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Process Optimization for the Extraction of Alginate, Derived from Sargassum Fluitans

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Abstract

Brown algae is the class of seaweed which is widely used in industrial, food and medical sector. Sargassum is the major type of brown algae with furthermore than 300 sub species and Sargassum fluitans is one of them. The extraction of alginate from brown algae: Sargasssum fluitans was studied at boiling temperature from 90 to 120 °C, alkaline concentration from 4 to 10 w/w % and boiling time from 2 to 8 hours. The alginate is extracted from each experiment and studied the impact of boiling temperature, alkaline concentration and boiling time on yield one by one. The maximum yield of alginic acid was determined by evaluating the effect of three parameters independently. In addition, the optimum yield of alginate was investigated from the influence of three variables by using Box-Behnken experimental design. From the experiments, the maximum yield 0.272 (g/g) of alginate was obtained at 10 % alkaline concentration with extraction time of 6 hours at 120°C. The recommended optimum parameters for commercial production of alginate from the sargassum fluitans are 110 °C, 6 w/w % of alkaline concentration with 4 hours of boiling and the optimum yield at defined optimum conditions was 0.26 (g/g). The maximum yield can be achieved at extreme conditions but that is not commercially viable.

Key words: Brown Algae, Sargassum fluitans, Alginate, Extraction, Process Optimization

1. Introduction:

Brown algae is the major class of macro algae which mainly consisting of alginates. Alginates exist naturally as polysaccharides in the cellular brown matrix of macroalgae of class phaeophyceae [1] [2]. Alginic acid is present in the form of calcium/magnesium salt in the cell wall of macro algae, which increases its flexibility and robustness [3] [4], [5]. The alginate seaweed naturally grows in deep sea, on seashore and rocky surfaces, which are collected and utilized for producing derivatives and food products. [5] [6] [7] [8, 9]. Alginates are also present in the form of extracellular polysaccaharides in bacteria such as *Azobacter*, *Pseudonmonas* and *Vinelan* [10]. Alginate is a polymer consist of M (- D manuronnic acid) and G (-L Guluronic acid) blocks in heterogenous proportions [2] [8] [7]. A variety of arrangements of M and G to form homo-polymer and block-copolymer are possible in alginate structure [3].

For the last two decades, the consumption of seaweed and their derivative products have raised significantly. The aquaculture growth of seaweed is the only way to maintain the consistent raw

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material supply and to meet the market consumption [2] [11] [12] [13]. Alginates have wide range of commercial applications. They are being used in food industry as emulsifiers, stabilizers and thickening agents [9] [5]. In medical industry they are used in tissue engineering and microencapsulation for drug delivery and for cancer therapy [14], [15]. In textile industry, the sodium alginate is an essential hydrocolloid in reactive printing. [16], [17]

Pakistan is one of the largest consumers of sodium alginate in textile sector and imports around 6000 MT/year. In Pakistan the wild brown algae is available on seashores, but it is not being utilized for any valuable product. The possible abundance of wild brown algae is estimated around 3500 MT/year in which five different types are included. On dry basis around 2000 MT/year of Sargassum, it is estimated that the most abundant wild brown algae are available on seashore of Karachi [18].

The molecular mass and composition of alginate depends upon many factors such as nature of algal species, cultivation area, seasonal variations and kind of tissue taken for sampling [8]. In addition, processing conditions of extraction of alginate from algae biomass also have significant impact on the structure, composition and yield of the final product isolated which in turn effects its availability in market for industrial usage [19] [17] [20] [21]. Therefore, optimization of extraction process and conditions is one of the critical steps to obtain maximum yield of isolated alginate derivative.

