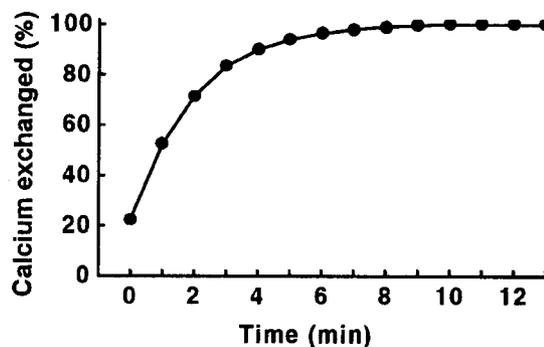


Figure 1. Percentage of calcium ion exchanged during acid pre-extraction, using the continuous flow system designed by Myklestad (1968).

Yields of sodium alginate were calculated as % w/w, based on the initial dry weight of the alga. Viscosities were measured on 1% sodium alginate solutions at 22 °C, using a Brookfield viscometer

model LVTDV-I, 60 rpm, with the appropriate spindle. Prior to viscosity measurement, the alginate solutions were treated with 0.5% sodium hexametaphosphate to sequester any calcium ions present. Color was measured reading the percentage of transmittance of the alginate solution in a spectrophotometer at 510 nm. The higher the value obtained, the lighter the alginate color.

### (ii) Pilot plant level

After the pre-extraction treatment, the extraction was carried out by transferring the alga to a tank with 166 L of water and adjusting the pH to 10 with powdered sodium carbonate. The sample was heated at 80 °C for 2 h with constant stirring. The paste obtained was transferred to another tank and diluted to 600 L with hot water (70 °C).

The solution was filtered in a rotary vacuum filter previously covered with a 15 mm layer of filter aid (diatomaceous earth). Samples (500 mL) of filtrate were taken when the volume in the dilution tank went down to 400, 300 and 200 L. This sampling method allows for any inhomogeneous distribution of the slurry in the tank that feeds the filter and allows the estimation of the average alginate content.

The three samples were taken to the laboratory and the rest of the process was followed using the method described in the laboratory level section above. Finishing the extraction process in the laboratory avoided any interference, on the effect of the pre-extraction treatments, from variables in the latter stages of the pilot plant process. The three samples collected from the filter effluent for each run were analyzed in the laboratory, the three results were averaged and considered as one replicate. Yield, viscosity and color were measured as stated above for the laboratory-scale samples.

All experimental treatments, at laboratory or pilot plant level, were conducted in triplicate and the averages for each treatment were calculated for (a) quantities of calcium removed and exchanged, (b) yield, (c) viscosity and (d) color of the extracted sodium alginate. A one way anova was used to detect significant differences among treatments. Tukey honest significant difference (HSD) test was used to determine the mean differences between the treatments.

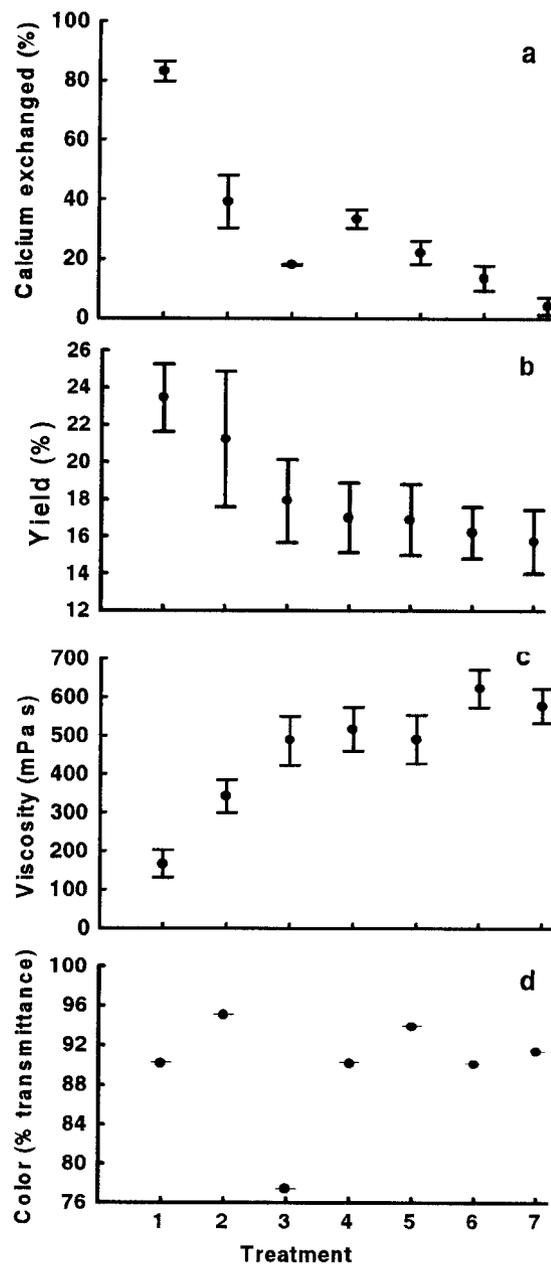


Figure 2. Effect of the pre-extraction treatment on the percentage of calcium exchanged, alginate yield, alginate viscosity and alginate color. Refer to Table 1 for treatment description.

