Supplementary Information

Greener citrate-assisted extraction of sodium alginate: Process optimization and mechanical performance of alginate-based films

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Table S1. Treatment conditions and corresponding uronic acid composition and block conformation for sodium alginate extraction.

	Factor			Responses								
Std. Order	X_1: Time (h)	X_2: Temp. (°C)	X_3: Sodium Citrate (M)	M/G ratio	Fraction of G blocks, F_G	Fraction of GG blocks, F_{GG}	Fraction of M blocks, F_M	Fraction of MM blocks, F_{MM}	Fraction of alternating MG, GM blocks, $F_{GM,MG}$	G Block length, $\bar{N}_G$	M Block length, $\bar{N}_M$	Block distribution, η
1	1	21	0.16	1.10	0.48	0.22	0.52	0.27	0.25	1.87	2.06	1.02
2	4	21	0.16	1.02	0.49	0.24	0.51	0.25	0.25	1.96	2.00	1.01
3	1	50	0.16	1.19	0.46	0.26	0.54	0.35	0.20	2.32	2.76	0.79
4	4	50	0.16	0.86	0.54	0.25	0.46	0.17	0.29	1.85	1.60	1.17
5	1	35.5	0.05	1.23	0.45	0.20	0.55	0.30	0.25	1.78	2.20	1.02
6	4	35.5	0.05	0.86	0.54	0.28	0.46	0.20	0.26	2.06	1.77	1.05
7	1	35.5	0.5	1.37	0.42	0.17	0.58	0.33	0.25	1.70	2.33	1.02
8	4	35.5	0.5	1.11	0.47	0.23	0.53	0.29	0.24	1.96	2.18	0.97
9	2.5	21	0.05	1.36	0.42	0.18	0.58	0.34	0.24	1.76	2.40	0.98
10	2.5	50	0.05	1.12	0.47	0.22	0.53	0.28	0.25	1.87	2.10	1.01
11	2.5	21	0.5	1.45	0.41	0.17	0.59	0.35	0.24	1.70	2.47	0.99
12	2.5	50	0.5	1.35	0.43	0.18	0.57	0.33	0.25	1.71	2.31	1.02
13	2.5	35.5	0.16	0.89	0.53	0.23	0.47	0.17	0.30	1.78	1.58	1.19
14	2.5	35.5	0.16	0.94	0.52	0.26	0.48	0.23	0.26	2.00	1.88	1.03
15	2.5	35.5	0.16	1.02	0.49	0.26	0.51	0.27	0.24	2.06	2.11	0.96
16	2.5	35.5	0.16	1.00	0.50	0.22	0.50	0.22	0.28	1.76	1.76	1.13
17	2.5	35.5	0.16	0.96	0.51	0.24	0.49	0.22	0.27	1.87	1.79	1.09

The M/G ratio, molar fractions of monads (F_G, F_M), and dyads (F_{GG}, F_{MM}, F_{MG}, F_{GM}) were derived from the integrated areas of the ^{1}H NMR signals. Calculations followed: FG = AI/(AII + AIII); $F_M = 1\text{-}F_G$; F_{GG} = AIII/(AII + AIII); $F_{GM} = F_{MG} = F_G\text{-}F_{GG}$; $F_{MM} = F_M\text{-}F_{MG}$; $M/G = (1 - F_G)/F_G$; $\eta = F_{MG}/(F_M \times F_G)$(1,2).

Table S2. Uronic acid composition of Commercial sodium alginate reference (KIMICA, Tokyo, Japan).

M/G ratio	Fraction of G blocks, F_G	Fraction of GG blocks, F_{GG}	Fraction of M blocks, F_M	Fraction of MM blocks, F_{MM}	Fraction of alternating MG, GM blocks, $F_{GM,MG}$	G Block length, $\bar{N}_G$	M Block length, $\bar{N}_M$	Block distribution, η
1.01 ± 0.03	0.50 ± 0.01	1.20 ± 0.00	0.50 ± 0.01	0.20 ± 0.02	0.30 ± 0.02	1.67 ± 0.03	1.67 ± 0.07	1.19 ± 0.04