The extraction process of alginate involves several steps such as treatment with mineral acid, alkaline boiling, alginate precipitation with solvent and drying [22] [20] [23] [24]. In the first step of acid pretreatment, alginic acid is precipitated by removal of counter ions by proton exchange [22]. In the second step alginic acid is neutralized by alkali such as sodium hydroxide or sodium carbonate [19]. Neutralization reaction results in formation of soluble sodium alginate. The alginate is then re-precipitated in the form of alginic acid using different precipitating agents such as mineral acid or calcium chloride. Finally, the product is dried and milled. Alkaline boiling during extraction protocol is the essential step as it greatly influences the yield and composition of the product. Solubilization of alginic acid in alkali medium is highly dependent upon concentration of alkali, temperature and extraction time. Studies have been conducted to optimize these extraction conditions by varying one parameter and keeping the other two parameters constant. Mazumdar *et al* obtained optimum alginate yield of 13 % with 3 % alkali concentration at 86 °C for *Sargassum muticum* [20]. Hernanadez *et al* studied the effect of temperature and time on *Macrocystis pyrifera* and extracted 21% alginate at 90 °C for 3.5 hours residence extraction time [25], [20]. Fawzy and coworkers achieved 40 % alginate from *Sargassum latifolium* with 40 ml/g (alkali/alga ratio) at 45 °C for 3 hours treatment time [19]. Kokilam G. was extracted alginate from Sargassum wightii at 80 °C in form of alginic acid and obtained 14.2 % yield after 1 hour and 21.7 % after 5 hours [26].

In present study, the effect of extraction time, temperature and alkaline concentration has beeninvestigated on yield of alginate extraction from *Sargassum fluitans*. The extraction process hasbeen optimized with respect to the three variables using Box-Behnken experimental design.

2. Material and method:

Sargassum fluitans was collected from coastal area of Karachi, Pakistan. Karachi is capital of Sindh province and geographically located between longitude 66°30' to 68°E and latitude 24°30'N to 26°N as shown in figure 1. The exact sample points are at seashore of Abdul Rehman Goath which is located on Hawke's Bay In Keamari Town. The sample area geographically located between longitude 66°49'45"E to 66°49'20"E and latitude 24°51'15"N to 24°50'55"N and highlighted in figure -1.