## Results

Using the continuous flow system, the percentage of calcium ions exchanged during the acid treatment followed a logarithmic relation as described by Myklestad (1968) (Figure 1). The amount of free salts

washed with water was 18.1 mg of calcium per gram of alga. When using hydrochloric acid, after 9 to 10 min all the calcium was extracted and the average total amount of calcium removed was 60.7 mg per gram of dry alga. Therefore the real amount of calcium exchanged during this reaction was the difference, 42.6 mg of calcium per gram of alga, and this value was considered as the maximum amount (100%) of calcium that could be exchanged during the pre-extraction step.

Table 1 shows the results for all treatments at laboratory and pilot plant levels respectively. Results of the control washings are also shown. Figure 2a–d shows the standard error for each value of the % calcium exchanged, the alginate yield, viscosity and color.

## Discussion

Calcium ion exchange increased with the number of acid washings and with increasing acid strength (Figure 2a). With the milder acid treatment, at pH 4, the maximum exchange of 33.5% was achieved after three washings whereas 39.3% resulted after just one washing with 0.1 M acid. A second washing with 0.1 M acid more than doubled the exchange to 83.2%. When the alga was not soaked in formalin overnight before acid treatment at pH 4, the exchange was reduced to 18.1%, presumably because it was more difficult for the acid to penetrate the algal tissues (McHugh, 1987). The two laboratory treatments (batch system) gave greater calcium exchange than the corresponding pilot plant treatments.

The yield of alginate was not affected by the degree of calcium exchange for the acid treatment at pH 4; there was no significant difference in yield for treatments 3 to 7 (Figure 2b). However, the treatments with two 0.1 M acid washings gave significantly higher yield, with a maximum difference of 7.4%. This means that, using the alkaline extraction conditions previously described, it is possible to reduce the calcium exchanged from 33.5% to almost zero without any loss in yield.

The cell walls of brown algae are composed of three kinds of polymers: alginates, cellulose, and complex heteroglycans (Painter, 1983). The alginate occurs also as extensive intercellular deposits. The structural integrity of the brown algal thallus depends primarily on alginate, and its ability to form gels and viscous solutions contributes to the flexibility of these algae (Clayton, 1990). This structural integrity

can be broken down with the use of strong extraction conditions (pH 10, 80 °C), allowing the direct transformation of the mixed salts ( $\text{Na}^+$ ,  $\text{Mg}^{2+}$ , and  $\text{Ca}^{2+}$ ) of alginate into sodium alginate, which dissolves in the water.

The alginate yields obtained with the experimental treatments at pilot plant level are higher than those reported by Duville et al. (1974) for *M. pyrifera*, from Argentina, but lower than those reported for the same species collected in Mexico (Hernández-Carmona & Aguirre-Vilchis, 1987; Hernández-Carmona et al., 1992; Reyes-Tisnado et al., 1992; Arvizu-Higuera et al., 1995). This is because industrial conditions (equipment and chemicals) were used which were expected to give lower results than those obtained at the laboratory level. However, even the lowest yield obtained is industrially acceptable.

The viscosity of the sodium alginate obtained after extraction was negatively correlated with the degree of acid treatment. Viscosity fell from 623 mPa s for the mildest acid treatment (6) to 168 mPa s for the strongest treatment (1). No significant viscosity differences were found among treatments 3 to 7 but treatments 1 and 2 were significantly lower than 3 to 7 (Figure 2c). This is because the alginic acid, formed by ion exchange, is less resistant to acid degradation than the original calcium alginate. So the greater the ion exchange, the more vulnerable is the alginate to degradation by the acid being used for the exchange. A similar effect was found at the laboratory level; continuous washing with 0.2 M acid gave a much lower viscosity (406 mPa s) than the treatment at pH 4 (1016 mPa s).

The color of the alginate produced was reduced by the formalin hydration (Figure 2d). Treatment 3 gave a markedly darker product than any of the other treatments. There was no correlation between the number of acid washings and color, when formalin treatment was used. Even with no acid washing (7) the color was similar to that of the strongest acid treatment (1). It appears that most of the soluble phenolic compounds are removed by the formalin treatment.

Although the formalin treatment was previously proposed (Le Gloahec & Herter, 1938; Le Gloahec, 1939; McHugh, 1987), in this work the formalin treatment was carried out before the acid pre-extraction treatment, with the advantage of removing the phenolic compounds at the same time as the algae is rehydrated to facilitate the further reactions in the process.

The results demonstrate that, using industrial conditions, it is desirable to rehydrate the dried algae with 0.1% formalin solution overnight; this reacts with phenolic compounds in the algae and reduces the color in the alginate produced after extraction. A further washing with water or acid assists in the removal of soluble salts and any residual formalin solution that might contain unreacted phenolic compounds. One acid or water washing has the added advantage of removing sulfated polysaccharides of the fucoidan type (Percival & McDowell, 1967; Larsen, 1978). These polysaccharides produce mucilaginous or stringy solutions when dissolved in water and would otherwise make the separation of the insoluble residue from the alkaline extract more difficult and could interfere in later stages of the process.

For the alkaline extraction conditions used here, acid pretreatment resulted in a higher yield of alginate only if the calcium exchange was approximately 40% or higher. To achieve this degree of ion exchange, 0.1 M acid was used, and the algae washed for 15 min. However the higher yield was gained at the expense of a lowering in viscosity. A second acid wash reduced the viscosity further. In the production of alginate, controlled reduction of alginate viscosity might be achieved by varying the number of acid washes. The replacement of 0.1 M acid by one washing with water, or dilute acid (pH 4), resulted in a lower yield but the viscosity of the alginate was higher and more acceptable for many applications.

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