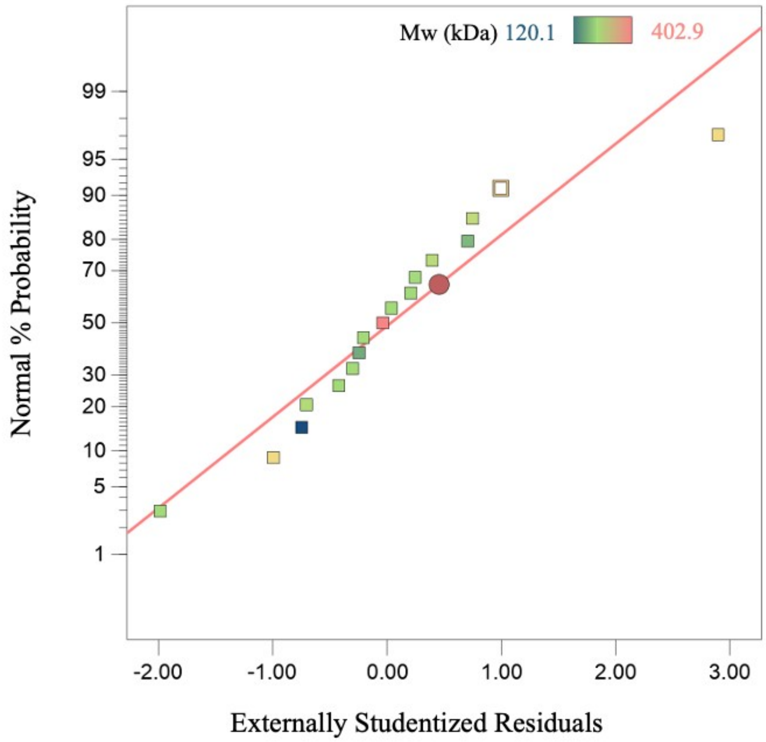

Fig. S1. Normal probability plot of externally studentized residuals for the molecular weight (Mw) model. The red line represents the expected trend if the residuals follow a normal distribution. Deviations from the line indicate minor departures from normality, prompting further investigation and transformation of the data.

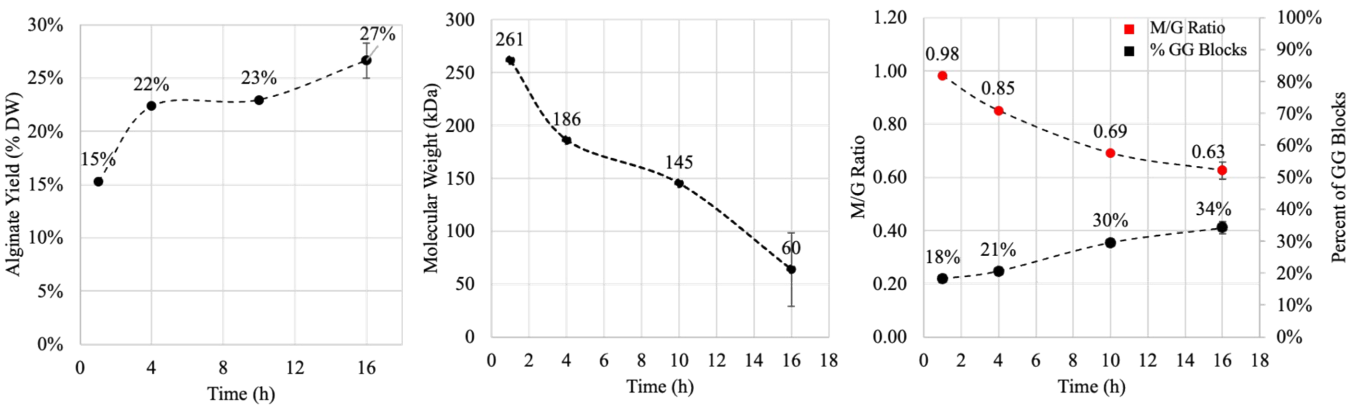

Fig. S2. Investigating the initial condition of chelate-assisted extraction time and the effect on yield, M/G ratio, and molecular weight of alginate extracted from *Macrocystis pyrifera*. These pre-experimental results informed the selection of time ranges for optimization in the main study. Molecular weight and block structure data were obtained using GPC and ^{1}H NMR, respectively. (16 h data points n = 8; other time internals n = 1). Lines are included only to aid visualization and do not imply interpolated trends between time points.