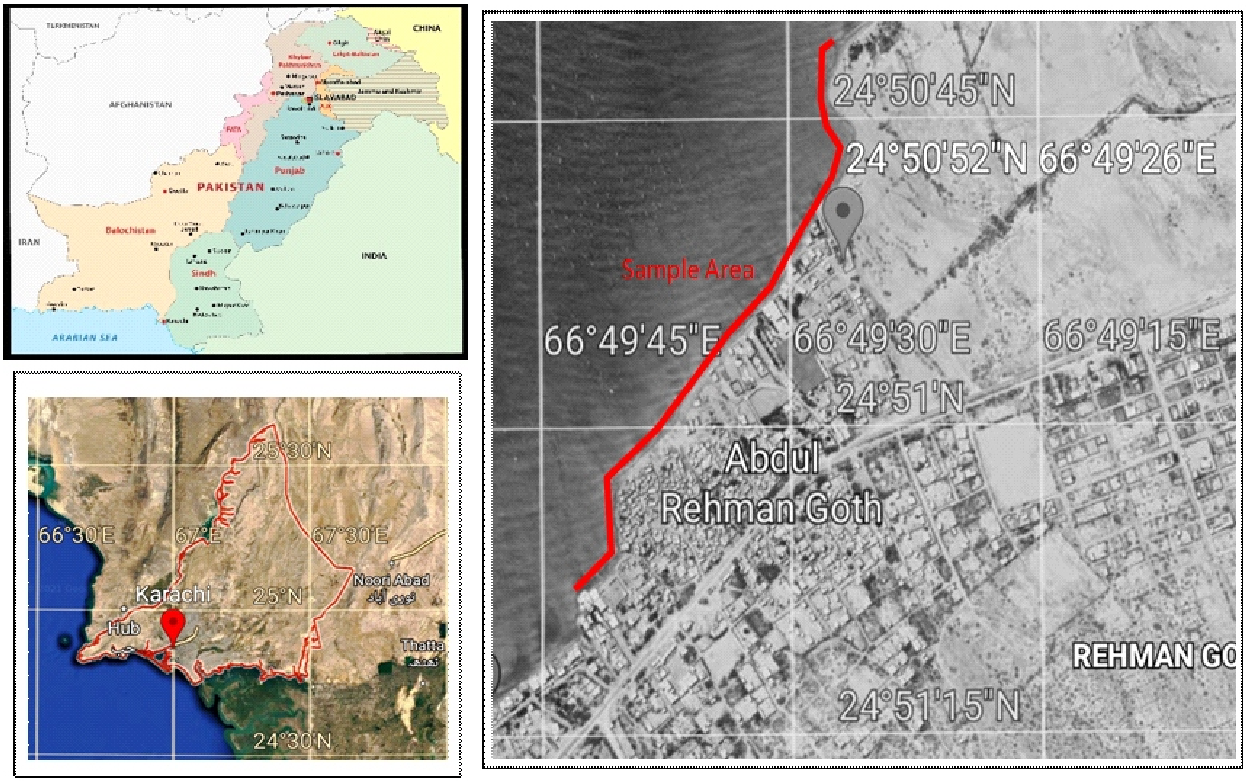

Figure 1: Geographical location of Pakistan and Karachi city (Left) and location of Abdul Rehman Goth, coastal area of Karachi for sargassum sample collection area (Right)

For study of alkaline boiling sodium carbonate (Merck 99.9 % Assay), hydrochloric acid fuming (Merck 37.0 % purity), and calcium chloride (Merck 98 % purity) were used. The whole experimentation was studied in a closed vessel having dimensions of 1liter made of Stainless steel, which were composed of agitator and electronic heating coils and a pressure gauge were fixed on the extractor to maintain the pressure of 1 to 2 bar.

2.1 Extraction process

(a) Washing and meshing

Macro particles were removed by simple washing and rinsing. A screw kneader was used for meshing. After first meshing, product was passed through 10 mm sieve, and again passed through screw kneader. Same process was repeated for 5 mm and 3 mm sieve sizes. The 3 mm particle size was used for further treatment. The overall process of extraction is shown in figure 2.

(b) Acid treatment (swelling):

The algal mass (3 mm) was swelled for 3 to 4 hours with 1% hydrochloric acid solution with pH maintained at 1- 2 to turn the insoluble algal mass to alginic acid [13], [21].

(c) Alkali treatment:

Acidified algal mass was treated with sodium hydroxide at different conditions of temperature, time and alkali concentration. For this purpose, acidified algal solution was divided into 3 portions. For the first portion, boiling time & temperature were kept constant, while the alkali concentration was varied. In second portion, the effect of temperature was studied while the concentration & boiling time were kept constant. In third portion, temperature & concentration were kept constant and effect of extraction time was studied.

(d) Acid precipitation and purification:

All the sodium alginate solution samples were precipitated with hydrochloric acid solution (10 %)

with drop wise dosing up to the pH 5 as shown in figure 2. [27]. The alginic acid after filtration and removal of water further reacts with sodium hydroxide solution to convert into sodium salt to avoid the gel formation due to the presence of water. In the end all samples were dried in oven at 120 ºC for one hour. Cloth filter was used for separation of cellulosic and fibrous material from extracted water [21].

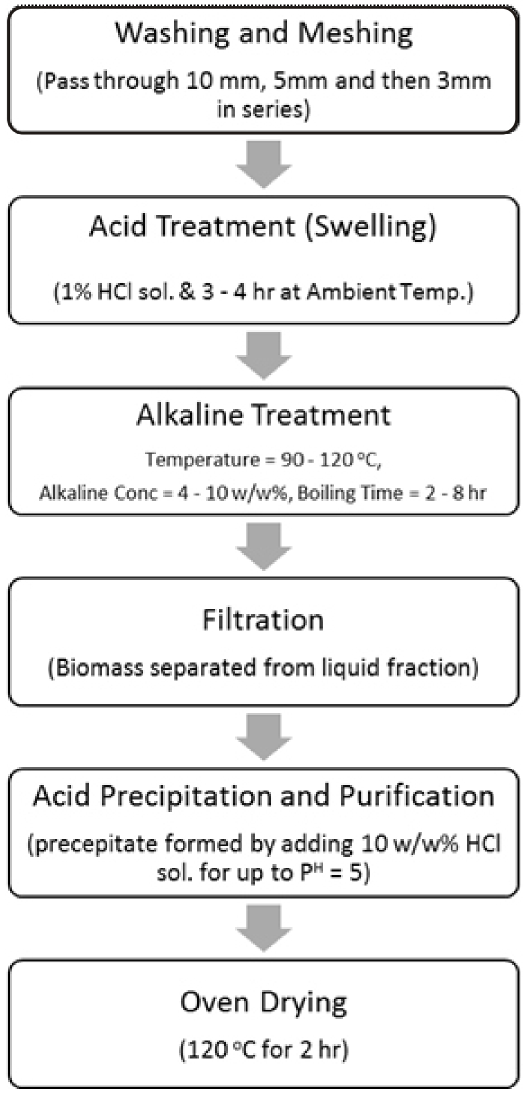

Figure 2: Process for alginate extraction process

2.2 Determination of alginate yield:

Overall yield is the function of alkaline concentration, boiling temperature and boiling time, and experimentally extracted from the sargassum fluitans which is measured in terms of the alginic acid mass extracted per mass of seaweed on dry basis as given in the equation 1.

$$Y = \frac{m_{ae}}{m_{sw}} \tag{1}$$

Where:

Y = Extraction yield of alginic acid (gram of alginic acid / gram of Sargassum on dry basis)

m_{ae} = mass of alginate extracted from seaweed (g)

m_{sw} = mass of seaweed on dry basis (g)

3 Results and discussion:

3.1 Effect of boiling temperature:

Boiling temperature is an important factor which directly depends on the concentration of alkaline solution and pressure of the alkaline extractor. It is clear from the graph that by increasing the temperature from 90 °C to 120 °C for constant boiling time of 4 hours and alkaline concentration maintained at 6%, the yield of the alginic acid was increased till 110°C as given in figure 3. It was observed that there is no increase after 110 °C. As we increase the temperature the solubilization of the seaweed will increase in alkaline solution so yield will remain same or will decrease slightly. Required reaction is simple ion exchange reaction in which acid swelled due to solubilization and calcium salt of alginic acid converts into water soluble sodium salt which was removed from insoluble algae residual biomass by simple filtration. Higher boiling temperature gave higher yield of alginic acid but there is no significant impact on yield below the boiling point of alkaline solution. Above the boiling temperature significant change observed in yield and the yield approached to its maximum value with 10 to 11% increase from 100 °C to 110 °C for 6% (by weight) of alkali concentration and similar increasing trend is continue up to 120 °C as shown in figure 3.