Preliminary single replicate trials were conducted using *Macrocystis pyrifera* biomass sourced from Canadian Pacifico Seaweeds Ltd. under extraction conditions: 0.2 M sodium citrate, 21°C, and 7.5 g dried feedstock. Extraction durations of 1, 4, and 10 h were compared, and a 16 h data point from (3) using the same kelp source. These screening experiments indicated that longer extraction times improved alginate recovery but were associated with reduced molecular weight (as low as 60 kDa at 16 h). Additionally, prolonged extraction increased G-block content, reflected by a lower M/G ratio.

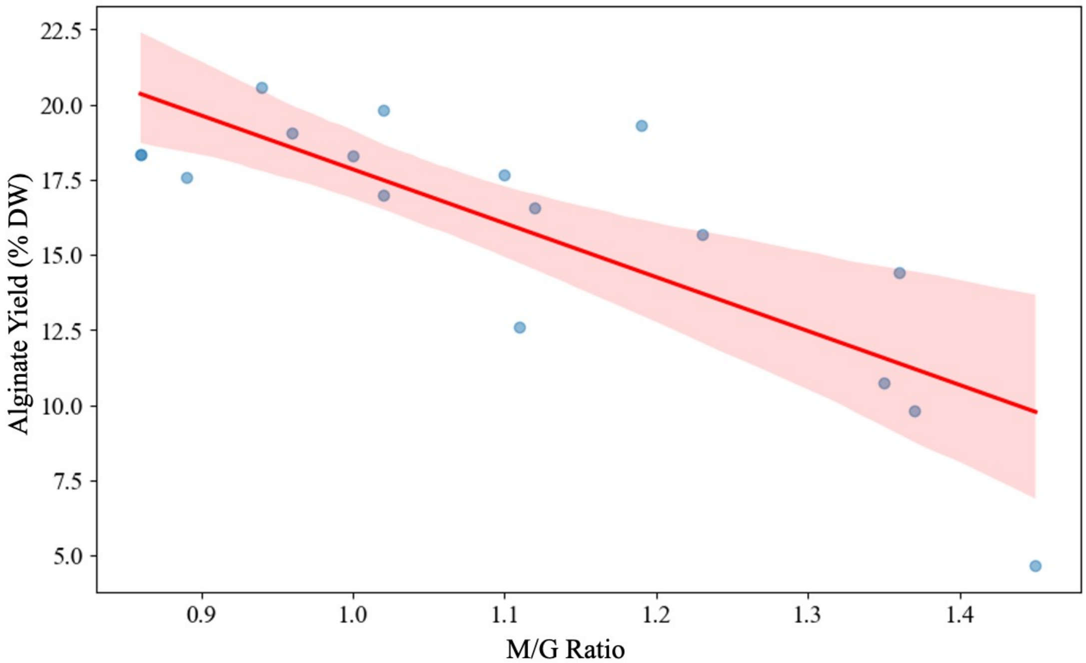

Fig. S3. Correlation between M/G ratio and alginate yield from *Macrocystis pyrifera* in chelate-assisted extractions.

A trend was observed in which alginate samples with higher yields also exhibited lower M/G ratios, indicating an increased proportion of G-blocks. The red line represents the linear regression fit (R^2 = 0.65), and the shaded region denotes the 95% confidence interval.

Environmental Assessment

This Supplementary Information outlines the calculations, assumptions, and data sources used to derive the chemical, water, and energy consumption values reported in the main text. The assessment compares the environmental performance of optimized and non-optimized sodium citrate extraction methods, focusing on water, chemical, and energy use.

The functional unit is 1 kg of sodium alginate product, consistent with Sterner et al. (2017) and Smith et al. (2024) (3,4). The system boundary encompasses alginate extraction from *Macrocystis pyrifera* (pretreatment through sodium alginate precipitation), representing a gate-to-gate assessment. Upstream and downstream processes, such as kelp drying and milling, product drying, equipment construction, and chemical transport, were excluded. As sodium citrate inventory data was unavailable in Ecoinvent v3.9(5), the compound was modeled as produced on-site from citric acid and sodium hydroxide, following the approach of Sterner et al. (2017) and Smith et al. (2024)(3,4).