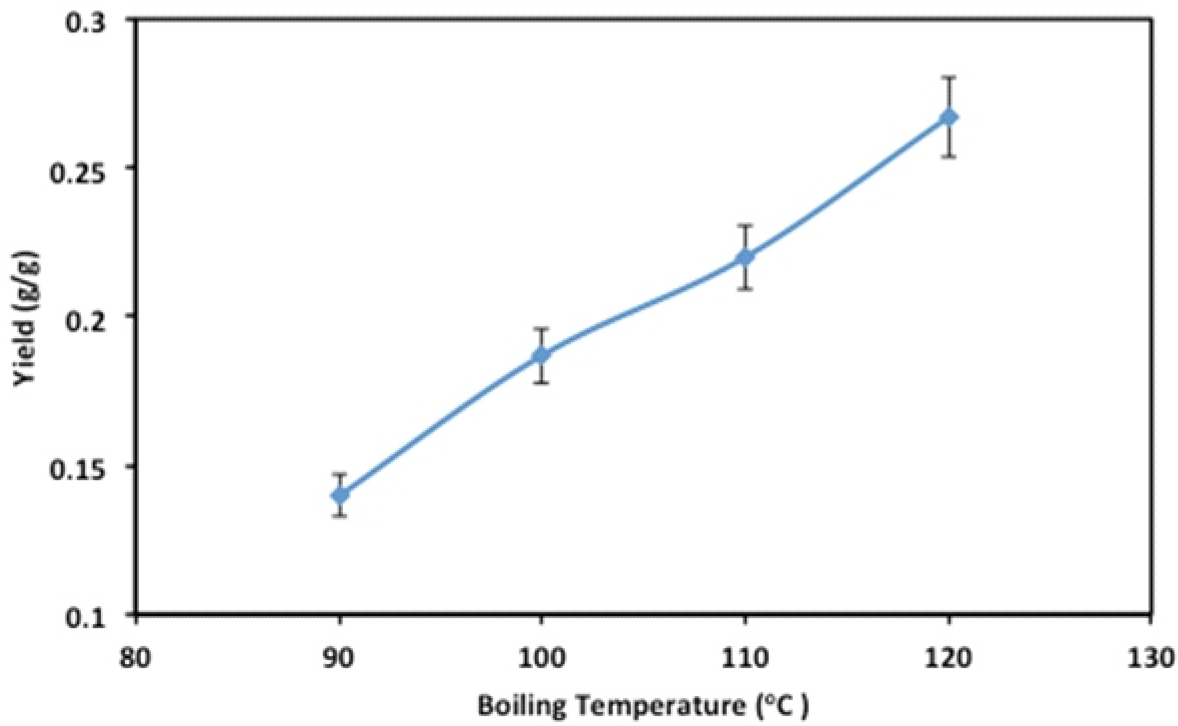

Figure 3: Alginic acid yield vs boiling temperature effect (6% alkaline concentration, and 4 hours boiling time)

3.2 Effect of alkali concentration:

Effect of alkali concentration on alginate yield was investigated at constant temperature and constant extraction time. Graph was plotted between the alkaline concentration and the yield of the alginic acid as shown in figure 4. It is observed from the figure 4, by increasing the alkali concentration from 3% to 10% at constant temperature of 110 ° C and residence time of 4 hours, the yield of the alginic acid increased up to 8%. However, there is no significant change in the yield of the alginic acid above 8% alkali concentration. As the concentration of the alkali increases, the concentration of sodium ions also increases consequently, which easily replaces calcium ions from cell wall of Sargassum. As a result, more alginate is isolated.

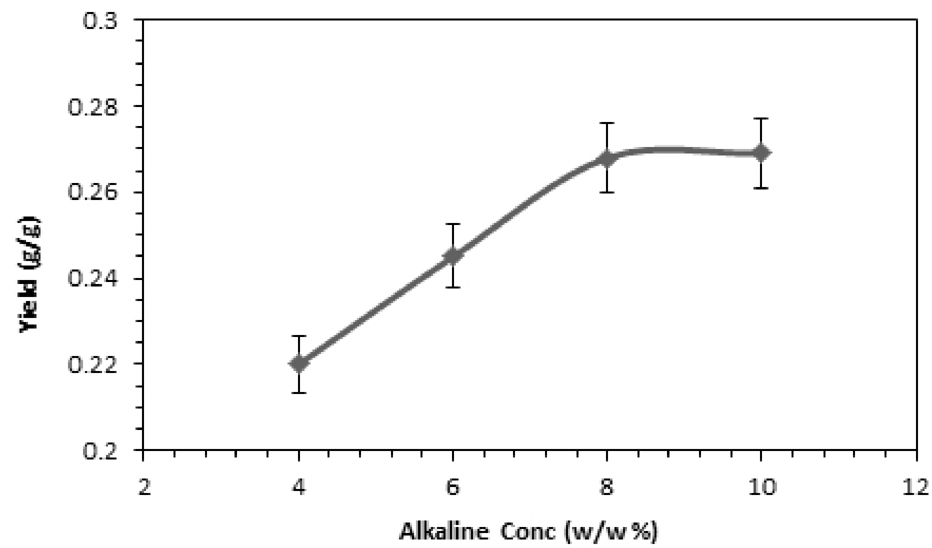

Figure 4: Alginate yield vs. alkali concentration profile at 110 °C for 4 hours boiling time.

The extreme value may not be more viable and economical due to high thermal load and the highest chemical cost at moderate operating conditions.