1. Chemical and Water Consumption

Chemical and water use for each extraction protocol were determined from literature data(3,6). Reported concentrations and solution volumes were scaled to 1 kg alginate product, using the experimental yield from the upscaled optimized extraction (19.78%).

$$Yield\ \% = \frac{Alginate\ extract\ (kg)}{Algal\ biomass\ (kg)\ feedstock} \times 100$$

$$Algal\ biomass\ feedstock\ to\ produce\ 1\ kg\ alginate = \frac{Alginate\ extract\ (kg)}{Yield\%} \times 100$$

$$Scale\ up\ conversion\ factor = \frac{Algal\ biomass\ feedstock\ to\ produce\ 1\ kg\ alginate}{Experimental\ algal\ biomass\ feedstock}$$

Table S3. Experimental average extraction yield from upscaled optimized extraction, corresponding feedstock required to produce 1 kg alginate, and calculated scale-up factor from 24 g kelp feedstock.

Average Experimental Yield (%)	Feedstock to Produce 1 kg of Alginate (kg)	Scale-up Conversion Factor
19.78	5.06	210.65

Table S4. Summary of chemical and water inputs to produce 1 kg of alginate (based on upscaled optimized yield).

	Optimized (This Study)	**Non-Optimized (Smith et al., 2024; equivalent yield to this study – 19.78%)**
Deionized Water (L)	197.0	197.0
Ethanol (96%) (L)	51.8	51.8
HC1 (12M) (L)	10.7	10.7
Na_2CO_3 (kg)	1.1	1.1
$Na_3C_6H_5O_7$ (kg)	4.9	7.8
NaOH (kg)	0.1	0.1

Values represent total inputs per kilogram of alginate produced. Only Step 1 (extraction) was optimized; all subsequent steps, acidification, washing, and ethanol precipitation, remained identical across both protocols.

Tables 5 and 6 provide detailed material balances for both optimized and non-optimized extractions. Experimental inputs (based on 24 g kelp) were multiplied by the scale-up factor (210.65) to obtain total chemical and water requirements per 1 kg alginate.

Table S5. Detailed chemical and water use per process step for the optimized upscaled sodium citrate extraction process (this study), scaled from 24 g experimental feedstock to the functional unit of 1 kg sodium alginate.

	Experimental Inputs based on 0.024 kg Algal Biomass Feedstock					**Inputs to Produce 1 kg of Alginate (kg or L)**	
Process Step	**Solution Volume (L)**	**Chemical Concentration**	**Chemical**	**Chemical Input (kg or L)**	**Water Input (L)**	**Chemical Input (kg or L)**	**Water Input (L)**
1	0.72	0.125 M $Na_3C_6H_5O_7$	Na3C6H5O7 (kg)	0.02	0.72	4.89	151.67
1	0.01	1 M NaOH	NaOH (kg)	0.00	0.01	0.06	1.49
2	0.06	10 M HCl	12M HCl (L)	0.05	0.01	10.53	2.11
3	0.10	0.1 M HCl	12M HCl (L)	0.00	0.10	0.18	20.89
4	0.05	1M Na_2CO_3	Na2CO3 (kg)	0.01	0.04	1.12	9.42
5	0.10	96 % EtOH	96% EtOH (L)	0.10	0.00	21.07	0.00
6	0.20	70% EtOH	96% EtOH (L)	0.15	0.05	30.72	11.41

Table S6. Detailed chemical and water use per process step for the non-optimized sodium citrate extraction (adapted from Smith et al., 2024**; equivalent yield to this study – 19.78%**), scaled from 24 g experimental feedstock to the functional unit of 1 kg sodium alginate.

	Experimental Inputs based on 0.024 kg Algal Biomass Feedstock					**Inputs to Produce 1 kg of Alginate (kg or L)**	
Process Step	**Solution Volume (L)**	**Chemical Concentration**	**Chemical**	**Chemical Input (kg or L)**	**Water Input (L)**	**Chemical Input (kg or L)**	**Water Input (L)**
1	0.72	0.2 M $Na_3C_6H_5O_7$	Na3C6H5O7 (kg)	0.04	0.72	7.83	151.67
1	0.01	1 M NaOH	NaOH (kg)	0.00	0.01	0.06	1.49
2	0.06	10 M HCl	12M HCl (L)	0.05	0.01	10.53	2.11
3	0.10	0.1 M HCl	12M HCl (L)	0.00	0.10	0.18	20.89
4	0.05	1M Na_2CO_3	Na2CO3 (kg)	0.01	0.04	1.12	9.42
5	0.10	96 % EtOH	96% EtOH (L)	0.10	0.00	21.07	0.00
6	0.20	70% EtOH	96% EtOH (L)	0.15	0.05	30.72	11.41

Optimization in this study reduced the sodium citrate concentration from 0.2 M (non-optimized) to 0.125 M, decreasing citrate use by 37%. All other material inputs and water consumption remained unchanged, as the optimization focused only on the extraction step.

2.0. Energy Consumption

Energy consumption was estimated for the three key unit operations in the sodium citrate extraction process: heating, mechanical mixing, and solid-liquid separation. All calculations follow the methodology established by Langlois et al. (2012)(7) for industrial-scale alginate production and adapted in Smith et al. (2024). Laboratory-scale conditions (24 g kelp feedstock) were scaled to the functional unit of 1 kg sodium alginate, using the experimentally validated yield from the optimized extraction (19.78%, corresponding to 5.06 kg algal feedstock).

The goal of this section is to compare energy requirements between the optimized citrate process (1 h extraction at 49.5°C) and the non-optimized baseline (16 h at room temperature), with consistent assumptions for downstream operations.

2.2 Data Sources and Assumptions

Industrial energy intensities were obtained from Langlois et al. (2012) and applied using the same methodological framework as Smith et al. (2024). These values represent industrial-scale operating conditions for alginate extraction. Heating was modeled as natural gas combustion in an industrial furnace operating between 50-60°C, with an intensity of 937 Wh/h per kg feedstock. Mechanical mixing was powered by electricity using a standard industrial agitator, consuming 463 Wh/L per kg feedstock. Solid-liquid separation was modeled using a filter press as a surrogate for centrifugation, requiring 0.015 kWh/L of liquid removed(7). Heat losses or idle dissipation were neglected, consistent with Langlois et al. (2012). The heating requirement for 49.5°C in the optimized process was assumed equivalent to the 50-60°C baseline. Mixing energy scaled linearly with feedstock mass and duration, and separation energy was calculated based on the total supernatant volume removed, assuming negligible viscosity effects. Short agitation periods (less than 2 min) during acid washing and ethanol precipitation were excluded from the energy balance due to their minimal contribution.

Table S7. Energy consumption to produce 1 kg of alginate

	Optimized (This study)				**Non-optimized (adapted from Smith et al., 2024; equivalent yield to this study – 19.78%)**			
	Process Step	**Volume (L)**	**Unit per kg of alginate produced**		**Process Step**	**Volume (L)**	**Unit per kg of alginate produced**	
Citrate-assisted Extraction of Alginate	**Sodium Citrate Treatment, 49.5C, 1 h**				**Sodium Citrate Treatment, room temp, 16 h**			
	Mechanical Mixing (E)		2341	Wh	Mechanical Mixing (E)		37452	Wh
	Heating (NG)		4737	Wh				
Solid-Liquid Separation	Centrifuge (Separation) (E)	152	2275	Wh	Centrifuge (Separation) (E)	152	2275	Wh
Purification/ Isolation	**Acid Precipitation/Purification**				Acid Precipititation/Purification			
	Centrifuge (Separation) (E)	34	506	Wh	Centrifuge (Separation) (E)	34	506	Wh
	Neutralization Na2CO3, 20 min				**Neutralization Na2CO3, 20 min**			
	Mechanical Mixing (E)		780	Wh	Mechanical Mixing (E)		780	Wh
	Ethanol Precipitation				**Ethanol Precipitation**			
	Centrifuge (Separation) (E)	30	457	Wh	Centrifuge (Separation) (E)	30	457	Wh
Total:	Electricity		11096	Wh	Electricity		41470	Wh
	Natural Gas		4737	Wh	Natural Gas		0	Wh
	Electricity		**40**	**MJ**	**Electricity**		**150**	**MJ**
	Natural Gas		**17**	**MJ**	**Natural Gas**		**0**	**MJ**
		Total:	**57**	**MJ**		**Total:**	**150**	**MJ**