3.3 Effect of boiling time:

In alkaline boiling, the boiling time is third important operating parameter which has significant impact on alginate extraction yield. Alginate extraction experiments were conducted at different durations including 2, 4, 6 and 8 hours. Figure 5 clearly reflects that by increasing the alkaline boiling time from 2 hours to 8 hours at constant alkaline concentration of 6% and constant temperature of 110 °C, the yield of the alginate increased. However, maximum yield was achieved with boiling time of 6 hours. It was also observed that in first 2 hours the extraction was negligible. After 2 hours, the yield rapidly increased up to 6 hours and after that, there was no significant change observed till 8 hours as shown in figure 5. By increasing the boiling time, the residence time increased and the conversion also increased upto a specified time. After that specified time most of the reactant has been consumed, therefore no further increase in the yield was detected.

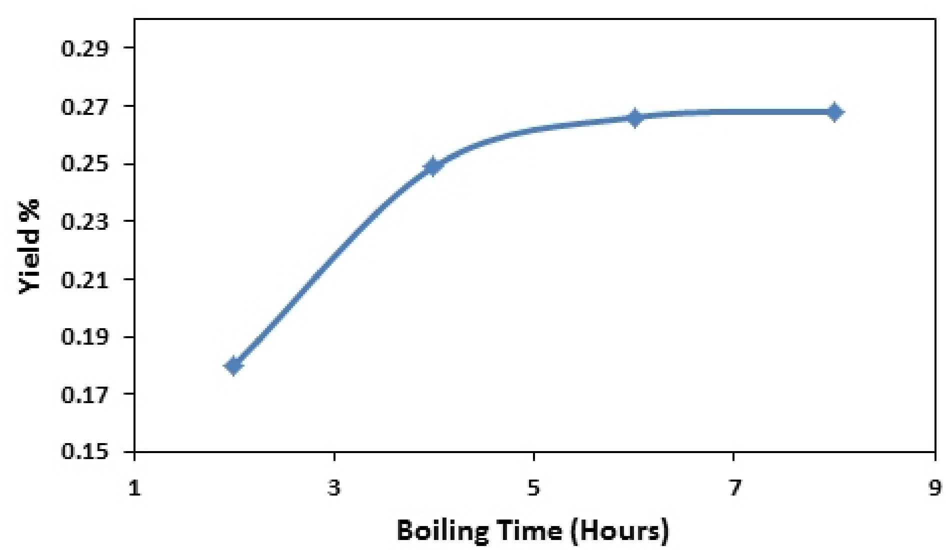

Figure 5: Alginic acid yield vs boiling time profile at 6% alkaline concentration and boiling temperature is 110 °C

3.4 Effect of alkali concentration at different boiling times

Different profiles are showing relation between alginate yield and alkali concentration for various boiling time durations at constant extraction temperature of 110 °C as shown in figure 6. The maximum yield can be obtained for residence time of 8 hours with 6% alkaline concentration at boiling temperature of 110 °C.

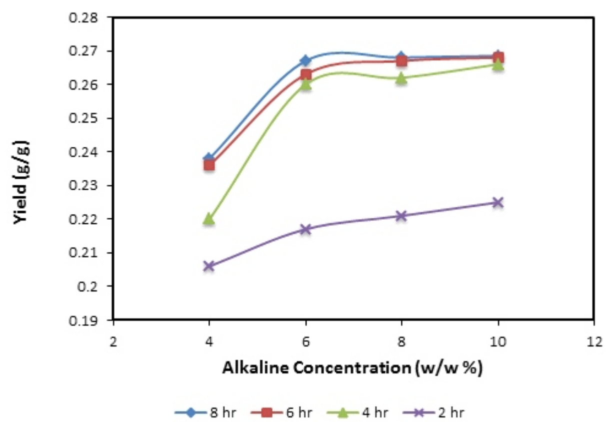

Figure 6: Alginate yield vs. alkali concentration curves at different boiling times (110 °C)

Alkaline concentration, boiling temperature and boiling time are the substantial and key factors in alkaline boiling to extract the alginic acid from the cell wall of. All these three parameters have direct influence on the extraction yield of alginic acid. The minimum temperature required to get major yield is exactly boiling or should be above the boiling temperature.

3.5 Effect of alkali concentration at different extraction temperatures:

Figure 7 shows the relation between alginate yield and alkali concentration for experiments conducted at temperatures of 90, 100, 110 and 120 °C, whereas the extraction time of 4 hours was kept constant. The figure clearly indicates that the maximum yield is achieved with 6% alkaline concentration at 120 °C.

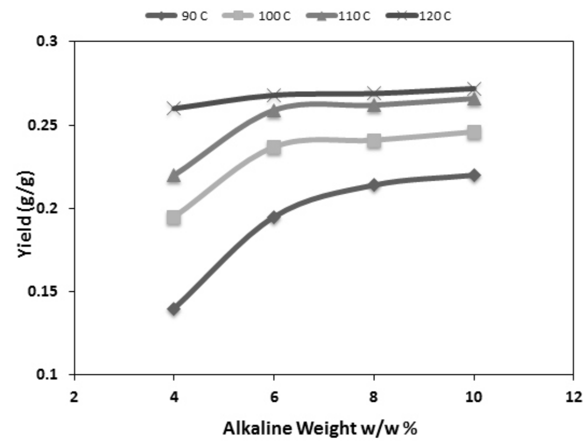

Figure 7: Alginate yield vs. alkali concentration curves at different boiling temperatures for 4 hours residence time

Here the peak surface of yield is placed between 100 to 120 °C and 6 to 10% alkaline concentration for 4-hour boiling. The boiling time is important but that should be in between of 1 to 4 hours and above that the change in yield in noteworthy.

The highest yield is achieved for 4hours boiling at 120 °C and 10% alkaline concentration which might be the viable set of operating condition for alkaline boiling of sargassum but that is only 1.4 % higher yield than yield achieved for 4 hour boiling at 120 °C and 6% alkaline concentration and only 4.7% higher value than the yield obtained for 4 hour boiling at 110 °C and 6% alkaline concentration.

3.6 Optimization of response variables by Box-Behnken model:

The three variables; alkali concentration, extraction time and temperature were optimized using Box-Behnken model. In Figure 8, the 3D surface plot represents the influence of boiling temperature and alkali concentration on alginate yield. Whereas in Figure 9, the 3D surface plot represents the influence of extraction time and alkali concentration on alginate yield. The optimum yield is achieved at 10% alkali concentration and 120 ° C for 6 hours boiling. There is no significant change observed in extraction of alginic acid between 4 to 6 hours, so residence time can be recommended between these time intervals for commercial operation.