* E - electricity energy source, NG - natural gas energy (heating)

3.0. GHG Emissions

Data sources and analytical methods followed the approach of Smith et al. (2024). Greenhouse gas (GHG) emissions were characterized as Global Warming Potential over 100 years (GWP_{100}) using the ReCiPe 2016 Midpoint (H) impact assessment method(8). This method quantifies the integrated radiative forcing (W m-2-year kg^{-1}) of each emitted greenhouse gas over a 100-year time horizon and converts it into CO_2-equivalent units. The Hierarchist (H) perspective was selected as it reflects a consensus-based scientific interpretation that incorporates climate carbon feedback mechanisms, socio-economic baseline trends, and realistic adaptation potential.
Electricity and thermal energy generation were modeled using regional market datasets for British Columbia, Canada (BC-CA) within the Ecoinvent v3.9 database(5), ensuring that system boundaries captured region-specific energy mixes, grid emission intensity, and natural gas composition. This regionalization provides a more accurate representation of the environmental performance of the extraction processes under Canadian industrial conditions(3).

Table S8. Comparison of process input contributions to climate change impact, expressed as GWP_{100} (kg CO_2-eq per kg alginate), for optimized and non-optimized sodium citrate extraction processes.

	Optimized (This study) (kg CO_2-eq)	**Non-optimized (adapted from Smith et al., 2024; equivalent yield to this study – 19.78%) (kg CO_2-eq)**
Energy (total)	3.8	4.1
Heating (natural gas)	2.7	-
Electricity	1.1	4.1
Freshwater	0.3	0.3
Ethanol	55.0	55.0
Hydrochloric acid	1.2	1.2
Sodium carbonate	0.5	0.5
Sodium citrate	29.4	47.1
Sodium hydroxide	0.1	0.1
Total	90.3	108.3

References

1. Grasdalen H. High-field, 1H-n.m.r. spectroscopy of alginate: sequential structure and linkage conformations. Carbohydr Res. 1983 July;118:255–60.

2. Grasdalen H, Larsen B, Smidsrød O. A p.m.r. study of the composition and sequence of uronate residues in alginates. Carbohydr Res. 1979 Jan 1;68(1):23–31.

3. Smith HA, Cabling LPB, Leonard NA, Dubrawski KL, Buckley HL. Green Alginate Extraction from Macrocystis pyrifera for Bioplastic Applications: Physicochemical, Environmental Impact, and Chemical Hazard Analyses. ACS Sustain Resour Manag. 2024;

4. Sterner M, Ribeiro MS, Gröndahl F, Edlund U. Cyclic fractionation process for Saccharina latissima using aqueous chelator and ion exchange resin. J Appl Phycol. 2017 Dec;29(6):3175–89.

5. Wernet G, Bauer C, Steubing B, Reinhard J, Moreno-Ruiz E, Weidema B. The ecoinvent database version 3 (part I): overview and methodology. Int J Life Cycle Assess. 2016;21:1218–30.

6. Albers E, Malmhäll-Bah E, Olsson J, Sterner M, Mayers JJ, Nylund GM, et al. Influence of preservation methods on biochemical composition and downstream processing of cultivated Saccharina latissima biomass. Algal Res. 2021 May;55:102261.

7. Langlois J, Sassi J, Jard G, Steyer J, Delgenes J, Hélias A. Life cycle assessment of biomethane from offshore-cultivated seaweed. Biofuels Bioprod Biorefining. 2012;6(4):387–404.

8. Hauschild MZ, Goedkoop M, Guinée J, Heijungs R, Huijbregts M, Jolliet O, et al. Identifying best existing practice for characterization modeling in life cycle impact assessment. Int J Life Cycle Assess. 2013;18:683–97.