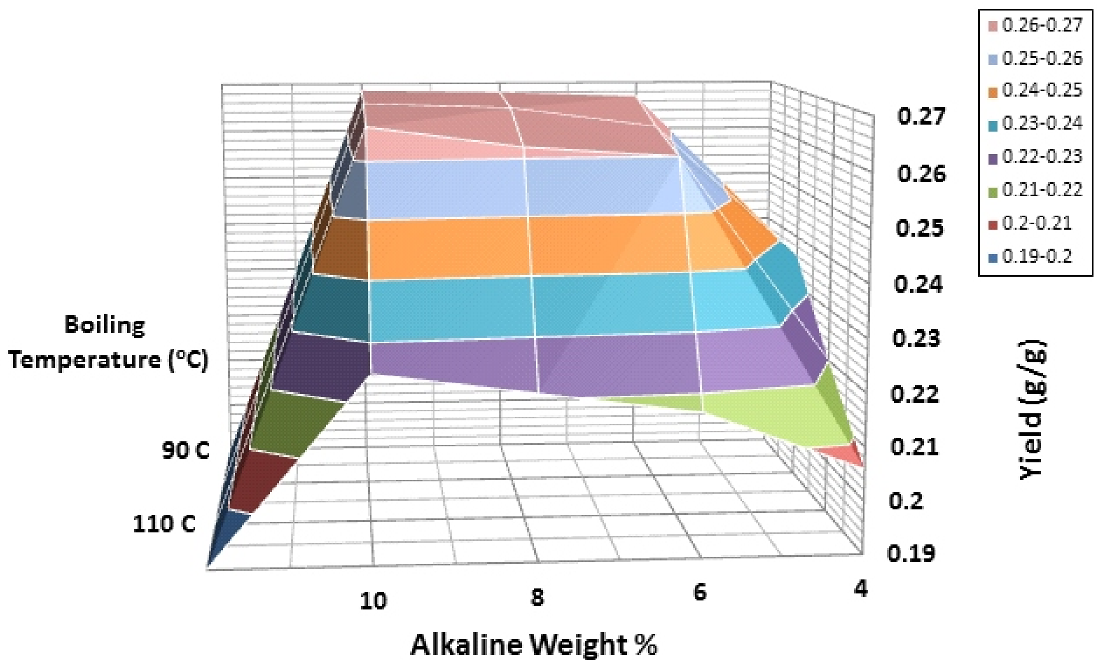

Figure 8: 3D surface plot; Alginate yield vs. alkali concentration and boiling temperature

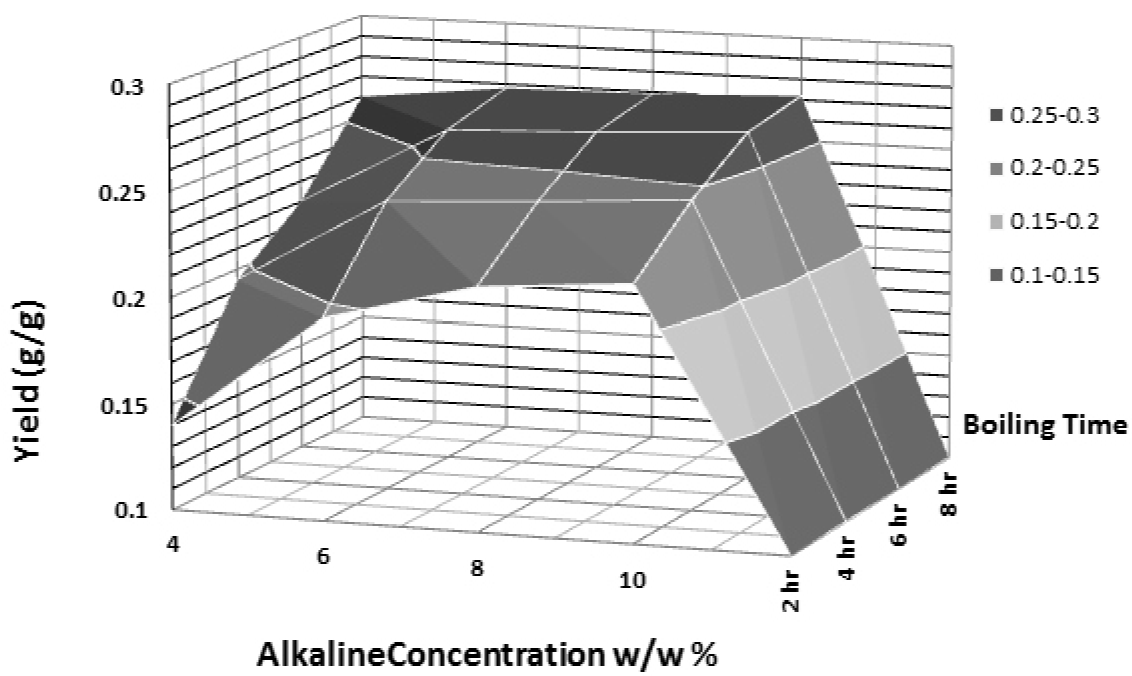

Figure 9: 3D surface plot; Alginate yield vs. alkali concentration and boiling time

Conclusion

Extraction of alginate from brown algae has been conducted. Product yield of the isolated derivative i.e. alginate is highly influenced by process parameters including alkali concentration, extraction time and temperature. Effect of each parameter has been evaluated individually. In addition, extraction process has been optimized for the three variables using Bex-Behnken approach. The optimized parameters are 10% alkali concentration for 6 hours' time at 120 °C. Brown algae offers significant potential for alginate production. Therefore, extraction methods can be further improved to enhance the extraction yield and minimize the operational cost. Moreover, new varieties of brown algae should be tested and applied for planned cultivation of specific type to maintain bulk production of alginic acid and sodium alginate.

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